CMLS, Cell. Mol. Life Sci. 60 (2003) 1333–1341
1420-682X/03/071333-09
DOI 10.1007/s00018-003-2310-X

CMLS Cellular and Molecular Life Sciences

Review

The free-radical hypothesis of aging goes prokaryotic

T. Nyström

Department of Cell and Molecular Biology – Microbiology, Göteborg University, Medicinaregatan 9C, 413 90 Göteborg (Sweden), Fax: + 46 31 7732599, e-mail: Thomas.Nystrom@gmm.gu.se

Received 7 November 2002; received after revision 7 January 2003; accepted 9 January 2003

Abstract. With respect to oxidative damage and its targets, growth-arrested bacterial cells show some of the same signs of senescence as aging insects, worms and mammals. In addition, the fact that the life span of growth-arrested *Escherichia coli* cells is greatly extended by limiting oxygen availability suggests that free radicals may be one causal factor behind bacterial senescence. Recent analysis reveals a novel culprit in this oxidation, namely the production of aberrant proteins, which are especially susceptible to oxidative attack. This route of oxidation appears to elude the classical oxidative defense proteins. In addition, the failure of growth-arrested cells to fully combat oxidative damage may be linked to a trade-off between proliferation activities and stress management. Even during stasis, *E. coli* cells maintain a basal transcription of reproduction-related genes, and resources are thus partly diverted from maintenance and stress defences to activities relating to proliferation. Thus, some aspects of bacterial senescence may lend support to contemporary theories of aging, including the free radical, antagonistic pleiotropy, and disposable soma theories.

Key words. *Escherichia coli;* free-radical hypothesis; protein oxidation; translational fidelity; transcriptional tradeoff.

The free-radical hypothesis of aging

The 'stochastic' as opposed to 'programmed' view of aging states that aging results from random deleterious events, and oxidative damage has been suggested to be a major contributor to such stochastic degeneration of cells and organisms [1–5]. Harman's original free-radical theory of aging [1] gained in credibility with the identification of superoxide dismutase, which provided compelling evidence of in vivo generation of superoxide anions [4]. The hypothesis was later supported by different experimental data using different model organisms. For example, it has been demonstrated that the levels of oxidatively damaged macromolecules increase with age in a large variety of species and that oxidatively modified proteins lose their catalytic activity and structural integrity [2, 3]. In *Drosophila melanogaster*, life span can be marginally prolonged by genetically increasing the copy number of their native Cu/Zn superoxide dismutase [5]. However, a more significant and impressive effect is seen with tissue-specific overexpression [6] or an ectopic, inducible overexpression [7] of the Cu/Zn superoxide dismutase. In addition, overproduction of the mitochondrial Mn superoxide dismutase extends the life span of adult fruit flies without affecting metabolic activity [8]. In *Caenorhabditis elegans,* identification of gerontogenes (genes whose alteration causes life extension) supports a strong correlation between longevity and oxidative stress defence also in worms [9]. For example, a mutation in the *C. elegans* iron sulfur protein (isp-1) of the mitochondrial complex III reduces oxygen consumption, decreases sensitivity to reactive oxygen species (ROS) and markedly extends the life span of the adult worms [10]. Interestingly,

* Corresponding author.

a mutation in *daf-2*, which extends life span in otherwise wild-type worms, has no effect in the *isp-1* mutant background, indicating that the *daf-2* pathway primarily controlls life span by affecting oxidation management [10]. Also, Daf-2 is a member of a conserved insulin-like signal transduction pathway [11], and the increased life span achieved by mutations in this pathway is associated with increased expression of the mitochondrial Mn superoxide dismutase [12].

With respect to life extension and free-radical biology, the filamentous fungi *Podospora anserina* provide one of the most impressive cases. The life span of *P. anserina* is, on average, around 25 days, depending on the strain [13]. The respiratory chain of this fungus can use two different terminal oxidases, COX and AOX. Mutations, which affect respiration such that the alternative oxidase, the iron containing AOX, is used preferentially over the copper-containing COX, tend to increase life span, and this effect is believed to be due to AOX being less prone to generate reactive oxygen species [13, 14]. Intriguingly, a mutant in which most of the *cox1* gene is deleted is essentially immortal (presently grown for 10 years in suspension) [13, 15].

While it appears clear that oxidative damage is one important factor in setting the pace of aging, the key task of pinpointing the causal factors behind the increased oxidation of macromolecules during aging has proven difficult. Some attempts have been made to correlate oxidation in aging cells with reduced activity (or abundance) of the antioxidant defence and repair systems. However, these attempts have generated conflicting results. For example, catalases have been demonstrated to either increase or decrease with age depending on the tissues analyzed [16], and in other studies it has been demonstrated that some antioxidant defence proteins may increase while others decrease with age in the same tissues [17]. In the prokaryotic model system *Escherichia coli,* the situation is paradoxical rather than conflicting since in a reproductively arrested population of *E. coli* cells, the levels of oxidative defence proteins increase markedly and the population becomes increasingly resistant to external oxidative stresses [18, 19]. Yet the levels of oxidation-damaged proteins in such an *E. coli* population increase [20, 21]. Recent analysis of this phenomenon has yielded results that may, at least in part, explain this apparent paradox, and these data will be reviewed here. Before doing this, I will briefly introduce the concept of conditional senescence in bacteria and some of the key players in the bacterial fight against stasis-induced death.

