

RNA polymerase and an activator form discrete subcomplexes in a transcription initiation complex

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Using high-resolution atomic force microscopy (AFM) we show that in a ternary complex of an activator protein, FIS, and RNA polymerase containing the σ^{70} specificity factor at the *Escherichia coli* *tyrT* promoter the polymerase and the activator form discrete, but connected, subcomplexes in close proximity. This is the first time that a ternary complex between an activator, a σ^{70} polymerase holoenzyme and promoter DNA has been visualised. Individually FIS and RNA polymerase wrap ~80 and 150 bp of promoter DNA, respectively. We suggest that the architecture of the ternary complex provides a general paradigm for the facilitation of direct, but weak, interactions between polymerase and an activator.

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Introduction

DNA–protein interactions are central to the regulation of the transcription of genetic information from DNA to RNA. The process of transcription initiation requires the binding of RNA polymerase to well-defined DNA promoter regions. This binding is in many cases facilitated by activator proteins that bind within the promoter regulatory region (Browning and Busby, 2004). Yet the general spatial organisation of single ternary complexes of RNA polymerase and activator proteins at a DNA promoter region is unknown.

The *Escherichia coli* *tyrT* promoter is typical of the highly active stable RNA promoters in this organism and contains three binding sites for the activating DNA-binding protein FIS upstream of the core promoter (Muskhelishvili *et al.*, 1997). The FIS sites are in helical phase and have been proposed to facilitate the wrapping of upstream DNA around RNA polymerase (Muskhelishvili *et al.*, 1997). In this paper we have visualised the wrapping of promoter DNA by FIS and RNA polymerase both individually and together in the ternary complex.

FIS wraps the *tyrT* upstream activating sequence (UAS)

We used atomic force microscopy (AFM) to visualise single protein–DNA complexes immobilised on mica. Imaging was

performed in air in tapping mode (see Materials and methods). For each type of complex several hundred images were recorded with different AFM cantilevers to obtain statistically valid information on the shape and structure of the different complexes.

The association of three FIS dimers with the three FIS binding sites in the *tyrT* UAS upstream of the core promoter (Lamond and Travers, 1983) is a highly cooperative event with a Hill coefficient of ~3 on linear DNA (Pemberton *et al.*, 2002), a value that is indicative of the formation of a highly organised complex. On visualisation we observed that FIS formed a compact complex at the UAS and that its binding reduced the contour length of the DNA by 29 nm, corresponding to ~87 bp, a value very similar to the length of the UAS (Figures 1A and D, 2, and 3A and B) and to the theoretically predicted value of 81 bp (Herman, 1996). We conclude that the FIS complex wraps DNA. To estimate more precisely the position of the FIS binding site, we determined the average lengths of the unbound DNA to the left and right arms of the complex (Figure 2A and B). These measurements in principle define two possible positions, one including the whole UAS region and a second downstream of the core promoter. However, since one boundary of the former position coincides with one of the ternary complex boundaries (see below), we conclude that FIS forms a compact complex at the UAS. Compared with RNA polymerase the approximate size of this complex is consistent with a content of at least three FIS dimers.

RNA polymerase wraps ~150 bp of *tyrT* promoter DNA

Previous studies have demonstrated that RNA polymerase containing the σ^{70} specificity factor interacts directly with the UAS of the *tyrT* promoter, both protecting at least 130 bp upstream of the transcription start site from DNase I cleavage (Travers *et al.*, 1983) and photosensitising specific bases between positions –120 and –130 (Pemberton *et al.*, 2002). Although the untwisting of DNA at the startpoint of the *tyrT* promoter and consequent formation of salt-stable complexes requires the initiating triphosphates the binding of the upstream region is independent of the presence of these nucleotides (Debenham, 1979; Travers *et al.*, 1983; Auner *et al.*, 2003). To visualise the enzyme bound at the *tyrT* promoter by AFM, we bound polymerase to the *tyrT*2 DNA fragment (Figure 1C) in the absence of nucleoside triphosphates at low temperatures. The appearance of these complexes was variable (Figures 3B, D, F and 4A). The complexes formed under these conditions would represent closed, or possibly nucleated, but not open, transcription initiation complexes. The polymerase–promoter complex reduced the contour length of DNA by 52 nm corresponding to ~150–160 bp (Figure 1D), a value that is similar to the complete *tyrT* promoter including the UAS region and is in good agreement with previous footprinting studies (Travers *et al.*, 1983;

