The Two DNA Binding Modes of RPA

Schematic representation of the trimeric RPA molecule (p70, p32, and p14 subunits) with the four DNA binding domains designated as A, B, C, and D. The binding of ssDNA (thick line) occurs via a multistep pathway. The initial, unstable 8-nucleotide binding is mediated by domains A and B. A conformational switch then reorients domain C, allowing it (and likely domain D) to make contact with the ssDNA protruding from domain B to attain the stable 30-nucleotide binding mode. The 5’ to 3’ polarity of DNA engagement by RPA was first reported by de Laat et al. [10].

Tandem DNA Binding of E. coli’s Transactivator PhoB

In this issue of *Structure*, Blanco et al. describe the first structure of a two-component response regulator effector domain bound to its target DNA, showing novel tandem binding to successive direct repeat sequences of pho boxes from the phoA operon promoter.

“Two-component” is an old moniker used to describe the vast group of bacterial signal transduction systems that respond to environmental cues [1]. It is the basic “stimulus-response” situation, where the stimulus is intercepted by a receptor (component one), which then phosphorylates a response regulator (component two) to execute the appropriate reaction, usually through transcriptional activation. The response regulators are typically two-domain proteins, where the conserved N-terminal domain experiences transient phosphorylation, and then somehow “activates” the C-terminal DNA binding domain. It is this transcriptional activation process that has held so much research interest for so long. How do these phosphorylation events manifest themselves in DNA recognition and transcriptional initiation? Recent structural reports have begun to reveal how these mechanisms may work.

The scores of response regulators identified through sequence homology in their N-terminal domains constitute a superfamily [2]. This superfamily can be subdivided into three families according to the sequences of their C-terminal, DNA binding domains. The dominant families are NtrC, FixJ, and OmpR, each named after its representative protein member. There may be a unique transcriptional activation mechanism for each family because of their different C-terminal domains. An understanding of the mechanisms for some of these families is now becoming possible through structural analysis. This was first done with the structural determination of the full-length two-domain NarL protein (important in the regulation of nitrite and nitrate uptake in *E. coli*) of the FixJ family, which showed control of DNA access through domain surface occlusion [3].

Recent structural work on PhoB, a member of the OmpR family, suggests that its mechanism of transcriptional activation is very different, and perhaps more complex [4]. OmpR family members have C-terminal domains that are structurally analogous to the winged helix family of DNA binding proteins [5], so they may employ similar transcriptional activation mechanisms. However, even winged helix domains come in a variety of forms. The first identified winged helix protein, the eukaryotic HNF-3γ [6], has a structure reminiscent of the familiar helix-turn-helix motif but with two loops called “wings” flanking the central DNA recognition helix [7]. The central helix H3 is usually the most important DNA binding component of the winged helix domain, while the wings can assume different functions in different cases. For example, most wings participate in minor groove interactions (e.g., the HNF-3/forkhead family, LexA), but some are involved in major groove recognition.
(e.g., RFX1), while in other cases the wings are used exclusively for protein-protein contacts (e.g., E2F4-DP2, HSF) [8, 9]. Now, in this issue of *Structure*, Blanco et al. show that the single wing of PhoB has specific minor groove interactions through Arg219 and a thymine base, as well as a nonspecific contact with a backbone deoxyribose.

The neatest twist in this recent analysis of PhoB DNA recognition and transcriptional control comes from their results on the PhoB effector domain bound to its specific DNA target sequence. PhoB was cocrystallized with a 23 base pair double-stranded DNA corresponding to PhoB’s natural target, including the direct repeats from within the *pho* boxes. Until now, a major outstanding question has been: how do response regulators self-associate upon binding to promoter regions that require tandem assembly? Some models for self-association in vivo have been suggested by homodimers of the N-terminal domains of response regulators found in the crystalline state, but those dimers may have been crystallization artifacts. It has been aptly pointed out that the isologous association observed in those dimers does not result in the proper geometry required for tandem assembly [10]. Blanco et al. resolve this question in their current work by clearly showing that heterologous self-assembly onto tandem promoters is directed solely by the C-terminal domains (see Figure). Their results beautifully show tandem PhoB oligomerization onto consecutive *pho* boxes. This has direct implications for the in vivo situation for the entire OmpR family, where all known DNA recognition sites of the family are direct repeats.

What do the results of Blanco et al. suggest for a mechanism of transcriptional initiation for PhoB and other OmpR family members? The authors have arrived at a mechanism quite different from that proposed earlier for NarL and the FixJ family [3]. By modeling the full-length PhoB homolog DrrD [10] onto their effector domain-DNA structure, Blanco et al. convincingly demonstrate steric clashes between the (unphosphorylated and unactivated) molecules. The logical conclusion from their model is that phosphorylation could cause subtle conformational changes to relieve these steric restrictions, enabling tandem assembly onto the direct repeats of the DNA. The likely site for release of the conformational stress is the small four-stranded β sheet platform at the interface between the two domains, which is conserved among all OmpR-PhoB members. This is a very insightful model of transcriptional activation for PhoB.

I am sure it will be put to the test in the near future.

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Selected Reading