

Progeroid syndromes: probing the molecular basis of aging?

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Abstract

A valid method of studying age related degenerative pathologies is to study human genetic diseases that appear to accelerate many, though not necessarily all, features of the aging process. Such diseases are described as progeroid syndromes because of their possible relevance to many aspects of aging and age related disease. This article describes the recent progress made at the cellular and molecular levels in understanding the pathogenesis of one of the best characterised of these disorders, Werner's syndrome. These observations are related to some of the less well characterised progeroid syndromes within the context of the cell senescence hypothesis of aging, a theory formulated to explain the aging of regenerative tissue in normal individuals.
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With the proportion of the elderly within the population increasing dramatically the effects of the aging process are already the major item of expenditure for the National Health Service and will become the major health care challenge of the next century.¹ Aging is associated with a subset of diseases including potentially fatal cardiovascular disorders, diabetes, and neoplasms, and crippling conditions such as cataract, macular degeneration, and auditory impairment, which greatly reduce the quality of later life. Understanding the mechanisms of human aging may therefore give important insights into the pathogenesis of a range of age related diseases. It may also allow age related diseases to be attacked at their roots by disabling the aging process itself, such a preventive approach being attractive for more than simple economic reasons.

Human aging, distal and proximal causes, and model systems

"Why do we die of old age?" is perhaps the most fundamental physiological question it is possible to ask. Before any discussion of this process is undertaken it is helpful to distinguish the separate questions of "why do we age?" and "how do we age?". To the question of why perhaps the most persuasive argument is that offered by the disposable soma theory of Kirkwood and Holliday² that explains aging as the inevitable result of the optimisation of evolutionary fitness in animal populations. In this

model, aging occurs because insufficient physiological resources are allocated by the body to somatic repair and maintenance. This model thus rules out a central "clock" that controls the process. Such a central programme would require resources to operate and this very process would compromise reproductive efficiency. It should however be emphasised that the lack of a mechanistic aging programme does not indicate the lack of involvement of programmed mechanisms in aging (such as development or tissue repair). Indeed, one such mechanism, the senescence of individual human cells, has been proposed as a key process in the age related failure of regenerative tissue³ and will be discussed in detail later.

As an evolutionary side effect of millions of years of selection for reproductive success the genetic basis of aging is potentially extremely broad. Estimates of the number of genes that may have a role in determining lifespan in *Homo sapiens* have ranged from several hundred to tens of thousands (upwards of 7% of the total human genome⁴). With such a highly polygenic system, to suggest that any single mechanism or simple network of mechanisms is responsible for the aging of a whole organism is rather naive. Applying the same logic the identification of candidate genes involved in successful aging through the study of normal centenarian "survivor" populations, while potentially feasible is likely to prove an extremely complicated task.^{5,6} An alternative approach, first articulated as a formal concept by George Martin, is the study of heritable genetic diseases that mimic some, but not all, the features of the aging process, to gain insights into how the aging process functions in normal individuals. The study of such progeroid syndromes (like premature senility) has the advantage that only a single gene is usually involved in each case. This renders hypotheses easier to frame and test and makes the potential identification of the defective allele amenable to modern reverse genetics and gene mapping studies in a way that the study of polygenic traits is not. The disadvantage of studying progeroid syndromes is that they are essentially phenocopies of normal aging rather than the genuine article, any observations must thus be evaluated within the context of theories designed to explain how normal aging operates. One such theory is the cell hypothesis of aging.

