Conditional and replicative senescence in *Escherichia coli*
Åsa Fredriksson and Thomas Nyström

Analysis of the molecular mechanisms underlying the cellular degeneration of bacteria in stationary phase (known as conditional senescence) reveals interesting similarities with the aging process of higher organisms. These similarities include the role of self-inflicted oxidative damage and the importance of protein quality control systems in retarding senescence. In addition, recent data suggests that *Escherichia coli* cells display signs of replicative senescence, or loss of fitness, during exponential growth and that this phenomenon targets the ‘older’ cells. Thus, bacterial physiology might entail both conditional and mandatory aging processes.

**Introduction**

The process of producing an entire, functional *Escherichia coli* cell from a preferred carbon source, amino acids and some minerals takes less than 20 min at 37 °C. The physiological and molecular mechanisms behind this remarkable metabolic and reproductive efficiency have been the major subject of the study of bacterial physiology. However, bacterial growth cannot proceed ad infinitum and bacteria eventually enter stationary phase, a phase of growth arrest resulting from nutrient depletion. The average life-span — measured as the time of sustained reproductive ability — of stationary phase *E. coli* cells is around three to five days when starved for exogenous carbon (for examples see [1–3]). This loss in plating efficiency has been described in microbial textbooks as the nearest bacteria come to a ‘natural’ death that is familiar among aging organisms. Even so, it is a form of accidental death conceptually distinct from mandatory aging. Therefore, the term conditional senescence has been used to make such a distinction. Yeast microbiologists have been less cautious and refer to stationary phase die-off simply as chronological aging (e.g. see [4]).

It has been assumed that binary fission of bacteria proceeds with a non-conservative dispersion of both undamaged and damaged constituents, and that in an exponentially growing bacterial population there is no adult form of bacterial cells. This has lead to the conclusion that bacteria are essentially immortal as long as the nutrient supply allows for growth and reproduction; this view has been challenged recently by data showing that bacteria, like the yeast *Saccharomyces cerevisiae*, show signs of reduced fitness, or deterioration, during growth and that this specifically affects the ‘replicatively older’ cells (i.e. old-pole cells in bacteria) [5**]. A brief overview of recent findings concerning conditional senescence and replicative senescence is given here, with the spotlight placed upon protein damage and quality control as a possible common denominator in these separate pathways of deterioration.

**Conditional senescence, oxidation, and protein quality control**

There are two major regulatory networks responsible for damage-protection during *E. coli* growth arrest; the σ^8^ (encoded by rpoS) and σ^32^ (encoded by rpoH) regulons. The σ^8^ system is called the general stress defence regulon, whereas the σ^32^ system is commonly known as the heat shock regulon. If either of these two sigma factors are deleted, the cells die off more rapidly in stationary phase (accelerated senescence) and the σ^8^ regulon has been shown to mitigate starvation-induced protein carbonylation [6,7], an irreversible modification that is widely used for the detection of oxidative damage to proteins in a variety of organisms [8]. In addition, heat shock proteins (HSPs) extend the life span of higher organisms when ectopically overproduced and such effects on senescence might be linked to a possible role of HSPs in countering protein oxidation. Indeed, overproduction of the HSP transcription factor σ^32^ was recently shown to mitigate protein carbonylation in *E. coli* cells entering a growth-arrested state [9].

Individual overproduction of different chaperone systems suggests that the DnaK/Dnaj (see Table 1 for this and other genes and proteins mentioned here) system is more important in the cellular defence against this oxidative modification than the GroEL/GroES system [9] (see also [10]). Defence against stasis-induced carbonylation also requires the Lon and HslVU proteases, whereas a role for ClpP was only observed in cells lacking Lon and HslVU [9]. However, it is clear that ClpPX and ClpPA have an important role in stationary phase proteolysis because many proteins, including LeuA, TrxB, GdhA, GlnA, MetK and alkyl hydroperoxide reductase, are significantly...
more abundant in stationary phase cells lacking these proteases [11]. In fact, Weichart et al., [11] suggest that the abundance of most major growth phase regulated proteins in *E. coli* are regulated by the activity of the ClpAP and ClpXP proteases.

