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Epigenetics and longevity

by Prof
Jane Mellor

Why do some organisms senesce and die, while others exhibit negligible senescence with no loss of fertility over time? My group is using the yeast *Saccharomyces cerevisiae*, the worm *Caenorhabditis elegans*, mammalian cell lines, and mouse models to develop new treatments for age-related conditions and to understand the conserved processes that contribute to ageing. To achieve this, we are collaborating with several research groups and Oxford-based Chronos Therapeutics.

Why do we age?

Only organisms that have a distinction between soma and gametes can age, as material capable of generating new individuals must not age in order for the species to persist. Hydra, for instance, can generate new individuals from any body part, thus appears not to age. This distinction between soma and gamete exists even in single-celled eukaryotes such as *S. cerevisiae*, making yeast an excellent model for animal ageing.

Yeast age over time (1). This is known as chronological lifespan (CLS) and acts as a model for quiescent, non-replicating cells such as neurons and adult stem cells. Yeast also show replicative ageing, or senescence, and are limited in the number of daughter cells they produce, acting as a model for proliferating stem cell ageing. As yeast is single-celled, both forms of ageing must result from the separation of gametes and soma in asymmetrical cell divisions.

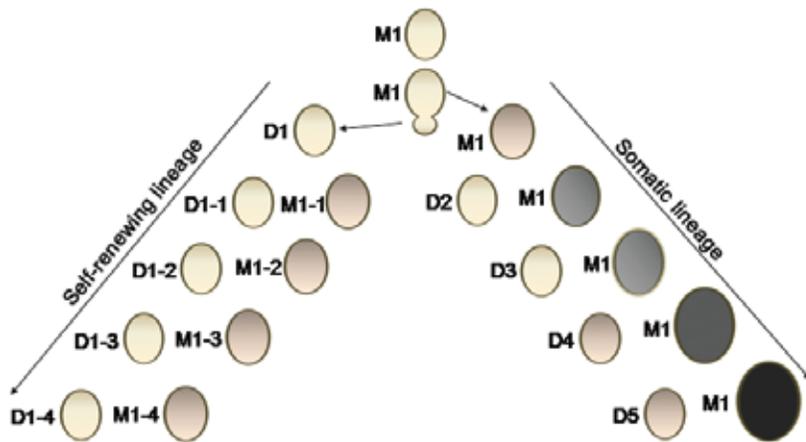


Figure 1. Asymmetric cell division in yeast results in a different fate for daughter (D) and mother (M) cells. Asymmetrical division of a virgin cell (M1) results in two lineages. The virgin daughter cells (D1 to D1-4) constitute a self-renewing germ lineage with infinite replicative potential. The mother cell M1 produces another four daughter cells (D2 to D5), accumulating more damaged protein and organelles at every division, represented by the darker larger cells. As M1 ages, the mechanism for segregating damage to the mother cells gets less efficient and the daughter cells (D4 and D5) also begin to age. Similarly, when D1 divides, the resulting mother cell (M1-1) accumulates damage from her daughter cell (D1-1). The fates of M1-1 to M1-4 and D2 to D5 are not shown for clarity.

A single yeast cell has two phases of life: gamete and mother cell (Figure 1). A gamete may give birth to a daughter gamete, thus becoming a mother cell. Mother cells resemble soma and age, showing senescence rather than quiescence, and a limited capacity to replicate. This is illustrated by the response of mother and daughter cells to glucose starvation, a model for chronological ageing (Figure 2). Mother cells senesce and eventually undergo apoptosis while virgin daughter cells become quiescent, retaining the ability to proliferate once nutrient availability returns. The nutrients released following apoptosis of the mother cells can be utilised by the daughters to aid survival. Non-random segregation of damaged DNA (2) or mis-folded protein (3) into the somatic cells may be used to keep the gametes' lineage 'forever young' (Figure 3).

Asymmetric division between yeast mother and daughter cells establishes a pseudo-programme which results in cell death. A pseudo-programme describes a process by which a secondary, 'unintended' action occurs after the programme has completed its 'intended' task (4). In this case, the intended task is to maintain the stem cell lineage by coordinating cell proliferation with environmental signals such as nutrient availability (Figure 4A). The unintended action is that, in the presence of nutrients, the programme continues to drive cell proliferation despite the fact that mother cells are accumulating damaged DNA, proteins and organelles (Figure 4B). Thus, continued proliferation occurs at the expense of longevity of the mother cells, leading to ageing and death.

Reversing the effects of ageing

Remarkably, this programme is reversible. Lifespan in yeast, flies and mammals can be extended by calorie restriction (CR) and interventions such as rapamycin addition that promote CR by down-regulating pro-growth signalling pathways (5) (Figure 4C). Genetic interventions that mimic CR from birth lead to smaller organisms, suggesting that normal growth is compromised. However, treating middle-aged mice with rapamycin resulted in reversal of the pseudo-programme after the growth phase had completed, improving longevity and alleviating age-related conditions in the mice.