Cell hypothesis of aging

A priori the simplest question that can be asked regarding the operation of the aging process is

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whether the mechanism of aging functions at the level of the organism or of the cells that compose it. For the first half of this century it was generally believed, based on experiments conducted in the laboratory of the Nobel laureate Alexis Carrel,⁷ that individual cells were immortal once placed into culture and that the mechanism of the aging process thus represented a failure of the organisation rather than the component parts. However, in the 1960s a series of experiments by Hayflick and Moorehead^{8,9} demonstrated that normal human fibroblasts would only proliferate for a fixed number of passages during which the population would double in number about 50 times. The number of "population doublings" a culture would undergo appeared to be fixed by a mechanism internal to the cells that composed it. This theory was based on experiments in which cells from populations with different growth abilities were cocultivated and on the observation that cryopreservation in liquid nitrogen did not cause the culture to "forget how old it was".¹⁰ After completing its quota of population doublings the culture would be entirely composed of cells in a non-dividing state, which Hayflick termed "senescence". Based in part on the observation that embryonic fibroblasts completed more population doublings than neonatal cells, Hayflick proposed that senescence was linked to the aging process. These observations have been confirmed by hundreds of reports involving every renewing or conditionally renewing normal mammalian cell type that has been subjected to rigorous investigation. In addition a number of observations has emerged that support Hayflick's original contention that cell senescence and aging are associated (table 1).

How do cells become senescent?

The original description of *in vitro* fibroblast growth formulated by Hayflick assumed that the cultures studied were composed of homogeneous populations of cells that were either all growing or all senescent and that the failure to grow was a result of cell death. Both these assumptions, while initially sensible, were rapidly shown to be incorrect. Early work on RNA synthesis in growing and senescent fibroblasts showed that tritiated uridine incorporation occurred in the cells regardless of age,²⁵ senescent fibroblasts were thus alive and metabolically active. Subsequent pulse labelling experiments performed at every passage with tritiated thymidine and designed to pick up viable cells that never divided showed that unlabelled cells were present in very young cultures and that labelled cells were present in very old cultures.²⁶ These cell kinetic experiments showed that primary cultures *in vitro* are bimodal mixtures of dividing and senescent cells. The proportions of which alter as the cells are serially passaged. A simplified version of the two kinetic models for cell senescence is shown in fig 1.

This behaviour and related work based on the analysis of clone sizes through the life spans of human fibroblast²⁷ and glial cell²⁸ cultures is consistent with a counting mechanism that

Table 1 Evidence linking senescence with aging

Evidence	Reference
Human cell cultures show a decline in growth potential with advancing donor age	11-17
The proliferative lifespan of fibroblasts in culture correlates strongly with the lifespan of the species from which they were taken	18
Calorie restriction extends the lifespan of whole organisms and leads to a reduced number of senescent cells in lens epithelium <i>in vivo</i>	19
Cultures of vascular endothelial cells derived from atherosclerotic arteries show a greatly reduced lifespan compared to autologous cultures derived from veins	20
Organ cultured corneas show a strongly age linked increase in the number of senescent cells in the endothelial layer	21
The pattern of gene expression in senescent cells <i>in vitro</i> is consistent with the development of age related degenerative disease <i>in vivo</i>	22, 23
The number of senescent cells observed within dermal tissue sections increases in a strongly age dependent manner	24
The proliferative lifespan of fibroblasts from donors with progeroid syndromes is significantly reduced	Present article

operates at each cell division. This mechanism contains within it a strong stochastic (chance) element. Essentially at each cell cycle the cell has a chance of committing to senescence or continuing to divide with the relative probability of each event dependent on the number of times the cell has divided before.^{29,30} The mechanisms responsible for this process are gradually being characterised and have mostly built upon a series of cell fusion experiments which demonstrated: (1) that the phenotype of senescence was dominant over that of growth in synkaryon fusions between growing and senescent human fibroblasts³¹; (2) that senescence was genetically active and not the result of random damage following fusions between senescent cells derived from different cultures,³¹ and heterokaryon experiments between young and senescent cells, which showed

