Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 11034–11036, September 1998
From the Academy

This paper is summary of a session presented at the Ninth Annual Frontiers of Science Symposium, held November 7–9, 1997, at the Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering in Irvine, CA.

Aging, life span, and senescence

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Research in the field of aging recently has entered a new era. Model systems have begun to provide insight into cellular, molecular, and organismal changes that are related intimately to aging. One important approach has been the identification of genes that determine the life span of an organism. The very existence of genes that when mutated can extend life span suggests that one or a few processes may be critical in aging and that a slowing of these processes may slow aging itself.

Yeast

In the budding yeast *Saccharomyces cerevisiae*, aging results from the asymmetry of cell division, which gives rise to a large mother cell and a small daughter cell arising from the bud. Much of the macromolecular composition of the daughter cell is newly synthesized, whereas the mother's composition grows older with each cell division. By microscopic analysis, a single mother cell may be followed through multiple rounds of cell division. Thus, it was shown that mothers divide a relatively fixed number of times and undergo morphological changes of aging, including a slowing of the cell cycle, cell enlargement, and sterility (1).

A long-term genetic study of mutations altering life span pointed to the importance of the Sir protein complex, which mediates chromosomal silencing in yeast (2). Sir4p, along with Sir2p and Sir3p silence genes at yeast telomeres (the ends of chromosomes) and also the extra copies of **a** and α mating type genes on chromosome III. By immunostaining, the Sir complex is normally found in several spots at the nuclear periphery corresponding to bundles of telomeres. Surprisingly, in old mother cells, the Sir complex redistributes from telomeres and the mating type genes to the nucleolus, and this process lengthens life span by 50% (3). The nucleolus is a nuclear structure containing ≈140 tandem copies of ribosomal DNA (rDNA) on chromosome XII. The redirected Sir complex may forestall aging by counteracting some kind of cumulative damage in the rDNA.

In a separate study, the yeast homolog of the human *WRN* gene, mutated in the disease Werner's Syndrome, was knocked out. In humans, homozygous young adults missing the activity of this gene undergo many symptoms that resemble premature aging. The sequence of WRN protein indicates that it belongs to a conserved family of DNA helicases (4). The single yeast homolog, SGS1, had been previously implicated in recombination and chromosome segregation (5).

The Δ*sgs1* strain also had a shortened life span and compressed several phenotypes of aging into this truncated time frame (6). Immunostaining showed that Sgs1p itself was concentrated in the nucleolus in all yeast cells. This result suggested that a conserved mechanism of cellular aging may involve damage in the rDNA, which occur more rapidly in the absence of the WRN-Sgs1p DNA helicase. Indeed, the nucleoli in old sgs1 mother cells were enlarged and fragmented, changes that are also found in very old wild type cells.

What is the molecular basis of this fragmentation and does it cause aging? Examination of the rDNA in old cells revealed an accumulation of extrachromosomal rDNA circles (ERCs) of discrete sizes representing oligomers of the rDNA unit (7). ERCs arise from recombination within the rDNA of chromosome XII in the early part of the life span (Fig. 1). Each subsequent cell cycle, the ERCs replicate via the replication sequence in the rDNA repeats and then segregate asymmetrically into mother cells. Thus, mothers undergo an exponential increase in the number of ERCs, which may reach a copy number of 1,000 in old cells. At this level, ERCs may arrest cell growth by titrating replication factors away from the yeast genomic origins of replication. ERCs were further shown to be a cause of aging in an experiment in which they were generated artificially by a site specific recombinase. By thus releasing ERCs in very young mothers, their life span was significantly shortened.

ERC accumulation is an attractive model for aging for several reasons. First, the replication of ERCs is coupled to the cell cycle and thus the ERC copy number would be a precise clock that times the number of cell divisions. Second, ERC accumulation is gradual, potentiating a progressive change in the phenotype of the organism. Finally, the model proposes that the maintenance of the stability of repeated DNA, such as rDNA, is the Achilles' heel of cells over time. The redistribution of the Sir complex to the rDNA may act to delay the formation of the first ERC as mother cells age. It will be of great interest to assess the generality of the above aging mechanism in higher organisms.

Caenorhabditis elegans

A *C. elegans* neurosecretory system regulates whether animals enter the reproductive life cycle or arrest development at the dauer diapause stage. Dauer larvae arrest is induced by a pheromone and involves behavioral and morphological changes in many tissues of the animal: (*i*) the molting cycle is suppressed, (*ii*) feeding and growth are arrested, (*iii*) metabolism is shifted to fat storage, and (*iv*) lifespan is four- to eightfold longer than the normally 3-wk lifespan of animals, which develop as nondauers. Declines in pheromone concentration induce dauer recovery to reproductive adults with normal metabolism and lifespan.

Genes that regulate the function of the *C. elegans* diapause and aging neuroendocrine pathway were identified by dauer defective (Daf-d) and dauer constitutive (Daf-c) mutants (8). For example, *daf-2* dauer constitutive mutant animals form dauers in the absence of high pheromone levels. Conversely, *daf-16* dauer defective mutants do not form dauers under normal dauer pheromone induction conditions and suppress

Abbreviations: rDNA, ribosomal DNA; ERC, extrachromosomal rDNA circle.

