PERSPECTIVES: AGING

Genomic Priorities in Aging

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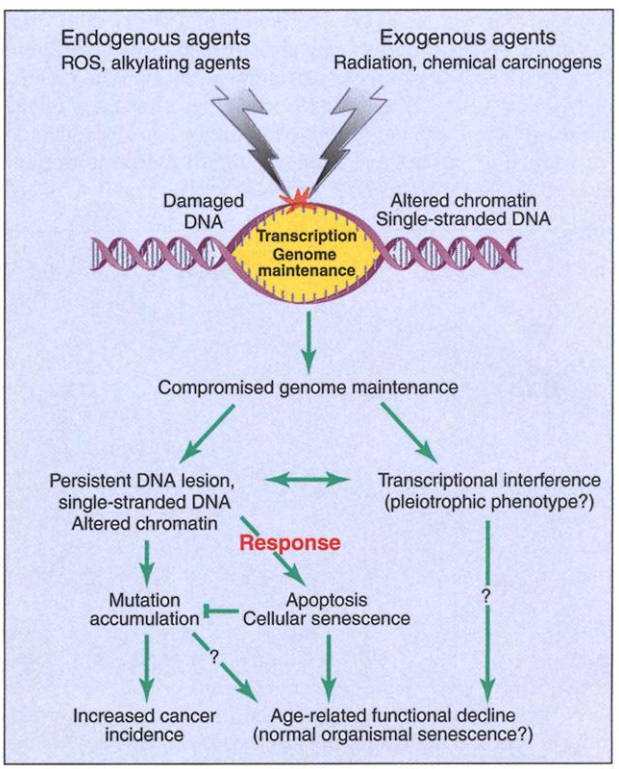
Understanding the deleterious processes that cause aging has been a human endeavor ever since we figured out that we grew old and that we didn't like it. Many hypotheses have been proposed to explain the root cause of aging (*1*). One broad-based hypothesis is that generalized homeostatic failure leads to age-related decline. Although notions of time- and use-related deterioration may be applicable to mechanical objects, they fall short as analogies to biological systems because energy input should theoretically maintain living systems indefinitely. Yet, despite the regenerative potential of biological organisms, progressive deterioration accompanies postmaturational aging. That the organism's repair capabilities cannot keep up with wear and tear is, according to evolutionary theory, explained by the inevitable declining force of natural selection with age. According to this reasoning, there is no selective advantage to maintaining somatic cells in perfect order much beyond reproductive maturation (*1*). Hence, a long life depends on the timing of maturation and the quality of somatic cell maintenance.

Wear and tear on the DNA often has been touted as a possible basis for our progressive age-related decline. Supporting this notion is the work of de Boer *et al.* (*2*) reported on page 1276 of this week's issue. They reveal important evidence for imperfect genome maintenance of DNA damage as a possible causal factor in aging. Harman, with his "free radical theory of aging" (*3*), was the first to propose that metabolic by-products called reactive oxygen species (ROS) continually damage cellular macromolecules, including DNA. Incomplete repair of such damage would lead to its accumulation over time and eventually result in age-related deterioration. A number of observations support the free radical theory, including the discovery that dietary restriction delays aging and extends life-span in a wide range of rodents and other species, possibly by reducing free radical damage. The notion that genomic DNA could be a major target of continual free radical attack over time is supported by the recent observation that genetic lesions accumulate with age and that dietary restriction reduces this accumulation in rodents (*4*). In addition, deletion of $p66^{shc}$, a signaling protein that maintains oxidant levels, increases resistance to oxidative damage and extends the life-span of mice (*5*).

De Boer and his colleagues (*2*) studied the phenotype of mice with an engineered mutation in *Xpd*, a gene that encodes one of the two helicase protein subunits of the core transcription factor IIH (TFIIH). TFIIH facilitates partial unwinding of the DNA duplex and is required for the initiation of transcription by the RNA polymerase II transcription machinery. In addition to basal transcription, Xpd is important for repairing a range of genetic lesions by nucleotide excision repair (NER). De Boer *et al.* originally generated these mutant mice to mimic a mutation that causes trichothiodystrophy (TTD), a heritable human disease (*6*). This mutation does not ablate but rather alters the normal activity of Xpd. In their initial description, the authors showed that TTD mice have impaired transcription and mildly impaired NER. The characteristics of TTD mice closely resemble those of humans with the disorder, including brittle hair. Their exhaustive characterization of the mouse model reported in the new study reveals that these TTD mice show signs of premature aging, including osteoporosis, osteosclerosis, early graying, cachexia, and reduced life-span (*2*). Thus, Xpd may be important for ameliorating senescence and extending life-span.



Aging and DNA damage. Defects in genome maintenance and their relation to various molecular and cellular endpoints over the life-span of an organism. Endogenous and exogenous agents continuously inflict damage on DNA. Normal genome maintenance, which is never perfect, allows certain lesions and intermediate genome structures to persist. This may interfere with transcription, decrease gene activities, and elicit cellular responses such as apoptosis and cellular senescence. Alternatively, errors during repair, replication, or recombination of a damaged DNA template may lead to the accumulation of mutations, which could increase the incidence of cancer and possibly other aging-related phenotypes. Interestingly, increased cellular responses, such as apoptosis, may ward off cancer at the expense of aging and vice versa.