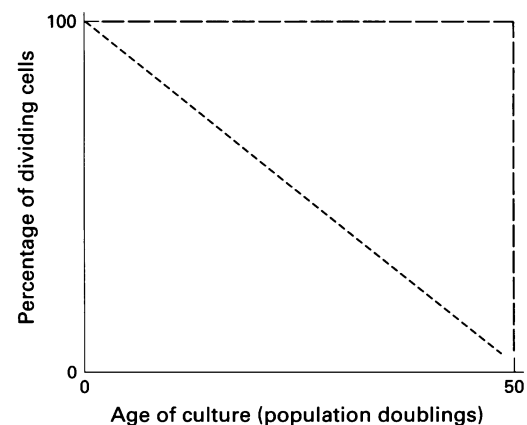


Figure 1 Simple schematic of the difference in kinetic behaviour between a unimodal (dashed line) and a bimodal (dotted line) population. A unimodal population would display a fixed fraction of dividing cells until the very last population doubling when they would enter senescence simultaneously. In contrast a bimodal population has senescent cells present from the start. Analyses of gene expression within the culture such as northern blots thus give an average for the population and can be misleading unless the fraction of dividing cells is known.

that senescent nuclei or cytoplasm could inhibit DNA synthesis in the young nucleus³²; (3) that synkaryon fusions of immortalised cells gave rise to clones that underwent senescence, and that the fusion partners in these studies could be assigned into four distinct complementation groups.³³

The principal molecule involved in bringing about senescence in fibroblasts appears to be the p21 inhibitor of the cdk2–cyclin complexes.³ p21 was originally identified as a cell senescence derived inhibitor in an expression screen.³⁴ Recently evidence has also emerged that the p16 INK4 inhibitor may also repress cdk4–cyclin D kinase activity.^{35 36} Both cyclin D kinase inhibitors and their upstream regulatory genes, such as p53, are involved in a wide range of cell death and differentiation processes.³⁷ However, cell senescence is not an apoptotic phenomenon and has recently been shown to be a distinct process from terminal differentiation.³⁸ These molecules appear to be the effectors of cell senescence rather than the trigger, which must be sought at a higher level of cell control.

One of the most plausible systems by which cells may count their divisions to have been

suggested in recent years is the progressive loss of telomeric sequences from the ends of chromosomes. In the absence of any mechanism to produce compensatory de novo synthesis of telomeric DNA, these chromosomes are faced with what has been termed the “end replication problem”.³⁹ This results from the inability of all known DNA dependent DNA polymerases to commence synthesis de novo. At the termini a region at least as large as the priming RNA primer for lagging strand DNA synthesis will systematically be deleted every time the cell undergoes division. In contrast, in most immortal human cell lines there appears to be compensatory de novo synthesis of telomeric DNA by the enzyme telomerase and a stable maintenance of telomere length.^{39–41}

In most primary human cells telomerase is absent, and telomere shortening with proliferation in vitro has been reported.^{40–43} This shortening is presumed to reflect losses from a failure in end replication, although the problem of C strand degradation may also be shown to contribute.⁴⁴ An analogous decline in telomere length with donor age has been reported for a variety of human tissues.^{40 45} As the loss of telomeric DNA is linked to passage through S phase it forms an attractive mechanism by which cells can count divisions. However, as all the components of the telomerase enzyme have yet to be cloned, interventional tests of the hypothesis are not yet possible.

Why does senescence matter?

Once senescent, human cells display a variety of characteristics that are best broadly described as a cessation of DNA replication under normal conditions, and an altered spectrum of gene expression. This is best illustrated in adherent cells where a dramatic shift in cell size usually also accompanies the onset of senescence (fig 2). It should be noted that senescence is a highly active but selective process, some genes are repressed, some genes are upregulated, and some totally novel messages are induced. This is perhaps best illustrated with respect to the *c-fos* proto-oncogene. Repression of *c-fos* is a characteristic event in the replicative failure of T cells⁴⁶ and fibroblasts⁴⁷ (although it does not occur in senescent melanocytes³). However, a number of other cell cycle associated genes (including *c-myc* and *H-ras*) are not repressed.⁴⁸ Table 2 lists a number of the principal changes identified in senescent cells. Many more have been reported.⁴⁹

