society for general
Microbiology

159th Meeting
11–14 September 2006
University of York

Abstracts

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Contents

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Plenary session

DNA replication, recombination, repair and the cell cycle

Initiation of DNA replication in chromosomes

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Duplication of chromosomes in eukaryotes is controlled in a cell cycle dependent manner by the establishment at origins of DNA replication of a licensed complex called the pre-replicative complex (pre-RC) as cells exit mitosis and proceed through G1 phase. The Origin Recogniton Complex (ORC), a six-subunit, ATP-dependent DNA binding protein is the central protein that recruits other pre-RC components including Cdc6, Cdt1 and MCM's. This complex is later activated by the cyclin-dependent protein kinases and Cdc7-Dbf4 kinase to put in motion a series of biochemical reactions that result in initiation of DNA replication at each origin in S phase. Structural studies show that the ORC-Cdc6 complex forms a ring-shaped complex that binds DNA. Both ORC and specific origin DNA sequences regulate the Cdc6 ATPase activity in this complex and specificity of DNA binding is determined by the ORC ATPase.

In addition to participating in the initiation of DNA replication, ORC subunits also participate in chromosome structure by forming heterochromatin and function in chromosome segregation by binding to centromeres and regulating centrosome activity. Depletion of ORC subunits not only compromises DNA replication, but affects chromosome structure and segregation of chromosomes in mitosis. The observations suggest an intimate link between the processes of DNA replication and segregation of the replication products.

Recombination at a site-specific replication stall

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We have established an assay to stall replication fork at a defined locus in the *S. pombe* model system. We use the RTS1 sequence (Replication Termination Site) that blocks the replication fork at the *MAT* locus in a uni-directional way. RTS1 was integrated on both sides of the *ura4*$^+$ gene on the chromosome III either as inverted repeats or within a palindrome. *rtf1*, a gene required for RTS1 stalling, has been placed under transcriptional regulation in order to control fork arrest.

2D gel analysis shows that replication can be stalled at the *ura4*$^+$ locus. The recombination machinery is required for normal growth in response to replication arrest and Rhp51 (Rad51) and Rad22 (Rad52 homologue) foci are induced. Quantitative PCR and Chromatin Immunoprecipitation have confirmed a direct association of the recombination protein with the stalled replication forks.

When the *RTS1* sequences are present as inverted repeats, intrachromosomal recombination leads to a switch of the *ura4* genes orientation. When the RTS1 sequence is present in a palindrome, chromosomal rearrangement occurs and this results in ~25% dicentric/acentric chromosomes through HR-dependent rearrangements. We will present a model that may explain the distinct outcome between these two systems.

Analysis of the archaeal / eukaryotic DNA replication machinery

S.D. Bell

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Abstract not received

Regulation of pre-RC assembly in yeast and human cells

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The eukaryotic cell cycle is designed to promote and coordinate the accurate duplication and apportionment of the genome during proliferation. The large genomes of eukaryotic cells are replicated from multiple replication origins during S phase. These origins are not activated synchronously at the beginning of S phase but, instead, fire throughout S phase according to a pre-determined, cell type specific program. Only after the entire genome is completely replicated do cells proceed into mitosis.

Ensuring that each origin is efficiently activated once and only once during each S phase is crucial for maintaining the integrity of the genome. This is achieved by a two-step mechanism. Pre-RCs, which are essential for initiation, can only assemble at origins during G1 phase when cyclin dependent kinase (CDK) activity is low. Initiation is then triggered by an increase in CDK activity at the end of G1 phase which also prevents new pre-RC assembly until CDKs are inactivated in the subsequent mitosis. The prevention of pre-RC assembly by CDKs is redundant: every component of the pre-RC is, in some way, inhibited by CDKs. Mechanisms regulating pre-RC assembly will be discussed. In particular, a novel mechanism by which Cdc6 function is inhibited by the mitotic CDK, Clb2/Cdc28, will be discussed in detail.

In human cells, CDKs including cyclin E-CDK2 can also play a positive role in pre-RC assembly in certain circumstances. We have found that phosphorylation stabilizes Cdc6 by preventing its association with the Anaphase Promoting Complex/ Cyclosome (APC/C). In cells re-entering the cell cycle, Cdc6 phosphorylation is executed primarily by Cyclin E-Cdk2. This stabilization by Cdk phosphorylation ensures that Cdc6 can accumulate prior to the licensing inhibitors geminin and cyclin A.

Interplay between DNA replication and recombination

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The DNA replication fork complexes that duplicate the genome with remarkable fidelity once every cell cycle are intrinsically processive but often encounter problems that damage the fork and provoke recombination. This recombination may be necessary in some cases to complete replication and maintain viability, but a number of studies have suggested that recombination proteins may also target stalled forks and initiate exchanges when there is no need. They may even cause harm by provoking unequal exchanges between repeated sequences. Faithful duplication of the genome may rely therefore not

only on the ability to minimize impediments to fork progression and to rescue any forks that have been damaged, but also to limit unnecessary recombination. A clear picture of how replication and recombination can become entangled is emerging through studies in *Escherichia coli*. Our recent studies have focussed on DNA replication in UV-irradiated cells. They indicate that replication forks stall at lesions blocking synthesis by the polymerase subunits and undergo extensive processing before being reassembled. During this period lesions are removed from the bulk of the genome by excision repair, clearing the way for the newly assembled replisomes to complete replication without much further hindrance, and with the minimum of recombination.

Visualizing steps of DNA recombination at the level of single molecule

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Abstract not received

Structure and mechanisms of RecBCD: a machine for processing breaks in DNA

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Double strand breaks in bacterial cells can result from a variety of things including collapsed replication forks or other DNA damage. One mechanism for repair of breaks involves the multifunctional enzyme complex, RecBCD. RecBCD comprises two distinct DNA helicase subunits, a number of differentially regulated nuclease activities, and the ability to recognize a recombinational hotspot called Chi. In order to understand more about the molecular basis of these activities we have determined the crystal structure of RecBCD complexed with DNA. The structure reveals the basis for the two different helicase activities and explains the regulation of nuclease digestion. The structure also suggests how the enzyme might be able to scan DNA for Chi sequences as the DNA passes through the protein complex. Recent work has determined the structure of a phage inhibitor of RecBCD and the likely mode of inhibition will be discussed.

Choreography of the DNA damage response in budding yeast

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The cellular response to DNA double-strand breaks (DSBs) involves early recognition by Ku70/80, the MRX (MRN) complex and RP-A, which leads to subsequent activation of checkpoint proteins. The coordinated recruitment of checkpoint and recombination proteins into repair centers at lesions demonstrates the orchestrated nature of DSB repair. By examining the movement of fluorescently marked proteins to repair foci in *S. cerevisiae*, we show that DSBs induced by ionizing radiation (IR) or I-*Sce*I endonucleases are efficiently processed for homologous recombination during S phase. However, in G1 cells, only IR-induced breaks are processed. This differential processing depends on the Ku70/80 complex showing that during G1, the DNA repair machinery distinguishes between DSB ends that require further processing for homologous recombination from those suitable for non-homologous end-joining.

To search for new mutations involved in DNA damage recognition and processing, we developed efficient methods to screen. We screened the yeast gene disruption library for mutations that alter spontaneous Rad52 focus formation and uncovered genes in the nuclear pore complex as well as genes involved in sister chromatid cohesion and chromatin remodeling. We have also identified 18 unknown genes that we have named *IRC1* to *IRC18*, which when deleted *i*ncrease *r*ecombination *c*enters.

Links between replication, recombination and chromosome segregation

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DNA replication divides the circular *Escherichia coli* chromosome into equal arms [replichores]. Visualization of pairwise combinations of multiple genetic loci reveals that the two replichores occupy separate nucleoid halves, with the replication origin between; positions of loci on each replichore recapitulate the genetic map. Sequential replication-segregation regenerates the <left-right> structure by sequentially layering newly replicated replichore DNA to specific inner and outer edges of the developing sister nucleoids. Replication fork-dependent locus positions are imprinted, so that in most generations the <left-right> chromosome orientation in a mother cell is recreated as a <left-right-left-right> arrangement of sister chromosomes in daughter cells. Co-visualization of genetic markers with a range of replisome components supports a model in which separation of sister replisomes on the long axis of the cell early in S phase establishes the observed segregation pattern. Replication forks can be blocked site-specifically by DNA-bound TetR repressor. Replisome and replisome-associated components remain at the stalled forks for extended periods, with replication-segregation resuming rapidly after the replication block is removed. The ways in which the DNA translocase, FtsK, links replication, recombination and segregation will also be discussed.

Molecular and functional insights into the DNAdamage response

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Work in my laboratory aims to decipher the mechanisms by which eukaryotic cells detect various forms of DNA-damage and then signal the presence of these structures to the DNA-repair and cell-cycle machineries. As many aspects of the DNA-damage response have been highly conserved throughout eukaryotic evolution, we are analysing proteins involved in these pathways both in mammalian cells and in the yeast *Saccharomyces cerevisiae*.

A major goal of our research is to understand in detail how cells respond to DNA double-strand breaks (DSBs). In this seminar, I will first provide an overview of how cells respond to DSBs and will describe the pathological consequences of such responses going awry. I will then explain how recent work in my laboratory has provided new molecular insights into how cells detect DSBs, trigger DNA-damage signalling events and mediate DNA DSB repair.

Mechanisms controlling the integrity of replication forks

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Abstract not received

Replication fork recovery in yeast

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Abstract not received

Checkpoint responses and repair of a broken yeast chromosome

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A chromosome double-strand break (DSB) activates the DNA damage checkpoint, including both phosphorylation of histone H2AX and inhibition of cell cycle progression. We are interested in defining in more detail how the checkpoint is activated and maintained, and how it is extinguished once DSB repair is completed. A combination of DNA analysis, chromatin immunoprecipitation of recombination proteins and microscopic visualization of fluorescently-tagged chromosome regions allows us to define many steps in the search for homology and the subsequent completion of recombination. A DSB can be repaired by gene conversion or in some cases by break-induced replication. The initial homologous pairing steps are similar, but in BIR there is a several-hour-long delay in initiating new DNA synthesis. The delay appears to reveal a topological sensing whether two DSB ends are oriented toward each other on the same template, as in gene conversion. One the DSB is repaired, the DNA damage checkpoint is turned off and the γ-H2AX over a >50-kb region is rapidly removed. Genes required for recovery from arrest include PP2C phosphatases, the Srs2 helicase and the retrograde golgi associated retrograde transport complex, GARP, which we speculate is involved in regulating transport of a key checkpoint component into the nucleus.

Effects of telomere length on telomerase action in Baker's yeast

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Baker's yeast telomerase is a multi-subunit complex: Est2p is the catalytic reverse transcriptase and Est1p plays roles in both recruitment and activation of telomerase. Yeast telomeric DNA is assembled into a non-nucleosomal protein-DNA complex called the telosome. Two components of the telosome, Cdc13p (which binds the single-strand G-tail) and the heterodimeric yKu complex (which binds the junction of single-stranded and double-stranded telomeric DNA) are positive regulators of telomerase since both play roles in recruiting telomerase to telomeres. Two other telosomal proteins, Rif1p and Rif2p, act synergistically to inhibit telomerase. In lower and higher eukaryotes, telomerase preferentially lengthens short telomeres. The mechanism(s) underlying this preference are unknown. We anticipate that a protein that is responsible for the length dependence of telomerase action will bind differentially to short versus wild type length telomeres. To determine if a given protein is associated preferentially with short telomeres, we used chromatin immuno-precipitation in combination with a genetic system developed in the Gilson lab. This system allows one to generate a single short telomere in G1 phase cells that have otherwise wild type-length telomeres. For these experiments, we monitor protein association at both a shortened telomere and an unmodified wild type telomere in the same cell population in the S phase after telomere shortening. In addition, association is monitored in a control strain in which the shortened telomere has been allowed to re-elongate to wild type length. Using this short telomere system, we find that Cdc13p, yKu, and the Rif proteins bind equally well to short and wild type-length telomeres. Thus, the binding or loss of binding of these proteins does not mark a short telomere for elongation. However, both Est1p and Est2p preferentially bind the shortened telomere. This preference is at least partially dependent on Tel1p, a yeast ATM checkpoint kinase that leads to telomere shortening when deleted. We are currently assessing the association of Tel1p with short versus wild type-length telomeres.

Control of DNA damage tolerance by ubiquitin and SUMO

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Tolerance to replication-blocking DNA lesions is achieved by the ubiquitylation of PCNA, the eukaryotic processivity clamp for replicative DNA polymerases. While monoubiquitylation in response to treatment with genotoxic agents induces mutagenic lesion bypass by recruitment of damage-tolerant polymerases, polyubiquitylation facilitates an error-free damage avoidance mechanism that makes use of the genetic information encoded by the undamaged sister chromatid. During S phase in the absence of exogenous DNA damage PCNA is modified by the ubiquitin-like protein SUMO, which – like ubiquitin – contributes to spontaneous mutagenesis. As both modifiers target the same attachment site on PCNA, an antagonistic relationship between ubiquitin and SUMO had previously been postulated.

Our recent analysis of PCNA modifications in the yeast *Saccharomyces cerevisiae* has revealed that SUMO and ubiquitin do not act as antagonists, but actually cooperate in responding to replication-stalling DNA lesions. I will describe the mechanisms by which the two modifiers change the properties of the clamp and will discuss our observations that give insight into the cellular signals necessary for PCNA modifications.

Genomic instability and cancer: insights from analysis of Bloom's syndrome

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Genomic instability appears to be a universal feature of cancers. Evidence that loss of genome stability can drive tumorigenesis comes from the existence of certain heritable disorders of man that are associated with inherent genomic instability and cancer predisposition. Our laboratory focuses on one of these disorders, Bloom's syndrome (BS), which is associated with proportional dwarfism, sunlight sensitivity and a greatly increased incidence of cancer. At the cellular level, BS is characterized by a high frequency of sister chromatid exchanges (SCEs) and other homologous recombination events that derive from crossing over. The BS gene product, BLM, is a member of the RecQ helicase family. BLM forms a functional complex with topoisomerase IIIα and RMI1 (BLAP75). One role for this complex is to catalyze the so-called 'dissolution' of recombination intermediates that contain a double Holliday junction. BLM, topoisomerase III and RMI1 are all conserved in *S. cerevisiae*, and our ongoing studies in that organism aim to identify other factors that assist this complex in the dissolution of recombination intermediates. We have also developed a model system for analysis of the process of replication fork regression, and will present evidence that BLM can promote fork regression *in vitro*.

Cells & Cell Surfaces Group session

Antigenic and phase variation

Epigenetic phase variation: DNA modification as a means to obtain population heterogeneity

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In *Escherichia coli*, one of the mechanisms of phase variation is epigenetic. This means regulation is not associated with a DNA sequence change but instead is mediated by Dam-dependent DNA methylation. This controls phase variation of the *pap* family of fimbriae and of a family of Ag43 outer membrane proteins at the level of transcription initiation. Post-transcriptional regulation of Ag43 expression appears to occur as well. Ag43 encoding genes are present in many *E. coli* isolates and the protein mediates autoaggregation, is antigenic and can affect biofilm formation. The molecular mechanism underlying Ag43 phase variation is Dam and OxyR-dependent. The On and Off state are mediated by methylation-dependent binding of OxyR. At *agn43* this binding site contains three Dam target sequences that are essential for this phase variation. Currently we are focusing on understanding how the DNA methylation states can be passed on to the next generation so efficiently that a change in only 1 in 1000 cells/generation occurs, but yet allow for that rare change. Our data suggest that differential interactions between Dam, SeqA and OxyR at hemimethylated DNA play an important role. A model for this regulation will be presented and the implications for Ag43 expression discussed.

Role of the type IV pili of pathogenic *Neisseriae* in the infection of human cells

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The pathogenic *Neisseriae*, including meningococci and gonococci, express several variable protein components on their surfaces. One of the striking examples is the pilin (PilE), the main subunit component of the type IV pili that play an essential role in the pathogens' mucosal adherence. Our laboratory has previously contributed to the clarification of the genetic mechanisms underlying the variation of pilin. However, we were also fascinated by the fact that such variable structures are capable of binding to an apparently conserved receptor present only on human cells. This was explained by identification of the pilus associated PilC protein as the key adhesion conferring the binding of piliated gonococci to human epithelial and endothelial cells (1–3). An intriguing phenomenon following the pilus mediated attachment however is the formation of bacterial microcolonies which seems to depend on host cellular functions. In this context we observed a recruitment of caveolar markers to the neisserial microcolonies. Caveolae are plasmalemmal invaginations implicated in signal transduction and vesicular transport. A principle protein of caveolae is caveolin-1. Perturbation of caveolar stability induced by cholesterol depletion, abolishing caveolin-1 recruitment or down-regulation of caveolin-1 expression prevent the microcolony formation on the surface of host cells and induce bacterial invasion, in a Src kinase activation-dependent fashion. Thus, caveolae function in the regulation of initial neisserial attachment to the host cells and control early bacterial entry.

References: (1) Rudel, T., Scheuerpflug, I., Meyer, T.F. (1995). *Neisseria* PilC protein identified as type-4 pilus tip-located adhesin. *Nature* **373**, 357–359. (2) Kirchner, M., Heuer, D., Meyer, T.F. (2005). CD46 independent binding of neisserial type IV pili and the major pilus adhesin PilC to human epithelial cells. *Infect Immun* **73**, 3072–3082. (3) Kirchner, M., Meyer, T.F. (2005). The PilC adhesin of the *Neisseria* type IV pilus – binding specificities and new insights into the nature of the host cell receptor. *Mol Microbiol* **56**, 945–957.

Cross-talk between fimbrial clusters in uropathogenic *Escherichia coli*

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Analysis of the annotated sequence of an *Escherichia coli* clinical isolate associated with pyelonephritis, *E. coli* CFT073, has revealed the presence of multiple fimbrial operons. These include two copies of pyelonephritis-associated pili (Pap), type 1 fimbriae, an Srf/F1C hybrid, the recently characterized F9 fimbriae and several uncharacterized chaperone/usher class fimbriae. This multiplicity is repeated across other *E. coli* pathotypes and related enteric pathogens such as *Salmonella*. In addition to fimbrial adhesins, these bacteria also use non-fimbrial adhesins or outer membrane proteins to interact with host factors and this is in addition to capsule and flagellal/type III secretion systems. At the level of the single bacterium, this surface presentation needs to be co-ordinated to limit antigen presentation and functional hindrance. While this is often achieved through 'global' environmental regulation, the expression of most fimbriae, and many other surface proteins, is phase variable and considered stochastic. This talk will cover research into regulatory mechanisms that limit co-expression or may favour sequential expression of fimbrial adhesins at the single cell level.

Phase variation in rhizosphere pseudomonads

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Abstract not received

Immune evasion in African trypanosomes

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The African trypanosome *Trypanosoma brucei* is a unicellular protozoan parasite which causes African Sleeping Sickness in subSaharan Africa. Trypanosomes are exposed to constant immune attack while multiplying in the mammalian bloodstream. We would like to understand the molecular mechanisms behind this immune evasion. Bloodstream form *T. brucei* is covered with a dense coat of Variant Surface Glycoprotein (VSG), which is switched during a chronic infection allowing escape from host antibodies. The active *VSG* gene is transcribed from one of many telomeric *VSG* expression sites. In order to investigate the role of the VSG itself, we have blocked VSG synthesis using inducible RNAi. We find that

VSGRNAi results in a specific precytokinesis cell-cycle arrest and then death. After the induction of RNAi against a given VSG variant, revertants eventually appear which have switched to a new VSG not recognized by the RNAi. VSGRNAi has allowed us to develop a powerful means for studying VSG switching in the absence of immune selection completely *in vitro*. These new technologies are providing us novel insights into the molecular mechanisms of antigenic variation in African trypanosomes.

Analysis of the repertoire and function of fimbrial operons in *Salmonella*

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Salmonella enterica is associated with food poisoning and over 2,400 serovars exist. The serovars are 85–100% identical at the genome level but infect different hosts, causing different diseases. We sought to determine if variation in the repertoire or sequence of fimbrial loci may explain these differences.

We examined the distribution of fimbrial loci in the sequenced strains of *S. Enteritidis*, *S. Typhi, S. Typhimurium, S. Choleraesuis, S. Gallinarum, S. bongori, E. coli* K12 and *E. coli* O157 using comparative genomic techniques. Up-to 13 conserved fimbrial operons were identified, but the host restricted serovars contained a higher proportion of pseudogenes.

The high number of fimbrial operons may provide flexibility to colonize different niches. Fimbriae are subject to phase variation which may assist in evading the host immune response.

We systematically mutated the fimbrial operons of *S. Enteritidis* and *S. Gallinarum* using lambda red mutagenesis and assessed their function using chick kidney cells, human epithelial cells, mucous secreting cell lines and a macrophage derived cell line.

The program for antigenic variation during relapsing fever

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The vector-borne bacterium *Borrelia hermsii*, a relapsing fever agent, switches expression of a surface protein between different antigenic variants. Analogous programmed systems of antigenic variation occur in African trypanosomes and *Plasmodium falciparum*. In these examples switch rates to individual variants differ over a wide range. We studied how *B. hermsii* determines switch rates in experimental infections. Unexpressed loci of variant antigens copy into a single expression site at rates determined mainly by extragenic features of silent loci rather than the sequence similarity between silent site and expression site. Two extragenic elements in particular determine switch rates. One set of elements overlaps the 5′ ends of silent loci and single expressed gene; greater sequence identity between a pair of elements was associated with a higher switch rate. The second set's elements flank the expression site on the 3′ side or occur at variable distances downstream from silent loci; the nearer the element to a silent locus, the greater the switch rate of that locus into the expression site. In combination, these two features of the genome provide a mechanism to modulate switch rate, whereby silent loci form a hierarchy of switch rates into the expression site. While the switching hierarchy causes changes in individual cells that are stochastic, ordering of antigenic variants within hosts is semi-predictable.

Phase variation rate confers an adaptive advantage on *Neisseria meningitidis*

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Rapid and reversible generation of phenotypic variants, phase variation, is mediated in many bacterial pathogens by simple sequence repeat tracts in specific loci. Variants are generated prior to, and selected for by, alterations in the environment. In the presence of human serum, mAb L3B5 is bactericidal for *Neisseria meningitidis* strains with a phosphoethanolamine moiety on a specific position of their lipopolysaccharide molecules. In strain 8047, attachment of this moiety is phase variable due to a mononucleotide repeat tract in *lgtG*. Repeated cycles of growth in the presence of mAb L3B5 and human serum leads to a rapid increase in the proportion of B5 non-reacting variants. The parental strain is out-competed in this assay by a *mutS* mutant, which exhibits an elevated phase variation rate. Additionally, in passive protection experiments with mAb L3B5, the *mutS* mutant is the predominant cause of bacteraemia following co-inoculation with
the wild-type strain. These assays demonstrate that phase variation rate is a critical determinant of the ability of bacterial populations to survive an adaptive immune response.

Helicobacter pylori lipopolysaccharide phase variation and interaction with the innate immune system

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Like several other pathogens, *H. pylori* displays phase variation in surface antigens. This stochastic ('random') process generates diversity within a single clonal population at little cost of energy; the outside circumstances then serve as Darwinian pressure to select for survival of the fittest variant.

We have discovered that *H. pylori* phase variation takes place through DNA slippage in C-tracts, present in several glycosyltransferase genes. Upon replication a Cn tract may yield both Cn, Cn+1 and Cn-1 tracts, which in the latter two cases cause down-stream frame-shifts. Through this mechanism, glycosyltransferases are reversible switched on-and-off, with concomitant differences in LPS structure. Within a single clone, at least five different LPS phase variants were isolated.

We have investigated the switch from Lewis x to i-antigen. This involved phase variation in an α3-fucosyltransferase (α3FT) that indeed was mediated by a C-tract.

The big question hence is: which pressure would select how for what LPS variant?

We discovered that the dendritic cell lectin DC-SIGN (as well as surfactant protein D), both calcium dependent (C-type) lectins, bind to fucosylated antigens like Lewis x but not phase variants expressing the i-antigen.

One variant expressing Lewis x bound well to DC-SIGN-Fc, to DC-SIGN-transfected Raji cells and human monocyte-derived dendritic cells. The frame-shifted (α3FT) variant did not interact at all. These differences in binding were reflected in differences in cytokine expression when incubated with human DC: as compared to the non-binding variant, the DC-SIGN binding variant induced relatively more IL-10; IL-12 induction did not differ between the variants. In a

more complex system, bacteria were incubated with DC and T-cells and intracellular IL-4 and IFNγwas determined by FACS analysis. As compared to the binding variant, the non-binding variant induced relatively more IFNγ and less IL-4. Thus, a difference of a single C, which led to the difference in presence of afucose, caused also the difference between an immunostimulatory Th1 variant (less IL-4 and IL-10, more IFNγ and a more immunosuppressive Th2 variant.

Based on data from KO studies in mouse cytokine genes we propose that the cytokine pattern of a given mammalian host constitutes the Darwinian pressure that selects the best fitting variant (with regard to cytokine induction). We plan to test this hypothesis by experimental infection in mice.

Clinical Microbiology Group / Systematics & Evolution Group joint session

Mycobacteria in clinical practice

Genealogy of currently circulating strains of *Mycobacterium tuberculosis*

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Recent genomic and genetic studies on *Mycobacterium tuberculosis* and other members of the *M. tuberculosis* complex have identified numerous genetic markers that now enable us to identify and differentiate the strains very efficiently. In this respect, the presence or absence of certain regions of difference (RD) have served to redefine the evolutionary pathway of the *M. tuberculosis* complex and this novel scheme has since then been completed by data on selected single nucleotide polymorphisms (SNP) and microdeletions, especially as the population structure of the *M. tuberculosis* comples is highly clonal. In addition, other markers that show greater variability like for example mycobacterial interspersed repetitive units-variable-number tandem repeats (MIRU-VNTR) and spoligotypes allow to refine the differentiation. In contrast to previous hypotheses these studies suggest that the agent of bovine tuberculosis *M. bovis* is not the ancestor of the human tuberculosis agent *M. tuberculosis*, as was thought for a long time. This perspective is further supported by recent results from paleo-microbiological investigations that demonstrate the presence of *M. tuberculosis* rather than *M. bovis* in ancient human remains. The same techniques are also applied to characterize the extant population of *M. tuberculosis* strains in the world, which is of great importance to understanding the transmission patterns of *M. tuberculosis* better and help to anticipate future trends in the spread of the disease.

The happy families of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis is characterized by extensive genetic clonality and extensive genomic conservation. Although SNPs are known, the majority of genomic polymorphisms are the result of deletions and of insertions, particularly of the transposable element IS*6110*. Whilst the predominant consequence of an IS*6110* insertion into a gene is likely to be its inactivation, IS*6110*–mediated transcription of adjacent genes has also been reported. Much is known about the relatedness of strains to each other, using spoligotyping and IS*6110* RFLP. However, little is known about the genetic diversity of the species from a phylogenetic perspective. Whether genomic polymorphisms have had a selective influence on the fitness of strains or have been largely neutral is unknown. We have examined the phylogenetic distribution of the more popular IS*6110* insertions in the genome in two different international collections of clinical isolates. Our results provide compelling evidence that IS*6110* insertions can be highly stable; that specific insertions correlate strongly with the well-known, geographically restricted *M. tuberculosis* families; that there are very few specific insertions common to different families; and that some loci are repeatedly and independently targeted by IS*6110* in a lineage specific way.

Phylogenetic analysis of *Mycobacterium tuberculosis* strains circulating in Leicester using Genome Level-Informed PCR

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Genomic deletions in *Mycobacterium tuberculosis* can be used in the phylogenetic analysis of strains since they represent irreversible evolutionary events. We have analysed ~100 local isolates of *M. tuberculosis* using Genome Level-Informed PCR (GLIP). This method involved prior microarray analysis of a strain, CH, which was responsible for an outbreak of tuberculosis in Leicester in 2001, detailed in a previous report. Compared to H37Rv, GLIP identified deletions of 6 large (>0.5 kb) sequences in the genome of CH and formed the basis of a genotyping assay that was used to interrogate local isolates. Strains were subjected to a panel of 6 PCR reactions to identify the presence or absence of each deletion and assigned to a specific genogroup. The information was used to construct a deletion-based phylogeny of local strains carrying a deletion in Rv1519 (RD750), which has been shown to define the East African-Indian lineage.

Systematics and practical identification of non-tuberculous mycobacteria

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The last decade of the 20th century has been characterized by an extraordinary progress of genetic techniques which has influenced every field of life sciences. For what concerns the mycobacterial taxonomy the keystone has been represented by the detection, within the genome, of variable nucleotide traits interspersed within highly conserved regions. The consistent correlations of the genetic sequences present in such traits, with known mycobacterial species, soon appeared an evident confirmation of the taxonomic knowledge based previously on phenotypic characters. As a consequence, many previously unrecognized species were described on the basis of the detection of isolates with new, unreported sequences. With the increase of species, which are now more than 120, the conventional identification methods, based on phenotypic characters, soon became inadequate and were rapidly replaced by genetic approaches. DNA-probes, PCR-restriction analysis and genetic sequencing are nowadays the most popular identification methods. Microarrays, which are essentially miniaturized multi-probe systems, seem addressed to pay a major role in the future of mycobacteriology.

Evaluation of INNO-LiPA *Mycobacteria* v2 for identification of aquatic *Mycobacterium* spp.

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Mycobacteriosis is a common disease in wild and captive fish. A rapid detection method capable of identifying multiple species of *Mycobacterium* in a single procedure was evaluated here for use with aquatic mycobacteria.

The INNO-LiPA *Mycobacteria* v2 Kit (Innogenetics, Ghent, Belgium)

was evaluated using a panel of cultures of known identity and diverse geographical origin. All *M. marinum* (n=14), *M. chelonae* (n=5), *M. gordonae* (n=3) and *M. fortuitum* (n=10) hybridized to their corresponding species- or complex-specific probes. However, sequence analysis of the the 16s-23s rDNA internal transcribed spacer (ITS) region revealed that 9/10 of the *M. fortuitum* isolates showed highest homology with *M. senegalense*, which to our knowledge has never been reported in fish. This cross-reaction has been reported by 2 other groups highlighting the need for revision of the *M. fortuitum* group II probe by the manufacturer.

In conclusion, the INNO-LiPA mycobacteria v2 kit is a rapid and reliable method for identification of *Mycobacteria* in aquatic environments. Laboratories without sequencing facilities would benefit in particular from this kit.

Bleach-sedimentation of sputum improves the safety and speed of microscopy but does not concentrate mycobacteria for the diagnosis of tuberculosis

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Background Direct-smear sputum microscopy for TB diagnosis is rapid but insensitive. Concentrating mycobacteria would be expected to increase sensitivity plus efficiency of slide-reading. We aimed to quantify the effect of bleach-sedimentation of sputum on these parameters, and determine the optimum conditions for bleach mycobactericidal activity.

Methods Microscopy-positive samples were treated with 5% bleach and sedimented for 16 h. The number of bacilli per 100 fields was counted in triplicate slides and compared with controls made from untreated sputum. Samples were also subjected to varying combinations of bleach concentration and exposure times, then cultured.

Results Bleach-sedimentation caused a significant (p=0.01) decrease in mycobacterial concentration. A weakly significant (p=0.06) decrease in slide-reading time was seen in paucibacillary samples. Treatment with 6% bleach for 5 min was sufficient to kill mycobacteria.

Conclusions Bleach-sedimentation does not concentrate mycobacteria, but increases slide-reading speed for paucibacillary samples. Bleach-treatment of sputum is an inexpensive, simple step with implications for biosafety.

Detection of serum biomarkers of paediatric TB using Surface Enhanced Laser Desorption Ionization (SELDI) mass spectrometry

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Paediatric tuberculosis (TB) is a real diagnostic challenge. A definitive diagnosis from a positive sputum culture is rarely achieved due to difficulties in obtaining samples from children and the paucibacillary load of the disease. This may lead to inappropriate treatment regimes being followed as a precautionary measure. The development of an accurate, rapid, serological diagnostic test for TB in young children would, therefore, be of enormous benefit.

SELDI was used to find novel biomarkers of TB disease in a cohort of children from Cape Town, South Africa. Serum proteomic profiles were obtained using CM10, Q10 and IMAC-Cu ProteinChip arrays for children with active TB (pulmonary and extra-pulmonary) and controls (TST negative; TST positive and other infections/illnesses). For each group n=45.

Using Ciphergen Express peak recognition software in conjunction with Biomarker Patterns software, 5 proteins (4.0 kDa, 4.1 kDa, 9.1 kDa, 14.9 kDa, 17.9kDa) were detected which distinguished between children with active TB from those with other infections/illnesses with a sensitivity and specificity of 96% and 64% respectively.

Identification of these markers may increase our understanding of Paediatric TB and could be useful in the development of a new diagnostic test for this disease.

Application of Interferon-gamma release assays to a very high HIV-TB burden setting

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Tuberculosis infection is conventionally inferred by means of delayed type hypersensitivity to purified protein derivative in the form of tuberculin skin testing (TST). The TST is imperfect operationally and also suffers deficits in sensitivity and specificity that are particularly important when considering its application in children and in HIV infected people. The potential to replace the TST by assays that determine the *in vitro* production of Interferon-gamma secreted in response to *M. tuberculosis* antigens became apparent in the early 90's. A major impetus to such approaches was provided by the discovery of 2 immunodominant antigens ESAT-6 and CFP-10 that are encoded on a genomic segment of *M. tuberculosis* that is absent from all *M. bovis*-Bacille Calmette Guérin (BCG) strains. Two commercially available tests based on these antigens (T-Spot.*TB*, Oxford Immunotec; and QuantiFERON-TB Gold, Cellestis) are now variously licensed for the detection of tuberculosis infection. Some national guidelines have been modified to incorporate the use of these tests. Neither test has been rigorously evaluated in comparable groups of HIV infected and uninfected people to determine whether they may have utility in this very high groups. This talk will discuss data from Khayelitsha, South Africa: a socio-economically deprived township in which 33% pregnant women are HIV infected and the overall tuberculosis incidence is 1611/100,000.

Microbial markers as surrogates of response to tuberculosis drug treatment

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Traditionally studies of new drugs use a combination of microbiological and clinical endpoints to evaluate safety and efficacy. For studies of TB treatment, the proportion of subjects with relapse 2 years after completion of therapy has been the key indicator of clinical outcome. Relapses occur in 3–5% of patients, as a result, statistical requirements for phase III efficacy trials are daunting, requiring at least 2500 subjects and 4 years for completion to determine if a new therapy reduces the relapse rate by half. These requirements have slowed the evaluation of new TB treatment protocols and increased the costs. In response to this conundrum, it has been suggested that surrogate endpoints, which do not directly measure clinical benefit to the patient, may function as substitutes for clinical endpoints in clinical trials. Presently, the best surrogate marker for relapse is the proportion of patients who convert their sputum mycobacterial cultures to negative after 2 months of anti-TB treatment. Less well studied surrogate markers for TB trials include sputum 85B protein and mRNA, serial sputum culture conversion, sputum cytokines, and whole blood bactericidal activity. Changes in

sputum mRNA and urinary DNA as detected by quantitative PCR are associated with the rate of bacterial clearance in sputum during the first 2 months of TB drug therapy, thus are promising surrogate markers. Evaluation and application of these methods in a field setting will be discussed.

Commercial blood tests for the diagnosis of infection with *Mycobacterium tuberculosis*

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Targeted testing and treatment of individuals with latent tuberculosis infection at increased risk of progression to active disease is a key element of tuberculosis control. This strategy is limited by the poor specificity of the tuberculin skin test in BCG-vaccinated populations and its low sensitivity in immunosuppressed persons, who are at highest risk of progression. Two blood tests (T-SPOT.*TB* and QuantiFERON-TB Gold), based upon detection of interferon-γ released by T-cells in response to *M. tuberculosis*-specific antigens, may offer an improvement on the skin test. However, validation is challenging due to the lack of a diagnostic gold standard. The blood tests have clear operational advantages over the skin test because no return visit is required, results are available by the next day and repeated testing does not cause boosting. Both tests are significantly more specific than the skin test in BCG-vaccinated populations. The data available suggest that T-SPOT.*TB* may also be more sensitive than the skin test. Data in groups at high risk of progression are scarce and more research is needed in these populations. Incorporation of these tests into programs for targeted testing of latent tuberculosis infection will likely reduce false-positive and false-negative test results inherent in tuberculin testing.

Bacillary dormancy and treatment of tuberculosis

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Dormancy is an anthropomorphic term that covers various microbial conditions. In the chemotherapy for tuberculosis (TB), it encompasses microbial persistence, the ability of drug-susceptible micro-organisms to survive prolonged drug exposure. MTB is so capable of persistence during treatment with the bactericidal combination of isoniazid (H), streptomycin (S) and PAS (P) that treatment requires 18 to 24 months for acceptable outcomes. With the addition of rifampicin (R) and pyrazinamide (Z), drugs that curiously have less potent bactericidal activity than H and S, stable cure is obtained in 6 months time, clearly indicating that persisters are more susceptible to R and Z as compared to H and S. A further reduction in the duration of curative (and preventive) therapeutic regimens will require greater understanding of the mechanisms involved in persistence and the discovery of new drugs with more potent activity against persisters. With this objective in mind, we have studied the main characteristics of the new antituberculosis drugs, moxifloxacin and the nitroimidazopyran PA-824, and compared them to the reported characteristics of two other new drugs, the nitroimidazo-oxazole OPC-67683 and the diarylquinoline R207910. At least some of these drugs have the potential for more rapid killing of persisters.

Lipid bodies, a marker for antibiotic tolerance in *Mycobacterium tuberculosis*?

M.R. Barer

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Mycobacterial lipid bodies (LBs) are triacylglycerol (TAG) containing inclusion bodies that are readily demonstrable in sputum microscopy positive cases of tuberculosis but strikingly absent from M. tuberculosis cells grown in vitro. Thus it appears that LB positive cells in sputum must be in a distinct physiological state from cultured cells and this could have significant implications for chemotherapy. Recent data from this and other labs connects LB formation with the non-replicating persistence (NRP) state described by Wayne and colleagues. If LB positive M. tuberculosis cells are in an NRP state, then they represent an antibiotic refractory population that will need to be separately targeted by novel therapeutic strategies. The evidence for this proposal and other antibiotic tolerant states in M. tuberculosis will be reviewed and the implications for chemotherapy considered.

Sputum induction allows immune and microbiological diagnosis of tuberculosis

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Introduction Blood-based interferon-γ (IFN-γ) secretion assays have uncertain utility in active TB. We have focussed on lung immune responses to enable integration of immunological techniques with microbiology. We have sought to adapt a method developed for broncho-alveolar lavage for use with induced sputum.

Methods: Nebulized 3% saline was inhaled for 20 minutes. Mucolysed sputum was incubated overnight with PPD and then IFN-γ synthetic CD4+ lymphocytes measured using flow cytometry.

Results 36 TB patients, 10 HIV co-infected, have undergone sputum induction at baseline. 23/36 had pulmonary TB (20/23 AFB-smear negative). 2/36 had miliary TB, 2/36 had mediastinal lymph gland TB and 2/36 pleural TB without parenchymal involvement. 7/36 had solely extra-thoracic disease. Median CD4+IFN-γ+ frequency response to PPD was 3.96% (range: 0–23.79%). Responses were >0.5% in 33/36 and >1% in 30/36. In 8 of 8 in BCG-vaccinated healthy controls responses were <0.5%.

