Stress responses of bacteria

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Summary of Recent Advances

Bacteria, irrespective of natural habitat, are exposed to constant fluctuations in their growth conditions. Consequently they have developed sophisticated responses, modulated by the re-modelling of protein complexes and by phosphorylation-dependent signal transduction systems, to adapt to and to survive a variety of insults. Ultimately these signaling systems affect transcriptional regulons either by activating an alternative sigma factor subunit of RNA polymerase, e.g. sigmaE (σE) of Escherichia coli and sigmaB (σB) and sigmaF (σF) in Bacillus subtilis or by activating DNA-binding two-component response regulators. Recent structure determinations, and systems biology analysis of key regulators in well-characterised stress-responsive pathways, illustrate conserved and novel mechanisms in these representative model bacteria.
Introduction

Bacteria have successfully colonised every niche on the planet, from the soil-dwelling Gram’
*Bacillus subtilis* to the Gram *Escherichia coli*, found in the lower intestines of mammals to
*Deinococcus radiodurans*, which persists in nuclear reactors and can survive radiation doses
sufficient to kill all other life forms [1]. In this vast range of different environments, bacteria are
exposed to wildly-fluctuating environmental stresses, including changes in temperature, pH,
osmolarity, radiation and the concentration of nutrients and toxins [2]. To ensure survival in the
face of these adversities, bacteria may move by 'swimming' using their molecular motor, the
flagellum, to more favourable locations [3], or the bacteria may adapt to changes in their immediate
vicinity by responding to the imposed stress. The response to the imposed stress is accomplished
by changes in the patterns of gene expression for those genes whose products are required to
combat the deleterious nature of the stress [4]. The up-regulation of the transcription of stress-
responsive genes is achieved by the activation of transcription factors that interact with RNA
polymerase to co-ordinate gene expression.

One family of transcription factors with a role in stress-resistance is a subunit of RNA polymerase,
the sigma factor, which is essential for transcription initiation by playing a key role in promoter
recognition [5]. Each of several sigma factors in the cell is required for the transcription of a
specific sub-set of genes/operons within their 'regulon' [6]. For instance, the $\sigma^B$ regulon in *B.
subtilis* comprises ~200 open reading frames (5 % of the genome) the products of which confer
general stress resistance to the cell, whereas most of the other ~17 sigma factors that have been
characterised in this bacterium each regulate fewer than 50 ORFs [7]. Since sigma factors can
regulate a significant subset of ORFs the modulation of their activity can be rather complex – for
instance the *B. subtilis* sporulation mother-cell specific $\sigma^F$, with a known regulon of 19 ORFs, is
controlled by a network of three other proteins [8], whereas for $\sigma^B$ there are over a dozen regulators,
which act in concert to provide tight control [9]. The availability of some alternative sigma factors
(e.g. $\sigma^d$ and $\sigma^n$) for forming productive complexes with RNA polymerase is, ultimately, controlled by a binding partner known as an anti-sigma factor that in turn can be regulated, for instance, by targeted proteolysis [10] or by phosphorylation [11].

Alternative stress-sensing responses are also phosphorylation-dependent and are maintained by two-component regulatory systems, which consist canonically of a membrane-embedded sensory kinase and a response regulator [12]. The kinase auto-phosphorylates a conserved histidine on the receipt of a stress-signal before transferring the phosphoryl group to an invariant aspartate in its cognate response regulator and in doing so activates its latent biological function [13]. Most frequently, but not exclusively, response regulators are transcriptional activators and bind upstream of the promoters of the ORFs they regulate and stimulate initiation of transcription by interacting with sigma-bound RNA polymerase. As the article by J. A. Hoch in this issue considers two-component signaling, the focus of this review will be on the regulation of sigma factor activity – by phosphorylation and by proteolysis - as a response to stress.