The DnaK/DnaJ system is not expected to repair or refold carbonylated proteins as, as far as we know, carbonylation is an irreversible modification [8]. The reduced carbonylation observed in cells that overproduce this chaperone system might instead result from a reduced abundance of aberrant proteins, and/or an increase in the DnaK/DnaJ-dependent solubility of carbonylated proteins (Figure 1). The former of these suggestions stems from data demonstrating that aberrant forms of proteins are more susceptible to oxidative carbonylation than are their native counterparts [12]. Thus, any condition that reduces the levels of such aberrant proteins, such as increased ribosomal proofreading [1] or elevated levels of DnaK/DnaJ [9] might also be expected to reduce cellular carbonyl levels. The second idea results from the observation that carbonylated proteins are susceptible to proteolysis as long as they remain soluble [13]. However, heavily carbonylated proteins are prone to aggregate, and such high molecular aggregates escape proteolysis [13]. Increased levels of DnaK/DnaJ might keep carbonylated proteins in a soluble protease-susceptible form, and thereby contribute to their degradation, for example by Lon and HslVU (Figure 1). However, whereas it is clear that mutant cells lacking specific members of the heat shock regulon, (e.g. DnaK, ClpP, HslVU and Lon [14]), die at an accelerated rate in stationary phase, it is uncertain whether the levels of HSPs constitute a bottleneck in the stationary phase survival of wild type *E. coli* cells [9]. In addition, whereas genes and regulons that are of importance to mitigating oxidative damage are required in order for the cells to survive stasis [3,15,16], it is not clear that oxidative damage are required in order for the cells to survive stasis [3,15,16], it is not clear that oxidative damage reduces the survival of wild type cells and, as yet, only circumstantial evidence supports oxidative damage as being a key factor in the loss of E. coli culturability in stationary phase [15–17]. By contrast, stationary phase die-off of *S. cerevisiae* has been more firmly linked to oxidative damage, and genetic alterations that affect the production and scavenging of reactive oxygen species are effective in retarding stationary phase death of this model system [18–21]. No such similar effects have, as far as we know, been obtained with respect to delaying conditional senescence in *E. coli*.

The functions of other proteins that are of importance in delaying conditional senescence, that is the universal stress proteins (USPs), have recently been linked to oxidative stress defence [22]. It had previously been observed that the *E. coli* USPs undergo coordinated induction in response to growth arrest [23]. There are

### Table 1

The genes and proteins mentioned in this review, with and their functions and induction pathways

