Aging in bacteria

Thomas Nyström

Analysis of senescent *Escherichia coli* cells reveals a link between protein oxidation and the fidelity of the translational apparatus. This model system has also provided a mechanistic molecular explanation for a trade-off between reproduction and survival activities, which may inspire proponents of the disposable soma theory and antagonistic pleiotropy hypothesis of aging.

Addresses
Department of Cell and Molecular Biology – Microbiology,
Göteborg University Medicinaregatan 9C, 413 90 Göteborg, Sweden;
e-mail: Thomas.nystrom@gmm.gu.se

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Introduction

Cytokinesis in bacteria such as *Escherichia coli* proceeds in an apparently symmetrical fashion. The components of the cytoplasm are dispersed non-conservatively during fission and damaged constituents are distributed equally to both cells produced. As a consequence, *E. coli* cells do not exhibit a 'Hayflick limitation' (a limitation in the number of divisions an individual cell can complete; [1]) in their reproduction or a mandatory replicative aging process. Moreover, evolutionary biologists have argued that biological aging is only applicable to organisms with a soma distinct from the germline [2]. If we accept this definition, then unicellular bacteria are clearly not members of the exclusive club of aging creatures. This is not to say that bacteria are immortal. Bacterial cells entering a non-proliferating state (stationary phase) because of nutrient depletion gradually lose their ability to recover and reproduce. These 'sterile' cells initially remain intact but may eventually lose their membrane integrity and life-supporting activities [3•]. This process has been referred to as conditional senescence elicited by growth arrest [4]. Recent analysis of conditional senescence in *E. coli* has revealed interesting similarities with the aging process of higher organisms and may, in fact, provide mechanistic support and inspiration for some contemporary aging theories, including the free radical hypothesis of aging and the disposable soma theory.

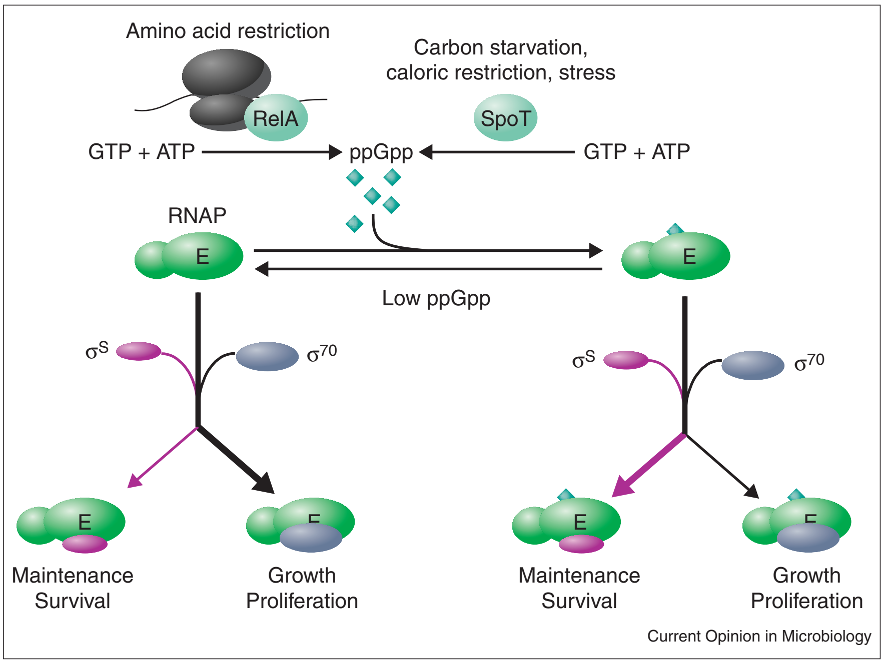
In this review, results demonstrating that there is a trade-off between reproduction and maintenance in *E. coli* are discussed in the context of aging theories. The molecular explanation for this trade-off includes sigma factor competition for RNA polymerase binding and explains how the quality of the environment can be sensed and translated to intracellular signals that control the allocation of resources between reproductive and maintenance activities. Furthermore, recent data pointing to a link between translational accuracy and protein oxidation in senescent *E. coli* cells highlights that there may be lessons to learn from this model system also in the context of free radical biology and aging.