Conclusion We have shown that a simple lung-orientated approach allows microbiological and rapid immunological investigation of a single sputum sample regardless of HIV status and site of disease.

Evaluation of molecular beacon, TaqMan, and FRET probes for the detection of antibiotic resistance conferring single nucleotide polymorphisms in mixed *Mycobacterium tuberculosis* DNA extracts

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The ability of FRET, molecular beacon and TaqMan probes to detect single nucleotide polymorphism (SNP) in the presence of a wild type allele was evaluated using drug resistance conferring SNPs in mixtures *Mycobacterium tuberculosis* DNA. It was found that both the absolute quantity and the ratio of alleles determine the detection sensitivity of the probe systems. Regardless of a second allele, with both TaqMan and molecular beacon probes consistent detection of both mutant and wild type alleles was achieved when the reaction mixtures contained between 0.126 to 3.4 ng DNA for wild type and 0.079 to 2.13 ng for the mutant allele, provided the ratios of the alleles did not exceed 1:5.6. With both system, in mixtures containing low quantities of templates, 0.0047 to 0.042 ng for wild type and 0.0029 to 0.026 ng mutant allele, both alleles could be

detected provided the ratio of alleles did not exceed 1:4.8. In all other cases only the higher quantity allele could be detected. On the other hand, with FRET probes it was only after a preamplification step that the detection of either alleles alone could be achieved to a similar sensitivity as the molecular beacon and TaqMan probes.

Expression of *Mycobacterium tuberculosis* DNA repair genes involved in the SOS response upon exposure to sub-lethal doses of quinolone

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Quinolones induce the SOS response with maximal induction occurring at about ten times the MIC. The correlation between SOS error prone repair and mutagenic effects indicate that quinolone-induced mutagenic effects in bacteria are almost entirely due to SOS-processed DNA damage; exposure to sub-inhibitory concentrations of quinolones can increase the mutation rate of mycobacteria by up to 120 fold. We have previously reported the use of microarray technology (supplied by BuG@S group) as a screen to identify SOS genes induced upon exposure to 1/2 and 1/4 MIC of ciprofloxacin. The results were inconclusive as many of the changes in gene expression were below the level of sensitivity of microarray. To validate the general observations made from the array data we adopted a RT-qPCR strategy to confirm the role of specific genes involved in the SOS response; *recA, lexA* and *dnaE2* were analysed and *sigA,* 16S rRNA and *rpoB* were used as reference genes for expression in all samples.

Coming soon to a clinic near you: genomics revolutionizes drug development for TB

C. Barry

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With the genomes of hundreds of pathogenic micro-organisms and dozens of their unfortunate hosts literally at our fingertips anti-infectives drug discovery is undergoing a renaissance. Mycobacterial species have been extensively sequenced and integrative tools are emerging with direct and immediate impact on every stage of the discovery pipeline. This lecture will highlight recent advances in 'omics' tools from the perspective of their potential impact on the pace of pre-clinical development and clinical evaluation of highly active new therapies for TB. Genomic tools are impacting informed selection of disease-relevant targets, making mechanism of action studies for new compounds routine, and identifying surrogate markers that make clinical evaluation of new agents rapid and reliable. These tools will be illustrated with examples from new agents currently in late preclinical and early clinical development for TB therapy and with examples from agents and strategies only now being implemented.

Environmental Microbiology Group / British Ecological Society joint session

Microbes, macrobes and ecology

Microbial ecologists and classical ecologists; uneasy bedfellows or a match made in heaven?

S. Nee

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Abstract not received

Microbial biogeography and why it matters

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A large body of research supports the idea that free-living microbial taxa exhibit biogeographic patterns such as those observed in larger organisms. If one accepts this premise, two large questions remain. First, what are the processes underlying the spatial variation in microbial community composition? To address this question, one can use a framework from traditional biogeography to test whether microbial biogeography is due to present-day environmental factors, historical contingencies, or both. As an example, I will present a study of bacterial composition in salt marshes. The second question is whether spatial variation in microbial composition matters. In particular, to what extent does variation in microbial composition have consequences for ecosystem processes? Field experiments demonstrate that the composition of plant and animal communities have effects on ecosystem functioning, but one can manipulate the composition of macro-organisms relatively easily. I will discuss an approach to address this question for micro-organisms in the field, again using an example from the salt marsh.

Neutral community assembly models: prospects and problems for microbial ecology

G. Bell

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Abstract not received

Neutral models and microbial community dynamics

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What constitutes a microbial community? In microbial ecology we rely entirely on small samples to determine community structure in what are ostensibly very large populations. Even using the most up-to-date molecular methods to characterize the genes in an environmental sample the disparity between the sample and community size is far greater than for surveys of macro-organisms. Furthermore, there has never been a complete census of any naturally occurring microbial community. So when inferring community structure from, for example, a clone library from a small soil sample do we extrapolate to a thimble full of soil or to a whole field? This is a fundamentally important and yet much neglected question in microbial ecology. Here we use a simple neutral community assembly model (NCM) to demonstrate how important answering this question is to assessing the dynamic response of microbial ecosystems. Calibrating the NCM on small environmental samples gives us the opportunity of rationally extrapolating to larger scales but the predicted community dynamics and structure are scale dependent. Therefore, to predict a microbial ecosystems response to disturbances will require either some method of partitioning the microbial population into effective communities or a scale independent model of community assembly and dynamics. These will only be achieved through an effective dialogue between theoretical ecologists and molecular microbial ecologists; a dialogue that may yield sobering predictions on the size of samples that are required to determine what constitutes a microbial community.

Spatial structure and function of micro-organisms in Arctic glacial ecosystems

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Glacial surfaces represent an important but relatively uncharacterized aquatic ecosystem, contributing to carbon and nutrient cycling in cold environments. Of these aquatic ecosystems, cryoconite holes (small water-filled depressions) comprise up to 6% of glacial surfaces and contain complex communities supported by microbial carbon fixation and nitrogen-cycling. Cryoconite holes represent both a model for island ecology, and additionally provide an excellent contained ecosystem to investigate microbially-mediated biogeochemical cycling. In this research, molecular (T-RFLP) fingerprinting analysis of both bacterial and picoeukaryal rRNA genes amplified from DNA extracted from cryoconite assemblages from the Midre Lovenbreen glacier, Svalbard (78°N) challenges the emerging orthodox of a taxa-area relationship for microbial groups seen in other environments. Additionally, PCR-based analysis of genes encoding carbon fixation functions suggests both bacteria and eukarya are involved in primary production within the cryoconite ecosystems.

What dictates endemism and cosmopolitan distribution of micro-organisms?

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Spatial distance (SD) and environmental heterogeneity (EH) are currently thought to be the main factors shaping prokaryotic biogeography. There is however little data available to compare the relative contribution of each factor and their interactions at different spatial and taxonomic scales. We therefore examined the abundance and diversity of soil-borne *Burkholderia cepacia* species from communities to single genotypes at different geographic scales. EH consisted of dissimilarities of soil physical and chemical parameters as well as the presence of different plant species for the different samples. By combining spatial decomposition techniques with multivariate canonical analyses, we determined the effects of SD and EH on biotic variables, which consisted of abundance, community structure and genomic diversity. Although total community and species abundance varied at the smaller scales of the sampling design, genomic similarities within the most abundant species responded to all spatial scales that were detected in the study. Whole-genome similarities may thus reflect the simultaneous effects of historical events and contemporary ecological adaptations in microbial populations. Those factors however contributed to a

small proportion of the complex variation in microbial diversity, suggesting a need for future biogeographical studies to shed more light on those poorly understood variations.

Species abundance models for microbial communities

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Abstract not received

Response of bacterial community structure to anthropogenic stresses

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Recent interest in applying classical ecological principles to microbial ecology has led us to explore the possibility that environmental perturbations such as pollution events result in similar patterns of succession in bacterial communities as have been observed for animal and plant communities. These successional patterns are typified by a decrease in the slope of rank abundance curves over time after a perturbation or vice versa during a perturbation. We have found that this is the case for natural bacterial assemblages and furthermore, following Robert May's prediction, we have observed that undisturbed communities follow a log normal distribution while perturbed communities follow log series distributions. These observations may prove invaluable for assessing habitat health and recovery from a pollution event. Especially, when combined with monitoring pollution load (chemical oxygen demand) and toxicity testing (biosensors).

Spatial modelling of microbial communities in soil

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Abstract not received

Being(s) in control: metabolic flux modelling applied to microbial ecosystems

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Identification of the functional groups of micro-organisms that are predominantly in control of fluxes through, and concentrations in, ecological networks should benefit the understanding on and manipulation of material fluxes in natural and human-made environments. We have developed ecological control analysis (ECA) as a versatile mathematical framework that allows for the quantification of the control of each functional group in an ecological network on its process rates and concentrations of intermediates. We illustrate ECA by a case study on the anaerobic syntrophic degradation of organic matter. In contrast to current views, degradation fluxes are not always limited by a single functional group; rather flux control can be distributed over several groups. Control over intermediate concentrations is always shared. Because of networking effects, the concentration intermediates can also be controlled by functional groups not producing or consuming it. ECA can give rise to counterintuitive results, for example we found that halorespiring micro-organisms do not control the rate of perchloroethylene and trichloroethylene degradation even though they catalyze those processes themselves.

Resource supplies and microbial community composition

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The structure of microbial communities in both natural and engineered ecosystems can be strongly influenced by resource availability. In lake plankton, for example, it has been well established that nutrient enrichment (eutrophication) results in profound shifts in microbial species composition, species diversity, and functional diversity. Comparative analyses of data from a variety of lakes worldwide revealed consistent patterns that were predictable from measurements of water column nutrient concentrations and biomass production. In contrast, the effects of nutrient availability on microbial community structure in terrestrial ecosystems are much less well characterized. However, comparative analyses of data from experimental studies of a series of grassy mountain ecosystems in Europe revealed not only very strong effects of nutrient enrichment on vascular plant diversity, but also striking relationships between nutrient enrichment level and the diversity of culturable soil microbes.

Biogeochemical potential of fungal-plant mutualism in metal-polluted soils

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There is a critical need for the development of cost-effective remediation technologies for metal-polluted soil. One of the most public-appealing biotechnologies is phytoremediation. More than 95% of land plants depend on symbiotic mycorrhizal fungi meaning that any bioremediation strategy involving mycorrhizal plants is dependent on fungal ability to transform toxic metal speciation. The main functions of mycorrhizal mycobionts are phosphorus acquisition, mineral nutrition, and contribution to plant adaptation to extreme environments including metal tolerance. In this work, it was observed that mycorrhizal fungi are capable of toxic metal mineral solubilization and this was related to fungal metal tolerance and the phosphorus status of the soil. In providing the plant host with phosphorus, fungal solubilization of inorganic phosphates (e.g. $Zn_3(PO_4)_2$) can result in release of associated toxic metals increasing metal toxicity. However, some mycobionts demonstrated the ability to re-precipitate released metals (Cu, Zn, Pb) within insoluble secondary mycogenic minerals making them chemically stable and unavailable for the plant host and soil biota. Introduction of metal stabilizing mycorrhizal associations could ensure success of phytoremediation and revegetation/reforestation of polluted sites.

The application of ecological and evolutionary theory to understanding the persistence of plasmids in bacteria

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The mechanisms that underlie the ability of plasmids to persist in bacterial populations, despite the costs associated with them, have been debated for some considerable time. A series of reciprocal 'invasion from rare' experiments with mercury resistance plasmids in *Pseudomonas* populations demonstrated that plasmid-free and plasmid-bearing cells can coexist under a range of intermediate mercury concentrations in both structured and homogeneous

environments. Interestingly, spatial structure did not enhance coexistence, but this can be attributed to the local effect of mercury removal in the structured environment. Analysis of this data and the application of ecological and evolutionary theory indicate that the presence of plasmids can be attributed to the frequency-dependent selection of group-beneficial traits. Moreover, the majority of traits commonly associated with plasmids are group-beneficial; the gene products are often excreted from the cell or they function to alter the external environment. Therefore our conclusions will be widely applicable to the understanding of plasmid biology.

Non-linear dynamics as a factor shaping microbial communities

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Considerable evidence suggests that ecosystems can display chaotic behavior, including microbial-scale systems. Recent work indicated that a protozoan predator displayed conditional chaos in a two prey-one predator axenic culture; however, natural and engineered microbial communities are rarely this simple. As such, a more critical question regarding non-linear dynamics and chaos in natural microbial systems is whether guilds of organisms that perform similar functions display chaos as groups. To assess whether 'functional chaos' exists within a multi-specie microbial community, we fed three chemostats simulated wastewater at three different dilution rates for 207 days. Total community biomass, ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), 'other' bacteria, and protozoan predators were monitored daily using real-time PCR and direct enumeration. The three dilution rates straddled conditions that would conditionally stress slower growing N-processing guilds, and guild and community dynamics were assessed. Both NOBs and predators had positive Lyaponov exponents at all three dilution rates (i.e. sensitivity to initial conditions); whereas, AOBs only had positive exponents when dilution rates were high. Results indicate that although some guilds within a microbial community can display functional chaotic behavior, the whole community and other guilds within the same community may not. Investigations are on going to determine the basis of conditional 'chaos' in guilds, and to assess relationships between chaotic instability and reactor performance.

Bacterial cooperation, competition and virulence

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Bacteria have complex social lives. They communicate, cooperate and compete with each other. Understanding the conditions that drive the evolution of these behaviours is a fundamental aim of evolutionary biology. Furthermore, these behaviours can have important implications for how pathogenic infections can be. I outline experiments we have carried out to address some of these issues.

Physiology, Biochemistry & Molecular Genetics Group / Education & Training Group joint session

with sponsorship from the Royal Microscopical Society

Imaging microbial systems: from whole micro-organism to single molecules

From chemosensory pathways to rotating motors-imaging functioning protein complexes in living bacterial cells

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The photosynthetic bacterium *Rhodobacter sphaeroides* has operons encoding 3 putative chemosensory pathways. *In vitro* analysis of phosphotransfer using purified proteins identified a possible complex pathway of phosphotransfers between the pathways, but there was no evidence of *in vivo* complementation. We replaced the genomic copy of each wt putative chemosensory gene in each pathway, singly and in pairs, with a gene encoding an C- or N-terminal GFP fusion. DIC imaging revealed that the proteins from each pathway did not complement *in vivo* because the proteins for two complete chemosensory pathways are physically separate, one pathway localized to the cell poles, the other to the cytoplasm. Using the GFP gene fusions we were identified the proteins required for cluster formation and follow protein segregation through the cell cycle, identifying a central role for a protein homologous to ParA (a protein involved in plasmid segregation).

In addition we replaced the genes encoding the flagellar motor proteins with ones encoding fluorescent fusions. By tethering the cell by its flagellum to a slide we brought the motor of a rotating bacterium within the evanescent field. We measured the rate of bleaching and following fluorescence recovery (FRAP) on single rotating motors. This showed that the motor has 22 MotB proteins (equivalent to 11 stators), and these are exchanging with a membrane pool, with the complete stator component of the motor turning over in about 1 minute.

Fluorescence microscopy and its application to imaging of the bacterial cytoskeleton

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Bacterial cells are small and for several decades, since the first application of electron microscopy in the 1940's, were thought to have a relatively simple organization. However, this view changed completely in the early 1990's, with the renewed application of immunofluorescence methods to bacteria, together with the advent of GFP and the availability of digital image capture and processing systems. The ability to determine the subcellular-localization of proteins in several model bacteria, provided researchers with a revolutionary new view of bacterial cell structure and effectively founded a new field of 'Bacterial Cell Biology'. One of the most surprising discoveries has been that bacteria possess all of the elements of the eukaryotic cytoskeleton; tubulin (FtsZ), actin (MreB) and intermediate filaments (Crescentin). Indeed, it is now clear that these proteins evolved first in bacteria. The talk will include an update of our current understanding of the MreB system and an indication of how various fluorescence microscopic methods have impacted on this problem.

Development of high throughput biosensors

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The rhizosphere is one of the most complex, yet important, of environments for both microbial and plant growth. One of the most powerful tools that could be developed to study this environment is a set of molecular biosensors to monitor environmental conditions temporally and spatially. Our genome analysis of *Sinorhizobium meliloti, Mesorhizobium loti* and *Rhizobium leguminosarum* reveals an explosive growth in the number of ABC (ATP binding cassette) uptake systems in these organisms, with around 160 systems present. The binding proteins of these transporters are highly solute specific and tightly induced in response to appropriate conditions (Hosie and Poole 2001, Hosie *et al.* 2001). We have developed a complete set of ABC-binding protein-Gfp fusions in a pOT vector that has been TOPO adapted by Invitrogen. This allowed the direct cloning of the PCR products of the promoter regions of all the ABC binding proteins. We were then able to identify the inducing solute for 90 binding proteins. This included a wide range of sugars, amino acids, organic acids, nucleotides and metal ions. These biosensors are now being introduced into the plant rhizosphere, using an agar sandwich technique developed here, and expression of fusions monitored by fluorescence microscopy.

New biophysical tools based on single molecule fluorescence and a scanned nanopipette

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One major challenge in biology is to understand how the individual molecules and complexes of the cell are organized and interact to form a functional living cell. To address this problem new biophysical tools are needed.

One method that we have developed for functional nanoscale mapping of the cell surface is based on a scanned nanopipette. This allows non-contact imaging of the soft and responsive cell surface using the ion current that flows between an electrode in the nanopipette and bath for distance feedback control with a resolution down to 10 nm (1). We have also combined high resolution topographic imaging with simultaneous recording of the fluorescence from the cell surface to follow viral entry (2). The pipette can be used for controlled voltage driven delivery and deposition of biomolecules down to the single molecule level1 and this is being used to probe the structure of the cell membrane using single molecule fluorescence tracking.

To determine the oligomerization state of proteins on the surface of living cells we have used two colour single molecule coincidence detection based on the excitation of two distinct fluorophore labels on proteins with two lasers focussed to the same spot.3 This method requires no prior knowledge of the structure of any complex formed or control of fluorophore position on the molecule. We have also used this method to probe the variation in the assembly of Herpes Simplex virus.

References: (1) L.M. Ying, A. Bruckbauer, D.J.Zhou, D.J. Gorelik, J. Shevchuk, A. Lab, Y. Korchev & D. Klenerman (2005). The scanned nanopipette: A new tool for high resolution bioimaging and controlled deposition of biomolecules. *Phys Chem Chem Phys* **7**, 2859–2866. (2) J. Gorelik, A. Shevchuk, M. Ramalho, M. Elliott, C. Lei, C. F. Higgins, M. J. Lab, D. Klenerman, N. Krauzewicz & Y. Korchev (2002). Scanning surface confocal microscopy for simultaneous topographical and fluorescence imaging: Application to single virus-like particle entry into a cell. *Proc Natl Acad Sci USA* **99**, 16018–16023. (3) H. Li, L. Ying, X. Ren, S. Balasubramanian & D. Klenerman (2004). Fluorescence studies of single biomolecules. *Biochem Soc Trans* **32**, 753–756.

Live-cell imaging of fungal hyphae using confocal microscopy

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In recent years, there has been a major renaissance in microscopic imaging for microbiology. A revolutionary new perspective of the cell biology of fungal hyphae is arising as a result of using live-cell imaging techniques to analyse organelle and molecular dynamics at high spatial resolution. This has come about particularly as a result of major innovations and developments in microscope technologies (e.g. confocal microscopy) and fluorescent probes (both recombinant probes and vital dyes). These innovations are having a profound impact on the experimental analysis of living fungal hyphae at the single cell level. In my presentation I will show how live-cell imaging using confocal microscopy is being used to analyse hyphal tip growth, hyphal fusion, and organelle organization and dynamics.

Microscale analyses of microbial communities

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One of the most versatile tools to investigate microbial communities in situ is laser scanning microscopy (LSM). LSM has become a routine approach to investigate a wide range of biological samples facilitating optical sectioning of fully hydrated living microbial communities such as flocs, mats and biofilms. By employing 1-photon and/or 2-photon excitation in combination with a step by step approach many parameters can be assessed. These include the inherent sample properties observed without staining and those obtained by using a wide range of fluorescent and reflective molecular probes. These techniques can be used to determine sample thickness, localize reflective material, detect autofluorescence, differentiate photosynthetic organisms, map cell distribution, quantify biomass, assess distribution, quantify and characterize exopolymeric substances, determine the viability, identity and activity of cells, enzyme activity and measure various parameters of the microenvironment. In many cases these parameters may be collected simultaneously or sequentially using multiple observation channels. Subsequent image analysis and rendering allow quantification and presentation of data collected in multiple imaging channels. A series of case studies will be presented to illustrate the application of LSM to a wide range of microbial samples.

Who you are and what you do: imaging complex microbial communities

Michael Wagner[1], Kilian Stoecker[1], Holger Daims[1], Andrew Whiteley[2] & Gerhard Schütz[3]

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Direct insights into the composition, ecophysiology and interactions of microbial communities are at the heart of microbial ecology and are also of major relevance for medical microbiology. Microbial ecologists have recently developed a battery of molecular microscopic tools which allow for simultaneous identification and functional analyses of single microbial cells within such communities. Most importantly, it is now possible to monitor uptake of labelled substrates and specific gene expression patterns of defined bacterial genotypes in the environment. Quantitative information on the abundance and activity of specific microbial populations can be inferred by analyzing micrographs with computer programs like *daime* (*Environ Microbiol* 2005 8, 200–213) which integrate digital image analysis and 3-D visualization functions. However, the available molecular techniques are still tedious, often require the use of radioactively labelled compounds and do not work for all species/environments. Here we present new approaches, including the combination of FISH and Raman spectroscopy and the use of single molecule optical microscopy, which aim to overcome these limitations for structure-function analyses of microbial communities.

Scanning probe microscopy of bacteria and their extracellular components

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Methods for imaging bacteria and extracellular components by probe microscopy will be described and discussed. Although emphasis will be placed on the use of atomic force microscopy the use of scanning near-field optical microscopy (SNOM) to locate fluorescently-labelled proteins within bacteria will also be described. Examples will be shown of the use of atomic force microscopy (AFM) to study the role of surface components in the adhesion of bacteria to surfaces prior to the formation of biofilms. Finally, data will be presented to show how AFM cam be used to image and characterize extracellular components such as polysaccharides and to investigate and explain their functional properties.

Using AFM to explore the surface of living microbial cells

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There is a need in current microbiological and biophysical research to develop new, high resolution tools for probing the structure, properties and interactions of microbial cell surfaces. The advent of atomic force microscopy (AFM) has recently opened a wide range of novel possibilities for probing microbial cell surfaces in their native environment (1–3). Using AFM imaging in aqueous solution, microscopists can visualize cell surface nanostructures (surface layers, appendages), follow physiological changes (germination, growth) and monitor the effect of external agents (antibiotics, metals) in real-time. Further, using force spectroscopy researchers can learn about local biomolecular interactions and physical properties. For instance, spatially-resolved force mapping offers a means to determine variations of elasticity and chemical properties at the subcellular level, thereby providing complementary information to classical characterization methods. Functionalizing the AFM tip with chemical groups or biomolecules enables quantitative measurements of surface charge, surface energy and receptor-ligand interactions. Finally, force spectroscopy can be applied to single cell surface molecules to gain insight into their mechanical properties. Clearly, these novel AFM-

based experiments contribute to improve our understanding of the structure-function relationships of microbial cell surfaces and open the door to new applications in biotechnology and medicine.

References: (1) Dufrêne, Y.F. (2004). *Nature Rev Microbiol* **2**, 451–460; (2) Dupres, V. *et al.* (2005). *Nature Methods* **2**, 515–520; (3) Hinterdorfer, P. & Dufrêne, Y.F. (2006). *Nature Methods* **3**, 347–355.

Using AFM to study DNA, RNA and proteins

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Proteins must fold and unfold during nuclear export, trafficking, regulation, sometimes function, and degradation. Studying the force dependence of protein stability permits the determination of unfolding transition states. From initial force experiments with the muscle protein titin, several other proteins have been studied, but in all cases to date only a single unfolding transition state has been observed. In the case of titin I27, for example, we predicted that at least two transition states are present on the unfolding landscape but only one has been seen experimentally. Why is this? Does only one transition state under force exist? Are the models wrong, and hence our interpretation of all the data?

I will show the success of the mathematical models through their application to force measurements of the interaction between 2F5, a broadly neutralizing monoclonal antibody against HIV-1, and two peptides encompassing the HIV-1 epitope. I show the use of dynamic force spectroscopy in dissecting complex bond architecture resulting from multiple intermolecular bonds loaded in parallel.

Force spectroscopy measurements of the dissociation of RNA duplexes suggest that the AFM is far from ideal in measuring biological interactions. I will show a comparison of AFM and biomembrane force probe (BFP) measurements on the force-induced dissociation of short nucleotide strands and the affect on the kinetic stability of a bulge.

So do multiple transition states exist in protein unfolding? I will present the results of studying protein unfolding with the BFP. The dynamic force-unfolding spectrum for the protein Barnase presents two clear unfolding transition states. These transition states are found to be on-pathway. The data shows the importance of studying such systems over a wide range of forces and the necessity for ultra-sensitive measurements. The presence of multiple transitions states implies that a single exponential is unable to describe unfolding kinetics at all forces, and that it is not possible to compare folding and unfolding kinetics at different levels of force.

Finally, to gain atomistic insight of the unfolding process it is desirable to undertake molecular dynamics simulations. I present the results of a new method of molecular simulation that closes the disparity in time between simulation and experiments and show a computer-predicted unfolding landscape validated by the comparison with experimental data.

These works have been submitted for publication elsewhere.

Using AFM to characterize the mechanical strength of proteins and their complexes

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Many proteins react or respond to a mechanical force as part of their function. Until recently the mechanical properties of proteins on the nanoscale were unknown as techniques that allowed their manipulation and measurement were not available. Over the last decade, development of robust instruments capable of picoNewton force sensitivity and sub-nanometre positional accuracy has allowed such experiments to be routinely undertaken. The wealth of experimental data now available in conjunction with computational simulation methods to visualize the process at molecular resolution and the development of a theoretical framework to underpin these observations has resulted in much progress in our understanding of the effects of a mechanical perturbation on proteins and their complexes. Experimental methods including the construction of suitable biomolecules and their measurement will be described and, by reference to examples, the information that can gained from this technique will be discussed.

High resolution visualization of microbial biofilms by freeze-substitution transmission electron microscopy

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Cryo-electron microscopy has become an optimal method in elucidating biological structures. However, owing to their size and difficult manipulation, microbes have been particularly challenging in this regard. To this end, a recent improvement to the field, high-pressure freezing, has allowed us to image these organisms after their remarkable preservation. In our studies, high-pressure freezing, freeze-substitution and transmission electron microscopy are used for high-resolution imaging of the natural structure of microbial biofilms, which is the preferred mode of bacterial growth in nature. Unlike conventional EM techniques, these methods confirm the observations seen by confocal microscopy but with finer structural detail. They reveal a structural complexity to biofilms at both the cellular and extracellular levels that has not been seen before. Different domains of healthy and lysed cells exist that are randomly dispersed within a single biofilm as well as heterogeneous structural organizations of polymeric substances and particulate matter. The O-side chains of lipopolysaccharides have also been found to extend from the cell surface and are integrated into the surrounding extracellular polymeric matrix. Together, these studies support the concept of disparate 'microenvironments' that exist within individual microbial biofilms that until now have been assumed, but not observed in such great detail.

Visualization of biological assemblies by cryoelectron microscopy

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Single particle cryo EM is an important tool in structural biology, complementary to the more established methods of X-ray crystallography and NMR spectroscopy. The single particle approach can tackle complexes that are too large for NMR and/or too flexible for crystallization. Cryo-microscopy allows the study of dynamic processes by taking 'snapshots' of a protein complex that is experimentally trapped in different conformational states by chemical or physical means. Using electron microscopy and single particle approach at image processing we were able to analyse structure of a small molecule like geminin. Geminin blocks the sequential assembly of pre-replicative complexes at replication origins during S and G2 phase thereby preventing re-replication within the same cell cycle. Another interesting molecule that was studied by cry-electron microscopy is p53 tumour suppressor involved in quality control of DNA in genes. The structure provides the first insight into the architecture of the p53 molecule /*in toto*/ and suggests mechanisms for p53's DNA binding, activation and function.

Cryo EM analysis of protein conformational changes in bacterial chaperonins and in the pore-forming toxin pneumolysin

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Cryo EM and single particle analysis have been used to follow the dynamics of large macromolecular complexes involved in protein folding and in membrane pore formation. The *E. coli* chaperonins GroEL and GroES form a container for folding of protein subunits. A series of dramatic conformational changes in GroEL is driven by the binding and hydrolysis of ATP, in which non-native substrate protein first binds at hydrophobic sites and then becomes encapsulated in a hydrophilic chamber. The interaction appears to involve forced unfolding of the substrate protein, potentially releasing it from trapped, misfolded states and allowing repeated attempts at correct refolding.

The cholesterol-dependent cytolysin family provides a bacterial virulence factor by creating large pores in the membranes of cholesterol-rich target cells. Monomeric toxin subunits released by the bacterium assemble into very large (~40 subunit) rings on the cell surface, and punch ~300 Å holes in the membrane. By imaging these assemblies on liposomes and combining the EM reconstructions with domain structures from earlier crystallographic analysis of the closely related toxin perfringolysin, we have characterized the conformational changes in pore formation.

Structure determination of integral membrane proteins in a membrane

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Structure determination of integral membrane proteins has been achieved by X-ray crystallography, NMR and electron crystallography. However, currently only electron crystallography has the capability of determining the structure of a protein actually embedded in a membrane, the other techniques determining the structure of detergent-solubilized membrane proteins. It has long been thought that detergents are a good membrane mimetic that can faithfully preserve the structure of membrane proteins, and on the whole this is still true for robust membrane proteins. However, there are now three examples of membrane proteins whose structure has been solved by X-ray crystallography and the structures have been shown to be in a non-native state. I will discuss the problems associated with the purification and crystallization of membrane proteins, along with the use of electron cryo-microscopy to collect high-resolution data for structure determination. The main example I will use is the work from our own laboratory on the structure of the Escherichia coli multidrug transporter EmrE.

Eukaryotic Microbiology Group session

Cell signalling: environmental and intercellular interactions

Transcription factors as direct nutrient sensors

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The recognition of changes in environmental conditions, and the ability to adapt to these changes, is essential for the viability of cells. There are numerous well-characterized systems by which the presence or absence of an individual metabolite may be recognized by a cell. However, the recognition of a metabolite is just one step in a process that often results in changes in the expression of whole sets of genes required to respond to that metabolite. Recent evidence from yeast suggests that complex signalling pathways may be circumvented through the direct interaction between individual metabolites and regulators of RNA polymerase II transcription. The yeast *GAL* genetic switch controls the expression of the genes encoding the enzymes of the Leloir pathway. When cells are grown in the presence of galactose is the sole carbon source the *GAL* genes are rapidly activated and expressed at a high level. The *GAL* switch is composed of three proteins (the activator Gal4p, the repressor Gal80p and the ligand sensor Gal3p) and two small molecules (galactose and ATP). The small molecules bind to Gal3p which subsequently renders Gal80p inactive and allows Gal4p-mediated activation of RNA polymerase II to occur. Continuing biochemical, structural and microscopic analyses are unravelling the molecular details of this elegant genetic control element.

Ion channels: What are these brainy molecules doing in microbes?

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Ion channels are not ion pumps or exchangers, and are not primarily concerned with ion uptake or metabolism. These are protein pores, the opening of which dissipates preformed ion gradients. The opening is directly triggered directly by physical or chemical stimuli: force, heat, voltage, or ligands. Therefore, channels are the frontline molecules that transduce environmental signals into ionic or electric messages for the cell.

Historically, ion-channel research originated from the study of the animal nervous system. However, modern biophysics and molecular biology now showed that ion channels exist in all forms of lives, including plants and microbes, and are even encoded in some viral genomes. With all its sophistications, it is ironic that ion-channel study gained most information in recent years from the crystal structures of a handful of ion channels from bacteria or archea, channels of unknown biological roles. (These microbial channels, revealed from genome sequences, allowed massive expression in *E. coli*, making crystallization practicable).

What we learned from animal physiology and biophysics seems to apply to microbes channels. These constitutive membrane proteins change from a closed to an open conformation upon the impact of membrane stretch force, heat, changes in transmembrane voltage, or extra- or intracellular ligand concentrations. MscL and MscS, the mechano-sensitive channels of *E. coli*, are the most well studied examples. What relatively little is known on other microbial channels will be summarized and described.

Acknowledgements: Supported by NIH GM054867 (Y.S.), GM047856 (C.K.) and the Vilas Trust of the University of Wisconsin–Madison

Survival in the host – specialized stress responses in human fungal pathogens

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Comparisons of conserved cellular processes in pathogenic organisms with those in closely related non-pathogenic species, provides an ideal opportunity to define particular specializations that enable virulence. An increasing body of work indicates that the ability of human fungal pathogens to sense and respond to environmental changes is essential for disease establishment and progression. Recent molecular and genomic studies have contributed significantly to our understanding of *Candida* stress responses and how they are regulated. Interestingly, it is now apparent that *Candida albicans and Candida glabrata* have diverged significantly from the benign model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* with respect to the nature and regulation of their stress responses. For example, studies from us and others have revealed that whilst key regulatory molecules are conserved in *C. albicans* and *C. glabrata*, their contributions to the regulation of stress responses have diverged. During this talk I will highlight such differences and discuss how pathogenic *Candida* species may exploit specialized stress responses to protect themselves during disease progression in the human host.

Interconnected feedback loops in the circadian clock of *Neurospora crassa*

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The *Neurospora* circadian clock protein Frequency (FRQ) is a component of interconnected negative and positive feedback loops. In the negative loop FRQ it inhibits its own transcriptional activator, the White Collar Complex (WCC). In the negative loop it supports expression of high levels of. These apparently contradictory functions of FRQ are confined to distinct subcellular compartments and coordinated in temporal fashion. Inactivation of WCC is mediated by FRQ in the nucleus early after the onset of FRQ expression. Support of WCC accumulation is observed when high amounts of hyperphosphorylated FRQ have accumulated in the cytosol. The transcriptional function of FRQ in the negative limb and its posttranslational function in the positive limb are independent and associated with distinct regions of FRQ. Phosphorylation of two serine residues within the PEST-2 region triggers its maturation from a nuclear repressor toward a cytoplasmic activator.

A whole-genome view of microbial signal transduction

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The complexity of microbial signaling systems makes comparative

genome analysis a particularly valuable tool for their studies, complementing the experimental approaches. A comprehensive census of environmental sensors and intracellular signal transduction proteins encoded in the completely sequenced genomes of more than 250 microbial species showed that the number of histidine kinases and response regulators encoded in any given genome grows approximately as a square of genome size. The same was true for the total number of alternative signal transduction components – chemotaxis sensors, diguanylate cyclases, c-di-GMP phosphodiesterases and Ser/Thr protein kinases. Most complex signal transduction machineries were found in environmental (e.g. soil) bacteria that have complex metabolic capabilities and can use a variety of terminal electron acceptors. Archaea, bacterial pathogens and environmental bacteria that inhabit stable ecological niches typically encode much simpler systems of sensory transduction. We argue that the evolution of signal transduction follows the 'Lego principle': almost any combination of sensory and output domains has a chance to be assembled, but only some of those remain fixed in evolution, usually the ones that confer a clear evolutionary advantage to the host cell. We plan to discuss to which extent these findings hold true for the eukaryotic signal transduction machinery.

CaMac1p – a copper responsive transcriptional activator in *Candida albicans*

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The transcriptional activator CaMac1p regulates the expression of the high affinity copper transporter gene *CaCTR1* in response to environmental copper concentrations (Marvin *et al*., 2004). Copper is an essential nutrient for the growth of *C. albicans* and is also required for high affinity uptake. Iron acquisition is an important virulence factor in many bacterial and fungal pathogens and *C. albicans* mutants with iron uptake defects are avirulent in mouse models (Ramanan & Wang, 2000).

Work in our laboratory has shown that CaMac1p regulates the expression of the ferric reductase gene *CaFRE12* in response to environmental copper concentrations. The *CaMAC1* gene is also self-regulated by CaMac1 protein in a copper-responsive manner. Putative binding sites for CaMac1p have been identified in the promoters of *CaCTR1, CaFRE12* and *CaMAC1* and point mutagenesis and electromobility shift assays used to investigate binding site function.

These three genes are homologous to *Saccharomyces cerevisiae* copper uptake genes but they are regulated differently, reflecting different responses of these two yeasts to their contrasting natural environments.

Potential cofactors and regulatory targets of the zinc finger protein (zfp) family in *Trypanosoma brucei*

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The parasitic kinetoplastids have a unique reliance on posttranscriptional control of gene expression because their genome is organized into polycistronic transcription units in which adjacent genes are not coordinately regulated. Although kinetoplastids are evolutionarily ancient, they conserve many of the core components of the eukaryotic machinery for mRNA turnover. We have discovered a novel small CCCH zinc finger protein, TbZFP3, that promotes differentiation between life cycle stages in *Trypanosoma brucei*. This protein interacts in a lifestage-specific manner with the translational machinery as well as the other small CCCH proteins of the ZFP family previously implicated in trypanosome developmental control. Additionally, we have isolated potential cofactors that lend insight into the functional role of this protein family. Investigation into the identity of specific regulatory targets is currently underway. The ZFP proteins are unique regulators of developmental events in kinetoplastids that act at the level of the posttranscriptional control of gene expression.

Systems-level analysis of yeast osmoregulation

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The ability to adapt to altered water availability is a fundamental property of living cells. The principles underlying osmoadaptation and the control of cellular water homeostasis are well conserved from yeast to human. Upon shift to high osmolarity, yeast cells stimulate the HOG (p38) MAPK system, which orchestrates the transcriptional as well as post-transcriptional responses.

Activation and deactivation of MAP kinase pathways is tightly controlled by feedback mechanisms because prolonged activation is detrimental. By employing a combination of experimental studies and mathematical modelling we show that the critical event in downregulation of the osmosensing yeast HOG pathway is signal cessation. For instance, inability to produce or to retain the osmolyte glycerol results in prolonged HOG pathway activation. Moreover, the HOG pathway is re-activatable by subsequent osmotic shock treatment. To illustrate the importance of turgor changes in the HOG pathway activation/deactivation profile we employed an artificial system in which yeast passively adapts to an osmotic shock. Taken together, the HOG pathway combines rigorous feedback control with competence for pathway reactivation. This is in accordance with its role in turgor regulation and achieved by mainly controlling pathway activity by turning on and off the osmosensors.

The HOG pathway consists of two apparently independent sensing branches that converge on activation of the MAPKK Pbs2 and eventually the MAPK Hog1. Recently we have started to dissect the relative contributions of these two branches to signalling. Initially, we study the branches individually in mutants that block one branch. Using computational modelling and specific experiments we try to reveal the individual contributions of each branch, and hence its specific role, in the overall response.

Part of the HOG-dependent response aims at increasing the capacity to produce and accumulate glycerol, the yeast osmolyte. Glycerol accumulation in yeast is controlled by at least four different mechanisms (1) Closing of the aquaglyceroporin Fps1 in order to retain glycerol; (2) stimulation of glycolysis by activation of fructose-2,6-kinase; (3) increasing the capacity for glycerol production by enhancing expression of relevant genes; (4) enhanced capacity for active glycerol uptake. We presently try to elucidate the contributions of these different mechanisms in a quantitative manner over time.

