# WEAR AND TEAR

## Cells and the biology of ageing

Can clues to how and why we age be found by looking at what happens when our cells wear out? By Richard Twyman.

It doesn't matter how careful we are with our bodies, our cells are subject to constant wear and tear. The simple act of eating a sandwich exposes millions of cells to physical damage, noxious chemicals and invading pathogens. In some cases cells are killed directly by the damage they sustain, a process known as **necrosis**. In other cases, damage accumulates over time and cells eventually go into retirement, commit suicide or get picked off by the immune system. In scientific terms, they enter a state known as **senescence**, where they stop dividing permanently, or they undergo a process termed **apoptosis**, a form of programmed cell death in which they are broken up and reabsorbed. These processes are in many ways analogous to the ageing of our bodies, but what triggers them and are they the basis of ageing?

### Senescence and ageing

Senescence was discovered in the early 1960s when two researchers studying human cells growing in culture made a startling and unexpected finding – after about 50 divisions, the cells stopped dividing and withdrew into a permanent quiescent state, with a strikingly different appearance compared with dividing cells. Senescence was subsequently documented in many types of cell and it occurred even under ideal culture conditions with plenty of nutrients available. This suggested that most of the body's cells have an intrinsic replication limit (named Hayflick's limit after its discoverer); once this limit is reached, a cell's self-renewing potential appears to be exhausted.

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Many of the body's tissues are made predominantly of dividing cells, so cells that are lost or destroyed can be replaced through the division of others. With age, senescent cells build up in older tissues, accumulating at sites such as arthritic joints and skin ulcers, and potentially contributing to the signs of ageing through their release of enzymes that break down the molecules holding cells together in tissues. But does this senescence arise simply because cells have replicated a set number of times?

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"When scientists delved deeper into the mechanisms of senescence, the relationship between cellular replicative capacity and ageing was found to be a lot more complex than originally envisaged," says Professor Judith Campisi (Lawrence Berkeley National Laboratory and Buck Institute for Age Research, both in California). "Many factors can trigger senescence, including stress, the activation of certain cancer-causing genes (oncogenes) and the expression of genes or the delivery of drugs that alter the structure of chromatin, the protein–DNA assembly that determines which genes are switched on and off in the cell."

Replicative senescence may therefore be part of ageing, but it is not the whole story. "In some but not all cells, replicative senescence is triggered by telomere shortening," says Dr Richard Faragher at the University of Brighton (see page 21). "In other cells, replicative senescence is triggered by a different mechanism, and still other pathways are involved in the senescence response to stress, oncogene activation and changes to chromatin. What seems clear, however, is that all these pathways converge

on two regulatory proteins known as p53 and pRB." These two molecules have the important function of protecting our cells from the consequences of DNA damage – such as uncontrolled cell proliferation and cancer – and do so by forcing cells into senescence or by causing them to undergo apoptosis.

**Damage limitation**

The involvement of p53 (and pRB) in the senescence response suggests that senescence, apoptosis and ageing may be related to the detection of DNA damage in the cell. Indeed, a long-standing theory of ageing is that it results from the accumulation of damage in cells, particularly in DNA, structural proteins and the fatty molecules of the cell membrane. While damage can be caused by physical and chemical insults such as radiation and toxic chemicals, researchers are particularly interested in 'reactive oxygen species'. These highly reactive molecules are produced in the cell's mitochondria as part of the process of respiration (and at other sites).

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Cells try to protect themselves by producing enzymes that convert reactive oxygen species into harmless compounds or repair the damage they cause, or by producing enzymes that synthesise antioxidant chemicals to counteract the harmful molecules. However, the impact of such enzymes on the ageing process has been hard to decipher. Mice engineered to express higher-than-normal amounts of such enzymes appear to be protected from cancer to a certain extent, so they are more likely to live to older ages, but ageing is not delayed.

"Researchers are becoming increasingly aware of the limits of these experiments," says Professor Malcolm Jackson (University of Liverpool). "Cells produce reactive oxygen species at multiple sites and they exert their effects in different compartments in the cell. Therefore, the expression of a particular enzyme in the mitochondria, for example, will not affect reactive oxygen species produced in another compartment, such as the cytosol, and will not modify the resulting ageing process." For example, mice engineered to lack one such enzyme, glutathione peroxidase 1, aged at the normal rate, whereas other mice lacking cytosolic superoxide dismutase aged prematurely. Both gene deletions led to increased damage to proteins, lipids and DNA but only one influenced ageing. "We really need to find ways to block reactive oxygen species activity at specific sites before we get a clear picture of their impact on the ageing of cells and organisms," says Professor Jackson.

Other forms of damage to cellular components have also been linked to ageing and metabolism. For example, proteins interact with sugars to produce 'advanced glycation end-products', which cause protein and lipid cross-linking. Cross-linked proteins and lipids are difficult for cells to get rid of, and tend to accumulate as a substance called lipofuscin in lysosomes, the cell's recycling centres for damaged molecules. This is not such a problem in dividing cells, where the lipofuscin is diluted after every cell division, but our bodies also contain many non-dividing cells – such as mature muscle cells and neurons – which are never replaced. In these cells, and in cells that have become senescent, dysfunctional lysosomes can build up to form visible granules that eventually cause the cells to swell up and die.