**Regulation by phosphorylation: $\sigma^B$**

The $\sigma^B$ pathway of *Bacillus subtilis* is a partner-switching cascade that utilises phosphorylation to alter the binding partner-specificity of the proteins that are ultimately responsible for the activation of $\sigma^B$. In unstressed cells the sigma factor is held in an inactive state by the anti-sigma factor RsbW. At the onset of either environmental or energy stress the anti-anti-sigma factor RsbV is dephosphorylated by a phosphatase, causing it to sequester RsbW and thus to allow the sigma factor to bind RNA polymerase and to activate gene transcription. The two distinct stress inputs to this system utilise different upstream activators of RsbV. In the case of energy stress (i.e. depletion of the ATP pool) an energy responsive phosphatase RsbP activates RsbV [14], while RsbP is itself activated by RsbQ [15]. The exact mechanism of this activation is unknown but structural studies of
RsbQ show that the protein is an α/β-hydrolase [16] that binds some small, as yet unidentified, hydrophobic molecule [17] that may be used to activate RsbP (Figure 1).

The environmental stress response is the subject of much current structural interest as it is controlled by a supramolecular complex known as the stressosome [18-20]. The stressosome complex was first identified by Chen et al [21] in a series of gel-filtration experiments on the interactions of RsbR, RsbS and RsbT. They found that the RsbR and RsbS proteins would form a high-molecular weight complex when the proteins were mixed that could also bind RsbT [21]. Initial electron microscopy studies using negatively-stained samples revealed the homogenous nature of these complexes indicating that the complexes are formed in a specific manner and are not the result of spontaneous and random aggregation [21]. These complexes were subsequently isolated from wild-type Bacillus subtilis [19]. The native stressosome consists of an unknown ratio of RsbS, RsbR and the four RsbR paralogues, YkoB, YojH, YqhA and YtvA [19]. RsbS consists of a single STAS domain (sulphate transporter and anti-anti-sigma factor [22]) and forms a scaffold with the RsbR paralogues that is assembled through their well-conserved C-terminal STAS domains. It is these STAS domains that are phosphorylated by RsbT under environmental stress. This description of stressosome structure is supported by limited proteolysis experiments carried out by the authors that show when minimal stressosomes consisting of RsbR and RsbS (sufficient to activate σ^B in vivo) are subjected to digestion with trypsin and subsequent separation by size exclusion chromatography, a fragment is liberated from larger molecular weight assemblies. This fragment has an amino acid sequence that corresponds to the N-terminal domain of RsbR (J Marles-Wright et al, unpublished). Cryo-electron microscopy studies of stressosomes formed with full length and a recombinant, N-terminally truncated version of RsbR indicate that the N-terminal domain of RsbR, which may function as a sensor, projects from the core of the complex (Figure 2, unpublished data). The STAS domains within the stressosome core are phosphorylated by the RsbT kinase at the onset of stress, and in doing so, RsbT dissociates from the complex [23]. RsbT then
binds to and activates the environmental phosphatase RsbU [24], which in turn dephosphorylates the RsbV anti-anti-sigma factor leading to activation of σ^B [25]. The purpose of phosphorylation in the σ^B response is to act as a steric and electrostatic ‘flag’ that alters the affinity of a protein for its choice of binding partner and thus facilitate the partner-switching cascade. This consequence of phosphorylation in σ^B signaling is reminiscent of that controlled by the σ^F system in B. subtilis. In this case, X-ray crystallography has revealed that the phosphorylation of SpoIIAA, the best characterized STAS domain, does not induce any significant conformational changes in the protein [26]. Phosphorylation, in this instance, also acts as a ‘flag’ to control protein complex re-modelling in cell-fate determination.

