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When is a transcription factor a NAP?

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Proteins that regulate transcription often also play an architectural role in the genome. Thus, it has been difficult to define with precision the distinctions between transcription factors and nucleoid-associated proteins (NAPs). Anachronistic descriptions of NAPs as 'histone-like' implied an organizational function in a bacterial chromatin-like complex. Definitions based on protein abundance, regulatory mechanisms, target gene number, or the features of their DNA-binding sites are insufficient as marks of distinction, and trying to distinguish transcription factors and NAPs based on their ranking within regulatory hierarchies or positions in gene-control networks is also unsatisfactory. The terms 'transcription factor' and 'NAP' are *ad hoc* operational definitions with each protein lying along a spectrum of structural and functional features extending from highly specific actors with few gene targets to those with a pervasive influence on the transcriptome. The *Streptomyces* BldC protein is used to illustrate these issues.

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Introduction

The analysis of transcription control networks in model bacteria shows that genes are organized for collective responses within regulatory groupings such as operons, regulons and stimulons [1–3,4•]. Regulons and operons are characterized by their dependence on a common regulator, usually a protein, which imposes collective control in response to signals such as quorum-sensing molecules, second messengers such as cAMP or c-di-GMP, metals, carbohydrates, or changes in a physical parameter such as temperature.

An important concept in the analysis of regulatory networks is the range of the regulator: how many genes does it control? Conventional transcription factors can govern the expression of very few or very many genes. For example, the LacI repressor in *Escherichia coli* controls the *lacZYA* operon negatively in the absence of its allolactose inducer, while the cAMP receptor protein (CRP) controls the same operon positively but also regulates the transcription of hundreds of other genes in response to the second messenger cAMP [5,6]. LacI targets the *lac* operator sequence and cAMP-CRP targets its own binding site within the same promoter. Thus, target genes can join or leave a regulon simply by gaining or losing sequences that resemble a transcription factor-binding site [7•,8,9].

Nucleoid-associated proteins (NAPs) are DNA-binding proteins that play a role analogous to that of histones in the chromatin of eukaryotes [10]. NAPs can bend, bridge, wrap and polymerize along DNA [10], allowing them to play an architectural role in the genome, but they can also influence transcription. The promiscuous nature of their binding activity throughout the genome allows NAPs to exert a pervasive effect on gene expression. Interestingly, several of the DNA-dependent activities associated with NAPs are shared with transcription factors, raising questions about the sharpness of the distinction between these two groups of DNA-binding proteins [11•]. Sequence specificity is not a reliable distinguishing feature. For example, Integration Host Factor (IHF) is a NAP that requires a relatively strict match to its consensus sequence for DNA binding [12]. In this respect, IHF seems to be equivalent to the transcription factors LacI and CRP. Conversely, LysR-type transcription regulators (LTTRs) rely heavily on DNA shape rather than on nucleotide sequence in recognising their DNA targets, something they share with NAPs such as H-NS and HU [13]. IHF bends DNA impressively, in some instances imposing bend angles of up to 180°, but transcription factors also bend their DNA targets, even if they do not introduce complete U-turns into the DNA duplex, for example, the *E. coli* purine repressor, PurR [14]. H-NS has a DNA-binding mode that involves the formation of DNA-protein-DNA bridges, but so too do the transcription factors LacI, LeuO and lambda CI [15,16]. Many transcription factors bind signalling ligands as part of their sensing-and-response activities, but the activity of Leucine-responsive Regulatory Protein (LRP),

often regarded as a NAP, is controlled by its binding to L-leucine and other branched-chain amino acids [17,18]. Finally, although FIS (the Factor for Inversion Stimulation) is viewed as a NAP, it can function as a conventional transcription factor by recruiting RNA polymerase via protein-protein contacts to initiate transcription [19]. These examples show the challenge in identifying clear distinctions between NAPs and transcription factors.

