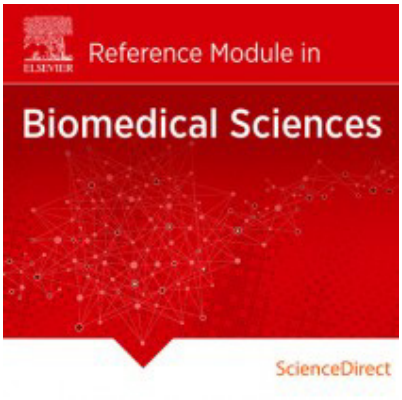

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Stress, Bacterial: General and Specific☆

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Glossary

Adapter and anti-adapter proteins Proteins that regulate protein degradation by the ClpXP protease.
Ancillary factors Proteins or other molecules that influence RNA polymerase activity.
Antiporter A protein in cytoplasmic membrane that brings about exchange of external protons and a cellular ion/compound.
Electrophiles Compounds that accept electrons.
Eutrophic environments Environments made nutrient-rich primarily through human activity.
Inclusion bodies Precipitated and denatured proteins inside a cell. These are usually formed in bacteria when a heterologous protein is overproduced.
Periplasm Space between outer and cytoplasmic membranes in Gram-negative bacteria.
Pex proteins The core set of proteins induced in response to diverse stresses.
Porins Proteins in the bacterial outer membrane that form water-filled pores, permitting transport.
Proteome Complete protein profile of a cell.
Redox cycle A reduction reaction that generates unstable radicals. These give their electrons to oxygen generating reactive oxygen species (ROS). The radical is changed back to the original compound and becomes available for further ROS generation.
Sigma factors Small proteins that combine with the RNA polymerase core enzyme. The resulting RNA polymerase holoenzyme can transcribe various genes. Each species of RNA polymerase generally recognizes specific promoter sequences.
Transcriptome Complete gene transcription profile of a cell.

☆*Change History:* August 2014. AC Matin added abbreviations, references, and further reading. The author made changes in sections 'General Stress Response' and 'Regulation of Stress Response.'

Abbreviation

cAMP	Cyclic AMP
GSR	General stress response
HARVs	High aspect to ratio vessels
HGH	Human growth hormone
HPK	Histidine protein kinase, the sensor kinase of thetwo component systems
IHF	Integration host factor
LpDH	Lipoyldehydrogenase
OMPs	Outer-membrane proteins
QH_2	Hydroquinone
RNAP	RNA polymerase
ROS	Reactive oxygen species
RR	Response regulator
RR	Response regulator protein, also, a part of the two component systems.
rRNA	Ribosomal RNA
sRNAs	Small RNA
TIR	Translational initiation region
UTR	Untranslated region

Defining Statement

Bacteria counter stress at two levels, specific and general, to escape a given stress and to acquire greater robustness. I will discuss here the mechanisms of escape, increased cellular robustness, and the molecular mechanisms that enable a bacterium to shift from rapid growth mode to stasis and enhanced resistance.

Introduction

Bacteria, like other living things, require certain physicochemical conditions in order to thrive. Usable nutrients need to be sufficiently available, temperature and pH maintained within specific limits, and toxic influences absent. Under such optimal conditions, bacteria grow at maximal rates of which they are genetically capable. The animal gut flora encounters such conditions after the host has taken a meal, intracellular pathogens often immediately after invasion, and environmental bacteria in, for example, eutrophic environments. But such conditions are rare and fleeting, and as a rule, bacteria in nature exist under conditions that are not only suboptimal but can be outright hostile to their survival, exposing them to diverse kinds of stresses.

A common stress is lack of food. Thus, the gut flora by its rapid growth soon exhausts the nutrients passed on to the host intestine and progresses from feast to famine, and the same is likely true of an intracellular pathogen. While eutrophic environments are on the rise due to human activities, much of the natural environment nevertheless remains severely nutrient-poor. Oceans are estimated to have 0.8 mg carbon nutrients per liter, and the concentration of individual carbon compounds in fresh water is often as low as 6–10 $\mu g\, l^{-1}$. Similarly, soils as rule possess little usable nutrients, as most of the 0.8–2.0% carbon in this environment is humus, which bacteria for the most part cannot use. In other natural environments, bacterial growth is restricted by the scarcity of other nutrients, such as nitrogen, phosphorus, and/or iron (Ghiorse and Wilson, 1988; Kaiser et al., 2014; Matin et al., 1989; Rohmer et al., 2011).

The fluctuating conditions in nature expose bacteria to additional stresses. Diurnal and seasonal changes in temperature can be significant, and a host of abiological and biological factors can result in exposure to a variety of insults, such as pH, osmotic, shear, and oxidative stresses. The pathogenic bacteria have not only to be adept at surviving these stresses during their extra-host existence but also to be able to cope with deleterious influences as they attempt to survive in the host in disease initiation. For example, to infect a host, *Salmonella enterica* serovar Typhimurium, which causes a typhoid-like disease in mice, has to survive passage through the stomach where the average pH over a 24-h period is as low as 1.5. It then invades the interior of the host by infecting the microvilli of the gastrointestinal tract, which are low-shear environments (Guo et al., 2000), and it is then ingested by the host macrophage, where additional insults await – oxidative stress, nutrient deprivation, and low pH. To meet such threats to survival, bacteria have evolved elaborate adaptive responses; these are the subject of this article with special emphasis on starvation, although other stresses are also considered.

The Stress Response is Two-Pronged

Bacteria meet the challenge to survival posed by stresses using a two-pronged strategy. One is aimed at neutralizing and escaping the specific stress that is encountered. This response tends to be unique to each stress; thus the proteins a bacterium needs to escape, for instance, oxidative stress are different from those it utilizes to escape starvation. This is termed the specific stress response. The second component of the stress response is aimed at preventing and repairing the damage that the stress might cause and is activated as an insurance policy, since there is no guarantee that the first response will succeed in preventing the deleterious effects of the stress. All stresses, if not neutralized, lead to a common outcome, namely damage to the cell biomolecules, and the second

tier of the stress response is aimed at preventing and repairing this damage. Thus, this facet of the stress response results in making bacteria resistant not only to the stress that is experienced but also to others, and is thus termed the general stress response (GSR).

Specific Stress Response

Starvation

The first definitive indication that bacteria respond to stresses by a two-pronged strategy came when the proteomes of bacteria subjected to different stresses were examined. For example, starvation for carbon, nitrogen, or phosphorus resulted in the induction not only of proteins unique to that starvation condition but also to that of a core set of proteins that was common to all the starvation conditions (referred to as Pex proteins) (Groat et al., 1986). Exposure to stresses mechanistically different from starvation, *viz.*, oxidative, osmotic, pH, and others, also led to the induction of unique and common proteins, many of the latter being the same as the core starvation (Pex) proteins (Jenkins et al., 1988a). Based on these findings, it was proposed that the proteins unique to a specific stress were concerned in enabling the bacteria to neutralize that particular stress, while the core set of proteins was concerned with conferring resistance to stresses in general (Matin, 1991a; Matin et al., 1989). This has been found to be the case (Peterson et al., 2005a). In this section, I will discuss the physiological role of selected proteins that are concerned with the escape response; the function of the Pex proteins that confer general resistance is discussed in subsequent sections.

Examples of proteins concerned with escaping stresses are provided in **Table 1**. Starvation-escape response consists in the synthesis by bacteria of enzymes that amplify their capacity to obtain the scarce nutrient (Harder et al., 1977). This is accomplished either by increasing the concentration of the relevant enzymes or by synthesizing a new set that possess a higher affinity for the nutrient. Either way, a superior capacity is acquired to scavenge the scarce nutrient. The proteins that are induced can concern every metabolic feature: transport through the outer and cytoplasmic membranes, enzymes involved in substrate capture, and those responsible for subsequent flux through the metabolic pathways. Thus, when phosphate concentration falls below some

Table 1 Selected escape-response proteins

Protein	*Function*
Phosphorous starvation	
Pst	High-affinity phosphate transport system
PstS (also called PhoS)	Periplasmic Pi-binding protein required for PstS function
PhoE	Porin that facilitates Pi transport through the outer membrane
PsiB and PsiC	Glycerol phosphate transport systems
Bacterial alkaline phosphatase	Carbon–phosphorus bond lyase
Carbon starvation	
Periplasmic-binding proteins (e.g., MalE)	Enhanced transport (e.g., maltose)
Glucokinase	Substrate capture (glucose)
Lactate dehydrogenase	Substrate capture (lactate)
β – Galactosidase	Substrate capture (lactose)/metabolic potential amplification
CstA	Substrate capture (peptides)/metabolic potential amplification
Glycerol kinase	Substrate capture (glycerol)
Glucose-6-phosphate dehydrogenase	Enhanced flux through catabolic pathways
Phosphofructokinase	Enhanced flux through catabolic pathways
Pyruvate kinase	Enhanced flux through catabolic pathways
Aconitase	Enhanced flux through catabolic pathways
Isocitrate dehydrogenase	Enhanced flux through catabolic pathways
Malate dehydrogenase	Enhanced flux through catabolic pathways
Other stresses[a]	
Aerobactin (iron starvation)	Iron chelator
Glutamine synthetase (nitrogen starvation)	Substrate capture
Kdp (potassium starvation)	High affinity K^+ transport
Superoxide dismutase (oxidative stress)	Decomposes superoxide
KatE (oxidative stress)	Catalase
KatG (oxidative stress)	Catalase
Thiol peroxidase (oxidative stress)	Thiol-dependent hydroperoxidase
Sulfate adenylyltransferase (oxidative stress)	Cysteine biosynthesis
Cysteine synthase (oxidative stress)	Cysteine biosynthesis
ChrR (oxidative stress)	H_2O_2 quencher
Lysine decarboxylase (acid stress)	Generates cadaverine that buffers the cytoplasm
CadB (acid stress)	Brings about exchanges of cellular cadaverine for medium lysine
UreI (acid stress)	Increases membrane permeability to urea which, through urease activity,buffers the cytoplasm

[a]Text in parentheses indicates the stress.