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein name</th>
<th>Function</th>
<th>Induced by</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ahpC</em></td>
<td>Alkyl hydroperoxide reductase</td>
<td>Redox regulation</td>
<td>Oxidative conditions</td>
</tr>
<tr>
<td><em>clpA</em></td>
<td>ClpA</td>
<td>Chaperone, specificity component of a Clp protease</td>
<td>Starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>clpP</em></td>
<td>ClpP</td>
<td>Proteolytic subunit of a Clp protease</td>
<td>Heat shock</td>
</tr>
<tr>
<td><em>clpX</em></td>
<td>ClpX</td>
<td>Chaperone, specificity component of a Clp protease</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>dnaJ</em></td>
<td>DnaJ</td>
<td>Chaperone</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>dnaK</em></td>
<td>DnaK</td>
<td>Chaperone, protein management, interacts with DnaJ and GrpE</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>ftsH</em></td>
<td>FtsH</td>
<td>AAA family protease</td>
<td>Heat shock</td>
</tr>
<tr>
<td><em>gdhA</em></td>
<td>NADP-specific glutamate dehydrogenase</td>
<td>Nitrogen metabolism</td>
<td>Nitrogen availability in growth medium</td>
</tr>
<tr>
<td><em>glnA</em></td>
<td>Glutamine synthetase</td>
<td>Nitrogen metabolism</td>
<td>Nitrogen starvation</td>
</tr>
<tr>
<td><em>groEL</em></td>
<td>GroEL</td>
<td>Chaperone, protein management, interacts with GroES</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>groES</em></td>
<td>GroES</td>
<td>Chaperone, modulates the ATPase activity of GroEL</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>grpE</em></td>
<td>GrpE</td>
<td>Nucleotide exchange factor</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>hsU</em></td>
<td>HsU</td>
<td>Chaperone subunit of the HsU protease</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>hsV</em></td>
<td>HsV</td>
<td>Proteolytic subunit of the HsV protease</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>iraP</em></td>
<td>IraP</td>
<td>Inhibitor of RssB activity during phosphate starvation</td>
<td>Leucine starvation</td>
</tr>
<tr>
<td><em>leuA</em></td>
<td>α-isopropylmalate synthase</td>
<td>Leucine biosynthesis</td>
<td></td>
</tr>
<tr>
<td><em>lon</em></td>
<td>Lon</td>
<td>Protease</td>
<td>Heat shock, starvation, aberrant proteins</td>
</tr>
<tr>
<td><em>metK</em></td>
<td>S-adenosylmethionine synthetase</td>
<td>Methionine biosynthesis</td>
<td>Amino acid starvation</td>
</tr>
<tr>
<td><em>rssB</em></td>
<td>RssB</td>
<td>Sigma-S adaptor protein</td>
<td>Stationary phase</td>
</tr>
<tr>
<td><em>trxB</em></td>
<td>Thioredoxin reductase</td>
<td>Redox regulation</td>
<td>Oxidative conditions</td>
</tr>
<tr>
<td><em>upsA</em></td>
<td>Universal stress protein A</td>
<td>Stress resistance</td>
<td>A large number of stresses</td>
</tr>
<tr>
<td><em>upsD</em></td>
<td>Universal stress protein D</td>
<td>Stress resistance, Iron metabolism</td>
<td>A large number of stresses</td>
</tr>
</tbody>
</table>
six *E. coli* USPs, and they can be divided into four classes on the basis of their structural motifs. A series of chromosomal deletion mutants were designed to analyze the function of each class and, contrary to expectation, this analysis showed that USPs have distinct, overlapping functions. Specifically, two of the three class I proteins, UspA and UspD, are involved in defence against oxidative stress [22]. In addition, UspD was found to be involved in intracellular iron scavenging [22]. Also, different *usp* mutants were greatly affected in their adhesion and motility capabilities. On the basis of these results it was suggested that USPs are involved in “reprogramming the cell towards stress defence and escape”, a bacterial version of the ‘fight or flight’ response [22].

**Protein oxidation as a signal for stationary phase induction of cross protection**

In addition to being involved in the mitigation of stasis-induced oxidation, efficient induction of the heat shock regulon upon starvation was recently shown to require the presence of oxygen [24]. It was proposed that the role of oxygen in triggering HSP expression is intimately linked to the observation that mistranslated proteins are susceptible to oxidation [1,12], and that this oxidation promotes a further loss of the structural integrity of the protein [13]. This, in turn, results in an increased exposure of hydrophobic surfaces, increasing the target sites for the DnaK/DnaJ chaperone system; the negative modulator of the HSP response (Figure 2).

The effect of oxygen on HSP gene expression might also be exerted through detrimental oxidation of DnaK itself. A larger fraction of DnaK is oxidized and shows signs of aberrant structural integrity in cells that have been starved in the presence of oxygen, when compared to those starved in the absence of oxygen [24]. Possibly, this aberrant and more open structure might render DnaK non-functional, which would lead to a higher level of HSP synthesis because of decreased degradation of α32. The evolutionarily conserved sensitivity of Hsp70 proteins to oxidative modification in distantly related organisms might point to a role of Hsp70 proteins in oxidation sensing and signalling. In line with this, a recent study shows that DnaK is reversibly inactivated upon heat stress in the presence of H2O2 [25*]. This inactivation was linked to significant reduction of cellular ATP-levels by H2O2, leading to nucleotide-deprivation of the N-terminal ATPase domain of DnaK, which, as a consequence, becomes thermolabile and unfolded. It is noteworthy that *in vitro* refolding of the inactivated DnaK
required the presence of a reducing agent; for example, stress-removal and addition of ATP was not enough, implying that the unfolded domain is oxidatively modified [25/26].