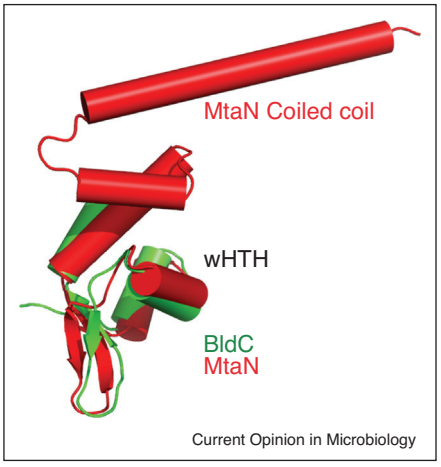
This distinction becomes even more blurred because NAPs are sometimes equivalent to functional domains within transcription factors: NtrC is a prominent example. NtrC is the response regulator partner of the cytosolic NtrB sensor kinase and it activates transcription at σ^{54}-dependent promoters in response to nitrogen stress [20]. These interactions require NtrC to bind to a well-defined enhancer sequence upstream of the target promoter. The DNA-binding domain of NtrC is closely related to the NAP FIS, leading to the proposal, supported by phylogenetic evidence, that FIS evolved from an NtrC-like transcription factor [21]. The *fis* gene probably arrived in *E. coli* at the same time as *dusB* (formerly *yhdG*), with which it now forms a *dusB-fis* operon. The DusB protein is related to NifR3 in *Rhodobacter*, *Rhizobium* and the *nifR3* gene is co-transcribed in those bacteria with *ntrB* and *ntrC*. It has been proposed that *dusB/yhdG* and *fis* evolved following horizontal gene transfer of the *nifR3 ntrB ntrC* nitrogen metabolism operon followed by deletion of all but the *fis* sequences from *ntrC* [21].

Here we consider the case of BldC, a recently characterized DNA-binding protein from the Gram-positive bacterium *Streptomyces*. Streptomycetes are filamentous bacteria that differentiate by producing spore-bearing reproductive structures called aerial hyphae [22,23]. The transition from vegetative to reproductive growth is controlled by the *bld* (bald) loci, which were identified in classical mutagenic screens. Mutations in *bld* genes prevent the formation of aerial hyphae, either by blocking entry into development (typically mutations in activators) or by inducing precocious sporulation in the vegetative mycelium (typically mutations in repressors) [24–26]. One of the classic *bld* genes, *bldC*, encodes a 68-residue DNA-binding protein related to the DNA-binding domain of MerR-family transcription factors. Recent transcriptional, biochemical and structural analyses have revealed the effect of BldC on global gene expression, how it binds DNA, its wider relationship to previously characterized transcription factors and NAPs, and the diverse modes of DNA binding found among BldC-related proteins. These observations raise further interesting questions about the distinction between NAPs and transcription factors, and the evolution of one from the other.

Diverse functions and modes of DNA binding in the MerR superfamily

Phenotypically, BldC acts to delay entry into development to produce a sustained period of vegetative growth,