The stressosome represents a very interesting case of what could be described as a signaling hub. In the unstressed cell, RsbT is sequestered by the complex and only in the event of an activating stress signal is the kinase activity of RsbT sufficient to phosphorylate RsbS and the RsbR paralogues and thus switch partners to RsbU. Why RsbT should be bound by a 1.5 MDa complex is currently unknown, but as environmental stress can encompass signals ranging from small molecules, light, protein and peptide fragments [4], it could be seen as a mechanism by which the cell can integrate multiple signals to effect a single signaling outcome. The stressosome contains five different RsbR proteins, each with distinct N-terminal domains [19] that project from the core of the stressosome. These projections may be utilised in sensing signals, for instance, the N-terminal, LOV-domain (LOV = light, oxygen, voltage) of YtvA has been shown to respond to blue light [27], and the globin fold of the N-terminal domain of RsbR [28], more commonly found in proteins that sense oxygen [29], suggests a sensory role. It is likely that RsbR is responsive to signals other than gaseous diatoms as the N-terminal domain of RsbR does not, and indeed cannot, bind a heme cofactor [28]. Indeed, in other bacteria that encode the RsbR-RsbS-RsbT signaling module, the N-terminal region of RsbR encodes a domain easily identifiable by sequence homology to genuine heme-binding globins [18]. That the stressosome contains these distinct protein-sensing modules is
consistent with the idea that the complex functions in the role of a signaling-centre. This idea has yet to be proven definitively.

**Regulation by phosphorylation: \( \sigma^F \)**

The past twenty years have seen a meticulous approach to the characterization of the \( \sigma^F \) signaling pathway and have provided almost all of the key parameters required for a systematic mathematical modeling of the interplay between \( \sigma^F \) and its three regulators: SpoIIE, SpoIIAB and SpoIIAA [30]. Sporulation, which is perhaps the most extreme example of a stress-survival strategy, is regulated by the \( \sigma^F \) response. In sporulation normal cell division is abandoned and, instead, a simple example of cellular-differentiation is followed, resulting in a hardy, environmentally resistant endospore. The spore can survive almost indefinitely until it receives a germination signal, where it will resume a vegetative lifestyle [31].

Spore formation is regulated by the interactions between \( \sigma^F \), SpoIIE, SpoIIAB and SpoIIAA. SpoIIAA is phosphorylated by SpoIIAB, which itself is controlled by conformational changes that result from the cycling of the bound adenine nucleotide during the phosphorylation of SpoIIAA. SpoIIE regulates SpoIIAA by acting as phosphatase against the phosphorylated protein [30]. The ~200 reactions (see supplementary Figure 2 in reference 29) involved in the \( \sigma^F \) activation pathway can be described by approximately 70 differential equations which, when solved simultaneously, can predict accurately the fate of the mother cell under different conditions and the can also predict the phenotypes of a number of previously-characterised mutant strains of *B. subtilis* [30]. This successful application of a systems approach to a relatively simple biological process re-iterates the importance of obtaining good quality kinetic and thermodynamic parameters on the key reactions of proteins involved in biological pathways by rigorous biochemical investigations.
Regulation by proteolysis: $\sigma^E$

Proteolysis has recently been identified as a modulatory mechanism for the activity of $\sigma^B$ in *Bacillus subtilis*, where the action of the protease ClpP, modulates the response to environmental stress [32]; but in *E. coli*, proteolysis is a well established mechanism for the regulation of $\sigma^E$ and genes that are responsive to this sigma factor. In *E. coli* $\sigma^E$ is activated in response to what is termed bacterial envelope stress [33] i.e. signals from within the envelope compartment, such as misfolded proteins, small molecules and other environmental stresses are sufficient to trigger the $\sigma^E$ response [34]. Two key components of this response are the RseA/B and CpxP systems, which act to modulate $\sigma^E$ [35,36] and specific, Cpx-activated genes [37], respectively.