Trade-off between reproduction and maintenance

Genes induced early upon cellular growth arrest have been recognized as the most important ones in the bacterial fight against stasis-induced senescence (see, for example, [5–7]). Many of these genes encode proteins with specific roles in protecting the cell against external stresses, such as heat, oxidants and osmotic challenge. As a consequence, growth-arrested cells are highly resistant to a variety of secondary stresses, a phenomenon known as stasis-induced crossprotection [5]. This crossprotection relies, to a large extent, on one single regulator, the sigma factor σ^S (see, for example, [6]). The σ^S transcription factor accumulates, binds and directs the RNA polymerase to more than 50 specific genes upon conditions of cellular starvation and stress [6]. The members of the regulon are a diverse set of proteins whose functions overlap significantly with those of the *daf-16*-regulated genes of *Caenorhabditis elegans* (see, for example, [8–10]). The Daf-16 fork-head transcription factor is a key regulator in the starvation-induced dauer formation and, like σ^S, this regulator directs the transcriptional apparatus to genes involved in protection against heat shock and oxidative agents (see, for example, [8,9]). Overexpression of *daf-16* extends the life span of adult nematodes, whereas *daf-16* inactivation accelerates aging and causes an increased oxidative damage of proteins [10]. Similarly, *E. coli* mutants lacking σ^S exhibit accelerated senescence during conditions of growth arrest [6], and elevated levels of oxidatively damaged proteins [11,12]. Apart from σ^S and the primary defense proteins, such as superoxide dismutases and catalases [11,12], glutaredoxin 2 has recently been shown to be required in the combat against protein oxidation, particularly in the stationary phase [13]. In *Salmonella*, both σ^S and σ^E have been shown to be required for protection against oxidative damage in stationary phase. Mutants lacking σ^E have reduced survival during stationary phase as well as increased susceptibility to oxidative stress [14•]. Cells of a *Salmonella* strain lacking both σ^E and σ^S become non-viable after 24 hours in stationary phase, but survival of these mutants is completely preserved under anaerobic stationary-phase conditions [14•]. This reinforces the argument that oxidative injury is one of the major mechanisms of reduced microbial viability during periods of nutrient deprivation.

Somewhat surprisingly, it has been demonstrated that mutations in the gene encoding σ^S are common in many natural and laboratory *E. coli* populations. This has recently been explained, in part, by the fact that there is a selective advantage of losing σ^S during growth under non-stressful conditions [15•]. The loss of σ^S in populations growing in

Figure 1



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A model for the trade-off between reproduction and survival. The model is based on the argument that RNA polymerase (RNAP) is limiting for transcription and that sigma transcription factors, such as σ^{70} and σ^{S}, compete for binding to RNAP. This competition is regulated by the nucleotide ppGpp, which accumulates during conditions, causing growth arrest. Thus, ppGpp primes the RNAP in accordance with environmental signals. As a result, the transcriptional apparatus is primarily occupied with transcription of σ^{70}-dependent housekeeping genes (proliferation) as long as the ppGpp levels are low, which signals that the nutritional status of the environment is favorable for growth. During growth arrest, elevated ppGpp levels allow the alternative sigma factor σ^{S}, required for expression of maintenance genes, to work in concert with σ^{70} by shifting the relative competitiveness of the sigma factors. RelA is ppGpp synthetase I responding to amino acid starvation (uncharged tRNA in the ribosomal A-site), whereas SpoT is ppGpp synthetase II responding to a variety of conditions, including carbon/energy limitation.

a glucose-limited chemostat is accompanied by an elevated expression of genes contributing to fitness, such as genes encoding glucose uptake systems [15•]. Thus, there appears to be a trade-off between the functions relating to reproduction and those concerned with maintenance and stress resistance. There are, in fact, other examples of such a trade-off. For example, Kurland and Mikkola [16] found the growth rates of natural and laboratory isolates of *E. coli* to differ significantly, and this difference correlated with altered kinetic properties of the translational apparatus. In general, 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 and a concomitant increased efficiency of translation. However, the pay-off for this increased rate of reproduction was a reduced ability to withstand starvation-induced stasis [16].

Trade-off as a consequence of sigma factor competition

The conflict between proliferation activities (primarily directed by the housekeeping sigma factor, σ^{70}) and maintenance (primarily directed by σ^{S}) might stem from the fact that sigma factors compete for polymerase binding (Figure 1). Even a subtle overproduction of σ^{70} effectively shuts down transcription from genes requiring σ^{S} and the cells become stress-sensitive [17]. Also, over-expression of *rpoS*, which encodes σ^{S}, attenuates the expression of genes requiring σ^{70} [17]. This antagonism between sigma factors has recently been shown to be highly regulated and is dictated by the nutritional quality of the environment and the hormone-like nucleotide ppGpp (see later and [18••]).