In conclusion, quantitative experimental studies combined with mathematical modelling advances our understanding of the mechanisms controlling osmoadaptation and water homeostasis in yeast, which has model character for other fungi and higher cells.

The Alpha project and the vision of a predictive biology

R. Brent

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Abstract not received

Eukaryotic-like signalling and gene regulation in bacteria that undergo development

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In response to starvation, certain bacteria undergo developmental processes that include intercellular signalling, cell fate determination, multicellular morphogenesis, and cellular differentiation. Outwardly, these processes resemble those of eukaryotic micro-organisms. Are similar molecular mechanisms used? Sometimes. For example, *Myxococcus xanthus* is a soil bacterium that undergoes a multicellular developmental process resembling that of *Dictyostelium discoideum*. A hundred thousand cells aggregate to form a fruiting body of uniform size and shape, within which some of the cells differentiate into spores. The mechanisms of motility and intercellular signaling during aggregation are quite different in the two organisms. On the other hand, some of the intracellular signal transduction pathways and gene regulatory mechanisms in *M. xanthus* are eukaryotic-like. There are 99 genes for eukaryotic-like Ser/Thr protein kinases in the 9 Mb *M. xanthus* genome and 52 genes for enhancer-binding proteins predicted to activate transcription *via* DNA looping. Gene proximity and other evidence suggests these eukayotic-like proteins function together in signaling pathways, or in pathways with the numerous His protein kinases (137 genes) and response regulators typical of bacteria. Genome analysis indicates that *M. xanthus* favors multi-component regulators over one-component regulators much more than other bacteria. This may endow *M. xanthus* with signal integration and gene regulation capability necessary for its social lifestyle. Other emerging examples of eukaryotic-like gene regulatory mechanisms in *M. xanthus* will be presented, as well as examples of eukaryotic-like signaling by regulated proteolysis during *Bacillus subtilis* sporulation.

Cyclic cAMP signalling in protist survival and evolution to multicellularity

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In contrast to solitary amoeba, which encyst individually in response to nutrient- or other stress, social amoeba aggregate to form multicellular fruiting structures where most of the cells differentiate into spores and a smaller proportion into stalk cells. Cyclic AMP (cAMP) controls the major life cycle transitions in the social amoeba *Dictyostelium discoideum*. Extracellular cAMP acts as a chemoattractant that mediates the aggregation of starving cells and induces entry into the spore pathway. Intracellular cAMP acting on PKA triggers maturation of stalk and spore cells, and regulates the germination of spores. The cAMP signalling pathways that regulate these processes contain both vertebrate-like and prokaryote-like components with a dominant role for sensor histidine kinases. By studying the presence and function of cAMP signalling genes throughout the Dictyostelid phylogeny and their ancestors, the solitary amoeba, we found that the intracellular role for cAMP in controlling spore formation and germination originates from a deeply conserved role in regulation of encystation and excystation in solitary amoeba. The role of extracellular cAMP in controlling cell aggregation in the derived species D.discoideum originates from an ancestral role in the sporulation process of basal Dictyostelid species.

Signals from hostile environments: the lucky life cycle of *Trypanosoma brucei*

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All parasites that thrive in the mammalian bloodstream face two major challenges: they have to effectively combat the host's immune response and they need to maximize their chance of transmission before death of the host. African trypanosomes provide an attractive model for studies on the molecular interface between extracellular parasites and mammals. Proliferating 'slender' cells of *Trypanosoma brucei* evade the host's immune attack by antigenic variation, i.e. by switching the expression of the predominant cell surface antigen. The growth of the parasite population, however, is not only limited by the host's immune response, but also by the parasite itself. *T. brucei* responds to increased cell density by differentiation to the cell cycle arrested 'stumpy' stage. Stumpy trypanosomes have lost a major line of defence, the capability of undergoing antigenic variation. At the same time, stumpy cells are the leading actors in a dramatic part of the parasite's life cycle, the infection of the transmitting tsetse fly. We postulate that *T. brucei* stumpy cells are not stranded in the mammalian bloodstream. They integrate in part distinctive molecular machineries that allow temporary survival in the host as well as successful infection of the insect vector. The cells are able to rapidly clear host-derived antibodies from their cell surface. We postulate that this unique phenomenon is accompanied by augmented endocytosis and changes in the control of surface protein expression and trafficking. The environmental signals that direct life cycle progression in African trypanosomes will be highlighted.

The role of cGMP and cGMP-dependent protein kinase in the sexual development of the malaria parasite *Plasmodium falciparum*

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The life cycle of the malaria parasite is complex; comprising an asexual blood stage that causes disease pathology and a sexual stage responsible for transmission to female *Anopheles* mosquitoes. The sexual stage of the life cycle is initiated in the bloodstream of the mammalian host in the form of male and female gametocytes. Mature gametocytes are taken up by a mosquito during a blood meal; on entering the midgut, they quickly escape from the encapsulating red blood cell prior to fertilization. Male gametogenesis is a metabolically and visually spectacular process called exflagellation resulting in the release of eight highly motile male gametes. Previous work has implicated cGMP in this process. The levels of cGMP in a cell are finely balanced between synthesis by guanylyl cyclase and breakdown by phosphodiesterase. One of the major intracellular receptors for cGMP is cGMP-dependent protein kinase. We have identified genes encoding these enzymes in the *P. falciparum* genome and have generated mutant *P. falciparum* cell lines to investigate the potential role of cGMP in sexual development. Our results suggest that cGMP is essential for the earliest events of gametogenesis in *Plasmodium falciparum*.

Inositol phosphate signalling and *Dictyostelium* chemotaxis

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Inositol, a 6-carbon sugar, provides the structural backbone for a series of lipid and water-soluble signal molecules in eukaryotic cells. These molecules are interconnected through a network of catabolic

and metabolic enzyme cascades, whose complexity we are only just beginning to understand. The membrane lipid Phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] is hydrolysed by phospholipase C (PLC) to release inositol (1,4,5) trisphosphate [Ins(1,4,5)P_3] into the cytosol, where it triggers a wave of higher intracellular Ca^{2+}. Traditionally, regulation of PLC is considered to function via Ca^{2+} change, however Ins(1,4,5)P_3 is rapidly converted to InsP_6, and it is becoming clear that this molecule and its intermediate metabolites also have cellular functions.

During *Dictyostelium* chemotaxis, cAMP stimulation generates a fast peak of Ins(1,4,5)P_3 production. However, single or double disruption of the *Dictyostelium* PLC gene and MIPP2, which generates Ins(1,4,5)P_3 from InsP_6, has no apparent effect on chemotaxis or development. Here, we resolve this paradox by examining the effect of lithium, a drug that attenuates Inositol Phosphate [InsP] signalling by reduction of inositol production. By investigating lithium sensitivity of these, and other, mutants we demonstrate that indeed inositol-based events are required during chemotaxis and present alternative mechanisms for the action of PLC and highly phosphorylated InsPs.

Fungal responses to microbial and environmental cues

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As with other microbes, fungi sense and respond to signals, stresses and other cues that are perceived in the environment. The nature of these cues and of the fungal response can have important consequences for the fungal life-cycle and physiology in ecological and clinical settings. Our group is particularly interested in how the model yeast *S. cerevisiae* and pathogenic *Candida* spp. respond to extracellular signals. In particular, we focus on secondary metabolites and signal molecules secreted by *Pseudomonas* bacteria, and on how yeasts perceive and respond to these. As with other research groups, we find that 12 carbon molecules related to bacterial N-acyl homoserine lactones influence morphology of *C. albicans* and are exploring this interaction in more depth using a bank of CF *P. aeruginosa* isolates. We are also examining the effect of metabolites from *P. fluorescens,* and have focused on 2,4 diacetylphloroglucinol (DAPG) using *S. cerevisiae* as a model. Using a proteomic approach followed by physiological assays, molecular probes, reporter constructs and mutant strains, we identified a number of key metabolic pathways that respond to DAPG treatment, including environmental stress response, calmodulin-regulated pathways, and multidrug exporters. We have explored the significance of these responses in more detail. Finally, in the opportunistic pathogen *C. glabrata,* we are addressing the importance of calcium-regulated signal transduction pathways for survival under stressed conditions, for example within host cells.

Signalling between marine roseobacters and dinoflagellates

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Members of the *Roseobacter* clade of the α-*Proteobacteria* are among the most abundant and ecologically relevant marine bacterial groups. One of the most salient features of the *Roseobacter* clade bacteria, such as *Silicibacter* sp. TM1040, is their ability to metabolize dinoflagellate-derived dimethylsulfoniopropionate (DMSP), a major source of organic sulfur in the ocean. *Silicibacter* sp. TM1040 forms an obligate symbiotic relationship with DMSP-producing dinoflagellates and phytoplankton suggesting that these bacteria are highly adapted to engage in both positive and negative interactions with other marine micro-organisms. *Silicibacter* sp. TM1040 actively metabolizes DMSP, and is chemotactically attracted to dinoflagellate homogenates, DMSP, amino acids, and other chemicals released by the dinoflagellate. Little is known about the cellular factors and molecular mechanisms required for roseobacters to establish and maintain their symbiosis with dinoflagellates. Recently, we discovered that *Silicibacter* sp. TM1040 produces an antibacterial compound, tropodithietic acid, which inhibits many other marine bacteria. A genetic analysis has revealed the biosynthetic pathway, as well as the regulatory circuit controlling tropodithietic acid expression. The implications of these data and the effect of the antibiotic on the interaction of roseobacters with their dinoflagellate hosts, the structure and composition of bacterial communities, and the sulfur cycle are discussed.

Protein tyrosine phosphatase TbPTP1: a molecular switch controlling life cycle differentiation in trypanosomes

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Differentiation in African trypanosomes (*Trypanosoma brucei*) entails passage between a mammalian host, where parasites exist as a proliferative slender-form or a G0-arrested stumpy-form, and the tsetse fly vector. Stumpy-forms arise at the peak of each parasitaemia and are pre-adapted for differentiation to procyclic-forms that inhabit the tsetse midgut. We have identified a protein tyrosine phosphatase (TbPTP1) pivotal in trypanosome differentiation. Consistent with a tyrosine phosphatase, recombinant TbPTP1 exhibits the anticipated substrate and inhibitor profile, and its activity is impaired by reversible oxidation. Remarkably, TbPTP1 inactivation in bloodstream trypanosomes by RNAi or pharmacological inhibition triggers spontaneous differentiation to procyclic forms in a sub-set of committed cells. Consistent with this, homogeneous populations of stumpy forms synchronously differentiate to procyclic forms when tyrosine phosphatase activity is inhibited. Our data invoke a new model for trypanosome development in which differentiation to procyclic forms is prevented in the bloodstream by tyrosine dephosphorylation. This model has implications for using PTP1B inhibitors to target trypanosomatid transmission.

Fermentation & Bioprocessing Group session

Continuous culture: revisiting from a post-genomic perspective

History and current perspectives of continuous culture

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The use of continuous culture techniques in studying microbial physiology dates back to the early 1950's and since then the technique has been accepted as an important tool by many microbiologists. Today the continuous culture is seeing something of a revival in post genomic studies. This talk will concentrate on some of the fundamental advantages and disadvantages of technique to such studies looking at aspects of the mathematical model that underpins the method.

One of the most important prerequisites to the application of this culture technique is the establishment of the 'steady state'. This term will be challenged during the talk using examples from our laboratory that suggest that the steady state is never achieved in a microbial population.

Chemostat-based transcriptomics: possibilities and pitfalls

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Excellent experimental (intra- and inter-laboratory) reproducibility and, in particular, the option to change individual culture parameters, make chemostat cultivation an ideal platform for high-information-density studies, such as transcriptome analysis with microarrays. Ongoing studies in this field have revealed a number of important methodological and conceptual constraints, that should be taken into account in the design and interpretation of transcriptome studies. Using examples from our own research on chemostat cultures of the yeast *Saccharomyces cerevisiae*, I will discuss the following issues:

1. Context dependency: the transcriptional response to any external stimulus (or genetic intervention) strongly depends on the experimental 'background' against which this response is recorded. Consequently, classical comparisons with a single reference condition are of limited value.

2. Transcriptional regulation and functional analysis: null mutations in genes with unknown function that are consistently up-regulated under a certain set of conditions do not always confer a selective disadvantage under the same set of conditions.

3. Post-transcriptional regulation: transcript levels of structural genes have little, if any, predictive value for flux through the metabolic pathway in which the encoded protein participates. Chemostat cultivation is, however, ideally suited to dissect and quantify the contribution of different levels of regulation.

Global physiological analysis of bacterial cultures

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During the last thirty years many sophisticated molecular methods have been developed that allow us to obtain a much more detailed insight into the life of microbial cells. This 'molecular focus' has led to an unfortunate disinterest in the rigorous control of cultivation conditions in laboratory experiments. Particularly continuous cultivation, which was used extensively in the 1960s for investigating fundamental aspects in microbiology including kinetics, genetics, biochemistry, physiology and ecology, was considered a difficult und unnecessary tool. Recently a revival in basic microbial physiology is seen and the developed molecular methods are now being applied to study microbial cultures at the transcriptome, proteome or metabolome level. Applying these sophisticated tools to physiological questions requires a strict control of experimental growth conditions in order to obtain reproducible data. This applies particularly when investigating metabolite and mRNA levels, both of which are highly dependent on environmental conditions and time. Many examples are found in the literature where the potential of molecular techniques was lost by applying them to cultures that had been grown under ill-defined conditions. Here, several examples will be given that illustrate the advantages of using continuous cultivation in global physiological studies. In particular the use of chemostats for investigating the role of the general stress response sigma factor RpoS will be discussed.

Elucidating the response of *Corynebacterium glutamicum* to ammonium limitation using global analysis techniques and continuous culture

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Nitrogen metabolism in the amino acid producer *Corynebacterium glutamicum* was subject of research for several decades (for review, see [1]). While previous studies focused on single enzymes or pathways, the publication of the *C. glutamicum* genome sequence gave fresh impetus to research and global studies of metabolism and regulation networks became possible.

In batch culture *C. glutamicum* reacts to nitrogen deprivation with a rearrangement of the cellular transport capacity, changes in metabolic pathways for nitrogen assimilation and amino acid biosynthesis, enhanced energy generation and increased protein stability [2]. Using chemostat experiments we were able to distinguish general starvation effects from specific nitrogen limitation-dependent changes [3]. The core adaptations of *C. glutamicum* to cope with poor nitrogen supply are controlled by the TetR-type transcriptional regulator AmtR [4].

References: [1] Burkovski (2005). In *Handbook of Corynebacterium glutamicum*, pp. 333–349. Boca Raton, FL: CRC Press LLC. [2] Silberbach *et al.* (2005). *J Biotechnol* **119**, 357–367. [3] Silberbach *et al.* (2005). *Appl Environ Microbiol* **71**, 2391–2402. [4] Beckers *et al.* (2005). *Mol Microbiol* **58**, 580–595.

Carbon flux analysis in *Clostridium cellulolyticum*: a continuous culture approach

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Compared to other saccharolytic clostridia and as most truly cellulolytic clostridia, *Clostridium cellulolyticum* is characterized by limited carbon consumption, and subsequent limited bacterial growth. Early metabolic studies performed in batch cultures suggested that it resulted from inefficient cellulolysis, nutrient(s)

limitation, and/or by-product(s) inhibition. Instead, metabolic flux analysis (MFA) in chemostat cultures, using either cellobiose (a soluble cellodextrin resulting from cellulose hydrolysis) or cellulose (an insoluble biopolymer) as sole carbon and energy source, suggests self-intoxication of bacterial metabolism resulting from inefficiently regulated carbon fluxes. In fact, MFA revealed that (i) in comparison to cellobiose, cellulose hydrolysis by cellulosomes introduces an extra regulation of entering carbon flow resulting in lower metabolic fluxes on cellulose than on cellobiose, (ii) glucose 1-phosphate/glucose 6-phosphate branch point controls the carbon flow directed towards glycolysis and dissipates carbon excess towards the formation of cellodextrins, glycogen and exopolysaccharides, (iii) the pyruvate/acetyl-CoA metabolic node is essential for regulating electronic and energetic fluxes. This in-depth carbon flux analysis on actively growing bacteria strengthens the idea of cellulolytic clostridia particularly well adapted, and even restricted, to a cellulolytic lifestyle, and further served as basis for the improvement of cellulose degradation by *C. cellulolyticum*.

Transcriptional profiling of bacterial responses to nitrosative, oxidative and metal stresses in a chemostat – the physiologist's Swiss army knife

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The chemostat has a long and distinguished history of service to microbial physiologists. A distinctive feature is the ability to control specific growth rate, despite changes in the medium or environmental perturbations, which is finding renewed usefulness in transcriptomics, where a frequent experimental design is to compare growth under 'control' and 'stress' states. Although post-genomic technologies offer unprecedented opportunities for observing global changes in physiological potential (as revealed, for example, in transcript populations) they also reveal changes unrelated to the primary experimental variable; thus, elimination or reduction of undesired growth perturbations is an experimental pre-requisite.

Here we illustrate recent applications of chemostats to understanding the stress and adaptive responses mounted by *Escherichia coli* or *Campylobacter jejuni* in the face of (a) short- and long-term treatment with elevated, growth-inhibitory concentrations of zinc, (b) continuous growth with limiting zinc concentrations, (c) short-term exposure to nitric oxide and nitrosating agents and (d) the transition between anaerobiosis and aerobiosis. Notable features of these experiments are the lack of transcriptional changes that can be attributed directly to growth rate and, by comparison with parallel datasets derived from batch cultures, relatively simple, and frequently comprehensible, transcriptome profiles.

This work was supported by the BBSRC.

Continuous cultures in metabolic engineering and modelling in *Streptomyces* (and other *Actinomycetes*)

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Continuous cultures are a key tool in the analysis of the micro-organism's physiological status, due to the possibility of obtaining steady state cultures under well defined growth conditions.

Streptomyces, producers of a large number of antibiotics and other bioactive molecules, is the best characterized genus of the actinomycete family, in terms of their genetics and physiology.

The recent sequencing of the genomes of *Streptomyces coelicolor* and *S. avermitilis* has resulted in the construction of models representing their metabolism. With appropriate modifications, those models can be extended to related species.

Stoichiometric models involving 100–120 reactions have been constructed for several actinomycetes, including *S. clavuligerus, S. coelicolor, S. lividans, Amycolatopsis orientalis* and *Saccharopolyspora erythraea*, and have been used for the design of bioprocesses and metabolic engineering strategies with the aid of metabolic modelling approaches such as Metabolic Flux Analysis (MFA) and Flux Balance Analysis (FBA).

MFA of chemostat cultures subject to a systematic variation of growth rates identified those reactions showing highest influence on clavulanic acid biosynthesis by *S. clavuligerus*, explaining how precursor availability is affected by the growth limiting nutrient. Similarly, the distribution of carbon flux through catabolic pathways in *S. lividans* was shown to be dependent on both the growth-rate and nutrient availability. Increasing growth rates lead to increased flux through glycolysis and the pentose phosphate pathway, affecting the synthesis of the antibiotics actinorhodin and undecylprodigiosin.

A genome scale metabolic network has been constructed for *S. coelicolor* and used to analyze the metabolic capabilities of the species. Using metabolic flux variability (MFV) analysis, an extension of FBA, it has been found that areas of metabolism from different pathways interconnect to form an actinorhodin-generating network. Also, it has been possible to predict the effect of gene deletions on the physiology and the biosynthetic capability of the resulting mutants.

The combination of metabolic modelling and chemostat experiments is a powerful tool for physiological and functional genomic studies, not only for basic biological studies but also for applied objectives, providing support in the design of bioprocesses and in the prediction of targets to improve the metabolic capabilities of micro-organisms.

In vivo molecular evolution of enzymes and metabolic pathways in continuous culture

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Metabolic Explorer is a leading company in the development of efficient micro-organisms for the production of chemicals and pharmaceuticals by fermentation or bioconversion. The different and complementary Metabolic Explorer platforms enable the construction of genetically optimized micro-organism based on a rational metabolic engineering approach. However, improved enzymes or new enzyme activities are needed that frequently can not be obtained sufficiently rapidly by either rational protein engineering or the now classical evolutionary methods like error prone PCR or DNA shuffling.

Metabolic Explorer has recently developed and patented an efficient in vivo molecular evolution method, MetEvol, to rapidly evolve enzymes or metabolic pathways. The simple principle of this method is to link the activity of the enzyme(s) or the metabolic pathway to evolve to the growth of the host organism. When such a strain is constructed, it is then easy to evolve the enzyme(s) (thanks to the 'natural mutation frequency' of living organisms) by selecting for growth improvement in a continuous culture. To highlight efficiency of this approach, we will present several examples where in less than a month improved or new enzymes were successfully obtained. In the context of developing new bioprocesses involving bioconversions, we will demonstrate how this method can really speed up the time to market of new 'biochemicals'.

Food & Beverages Group session

Disinfection in the food and beverage industry

Incidence of food poisoning related to fruit and vegetables

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In the last twenty years there has been in increase in recognized outbreaks of both bacterial and viral origin linked to the ingestion of contaminated seeds, fruit and vegetables including salads vegetables such as lettuce, tomatoes and herbs. Investigation of outbreaks has shown that the problem of contaminated food of plant origin irrigated with contaminated water has caused international outbreaks. Several outbreaks, due to, for example; *Salmonella*, *Shigella*, *Yersinia pseudotuberculosis*, *E. coli* O157 and *Hepatitis* A have been caused by eating contaminated salad vegetables. The globalization of the market means that fresh fruit and vegetables can arrive in this country and be distributed within hours of arrival. An overview of plant product outbreaks over the last twenty years will be presented together with new evidence gathered from the proactive sampling of imported foods by local authorities showing contamination of snack seeds, flour, and of imported fresh herbs flown in from the Far East.

Sources of the problem; the need for hygienic design

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Cleaning and disinfection are critical elements in assuring the production of safe and wholesome foods but are rarely given the attention they deserve during the engineering design of food processes and equipment.

Hygienic design is far too often exclusively associated with the design of equipment and this presentation aims to highlight the importance of other engineering related aspects of design such as process design, installation and layout, monitoring and control. Practical examples will be given to illustrate the impact of these engineering factors on the disinfection of food process plant.

Biofilm or not? The importance of attached micro-organisms on food contact surfaces

Joanna Verran

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The presence of viable micro-organisms on a surface presents a 'biotransfer potential'. These micro-organisms may be actively multiplying and colonizing the surface, or may merely be surviving, whilst retaining the ability to multiply when subsequently encountering a more favourable environment.

Micro-organisms which form part of a 'classical' biofilm are present at a dynamic solid-liquid interface, within an extracellular matrix. On hygienic food contact surfaces, this may not be the case. Attached micro-organisms may be retained in surface features, mixed with organic (eg food debris/soil) or inorganic (eg cleaning agent) debris. These non-microbial components will provide additional barriers to adequate cleaning and disinfecting regimes, and are less easily removed from surfaces than micro-organisms: indeed the material may accumulate during repeated fouling-cleaning cycles.

It is important to monitor the behaviour of both micro-organisms and non-microbial soil components on a surface, because the presence of the soil provides a challenge for cleaning procedures, and may affect the survival of any micro-organisms present, afford protection from disinfection, enhance attachment to the surface, and potentially provide nutrient. Differential fluorescent staining, and assessment of the contribution of both components to surface coverage provides a relatively simple approach. More sophisticated surface analytical techniques provide additional information on surface topography and the chemistry of the substratum and fouling layers, which in turn informs decisions concerning the hygienic status of a surface and the choice of an appropriate cleaning regime.

Non-microbial soil on surfaces affects cleanability, and its presence should be monitored along with the presence of microbial cells during cleaning and sanitising regimes.

The term 'biofilm' is used to describe diverse modes of microbial surface 'growth'. An understanding of this diversity is important when considering appropriate cleaning and sanitation approaches.

Aspects of disinfection resistance

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The terms resistance and susceptibility are relative ones and could be equally applied to collections of organisms that were variously but exquisitely responsive to disinfection treatment. 'Resistant' on the other hand is a term that is applied to those organisms that fail to respond to treatments that are normally effective against genetically related strains and species, and implies a genetic or phenotypic *change* that render the cells insusceptible. Resistance development (susceptibility change) towards disinfection agents is rarely of sufficient magnitude to render disinfection regimens ineffective, nevertheless in the general environment there are some species and phenotypic variants (endospores) that are intrinsically insensitive. Sadly, inappropriate use of the term 'resistance development' has led to misplaced concern that disinfection protocols are suffering the same assault on effectiveness as are antibiotic therapies for infectious disease.

Chronic, sublethal exposure of pure-cultures of bacteria towards a broad spectrum of antibiotics and antibacterial agents invariably leads to the selection of less susceptible phenotypes. These might reflect genotypic variation and be relatively stable in the absence of the selection agent. One might therefore logically predict that the chronic use and abuse of such agents over the last 100 years would have led to a lowering of their effectiveness. Whilst this is undoubtedly the case with antibiotics where resistant strains evolve during the treatment of essentially monoculture infections, and pass horizontally between patients, there is little or no evidence of parallel trends with respect to the environment and common actives of disinfectant products such as triclosan, quaternary ammonium compounds, oxidizers and biguanides. Products containing antibacterial agents such as these are invariably deployed in

situations where stable polymicrobial communities exist, *i.e. soil, oral cavity, skin,* or upon aesthetically clean hard surfaces (Food industry), where the susceptibility profile of resident / transient species is broad. Exposure of the community to antibacterial agents will therefore kill / inhibit the growth of some strains and reduce the growth efficiency of others, yet will leave many others unaffected. Whilst partially inhibited strains will be subject to a selection pressure towards less susceptible phenotypes, this will incur a fitness-cost and a temporary loss of competitive efficiency. Climax communities generally resist the influx of new species since adventitious arrivees must be able either to out-compete residents in terms of nutrient utilization and/or occupy vacant functional niches. During sub-lethal exposure to antibacterials, colonization resistance is lost and adapted strains must re-compete for their position in the community. Invariably this battle is lost with the effects of antibacterial use being a clonal expansion of pre-existing less susceptible strains with displacement of the susceptible ones. Microcosm studies will be described that illustrate these trends with respect to triclosan, quaternary ammonium biocides and biguanides.

Pulsed ultra-violet photoreactivation spectrum of *Listeria monocytogenes*

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A solid-state pulsed-power source is used to transfer stored electrical energy to a xenon flashlamp in a short duration but with high peak power. The flashlamp provides short-duration light pulses and high energy output over a broad spectral band from the ultraviolet to the infrared. The arrangement was used in conjunction with a 260 nm narrow band-pass filter (bandwidth 10 nm) to examine the inactivation of *L. monocytogenes* NCTC 11994 (serotype 4b). The wavelength of 260 nm is known to be close to the most efficient wavelength for the UV inactivation of pathogens. Photorepair of the inactivated samples was then examined at several wavelengths in the 300 nm – 500 nm range using the pulsed light from the xenon flashlamp. Separate wavelengths within this range were isolated using narrow-band filters, each of bandwidth 10 nm. A calibrated sensor allowed absolute measurement of light energy density falling on the bacterial samples during both inactivation and photoreactivation. This study provides the first data on the efficiency of UV inactivation at a particular wavelength, and the efficiency of photoreactivation as a function of wavelength, for *Listeria monocytogenes* – a food-borne pathogen of major concern to the food industry.

Atmospheric cold gas plasmas: a potential decontamination process for foods

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Gas plasmas comprise mixtures of ionized atoms and molecules and have been referred to as the 'fourth state of matter'. In the last 20 years methods have been developed for generating gas plasmas at atmospheric pressures and at temperatures that are near ambient. There has been growing interest in using such 'cold plasmas' for decontaminating surfaces that harbour micro-organisms, but to date little of this attention has focussed on foods. Our objectives are to establish whether cold plasmas can realistically be used to treat the surfaces of fresh foods such as salad vegetables and fruit that cannot be treated by conventional thermal methods without undergoing deterioration. We present microbial inactivation curves obtained on both abiotic surfaces (membrane filters) and food surfaces (melon discs) obtained using a cold gas plasma generated using He and O_2. In these experiments we employed a variety of spoilage micro-organisms (e.g. *Gluconobacter oxydans* and *Saccharomyces cerevisiae*) and pathogens (e.g. *E. coli* and *Listeria monocytogenes*) that are commonly associated with ready to use fruit and salad foods. We were further able to describe the inactivation curves obtained using the Weibull mathematical model and discuss the implications of our results for commercial processing systems.

The effect of surface topography and chemistry on microbial retention

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Much work has been carried out on the effect of microbial retention on surfaces with topographical features effectively randomized across a surface (i.e. size, orientation, shape of pits, scratches). To gain a better understanding of the fundamental cell: surface interactions, surfaces with defined features, (topography, hydrophobicity, chemistry) were produced in house using a combined physical vapour deposition/template technique. Surfaces with titanium dioxide chemistry were produced with features (pits 0.2, 0.5, 1, and 2 µm diameter (Ra 0.04–0.217 µm).

Retention assays were carried out using micro-organisms of different sizes/shapes (cocci, rods, *Bacillus* spores and yeast). The use of defined surfaces demonstrated that the shape and size of the micro-organism relative to that of the surface defect were significant features affecting retention.

Using the Atomic Force Microscope under liquid, attached *Pseudomonas aeruginosa* or *Staphylococcus aureus* were removed from a surface by applying increasing force. For *S. aureus* cells, this enhanced removal from the smooth titanium surface. In contrast, *P. aeruginosa* was removed more easily from the 0.5 µm featured surface. This effect is due to the amount of area contact between the cell: surfaces.

A fundamental understanding of the cell: substratum interface may lead to the production of more hygienic surfaces.

Strategies for control: development of appropriate methods

John Holah

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The presentation will initially review why disinfection is necessary in the food industry and where it is appropriate based on risk assessment. This will include what are our target micro-organisms (spoilage/pathogens), under what physiological status we expect to find them (in suspension, surface adhered, protected by organic matter, biofilms) and where they are likely to occur (food contact surfaces, hands, processing environment, air). The choice of disinfectants available and their application will also be considered, focussing on our knowledge of ratios of disinfectant volume to micro-organisms, i.e. how much disinfectant should we use. Finally, how we control the disinfection process in practice and how we determine its success will be described.

So is disinfection adequate in the food industry? In terms of controlling pathogenic micro-organisms in product overwhelming yes; but what about persistence? The industry is beginning to recognize the existence of persistent or 'house' strains, the presence of which our current cleaning and disinfection protocols cannot

control. This maybe due to a number of reasons; are the micro-organisms beyond the reach of our disinfectants, are our disinfectants unsuitable (what is the relationship between disinfectant approval schemes and real life) or is there any evidence for resistance. The impact of some future technologies including enhanced microbial removal, synergy between cleaning and disinfection compounds, antimicrobial surfaces and wholeroom disinfection techniques will be considered.

The impact of legislation: the Biocidal Product Directive

John Rigarlsford

John Rigarlsford & Associates Ltd

The Biocidal Product Directive (BPD) was published on the 16 February 1998 as Publication of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. It was implemented in all EU States by the 14th June 2000. It covers all biocidal products not covered by other directives such as the Pesticide Directive. As part of the registration the competent authorities will review the actives used in biocidal products, then the products themselves and the product applications. The deadlines for submitting dossiers on actives have been announced and currently the actives are starting to be reviewed. The active dossier has to include efficacy data and very comprehensive data on toxicology and the environmental impact of the active. Already some suppliers have withdrawn actives as sales of the active do not justify the cost of obtaining the data for the dossier. This will inevitably lead to fewer, more expensive disinfectants. However, disinfectants, and other biocides will have better efficacy data. The implications of the BPD will be discussed, particularly its impact on the Food and Beverage Industry.

Microbial Infection Group / Physiology, Biochemistry & Molecular Genetics Group / Cells & Cell Surfaces Group / British Society for Proteome Research joint session

From proteomics to pathogenesis

How proteomics has contributed to our understanding of pathogenesis

C. David O'Connor

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Proteins are the ultimate effectors of disease and host responses to it. As such, the study of their expression, functions and interactions – proteomics – is directly relevant to an understanding of pathogenesis and provides information that is unobtainable by other approaches. For example, microbial pathogenesis often involves the delivery of virulence factors into an infected cell and subversion of normal cellular processes by proteolysis or chemical modification of key host components. Conversely, binding of particular host proteins to the cell surface of a pathogen may determine whether it evades the immune system or is cleared by it. In such cases, DNA or mRNA analyses are largely irrelevant – the pathogenic mechanisms can only be truly understood at the protein level. While significant technological challenges remain, proteomics is already providing insights into pathogenesis, most notably regarding: (i) the discovery of virulence-associated proteins; (ii) definition of their roles; and (iii) characterization of host responses to infection. The talk will review progress in these areas and will also consider how proteomics is currently contributing to the identification of vaccine candidates, and the identification of biomarkers for more rapid and accurate diagnosis of infectious disease.

A combined genomic and proteomic approach to the investigation of host-pathogen interactions during infection by *Bacillus anthracis*

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Bacillus anthracis exists in the environment primarily as a dormant endospore, and this form of the bacterium is both infectious and highly resistant to harsh environments. In order to better understand this unique morphology, we have characterized its composition using shotgun proteomics. We have identified more than 750 proteins present within the spore, and statistical analysis of these data has revealed new insights into spore function. In a complementary study, we have also used DNA microarrays to define the gene expression patterns that occur in *B. anthracis* throughout its life cycle, and we have used these data to examine the temporal expression of the spore proteome. In doing so, we have found that the spore contents are not synthesized *de novo* during sporulation, but rather appear to be packaged from pre-existing stocks. We explored several different potential mechanisms by which the cell could control which proteins are packaged into the developing spore, and our analyses were most consistent with a model for sporulation in which *B. anthracis* selects spore contents based on protein stability. Our findings suggest a model for sporulation that has broad implications for *B. anthracis* biology, and offer new possibilities for microbial forensics and detection.

Proteomics of *Staphylococcus aureus*

M. Hecker

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Proteomics is an excellent approach to 'bring the genome sequence to life'. Because of their low complexity bacteria are useful model systems to transfer the 'virtual life of the genes to the real life of the proteins' shown for our model bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Gel-based proteomics is used in combination with gel-free approaches on the way towards the visualization of the entire proteome. Protein expression profiling in growing cells (vegetative proteome) and non-growing cells of bacteria is the first step in this approach. In kinetic studies the reprogramming of the protein expression profile of growing to non-growing cells can be followed at a proteomic scale. Proteomics signatures for stress and starvation stimuli are useful tools for the prediction of the physiological state of growing and non-growing cells.

Because 'life is more than a mixture of proteins' it will be demonstrated how proteomic approaches can be used for analysing global control of protein stability, protein secretion, posttranslational modifications or protein damage. At the end of this part the physiological state of cells is visualized by 'comprehensive proteomic signatures' that include protein synthesis, protein level, protein stability, protein phosphorylation and protein damage.

In the main part of the talk it will be shown how to transfer our proteomics expertise from *B. subtilis* to *S. aureus*, a human pathogen which has become a threat to the human race because of its antibiotic resistance. A proteomic approach has been used to come to a comprehensive understanding of cell physiology focussing on metabolism, stress and starvation responses, extracellular proteins and protein expression networks.

Proteomics as a tool for studying pathogenesis

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Quantitative proteomics investigates cellular mechanisms at a molecular level by measuring the relative differences in protein abundance under different experimental conditions. Measurement of these changes is fundamental to many branches of biology, and can deliver insight into mechanisms of pathogenesis if utilized in an appropriate manner.

In order to achieve useful data sets using quantitative proteomics, it is necessary to carry out comparisons across large numbers of biological samples. All comparative experiments are subject to many degrees of variation such as technical experimental noise and biological variation (genetic, growth conditions). The challenge for researchers is to be aware of the degree of variation within and across experiments, in order that experiments can be adequately designed.

Currently there are many approaches to studying the proteome in a quantitative manner. One of the fundamental differences between these is the point within the experimental schema that quantitative measurements are made. These approaches and their relative power will be described with reference to their application to the study of pathogenesis.

Rapid single-dimensional monolithic liquid chromatography and ultra-fast MS/MS scanning for the analysis of proteins of *Escherichia coli* O157:H7

Neil F. Inglis[1], Benedikt M. Kessler[2], Douglas Fraser-Pitt[1], Ken Cook[3], Laura C. Main[4], Anneke Lubben[4], Andrea Kiehne[4], Lisa H. Imrie[1], Kevin Mclean[1] & David G.E. Smith[1]

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Rapid single-dimensional monolithic liquid chromatography (1D-LC) interfaced directly with electrospray ionization tandem mass spectrometry (ESI-MS/MS) has been applied to the identification of proteins comprising different fractions of *Escherichia coli* O157:H7 (*E. coli*) cell lysates as resolved by 1D-sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Lanes of resolved proteins were divided into equal (2.5mm) gel slices and processed as individual tryptic digests for downstream reversed phase LC. Using elution gradients of as little as 4min duration and ultra-fast (26,000 amu/sec) MS/MS scanning, this approach identified 125 and 119 proteins respectively in cell membrane extracts and TCA-precipitated secretory protein preparations. Similar results were obtained using a C18 Pepmap column but it was necessary to extend the duration of the elution gradient to 30min. This combined approach provides a means of identifying proteins in simplified biological mixtures rapidly and at higher levels of resolution than those offered by more conventional C18 LC.

The growth of group A streptococcus in hyaluronan-rich media results in the up-regulation of virulence factors and proteins of unknown function

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Group A streptococcus (GAS, *Streptococcus pyogenes*) is a Gram-positive human pathogen. GAS infections affect more than 18 million people per year with more than 0.5 million deaths. To invade the human tissue, GAS produces several virulence factors, and the degradation of hyaluronan in connective tissue is a key element of invasion infection. We investigated the molecular basis of infection using proteomics technology. GAS was grown in hyaluronan-rich media, which was used to create a simple system that reflected some elements of infection. The cell-associated proteins were separated by two-dimensional gel electrophoresis, fragmented using in-gel tryptic digestion, and analysed using ion trap mass spectrometry with static nanospray source. We identified several up-regulated proteins that included virulence factors and proteins of unknown function.

Mass spectral profiles of intact cells and membrane-bound/intracellular proteins of clinical isolates of *Staphylococcus aureus*

Lakshani Rajakaruna[1], Haroun Shah[1], Renata Culak[1], Vesela Encheva[1], Gill Hallas[1] & Graham Ball[2]

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Infections caused by *S. aureus* have been increasing with the emergence of multidrug resistant strains. Typing of isolates is currently based upon phenotypic and genotypic methods. Here, a new approach involving mass spectral analysis of both surface-associated (MALDI-TOF-MS) and membrane-bound/intra cellular proteins (SELDI-TOF-MS) is being investigated using 111 strains, presumptively identified as *S. aureus*. Results obtained indicate that clinical isolates share many common mass ions with type/reference strains but the former possess more complex mass spectra. The MicrobeLynx software is employed to analyse the intact cell data while Ciphergen Express Software is used to produce 'protein expression dendrograms'. In addition, a panel of 50 authentic strains has been used to construct an Artificial Neural Networks model which will be used analyse the data from clinical isolates. Once derived the data will provide a new means of analysing the intraspecies diversity of *S. aureus* and help to shed light on the virulence and pathogenicity of this species.