1 mmol l^{-1} in the environment, cells increase the protein PhoE, which is a porin facilitating the passage of phosphate compounds through the outer membrane into the periplasmic space of *Escherichia coli*. Here, it interacts with a high affinity-binding protein (PstS), also induced under these conditions, promoting efficient functioning of PhoE. The compounds thus transported to the periplasm are hydrolyzed by another protein induced by phosphate starvation, the bacterial alkaline phosphatase, generating Pi. Rapid transport of the latter across the cytoplasmic membrane is ensured by the fact that a high affinity Pi transport system, Pst (energized by ATP; K_m for Pi, 0.16 μmol l^{-1}), is concomitantly induced under these conditions, replacing the low affinity Pit system (energized by proton motive force; K_m for Pi, 25 μmol l^{-1}) that operates under phosphate-sufficient conditions (Harris et al., 2001; Matin et al., 1989).

This pattern has been demonstrated in several bacteria also when limitation for other nutrients is encountered. Carbon-scarce cells often also synthesize high affinity-binding proteins, for example, MalE, which binds maltose facilitating its transport into the cell. When *Pseudomonas* or enteric bacteria utilizing lactate or glucose as carbon source were subjected to the limitation of these substrates, they greatly increased the synthesis of lactate dehydrogenase or glucokinase, respectively. Concomitantly, there was a marked induction of several enzymes of glycolysis and tricarboxylic acid cycle, ensuring effective channeling of low levels of catabolites through them (Matin et al., 1976). Large amounts of glutamine synthetase, which catalyzes the first step in ammonium assimilation, are synthesized during ammonium limitation (Reitzer and Magasanik, 1986), and induction of high affinity substrate-capturing proteins occurs also during potassium and glycerol scarcity. In the former case, the cells shift to the Kdp system (high affinity; energized by ATP) from the Trk transport system (low affinity; energized by proton motive force) that is used when potassium is plentiful (Rhoads et al., 1976). Cells grown on nonlimiting concentrations of glycerol utilize a low affinity pathway for its catabolism whose initial step is catalyzed by glycerol dehydrogenase; under glycerol scarcity on the contrary, a high affinity pathway initiating with glycerol kinase is utilized (Martinez-Gomez et al., 2012). Iron-challenged cells increase the synthesis of the iron siderophore, aerobactin (Hantke, 1981). Thus, a combination of the synthesis of high affinity transport and other proteins coupled with a general increase in the level of metabolic enzymes ensures that the cells can effectively scavenge and utilize the scarce nutrient from the environment.

These measures can of course not always succeed in alleviating starvation. For instance, cells growing on glucose can synthesize any amount of enzymes to facilitate its utilization, but this would not help if this substrate becomes completely absent from the environment. An additional measure is therefore employed, which is to de-repress the synthesis of enzymes for substrates other than glucose counting on the chance that the constantly fluctuating conditions might promote their appearance in the environment. Thus, cells subjected, for instance, to glucose starvation also synthesize enzymes such as β-galactosidase and CstA, which confer on them the capacity to utilize lactose and peptides, respectively, thereby acquiring the capacity to cast a wider net for alleviating carbon starvation (Schultz and Matin, 1991).

Oxidative Stress

Ground state oxygen has two unpaired spins, and the constraints of quantum mechanics, and the resulting spin restriction, hinder its divalent reduction. This favors the univalent pathway that generates highly reactive (and toxic) oxygen species (ROS). Consequently, oxidative stress from ROS is a constant threat to bacteria and other living entities. Bacterial respiratory chains (like those of mitochondria) leak ROS (Imlay, 2008). Phagocytes possess a membrane-bound NADPH reductase, whose function is to catalyze one-electron reduction of O_2 to generate ROS so as to kill the invading bacteria (Babior, 1984). When plant cells come in contact with soil-dwelling bacteria, such as *Pseudomonas putida*, they release an immediate burst of H_2O_2. Many electrophiles generated internally by bacteria or those found in the environment are also a source of oxidative stress. Examples are quinones, nitro-compounds, chromate, and several dyes; quinones such as plumagin and juglone are secreted by plants as defense mechanisms against bacteria (Lin et al., 2010). These compounds are vicariously attacked by cellular metabolic enzymes such as glutathione and cytochrome *c* reductases, and lipoyl dehydrogenase (LpDH), which reduce them by one-electron transfer. The result is the generation of reactive radicals, such as semiquinones and Cr(V), which set up a redox cycle. In this process, the radical (e.g., semiquinone) transfers its electron to O_2 or, depending on the conditions to another molecule (e.g., NO_3), regenerating quinone and producing ROS or other equally destructive oxidizing agents (e.g., nitrosative radicals). With the continued activity of one-electron reducers, the quinone (or other such electrophiles) shuttles back and forth between its quinone and semiquinone valence states, producing large quantities of ROS. These compounds are referred to from here on as 'univalent reduction-prone' electrophiles.

That bacteria do indeed experience severe oxidative stress when exposed to univalent reduction-prone compounds was demonstrated by the use of the intracellular oxidative stress sensor 2′, 7′-dihydrodichlorofluorescein (H_2DCFDA), which is taken up by the cells and emits green fluorescence in the presence of ROS. For instance, *E. coli* cells exposed to chromate do indeed emit green fluorescence (**Figure 1**). Proteome analysis showed that these cells induced several proteins concerned with combating oxidative stress, for example, superoxide dismutase, which decomposes the superoxide radical, and those concerned with cysteine and thiol biosynthesis, which are ROS quenchers. Mutants unable to synthesize these proteins proved more sensitive to chromate killing, and strains with bolstered capacity to synthesize antioxidant defense proteins, such as ChrR (**Table 1**; see below) less so compared to the wild type (Ackerley et al., 2006). Other examples of proteins that permit escape from oxidative stress are given in **Table 1**.

ChrR, mentioned above, belongs to a recently discovered class of enzymes that combat oxidative stress in a variety of ways. These enzymes bring about a simultaneous two-electron reduction of univalent reduction-prone electrophiles. Thus, for example,

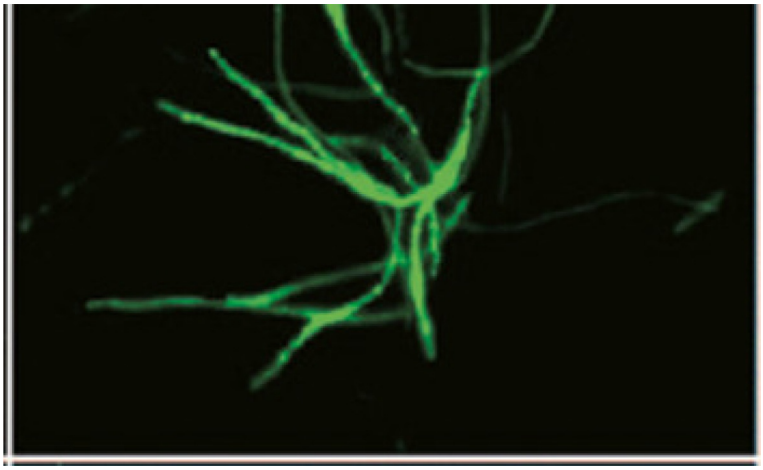

Figure 1 *Escherichia coli* cells exposed to 250 μmol l^{-1} chromate and treated with intracellular ROS sensor 2′, 7′-dihydrodichlorofluorescein. Cells were examined at × 1000 magnification with an Olympus BX60 upright fluorescence microscope. Note that the cells form snakes and fluoresce green; both are indicative of oxidative stress. Reproduced from Ackerley DF, Barak Y, Lynch SV, et al. (2006) Effect of chromate stress on *Escherichia coli* K12. *Journal of Bacteriology* 188: 3371–3381.

they convert in one step quinone into fully reduced and stable hydroquinone (QH_2), bypassing semiquinone formation. The experimental approach to determine if an enzyme reduces the univalent reduction-prone electrophiles by one- or two-electron pathway utilizes pure proteins and a source of electrons, namely NADH or NADPH. It takes advantage of the fact that cytochrome *c* is reduced by semiquinones but not by hydroquinones, and since reduced cytochrome *c* absorbs light of 550 nm wavelength, its reduction can easily be monitored in a spectrophotometer, serving as a facile probe for semiquinone formation. It was found that when quinone was reduced by a number of different cellular enzymes, such as LpDH, large amounts of reduced cytochrome *c* were generated, indicating that the quinone was reduced by one-electron transfer and generated semiquinone. However, when the reduction was catalyzed by the enzyme ChrR, no reduction of the cytochrome was seen (**Figure 2(a)**). Thus, the latter enzyme bypassed semiquinone formation resulting in direct conversion of the quinone to QH_2 (Gonzalez et al., 2005).

In an extension of this experimental approach, limiting concentrations of quinone were used, which ensured that the reaction ceased because all the available quinone in the reaction mix was exhausted. **Figure 2(b)** shows that in such a situation when ChrR is added to an in-progress LpDH-catalyzed quinone reduction, cytochrome reduction is swiftly halted, indicating that the LpDH is no longer generating semiquinone. Addition of further quinone to the reaction mix reinitiated cytochrome *c* reduction but at a very low rate and this too was soon halted. The experiment thus indicated that when ChrR is present, quinone is made largely nonavailable to LpDH, so semiquinone formation ceases. Experiments using other single-electron reducing enzymes have given similar results. Thus, not only ChrR constitutes a safe pathway for the reduction of univalent reduction-prone electrophiles, such as quinones, it is also effective in preempting their reduction by the one-electron reducers, thereby affording a two-way protection to the cell exposed to such electrophiles (Gonzalez et al., 2005).

There is in fact another level at which ChrR protects the cell against oxidative stress and that is by virtue of the fact that QH_2, which it generates, is an effective quencher of ROS, such as H_2O_2. Strains of *P. putida* devoid of ChrR and those overproducing this enzyme were grown in the presence of 3 mmol l^{-1} H_2O_2. The different cell cultures exhibited lag phases of varying duration, following which normal growth was seen (**Figure 3**). The ChrR overproducing strain was the first to recover, followed by the wild type, and finally the ChrR mutant. The recovery correlated with the ability of each strain to remove H_2O_2 from the medium, indicating that the cellular ChrR bolsters this capacity. Protein carbonylation, which is an indication of oxidative damage, was greatest in the strain devoid of ChrR and least in the one overproducing this enzyme (Gonzalez et al., 2005).

