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A Master Regulator σ^B Governs Osmotic and Oxidative Response as well as Differentiation via a Network of Sigma Factors in *Streptomyces coelicolor*

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Alternative sigma factors in bacteria coordinate gene expression in response to various environmental and endogenous signals. Current microbial genome sequence data reveal a variety of alternative sigma factors, ranging from one or two sigma factors to about 66 sigma factors in *Streptomyces coelicolor*. Most alternative sigma factors are related in sequence to σ^{70} (σ^D) of *Escherichia coli*. Based on phylogenetic relatedness, σ^{70} family has been sub-divided into several groups; group 1 for the σ^{70} orthologs, the essential house-keeping sigma factors, group 2 for nonessential close relatives of group 1 members, group 3 for more divergent but evolutionarily related sigma factors, and group 4 for distantly related σ^{70} family members originally termed extracytoplasmic function (ECF) sub-family.

Among these sigma factors, those that respond to diverse growth-limiting stresses and protect bacteria against further stresses are known as general stress response sigma factors. The best studied examples are σ^S (group 2) of *Escherichia coli* representing γ-proteobacteria and σ^B (group 3) of *Bacillus subtilis* representing low G+C Gram-positive group of bacteria. σ^B in *B. subtilis* regulates the expression of more than 200 genes involved in heat, acid, ethanol, salt, and oxidative stress resistance. Its homologue also regulates stress resistance, virulence, adherent biofilm formation in various bacteria. In *Mycobacterium tuberculosis*, a high G+C Gram-positive bacterium, a σ^B-like factor contributes to its virulence.

The genome sequence of *S. coelicolor* A3(2) M145, containing 7825 protein-coding genes, revealed ten group 3 sigma factors; σ^B (SCO0600), σ^L (SCO7278), σ^I (SCO3068), σ^N (SCO4034), σ^F (SCO4035), σ^H (SCO5243), σ^K (SCO6520), σ^M (SCO7314), σ^G (SCO7341), and σ^{WhiG} (SCO5621) in the order of similarity to σ^B. Of these, at least three (σ^B, σ^H, σ^I) are specifically induced by osmotic stress, suggesting that osmotic stress response of *S. coelicolor* is mediated by multiple σ^B-like sigma factors. They are regulated in diverse ways. σ^B is regulated at transcriptional level for its synthesis and

post-translationally for its activity through interaction of its anti-sigma factor (RsbA) with an anti-anti-sigma factor (RsbV), involving a phospho-relay mechanism. σ^H is regulated at levels of transcription, translation start site selection, protein processing, and possibly interaction with an anti-sigma factor (PrsH/UshX). σ^I increases rapidly upon osmotic stress, most likely via increased transcription. σ^B, σ^H, σ^F, and σ^{WhiG} have been also implicated in controlling proper differentiation.

Microarray analysis revealed σ^B-dependent induction of more than 280 genes by 0.2 M KCl. These genes encode several sigma factors, oxidative defense proteins, chaperones, and systems to provide osmolytes, cysteine, mycothiol, and gas vesicle. σ^B controlled induction of itself and its two paralogues (σ^L and σ^M) in a hierarchical order of $\sigma^B \rightarrow \sigma^L \rightarrow \sigma^M$, as revealed by S1 mapping and Western blot analyses. The phenotype of each sigma mutant suggested a sequential action in morphological differentiation; σ^B in forming aerial mycelium, σ^L in forming spores, and σ^M for efficient sporulation. σ^B was also responsible for the increase in cysteine and mycothiol, the major thiol buffer in actinomycetes, upon osmotic shock, revealing an overlap between protections against osmotic and oxidative stresses. Proteins in *sigB* mutant were more oxidized (carbonylated) than the wild type. These results support a hypothesis that σ^B serves as a master regulator that triggers other related sigma factors in a cascade, and thus regulates differentiation and osmotic and oxidative response in *S. coelicolor*.