Developing a proteomic/molecular algorithm for the rapid characterization of *Bacillus* sp.

Nadia Ahmod, Gillian Hallas, John Hookey, Anthony Underwood & Haroun Shah

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Members of the genus *Bacillus* are widely distributed in nature and consequently encompass a broad and heterogeneous range of phenotypes. Attempts to redefine the genus have led to a restricted number of species but there is still a paucity of reliable characters to circumscribe each taxon.

This study focused on clinically important species and utilized a combination of MALDI-TOF-MS and comparative gene sequence analysis. 16S rRNA sequence analysis provided good presumptive identification but among closely related species results were often equivocal. However the *rpo*B gene, increasingly being used as a molecular chronometer in microbiology, yielded unambiguous identification of isolates studied so far. The mass spectral profiles derived from the surface-associated molecules of intact cells provided a good adjunct to the sequence data and facilitated separation of closely related species.

These techniques although still in their infancy are likely to have a profound effect on the speed and accuracy in the identification of clinical isolates in the near future.

Acknowledgement: Supported by a HPA PhD studentship.

Response of *Bacillus anthracis* to oxidative stress: a combined proteome and transcriptome analysis

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The response of *B. anthracis* to superoxide and peroxide stress were characterized as part of our work to understand interactioning between *B. anthracis* and host macrophages. 2D PAGE analysis shows distinct protein profiles in the presence of paraquat and hydrogen peroxide. In the case of hydrogen peroxide, four major proteins were identified by peptide mass fingerprinting: alkyl-hydroperoxide reductase, glyceraldehyde-3-phosphate dehydrogenase, RecA and catalase B. In contrast only two major proteins were induced in the presence of paraquat: an acyl carrier protein phosphodiesterase and a glyoxylase family protein.

Preliminary transcriptome analysis using Northern blots showed that some of the oxidative stress marker genes (*e.g. sodA,* encoding superoxide dismutase, and *trxA,* encoding thioredoxin) were induced by both types of oxidative stress. In contrast to *B. subtilis, katB,* encoding a catalase, was only induced by hydrogen peroxide. These data have been extended, in collaboration with Dr. T. Read of the

in US Navy, by a full transcriptomic analysis using tiled DNA arrays containing approximately 300,000 probes.

In vivo proteomic analysis of the intracellular bacterial pathogen, *Francisella tularensis*, isolated from mouse spleen

Susan M. Twine[1], Nadia C.S. Mykytczuk[1], Mireille D. Petit[1], Hua Shen[1], Anders Sjostedt[2], J. Wayne Conlan[1] & John F. Kelly[1]

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Understanding the pathogenesis of infectious diseases requires comprehensive knowledge of proteins expressed by the pathogen during *in vivo* growth in the host. Proteomics provides the tools for such analyses but the protocols required to purify sufficient quantities of bacteria from the host organism are currently lacking. Here we present a rapid immunomagnetic protocol for the separation of *Francisella tularensis*, a highly virulent bacterium and potential biowarfare agent, from spleens of infected mice. In one hour, bacteria can be isolated in quantities sufficient to carry out meaningful proteomic comparisons with *in vitro* grown bacteria. Furthermore, the isolates were virtually free from contaminating host proteins. 2D-PAGE revealed a host induced proteome in which 78 proteins were differentially expressed compared to *in vitro* controls. The results obtained demonstrate the complexity of the adaptive response of *F. tularensis* to the host environment, and the difficulty of mimicking such behaviour *in vitro*.

Mining quorum sensing regulated proteins – role of bacterial cell-to-cell communication in global gene regulation as assessed by proteomics

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Many Gram-negative bacteria utilize cell-to-cell communication systems that rely on diffusible *N*-acyl homoserine lactone (AHL) signal molecules to monitor the size of the population in a process known as quorum sensing (QS). In human pathogens this form of gene regulation ensures that the cells remain invisible to the immune system of the host until the pathogen has reached a critical population density sufficient to overwhelm host defenses. The QS regulon of *Pseudomonas aeruginosa* and *Burkholderia cepacia*, two important pathogens for cystic fibrosis patients, has been studied by proteome analyses. Comparative two-dimensional gelelectrophoresis coupled to mass spectrometry analysis or N-terminal sequencing has been employed to recognize and identify QS-controlled proteins. Our findings strongly support the importance of AHL-mediated cell-cell-communication as a global regulatory system and suggest that QS control also operates via post-translational mechanisms. As QS has been proven to be a central regulator for the expression of pathogenic traits in opportunistic human pathogens it represents a highly attractive target for the development of novel anti-infective compounds. Functional genomics technologies have been exploited to validate the target specificity of natural and synthetic QS inhibitors, thus having a great potential as alternative therapeutics for the treatment of bacterial infections.

Iron-regulated surface proteins of *Francisella*

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The Gram-negative facultative intracellular bacterium *Francisella tularensis* is the causative agent of tularemia, a highly infectious disease known to infect a range of vertebrates and invertebrates throughout the Northern Hemisphere. Human vaccination against tularemia was previously achieved with an attenuated *F. tularensis* Live Vaccine Strain (LVS). However, due to the undefined nature of both the protective response and the basis of attenuation, the production of a characterized tularemia vaccine remains the target of much current research. Although little is known about the virulence traits of the *Francisella* species, the implications are that the acquisition of iron is integral to the onset of infection. For this study a series of proteomic investigations using GeLC and iTRAQ were performed, with the aim of identifying putative iron uptake systems expressed during iron starvation by *F. tularensis* subspecies *novicida*. To complement this work, a series of microarray experiments were performed to identify iron-regulated genes. It is hoped these findings will provide more targets for the production of rationally attenuated *Francisella* mutants, helping the on-going search for a licensable tularemia vaccine.

Role of glycosylation in virulence of *Campylobacter jejuni*

John Kelly

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Although *Campylobacter jejuni* is a major cause of diarrheal disease worldwide, very little is known about how the organism persists in the environment and triggers human disease. Genome sequencing of NCTC11168 has demonstrated that *C. jejuni* devotes a relatively large proportion of its small genome to carbohydrate biosynthesis. This includes gene clusters involved in the biosynthesis of lipo-oligosaccharides, capsular polysaccharides, flagellar *O*-linked protein glycosylation and the first demonstration of a general *N*-linked protein glycosylation pathway. In order to fully understand the *C. jejuni* glycome, sophisticated analytical techniques are being employed to functionally characterize these pathways and continue to reveal unexpected complexities in the carbohydrate composition of this organism. This presentation will describe the flagellar *O*-linked and general *N*-linked protein glycosylation pathways highlighting the methods used to characterize these systems, the biological relevance the sugars play in *Campylobacter* survival and pathogenesis and the potential exploitation of the glycome for novel therapeutics against this common food-borne pathogen.

Host cell cytoskeletal target proteins affected by type IV secreted CagA protein of *Helicobacter pylori*

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Expression of the *Helicobacter pylori* CagA protein has been linked with an increased risk of gastric ulcer and adenocarcinoma. We and others have previously demonstrated tyrosine phosphorylation of CagA protein following its translocation into the host cell via type IV secretion. CagA tyrosine phosphorylation occurs at distinct sites resembling a Src-family kinase (SFK) specific consensus sequence (1). Interestingly, SFKs themselves become inactivated upon CagA

tyrosine phosphorylation via a negative feedback loop. As a consequence of SFK inactivation, several host cell cytoskeleton-associated proteins become dephosphorylated, which have been revealed as cortactin, ezrin and vinculin (2, 3). We can now show that CagA disrupts adhesion of AGS cells to the extracellular matrix via dephosphorylation of vinculin. Vinculin dephosphorylation causes a loss of interaction with p34Arc of the Arp2/3 complex and a reduction in the number of focal adhesion complexes. In consequence, vinculin tyrosine dephosphorylation affects severe cellular functions including cell matrix adhesion, cell spreading and wound-repair. Thus, CagA-mediated inactivation of vinculin is considered to play a key role in *H. pylori* induced damage of the gastric epithelium.

References: (1) Moese, S., Selbach, M., Zimny-Arndt, U., Jungblut, P., Meyer, T.F., Backert, S. (2001). Identification of a tyrosine-phosphorylated 35 kDa carboxy-terminal fragment (p35CagA) of the *Helicobacter pylori* CagA protein in phagocytic cells: processing or breakage? *Proteomics* **1**, 618–629. (2) Selbach, M., Moese, S., Hauck, C.R., Meyer, T.F., Backert, S. (2003). The *Helicobacter pylori* CagA protein induces cortactin dephosphorylation and cell scattering by inactivation of c-Src. *EMBO J* **22**, 515–528. (3) Selbach, M., Moese, S., Backert, S., Jungblut, P.R., Meyer, T.F. (2004). The *Helicobacter pylori* CagA protein induces tyrosine dephosphorylation of ezrin. *Proteomics* **4**, 2961–2968.

Using surface enhanced laser desorption/ionization-proteinchip (SELDI) technology for rapid characterization of pathogens

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SELDI TOF MS and the ProteinChip arrays provides the opportunity for rapid chromatography based purification and detection of proteins and peptides from complex biological mixtures. The technology is suitable for high-throughput applications and has been widely used in the field of disease diagnostics. However, the potential of the technique as a general typing tool in Microbiology has not been investigated.

In our laboratory SELDI TOF MS has been used in combination with other protein separation technologies such as SDS PAGE, 2D GE and DIGE for the characterization of microbial diversity and mechanisms of adaptation. The protein expression patterns of various gastrointestinal and respiratory pathogens such as *Neisseria* spp, *Enterococcus* spp, *Salmonella enterica*, *Streptococcus pneumoniae* and *Staphyloccoccus aureus* have been characterized to identify protein factors associated with virulence and potentially useful for their diagnosis. SELDI TOF when combined with suitable data analysis method has proven useful when characterizing the diversity of microbial populations and in differentiating bacterial isolates at the species or subspecies level. With consistent improvement of data analysis, the small sample volume required and the high-throughput nature of the technique, it is likely that SELDI TOF will have a prominent place as a tool for the characterization of microbes.

Proteomic comparisons of *Mycobacterium avium* subspecies *paratuberculosis*

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Paratuberculosis (Johne's disease) poses a significant economic problem to beef, dairy and sheep industries in the United Kingdom and worldwide and is caused by *Mycobacteriun avium* subspecies *paratuberculosis* (*Map*). *Map* can be subdivided into three types as defined by their pulsed-field genotypes and physiological characteristics and host preferences. We have used 2D gel electrophoresis to characterize the 'type proteomes' and their analysis has revealed type-specific proteins. The relevance of these proteins to phenotype will be discussed.

In an attempt to understand the interaction between *Map* and its host at the molecular level we have used 2-D PAGE as a tool to investigate the virulent state of *Map*. A direct comparison of the proteomes of *Map* scraped from the terminal ileum of ovine paratuberculosis cases and the identical strain grown *in vitro* is presented. These analyses have identified a set of proteins whose expression is up-regulated during natural infection.

Proteomic and functional genomic analysis of the plant pathogen, *Erwinia carotovora*

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The enteric phytopathogen, *Erwinia carotovora*, causes soft-rot disease of several important crops, including potato. The pathogenicity of this organism depends on the secretion of large quantities of multiple plant cell wall degrading enzymes, as well as production of other, more subtle, virulence factors. Production of secreted virulence factors by *Erwinia carotovora* is subject to a complex network of regulatory inputs, including quorum sensing (regulation according to cell density). In this work we have examined the cell-associated and secreted proteome of *Erwinia carotovora* in order to enhance our understanding of the mechanisms and regulation of pathogenicity. We have used proteomic techniques including 2D-Difference in Gel Electrophoresis (2D-DiGE) and mass spectrometry in combination with defined regulatory and secretion mutants. This has led to the identification of novel virulence factors and provided insight into the regulation and/or the secretion pathways of both novel and previously-described proteins of *Erwinia carotovora*.

Use of proteomics to study pathogenic effects of *Wolbachia* on fly sperm

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Wolbachia are obligate intracellular alpha-proteobacteria that infect a diverse array of arthropod hosts. Wolbachia are not generally pathogenic in the traditional sense, instead enhancing their own survival by disabling normal sperm function during fertilization. Thus Wolbachia are 'pathogenic' in a context-dependent fashion only in crosses between infected males and uninfected females through the expression of cytoplasmic incompatibility (CI). Because only Wolbachia infected males can express CI, the search has been on for over a decade to define the molecular mechanism(s) involved. One approach has been to identify biochemical differences in mature sperm from infected vs. uninfected males. I will discuss our recent efforts to identify such differences using 2D gels and mass spectrometry of sperm.

Genomics and proteomics approaches to investigate host–pathogen responses in the pathogenesis of listeriosis

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Internalization of the food-borne pathogen *Listeria monocytogenes* into non-phagocytic cells is mediated by the interactions between the surface proteins InlA (internalin) or InlB and their cellular surface receptors, E-cadherin and c-Met. To identify novel bacterial and cellular components involved in the infectious process, we undertook genomics and proteomics approaches. On the bacterial side, we focused our studies on surface proteins of the LPXTG family, exemplified by InlA, and that are predicted to be anchored to the proteoglycan by sortase A. Animal studies using a *srtA* mutant demonstrated the involvement of SrtA substrates in orally acquired listeriosis. By using a non-gel proteomics approach, we showed that the cell wall fraction of bacteria grown in rich medium contains at least 13 LPXTG proteins anchored by sortase A. By using a comparative genomics approach we were able to identify new virulence factors among LPXTG proteins. On the mammalian host side, to search for eukaryotic proteins involved in internalization or early events during infection, we performed a global proteomic characterization of the early listerial phagosome in the human epithelial cell line LoVo. Two-dimensional gel electrophoresis coupled to mass spectrometry identified several host proteins, among which a protein called septin 9. Septins are members of a novel family of GTPases involved in cytokinesis/septum formation, exocytosis and other trafficking events. Using immunofluorescence or immunogold labeling, we showed that during entry septin 9 is recruited transiently after the initial burst of actin polymerization and is associated with *Listeria* containing vacuoles. Three other pathogens, *Shigella flexneri*, *Rickettsia conorii* and *Salmonella typhimurium* also recruit septin 9 after entry onto cells. Our results point to the possible contribution of septins as factors controlling vacuolar escape of intracellular pathogens. In conclusion, combined genomics and proteomics approaches offers new opportunities to better characterize the complex interplay between *Listeria* and the mammalian cells.

Peter Wildy Prize for Microbiology Education

Not just germs – bringing bacteria to life.

Professor Liz Sockett

University of Nottingham

Professor Liz Sockett will share her experiences as a microbiology educator of University undergraduates, retired people from University of the 3rd Age, school children and teachers. We all face challenges in getting important messages across about micro-organisms that are too rarely explained in school curricula and often derogated as 'germs' on TV. Liz takes a popular approach to her task using home made 'props' and anecdotal stories to enliven her teaching. She knows that this works as years later graduates remember 'microbiology lectures' with fondness. Bacterial genomics also provides an excellent vehicle to show, in the public arena, that evolution is much more than theory! She will show some of the props and adaptations that make microbiology accessible to students with visual impairments and those than enliven any microbiology lecture. She will also share some of the responses that the 'public' make to such lectures showing what an appetite there is for bacterial learning and that we should all get out there and evangelize that bacteria are fab! She will draw on her research into swimming bacteria, including the predatory *Bdellovibrio*, to show how she brings bacteriology to life in the lecture hall.

Liz obtained a BSc in Biochemistry and Microbiology at Leeds, and a PhD in Microbiology at University College London. Following postdocs in the USA and at Oxford, in 1991 she became a lecturer at the University of Nottingham. Since 1999 she has worked in the Institute of Genetics in the Medical School where her group researches bacterial flagellas as well as *Bdellovibrio*. She was appointed to a chair in 2006.

Young Microbiologist of the Year Competition

Glycoprotein biosynthesis in *Streptomyces coelicolor*

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The streptomycetes are a group of soil bacteria that produce two-thirds of the world's clinically important antibiotics. They have a mycelial growth habit and a developmental phase during which they produce spores. The nature of the *Streptomyces* cell wall is poorly understood. Enzymes that are predicted to be required for protein glycosylation have been found to be essential for phage infection. We therefore propose that *S. coelicolor* contains within its cell wall one or more glycoproteins that can act as a phage receptor. The pathway is predicted to be similar to the O-glycosylation pathway in yeasts, humans and other Actinomycetes, in particular *Mycobacterium tuberculosis*. Polyprenol phosphate mannose synthase (Ppm) synthesizes polyprenol phosphate mannose, which donates mannose to secreted proteins by protein mannosyl transferase (Pmt). The phage may then interact with the glycan moiety on the glycoprotein. Knock out mutants in genes encoding Ppm and Pmt have been isolated and are phage resistant. In addition the Pmt and Ppm mutants have a reduced growth rate, in particular a small colony size, compared to the wild type strain. Complemented mutants behave like the wildtype, producing large colonies and showing phage sensitivity. In addition there appears to be a general increase in sensitivity to the ionophore monensin in both the Pmt and the Ppm mutants. There may therefore be subtle differences in membrane and cell wall functions, possibly ion permeability. Although microscopy has not yet revealed obvious differences in morphology between the wild type and the mutants, it is clear that glycosylation plays a role in the vegetative growth of *Streptomyces*.

Topology modelling of the Pet autotransporter translocator domain

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Autotransporters are arguably the simplest of the five major recognized Gram-negative bacterial secretion systems, whereby all the information necessary for the secretion of the effector protein is encoded within a single transcript. Their conserved domain architecture consists of an N-terminal signal sequence, which directs export across the inner membrane, the effector domain, and a C-terminal β-barrel domain which translocates the secreted effector through the outer membrane. This translocation event is the subject of much controversy. Homology modelling showed that the C-terminal 299 amino acids of the Enteroaggregative *Escherichia coli* Plasmid encoded toxin (Pet) autotransporter has a high degree of similarity to the known structure of NalP, despite a lack of sequence identity. The effects of random and directed peptide linker insertions in-frame within the C-terminal domain were used to test the model. Insertion sites of secretion competent and incompetent clones were identified on the model, which then helped to discern the topology of the b-barrel. This work provides further evidence for a monomeric translocator domain and the hairpin model of translocation, and also provides a framework for research into translocator domain structure and function.

Exploring inter-kingdom signalling interactions between clinical *Pseudomonas aeruginosa* strains and the fungal pathogen *Candida albicans*

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Effects of *P. aeruginosa* signalling molecules on eukaryotic organisms are well documented with recent research concentrating on interactions between *P. aeruginosa* and *C. albicans*. *P. aeruginosa* attaches to and kills *C. albicans* filaments while having no effect on its yeast form. Instrumental in these interactions are 12-carbon molecules including those produced by bacteria (e.g. 3-oxo-C12 HSL). Our work concentrates on such interactions where, unlike previous studies with well-characterized strains, we use *P. aeruginosa* clinical isolates recovered from CF patient sputum. We examined the production of secreted signal molecules by a number of these isolates and found that there are significant differences in types, levels and timing of HSL molecules produced by different strains. This is likely to significantly impact on how these strains interact with *C. albicans* in the co-infected CF lung. In particular, it seems likely that mixtures of secreted bacterial signals and metabolites have different effects on the yeast. In support of this hypothesis, we find that whereas some of our strains affect *C. albicans* morphology, others do not. Currently, work is being carried out to investigate the differential effects of concentrated supernatants from three clinical *P. aeruginosa* isolates on *C. albicans* using transcriptomics and other techniques.

Methylotrophs in the spotlight: stable-isotope probing illuminates carbon metabolism in the marine environment

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Marine microbial communities harbour an enormous collection of uncultivated micro-organisms with metabolic capabilities that continue to elude description. Despite their global significance, marine micro-organisms and enzymes involved in the metabolism of one-carbon compounds remain poorly understood. Here we link the metabolic function and diversity of microbial communities in coastal seawater by increasing the sensitivity of DNA stable-isotope probing (SIP) for the analysis of marine bacteria that consume methanol, methylamine, and dimethylsulfide. Clone libraries of 16S rRNA and methanol dehydrogenase genes, in addition to the first-ever collection of PCR-amplified methylamine dehydrogenase sequences, identified the moderately halophilic *Methylophaga* spp. and members of uncultivated clades of *Gammaproteobacteria* in the metabolism of these compounds. Combining the strengths of SIP, cultivation and metagenomics will further link marine micro-organisms and their corresponding metabolic functions, offering new insight into carbon cycling in the ocean.

Pseudomonas putida sulfonatase and sulfatase genes in the rhizosphere and their role in plant growth promotion

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Pseudomonas putida S-313 is a plant growth promoting bacterium and has been shown to promote the growth of *Arabidopsis thaliana*,

barley and tomato. This plant growth promotion property has been linked to organosulfur metabolism by the bacterium, which is hypothesized to mobilize plant-available sulfur from organically bound sulfur in the soil. Strains of *P. putida S*-313 that carry a mutation in the *asfA* gene are unable to desulfurize aromatic sulfonates, and are also deficient in the promotion of tomato plant growth observed with the wild-type strain. Reporter strains of *P. putida* S-313 were generated in which the promoters for known sulfonate and sulfate ester utilization genes (*asf*, *ats*, *ssu* and *sft*) were linked to the *gfp*mut3* gene as transcriptional fusions. The fusion constructs were introduced into the *Arabidopsis* rhizosphere on a medium-copy vector (pBBR1MCS-3) in *P. putida* S-313. Levels of expression of the *gfp* fusions varied with sulfur supply and time post-transplantation. The combined use of confocal microscopy and qRT-PCR has revealed that expression of the organosulfur utilization genes of *P. putida* S-313 is controlled *in rhizo* by sulfur supply and that they are differentially expressed along the length of the root. Confocal microscopy has revealed that the organosulfur utilization genes are expressed on the *Arabidopsis* root surface, suggesting that the root surface is sulfate-limited regardless of the sulfate availability in the surrounding soil.

Investigations into the biological succession of the infant faecal microbiota

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From birth onwards, infants are challenged by a vast quantity and variety of micro-organisms. The impact of diet on the gut microbiota is of particular interest, especially in relation to the health of the infant. The objective of this work was to monitor the bacterial succession of the infant GI tract during the developmental stages of gut physiology and microbiology. The diversity and dynamics of the infant gut microbiota were analysed, both at the community level and with regard to specific populations of interest (including *Bifidobacterium*). Regular faecal samples were collected from eight breast-fed infants, from 4 weeks of age to 12 months of age. Predominant bacterial groups were monitored using modern molecular techniques such as fluorescence *in situ* hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE). The data demonstrated both inter- and intra-individual differences in microbiological profiles. Furthermore, the introduction of solid foods in the diet (i.e. weaning) was shown to elicit modulation of the faecal microbiota of breast-fed infants.

Detection of anaerobic bacteria in the sputum of patients with cystic fibrosis

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Pseudomonas aeruginosa pulmonary infection is the leading cause of morbidity and mortality in Cystic Fibrosis (CF) patients. The reduced oxygen concentration observed in sputa coupled with respiration of *P. aeruginosa* creates anoxic zones within the CF lung that could allow the growth of anaerobic bacteria .In this study we used strict anaerobic bacteriological culture techniques to detect anaerobic bacteria in sputa samples from CF patients. Potential anaerobes were checked for oxygen sensitivity and identified by sequencing of the 16S rRNA gene. Anaerobes were detected in 67% of the 67 samples examined representing 50 patients. The total viable count of the majority of anaerobes isolated from each individual patient equalled or exceeded the total viable count of *P. aeruginosa* isolated from that same patient. Anaerobes isolated belong primarily to the genus's *Prevotella, Veillonella* and *Propionibacterium*. These results indicate that anaerobes are present in the lungs of CF patients in significant numbers and may, therefore, contribute to a polymicrobial infection in the lungs of these patients.

Mutational analysis of ϕC31 integrase

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The temperate bacteriophage ϕC31 encodes a large-serine recombinase that is required for integration of the bacteriophage genome into the *Streptomyces* chromosome. Control of the recombinases integrase and excisionase activities is essential for the bacteriophages viability and influences its spread through its bacterial host. *In vitro* it has been demonstrated that integrase can only recombine *attB* and *attP* (i.e. integration) with the block on excision (recombination involving *attL* and *attR*) being due to integrase failing to form a synapse.

To fully understand the process of synapsis and directional control of ϕC31 integrase we have directed our attention to elucidating residues that are important in forming the synaptic interface. We propose that amino acids required for synapsis in ϕC31 integrase may be in equivalent positions to those that form the synaptic interface in γδ and Tn*3* resolvases. To test this we isolated a range of ϕC31 integrase mutants that are either defective in *attP* and *attB* recombination or hyperactive by demonstrating recombination with *attL* and *attR* (excision). The hyperactive mutants isolated to date all map to a region in the uncharacterized and non-conserved C-terminus of ϕC31 integrase. The most active of these mutants has shown the ability to recombine *attB* with *attP*, *attL* with *attR* as well as other combinations of *att* sites including *attL* with *attL* and *attR* with *attR*. These observations suggest that the C-terminal domain is directly or indirectly involved in forming the synaptic interface and we are currently testing this with further biochemical characterization.

The GGDEF/EAL domain protein, PigX, regulates the biosynthesis of prodigiosin, swarming and virulence of *Serratia* sp. ATCC 39006

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Bacteria of the genus *Serratia* are opportunistic human, plant and insect pathogens. *Serratia* sp. ATCC 39006 secretes plant cell wall degrading enzymes and produces the secondary metabolites, carbapenem and prodigiosin. Prodigiosins display immunosuppressive and anticancer properties. Mutations in *pigX* resulted in increased swarming and virulence in a potato tuber assay and the production of pectinases, prodigiosin and a surfactant-like compound was elevated. PigX is predicted to encode a protein containing GGDEF and EAL domains that might be involved in cyclic-di-GMP signalling. The transcription start of *pigX* was mapped and *pigX* was demonstrated to be regulated by the pleiotropic 'master' regulator, PigP. Furthermore, *pigX* upregulates the prodigiosin biosynthetic operon at the level of transcription. Swarming motility was flagella-mediated and was controlled by a two-component system, quorum sensing and addition regulators. A genetic screen identified numerous genes involved in the regulation and production of the surfactant, and hence swarming motility. Therefore, the GGDEF and EAL domain protein, PigX, has an important role in the regulation of swarming motility, virulence and the production of exoenzymes and prodigiosin in *Serratia* 39006.

Horizontal gene transfer and evolution of the *Streptomyces* chromosome

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Streptomyces species are well known for their ability to produce an array of secondary metabolites which have antibacterial, antifungal and antitumour properties. The ability of one strain to produce different compounds which have both clinical and agricultural importance led to over speciation of the genus. A number of classification methods were developed to overcome this problem. Williams *et al.,* (1983), carried out a comprehensive study of the genera using numerical taxonomy. This enabled *Streptomyces* species to be clustered based on the phenotype, however this method was unable to accurately resolve the relationships between closely related strains. It is widely accepted that molecular methods are required to define the intrageneric relationships between closely related strains and to improve the understanding of species relatedness within the *Streptomyces* genera.

The linear chromosome of *Streptomyces* species is genetically unstable and is highly prone to genomic rearrangements including insertions, deletions, amplifications and horizontal gene transfer events which occur mainly in the terminal regions. The aim of this research is to define the phylogenetic structure of the Cluster 21 *S. violaceoruber* clade by carrying out an extensive phylogenetic analysis using house-keeping genes (16S rDNA, *recA*, *trpB* and *gyrB*) and adaptive genes (*strA*). This study has identified the transfer of the streptomycin gene cluster from a putative *S. griseus* (Cluster 1) source to ASB37, a Cluster 21 soil isolate. The possible location of the cluster was determined in ASB37 by PCR and sequencing. This analysis identified the presence of a number of genes showing high sequence similarity to membrane proteins of *S. coelicolor* M145 being located in the middle of the Sm cluster in ASB37. The flanking regions of the cluster in ASB37 have been determined giving a possible insertion point of the cluster. RT-PCR and Western blot analysis has shown this cluster to be silent and DNA/DNA microarrays have been carried out to determine the structural integrity of ASB37 chromosome. This has revealed a number of significant differences between this strain compared to *S. coelicolor* M145.

Regulation of foot-and-mouth disease virus infection by cellular rab proteins

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Infection by foot-and-mouth disease virus requires endocytosis and acidic endosomes. However, the site of membrane penetration is unknown. Rab proteins are small GTPases that regulate vesicle formation, trafficking and fusion events. Each rab protein regulates a specific stage of endocytosis. A number of GFP-tagged dominant-negative rab proteins were expressed in pig kidney cells (IBRS-2). FMDV infection is inhibited by a dominant-negative rab5 (which impairs formation of early endosomes) but not by dominant-negative rab4 or rab11 (which regulate trafficking through recycling endosomes) or by a dominant-negative rab9 (which inhibits late endosome to golgi trafficking). Rab7 regulates trafficking between early and late endosomes. Dominant-negative rab7-T22N binds membranes, whereas dominant-negative rab7-N125I is primarily cytosolic. rab7-T22N has no effect on FMDV infection, but rab7-N125I inhibits by ~80%. We have shown this inhibition is at the replication stage rather than entry. These studies strongly suggest that the early endosome is the critical compartment required for infection and that the availability of a membrane-bound rab7 may be required for intracellular virus replication.

Posters

Cells & Cell Surfaces Group

CCS 01 Environment influences phase variation frequencies in *Haemophilus influenzae*

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Haemophilus influenzae (*Hi*) colonizes the upper human respiratory tract and also enters the inner ear, lungs, bloodstream and meninges to cause disease. Survival of the organism in each of these environments requires rapid adaptation. *Hi* has several phase variable contingency loci, important for commensal and virulence behaviour, that are associated with dinucleotide or tetranucleotide repeat tracts. The frequency of slippage events at these loci influences the rate of adaptation and survival. We have investigated the influence of environmental factors on phase variation (PV) frequencies mediated by an AGTC or AT repeat tract following growth on different media. PV frequencies were lowest on rich media, higher on defined media and highest on minimal media. PV frequencies were lowered to the levels observed for rich media when glutathione was added to minimal media, suggesting that oxidative damage may be a significant factor in altering PV frequencies. The effect of oxidative damage on PV frequencies in *Hi* has been further investigated through mutation of the *mutY* gene, the addition of exogenous peroxide and changes from aerobic to microaerophilic environments.

CCS 02 Lipopolysaccharide phase variation in *Haemophilus influenzae*

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Unencapsulated (non-typeable) *Haemophilus influenzae* (NT*Hi*) is an obligate human commensal bacterium and a frequent pathogen responsible for respiratory tract infection and non-invasive diseases e.g. otitis media. All NT*Hi* express membrane-anchored lipopolysaccharide (LPS) which acts as a major virulence factor. NT*Hi* LPS exhibits wide structural heterogeneity both between, and within strains, largely directed by the phase variable expression of a number of LPS biosynthetic genes. Changes in the number of tetranucleotide repeat units within the ORF can shift translation in or out of frame. The reversible expression of these genes results in the presence or absence of a number of biologically relevant epitopes on the LPS, correlating with potential changes in virulence.

The repertoire of phase variable LPS genes has been determined in a set of disease-causing NT*Hi* strains. To analyse the role of the reversible expression of these genes and respective epitopes, mutants lacking the ability to phase vary LPS gene expression have been generated in this set of strains. These phase-locked genes have been examined individually and in combination, and their effects investigated in biologically relevant assays.

CCS 03 Characterization of cis-active elements required for distant regulation of the *Escherichia coli* recombinase gene, *fimB*

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Expression of the type 1 fimbrial adhesin of *Escherichia coli* is phase variable. This reversible on-off switching in expression is dependent, in part, upon the orientation of a 314 bp invertible element. DNA inversion at *fim* is catalysed by two site specific recombinase proteins, *fimB* and *fimE*, and whilst the former facilitates recombination in both directions, the latter does so predominantly on-to-off (fimbriate-to-afimbriate). The *fimB* recombinase gene is separated from the divergently transcribed *nanC* by a 1.4 kb intergenic region. Two regulatory proteins, NanR and NagC bind to sequences far (> 700 bp) upstream of *fimB* and regulate its expression in response to *N*-acetylneuraminic acid and *N*-acetylglucosamine (respectively). Using site-directed mutagenesis and allelic exchange, we have (i) identified additional cis-acting elements and factors required for control of fimB by these aminosugars and (ii) analysed the effect of distance of the control elements from the fimB promoter on this regulation.

CCS 04 Polar targeting of FlhF: a putative GTPase controlling flagellum localization in *Vibrio cholerae*

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Vibrio cholerae swims by rotating a single flagellum located at its old cell pole. Flagellum biogenesis requires correct targeting of flagellar proteins to the appropriate pole and must be closely coupled to cell division to ensure that only one flagellum is built per cell. The mechanisms controlling correct flagellum polar localization are poorly understood. Our current work focuses on a novel flagellar protein, *flhF*, which has been implicated in controlling flagellum placement.

Deletion of *flhF* was found to severely reduce, but not abolish, *V. cholerae* motility. Electron microscopy revealed that *flhF* null cells either did not assemble a flagellum or had a flagellum that was randomly localized rather than specifically targeted to the cell pole. Cells over-expressing *flhF* frequently produced two flagella that were either polar or bipolar. FlhF contains a region with similarity to signal recognition particle (SRP) GTPases, and site-directed mutagenesis revealed that the GTPase motifs are critical for FlhF function. The cellular localization of FlhF was investigated using a functional GFP fusion. FlhF-GFP was found to co-localize with the flagellum at the old cell pole and studies in the aflagellate *flrA* null, which lacks the master regulator of flagellar gene transcription, demonstrated that FlhF-GFP polar localization occurs independently of other flagellar proteins. Deletion studies identified a ca 40 residue region of FlhF that is critical for polar targeting.

CCS 05 Control of flagellar number in *Vibrio cholerae* by the putative ATPase FlhG

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The bacterial pathogen *Vibrio cholerae* relies on a single cell-surface flagellum for motility and thus for its survival, colonization and transmission in humans. The flagellum is located at the old cell division pole and over 50 genes, arranged in a four-tier transcriptional hierarchy, co-ordinate flagellum biogenesis and localization. One of these genes, *flhG*, encodes a 34kDa putative ATPase that has a critical role in flagellum assembly and control of flagellar number. Overexpression of *flhG* results in non-flagellated

cells, while *flhG* null mutants possess up to fifteen flagella. Mutagenesis studies have revealed that the FlhG ATPase motif is critical for function. To identify FlhG binding partners, bacterial two-hybrid and *in vitro* pull-down assays were carried out. These studies detected a FlhG interaction with FlrA, the flagellar master regulator that is thought to activate σ^{54}-dependent transcription of class 2 flagellar genes. Using fluorescence microscopy we have determined FlhG and FlrA cellular localization and we are further investigating the functions of FlhG and FlrA in flagellar gene transcription. Our results suggest that FlhG might couple flagellar gene transcription to cell division, ensuring that only one flagellum is produced during each cell division cycle.

CCS 06 DNA methylation-dependent regulation of *Escherichia coli* Ag43 phase variation

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DNA methylation plays a role in various cellular processes including epigenetic control of gene transcription. A model for methylation-dependent regulation is transcription of agn43 encoding phase variable E. coli protein Ag43. Phase of agn43 transcription is heritable and determined by a methylation state of Dam methylase target sequences that affects DNA binding of the global regulator OxyR. OxyR binds to unmethylated GATC sites downstream of the agn43 and represses transcription (OFF). Dam methylation prevents repressor binding and allows the ON phase of agn43 transcription. Previous studies have suggested that key to understand mechanism of inheritance of DNA methylation state and ON phase of transcription are protein-DNA interactions at hemimethylated agn43 (HDNA) that is formed after chromosome replication in an ON phase cell. Here we describe the competition between Dam, OxyR and the sequestration protein SeqA for the hemimethylated agn43 in vitro. Dam can destabilize SeqA-HDNA complexes more rapidly than those formed by OxyR. Formation of the more stable OxyR-HDNA complexes may facilitate the unmethylated (OFF) phase after an additional DNA replication round.

CCS 07 The effect of tunicamycin on incorporation of GlcNAc into membrane lipids catalysed by KfiB: *in vitro* & *in vivo* analysis

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Tunicamycin is an analogue of UDP-GlcNAc and has been shown to inhibit several enzymes that catalyse GlcNAc-1-P transfer to polyprenyl-P. To investigate the effect of tunicamycin on incorporation of GlcNAc into membrane lipids catalysed by KfiB, an experiment based on the modified method of Lehrman *et al.*, (1988) was performed. Membranes from strain PA360[pPC6::6][pMA1] induced with IPTG, were incubated with radiolabelled UDP-GlcNAc in the absence and presence of tunicamycin prior to extraction with 1-butanol. The presence of tunicamycin reduced the incorporation of GlcNAc to <88.2% at 0.2µg/ml (no significant, P=0.1) and to <66% at 1mg/ml (significant, P<0.02).

In an *in vivo* attempt, pPC6 was transformed into the deep rough *E. coli* X711 strain, thus enabling this strain to express K5 polysaccharide. Transformants expressing the K5 polysaccharide were grown in broth in the absence and presence of tunicamycin. There were no changes in susceptibility to K5-specific bacteriophage at different concentrations of tunicamycin suggesting that the initiation of K5 polymerization was not inhibited by tunicamycin.

CCS 08 Genetic characterization of the phase variable lipoligosaccharides of *Campylobacter jejuni*

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Lipooligosaccharides (LOS) have a role in *Campylobacter* virulence and host ganglioside mimicry is the aetiological basis for *C. jejuni*-associated Guillain – Barré syndrome. LOS structural and antigenic variation arises from gene sequence and content variation and homopolymeric tract changes within the LOS biosynthesis gene cluster. A strategy is required to enable the identification of novel LOS gene content in a genomic context.

A PCR-based method was used to characterize 50 clinical strains for LOS gene content; these strains form part of the 'Campynet' strain set. Most strains belonged to the NCTC11168-like classes A, B and C (groups 1a, 1b and 1c). Strains with potentially new class structure and strains containing a novel combination of genes from different known classes were found. Experiments are ongoing with a 70-mer oligonucleotide microarray with gene specific and gene boundary elements to assess its effectiveness for the determination of LOS gene organization.

CCS 09 The multiple flagellins of group I and group II *Clostridium botulinum*

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C. botulinum is a Gram positive anaerobe that produces the potent neurotoxin, BoNT, the causative agent of botulism. Isolation of flagella from Group I and Group II strains, followed by SDS-Page, identified 1–4 flagellin of 27–50 kDa, depending on the strain examined. Tryptic digests and mass spectrometry indicated that the major structural protein was encoded by one of two tandem flagellin genes which we have designated *flaA1* and *flaA2*. PCR and sequencing of *flaA1/A2* predicted molecular weights smaller than that observed by SDS-PAGE suggesting the proteins are post-translationally modified. A 50 kDa flagellin was observed only in type E strains. PCR, cloning and sequencing identified *flaB*, a flagellin gene not present in the Hall A genome sequence. FlaB does not appear to be post-translationally modified. Sequencing of *flaA1/A2* from 75 strains had identified 12 allelic *flaA1/A2* variants; however, it appears to have underestimated the structural diversity present in the flagellin proteins.