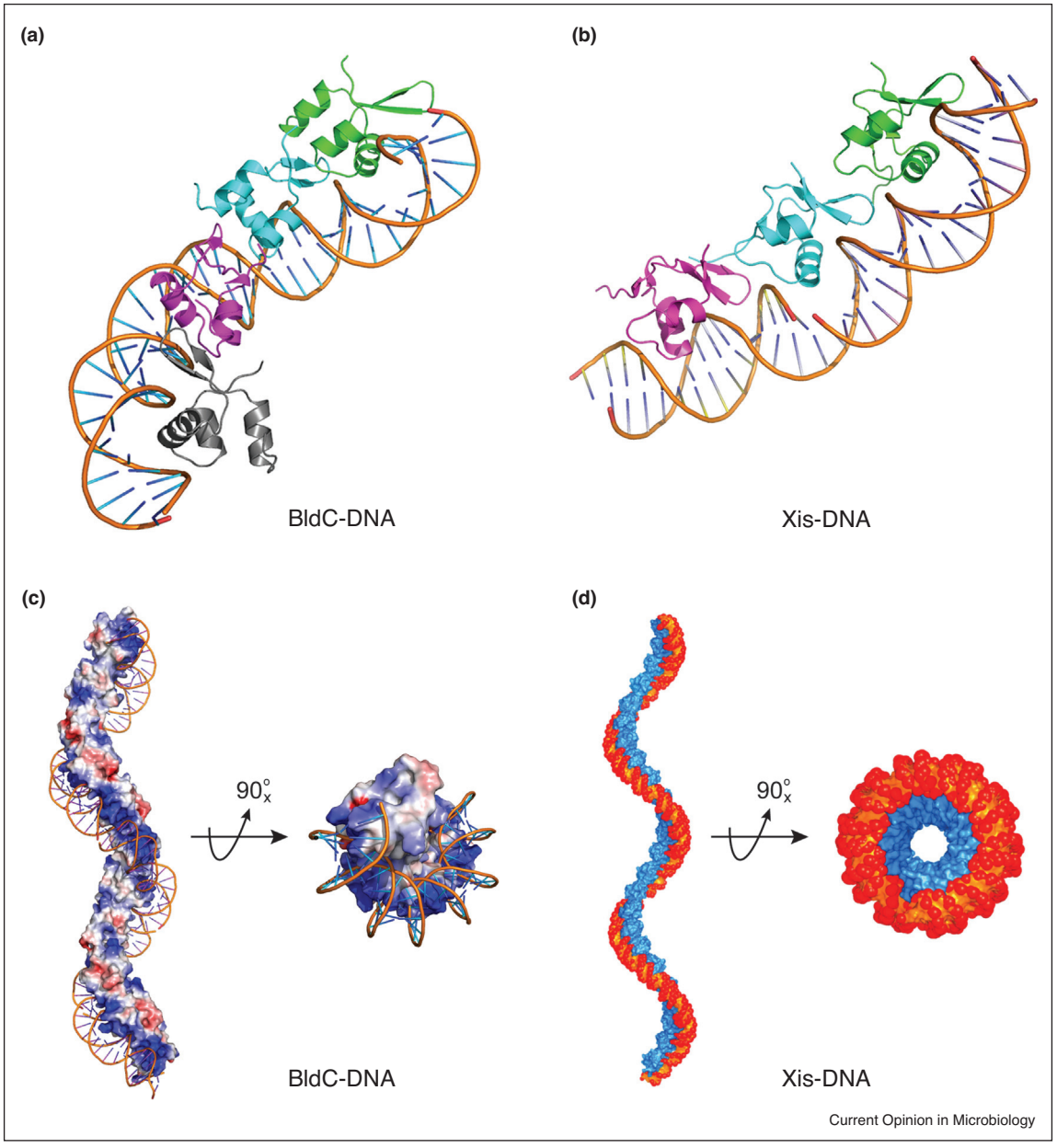
Figure 1



BldC contains a MerR-like DNA-binding domain. Superimposition of the BldC DNA-binding domain (green) [29••] onto that of the MerR-family transcription factor MtaN from *B. subtilis* (red) [30]. The domains can be overlaid with an rmsd = 1.7 Å for 45 corresponding Cα atoms. Note the lack of the dimerization coiled coil in BldC, which is a monomer in solution.