Linking cell senescence and progeroid syndromes

Given the contention that cell senescence is linked to the normal aging process the ability of cells from progeroid donors to grow in culture has been studied for over 30 years. These studies have principally involved the segmental progeroid syndromes (that subset of progeroid syndromes in which multiple aspects of aging are mimicked) rather than the unimodal type (in which a single tissue or process is subjected to accelerated “aging”, exemplified by Alzheimer’s disease). A reduction in the ability of

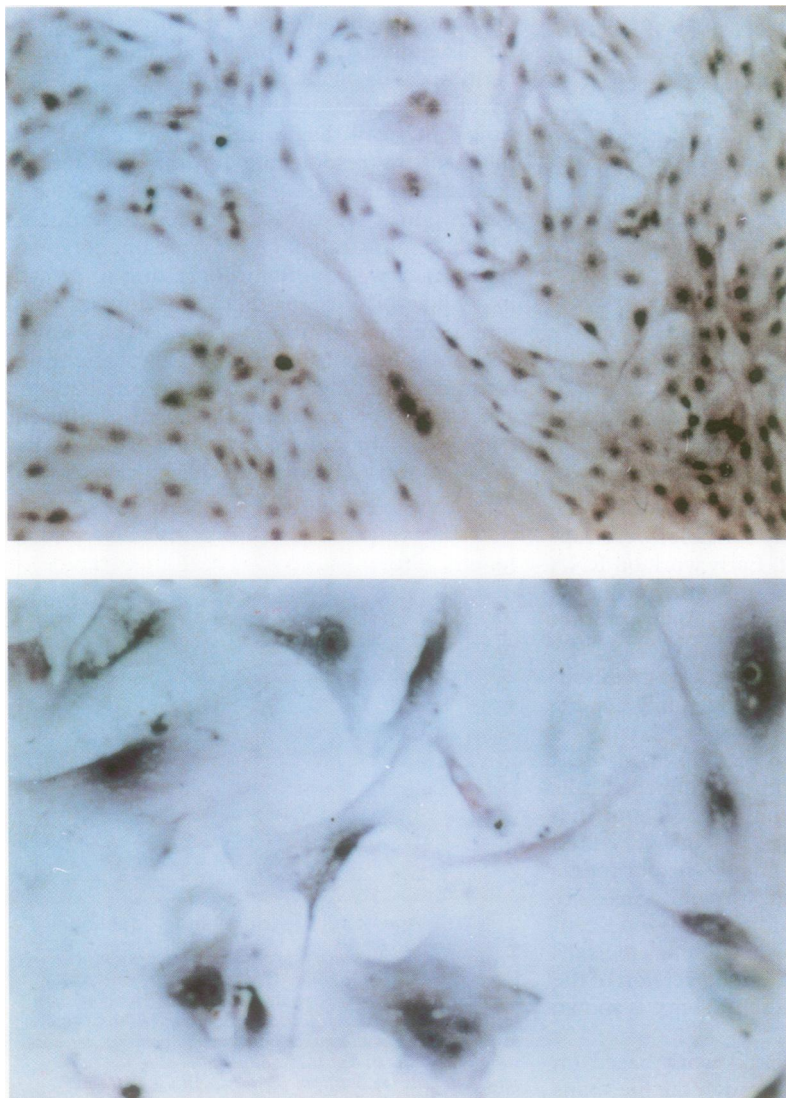


Figure 2 Haematoxylin and eosin staining of (top) young and (bottom) senescent mesothelial cells (original magnification $\times 400$).

Table 2 Selected alterations in cell phenotype with the onset of senescence

Phenotypic alteration in senescence	Cell type	Reference
Repression of c-fos	Fibroblasts, T lymphocytes	46, 47
Repression of cyclin A & B	Fibroblasts	35
G ₂ arrest on restimulation without division	Fibroblasts, T lymphocytes	50, 51
Elevated collagenase	Fibroblasts	52
Elevated TIMP 2	Fibroblasts, endothelial cells	53, 54
Elevated PAI-1	Fibroblasts, endothelial cells	54
Elevated ceramide	Fibroblasts	55
Transcriptional repression of IGF-1 gene.	Fibroblasts	56
Specific induction of Ws3.10 inhibitor of Ca ²⁺ dependent membrane currents	Fibroblasts	57
Elevated IL-1a expression	Fibroblasts	58
Senescence associated β galactosidase activity	Fibroblasts, keratinocytes, mammary epithelial cells, endothelial cells, neonatal melanocytes	24
Specific induction of SAG gene	Fibroblasts	59
Elevation of cytochrome b and NADH 4/4L subunit	Fibroblasts	60