RseA is a negative regulator of $\sigma^E$. In unstressed cells, RseA sequesters the sigma-factor in an inactive state by binding directly to sites on the protein that are involved in the interaction with RNA polymerase [38], in a manner reminiscent of that described above for RsbW and $\sigma^B$. The crystal structure of RseA in complex $\sigma^E$ with illustrates the molecular mechanism by which the protein sequesters $\sigma^E$ [39]; sixty-six amino acids at the N-terminus of the protein bind the sigma factor through a mixture of hydrophobic and electrostatic interactions on surfaces that are required for the formation of the RNAP-holoenzyme [39] (Figure 3). The release of $\sigma^E$ is facilitated by the proteolytic degradation of RseA by the ClpAP/XP proteases [10]. Proteolytic degradation of the RseA anti-sigma factor is facilitated by the DegS-dependent targeting of RseA by ClpX for subsequent degradation by the ClpP protease. The ClpXP protease complex belongs to the family of molecular machines called AAA+ (associated with assorted cellular activities) ATPases. The AAA+ ATPases form multimeric ring structures that can be utilised for a variety of purposes [40]. For instance, recent structural studies of the ClpX protein complex have shown the hexameric ring-structure forms a central pore [41] through which protein chains are translocated to the heptameric ClpP protease in an ATP dependent manner [41], and although the subunits are intimately linked,
they do not appear to display co-operativity in their action [42]. By contrast, a structurally related
AAA+ ATPase, the enhancer binding protein PspF, binds directly to the $\sigma^{54}$ class of sigma factors
[43]. The ATPase activity of hexameric PspF is required for the formation of the transcription
bubble [42], a pre-requisite for the initiation of transcription by the holoenzyme RNAP complex.
From a 20 Å cryo-EM reconstruction, loop regions are seen projecting from the hexameric ring to
bind to the sigma factor [44]. It has been suggested that on ATP hydrolysis the loops change
orientation and allow the remodelling of the holoenzyme activation complex and thus initiate gene
transcription [44].

The RseA response is fine-tuned by the regulator RseB, which binds to the periplasmic C-terminal
domain of RseA through mainly electrostatic interactions [38]. The interaction of RseA with RseB
prevents the proteolytic cleavage of RseA, between residues 148 and 149, by DegS [39]. RseB
binding to RseA is thought to be affected by the presence of denatured outer-membrane proteins or
other peptide fragments [35], but the exact means by which RseB disengages RseA remains to be
elucidated.

**Regulation by proteolysis and phosphorylation: $\sigma^8$**

In *E. coli* the general stress sigma factor $\sigma^8$ is controlled by what has been termed a 'three-
component system' that responds to energy stress in the form of the redox state of the cellular
quinone pool [45]. The ArcB protein autophosphorylates in the presence of quinol and
subsequently phosphorylates both ArcA and RssB [46]. The ArcA protein acts to repress the
transcription of *rpoS* genes, one of which is $\sigma^8$ itself; while activated RssB marks $\sigma^8$ for proteolytic
degradation [47]. Under oxidative stress ArcB is dephosphorylated, as are RssB and ArcA,
relieving both the repression of $\sigma^8$ expression and proteolysis [45]. What is interesting about this
two-pronged pathway is that the ArcA protein acts as both a repressor and activator of $\sigma^8$,
depending upon the status of the cell, due to its ability to inhibit the phosphoryation of RssB in cases of limiting energy resources [46].

**Concluding remarks**

One of the more intriguing aspects to have come emerged from recent research into the stress responses of bacteria is the involvement of large multi-protein complexes in several signalling pathways. For instance in the σB response, the stressosome presents an unusual means by which stress signals can be integrated and propagated to the sigma factor. Yet we currently know little about how the stress signals are sensed or the manner in which they are used to activate the signaling cascade and much work is yet to be done to elucidate these signals. The application of what have traditionally been very distinct structural biology techniques, protein crystallography and single particle reconstruction from cryo-EM, has proven to be very valuable in answering key biological questions. For instance, the structure and mechanism of action of PspF has been elucidated through the marriage of cryo-EM envelopes with crystallographic models [44]. The cryo-EM envelope of the stressosome is being used in the authors’ laboratory to inform both biochemical and crystallographic experiments (J Marles-Wright et al, unpublished data). What may be seen as the next logical progression in the marriage of structural methods to the study of high molecular weight complexes has already taken place with the recent application of NMR to the 300 kDa ClpP complex in the discovery of new facets of proteasome biology [48].