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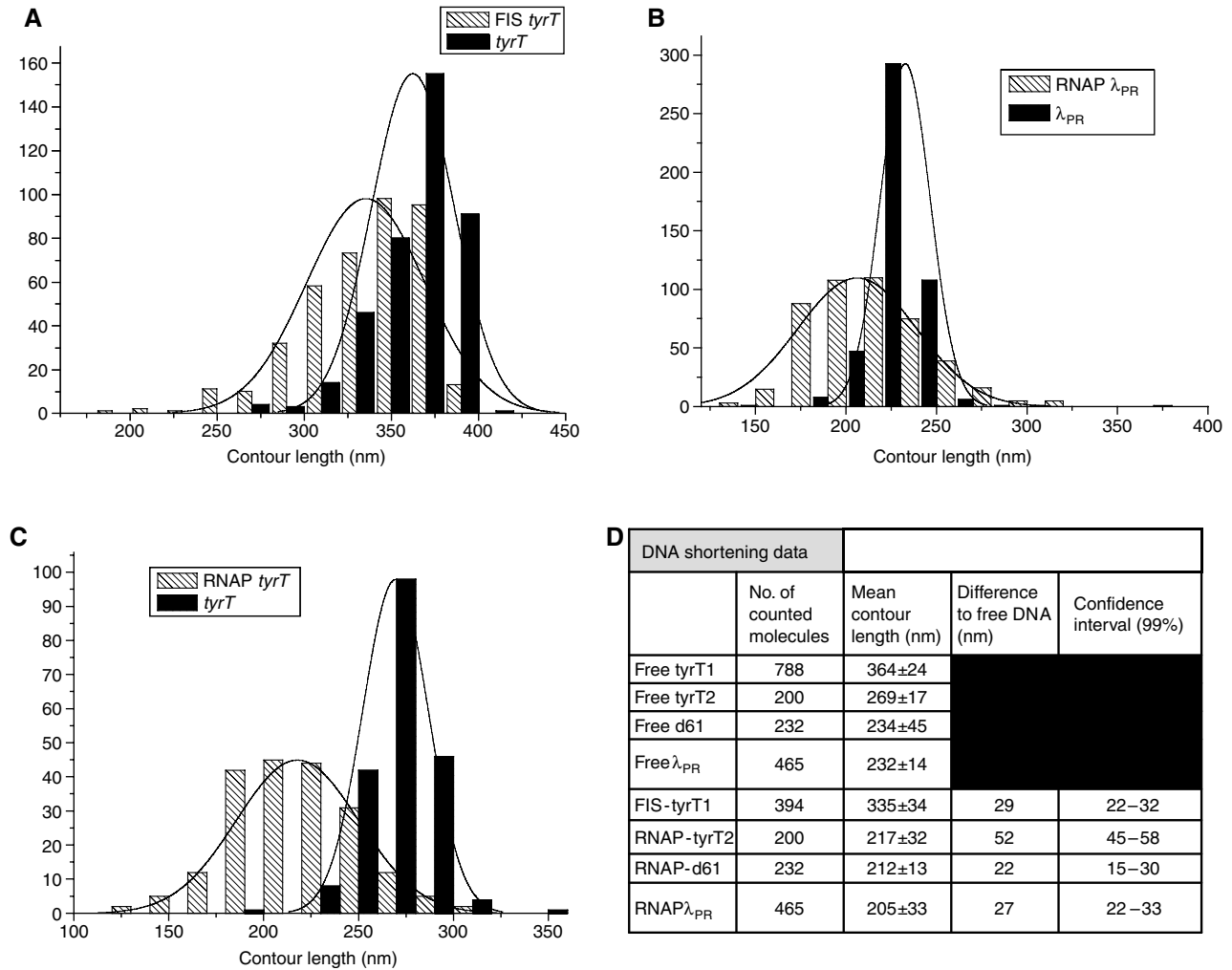


