DNA damage responses: a combination of maintenance and fire-fighting

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The DNA in every living cell is constantly subjected to damaging events. Fiona Benson and Tony Carr describe how the integrity of genes is maintained.

All cells rely on DNA to transmit genetic information and it is vital to maintain the integrity of this information. This is underlined by the fact that in both prokaryotes and eukaryotes around 5% of the genes are devoted to encoding DNA repair and DNA damage response proteins.

DNA itself is fragile and undergoes a host of interactions with endogenous molecules in the cell. It has been estimated that every cell in our body suffers 10,000 DNA damaging events every day. Add to this the intrinsic infidelity of DNA replication and the exogenous damage induced by exposure to sunlight and other genotoxic treatments (smoking, X-rays, etc.) and it is clear that DNA damage responses are key to the survival of an unaltered genome. Preservation of a stable genome is critical; genome alterations have a fundamental role in initiation of cancer in higher eukaryotes.

The genome has often been likened to an encyclopaedia of technical information for building a complicated machine. The entries in the encyclopaedia are composed of just a four-letter base alphabet, comprising A for adenine, G for guanine, T for thymine and C for cytosine. It is the precise ordering of these four letters of the alphabet in each individual encyclopaedia entry, or gene, that provides the instructions for making the proteins that are the key cellular building blocks. In a simple eukaryote such as yeast there are approximately 12 million bases encoding 6000 genes. That's 6000 individual entries in a hypothetical encyclopaedia just to make a yeast!

In humans there are about 3000 million letters of DNA and about 30,000–40,000 genes. Now, if there are 10,000 spontaneous DNA damage events in each cell, then it's like somebody randomly erasing 10,000 letters in the encyclopaedia every day. Even though less than 2% of the human genome actually encodes proteins, a large number of these erasures will still affect an entry in our hypothetical encyclopaedia.

Fortunately, the double-stranded nature of DNA allows single base erasures to be fixed with high fidelity – information lost from one strand can be replaced by referring to the alphabet order on the other strand. Some forms of damage are more severe and delete letters opposite each other. This is a much more severe problem for a cell.

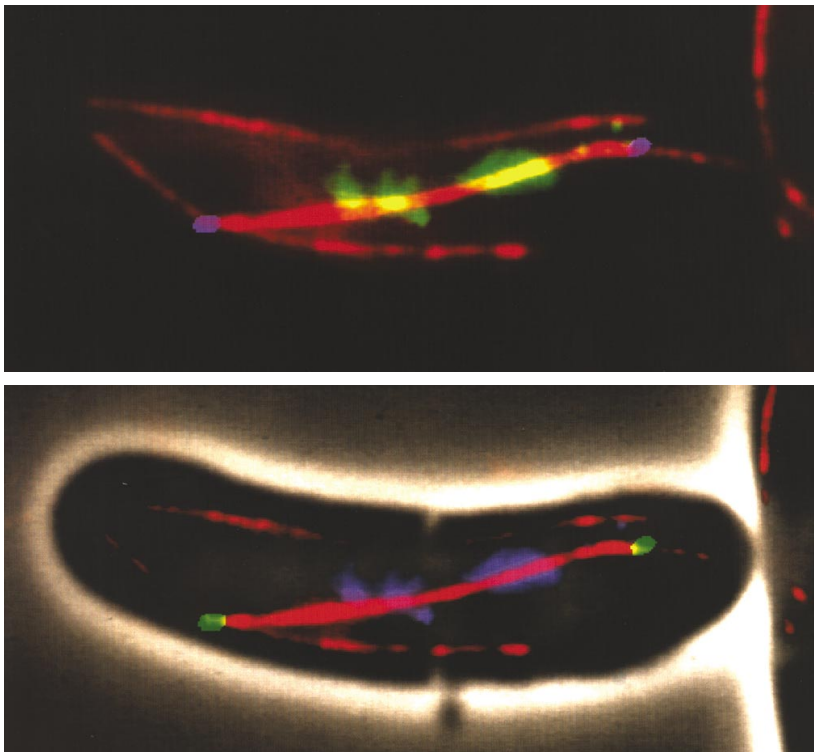
DNA repair pathways

How does a cell deal with all these problems? First, intrinsic damage to single letters is repaired by a variety of mechanisms designed to housekeep the encyclopaedia. These DNA repair pathways, which include base excision repair and mismatch repair, can essentially be thought of as maintenance workers which recognize inappropriate changes in the double-stranded DNA and put these right by copying from the undamaged strand. Probably these routine maintenance operations occur unseen, i.e. they are not monitored by the cell as a sign that something is wrong. However, induced DNA damage, such as distortions in the double helix which may result from exposure to the ultraviolet component of sunlight, or DNA strand breaks induced by ionizing radiation, require more complex repair pathways such as nucleotide excision repair (NER), homologous recombination repair (HR) and non-homologous end joining (NHEJ). These pathways can perhaps be considered as the emergency services, being recruited to sort out problems that hopefully do not occur too often and which, when they do occur, can have fatal consequences. Certainly it seems that these pathways are not silent, but are monitored in the cell by a series of mechanisms which are collectively called DNA-integrity checkpoints.