fibroblasts to proliferate relative to normal controls has been reported for trisomy 21, Rothmund-Thompson syndrome, ataxia telangiectasia, and Hutchinson-Gilford progeria.⁶¹⁻⁶³ The failure of fibroblasts to grow has however been most heavily studied in Werner's syndrome where the reduction is particularly severe and the molecular basis of the disease is now especially well understood.

The principal features of Werner's syndrome are shown in table 3 and have been reviewed previously.⁶⁴ The clinical changes are accompanied by a truly striking reduction in growth potential best exemplified by a study of 20 Werner's syndrome cell strains derived from three patients that demonstrated that the average population doublings of the strains were only 27% of the average population doubling obtained from 10 control strains. No overlap was observed between the population doubling of the longest lived Werner's syndrome strain and the shortest lived normal strain.⁶⁵ Literature review and a range of other studies confirmed these results and showed that Werner's syndrome cell strains have a 70% reduction in their expected lifespan in culture, it was also observed that more than 90% of Werner's syndrome cell cultures fail to accomplish 20 population doubling in vitro.⁶³

The mechanistic basis of this behaviour remained unclear until the cloning of the Werner's syndrome gene (WRN) in 1996. However, molecular cell biology studies provided two complementary pieces of data. Kinetic studies, which compared the rate of loss of the fraction of dividing cells in Werner's syndrome and normal fibroblast cultures, showed that the cause of the short culture lifespan in Werner's syndrome was a threefold to fivefold accelerated rate of exit from the cell cycle.^{66, 67} Put simply, the probability of any dividing Werner's syndrome cell entering senescence was three to five times more probable than an equivalent control fibroblast. Simultaneously it was demonstrated that both immortalised Werner's syndrome cell lines and primary cell strains displayed a "mutator phenotype", a greatly elevated mutation rate when placed under selection conditions for the emergence of HPRT[-] clonal variants. Further analysis showed that the HPRT[-] muta-

tions in Werner's syndrome were characterised by extremely large DNA deletions compared with controls.^{68, 69} This evidence suggested a loss of function mutation which gave a detectable in vitro phenotype involving DNA recombination.

A systematic attempt to clone the gene responsible for Werner's syndrome was undertaken in the late 1980s. Initial efforts based on linkage analysis localised the disorder to chromosome 8p12.⁷⁰ Subsequent positional cloning identified the WRN gene⁷¹ in 1996. These studies demonstrated that WRN encoded a putative protein of 1432 amino acids with a central region approximately 60% homologous to the recQ gene of *Escherichia coli*, the F18C5C gene of *Caenorhabditis elegans*, and the human RECQL gene. On this evidence, WRN is believed to encode a novel helicase. WRN is expressed fairly widely in muscle, fibroblasts, transformed B cell lines, heart, pancreas, and placenta. Neural expression was not demonstrated correlating with a lack of unusual neurological pathology in Werner's syndrome patients.⁶³ Most recently the complete intron-exon structure of WRN was determined.⁷² The gene has 35 exons and an unusual exon duplication within the helicase domain that appears to be a relatively recent event. WRN can essentially be broken down into three regions; a 5' region (codons 1-539) that contains the duplication and has no significant homology to any other genes (although it contains long acidic stretches similar to those seen in the XPB helicase); a central region (codons 540-963) that is highly homologous to the DExH-box DNA and RNA helicases; and a 3' region (codons 964-1432) with no close homology to any other gene and as yet no clear potential function. Eighty three per cent of the mutations within the Japanese Werner's syndrome population (two splice junction and two nonsense mutations) were located within this region and left the helicase domain intact.⁷² More recently, mutations within the helicase region have been identified. The cell strain (2Br.WS) carrying one of these mutations (a CGA→TGA nonsense at codon 889) was previously used in kinetic studies and gave an extremely rapid rate of senescence.⁶⁶ A series of subtractive hybridisation studies have identified a number of genes in senescent Werner's syndrome cells that are also upregulated in senescent normal fibroblasts.^{73, 74} It thus appears that loss of function mutations within the WRN helicase can induce premature entry into senescence in fibroblasts. The presence of such senescent