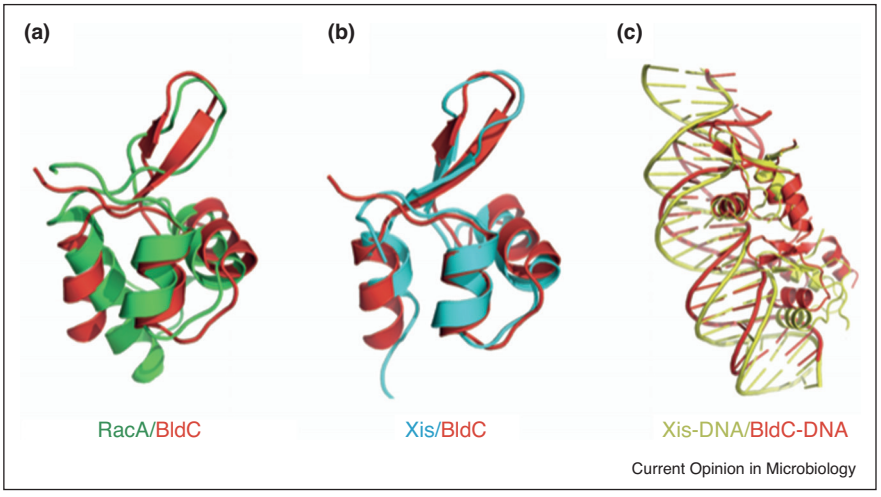
and so *bldC* mutants initiate development prematurely [27••]. BldC binds to hundreds of promoter regions and has a global influence on the transcription profile of the cell, exerting positive effects on the activity of some of its target promoters and negative effects on others [27••]. Bioinformatic analysis suggested [28] and structural studies demonstrated [29••] that BldC is related to the DNA-binding domain of MerR family transcription factors like MtaN (Figure 1) [30], consistent with the transcription factor-like behaviour of BldC. However, classical MerR transcription factors bind to palindromic DNA sequences as homodimers, whereas BldC is a monomer that lacks the effector domain and dimerization helix of typical MerR family proteins, raising interesting questions as to how BldC might bind DNA. Biochemical and structural analyses answered these questions, showing that BldC binds DNA in a completely different way to classical MerR transcription factors, instead involving asymmetric, cooperative, head-to-tail oligomerization of BldC on DNA direct repeats, inducing pronounced DNA distortion (Figure 2a) [29••]. The number of direct repeats present in BldC target promoters is variable, allowing the cooperative, head-to-tail binding of additional BldC monomers [29••]. In this way, BldC binding to DNA results in the formation of a continuous nucleoprotein filament of variable length (Figure 2c). The BldC-DNA structures identified two major elements that define the specificity of BldC binding, a 4-bp AT-rich sequence followed by a C or G four to five nucleotides downstream. The consensus direct repeat is 5′-AATT(N_{3-4})(C/G)-3′, but even this degenerate consensus is not critical for BldC binding. Rather, the AT-rich portion of this direct

Figure 2



BldC and Xis bind to DNA by cooperative, head-to-tail oligomerization on direct repeats, producing a continuous nucleoprotein filament of variable length, accompanied by pronounced DNA distortion.
(a) Head-to-tail oligomerization of 4 BldC monomers on the *smeA* promoter. Each BldC monomer in the nucleoprotein filament forms identical interactions with the next [29••]. **(b)** Head-to-tail oligomerization of Xis on the 3 direct repeats present in its binding site. Xis monomers bound to the X1, X1.5, and X2 sites are coloured dark salmon, green, and blue, respectively [33]. **(c)** Structure-based model of an extended BldC–DNA filament. Crystallization of BldC bound to a double-stranded oligonucleotide carrying 2 of the 4 direct repeats found in the BldC-binding site from the *smeA* promoter generated a pseudo-continuous helix in the structure [29••]. Two views are shown. Left is an electrostatic representation showing electropositive and electronegative regions of BldC in blue and red, respectively. Note the continuous protein superstructure also forms a continuous electropositive stripe that tracks along the DNA. Right is a view looking down the axis of the structure. **(d)** Structure-based model of an extended Xis–DNA filament [33] Copyright (2007) National Academy of Sciences, U.S.A. Units of the Xis–DNA crystal structure were stacked end-to-end to assemble a pseudo-continuous helix with a pitch of ~22 nm. Xis is shown blue and the DNA in orange and red.

Figure 3



Results of structural homology searches with the BldC structure.
(a) Overlay of the BldC DNA-binding domain (red) with the DNA-binding domain from the sporulation-specific *B. subtilis* RacA protein (green) (rmsd = 2.0 Å for 50 similar Cα atoms) [29••]. **(b)** Superimposition of the BldC DNA-binding domain (red) with that of the *Mycobacteriophage pukovnik* Xis protein [60] (rmsd = 1.6 Å for 41 similar Cα atoms). **(c)** Overlay of DNA-bound BldC (red) [29••] and DNA-bound lambda Xis (yellow) [33], showing that BldC and Xis bind DNA in the same head-to-tail manner.

repeat sequence is crucial for binding as BldC recognizes AT-induced narrowing of the minor groove in lieu of direct readout of these base pairs [29••]. Further, BldC binding sites that contain multiple direct repeats can tolerate individual repeats that diverge from the consensus provided they are flanked by consensus repeats [29••]. Such plasticity makes it impossible to predict BldC-binding sites bioinformatically.