Many genes requiring alternative sigma factors have been shown to depend on ppGpp for their induction. For example, the inducers of the σ^{54}-dependent promoters Po and Pu are effective only when ppGpp levels are elevated [19,20]. Similarly, mutant cells with no or low levels of ppGpp exhibit an attenuated and sluggish expression of σ^{32}-dependent heat shock genes [21,22]. In addition, mutants lacking ppGpp fail to induce σ^{S}–dependent genes upon imposition of stress and starvation [23,24]. The fact that σ^{S} itself requires ppGpp for its production [23–25] initially appeared to explain this. However, it was later demonstrated that σ^{S}–dependent genes require ppGpp even in the presence of wild-type levels of σ^{S} [26•]. In other words, ppGpp exerts a dual control on the RpoS regulon by affecting the levels of the required sigma factor and its activity. A recent report has presented evidence for a role of ppGpp in facilitating the ability of σ^{S} and σ^{32} to compete with σ^{70} for RNA polymerase binding [18••]. The data suggests that ppGpp-dependent alteration in sigma factor competition for RNA polymerase binding is an integral part of the typical stringent response that allows alternative sigma factors to operate successfully in concert with σ^{70} during increased maintenance requirements. In other words, ppGpp primes the RNA polymerase in accordance to environmental signals. As a result, the transcriptional apparatus is 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.

The results are, in a sense, in line with the disposable soma theory of aging. This theory of aging was developed from a molecular theory about the fidelity of macromolecular synthesis, specifically the process of translation [27]. Originally, the disposable soma theory stated that there is a trade-off between the energy investments required in obtaining a given level of accuracy in protein synthesis and the production of offspring. Later, the theory included all kinds of macromolecules and maintenance mechanisms, such as macromolecular repair and stress defence pathways, in the trade-off equations. The assumption, which this theory is based upon, is that the resources are limited in any one individual and that these resources may be channeled into two main activities, survival and reproduction. Furthermore, it is argued that a high level of homeostatic stress defense activities will promote long survival of the individual (or soma) but redistribute resources away from reproduction activities or Darwinian fitness. There is, of course, no distinction between a soma and a germline in bacteria but the *E. coli* sigma-factor-competition model nevertheless provides one of very few mechanistic molecular explanations for a trade-off between reproduction and maintenance activities, and puts the spotlight on RNA polymerase as the key player in the allocation of cellular resources.

Caloric restriction and sigma factor competition

One efficient way to increase the life span of rodents, worms, fruit flies and yeast cells is to subject them to caloric restriction, a diet in which calories are limited by 30–40% compared with animals fed *ad libitum*. However, the mechanism by which caloric restriction retards aging is unclear. Extension of yeast replicative life span by caloric restriction depends on the status of the Ras-cAMP-dependent protein kinase A (PKA) pathway [28]. Ras is a key regulator of this pathway that links nutritional status to cAMP levels by controlling the activity of adenylate cyclase. Disruption of *RAS* reduces cAMP levels and upregulates genes containing a stress response element (STRE) in their promoter region (see, for example, [29]). Caloric restriction, such as glucose limitation, converts Ras to the inactive, GDP-bound form, which in turn reduces cAMP levels and elevates the expression of such STRE element genes (for example, genes encoding heat shock proteins, catalase and CuZn superoxide dismutase; [30]). In *E. coli*, glucose restriction activates, in some as yet unknown way, the SpoT protein, which then catalyzes the production of ppGpp. As elaborated above, this allows an elevated expression of stress defense genes requiring alternative sigma factors (Figure 1). In other words, the ppGpp/sigma factor competition model links the trade-off between reproduction and maintenance with nutrient availability and caloric restriction [18••]. Notably, increased production of stress defense genes (in particular, heat shock and oxidation defense genes) is accompanied by increased longevity in many genetic model systems (see also the Daf-3/Daf-16 story of *C. elegans* [8–10]) and it is tempting to speculate that there is a causal link between the two phenomenon. Possibly, caloric restriction causes a reallocation of resources via different signal transduction systems (for example, Ras, Daf-16 or SpoT/RelA/σ^S) and hormone (insulin) and alarmone (ppGpp) control, such that expression of genes required for maintenance is favored at the expense of reproductive activities. The question of how SpoT is sensing carbon/energy restriction is a key question of bacterial molecular biology and physiology that remains to be answered.