Table 3 Clinical feature of Werner's syndrome

Diagnostic inclusion criteria for Werner's syndrome
Autosomal recessive inheritance
Bilateral cataracts
Dermatological pathology (tight skin or ulceration)
Short stature
Premature greying/thinning of hair
Diabetes mellitus
Hypogonadism
Osteoporosis
Mesenchymal or other rare neoplasms
Flat feet
High pitched or hoarse voice

cells within the bodies of Werner's syndrome patients would explain many of the pathological features of the disorder, and strongly support the cell hypothesis of aging.⁷⁵ However, while the identification of the defective Werner's syndrome gene is a triumph of molecular biology, there are some provocative areas of Werner's syndrome cell biology to be investigated.

Why was WRN never complemented in cell fusion experiments?

Werner's syndrome is a classic autosomal recessive mutation, and cells from obligate Werner's syndrome heterozygotes show a normal rate of exit from the cell cycle when analysed in kinetic studies, indicating that a single wild-type WRN allele is sufficient for normal function. However, cell fusion experiments between early passage young and Werner's syndrome fibroblasts failed to produce growing hybrids, instead a lifespan closer to the Werner's syndrome parent was obtained. While many of the fusions in these studies would have involved the fusion of senescent Werner's syndrome fibroblasts to growing young cells (due to the bimodal nature of the populations and the accelerated exit rate of Werner's syndrome cultures) several of the studies were conducted with Werner's syndrome cultures of such low population doubling that a high fraction of cycling cells should have been present.⁶⁵ Why was WRN not complemented?

Recent patch clamp analysis of Ca²⁺ dependent potassium currents of individual growing Werner's syndrome and normal fibroblasts from our laboratory suggest that growing Werner's syndrome cells are not exactly the same as their normal counterparts.⁷⁶ Evoked currents in the Werner's syndrome fibroblasts displayed a different reversal potential to that of the normal controls, and the cells registered a larger capacitance (size) although they were not senescent. A possible explanation for this behaviour is that mutation of WRN increases the rate at which cells enter "commitment"—a cell kinetic phase between simple growth and senescence that has been postulated but never formally demonstrated.⁷⁷ Alternatively, WRN may effect some unknown characteristic that prevents simple complementation of the helicase. A failure to repress *c-fos* at senescence has been reported in 2Br.WS,⁷⁸ which suggests that senescence in Werner's syndrome cells may be subtly different from that in normal cells (although the presence of a transient "restimulatable" phase at senescence renders this experiment difficult to interpret⁷⁹). A series of subtractive cloning studies carried out in senescent Werner's syndrome fibroblasts have identified genes also overexpressed in senescent normal cells, which strongly suggests that the Werner's syndrome growth arrest is essentially true cell senescence.^{57 73 74} It is thus pertinent to enquire how a helicase-type mutation may trigger cell senescence.

Does DNA damage cause senescence?