Figure 1 Histograms of the DNA contour length distributions in nucleoprotein complexes. (A) DNA contour length distributions of the FIS-*tyrT* promoter DNA complexes using the 1167 bp *tyrT1* template. (B) DNA contour length distributions of the RNAP-λ_{PR} DNA complexes using the 735 bp λ_{PR} promoter fragment. (C) DNA contour length distributions of the RNAP-*tyrT* promoter DNA complexes using the 819 bp *tyrT2* template. The number of analysed complexes is indicated on the ordinate. The black bars—free DNA, grey bars—bound DNA. (D) Summary of the DNA contour length distributions and DNA shortening data. RNAP-d61 indicates the contour length distributions for complexes of RNAP with the 750 bp *tyrT* construct comprising the core promoter but with internally deleted UAS region. The histograms were generated by *Originlab* Pro 7.5 software. The curves in (A)–(C) represent the Gaussian fitting over the distribution of classes. The *t*-test assuming a Gaussian distribution confirmed that in all cases the shift in the contour lengths is highly significant.

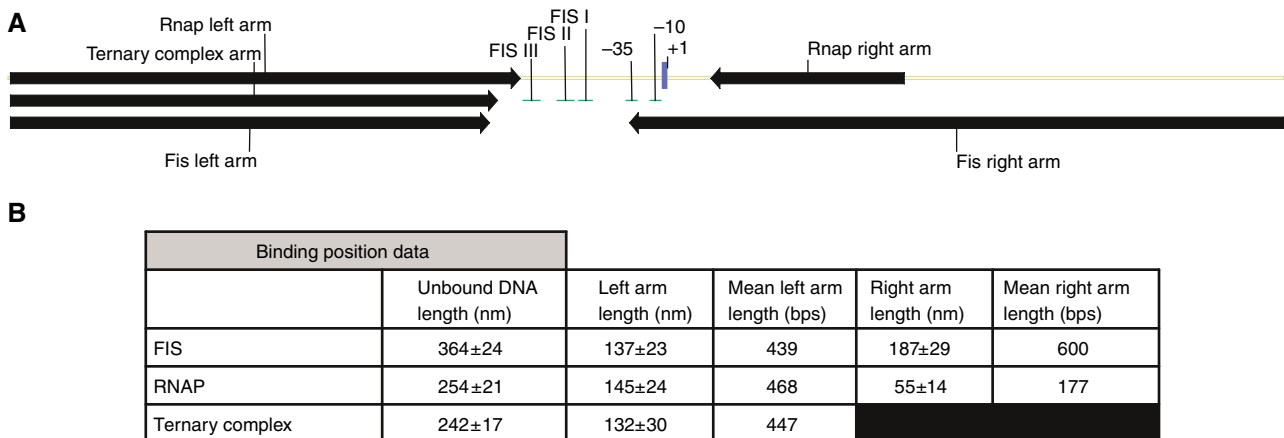


Figure 2 Determination of the binding positions for FIS, RNAP and the ternary complex on the *tyrT* promoter DNA. (A) Spatial localisation map. The left (promoter upstream) and right (promoter downstream) arms are indicated for each the FIS and the RNAP complexes. For the ternary FIS-RNAP-promoter DNA complex, for determination of binding position only the long left arm was used. The spatial organization of the initiation start point, the -10 and -35 hexamers and the three FIS binding sites is indicated. Promoter fragments of different lengths were used for right arm measurements for FIS (*tyrT1*) and RNAP (*tyrT2*). (B) Summary of the binding position determination data.