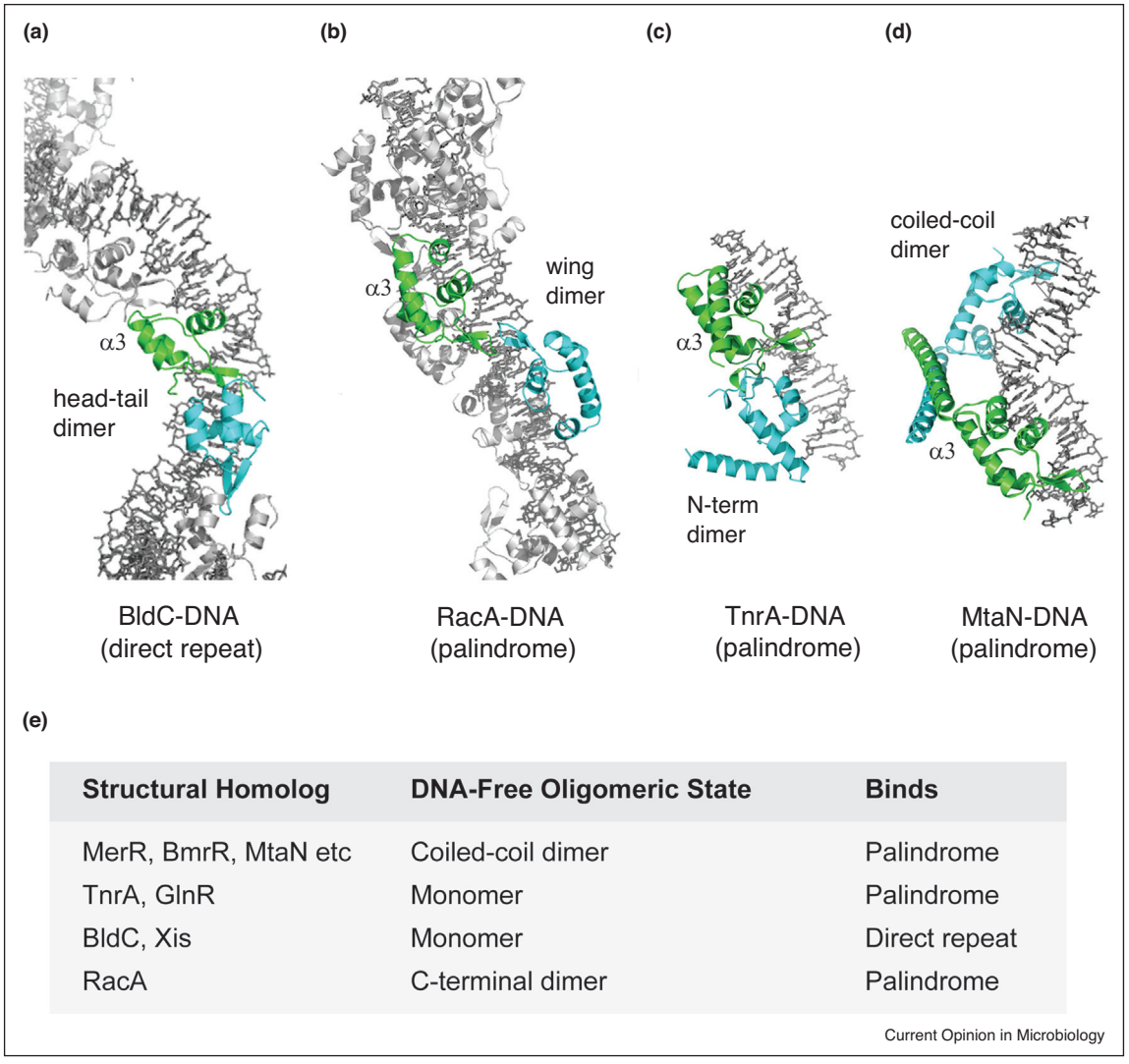
Once the structure of BldC was solved, structural homology searches revealed that, beyond classical MerR transcription factors, BldC has high structural similarity to proteins regarded as NAPs or DNA architectural proteins. These proteins include RacA (Figures 3a and 4 b), which serves to anchor the chromosome to the cell pole in *Bacillus subtilis* [31]. RacA is similar to canonical MerR proteins in having a C-terminal dimerization coiled coil; however, its coiled coil is attached to the N-terminal DNA-binding domain by a long, flexible linker [32]. RacA is not a transcription factor, but like canonical MerR proteins, it too binds palindromic DNA sites as a symmetric dimer [32]. With respect to its mode of DNA binding, the closest relative of BldC is Xis from bacteriophage lambda (Figures 2b, d, 3b and c). Xis is a DNA architectural protein that mediates the formation of a nucleoprotein complex required for the phage-encoded Int recombinase/integrase to catalyse the site-specific recombination event that permits excision of phage lambda from its integration site on the *E. coli* chromosome. BldC (68 residues) consists exclusively of a DNA-binding domain while lambda Xis (72 residues) consists of a winged helix DNA-binding domain and a C-terminal module that recruits the lambda integrase protein. Like BldC, Xis binds to direct repeats in a head-tail fashion to generate a nucleoprotein filament with pronounced DNA distortion (Figures 2b, d and 3c) [33]. In the case of Xis, there are 3 direct repeats in its only binding site on the *E. coli* chromosome (Figure 2b). Thus, in the way that it binds DNA, BldC is more similar to the architectural protein Xis than to classical MerR transcription factors. Nevertheless, BldC has many of the characteristics of a transcription factor, binding almost exclusively to intergenic regions, positively influencing roughly half of its target promoters and negatively influencing the other half [27••].