Another link between the σ^S regulon and carbon/caloric availability has recently been discovered by Ueguchi *et al.* [31•], who demonstrated that Crr ($EIIA_{Glc}$) plays an important role in the translational control of *rpoS* expression. $EIIA_{Glc}$ is a component of the phosphoenolpyruvate–carbohydrate phosphotransferase system and is involved in inducer exclusion and regulation of adenylate cyclase activity.

The free radical hypothesis of aging and bacterial senescence

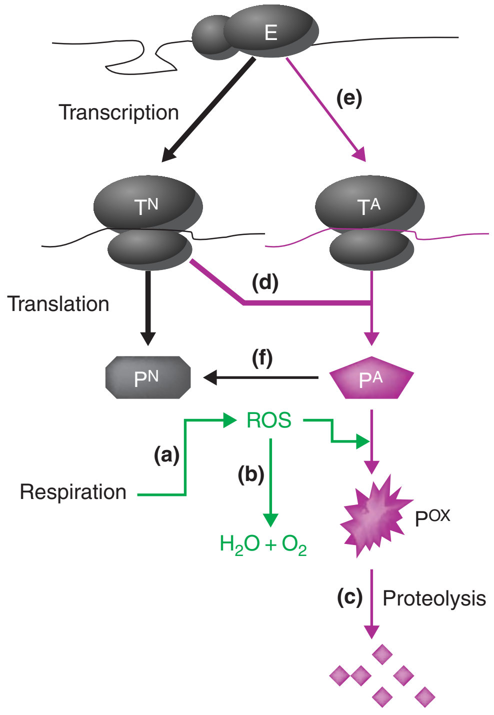
The free radical hypothesis states that aging results from random deleterious events, and that self-inflicted oxidative damage is the primary contributor to such a stochastic degeneration of organisms. The hypothesis has been supported by experimental data that demonstrate that steady-state levels of oxidation-damaged macromolecules increase with age. Moreover, support for the theory comes from the identification of alleles causing life extension in *C. elegans* [9] and experiments demonstrating that the life-span of fruit flies can be prolonged by overproducing antioxidants, specifically superoxide dismutase [32–34].

The task of elucidating the mechanism behind the increased oxidation of macromolecules during aging has proven difficult. Some attempts have been made to correlate oxidation with a reduced activity of the oxidative defense systems. However, these attempts have generated conflicting results and catalases have been demonstrated to either increase or decrease with age, depending on the tissues or organisms analyzed. Other studies have demonstrated that the abundance of some antioxidant defense proteins may actually increase with age in some tissues. Similarly, in a reproductively arrested population of *E. coli* cells, the levels of oxidative defense proteins increase and the population becomes increasingly resistant to external oxidative stresses [5,6]. Yet, the levels of oxidation-damaged proteins in such an *E. coli* population increase [11,12]. In addition, it has been demonstrated that no strict correlation exists between respiratory activity and protein oxidation (or life-span) in growth-arrested *E. coli* cells [35•]. Similar results have been obtained with growth-arrested G_0 cells of the yeast *Saccharomyces cerevisiae* [36•]. Thus, the rate of respiration in a non-growing aerobic system does not, *per se*, determine the degree of oxidative damage to the proteins of the system.

Instead, the use of diagnostic proteomics demonstrated that the sudden increase in protein oxidation during the early stages of stasis in *E. coli* is strongly associated to the production of aberrant protein isoforms; this is seen as protein stuttering on two-dimensional gels [35•]. (The phenomenon called protein stuttering has been shown to be the result of erroneous incorporation of amino acids into proteins and can be detected on autoradiograms of two-dimensional gels as satellite spots with similar molecular weights to the authentic protein but separated from it in the isoelectric focusing dimension [37].) Moreover, the level of protein carbonylation has been found to increase upon treatment of cells with antibiotics, such as streptomycin, causing mistranslation [38••]. Other means of producing aberrant proteins generated similar increased oxidation of proteins. The conditions tested include: addition of puromycin, which causes premature translation termination; overproduction of a mutated 16S rRNA, which, when incorporated into ribosomes, render them prone to mistranslate; and introduction of a mutation in *mutT*, causing decreased transcriptional fidelity [38••]. During these treatments, the rate of superoxide production and the activity of the superoxide dismutases and catalases were unchanged and the expression of oxidative stress defense genes did not increase [38••]. In other words, protein oxidation of aberrant proteins is not sensed by the oxidative defense regulons and does not appear to require increased generation of reactive oxygen species.

