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TRANSCRIPTION FACTOR AS A TOPOLOGICAL HOMEOSTAT

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1. ABSTRACT

Abundant prokaryotic chromatin architectural proteins often function also as global transcriptional regulators. In addition, some of this class of proteins modulate the activity of cellular topoisomerases and hence, the superhelical density of DNA. The relationships between the global effect of these proteins on DNA topology and their local effects exerted on particular promoter regions remain largely unexplored. One of the best-characterised examples of this class of proteins is the pleiotropic regulator of metabolism FIS, which reduces the activity of DNA gyrase and counteracts the increase of the overall superhelicity of DNA during early exponential growth phase. Binding of FIS to supercoiled DNA molecules *in vitro* leads to the formation of branched structures and consequent multiplication of apical loops, whereas on bending the upstream regions of stable RNA promoters FIS acts as a topological homeostat maintaining high local levels of supercoiling required for promoter activity. We argue that the coordinated effects of FIS on the global and local DNA architecture optimise gene expression by channelling the free energy of negative supercoiling to specific, biologically relevant sites.

2. INTRODUCTION

In the enterobacterium *Escherichia coli* certain global regulators of gene transcription both function as chromatin architectural factors and also affect the topology of DNA by modulating the activity of cellular topoisomerases (1 - 8). This means that in general these abundant DNA binding proteins may influence the architecture of chromatin in two ways: by directly constraining DNA and by modulating DNA superhelical density. Under laboratory conditions, both the relative abundance of the chromatin architectural factors and the superhelical density of DNA change in an orderly manner with growth phase (9 - 15). Since the pattern of cellular transcription also changes with growth phase (16), this suggests a coupling between the effects of global transcriptional regulators on DNA topology and their local effects on DNA geometry of cellular promoters.

To a first approximation the effect of a global transcriptional regulator could be considered as an average of multiple local effects elicited directly from cognate binding site(s) in the vicinity of the promoters affected. However, many of the *Escherichia coli* promoters which are regulated by growth phase, including the very strong stable RNA (tRNA and rRNA) promoters and other promoters of the "stringent control" regulon (8), are highly sensitive to the superhelical density of DNA (17 - 24). Whilst supercoiling can selectively increase the strength of certain promoters but not of others, the promoter strength itself can modulate the efficiency of action of a transcription factor. In this way promoters can be fine-tuned to respond to a number of different regulatory variables depending on the precise physiological requirements for the expressed gene (25, 26). More specifically, the activity of a particular promoter will ultimately be approximated by the global effect of the transcription factor on DNA topology and its local *cis*-effect on the promoter elicited from cognate binding site(s). Notably, these two effects do not necessarily reinforce each other and could affect the promoter activity in opposite ways, or may compensate for each other, resulting in a homeostatic mode of regulation (22 - 24). It is apparent

therefore, that consideration of the directional effects of these two variables (i.e. DNA topology and *cis*-acting sites) on formation of transcription complexes is pivotal for the understanding of the mechanisms coordinating the activity of cellular promoters with growth conditions.

In this review we focus on the effects of the chromatin architectural protein FIS, which appears to optimise gene expression by coordinated changes of global DNA topology and local DNA geometry of the promoter regions.

3. EFFECTS OF FIS BINDING ON DNA STRUCTURE

3.1. Global effect of FIS binding to supercoiled DNA

FIS is the predominant chromatin architectural factor (about 50,000 molecules per cell), which reorganises cellular metabolism in order to facilitate the entry into the exponential growth phase (27, 28). During stationary phase the concentration of FIS drops to very low levels (29, 30). Like other abundant chromatin architectural factors, FIS binds promiscuously to DNA due to its ability to recognize local DNA geometry rather than primary sequence (31, 32). *In vitro* FIS binds to DNA sites with affinities differing by about three orders of magnitude (31). This large difference in affinity is correlated with induced DNA bend angles ranging from 50° to 90° (31, 33). In supercoiled plasmid DNA *in vitro* FIS binds selectively to distinct regions apparently containing clustered low affinity sites (34). This selectivity of binding is thought to reflect intrinsic differences in the bendability of particular DNA sequences (35). On binding to supercoiled DNA *in vitro* FIS induces DNA branching with resultant multiplication of apical loops (34). Although nothing is yet known about the chromatin structures stabilised by FIS, the protein appears to bind at confined chromosomal loci *in vivo*, consistent with predicted clustering of strong FIS binding sites on the chromosome of *E. coli* (36, 37). The branching of DNA on binding of FIS is presumably due to stabilisation of multiple bends, which in turn affect the reshaping dynamics of the supercoiled DNA molecule. In general the DNA of the core promoter region is bendable (38, 39), while the upstream regions of strong promoters are often curved (40, 41). The latter would be expected to be preferentially located at thermodynamically favourable locations at the apices of the loops in branched DNA molecules (41 - 43). In these locations the promoters would be accessible to transcriptional machinery, consistent with the role of FIS as a transcriptional activator. Notably, another abundant chromatin architectural factor H-NS implicated mainly in gene silencing, reduces the accessibility of DNA by bridging two duplexes and packaging the supercoiled DNA molecule into long filaments *in vitro* (34, 44).