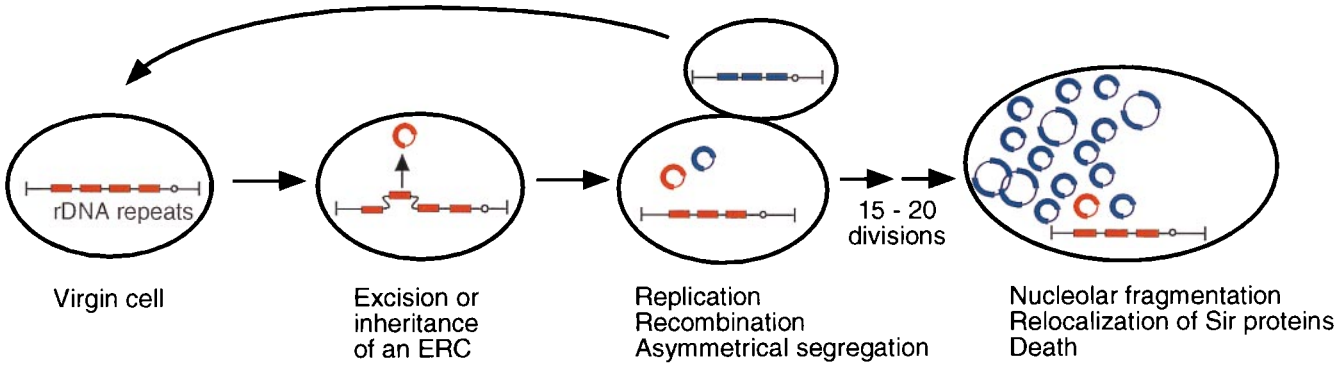

FIG. 1. Aging in yeast mother cells. ERCs excise from the rDNA array in chromosome XII, replicate in subsequent cell cycles, and segregate asymmetrically to mother cells. Aging mother cells thus experience an exponential increase in ERCs.

the dauer constitutive phenotype induced by *daf-2* mutations. These and other data indicate that *daf-16* lies downstream of *daf-2* in an ordered genetic pathway.

daf-2 encodes an insulin receptor family member (9). *age-1* is another Daf-c mutation that acts at the same point in the genetic epistasis pathway as *daf-2*. AGE-1 is closely related to a family of mammalian phosphatidylinositol 3-kinase p110 catalytic subunits (10). Phosphatidylinositol 3-kinases generate a membrane-localized signaling molecule, phosphatidylinositol P_3 that is thought to transduce signals from upstream receptors to effector molecules such as AKT/PKB. *age-1* alleles cause a dauer constitutive phenotype, which can be rescued by wild type maternal gene activity. These maternally rescued animals (genotypically *age-1*/*age-1*) show a dramatic longevity increase (11). The similar genetic epistasis behavior or *daf-2* and *age-1* mutants and the precedent from biochemical studies of mammalian homologues suggest that the DAF-2 insulin-like receptor and the AGE-1 phosphatidylinositol 3-kinase associate to transduce insulin-like signals, which induce reproductive development and a short lifespan.

Diapause arrest in general and dauer arrest in particular are associated with major metabolic changes. In wild-type animals, DAF-2 signaling activates reproductive growth, which is associated with the use of food for growth in cell number and size and small stores of fat (12). In daf-2 mutant animals, metabolism is shifted after the Larval 1 stage to the production of fat. Even when daf-2 function is decreased at the Larval 4 or adult stage (by shifting a temperature sensitive daf-2 mutant allele to the nonpermissive temperature), metabolism is shifted toward storage of fat without arrest at the earlier dauer stage.

The increase in longevity of both *daf-2* and *age-1* mutants is suppressed by mutations in *daf-16*. At the nonpermissive temperature for a *daf-2* ts allele, *daf-16*; *daf-2* double mutant animals store dramatically less fat than *daf-2* single mutant animals. Thus *daf-16* gene activity is necessary for the dauer arrest, metabolic shift, and longevity increase induced by declines in DAF-2/AGE-1 signaling. *daf-16* encodes two proteins with extensive sequence identity to three human proteins, AFX, FKHR, and AF6 of the so called Forkhead family (12, 13). Based on DAF-16 function in *C. elegans* metabolic and longevity control, FKHR, AFX, and AF6 may constitute the major transcriptional output of insulin or IGF-1 receptor engagement in mammals.

A simple model for this regulation of DAF-16 transcriptional activity is that in the absence of DAF-2/AGE-1 signaling, DAF-16 acts as a repressor of metabolic genes that mediate energy usage, molting, and growth. The repression of these genes causes a metabolic shift to energy storage and arrest of development. When the DAF-2/AGE-1 signaling cascade is activated under reproductive growth conditions, DAF-16 is phosphorylated either inactivating this repressor or changing it to an activator to now allow the expression metabolic genes necessary for reproductive development and short lifespan.