Conditional senescence

Cytokinesis in simple unicellular bacteria such as *Escherichia coli* proceeds in a symmetrical fashion, with a nonconservative dispersion of cytoplasmic material. During division, potentially damaged constituents are distributed equally to both daughter cells, and the two cells are said to be of the same age. As a consequence, *E. coli* cells do not exhibit a mandatory replicative aging process and are, in principle, immortal creatures. However, bacterial cells entering a nonproliferating state due to starvation-induced stasis become unable to reproduce on standard nutrient plates and, if stasis proceeds, eventually lose their membrane integrity and life-supporting activities [22]. The death phase following starvation has been argued to be the nearest bacteria come to a 'natural' death of the kind familiar among higher organisms [23, 24], and this has more recently been referred to as conditional senescence of bacteria [25, 26].

It has been suggested that prolonged survival of bacterial cells starved for exogenous nutrients is dependent upon regulation of endogenous metabolism in accordance with the energy of maintenance requirement. Endogenous metabolism is defined as 'the total metabolic reactions which occur within the living cell when it is held in the absence of compounds or elements which serve specifically as exogenous substrates'. It has been shown that the endogenous metabolism of starved bacteria encompasses de novo production of macromolecules, including proteins. Specifically, Matin and co-workers [18] demonstrated that cells of *E. coli* continue to synthesize proteins for an extended period of time when held in the absence of exogenous nutrients, and the synthesis of proteins belonging to the early class of starvation proteins is required for long-term survival. Several of these proteins have now been identified, and many of these have specific roles in protecting the cell against heat and oxidative stress [18, 26–28]. Their expression relies, to a large extent, on one transcription factor, the sigma factor σ^S [27, 28].

σ^S and its role in oxidative stress defence

The σ^S transcription factor accumulates during stasis and directs the RNA polymerase to a large number of genes with diverse functions [27, 28]. However, there is a significant bias towards stress defence functions. Interestingly, these functions overlap with those of the *daf-16*-regulated genes of *C. elegans* and the *RAS*/cAMP/PKA-regulated genes in yeast [9, 29, 30]. The Daf-16 fork-head transcription factor is a key regulator in the starvation-induced dauer formation in the nematode, and like σ^S, this regulator directs the transcriptional apparatus to genes involved in protection against heat shock and oxidative agents [29]. Overexpression of *daf-16* (achieved, for example, by mutating *daf-2*) extends the life span of adult nematodes, whereas *daf-16* inactivation accelerates aging and causes increased oxidative damage of proteins [31]. The *RAS*/cAMP/PKA regulatory pathway of *Saccharomyces cerevisiae* is similarly involved in general stress

defence and longevity. Disruption of *RAS* alleviates PKA-dependent repression of genes (e. g. genes encoding heat shock proteins, catalase and CuZn superoxide dismutase) containing a stress response element (STRE) in their promoter region, resulting in increased resistance to oxidative agents and heat [30, 32]. Moreover, the replicative life span of yeast is extended in mutants with low protein kinase A (PKA) activity, whereas the opposite is true for mutants with constitutive PKA activity [33]. Like the σ^S and Daf-16 pathways, the *RAS*/cAMP/PKA pathway responds to starvation, such that glucose limitation converts Ras to the inactive GDP bound form, which in turn reduces cyclic AMP (cAMP) levels and elevates the expression of STRE element genes. The σ^S, Daf-16 and *RAS*/cAMP/PKA regulatory systems are thus functionally analogous; they all respond to starvation, they are all required to mount a general stress defence and they are longevity determinants.

It is not clear which members of the σ^S regulon are most important in slowing down senescence, but σ^S mutants exhibit elevated levels of oxidatively damaged proteins during stasis, suggesting that oxidative stress defence proteins, as in the *C. elegans* Daf-16 network [31], might be key members of the regulon [20, 21]. In addition, the role of σ^S in the survival of stationary phase *Salmonella enterica* serovar Typhimurium has recently been intimately linked to oxidative stress defence [34], reinforcing the argument that oxidative injury is one of the major mechanisms of reduced microbial viability during periods of nutrient deprivation and that one important, if not primary, role of σ^S in stasis survival is to prevent such damage. However, another alternative sigma factor, σ^E, appears to aid and partially complement σ^S in this function [34]. In addition, it should be noted that the bacterial defence against stasis-induced oxidative deterioration proteins also encompasses the heat shock sigma factor σ^{32} and the Hsp60 (GroEL) and Hsp70 (DnaK) chaperones (fig. 1) [18].

Reorganization of catabolism in senescing cells; an oxidative stress defence?

The response of *E. coli* to starvation also includes an increased synthesis of specific glycolysis enzymes, concomitantly with reduced production of tricarboxylic acid cycle (TCA) cycle enzymes [35]. Thus, modulation of the synthesis of catabolic enzymes during aerobic carbon/energy starvation is remarkably similar to the response of cells shifted to anaerobiosis. *E. coli* can grow under aerobic and anaerobic conditions, deriving energy from a number of different respiratory pathways or from fer-