The concept that defective DNA repair is linked to the aging process is not a new idea.⁷⁹

The discovery that WRN is a mutant helicase associated with a mutator phenotype potentially strengthens the association. However, defective DNA repair has not been unequivocally observed in Werner's syndrome either in studies using classic DNA repair assays such as unscheduled DNA synthesis⁸⁰ and mutagen survival studies,⁸¹ or more sophisticated assays for strand specific or gene specific repair.⁸² Similarly, cells from donors with classic DNA repair defects such as xeroderma pigmentosum do not show accelerated senescence, although a mutator phenotype is present in at least some complementation groups.⁸³ Instead a defective ability to repair ultraviolet damage products is present. Cockayne's syndrome, a highly placed candidate for a segmental progeroid syndrome, has three distinct complementation groups, one of which (group B) is due to a defective helicase (ERCC6).⁸⁴ In this syndrome excision repair of ultraviolet damage is essentially normal but the cells show a failure of RNA synthesis recovery following ultraviolet exposure.⁸⁵ To complicate matters further overlapping xeroderma pigmentosum and Cockayne's syndrome phenotypes exist⁸⁶; and while some Cockayne's syndrome fibroblast strains show a slightly reduced lifespan *in vitro* the number of population doublings achieved are very close to the normal range, unlike Werner's syndrome in which there is no overlap. Similarly some ataxia telangiectasia fibroblasts demonstrate a reduced lifespan *in culture* but the syndrome has at least nine recognisable complementation groups rendering analysis complex. Cells from trichothiodystrophy, a disorder with two complementation groups one of which shows DNA repair defects, has never been reported to show accelerated cell senescence.⁸⁴ Hutchinson-Gilford progeria, perhaps the best known of all progeroid syndromes, is associated with severely reduced fibroblast growth *in vitro*; however, its DNA repair features are variable, which suggests that different complementation groups may also exist within the disease.⁶¹ It is clear that any relation between aging and DNA damage will not be a simple affair and will require rigorous study to validate.

Is senescence the result of altered telomere metabolism?

Do any of these premature aging syndromes shed light on the normal process of aging, and in particular do any critically address the normal mechanism leading to replicative senescence? One of the more plausible models for the trigger to replicative senescence is that progressive telomere shortening in somatic cells that do not express telomerase eventually leads to one or more critically short telomeres, which in some way signals cell cycle arrest and senescence.⁴¹⁻⁴⁶ In human fibroblasts division potential in culture correlates well with initial telomere length both for bulk cultures from different individuals⁸⁸ and for subclones derived from the same culture.⁸⁹ Telomere loss in fibroblasts only occurs when the cells are dividing and not when held quiescent by density dependent contact inhibition.⁹⁰



Figure 3 Human metaphase chromosomes (red) and their telomeres detected by *in situ* hybridisation with a (TTAGGG)*n* probe (yellow). (Reprinted from reference 39 with permission of Oxford University Press.)

However, human fibroblasts will also enter a senescent-like state within a few days of infection with a retroviral vector expressing activated (V12) H-ras.⁹¹ This raises the possibility that senescence can be induced by aberrant mitogenic pathway stimulation, and that replicative lifespan represents a response to chronic mitogen load.⁹¹ It is difficult experimentally to separate mitogenic stimulation from cell division, and one might argue that the fact that quiescent cells do not undergo senescence is related more to their lack of exposure to mitogenic stimulation; in this example⁹¹ suppression by contact inhibition. This would open the way for mutations that affect cell cycle progression but leave the mitogen stimulation pathway intact to increase the rate of senescence as measured on a per cell division basis. In this respect it is interesting to note that one pleiotropic effect of the WRN mutation is increased cell cycle time.⁹²

Telomere dynamics have been measured for a number of progeroid syndromes. For both ataxia telangiectasia⁹³ and Hutchinson-Gilford progeria,⁸⁸ telomeres are shorter than for age matched normal controls, but in neither case have data on the per cell division rate of telomere shortening been presented. Although altered telomere dynamics cannot be ruled out, it is also possible that the increased telomere loss rates act simply as a biomarker for increased cellular turnover in these cases.

