PERSPECTIVE

Link between aging and the nucleolus

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Our ancient ancestors recognized events of nature, like the sporadic appearance of comets and lightning, the periodic display of stars, planets, and the moon, and biological processes like birth, development, and aging. The rapid progress of science has formulated a firm basis for understanding many of these phenomena, but aging has remained a conspicuous exception. Recently, however, a concerted effort has been made to get at the scientific basis of what cellular and molecular events might *cause* aging. This perspective will begin with an introduction into new approaches being used to study aging. The main body will focus on two independent studies in a model system that both point to the cell as a probable unit of aging and the nucleolus as a key player in the aging process. Finally, these recent findings will be placed into a context of human aging, which considers a possible interplay between telomeres and the nucleolus, as well as the relationship between dividing and nondividing (postmitotic) cells.

Background

How do we define aging? There are two reliable measures of aging, the first statistical, and the second descriptive (but amenable to quantitation). In the 1820s, a statistician, Gompertz (1825), noted that human mortality rates increase exponentially with age, giving rise to a survival curve depicted in Figure 1A. These kinetics are indicative of a progressive degenerative process and are observed in many organisms, including mammals, flies, worms, plants, and yeast mother cells. These kinetics contrast with stochastic death, for example, by predation, which would give rise to a curve similar to the decay of a radioisotope (Fig. 1B). The second definition of aging is changes in phenotype that occur in all of the individuals over time. In humans it is relatively clear whether someone is young, middle aged, or elderly by their appearance. The above definitions apply to organisms that age, and they both will be used in this paper.

How can one study aging to identify root causes? A useful analogy here is cancer. Twenty years ago, many differences between cancer cells and normal cells had been cataloged, but a fundamental cause of cancer remained elusive. Molecular causes of cancer were brought to light by the identification of oncogenic mutations in

normal cellular genes that caused a loss of growth control. Likewise in aging research, there has recently been a concerted effort to identify genes that alter the rate of aging, when mutated.

Studies of the genetics of aging are possible in model systems. In *Caenorhabditis elegans*, mutations have been identified that allow the animal to live longer than wild type. These mutations fall into two categories. The first is mutations that activate the Dauer larvae pathway (Kenyon et al. 1993). Typically, worms will enter a dormant state termed Dauer when they are nutritionally limited at a time early in development. They can exist in this state for months, much longer than their typical 2-week life span. Mutations in *daf-2* or *age-1/daf-23* (Friedman and Johnson 1988; Morris et al. 1996) can turn on the Dauer pathway at a time in development of the experimenter's choice. By activating the Dauer pathway in adults, worms can be made to live twice as long as wild type. The sequence of *daf-2* and *daf-23* reveal an insulin-like receptor (Kimura et al. 1997) and a phosphatidylinositol-3-OH (PI3) kinase (Morris et al. 1996), respectively, suggesting that the Dauer pathway may be determined by an intercellular signaling pathway that slows metabolism in Dauer animals. It is thus possible that the activation of this pathway in adults also slows metabolism, giving rise to a long life span, although metabolic effects are not readily observable in these *daf* adults.

Another class of *C. elegans* mutants affects genes termed *clk* (Wong et al. 1995). *clk-1* mutations slow down development and rhythmic movements in adults, and these mutants live longer (Lakowski and Hekimi 1996). The sequence of *clk-1* (Ewbank et al. 1997) is similar to a yeast gene, *CAT5* (Proft et al. 1995), that activates oxidative metabolism, again suggesting that the *clk-1* mutant owes its longevity to a slowing in metabolism. It is of interest that all of the cells of the adult worm are postmitotic; thus this system may be an excellent model for aging in mammalian organs consisting of nondividing cells (e.g., brain and heart).

Another model system is the budding yeast *Saccharomyces cerevisiae*. The asymmetric cell division in budding yeast gives rise to a large mother cell and a small daughter cell arising from the bud (Fig. 2). At least some of the components of the daughter, such as the cell surface, are newly synthesized during the budding process. Thus, the mother is a repository for many cellular constituents that get older with each budding. By following

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Figure 1. Kinetics of aging. (A) The survival curve of a cohort of individuals over time. The precipitous decline is attributable to the exponential increase in mortality rates over time, as first described by Gompertz (1825). (B) The curve for an organism that dies stochastically.

the mother cell through multiple rounds of division microscopically, it was demonstrated that they divided a relatively fixed number of times (Mortimer and Johnston 1959) and that they displayed Gompertz kinetics of death (Pohley 1987). Yeast mother cells may therefore be assigned an average and maximum life span, which varies from strain to strain. Furthermore, mother cells adopt characteristic phenotypes with age, such as an increase in cell size and a slowing of the cell cycle. Thus, mother cells meet both the statistical and phenotypic definitions of aging.

