Alternative sigma factors: the master regulators

LS Thompson & EJ Harry

Institute for the Biotechnology of Infectious Diseases
University of Technology
Sydney NSW 2007
Tel: (02) 9514 4173
Fax: (02) 9514 4201
E-mail: liz.harry@uts.edu.au

When a bacterial cell encounters a change in environmental conditions, it responds by producing a different complement of cellular proteins. Which proteins are produced and maintained is regulated in a number of ways, including regulation of gene transcription, stabilising or degrading mRNA transcripts, post translational modifications and targeted degradation of proteins.

Regulation of transcription occurs through the recognition of promoter sequences that lie upstream of each gene or operon and are recognised primarily by the sigma factor of RNA polymerase (RNAP). In addition, the activity of the RNAP is controlled by other transcriptional regulators.

Sigma is a protein required by RNAP to initiate transcription by recognising the promoter sequence, mediating specific binding of RNAP to the DNA and melting it, forming an open complex and allowing transcription to proceed [1]. During exponential growth the house keeping sigma factor, σ^{70}, is responsible for transcription. However, when conditions change, a cohort of alternative sigmas are available that allow specific sets of genes (termed regulons) to be expressed [2].

Comparisons between different sigma families

In general, organisms with more varied lifestyles contain more sigmas. *Escherichia coli* contains six alternative sigma factors, *Bacillus subtilis*, 18, and *Streptomyces coelicolor*, 63, the largest number found in a bacterium to date.

Most alternative sigma factors are similar in sequence and structure to σ^{70}, thus belonging to the σ^{70} family. A second family of alternative sigma factors, the σ^{54} family, is also found in bacteria. Whilst most bacteria contain only one representative of the σ^{54} family, most contain multiple representatives from the σ^{70} family. The σ^{70} family is divided into four groups depending on phylogenetic similarities; Table 1 describes these and gives representatives from the well characterised Gram-positive bacterium, *B. subtilis*.

How sigma factors compete for RNA polymerase

Since all sigmas interact with the same RNAP, what confers which sigma will interact under a particular environmental condition? The pool of sigma factors can be adjusted by synthesis or degradation, activation or deactivation, or through changing the competition parameters for RNAP. Because many of the alternative sigma factors are themselves transcribed by the constitutively expressed σ^{70}, novel methods of changing protein levels within the cell have arisen to ensure independent and differential availability of alternative sigma factors.

For example, σ^{S}, the alternative sigma factor in *E. coli* that is induced under conditions of stress and starvation, has an mRNA secondary structure inaccessible to ribosomes. A number of factors regulate the translation of *rpoS* mRNA and it is only when these factors are expressed that σ^{S} is produced [2]. Furthermore, σ^{S} is targeted by ClpXP protease, further influencing its levels within the cell.

Alternative sigma factors can also be regulated by an anti-sigma factor. This kind of regulation is key to the rapid activation of the alternative sigma factor in response to a specific cue, since the alternative sigma factor has already been synthesised but is held in an inactive complex by the anti-sigma factor. This is illustrated in the switching on of cell-specific gene expression during starvation induced sporulation in *B. subtilis*. The earliest event in establishing differential gene expression during sporulation in *B. subtilis* is the activation of the transcription factor σ^{F} in the forespore (Figure 1).

Table 1. Different groups of the σ^{70} family and σ^{54} family of sigma factors and representatives found in *B. subtilis*.

Descriptive characteristics		Representatives in *B. subtilis*
σ^{70} family		
Group 1:	Required for transcription of house keeping genes	σ^{A}
Group 2:	Closely related to Group 1 but not essential for growth	None found in *B. subtilis*
Group 3:	Divergent in sequence. Divided into evolutionarily related proteins involved in sporulation, flagellar synthesis etc.	σ^{E}, σ^{F}, σ^{G}, σ^{H}, σ^{K}, σ^{B}, σ^{D}, σ^{ykoZ}, σ^{Xpf}
Group 4:	The extra cytoplasmic family, usually involved in some aspect of the cell surface or transport	σ^{X}, σ^{W}, σ^{M}, σ^{Y}, σ^{V}, σ^{Z}, σ^{ylaC}
σ^{54} family		σ^{L}

spo0F, the gene encoding σ^F, is expressed prior to asymmetric division by σ^A, the σ^{70} house keeping sigma factor in *B. subtilis*. σ^F is produced in both the forespore and mother-cell; however, it is held inactive by the anti-sigma factor SpoIIAB. Only when the anti-anti-sigma factor SpoIIAA complexes with the anti-sigma factor SpoIIAB, is active σ^F released in this compartment.