3.2. Local effect of FIS binding to promoter upstream regions

The upstream activating sequences (UAS) of stable RNA promoters, which are specifically activated by FIS, contain several FIS binding sites often arranged in helical register (45 - 50). This arrangement of binding sites on the same face of the helix is consistent with stabilisation of bent “DNA microloops” involving the entire UAS region, as inferred from biochemical studies on the *tyrT* and *rrnA* P1 promoters (24, 48, 49, 51). Our preliminary results obtained by circular permutation and electron microscopy studies also suggest that binding of FIS at the UAS of the *tyrT* promoter constrains tightly bent DNA structures (Rudolf Lurz, A.T. and G.M., unpublished data). Since apical loops in supercoiled DNA are shown to strongly attract RNA polymerase (RNAP), it is apparent that the stabilisation of DNA microloops by FIS could direct the polymerase to the promoter (52). Thus, there seems to be coherence in the global indirect effect (multiplication of apical loops consequent on DNA branching) and the local direct effect (stabilisation of DNA microloops in upstream regions of certain promoters) of FIS binding, both of which would facilitate the interaction of promoters with transcriptional machinery. However, the stability of the DNA microloops constrained on direct binding of FIS to phased sites in UAS may differ from that of apical loops induced indirectly, as a result of FIS-dependent branching of DNA (34). The stability of these latter will strongly depend on flexural and torsional features of DNA, which vary with superhelical density (53). By contrast, the DNA microloops stabilised by binding of FIS to high affinity sites in UAS are less dependent on the DNA topology, perhaps because binding of FIS at the phased high affinity sites in UAS can be highly cooperative, allowing their occupation over a range of FIS concentrations (54). Taken together, these observations suggest an experimentally testable simple mechanism of coordinated changes of cellular promoter activities, governed by growth phase-dependent differences in the stabilities of distinct apical loops.

4. REGULATION OF PROMOTER ACTIVITY BY FIS

The DNA microloops stabilised by direct binding of FIS at the phased sites in the UAS are thought to represent topologically isolated domains capable of storing torsion (51, 55). Therefore in the presence of FIS the activity of stable RNA promoters would be expected to be less sensitive to the fluctuations in the superhelical density of DNA. This is indeed the case. In cells lacking *fis* both induced relaxation and hyper-negative supercoiling of DNA strongly reduce the activity of the *rrnA* P1 promoter, whereas in wild-type cells no such effect is observed (24). Furthermore, deletion of the distal FIS binding sites in the UAS abolishes this protecting effect in wild-type cells, indicating that binding of FIS at multiple phased sites is required for the rescue of promoter activity at both the sub- and supra- optimal superhelical densities (24, unpublished data). FIS and the superhelical density activate the *tyrT* promoter reciprocally *in vitro*. The activating potential of FIS is low at optimum superhelical density but increases on deviations from this optimum level (unpublished data). Similarly, the activating potential of FIS at the *rrnA* P1 promoter is higher at suboptimal superhelical densities *in vitro* (24). These data are consistent with an essentially homeostatic regulation mechanism preserving high levels of stable RNA transcription under a variety of conditions. It also explains why there is only a small difference in the

activities of *rrnA* P1, *rrnB* P1 and *tyrT* promoters between wild-type and *fis* cells (24, 48, 56). In *fis* cells the overall negative superhelical density increases due to increased gyrase activity and could thus compensate for the absence of a *cis*-acting factor FIS (24, 57).

4.1. Mechanistic relationship between the effects of FIS and supercoiling

How are the reciprocal effects of FIS and superhelicity mechanistically related? The coherently bent DNA microloop stabilised on binding of FIS traps the polymerase at the promoter (48, 49, 58). Indeed, the disruption of the helical arrangement of FIS binding sites in *tyrT* promoter causes a ~10-fold reduction of RNAP affinity in the absence of FIS and also, impairs the activation (49). On this latter promoter RNAP itself forms a far upstream contact with UAS DNA, consistent with the bending of DNA towards polymerase, whereby DNA supercoiling increases the rate of interaction with this far upstream site by about two orders of magnitude (54). These data suggest that the UAS, which itself is anisotropically flexible (59, 60) wraps around polymerase, whereas the binding of activator FIS at phased sites stabilises this wrapped UAS microloop. In the absence of FIS increased supercoiling would facilitate wrapping and also affect the stability of the microloop. Thus, the effects of FIS and supercoiling can be understood on a common mechanistic basis.