Using this system, Jazwinski and colleagues have identified genes that are preferentially expressed in young or old cells, and some of these, such as *LAG1* (D'Mello et al. 1994), are important determinants of life span, although their function is not yet known. The yeast *RAS* genes are another determinant of life span, suggesting that a link between metabolism and life span is also present in yeast (Sun et al. 1994).

In addition to model systems, nature may have provided another genetic clue about aging. Certain human diseases cause a phenocopy of premature aging in a variety of organ systems. These include Hutchinson–Guilford's progeria, a sporadic disease affecting young children, and Werner's Syndrome, a heritable disease affecting young adults (Salk et al. 1985). Werner's Syndrome is attributable to recessive, loss-of-function mutations in the *WRN* gene (Epstein et al. 1966; Goto et al. 1992). Werner's individuals display phenotypes including cataracts, hair loss and graying, loss of subcutaneous fat cells, aging of the skin, atherosclerosis, osteoporosis, diabetes, and cancer (Salk et al. 1985). Their average life span is ~45 years. The *WRN* gene (Yu et al. 1996) is homologous to a group of DNA helicases, including *recQ* of *Escherichia coli*, *SGS1* of *S. cerevisiae* (Gangloff et al. 1995), *rqh1* of *Schizosaccharomyces pombe* (Stewart et al. 1997), and *BLM*, mutations of which cause Bloom's Syndrome (Ellis et al. 1995). The symptoms of Werner's and Bloom's Syndromes are quite different, suggesting that some aspect of specificity of the *WRN* helicase may

lie at the core of the aging phenotypes (Lombard and Guarente 1996).

This paper will focus on one of the many promising new directions in the field of aging—a possible link between aging and changes in the nucleolus. This study is but one of many that use model systems to reveal molecular features of aging that may be relevant to human aging.

Two independent experimental paths to the nucleolus

Redistribution of the Sir complex to the nucleolus

Mutations in two genes that extend yeast mother cell life span have been characterized. One gene, *SIR4*, encodes a protein that exists in a complex with Sir2p and Sir3p, which mediates transcriptional silencing at telomeres and *HM* loci (Rine and Herskowitz 1987; Gottschling et al. 1990; Aparicio et al. 1991). Immunolocalization in yeast nuclei displays the Sir2/3/4p complex bound to telomeres in several perinuclear clusters (Palladino et al. 1993). In addition, Sir2p functions alone at the rDNA repeats to lower the rate of recombination in that tandem array (Gottlieb and Esposito 1989). Immunolocalization shows that Sir2p is present at both the rDNA and telomeres (Gotta et al. 1997).

The *SIR4-42* mutation extends life span 50% and

Figure 2. Aging in yeast mother cells. Budding yeast divide asymmetrically giving rise to a large mother cell (M) and a smaller daughter cell (D). By microscopic manipulation, the same mother can be followed through repeated divisions, and after ~20 generations, cell division ceases and the senescent cells display phenotypes of aging. Shown here are cell enlargement and the accumulation of intracellular blebs of unknown composition.

causes sterility due to loss of silencing of α and **a** mating genes at *HML* and *HMR* (Kennedy et al. 1995). A null mutation in *SIR4* also causes sterility, but actually shortens life span (Table 1). Thus, *SIR4-42* is a gain-of-function mutation for life-span extension. What function has this mutant gained? We initially argued on genetic grounds that the *SIR4-42* mutation prevented recruitment of the Sir complex to telomeres and *HM* loci and freed the complex to go to a novel genomic site important in life span. Consistent with this view, the mutation truncated the protein to remove a carboxyl terminus that may anchor the wild-type Sir complex to telomeres and *HM* loci.

Subsequent immunolocalization revealed that the *SIR4-42* complex no longer resided at telomeres but was relocalized to the nucleolus (Kennedy et al. 1997). The nucleolus is a region of the nucleus containing rRNA, proteins, and ~120–200 tandem copies of rDNA on chromosome 12. It is the site of ribosome synthesis and assembly. This finding provided an initial clue that nucleolar changes might be important in aging.