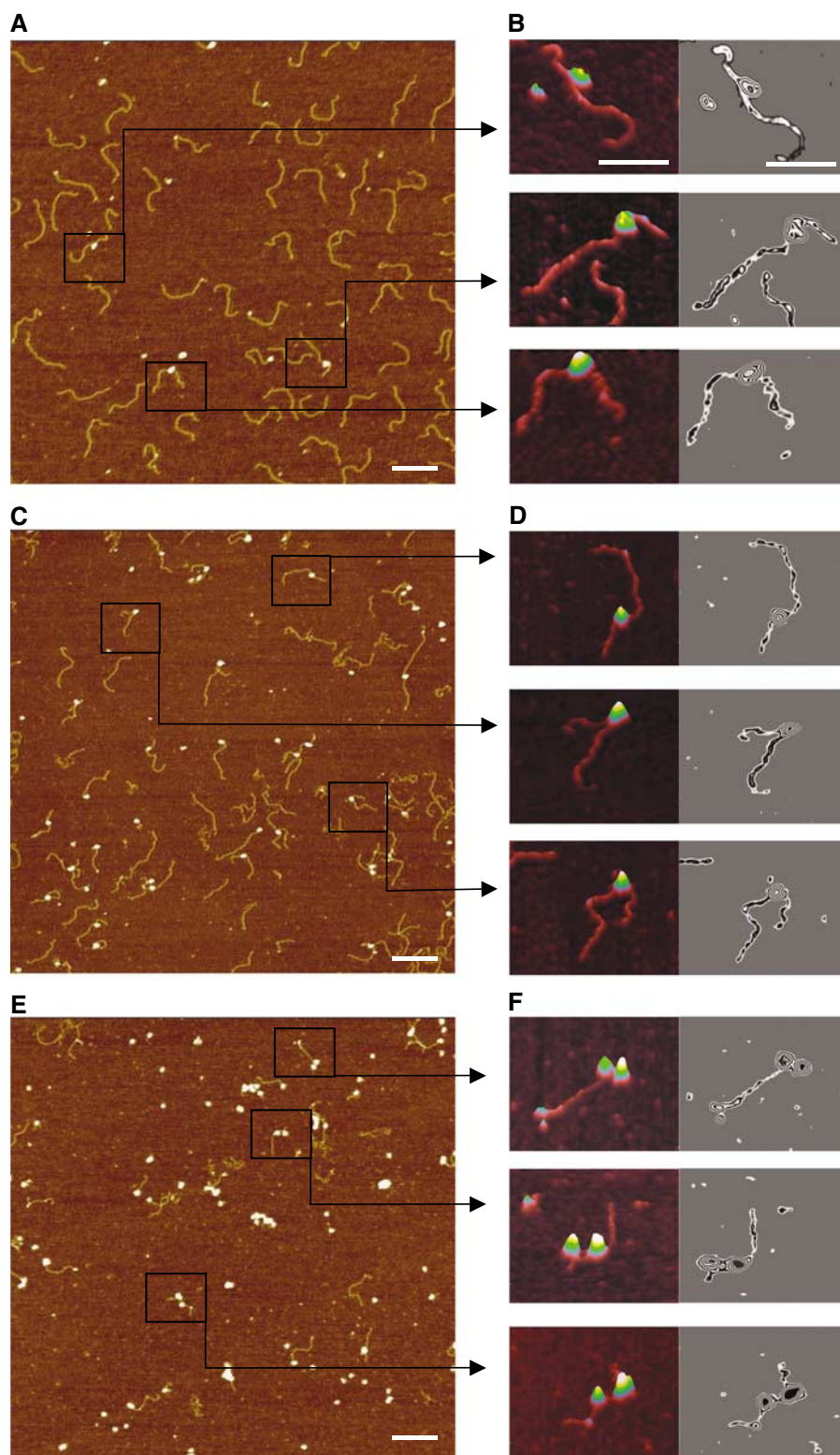


Figure 3 AFM images of the nucleoprotein structures formed by FIS, RNAP and the ternary polymerase-FIS-promoter complexes. The respective $2 \times 2 \mu\text{m}$ overviews (A, C, E—the scale bar is 200 nm) and $240 \times 240 \text{ nm}$ magnified images (B, D, F—the scale bar is 100 nm) in an angled view (left panel) and top view (grayscale, right panel) are shown. Different tips were used.

Pemberton *et al*, 2002) but is considerably larger than values observed at other promoters (Rivetti *et al*, 1999; Shin *et al*, 2005). Measurements of the unbound DNA arms of the polymerase-promoter complex indicated that RNA polymerase binding site included the core promoter and the upstream

region in the vicinity of FIS binding site III (Figure 2A and B). Under our conditions, we could accurately reproduce the reduction in contour length consequent on binding polymerase to the λP_R as recently determined (Rivetti *et al*, 1999; Figure 1B). In addition, we measured the contour

lengths of complexes formed by RNAP with a *tyrT* construct in which the sequence upstream of position -61, that is including the UAS, is deleted. We observed that for this

construct the reduction in contour length was only 22 nm corresponding to ~66 bp (see RNAP-d61 in Figure 1D). This value corresponds well to the length of the core promoter