Acid Stress

Escape from acid stress involves a combination of physicochemical processes as well as the use of special enzymes to ensure that the cytoplasm is not acidified. The former mechanisms include making the cytoplasmic electric potential ($\Delta\psi$) positive, so as to oppose the entry of protons that, of course, are positively charged. It also includes changes in the composition of cytoplasmic membrane so as to render it less permeant to protons. In *Clostridium acetobutylicum*, for example, exposure to low pH results in a decrease in the ratio of unsaturated to saturated fatty acids and an increase in cyclopropane fatty acid content. An increase in phospholipids with amino acid head groups is another measure that appears to be aimed at decreasing proton permeability of the cytoplasmic membrane (Baumeister and Lembcke, 1992; Matin, 1999; Sunamoto et al., 1982).

The enzymes involved are amino acid decarboxylases. A well-studied system involves lysine decarboxylation, which removes CO_2 from lysine and generates cadaverine. Cadaverine picks up a proton, thereby contributing to the de-acidification of the cytoplasm (Merrell and Camilli, 1999). The protonated cadaverine is exchanged for external lysine by the antiporter CadB. Another

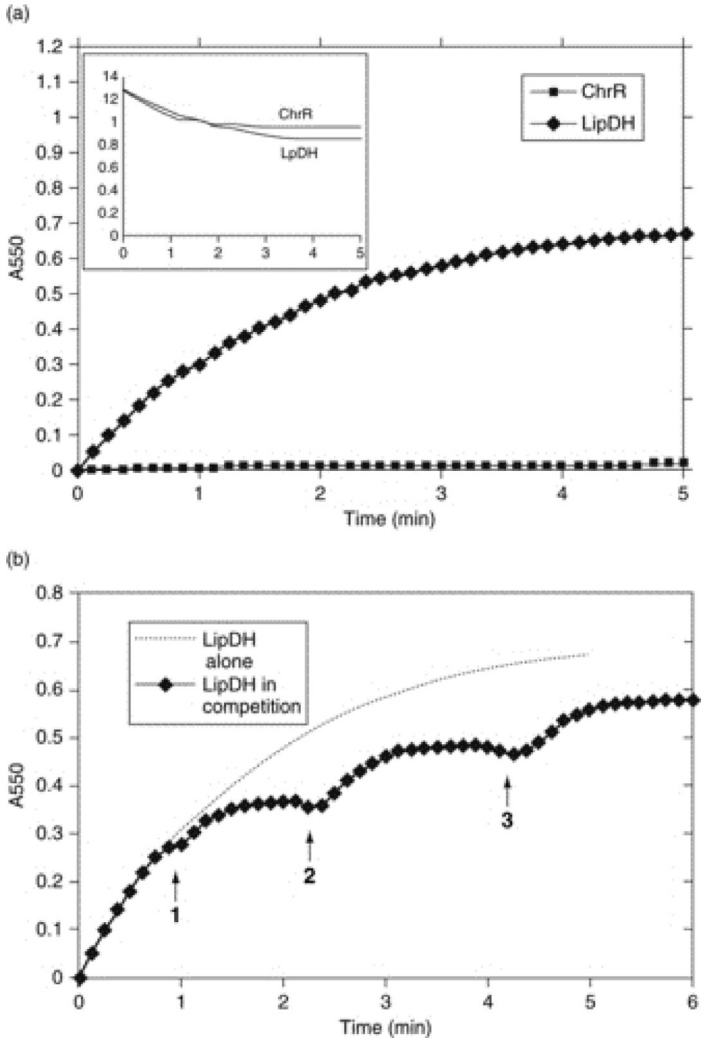

Figure 2 (a) Reduction of cytochrome *c* monitored spectrophotometrically at 550 nm during LpDH- or ChrR-catalyzed reduction of 50 μmol l^{-1} of a quinone species, benzoquinone. The appearance of reduced cytochrome *c* during the LpDH-catalyzed reaction indicates one electron transfer and generation of semiquinone, whereas the lack of this species in the ChrR-catalyzed reaction signifies a divalent mode of quinone reduction that generates QH_2 bypassing semiquinone generation. (b) Addition of ChrR to an LpDH-catalyzed reduction of limiting benzoquinone, at the point marked by *arrow* 1, rapidly arrested the reduction of cytochrome *c* relative to LpDH alone (dashed line). The addition of fresh benzoquinone (arrows 2 and 3) reinitiated cytochrome *c* reduction, but with ChrR now present, only little semiquinone is generated as indicated by very limited cytochrome *c* reduction. This indicates that the presence of the two-electron reducer, ChrR, preempts quinone reduction by the one-electron reducer, LpDH. Reproduced from Gonzalez CF, Ackerley DF, Lynch SV, et al. (2005) ChrR, a soluble quinone reductase of *Pseudomonas putida* that defends against H_2O_2. *Journal of Biological Chemistry* 280: 22590–22595.

enzyme involved in the buffering of the cytoplasm is urease, which is thought to be critically important in the ability of the gastric ulcer/carcinoma-causing bacterium *Helicobacter pylori* to colonize the stomach. This bacterium synthesizes a special membrane protein called UreI that enhances urea transport into the cell. Urea is present in the gastric juice, but its permeation into the cell without UreI is too slow to be effective in enabling *H. pylori* to keep a neutral cytoplasm (Sachs et al., 2000).

General Stress Response

Cross-Protection

As mentioned above, cells respond to different insults not only by measures aimed at escaping a particular stress, but also by bolstering the cellular machinery meant to prevent and repair damage to biomolecules that may result if the escape response fails. The evolutionary basis for this is obvious: the external environment is often so unforgiving that the escape response strategies can

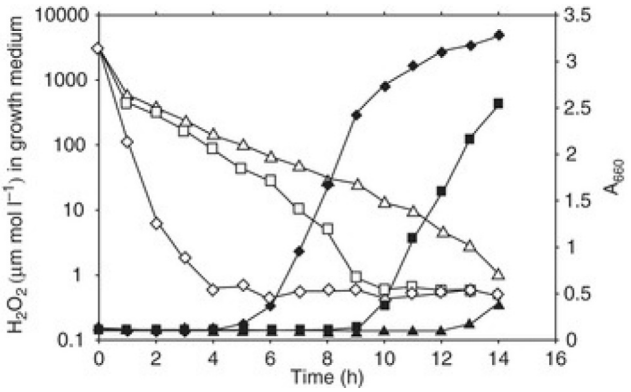

Figure 3 H_2O_2 scavenging (open symbols) and growth (as measured by increase in absorbance at 660 nm, solid symbols) of ChrR-overproducing (♦), wild-type (■), and ChrR-deficient (▲) strains of *P. putida*. Note that the overproducing strain is most efficient in decomposing H_2O_2. Reproduced from Gonzalez CF, Ackerley DF, Lynch SV, et al. (2005) ChrR, a soluble quinone reductase of *Pseudomonas putida* that defends against H_2O_2. *Journal of Biological Chemistry* 280: 22590–22595.

Table 2 Stress-induced resistances

Starvation
Heat
Cold
pH extremes
Oxidation
Hyperosmosis
Cl_2
ClO_2
Ethanol
Acetone
Deoxycholate
Toluene
Irradiation
Antibiotics and other antimicrobials

Source: Reproduced from Matin A (2001). Stress response in bacteria. In: Bolton S (ed.) *Encyclopedia of Environmental Microbiology*, vol. 6, pp. 3034–3046. New York: John Wiley and Sons.

often at best have only a partial success and survival necessitates that measures be activated to deal with the damaging effect of stresses. This is the function of the (Pex) core set of proteins that are synthesized regardless of the nature of stress, and they confer on the cell a robustness enabling it to withstand stresses in general.

Proteome analysis of cultures starved for glucose or other nutrients showed that the proteins synthesized fall into different temporal classes and that this synthesis program is essentially complete in 4 h after the onset of starvation (Schultz et al., 1988). The Pex proteins for the most part exhibit a sustained pattern of synthesis through this period, leveling off at its end. Consistent with their role in enhancing cellular robustness, it was found that inhibition of protein synthesis in a starving culture had a time-dependent effect on starvation survival, with maximum resistance developing after 4 h of protein synthesis during starvation (Reeve et al., 1984). That the core proteins are involved in conferring general resistance on the cell is further indicated by the fact that the cross-protection that starvation confers on cells against unrelated stresses, for example, heat, oxidation, hyperosmosis, and others (**Table 2**), is also dependent on the time, up to 4 h, for which they have been starved. This phenomenon is illustrated in **Figure 4(a)** for the starvation-mediated cross-protection against heat, involving exposure to the normally lethal temperature of 57 °C. For the first 4 h after the onset of starvation, increasing resistance to heat is exhibited the longer the cells are starved, with maximal resistance being acquired within this period. The phenomenon is completely dependent on protein synthesis during starvation, since its inhibition by inclusion in the starvation regime of chloramphenicol or by other means prevents resistance development (Jenkins et al., 1988b).

Since the core protein set is synthesized regardless of the nature of stress, it follows that exposure to any stress and not just starvation should confer general resistance. This is indeed the case as is illustrated in **Figure 4(b)**, which shows that cells exposed to adaptive doses of a variety of mechanistically unrelated stresses become more resistant to lethal concentrations of H_2O_2.

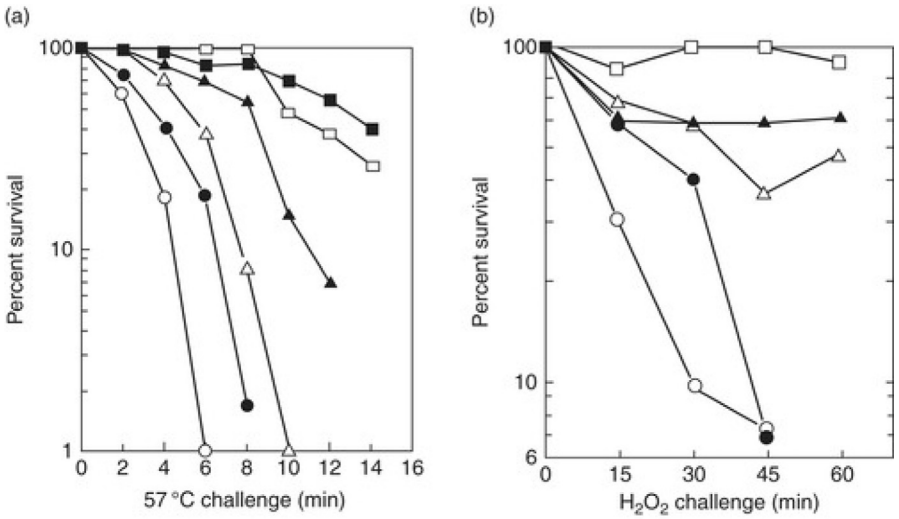

Figure 4 (a) Induction of thermal resistance in *Escherichia coli*. Cells grown at 37 °C were exposed to 57 °C during exponential growth (o), or at 1 h (Δ), 2 h (▲), 4 h (□), or 24 h (■) after glucose exhaustion from the medium. (●) Represents culture starved in the presence of chloramphenicol. (b) Comparison of the H_2O_2 resistance of glucose-starved *E. coli* cultures to growing cultures adapted by heat, H_2O_2, or ethanol. Symbols: (o) untreated; (●) ethanol-adapted; (Δ) heat-adapted; (▲) H_2O_2-adapted; (□) glucose-starved. Reproduced from Jenkins DE, Schultz JE, and Matin A (1988) Starvation-induced cross protection against heat or H_2O_2 peroxide challenge in *Escherichia coli*. *Journal of Bacteriology* 170: 3910–3914.