CCS 10 Proposed mechanism of cross-talk between pyelonephritis-associated pili and type 1 fimbriae in uropathogenic *Escherichia coli*

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Type 1 fimbriae, encoded by the *fim* cluster, are a common fimbrial adhesin expressed by most *E. coli* strains and other Gram negative enteric bacteria. Pyelonephritis-associated pili (Pap) are a fimbrial adhesin associated with more severe urinary tract infections (UTI) caused by *E. coli*. Most *E. coli* clinical isolates associated with symptomatic UTIs can express both adhesins. Expression of both clusters is under phase variable control but by two distinct mechanisms. Fim expression is regulated by an invertible genetic

element whereas Pap phase variation is controlled by the formation of two mutually exclusive DNA protein complexes that determine, but are also influenced by local GATC methylation patterns. Previous research has demonstrated that the Pap regulator, PapB, represses *fim* expression by inhibiting the activity of the FimB recombinase that 'switches on' type 1 fimbriae. The molecular basis to this inhibition was investigated using site-specific mutagenesis of the *fim* switch region and gel mobility and footprinting techniques to analyse PapB interactions. Current evidence favours an interaction of PapB with the recombinational complex rather than direct inhibition of FimB binding to the *fim* switch.

Posters

Clinical Microbiology / Systematics & Evolution Groups

CM/SE 01 *Borrelia duttonii* and *Borrelia recurrentis* – clones of the same species?

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Relapsing fever *Borrelia* challenge microbiological typing, as unlike other microbes, they possess segmented genomes maintaining essential genes on large linear plasmids. Antigenic variation further complicates typing. Intragenic spacer (IGS, between 16S-23S genes), provides resolution among Lyme-associated and some relapsing fever spirochaetes. When applied to East African relapsing fever borreliae two and four types were found respectively among *Borrelia recurrentis* and *Borrelia duttonii*. However, IGS typing was unable to discriminate between the tick and louse-borne forms of disease, raising the question as to whether these are indeed separate species? To address this question, further genes were sequenced to produce a multi-locus approach to resolve whether these are either a single or different species. Various house-keeping genes were selected from data deposited for *B. hermsii* (limited sequence information exists for either B. recurrentis or *B. duttonii*). Of selected targets, only sufficient data was produced from glpQ. Further genes analysed included flaB, rrs rDNA and P66 outer membrane protein.

Sequence comparison of multiple genes was undertaken, but restricted through the limited number of available isolates of these notoriously fastidious organisms which until recently were considered non-cultivable. Whereas the IGS typing was applied to a range of clinical isolates, patient blood samples and arthropod vectors, other genes were only sequenced from cultivable strains, potentially introducing a bias to the results. Our data highlights the remarkable similarity between these Borrelia with only minor differences at the nucleotide level. Collectively, this suggests a common ancestral lineage for these spirochaetes, with the limited differences revealed at the nucleotide level from these cultivable strains being able to divide both 'species' into separate clades, however it must be stressed that these differences ranged from 2–10 nucleotides depending on the gene used. It is more likely that these are clades of the same species, which have accumulated adaptive changes through time and pressures of different vector transmission. In contrast, the IGS sequence, being non-coding, is not under such selection and in consequence probably reflects changes accumulated over time alone, but without the constraints of producing functional gene products. Full genomic sequence analysis should reveal further insights into the taxonomic relationship between these microbes, elucidate the molecular basis of arthropod competence and pathogenicity among these spirochaetes.

CM 02 Mutations in genes involved in mutation repair and their association with drug resistance in *Mycobacterium tuberculosis*

Jane Ashby, **Claire Jenkins**, Denise O'Sullivan, Robert J. Shorten, Stephen H. Gillespie & Timothy D. McHugh

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In *Mycobacterium tuberculosis* (TB), the resistance to anti-mycobacterial drugs is due to genomic mutations in specific genes. Mutated phenotypes commonly result from defects in DNA repair and it has been suggested that strains with defective DNA repair systems may be associated with increased drug resistance. Three mutation repair genes, *mutT*2, *mutT*4 and *ogt* were sequenced to look for mutations in 24 strains of drug resistant TB and 34 fully sensitive strains. Interestingly, 14% of the strains in our study had mutations in their *mutT*2 and *ogt* genes. Two fully sensitive isolates had a mutation in the *mutT*2 gene and six isolates had a mutation in the *ogt* gene, two of these were multi-drug resistant strains and four were fully sensitive. The results of this study showed that there was no association between strains with mutations in their DNA repair genes and increased drug resistance.

CM 03 A 5 year prospective analysis of the population structure of *Mycobacterium tuberculosis* in North London

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Following the completion of the 1995–1997 Molecular Epidemiology of TB in London Study, routine IS*6110* RFLP typing of a single *M. tuberculosis* isolate from all TB cases presenting to the TB Service of the Royal Free Hospital was instituted as a tool to monitor cross contamination and aid outbreak investigations. MIRU typing was used for rapid investigations.

During the period 2001 to 2006 over 400 strains were characterized by IS*6110* RFLP and a Bionumerics (version 3.0) database created. 62 strains were low copy number (<5 copies). Cluster analysis of the high copy number strains revealed 21 clusters (100% identity) of which 3 had >2 members (4, 6 and 10 respectively). Risk factors associated with clustering, including the presence of cavitatory TB, HIV status and drug resistance, were assessed. The largest cluster (10 isolates) is known to be associated with an ongoing outbreak of isoniazid resistant tuberculosis in North London. Other clusters revealed evidence of laboratory cross contamination, the overall rate was 1%.

CM 04 Sputum *Mycobacterium tuberculosis* (M.tb) mRNA to monitor treatment in pulmonary tuberculosis (PTB)

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Rapid markers of treatment response in PTB are necessary for treatment and evaluation of new drug regimens. Expression of *M. tb* specific mRNA is an attractive target as it identifies viable organisms. We have undertaken an exploratory study of 7 patients with PTB on standard therapy, and compared colony count with mRNA expression.

Sputum samples were collected prior to therapy, and serially for the first week of treatment, at 2 weeks, 3–4 months and 6 months. Viable count was calculated on selective agar plates. mRNA was detected using quantitative real-time reverse transcribed PCR using dual labelled probes for *rpo*B, *sig*A and 16S rRNA genes.

Bacterial load fell 3–4 logs over the first 2 weeks of treatment which correlated to the Δct observed for each gene. *rpoB* and *sigA* signals declined more rapidly than the 16S rRNA signal. No mRNA signal was found after month 3–4.

This data demonstrates that change in mRNA expression correlates with viable bacterial load providing an early assessment of treatment response.

CM 05 Real-time PCR assay for the detection of *Mycobacterium avium* complex in clinical specimens

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Patients infected with HIV are at an increased risk of developing disseminated disease caused by *Mycobacterium avium* complex (MAC). There are currently no commercially available molecular assays for the detection of MAC directly from clinical samples. To improve the diagnosis and management of HIV patients with MAC at the RFH our aim was to design a real-time PCR assay for the detection of MAC from clinical samples. Primers were designed to bind specifically to the ITS region of the MAC genome. A total of 42 MAC strains and 15 non-MAC mycobacterial species were tested. DNA was extracted from bacteria growing in culture using a glass bead extraction method. The resulting assay correctly identified all 42 MAC isolates and did not amplify other mycobacterial species including *M. tuberculosis, M. kansasii* or *M. fortuitum*, confirming that the assay is capable of identifying MAC cultures and does not cross-react with other *Mycobacterium* sp. Studies are underway to assess the sensitivity and specificity of the assay for respiratory samples.

CM 06 Lipid bodies in acid fast bacilli in tuberculous sputa

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Lipid Bodies are a morphological feature observed in numerous species of mycobacterium. *Mycobacterium tuberculosis* (MTB) does not make LBs under normal growth conditions *in vitro* but strikingly LBs are present in acid fast bacilli (AFB) in sputum (Garton *et al,* 2002). This is the first evidence of a heterogeneous population of MTB in human infection. We assessed the occurrence of LBs in AFB in sputum from patients in The Gambia and Leicester. Markedly LBs were an almost universal feature of AFB in sputum. The number of LB positive cells varied substantially from 3–80% of the population of AFB. Increasing numbers of LB positive cells in consecutive sputum specimens from patients undergoing chemotherapy may indicate that these cells form a persistent and drug tolerant population. If this is the case, the enzymes involved in LB metabolism may be important antibiotic targets in the future.

CM 07 Transcription analysis of tubercle bacilli in human sputum

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Expectorated cells of *Mycobacterium tuberculosis* reflect conditions in the human lung. Identification of genes differentially expressed in such bacilli will provide insights into the metabolic state resulting from adaptation to this environment and has the potential to identify critical determinants of infection. Sputum samples were collected from twenty known microscopy positive Gambian patients prior to commencement of chemotherapy. Extraction of sufficient mycobacterial mRNA for global transcriptome analysis was difficult. Only one sample yielded sufficient *M. tuberculosis* RNA for a single microarray. Cautious interpretation of this microarray, combined with confirmatory QRT-PCR analysis in this and eight further samples, suggested that the *M. tuberculosis* population within the lung is heterogenous. Gene expression signatures consistent with *in vitro* non-replicating persistence phenotype (*ie.* adaption to a microaerophilic environment and use of fatty acids as a carbon source) have been demonstrated.

CM 08 Laboratory experience with the In-Tube QuantiFERON test for *Mycobacterium tuberculosis* immune responses in Leicester

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Many recent studies have indicated the value of blood tests that detect interferon gamma produced by peripheral blood cells in response to *M. tuberculosis* specific antigens. These tests have been shown to be more specific than the tuberculin skin test (TST), which, nonetheless, remains the standard used by many tuberculosis control programmes. While several reports document results from tests in which peripheral blood monocytes are exposed to antigens in the diagnostic lab, we report here our experience with a test format in which antigen exposure occurs directly at the time of venesection. The QuantiFERON-TB (QF) Gold 'In-tube' test has been used at this laboratory since January 2005. Of the 660 samples tested by the three tube, mitogen controlled method, we have observed 223 (34%) positive, 411 (62%) negative and 26 (4%) indeterminate results. Most of the 'indeterminate' values, 77%, were obtained from immunosuppressed patients. We note that the 'In tube' test yielded a lower indeterminate rate than previously recorded for the QF method (4 vs. 11%) and speculate that this may reflect immediate rather than deferred exposure to antigens.

CM 09 Transcriptome analysis of the Leicester outbreak strain *Mycobacterium tuberculosis* CH

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Comparative genomic hybridization analysis of *Mycobacterium tuberculosis* CH (a highly virulent strain) against the sequenced reference strain *M. tuberculosis* H37Rv revealed 6 large sequence polymorphisms (LSP) which involved 10 open reading frames (ORF). One of these LSPs, *Rv1519-1520* has recently been recognized by Gagnuex *et al**, who have demonstrated that this LSP defines the East African-Indian lineage of *M. tuberculosis*.

We have observed that CH possesses an immune suppressive phenotype with similarities to that effected by strains producing phenolic glycolipid (e.g. Beijing). Our preliminary results indicate that deletion of *Rv1519* makes a major contribution to this effect but that this is not directly attributable to loss of the polypeptide encoded by *Rv1519* on the immunostimulatory phenotype of CH. We have therefore examined the transcriptome of CH to determine whether there are polar effects attributable to the deletion of *Rv1519-20* or whether other features in the pattern of transcription as compared to H37Rv might explain the immune suppressive phenotype of CH. Polar effects were excluded and a distinct CH transcriptome defined.

Reference: *Gagneux *et al.* (2006) *PNAS* **103**, 2869–2873.

CM 10 Adding value to the analysis of AFB smear-positive respiratory specimens by real-time PCR analysis

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In 2001 the largest recognized outbreak of tuberculosis in a UK school occurred in Leicester. Microarray analysis revealed five deletions in the outbreak strain (CH) compared to H37Rv and this information used to design a Genome-Level Informed PCR (GLIP). GLIP analysis applied to local isolates revealed that one of the deletions divided the Leicester population: 58% (Rv1519+): 42% (Rv1519-). It was hypothesized that this polymorphism could be used as the basis for a rapid, in-house, preliminary typing system to aid the public health team. A SYBR Green real-time PCR assay was designed and applied to 99 isolates. The accuracy of the Rv1519+/- assay was 84% and provides a platform for further optimization. Together with a second assay that combines SYBR Green detection for mycobacterial 16S and a TaqMan probe for *M. tuberculosis* complex we have established a rapid, real time system enabling identification and preliminary typing of mycobacterial infections.

CM 11 Application of generic PCR primers and DNA sequencing for developing species – specific probes to identify arthropod-borne haemopathogens of domestic animals

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Arthropod- borne haemopathogens of domestic animals in the Caribbean cause significant morbidity and are fatal if undiagnosed. To screen for arthropod-borne haemopathogens, 95 EDTA blood samples from domestic animals submitted to a veterinary diagnostic laboratory in Trinidad were analysed. Extracted DNA was amplified using generic primers for the 16S and 18S rRNA genes to detect bacteria and protozoa respectively. Positive samples with amplified PCR products of approximately 400 base pairs were sent for sequencing and the results aligned using the NCBI- BLAST2 programme. *Candidatus Mycoplasma haemominutum* (*Haemobartonella felis*) and an unknown organism showing 96% homology with *Babesia sp* was sequenced from cats. *Babesia canis vogeli* and *Ehrlichia canis* were sequenced from dogs and *Babesia bovis* was sequenced from cattle. The results will be discussed in relation to developing sensitive macro-arrays to screen for arthropod-transmitted haemopathogens to facilitate early diagnosis and hence improve animal care.

CM 12 RFLP and MIRU typing of *Mycobacterium tuberculosis* isolates in the investigation of a significant rise in cases

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Restriction fragment length polymorphism (RFLP) typing is seen as the gold standard method for typing *Mycobacterium tuberculosis* (MTB). Mycobacterial interspersed repetitive unit (MIRU) typing is a newer PCR-based method. It involves amplification of 15 independent tandem repeat loci and evaluation of the number of repeats present at each locus.

The SMRL was asked to investigate Lothian TB isolates in 12 months from 2004–2005 because of a 50% increase in new cases. Seventy three individual patient MTB isolates were investigated. All isolates were typed by RFLP and MIRU.

There were eight clusters which included 25 patients. The discrimination of MIRU was better for the low IS*6110* copy number (LCN) strains and comparable to RFLP for the high copy number strains.

Conclusion MIRU is now the routine first line method for typing MTB. It is better than RFLP for LCN strains, uses less DNA and has shorter turnaround times. The results contribute in a more timely manner to better informed contact tracing.

CM 13 Investigation on inhibition effect of *Teucrium polium* on different growth phases of *Escherichia coli* O157

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Teucrium polium commonly named as Kalbporeh is a shrub plant. A liquid extract of the plant has been used in the treatment of fungal diseases and abscesses. Regarding its potential treatment, alcoholic extract of plant was prepared by uses of oven dried plant particles suspended in alcohol solution, shaked and extracted by evaporation all sterile. Appropriate concentration was determined by observing inhibition zones on plates inoculated with both *E. coli* 0157 and plant extract. Bacterial growth behavior was monitored at presence of selected concentrations using spectrophotometer. Spectrophotometery results showed that in all three concentrations (0/1, 0/2, 0/3 mg/ml) of plant extracts, absorbance decreased during first hours of inoculation especially in 2nd hrs and then bacterial growth increased and reached to normal in 8th hrs. Comparison of growth curves showed that higher concentration (0/3 mg/ml) had more effect where effect of 0/2 and 0/1 mg/ml were more or less similar. Results also suggested that other concentrations of plant extracts have to be considered for more effect on bacterial growth although toxicity testing must be checked before usage.

CM 14 Investigation of *Chlamydia trachomatis* infections in asymptomatic women in Tehran

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Chlamydia trachomatis is one of the most important sexually transmitted disease in the world. Infection by this organism is insidious. Symptoms are absent or minor among most infected women. Commonly, unrecognized or inadequately treated can cause serious sequelae such as infertility, ectopic pregnancy and chronic pelvic pain. In this study, serological tests were used to determine the prevalence of *Chlamydia trachomatis* infections in asymptomatic women that not showing any kind of genital infections attending to hospitals in Tehran. By using commercial ELISA kit, the presence of *Chlamydia trachomatis* MOMP immunoglobuline IgA and IgG in women sera were determined. Our results from 70 asymptomatic women showed that the prevalence of MOMP IgA and IgG were 2.8% and 4.3% respectively. This is the first study to report the *Chlamydia trachomatis* prevalence rate among asymptomatic women in Iran. Evaluation of screening efforts of sexually active asymptomatic women is recommended as a part of routine health care.

CM 15 Evaluation of buffy coat in diagnosis of pulmonary tuberculosis

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Besides charging the patients with outstanding costs, tuberculosis (TB) causes high mortality and morbidity in a country. We studied the sensitivity, specificity, positive and negative predictive values as well as the efficiency of buffy coat smear test in patients who were pulmonary TB suspects. This research was conducted at Massih Daneshvari Hospital. According to clinical and radiographic records of 50 pulmonary TB suspects, five ml of blood along with smear and culture of sputum were collected. The research method was a clinical trial (Diagnostic test type) and the technique was of observational-interview type. Six buffy coat smears were obtained by Ficoll-Hypaque sedimentation method while the samples were stained by Ziehl-Neelsen stain On sputum examination, 32 patients (64%) were B.K positive while 22 (44%) had positive sputum culture for *Mycobacterium tuberculosis*. Buffy coat was positive in 4 patients (8%). In comparison with sputum smear and culture, buffy coat had sensitivity of 12.5% and 13.6%, specificity of 100% and 96.4%, positive predictive value of 100% and 75%, negative predictive value of 39.4% and 58.7%, efficiency of 44% and 60% respectively. In regard to the high specificity of buffy coat as compared to sputum smear (100%) and sputum culture (96.4%), it is possible to consider buffy coat as a method for screening tuberculosis patients that cannot expectorate sputum. Since buffy coat method has a high positive predictive value as compared to sputum smear (100%), it could replace other expensive accurate methods like sputum culture and PCR and be used as a substitution for sputum smear.

CM 16 Rapid detection and identification of viable *Mycobacterium paratuberculosis* using a combined Phage-PCR assay

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The *FASTPlaque*TB™ assay is a well-established diagnostic aid for the rapid detection of viable *Mycobacterium tuberculosis* from human sputum samples. The end-point of the assay is the development of plaques following successful infection of the target cell by a bacteriophage. We have now shown that the *FASTPlaque*TB™ assay reagents can be used to detect viable *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in just 24 h. To add specificity to this assay, a PCR-based identification method was developed to amplify species-specific sequences from the DNA of the target cell present in the plaque. Using this combined Phage-PCR assay we have shown that viable MAP cells can be detected and identified in naturally contaminated milk samples within 48 h. In addition we shown that the assay an be used to enumerate MAP cells in milk samples for rapid determination of D-values, demonstrating that the assay can be used to replace culture techniques for physiological studies of this slow growing organism.

CM 17 Specific mutations in the *Mycobacterium tuberculosis rpoB* gene are associated with increased SOS-responses

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Tuberculosis (TB) is responsible for two million deaths each year. Standard prophylactic regimens to control Mycobacterium tuberculosis (MTB), the causative agent of TB, include at least a rifamycin and isoniazid. Over the years, (multi)drug-resistant TB has been on the rise due to inadequate treatment and patient's non-compliance. Drug resistance in MTB is the consequence of spontaneous mutations. Initially, most of these mutations impair the fitness of the bacteria, but they provide a benefit when antibiotic pressure is exerted. In MTB resistance to rifamycins is caused by mutations in *rpo*B. The general consensus is that rifamycin-resistant bacteria are more likely to become multi-drug resistant than sensitive bacteria. We hypothesize that a molecular mechanism triggered by the presence of an *rpo*B-mutation increases the risk of further drug resistance mutations emerging in *rpo*B mutants.

Using real time PCR to measure mRNA expression in MTB strains with mutations in *rpo*B, specific mutations were consistently associated with a moderate but persistent SOS-response, possibly responsible for an increased mutation rate. A propensity to acquire mutations may have major implications for the evolution of multidrug-resistant MTB. Moreover, this mechanism may facilitate the acquisition of adaptive mutations, thereby restoring the fitness of the bacteria and consequently fixing drug resistance in the population.

CM 18 Illumination with Light Emitting Diodes (LEDs) for fluorescent microscopic screening of *Mycobacterium tuberculosis*

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Direct sputum microscopy remains a critical component of tuberculosis diagnosis and thus control programs. Microscopy can either be performed using light microscopy and acid fast staining (Ziehl-Neelsen) or fluorescent microscopy using Auramine O. Fluorescent microscopy (FM) is more sensitive and the higher contrast obtained allows the use of lower power magnification, reducing the time needed to screen each slide. Unfortunately the use of Hg-vapour short arc lamps is problematic, particularly in developing countries, requiring not only high initial investment but also incurring significant ongoing maintenance costs. Hg-vapour lamps are expensive, inefficient requiring an expensive power supply, and have a short usable life (typically 200 to 300 hours).

We demonstrate the simple adaptation of a standard fluorescent microscope for illumination using a 'Royal Blue' LED. The adapted microscope was suitable for the detection of auramine O stained *Mycobacterium* species. Illumination with the LED compared favourably with that of the Hg-vapour lamp. The low cost, power consumption, safety, and reliability of LEDs, makes LEDs attractive alternatives to mercury vapour lamps. We believe that the use of LEDs has the potential to greatly simplify the provision of fluorescent microscopy for *M. tuberculosis* screening in developing and high burden countries. Microscope manufactures will begin to use this form of illumination in the next generations of their microscopes but simple adaptation of existing high quality fluorescent microscopes when currently available should be considered in the short term to facilitate the rapid cost effective provision of optimal microscopic screening in high burden countries.

Posters

Environmental Microbiology Group

EM 01 Repair of UV damage in *Halobacterium* sp. NRC-1

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The extremely halophilic archaea are among the few archaea that have to tolerate high levels of sunlight in their natural environment. The laboratory model archaeon, *Halobacterium* sp. NRC-1, is relatively UV-resistant and its genome sequence reveals homologues of both bacterial and eukaryotic excision repair genes. These include homologues of bacterial *uvrA*, *uvrB*, *uvrC* and *uvrD*; *Saccharomyces cerevisiae RAD1*, *RAD27*, *RAD3* and *RAD2*; and *XthA*, which has some homology with *uvde* in *Schizosaccharomyces pombe*. There is also a photolyase, Phr2, for light-dependent repair of cyclobutane pyrimidine dimers (CPDs). We know that *Halobacterium* is able to carry out excision repair of CPDs and 6-4 photoproducts (6-4pps) in the dark and, to find out which genes are involved and whether there are multiple excision repair pathways, we have deleted genes encoding the bacterial nucleotide excision repair genes, *uvrA* and *uvrC*. The deletion mutants are significantly UV sensitive and using immunoassays for UV damage, we detect no repair of CPDs or 6-4pps in either mutant or in the *uvrA uvrC* double mutant during 3 hours post-irradiation incubation. We conclude that a bacterial nucleotide excision repair system is the sole excision repair pathway in *Halobacterium* sp. NRC-1.

EM 02 UV responses in *Halobacterium* sp. NRC-1 and *radA1* UV induction kinetics

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Halobacterium sp. NRC-*1* is a useful model for studying repair of UV damage in archaea. It has homologues of bacterial and eukaryotic DNA repair genes and it is also highly UV resistant.

In this study, DNA microarrays were used to monitor UV-inducible responses via genome-wide expression screening. Cells were irradiated with various doses of UVC and UVB and allowed to recover for 1hr and 3hrs. Interestingly, no SOS response was observed and no bacterial or eukaryotic nucleotide excision repair gene homologues were up-regulated.

From 8 microarray experiments, *radA1*, *nrdB2* and *vng1642* were the most highly up-regulated genes (at least 3-fold by UVC and UVB). RadA1 is the archaeal homologue of Rad51/RecA, a protein involved in homologous recombination and stabilizing stalled replication forks in damaged DNA; *nrdB2* encodes a subunit of ribonucleotide reductase; and *vng1642* is a gene of unknown function that is co-expressed with *nrdB2*.

UV induction kinetics of *radA1* were determined by quantitative real time PCR. UV induction for UVB and UVC was detectable after 20 min, and transcripts were induced to 8-fold by 45 minutes. *radA1* transcript levels continued to increase up to 5 hours after UVC irradiation. After UVB, levels began to decrease after 45 minutes.

EM 03 Identification of biofilm specific genes in *Rhodobacter sphaeroides* Ws8n – identified using recombination-based *in vivo* expression technology (RIVET)

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We have developed recombination-based *in vivo* expression technology (RIVET) to identify *Rhodobacter sphaeroides* WS8N fusions that are transcriptionally induced during cell attachment and biofilm development (within 48hr). Using green fluorescent protein (GFPmut3) and kanamycin antibiotic sensitivity as heritable reporter genes, a transcriptional fusion library (15,000 isolates) of *R. sphaeroides* WS8N was screened within microcosms to identify genes specifically induced during initial attachment and development processes during biofilm formation (termed biofilm induced RIVET (*bir*) fusions). Seventy-two transcriptionally induced *bir*-fusions were identified. Here we describe the development of the RIVET method and demonstrate that the resulting *bir*-fusions are either i) consistent with previously identified genes from other organisms involved in biofilms or ii) have novelty as they are previously unassociated with biofilm formation. Also presented are 3D confocal image analyses of *R. sphaeroides* WS8N strains containing deletant-mutations of biofilm genes that show overall effect on mature biofilm formation.

EM 04 The role of quorum sensing in *Rhodobacter sphaeroides* WS8N

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Rhodobacter sphaeroides is a free-living photoheterotrophic bacterium. It has a single characterized *luxI* homolog called *cerI* that synthesizes the long chain acylhomoserine lactone signal, 7,8-*cis*-*N*-(tetradecenoyl) homoserine lactone. The insertional inactivation of *cerI* with a ΩKm cartridge causes a mutant phenotype in which cells clump in liquid culture. A predicted *luxR* homolog, *cerR* and a small ORF of unknown function, *cerA* lie immediately upstream of *cerI* (Puskas *et al.* 1997). *CerI, cerR* and *cerA* have being deleted in-frame. Deletion of *cerI* caused the mutant clumping phenotype previously described, but deletion of *cerR* did not. The role of *cerI*, *cerR* and *cerA* on biofilm formation, extracellular polysaccharide (EPS) production and motility were investigated. Fluorescently tagged *R. sphaeroides* biofilms were grown in flow chambers and biofilm development was measured by confocal laser scanning microscopy. Variations in EPS production between wild type *R. sphaeroides* and the mutants were detected by calcofluor white staining. Differences in the motility of planktonic cells were measured on swarm plates.

Reference: Puskas, A., Greenberg, E.P., Kaplan, S. & Schaefer, A.L. (1997). A quorum-sensing system in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. *J Bacteriol* **179**, 7530–7537.

EM 05 Effect of spatially variable mercuric selection on *Pseudomonas* sp. in a bio-layer

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Previous studies have investigated bacterial community dynamics at the micro-scale using epifluorescence microscopy. Grown as a bio-layer with and without uniform mercuric (Hg(II)) selection, the model community comprised two *Pseudomonas fluorescens* strains; (i) Hg sensitive, chromosomally labelled with red fluorescent protein, and (ii) Hg resistant, carrying plasmid pQBR103 conferring Hg resistance, labelled with green fluorescent protein. In the environment selection is rarely uniformly distributed: we have, therefore, extended these studies to investigate the impact of spatially variable mercuric selection.

A novel method, in which foci of particle-associated mercuric chloride ($HgCl_2$) are sprayed onto a filter membrane bio-layer, has enabled us to study bacterial interactions using spatial analysis software. The trade-off between cost and benefit of plasmid carriage results in significantly different bacterial community structures according to local Hg conditions and competition. This study demonstrates an evaluation of community processes at a previously intractable scale.

Supported by the Natural Environment Research Council.

EM 06 The effect of heterospecific competitors on diversification of experimental *Pseudomonas fluorescens* populations

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It is traditional wisdom that the absence of inter-specific competition creates the ecological opportunities for diversification of natural populations, whereas competition from other taxa should hinder diversification. There is some empirical evidence for this hypothesis, which often involves comparison of diversification rate between island and mainland populations of sister taxa, but direct experimental tests are scarce. However, we argue that the presence of competitors can either impede diversification by decreasing its potential niche width, or enhance diversification by imposing biased influences on different genotypes in a population and preventing dominance of particular genotypes. Microbial microcosms provide the ideal experimental set-up in which to test these ideas. In spatially heterogeneous environments *Pseudomonas fluorescens* evolves into three classes of variants with distinct colony morphologies and spatial niches. Results from our initial experiments indicate that the presence of a competitor species, *P. putida*, lead to an early on-set of diversification combined with increased diversity when compared to the monoculture microcosms. Current experiments aim to determine if the underlying mechanism is a result of niche partitioning by comparison of spatially heterogeneous with homogeneous environments.

EM 07 Enteric pathogen decay kinetics and microbial biomass dynamics in biosolids-amended agricultural soil

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Natural decay of enteric micro-organisms in soil following application of biosolids provides a final environmental barrier to the potential transmission of infectious disease. Although general factors governing loss of viability are well known, the ecological variables involved in pathogen decay are not well defined. Three field trials, involving the amendment of two agricultural soils with a range of biosolids, were set up to investigate the environmental interactions involved in the decay process. The indicator bacterium *E. coli* decayed in two phases in soils amended with conventionally treated biosolids, and numbers remained slightly higher than those in unamended soil. *E. coli* O157 also decayed in two phases. Soil microbial biomass concentrations were linked to organic matter content of the soils, and soil properties had a profound influence on the microbial response to the biosolids. A novel method for enumeration of protozoa, based on predation of luminescent bacteria, was employed. Nitrogen mineralization was also influenced by soil ecological dynamics.

EM 08 Microbial community composition of a trichloroethene (TCE)-contaminated aquifer undergoing phytoremediation

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TCE is used as an industrial solvent and contaminates vast volumes of groundwater worldwide. The microbial community composition of a long-term TCE-contaminated aquifer undergoing phytoremediation was investigated. A combination of genetic, functional and physiological analyses was used. Chemical analyses of the site revealed that transformation of TCE had occurred with the main contaminant being cis 1,2-dichloroethene (cis 12-DCE). The concentrations varied across the site with the highest concentrations of TCE and its metabolites at the source area. The phytoremediation strategy involved the planting of poplar trees across the site. Samples were taken from the root zone of the poplars as well as from boreholes between the poplar trees. In general, bacterial numbers, as well as the genetic and functional diversity of the bacterial community structure were all higher in the root zone of the trees. The concentration of volatile organic carbon compounds (VOCs) was shown to impact negatively on the overall numbers of bacteria. Differential effects of the VOCs on specific sub groups of bacteria were observed. High concentrations of VOCs impacted on the genetic and functional composition of the microbial community. The presence of genes encoding aromatic oxygenases, known to be capable of aerobically transforming TCE, was confirmed across the site.

EM 09 Plant secondary metabolite amendment affects soil microbial community composition

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Plant secondary metabolites (PSMs) have been proposed to stimulate the microbial transformation of TCE since aerobic dehalogenation is a cometabolic process that requires a primary substrate in order to induce the responsible monoxygenase enzymes. The effect of PSMs on the soil microbial community has not previously been determined. 13 different PSMs were added to the soil at a range of concentrations and the effect on the physiological, functional and genetic composition of the microbial community investigated. The effect of the PSMs varied dependent on the chemical and on the concentration, with stereoisomers exhibiting differential effects. The PSMs judged of most potential interest for the purpose of TCE bioremediation were R-carvone, limonene and thymol. R-carvone had a big stimulatory effect on the overall bacterial numbers and carbon

substrate utilization, limonene preferentially induced the pseudomonad population and thymol induced overall numbers and the actinomycetes. Genetic analysis using denaturing gradient gel electrophoresis confirmed these shifts.

EM 10 The nitroaromatics trinitrotoluene (TNT) and dinitrotoluenes (2,4-DNT and 2,6-DNT) exert differential effects on the soil microbial community

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TNT and the dinitrotoluenes are priority pollutants found in sites across the world. TNT is a high explosive, and 2,4-DNT and 2,4-DNT are also explosives, as well as being used for dyes and plastics. The effect of amending soil with a range of concentrations of TNT, 2,4-DNT or 2,6-DNT on the bacterial community was investigated. We used a combination of genetic, functional and physiological analyses. All three compounds were toxic to the overall bacterial numbers at high concentrations. With TNT, however, at high concentrations the numbers of pseudomonads increased. This effect was not observed with either 2,4-DNT or 2,6-DNT. The functional capacity of the soil bacterial community was affected by both the concentration of amendment and the chemical used, as judged by carbon substrate utilization patterns. The genetic composition also varied dependent on the concentration of amendment and the chemical used as judged by denaturing gel gradient electrophoresis. Of particular note was the prevalence of bands relating to pseudomonad species at high concentrations of TNT.

EM 11 Investigation of the effect of plant secondary metabolite addition on trichloroethene -contaminated soil

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Trichloroethene (TCE) is one of the most prevalent soil and groundwater contaminants due to its use as a metal degreaser, dry cleaning agent and household cleaning solvent. TCE is very stable in the environment. It is known to be hepatotoxic and there is a growing body of evidence suggesting that it may be a carcinogen. Finding innovative ways to clean up TCE contaminated sites has become a priority in the remediation field since the physical characteristics of TCE make it difficult to remove from soil and groundwater using traditional technologies. Thus, the use of bioremediation has been suggested as a more cost effective remediation solution. Plant secondary metabolites have been added to TCE contaminated soil in an attempt to stimulate microbial transformation of TCE since aerobic dehalogenation is a cometabolic process requiring a primary substrate in order to induce the responsible monoxygenase enzymes. The physiological, functional and genetic composition of the microbial community was observed to vary dependent on the concentration of TCE as well as with the addition of the plant secondary metabolites thymol and R-carvone.

EM 12 The distribution ecology of a model micro-organism along an estuarine gradient

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Microbial ecology has, to date, been primarily descriptive in nature with a limited grounding in ecological theory, the result being that the structure and function of microbial communities and their importance in the global ecosystem are poorly understood. One aim of this project is to start to address this issue by using an indigenous model organism as a tool to investigate the relationship between microbial community structure and function.

The sulfate-reducing bacteria (SRB) play a vitally important role in the global carbon and sulfur cycles (Postgate, 1984; Purdy *et al.*, 2002). One genus of the SRB – *Desulfobulbus* – is thought to be ubiquitous in its range and is a major propionate utilizing micro-organism in the environment. It is also unknown what else, if anything, these organisms are doing in the environment but evidence suggests that they utilize sulfate as an electron acceptor in the marine end of the estuary but not at the freshwater end (Purdy *et al.*, 2003).

The major aim of the project is to investigate the effects on the bacteria that changes in the dynamic environmental factors found in the estuarine environment cause as they fluctuate down an estuarine gradient and determine what the bacteria are capable of doing in the environment.

The River Colne estuary, Essex, UK was the chosen project site, of which a body of background information is already known (Purdy *et al.*, 2003). DGGE profiling at strategic points along the estuary has produced community fingerprints which reveal that different bacterial communities exist at opposing ends of the watercourse. Phylogenetic analysis of cloned PCR products indicate that distinct groups of *Desulfobulbus* are found in different stretches of the estuary.

References: Postgate, J. (1984). *The Sulphate-Reducing Bacteria*, 2nd edn. Cambridge: Cambridge University Press; Purdy, K.J., Embley, T.M. & Nedwell, D.B. (2002). *Antonie van Leeuwenhoek* **81**, 181–187; Purdy, K.J., Munson, M.A., Embley, T.M. & Nedwell, D.B. (2003). *FEMS Microbiol Ecol* **44**, 361–371.

EM 13 Role of methanogenic archaea in termite evolution

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Termites are one of the dominant animals in tropical regions, where they function as 'ecosystem engineers' affecting the structure and nutrient content of soil by helping to degrade plant matter. The evolution of soil-feeding by termites is associated with the phenomenal success and diversity of this insect family. Distinct differences can be seen in the euryarchaeal communities in a small sample of termites from across the feeding guilds. Gut euryarchaeal communities in wood-feeding termites from the lower and higher termites are most similar to each other while soil-feeding termite gut communities clearly distinct. There is at least one methanogen-related clade that appears to be specific to the soil-feeding termites. This group is related to the *Methanomicrobiales* and probably maintains the H_2 partial pressure within the guts of soil-feeding termites at significantly lower levels than that seen in wood-feeders. This would make the complete fermentation of the more reduced components of organic matter in soil energetically favourable and thus makes soil a viable food source for termites. We propose that the evolution of soil-feeding in termites is associated with the acquisition of specific methanogenic archaeal clades and that the evolution of this important insect family has a distinct microbial driver.

EM 14 Metagenomic analysis of human tongue using phage display

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Microbial infections are dependent on a series of changes in both host and bacterium, in response to bacterial attachment. Commensal

bacteria also develop and maintain an intimate relationship of mammalian cells, without invasion. Phage display is a simple genetic technique for the identification of protein-ligand interactions, such as microbial attachment to cells, and is currently used successfully in epitope mapping, antibody tailoring and receptor agonist and antagonist screening. It is well established that the adhesion of enteric, oral and respiratory bacteria is required for colonization and, once bound, they are less likely to succumb to host defences. This study aims to outline the identification of bacterial adhesins which are vital for bacterial colonization of the human tongue, using phage display techniques. Screening of the phage display library is expected to yield one or more bacterial adhesins.

EM 15 Identification of markers of genetically stable clones of *Campylobacter jejuni* and tracking their sources and routes of transmission through the food chain

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Campylobacter jejuni is the leading cause of foodborne bacterial enteritis in the industrialized world. Genomic instability and the lack of standardization among laboratories in phenotypic and genotypic characterization has largely hindered the epidemiological tracking of campylobacter infections. However, clonal *C. jejuni* populations have been identified to exist in Penner serotypes O:6, O:19 and O:41. Therefore, the aim was to develop genetic markers, specific to stable Penner groups for the epidemiological tracking of *C. jejuni* through the food chain. The Penner O:6 stable group is represented by the *C. jejuni* strain 81116, which has been observed to be genetically stable for over 24 years. This group, collected from various sources and European countries are resistant to the *Sma*I restriction endonuclease. PFGE, AFLP, and *fla*A analysis support the clonality of these clonal Penner groups, but surprisingly intra-genetic rearrangements were observed following freeze-thaw stress passaging of some strains within Penner O:6 and O:19 groups. Interestingly, such rearrangements often included an approximate 50 kb gain in genome size. Sequence data from MLST and the Short Variable Region of the *fla*A gene, excluding genetically-unstable clonal strains, further suggest the genetically stable, clonal Penner groups are tightly clonal. Mechanisms for genetic stability, such as Restriction Modification, and repair and recombination genes revealed these to be poor markers for the Penner clonal groups. Comparative genomics by microarray, is being explored for development of markers to these genetically stable clones.

EM 16 Effect of pH on biodegradation of polycyclic aromatic hydrocarbons

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The purpose of this study was to investigate the effect of pH on biodegradation of polycyclic aromatic hydrocarbons (PAHs) and their mobility. Four different type of soil were used in these experiments. The soil pH was decreased by adding HCL and increased by sodium carbonate. Our investigations suggest that pH has a huge impact on PAHs biodegradation and mobility. While at low pHs there was a little rate of degradation, breaking down of pollutant was accelerated by increasing pH up to 7.0. Although at basic pH degradation rate slows down, it was still quite high and it was comparable with neutral pH. The mobility rate, however, was vice verse. At neutral pH the mobility of pollutant was lower in comparison with acidic and basic pH. It was therefore concluded that the ideal pH for degradation of these pollutants was neutral pH due to high level of degradation and low level of pollutant mobility which helps to keep the pollutants on site and minimize the leaching of pollutants to the underground water.