The anti-anti-sigma factor SpoIIAA exists in both an inactive, phosphorylated state, and an active, non-phosphorylated, state. The level of active SpoIIAA becomes high enough only in the smaller forespore cell when the asymmetric sporulation septum has been completed, hence releasing σ^F from SpoIIAB and initiating the first step in differential gene expression required for sporulation [3]. The remainder of this review will describe the complex pathways and regulatory cascades that control some of the σ^{70}-type sigma factors of *B. subtilis*.

Sporulation

B. subtilis is the most extensively studied Gram-positive organism to date. Years of excellent work have demonstrated the diversity of sigma factor activation mechanisms employed by this organism. Some of the best work has been in characterising the sigma factor cascade that regulates differentiation of this bacterium under conditions of nutrient limitation, resulting in the formation of highly resistant, dormant spores. For an overview of sporulation in *B. subtilis,* see Figure 1 [4, 5].

Five σ^{70}-type alternative sigma factors are dedicated to the sporulation process. The spatial and temporal control of these alternative sigma factors forms a regulatory cascade where the next stage in sporulation is initiated only after the previous morphological check point has been reached (Figure 2 [5, 6]). Transcription of the genes encoding σ^F and σ^E, required for forespore specific and mother-cell specific gene expression respectively, is directed by σ^A prior to asymmetric cell division. These two sigma factors are, however, held inactive until the sporulation septum has been completed.

The release of active σ^F, the first step in differential gene expression, is linked with the morphological check point of septum formation (see above) [7]. Regulation of σ^E activity is different to that seen for σ^F, in that it is translated as an inactive precursor, pro-σ^E, with an additional 27 amino acids at its N-terminus. Proteolytic processing of pro-σ^E depends on the signalling protein SpoIIR, the product of a gene transcribed by σ^F RNAP in the forespore. SpoIIR contains a putative signal sequence and is thought to be secreted into the space between the septal membranes, where it activates SpoIIGA a probable aspartyl protease responsible for processing σ^E to its active form [6].

Both σ^F and σ^E are required for the phagocytic-like process of engulfment where the mother-cell engulfs the forespore (Figure 1). Once engulfment is complete, σ^G is activated in the forespore. The exact regulation of σ^G is not fully understood; however, σ^G is initially transcribed by RNAP σ^F and later auto-transcribed by RNAP σ^G, with other transcription factors also being required for its full expression [8].

A factor under the control of σ^E is also necessary for σ^G activation. Hence, it is apparent that both σ^E activity and completion of engulfment are required to activate σ^G, once again linking this sigma regulatory cascade to a morphological checkpoint [6].

Shortly after σ^G becomes active in the engulfed forespore, σ^K becomes active in the mother-cell. σ^K displays a unique form of regulation in that it is encoded as two separate loci located 48 kb apart on the chromosome. This 48 kb intervening sequence is removed by a site specific recombinase only on the non-inheritable mother-cell chromosome, allowing the *sigK* gene encoding σ^K to be transcribed [6, 9].

Like σ^E, σ^K is synthesised as an inactive precursor, pro-σ^K with a 20 amino acid pro-σ^K sequence inhibiting binding to core RNAP. Similar to pro-σ^E, pro-σ^K is thought to be processed by a protease expressed in the mother-cell, activated through a signal produced by RNAP σ^G in the engulfed forespore.

How the alternative sigma factors activated earlier in the sporulation cascade are repressed at the later stages of sporulation is proposed to be through negative feed back loops. For example, it has been shown that the mother-cell expressed σ^K inhibits the σ^A-dependent expression of *sigE* encoding pro-σ^E. It has also been shown that feedback loops negatively regulate the expression of each sigma factor in the cascade. For example GerE, a product of the σ^K regulation, represses the transcription of *sigK*, the gene encoding σ^K. Similarly, SpoVT, a product of the σ^G regulon, inhibits transcription of the gene encoding σ^G [6].

It is evident from the many studies of the sporulation sigma factor cascade in *B. subtilis* that the activation and inactivation of each sigma is carefully choreographed in a complex programme that couples gene expression to morphogenesis. There are still more questions, however, that need to be answered to understand this complex regulatory pathway.