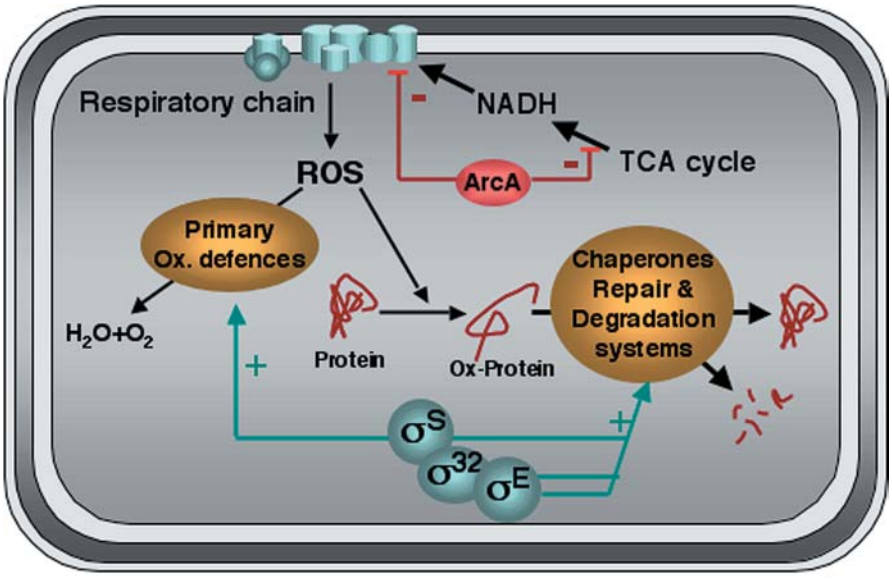

Figure 1. Schematic representation of the bacterial defence against starvation-induced oxidative deterioration of proteins. The ArcA-dependent downregulation of respiration and the production of reducing equivalents derived from the TCA cycle is suggested to reduce generation of reactive oxygen species (ROS) during starvation, and the poor survival of an *arcA* mutant can be suppressed by overproducing Mn-superoxide dismutase [35]. Primary oxidative defence proteins, including the superoxide dismutases (SodA and SodB), and catalases (KatE and KatG), are induced in growth arrested cells, which further reduces ROS levels catalytically. At least some of the corresponding oxidative stress defence genes require the alternative sigma factor σ^S. The elevated production of proteins involved in the reduction, repair and proteolysis of oxidized proteins is a third mode of action in the oxidative stress defence. The proteins of this defence system include peptide methionine sulfoxide reductase, glutathione reductase in concert with glutathione, thioredoxin, glutaredoxin and heat shock chaperones. At least three alternative sigma factors, σ^S, σ^E and σ^{32} are required for this response. The proteases involved in this process have not been identified but may be members of the heat shock regulon requiring σ^{32} for transcription.

mentation. A number of genetic regulatory programs coordinately direct the cells' selection of the most efficient metabolic system in a particular environment. This selection ensures that electrons are channeled from donors to a terminal acceptor such that the drop in Gibbs energy is the maximum allowed under the particular growth condition [36]. This regulation, presumably, optimizes the metabolic systems used to maximize growth rate in a given environment. The ArcA/ArcB regulon is one of several global regulatory systems involved in this metabolic regulation [36]. The ArcA/ArcB pair makes up a two-component regulatory system which is activated when the environment contains no electron acceptors or only poor ones, ArcA being the regulator and ArcB the sensor component [36].

Interestingly, the ArcA regulator is also a key player in starvation-induced modulations of gene expression, and an Δ*arcA* mutant is impaired in several activities associated with the *E. coli* starvation response [35]. Specifically, the ArcA modulon appears to be involved in checking the rate of catabolic degradation of endogenous biomolecules. The respiratory activity is significantly higher during stasis in the Δ*arcA* mutant than the wild-type parent, as is the total metabolic activity and the fraction of total activity derived from aerobic respiration [35]. The ArcA-dependent reduced production and activity of the aerobic respiratory apparatus during starvation may prevent uncontrolled drainage of endogenous reserves. In addition, this response may be an additional defence mechanism mustered by the cell to protect itself against self-inflicted oxidative damage (fig. 1; in support of this notion, the reduction in the life span caused by the *arcA* mutation can be suppressed by overproducing the superoxide dimutase SodA [35]). This notion highlights an important coupling between energy efficiency, catabolic flux and metabolic stress. Thus, it is possible that the reorganization in catabolic flux during starvation for exogenous nutrients is dictated by stress sensing rather than, or in addition to, sensing energy efficiency and a maximal drop in Gibbs energy.

A cause of oxidation I: translation errors

As described, a significant number of the genes and regulons induced by stasis are part of an oxidative stress defence machinery (shown schematically in fig. 1). However, as in aging organisms of eukaryotic origin, this system fails to fully combat stasis-induced oxidation of proteins. A possible explanation for this enigma comes from recent results that demonstrate that stasis-induced oxidation might occur by a route that eludes the classical oxidative defence pathways.

The level of oxidized proteins increases upon treatment of cells with antibiotics and mutations causing increased mistranslation [37]. Interestingly, during these treatments, the rate of superoxide production and the activity of the superoxide dismutases and catalases are unchanged, and the expression of oxidative stress defence genes does not increase [37]. In addition, it was demonstrated that increased oxidation during these treatments was primarily the result of aberrant protein isoforms being oxidized [37]. In other words, increased protein oxidation can be the result of increased production of aberrant proteins, and this does not appear to be sensed by the oxidative defence regulons and does not require increased generation of reactive oxygen species [37]. Moreover, two-dimensional gel electrophoresis of proteins demonstrated that the sudden increase in protein oxidation during the early stages of stasis in *E. coli* is strongly associated with the production of aberrant protein isoforms that appear to be specific targets for oxidative modifications [38]. Also, results showing that frameshifting [39, 40], missense errors [41] and stop codon read-through [38] increase in response to stasis in *E. coli* cells raises the possibility that protein oxidation in nonproliferating cells might be caused by an increased mistranslation. Indeed, protein oxidation is drastically attenuated in the early stages of stasis in *E. coli* cells harboring intrinsically hyperaccurate ribosomes (carrying the *rpsL141* allele; [38]). Thus, elevated oxidation of proteins in nonproliferating cells might be due to the abundance of substrates (aberrant proteins) available for oxidative attack surges during stasis due to reduced fidelity of the translational apparatus.