EM 17 Prediction of fusarium foot rot disease of peas in agricultural soils: a molecular approach

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Foot rot disease due to *Nectria haematococca* (anamorph *Fusarium solani* f.sp *pisi*) is a globally, economically important disease of peas. However, our ability to accurately predict the likelihood of foot rot infections is limited because culture-based assays do not discriminate between non-pathogenic and pathogenic strains. Hence, in this research, PCR-based detection assays were developed to detect and quantify three *N. haematococca* pathogenicity determinants (PDA, PEP3 and PEP5) both from isolates and DNA extracted from pea field soils with a foot rot history. Results showed that the PDA^H allele of the PDA gene, responsible for rapid demethylation of the phytoalexin pisatin, together with PEP3 and PEP5 genes promotes maximal foot rot disease in peas. Secondly, there was a positive correlation between numbers of pathogenic *N. haematococca* spores and numbers of pea pathogenicity genes in soil. Moreover, soils with higher numbers of pathogenicity genes had a correspondingly greater pathogenic effect on peas. Molecular approaches may therefore enable quantitative prediction of foot rot infections in agricultural soils prior to pea cultivation.

EM 18 Analysis and monitoring of a faecal bacterial succession in pigs by a reverse line blot hybridization assay

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Pig faecal bacterial changes during a period before and after weaning together with the effect of micronized whole rapeseed in the diet were analysed using a reverse line blot (RLB) hybridization assay. Faecal samples from 12 piglets were collected at 2 weeks of age and when the piglets were weaned at 4 weeks old. After weaning, the piglets were housed in litter matched groups of 4 and received 3 levels of inclusion of micronized whole rapeseed in the diet (0, 50 or 100 g/kg). Faecal samples were taken at 5, 14 and 26 days after weaning. When comparing the results of samples obtained before weaning, *Lactobacillus reuteri* and *L. acidophilus* number were similarly detected in samples taken at 2 and 4 weeks of ages. *Bifidobacteria* spp. and *Streptococcus* spp. presented in a higher number in 2- and 4-week-old piglets, respectively. At 5 days after weaning, *Streptococcus* spp. numbers found in each groups decreased corresponding to the increasing of inclusion level of rapeseed. However, *Streptococcus* spp. reappeared in all samples taken from all groups of pigs at 14 and 26 days after weaning. Weaning as well as the rapeseed inclusion level in the diet had no impact on *L. acidophilus*. According to this study, the diet of weaned pigs favours a growth of *L. animalis* and *L. murinus* but does not affect *L. acidophilus*. Also, different inclusion levels of rapeseed in diet have an impact on *Streptococcus* spp.

EM 19 Competitiveness of a ciprofloxacin hyper-resistant strain of *Pseudomonas aeruginosa*, grown in chemostat culture with minimal antibiotic selection pressure

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Sub-inhibitory concentrations of antibiotics may confer a selective advantage to antibiotic resistant micro-organisms. A hyper-resistant derivative of *Pseudomonas aeruginosa* NCIMB 10421 (MIC ciprofloxacin: >32 mg/ml) was isolated after 456 h of selective chemostat culture (D=0.04 h^{-1}) with ciprofloxacin selection pressure. Whilst the derivative (PMC456) had a maximum specific growth rate analogous to the original strain, it out competed the nascent micro-organism during chemostat competition studies conducted in the absence and presence of ciprofloxacin concentrations deemed sub-inhibitory to the parent strain. The rate of displacement of the parent strain increased with increasing concentrations of antibiotic.

EM 20 Dynamics and interactions within a microbial community on model cheese

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Background and objectives Little is known about the microbial dynamics and interactions which occur during the ripening of smear-ripened cheeses. The aim of this study was to monitor the growth of 3 yeast and 6 bacteria on experimental curd made under aseptic conditions. These data were processed using the generalized Lotka-Volterra (GLV) model and the type of interactions occurring between these organisms was identified.

Results The deacidifying yeasts, *D. ebaryomyces hansenii* and *Yarrowia lipolytica* grew first on the cheese surface while *Geotrichum candidum* grew only after 2 days of ripening. *Arthrobacter arilaitensis*, *Hafnia alvei* and *Leucobacter* sp. dominated after 7 days while the 3 other bacteria were subdominant. GLV analysis showed that 10 interactions were significant. 'Trophic' interactions were identified between the yeast while mutualistic or competitive interactions were observed between 3 bacteria and the yeast. Interactions among the bacteria were not significant. Surprisingly, 3 bacteria did not interact with any of the other micro-organisms.

Conclusions The GLV model showed that yeast interacted significantly within the cheese microbial community. However, further work is necessary to confirm these interactions. Because cheese ecosystems are composed of a limited number of micro-organisms, cheese may be a model of particular value to study microbial interactions.

EM 21 Isolation of biofilm-forming bacteria from marine environments

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In recent years, increasing interest in the bacteria from saline environments has led to the discovery of novel species and genera belonging to the order '*Rhizobiales*'. Almost of the *Rhizobiales* had been isolated from terrestrial rhizosphere or fresh water. Here, we isolated a novel marine bacterium C2304 from the biofilms formed on an inner wall of seawater pipelines of the Seto Inland Sea. C2304 is strictly aerobic, chemoheterotrophic, non-motile short rods, and forms brownish-yellow colonies. The cell grew optimally at NaCl concentration of 3% and it also grew up to 5% NaCl. The predominant cellular fatty acid was C18:0 and the optimum growth temperature was 30C. A most significant feature of C2304 is the ability to form robust biofilms in the proteineous media. Another isolate belonging to the genus *Mesorhizobium* formed moderate biofilms. NaCl concentrations above 5% inhibited biofilm formation of C2304. The 16S rRNA gene sequence of C2304 showed 90 and 89% similarity to the most closely related *Aurantimonas coralicida* and *Fulvimarina pelagi*, respectively. Phylogenetic analysis of C2304 suggested a novel genus that forms a deep evolutionary lineage in the order '*Rhizobiales*'.

EM 22 Construction of a device containing immobilized consortia of *Thiobacillus* and *Spirogyra* for air decontamination

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Microbial biofilters decontaminate large air streams by forcing the polluted air through a filtering bed with naturally immobilized micro-organisms. As a result of microbial metabolism, the absorbed toxic pollutant is converted to nontoxic components. The work presented here addresses microbial phenomena and biofiltration performance in immobilized surfaces. The micro-organisms used were *Thiobacillus* sp. And *Spirogyra* isolated from deep wells around old public bathrooms and pounds. For this selective medium were prepared and microbial biomass were immobilized using Sol-Gel silica method. The air used in these experiments was introduced to the system by pumping device. In first experiment handy monitoring systems were used and results showed that concentration of carbon monoxide (CO) decreased from 25 ppm in time of start to 23, 20 and 14.83 ppm in time intervals 10, 20 and 30 minutes respectively. It showed to near 60% decrease in the amount of CO. At the same method SO_2 concentration were 100 ppm at the start point, however after biofiltration, SO_2 concentration were decreased to 36,16 and 10 ppm at the same time intervals indication 90% of depletion. In second monitoring experiment CO and SO_2 concentration were changed by 22 and 60% respectively. In both experiment significant amount of air pollutant were removed using this invented devices promising application of that for decontamination of polluted air of factories and other chemical bodies. Optimization of device using most characterized micro-organisms is underway and will be presented in near future.

EM 23 Analysis of combined gamma radiation and hiperthermic treatments on *Microcystis panniformis* Komárek et al (*Cyanobacteria*)

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Microcystis panniformis are cyanobacteria that seem blooms formation produce a toxin from microcistin that cause poisoning and promote liver tumors. The blooms are relacionated with artificial eutrophyzation caused by excess of nutrients from domestical effluents and industrial waste, that in large amount cause extensive death of fishes and other animals. The reservoirs of water used for people supplying are subjected to blooms appearance of cyanobacterias the needs to be monitored to avoid human health risks.

In the midst of non chemical methods of both water treatment and sterilization for human consume, the irradiation process with beam of gamma ration is the object of this work.

Specifically, the aims of this work were: to value the radiosensitive of the *Microcystis panniformis* Komárek *et al.* (*Cyanobacteria*) to the

gamma radiation of 5KGy, as well as the combined effects of irradiation and heat.

In the synergy of studs between irradiation and thermal treatment, the cultures were irradiated with doses of 5KGy with and without heating at 40°C for 2 hours. In this case, was cheek that the consumed treatment (irradiation and heating) increases the cellular non viability of 97%, after 48 hours. The heating of the central, in these conditions, presented a discreet fall (16%) in the viable cellules number, after 24 hours later of treatment end, but with fast recuperated in the subsequent periods. The treatment with gamma radiation only presented a cellular drop of 67%.

The global results shows: with an action of physical agents the considerable reduction of the doses required for the population central of the cyanobacteria is possible. Here with, the processes will be cheaper and safer.

Posters

Eukaryotic Microbiology Group

EukM 01 Entrainment of the *Neurospora* circadian clock: a vivid story!!

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In *Neurospora crassa*, frequency (*frq*), white-collar1 (*wc-1*), white-collar2 (*wc-2*) and their corresponding proteins are essential for circadian rhythmicity. Although not essential for circadian rhythmicity, the PAS/LOV protein VIVID (VVD) is a WC-1 dependent photoreceptor that modulates light responses in *Neurospora*, influencing light sensitivity and the phase of the clock. *Neurospora* synchronizes its circadian clock to the external environment via a process called entrainment, for which light and temperature are important cues. Recently we have shown that VVD modulates clock resetting both at dawn and dusk with implications for entrainment of the *Neurospora* clock to light-dark cycles. In *vvd* knockout strains the phase of clock-controlled conidiation is altered when compared to wild-type strains and a second round of conidiation is initiated. The cause for this second peak of conidiation in *vvd* knockout strains is unclear but may be due (amongst other possibilities) to a reduction in the threshold for clock-independent light-induced conidiation, repression of conidiation, or unmasking of a second pacemaker that controls a second round of conidiation in light-dark cycles.

EukM 02 Investigation of two putative *Neurospora crassa* heat shock transcription factors

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Heat shock transcription factors (HSFs) play important roles in the environmental stress response, allowing organisms to cope with a number of stresses including sudden temperature change. In the absence of complex temperature sensing organs, the filamentous fungus *Neurospora crassa* may also use HSFs to respond to small subtle changes in temperature. To investigate this hypothesis we obtained strains in which genes encoding putative HSFs (NCU08512 and NCU08480) have been deleted. Our results indicate that NCU08512 is an essential gene, since no NCU08512 deletion strains were recovered from a sexual cross of a ΔNCU08512 heterokaryon to a wild-type. Information on the transcripts produced from NCU08512 and NCU08480 obtained from cDNA shows that several splice variants of NCU08480 are synthesized that encode altered forms of the predicted protein. Computer analysis of the sequences shows that the encoded proteins share structural similarities to other HSFs. We are currently investigating whether or not the Neurospora HSFs are able to functionally substitute for the *Saccharomyces cerevisiae* HSF1.

EukM 03 The role of a natural cis-antisense clock gene specific RNA in *Neurospora crassa*

Simon Anson & Susan K. Crosthwaite

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From simple bacteria to complex eukaryotes, circadian clocks control changes in metabolism, physiology and behaviour. The filamentous fungus *Neurospora crassa* requires rhythmic levels of *frequency* (*frq*) mRNA to maintain circadian rhythmicity. It has been found that in addition to *frq* RNA an antisense RNA, *qrf,* is transcribed from the *frq* locus. Like *frq, qrf is* rapidly light induced and if this light response is abolished light resetting of the clock is dramatically altered. Moreover, levels of *qrf* in the dark cycle in antiphase to *frq* RNA. We constructed a vector, pSA1, containing an altered *frq* locus in which *qrf* is fused to the inducible *quinic acid-2* promoter whilst the natural *frq* promoter remains intact. In a *frq* null strain transformed with pSAI low concentrations of inducer quinic acid restored rhythmic clock-controlled spore production whilst higher levels resulted in its loss, suggesting that *qrf* may be important for clock function in the dark.

EukM 04 Iron acquisition in *Candida albicans*: the ferric reductases

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Iron is an essential element for the survival of all cells, and acquisition of iron is important to the survival of any organism. In the opportunistic pathogen *C. albicans* iron is first reduced to the soluble ferrous form by the ferric reductase proteins. The ferrous iron is then taken up by a protein complex involving the multicopper oxidase proteins, as such the uptake of copper and iron in *C. albicans* are inextricably linked. In our lab we research the genes involved in iron and copper acquisition in *C. albicans*. There are sixteen ferric reductase genes in *C. albicans*, this indicates that they may possess a variety of functions in the cell. I am using GFP techniques to localize *CFL95*, the major ferric reductase, and *FRE12*, a copper regulated ferric reductase. Lan *et al.*, (2004) implicated Sfu1p as being involved in the regulation of the ferric reductase genes *CFL1, CFL2, CFL4* and *CFL5* in a microarray study. I am using Northern blots to determine the role of Sfu1p in the regulation of the other ferric reductases.

EukM 05 Mechanisms of break-induced loss of heterozygosity in fission yeast

Anoushka Davé, Jason Cullen, John Prudden, Carol Walker, Boon-Yu Wee, Sharon Hussey & Tim Humphrey

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Loss of heterozygosity (LOH) is considered a causal event in tumorigenesis, yet the mechanisms leading to LOH are poorly understood. We have investigated the mechanisms of DNA double-strand break (DSB) induced LOH by screening for auxotrophic marker loss ~25 kb distal to an HO-endonuclease break-site within a non-essential minichromosome in *Schizosaccharomyces pombe*. Extensive break-induced LOH was infrequent, resulting predominantly from large non-reciprocal translocations arising through both allelic crossovers and break-induced replication. These events required homologous recombination (HR). In HR mutants, extensive LOH resulted predominantly from *de novo* telomere addition, and was facilitated by end resection. We have further sought genes that suppress break-induced LOH. Surprisingly, disrupting Rqh1, the *S. pombe* homolog of the human BLM and WRN recQ helicases in an HR-deficient background resulted in a dramatic increase in LOH levels through *de novo* telomere addition compared to the corresponding single mutants. These findings identify dual mechanisms of suppressing *de novo* telomere addition at break-sites, and further suggest a novel role for BLM in maintaining genome stability.

Posters

Fermentation & Bioprocessing Group

FB 01 Global responses of *Campylobacter jejuni* to nitric oxide-releasing compounds reveal that the single domain globin, Cgb, is the major NO detoxification mechanism

Claire Monk & Robert K. Poole

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The ability to control growth rate in sub-optimal conditions is finding renewed usefulness in transcriptomics, where a frequent experimental objective is to compare growth under 'control' and 'stress' states. In this contribution we describe the application of simple home-built chemostats operating under microaerobic conditions to understanding the stress responses mounted by *Campylobacter jejuni* in the face of short-term exposure to nitric oxide. *Campylobacter jejuni* regularly encounters reactive nitrogen species (RNS) as a result of its interaction with host organisms but to date only the single domain globin, Cgb, has been identified as having a role in NO tolerance and detoxification. This work describes and compares the transcriptomes of both wild type *C. jejuni* and the *cgb* mutant to identify alternative and additional mechanisms of RNS tolerance in this species. We show through respiratory analysis that *C. jejuni* is capable of an oxygen dependent adaptive response to NO and that this is predominantly mediated by Cgb, although the truncated globin, Ctb, may also have a lesser role. The dominance of Cgb and subsidiary role of Ctb are further enforced by the microarray analysis and protein expression studies, which suggest that Ctb may be used by the organism to compensate in part for a loss of Cgb. In addition, evidence is presented that may implicate the iron-responsive Fur repressor in transcriptional regulation of *C. jejuni* under conditions of nitrosative stress. Increasing our understanding of how *C. jejuni* survives RNS will allow better understanding of host/pathogen interactions, and suggest potential targets for pharmaceuticals.

This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC).

FB 02 The global transcriptional responses of chemostat-cultured *Escherichia coli* to nitric oxide and *S*-nitrosoglutathione are markedly different: inactivation of global regulators by NO but lack of evidence for nitrosation reactions

Steven T. Pullan, Jason Barrett, Jeffrey Green & **Robert K. Poole**

Dept of Molecular Biology & Biotechnology, University of Sheffield, S10 2TN

The ability to control growth rate in sub-optimal conditions is finding renewed usefulness in transcriptomics. We have previously used well-defined chemostat cultures to elucidate the global transcriptional responses of *E. coli* to the nitrosating agent *S*-nitrosoglutathione (GSNO) under both aerobic and anaerobic conditions. Here we adopt an identical experimental design to define the response to nitric oxide (NO) *per se* and demonstrate marked differences at the level of transcription in the responses to these two distinct nitrosative stresses. Exposure to NO, in contrast to GSNO, did not lead to the up-regulation of any genes involved in the methionine biosynthetic pathway and exogenous methionine afforded no protection from NO-mediated killing. Anaerobic exposure to NO led to the up-regulation of several FNR-repressed genes and the down-regulation of many FNR-activated genes, including those encoding the cytochrome *c* nitrite reductase, NrfA, providing strong evidence for the *in vivo* inactivation of FNR by NO. The other regulators affected by NO were IscR, Fur, SoxR, NsrR and NorR.

This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC).

FB 03 Transcriptional profiling of *Escherichia coli* responses to zinc-inhibited and zinc-limited growth

Lucy Lee[1], **Alison Graham**[1], Cameron McLeod[2], Alan Cox[2] & Robert K. Poole[1]

[1]Dept of Molecular Biology and Biotechnology, University of Sheffield, S10 2TN; [2]Dept of Chemistry, University of Sheffield, S10 2TN

The chemostat has long been employed to investigate physiological changes arising from growth limitation by various nutrients while maintaining specific growth rate. The ability to control growth rate even in sub-optimal conditions is finding renewed usefulness in transcriptomics, where a frequent experimental objective is to compare growth under 'control' and 'stress' states. In this contribution we describe the use of simple home-built chemostats constructed from non-metal parts to understand the stress responses mounted by *Escherichia coli* in the face of (a) both short- and long-term treatment with elevated, growth-inhibitory concentrations of zinc (200 μM) and (b) continuous growth with limiting concentrations of zinc (<0.1 μM). In the former, genes up-regulated included those known to be involved in zinc tolerance (*zntA*, *zraP*), but also genes not previously implicated in zinc homeostasis. These include the *cus* operon (encoding a copper export system) and *basRS* encoding a two-component regulatory system implicated in sensing iron and magnesium. In the case of zinc limitation, rigorous precautions are required to limit metal contamination, including the use of Chelex-treated defined growth media, acid-washed glassware, ultra-pure chemicals and dedicated Teflon plasticware. Significantly, continuous culturing in the same vessel progressively reduced the amount of zinc leached from the glass culture vessel.

This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC).

FB 04 *Escherichia coli* transcriptome dynamics during the transition from anaerobic to aerobic conditions

Jonathan D. Partridge, Colin Scott, Yue Tang, Robert K. Poole & Jeffrey Green

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Escherichia coli is a metabolically versatile bacterium that is able to grow in the presence and absence of oxygen. Previous transcript-profiling experiments have compared separate anaerobic and aerobic cultures usually in batch conditions, not exploiting the advantages of the continuous culture system to observe a temporal response to environmental changes. The process of adaptation to the presence of oxygen was investigated by determining changes in transcript profiles when anaerobic steady-state chemostat cultures were perturbed by the introduction of air. Snapshots of the transcript profile of the adapting culture 5, 10, 15 and 60 min after introducing air into the system were obtained. After 5 min of culture aeration the abundances of transcripts associated with anaerobic metabolism were

decreased, whilst transcripts associated with aerobic metabolism were increased. In addition to the rapid switch to aerobic central metabolism, transcript profiling, supported by experiments with relevant mutants, revealed transient changes suggesting that the peroxide stress response, methionine biosynthesis, and degradation of putrescine play important roles during the adaptation to aerobic conditions.

This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC).

FB 05 The role of lipid utilization in antibiotic synthesis by *Streptomyces clavuligerus*

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Streptomyces clavuligerus is a gram-positive filamentous bacterium that produces clavulanic acid, a very important antibiotic. Clavulanic acid is a β-lactamase inhibitor that prevents the development of resistance to β-lactam antibiotics by pathogenic bacteria. The metabolic pattern of *S. clavuligerus* is uncommon, since it is unable to utilize glucose and other sugars and presents a very active urea cycle. The hypothesis to be tested in this study is that food oils can be used as an alternative carbon source to glycerol for the production of clavulanic acid. Three different oils were tested (sunflower, olive and cod liver oil) in shake flasks, and *S. clavuligerus* was able to use all the oils to produce clavulanic acid, albeit at different levels, with olive and cod liver oil producing significantly higher levels than the glycerol control. Currently, the culture method is being optimized in 2-litre batch fermentation, using an olive oil-containing medium. Future work will involve the analysis of gene transcription levels in *S. clavuligerus* cultured in the presence of glycerol or olive oil, with subsequent metabolic modeling to define a mechanism for the utilization of olive oil in the production of clavulanic acid.

FB 06 An eternal clock? – *Neurospora crassa* in the chemostat

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The filamentous fungus *Neurospora crassa* is a model organism for studying biological clocks. To date key circadian clock genes (frequency, white collar-1, white collar-2) and their protein products have been investigated using batch cultures. This sets a time limit on observations of the molecular clockwork and culture conditions change as the organism metabolizes the carbon source. Here we report for the first time conditions for chemostat cultures of *Neurospora*.

Neurospora was grown in steady state in constant darkness at 25°C for 8 days (D=0.074/h). Samples were taken every 4 h for the extraction of RNA and protein and the state of the clock was monitored by following clock gene expression and clock-controlled sporulation. The clock continued to run in liquid culture throughout the experiment. These results open up the possibility of studying the response of *Neurospora* to a range of stimuli in the absence of confounding effects due to; alterations in growth rate, aging and changing conditions of the growth media.

FB 07 Analysing gene expression profiling data of *Escherichia coli* on biochemical networks with wavelet transformations

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Microarray technology produces gene expression data on a genomic scale for an endless variety of organisms and conditions. However, this vast amount of information needs to be extracted in a reasonable way and funneled into manageable and functionally meaningful patterns. Genes may be reasonably combined by the knowledge about their interacting behaviour. On a proteomic level, biochemical research has elucidated an increasingly complete image of the metabolic architecture, especially for less complex organisms like the well-studied entero-bacterium *Escherichia coli*. We want to discover central parts of the metabolic network that are regulated by the expression of the corresponding genes under changing conditions. As a case study, we mapped gene expression data from *E. coli* under aerobic and anaerobic conditions onto the enzymatic reaction nodes of its metabolic network. Wavelet transforms were applied on entry enriched blocks of the clustered adjacency matrix to collect features for the classifier (Support vector machines). The most discriminating features were selected and given out. With this, we yielded a sub-graph representing formate fermentation, a switching of the starting point for NAD biosynthesis, and an adaptation of the l-aspartate metabolism in accordance to its higher abundance under anaerobic conditions [*]. We extended our method by applying simple Haar-wavelet transforms to reaction pairs being adjacent in the metabolic network. We assembled all significantly discriminating pairs and revealed e.g. the up-regulation of the entire histidine biosynthesis pathway during oxygen deprivation. Functionally, this may be in accordance to higher needs of histidine for buffering acids coming from fermentation. We developed a novel method to analyse gene expression data on the basis of the metabolic network by combining well-chosen machine learning methods in an effective way. As a case study, we applied our method to *E. coli* under oxygen-deprived conditions and could extract physiological relevant patterns and pathways describing an adaptation of the cells according to a changing environment. The data was taken from batch cultured cells. We are now applying our system to chemostat-cultured *Escherichia coli* cells exposed to toxic media, i.e. excess Zinc concentrations and will present first results for this data-set.

Reference: *König, R., Schramm, G., Oswald, M., Seitz, H., Sager, S., Zapatka, M., Reinelt, G. & Eils, R. (2006). Discovering functional gene expression patterns in the metabolic network of *Escherichia coli* with wavelets transforms. *BMC Bioinformatics* **7**, 119.

Posters

Food & Beverages Group

FdBev 01 The effect of defined linear features on surface hygiene and cleanability

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Previous work has demonstrated that wear of hygienic food contact surfaces does not necessarily affect their cleanability in terms of removal of micro-organisms, but changes in surface topography may enhance the retention of organic material derived from food soil. This may affect the hygienic status of the surface, thus providing a biotransfer potential. The diameter, depth and shape of surface features and the removal force of cleaning, will affect the retention of micro-organisms, but limits have not yet been defined.

Fabrication of defined linear features in stainless steel from 0.2 µm to 5 µm across, with varying profiles and densities, will be coated with titanium via PVD, to assess the effect of topography and chemistry on fouling and cleanability. The effect of these features on the retention of *Comamonas*, *Pseudomonas fragi* and *Listeria monocytogenes* will be monitored. A number of different analytical techniques (AFM, DCA, XPS) will allow characterization of surfaces and assess fouling and cleanability.

Acknowledgement: This work is part of the EU PathogenCombat project.

FdBev 02 A method for monitoring substratum hygiene using a complex soil: the human fingerprint

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Our study brings together new and existing techniques to allow the assessment of a range of hygienic surfaces, initially using fingerprint residue as the test soil.

Human fingerprint residue is an example of a specific and complex organic-material/micro-organism soil which is often present in a variety of environments that are required to be hygienic. When applied onto surfaces a fingerprint may affect cleanability and influence bacterial retention, alter topography and in some cases, may even compromise the aesthetic qualities of the material. One of the most common modes of cross contamination in hygienic environments is via the human hand but this soil/micro-organism matrix is rarely studied in controlled conditions.

In our study slight modifications were made to published sweat and sebum standard formulations which enabled survival of Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) micro-organisms in a synthetic fingerprint soil which will then allow hygienic assessment of a range of surfaces.

Alongside this, a method for depositing a range of organic soils in specific quantities and arrangements (e.g. the synthetic fingerprint soil in a fingerprint pattern) onto a range of substrata has been developed to allow cleanability to be assessed. The method to assess cleanability was developed by identification and quantification of individual chemical components of a given soil and their ease of removal from the surface. After a cleaning procedure, the quantity of each component was measured using the appropriate analytic technique and in the case of the fingerprint soil gas chromatography-mass spectroscopy (GC-MS) was utilized.

The effectiveness of anti-bacterial surfaces against cell suspensions in water was demonstrated over time using the respiratory dye nitro blue tetrazolium (NBT) and live/dead staining.

The techniques described, when combined, can potentially be used to assess bacterial survival and retention and the cleanability of surfaces using a variety of soils, micro-organisms and surfaces. Combined with methods to measure surface topography and surface free energy this study presents a comprehensive guide to the relevant techniques when quantifying the hygienic performance of a surface.

Acknowledgement: EPSRC CASE studentship with Outokumpu Ltd.

FdBev 03 Investigation into the mechanisms involved in detergent induced changes in disinfectant susceptibility

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Food industry cleaning procedures involve the use of detergents to remove food soil followed by disinfectants to reduce the viability of the remaining organisms. Pathogenic micro-organisms attached to stainless steel surfaces, and in suspension, have been observed to become more susceptible or more resistant to disinfectants after treatment with commonly used detergents. This work is investigating the underlying mechanisms that lead to increased resistance or increased susceptibility.

Following 20 minutes exposure to the detergents, standard plate counts have shown that *Escherichia coli* became more susceptible and *Listeria monocytogenes* became more resistant to Benzalkonium chloride (0.87 and 3.45 $\log_{10}$ difference respectively). *E. coli* and *L. monocytogenes* became more hydrophilic following treatment with the anionic detergent as measured by hydrophobic interaction chromatography. Flow cytometric analysis indicates significant changes in cell membrane permeability of *E. coli* compared to that seen in *L. monocytogenes*, which may relate to the observed changes in susceptibility.

In conclusion, increased susceptibility or increased resistance to disinfectants may be effected by changes in cell surface hydrophobicity and/or changes in membrane permeability.

FdBev 04 A role for SigmaB in surfactant stress response of *Listeria monocytogenes*

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Listeria monocytogenes is a Gram positive, facultative intracellular pathogen which is responsible for approximately 35% of known food poisoning related deaths in the U.S. The stresses imposed by industrial cleaning agents are of interest both medically and economically due to persistence of *L. monocytogenes* in the food-processing industry.

The objective of this study was to investigate the role of the alternative sigma factor Sigma B in the listerial stress response imposed by industrial surfactants. Surfactant stress in *L. monocytogenes* 10403S and a Δ*sigB* mutant of 10403S was investigated by lethality, impact on growth and by transcriptional analysis. A significant 1–3 log decrease in the viability of the Δ*sigB*

mutant in response to lethal levels of surfactants was observed. Growth analysis upon exposure to sub-lethal levels of surfactant demonstrated a role for SigmaB in adaptation of exponential growth phase cells and mirroring these findings transcriptional (RT-PCR) analysis revealed the induction of *sigmaB*. Sigma B-regulated loci previously identified could also potentially play a role in surfactant stress response, these loci include putative general stress proteins and predicted transport systems, a number of which are currently the subject of further analysis in our laboratory.

These results demonstrate that Sigma B plays a significant role in the surfactant stress response resulting from industrial cleaning regimes and therefore may provide a target for novel cleaning agents.

Posters

Microbial Infection Group

MI 01 Cyclo-oxygenase (COX)-2: expression and signalling pathways in an in vitro model for meningococcal meningitis

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COX-2 is the inducible isoform of the cyclooxygenase enzyme and its expression is commonly stimulated by inflammatory mediators during infection. Previously, our research using expression arrays found that COX-2 expression was disproportionately up-regulated (~2000 fold) in human meningeal epithelial cells exposed to viable whole meningococci and/or their secreted protein products.

The aims of this current research were firstly to investigate whether the high-level COX-2 expression is unique to meningeal cells, and secondly to identify the signalling pathways involved in its expression.

Using Western Blot analysis, no COX-2 expression was found in other biologically relevant cell lines including HBMEC (human brain microvascular endothelial) and BEAS-2B (bronchial airway epithelial) cells when exposed to meningococci. Initial assays using a COX-2 luciferase reporter system in meningeal cells showed that there was a brief initial response to the bacterial stimulus after one hour, followed by a later and more sustained response.

These data imply that the increase in COX-2 expression could indeed be a meningeal cell-specific phenomenon, possibly regulated by more than one signalling mechanism.

MI 02 An improved *in vitro* model for the study of meningococcal pathogenesis

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Background The mechanism(s) by which meningococci cross the Blood Brain Barrier (BBB) are not completely understood. This study aims to construct a bilayer model of the BBB for infection studies that mimics *in vivo* conditions more closely than simple monolayer cultures.

Results Immortalized human brain microvascular endothelial cells (HBMEC) and human tumour-derived meningeal cells (MC) were successfully co-cultured on cell culture inserts and infected with meningococci. Infection was followed by electron and confocal microscopy showing bacteria interacting with the HBMEC cells. The production of the pro-inflammatory cytokines IL-6, IL-8 and TNF-alpha in response to infection showed differences between the monolayer and bilayer systems, suggesting a cross-talk between the HBMEC and the MC cells.

Conclusion This system provides an excellent *in vitro* model for studying meningococcal pathogenesis and is expected to shed new light on how meningococci in the bloodstream pass through the BBB.

MI 03 *Exiguobacterium* species; tracing the phenotypic variation of an emerging pathogen through proteomic analysis

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Many pathogens, particularly those from soil and water, often infect multi-host species. *Exiguobacterium* normal habitat is the sea/salt lakes but clinical reports over the last two decades suggest that this species is gaining entry to man. Accurate identification and appreciation of the diversity of the species is crucial to understanding which ecotype gains entry to the human ecosystem. Here strains collected over the last few years have been subjected to proteomic profiling using MALDI-TOF-MS and SELDI-TOF-MS.

Comparative 16S rRNA sequencing provided identification to a genus level but failed to delineate species. MALDI-TOF-MS profiles helped to cluster strains but even with a reference database lacking with this organism, unique mass ions are apparent of the species level. SELDI-TOF-MS, in tandem with SDS-PAGE profiles will enable the grouping of isolates into coherent profiles. As more strains are investigated, biomarker proteins of each species will be analysed by tandem MS-MS to identify the nature of their proteins. We envisage that these databases will provide a sound basis for tracing the adaptation of this species.

MI 04 *Francisella tularensis*: rationally generated live vaccines

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A spontaneously attenuated mutant of the biowarfare agent, *Francisella tularensis* subsp. *tularensis,* designated FSC043, was demonstrated to protect mice against intradermal or aerosol challenge with a highly virulent strain of subsp. *tularensis*, FSC033. The degree of protection was as good as that of *F. tularensis* LVS, an empirically attenuated strain which has been used as a human vaccine. In order to develop a defined live vaccine, comparative proteomics was used to identify two proteins found to be defective in LVS and FSC043. Mutant strains of SCHU S4 were generated by allelic replacement of the encoding gene, *FTT0918*. The Δ*FTT0918* mutant failed to express a 58-kDa protein and was attenuated in mice. Mice that recovered from sublethal infection with Δ*FTT0918* survived when challenged 2 months later with >100 LD_{50} of FSC033. This is the first report of the generation of defined mutants of *F. tularensis* subsp. *tularensis* and their use as live vaccines.

MI 05 Proteomic and immunoproteomic analysis of *Lawsonia intracellularis*

Eleanor Watson, M. Pilar Alberdi, Neil F. Inglis, Gina E.M. McAllister & David G.E. Smith

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Lawsonia intracellularis is the aetiological agent of proliferative enteropathy in pigs, a commercially significant disease with a worldwide distribution. This gram-negative, obligate intracellular bacterium has been largely uncharacterized due to its fastidious nature in the laboratory. *L. intracellularis* whole cell extracts from heavily infected *in vitro* cultures were separated by SDS-PAGE then fractions were analysed by LC-ESI-MS/MS. Protein identification was achieved by searching mass spectra against a *L. intracellularis* MASCOT database. In addition, *L. intracellularis* cell extracts were probed on Western blots with a panel of sera from pigs naturally

infected with *L. intracellularis* to identify immunogens. Several different proteins were recognized and these are being further investigated. This shotgun approach to characterize the *L. intracellularis* proteome, coupled with the evidence of immunological recognition of several proteins identifies novel determinants expressed during interaction of this unusual bacterium with its mammalian host.

MI 06 The *Lawsonia intracellularis* genome encodes typical Gram-negative bacterial secretion systems

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Lawsonia intracellularis is an obligate intracellular gram-negative bacterium which has been identified as the aetiological agent of proliferative enteropathies. *Lawsonia intracellularis* is phylogenetically distinct from other pathogens, its closest relatives being *Bilophila wadsworthia* and *Desulfovibrio* spp. Its complete genome has been recently made publicly available through the NCBI. Analysis of the 1.5 Mbp genome and three plasmids (a total of 1718014 bp) indicate several determinants typically involved in pathogenesis. Among these are a Type III Secretion System (TTSS) and a flagellar locus which show similar gene arrangements to that of *D. vulgaris*. Several ORF's resembling autotransporters are also present. Confirmation that TTSS genes are expressed during infection indicates that this apparatus may play a significant role in the virulence of this unusual pathogen.

MI 07 Proteomic analysis of sub-cellular fractions of *Escherichia coli* O157:H7

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Enterohaemorrhagic *E. coli* O157 pathogenicity determinants have been extensively investigated. Exported proteins are known to contribute to the establishment of infection and the characteristic localized attachment and enterocyte effacement. Characterization of the bacterial 'secretome' is therefore crucial to further understanding the carriage and pathogenesis of this organism.

A shotgun approach employing SDS-PAGE separation in conjunction with LC-ESI-MS/MS (Dionex Ultimate 3000 LC and Bruker Daltonics HCT plus instruments) was used to assess *E. coli* O157 secreted proteins. Comparative analysis of secreted protein profiles on SDS PAGE gels of different EHEC strains indicates possible strain-related differences in the secretome. EHEC strains have also been compared under conventional conditions and in response to contact with epithelial cells.

MI 08 Proteome analysis of *Helicobacter pylori* supports a role for TlyA in the regulation of specified virulence factors

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Several possible roles have been hypothesized for TlyA family members which have been found in many pathogenic species including *Helicobacter pylori, Campylobacter jejuni, Brachyspira hyodysenteriae* and *Lawsonia intracellularis*. Postulated roles include involvement as a cytotoxin/enterotoxin or in adherence. However, the existence of an S4 RNA binding domain in conjunction with a methlytransferase domain in most TlyA members suggests they may function as regulators of gene expression.

2D electrophoresis was employed to compare whole cell and outer membrane protein profiles from *H. pylori* wild-type strain SS1 and *tlyA* mutant strain RS7.

Results 2D electrophoresis of whole cell protein extracts from RS7 and SS1 showed some differences in protein expression. Proteins of interest were selected and analysed by MALDI-ToF. Most notably, both flagellin B and catalase were absent from protein profiles of RS7 compared to SS1. This correlates with attenuation of RS7 shown previously.

TlyA may not play a direct role in adherence, but act indirectly via regulation of virulence factors including those involved in motility and survival mechanisms in the host.

MI 09 Investigating the role of Ca^{2+} as a second messenger in *Candida glabrata*

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In recent years *Candida glabrata* has emerged as a serious concern in the context of a general increase in invasive candidosis. While it forms part of the normal micro flora of healthy individuals, the increased use of immunosuppressive agents has led to high rates of disease complication and mortality. In conjunction with this, its intrinsic resistance to azole antimycotic therapy and its rapid rise in incidence has warranted a better understanding of the biology of this fungus. Importantly, phylogenetic analysis has shown that *C. glabrata* is distinct from the main pathogens in the *Candida* genus.

Calcium is an important second messenger which has been seen to play a role in resistance to azoles by *C. glabrata*. Further elucidation of the role of calcium as a mediator of environmental stress responses is underway using strains mutated in components of the calcium regulated signal transduction pathway. In order to characterize the physiological response to environmental stress, *C. glabrata* and newly constructed mutants are being compared to other pathogenic and non-pathogenic yeasts. An investigation into the survival of *C. glabrata* in the macrophage will also provide further knowledge of the biology of infection.

MI 10 Study of awareness of methicillin resistant *Staphylococcus aureus* among non-consultant hospital doctors

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Introduction Methicillin resistant Staphylococcus aureus (MRSA) is increasingly seen in Irish health units. Its impact is significant. Mortality of patients with MRSA bacteremia is twice that of methicillin sensitive *S. aureus*.

Doctors play a major role in the control and prevention of MRSA. To step up to this part, they will need good awareness of MRSA. We undertake a survey to study awareness of MRSA among non-consultant hospital doctors (NCHD).

Method The survey was conducted in a university hospital. NCHD from various specialties and of different grades responded to a questionnaire on their perception of MRSA and various methods of control.

Results 46 doctors responded – 19 interns, 18 senior house officers and 9 registrars.

42 are aware of a hospital protocol for managing MRSA and 40 felt they can manage MRSA competently.

All 46 identified hand washing as crucial in MRSA control. 87% (40)

are aware that appropriate isolation is just as important. 83% (38) recognized there is no strong indication to screen all patients and staff routinely. 36 (78%) are correct about MRSA eradication agents.

