ESRF	**Experiment title:** **The crystal structure of the trans-activation domain of Spo0A**	**Experiment number**: LS-1532
Beamline: BM14 ID14-2	**Date of experiment**: from: 7/12/99 to: 9/12/99 from: 10/12/99 to: 12/12/99	**Date of report**: 28/08/00
Shifts:	**Local contact(s)**: V. Stojanoff S. Wakatsuki	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

R. J. Lewis[1], S. Krzywda*[1], J. A. Brannigan[1], J.P. Turkenburg*[1] , K. Muchová[2], E. J. Dodson[1], I. Barák[2] & A Wilkinson[1]

[1]YSBL, Department of Chemistry, University of York, York, YO10 5DD, UK
[2]IMB, Slovak Academy of Science, Dubravska cesta 21, 842 51 Bratislava, Slovak Republic

Report:

The process of sporulation in *Bacillus subtilis*, the most extreme survival strategy of Bacilli, involves the induction of scores of genes in a carefully controlled manner. Sporulation commences with an asymmetric division of the cell producing two progeny of unequal size but with identical chromosomes. The smaller cell, the forespore, is engulfed by the mother cell and the two compartments collaborate in the maturation of the developing spore. Finally, the mother cell lyses and in doing so releases the fully developed spore, which can lie dormant indefinitely. When favourable conditions for growth are restored, the spore may germinate. This simple model of cellular development is temporally and spatially co-ordinated, with the initiation under the control of an expanded two-component signal transduction system, called the phosphorelay.

Two-component signalling systems consist of a sensor histidine kinase and a cognate response regulator. Firstly, on the receipt of cellular signals the sensor kinase autophosphorylates on a histidine residue using ATP. Secondly, the phosphoryl group is transferred to a conserved aspartic acid residue in the response regulator, activating its latent function. In the case of the sporulation phosphorelay, the (ultimate and key) response regulator is Spo0A. Phosphorylation of Spo0A activates its DNA transcription activation and repression properties, by stimulating its binding to consensus DNA sequences present in multiple copies at Spo0A-regulated promoters. Thus if a threshold level of phosphorylated Spo0A accrues, sporulation commences.

Previously, we have determined the structure of the N-terminal, phosphoacceptor domain of Spo0A in the phosphorylated state, in the presence of divalent cations, revealing the stereochemical basis for aspartic acid phosphorylation and suggesting a common activation mechanism for all response regulators. Here we present the

structure of the C-terminal, trans-activation domain of Spo0A, which with our two structures of the receiver domain provides a framework for understanding the function of this master control element.

The structure of the trans-activation domain of Spo0A from *B. stearothermophilus* was determined from crystals of selenomethionine-substituted protein using MAD on beamline BM14 of the ESRF. The structure has now been fully refined against diffraction data extending to 2.0 Å, collected on beamline ID14-EH2. It forms an all α-helical assembly with no extended structural similarity to other structures in the PDB. Helices B, C and D form a three-helical bundle, somewhat reminiscent of CAP, where helices C and D form an archetypal helix-turn-helix DNA-binding motif. The structure suggests a mode of sequence-specific DNA binding.

Mutations in Spo0A, which affect the transcription activation properties, cluster in a flexible region of the structure, around helix E. The structural plasticity of helix E is an important aspect of the positive regulation of transcription. This area also forms a significant negatively-charged surface, which is pertinent with regard to transcription activation since Spo0A contacts RNA polymerase through a highly positively-charged sequence in the sigma subunit.