DNA-integrity checkpoint pathways

When the cell detects DNA damage or its repair (we still do not know precisely how this occurs), it responds in different ways, depending on the circumstances it finds itself in. For simple eukaryotes, such as yeast, every cell is going to reproduce, so the different responses are largely geared to keeping the encyclopaedia intact, thus ensuring that the blueprint remains unchanged. To do this it is necessary to arrest the cell cycle and co-ordinate DNA repair with whatever is on-going at the time. For example, if the cell is undergoing DNA replication, equivalent to making a copy of our entire hypothetical encyclopaedia, then the repair apparatus must interact with the replication apparatus and stop the latter temporarily, since copying damaged information is likely to exacerbate the problem. If however the DNA replication is complete, then it is important not to try and separate the replicated copies (i.e. undergo mitosis) until repair is complete. This is important for two reasons. First, if the DNA is physically broken (analagous to tearing a page out of our encyclopaedia) then pulling the two DNA molecules apart is going to result in the information (i.e. the page) being left behind. Second, if the DNA is broken, and still side by side with an intact copy, it is possible to use the information from the intact volume to effect repairs to the broken one. This process, homologous recombination, requires that the two identical volumes of the encyclopaedia are in close proximity until the repair is completed. Recent data suggests that co-ordination of homologous recombination is controlled by the checkpoint proteins.

In multicellular organisms, many cells are terminally differentiated and are not going to divide again. It is not clear exactly what the DNA damage responses are in such cells, since most work in laboratories is performed on dividing cultures. Clearly, stationary phase – or quiescent – cells can repair DNA damage and their



RIGHT:
A *Schizosaccharomyces pombe* checkpoint-defective cell following abortive mitosis. Top: DNA false-coloured in green, microtubules in red and spindle pole bodies in blue. Bottom: the same image superimposed onto an optical image of the cell (DNA blue, microtubules red, spindle poles green).
WITH THANKS TO IAIN HAGAN

checkpoint pathways still operate. Obviously, arresting the cell cycle is not an issue in a stationary-phase cell, and in this case checkpoint pathways seem to act mainly to induce other responses, for example, initiating transcription to ensure an adequate repair response and to make sure repair pathways are properly controlled. Another function of the checkpoint pathways is to help co-ordinate a cell suicide response, termed apoptosis. This response is thought to remove cells which have received particularly dangerous levels of DNA damage. It ensures that damage does not become fixed as a mutation, or permanently changes an encyclopaedia entry, which might result in a cell beginning to cycle again and potentially initiating tumour formation by inappropriate division.

In contrast to quiescent cells, stem cells are programmed to divide, primarily to give rise to differentiated cells of specific cell types, but also to self-renew, as part of the growth and maintenance of the whole organism. In these cells, it is particularly important to repair DNA damage and to monitor this so that the cell can respond appropriately. A dividing cell must be kept under strict control in a multicellular organism, since it is one step closer to forming a tumour than a quiescent cell simply by virtue of being programmed to divide. Perhaps unsurprisingly, stem cells appear to have a lower threshold of DNA damage for the cell suicide response than quiescent cells. The logic of this may be that, as dividing cells are more dangerous, it is better to sacrifice the individual cell when there is a chance of corrupting the blueprint, rather than potentially risking the integrity of the whole organism. The critical role of DNA repair and DNA damage response pathways is emphasized by the observation of the devastating consequences of inherited genetic defects in these pathways in diseases such as Xeroderma pigmentosum and Ataxia telangiectasia.

Similarities between prokaryotic and eukaryotic microbes

Prokaryotic organisms face a similar challenge to simple eukaryotes in the event of damage to their DNA. As each prokaryotic cell is destined to divide, the focus of the DNA repair and damage response is in protecting the integrity of the genome. Suicide is not an option! In bacteria DNA damage and repair are continually monitored and a primitive damage response, termed the SOS response, is activated to co-ordinate repair and cell division. Emergency repair pathways of nucleotide excision repair, homologous recombination and lesion bypass are induced as part of the SOS response, in parallel with temporary cessation of cell division. Once the DNA is repaired, the emergency repair systems are turned off and cell division is allowed to proceed. Remarkably, the mechanisms of DNA repair processes are essentially conserved from prokaryotes, through simple eukaryotes to complex multicellular organisms, such as man. In many cases, pivotal repair enzymes show a high level of conservation of sequence and structure from bacteria, through simple eukaryotes, such as yeast, to man. In view of the increased complexity of eukaryotic organisms it is perhaps not surprising, however, that the complexity of the enzymatic machinery that mediates repair is increased approximately 10-fold between bacteria and man.

From microbes to man

Study of prokaryotes has been important in elucidating repair pathways and providing a paradigm in the SOS response for study of eukaryotic DNA damage responses. However, studies of single-cell eukaryotes have proved to be of increased importance in delineating DNA repair and checkpoint responses of relevance to multicellular eukaryotes such as man. Clearly, such cells do not have a suicide response (it's better to try and divide, since there is nothing to lose). However, it is clear that the same fundamental eukaryotic surveillance pathways characterized in yeasts and other model eukaryotes report both to cell cycle regulators and the apoptosis system in multicellular systems.

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