Frameshifting [39,40], missense errors [37] and stop codon read-through [35•] increase in response to stasis in *E. coli* cells. This fact, together with results showing that aberrant proteins are more susceptible to oxidation, raises the possibility that carbonylation in non-proliferating cells may be caused by an increased mistranslation. This notion was tested directly by assaying protein oxidation in a mutant strain (*rpsL141*) that harbors intrinsically hyperaccurate ribosomes. Notably, this mutant retains its translational fidelity during stasis and it was demonstrated that protein carbonylation is drastically attenuated in the early stages of stasis in the cells carrying the *rpsL141* allele [35•]. Thus, the elevated oxidation of proteins in non-proliferating cells may be due to an increased availability of substrates (aberrant proteins) available for oxidative attack and these substrates surge during stasis, because of a reduced fidelity of the translational apparatus (Figure 2). It is not, at present, clear why aberrant proteins are more susceptible to carbonylation. Possibly, a slight misfolding of the corrupted polypeptide exposes oxidation-sensitive targets that are normally hidden during the coupled translation-folding process. This, and other possibilities, awaits experimental scrutiny.

Figure 2



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Activities of potential importance for stasis-induced oxidation of proteins. Traditionally, increased protein oxidation has been argued to be an effect of **(a)** increased production of reactive oxygen species (ROS), presumably derived from respiratory activity, **(b)** diminished activity or abundance of the antioxidant systems, or **(c)** reduced activity of the proteolysis or damage repair systems. Work on *E. coli* has highlighted the role of some alternative pathways in protein oxidation. These pathways relate to the production of aberrant proteins, which are highly susceptible to oxidative modification (carbonylation). Increased levels of such aberrant, malformed polypeptides can be the result of **(d)** reduced translational fidelity, **(e)** reduced transcriptional fidelity, or **(f)** diminished activity of the repair refolding apparatus. In the early stages of *E. coli* growth arrest, reduced translational fidelity appears to be the most important contributing factor to the elevated levels of oxidatively modified aberrant proteins. E, core RNA polymerase; P^A, aberrant protein; P^N, native protein; P^{ox}, oxidized protein; T^A, aberrant transcript; T^N, native transcript.

Protein oxidation and fed-back catastrophe

Orgel [41] presented a conceptual and mathematical account for how an error feedback loop in macromolecular synthesis may cause an irreversible and exponential increase in error levels, leading to an 'error catastrophe'. The feedback loop in Orgel's original model concerned ribosomes and translational accuracy such that errors in the sequences of proteins that themselves functioned in protein synthesis (such as ribosomal proteins and 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, cellular senescence. The hypothesis is thus based on the assumption that mistranslated proteins can escape degradation and be incorporated into functional (but less accurate) ribosomes. A later model, called the 'Network theory of aging', integrates the 'Free radical theory of aging' with the 'Protein error theory' [42]. Briefly, the model is based on a mathematical simulation aimed at showing how an increased radical production (or insufficient radical protection) can destabilize the translation system and give rise to an error propagation loop. 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 proof-reading capacity of the ribosomes (see [43]). The susceptibility of mistranslated proteins to carbonylation may provide a molecular explanation for this. It has been shown that carbonylated proteins are more susceptible to proteolytic degradation than their non-oxidized counterparts (see, for example, [38••,44]). Thus, the rapid carbonylation of an erroneous protein may ensure that such a polypeptide is directed to the proteolysis apparatus. This will effectively reduce the likelihood of mistranslated proteins being incorporated into mature machines (for example, 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 tRNAs (empty A-sites are known to be slippery) rather than being intrinsically error-prone. However, this notion awaits experimental support.