The σ^S regulon might be ineffective in counteracting such mechanisms of oxidation. In addition, this type of oxidation occurs as an immediate response to growth arrest before σ^S has reached its maximal concentration. It should be noted, however, that the gradual increase in protein oxidation levels observed during prolonged stasis is counteracted by σ^S, since this increase is much more pronounced in *rpoS* mutants [21]. It is not clear why aberrant proteins are more susceptible to oxidation. Possibly, a slight misfolding of the corrupted polypeptide might expose oxidation-sensitive targets that are normally hidden during the coupled translation-folding process.

An alternative idea holds that continued respiration in somatic G_0 cells or growth-arrested bacteria will inevitably increase the levels of oxidized macromolecules because such cells have little ability to dilute any damage with de novo macromolecular synthesis. This proposal is in line with the rate-of-living hypothesis. In its simplest form, this model predicts that the higher the metabolic activity (i.e. respiration) in a nongrowing system, the higher the protein oxidation and the shorter the life-span. However, data concerning nonproliferating *E. coli* and yeast G_0 cells does not support this notion, since the correlation between respiratory activity and protein oxidation in growth-arrested cells is poor or nonexistent in the set of

starvation experiments performed [8, 42]. For example, phosphate-starved cells exhibited the highest rates of respiration during growth arrest, yet protein oxidation is only marginally increased. In addition, the culture half-life is longer in the phosphate-starved cultures despite the continued high metabolic activity in these nonproliferating cells [38]. Again, this result is at odds with the rate-of-living hypothesis but not the free-radical hypothesis of aging, since phosphate-starved cells exhibited very low levels of oxidized proteins. Thus, it can be concluded that the rate of respiration in a nongrowing aerobic system does not, per se, determine the degree of oxidative damage to the proteins of the system.

A cause of oxidation II: trade-off between stress defence and proliferation

Some evolutionary models of senescence propose that there is a trade-off between the resources an organism devotes to reproduction and growth and those devoted to cellular maintenance and repair [43]. As an inevitable consequence, an optimal life history entails an imperfect ability to resist stress. In *C. elegans,* this trade-off can be altered by mutations in *DAF-16* such that transgenic animals carrying *DAF-16* alleles that slow down growth and reproduction live longer and are more resistant to extrinsic stresses [44]. There are examples of such a trade-off also in *E. coli.* For example, Kurland and Mikkola [45] found that in general, natural and laboratory *E. coli* isolates exhibiting fast growth and efficient ribosomes died more rapidly during starvation-induced stasis. Continuous cultivation in chemostats effectively selected for cells with faster growth rates with a concomitant increased efficiency of translation. However, the trade-off for this increased rate of reproduction was a reduced ability to withstand starvation-induced stasis [45].

The *E. coli* trade-off phenomenon has more recently been linked to the status of the *rpoS* gene encoding σ^S. It is known that mutations in *rpoS* are common in many natural and laboratory *E. coli* strains, and it was demonstrated that there is a selective advantage of losing σ^S function during growth under nonstressful condition [46]. The loss of σ^S in populations growing in a glucose-limited chemostat is accompanied by an elevated expression of genes contributing to fitness; e. g. genes encoding glucose uptake systems that require the housekeeping sigma factor σ^{70} [46]. However, increased fitness is traded for reduced stasis survival and stress resistance, since σ^S is a master regulator required for these functions. The effect of mutations in *rpoS* a bacterial example of antagonistic pleiotropy, in which mutations that are beneficial for reproduction may be harmful during old age or stasis. This kind of antagonistic pleoitropy has been suggested to be a major factor in the evolution of aging [47].

A molecular model for this antagonism, or trade-off, in *E. coli* has recently been suggested that includes sigma factor competition for RNA polymerase binding and explains how the quality of the environment can be sensed and translated to intracellular signals that control the allocation of resources between reproductive and maintenance activities. The model argues that the conflict between proliferation activities and maintenance could stem from the fact that RNA polymerase may be limiting for transcription and that sigma transcription factors compete for polymerase binding. It has been shown that even a subtle overproduction of σ^{70} effectively shuts down transcription from genes requiring σ^S, and the cells become sensitive to stresses [48]. Also, overproduction of σ^S attenuates the expression of genes requiring σ^{70}, [48]. This antagonism between sigma factors is highly regulated and is dictated by the nutritional quality of the environment and the alarmone ppGpp [49]. Mutants lacking ppGpp fail to induce σ^S-dependent genes upon the imposition of stress and starvation, a phenomenon that is explained by the fact that σ^S itself requires ppGpp for both its production [50, 51] and activity [52]. This activity appears to be linked to ppGpp, facilitating the ability of σ^S to compete with σ^{70} for RNA polymerase binding [49]. Thus, ppGpp is priming the RNA polymerase (by direct binding to the β and β' subunits of the polymerase) in accordance with environmental signals such that the transcriptional apparatus will be primarily occupied with transcription of σ^{70}-dependent housekeeping genes as long as the ppGpp levels are low, which signals that the nutritional status of the environment is favorable for reproduction. When conditions are less favorable for proliferation, elevated ppGpp levels allow the alternative sigma factors to work in concert with σ^{70} by shifting their relative competitiveness (fig. 2). Thus, the antagonistic pleiotropy observed by Notley-McRobb and co-workers [46] could be explained by the fact that more σ^{70} proteins are allowed to bind RNA polymerase core in the total absence of any competing σ^S, and more resources are, thereby, directed towards growth and reproduction-related activities.

