



# Control of transcription by nucleoid proteins

Although not confined to a membrane-bound organelle such as the eukaryotic nucleus, the chromosome(s) of bacterial cells are compacted into a DNA-protein complex termed the nucleoid<sup>1</sup>. Many different proteins appear to be associated with the nucleoid, but we understand the function of just a few of these.

Because of their non-specific DNA binding ability, these proteins have also been termed histone-like<sup>2</sup>. This term is somewhat misleading because there is no amino acid sequence or structural similarity between nucleoid proteins and eukaryotic histones<sup>3</sup>.

The best-characterised nucleoid proteins are all small basic DNA binding proteins that can be present in tens of thousands of copies per cell<sup>3</sup>. Typically, they are active as hetero- or homodimers<sup>3</sup>. All are capable of binding DNA non-specifically, although some show a preference for particular sequences or structures<sup>3</sup>. Most were originally discovered because of their effect on specific recombination reactions in *Escherichia coli* and related bacteria, but it is now clear that, besides their roles in nucleoid compaction, these proteins can affect a variety of DNA-related processes – recombination, replication, DNA repair, and regulation of transcription, the latter being the subject of this review.

The number of copies of each protein

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present is heavily influenced by both growth rate and growth phase<sup>1</sup>. As each of the nucleoid proteins has a distinct effect on DNA architecture, the nucleoid is not a static structure. The level of compaction of the nucleoid, and supercoiling of the DNA, will vary depending on the predominant nucleoid proteins present<sup>1</sup>. From genome sequencing projects, we know that not all the nucleoid proteins characterised in *E. coli* are present in other bacterial species.

## Nucleoid proteins

Some properties of four of the best-studied nucleoid proteins of *E. coli* are summarised in Table 1. Fis increases in abundance during exponential growth, and binds to a poorly conserved core binding motif, bending the DNA 50-90°. This results in extrusion of supercoiled DNA branches<sup>2,3</sup> (Figure 1).

HU is composed of two subunits that show a high degree of amino acid sequence identity<sup>4</sup>. However, not all species have genes encoding two separate subunits. HU displays no sequence specificity but binds to nicks, gaps and junctions in the DNA.

At low concentrations, it forms flexible bends of approximately 70° in the DNA, compacting the nucleoid<sup>4</sup>. Accordingly, HU mutants have partially relaxed chromosomes<sup>4</sup>. At higher concentrations, and in an alternative binding mode, it opens up supercoiled DNA to form rigid helical fragments<sup>4</sup> (Figure 1). In this mode it effectively acts to reverse H-NS condensation (see below).

Again, IHF is composed of two subunits that have a high degree of sequence identity<sup>4</sup>. In contrast to HU, it is only active as a heterodimer. IHF has amino acid sequence, and three dimensional structural, similarity to HU<sup>4</sup>. However, IHF binds preferentially to a specific nucleotide sequence, and bends the DNA through 160°. The effect of IHF binding is to condense the DNA<sup>4</sup> (Figure 1).

The H-NS protein contains an amino-terminal oligomerisation domain, joined to a carboxy-terminal DNA binding domain by a flexible linker<sup>5</sup>. H-NS is not nucleotide sequence specific, but preferentially binds to curved DNA<sup>5</sup>. Over-expression of H-NS leads to compaction of the nucleoid. It can oligomerise to form nucleoprotein complexes. These patches of oligomers can interact with each other to form bridges that eventually lead to the formation of rod-like filaments<sup>5</sup> (Figure 1).

## Supercoiling and transcription

The variation in nucleoid protein

Table 1. Properties of some nucleoid proteins.

Protein	Derivation of name	Active structure	DNA target	Maximal expression
Fis	Factor for inversion stimulation	Homodimer	GNtYaaWWwtTrANC	Exponential phase
HU	Heat unstable	Hetero- or homodimer	Distortions, gaps or junctions in DNA	Exponential phase
IHF	Integration host factor	Heterodimer	WATCAANNNTTTR	Stationary phase
H-NS	Histone-like nucleoid-structuring	Homodimer or oligomer	Curved DNA	Exponential phase



abundance in different growth phases affects the degree of supercoiling, at least in localised regions of the chromosome, as well as nucleoid compaction. This can, in turn, affect the transcription of specific genes. In particular, transcription of many of the genes encoding components of the translation machinery (rRNA, tRNA, ribosomal proteins) are particularly sensitive to changes in supercoiling<sup>2,3</sup>.