Because insulin-like DAF-2/AGE-1 signaling mediates *C. elegans* diapause longevity control, the mammalian insulin-signaling pathway also may control longevity homologously. In fact, the increase in longevity associated with decreased DAF-2 signaling is analogous to mammalian longevity increases associated with caloric restriction. It is possible that caloric restriction causes a decline in insulin signaling to induce a partial diapause state, like that induced in weak *daf-2* and *age-1* mutants.

If the association of longevity and diapause arrest is general, it is possible that polymorphisms in the human insulin receptor-signaling pathway genes, including the insulin receptor, the associated PI-3 kinase, AKT, and the human DAF-16 homologues may unlie the genetic variation in human longevity that has been noted. The association of particular alleles of these genes with long lived siblings would support this model. In this way, molecular genetic analysis of the *C. elegans* dauer pathway may identify promising candidates for the molecular determinants of human longevity.

Plants

There is a large range of lifespans in the plant kingdom. Certain tree species can live for well over a century whereas other plants complete their life cycle in a few weeks. When considering the lifespan of a particular plant species, it is important to keep in mind that there is a high rate of cell death occurring throughout the life of the plant. For example, much of the mass of a tree consists of wood, which is dead vascular tissue. Individual organs of a plant such as leaves have lifespans that can substantially differ from that of the whole organism. Moreover the lifespan of leaves from different species varies.

It is advantageous to maintain leaves only for as long as they contribute to the survival of the plant. In temperate climates, for example, the shortening days and colder temperatures of the approaching winter limit productivity and in deciduous plants triggers a massive programmed cell death that often leads to beautiful autumn colors and ultimately death and loss of the leaves.

This "yellowing" of leaves is often referred to in the plant biology literature as leaf senescence or the senescence syndrome (14). Specifically the senescence syndrome refers to the process by which nutrients are mobilized from the dying leaf to other parts of the plant to support their growth. Nutrient availability, particularly nitrogen, has been a major limit to growth and reproductive success throughout plant evolution. Moreover, plants are fixed in a particular location in the soil and deplete their local environment. Thus, there has been strong selective pressure to evolve systems to retain and recycle nutrients.

There are many factors that can initiate the nutrient-recycling program. In some species, a need for nutrients elsewhere, such as for developing seeds, can trigger the senesence syndrome (15). Another likely factor is the loss of photosynthetic productivity due, e.g., to shading by upper parts of the plant or to the accumulation of damage to leaf cells. Certain reactions of photosynthesis can generate oxidative damage, and, as in animals, different species have invested to differing extents in systems to prevent and repair such damage (14). To be consistent with the literature on animal aging, the age-related declines in leaf productivity caused by metabolism and other types of cumulative damage would constitute leaf senescence and the recycling program to salvage nutrients from the senescing leaf is the senescence syndrome.

The senescence syndrome is characterized by distinct cellular and molecular changes. The chloroplast is the first part of the cell to undergo ultrastructural changes resulting from its disassembly (the concomitant loss of chorophyll results in the characteristic color changes of leaves). Because the chloroplast contains the bulk of the nutrients in a leaf cell, it is not surprising that it is the target of the recycling program. During senescence, the mRNA level of most genes declines rapidly, but the mRNA level of specific genes (senescence-associated genes) increases during this process. Many senescence-associated genes encode proteins that accomplish parts of the recycling program such as proteases, nucleases, and proteins involved in metal binding and transport (16).

In many plant species, certain hormones can either enhance or delay senescence. For example, delayed leaf senescence is observed in *Arabidopsis* plants containing a mutation that confers ethylene insensitivity (17). Cytokinin levels decline in senescing leaves (18), and treatments that reverse or prevent senescence such as the removal of seed pods restores the flux of cytokinins to leaves (19). Thus, changes in levels of or sensitivity to the hormones ethylene and cytokinin may be part of the normal system that regulates leaf senescence.

Further support that cytokinin regulates senescence is provided by transgenic plant studies. To deliver cytokinin specifically to leaves at the onset of senescence, the promoter of one of the senescence-associated genes that is uniquely active during senescence was used to drive expression of the structural gene for an enzyme of cytokinin synthesis. When leaves of plants that contain this "engineered" gene initiate senescence, the promoter is activated, cytokinin is produced, and the leaves do not senesce (20). The system is autoregulatory: just enough cytokinin is produced to block leaf senescence because the prevention of senescence attenuates promoter activity. This autoregulatory system avoids the toxic effects that result from high levels of cytokinin.

Although genes that are expressed during the senescence syndrome as well as ways to manipulate senescence have been identified, much remains to be done to understand the molecular basis of aging in plants. For example, nothing is known about the signal transduction pathways that lead to altered gene expression during senescence or how hormones such as cytokinin influence senescence. However, we now have many of the tools to explore this process.

Perspective

It remains to be seen whether common mechanisms link the aging process in diverse organisms. We, however, can depend on the knowledge that aging mechanisms will be worked out in several systems in the relatively short term. Over the longer term we can hope to continue the tradition of biomedical research by intervening in this process to maintain our vitality and quality of life for a greater fraction of our life time.

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