Epigenetics and aging

Jane Mellor
(University of Oxford)

Aging is the accumulation of changes in an organism over time that leads to reduced viability. Studies in model organisms indicate that aging is genetically determined and that alterations to specific genes can extend or shorten lifespan. Some organisms exhibit negligible aging with no loss of metabolic functions or fertility over time, despite a high metabolic rate, raising the question as to why and how some organisms age and die. A number of theories of aging have been proposed, including telomere shortening, wear and tear (somatic mutations, error accumulation, loss of protein function, etc.), autoimmune disease and reduced mitochondrial function leading to oxidative stress and damage to DNA and proteins[1].

While the debate about the relative importance of these mechanisms continues, there is a real need to understand the molecular mechanisms that initiate the aging process. Much can be learned about the normal aging processes by studying model organisms, cancer cells, cells of organisms that show negligible aging, such as the Red Sea urchin (*Strongylocentrotus franciscanus;* Figure 1), or cells that retain infinite replicative capacity, such as those of the germline. These studies strongly implicate epigenetic processes in the control of cellular and organismal aging and a few examples are discussed in this article.

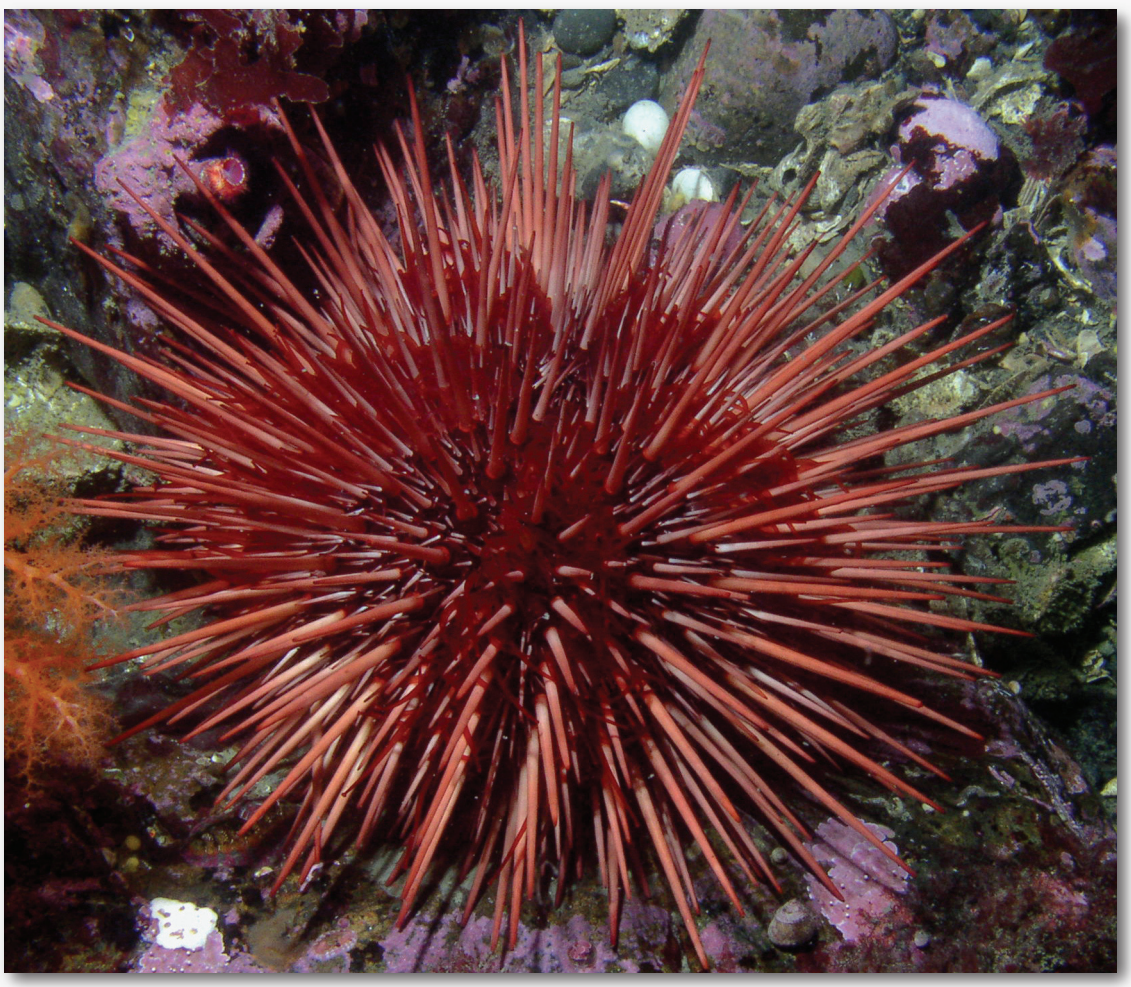

Figure 1. Red Sea urchin (*Strongylocentrotus franciscanus*)

Key words: *aging,* Apis mellifera, *chromatin,* Saccharomyces cerevisiae, *sirtuin*

Epigenetic changes

Cells that change their state through development, differentiation or aging are characterized by persistent non-genetic (epigenetic) alterations to their chromatin. There are three different components that are generally considered to contribute to the epigenome: non-coding (nc) RNA[2], histone post-translational modifications (PTMs) and, in some metazoans, DNA (CpG) methylation[3]. In this article, lysine acetylation and deacetylation by lysine acetyltransferases (KATs) and histone deacetylases (HDACs), as well as control of these enzymes by nutrient-dependent cofactors and non-coding RNA are discussed as epigenetic modulators of aging. These are illustrated using examples from the model organism budding yeast (*Saccharomyces cerevisiae*). Epigenetic control of lifespan in the honeybee (*Apis mellifera*) by the DNA methyltransferase Dmnt-3 is also discussed.