Conclusions

Recent studies of bacterial physiology during starvation-induced stasis has raised the question of whether the free-radical hypothesis of aging is relevant also for explaining the progressive decline in the culturability of growth-arrested bacterial cells [4]. Indeed, with respect to protein oxidation and its targets, growth-arrested *E. coli* cells show many of the same signs of senescence as aging eukaryotes do [11]. Work on this simple prokaryotic model system implicates a novel culprit in the accumulation of oxidatively damaged proteins. Elevated levels of oxidized proteins may not necessarily stem from an increased production of free radicals or a diminished defense system but may be caused by an increased production of misfolded or malformed polypeptides. These aberrant proteins are highly susceptible to oxidative modifications and the number of such polypeptides surges in senescent *E. coli* cells because of a decline in ribosome fidelity. In addition, work on *E. coli* has provided a novel mechanistic explanation for a trade-off between reproduction and survival. Transcription factors directing functions relating to reproduction, on the one hand, and stress resistance and survival, on the other, compete for a limiting amount of RNA polymerases in the cell. This limitation in transcriptional capacity results in the antagonism between survival activities and reproduction. The trade-off between these activities is stringently regulated by environmental cues acting through the hormone-like second messenger, ppGpp, such that RNA polymerase is redistributed from proliferating activities to maintenance when the environment is no longer favorable for growth. Future research may establish whether the described features of *E. coli* senescence are strictly prokaryotic in nature or if similar mechanisms operate during aging of higher eukaryotes.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest

1. Hayflick L: **The limited *in vitro* lifetime of human diploid cell strains.** *Exp Cell Res* 1965, **37**:614-636.

2. Williams GC: **Pleiotropy, natural selection, and the evolution of senescence.** *Evolution* 1957, **11**:398-411.

3. • Ericsson M, Hanstorp D, Hagberg P, Enger J, Nyström T: **Sorting out bacterial viability with optical tweezers.** *J Bacteriol* 2000, **182**:5551-5555.

This paper reports on the use of laser tweezers and microchambers to evaluate bacterial viability by two independent measurements: cell membrane integrity and reproductive ability. It is shown that, contrary to previous belief, a stationary-phase *E. coli* population does not harbor a large fraction of intact but non-culturable cells.

4. Nyström T: **Starvation, cessation of growth and bacterial aging.** *Curr Opin Microbiol* 1999, **2**:214-219.

5. Matin A: **The molecular basis of carbon-starvation-induced general resistance in *Escherichia coli*.** *Mol Microbiol* 1991, **5**:3-10.

6. Hengge-Aronis R: **The general stress response in *Escherichia coli*.** In *Bacterial Stress Responses.* Edited by Storz G, Hengge-Aronis R. Washington DC: American Society of Microbiology Press; 2000:161-179.

7. Nyström T: **Not quite dead enough.** *Arch Microbiol* 2001, **176**:159-164.

8. Larsen PL: **Aging and resistance to oxidative damage in *Caenorhabditis elegans*.** *Proc Natl Acad Sci USA* 1993, **90**:8905-8909.

9. Johnson TE, Cypser J, de Castro E, de Castro S, Henderson S, Murakami S, Rikke B, Tedesco P, Link C: **Gerontogenes mediate health and longevity in nematodes through increasing resistance to environmental toxins and stressors.** *Exp Gerontol* 2000, **35**:687-694.

10. Yasuda K, Adachi H, Fujiwara Y, Ishii N: **Protein carbonyl accumulation in aging dauer formation-defective (*daf*) mutants of *Caenorhabditis elegans*.** *J Gerontol* 1999, **54**:47-51.

11. Dukan S, Nyström T: **Bacterial senescence: stasis results in increased and differential oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon.** *Genes Dev* 1998, **12**:3431-3441.

12. Dukan S, Nyström T: **Oxidative stress defence and deterioration of growth-arrested *Escherichia coli* cells.** *J Biol Chem* 1999, **274**:26027-26032.

13. Vlamis-Gardikas A, Potamitou A, Zarivach R, Hochman A, Holmgren A: **Characterization of *Escherichia coli* null mutants for glutaredoxin 2.** *J Biol Chem* 2002, **277**:10861-10868.

14. • Testerman TL, Vazquez-Torres A, Xu Y, Jones-Carson J, Libby SJ, Fang FC: **The alternative sigma factor σ^E controls antioxidant defences required for *Salmonella* virulence and stationary-phase survival.** *Mol Microbiol* 2002, **43**:771-782.

The authors of this paper show that *Salmonella* cells deficient in both σ^E and σ^S are unable to survive stasis, but survival of these mutants is completely preserved under anaerobic stationary-phase conditions. The data pinpoint self-inflicted oxidative injury as one of the major mechanisms of reduced microbial viability during periods of nutrient deprivation and indicate that perhaps the most important function of the σ^E and σ^S regulons during stasis is intimately connected to free radical defence.