Role of σ^s in Resistance to Bactericidal Antibiotics.

Recent studies have shown that the loss of σ^s renders stationary phase *E. coli* highly sensitive to the bactericidal antibiotics, gentamicin, ciprofloxacin, and ampicillin. The *rpoS* mutant experienced greater oxidative stress than the wild type as determined by the activation of the SOS response, and measurement of O●, and OH● by appropriate dyes. Further investigation showed that mutational loss of antioxidant proteins, those that decompose ROS or supply NADPH for their activity namely the pentose pathway proteins (Grant, 2008; Ralser et al., 2007) – also results in the generation of greater oxidative stress in the mutants and heightened sensitivity to these drugs (Wang JH, Singh R, Benoit M, Keyhan M, Sylvester M, et al. (2014)). These findings promise to lead to measures for increasing the effectiveness of these antibiotics, an important result, given the serious threat posed by increased bacterial antibiotic resistance.

Biochemical Basis

The comprehensive resistance that stresses confer on cells is due to the fact that the core set of proteins are concerned with protecting vital cell biomolecules – proteins, DNA, cell envelope – from damage as well as to bring about repair of any damage that may still result. Envelope protection and reinforcement is afforded by proteins such as D-alanine carboxypeptidase, which likely increases peptidoglycan cross-linkage (Ghosh et al., 2008), and the products of the *otsBA* (*pexA*) genes which protect the cell membrane by promoting trehalose biosynthesis (Hengge-Aronis, 2002a). Furthermore, several periplasmic proteins concerned with the proper folding of proteins in this cell compartment are upregulated by stress; these include Dsb proteins that play a role in the formation or isomerization of disulfide bonds in proteins secreted into the periplasm, and peptidyl-prolyl isomerases concerned with the proper folding of proline-containing substrates. A consequence of stress is the accumulation in the periplasm of misfolded outer-membrane proteins (OMPs) due to the stress and excessive OMP synthesis. The OMP mRNAs are unusually stable. Two small noncoding RNAs, RybB and MicA, are induced under stress. These function as global mRNA repressors (Gogol et al., 2011a), and accelerate the decay of OMP mRNAs, thereby minimizing stress-induced damage by preventing excessive OMP production (Papenfort et al., 2006).

Protein repair

This is brought about by proteins called chaperones, which are a large and diverse group with indispensable physiological roles under all growth conditions, but which become more important under stress. Apart from conferring stress resistance, the chaperones are responsible for proper folding of nascent proteins and protein translocation across membranes. The chaperones DnaK, DnaJ, and GrpE, as well as GroEL and GroES are among the most extensively studied. These proteins are widely conserved through evolution: hsp70 is the eukaryotic homologue of the bacterial chaperone DnaK and hsp60 that of GroEL (Hendrick and Hartl, 1993).

It is thought that the nascent polypeptide chains or denatured proteins (referred to from here on as 'substrate proteins') bind DnaK and DnaJ (**Figure 5**). Interaction between the chaperones in the presence of ATP results in the formation of a ternary complex

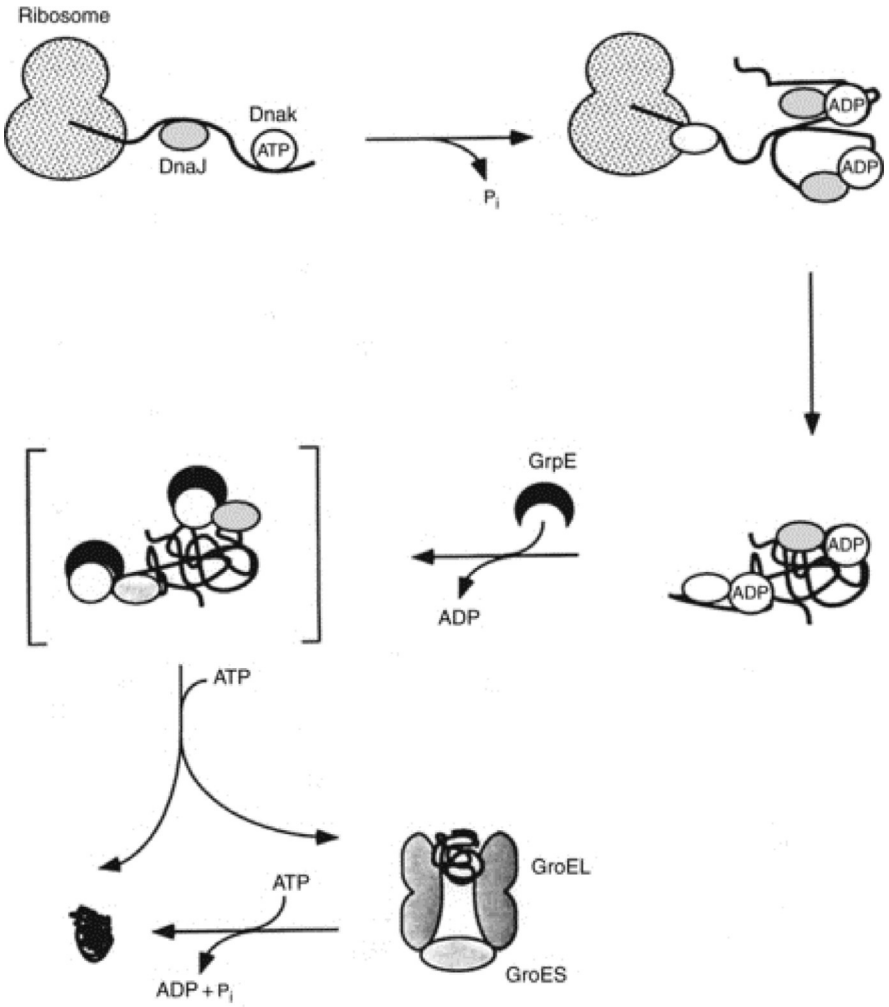

Figure 5 Schematic of the two-step pathway involved in the folding of nascent proteins and repair of damaged proteins. Reproduced from Mayhew M and Hartl F (1996) Molecular chaperone proteins. In: Neidhardt F et al. (eds.) *Escherichia coli and Salmonella typhimurium*: *Cellular and Molecular Biology*, pp. 922–937. Washington, DC: American Society for Microbiology.

consisting of the substrate protein, DnaK–ADP, and DnaJ. Dissociation of this complex is mediated by interaction with GrpE and by binding of ATP. The final stages of folding/repair in most cases involve GroEL and GroES. This model is supported by several lines of evidence. For example, the denatured enzyme rhodanese aggregates in a buffer solution, but not in the presence of DnaK, DnaJ, and ATP, as the protein is protected by the ternary complex formation. Addition of GrpE, GroEL, and GroES results in efficient refolding and activation of the enzyme. In bacteria lacking these chaperones, newly synthesized proteins aggregate *in vivo*. However, this aggregation is prevented if the chaperone production is restored. Similarly, proteins imported into the yeast mitochondria from the cytosol show defective assembly in mutants missing hsp60 (GroEL homologue), and most soluble denatured proteins of *E. coli* form complexes with GroEL as a prelude to their repair. Strikingly, proteins in their native state do not interact with the chaperones. Exposure to stresses results in association of a large number of proteins *in vivo* with chaperones presumably to escape damage. In essence, chaperones are slow ATPases, which, when bound to ADP, have a high affinity for denatured proteins, but a low affinity for them when bound to ATP. These characteristics determine the duration of their action on an unfolded part of a protein and ensure the continuation of the process until renaturation is complete.

Bacteria are often used in industry and laboratory to overproduce heterologous proteins as the process is fast and economical. However, often the overproduced protein is denatured within the cell and precipitates, resulting in the formation of inclusion bodies. A protective role against this denaturation for DnaK was demonstrated by its overproduction in the cells. Human growth hormone (HGH) is produced industrially using *E. coli* transformed with a high copy number plasmid containing the *hgh* gene that encodes this hormone. In control cells producing normal levels of DnaK, the HGH produced in the cell formed massive inclusion bodies, but in cells overproducing this chaperone there was marked breakup of these bodies (**Figure 6**) and a corresponding increase in the soluble hormone (Blum et al., 1992). Recently, CysQ, another stress response protein has been shown to play a similar role (Lee et al., 2014).

DNA repair

Several enzymes induced by stresses are concerned with DNA repair. Examples are endonuclease III and IV, Dps (PexB), and AidB, which reverse DNA methylation. A role for DnaK in DNA repair has also been reported. A major mechanism for DNA repair is the SOS response, which is activated by many different stresses, such as starvation, oxidative stress, irradiation, and treatment with antibiotics that damage DNA. This response promotes various kinds of DNA repair such as excision repair. This is aimed at excising pyrimidine dimers and other bulky lesions found in damaged DNA. The enzymes involved are UvrABC endonuclease, which is made up of proteins encoded by the *uvrA*, *uvrB*, and *uvrC* genes, helicase II (encoded by *uvrD* gene), DNA polymerase I, and DNA ligase. The UvrABC endonuclease makes incisions on each side of the lesion, generating a 12 to 13 base pair oligonucleotide. Different components of the enzyme act separately in this process. UvrA and UvrB interact to form a $UvrA_2UvrB$ complex, which identifies the DNA lesion and locally unwinds it, producing a kink in the DNA of 130°. This is followed by dissociation of the UvrA protein and formation of a stable UvrB–DNA complex, which is acted upon by UvrC to make the incision. The function of helicase II is to release the oligonucleotide and to free UvrC after the excision of the nucleotide. The gap generated by the incision is filled by DNA polymerase I, which carries out the repair synthesis, and DNA ligase, which fills the remaining nick (Hoeijmakers, 1991).

