Study of Environmental Stress Signaling in Bacillus subtilis via components of RsbR paralogues

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Abstract: *Bacillus subtilis* has found to respond the signals of environmental and metabolic stress by inducing over 40 general stress genes which are under the control of the sigma B transcription factor. Sigma B is an alternative sigma-factor in *Bacillus subtilis.* It mediates the response of the cell to a variety of physical insults. General stress response of *Bacillus subtilis* is regulated directly by a partner-switching mechanism via key protein interactions and transcription factor sigma B expression plays important role on it. Physical stress is communicated to sigmaB via a large-molecular-mass (>106-Da) structure (i.e. called the stressosome) formed by one or more members of a family of homologous proteins (RsbR, YkoB, YojH, YqhA). Signals of energy or environmental stress are conveyed to sigma B by independent pathways, each terminating with a differentially regulated serine phosphatase (i.e. for serine phosphorylation), whose activity is required to control the partner-switching regulators. In *B. subtilis* genome, six paralogous proteins such as YetI, YezB, YkoB, YojH, YqhA, and YtvA are found which has significant similarity to RsbR.

Key word: *Bacillus subtilis*, RsbR, environmental stress, sigma B genes, pathways

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I. Introduction:

Bacillus subtilis is a Gram-positive and catalase-positive bacterium and catalase enzymes convert hydrogen peroxide to water and oxygen molecules. It is also known as the hay bacillus or grass bacillus. It is mostly found in soil and the gastrointestinal tract of ruminants and humans. A member of the genus *Bacillus*, *B. subtilis* is found in rod-shaped morphology and it can form a tough, protective endospore which is allowing it to tolerate extreme environmental conditions. *B. subtilis* has historically been classified as an obligate aerobe, though evidence exists that it is a facultative aerobe. *B. subtilis* is Gram-positive bacterium and this model organism has shown to study bacterial chromosome replication and cell differentiation. It has capability to secrete various enzyme production and used on an industrial scale by biotechnology companies (You et. al., 2013).

Bacteria have capability to manifest diverse adaptive responses to adjust their physiology by changing surroundings. In *Bacillus subtilis* and the related pathogens *Listeria monocytogenes* and *Staphylococcus aureus*, the general stress regulon is controlled by σB and plays a prominent role in this strategy, interacting with other response systems to form flexible adaptive networks. The stressosome *of Bacillus subtilis* has very complex structure with weight of 1.8 MDa and it help in activation of the σB transcription factor by environmental stress. This complex structure is made up of members of the RsbR coantagonist family and the RsbS antagonist, which together form an icosahedral core. It sequesters the RsbT serine-threonine kinase. Phosphorylation of this core by RsbT is associated with RsbT release. It has capability to activate downstream signaling. RsbRA, the prototype co-antagonist, is phosphorylated on T171 and T205 in vitro. In unstressed cells T171 is already phosphorylated; this is a prerequisite but not the trigger for activation, which correlates with stress-induced phosphorylation of RsbS on S59 (Macek et al 2007). Phosphorylation of RsbRA T205 has not been detected in vivo. But report and study has done on following (i) RsbRA is additionally phosphorylated on T205 following strong stresses; (ii) this modification requires RsbT; and (iii) the phosphorylation-deficient T205A substitution greatly increases post-stress activation of σ B (Eymann et al., 2011). We are observing from different literature that T205 phosphorylation constitutes a second feedback mechanism to limit σ B activation, operating in addition to the RsbX feedback phosphatase. Loss of RsbX function increases the fraction of phosphorylated RsbS and doubly phosphorylated RsbRA in unstressed cells (Kang et al., 1996). We conclude that RsbX can maintain the ready state of the stressosome prior to stress, and it can restore it post-stress.

General stress system in *Bacillus subtilis*

σB activity in *Bacillus subtilis* is regulated by a signaling network that employs the partner switching mechanism, in which interactions between alternative binding partners are

governed by serine and threonine phosphorylation. General stress mechanism in *Bacillus subtilis* contains two independent branches that converge on the RsbV and RsbW regulators of σB (Hecker et al., 2009). Null mutations in four of the six paralogous genes have marked effects on the sigmaB environmental signaling pathway, either singly or in combination. The paralogous proteins RsbRA (formerly RsbR), RsbRB (YkoB), RsbRC (YojH), and RsbRD (YqhA) has capability to possess two conserved threonine residues in their STAS domains. It can determine the growth conditions of B. subtilis under which these conserved residues were phosphorylated in vivo, the contribution of the RsbT kinase and RsbX phosphatase, and the effects of these modifications on signaling. The four co-antagonists are functionally similar but are not identical (Kim et al ., 2004 a.b). RsbT serine-threonine kinase is required to convey environmental stress signals to sigmaB, and this kinase activity is magnified in vitro by the RsbR protein, a positive regulator important for full in vivo response to salt or heat stress (Akbar et al 2001). A model has been presented in which negative regulation of the RsbU phosphatase depends solely on the RsbS antagonist protein. It has also demonstrated that the RsbS antagonist alone is insufficient to prevent environmental signaling. It requires one of a family of four co-antagonist proteins, renamed RsbRA, RsbRB, RsbRC, and RsbRD, each with a carboxyl-terminal domain closely resembling the entire RsbS protein. Any single member of the RsbR family, together with RsbS, is sufficient for environmental signaling. RsbR proteins serve as redundant co-antagonists, necessary for RsbS antagonist function (Kim et al 2004). Phosphorylated in vitro by the RsbT environmental signaling kinase, as has been previously shown for RsbR, which is phosphorylated on two threonine residues in its C-terminal region. It had been shown that (i) RsbR, YkoB, YojH, YqhA, and YtvA function in the environmental stress signaling pathway; (ii) YtvA acts as a positive regulator; and (iii) RsbR, YkoB, YojH, and YqhA collectively act as potent negative regulators whose loss increases sigmaB activity more than 400-fold in unstressed cells (Akbar et al 2001)