Replicative and chronological aging

In humans and other animals, aging has been attributed to the shortening of telomeres, the ends of chromosomes, eventually leading to cell death. The capacity of cancer cells for unregulated growth and division is associated with reactivation of telomerase genes and lengthening of telomeres. The length and stability of telomeres is therefore considered to be one of the features of the 'molecular clock' of replicating cells, determining the number of divisions of which they are capable. This is also called their replicative lifespan (RLS). Factors known to affect the RLS in model organisms such as yeast and the nematode worm include the sirtuins, with NAD^+-dependent protein deacetylase activity, and components of signalling pathways that respond to nutrients, particularly glucose. It is therefore not surprising that diet, specifically caloric restriction*, which leads to down-regulation of the nutrient-dependent signalling pathways, has been shown to increase lifespan in organisms ranging from yeast to mice. As discussed below, sirtuins, together with other nutrient-regulated factors, link nutrient availability and telomere stability to aging. Increased lifespan can be induced by treatment of yeast, fruitflies and mice with the macrolide antibiotic rapamycin that directly inhibits the nutrient signalling pathway regulated by the target of rapamycin (TOR) kinase, thus mimicking CR. At the molecular level, age is not measured by time, but by the number of cell doublings, so it is possible that the effects of CR could be mediated by slow cellular growth and therefore the increasing time between cell divisions.

*CR; restricting calories to 30–50% less than an *ad libitum* animal would consume, while still maintaining proper nutrient intake.

Figure 2. Budding yeast (*Saccharomyces cerevisiae*)

Indeed, in yeast, CR is known to increase survival time in non-dividing cells, known as chronological life span (CLS). Although there are some yeast-specific aspects to CLS, many features, including improved mitochondrial respiration and efficient autophagy (the process of degrading and resynthesizing cellular components), are also common features of long-lived non-dividing cells, such as neurons and muscle cells. Thus it is likely that aspects of both RLS and CLS contribute to the overall longevity of an organism.