More data are available for Werner's syndrome, where cultured fibroblasts show an increased rate of telomere loss.⁹² However, they senesce at a point where their telomeres are

considerably longer than would normally be expected for a senescent fibroblast.⁸⁹ Schulz *et al* argue that this is not compatible with a simple telomere driven senescence in these cells.⁹² However, a number of points need to be addressed before altered telomere dynamics can be fully excluded as the underlying cause of the premature senescence of Werner's syndrome cells. First, although the amount of telomeric DNA at senescence is longer than in normal senescent fibroblasts, it is possible that the length at which a telomere is recognised as critically short is different in Werner's syndrome cells. The ability of the cell to recognise a normal chromosome end as a telomere rather than a double strand DNA break, reflects not simply the terminal DNA sequences (fig 3) but also the altered chromatin structure at the natural end caused by various telomere binding proteins. A helicase such as WRN could have pleiotropic effects on the assembly and maintenance of the terminal chromatin structure, leading to compromised telomere behaviour and its signalling as critically short at an increased telomere length. Indeed, compromised terminal chromatin structure is one of a number of explanations for why histone deacetylase inhibitors cause a dramatic decrease in proliferative lifespan without apparently altering the rate of telomere loss.⁹⁴

Another caveat regarding the data on telomere loss rates in Werner's syndrome cells is that the hypothesised most important biological variable is not the average telomere length, but rather that of the shortest telomere in the nucleus. It is possible, therefore, that the distribution of telomere lengths might be altered in Werner's syndrome cells (because of alterations in telomere metabolism), leading to a situation where bulk telomere length is somewhat larger than normal when the first critically short telomere arises. This is a possibility that requires further attention as there clearly is some form of alteration in telomere dynamics in Werner's syndrome cells (the rate of loss is greater than in normal cells).

How might this arise? It seems at first paradoxical that telomere loss rates could vary between cells that express no detectable telomerase activity. Certainly there will be a basal level of telomere loss because of the end replication problem, but there is increasing data for an additional route to telomere shortening involving degradation of the C-strand of the telomere, the strand complementary to that synthesised by telomerase. This stems from studies in budding yeast where certain *cdc13* and *stn1* alleles on the appropriate genetic background led to the accumulation of extensive regions of single stranded DNA extending from the end of the chromosome, reflecting degradation of the C-strand in a cell cycle controlled fashion.^{44 95-98} Mutations in *RAD24* can suppress this phenotype—of interest as the product of this gene regulates the activity of *RAD17*, an exonuclease whose known directionality (3→5) is just that necessary to produce C-strand degradation from the telomere.⁹⁹ This model illustrates the potential to modulate telomere loss rates independent of

telomerase. Although mammalian homologues of CDC13 and others have yet to be identified, a structural analysis of human telomeres is consistent with the existence of a C-strand degradation pathway in human cells.¹⁰⁰ It is interesting to note that human fibroblasts grown in 40% partial pressure of oxygen show a marked increase in both the rate of senescence and of telomere loss, together with an accumulation of single stranded DNA at the telomeres.¹⁰¹ Although consistent with the available data, whether hyperoxia exerts its effects by modulating C-strand degradation remains to be determined.

The mechanism whereby senescence is activated in Werner's syndrome cells remains a mystery. The altered telomere dynamics⁹² suggest some altered behaviour in these regions, but whether this is causal in the premature senescent phenotype, or whether it is simply a side effect of a mutation potentially capable of modulating DNA and chromatin structure, is far from clear. It is important to note that the mechanism whereby senescence is activated in Werner's syndrome cells is not necessarily the same as that normally used by fibroblasts at the end of their lifespan, as the cell senescence hypothesis of aging is concerned with the occurrence and phenotype of senescent cells rather than the details of the mechanism that led to them arising. Indeed, the ability of H-rasV12, hyperoxia, and histone deacetylase inhibitors^{91 94 101} all to trigger apparently indistinguishable states of cellular senescence seems to indicate that multiple effectors can lead to the same final outcome. With so many potential triggers there is ample room for genetic lesions such as WRN to lead to senescent cells by activating an alternative route, as opposed to modulating the normal effector route to senescence. Perhaps the characterisation of other progeroid syndromes to the level of detail now available for Werner's syndrome will shed additional light on the control of these processes and ultimately on aging itself.

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