II. Function of Stresssome

Biochemical analysis has shown that YkoB, YojH, and YqhA play important role on general stress response of *B. subtilis* and possess two conserved threonine residues in their STAS domains. Three phosphorylated paralogs such as YkoB, YojH, and YqhA but are absent in the ones that were not substrates of RsbT. YetI and YezB, each of which bears only one of the conserved residues and YtvA, which lacks both residues and instead possesses an N-terminal PAS domain (Chen et al., 2003). Yeast two-hybrid system has suggested that all six paralogs interact with each other and with the RsbR and RsbS environmental regulators. Exposure to stress empowers an RsbT-dependent phosphorylation of RsbR and RsbS, with the subsequent release of RsbT to activate downstream processes (Akbar et al 2001). Changes in stressosome components could lead to sigmaB activation. Eight mutations have done to heightened sigmaB activity in the presence of their wild-type counterparts. Two of the mutations that are missense changes in rsbR, and six are amino acid changes in rsbS. RsbR and rsbS mutations have enhanced sigmaB activity by elevating the level of RsbS phosphorylation. Changes in RsbR family can initiate the downstream events that lead to sigmaB activation and that RsbR, RsbS, and RsbT likely interact with each other concomitantly with their synthesis (Reeves and Haldenwang 2007). RsbT's has ability to function strongly which is influenced by coexpression with these adjoining genes. When rsbT is expressed at a site, displaced from rsbS and rsbU, rsbT accumulates but it is unable to activate sigmaB following stress. RsbT activity is restored if rsbT is co-transcribed at the alternative site with the genes that normally about it. Gene rsbT is located within an operon that includes the genes for its principal negative regulator (RsbS) and the stress pathway component that it activates (RsbU), as immediate upstream and downstream neighbor. RsbS and RsbT are synthesized in equivalent amounts and interact coincidently with their synthesis to form stable regulatory complexes that maintain RsbT in a state from which it can be stress activated. s. They have demonstrated that. (Zhang et al 2005).

Sigma B activity is controlled by protein-phosphorylation-dependent interactions of anti-sigmaB with anti-anti-sigma factors. Under stress conditions, the phosphatase RsbU triggers release of sigma B and thus induces the expression of stress genes. RsbU activity is controlled by three proteins, RsbR, RsbS and RsbT which form a supramolecular complex called the stressosome. They have reviewed the occurrence of the genes encoding the stressosome proteins (called the RsbRST module) in a wide variety of bacteria (Pané-Farré et al., 2005). While this module is linked to the gene encoding sigmaB and its direct regulators in *B. subtilis* and its close relatives, genes encoding two component regulatory systems and more complex phosphorelays are clustered with the RsbRST module in bacteria as diverse as cyanobacteria, bacteroidetes, proteobacteria, and deinococci. It cleared that stressosome proteins form a signal sensing and transduction unit that relays information to very different output modules.

In control of sigmaB activity, the RsbR-RsbS complex plays an important role by trapping RsbT. At the onset of stress, RsbR becomes phosphorylated, resulting in an enhanced activity of RsbT towards RsbS. RsbT is then free to interact with and activate RsbU, which in turn ultimately activates sigmaB. In this study with purified proteins, they used mutant RsbR proteins to analyze the role of its

phosphorylatable threonine residues. They found results that showed that phosphorylation of either of the two RsbT-phosphorylatable threonine residues (T171 and T205) in RsbR enhanced the kinase activity of RsbT towards RsbS. However, it appeared that RsbT preferentially phosphorylates T171. They also presented in vitro evidence that identifies RsbX as a potential phosphatase for RsbR T205 (Chen et al., 2004).

III. Conclusions

Bacillus subtilis is a Gram-positive. General stress response of *Bacillus subtilis* is regulated directly by a partner-switching mechanism via key protein interactions and transcription factor sigma B expression. RsbR, RsbS and RsbT is important components in stressosome proteins and is work as major structural component and it helps the organism via forming a signal sensing and transduction unit and then it relays information to very different output modules. RsbT's has ability to function strongly which is influenced by coexpression with these adjoining genes T171 and T205 and preferentially phosphorylates T171 threonine residues. RsbR and rsbS mutations have enhanced sigmaB activity by elevating the level of RsbS phosphorylation.

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