Another gene identified in the screen was *UTH4* (Kennedy et al. 1995). The expression level of *UTH4* dictates life span; null mutants have short life spans, and hyperexpression strains have unusually long life spans (Table 1) (Kennedy et al. 1997). A genetic link exists between *UTH4* and *SIR4*. The *SIR4-42* mutation is an allele-specific suppressor of a frameshift mutation in *UTH4* that is a naturally occurring polymorphism in some yeast strains. *UTH4* is homologous to two other genes in the yeast genome, *YGL023* and *YLL013*, as well as to the *Drosophila* gene *pumilio*. *UTH4* requires the integrity of the Sir complex to affect life span, indicating that it functions by regulating some aspect of the Sir complex. Indeed, the nucleolar localization of the *SIR4-42* complex is abolished in strains mutant in both *UTH4* and *YGL023* (Kennedy et al. 1997). In another study, it was found that Sir3p goes to the nucleolus when *SIR4* is deleted and that this nucleolar localization also requires *UTH4* (Gotta et al. 1997). Thus, our findings on *SIR4-42* and *UTH4* suggest the following model. Damage to the nucleolus might accumulate in mother cells to cause aging. This may be partially counteracted by the redistribution of the *SIR4-42* complex to promote longevity.

What function might nucleolar localization of Sir

Figure 3. The localization of the Sir complex (as stained with anti-Sir3p) and the nucleolus (as stained with anti-Nop1p) in mother cells of various ages. In nuclei of young cells (A), the Sir complex (green) is at the perimeter of the nucleus and is bound to bundles of telomeres (T). The nucleolus (N, red) is a discrete crescent at one side of the nucleus. After cells have completed one-half to two-thirds of their life spans and are now in middle age (B), the Sir complex has redistributed to the nucleolus (yellow in the merged image). In very old cells (C), the nucleolus enlarges and fragments into several nucleolar bodies (N') around the perimeter of the nucleus. The Sir complex is found in all of these bodies.

complexes play in the life span of wild-type cells? We speculated that the *SIR4-42* mutation mimicked and strengthened a response that normally occurred in wild-type old mother cells—redistribution of the Sir complex to the nucleolus to promote longevity. Strikingly, this turned out to be the case. When wild-type cells that had completed about two-thirds of their life span were isolated and immunostained, Sir3p was found to be redistributed from telomeres and *HM* loci to the nucleolus (Fig. 3A,B) (Kennedy et al. 1997). This finding explained our earlier observation that old cells adopt the aging phenotype of sterility as a result of a loss of silencing at *HM* loci (Smeal et al. 1996). Thus, both aging-specific sterility and longevity appear to be attributable to the same molecular event—the redistribution of the Sir complex to the nucleolus.

The yeast WRN homolog SGS1 and life span

Why do Werner's individuals show symptoms that phenocopy premature aging? It is likely that these symptoms arise at the cellular level because of one or more

Table 1. Properties of *SIR4* and *UTH4* alleles

	Sterility	Location of Sir complex	Life span
Wild type	no	telomeres	medium
$\Delta sir4$	yes	none	short
<i>SIR4-42</i>	yes	nucleolus	long ^a
$\Delta uth4$	no	telomeres	short
$\uparrow UTH4$	no	telomeres	long

^aThe extension of life span by *SIR4-42* occurs in a strain with a partially defective allele of *UTH4*, i.e., *SIR4-42* is a suppressor of this *uth4* allele (Kennedy et al. 1997).

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defects in chromosome biology (Guarente 1996). This view suggests that aging may be caused by defects that occur at the cellular level rather than a breakdown solely in some organismal (e.g., endocrinological or immunological) process. As one approach to identifying the cellular function of *WRN*, the study of *WRN* homologs in model systems offers promise. The yeast homolog *SGS1* was identified because loss-of-function mutations suppress the slow growth in *top3* (topoisomerase 3) mutants (Gangloff et al. 1994). Sgs1p was shown to bind to topoisomerases 2 and 3 and to affect recombination (including at the rDNA loci) and chromosome segregation (Gangloff et al. 1994; Watt et al. 1995, 1996).

To determine whether *SGS1* was involved in yeast life span, the gene was disrupted (Sinclair et al. 1997). Mutants were found to age prematurely by three criteria: (1) Their average life span was shortened by 60%; (2) mother cells became sterile over their shortened life span as a result of loss of silencing at *HM* loci; and (3) the Sir complex redistributed to the nucleolus in old cells. Premature aging was specific to *sgs1* mutants because other randomly chosen yeast mutations either did not shorten life span, or, if they did, did not cause the appearance of sterility in aging mother cells.