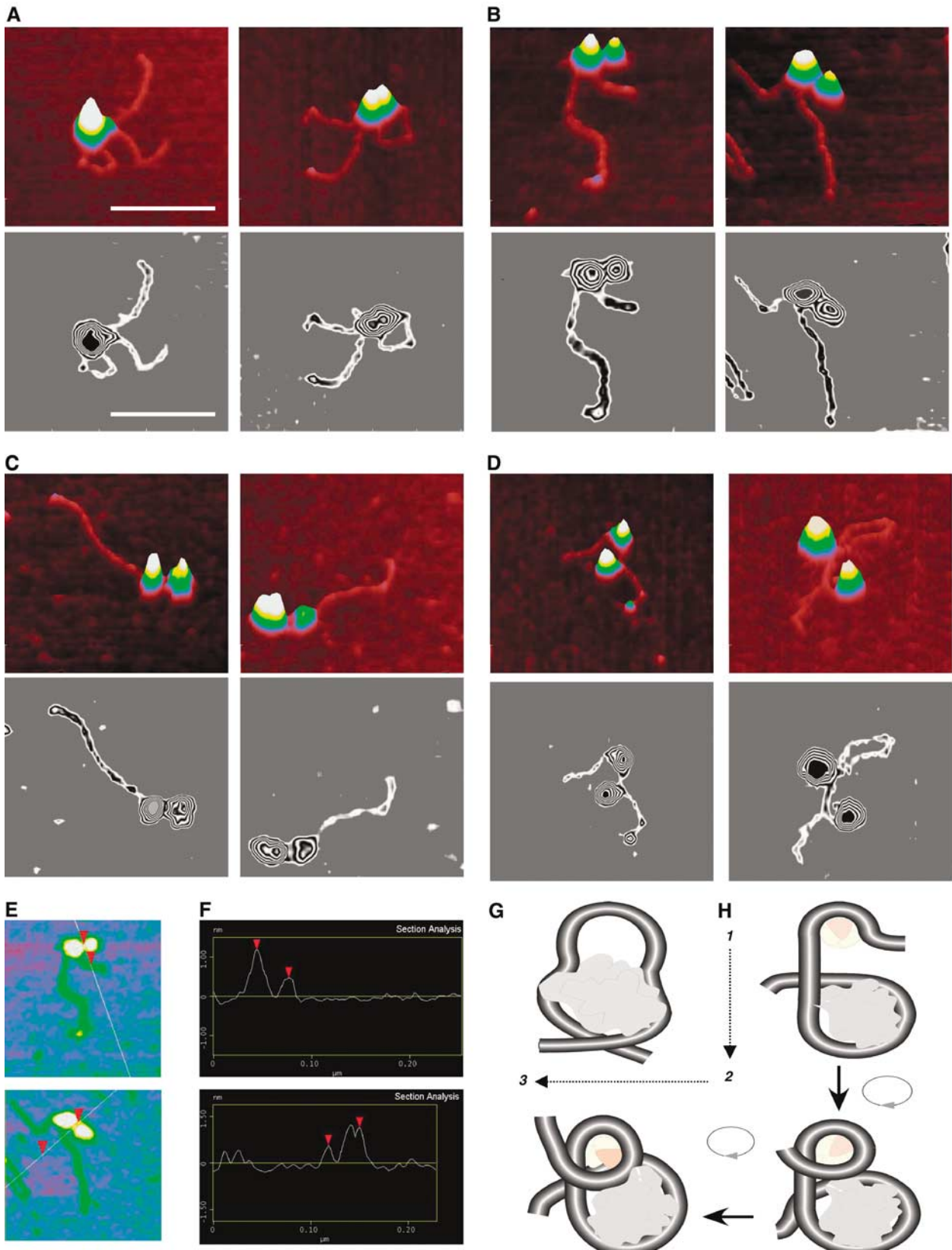


Figure 4 Caption on page 3788.

including the UP element between -48 and -56 . We conclude that the complete *tyrT* promoter binds a single molecule of polymerase holoenzyme and at this promoter the DNA is wrapped around the enzyme as originally hypothesised by Buc (1986). The data indicate the extensive DNA wrapping by RNA polymerase requires the presence of the UAS.