Structural analyses have revealed yet further modes of DNA binding in the MerR superfamily (Figure 4). The master regulators of nitrogen metabolism in *B. subtilis*, TnrA and GlnR, both have a DNA-binding domain similar to classical MerR proteins, but they lack the dimerization coiled coil and behave as monomers in solution. However, TnrA and GlnR both bind palindromic DNA sequences (Figure 4c and e), dimerizing only upon DNA binding through residues in their N-terminal regions [34]. In summary, different members of the MerR superfamily exhibit a diverse range of oligomeric states, modes of DNA binding, and functions (transcription factors, NAPs, architectural proteins) (Figure 4).

When is a transcription factor a NAP?

The difficulty in answering this question satisfactorily suggests that it may address a false dichotomy. Historically, investigators who were studying processes other than transcription discovered the NAPs [35]. For example, FIS was characterized initially as an architectural protein that promotes DNA inversions through site-specific

Figure 4



(e)

Structural Homolog	DNA-Free Oligomeric State	Binds
MerR, BmrR, MtaN etc	Coiled-coil dimer	Palindrome
TnrA, GlnR	Monomer	Palindrome
BldC, Xis	Monomer	Direct repeat
RacA	C-terminal dimer	Palindrome

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Comparison of the DNA-bound structures and modes of DNA binding among MerR-related proteins.
(a) BldC-DNA [29••]. **(b)** RacA-DNA [32]. **(c)** TnrA-DNA [34]. **(d)** MtaN-DNA [30]. The green subunits are shown in the same orientation to highlight the very different dimers and DNA-binding modes for each protein. Note that in the case of the RacA-DNA structure, the RacA dimerization domain has been omitted so the DNA-binding domain can be seen clearly. **(e)** Summary of the oligomeric states and modes of DNA binding of different MerR-family proteins.

recombination [36,37]. IHF was discovered by investigators of the life cycle of bacteriophage lambda because this protein is essential for the integration into and excision from the *E. coli* chromosome through site-specific recombination at the *att* λ site [38]. Both proteins also play important structural roles in the initiation of chromosome replication [39,40]. These structural observations predisposed the field to consider NAPs from an architectural perspective first, with discoveries of their involvement in transcription control following later. The history of our understanding of the CRP transcription factor follows this path in reverse: its contribution to the control of transcription initiation preceded the observation that its mechanism of action involves DNA remodelling [41]. The same is true of many other transcription factors that are now known to remodel DNA structure through bending (CRP), kinking (BmrR), wrapping (VirB) or bridging (LacI) [41–44].