The trade-off model could also explain why σ^S and its regulon genes are not able to fully combat stasis-induced deterioration, e. g. oxidative damage to proteins and other macromolecules. The model argues that sigma factors work in concert in a ppGpp-regulated fashion and that the housekeeping sigma factor competes with alternative sigma factors even during severe stress and growth arrest. As a consequence, a certain fraction of the cell's resources is therefore allocated to activities related to proliferation rather than survival and oxidative stress defence (fig. 2). The benefit of such a regulatory system might be that the growth-arrested cell maintains the potential to respond rapidly, grow and initiate proliferation should nutrients become available.

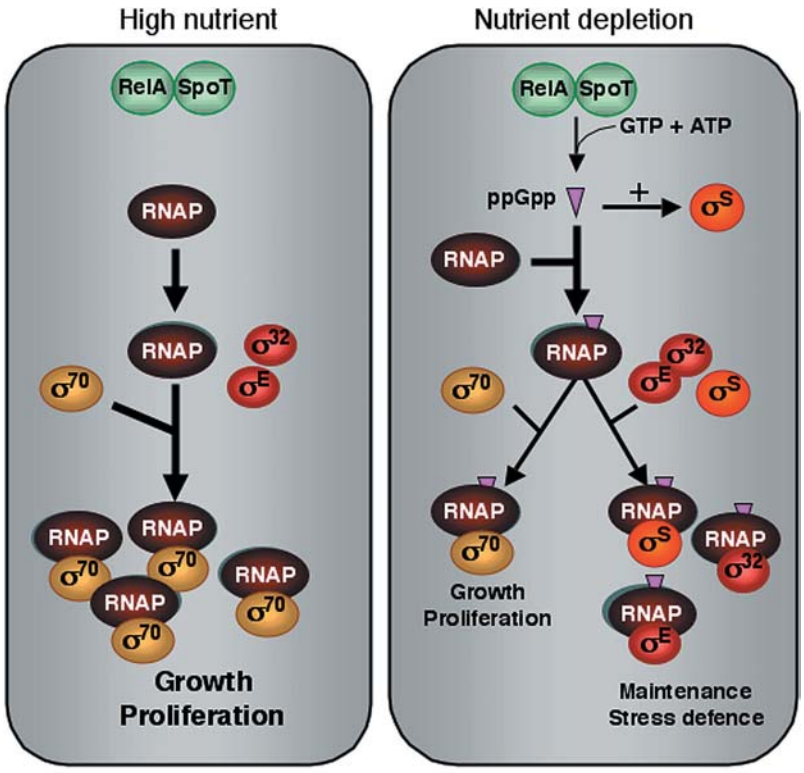

Figure 2. Transcriptional trade-off between reproduction and survival/stress defence. The model is based on data suggesting that RNA polymerase (RNAP) is limiting for transcription and that sigma transcription factors, e.g. σ^{70} (housekeeping sigma factor) and alternative sigma factors, such σ^{S}, σ^{32} and σ^{E}, compete for RNAP binding. This competition is regulated by the nucleotide ppGpp, which accumulates during starvation and stress. Two proteins, RelA and SpoT, are responsible for the production of ppGpp, and these proteins are activated by different conditions. The nucleotide binds the core RNAP and primes the RNAP in accordance with environmental signals such that the transcriptional apparatus will be primarily occupied with transcription of σ^{70}-dependent housekeeping genes (proliferation) as long as the ppGpp levels are low (left panel: nutritional status of the environment favorable for growth). During growth arrest (right panel), elevated ppGpp levels allow the alternative sigma factors σ^{S}, σ^{32} and σ^{E} required for expression of many maintenance genes, including those encoding oxidative stress defence proteins, chaperones and proteases, to work in concert with σ^{70} by shifting the relative competitiveness of the sigma factors. In addition, ppGpp is required for the productions of σ^{S}. However, even during growth arrest, a certain fraction of the RNAP is allocated to σ^{70}-dependent, housekeeping gene expression, and genes requiring alternative sigma factors might not be saturated.

Protein oxidation and feedback catastrophe

Orgel [53] has presented a conceptual and mathematical model explaining how an error feedback loop in macromolecular synthesis may cause an irreversible and exponential increase in error levels leading to an 'error catastrophe' (fig. 3). The feedback loop in Orgel's original model concerned ribosomes and translational accuracy such that errors in the sequences of proteins, which themselves functioned in protein synthesis (e.g. ribosomal proteins, elongation factors) might lead to additional errors. Such a positive feedback loop was argued to lead towards an inexorable decay of translational accuracy and, as a result, aging. The hypothesis is thus based on the assumption that mistranslated proteins can escape degradation and be incorporated into functional (but less accurate) ribosomes. However, several experimental and theoretical approaches, primarily using *E. coli* as a model system, have indicated that increased mistranslation does not cause a progressive decay in the proofreading capacity of the ribosomes (see [54]). The susceptibility of mistranslated proteins to oxidation may provide a molecular explanation for the maintenance of translational fidelity. It has been shown that oxidized proteins are more susceptible to proteolytic degradation than their nonoxidized counterparts [37, 55]. Thus, the rapid oxidation of an erroneous protein may ensure that such a polypeptide is directed to the proteolysis apparatus (fig. 3). This proteolysis will effectively reduce incorporation of mistranslated proteins into mature machines (e.g. ribosomes and RNA and DNA polymerases) involved in information transfer. In this context, it should be pointed out that the reduced translation fidelity of growth-arrested cells is most likely the result of ribosomes being increasingly starved for charged transfer RNAs (tRNAs) (empty A sites are known to be slippery) rather than being intrinsically error prone. In addition, increased mutagenesis and the SOS response are probably not contributing to stasis-induced