15. • Notley-McRobb L, King T, Ferenci T: ***rpoS* mutations and loss of general stress resistance in *Escherichia coli* populations as a consequence of conflict between competing stress responses.** *J Bacteriol* 2002, **184**:806-811.

This paper demonstrates that continuous cultivation under nutrient limitation leads to selection of *rpoS*-null mutations and loss of general stress resistance. The rate of takeover by *rpoS* can be extremely rapid – within 10 generations of culturing. The authors suggest that *rpoS* polymorphism in *E. coli* populations may be viewed as the result of competition between the hunger response (which, to a large degree, requires the housekeeping sigma factor for expression) and the maintenance of the ability to withstand external stresses (which requires σ^S).

16. Kurland CG, Mikkola R: **The impact of nutritional state on the microevolution of ribosomes.** In *Starvation in Bacteria*. Edited by Kjelleberg S. New York: Plenum Press; 1993:225-238.

17. Farewell A, Kvint K, Nyström T: **Negative regulation by RpoS: a case of sigma factor competition.** *Mol Microbiol* 1998, **29**:1039-1052.

18. •• Jishage M, Kvint K, Shingler V, Nyström T: **Regulation of sigma factor competition by the alarmone ppGpp.** *Genes Develop* 2002, **16**:1260-1270.

Using *in vitro* and *in vivo* competition assays, this paper demonstrates that the alarmone ppGpp primes the RNA polymerase in accordance with environmental signals. As a result, the transcriptional apparatus is 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 favourable for reproduction. During growth arrest or growth under stress, elevated ppGpp levels allow the alternative sigma factors to work in concert with σ^{70} by shifting the relative competitiveness of the sigma factors.

19. Sze CC, Shingler V: **The alarmone (p)ppGpp mediates physiological-responsive control at the σ^{54}-dependent Po promoter.** *Mol Microbiol* 1999, **31**:1217-1228.

20. Carmona M, Rodriguez MJ, Martinez-Costa O, De Lorenzo V: ***In vivo* and *in vitro* effects of (p)ppGpp on the sigma(54) promoter Pu of the TOL plasmid of *Pseudomonas putida*.** *J Bacteriol* 2000, **182**:4711-4718.

21. Grossman AD, Taylor WE, Burton ZF, Burgess RR, Gross CA: **Stringent response in *Escherichia coli* induces expression of heat shock proteins.** *J Mol Biol* 1985, **186**:357-365.

22. VanBogelen RA, Neidhardt FC: **Ribosomes as sensors of heat and cold shock in *Escherichia coli*.** *Proc Natl Acad Sci USA* 1990, **87**:5589-5593.

23. Gentry DR, Hernandez VJ, Nguyen LH, Jensen DB, Cashel M: **Synthesis of the stationary phase sigma factor σ^S is positively regulated by ppGpp.** *J Bacteriol* 1993, **175**:7982-7989.

24. Lange R, Fisher D, Hengge-Aronis R: **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* 1995, **177**:4676-4680.

25. Zgurskaya HI, Keyhan M, Matin A: **The σ^S level in starving *Escherichia coli* cells increases solely as a result of its increased stability, despite decreased synthesis.** *Mol Microbiol* 1997, **24**:643-651.

26. • Kvint K, Farewell A, Nyström T: **RpoS-dependent promoters require guanosine tetraphosphate for induction even in the presence of high levels of σ^S.** *J Biol Chem* 2000, **275**:14795-14798.

This work demonstrates that σ^S–dependent genes require ppGpp for their induction even in the presence of wild-type levels of σ^S. It is suggested that ppGpp exerts a dual control on the RpoS regulon by affecting both the production of the required sigma factor and its activity.

27. Kirkwood TB: **Evolution of ageing.** *Nature* 1977, **24**:301-304.

28. Chen JB, Sun J, Jazwinski SM: **Prolongation of life span by the v-Ha-*RAS* oncogene.** *Mol Microbiol* 1990, **4**:2081-2086.

29. Marchler G, Schuller C, Adam G, Ruis HA: **A *Saccharomyces cerevisiae* UAS element controlled by protein kinase A activates transcription in response to a variety of stress conditions.** *EMBO J* 1993, **12**:1997-2003.