Sgs1p was immunolocalized to the nucleus, with a concentration in the nucleolus (Sinclair et al. 1997). This prompted an examination of the nucleoli in old *sgs1* mothers. Whereas in young *sgs1* or wild-type cells the nucleolus is a crescent-shaped structure at one side of the nucleus, strikingly, in old *sgs1* mother cells the nucleolus was enlarged and fragmented (Fig. 3C) (Sinclair et al. 1997). These changes caused the nucleolus to occupy at least 50% of the nuclear volume in most cells. In cells with these fragmented nucleoli, the Sir complex redistributed to all of the nucleolar bodies. Significantly, similar changes were found in nucleoli of very old wild-type cells, indicating that they are not a consequence of the *sgs1* mutation per se. Thus, like the analysis of the Sir complex above, the study of *SGS1* also suggests that nucleolar changes might be important in the aging of yeast mother cells.

Finally, the relationship between the fragmentation of the nucleolus and the redistribution of the Sir complex was examined. Assuming that the fragmentation of the nucleolus is relevant to aging, there are two extreme possibilities. The first is that the redistribution of the Sir complex *delays* the fragmentation of the nucleolus and thus provides longevity. The second possibility is the redistribution of Sir proteins *causes* the fragmentation of the nucleolus, and these fragments do not cause aging but promote longevity. Two experiments suggest that the first possibility is correct (Sinclair et al. 1997). In one experiment, old cells were examined in a *sir3* mutant, and the nucleoli were found to be fragmented. Thus, fragmentation does not require the Sir complex. In the second experiment, age-matched cells of 12 generations were obtained from wild-type and *sir3* mutant strains, and fragmentation was found much more commonly in the *sir3* mutant. This finding suggests that the Sir complex could function to delay fragmentation in wild-type cells.

A model for aging in dividing cells—the nucleolus and telomeres

The above findings begin to provide a model for aging that draws on the idea that cells undergo cumulative damage to chromosomes, most likely focused on the rDNA (Guarente 1996). The rDNA may be the Achilles' heel of the cell because it comprises a potentially unstable array of tandemly repeated DNA sequences. A loss of the ability to maintain this stability may lie at the heart of aging. Evolution may have selected for counteracting response mechanisms to forestall this damage and promote longevity, as exemplified by the redistribution of the Sir complex to the nucleolus in aging yeast mother cells. The life span of an organism is then the sum of the rate of accumulation of this damage and the robustness of the response mechanism (Guarente 1997).

Three key questions must be addressed in the near term regarding this mechanism of aging. First, what is the precise nature of the changes that occur in the yeast nucleolus, and how might these alterations cause aging of mother cells? Second, how does the redistribution of the Sir complex to the rDNA forestall these changes? Third, how general are these mechanisms in the aging of other organisms, particularly in mammals?

A telomere-rDNA balancing act

The functional role of Sir protein positioning at yeast telomeres has remained obscure. No telomeric yeast genes are known to be subject to silencing in wild-type strains. In contrast, silencing at the rDNA does occur and is mediated by Sir2p (Bryk et al. 1997; Smith and Boeke 1997). It has been proposed that telomeres might serve as a reservoir or sink for the silencing factors (Maillet et al. 1996; Marcand et al. 1996). Our findings suggest that these reserves of the Sir complex are released from telomeres as yeast cells age to provide longevity (Fig. 4). Indeed, if telomere length is manipulated to a new steady-state length by genetically altering telomerase or the telomeric protein Rap1p, we find that shortened telomeres *increase* life span and elongated telomeres *decrease* life span (Austriaco and Guarente 1997). We imagine that the shortened telomeres facilitate the redistribution of the Sir complex, whereas elongated telomeres have the opposite effect. However, because yeast telomeres do not normally shorten in aging mother cells (D'Mello and Jazwinski 1991; Smeal et al. 1996), we imagine that there must be some novel mechanism causing redistribution of the Sir complex. One possibility is that old cells signal a post-translational modification of one or more Sir proteins that prevents their recruitment to telomeres and *HM* loci and thus elicits redistribution to the rDNA (Fig. 4).