Are the accelerated aging characteristics observed in the TTD mice due to defective DNA repair alone? Xpd is important for repairing DNA in association with transcription, so the exact way that it delays age-related decline is difficult to determine. Indeed, the phenotype that is the namesake of trichothiodystrophy appears to result from a transcriptional defect (*6*). Furthermore, complete abrogation of NER, accomplished by mutating Xpa, has not yet been shown to cause premature aging in mice (*7*). Thus, defective NER, which leads to an accumulation of DNA mutations (*8*), is unlikely to be the sole cause of early senescence in TTD mice. However, defective DNA repair apparently does contribute to premature senescence in these animals. This can be derived from the authors' observation that a much more severe phenotype is obtained by bringing the Xpd mutation into an NER-null background, that is, by crossing the TTD mouse with an Xpa-knockout mouse. It is noteworthy that only cells from the hybrid, but not

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those from the TTD mouse or the Xpa mouse separately, have increased sensitivity to the poison paraquat or ionizing radiation. Both of these agents damage DNA, generating some of the same lesions as ROS, which lends support to the possibility that ROS-induced lesions initiate the age-related decline in TTD mice.

The authors propose that a response to stalled transcription at sites of DNA damage is responsible for early aging in TTD mice. In this scenario, a defective TFIIH results in stalled transcription that decreases gene activity and leads to a mild accumulation of DNA damage. Cells respond by undergoing either apoptosis (programmed cell death) or cellular senescence (irreversible cessation of cell division), both of which may contribute to early organismal senescence in TTD mice. In the absence of Xpa alone (that is, with a fully functional Xpd protein), transcription from a damaged DNA template can still take place, possibly because the amount of spontaneous damage subject to NER is low. In combination with TTD, however, the complete absence of Xpa would exacerbate the suboptimal performance of TFIIH, leaving the DNA lesion exposed for a greater period of time; this would result in a further decline in gene activities and an enhanced response. Thus, early senescence in the TTD mouse may be primarily the result of a cellular response to impaired TFIIH at the site of a spontaneous DNA lesion, rather than the accelerated accumulation of DNA damage or mutations.

Although the true nature of the premature aging phenomenon in the TTD mouse is not yet completely understood, there is support for the scenario sketched above. For example, Ku86 knockout mice, which are completely defective in nonhomologous end joining of double-strand DNA breaks, display various symptoms of accelerated aging (*9*). At least part of this phenotype is dependent on p53 (*10*), a tumor suppressor protein crucial for the cellular response to DNA damage that leads to apoptosis or cellular senescence. Interestingly, mice with increased p53 activity exhibit premature senescence (*11*). This supports the notion that at least a part of organismal aging is due to cell death or cellular senescence in response to imperfect genome maintenance.

What does this tell us about normal aging? Although there is no doubt that a single-gene mutation, such as the TTD defect, merely exaggerates certain components of aging processes, these mutations do indicate various molecular and cellular endpoints of normal aging. The continual induction of DNA damage during normal aging ultimately results in genomic instability as exemplified by persistent DNA lesions, various types of mutations, stalled intermediate repair complexes, and transcription interference (see the figure). In addition, this state of genomic instability activates a variety of cellular response pathways. The frequency and rapidity with which these various molecular and cellular endpoints occur over a normal life-span reflect the proficiency of the organism's multiple genome maintenance pathways. Animal models such as the TTD mouse should help us to unravel the various pathways of genome maintenance and their contributions to organismal longevity, while also shedding light on the great diversity of aging-related phenotypes.

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PERSPECTIVES: PALEOCLIMATE

A Fresh Look at Glacial Floods

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We tend to think of continental-scale ice sheets as rather ponderous affairs, inexorably advancing southward over the landscape and then slowly retreating to the north at the end of each ice age. Over the last 20 years, however, evidence has accumulated that this is a misconception. We now know that the Laurentide Ice Sheet—the largest ice-age glacier—was characterized by thin, marginal ice streams flowing rapidly on low-friction beds and was unstable through much of its history (*1–3*). The ice sheet periodically and abruptly discharged massive amounts of ice into the North Atlantic (*4*), and abrupt coolings and warmings occurred throughout the last ice age (*5*).

Two recent papers (*6, 7*) investigate the roles of glacial meltwater and continental drainage in this glacial and climatic instability. Both papers review numerical modeling studies of ocean circulation. These and another recent paper (*8*) suggest that the thermohaline circulation of the oceans is sensitive to changes in the amount and location of freshwater discharge.

Each time the ice sheet advanced beyond the Canadian Shield, it confined large volumes of water in proglacial lakes at the ice margin. This occurred because the ice sheet flowed over low-relief terrain containing large basins (such as those occupied by the modern Great Lakes), because ice flowed up-slope or against preexisting drainage, and because the glacier depressed Earth's crust under its own weight. As various lobes of the ice sheet slithered forward or back, two major types of drainage event occurred.

First, the overall drainage of much of the continental interior was periodically rerouted to different parts of the ocean—the Gulf of Mexico (via the Mississippi River), the Arctic Ocean (via the Mackenzie River), the North Atlantic (via the St. Lawrence and Hudson Rivers), and the Labrador Sea (via the Hudson Strait) (*9*). Second, large proglacial lakes, such as Lake Agassiz, catastrophically drained in conjunction with some rerouting events.

The amounts of fresh water involved in these drainage changes were enormous (see the figure). Baseline flow (that excluding the sudden drainage of proglacial lakes) of some river systems changed by a factor of 2. Lake-drainage floods ranged from ~0.05 to ~0.30 sverdrups (1 sverdrup = 10^6 m^3 s^{-1}) above baseline flow, averaged over a year. The flood that accompanied the final collapse of the ice sheet may have been as much as 4 to 5 sverdrups.

Building on recent reconstructions of ice-age runoff in North America (*9*), Clark *et al.* (*6*) show that as the Laurentide Ice Sheet retreated, each major change in drainage routing coincided with a change in thermohaline circulation, as indicated by changes in the radiocarbon content of the atmosphere and by ocean-circulation proxies in marine sediment cores. The authors focus on the first type of drainage change—the routing of baseline flow—but suggest that catastrophic lake-drainage events during some of the later reroutings

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