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Figure legends

Figure 1. The $\sigma^B$ cascade of *Bacillus subtilis*

Pre-stress, the anti-sigma factor RsbW sequesters $\sigma^B$ and prevents it from directing RNA polymerase to $\sigma^B$ controlled promoters. RsbW also inactivates RsbV through its kinase activity. Under stress conditions, RsbW is sequestered by the anti-anti-sigma factor RsbV. RsbV is the point at which the environmental and energy stress responses converge. Under energetic stress, the energy photphatase RsbP is activated by RsbQ and is able to dephosphorylate RsbV and thus allow it to bind RsbW. The environmental stress response is somewhat more complicated, with a large protein complex termed the stressosome, acting to sequester the phosphatase activator RsbT in the absence of stress. Under stress conditions RsbT phosphorylates the STAS domains of the stressosome proteins and dissociates due to a reduced affinity for the phosphorylated protein, switching partners to the environmental phosphatase RsbU, which activates RsbV. The phosphatase RsbX acts to remove phosphoryl groups from the stressosome and to mediate the duration of the stress response by ‘resetting’ the system. Ringed plus signs indicate positive regulators of $\sigma^B$ activity, while ringed minus signs indicate those that are negative regulators.

Proteins for which a crystallographic model is available, are shown as a cartoon representation, depicted in colour for those with the *Bacillus subtilis* structure and in grey for homologous structures: nRsbR [28]; RsbS, cRsbR, STAS domain homologue SpoIIAA [49]; RsbT, RsbW, kinase domain homologue SpoIIAB [49]; nRsbU [24]; cRsbU, cRsbP [50]; RsbQ [17]; RsbV, *Thermotoga maritima* homologue [51]; RNA polymerase. This figure is adapted from [24].

Figure 2. Stressosome class averages

Class averages of single particle views from RsbR:RsbR stressosomes (a,c) and RsbR$^{[137-274]}$:RsbS (b,d). (a) represents a view of the stressosome down a two-fold symmetry axis while (c) shows a
three-fold axis with ‘turrets’ projecting from the central core of the structure (black arrows). These images show a clear symmetry mismatch between arrangement of the turrets and the core, the nature of which will be revealed on completion of the structural analysis. (b) and (d) show the same views as (a) and (c) respectively, but in this case the RsbR is N-terminally truncated so no ‘turrets’ are visible. The diameter of the core of the particle is 180 Å with the projecting ‘turrets’ giving a total diameter of 280 Å.

Figure 3. Structure of RseA in complex with $\sigma^E$

The structure of the *E.coli* $\sigma^E$ in complex with the cytoplasmic domain of the anti sigma factor RseA. The two domains of $\sigma^E$ are shown in surface representation in green and cyan with the N-terminal sixty-six residues of RseA shown in an orange cartoon representation. There are extensive contacts between the RseA protein and $\sigma^E$ with the $\alpha3$ helix of RseA almost completely buried between the two $\sigma^E$ domains preventing the interaction of the sigma factor with RNA polymerase [39].
References


This review of the stress response of E.coli gives a good picture of the key proteins involved in envelope stress. Their figure of the CpX and Rse pathways gives a clear schematic for these pathways.


This recent review gives an excellent overview of the proteins and gene products involved in the stress response in Bacillus subtilis.


The authors of this paper determine the proteins that make up the stressosome of Bacillus subtilis. They show that it consists of RsbS, and the RsbT phosphorylatable RsbR proteins.


The authors present the structure of the first signaling domain from an RsbR protein. The protein adopts a unique non-heme globin fold and poses questions as to the function of such modules in stress signalling.


Using a wealth of biochemical data the authors present an elegant mathematical model for the
activation of σF in Bacillus subtilis. Of particular interest is supplementary Figure 2 which shows the interaction network for all the proteins involved in the pathway.


In this structural paper the authors present the structure of the anti-anti-sigma factor RseB and model its interactions with RseA presenting a clear account of how the σS signalling cascade is activated.


46. Malpica R, Sandoval GR, Rodriguez C, Franco B, Georgellis D: Signaling by the arc two-
component system provides a link between the redox state of the quinone pool and gene expression. Antioxid Redox Signal 2006, 8:781-795.


The authors of this paper use NMR spectroscopy to investigate the dynamics of the ClpP proteasome and show that side-pores in the structure are necessary for the release of degraded protein fragments.