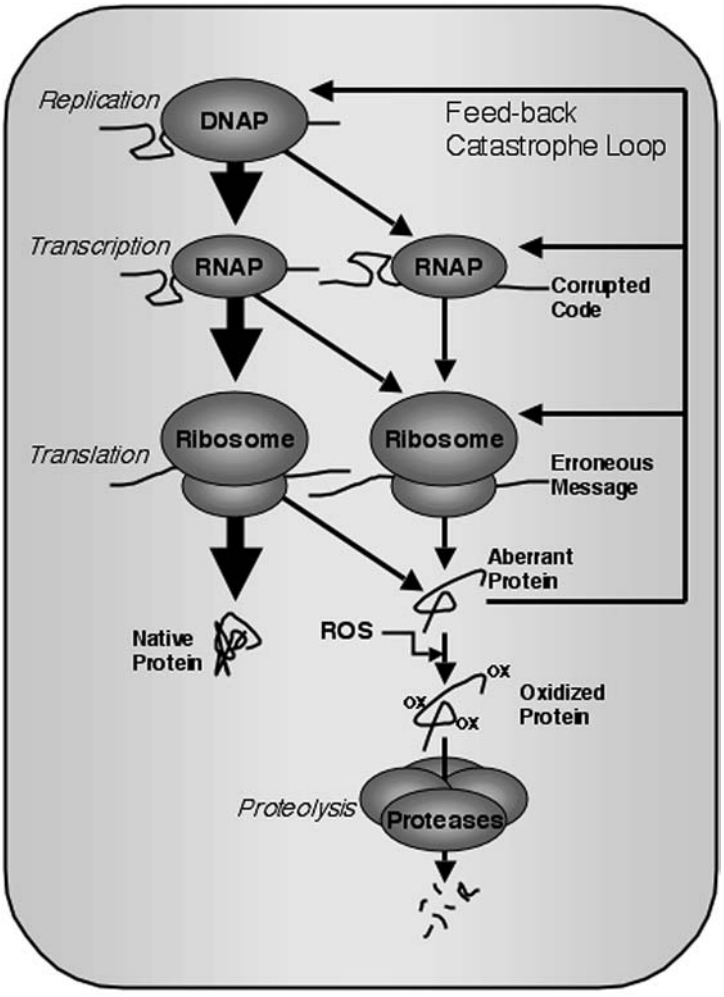

Figure 3. The principle of the feedback catastrophe hypothesis. The feedback loop of this hypothesis concerns the central dogma such that errors in the sequences of proteins (aberrant proteins), which themselves function in information transfer (e. g. DNA polymerase [DNAP], RNA polymerase [RNAP], and ribosomes) might lead to additional errors. These aberrant proteins might be the stem from a corrupted code, an erroneous transcript and/or errors in the translational process. The hypothesis is thus based on the assumption that mistranslated proteins can escape degradation and be incorporated into functional (but less accurate) machines involved in information transfer. However, increased mistranslation does not appear to cause a progressive decrease in the fidelity of ribosomes (see [54]), and the susceptibility of mistranslated proteins to oxidation may provide a molecular explanation for this effect since oxidized proteins are more susceptible to proteolytic degradation than their nonoxidized counterparts [37, 55].

production of erroneous proteins, since specific mutations that render the ribosomes hyperaccurate counteracted aberrant protein production [38].

Conclusion

Gerontologists have proposed that the progressive decline in the functional capacity of aging eukaryotes may be a consequence of self-inflicted damage caused by reactive oxygen species. This is the postulation of the free-radical hypothesis of cellular aging. Recent studies of bacterial culturability and physiology during starvation-induced growth arrest have raised the question whether the free-radical hypothesis of aging is relevant also for explaining the progressive decline in the culturability of growth-arrested bacterial cells [25]. The work on bacteria as model systems reveals that there might be a novel mechanism behind protein oxidation in nonproliferating cells. This oxidation may occur in the absence of increased oxidative stress and may instead be due to increased concentration of substrates available for oxidative attack. Recent data suggest that aberrant and misfolded proteins are such substrates that are more susceptible to oxidation than their native counterparts, and the concentration of these aberrant proteins increases during growth arrest due to reduced translational fidelity. Thus, the potential to oxidize proteins may be exceedingly high in the cell, but the process of coupled translation and folding may have evolved to escape such oxidation, for example by rapidly hiding oxida-

tion-sensitive domains in the peptides being produced. Any condition causing increased production of aberrant, misfolded proteins may also cause elevated levels of oxidized proteins. It is possible also that increased levels of oxidatively damaged proteins is a consequence of growth arrest due to the fact that erroneous products cannot be diluted in such a system. However, growth arrest elicited by phosphate starvation does not result in significantly elevated levels of oxidized proteins despite a high respiration rate [38], indicating that elevated damage is not an inevitable consequence of growth arrest. Interestingly, phosphate-starved cells exhibited a higher translational fidelity than carbon- or nitrogen-starved cells [38].

