Primary Sporulation Response Regulator Spo0A

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Key words: Spo0A, crystallisation, phosphorelay, helix-turn-helix

Sporulation of *Bacillus* is a simple example of cell differentiation Under conditions of nutrient deprivation *Bacillus* undergoes a complex differentiation process that involves biochemical, physiological and morphological changes and culminates in the formation of a resistant spore that can lay dormant until more favourable conditions are restored This process of an extreme adaptive response involves the activation of a number of genes and the expenditure of a vast amount of energy The decision to switch from vegetative growth to sporulation is stringently controlled by a complex series of internal and external signals Primarily, the initiation of sporulation is determined by the phosphorylation of a response regulator Spo0A, an ambiactive transcriptional regulator, which activates transcription of some genes while repressing others (Errington 1993)

Spo0A is phosphorylated by a phosphorelay, an extensive version of two-component signal transduction system (Hoch 1993) The most important advantage of this multistep relay may be that it provides the potential for multiple regulatory checkpoints The relay begins with the activation of one of three kinases KinA, KinB and KinC These kinases are activated by different input signals and also differ in their structural properties, the cellular localisation and in their respective contribution to the sporulation process (Ledeaux *et al* 1995) In this cumulative environsensory mechanism several kinases receiving different metabolic signals function to phosphorylate Spo0F Response regulator Spo0F is comprised only of a conserved N-terminal domain and serves as a phosphodonor for the phosphotransferase Spo0B that finally transfers phosphate to the Spo0A Recently several independent protein phosphatases that control initiation of sporulation were found RapA and RapB phosphatases specifically dephosphorylate Spo0F and Spo0A is dephosphorylated by Spo0E Their activities are regulated by signals that differ from those regulating the kinases It seems likely that the competition between the opposing activities of kinases and phosphatases that manifests itself in the level of Spo0A~P is the basis for the integration of diverse signals that affect the initiation of sporulation (Perego and Hoch 1996) The activity of Spo0A is not only regulated through the phosphorylation but also on the level of expression Transcription from vegetative promoter occurs during exponentional growth at a low level, but is sufficient to provide an initiation-sensing concentration of intracellular Spo0A protein As the cell begins to deplete the available nutrients, unknown signals trigger the activation of the kinases and then the phosphorylation of the available Spo0A molecules by phosphorelay Spo0A~P activates its own transcription from the sporulation promoter by σ^H RNA polymerase Concomitantly represses its own transcription from the vegetative promoter At the same time Spo0A~P represses *abrB* transcription and drop in AbrB level leads to derepression of the *spo0H* gene which results in the presence of

more RNA polymerase containing σ^H, the first sporulation specific σ factor σ^H is also required for the transcription of *spo0F* gene from the sporulation promoter Thus the initial amount of Spo0A~P formed quickly leads to a positive feedback loop in which not only more Spo0A is made but also the ability to phosphorylate is increased due to the increased Spo0F level (Strauch *et al* 1992) This transcription regulatory scheme can be viewed as a complex system of autoregulation, where Spo0A~P is the product and the major controller For the entry into sporulation a threshold level of Spo0A~P in the cell has to be achieved As the cell progresses through the sporulation programme Spo0A~P is no longer needed and its synthesis is turned off