Less is known about the sensing–signal transduction pathway that is behind stationary phase accumulation of $\sigma^S$. A large number of cis-regulatory determinants and trans-acting regulatory factors involved in $\sigma^S$ transcription and translation have been identified [26]; yet, the key process responsible for the accumulation of $\sigma^S$ during carbon depletion is regulated $\sigma^S$ proteolysis. The protease ClpXP and the two-component orphan response regulator RssB (also known as SprE), a specific $\sigma^S$ recognition factor, are essential for the process of $\sigma^S$ degradation. The affinity of RssB for $\sigma^S$ is modulated by phosphorylation of the RssB receiver domain [27,28]. It is tempting to speculate that stabilization of $\sigma^S$ during starvation is a result of dephosphorylation of the RssB response regulator, and it has been proposed that the ArcB two-component sensor might be involved in such a mechanism [29]. However, mutations (RssB Asp58Ala) in the conserved phosphorylation site of RssB affect basal levels of $\sigma^S$, whereas its accumulation and increased stability upon starvation or stationary phase remains regulated [30]. Thus, it appears that the sensing–signaling device used by *E. coli* to stabilize $\sigma^S$ upon carbon starvation operates independently of RssB phosphorylation/dephosphorylation [30]. Interestingly, $\sigma^S$ accumulation during phosphate starvation involves a novel protein, IraP, which interferes with RssB-dependent degradation of $\sigma^S$ during phosphate starvation, but not during carbon starvation [31]. In addition, increased translation of the rpoS transcript appears to be more important for $\sigma^S$ accumulation during phosphate starvation than during carbon starvation [32].

In view of the importance of the RpoS regulon in defending the cell against stationary phase induced oxidation [7], it would be interesting to determine whether such oxidation, such as is the case for the $\sigma^{32}$ regulon [24], is involved in triggering $\sigma^S$ accumulation.
Replicative senescence in E. coli — a concept revisited

In contrast to the case of asymmetrically dividing organisms, such as S. cerevisiae [33] and Caulobacter crescentus [34], the more-or-less symmetrical cytokinesis of E. coli cells is believed to be incompatible with replicative aging or, for that matter, with asymmetrical fitness (or growth rate) of the produced daughter cells. However, there was one report in 1999 [35] on a phenomenon akin to replicative senescence in a symmetrically dividing organism, Schizosaccharomyces pombe. More recently, Stewart et al. [5**,36] reported on a similar phenomenon in E. coli. By tracking the poles of the cells and by measuring the increase in length of cells (aided by the cells being YFP-labelled) in micro-colonies, it was possible to calculate the generation time of individual cells. By doing so, the authors found that the growth rate decreased in cells that inherited old poles. This prompted the authors to suggest that E. coli cells, like those of S. cerevisiae, are subject to replicative aging. A counterpoint was presented by Woldringh [37] who suggested that the rather modest effects observed (old-pole cells grew on average 2% more slowly than new-pole cells) falls within the expected stochastic variation and is sufficiently far from eliciting catastrophic cell death through an aging-like mechanism. Another point is that the new-pole cells were on average 0.9% larger than old-pole cells, which might account for the differences in growth rates as measured by the increase in length [38]. However, the reduction in growth rate of old-pole cells observed by Stewart et al. [5**,36] were cumulative. Thus, some aspects of E. coli cytokinesis might be similar to that of S. cerevisiae, in which damaged proteins are asymmetrically distributed such that oxidatively damaged proteins are retained in the old mother cell [39].