In this context, it is interesting that BldC is related structurally to Xis [38]. Despite being named 'excisionase', Xis is not an enzyme and it is not a transcription factor: it is a structural element in a site-specific recombination reaction [45] and its function can be partially replaced by the FIS NAP [38]. Does this define Xis as a NAP? Considering its involvement in one recombination system, probably not, but it illustrates the difficulty in assigning a protein with a

NAP-like function (DNA architectural element) to one functional class. Its similarity to BldC is probably a more reliable guide to its biological significance: that of a jobbing DNA-binding domain.

There is an interesting analogy between the relationship of BldC to MerR and the relationship of FIS to NtrC. As noted, FIS is a 98-residue nucleoid-associated protein that is closely related to the DNA-binding domain of the much larger NtrC protein [21,46,47]. Like BldC, FIS prefers binding to A + T-rich DNA and its interaction with DNA is affected by the width of the minor groove [48]. FIS can function in the cell as an architectural protein in the nucleoid, but it can also function as a transcription factor [49,50]. Like BldC, FIS exerts a global influence on the transcription profile of the cell and can have positive or negative effects on the activity of its target promoters [51]. FIS does not bind a ligand and it is not known to be controlled by post-translational modification. Instead, its influence appears simply to reflect FIS protein concentration, which is high in early log phase but low at other growth stages. In the future, it will be interesting to determine if the activity of BldC is controlled post-translationally, or whether BldC function is more akin to that of nucleoid-associated proteins like FIS.

The evolution of one from the other

FIS, HU, IHF, H-NS and other NAPs are important contributors to the life cycles of mobile genetic elements in addition to bacteriophage [35]. These include transposons, conjugative plasmids, horizontally acquired genetic islands and cellular defence systems that limit their spread, such as CRISPR-Cas [52••,53]. Like the boundary between transcription factors and NAPs, the distinctions between many of these genetic elements are also blurred: for example, bacteriophage Mu replicates via transposition [54], bacteriophage P1 replicates as an autonomous plasmid [55], transposons of the Tn7 class harbour CRISPR systems [56]. All these mobile elements possess site-specific recombination systems that depend on NAPs to function. The involvement of NAP-like proteins in the management of gene flow in bacterial populations points to a role for them in guiding the processes that underlie genome evolution. This point is reinforced by the observation that mobile genetic elements often encode their own NAP-like proteins that facilitate the establishment of the newcomers in a novel host background [52••,57]. MerR, the transcriptional regulator that resembles BldC, is encoded by self-transmissible plasmids and by transposons that specify resistance to mercury [58]. As noted, BldC also resembles the chromosome anchoring protein, RacA (Figures 3a and 4b).

Taken together, the proteins that structurally resemble BldC and their functional analogs comprise a collection of DNA-binding molecules involved in important aspects of genome management. Some are very specific, such as the Xis directionality determinant that guides lambda excision but has no known influence on gene expression and is not known to bind a signalling ligand. In contrast, MerR can restructure DNA through altering its twist in response to binding a heavy metal and thus toggle a promoter between its inactive and active conformations. Xis has some NAP-like features: it is expressed to high levels at prophage induction (although its DNA interactions are restricted to lambda) and it is recruited to its functional binding site by FIS [59]. One of the motivations for considering BldC as a potential NAP is its promiscuity as a regulator of transcription in *Streptomyces*. NAPs like FIS, H-NS and IHF control very large regulons while conventional transcription factors are regarded as being more circumspect in their regulatory relationships with the genome. However, the classic transcription factor CRP has been found to control a large regulon yet exhibits a NAP-like degree of promiscuity in its genome-wide DNA-binding pattern [5].

In summary, attempting to place DNA-binding proteins that have the potential to influence transcription into strictly circumscribed categories is likely to be futile. A capacity for flexible reassignment of DNA-binding proteins across regulatory and structural roles has potential selective advantages, perhaps especially in genomes evolving rapidly thanks to mobile DNA elements and horizontal gene transfer. Better to have on hand a set of highly adjustable tools than a toolkit composed of single-purpose items of limited functional flexibility!

Conflict of interest

The authors declare that they have no conflicts of interest.

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This article links, for the first time, the contributions of NAPs, variable DNA topology and bacterial physiology to the operations of CRISPR-Cas systems in bacteria, identifying-specific periods in the growth cycle when these defense systems are optimally active and optimally receptive to accepting novel spacer sequences in their molecular memory arrays.

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