Spo0A is a transcription activator and repressor of a number of genes Among these are three key sporulation-specific promoters that are activated by Spo0A~P *spoIIG*, *spoIIA* and *spoIIE* Spo0A~P by virtue of its increased affinity for binding to DNA binds at the specific DNA sequence 5'-TGTCGAA 3' called "0A box" The "0A-boxes" appear 5' to transcriptional start for all promoters that are stimulated by Spo0A (*spoIIA*, *spoIIE*, *spoIIG*, *spo0A*) At promoters repressed by Spo0A (e g *abrB*) the sequence appears downstream of the transcription start Near several promoters there are multiple "0A-boxes" Spo0A is unique in that it stimulates transcription initiation by RNA polymerase containing different σ-subunits The stimulation may result from the interaction of Spo0A with σ-subunit in some cases and the α-subunit in other cases Also the orientation of Spo0A binding sites differs at different promoters The different orientations may also reflect interaction with different polymerase subunits (Spiegelman *et al* 1995) In the case of *spoIIE* promoter region one "0A box" is situated in the -35 region, so it is possible that Spo0A could interact with σ^A subunit of RNA polymerase There is an evidence for the interaction of Spo0A with σ^A and σ^H (Baldus *et al* 1995) The region of the Spo0A protein which is contact with the σ factor is not known It is also not known whether the same region of Spo0A is in contact with both σ factors σ^A and σ^H The ability of Spo0A to interact with σ^A and σ^H raises the possibility that Spo0A may interact also with other σ factors

Spo0A contains an N-terminal phospho-acceptor domain that shows sequence identity to other response regulators and a C-terminal DNA-binding domain that is unique An alignment of Spo0A homologues from diverse *Bacillus* and *Clostridium* species revealed three highly conserved regions in the C-terminal domain (Brown *et al* 1994) The most highly conserved of these corresponds to the recognition helix of a putative helix-turn-helix motif Several mutations in the recognition helix were prepared that led to the inhibition of activation and repression of transcription from promoters containing "0A boxes" Therefore these sites may represent the actual DNA contacting surface of the protein It is not exactly known which functions might be associated with the two other regions A missense mutation called *spo0A9V* in the last conserved region of the C-terminal domain gave rise to a protein active as a negative regulator of *abrB* but unable to activate transcription of the *spoIIA* operon (Perego *et al* 1991) It was suggested that the C-terminus of Spo0A interacts with RNA polymerase complex to activate transcription Intragenic suppressors of the *spo0A9V* mutation called *suv-4* and *suv-3* have been isolated in the first conserved region of the C-terminal domain Another mutation in the last conserved region of the C-terminal domain that has the same *in vivo* suppress mutations in the first conserved region has been recently prepared These results suggest that these regions of protein may interact in some way

In order to better understand the action of Spo0A it would be advantageous to solve its structure by X-ray crystallography Therefore *spo0A* gene from *Bacillus stearothermophilus* has been cloned and the encoded protein purified Proteolytic digestion of intact

Spo0A yields initially two N-terminal and two C-terminal fragments Mass spectrum analysis determined the M_r' s of these fragments and amino acid sequencing of the C-terminal fragments led to the positioning of the tryptic cleavage sites The trypsin cleavage occurs in two positions in the non conserved region between the two domains of Spo0A and is consistent with the delineation of two discrete functional domains (Grimsley *et al* 1994) Based on these results, fragments of *spo0A* that encode solely the N-terminal domain and C-terminal domain were amplified by PCR and subcloned into pET26b for high level expression Proteins were purified to a single band and crystallised

The DNA-binding domain crystals belong to the triclinic spacegroup P1 and diffract X-rays generating by a rotating anode source of X-rays to beyond 2 5 Å spacing The crystals of the phospho-acceptor domain are of primitive orthorombic spacegroup $P2_12_12_1$ and using synchrotron radiation diffract X-rays beyond 2 Å spacing

A search for heavy metal derivative of the DNA-binding domain of Spo0A and molecular replacement studies of the phospho-acceptor domain are now underway The structure of the two domains of Spo0A should provide detailed information about the way how Spo0A regulates late growth responses in *Bacillus,* how specific DNA sequences are recognised and how its function is regulated by phosphorylation

Acknowledgements. This work has been supported by the Wellcome Trust, grant 047031/Z/PMG/MJD, by grant 2027 from the Slovak Academy of Sciences, the EU Programme Copernicus CIPA-CT94-0189 and the EU HCMP Access to large scale facilities grant, CHGE CT93 0040

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