Fis is maximally expressed in the early exponential phase of growth, when maximal protein expression is also required. It has been suggested that the branching induced by Fis binding, combined with Fis binding sites located adjacent to the promoters of this set of genes, results in a situation where the degree of supercoiling is optimal for maximal transcription<sup>2</sup>.

In addition to the direct consequences of their DNA binding, the nucleoid proteins can influence nucleoid structure in other ways. As discussed below, nucleoid protein binding can directly affect transcription of specific genes. Amongst the genes that are directly regulated in this fashion are those encoding DNA gyrase and topoisomerase, which in turn affect supercoiling, and therefore transcription<sup>6</sup>.

## Repression

Nucleoid proteins can also act directly to repress transcription of specific genes by several different methods. The most common means of repression is that used by 'classical' repressor proteins, where DNA binding occludes RNA polymerase

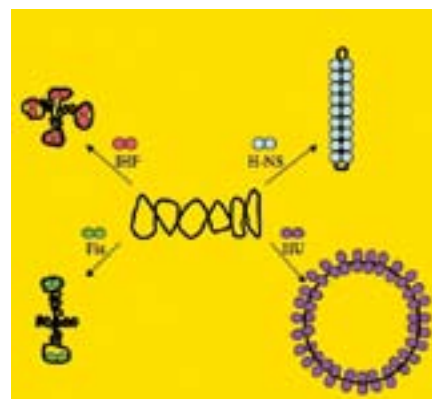


Figure 1. Schematic representation of the effect on nucleoid structure of DNA binding by various nucleoid proteins.

from the promoter. For instance, Fis represses transcription of the *gyrA* gene in this way<sup>7</sup>. Fis also represses transcription of *gyrB*, but here the binding site is upstream of the promoter and does not interfere with the binding of the RNA polymerase<sup>7</sup>. However, Fis binding to this upstream site severely inhibits the initiation of transcription, by a means that is not understood.

Secondly, several examples are known where nucleoid proteins repress transcription by promoting the formation of DNA loops in the promoter region. One example involves the *E. coli galP2* promoter, the GalR repressor protein, and HU<sup>8</sup>. GalR binds as a dimer to sites approximately 50 nt upstream and downstream of the promoter, but an effective repression complex is only formed in the presence of HU. HU binds to a central site and bends the DNA such that the two GalR binding sites are juxtaposed, allowing the formation of tetrameric GalR complex<sup>8</sup> (Figure 2A). In another example, H-NS acts alone to promote DNA loop formation at the *rrnB* promoter<sup>9</sup>. The *rrnB* promoter is flanked by curved DNA, to which H-NS preferentially binds<sup>9</sup>. Interaction between the two patches of H-NS promotes the formation of a DNA loop containing the promoter (Figure 2B). In both cases, the role of the nucleoid protein is architectural in that it promotes loop formation. The DNA loop effectively traps the RNA polymerase, repressing transcription from the relevant promoter.

## Activation

Some nucleoid proteins can act as conventional activators, binding just upstream of the promoter to facilitate promoter-RNA polymerase interaction. For instance, Fis appears to play such a role at the *proP* P2 promoter<sup>10</sup>. Again, binding of these proteins can influence promoter architecture, but in this case in ways that boost the level of transcription. In most cases, the induced DNA bends allow interactions with the carboxy-terminal domain of the alpha subunit of RNA polymerase in a manner that increases initiation of transcription.

For instance, IHF-induced bending of the promoters of several sigma-54 dependent promoters has been shown to promote direct interaction between RNA polymerase and an activator protein bound at an upstream site<sup>11</sup> (Figure 3A). IHF binding to the P<sub>L</sub> promoter of bacteriophage lambda allows the RNA polymerase to bind an upstream UP element, stabilising the RNA polymerase-promoter complex<sup>12</sup> (Figure 3B).

