Genetics and the Specificity of the Aging Process

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The identification and study of long-lived mutant animals has provided valuable insights into the mechanisms that limit the life-span of organisms. Findings with the gene Sir2 suggest that the rate of aging can be regulated under certain conditions. Indeed, increased expression of Sir2 lengthens life-span by acting on biological processes that promote survival under conditions of scarcity. In addition, studies of mutant strains of Caenorhabditis elegans, in particular daf-2, clk-1, and isp-1 mutants, suggest that the biology of reactive oxygen species in the mitochondria and elsewhere might be the main determinant of life-span in this organism. Thus, the aging process may be more specific than previously anticipated on evolutionary grounds.

Evolutionary biology teaches that natural selection is most effective in ensuring robustness in the developmental and reproductive phases of life but less powerful in the post-reproductive phase, when its action on the production and survival of offspring is indirect at best. Furthermore, owing to the hardships of feral life, the average life-span of a species is much shorter in the wild than under laboratory conditions. Thus, natural selection cannot act to prolong a life-span that is only observed under protected conditions. These considerations suggest that, under such conditions, many vital processes will decline concurrently because none could have been selected specifically to last.

Another way to look at the problem of aging is to consider medical records, which document the increase over time of the prevalence of lethal diseases in people. Although adequate nutrition and freedom from infectious agents can affect many aspects of health, medical records nonetheless give the impression that aging is a collection of mostly independent degenerative processes leading to disease and, ultimately, death. For example, although the development of atherosclerosis very much mirrors aging because it manifests itself first early in life but leads to overt disease only relatively late, it has nothing obvious in common with other processes linked to the passage of time, for instance, the increased probability of developing cancer. In fact, if a cure for cancer that killed transformed cells were found, it would increase the average life-span of the human population but would not prevent the processes that lead to atherosclerosis. It would seem that to increase human life-span substantially, medicine has to find cures for every human disease.

Very Long-Lived Mutants

Although aging may appear to be multifactorial, findings with forward genetics in simple organisms modulate this view. Indeed, single-gene mutations can extend life-span in worms, yeast, and mice, and simple combinations of these mutations can produce truly dramatic effects. In worms, for example, daf-2 clk-1 double mutants can live up to five times longer than wild-type animals (Fig. 1A) (1, 2). A similarly large increase in life-span is brought about by simultaneous mutations in daf-2 and daf-12 (3) as well as by the laser ablation of the germ line in daf-2 mutants (4).

The magnitude of these effects points to the existence of a central process of aging. Indeed, in these mutants, all the processes that allow the worm to remain alive can function effectively for five times longer than they do in the wild-type animals. These processes include resistance to infections (5); resistance of the cuticle to environmental damage; resistance to mechanical stresses produced by behaviors such as egg-laying, pharyngeal pumping, defecation, and movement; resistance to muscle degeneration (6); and clearance of degradation products such as lipofuscin (7). Thus, it appears that, in these very long–lived mutants, the degradation of all (or most) vital processes can be slowed down by affecting only very few genes at a time. These findings appear to contradict the notion that aging has many independent causes.

Sir2: A Targeted Deacetylase

Another insight into how single genes affect life-span comes from studies of the gene Sir2 in yeast and worms (8–10). In both organisms, life-span is increased with higher doses of Sir2 (sir-2) in worms. In budding yeast, aging is determined by how many times mother cells divide to give rise to daughter progeny (11). Upon division, cells segregate old material to the mother, thereby assuring that daughters are renewed (Fig. 2). Mother cells become enlarged and slowed in their division time, then reach senescence after 20 to 30 divisions. In contrast, worms grow old because of the decay of the 959 nondividing cells composing the soma (Fig. 2). It thus...
SIR2 genes affect life-span in these very diverse contexts. How can this regulator possess such flexibility? The answer to this question may lie in the fact that SIR2 genes encode a simple enzymatic activity, that of a deacetylase, which allows genes to regulate the expression of other genes (12–14).