In human dividing somatic cells, telomeres *do* shorten with age because the soma lacks telomerase (Alsopp et al. 1992). We have suggested that this shortening may aid in the release of telomere-bound proteins to another chromosomal locus, perhaps the nucleolus, to promote longevity (Austriaco and Guarente 1997; Kennedy et al.

Figure 4. Model for redistribution of telomeric proteins to the rDNA (at the nucleolus) in yeast and humans. In young yeast cells, the Sir complex (green ovals) is located at telomeres (broken line) and *HM* loci. In old cells, these proteins redistribute to the rDNA, perhaps because of some post-translational modification in a Sir protein (black dots). This redistribution causes a lengthening in life span, attributable to localization at the rDNA, and sterility, because of departure from *HM* loci. In humans, we hypothesize that telomere-binding proteins (red rectangles) might also redistribute from telomeres to the rDNA in aging individuals because of telomere shortening. By analogy to yeast, this could promote longevity and elicit phenotypes of aging.

1997) (Fig. 4). It is also likely that very long-lived animals lack telomerase (and thus undergo telomere shortening) as a safeguard against the unrestrained growth of tumor cells. In mice, which only live about 2–3 years, telomeres can be very long and shortening has not been observed in aging animals. It would be of interest to investigate whether telomere shortening occurs in other very long-lived animals, such as Galapagos turtles.

Aging in mammals—dividing vs. nondividing cells

What does the information that has emerged from yeast and *C. elegans* tell us about human aging? It is possible that aging in humans will be divided into two mechanistic categories that apply to organs with dividing cells versus postmitotic cells. In *C. elegans*, in which all of the adult cells are postmitotic, aging studies have pointed to the importance of metabolic rate and, perhaps, oxidative damage (Johnson 1996; Ewbank et al. 1997). Thus, it is possible that aging in the mammalian brain and heart is elicited by oxidative damage that accumulates over time, perhaps provoking the gradual death of cells.

Aging in yeast mother cells provides a rather different picture, in which changes in the nucleolus appear to be paramount. The fact that mutation in the yeast *SGS1* gene, a homolog of *WRN*, causes premature aging and nucleolar changes in yeast mother cells (Sinclair et al. 1997) suggests that phenocopies of premature aging can be elicited in both yeast and humans by the same mechanism. Werner's Syndrome has been termed a segmental aging disease because only some organs are affected (Salk et al. 1985). Interestingly, affected organs generally consist of dividing cells, and at least one organ with post-mitotic cells, the brain, is not affected in Werner's individuals. Thus, aging in dividing cells of normal mammals may be caused at least in part by a mechanism involving changes in the rDNA.

Finally, it has been noted that cultured human cells (fibroblasts) divide a relatively fixed number of times and then senesce (Hayflick 1965). Interestingly, fibroblasts from a variety of different animals divide a number of times roughly proportional to the life span of the animal (Finch 1990). Thus, it has been proposed that in vitro senescence of primary fibroblasts is a model for aging. This view is buttressed by the fact that cells from Werner's individuals divide fewer times in culture (Tollefsbol et al. 1984; Salk et al. 1985). In addition, cellular markers of senescence have been reported in both senescent fibroblasts and elderly animals (Campisi 1996). Although aging in cultured cells is an attractive model, there are reasons to believe that fibroblast senescence may be mechanistically distinct from aging in the animal. One incongruity is that cells from the severe Hutchinson-Guilford's progeroids divide more times in vitro than cells from Werner's individuals (Tollefsbol et al. 1984). Furthermore, fibroblasts from individuals with other nonaging-related diseases can display a reduced number of cell divisions in vitro (Weksberg et al. 1979). Thus, it remains to be demonstrated that there is a mechanistic relationship between in vitro senescence and aging.

Perspective

A pervasive view holds that aging is a many-faceted process that will not conform to any single mechanistic cause. A corollary of this view is that many cellular processes break down at roughly the same time because there has been no selection for any greater duration of maintenance. Studies in the model systems of yeast and *C. elegans* argue against this idea because mutations in single genes can exert substantial extensions in the life span of the organism. Thus, there may be a limited number of cellular events that figure most prominently in aging, perhaps including fragmentation of the nucleolus. Once the Achilles' heel(s) of the cell has been precisely identified, one can imagine interventions to slow changes that occur with aging. Meeting the challenges of aging and its accompanying infirmities has been a primary undertaking of biomedical research in the twentieth century. A search for the causes of aging will continue this mission into the next century. The spirit of Ponce de Leon lives on!

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