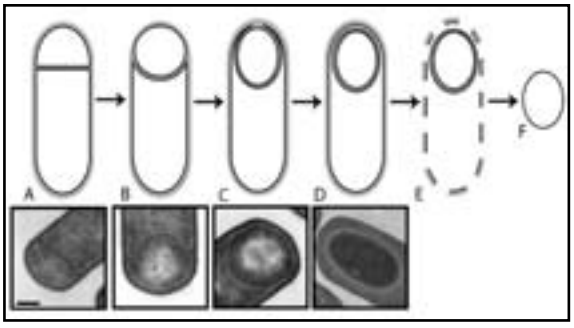

Figure 1. The sporulation pathway of *B. subtilis,* initiated in response to nutrient limitation. Vegetative cells divide at mid-cell.
(A) The first morphological step in sporulation is formation of a division septum, not at mid-cell, but near one cell pole, dividing the cell into two unequal compartments, the forespore (smaller cell) and mother-cell.
(B) Once the polar septum is complete the mother-cell membrane begins to migrate around the forespore, engulfing it.
(C) When the mother-cell membrane reaches the pole of the cell, the membrane fuses, releasing the engulfed forespore into the mother-cell (D). Once engulfment is complete, the spore cortex and spore coat are synthesised before the mother cell is lysed (E) and the mature spore is released into the environment (F).
The lower panel of images show electron micrographs of cells undergoing the stage of sporulation represented above in the corresponding cartoon. The scale bar in the lower left image represents 200nm. Chromosomes (1 in each compartment) are omitted for simplicity.

General stress response

σ^B directs RNA polymerase to promoters of the *B. subtilis* general stress regulon (127 genes), conferring multiple resistances on the cell [10]. The general stress regulon of *B. subtilis* is responsive to a wide range of stress conditions including energy stress such as carbon, oxygen and phosphorus starvation as well as environmental stress including heat shock and changes in pH and salinity. σ^B also functions as a central regulator of the stress response in human pathogens: *Bacillus cereus, Listeria monocytogenes* and *Staphylococcus aureus* [11].

In the absence of stress, the activity of σ^B is inhibited by a co-transcribed anti-sigma factor, RsbW [12]. In turn, the ability of RsbW to inhibit σ^B is dependent on the phosphorylation state of its complexing partner RsbV, which is phosphorylated by the kinase domain of RsbW, and dephosphorylated by a number of PP2C phosphatases depending on the stress experienced (Figure 3 [13]).

σ^B also functions in the resistance against antibiotics. In *B. subtilis*, a mutant devoid of σ^B cannot resume growth after exposure to rifampin as quickly as the parental strain. Importantly, σ^B plays a role in the resistance of *S. aureus* to several antibiotics, including methicillin and vancomycin [11].

Invasion of a host by a pathogen often requires the ability to mount a stress response; however, there is little evidence to suggest that σ^B is required for virulence in *L. monocytogenes* or *S. aureus*. There is, however, a clear link between σ^B and the virulence of *B. anthracis* [11]. Interestingly, other alternative sigma factors in bacteria have been shown to contribute both directly and indirectly to virulence [14], such as σ^E of *Salmonella typhimurium* which regulates expression of genes that provide resistance to oxidative stress, aiding bacterial survival in macrophages [15].

Conclusion

The activity of alternative sigma factors in bacteria is necessarily tightly controlled. The level of complexity observed in sigma factor regulation is needed for integrating between different pathways, as well as being able to fine tune the spatial and temporal control of cellular processes.

References

1. Mooney RA, Darst SA & Landick R. Sigma and RNA polymerase: an on-again, off-again relationship? *Mol Cell* 2005; 20:335-45.
2. Gruber TM & Gross CA. Multiple sigma subunits and the partitioning of bacterial transcriptional space. *Ann Rev Microbiol* 2003; 57:441-66.
3. Carniol K, Eichenberger P & Losick R. A threshold mechanism governing activation of the developmental regulatory protein σ^F in *Bacillus subtilis*. *J Biol Chem* 2004; 279:14860-70.
4. Errington J. Septation and chromosome segregation during sporulation in *Bacillus subtilis*. *Curr Opin Microbiol* 2001; 4:660-6.
5. Piggot PJ & Hilbert DW. Sporulation of *Bacillus subtilis*. *Curr Opin Microbiol* 2004; 7:579-86.
6. Hilbert DW & Piggot PJ. Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol Mol Biol Rev* 2004; 68:234-62.
7. Feucht A, Daniel RA & Errington J. Characterization of a morphological checkpoint coupling cell-specific transcription to septation in *Bacillus subtilis*. *Mol Microbiol* 1999; 33:1015-26.
8. Jiang X, Rubio A, Chiba S, & Pogliano K. Engulfment-regulated proteolysis of SpoIIQ: evidence that dual checkpoints control σ^K activity. *Mol. Microbiol.* 2005; 58:102-15.
9. Stragier P, Kunkel B, Kroos L, & Losick R. Chromosomal re-arrangement generating a composite gene for a developmental transcription factor. *Science* 1989; 243:507-12.
10. Petersohn A, Brigulla M, Haas S, Hoheisel JD, Volker U, & Hecker M. Global analysis of the general stress response of *Bacillus subtilis*. *J Bacteriol* 2001; 183:5617-31.
11. van Schaik W & Abee T. The role of σ^B in the stress response of Gram-positive bacteria: targets for food preservation and safety. *Curr Opin Biotech* 2005; 16:218-24.
12. Benson A & Haldenwang W. *Bacillus subtilis* σ^B is regulated by a binding protein (RsbW) that blocks its association with core RNA Polymerase. *Proc Natl Acad Sci USA* 1993; 90:2330-4.
13. Chen C-C, Yudkin MD & Delumeau O. Phosphorylation and RsbX-dependent dephosphorylation of RsbR in the RsbR-RsbS complex of *Bacillus subtilis*. *J Bacteriol* 2004; 186:6830-6.
14. Kazmierczak MJ, Wiedmann M & Boor KJ. Alternative sigma factors and their roles in bacterial virulence. *Microbiol Mol Biol Rev* 2005; 69:527-43.
15. Humphreys S, Stevenson A, Bacon A, Weinhardt AB & Roberts M. The alternative sigma factor, σ^E, is critically important for the virulence of *Salmonella typhimurium*. *Infect Immun* 1999; 67:1560-8.