Structure of ternary polymerase–FIS–promoter complex

Not only is the binding of FIS to the *tyrT* UAS highly cooperative but also the dependence of transcription on FIS concentration *in vitro* is sigmoidal, again with a Hill coefficient of ~ 3 (Pemberton *et al*, 2002). This correspondence implies that the structure of the FIS subcomplex is either maintained in the polymerase–FIS–promoter ternary complex or is at least an obligate intermediate in the formation of this initiation complex. Visualisation of the ternary complex revealed that the FIS subcomplex remained as a discrete assembly but in close proximity to an RNA polymerase molecule (Figures 3E and 4B). The protein components of these ternary complexes were identified by height, that of the FIS subcomplex being normally approximately half that of the polymerase. The location of this ternary assembly corresponds well to the locations of the complexes of FIS and polymerase by themselves (Figure 2A). In several examples of this complex, we see distinct single or double connections between the polymerase and the FIS assembly (see e.g. Figures 4E and F), but we have no indication of their nature except that in all ternary complexes their height is greater than that of a single DNA duplex. The morphology of the ternary complexes is more variable than that of either the polymerase–DNA or the FIS–DNA complexes alone. In many images of the ternary complex, the entering and exiting arms of DNA emerge from the combined polymerase–FIS complex suggesting that in these complexes the promoter DNA is wrapped around the whole complex. However, in others one of the contacts, probably the upstream one, between the polymerase and the DNA is no longer apparent and the DNA separates from the complex in the vicinity of FIS. In other complexes we observe completely separate complexes of FIS and polymerase (Figure 4D), while in yet others the height of the FIS peak differs from that in other ternary complexes (Figure 4C). While this variability in detail could be in part due to the variations in the AFM tip shape, it would also be consistent with previous results indicating that the ternary complex, even in the absence of the nucleoside

triphosphates, is highly dynamic (Muskhelishvili *et al*, 1997; Pemberton *et al*, 2002) and that FIS can accrete to an already established complex of three FIS dimers with the UAS (Muskhelishvili *et al*, 1995). Nevertheless, the primary organisation into two discrete complexes is invariant and we infer that this represents the structural basis for transcriptional activation at the *tyrT* promoter. The formation of the ternary complexes was dependent on the prior or simultaneous addition of polymerase relative to FIS and was not observed when FIS was added before polymerase (data not shown).

Implications

We have shown that FIS and RNA polymerase form discrete, but connected, complexes at the *tyrT* promoter. To our knowledge this is the first time that a complex between an activator and a σ^{70} polymerase holoenzyme has been visualised. Nevertheless, the discrete character of the activator and polymerase complexes is similar in principle to that observed at the *glnA* promoter between the NtrC activator and a σ^{54} polymerase holoenzyme (Rippe *et al*, 1997). We conclude that by positioning the activator in close proximity to the polymerase, this architecture provides a general paradigm for the facilitation of direct, but weak, interactions between polymerase and an activator.

The cooperative formation of the FIS–UAS complex and its apparent conservation in the ternary complex argue strongly that the assembly of three FIS dimers constitutes the functional activating unit at the *tyrT* promoter. This conclusion is supported by previous demonstrations that a single proximal FIS site is insufficient for optimal activation of this promoter (Muskhelishvili *et al*, 1997). RNA polymerase cooperatively recruits FIS to this proximal site (Muskhelishvili *et al*, 1995), suggesting that the polymerase itself can facilitate, but is not essential for, the formation of the FIS subcomplex containing three dimers. *In vivo* it is likely that other abundant nucleoid-associated proteins bind to the UAS so that the occupancy of the UAS by FIS is determined by dynamic competition between activating (polymerase and FIS) and repressing proteins.

In vivo and *in vitro* the *tyrT* promoter as well as the similar *rrnA* P1 promoter are strongly dependent on high negative superhelicity of DNA for optimal transcription (Lamond, 1985; Free and Dorman, 1994; Rochman *et al*, 2002). *In vivo* in the presence of FIS the activity of these promoters is buffered against variations in superhelical density, a phenomenon that requires the binding of FIS to multiple sites in the UAS (Rochman *et al*, 2002; Auner *et al*, 2003). We suggest