In addition, increased oxidation during stasis might stem from the fact that maintenance and stress defence activities are partly traded for continued transcription of genes involved in proliferation and growth. Sigma factors directing functions related to reproduction, on the one hand and stress resistance and survival on the other compete for RNA polymerases binding. This competition results in the antagonism between survival activities and reproduction. Even during growth arrest, a certain fraction of the cell's transcriptional resources is allocated to activities related to proliferation rather than stress defences and survival, and as a consequence, stress defence genes are not allowed to operate at their maximal capacity.

In summary, some contemporary models of conditional senescence in bacteria share concepts and ideas from the free-radical, antagonistic pleiotropy, and disposable some theories of aging. On the other hand, the data derived from prokaryotic model systems do not support the rate-of-living hypothesis or the feedback catastrophe theory of aging. The future might show to what extent the molecular mechanisms underlying bacterial senescence are strictly prokaryotic in nature or whether similar mechanisms operate in aging eukaryotes.

Acknowledgements. Work in my laboratory was supported by the Swedish Natural Science Research Council, NFR, and in part by the Swedish Strategic Research Foundation, SSF.

1 Harman D. (1956) Aging: a theory based on free radical and radiation chemistry. J. Gerontol. **2:** 298–300
2 Stadtman E. R. (1992) Protein oxidation and aging. Science **257:** 1220–1224
3 Berlett B. S. and Stadtman E. R. (1997) Protein oxidation in aging, disease and oxidative stress. J. Biol. Chem. **272:** 20313–20316
4 McCord J. M. and Fridovich I. (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. **244:** 6049–6055
5 Orr W. C. and Sohal R. S. (1994) Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science **263:** 1128–1130
6 Parkes T. L., Elia A. J., Dickinson D., Hilliker A. J., Phillips J. P. and Boulianne G. L. (1998) Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons. Nat. Genet. **19:** 171–174
7 Sun J. and Tower J. (1999) FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. Mol. Cell. Biol. **19:** 216–228
8 Sun J., Folk D., Bradley T. J. and Tower J. (2002) Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult *Drosophila melanogaster.* Genetics **161:** 661–672
9 Larsen P. L. (1993) Aging and resistance to oxidative damage in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **90:** 8905–8909
10 Feng J., Bussiere F. and Hekimi S. (2001) Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans.* Dev. Cell **1:** 633–644
11 Kenyon C. (2001) A conserved regulatory system for aging. Cell **105:** 165–168
12 Honda Y. and Honda S. (1999) The *daf-2* gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. FASEB J. **13:** 1385–1393
13 Osiewacz H. D. (2002) Aging in fungi: role of mitochondria in Podospora anserine. Mech. Aging Dev. **123:** 755–764
14 Dufour E., Boulay J., Rincheval V. and Sainsard-Chanet A. (2000) A causal link between respiration and senescence in Podospora anserine. Proc. Natl. Acad. Sci. USA **97:** 4138–4143
15 Schulte E., Kück U. and Esser K. (1988) Extrachromosomal mutants from *Podospora anserine*: permanent vegetative growth in spite of multiple recombination events in the mitochondrial genome. Mol. Gen. Genet. **211:** 342–349
16 Sohal R. S., Agarwal S. and Sohal B. H. (1995) Oxidative stress and aging in the Mongolian gerbil (*Meriones unguiculatus*). Mech. Ageing Dev. **81:** 15–25
17 Ji L. L., Dillon D. and Wu E. (1990) Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. Am. J. Physiol. **258:** 918–923
18 Matin A. (1991) The molecular basis of carbon-starvation-induced general resistance in *Escherichia coli*. Mol. Microbiol. **5:** 3–10
19 Hengge-Aronis R. (1993) Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli.* Cell **72:** 165–168
20 Dukan S. and Nyström T. (1998) Bacterial senescence: stasis results in increased and differential oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon. Genes Dev. **12:** 3431–3441
21 Dukan S. and Nyström T. (1999) Oxidative stress defense and deterioration of growth-arrested *Escherichia coli* cells. J. Biol. Chem. **274:** 26027–26032
22 Ericsson M., Hanstorp D., Hagberg P., Enger J. and Nyström T. (2000) Sorting out bacterial viability with optical tweezers. J. Bacteriol. **182:** 5551–5555
23 Burleigh I. H. and Dawes J. R. (1967) Studies on the endogenous metabolism and senescence of starved *Sarcina lutea.* Biochem. J. **102:** 236–250
24 Postgate J. R. (1976) Death of microbes and microbes. Symp. Soc. Gen. Microbiol. **26:** 1–18
25 Nyström T. (1999) Starvation, cessation of growth and bacterial aging. Curr. Op. Microbiol. **2:** 214–219
26 Nyström T. (2002) Translational fidelity, protein oxidation and senescence: lessons from bacteria. Ageing Res. Rev. **1:** 693–703
27 Kolter R., Siegele D. A. and Tormo A. (1993) The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47:** 855–874
28 Hengge-Aronis R. (2002) Signal transduction and regulatory mechanisms involved in control of the sigma(S) (RpoS) subunit of RNA polymerase. Microbiol. Mol. Biol. Rev. **66:** 373–395
29 Johnson T. E., Cypser J., de Castro E., de Castro S., Henderson S., Murakami S. et al. (2000) Gerontogenes mediate health and