A question of interest is whether asymmetrical cytokinesis and segregation is accidental or selected. In other words, is there an advantage of producing daughter cells of unequal reproductive potential or is asymmetry caused by physical or metabolic constrains that have no obvious bearing on fitness (see [40]). It might seem that symmetrical segregation of damage is the most efficient strategy of cytokinesis for unicellular organisms, at least during steady-state conditions. However, it has been suggested that asymmetrical segregation of damage that cannot be repaired — production of protein carbonyls is one example of such damage — might be beneficial at high cell densities and slow rates of replication [38]. In addition, during conditions of fluctuating external stress, which might occasionally reach lethal levels, an asymmetrical segregation of irreparable damage might permit survival of the clone at the expense of the ‘mother-type’ cells in which most of the damage is retained.

Conclusions

Studies of microbial models systems, such as those of S. cerevisiae and E. coli, have identified oxidative damage as a potentially important culprit in limiting the life-span of these organisms during growth arrest. A few key signalling pathways that regulate general stress resistance and damage protection are required for longevity under growth arrest. These systems might, in fact, have evolved and been retained as they enhance the maintenance capacity of the cell and promote survival during periods of starvation, and then later become crucial in retarding aging in higher, mandatory aging, organisms. Such arguments could be made for the systems, for example FOXO (Forkhead) transcription factors working in concert with insulin and insulin-like signalling, involved in postponing aging in worms and flies [41]. Whereas different organisms have evolved different regulatory circuits and factors (e.g. alternative sigma factors in bacteria and Forkhead transcription factors in worms) for controlling these systems, the target genes appear to be conserved and encode a variety of stress-defence proteins, including chaperones and scavengers of reactive oxygen species [42].

Recent data also point to the possibility that E. coli, like S. cerevisiae, is subjected to replicative aging, that is the two daughter cells produced show an unequal fitness and life expectancy [36]. Whereas unequal segregation of damaged proteins during cytokinesis has as yet only been demonstrated for S. cerevisiae [39], it is possible that a similar phenomenon is causing asymmetries in the fitness of dividing E. coli cells. In fact, E. coli cells subjected to growth-arrest exhibit markedly different loads of carbonylated proteins [17]. The cells from the population displaying low carbonyl levels remain reproductively competent, whereas cells with a high carbonyl load become genetically dead (or nonculturable). Whether this starvation-induced heterogeneity in carbonylation and fitness is programmed and involves damage segregation during cytokinesis remains to be elucidated. In addition, asymmetrical distribution of irreversible damaged proteins has recently been observed during embryonic development in mice [43**]. Unexpectedly, undifferentiated mouse embryonic stem (ES) cells contain high levels of both carbonyls and advanced glycation end-products, but rid themselves of such damage upon differentiation [43**]. On the basis of these results, it is worth considering that mammalian offspring might initially be free of cytosolic damage because of elimination of damage by early development rather than by a mechanism keeping the germ line cells free of deteriorated macromolecules. However, it is not yet clear to what extent this elimination of damage involves asymmetrical segregation, but it will be intriguing to elucidate if the genetic determinants involved in microbial damage segregation have been conserved to provide higher, multicellular, organisms with cellular rejuvenation potential.

Acknowledgements

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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


25. Winter J, Linke K, Jatzek A, Jakob U: Severe oxidative stress causes inactivation of DnaK and activation of the redox-regulated chaperone Hsp33. *Mol Cell* 2005, 17:381-392. This paper shows that upon exposure of cells to reactive oxygen species and elevated temperatures, the DnaK chaperone system fails to function. Inactivation of DnaK is argued to result from a dramatic decrease in intracellular ATP levels, rendering the ATPase domain of DnaK nucleotide-depleted and thermostable.


These authors demonstrate that undifferentiated mouse ES cells and cells of the inner cell-mass of blastocysts, unexpectedly contain high levels of damaged proteins. This damage is rapidly eliminated upon differentiation of ES cells *in vitro* and during normal embryonic development *in vivo*. These results might call for re-evaluation of the notion that the offspring of mammals start out with low levels of (cytosolic) damage because of a mechanism that keeps the germ-line cells free of deteriorated macromolecules.