4.2. Role of the DNA microloop

Transcription initiation is conventionally divided in several steps and structurally distinct complexes pertinent to each of these steps have been described (61, 62). Potentially, each of the postulated steps could be subject to regulation and corresponding "snapshots" of interactions between RNAP and the transcriptional regulator within ternary complexes have been reported (63). However, what we still need to understand is the *dynamic* mechanism of transcription activation, involving coordinated conformational rearrangements of interacting molecules, which ultimately channel the reaction towards productive initiation.

FIS has been shown to activate sequential steps in transcription initiation *in vitro* (49). Previous studies indicated that transcription initiation is associated with changes of promoter DNA curvature and constraint of writhe, which precedes DNA untwisting and open complex formation (64 - 66). Indeed, recent crystallographic data indicate a significant change in the trajectory of the DNA on transition from closed to open complex, resulting in an increased wrapping of the promoter DNA around the polymerase (67, 68). In addition, in the intermediate complex the alignment of the sigma^{70} holoenzyme –10 and –35 promoter recognition elements can induce a local negative DNA twist in the spacer region separating the –10 and –35 recognition elements (69). Thus local changes of both DNA twist and writhe are associated with the initiation complex formation. These changes are paralleled by alterations in protein conformation (68, 70).

DNA bending transcriptional activators usually adopt a specific rotational orientation with respect to polymerase in order to make energetically favourable protein-protein interactions with the surface of RNAP (63, 71). Yet, much evidence suggests a distortion of the DNA in the region between the bound activator and polymerase indicative of accumulation of torsion (54, 58, 72, 73). Notably, the introduction of a nick or gaps in this region respectively impair or abolish activation (74), suggesting that the torsion stored in this DNA microdomain is important for activation. Similarly, at the *malT* promoter the DNA geometry of the upstream region is crucial for activation by CRP (75).

DNA wrapping around polymerase appears to be a feature common to transcription initiation complexes (51, 76). In *tyrT* UAS DNA the upstream contact by RNAP occurs around positions –122 and –129 within FIS binding site III (54) indicative of extensive wrapping, which exceeds the length of DNA wrapped by *lac* and *malT* promoters, or in the open complex of the phage lambda P_R promoter as observed by AFM (67, 72, 77). Is there any rationale for a more extensive wrapping of DNA in the case of the *tyrT* promoter? Generally, the extent of available change of twist is proportional to the extent of stored writhe. DNA supercoiling not only stabilises the DNA microloop in *tyrT* UAS as indicated by the remarkably increased rate of the formation of the upstream contact by RNAP, but also helps to override the block imposed by the GC-rich discriminator sequence located between the –10 element and the transcription startpoint (21, 78). Thus the torsion stored as writhe in the DNA microloop might be required to untwist efficiently the GC-rich discriminator region of stable RNA promoters.

5. MECHANISM OF TRANSCRIPTIONAL ACTIVATION BY FIS

FIS has been shown to affect the rate of polymerase-promoter complex formation (49). Rapid photo-footprinting techniques revealed that in the presence of FIS the rate of appearance of the RNAP contact in the –35 region is increasing by two-threefold (54). Furthermore, coordinated changes in the pattern of interaction of polymerase and FIS with the *tyrT* promoter DNA during initiation have been observed. In the presence of polymerase the sequential order of binding of FIS at the three phased sites in *tyrT* UAS is altered, such that the promoter-proximal FIS binding site I is occupied first (48, 54, 58). FIS bound at site I can contact the C-terminal domain of one of the two alpha? subunits of RNAP, consistent with the reported cooperativity of binding of RNAP and FIS at site I (54, 58, 79). In the ternary complex, the upstream contact of RNAP with DNA within FIS site III is apparently replaced by a direct protein-protein contact between bound FIS dimer and polymerase. This accelerated binding of polymerase is accompanied by initial vacation and subsequent reoccupation of FIS binding site II. Notably, such a transient vacation-reoccupation of a binding site has been invoked for another chromatin architectural protein, IHF, to explain the directionality of the Tn10 transposition reaction by a mechanism involving the rotation of a supercoiled DNA loop (80). The data obtained on the *tyrT* promoter are strongly suggestive of