With age, the cell's repair mechanisms for proteins may also begin to fail, accelerating the accumulation of damage. "One repair mechanism which is important, particularly in cells such as skeletal muscle, is the family of stress proteins known as 'heat shock proteins'," says Dr Anne McArdle (University of Liverpool). "These accumulate in response to stress to help repair proteins and recover their function." As well as ensuring that new proteins are folded in the correct way, chaperones can also refold partially denatured proteins back into their functional shape. Muscles in older mice cannot respond to stress by upregulating this repair mechanism, research by Dr McArdle and Professor Jackson has shown, but when mice were engineered to make large amounts of a heat shock protein called HSP70, throughout life, skeletal muscles did not weaken with age and muscle damage could be repaired quickly and successfully – a process that is defective in older mice.

**DNA integrity**

The human genome encodes about 100 enzymes whose sole function is to repair damaged sections of DNA in the nucleus, either by reversing specific types of damage directly, or by cutting out damaged and unreadable sections of DNA and allowing their replacement by normal DNA copying. In young cells, this may be a highly efficient process, but if the genes encoding the repair enzymes themselves become mutated, the efficiency of repair may begin to diminish. If damage accumulates to the point at which the cell can no longer replicate its DNA, p53 (or pRB) would be activated and the cell forced into senescence or apoptosis. Evidence in support of this includes experiments in which mice have been engineered to make extra p53. Although these mice are less susceptible to cancer – a disease driven by DNA mutations – they age faster than normal, possibly because more cells than usual become senescent or undergo apoptosis.

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There is also evidence that the integrity of the DNA in cells' mitochondria is important in ageing. (Each mitochondrion contains a small circular DNA that encodes 37 genes.) Damaged mitochondria might


be expected to produce more reactive oxygen species, resulting in more mutations and so on. Furthermore, mice engineered to produce an inaccurate version of the enzyme responsible for replicating and repairing mitochondrial DNA showed many signs of premature ageing, and their cells were more likely to apoptosis. Suicidal cells accumulated in all the tissues examined, although this process was delayed in muscles and in the brain. In proliferative tissues, lost cells would presumably be replaced by cell division, leading to increased senescence, whereas in tissues such as muscle and brain the lost cells would be gone for ever. In both cases, progressive tissue deterioration would be the expected result.

**Full circle**

During our lives, our cells replicate and divide, are subject to stress, and accumulate damage both through contact with chemical and physical insults and through the intrinsic production of damaging molecules. All these processes naturally lead to two outcomes – senescence or death – to prevent damaged and exhausted cells losing integrity and proliferating uncontrollably.

Cell death, through necrosis or apoptosis, results in depletion. In muscles, heart and brain, where critical cells are never replaced, depletion leads to functional decline. In other tissues, where cells can be replaced by division, depletion brings the remaining cells closer to their replicative limit and increases the likelihood of senescence, which in turn changes the character of tissues and results in the effect we know as ageing.

Dr Richard Twyman is a science writer based in York.

**Further reading**

**Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 2005;120(4):513–22.**

**Balaban RS et al. Mitochondria, oxidants, and aging. Cell 2005;120(4):483–95.**

## END STORY

**Once regarded as counters that keep track of how many times a cell has divided, telomeres now appear to play a more complex and involved role in cell survival and ageing.**

Short telomeres signal danger for cells. If the caps on the ends of chromosomes are lost, crucial genes are no longer protected and the risk of mutation increases. “When telomeres become short, a normal cell has to assume that there is a lot of damage,” says Professor Thomas von Zglinicki at the University of Newcastle upon Tyne. “To minimise risk, the cell triggers a signalling pathway that includes p53 and p21 (tumour suppressor genes) and goes into senescence or apoptosis.”

Because telomeres get shorter when cells divide – DNA polymerases struggle to copy the ends of linear pieces of DNA – it was thought that telomeres were a counting mechanism: after a certain number of divisions, the cells would shut down. But why does telomere shortening vary so much? It differs between cell types; even in the same cell, they do not shorten by the same amount on every chromosome.

Stress is the key, argues Professor von Zglinicki: “We find that cells that produce more oxidative stress, or that have reduced antioxidant defences, lose their telomeres faster. Therefore telomeres are not a regular clock – we suggest that they are measuring the probability of damage, and have a sentinel function, looking for the risk of mutation and damage to the genome.”

“We looked at people who have had stroke-related dementia, and found that they had shorter telomeres. This makes sense, as the quality of defences against oxidative stress play a huge role in determining the consequences of stroke. Short telomeres have also been found to be associated with cardiovascular disease and survival after certain cancers.”

Thomas von Zglinicki is Professor of Cellular Gerontology at the Henry Wellcome Laboratory for Biogerontology, University of Newcastle.

**Further reading**

**Martin-Ruiz C et al. Telomere length predicts poststroke mortality, dementia, and cognitive decline. Ann Neurol 2006 [epub ahead of print].**

**von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci 2002;27(7):339–44.**