These interventions do not target a single 'longevity gene' but promote small changes in the expression of a large number of genes. Both intrinsic and environmental stressors, including ageing, genome instability and

food shortage, induce survival responses to overcome the crisis and extend lifespan. This is illustrated by recent research with mouse models of DNA repair-deficient progeroid syndromes, which cause premature ageing and death. Long-lived mice share similar patterns of gene expression with progeroid mice, both showing suppression of endocrine and energy pathways in response to stress (7). Interestingly, treatment with rapamycin, inducing CR, reduces progeroid symptoms (8).

One paradox in ageing research is that exceptionally long-lived people do not have 'good genes' to explain their longevity. In fact, they have more disease-related genes in their genome than those with normal lifespans, suggesting that something is buffering or cancelling out the negative effects of these harmful genes, allowing them to accumulate. A likely protective candidate is the individual's epigenome. This is consistent with ageing being a result of a reversible pseudo-programme controlling how genes are expressed over a lifetime.

Epigenetics in longevity

The epigenome controls gene expression and DNA repair, thus influencing cell phenotype (9). Three components generally contribute to the epigenome: methylated cytosine residues; histone protein modifications; and RNA molecules. Core histone proteins in the nucleosome, the basic building block of chromatin, are subject to post-translational modifications, including reversible acetylation, methylation, phosphorylation and ubiquitylation at over 100 different sites (10). These modifications guide proteins to specific sites on the chromatin to influence gene expression or DNA repair (11). Non-coding RNA provides an alternative way to guide the modifying enzymes (12).

The enzymes that reversibly acetylate and methylate proteins, DNA and RNA require co-factors that are central intermediates in metabolism or synthesised from essential amino acids and thus are influenced by diet. These cofactors include acetyl coenzyme A (CoA), nicotinamide adenine dinucleotide (NAD⁺), S-adenosyl methionine and α -ketoglutarate. Limited periods of extreme CR, used to treat type II diabetics, change energy balances and levels of metabolic intermediates, thus influencing the epigenome and patterns of gene expression. Thus, periodic ritual fasting may reset epigenomes and contribute to better health.

Dietary quality is also likely to influence ageing and behaviour. Newborn rats deprived of maternal care show changes in patterns of DNA methylation linked to anxiety. Anxiety in these rats can be reduced by flooding their brains with nutrients that directly alter the epigenome and patterns of gene expression in the brain. Diet may therefore affect the phenotype by shaping the epigenotype.

Acetylation and ageing

In yeast, reversible protein acetylation is an important component of ageing. The NAD⁺-dependent yeast

deacetylase sirtuin, Sir2, plays an important role in replicative longevity. Sir2 deacetylates lysine (K) 16 on histone H4 to promote nucleosome stability, particularly at telomeres (13). Age-related decreases in Sir2 levels may thus lead to breakdown in chromatin structure. Interestingly, three mammalian sirtuins also deacetylate H4K16, suggesting that regulated histone deacetylation and nucleosome stability will feature in anti-ageing mechanisms in many eukaryotes. Histone acetylation is also important for nucleosome stability in the wake of elongating DNA and RNA polymerases (14). This requires dynamic acetylation at K56 on histone H3 (H3K56ac) by the Rtt109 lysine acetyltransferase (KAT) using acetyl-CoA as cofactor and the histone chaperone Asf1.

Sir2 also controls asymmetric cell division and resulting fates of mother and daughter cells (3). Here, Sir2 acts on a chaperone required for the formation of actin cables. Daughter cells are kept young by retrograde transport of damaged proteins along actin cables into the mother cell where they accumulate in aggregates (Figure 2). Extrachromosomal ribosomal DNA circles (ERCs), a toxic product of genome instability in ageing mother cells, are also inherited asymmetrically so that daughters remain ERC free. Sir2 maintains the chromatin structure at the rDNA locus to suppress the formation of ERCs, but not via H4K16 deacetylation.

The co-activator complex SAGA contains a KAT activity (Gcn5) for H3K18. Amino acid substitutions at K18 suggest that acetylation here is detrimental for chronological longevity and that control of KAT activity of the SAGA complex is important during ageing. Acetylation by the Gcn5 KAT promotes growth at the expense of longevity similarly to adenoviral-induced increases in H3K18ac in mammalian cells that promote cell cycling and transformation (15). The integrity of the SAGA complex, and thus its ability to regulate Gcn5-dependent K18ac, is controlled by Spt7. Spt7 is sensitive to oxidation and other stresses in cells. CR reduces loss of Spt7 and improves longevity.

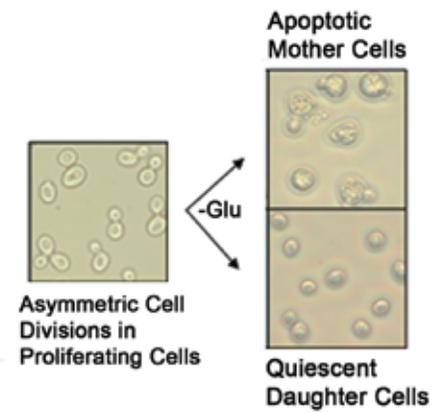


Figure 2. Glucose starved (-Glu) mother and daughter yeast cells form two distinct populations. The daughter cells become quiescent (G₀) and are able to survive for extended periods. The aged mother cells become apoptotic.