Regulation of Stress Response

Shift in the cellular gene expression and protein synthesis profile under stressful conditions involves several factors, *viz.*, changes in the concentration of sigma factors, ancillary regulatory molecules, and chemical alteration in certain proteins. Salient examples of each will be discussed.

Sigma Factors

Sigma (σ) factors are small proteins that associate with the RNA polymerase (RNAP) 'core' enzyme and determine what promoter the resulting 'holoenzyme' will recognize (**Figure 7**). The core RNAP (abbreviated as E) is made up of four polypeptides, $\alpha_2\beta\beta'$. Examples of sigma factors that play a role in stress response are σ^{70}, σ^{s}, σ^{32}, σ^{E} and σ^{54}; their holoenzymes recognize specific DNA sequences present in a region called the promoter that is located, as a rule, 10 and 35 nucleotides upstream of the transcriptional start site. The σ^{70} holoenzyme $E\sigma^{70}$ is indispensable under all growth conditions and is referred to as the vegetative sigma factor. The consensus promoter sequences recognized by three of these holoenzymes are $E\sigma^{70}$: −10: TATAAT, −35: TTGACA; $E\sigma^{32}$: −10: CATNTA, −35: CTTGAA; and $E\sigma^{54}$: GG-N_{10}GC. ($E\sigma^{s}$-recognized promoters are discussed below.) It should be noted that considerable variations from these sequences are tolerated by different species of RNAP, the enzyme species differ in their promiscuity in this respect, and a given promoter sequence can be recognized by different RNAP depending on specific conditions. For example, during starvation or osmotic stress, the transcription of the gene encoding an oxidative stress protection protein, Dps (also known as PexB), depends upon increased cellular levels of $E\sigma^{s}$ (Lomovskaya et al., 1994). However, under oxidative stress, $E\sigma^{70}$ with the help of the ancillary factor, called the integration host factor (IHF), allows transcription of *pexB* without $E\sigma^{s}$ (Kolter et al., 1993). Other genes are also transcribed by different RNAP species depending upon the presence of modifying conditions.

While all of these holoenzymes have a role in different stresses, their major role is concentrated on particular conditions. Thus, $E\sigma^{70}$ primarily transcribes the exponential phase genes and those concerned with the stress-escape response; $E\sigma^{32}$, the heat shock

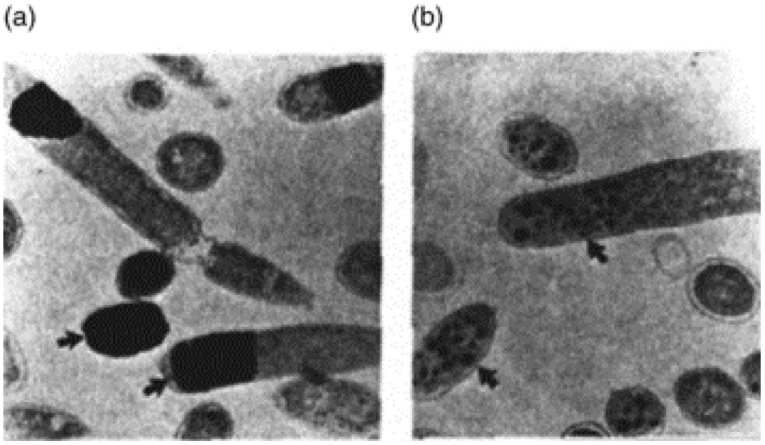

Figure 6 Transmission electron micrographs of *Escherichia coli* cells fixed in late exponential phase growth from cultures overproducing HGH protein. (a) Overproduction of HGH alone; (b) HGH overproduction along with that of DnaK. Note that in the latter, the HGH inclusion bodies are much smaller; and that a corresponding increase in soluble HGH was seen. Magnification, ×26000. Reproduced from Blum P, Velligan M, Lin N, et al. (1992) DnaK-mediated alterations in human growth hormone protein inclusion bodies. *Biotechnology* 10: 301–303.

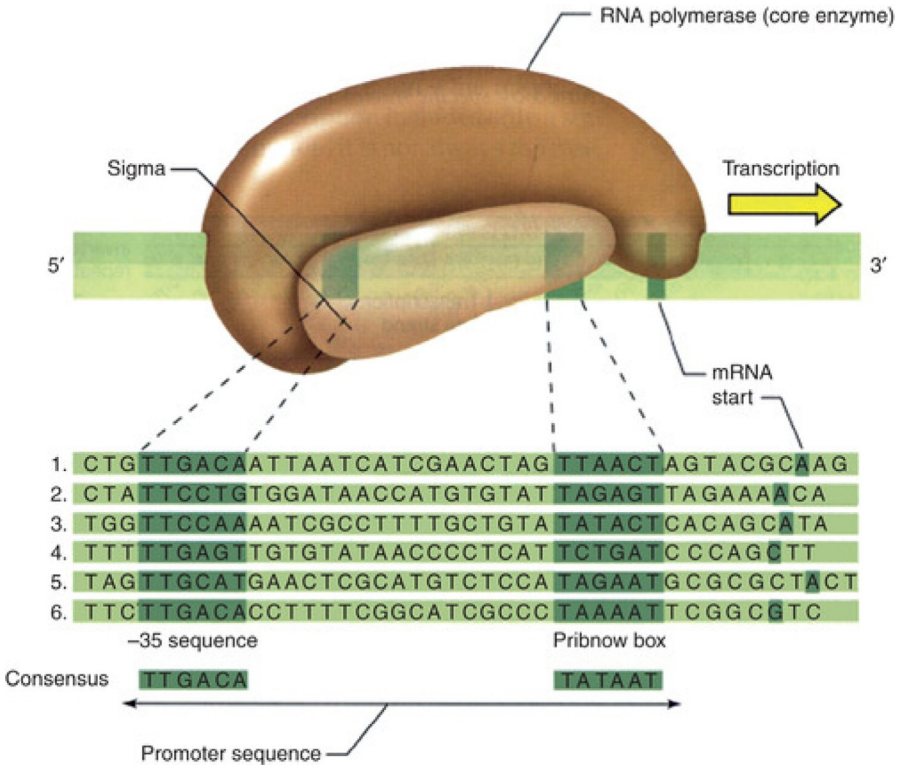

Figure 7 Schematic representation of RNA polymerase holoenzyme showing the 2.4 and 4.2 regions, which recognize respectively the −10 and −35 promoter elements. Reproduced from Madigan MT and Martinko JM (2006) *Brock Biology of Microorganisms*, p. vi. Upper Saddle River, NJ: Prentice Hall.

and starvation genes; $E\sigma^s$, the genes that are commonly expressed under stresses in general; and $E\sigma^{54}$, genes of diverse functions including those involved in starvation, flagellar synthesis, and in cell growth on nonpreferred substrates, such as environmental pollutants.

The RNAP holoenzyme most important in inducing the GSR in bacteria is $E\sigma^s$, as it controls the expression of some 140 core stress genes that are induced by diverse stresses and are responsible for this response. σ^s bears close homology with σ^{70} in critical regions of the sigma protein referred to as regions 2.4 and 4.2, which recognize respectively the −10 and −35 promoter elements. Indeed, $E\sigma^{70}$ and $E\sigma^s$ recognize many of the same promoters *in vitro*. *In vivo* however, under stresses such as starvation, despite the fact that σ^{70} is more abundant in the cells than σ^s, $E\sigma^s$ specifically targets the stress genes. Subtle differences in the promoter sequences and the role of ancillary factors account for this specificity.

Specific features of σ^s-recognized promoters

$E\sigma^s$-recognized promoters differ from those that $E\sigma^{70}$ recognizes in following respects. (1) They possess special features around their −10 region. Thus, a cytosine (C) at −13 position (i.e., 13 nucleotides upstream of the transcriptional start site) and a thymidine (T) at −14 facilitate $E\sigma^s$ binding to the promoter. Indeed, the −13 C may antagonize $E\sigma^{70}$ binding due to the differences in charged amino acids in the two sigma factors. In one instance, introduction of C at this position in a $E\sigma^{70}$ promoter improved its recognition by $E\sigma^s$. Adenine (A)/T-rich stretch is also involved, TAA at positions −6 to −4 being a common feature of $E\sigma^s$-recognized promoters; this feature may allow easier promoter melting (i.e., unwinding of the DNA strands to permit transcription). (2) $E\sigma^s$ can tolerate much wider deviations from consensus promoter sequences than $E\sigma^{70}$ and can, for example, recognize promoters with degenerate −35 sequences, possibly because it does not need such a sequence *in vivo*, or is able to recognize other sequences in place of this sequence. (3) While the requirement of a 17 base pair space between the −10 and −35 region is a strong preference of $E\sigma^{70}$, $E\sigma^s$ is more relaxed in this requirement. Indeed, many $E\sigma^s$-recognized promoters exhibit −35 like elements at other locations. (4) Certain AT-rich sequences present upstream of the −35 region favor $E\sigma^s$ binding to the promoter; the C-terminal domains of the RNAP α subunit play a role in this. (5) Both $E\sigma^{70}$- and $E\sigma^s$-recognized promoters tend to possess −10-like elements downstream of the transcriptional start site. Since early transcript complexes retain the sigma factors, these sequences cause the transcription to pause. σ^s is released more rapidly than σ^{70} from these complexes; thus the pause is shorter when $E\sigma^s$ is the transcriber, and this may facilitate $E\sigma^s$-mediated transcription of promoters that are recognized by both $E\sigma^{70}$ and $E\sigma^s$ (Hengge-Aronis, 2002b).