Sir2 proteins do not bind to DNA on their own. In yeast, Sir1p targets Sir2p to the ribosomal DNA (rDNA) repeats (15, 16), where it silences gene transcription by deacetylating the histones. This silencing reduces gene expression and genomic instability in the nucleus and thereby promotes longevity by reducing the production of toxic extrachromosomal rDNA circles. Sir2p is also targeted to silent copies of mitochondrial DNA (mtDNA). How might Sir2p genes sense environmental conditions and, in the face of scarcity or stress, trigger specialized survival forms (Fig. 3). This mode of regulation may have been present in the common ancestor of yeast and worms one billion years ago, and its adaptive nature might explain its pervasiveness.

In yeast, Sir2p promotes the yeast dauer (25), a specialized, very long–lived cells: the spores (Fig. 3). Because Sir2p regulates the sexual cycle in budding yeast, it mediates the differentiation of specialized, very long–lived cells: the spores (Fig. 3). Because Sir2p regulates the sexual cycle in budding yeast, it mediates the differentiation of specialized, very long–lived cells: the spores (Fig. 3). In summary, the findings with SIR2 genes suggest that nature has evolved a conserved survival mechanism in times of scarcity that results in slow aging. Unravelling the molecular basis of such mechanisms in mammals is a present challenge.

Worms, Reactive Oxygen Species (ROS), and Mitochondria

Protecting from mitochondrial ROS production: daf-2 and isp-1. daf-2 (29) encodes an insulin receptor-like transmembrane tyrosine kinase (20) and has a powerful effect on life-span (19). daf-2 mutants live a long time and are resistant to a variety of stresses, including oxidative (30), heat (31), ultraviolet (32), and heavy metal stresses (33), all of which are known to be mediated at least in part by the production of ROS (34–36). This requirement may link the activity of Sir2 proteins to the metabolic status of the cells in which they act. NAD$^+$ and its reduced form, NADH, serve as cofactors in a plethora of metabolic reactions involving oxidation and reduction.

A well-known phenomenon connects metabolism and aging. Diets low in calories, a regimen termed calorie restriction (CR), alter metabolism to promote a longer life-span (26) across a broad spectrum of organisms from yeast to mammals. Mutating the SIR2 gene or lowering the synthesis of NAD$^+$ by mutating its pathway of synthesis prevents the longevity conferred by CR (27). Sir2p may therefore sense CR through a change in the NAD$^+$/NADH ratio and then promote silencing in the rDNA, which extends life-span (28). It remains to be seen what kinds of physiological changes elicited by CR in mammals are most important for longevity. In this regard, it will be interesting to probe whether mammalian Sir2 proteins sense low calories and trigger these changes, thereby delivering the benefits of CR by slowing aging.

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stress resistance appears to be mediated by the up-regulation of enzymatic activities that detoxify (30, 37) ROS, which are short-lived toxic molecules that react with macromolecules, including nucleic acids, proteins, and lipids, and inhibit or destroy their functions. ROS include superoxide, peroxide, the hydroxyl radical, and several nitrogenous species. The main source of ROS is in the mitochondria, where superoxide is produced during the process of electron transport and can give rise to other reactive species through reactions with detoxifying enzymes (38). Superoxide is produced during the reduction and subsequent oxidation of the redox-active lipid ubiquinone (UQ; co-enzyme Q). UQ, which is an integral part of the electron transport chain (ETC), transports electrons from complexes I and II to complex III. ROS are also produced in the cytoplasm by processes that include redox reactions in other membrane-bound organelles, such as the peroxisomes and the endoplasmic reticulum (39–41), and at the plasma membrane (42, 43) (Fig. 4). In addition, ROS are bona fide intracellular second messengers (44).