Figure 4 Heterogeneity of binary polymerase–promoter and ternary polymerase–FIS–promoter complexes. (A) Binary RNAP–promoter complexes with protruding DNA loops (cf Figures 3B, D and F). (B–D) Three different types of ternary complexes. In (B, C), the promoter–downstream DNA is wrapped to different extents but the promoter–upstream DNA lengths are similar and the FIS and RNAP subcomplexes are closely associated. In (D), the path of DNA connecting the two subcomplexes is seen. Note the left-handed toroidal supercoil stabilised by smaller subcomplex (D, right panel). All magnified images (240×240 nm, the scale bar is 100 nm) are shown in both an angled view (top panels) and top view (grayscale, bottom panels). (E) Section measurements of the height of the connections between the FIS and RNAP subcomplexes shown in (B). The white line represents the path of the section. (F) The red arrows indicate the peaks corresponding to free DNA and the connections between the subcomplexes. In both cases the connections between the subcomplexes have greater height and both single and double peaks indicative of single (upper panel) or double (lower panel) connection can be seen (240×240 nm² magnification). (G) The model of the binary RNAP–promoter complex with protruding DNA loop shown in (A). (H) A model of the ternary FIS–polymerase–promoter DNA complex. (1) Binding of several FIS dimers (pink and yellow) bends the DNA (grey rod) in the subcomplex; (2) rotation of the FIS subcomplex relative to RNA polymerase (grey) brings the upstream DNA in close vicinity of polymerase; (3) binding of polymerase to upstream DNA and formation of FIS polymerase contacts stabilises a DNA microloop. Formation of this latter in absence of FIS can be facilitated by negative supercoiling of DNA, leading to structures exemplified in (G).

that FIS stabilises the left-handed wrapping of DNA in the ternary complex and activates initiation by a slight rotation of the FIS complex relative to polymerase thereby applying torque to the enzyme (Figure 4H). This would result in the untwisting of the -10 region as envisaged in our model for torsional transmission (Muskhelishvili and Travers, 1997; Muskhelishvili *et al*, 1997).

The promoters of several other stable RNA genes, including most of those encoding rRNA (Hirvonen *et al*, 2001) contain multiple FIS sites in the UAS region. These are often, as in *tyrU* and *rrnB* P1, in helical register and would be expected to form compact structures related to those we observe at the *tyrT* promoter. Nevertheless some of these promoters, for example *leuV*, contain only a single FIS binding site in the UAS (Ross *et al*, 1999). In such cases we would expect that a compact assembly cannot be formed by FIS alone. In contrast at the *rrnA* P1 promoter, FIS binds to a strong far upstream site at -225 and in so doing constrains an additional negative supercoil (Rochman *et al*, 2002). We suggest that in this case the FIS assembly contains more FIS dimers than at the *tyrT* UAS.

Another transcriptional activator that, like FIS, bends DNA strongly is the CRP dimer (catabolite repressor protein). At the *lac* promoter, CRP binds in the same helical register as FIS (Gaston *et al*, 1990) and also, like FIS, stabilises a DNA bend between the polymerase contacts at the -35 hexamer and in the upstream region (Buckle *et al*, 1992). CRP has been inferred to stabilise a similar structure at the *malT* promoter *in vivo* (Eichenberger *et al*, 1997). Contacts between FIS and RNA polymerase as well as those between CRP and polymerase are known to be mediated, at least in part, by the CTDs of the α -polymerase subunits (Murakami *et al*, 1997; Meng *et al*, 2001; Benoff *et al*, 2002). We suggest that in the absence of an activator an α -CTD contacts FIS site III in an analogous manner to its upstream contacts at the *lacUV5* promoter (Davis *et al*, 2005; Ross and Gourse, 2005). In accord with this view, it has recently been shown by AFM that the extensive wrapping of RNA polymerase with the λP_R promoter requires the interaction of an upstream sequence with the α -CTD (S Cellai, N Vannini, N Naryshkin, RH Ebright, and C Rivetti, personal communication). In contrast in the presence of an activator the α -CTDs bridge between polymerase and activator bound as distinct protein-DNA complexes.