Other factors involved in favoring $E\sigma^s$-mediated transcription

Several *trans*-acting proteins seem to favor $E\sigma^s$-mediated transcription over that of $E\sigma^{70}$. Examples are H-NS, IHF, and Lrp. The mechanisms are not understood. In the case of H-NS, one possible mechanism is that the binding of this protein to a promoter interacting with $E\sigma^{70}$, but not $E\sigma^s$, renders the promoter unavailable for transcription. Changes in core RNAP, cytoplasmic ionic composition, as well as DNA supercoiling can also influence what RNAP species will transcribe a given gene.

A major factor responsible for a shift to different RNAP species under stress is competition for the RNAP core enzyme. The core RNAP concentration in bacterial cell is limiting and different sigma factors have to compete for it. σ^{70} possesses highest affinity for the core enzyme of all sigma factors and is present in excess; this accounts for the predominance of $E\sigma^{70}$ in unstressed cells. In stressed cells, even though σ^{70} retains its quantitative dominance, the balance shifts to RNAP species containing the alternate sigmas. Several factors account for this. $E\sigma^{70}$ dissociates so that core RNAP concentration goes up. The effectiveness of σ^{70} to bind to core RNAP is impaired due to the activity of the stationary phase-specific protein Rsd, and the small 6S RNA. σ^s has the lowest affinity of all sigma factors for RNAP and its increased synthesis under stress notwithstanding, it never attains more than one-third the level of σ^{70}. Nevertheless, it becomes the most active sigma factor in stressed cells because proteins like Crl, by binding to $E\sigma^s$, greatly enhance its activity. The small nucleotide, guanosine tetraphosphate (ppGpp), has a similar role; this is discussed further below. Certain cell metabolites such as glutamate and acetate may also have a role in stimulating $E\sigma^s$ efficiency (Foster, 2007; Paul et al., 2004). The mechanism by which σ^s concentration increases under stress has received a lot of attention and is discussed below.

Ancillary Regulatory Molecules

Cyclic AMP (cAMP)

As stated above, the core stress genes responsible for general resistance are transcribed mainly by $E\sigma^s$ and other species of RNAP bound to alternate sigma factors. However, $E\sigma^{70}$ does have a role in stress gene expression. The stress genes that this polymerase species transcribes tend to have weak promoters, that is, they deviate from the canonical promoter sequence that $E\sigma^{70}$ recognizes. Consequently, the transcription of these genes depends on the availability of ancillary transcriptional factors. This is the case with several starvation genes concerned with uptake of different compounds, and their efficient metabolism when they are present at low concentration. These genes are transcribed if cAMP is available. cAMP binds a protein called CRP, and the resulting complex binds to a specific sequence ($AGTGAN_6TAACA$) present upstream of the promoters of these genes, facilitating transcription by $E\sigma^{70}$. cAMP is present in cells at low concentration under nutrient-sufficient conditions but is increased dramatically during starvation, thereby promoting the transcription of these genes by $E\sigma^{70}$. The cAMP-dependent stress genes, however, play no role in enhanced general resistance, since starved cAMP-deficient strains exhibit the same degree of cross-protection against stresses in general as do cAMP-proficient strains. The role of these genes appears to be confined to the escape response by encoding proteins that enhance the cellular scavenging capacity by improving cellular uptake and metabolic functions.

Given the similarity between the $E\sigma^{70}$and $E\sigma^s$ promoters, the following finding is of interest: changing the position of the CRP-binding site in certain genes can alter promoter preference from $E\sigma^s$ to $E\sigma^{70}$ and vice versa.

Guanosine tetraphosphate (ppGpp)

The small nucleotide ppGpp has been studied intensively in the context of the stringent response, which refers to the phenomenon whereby amino acid starvation results in rapid downregulation of ribosomal RNA (rRNA) biosynthesis and ribosomes. It is now known that the concentration of this nucleotide goes up also in response to starvation for other nutrients as well as in stresses. Its synthesis, initially as pppGpp (which is later dephosphorylated to ppGpp), involves two pathways in *E. coli*: by the ribosome-associated protein RelA, when the ribosome A-site contains an uncharged tRNA during amino acid starvation, and by the protein SpoT, which is responsible for ppGpp synthesis in most other stresses. SpoT can also degrade ppGpp and thus has a dual role. A strain of *E. coli* missing both RelA and SpoT (referred to as ppGpp° strain) cannot synthesize this nucleotide and fails to lower its ribosome production under starvation conditions; such strains are referred to as relaxed strains. In other bacteria, for example, *Streptococcus mutans*, additional enzymes appear to be involved in ppGpp synthesis, such as RelP and RelQ.

In general, ppGpp positively affects the transcription of stress-related genes and negatively those related to growth. It exerts its regulation by binding to ββ′ subunits of RNAP near its active site, as has recently been confirmed by crystal structure. This regulation is affected by several mechanisms, such as direct effect on the rate of formation and stability of the open complex, interference with promoter clearance (which obstructs further rounds of transcription), and competition with nucleotide triphosphates used in mRNA synthesis.

A major role of ppGpp in the stress response is that it increases the ability of σ^s (and that of other minor sigma factors) to compete with σ^{70} for binding to the core enzyme. This has been shown in *in vitro* transcriptional assays and is supported by the finding that ppGpp-deficient cells exhibit decreased fractions of both σ^s and σ^{54} bound to the core polymerase. The protein DksA may have a role in augmenting this effect. As can be expected from these findings, absence of ppGpp greatly compromises starvation survival, and proteome and transcriptome analyses have shown that this is because of the lack of stress protein synthesis; instead, the cells continue to express growth-specific proteins. Thus, ppGpp is a necessary adjunct to σ^s for stress survival, and although much of this effect is likely to be affected by ensuring σ^s function, some are likely to be directly due to ppGpp activity.

ppGpp has important roles also in growing cells, where it is required for amino acid synthesis – a deficient strain cannot grow in the absence of exogenously provided amino acids. Further, ppGpp deficiency affects bacterial virulence, for example, expression of genes of pathogenicity islands (Magnusson et al., 2005).

Chemical Alteration in Proteins

Protein phosphorylation

An important mechanism in bacteria for sensing starvation and other stresses, which involves chemical alteration of proteins, is the so-called two-component system. One component of this pair is a histidine protein kinase (HPK) that autophosphorylates at a conserved histidine residue. In response to specific stimuli, the phosphorylated form is stabilized; for this reason, it is also called the 'sensor kinase.' In turn, the HPK phosphorylates the response regulator (RR) protein at a conserved aspartic acid residue. This phosphorylated form of the protein then activates transcription of the target loci. Several pairs of such proteins have been found; these initiate special adaptive strategies in response to specific environmental cues. The HPKs of different systems share homology of about 100 amino acids at their C-terminus; the RRs share homology in the 130 amino acid segments of their N-terminal ends. Among the environmental stimuli sensed by the different two-component systems are phosphate and nitrogen starvations, osmotic changes, and chemotactic stimuli. Here, the phenomenon is illustrated in the context of sensing phosphate starvation.

As stated above (**Table 1**), several genes are induced in response to phosphate starvation; together these genes are referred to as the phosphate regulon. This regulon is under the control of the *phoBR* operon encoding the PhoB and PhoR proteins. The PhoB protein is a positive regulator of this regulon, since:

1. Mutations in *phoB*, which inactivate the protein, or deletion of this gene, render the phosphate regulon noninducible.
2. Sequence analysis shows that upstream of the *phoA*, *phoBR*, *phoE*, and *pstS* (*phoS*) promoters is a highly conserved 18-bp region (CTNTCATANANCTGTCAN) called the phosphate box. *In vitro* studies demonstrate that purified PhoB protein binds to the phosphate box and that this binding is required for the transcription of the phosphate regulon genes.
3. PhoB bears close homology to the RRs in other systems, such as NtrC (involved in sensing nitrogen starvation) and OmpR (involved in sensing osmotic stress).

The *phoR* gene has a hydropathy profile typical of a membrane protein, and it shows homology to the HPK family of proteins. Like other sensor kinases, it autophosphorylates, a condition that is stabilized by phosphate starvation. It then phosphorylates PhoB, which activates the transcription of the phosphate regulon as discussed above.

Protein oxidation

This type of chemical alteration is involved in activating genes that protect against oxidative stress specifically in response to the ROS, H_2O_2, and O_2^-. A more general mechanism that activates many of the same genes in response to diverse stresses is controlled by σ^s, as discussed above.

H_2O_2 is generally sensed by the transcriptional factor OxyR and O_2^-, by the SoxR/Sox S proteins, although the two systems probably overlap. H_2O_2 directly oxidizes OxyR. The conserved cysteines, at positions 199 and 208, are in free thiol form in OxyR; H_2O_2 converts them to disulfide form. The resulting conformational change, which has been documented by crystal structure, enables OxyR to activate the transcription of genes involved in escape from oxidative stress (**Table 1**). Upon removal of the H_2O_2 stress, OxyR is reduced by glutaredoxin 1.

The SoxR protein is constitutively synthesized and also becomes activated by direct oxidation, in this case by O_2^-. The protein is a homodimer with two [2Fe-2S] centers per dimer; these centers are the loci of redox changes, that is $[2Fe\text{-}2S]^{1+} \rightleftarrows [2Fe\text{-}2S]^{2+}$ conversion. The oxidized SoxR activates *soxS* gene transcription, which in turn induces a collection of genes called the *soxRS* regulon (**Figure 8**). These genes encode enzymes that can decompose O_2^- (**Table 1**) as well as repair the damage to DNA that may result from oxidative stress, such as the endonuclease IV, mentioned above. At the termination of the stress, SoxR is reduced by an NADPH-dependent SoxR reductase (Storz and Imlay, 1999).

Regulation of σ^S Synthesis

As stated above, σ^s is the most important regulatory element in the GSR. Its cellular levels and/or activity increase in response to starvation for diverse individual nutrients as well as other stresses, and how this is accomplished is now understood in some detail at all three levels of control – transcriptional, translational, and posttranslational. I will discuss the results mainly in the context of starvation stress, unless the available information is confined to another stress.