Caenorhabditis elegans isp-1(qrt150) mutants (45) carry a missense mutation in the “Rieske” iron sulfur protein of complex III of the ETC that leads to a large decrease in oxygen consumption and a large increase in life-span (46). The phenotypes of isp-1 mutants, which show slow development, behavior, and reproduction, can be partially suppressed by a missense mutation in ctb-1 (cytochrome b), another catalytic subunit of complex III. Although ctb-1 brings the phenotype of isp-1 much closer to that of the wild type, it does not affect life-span, suggesting that the increased life-span of the isp-1 and ctb-1 mutants is not the indirect consequence of their slow phenotype. As it slows down electron transport, the isp-1 mutation might be expected to increase ROS formation by increasing the half-life of the semiquinone intermediate that is responsible for superoxide formation (38). However, isp-1; ctb-1 double mutants are as resistant to oxidative stress induced by paraquat treatment as daf-2 mutants. Thus, this particular mutation in isp-1 modifies the function of complex III in a subtle, not-yet-understood way that reduces ROS production (45). This would explain the increased resistance to oxidative stress, because increased production of ROS induced by an exogenous agent will be better tolerated if endogenous ROS production is low.

The resistance to ROS associated with the high activity of detoxifying enzymes in the daf-2 mutants, and the resistance to ROS associated with the low respiration of the isp-1 mutants, suggest that the long life-span of these mutants is due to high ROS detoxification and low ROS production, respectively. Strong additional evidence for this is provided by the observation that the double mutants daf-2; isp-1 do not live any longer than either of the single mutants (45) (Fig. 1B). Given that these mutants have very little in common except low ROS, it is likely that they both live longer because of their low levels of ROS. These findings also suggest that the maximum life-span benefit that low mitochondrial ROS levels can bring about is reached in both isp-1 and daf-2 mutants.

Other ways to affect ROS. The gene clk-1 (47) encodes an enzyme that is necessary for the biosynthesis of UQ, a compound that is an antioxidant in all cellular membranes (48) as well as a redox cofactor in the mitochondrial ETC and elsewhere. In the absence of clk-1, yeast, worms, and mice accumulate demethoxyubiquinone (DMQ), a biosynthetic precursor and analog of UQ that can replace it functionally, at least in part (49–52). Interestingly, the redox properties of DMQ differ from those of UQ. In fact, DMQ might be a better antioxidant, and its redox cycle appears less prone to the production of ROS (53). clk-1 mutants show a normal level of respiration (54–56) but have low levels of detoxifying enzymes and accumulate by-products of oxidative damage, such as lipidfuscin, more slowly than the wild type (56). These observations suggest that the increased life-span of clk-1 mutants results from a decrease of ROS due to the presence of DMQ. However, given the normal mitochondrial respiration of clk-1 mutants and the numerous functions of UQ throughout the cell, part of the effect of DMQ in reducing ROS production may be by reducing extramitochondrial ROS. These findings are consistent with a model for the very long life-span of daf-2 clk-1 mutants in which daf-2 protects cells from mitochondrially produced ROS and clk-1 reduces extramitochondrially produced ROS (Fig. 4). This model is also consistent with the observation that clk-1 mutations increase life-span only moderately by themselves but much more dramatically in a daf-2 background (Fig. 1A). Indeed, as mitochondria are the major source of ROS, it has often been argued that damage to mitochondria is likely one of the first factors that limits life-span (57). In the daf-2 background, however, in which mitochondrial damage is reduced, the ROS damage to the rest of the cell might then become limiting for life-span, which allows the replacement of UQ by DMQ to have a large effect.

The free-radical theory of aging (58, 59), which states that damage from ROS is the cause of aging, is based on the observation that many age-related pathologies are associated with macromolecules by ROS. In fact, the correlation between the level of oxidative damage and the rate of aging of cells, tissues, and individuals is extremely good (58). Demonstrating an involvement of ROS by the genetic approach, with the end point of life-span rather than pathology, is a strong corroboration of this theory.

