Structure and function of AhrC, the arginine transcription factor from *Bacillus subtilis*

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Introduction

In *Bacillus subtilis* the concentration of L-arginine is controlled by the hexameric transcriptional regulator AhrC, which interacts with 18 bp pseudo-palindromic ARG boxes in the promoters of arginine biosynthetic and catabolic operons. The biosynthetic promoters contain two or three binding sites and a unique property of AhrC is its apparent ability to also stimulate the catabolic pathway through a single operator site.

The crystal structure of the apo-form of AhrC, in the absence of the arginine corepressor, was solved in Leeds (**Fig. 1a**). Each subunit has two domains, with the C-terminal domains forming the core of the hexamer. The N-terminal domains contain a winged helix-turn-helix DNA-binding motif, and are arranged around the periphery.

Crystallographic studies

To study the effects of ligand binding in the C-terminal hexamer (CAhrC) and ARG box interactions with the N-terminal domains of AhrC (NAhrC), the two independent domains were expressed, purified and crystallized, with CAhrC in the presence of L-arginine. In addition we have crystallized a complex between a dimer of NAhrC and an 18 bp blunt ended oligonucleotide, corresponding to an ARG box. The three crystal structures have been solved at 1.00 Å (NAhrC), 1.95 Å (CAhrC) and 2.85 Å (NAhrC operator complex).

The structures reveal how AhrC functions as a transcriptional repressor. AhrC has 32 symmetry and L-arginine binding to AhrC triggers an approximate rotation of 15˚ of one trimer with respect to the other, when compared to the core of apo-AhrC. When NAhrC domains recognise and bind to their ARG box, the flexible wing of the DNA binding motif is stabilised across the minor groove and the recognition helices (α3) lie in the major groove, bending the ARG box by approximately 30° towards the NAhrC dimer.

We have modelled the effects of L-arginine and operator binding to intact AhrC using the structures of the domains and the operator complex (**Fig. 1a,b,c**). These models suggest that the rotation induced by ligand binding results in the DNA binding domains coming together, although this alone will not bring the N-terminal domains into the correct arrangement. A reorientation of these domains is needed to reproduce the NAhrC dimer observed in the operator complex and create the fully activated transcription factor. We have also constructed models of the repression complexes at the *argC* biosynthetic operator, where the promoters are bent around AhrC, disrupting any attempts by RNA polymerase to bind and initiate transcription.

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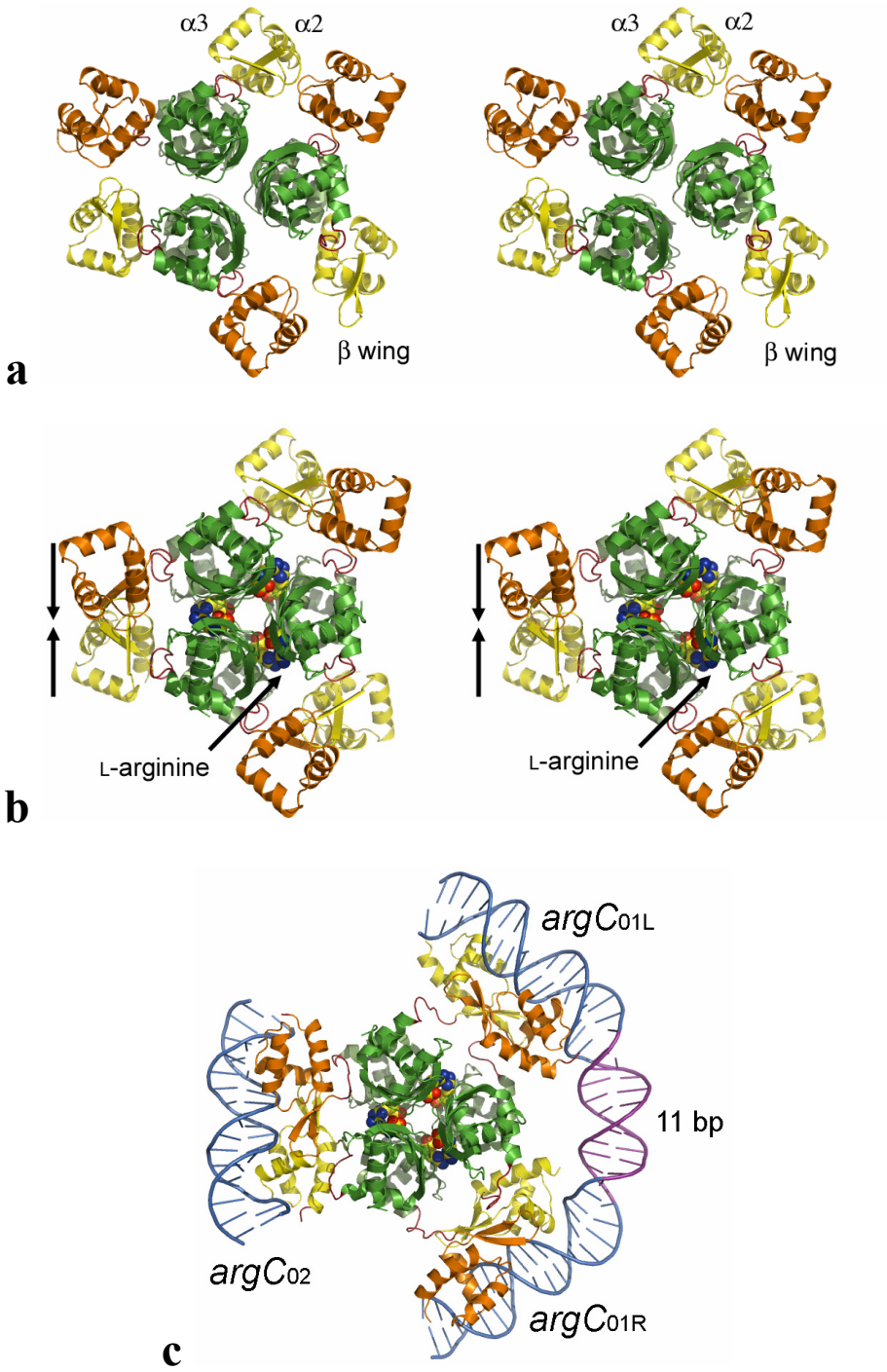

Figure 1: Models of L-arginine activation of AhrC and the consequent binding to its *argC* biosynthetic operator site. The C-terminal domains of AhrC are coloured light green (top trimer) and dark green (lower trimer). L-arginine ligands are shown as spheres. The N-terminal domains of AhrC are coloured yellow (associated with the upper trimer) and orange (associated with the lower trimer), with the flexible linker connecting the N- and C-terminal domains coloured red. (**a**) Crystal structure of apo-AhrC[1]. The N-terminal domains lie around the periphery of the protein core with imperfect symmetry. The DNA binding structures are labelled. (**b**) Model of holo-AhrC. Binding of L-arginine causes a rotation between the trimers, bringing the N-terminal domains together. (**c**) Model of holo-AhrC in complex with its *argC* operator. ARG boxes are coloured blue and the intervening DNA spacer is purple. For AhrC to bind to its operator site there must be a rearrangement in the N-terminal domains, orientating the wings and recognition helices into the correct position for DNA binding. The third ARG box is believed to be contacted through looping out the intervening DNA.