Nucleoid proteins can also activate transcription by an entirely different method. For instance, IHF binds to a site upstream of the *E. coli ilvP<sub>G</sub>* promoter<sup>13</sup> (Figure 3C). The binding site overlaps an AT-rich region where strand separation has been driven by superhelical stress<sup>13</sup>. IHF binding stabilises this region, but the increase in superhelical tension is driven downstream to the -10 box of the promoter, greatly assisting open complex formation<sup>13</sup>.

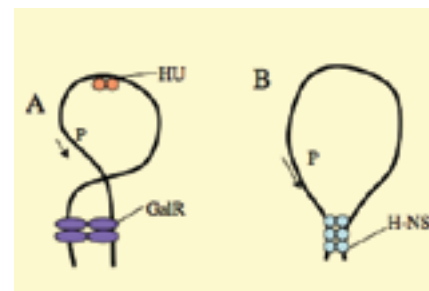


Figure 2. Repression of transcription by DNA loop formation involving a promoter.

A. HU binds and bends the *E. coli galP2* promoter region such that two binding sites for the regulatory protein GalR are brought together. The formation of a tetrameric GalR complex stabilises the DNA loop.

B. Interaction between two patches of H-NS, preferentially bound to two regions of curved DNA upstream and downstream of the *rrnB* promoter of *E. coli*. In both cases DNA loop formation traps the RNA polymerase at the promoter (P).

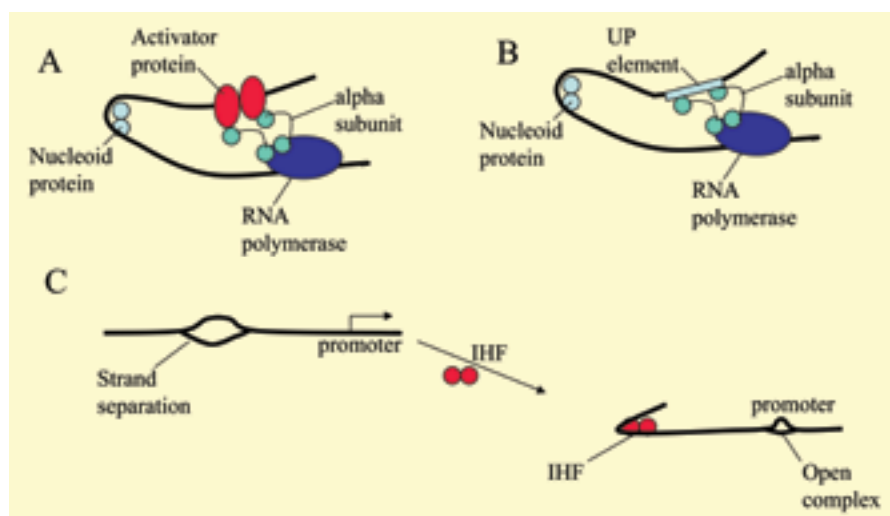
### MICRO-FACT

The largest known bacterium, *Thiomargarita namibiensis*, is the size of the full stop at the end of this sentence.



## Combinatorial control of transcription

The examples used above have described the action of a specific nucleoid protein at a specific promoter. In many, if not most, promoters of several nucleoid proteins can interact. To give just one example, the *rrnB* promoter is subject to regulation by both H-NS and Fis<sup>5</sup>. An additional layer of complexity is added by the fact that the gene(s) encoding a specific nucleoid protein can be regulated by another protein. For example, Fis activates the genes encoding H-NS and IHF, but represses those encoding HU<sup>6</sup>.



**Figure 3.** Activation of transcription by nucleoid proteins. DNA bending induced by a nucleoid protein allows interactions between the carboxy-terminal domain of the alpha subunit of RNA polymerase and either: A. activator proteins bound to an upstream activator sequence or B. an UP sequence. C. IHF binding to a region upstream of the promoter that is susceptible to strand separation, driven by helical stress. IHF stabilises the upstream region, but helical stress then assists the formation of an open complex at the promoter (P).

## Conclusion

Besides their various other functions, the nucleoid proteins have been used by the bacterial cell to regulate the expression of sets of genes in a manner that allows them to respond to changes in the growth rate and growth phase.

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