Sirtuins, lysine deacetylation and longevity

Sirtuins are found in organisms from bacteria to humans. In mammals, sirtuins have emerged as broad regulators of cell fate and physiology with important roles in longevity and aging[4]. Their NAD^+-dependent activity links sirtuins to the metabolic state of the cell. Interestingly, sirtuins that function as deacetylases act on acetylated lysine deposited by KATs using the cofactor acetyl-CoA, another intermediate of metabolism that reflects the energy state of the cell. Acetyl-CoA synthases catalyse the conversion of acetate, ATP and coenzyme A into acetyl-CoA and AMP and are themselves regulated by reversible acetylation. The sirtuins deacetylate and activate acetyl-CoA synthases. Thus acetylation and NAD^+-dependent deacetylation co-ordinate intracellular energetics with intracellular fate, in effect linking what we eat to what we are. In yeast and fruitflies, levels of the sirtuin Sir2 increase on CR and, in sirtuin-deficient yeast and mice, extension of lifespan by CR is abolished. Studies on yeast Sir2 give a molecular insight into how this is achieved and demonstrate that Sir2 is a key regulator of lifespan.

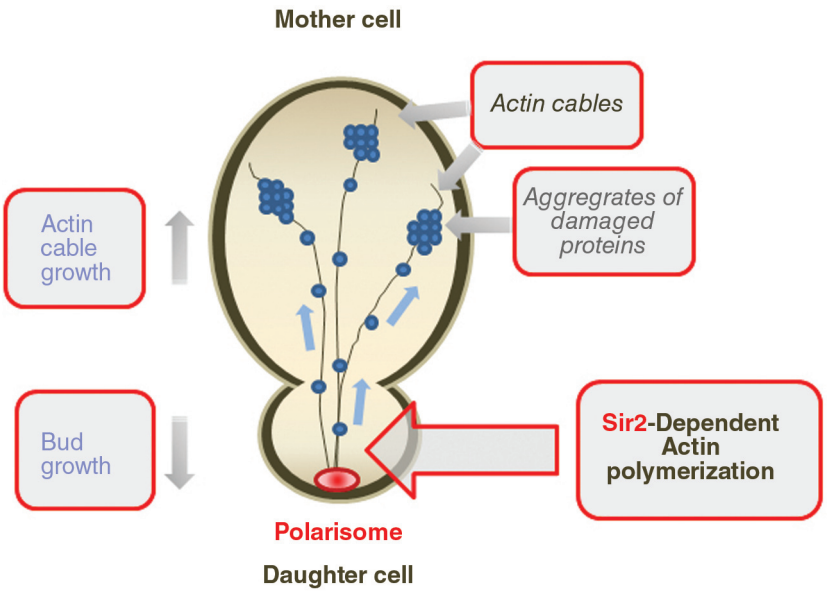

Figure 3. SIR2 protects daughter cells from aging-induced damage. Sir2-dependent deacetylation of an actin-folding chaperone is required for the formation of actin cables. Daughter cells (the bud) are kept young by retrograde transport of damaged proteins along actin cables into the mother cell where they accumulate in aggregates. In addition to damaged proteins, ERCs, a toxic product of genome instability in aging 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. This type of mechanism may be used to purge cells of the germline in animals to maintain ever youthful cells. Figure adapted from Guarente[5].

Yeast Sir2 as a regulator of cell fate

Budding yeast cells (Figure 2) divide asymmetrically to give a young daughter cell that arises from the bud (the analogue of a germ cell) and a mother cell that grows older with each budding event (the analogue of an aging somatic cell). Part of the mechanism that maintains the youthfulness of the daughter cells involves the transport of damaged protein aggregates out of the daughter bud along actin cables into the mother cell, which involves Sir2 (Figure 3[5]). Sir2 deacetylates and thereby activates a chaperone involved in actin folding, promoting the formation of actin cables[6]. The components of this mechanism are required for a long replicative lifespan. It remains to be seen whether a retrograde transport mechanism functions in organisms with *bona fide* germ cells, for example purging zygotes of the detritus of aging in each generation by the formation of polar bodies from oocytes. Sir2 also protects daughter cells from other types of aging-induced damage, including the accumulation of extrachromosomal ribosomal DNA (rDNA) circles (ERCs), a toxic product of genome instability that accumulates in aging mother cells[5]. Sir2 functions here by silencing the chromatin at the rRNA locus to suppress ERC formation, but the mechanism remains to be determined.