Transcriptional control

The *rpoS* gene is located in an operon downstream of the *nlpD* gene and is transcribed from two promoters, one within the *nlpD* gene and the other upstream of this gene. Use of transcriptional fusions suggested regulation in *E. coli* at this level under starvation, and by ppGpp. However, direct measurement of *rpoS* transcription in *E. coli*, by quantifying the *rpoS* mRNA levels and determination of its half-life, indicated that enhanced transcription has no role in the observed increased levels of this sigma factor under carbon starvation. More recently, the BarA-UvrY two-component system has been found to stimulate *rpoS* transcription

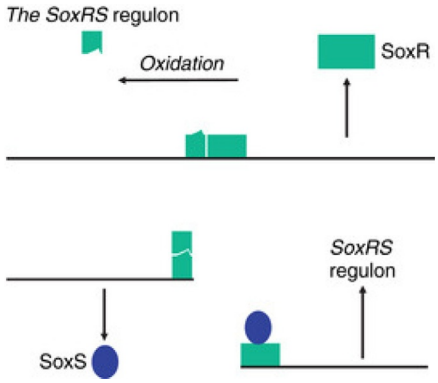

Figure 8 Schematic of SoxRS regulation of the genes involved in defense against O_2^- radical. The change in the configuration of the SoxR protein upon oxidation by O_2^- is schematically represented to show that in its altered configuration, it can activate SoxS transcription, which in turn activates the individual genes of the SoxRS regulon. Reproduced from Matin A (2001) Stress response in bacteria. In: Bolton S (ed.) *Encyclopedia of Environmental Microbiology*, vol. 6, pp. 3034–3046. New York: John Wiley and Sons.

(Mukhopadhyay et al., 2000). Also, proteins have been identified that negatively regulate the transcription of this gene; these include the Fis and the ArcA-P proteins; cyclic AMP too has a similar role (Hirsch and Elliott, 2005). K173 of the RpoS protein may also play a role in regulating *rpoS* transcription.

Translational control

The main *rpoS* transcript contains an unusually long untranslated region (UTR), which is central to its translational control. The UTR may form two types of hairpin structures. One of these sequesters the translational initiation region (TIR) by pairing with a complementary sequence present within the coding region of the *rpoS* mRNA (called the antisense element), thereby making it unavailable to the ribosomes for translation. Other hairpins may form due to complementary sequences within the UTR. It is possible that both types of secondary structures have a role in regulating *rpoS* mRNA translation, although the involvement of the antisense element-mediated secondary structure in this regulation has not been documented yet. But considerable evidence is available indicating that secondary structures within the UTR minimize *rpoS* translation in unstressed cells and that their relaxation under certain stresses is the major reason for increased cellular σ^s concentration (**Figure 9**). Small noncoding RNAs (sRNAs) and the RNA-binding protein, Hfq, play a role in this phenomenon. For example, the sRNA, RprA, possesses a complementary sequence to the UTR stretch of *rpoS* mRNA, which is involved in hairpin formation. Base pairing and hydrogen bonding by this sRNA is able to unfold the hairpin, free TIR, and permit translation to proceed. Another sRNA, DsRA, is induced under cold stress and promotes *rpoS* translation by a similar mechanism. ArcZ and GcvB sRNAs also perform similar roles; the latter is encoded by the intergenic region of the *pst* operon. The CsdA protein and the CspA family of RNA-binding proteins likely assist in the DsrA annealing with the UTR while also stabilizing the *rpoS* mRNA; the CspC and CspE proteins stimulate *rpoS* translation possibly by affecting the mRNA structure, and the histone-like protein, HU, likewise stimulates *rpoS* translation. In contrast, the OxyS sRNA, down-regulates *rpoS* translation, and so do a number of proteins, e.g., the LrhA protein, and RNase III, which degrades the *rpoS* mRNA (Battesti et al., 2011; Hussein and Lim, 2011).

Under phosphate starvation, the synthesis of σ^s is thought to be regulated at the translational level (Mandel and Silhavy, 2005), but its mechanism is not known. Some five other sRNAs are known to affect *rpoS* translation, but none of these appears to have a role under these starvation conditions. It is possible that an as yet undiscovered sRNA is involved or that the control is exerted through modulation of the antisense element-mediated hairpin. Additional possibilities involve regulation through a variety of proteins that are known to regulate *rpoS* translation. These include the nucleoid protein HU that binds two regions in the *rpoS* mRNA and may influence its secondary structure; the histone-like protein StpA; the cold shock proteins CspC and CspE; a PTS protein; and DnaK. It is known that PhoB-P stimulates translation of *rpoS* mRNA, and it is surmised that the former may activate the transcription of an as yet unknown sRNA that is responsible for facilitating *rpoS* mRNA translation. An additional reason for the increase in RpoS levels under phosphate starvation is stabilization of this protein as is discussed below.

Posttranslational control-increased RpoS stability

It was thought that the control of σ^s synthesis in carbon starvation in *E. coli* occurred at the translational level. Direct measurements of *rpoS* mRNA translational efficiency, however, disproved this notion and showed that the increase under these conditions is solely due to enhanced stability of the sigma protein (**Figure 10**). The experimental results shown in **Table 3** indicate this fact. In this experiment, the rates of *rpoS* mRNA and σ^s synthesis and their half-lives were measured, which permitted calculation of the *rpoS* mRNA translational efficiency, that is, the σ^s protein synthesized per unit of its mRNA. *E. coli* cells were cultured in a glucose-limited chemostat in order to precisely establish the relationship between dwindling glucose concentration in the medium (with decreasing dilution rate) and the above mentioned parameters (**Table 3**). As the available glucose diminished, both σ^s synthesis rate and *rpoS* mRNA translational efficiency declined. Meanwhile, however, the stability of the sigma protein increased from 7- to

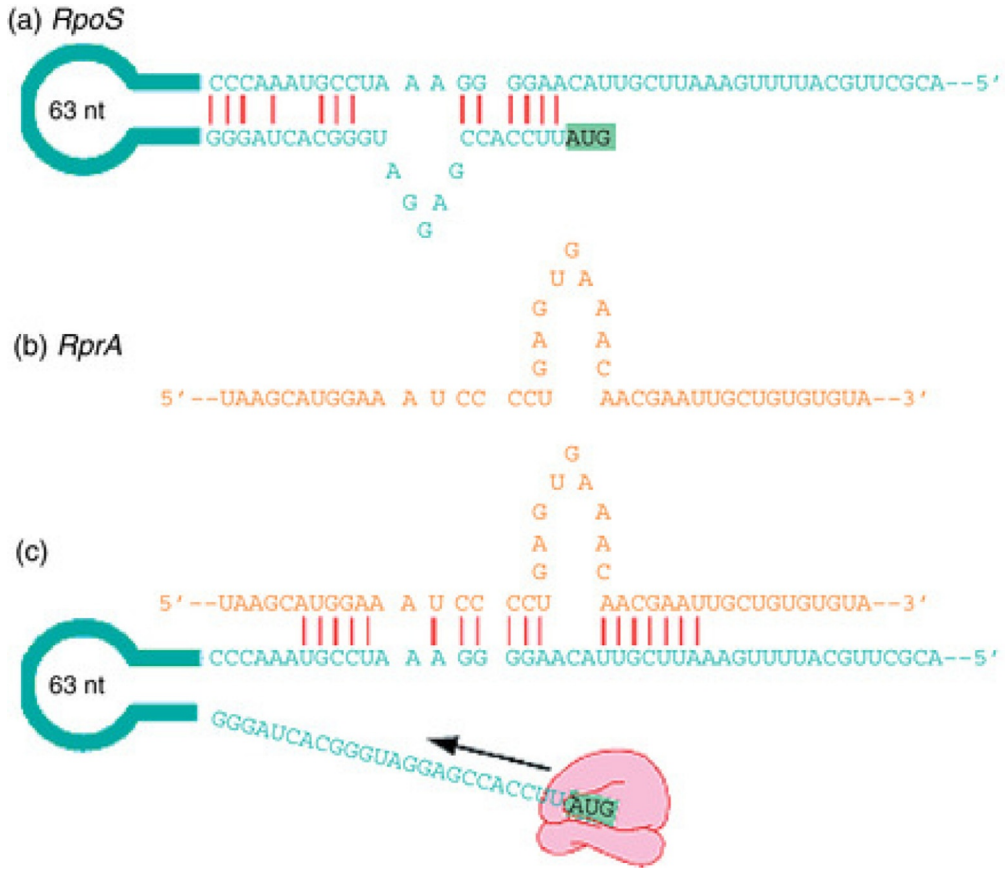

Figure 9 The untranslated region (UTR) of the *rpoS* mRNA that encodes σ^s. Note that the sequences upstream of the translational initiation codon (ATG) of the RNA includes regions of internal complementarity that result in the formation of a hairpin structure. This prevents the availability of the initiation codon. The small noncoding RNA, RprA, has regions of homology to the UTR of the *rpoS* mRNA (shown in red; B). Hydrogen bonding between the homologous regions of RprA and *rpoS* mRNA opens the hairpin, permitting translation (C). Reproduced from Matin A and Lynch SV (2005) Investigating the threat of bacteria in space. *ASM News* 71(5): 235–240. Washington, DC: American Society for Microbiology.

Table 3 σ^s synthesis rate and *rpoS* mRNA translational efficiency in glucose-sufficient cells and those subjected to increasing degree of glucose starvation (last three rows)

Glucose concentration (M)	*σ^s concentration*[a]	*σ^s half-life (min)*	*σ^s synthesis rate*[b]	rpoS *mRNA concentration*[c]	rpoS *translational efficiency*[d]
10^3 (glucose sufficiency)	190	5	55	1.0	1.0
2.2×10^6	270	11	34	0.75	0.75
1.3×10^6	300	34	13	0.52	0.52
1.2×10^6	570	>60	ND	0.5	0.5

ND, not determined.
[a]pmol mg^{-1} cell protein.
[b]pmol mg^{-1} cell protein per min.
[c]Relative units.
[d]σ^s synthesis rate/*rpoS* mRNA concentration.
Source: Reproduced from Zgurskaya HI, Keyhan M, and Matin A (1997). The σ^s level in starving *Escherichia coli* cells increases solely as a result of its increased stability, despite decreased synthesis. *Molecular Microbiology* 24(3): 643–651.

16-fold, accounting for the observed overall increase in the cellular levels of σ^s (Zgurskaya et al., 1997); this was subsequently conformed (Mandel and Silhavy, 2005).