longevity in nematodes through increasing resistance to environmental toxins and stressors. Exp. Gerontol. **35:** 687–694
30 Marchler G., Schuller C., Adam G. and Ruis H. A. (1993) A *Saccharomyces cerevisiae* UAS element controlled by protein kinase A activates transcription in response to a variety of stress conditions. EMBO J. **12:** 1997–2003
31 Yasuda K., Adachi H., Fujiwara Y. and Ishii N. (1999) Protein carbonyl accumulation in aging dauer fromation-defective (*daf*) mutants of *Caenorhabditis elegans*. J. Gerontol. **54:** 47–51
32 Martinez-Pastor M. T., Marchler G. S. C., Marchler-Bauer A., Ruis H. and Estruc F. (1996) The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress-response element (STRE). EMBO J. **15:** 2227–2235
33 Lin S. J., Defossez P. A. and Guarente L. (2000) Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science **289:** 2126–2128
34 Testerman T. L., Vazquez-Torres A., Xu Y., Jones-Carson J., Libby S. J. and Fang F. C. (2002) The alternative sigma factor sigmaE controls antioxidant defenses required for *Salmonella* virulence and stationary-phase survival. Mol. Microbiol. **43:** 771–782
35 Nyström T., Larsson C. and Gustafsson L. (1996) Bacterial defense against aging: role of the *Escherichia coli* ArcA regulator in gene expression, readjusted energy flux, and survival during stasis. EMBO J. **15:** 3219–3228
36 Iuchi S. and Lin E. C. C. (1991) Adaptation of *Escherichia coli* to respiratory conditions: regulation of gene expression. Cell **66:** 5–7
37 Dukan S., Farewell A., Ballestreros M., Taddei F., Radman M. and Nyström T. (2000) Proteins are oxidatively carbonylated in response to reduced transcriptional or translational fidelity. Proc. Natl. Acad. Sci. USA **97:** 5746–5749
38 Ballesteros M., Fredriksson Å., Henriksson J. and Nyström T. (2001) Bacterial senescence: protein oxidation in non-proliferating cells is dictated by the accuracy of the ribosomes. EMBO J. **20:** 5280–5289
39 Wenthzel A. M., Stancek M. and Isaksson L. A. (1998) Growth phase dependent stop codon readthrough and shift of translation reading frame in *Escherichia coli.* FEBS Lett. **421:** 237–242
40 Barak Z., Gallant J., Lindsley D., Kwieciszewki B. and Heidel D. (1996) Enhanced ribosome frameshifting in stationary phase cells. J. Mol. Biol. **263:** 140–148
41 O'Farrell P. H. (1978) The suppression of defective translation by ppGpp and its role in the stringent response. Cell **14:** 545–557
42 Aguilaniu H., Gustafsson L., Rigoulet M. and Nyström T. (2001) Protein oxidation depends on the state rather than rate of respiration in *Sacchormyces cerevisae* cells in the G_0 phase. J. Biol. Chem. **276:** 35396–35404
43 Kirkwood T. B. (1977) Evolution of ageing. Nature **24:** 301–304
44 Henderson S. T. and Johnson T. E. (2001) daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans.* Curr. Biol. **11:** 1975–1980
45 Kurland C. G. and Mikkola R. (1993) The impact of nutritional state on the microevolution of ribosomes. In: Starvation in Bacteria, pp. 225–238, Kjelleberg S. (ed.), Plenum Press, New York
46 Notley-McRobb L., King T. and Ferenci T. (2002) *rpoS* mutations and loss of general stress resistance in *Escherichia coli* populations as a consequence of conflict between competing stress responses. J. Bacteriol. **184:** 806–811
47 Williams G. C. (1957) Pleitropy, natural selection and the evolution of senescence. Evolution **11:** 398–411
48 Farewell A., Kvint K. and Nyström T. (1998) Negative regulation by RpoS: a case of sigma factor competition. Mol. Microbiol. **29:** 1039–1052
49 Jishage M., Kvint K., Shingler V. and Nyström T. (2002) Regulation of sigma factor competition by the alarmone ppGpp. Genes Develop**. 16:** 1260–1270
50 Gentry D. R., Hernandez V. J., Nguyen L. H., Jensen D. B. and Cashel M. (1993) Synthesis of the stationary phase sigma factor σ^S is positively regulated by ppGpp. J. Bacteriol. **175:** 7982–7989
51 Lange R., Fisher D. and Hengge-Aronis R. (1995) Identification of the transcriptional start sites and the role of ppGpp in the expression of *rpoS*, the structural gene for the σ^S subunit of RNA polymerase in *Escherichia coli*. J. Bacteriol. **177:** 4676–4680
52 Kvint K., Farewell, A. and Nyström T. (2000) RpoS-dependent promoters require guanosine tetraphosphate for induction even in the presence of high levels of σ^S. J. Biol. Chem. **275:** 14795–14798
53 Orgel L. E. (1963) The maintenance of the accuracy of protein synthesis and its relevance to ageing. Proc. Natl. Acad. Sci. USA **49:** 517–521
54 Gallant J., Kurland C. and Parker J. VERSUS Holliday R. and Rosenberger R. (1997) The error catastrophe theory of aging: point counterpoint. Exp. Gerontol. **32:** 333–346
55 Starke P. E., Oliver C. N. and Stadtman E. R. (1987) Modification of hepatic proteins in rats exposed to high oxygen concentration. FASEB J. **1:** 36–39