Yeast Sir2 as a chromatin modulator of telomere function

Sir2 also regulates the chromatin in subtelomeric regions. Interestingly, this mechanism is distinct from the role of Sir2 in suppressing ERC formation and relies on Sir2 deacetylating Lys[16] on histone H4 (H4K16)[7]. A failure to deacetylate H4K16 compromises transcriptional silencing, resulting mainly from loss of histones in subtelomeric regions, particularly in replicatively old yeast. One explanation for the breakdown in chromatin structure is an aged-related decrease in Sir2 protein levels, leading to an increase in global levels of acetylation at H4K16 by the Sas2 KAT (Figure 3). Intriguingly, three of the mammalian sirtuins also function as deacetylases of H4K16, raising the possibility that regulated histone deacetylation will also feature in anti-aging mechanisms in many eukaryotes.

The TOR signalling pathway, lysine acetylation and longevity

Down-regulation of the nutrient-dependent TOR signalling pathway by CR or rapamycin extends both RLS and CLS in yeast[8]. Although the translation machinery is likely to be a major target of the TOR pathway, balancing growth and longevity with nutrient availability, there are also significant changes in patterns of gene expression in rapamycin-treated cells, particularly up-regulation of genes promoting autophagy, stress resistance or altering mitochondrial function. The activity and composition of the Spt–Ada–Gcn5–acetyltransferase (SAGA) complex is controlled by TOR signalling and components of the complex influence CLS. As observed with Sir2, it is likely that part of SAGA's action to balance growth with longevity in response to nutrients is mediated directly through levels of acetylation of histone H3 at Lys[18] (Figure 4). Cells expressing H3K18Q, a mimic of the acetylated state, show a marked reduction in chronological longevity compared with wild-type (WT). This suggests that reduced acetylation at Lys[18] is necessary for enhanced longevity upon nutrient deprivation indicating that epigenetic modifications also function to control CLS.

Age-dependent activity of Rrp6 regulates levels of non-coding RNA, histone acetylation and gene expression

The regulated production of non-coding RNA (ncRNA), particularly antisense ncRNA, is becoming increasingly recognized as a significant factor controlling the production of sense transcripts, and thus mRNA, through epigenetic mechanisms. Many ncRNAs are subject to

degradation by the Rrp6-containing nuclear exosome. In chronologically aged yeast, there is reduced association of Rrp6 with genes such as *PHO84*, encoding a phosphate transporter, leading to increased levels of ncRNA and changed patterns of gene expression. Studies on *PHO84* indicate an epigenetic change in histone acetylation mediated by ncRNA at the promoter as a likely mechanism[9]. The increased level of antisense ncRNA leads to histone deacetylation at H3K18 by the Hda1 HDAC complex and repression of the promoter. This sort of gene-specific epigenetic mechanism is likely to compound the global changes in gene expression that result from reduced SAGA function that characterize aging cells.

Epigenetic regulation of lifespan by DNA methylation in honeybees

Honeybees show developmental plasticity in response to environmental and dietary cues that influence epigenetic mechanisms to determine whether they become workers or queens. The superior nutrient-rich diet of royal jelly fed to the larva destined to become a queen leads to increased insulin and TOR signalling, rapid utilization of nutrients and high demands for even more food. This might be expected to lead to a short lifespan for the queens, but queens survive 20 times longer than workers who develop from larvae fed on jelly with fewer nutrients. Longevity is under the control of juvenile hormone, a downstream metabolic regulator under the control of these signalling pathways that influences levels of DNA methylation at CpG islands by DNA methyltransferase 3 (Dnmt3), which in turn determines lifespan[10]. Levels of Dnmt3 are lower in queens than in worker larvae. Silencing of Dnmt3 induces queen-like traits in larvae designated to be workers, illustrating the importance of Dnmt3 in regulation of caste and longevity. Thus epigenetic regulation by DNA methylation, influencing patterns of gene expression and the quality and quantity of food play a key role in the determination of the lifespan of honeybees.