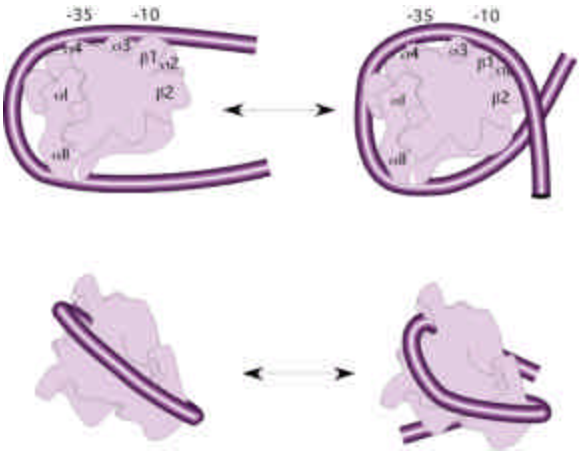

Figure 1. Rotation of the UAS microloop during transition from the flat, untwisted to writhed configuration. In the upper panel the location of RNAP subunits and of the –10 and–35 elements interacting respectively with σ2 and σ4, are approximated from the structure of Murakami et al. (68). DNA is indicated as a grey flexible rod. Lower panel shows the backside of polymerase to illustrate the transition in the geometry of the microloop. Only the N-terminal domains (NTD) of the α2subunits (αI and αII) are indicated. FIS is omitted from the drawing for clarity. For details see the text.

changes in the composition of the nucleoprotein complex with time (49, 54).

Thus, it appears that the ternary initiation complex containing FIS, RNAP and the *tyrT* promoter DNA is structurally dynamic and is characterised by extensive constraint of DNA. Both these features are consistent with the "torsional transmission" model proposed to explain the sequential effect of FIS during transcriptional activation of the *tyrT* promoter (49, 51, 55). According to this hypothesis, the primary function of FIS is to facilitate the formation of DNA microloops and to maintain their integrity throughout the course of the initiation reaction thereby acting sequentially on potential rate-limiting steps. The constraint of DNA around the polymerase-activator complex (comparable in extent to DNA wrapped by a nucleosome particle) would allow the accommodated torsion to be stored within a topologically isolated domain, delimited by protein contacts with the entry and exit sites of the constrained DNA microloop. Yet, the UAS microloop is large enough to allow repartitioning of writhe and twist and thus impart flexibility on the complex. These topological transitions in the microloop could be reinforced by the changes in the occupation of protein binding sites or contact configurations of proteins interacting within the nucleoprotein complex. The torsion in the microloop can be accumulated by rotation of the polymerase during the alignment of the –10 and –35 elements of the promoter. In this configuration the microloop would be more untwisted (Figure 1). However, this motion of the loop would also affect the interactions between FIS dimers, which are dynamic, as evidenced by the observed release from a specific binding site and then rebinding of FIS in the complex. Rebinding of FIS would than favour the backward rotation of the microloop to adopt a more "writhed" configuration. By this motion the accumulated torsion could be transmitted to the polymerase through protein-protein and/or protein-DNA contacts to facilitate local structural alterations of DNA required for promoter opening and initiation. For example, one possible mechanism by which the constrained upstream torsion could be coupled to promoter opening would be to facilitate any realignment of the –35 and –10 reading heads in the sigma subunit during this process. Thus, the flexible DNA microloops stabilized by FIS in the vicinity of certain supercoiling-dependent promoters function as local topological homeostats compensating for both sub- and supra- optimal levels of superhelicity (24).

6. FIS AS A TOPOLOGICAL OPTIMISER

Promoter regions are generally characterised by high deformability, being susceptible to duplex destabilisation under conditions of superhelical stress (41, 81). The cellular promoters can be thus understood as devices channelling the free energy of negative supercoiling to localised, biologically relevant sites in DNA. Several studies using different promoters and promoter derivatives revealed that there is a distinct, yet characteristic, coupling between the superhelical density of DNA and the activity of a particular promoter (22 - 25, 82). A change of superhelical density could thus differentially affect the efficiency of channelling at distinct promoters, allowing coordinated change of activities to occur.

In rich medium the increased ATP/ADP ratio activates DNA gyrase, which then converts the energy equivalents into negative supercoiling, consistent with the rapid increase of the level of superhelicity observed on nutritional shift-up (10, 83 - 85). At the stable RNA and related promoters increased supercoiling could facilitate the formation of DNA microloops to effect torsional transmission, whereas at the others (e.g. the *gyrA/B* type promoters) the same increase would reduce promoter activity (82, 87). Notably, the *fis* promoter activity requires high levels of negative supercoiling and the production of FIS is correlated with the richness of the medium (22, 86). FIS is reducing gyrase activity, probably to counteract excessive and potentially deleterious supercoiling (22), but the same effect will reduce the activity of promoters which require high superhelical density. However, the gene promoters required at that stage, as exemplified by stable RNA promoters here, would be then selectively activated by stabilisation of DNA microloops and FIS-dependent channelling of the torsional energy of DNA towards initiation. Thus the shallow reorganisation of gene expression by changes of negative supercoiling could be further optimised by FIS-dependent coordination of global and local conformational transitions in DNA, favouring selective utilisation of physiologically relevant cellular promoters.

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