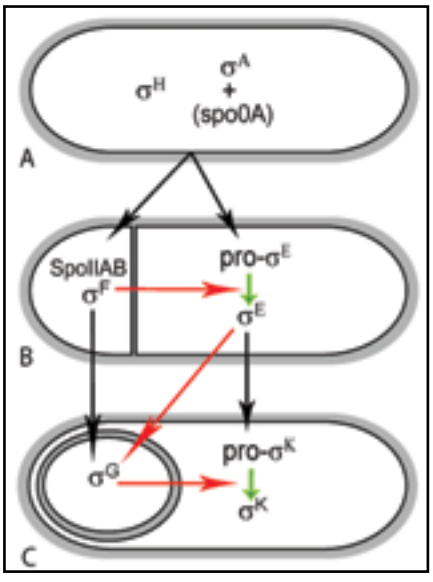

Figure 2. Morphogenesis and gene regulation during spore formation in *B. subtilis*.

(A) Activation of Spo0A (utilising σ^A) and σ^H in the pre-divisional cell leads to asymmetric division.

(B) σ^A along with the transcription factor Spo0A transcribes both σ^F and σ^E; however, both are held inactive. σ^F by its anti-sigma factor SpoIIAB and σ^E as a pro-σ^E product unable to bind RNAP. σ^F is released from SpoIIAB inhibition, only in the smaller forespore compartment, once the polar septum has been completed. Once active, σ^F transcribes the signalling gene that results in the cleavage of pro-σ^E to active σ^E. Spatial positioning of the partially segregated chromosome is thought to inhibit activation of σ^E in the forespore. Both σ^F in the forespore and σ^E in the mother-cell are required to direct engulfment.

(C) Transcription of σ^G is initially driven by σ^F; however, it directs its own transcription when engulfment is complete. Other factors under the control of both σ^F and σ^E are required for σ^G to be activated. Although the transcription of pro-σ^K is directed by σ^E, σ^K is not active until its pro sequence has been cleaved, allowing σ^K to bind to core RNAP. The signal for pro-σ^K cleavage is transcribed by σ^G in the engulfed forespore.

Black arrows represent transcriptional regulation, red arrows represent a signal transfer and green arrows represent a proteolytic event.

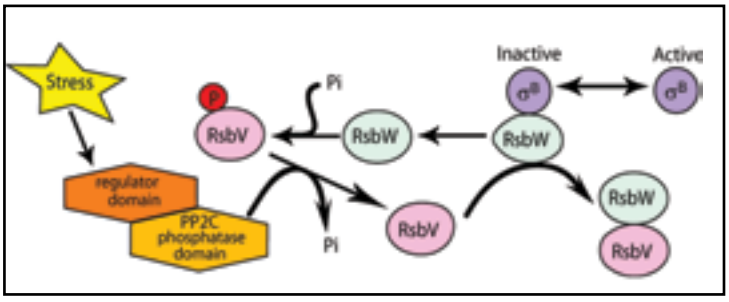

Figure 3. Regulation of σ^B activity by association with its anti-sigma factor, RsbW.

Under non-stress conditions, σ^B is bound to its cognate anti-sigma factor RsbW, holding it in an inactive state. RsbW also functions as a kinase on the anti-sigma factor antagonist RsbV. When RsbV is dephophorylated by the action of a phosphatase with a PP2C domain, RsbV can bind to RsbW. This results in the release of σ^B from its complex with RsbW and, upon association of σ^B with core RNAP, to the transcription of the σ^B regulon.