Summary

This overview provides an insight into the role played by epigenetic mechanisms in the regulation of longevity and aging by histone modifications, ncRNA and DNA methylation. All three mechanisms are known to change patterns of gene expression, but may also influence repair and recombination pathways important in longevity. These examples illustrate the plasticity in our genomes and how information from the environment that modulates our epigenome, together with genetically encoded differences in gene function, combine to determine longevity. ■

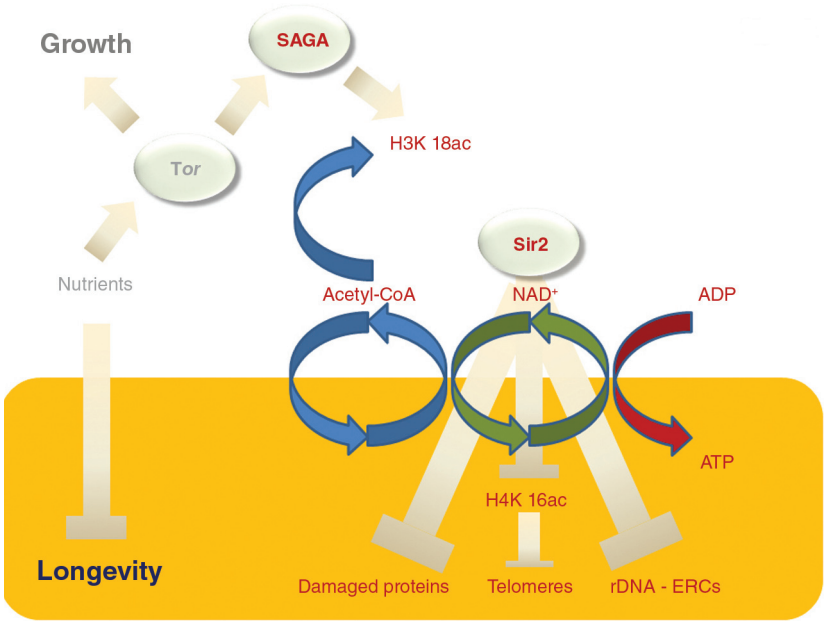

Figure 4. The metabolic state of a cell is linked to the acetylation and deacetylation of proteins, including histones, and balances growth and longevity. Two intermediates of metabolism, acetyl-CoA and NAD^+, act as cofactors for KATs and the sirtuin family of HDACs respectively. Acetylation of Lys^{18} on histone H3 by the SAGA and Lys^{16} on histone H4 by the Sas2 KAT are detrimental to chronological and replicative longevity respectively. Sir2 is required to deacetylate H4K16 to maintain telomeres and repress subtelomeric gene expression. Levels of H4K16ac increase in replicatively aged cells as the levels and activity of Sir2 decrease. In contrast, H3K18ac is controlled by the TOR nutrient-dependent signalling pathway which promotes growth by maintaining the activity of the SAGA complex. CR or the drug rapamycin lead to down-regulation of TOR and increased longevity in non-dividing cells.

Jane Mellor is a University Lecturer in Biochemistry at the University of Oxford and a scientific advisor to Oxford Biodynamics and Chronos Therapeutics Ltd. Her research focuses on the relationship between the environment and the chromatin in Saccharomyces cerevisiae *and is funded by The Wellcome Trust and Cancer Research UK. email: jane.mellor@bioch.ox.ac.uk*

References

1. Bowen, R.L. and Atwood, C.S. (2004) Gerontology **50**, 265–290
2. Bernstein, E. and Allis, C.D. (2005) Genes Dev. **19**, 1635–1655
3. Choi, J.K. and Kim, Y.J. (2008) Nat. Genet. **40**, 141–147
4. Finkel, T., Deng, C.X. and Mostoslavsky, R. (2009) Nature **460**, 587–591
5. Guarente, L. (2010) Cell **140**, 176–178
6. Liu, B., Larsson, L., Caballero, A. et al. (2010) Cell **140**, 257–267
7. Dang, W., Steffen, K.K., Perry, R. et al. (2009) Nature **459**, 802–807
8. Kaeberlein, M. (2010) Nature **464**, 513–519
9. Camblong, J., Iglesias, N., Fickentscher, C., Dieppois, G. and Stutz, F. (2007) Cell **131**, 706–717
10. Robert, L., Labat-Robert, J. and Robert, A.M. (2010) Biogerontology **11**, 387–399