However, merely 13 (28%) are aware that adequate spacing between beds is important. Less than 50% list prudent antibiotic use and antibiotic stewardship programme as a vital step in MRSA control.

Also only 6 (13%) are correct in identifying that nearly 50% of *S. aureus* isolated are MRSA.

Majority (60%) feel that the hospital does not provide adequate education and promotion on the issue.

Conclusion The findings suggest a reasonably good level of awareness among NCHDs of MRSA prevention. However the survey also highlights that lack of knowledge in certain areas of MRSA management and the need for better support from hospital to help doctors keep up to date with MRSA control so as to improve patient care, reduce mortality and contain healthcare costs.

MI 11 Genomic analysis of protein secretion in *Listeria*: new insights in the origin of bacterial virulence and biofilm formation

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Listeria monocytogenes, the etiologic agent of listeriosis, remains a serious public health concern when considering its frequent occurrence in food coupled with a high mortality rate. Capacity of a bacterium to secrete proteins to or beyond the bacterial cell surface is of crucial importance in the understanding of biofilm formation and bacterial pathogenesis to further develop defensive strategies. However, consistent information about protein secretion in Monodermata is essentially restricted to the model organism *Bacillus subtilis*. By using a variety of bioinformatics tools, both secreted proteins and protein secretory systems present in seqenced *Listeria* species could be identified and characterized based on their sequence and structural features. From this genomic analysis, protein secretion appears to rely essentially on the Sec (Secretion) pathway (TC #3.A.5). Contribution of Tat (Twin arginine translocation;TC #2.A.64) to protein secretion seems limited and this pathway appears encoded in all but one sequenced *Listeria*. In addition, a functional flagella export apparatus (TC #3.A.6.), a fimbrilin-protein exporter (TC #3.A.14), some holins (TC #1.E.) and a WXG100 (proteins with WXG motif of ~100 residues) secretion system are encoded in all listerial genomes. This investigation provides new insights into the physiology and virulence of *Listeria* species.

MI 12 Occurrence and expression of the lateral flagella (Flag-2) locus in enteroaggregative *Escherichia coli*

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A minority of *E. coli* and *Shigella* strains have been shown to possess a 44 gene cluster theoretically carrying the genes encoding a novel lateral flagella system (Flag-2). In other pathogenic and non-pathogenic strains of these species, two promotorless genes, *fhiA* and *mbhA* represent a 'scar' left by the ancestral deletion of this gene cluster. No strain has so far been shown to produce functional lateral flagella which would impart swarming motility: bioinformatics analysis of enteroaggregative *E. coli* 042 in which the Flag-2 locus was first identified has revealed a frame shift mutation in one gene of the cluster which is predicted to prevent the production of the lateral flagella.

In this study a reference collection of 24 strains of Enteroaggregative *E. coli* along with 16 strains of *E. coli* isolated from travellers with diarrhoeal illness were analysed for the presence of inserted sequences at the *fhiA-mbhA* locus by PCR. We found that 11 of these strains failed to give an amplification product with primers designed to prove the contiguousness of these genes. Additionally, for these 11 strains in all but two cases amplification of one gene predicted to be present in the Flag-2 locus was achieved. Motility assays were developed using low concentration agar plates containing variable amounts of Tween-20. In this system one strain predicted to carry the Flag-2 locus demonstrated swarming motility, indicating it was capable of expressing functional lateral flagella. Transmission electron microscopy of this strain revealed the presence of long filamentous structures arising from the medial region of the cell. We conclude that this is the first evidence for the production of functional lateral flagella by *E. coli*, but confirm that this is a rare event even in those strains which harbour the Flag-2 gene cluster.

MI 13 Two component regulators of *Francisella tularensis*

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The Gram negative coccobacillus *Francisella tularensis*, the etiological agent of tularaemia, can cause serious disease in humans. The low infectious dose of *F. tularensis* has led to concerns about the potential use of this organism as a bioweapon. A live vaccine strain, *F. tularensis* subspecies *holarctica* strain LVS, was developed but remains unlicensed due to a range of problems with the strain. Thus a licensable vaccine is required. The aim of the project is to produce a characterized defined two-component regulatory systems (TCS) mutant of *F. tularensis* that is attenuated and protective, and that could form the basis of a new live attenuated tularemia vaccine.

TCS are widespread signal transduction devices in prokaryotes. TCS have been well studied in many pathogenic bacteria, and they are known to regulate expression of many key virulence factors. Inactivation of these genes is likely to result in gross effects, including attenuation. There are currently three TCS annotated in the *F. tularensis* SchuS4 genome sequence determined by simple BLAST analysis. Therefore a more in-depth analysis of the genome sequence will be performed to ensure that these are the only TCS possessed by the pathogen. Isogenic mutants in the annotated TCS and in any we subsequently identify by this bioinformatic analysis will be produced and assessed.

 This contract has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Humans Services under Contract No. NO1-AI-50041.

MI 14 Cloning and expressing immunoreactive proteins of *Burkholderia pseudomallei*

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Burkholderia pseudomallei, the causative agent of the disease melioidosis, is a micro-organism endemic in South-East Asia and Northern Australia, where it causes fatal pneumonia and septicaemia. These infections are difficult to treat as the bacterium is resistant to several antibiotics and there is currently no vaccine available. Protein antigens present on the cell surface of bacteria are potential candidates for a sub-unit vaccine. These proteins are exposed to the immune system upon infection, and have been used successfully as vaccine candidates against a variety of micro-organisms. Using a proteomics based approach we have identified a panel of proteins

that were found to be immunoreactive with human convalescent sera. Here, we present work on the cloning and expression of these proteins. It is possible that one of these immunoreactive proteins could form a component of a new subunit vaccine against *B. pseudomallei.*

MI 15 Sequence variation in the *pap* epigenetic switch of uropathogenic *Escherichia coli* and impact on P fimbrial expression

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Pyelonephritis-associated pili (Pap) facilitate binding of uropathogenic *E. coli* to digalactoside-containing glycolipids and are associated with acute kidney infection. Pap expression is phase variable. The mechanism involves the leucine-responsive regulatory protein (Lrp), the *pap*-encoded regulator PapI, and DNA adenine methylase (Dam) forming complexes and distinct methylation patterns on *pap* regulatory DNA (the *pap* switch). In this study we sequenced 76 *pap* switches and their regulators (*papI* and *papB*) from 54 UPEC isolates of different clinical origin. Sequence conservation was observed in sites important for the reversible operation of the switch. PapI contained unique point mutations resulting in distinct allelic variants. Some variants correlated with symptomatic disease and differed in their ability to regulate *pap* expression. Sequence variation within the *pap* switch was mainly present in a high affinity binding site for PapB. The site contained a variable number of $(T/A)_3$ repeats occurring every 9 bp. Under particular environmental conditions and depending on the genetic background, the number of repeats altered the frequency of off to on phase transition. From this we suggest that this variation could affect the pathogenic potential of UPEC isolates.

MI 16 Comparison analysis of antimicrobial effect of *Eugenia caryophyllata* alcoholic extract at different concentrations on growth model of *Escherichia coli* 0157 using spectrophotometery

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Escherichia coli serotype O157 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. There is no evidence that antibiotics improve the course of disease. Therefore plant medicine could be promising for treatment of this fatal infection. For this purpose, alcoholic extract of *Eugenia caryophyllata* at concentrations of 0.2, 0.3 and 0.4 mg/ml was prepared using standard methods and added to NB culture medium inoculated with *E. coli 0157*. Then bacterial growth was monitored during 12 triplicate sampling using UV/visible Spectrophotmeter. Data was plotted and compared using Excel software program. No significant difference observed between 0.1, 0.2 and 0.3 mg/ml concentrations. Results also indicated that advanced statistical program such as SPSS has to be used to find out exact growth differences at various phases of bacterial growth. Usage of wide variety of concentrations could also be use to see more effective growth inhibition.

MI 17 Efficacy of immunization with purified outer membrane proteins to induce pulmonary clearance of nontypeable *Haemophilus influenzae* in a rat respiratory model

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Three strains of nontypeable *Haemophilus influenzae* namely NTHi-I, NTHi-II and NTHi-III were isolated from the sputum of patients with bronchitis and identified by biochemical, serological and electron microscopy. The polypeptide patterns of isolates were compared and found to have similar sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) polypeptide patterns, although some of the bands were specific in some strains. A similar comparison was made on extracted outer membrane proteins (OMPs) on the above mentioned strains, using Triton X-100 and sodium dodecyl sulfate (SDS). It was found that the polypeptides with molecular weights of 70, 42, 33 and 27 KDa were identified as P1, P2, P4 and P5 respectivly. The protein estimation of crude OMPs from the three strains were calculated, and OPM-I prepared from NTHi–I showed the highest amount of protein and was chosen for its immunogenicity in a rat respiratory model.

The efficacy of immunization with OMP was determined by enhancement of pulmonary clearance of live bacteria in the rat lung. A significant protective immune response induced by OMP was observed by enhanced respiratory clearance of nontypeable *H. influenzae* following mucosal immunization.

MI 18 Fast seeking to acquire reliable single monospecific antibody from special whole bacterial cells

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Monovalent and monospecific antisera are valuable tools for serodiagnostic infectious agents in health and epidemiological studies. The production procedures of previous monospecific antisera have some disadvantages. Heat killed micro-organisms from all of 13 identified Enteropathogenic *E. coli* members were injected to marginal ear vein of rabbits in an optimized schedule. Immune sera were partially purified by very fast, cheep and reliable precipitation method and specific predominant IgG showed by electrophoresis. By this way, 13 distinct solutions with the following specifications were obtained:

1.Without general agglutinating agents and shared antigenic factors.

2 With minimum requirement of heterologous antigens density (2×109 cells/ml) in absorption steps.

3. With low concentration of common sera proteins that could interact with bacterial LPS. So by this method, buoyant density of LPS reaches up to 1.4 g/cm^3. As a result, LPS is presented for combination with antibody molecules as well.

4. Colorless even in high concentration in use. The resultant monospecific antisera are specific and economic which could guarantee the objectives of relative serodiagnostic and epidemiologic studies, and also are applied as high quality for immunochemistry and immunoelectrophoresis studies.

Posters

Physiology, Biochemistry & Molecular Genetics / Education & Training Groups

PBMG 01 PigZ differentially regulates prodigiosin and carbapenem biosynthesis in *Serratia* sp. ATCC 39006

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Serratia sp. ATCC 39006 (39006) is a Gram-negative enterobacterium which produces a carbapenem antibiotic, pectinases, cellulases and an intracellular red pigment, prodigiosin. Prodigiosins have immunosuppressive and anti-cancer properties although the physiological role in 39006 is not yet known. Regulation of exoenzyme and secondary metabolite production involves numerous factors that form a complex hierarchal regulatory network. We have identified a hyper-pigmented mutant containing a transposon insertion in *pigZ*, encoding a TetR family transcriptional regulator. The *pigZ* mutant also shows reduced levels of carbapenem, cellulase and pectinase. We have shown that *pigZ* regulates both the prodigiosin and carbapenem biosynthetic operons at the level of transcription but does not affect transcription of known secondary metabolite regulators. It is predicted that *pigZ* exerts its effects, at least in part, via an influx/efflux pump. To date, *pigZ* is the only regulator identified that differentially regulates prodigiosin and carbapenem production. This differential regulation may imply a different 'fitness role' for the two secondary metabolites. Therefore, determining the physiological signals to which *pigZ* is responding may help to shed light on the biological role of prodigiosin.

PBMG 02 PigP a pleiotropic master regulator in *Serratia*: a proteomic analysis

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Gram-negative bacteria of the genus *Serratia* are opportunistic human, plant and insect pathogens and are members of the Enterobacteriaceae. *Serratia* sp. ATCC 39006 secretes pectinases, cellulases and produces the secondary metabolites, carbapenem and the red pigment antibiotic, prodigiosin. Prodigiosins are of interest because they show immunosuppressive and anticancer activities. Regulation of secondary metabolites in *Serratia* is complex and involves numerous environmental cues such as carbon source, inorganic phosphate, copper concentration and via a cell density dependent lactone (butanoyl homoserine lactone, BHL) based quorum sensing system. Recently we identified PigP, a pleiotropic master regulator of 204 amino acids with an N-terminal XRE-Like helix-turn-helix DNA binding domain. A *pigP* mutant is affected in the production of prodigiosin, carbapenem, exoenzymes and in motility.

In this study we used 2D-DiGE to investigate the PigP regulon. We also demonstrate that *pigP* is widely distributed in both clinical and environmental strains of *Serratia marcescens* (*Sma*) including the recently sequenced strain *Sma* Db11. Finally we have inactivated the *pigP* homologue in *Sma* Db10 and showed that the resulting mutant had reduced haemolytic activity and increased swarming motility.

PBMG 03 The ser-thr-tyr phosphoproteome of *Streptomyces coelicolor*

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Phosphorylation by serine (Ser) and threonine (Thr) kinases (STPKs) is an essential mechanism for cellular regulation in eukaryotes. More recently its significance has been discovered in a number of complex bacteria, including the identification of 34 STPKs in *Streptomyces coelicolor* A3(2). Thus far only AfsK and RamC have had their functions elucidated, with studies showing their involvement in secondary metabolism and morphological differentiation, respectively. This project focuses on the phosphoproteome of *S. coelicolor,* and comparison of the wild-type strain with STPK knockout mutants. Ultimately this may allow the identification of the target proteins of individual STPKs. To date, 14 mutants have been generated and their phenotypes examined on complex and minimal solid media. Initial results show that a number of these mutants behave differently to the wild-type strain, producing pigmented antibiotics at slightly earlier or later stages of development. Bioinformatic analysis to identify proteins with fork-head associated (FHA) domains, which recognize phosphothreonines, has led to the recognition of a number of possible substrates for the 34 STPKs that will be subject to confirmation by proteome analysis.

PBMG 04 Ammonium chemotaxis in bacteria

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Chemotaxis enables motile bacteria to respond to changes in the chemical composition of their surroundings by biasing their swimming pattern towards a preferred microenvironment. Chemotaxis towards ammonium has been previously reported in *E. coli*, *Rhodobacter sphaeroides* and *Synechococcus* spp. However understanding of the molecular mechanism of this process remains incomplete and the chemoreceptors responsible for ammonium detection have not been explicitly identified. In this study we have used the model organism *E. coli* to carry out a detailed investigation of the signalling pathway involved in the chemotactic response to ammonium. Responses have been quantified using a capillary assay. Using isogenic strains with defined deletions of each of the genes for the individual chemotaxis proteins we have demonstrated that the response to ammonium is dependent on CheW, CheA and CheY. This suggests that a classical transmembrane chemoreceptor senses ammonium. Of the five known *E. coli* receptors two, Tsr and Tap, were found to influence ammonium chemotaxis. Studies using chimeric genes are in progress to determine which parts of these proteins act as ammonium sensors.

PBMG 05 Reconstruction of genome scale metabolic network in *Streptomyces coelicolor*

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The value of building mathematical and metabolic models for both prokaryotes and eukaryotes and simulating their integrated behaviour has long been recognized. The application of data-rich genome sequencing and annotation combined with the well-established physiology and biochemistry information enables the construction of several genome scale metabolic network (GSMN) including the one for *S. coelicolor* A(3)2 (Boridina et al, 2005). The former GSMN of

S. coelicolor is very comprehensive. But it is not visually acceptable. Based on this model and the databases such as KEGG, ScoDB, ScoCyc, a modified network for *S. coelicolor* has been created in SimPheny in our study. SimPheny is a premiere software platform enabling the reconstruction of genome scale network, *in silico* metabolic modelling, high throughput data integration and visulization. SimPheny has the abilities to show the results in chart, table or on a map. Map view (colour indicated) of the simulation result clearly shows the flux distribution through each reaction. Transcriptional analysis and fermentation data are also imported as the constraints for the model simulation to optimize the network. The GSMN of *S. coelicolor* allows the prediction of the phenotype of the whole cell and the opportunities to mimic the real growth/metabolic scenarios.

PBMG 06 Regulation of *Streptomyces* development: reach for the sky!

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Streptomyces coelicolor is characterized by a complex life cycle and serves as a model system for bacterial development. After a feeding substrate mycelium has been formed, this filamentous bacterium differentiates by forming aerial hyphae that septate into spores. The *bld* cascade regulates initiation of aerial growth, whereas the *whi* genes control spore formation. Recent findings indicate the existence of another regulatory pathway that operates after aerial hyphae have started to grow into the air and which we call the sky pathway. This pathway controls the expression of the chaplin and rodlin genes. These genes encode proteins that assemble into a rodlet layer, consisting of pairwise aligned amyloid-like fibrils, that provides surface hydrophobicity to aerial hyphae and spores.

PBMG 07 Environmental electrolyte system changing on whole bio-specimen cells and applied microbial urease function

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Rapid urease test (RUT) is the initial choice due to fast, cheap and high validity for *H. pylori* detection in biopsy specimens. Most of the kinetic works have been carried out by using of phosphate buffer, which contain sodium and potassium ions, acts as enzyme inhibitors. An electrolyte system which has no inhibitory effects (Tris/HCl) in mentioned micro-environment was used. In this new condition, the enzyme should be obeyed the Michaelis-Menton law. Reliability of microbial urease in whole bio-specimen Cells was compared by using phosphate and Tris/HCl electrolyte systems. Usual pathologic biopsy examination used as a gold standard diagnostic method. On 91 specimens from suspicious patients with H.pylori infection, sensitivity, specificity and accuracy of both electrolyte systems were compared. After statistical evaluation of RUT with phosphate and Tris/HCl buffer systems, sensitivity of tests are 78.8%, 78.8%, with specificity of 84.6%, 89.7% and accuracy of 81.3% , 83.5% respectively. Enzyme kinetic behaviors showed (Km=1.1 mM, Vmax=13) and (Km =10.0 mM, Vmax= 880) respectively. So using of Tris/HCl electrolyte system make to proven.

PBMG/ET 01 Fluorescence imaging of colicin E9 binding by *Escherichia coli*

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Nuclease type E colicins target susceptible *E. coli* cells through binding of the vitamin B_{12} outer membrane receptor BtuB. Colicin E9 (colE9) binds BtuB in the form of a complex with its immunity protein: Im9. It is suggested that receptor binding triggers loss of the immunity protein from the complex prior to translocation of the colicin into the cytoplasm. Our aim was therefore to design a FRET construct which would enable us to follow the removal of Im9 from the colE9/Im9 complex upon BtuB binding.

Single cysteine mutants of colE9 and Im9 were labelled with Alexa Fluor 488 (FRET donor) and Alexa Fluor 546 (FRET acceptor) respectively and the biological activity of the labelled complex was characterized. FRET efficiency upon colE9/Im9 complex formation was ~ 30%. Loss of Im9 from the labelled complex in the presence of 3M GnHCl abolished FRET. Receptor binding *in vivo* of the labelled constructs was visualized using BtuB-overexpressing *E.coli* in combination with *wi*defield timelapse microscopy.

PBMG/ET 02 Observing RepD-PcrA-mediated plasmid unwinding by AFM and stopped-flow fluorescence

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Atomic force microscopy (AFM) and stopped-flow fluorescence were used to observe the RepD-PcrA-mediated plasmid unwinding. pCERoriD plasmid DNA has been unwound in the presence of RepD, PcrA and ATP. Without SSB the unwound plasmid forms multi-loop containing structures due to the (partial) re-annealing of the separated strands and the interaction between RepD and PcrA. In the presence of SSB, the plasmid is unwound and separated into two identical ssDNA protected by SSB. Comparative studies on linearized pCERoriD by different restriction enzymes show that PcrA translocates in 3′→5′ direction along the (–) strand of the dsDNA.

In situ AFM imaging indicates that the molecular motor, PcrA-RepD complex, is stationary during the unwinding process. Stopped-flow fluorescence results further support our conclusions from AFM and provide some useful kinetic information about the RepD-PcrA-mediated plasmid unwinding.

PBMG/ET 03 Super-STEM microscopy incorporating electron energy loss spectroscopy suggests the presence of cobalt in bacterial enterosomes

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In a scanning transmission electron microscope (STEM), images are formed by raster-scanning an electron beam across the sample and collecting transmitted or scattered electrons. Electron energy loss spectroscopy (EELS), can now be recorded simultaneously downstream of the fine electron probe scanned over the sample revealing aspects of chemical composition, electronic structure and coordination in the sample. The Super-STEM instrument also

incorporates magnetic feedback correction to the electron beam, removing spherical aberration, bringing resolution to picoscale. This has provided unprecedented insight into complex metal-containing structures such as semiconductors. We report preliminary observations with a Super-STEM microscope on a biological structure containing a metal cofactor. Enterosomes in heterotrophic bacteria are 100–150 nm protein assemblies containing cobalamin-dependant enzymes of various specificities. We imaged recombinant enterosomes produced by expressing the *pdu* operon from *Citrobacter freundii* in *E. coli*. Because of the known cobalt content of the cobalamin cofactor essential for enterosome function, low and high energy electron loss signatures for cobalt were sought within the enterosome. Both were found within specific areas of the enterosomes and not within adjacent areas of similar size on the grid.

Conclusion Super-STEM microscopy with EELS can provide structural insights into metal-containing biological structures.

Posters

Plenary

PS 01 Influence of the global regulator FIS on high copy number plasmid stability

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In the absence of active partition systems multicopy plasmids are thought to rely on random distribution to daughter cells at division, and as long as the copy number remains high, plasmids are lost at low frequencies. The formation of multimers by homologous recombination acts to decrease the total number of independent plasmid molecules thereby increasing the probability that one or the other of the daughter cells will fail to receive a plasmid. Multimeric plasmids can be resolved to monomers by site-specific recombination at the *cer* site. At least four host-encoded proteins are essential for *cer*-mediated dimer resolution *in vivo* and *in vitro*: ArgR, PepA, XerC and XerD.

Although necessary for stable plasmid inheritance *cer*-mediated multimer resolution alone is not sufficient to ensure plasmid stability. Rcd, a small regulatory RNA transcribed from P_{cer} within the *cer* site of ColE1 and related plasmids, also appears to have a role in plasmid maintenance. It has been proposed that Rcd is synthesized in response to plasmid multimerization and is part of a checkpoint which delays host division until after multimer resolution by Xer-*cer* recombination is complete.

In addition to the four proteins which are essential for ColE1 dimer resolution, FIS (Factor for Inversion Stimulation) has also been implicated in the process. Sequence analysis of *cer* and related multimer resolution sites has revealed the presence of two putative FIS binding sites. Purified FIS protein has been shown by EMSA to bind specifically to these sites and stability assays have demonstrated that mutation of *fis* leads to increased plasmid loss. We have identified a role for FIS on the *cer* site in the regulation of P_{cer}. Northern analysis has revealed Rcd is transcribed constitutively at a low level from plasmid monomers in a *fis*$^-$ host.

To determine whether there is a direct effect between FIS binding to *cer in vitro* and plasmid stability *in vivo* a mutational analysis of FIS binding sites was conducted. This has demonstrated that reduced binding of FIS to its cognate sites *also* reduced plasmid stability. These results suggest FIS is directly involved in the Xer-*cer* nucleoprotein complex and that its main role is in the regulation of transcription rather than in site-specific recombination.

PS 02 Transcription enhanced homologous recombination in mammalian cells

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Transcription can enhance recombination; this is a ubiquitous phenomenon from prokaryotes to higher eukaryotes. However, the mechanism of transcription enhanced recombination (TER) is not understood. Here, we have developed a construct containing a recombination substrate with which recombination levels can be studied in the presence or absence of transcription. It consists of a duplicated neomycin repeat, one copy of which is controlled by an inducible bi-directional promoter using the Tet-off system, which also controls the expression of luciferase gene on the other side, thus enabling the quantitative measurement of transcription. This construct was stably integrated into the SPD8 Chinese hamster cell line, which contains a separate system to assay for homologous recombination (HR) in the hprt gene. We found that there was a 30 fold increase in recombination when transcription was turned on as compared to when transcription was turned off. However, HR at the unrelated hprt gene was unaffected. We further found that transcription preferentially enhances short tract gene conversion. We also found that, this phenomenon, transcription enhanced recombination, is s-phase associated and depends on replication. In conclusion, we have developed a system in which to study TER and confirmed that it is a conserved mechanism, which exists in mammalian cells. Moreover, TER primarily involves short tract gene conversion. It is s-phase associated and is dependent on replication.

PS 03 Further candidates for PARP inhibitor therapy?

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Background PARP and ATM are involved in responding to DNA damage. We believe PARP inhibition causes an increase in unresolved spontaneous DNA single-strand breaks (SSBs), which collapse replication forks and trigger homologous recombination repair (HRR). While single knockouts of either PARP-1 or ATM are normal, double knockouts are embryonic lethal. Here we investigate the relationship between ATM and PARP with respect to HR.

Results PARP-1$^{-/-}$cells are sensitive to the ATM inhibitor KU55933 and conversely AT cells are sensitive to PARP inhibition. ATM is activated following PARP inhibition. PARP inhibitor-induced HRR is abolished in ATM inhibited cells.

Conclusion ATM is activated by PARP inhibitor induced collapsed replication forks and may function upstream of HRR in the repair of certain type of double-strand breaks (DSBs). We have previously reported that PARP inhibitors alone are efficient to treat tumours which are deficient in HR, such as BRCA2 defective cancers. This data reveals a relationship between PARP-1 and ATM which suggests that PARP inhibitors may also be useful in the treatment of other types of cancer. Mutations in the ATM gene have been found in T-cell prolymphocytic leukaemia, mantle cell lymphoma, and B-cell chronic lymphocytic leukaemia. Such cases would be ideal candidates for PARP inhibitor therapy, as the cells containing the ATM mutation would be expected to be more sensitive to PARP inhibitors than the surrounding ATM proficient tissue and thus the side effects seen with classical cytotoxic anti-cancer drugs minimal.

PS 04 Defects in the rescue of stalled replication fork by Mre11 complex in colon cancer cells

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Mre11/Rad50/NBS1 (MRN) complex is mutated in an inherited genomic instability syndrome featuring cancer predisposition, mental retardation and immunodeficiency. It functions in many aspects of DNA synthesis and repair, such as cell-cycle checkpoints, DNA repair,

recombination and telomere maintenance. Studies have shown that the MRN complex is essential for ATM activation (Uziel *et al.* 2003; Lee and Paull 2005), a central controller of cellular responses of DNA damage. Previously we have shown that a splicing mutant Mre11 found in colorectal cancer cell line (HCT116) suppresses the function of wild-type Mre11 in the rescue of stalled replication forks induced by DNA replication inhibitor (thymidine) (Scorah *et al.* in preparation). Cells expressing the mutant Mre11 become sensitive to the replication inhibitor, while the function of wild-type Mre11 is compromised. ATM activation is also absent in those cells. Here we have uncovered the mechanism by which function of wild-type Mre11 is impaired in these cells in the presence of the mutant protein. As suggested by recent studies (Giannini *et al.* 2002, 2004; Ottini *et al.* 2004), this impairment may contribute to colorectal and gastric cancer formation in mismatch deficient-tumour. Our studies have shown that although the 3rd and 4th phosphodiesterase motifs of Mre11 were deleted, the mutant Mre11 binds with increased affinity to ssDNA, dsDNA with ssDNA tail, which are likely to be substrates generated by replication inhibitor (thymidine). The nuclease activity characteristic of the protein is completely lost. Thymidine slows down replication fork progression and generates a specific substrate, which requires function of Mre11 to rescue. The mutant Mre11 is likely to block wild-type Mre11 and maybe other repair proteins to access the damaged replication forks generated by thymidine, thus blocking repair process. Given that the splicing various mutation of Mre11 occurs in 83~93% of MSI+ tumours (Giannini *et al.* 2002, 2004), this study has important implications for treatment strategies directly against this subset of tumours and also sheds light on understanding the role of MRE11 in this important damage response pathway.

Acknowledgement: This work is supported by a programme grant from Yorkshire Cancer Research

PS 05 Analysis of DNA double strand break repair, during meiosis, at the resolution of the single nucleotide

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In order to prevent the production of aneuploid gametes from non-disjunction of homologous chromosomes during meiosis, crossovers are used to create physical interactions between the maternal and paternal chromosomes. Crossovers are formed by homologous recombination, which is used to repair programmed DNA double strand-breaks (DSBs) generated by Spo11p. DSBs are processed to generate 3′ ended ssDNA tails that invade a homologous duplex, which through further processing and resolution of Holliday junctions yields crossovers.

In order to learn more about the fine detail processing of DSBs before repair, we are setting up a new protocol to analyse the ssDNA intermediates at single-nucleotide resolution. To this we are exploiting a site specific-DSB created by the meiosis specific homing endonuclease VDE. Using biotinylated probes and streptavidin coated magnetic beads, the resected strands on each side of the DSB can be isolated from each other and labelled specifically for visualization on a sequencing gel. The nucleotide resolution of the technique may distinguish for example, the roles of Mre11p and Exo1p in this pathway.

PS 06 The DNA repair helicase *Xeroderma pigmentosum* factor D

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Xeroderma pigmentosum factor D (XPD) is a 5′-3′ DNA helicase involved in both transcription and Nucleotide Excision Repair (NER). In eukarya XPD is part of the multi subunit complex TFIIH and its helicase activity is essential in NER for DNA opening at the site of bulky DNA lesions such as photoproducts. Mutations in the *xpd* gene lead to three distinct genetic disorders: *Xeroderma pigmentosum* (XP), Cockayne Syndrome (CS) and Trichothiodystrophy (TTD). Most of the disease causing mutations in the human *xpd* gene could be linked directly to either a disruption of the helicase/ ATPase activity or to attenuated or abolished interactions with TFIIH. An explanation for other mutations, for example R112H which abolished helicase activity and causes TTD, remains elusive, since they are not located in one of the seven conserved helicase motifs or in the domains that take part in the interaction with TFIIH. We purified the XPD homologue from the archaeon *Sulfolobus acidocaldarius* and revealed the presence of an unexpected N-terminal Iron-Sulfur (FeS) cluster that is conserved in the eukaryal XPD protein. Mutagenesis studies demonstrate the importance of the FeS cluster for the helicase activity of XPD. We have also shown that the loss of helicase activity in the human R112H mutant derives from disruption of the FeS cluster. The FeS domain of XPD is reminiscent of those found in the Base Excision Repair (BER) enzymes MutY/EndoIII. Published data for these enzymes suggests that the FeS cluster is redox active on binding DNA, and is involved in scanning the DNA for damaged sites by DNA charge transport chemistry. This possibility is currently being assessed for XPD.

PS 07 Investigating the role(s) of Sgs1-Top3 in the maintenance of genomic stability

Hocine W. Mankouri & Ian D. Hickson

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The RecQ family of DNA helicases have received considerable interest due to their roles in the maintenance of genomic stability and suppression of premature ageing. *S. cerevisiae* (budding yeast) possesses only one RecQ helicase, called *SGS1*. Interestingly, in both yeast and human cells, RecQ helicases physically interact with a type IA topoisomerase (Sgs1 with Top3 in yeast; BLM with hTOPIIIα in humans). Current theory suggests that, in both these organisms, these proteins act together to resolve repair intermediates formed during S-phase by the homologous recombination repair machinery.

To further investigate the cellular role(s) of type IA topoisomerases, we have investigated the effects of ablating Top3 function in *S. cerevisiae* using an inducible dominant negative allele of *TOP3* (*TOP3*^{Y356F}). We demonstrate that overexpression of *TOP3*^{Y356F} affects various aspects of DNA replication and homologous recombination repair following exposure to DNA damage. Our results provide novel insights into how Top3 functions to preserve genome integrity in replicating cells and suggest that hTOPIIIα could be an attractive anti-cancer therapeutic target in human cells.

PS 08 Investigating the role of Rmi1 in the maintenance of genome integrity

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In budding yeast, *SGS1* encodes for a RecQ family DNA helicase whose human homologues include *BLM*, the gene mutated in the cancer prone disorder, Bloom's Syndrome. Extensive studies of RecQ helicases have suggested a role in processing homologous recombination intermediates that arise during DNA replication. Rmi1 has been recently identified as a novel protein which forms a complex with Sgs1 and Top3 *in vivo*. The human homologue of Rmi1, BLAP75, also interacts with BLM, suggesting this association is conserved in evolution.

To further investigate the role(s) of Rmi1, we analysed the effects of deleting *RMI1* on DNA replication. Loss of *RMI1* function has severe consequences for cell survival in an unperturbed cell cycle and following DNA damage. We have also characterized suppressors of the *rmi1*Δ phenotype. Our results provide novel insights into the roles of the Rmi1-Sgs1-Top3 complex in the maintenance of genome integrity.

PS 09 Genetic interactions of the PSO2 and MGM101 DNA repair factors in *Saccharomyces cerevisiae*

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Our laboratory previously reported that the DNA factor Pso2 and the yeast MutS mismatch repair factors functionally overlap during the repair of interstrand cross-links (ICLs). Furthermore, we have shown that this overlap is epistatic with homologous recombination in asynchronous cells. Here, we further characterize this overlap and suggest that Mgm101, a mitochondrial-DNA repair and maintenance factor, may also influence the repair of nuclear DNA. We show that *pso2 msh2* and *pso2 mgm101* disruptants are epistatically sensitive to the cross-linking agents nitrogen mustard and cisplatin. Immunoprecipitation experiments suggest that Mgm101 interacts with members of the mismatch repair and homologous recombination pathways. This would suggest that there is an overlapping role between Pso2 and this putative complex. Moreover, we show that disruption of *mgm101* reduces survival of cells challenged with hydrogen peroxide, but not ionizing radiation, suggesting a role in global oxidative damage repair, but not double-strand break repair.

PS 10 Replication fork blockage by transcription factor-DNA complexes in *Escherichia coli*

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All organisms require mechanisms that resuscitate replication forks when they break down, reflecting the complex intracellular environments within which DNA replication occurs. Here we show that *lac* repressor-operator complexes blocked *E. coli* replication forks both *in vitro* and *in vivo*. Blockage *in vitro* occurred on both supercoiled and relaxed template DNA, demonstrating that alteration of DNA topology did not play a major role in the ability of repressor-operator complexes to halt replication forks. Thus replisomes have a limited ability to translocate through high affinity protein-DNA complexes. The effects of blockage were abrogated by RecA, RecBCD and RecG *in vivo*, suggesting that processing of blocked forks facilitates replication through protein-DNA complexes. We conclude there is a trade-off between efficient genome duplication and other aspects of protein-DNA metabolism such as transcriptional control and that processing of blocked forks by recombination enzymes are required for tolerance of such events.

PS 11 Precise genome-wide identification of replication origins in *Saccharomyces cerevisiae* by comparative genomics

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Replication of eukaryotic chromosomes initiates at multiple discrete sites called origins. The approximate location of origins has been determined genome-wide in several eukaryotes; however, no study has provided sufficient resolution to determine the elements bound by replication proteins at each origin. To determine the exact locations of *S. cerevisiae* origins, we examined the corresponding genomic regions in closely related *Saccharomyces* species. We find that origin elements are frequently evolutionarily conserved, and this conservation allowed us to identify origin sequences throughout the *S. cerevisiae* genome at base pair resolution. Origin activity was confirmed for 228 predicted sites—representing 86% of apparent origin regions.

Analysis of chromatin conformation reveals that origins elements exclude nucleosomes and that flanking nucleosomes have characteristic patterns of modification. Surprisingly, these patterns of chromatin modification do not appear to be related to replication time, calling into question a favoured current model for control of origin initiation time. Our investigation represents the first precise genome-wide identification of functional replication origin elements, and demonstrates that phylogenetic conservation can identify the sequences responsible for replicating a eukaryotic genome.

PS 12 DNA damage checkpoint signalling in *Caenorhabditis elegans*

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DNA damage checkpoint pathways are needed to detect DNA damage and transduce a signal that elicits cell cycle arrest, DNA repair, and/or programmed cell death. The nematode *C. elegans* has been recently shown to be a relevant genetic model for DNA damage response studies. Germ line cells respond to DSB by promoting cell cycle arrest and apoptosis which rely on a battery of genes conserved in high eukaryotes.

A Radiation sensitive screen has led to the isolation of a set of mutants defective in DSB-induced cell cycle arrest. Based on complementation testing none of them is allelic to a locus corresponding to an already described gene. Furthermore they fall into two allelic groups. Using quantitative trait mapping, both have been mapped to chromosome III, and been confirmed by three points factor to map to position -2,0 and -1,65 and will be cloned during the next months.

Using immuno-fluorescence as well as GFP tagging proteins, we show that the early events of DSB processing are not affected; however accumulation of CYB-1 (Cyclin B homolog) in G2/M arrested cells following DSB is abolished. Thus, we are describing new features of the DNA damage checkpoint pathway, potentially relevant for genomic instability understanding.

PS 13 Uncovering novel S-phase checkpoint factors by *Caenorhabditis elegans* genetics

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In response to a block in replication fork progression, artificially induced by HydroxyUrea (HU) treatment, the S-phase checkpoint is activated, resulting in replication forks stabilization, in the inhibition of late origin firing and in cell cycle arrest. We are employing an unbiased genetic approach to identify novel factors involved in S-phase checkpoint using *C. elegans* as genetic model system. We followed a forward genetic screening procedure to select for 1) mutants that die in otherwise non lethal concentrations of HU or 2) for mutants that can grow in otherwise lethal doses of HU. Up to now we isolated several docent mutants obtained from both screens and we focus on those mutants unable to arrest cell cycle progression

upon HU treatment. We will further characterize the panel of our mutants by a battery of cytological assays, indicative of cell cycle stages, DNA repair defect and replication defect to select the most interesting mutants for positional cloning of the corresponding genes. Finally we will embark on a genome wide RNAi screening to rapidly identify new genes whose inactivation confers defects in the S-phase checkpoint.

PS 14 Coordination of chromosome segregation with hyphal growth in *Streptomyces coelicolor*

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The filamentous soil prokaryote, *Streptomyces coelicolor*, undergoes an elaborate life cycle that is reflected in its morphological complexity. When grown on solid or liquid media, an *S. coelicolor* spore germinates to form substrate hyphae consisting of multinucleated, long branching filaments with infrequent septa. On solid media, aerial hyphae begin to protrude from the plain of vegetative growth before undergoing synchronized septation to form compartments that mature to create new spores for dispersal. Fundamental to understanding the depth of morphological complexity is the relationship between DNA replication and peptidoglycan synthesis. We have used fluorescence *in situ* hybridization and time lapse microscopy to investigate the coordination of chromosome organization with hyphal growth and sporulation. This technology is now being adapted to characterize the sequence of events during growth and cell division with the aim of reconciling chromosome segregation with apical hyphal growth as a primary goal.

PS 15 Histone acetylation and the control of replication timing

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DNA replication of eukaryotic chromosomes starts at multiple loci. These origins of replication are activated at distinct times during S-phase. Previous studies have shown that the acetylation state of the surrounding chromatin is part of the mechanism that controls replication timing (Vogelauer, Rubbi *et al.* 2002; Aparicio *et al.*, 2004). Histones carry a myriad of posttranslational modifications, which together regulate the binding of proteins to chromatin (reviewed in Peterson and Laniel, 2004). Our aim is to understand first the exact nature of the acetylation signal in order to determine next how this is read by the replication machinery.

We are taking advantage of the well characterized replication origins of the yeast *Saccharomyces cerevisiae*. Artificial recruitment of different histone acetyltransferases close to a late origin allows us to acetylate only a subset of lysine residues and investigate their importance in determining the replication timing. Preliminary data will be discussed.