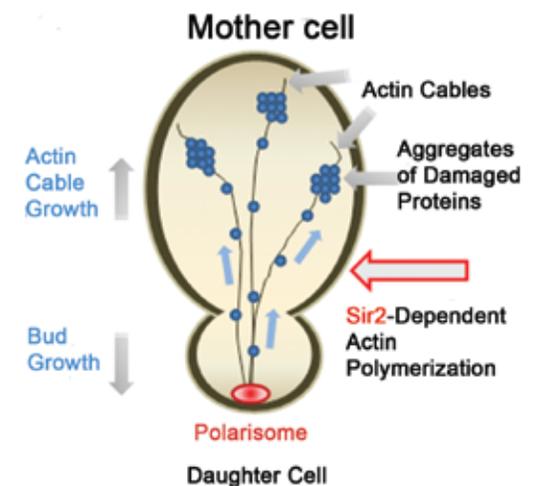


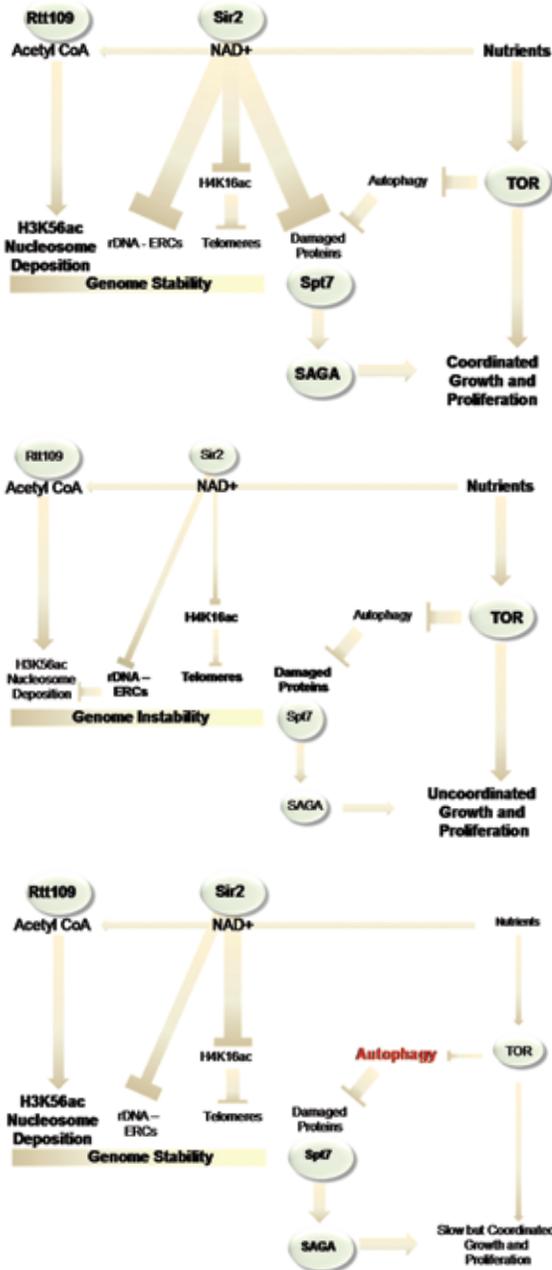
Figure 3. Asymmetric segregation of damaged protein: Sir2-dependent deacetylation of an actin chaperone facilitates the formation of actin cables and movement of damaged and aggregated proteins from the daughter cell into the mother cell.

Figure 4

A. Coordination of replicative and chronological longevity with the target of rapamycin (TOR) mediated growth programme in stem cell lineages.

B. The TOR pseudo-programme in somatic cells enhances the accumulation of damaged proteins and genome instability, contributing to ageing and death.

C. Calorie restriction increases autophagy and genome stability by down-regulating the pseudo-programme in soma and improves longevity.



Concluding remarks

In conclusion, cells that change their state, through development, differentiation or ageing, are characterised by persistent epigenetic alterations to their chromatin. An epigenetic state may remain for the life of a cell, surviving cell division and lasting for many generations, but is also changeable in disease states or during ageing. In ageing organisms, high levels of energy intake and poor levels of nutrients, in combination with the pseudo-programme promoting growth, may contribute to senescence and the onset of age-related conditions. The interesting aspect of these conditions is their reversibility, consistent with epigenetic rather than genetic changes.

A number of important questions remain to be addressed. Is there asymmetric, non-random segregation of chromosomes that contributes to fitness of the self-renewing daughter lineage? Are these marked epigenetically? Can we isolate novel small molecules to modulate the enzymes that are implicated in ageing? Exciting times lie ahead.

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