Indirect effects of manipulating mitochondrial function. Mutations are a major source of ROS and participate in life-span determination in this way. However, because they are crucial organelles for the overall function of the cell, in particular by producing adenosine triphosphate (ATP), mitochondria can also affect life-span indirectly. For example, two recent studies (60, 61) find that disrupting the synthesis of major subunits of the mitochondrial ETC complexes by RNA interference (RNAi) produces a reduction of oxygen consumption and ATP levels as well as increased life-span. This summary is extended in the remaining sections of this Review.
as an increase in life-span. These changes are accompanied by severe developmental defects that result in small, mostly sterile worms. These developmental changes are responsible for the increase in life-span, because RNAi against the ETC subunits in adults reduces ATP but does not increase life-span (61) and because adult life-span remains extended after RNAi treatment early in development, when normal ETC subunit synthesis is restored in adults. These results suggest that the low level of ATP experienced during development induces a morphological and physiological state that leads to increased life-span.

The long-lived RNAi-treated animals are hypersensitive to the superoxide-generating agent paraquat (60), likely a reflection of an increased level of ROS due to the disruption of the ETC, in particular at complex I (38). Thus, it is possible that the irreversible developmental alterations are a result of ROS acting as intracellular second messengers (44) rather than a result of low ATP.

This development-dependent life-span increase is quite different from that observed with isp-1 and clk-1 (Fig. 4), although these genes also encode mitochondrial proteins. First, unlike the animals in which the synthesis of ETC subunits is inhibited, isp-1 and clk-1 mutants have normal body size (56) and are quite fertile (45, 62). Second, the life-span effects of isp-1 and daf-2 mutations are not additive (Fig. 1B), whereas the life-span effect of inhibited synthesis of ETC subunits is additive with that of daf-2 (61). Third, in contrast to what happens with RNAi against ETC subunits, adult life-span increases can be obtained by reducing the function of daf-2 or clk-1 in adults only (63, 64).

Finally, the disruption of the expression of a number of genes coding for mitochondrial proteins other than ETC components (in particular two uncharacterized members of the mitochondrial carrier family) decreases ATP levels and increases life-span, whereas development, oxygen consumption, and stress resistance are minimally affected (60). It is not yet clear whether inhibition of the synthesis of these proteins extends life-span by any of the mechanisms discussed above.

Conclusion

Do these findings on the mechanisms and the regulation of aging in lower organisms shed light on age-dependent diseases? In fact, oxidative damage has been linked to many major degenerative diseases, including atherosclerosis (65), diabetes (66), Parkinson’s disease (67), and, more controversially, Alzheimer’s disease (68) and cancer (69). This is consistent with the genetic results showing that ROS are intimately involved with life-span determination and with the classical finding that ROS cause damage. The regulation of aging by CR, SIR2, and ROS acting as regulatory molecules may also relate to age-related diseases. CR can extend the life-span of several strains of mice and rats, even though their life-span is limited by strain-specific patterns of diseases (for example, cancer in C57Bl6 mice and kidney disease in Fischer rats). Thus, a detailed understanding of the pathways that mediate the beneficial effects of CR and the mechanisms by which ROS modulate the levels of ROS may lead to novel therapies for a wide range of age-related diseases.

Evolutionary theory correctly asserts that aging is not an adaptive trait but that many organismal functions are bound to fail with time, because none could have evolved to last indefinitely. However, the mechanisms of aging are nonetheless more specific than previously thought. The findings on life-span determination in C. elegans suggest that ROS, which are an inevitable consequence of life in an oxygen-rich world, are a leading proximal cause of aging. The results with SIR2 indicate that organisms have evolved ways to endure times of environmental stress and so have developed regulatory processes that implement survival strategies. The physiological changes that allow for survival must impinge on the processes limiting life-span, in particular ROS production and detoxification. Life-span, therefore, appears to be regulated in these situations in spite of the fact that it is not the feature shaped adaptively by natural selection.

References and Notes