30. Martinez-Pastor MT, Marchler GSC, Marchler-Bauer A, Ruis H, Estruc F: **The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress-response element (STRE).** *EMBO J* 1996, **15**:2227-2235.

31. • Ueguchi C, Misonou N, Mizuno T: **Negative control of *rpoS* expression by phosphoenolpyruvate: carbohydrate phosphotransferase system in *Escherichia coli*.** *J Bacteriol* 2001, **183**:520-527.

A series of genetic analyses reveals that Crr negatively controls *rpoS* translation and transcription. It is argued that, whereas the observed transcriptional control by Crr appears to be mediated by cyclic AMP, the negative control of *rpoS* translation is governed by a more direct Crr-dependent mechanism.

32. Orr WC, Sohal RS: **Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*.** *Science* 1994, **263**:1128-1130.

33. Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL: **Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons.** *Nat Genet* 1998, **19**:171-174.

34. Sun J, Folk D, Bradley TJ, Tower J: **Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult *Drosophila melanogaster*.** *Genetics* 2002, **161**:661-672.

35. • Ballesteros M, Fredriksson Å, Henriksson J, Nyström T: **Bacterial senescence: protein oxidation in non-proliferating cells is dictated by the accuracy of the ribosomes.** *EMBO J* 2001, **18**:5280-5289.

Using a proteomic/immunochemical approach to analyse protein oxidation, the authors of this paper demonstrate that the increased oxidation of proteins in growth-arrested cells is intimately linked to the production of aberrant protein isoforms. Stasis-induced oxidation is drastically attenuated in mutants with hyperaccurate ribosomes, whereas oxidation is enhanced in mutants with error-prone ribosomes. The data point to alternative ways of approaching oxidation in growth-arrested and aging cells.

36. • Aguilaniu H, Gustafsson L, Rigoulet M, Nyström T: **Protein oxidation depends on the state rather than rate of respiration in *Sacchormyces cerevisiae* cells in the G_0 phase.** *J Biol Chem* 2001, **276**:35396-35404.

This work demonstrates, by online measurements of total metabolic and respiratory activity, that the rate of respiration is not correlated to the degree of oxidative damage of arrested G_0 cells. Instead, it is shown that increased oxidation of target proteins occurs during a state transition in the respiratory apparatus and that this oxidation can be mitigated by blocking the quinones in the reduced form by Myxothiazol.

37. O'Farrell PH: **The suppression of defective translation by ppGpp and its role in the stringent response.** *Cell* 1978, **14**:545-557.

38. •• Dukan S, Farewell A, Ballestreros M, Taddei F, Radman M, Nyström T: **Proteins are oxidatively carbonylated in response to reduced transcriptional or translational fidelity.** *Proc Natl Acad Sci USA* 2000, **97**:5746-5749.

Several different means of increasing error rates in protein biosynthesis is reported to cause an increase in total protein oxidation without an apparent increase in reactive oxygen species (ROS) production or a decrease in ROS defences. The use of diagnostic proteomics demonstrated that protein oxidation is strongly associated to the production of aberrant protein isoforms. The authors of this paper hypothesize that oxidation of proteins occurs in the absence of increased oxidative stress, elevated levels of ROS, or a diminished defence system, but may instead be due to increased concentration of substrates (misfolded proteins) available for oxidative attack.

39. Wenthzel AM, Stancek M, Isaksson LA: **Growth phase dependent stop codon readthrough and shift of translation reading frame in *Escherichia coli*.** *FEBS Lett* 1998, **421**:237-242.

40. Barak Z, Gallant J, Lindsley D, Kwieciszewki B, Heidel D: **Enhanced ribosome frameshifting in stationary phase cells.** *J Mol Biol* 1996, **263**:140-148.

41. Orgel LE: **The maintenance of the accuracy of protein synthesis and its relevance to ageing.** *Proc Natl Acad Sci USA* 1963, **49**:517-521.

42. Kowald A, Kirkwood TB: **Towards a network theory of ageing: a model combining the free radical theory and the protein error theory.** *J Theor Biol* 1994, **21**:493-508.

43. Gallant J, Kurland C, Parker J, Holliday R, Rosenberger R: **The error catastrophe theory of aging: Point Counterpoint.** *Exp Gerontol* 1997, **32**:333-346.

44. Starke PE, Oliver CN, Stadtman ER: **Modification of hepatic proteins in rats exposed to high oxygen concentration.** *FASEB J* 1987, **1**:36-39.