What accounts for the instability of the σ^s protein under carbon-sufficient or other non-starvation/stress conditions? The answer to this question was provided by the discovery that a specific protease, called ClpXP, which is composed of two proteins, ClpX and ClpP, is involved in this regulation (Schweder et al., 1996). It rapidly degrades σ^s in unstressed cells, but not in those experiencing

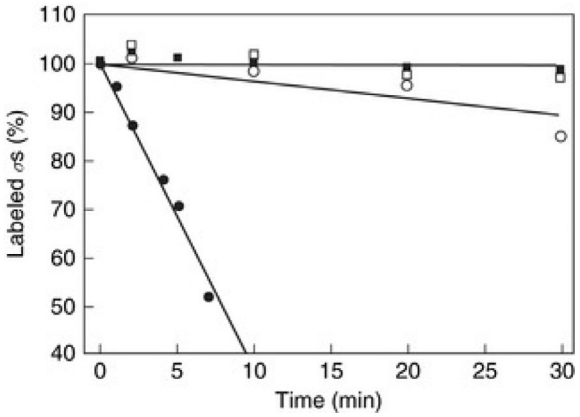

Figure 10 Comparison of σ^s stability in exponential phase (solid symbols) and stationary phase (open symbols) cultures in *clpP*-proficient (circles) and *clpP*-deficient (squares) backgrounds. Note that in a wild-type background, σ^s is stable only in the stationary phase, but in a mutant missing the Clp protein, it is stable in both the phases of growth. Reproduced from Schweder T, Kyu-ho L, Lomovskaya O, et al. (1996) Regulation of *Escherichia coli* starvation sigma factor (σ^2) by ClpPX protease. *Journal of Bacteriology* 178(2): 470–476.

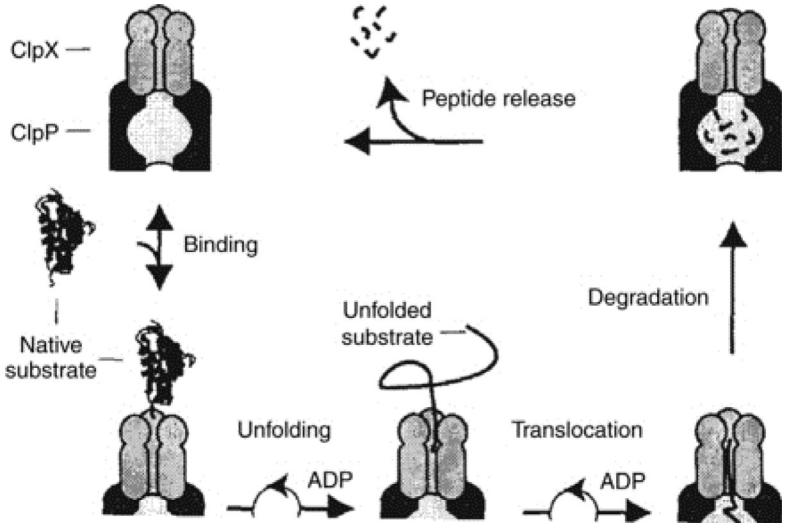

Figure 11 Schematic representation of native protein degradation by ClpXP protease. The ClpX component of the protease binds the substrate protein and unfolds it by its ATPase activity. The unfolded protein is translocated through the ClpP chamber, a process that also requires ATP, and is degraded; the resulting peptide fragments are released. Reproduced from Kenniston JA, Burton RE, Siddique SM, et al. (2004) Effect of local protein stability and the geometric position of the substrate degradation tag on the efficiency of ClpXP denaturation and degradation. *Journal of Structural Biology* 146: 130–140.

starvation (Figure 10). ClpP is a tetradecameric peptidase and has 14 active sties. ClpX is a hexameric ATPase, which binds to one or both ends of the ClpP chamber (Figure 11). ClpX binds directly or indirectly to proteins to be degraded and, using the energy provided by its ATPase activity, feeds them into the ClpP chamber. ClpX belongs to a family of proteins referred to as AAA + ATPase (ATPases associated with diverse cellular activities), and directly binds to proteins containing a short C-terminal sequence ('tagon'). The tagon is added by SsrA, or tmRNA to, for example, proteins with defects due to stalled ribosomes or other factors, causing these proteins to be rapidly degraded by the ClpXP protease. (It is estimated that some 5 proteins per thousand that *E. coli* synthesizes are destroyed by this quality control mechanism (Baker and Sauer, 2012; Moore and Sauer, 2007a).)

If ClpXP protease can degrade σ^s in exponential phase cells, why does this protein become resistant to this protease in the stationary phase? The answer to this and related questions has revealed involvement of a control mechanism involving adapter and anti-adapter proteins. While, as mentioned above, ClpXP can recognize proteins that are candidates for degradation, it does not recognize RpoS and requires the adapter protein RSSB for gaining access to it. Schweder et al. (1996) showed that a stretch of amino acids spanning K173 to K188 residues in RpoS protein was required for its cleavage by the ClpXP protease. Subsequent work has shown that K173 is essential for RpoS cleavage: K173/E mutation abolishes RpoS proteolysis. Also, E174 and V177, although not

absolutely required, facilitate this degradation (Becker et al., 1999). It is likely that RssB binding alters the conformation of RpoS, thereby exposing the site situated close to the N-terminus of this protein that binds to ClpX.

Under non-starvation conditions RSSB maintains an active configuration promoting RpoS degradation, and its inactivation under starvation accounts for the increased stability of σ^s. RssB is a homologue of RR proteins, mentioned above, but is unique in its C-terminal output domain. By analogy to other RRs, its activation may require phosphorylation, and it may be dephosphorylated, for instance, in starving cells due to the ArcB/ArcA two component system. ArcB senses the redox status of quinones; when these are oxidized, disulfide bond formation within ArcB results in its inability to autophosphorylate (Malpica et al., 2004) resulting in dephosphorylation of ArcA, which is thought to donate the phosphate group to RssB. Thus, RssB would be primarily dephosphorylated and may be inactive for this reason in starving cells leading to increased RpoS stability. However, it is not certain that phosphorylation of RssB is essential for its activity, since removal of the aspartate residue from RssB, which is thought to be the phosphorylation site, does not inactivate it. Another explanation for RSSB inactivation involving anti-adapter proteins has emerged. Three such proteins are known, IraP, IraM and IraD, which are induced in response to different stresses. Thus, IraP is induced under phosphate starvation, and IraD under conditions leading to DNA damage (Battesti and Gottesman, 2013). RSSB has low cellular levels and the antiadapters are able to saturate it. Despite this shared activity, they do not bear structural homology, although several contain conserved RR domains, and their activity involves interaction with the flexible N-terminal domains of the ATPase components of the proteases. ppGpp, a global regulator of stringent response (see above), is a positive regulator of the *ira*P promoter; this may further explain the observed up-regulation of σ^s by ppGpp (Gentry et al., 1993). Additionally, H-NS, a histone-like protein, negatively regulates σ^s by modulating the translational efficiency of *rpo*S mRNA and the stability of newly synthesized σ^s. H-NS is a DNA-binding protein known to regulate genes at transcriptional level. The precise molecular mechanisms by which it controls σ^s at the post-transcriptional level remain to be fully elucidated (Barth et al., 1995; Yamashino et al., 1995). Its negative influence on σ^s stability is likely mediated by the suppression of *ira*M and *ira*D expression.

It is interesting to note that the K173-K188 region corresponding to 519–564 nucleotides of the *rpoS* mRNA constitutes the antisense element, which as mentioned above, may also have a role in translational regulation of Sigma S synthesis, and that, as already mentioned, K173 may be important also in transcriptional control. Thus, the K173-K188 stretch of the RpoS protein could be involved in regulating Sigma S synthesis at all three levels of regulation.

Activity control

Control at the level of activity of σ^s evidently operates in nitrogen starvation. Under these conditions, the core set of proteins are still synthesized even though σ^s levels show only a very modest increase. Thus, it is thought that the sigma protein is more active under these conditions. The factors that may account for this are hypothesized to be those that increase the competitiveness of σ^s for RNAP. These have been discussed above.

Regulation under low-shear/simulated microgravity conditions

Low-shear environments, such as brush border microvilli of the gastrointestinal, respiratory, and urogenital tracts (Guo et al., 2000), are common routes of microbial infection. Low shear environments closely resemble microgravity conditions experienced by astronauts during space flight. There has therefore been considerable interest in studying the biological effects of these conditions. On Earth, the effects of such environments are simulated by the use of a special cultivation equipment that utilizes high aspect to ratio vessels (HARVs). There is strong evidence that these conditions weaken the human immune response (Stowe et al., 2011) and make bacteria more virulent and stress-tolerant (Lynch et al., 2004; Wilson et al., 2002); these have obvious implications for the control of disease on Earth and on astronauts' health. Studies on the regulation of this phenomenon have resulted in some intriguing findings. Thus, the increased bacterial resistance that low-shear environments confer on bacteria appears to be independent of σ^s in exponential but not in stationary phase. Further, these environments markedly enhance *rpoS* translational efficiency regardless of the growth phase and promote σ^s instability, especially in the exponential phase. Since both these regulatory phenomena involve macromolecular folding pattern, the findings raise the possibility that low-shear/microgravity environments can influence these patterns.

Sensing starvation

Given that the regulation of the starvation response differs depending on the missing nutrient, it seems likely that the dearth of different nutrients is sensed by different mechanisms. The sensing mechanism in the case of carbon starvation could be an effector that inactivates RssB or ClpXP. Recent reports indicate that an increase in denatured proteins may have a role. Starvation affects fidelity of ribosomes, resulting in the synthesis of abnormal proteins with a proclivity for oxidation. The latter sequester Clp, impairing ClpXP activity, resulting in the stabilization of σ^s. In this view, starvation is sensed by the increase in aberrant proteins. Phosphate and nitrogen starvations may involve the PhoBR- and NtrBC-sensing systems mentioned above (Battesti et al., 2011). In *P. putida*, a G-protein, called FlhF, which is situated at the cell pole and controls flagellar localization on the cell, may be involved in sensing stress, as its absence robs the cell of the capacity to develop the general stress resistance (Pandza et al., 2000).

Concluding Remarks

It is evident that in response to hostile and frequently fluctuating conditions in nature, bacteria have evolved highly sophisticated mechanisms that permit them to swiftly shift between rapid growth and static survival modes. Our understanding of this phenomenon has enhanced greatly in the last two decades, and further progress is likely to yield information that will permit better control of bacterial growth – its enhancement toward beneficial ends, such as ecosystem management, industrial processes, and bioremediation, as well as its mitigation, as in disease.

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