PS 16 Investigating the pathways of DNA double-strand break repair in *Escherichia coli*

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Double-strand DNA breaks are lethal unless repaired and the mechanisms that afford repair of these lesions have been conserved through evolution. *E. coli* provides an ideal model system in which to study these mechanisms and we have employed a novel method by which to induce the formation of dsDNA breaks in order to study their repair by recombination. The mutagen 2-aminopurine (2-AP) is used to create unmethylated binding sites for EcoKI, an endogenous endonuclease of *E. coli* that cleaves DNA to produce dsDNA breaks. By assaying the viability of recombination repair mutants cultured in the presence of 2-AP over time, relationships between pathways mediated by various recombination proteins at dsDNA break sites have been elucidated.

Using this assay, the propensity for the RecG and the RuvABC Holliday junction resolution pathways to form crossover products following DSB repair has been investigated. This was achieved by combining *recG* and *ruvAC* mutations with mutations in the XerCD/*dif* site-specific recombination pathway responsible for separating chromosome dimers produced by the formation of cross-over products in DSB repair.

PS 17 The processing and repair of palindrome-induced DNA double-strand breaks in *Escherichia coli*

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Long DNA palindromes are sites of genome instability (deletions and translocations) in eukaryotic cells. In both prokaryotic and eukaryotic cells they are sites of DNA breakage. Previously, we had obtained genetic evidence that cleavage of long palindromes in *E. coli* was mediated by the SbcCD (Rad50/Mre11) complex and that repair was mediated by homologous recombination. This work led to the proposal of a model where a misfolded palindrome was digested to generate a two-ended break implying that cleavage was a post-replicative event (rather than an event occurring at an arrested replication fork, which would give rise to only one end). This model proposed roles for RecA, RecBCD and RecF in the processing of the ends by homologous recombination.

We have set up an SbcCD-inducible system to test the predictions of this model. We show that *recA, recBCD, recG* and *ruvABC* genes are required for repair of the breaks, consistent with a double-strand break repair pathway. However, we show that *recF* is not implicated in this repair. In addition, we show a requirement for the SOS system for cell survival following palindrome cleavage. We have obtained *in vivo* physical evidence, from pulsed-field gel electrophoresis, concerning the nature of the break and are investigating its processing. We show that the break is indeed two-ended. This argues that a replication fork can traverse a palindrome leaving a structure that can be processed to generate these two ends. We will present the *in vivo* physical analysis of end-processing after cleavage with SbcCD of a misfolded palindrome and relate this to the genetic requirements for repair.

PS 18 CAG.CTG repeat instability in *Escherichia coli*: insight into the link between instability and repeat orientation

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CAG·CTG repeat instability has been associated with a number of human diseases such as Huntigton's disease, myotonic dystrophy and spinocerebellar ataxias. To understand the processes involved in instability, we have analysed the behaviour of repeat tracts both in wild type and mutants affected in pathways of DNA replication and repair. This is the first CAG·CTG repeat instability study carried out in the *E. coli* chromosome and we confirm previous observations made using plasmid systems that instability is length and orientation dependent. Further we show that SbcCD nuclease contributes to

instability in an orientation dependent manner causing more deletions when CAG is on the leading strand template. We suggest that a hairpin structure formed by CTG repeats on the lagging strand template can be an intermediate in the instability pathway(s). Instability is also increased in proofreading mutants with more profound effects on the CTG orientation. We propose that the CTG orientation is more stable because it is effectively proofread and is less accessible to attack by SbcCD.

PS 19 Repair of DNA double-strand breaks in the opportunistic pathogen *Bacteroides fragilis*

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Repair of DNA double-strand breaks (DSBs) in *Escherichia coli* has been accepted as the paradigm for recombinational repair in bacteria. Processing of DSBs is initiated by the helicase and exonuclease functions of the RecBCD complex. In many other bacteria, such as Gram positives and members of the α-proteobacteria, DSBs are re-sected by the AddAB (RexAB) complex that has analogous function to RecBCD but no significant homology to the *E. coli* proteins. Pre-synaptic processing of DSBs has not been studied within the broader context of bacterial diversity. The *Bacteroidetes/Chlorobi* are an evolutionary distinct group which diverged prior to the split of the Gram positives and the classical Gram negatives. *Bacteroides fragilis* is a Gram negative obligate anaerobe which is a resident bacterium of the human gastrointestinal tract but is also an opportunistic pathogen. If *B. fragilis* colonizes normally sterile sites within the body it can cause serious infections, such as peritonitis following bowel surgery. The drug of choice for treatment of anaerobic infections is metronidazole, which causes DSBs in the bacterial chromosome following reduction of the prodrug. We have identified distantly related homologues of the r*exA* and *rexB* genes in the *B. fragilis* genome, which implies that the AddAB/RexAB system may be more widespread in eubacteria. The biochemcial roles of the *B. fragilis* RexAB homologues in DSB repair will be discussed.

PS 20 Investigating a structural and functional model for the Helicase (DnaB) – Primase (DnaG) interaction in *Bacillus stearothermophilus*

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The interaction between the helicase (DnaB) and the primase (DnaG) is essential for bacterial DNA replication. It tethers DnaG at the replication fork, completing assembly of the primosome, modulates activities of both proteins, directs initiation of leading strand synthesis and repeatedly regulates lagging strand synthesis. Transient nature of DnaB-DnaG complex in *E. coli* and partial structural data of both proteins has hindered understanding the molecular details of this interaction. Recent, studies on the stable complex in the Gram-positive thermophilic bacterium.

B. stearothermophilus and more structural information on individual domains of two proteins have led to a putative model for this complex. Model predicts a structural homology between the N-terminal subdomain of P16 (DnaG) and the N-terminal domain of DnaB. Homology between domains suggests these modules should show some structural interchangeability and be functionally active. Here biochemical characterization of fusion proteins of DnaB and DnaG has been used to investigate the structural homology.

PS 21 The *Bacillus subtilis* DnaD exhibits DNA remodelling activity due to oligomerization and DNA-binding activities on separate domains

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DNA replication is the most fundamental of all biological processes. *Bacillus subtilis* DnaD is an essential protein that has been implicated in the initiation of DNA replication and more recently in global DNA remodelling. To fully understand replication it is important to establish the role played by DnaD on its initiation. Here we present DnaD remodelling activity and its impact on bacterial nucleoid architecture as well as the molecular details that underpin this activity. Our data shows that DnaD converts the supercoiled plasmid to an open circular form without nicking the DNA. We have also identified two domains with distinct activities; an N-terminal domain with oligomerization activity and a C-terminal domain with DNA-binding activity and a second DNA-induced oligomerization activity. Our results suggest not only has DnaD global DNA-remodelling activity, but also, this is the outcome of the sum of three separate oligomerization and DNA-binding activities residing on two distinct but linked domains.

PS 22 Mre11-Rad50 inhibits homologous recombination in the archaeon *Haloferax volcanii*

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Mre11 and Rad50 proteins form an evolutionarily conserved complex that binds to and processes DNA double-strand breaks (DSBs). However, depletion of this complex has varying effects on DNA repair, depending on the organism examined. In eukaryotes, mutations in *mre11/rad50* result in DNA damage sensitivity and recombination defects, while mutation of the *Escherichia coli* homologs has no such effect. Homologs of Mre11-Rad50 are present in archaea, but their function is currently unknown.

We generated *mre11/rad50* mutants of the archaeon *Haloferax volcanii*. In contrast to yeast or bacterial mutants, these strains exhibit significantly increased homologous recombination at DSBs. Furthermore, *H. volcanii mre11/rad50* mutants are more resistant to DNA damage than the wild-type. However, *H. volcanii* wild-type strains undergo accelerated cell division in response to UV irradiation, which is not seen in the *mre11/rad50* mutants.

Our results indicate that Mre11-Rad50 plays a central role in the *H. volcanii* DNA damage response, by inhibiting recombination at DSBs and promoting a program of rapid cell division. We suggest that the function of this DNA damage response is to reduce the genome copy number (which in *H. volcanii* is high), to a level where recombination can repair DSBs with less risk of generating deleterious chromosome concatemers.

PS 23 Hel308 helicases: roles in genome stability at replication forks through interaction with PCNA

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Hel308 helicase promotes genome stability in *Drosophila* and mouse.

This enzyme is conserved throughout metazoans and archaea but is absent from bacteria and yeasts. Very little is known about how metazoan Hel308 functions, but we have characterized the action of the archaeal orthologue (Hel308a), which targets lagging strands for dissociation from model replication forks. Hel308a can also dissociate strands from DNA backbone nicks, and its actions by are reminiscent of bacterial UvrD and yeast Srs2 helicases. Hel308a has low apparent processivity, but this is greatly enhanced by interaction with PCNA. A potential PCNA interaction motif has been detected, which is conserved in all archaeal and metazoan Hel308 helicases. We conclude that the biochemical and genetic characteristics of Hel308 indicate actions at replication forks, rather than at other downstream repair intermediates such as Holliday junctions. From this data and their evolutionary distribution, we hypothesize that in archaea and metazoans, Hel308 helicases may be analogous to UvrD and Srs2 in bacteria and yeasts.

PS 24 DNA binding specificity of the Werner helicase interacting AAA+ protein WHIP

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The Werner Helicase Interacting Protein (WHIP) in humans is a highly conserved member of the AAA+ protein family. Its bacterial homologue, RarA associates with the replisome while the *S. cerevisiae* homologue MGS1 is essential for genome stability. *In vitro* yeast Mgs1p was found to stimulate the activity of yFen1p while the human WHIP stimulated the activity of DNA polymerase δ. All these results indicate that WHIP activity is targeted towards the process of DNA replication, and/or replication fork repair.

Human histidine$_6$-tagged WHIP protein was expressed and purified from bacteria. The DNA-binding properties of WHIP were investigated using electrophoretic mobility shift assays and small synthetic model DNA substrates. The DNAs were tagged with infrared fluorophor IRD700 which showed sensitivity that was comparable to ^{32}P-labelled DNA. The results show that the oligomeric WHIP binds preferentially to forked DNA substrates and has the highest affinity for replication forks and Holliday junctions. WHIP did not bind duplex DNA and bound poorly to substrates with 3′ or 5′ overhangs. The result is indicating that WHIP may act directly on replicating DNA.

PS 25 Nse3/YDR288w, a conserved component of the essential SMC5-6 DNA repair complex

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Nse4 (non-Smc element 4) is a protein which has recently been identified as a component of the Smc5/6 DNA repair complex (Hu *et al.*, 2005). *Nse4^{ts}* mutants have been shown to arrest after S phase prior to mitosis. This arrest activates the DNA damage checkpoint, and is accompanied by the phosphorylation of Rad53. Mutants of Nse4 display sensitivity to DNA damaging agents, such as HU and MMS. Two-hybrid screens and co-purification experiments have identified an interaction between Nse4 and YDR288w, an open reading frame of unknown function (Hazburn *et al.*, 2003; Hu *et al.*, 2005). This protein is conserved across evolution, and its human orthologue is MAGE-G1 (*m*elanoma *a*ntigen-encoding *g*ene). Given that Nse4 interacts with YDR288w, it can be predicted that the product of YDR288w will play a role in the cell cycle and DNA repair. We generated temperature sensitive (*ts*) mutants of YDR288w. At the non-permissive temperature, these mutants have similar phenotypes to those displayed by *nse4^{ts}* mutants, arresting at a point after S phase but prior to the metaphase to anaphase transition. The mutants are also sensitive to damage DNA, using agents such as MMS, HU and UV. The *S. pombe* orthologue of YDR288w has been named Nse3 (non–SMC element 3) in keeping with the established nomenclature for Smc5/6 components (Pebernard *et al.*, 2004).

PS 26 Homologues of *umuC/D* on transposons in *Streptococcus pneumoniae* and oral streptococci

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DNA polymerase UmuC/D is capable of error-prone DNA repair, and is required for UV-resistance and SOS-induced mutagenesis in *Escherichia coli*. Pneumococci lack the classic SOS response, and the sequenced genomes do not encode a UmuC/D homologue. However, pneumococcal antibiotic resistance transposon Tn*5252* includes a UmuC/D-like locus, which has been reported to increase both resistance to UV-irradiation and UV-induced mutagenesis.

Five of 77 pneumococcal isolates carried *umuC/D* homologues with 89–99% identity to the Tn*5252* loci, and integrase with 98–100% identity. Restriction mapping and resistance profiles suggest these isolates carry elements similar, but not identical, to Tn*5252*. Nine of 13 oral streptococci carried *umuC* homologues with lower identity to Tn*5252* loci; some also had *umuD* homologues but integrase was not detected by PCR. Mutation frequency to rifampicin resistance for pneumococci carrying *umuC/D* homologues did not differ from those without. Two of four carriers had increased resistance to UV-irradiation, although the role of *umuC* in this phenotype remains to be determined.

PS 27 Functional similarities between phage Orf and *Escherichia coli* RecFOR in initiation of genetic exchange

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Genetic recombination in bacteriophage lambda relies on DNA end processing by Exo to expose 3' tailed strands for annealing and exchange by Beta protein. Phage lambda encodes an additional recombinase, Orf, which participates in the early stages of recombination by supplying a function equivalent to the *E. coli* RecFOR complex. These host enzymes assist loading of the RecA strand exchange protein onto ssDNA coated with SSB. We have purified distantly-related Orf proteins from lambda and *E. coli* DLP12 prophage. Both Orf proteins bind DNA, favouring single-stranded over duplex and show no obvious preference for gapped, 3′ or 5′ tailed substrates. The crystal structure of lambda Orf reveals a homodimer arranged as a toroid with a shallow U-shaped cleft, lined with basic residues, running perpendicular to the central cavity. Gapped duplex DNA binding experiments suggest that DNA is accommodated on the surface cleft. Both Orf homologs appear to interact with *E. coli* SSB and we present evidence that they associate together on DNA. The functional similarities between Orf and RecFOR are discussed in relation to the early steps of recombinational exchange and the interplay between phage and bacterial recombinases.

PS 28 Motifs involved in controlling minichromosome maintenance helicase activity

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Minichromosome maintenance (MCM) proteins are essential for DNA replication in eukaryotes and archaea, and are believed to form the replicative DNA helicase required for genome duplication. Eukaryotes possess six highly related MCM proteins that form high molecular weight complexes, whereas most archaeal species have only one, homohexamer-forming, MCM. We are using the MCM from *Methanothermobacter thermautotrophicus* (*Mth*MCM) as a model system to investigate the molecular mechanisms associated with DNA unwinding. We have identified N- and C-terminal motifs in *Mth*MCM that modulate ATP hydrolysis, DNA binding, and duplex unwinding. These motifs also influence the helix-2 insert (h-2i) in the ATPase domain of MCMs: a Δh-2i mutant showed a ~12-fold increase in dsDNA-stimulated ATP hydrolysis, and increased affinity for ssDNA and dsDNA. The Δh-2i mutant also showed complete abrogation of DNA unwinding activity. Our results suggest that the h-2i is key in regulating ATP hydrolysis in MCM proteins, and that this structure may act to transduce the chemical energy of ATP hydrolysis into the mechanical energy required for duplex unwinding.

PS 29 Dot1 and telomere uncapping

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Telomeres are nucleoprotein complexes that cap chromosome ends and prevent ends of chromosomes to be recognized as double strand breaks. Chromatin state plays a structural as well as a functional role in telomere maintenance. Dot1 is a nuclear histone methyltransferase in *S. cerevisiae*. Dot1 modifies histone H3-Lys79 thus regulating transcription, but also recruiting the checkpoint adaptor Rad9, which in turn elicits a checkpoint response.

We investigated the role of Dot1 responding to telomere uncapping. Cdc13 is an essential telomere capping protein in *S. cerevisiae*. *cdc13-1* mutant strains are temperature sensitive and cannot survive temperatures above 28°C. Inactivation of checkpoint proteins or nucleases permits *cdc13-1* strains to grow at 28°C. Here, we show that *dot1*Δ also enables *cdc13-1* strains to from colonies at 28°C, suggesting that Dot1 inhibits growth after telomere uncapping, possibly via a Rad9-regulated checkpoint arrest. We are currently investigating this hypothesis further and will present our most recent data.

PS 30 Mrc1 contributes to the vitality of cdc13-1 and yku70Δ telomere capping mutants

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Mrc1 (Mediator of Replication Checkpoint 1) is implicated in two independent roles in budding yeast *S. cerevisiae* i) checkpoint activation under replication stress and ii) the DNA replication machinery. *mrc1*^{AQ} is an allele with intact replication function, but defective in activation of Rad53, a checkpoint kinase. In this study, we addressed the role of Mrc1 at uncapped telomeres. We used the temperature sensitive *cdc13-1* and *yku70*Δ mutants to uncap telomeres.

Our experiments show that Mrc1 contributes to the vitality of both *cdc13-1* and *yku70*Δ mutants. This does not seem to be a result of enhanced ssDNA accumulation at uncapped telomeres, since growth was similar in *cdc13-1 mrc1*Δ and *cdc13-1 mrc1*Δ *exo1*Δ (Exo1: exonuclease 1) strains. At semi-permissive temperatures, *cdc13-1 mrc1*^{AQ} excibits better growth than both *cdc13-1 MRC1* and *cdc13-1 mrc1*Δ, possibly because of a defect in checkpoint activation. This observation suggests an Mrc1-dependent Rad53 activation after telomere uncapping. Interestingly, *cdc13-1 mrc1*^{AQ} and *cdc13-1 mrc1*^{AQ} *chk1*Δ strains display similar growth. We will discuss our latest experiments and their implications for telomere uncapping and checkpoint activation.

PS 31 The role of MRX at uncapped telomeres

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MRX, an evolutionally conserved DNA damage response complex composed of Mre11, Rad50 and Xrs2, is involved in DNA double strand break (DSB) repair, collapsed replication fork restart, checkpoint activation and telomere maintenance. At DSBs, MRX plays a role generating single stranded DNA (ssDNA) and signalling cell cycle arrest. Here we investigated whether MRX also contributes to generating ssDNA or signalling cell cycle arrest at uncapped telomeres. To investigate the role of MRX, we generated a conditionally degradable Rad50 protein and combined this with *cdc13-1*, a temperature sensitive mutation in a telomere capping protein. We show that Rad50 does not contribute to ssDNA generation or cell cycle arrest in response to *cdc13-1* uncapped telomeres. Instead we find that Rad50 inhibits ssDNA accumulation and promotes *cdc13-1* cell viability, consistent with a major role for MRX in telomere capping. Recent experiments suggest the protective role of MRX also applies to *yku70*Δ induced telomere uncapping and that Tel1 (ATM) functions in a protective pathway that is parallel, rather than in series, to MRX.

PS 32 Proliferating with genomic damage: which protection mechanisms have failed?

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Dividing cells have developed protection mechanisms against genomic instability, such as the checkpoint pathways, which stop cells with genomic damage from proliferating. Recently, we have shown that budding yeast can resume proliferation and generate immortal progenies, despite persistent genomic damage, triggered by the absence of telomere maintenance pathways. However, it was not clear which protection mechanisms have failed to stop the damaged cells from proliferating. To understand the nature of this failure, we generated hundreds of yeast strains lacking checkpoint genes (Rad9, Rad24 or Tel1), nucleases (Exo1, Mre11) and telomere maintenance pathways (Tlc1 and Rad52) and analyzed the fraction of these that survived, growth rates of survivors, viability after re-introduction of checkpoint/nuclease genes and also the checkpoint activation in strains proliferating with chronic genomic damage. We find that the Exo1 nuclease confers a better protection against proliferation with genomic damage than the DNA-damage checkpoint genes. This study gives insight into how cells with genomic instability, such as cancer cells, can overcome protection mechanisms, such as checkpoint proteins and nucleases.

PS 33 The RTS1 site-specific DNA replication fork barrier functions as a meiotic recombination hot spot in *Schizosaccharomyces pombe*

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Homologous recombination during mitosis and meiosis differ in a number of ways. One marked feature of meiotic recombination is that it is programmed to occur between homologues to ensure acquisition

of a physical link between homologue pairs prior to meiosis I, whereas the majority of homologues recombination during the mitotic cycle will be between sister chromatids to repair random DNA breakage. Meiotic recombination between homologues chromosomes occurs more frequently at some positions in the genome than at others; such positions are termed recombination hot spots. The chromosomal features which contribute to hot spot activity at a given location remain incompletely understood. Specific DNA sequences have been demonstrated to confer hotspot activity to a region, but are not sufficient and other factors, most probably chromatin context, play an important role in hot spot generation.

A number of studies have shown that elevated levels of mitotic recombination can be induced by factors which perturb the progression of the DNA replication machinery, mostly likely due to the collapse of the replication fork resulting in a recombination-inducing lesion. However, little is know about how such fork blockage is dealt with during pre-meiotic DNA replication or the relationship between meiotic recombination hot spots and pre-meiotic DNA replication.

In this study we have introduced a well characterized site-specific DNA replication block element, *RTS1*, into the *ade6* locus of *S. pombe*. During mitotic growth we demonstrate that this element function as an orientation-dependent recombination hot spot. During meiosis, we find that this element serves as a meiotic recombination hot spot, but that the orientation specificity is lost. These observations demonstrate that *RTS1* can serve to confer interhomologue meiotic recombination hot spot activity. The differential orientation-dependence between meiosis and mitosis indicates that inherent differences exist in the behaviour of *RTS1*-induced recombination during these distinct cell division events.

PS 34 Biochemical characterization of an ATP-dependent DNA ligase from the acidophilic archaeon '*Ferroplasma acidarmanus*' Fer1

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'*Ferroplasma acidarmanus*' Fer1 is an iron-oxidizing archaea that was isolated at Iron Mountain in California where it is the dominant prokaryote in its environment. Its natural habitat is acidified underground slime streamers, which results in *F. acidarmanus* having a growth optimum of pH 1.2. In this extreme environment DNA damage is likely to occur more frequently and more aggressively.

DNA ligases are enzymes that seal nicks in the phosphodiester backbone of DNA and have important roles in all aspects of DNA metabolism, including replication, recombination and repair. Analysis of the completed genome of *F. acidarmanus* identified a single potential DNA ligase. The gene was cloned into a bacterial expression vector to allow inclusion of an N-terminal His-tag, and the protein was over-expressed and purified using affinity chromatography. Biochemical experiments showed that FaLig can ligate nicks in DNA in the pH range 5–7, with specificity for ATP and a requirement for Mg^{2+}. This is the first characterization of a DNA ligase from an extreme acidophile.

PS 35 Characterization of DNA ligases from *Streptomyces coelicolor*

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DNA ligases are essential enzymes in all cells due to their involvement in DNA replication, recombination and repair. The biochemical reaction mechanism is a three-stage event that occurs via a covalent enzyme-adenylate intermediate, with either NAD^+ or ATP acting as the donor of the adenylate group.

The publication of the genome sequence of *Streptomyces coelicolor* identified the presence of predicted genes for two NAD^+-dependent and three ATP-dependent DNA ligases. The potential for multiple DNA ligases to be present in the cell questions whether they each have specific roles in distinct processes involved in DNA metabolism.

We report the characterization of all five predicted DNA ligases of *S. coelicolor*. Each of the genes was cloned into a bacterial expression vector to allow inclusion of an N-terminal His-tag, and the proteins were over-expressed and purified using affinity chromatography. Biochemical analysis verified that each protein is capable of *in vitro* DNA ligation. Microbiological experiments are being used to assess whether each of the enzymes functions in DNA replication or specific DNA repair processes.

PS 36 Evolution of a phage ruvc endonuclease for resolution of both Holliday and branched DNA junctions

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Resolution of Holliday junction recombination intermediates in most Gram-negative bacteria is accomplished by the RuvC endonuclease acting in concert with the RuvAB branch migration machinery. Gram-positive species, however, lack RuvC, with the exception of distantly-related orthologs found in phage genomes. We purified one of these proteins from *Lactococcus lactis* phage bIL67 and demonstrated that it functions as a Holliday structure resolvase. Differences in the sequence selectivity of resolution between67RuvC and *E. coli* RuvC were noted, although both enzymes prefer to cleave3? of thymidine residues. However, unlike its cellular counterpart, 67RuvC readily binds and cleaves a variety of branched DNA substrates in addition to Holliday junctions. Plasmids expressing 67RuvC induce chromosomal breaks, probably as a consequence of replication fork cleavage, and cannot be recovered from recombination defective *E. coli* strains. Despite these deleterious effects, 67RuvC constructs suppress the UV sensitivity of ruvABC mutant strains. The characterization of 67RuvC offers an insight into how a Holliday junction-specific resolvase can evolve into a debranching endonuclease tailored to the requirements of phage recombination. It also provides a powerful tool to explore the architectural differences in RuvC proteins responsible for DNA branch recognition and sequence-specificity of resolution.

PS 37 Chk1 requirement for normal replication fork progression during an unperturbed vertebrate S phase

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Metazoan Chk1 protein kinase maintains replication fork stability in response to DNA damage and replication inhibitors. We employed DNA fibre labelling to quantify to which extent Chk1 maintains replication fork rates during normal vertebrate S phase. Average replication fork rates in *Chk1*$^{-/-}$ chicken DT40 cells are half of those observed with wild-type cells. Similar results were observed if Chk1 was inhibited or depleted in wild type DT40 or HeLa cells using Chk1

inhibitor or siRNA. Reduced fork extension rates were also observed with permeabilized *Chk1*$^{-/-}$ cells in vitro. The requirement for Chk1 was not to suppress homologous recombination at replication forks, because inhibition of Chk1 slowed fork progression in *XRCC3*$^{-/-}$ DT40 cells. Rather, increased numbers of replication fibres in *Chk1*$^{-/-}$ cells displayed single-stranded nascent strands, indicating that slow fork rates in unperturbed *Chk1*$^{-/-}$ cells are associated with accumulation of aberrant replication fork structures.

PS 38 *xlf1*, a homologue of mammalian XRCC4-like factor, is a core NHEJ factor in *Schizosaccharomyces pombe*

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DNA double strand breaks (DSB), one of the most lethal forms of DNA damage, can be repaired by two different cellular pathways: homologous recombination (HR) and the more error-prone non-homologous end-joining (NHEJ). While mammalian cells use NHEJ as the predominant DSB repair mechanism, the yeasts *S. cerevisiae* and *S. pombe* repair DSBs almost exclusively by HR in their vegetative state. In order to gain further insight into the importance of NHEJ for fission yeast, we sought to identify new components of the *S. pombe* NHEJ pathway. We describe novel factors required for *S. pombe* NHEJ, including *xlf1*, a homologue of mammalian XRCC4-like factor (XLF) recently discovered in humans. Fission yeast *xlf1* is required for the religation of linearized plasmids by NHEJ and interacts with DNA ligase IV. In addition, *xlf1* is required for the survival of DSB under certain physiological conditions.

PS 39 Interactions of *Schizosaccharomyces pombe* Mcm10 with DNA replication factors

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Schizosaccharomyces pombe mcm10 encodes an essential protein of 593 amino acids that binds to chromatin. Mcm10 homologues are found in all eukaryotes where they function in DNA replication. Temperature sensitive *mcm10* mutants exhibit a characteristic cell division cycle arrest phenotype at 36°C with elongated cell morphology indicative of an intact checkpoint. SpMcm10 is required for both the initiation and elongation phases of DNA replication, and although it is not necessary for pre-replicative complex formation it is essential for its subsequent activation and Cdc45 loading onto chromatin. Consistent with a role at initiation SpMcm10 interacts in yeast 2- hybrid assays with four subunits of the origin recognition complex (Orc1, 2, 5 and 6), three subunits of the Mcm2-7 replicative helicase (Mcm4, 5 and 6), two GINS subunits (Psf2 and Sld5) and Rad4/Cut5. Genetic interactions have also been demonstrated with mcm2 as well as mcm4, mcm5 and mcm6. SpMcm10 is required for DNA polymerase α-primase stability *in vivo* but over-expression of mcm10 is insufficient to rescue mutations in the DNA polymerase or primase subunits.

PS 40 Versatile restriction-modification systems as models for replication, recombination and repair

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Scanning and specific recognition of DNA sequences, the ability to open up, unwind, base flip, methylate and/or cut the DNA, and methyl-directed strand discrimination, are all key issues in understanding replication, recombination and repair. Restriction-modification (R-M) enzymes prove superb model systems to study biochemical and biophysical aspects of such DNA-protein interactions. In addition to well-known enzymes such as EcoRI, thousands of other R-M systems in eubacteria and archaea have been discovered that reveal an ancient common origin with enzymes involved in DNA metabolism and maintenance of genome integrity. This presentation highlights one such enzyme, EcoKI, that distinguishes unmethylated DNA from hemi-methylated DNA because binding of S-adenosylmethionine (SAM) alters the DNA contacts of the protein with the DNA. In this way the enzyme switches between site-specific methylation or cutting at a distant site after DNA translocation. As this intricate pas de deux of EcoKI and SAM may date back to the last universal common ancestor between bacteria and man, this finding has potentially important implications for the action of nucleases involved in eukaryotic replication, recombination and repair.

PS 41 Identification of a *Bacteroides fragilis* transcriptional regulator involved in metronidazole resistance

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Infections by *Bacteroides fragilis*, an anaerobic opportunistic human pathogen, are treated with the DNA-damaging agent, metronidazole. In this study, genes involved in *B. fragilis* metronidazole resistance were investigated. It was found that a *B. fragilis* gene, encoding a putative AraC transcriptional regulator, conferred resistance to metronidazole and mitomycin C when expressed in *Escherichia coli uvrA*$^{-}$ and *uvrB*$^{-}$ DNA repair mutants under aerobic and anaerobic conditions. A putative *recQ* homologue, with 59% similarity to the RecQ protein involved in DNA replication and repair in *E. coli*, was identified upstream of the *araC* gene and expressed divergently from it. In order to define the role of the putative transcriptional regulator, a *B. fragilis* AraC mutant was generated by insertional inactivation of the *ara*C gene. The AraC mutant had increased sensitivity to the DNA damaging agents, metronidazole and hydrogen-peroxide, as compared to the wild-type *B. fragilis*. It also showed cell elongation after metronidazole treatment. These results indicate the involvement of the *ara*C homologue in the cellular response to metronidazole treatment.

PS 42 The role of recA in *Bacteroides fragilis* 638R

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The effect of a *recA* mutation on DNA repair was investigated in the anaerobic opportunistic pathogen, *Bacteroides fragilis* with a view to elucidating cellular responses to the DNA damage caused by the drug metronidazole. A *recA* mutant was generated by insertional inactivation of the *recA* gene using the pGERM plasmid. The *recA* mutant was sensitive to temperature with growth of the mutant on solid media inhibited at 37°C but not at 25°C. The MIC of metronidazole, the drug of choice for the treatment of *B. fragilis* infections, was 0.125 µg/ml for the *recA* mutant compared to 1.00 µg/ml for the wild type *B. fragilis*. In the disk diffusion assay, the mutant was more sensitive to hydrogen peroxide and superoxide than the wild-type. Microscopically, the mutant produced elongated cells as compared to the wild type, and this phenotype was enhanced following exposure to the stress conditions described above. These results indicate the involvement of the *B. fragilis* RecA protein in cell responses to a range of DNA damaging agents.

PS 43 Possible role of transcription and translation in the activation of the *oriV* of *repABC* plasmids

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The *repABC* replicons consist in an operon containing *repA*, *repB* and *repC* genes, a *par*-site and an antisense RNA. The *repC* gene under the P_{lac} promoter with an *E. coli* Shine-Dalgarno (pDO-CE) is the minimal replicon, demonstrating that RepC is essential for replication and that the *oriV* resides within *repC*. *repC* frame-shift mutations or deletions in its 5′ or 3′ ends destroy this ability.

pDO-CE replicates in a strain lacking the parental plasmid (CFNX107) but not in a strain containing it (CFNX101), indicating that RepC is a *trans*-incompatibility factor. Clones containing a promoter-less *repC* (in a replicable vector) do not displace the parental plasmid indicating that the *oriV* is not an incompatibility factor. A construct containing *repC* under the P_{lac} promoter, and a *R. etli* Shine-Dalgarno (pDO-CR) do not replicate in strain CFNX107, but is capable to do it in CFNX101. A mutant derivative of pDO-CR with a stop-codon instead of the initial ATG is incapable to replicate in both CFNX101 and CFNX107, suggesting a possible role of transcription-translation in *oriV* activation.

PS 44 Investigating long 'Eclipse' by thymine limitation

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A bacterial cell manages to multiply faster than duplicate its chromosome by initiating a successive replication cycle before the previous has terminated [1]. Initiation occurs upon doubling in cell mass or volume, at a constant value of mass per *oriC*, *Mi* [2]. At optimal conditions (37°C in rich medium), doubling time (t) of *Escherichia coli* is about twice the replication time (C) – 20 and 40 min, respectively, hence initiation of a new round occurs when the bifurcating replication fork assemblies are midway between *oriC* and *terC* ($L_{0.5}$, half chromosome). A delay between two successive initiation events was predicted, then found [3] following inhibition of replication for two mass doublings, during which the cells have accumulated enough potential to initiate twice. This so-called 'stacking' phenomenon, later termed 'eclipse', ensures that no further initiations occur for a substantial fraction of the cell cycle; several mechanisms have been postulated to explain the eclipse [4]. An open question thus arises: does it result of a time-consuming process or of a steric hindrance that forbids another replication fork to form before the previous one has traversed a presumed minimal distance l_{min} away from *oriC*. Assuming that such an l_{min} exists, specific questions arise such as 'What is its value?' and 'How would it affect cell physiology?'

If l_{min} is below half the distance *oriC*-*terC* ($L_{0.25}$), it cannot be observed in Thy$^+$ cells, where the number of replication 'positions' ($n=C/t$ [5]) never exceeds 2. To reach $n > 2$, C must extend beyond $2t$, achievable by 'thymine limitation' [6]: cultivating Thy$^-$ mutants at low thymine concentrations without affecting the growth rate ($m=1/t$). If the forks do not reach l_{min} when a new cycle should inaugurate to preserve steady state [7], the initiation event would be delayed by a period Dt necessary for the previous position of forks to reach l_{min}. Thus, when $C/t > L_{0.5}/l_{min}$, the next initiation event would occur Dt later, during which cell mass accumulates at the same rate to reach $Mi_1=Mi.2^{Dt/t}$, resulting in a larger cell mass at division ($Md_1=Mi_1.2^{(C+D)/t}$, D being the time between termination and the subsequent cell division [1]). When $C > tL_{0.5}/l_{min}$, a new steady state [7] can therefore **not** be reached: average cell mass M and DNA content G rise and DNA concentration G/M drops with time, depending on n and l_{min}/L. Continuous changes of M and G have been observed under such circumstances [8], but G/M seemed to remain unchanged. However, G/M was measured for a time much shorter than necessary to flocculate the cultures [8], and the question of how D change with C remains moot [9 vs 8].

Various physiological consequences of the relationship between Mi, t, C, D, M_d and $l_{min}/L_{0.5}$ will be described and discussed. Careful tests of the predictions of this model may distinguish between temporal/chemical and spatial/structural control of the eclipse [3, 4]. It is likely that both mechanisms simultaneously prevail and are related.

References: Helmstetter *et al.* (1968). *Cold Spring Harbor Symp Quant Biol* **33**, 809; Donachie (1968). *Nature* **219**, 1077; Pritchard *et al.* (1969). *Symp Soc Gen Microbiol* **19**, 263; Helmstetter (1971). In *Drugs and Cell Regulation: Organizational and Pharmacological Aspects on the Molecular Level*, pp. 1–20. NY: Academic Press; Zaritsky (1975). *J Bacteriol* **122**, 841; Helmstetter *et al.* (1979). In *The Bacteria* **7**, 517; Boye *et al.* (2000). *EMBO Rep* **1**, 479; Olsson *et al.* (2002). *Mol Microbiol* **44**, 1429; Molina & Skarstad (2004). *Mol Microbiol* **52**, 1597; Sueoka & Yoshikawa (1965). *Genetics* **52**, 747; Pritchard & Zaritsky (1970). *Nature* **226**, 126; Zaritsky *et al.* (2006). *J Bacteriol* **188**, 1667; Fishov *et al.* (1995). *Mol Microbiol* **15**, 789; Zaritsky & Pritchard (1973). *J Bacteriol* **114**, 824; Meacock & Pritchard (1975). *J Bacteriol* **122**, 931; Zaritsky *et al.* (1999). *Biochimie* **81**, 897.

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Environmental Microbiology Group / British Ecological Society joint session

Microbes, macrobes and ecology

Island size and bacterial diversity

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The relationship between the number of species and area sampled [species-area relationship (SAR)] is a fundamental pattern in ecology. The SAR is one of the oldest biological rules and has been extensively used for estimating population distributions, numbers and species diversity for animals and plants. It was only recently that SAR have been reported for bacterial taxa in contiguous habitats (e.g. soil) and in insular island communities such as treeholes, sump tank reservoirs, wastewater treatment plant reactors, and lakes. Here I will discuss: 1) observations on how bacterial diversity scales with island size; 2) questions of scale that have to be considered for contiguous bacterial SAR studies; and finally 3) possible implications for bacterial SARs once high throughput sequencing becomes routinely available.

Posters

Plenary

PS 45 Single molecule analysis of DNA translocation by the molecular motor R.EcoR124I

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The Type I restriction-modification enzyme R.*Eco*R124I binds to the DNA sequence GAA$(N)_6$RTCG and reads the methylation status of this recognition sequence. If both strands of the DNA are unmethylated, the enzyme undergoes an ATP-dependent conformational change that switches the function of the enzyme from DNA methyltransferase to restriction endonuclease. Addition of an excess of ATP results in DNA translocation, in which the enzyme remains bound to the recognition sequence, but 'pulls' the rest of the DNA through the bound complex until either stalling or a blockage prevents further translocation.

Therefore, Type I R-M enzymes are molecular motors that are potentially useful biological nanoactuators capable of 'pulling' objects attached to the DNA over great distances. However, initiation of translocation requires that the motor overcomes the problem associated with the persistence length of DNA – 50 nm – which requires localised 'melting' of the DNA before the motor can initiate translocation [1]. The motor can 'pull' the DNA at 550 bp s^{-1} (0.2 µm s^{-1}), over distances of several microns and is capable of also 'pulling' a micron sized magnetic bead attached to the end of the DNA [2]. The movement of this magnetic bead, as well as providing an example of the nanoactuator, can generate an electronic output from a suitable sensor leading to the possibility of a useful single molecule sensing system.

The subunit responsible for the molecular motor activity (HsdR) can readily dissociate from the restriction enzyme, which leads to loss of motor activity, but the core DNA-binding DNA methyltransferase can 're-load' HsdR and re-initiate translocation. Therefore, the motor is a very dynamic system [3].

In this poster we will show the single molecule measurements that have led to the above observations and present resent work showing how the motor discriminates polarity within the DNA and which of the two DNA strands is used for translocation.

References: 1. van Noort, J. *et al.* (2004). Initiation of translocation by Type I restriction-modification enzymes is associated with a short DNA extrusion. *Nucleic Acids Res* **32**, 6540–6547. 2. Seidel, R. *et al.* (2004). Real-time observation of DNA translocation by the Type I restriction-modification enzyme *Eco*R124I. *Nature Struct Mol Biol* **11**, 838–843. 3. Seidel, R. *et al.* (2005). Dynamics of initiation, termination and reinitiation of DNA translocation by the motor protein *Eco*R124I. *EMBO J* **24**, 4188–4197.