References

- Auner H, Buckle M, Deufel A, Kutateladze T, Lazarus L, Mavathur R, Muskhelishvili G, Pemberton I, Schneider R, Travers A (2003) Mechanism of transcriptional activation by FIS: role of core promoter structure and DNA topology. *J Mol Biol* **331**: 331–344
- Benoff B, Yang H, Lawson CL, Parkinson G, Liu J, Blatter E, Ebright YW, Berman HM, Ebright RH (2002) Structural basis of transcription activation: the CAP- α CTD-DNA complex. *Science* **297**: 1562–1566
- Browning DF, Busby SJ (2004) The regulation of bacterial transcription initiation. *Nat Rev Microbiol* **2**: 57–65
- Buc H (1986) Mechanism of activation of transcription by the complex formed between cyclic AMP and its receptor in *Escherichia coli*. *Biochem Soc Trans* **14**: 196–199
- Buckle M, Buc H, Travers AA (1992) DNA deformation in nucleoprotein complexes between RNA polymerase, cAMP receptor protein and the *lac UV5* promoter probed by singlet oxygen. *EMBO J* **11**: 2619–2625
- Davis CA, Capp MW, Record Jr MT, Saecker RM (2005) The effects of upstream DNA on open complex formation by *Escherichia coli* RNA polymerase. *Proc Natl Acad Sci USA* **102**: 285–290

Materials and methods

Proteins

FIS was purified according to the protocol of Koch and Kahmann (1986). RNA polymerase was obtained from Epicentre.

DNA fragments

The template DNA fragments were generated from plasmids pTyrTlac and pTyrTd61 (Auner *et al*, 2003) by PCR using *Pfu* polymerase (Promega). PCR products were purified with Qiagen Qiaquick[®] Gel Extraction kit. The DNA was eluted with a 20 mM HEPES buffer, pH 8.0.

Protein-DNA binding

RNAP-DNA complexes were formed by mixing equimolar amounts of protein and DNA (1.7 μ M final concentration) in 20 μ l AFM-buffer (20 mM HEPES pH 8.0, 50 mM KCl, 0.005% Tween, 2 mM NiCl₂) at room temperature. In all reactions containing FIS, the used molar ratio of FIS to DNA was 2.65:1. To facilitate ternary complex formation (Muskhelishvili *et al*, 1995), the RNA polymerase was added first to the DNA (RNAP to DNA molar ratio of 0.8:1) and FIS was added afterwards (FIS to DNA molar ratio of 2.65:1). The reaction mixture was incubated for 5 min at 4°C and subsequently transferred to a freshly cleaved mica disc (Plano GmbH, Wetzlar). After 10 min at 4°C, the mica disc was rinsed three times with 1 ml distilled water and dried for 20 s under a weak flux of nitrogen.

AFM imaging

Images were acquired with a Multimode atomic force microscope equipped with a Nanoscope IIIa controller (Veeco Instruments GmbH, Germany), operating in Tapping Mode in air using a J-scanner and RTESP silicon cantilevers. Images of 512 \times 512 pixels with a scan size of 2 \times 2 μ m were acquired at scan frequencies between 2 and 3 Hz. AFM images were processed by using the NanoScope Image software (version 5.12r5; Veeco Instruments Inc., Santa Barbara, CA, USA). Contour lengths of DNA molecules were determined manually using Image J software (version 1.32j) by Wayne Rasband, NIH, USA).

Statistical analysis

A Gaussian fitting over the distribution of classes and Student's *t*-tests to verify the significance of DNA contour length change were calculated for all data sets. The confidence limits are presented in Figure 1D.

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- Debenham P (1979) The influence of ribonucleoside triphosphates, and other factors, on the formation of very-salt-stable RNA-polymerase su⁺III-tRNA(tRNA^{Tyr})-promoter complexes. *Eur J Biochem* **96**: 535–543
- Eichenberger P, Dethiollaz S, Buc H, Geiselmann J (1997) Structural kinetics of transcription activation at the *malT* promoter of *Escherichia coli* by UV laser footprinting. *Proc Natl Acad Sci USA* **94**: 9022–9027
- Free A, Dorman CJ (1994) *Escherichia coli tyrT* gene transcription is sensitive to DNA supercoiling in its native chromosomal context: effect of DNA topoisomerase IV overexpression on tyrT promoter function. *Mol Microbiol* **14**: 151–161
- Gaston K, Bell A, Kolb A, Buc H, Busby S (1990) Stringent spacing requirements for transcription activation by CRP. *Cell* **62**: 733–743
- Herman T (1996) *Molekülmodellierung—ein Werkzeug der Molekularbiologie: Anwendung auf Nukleinsäuren und Nukleoproteinkomplexe*. München: Shaker Verlag
- Hirvonen CA, Ross W, Wozniak CE, Marasco E, Anthony JR, Aiyar SE, Newburn VH, Gourse RL (2001) Contributions of UP elements

- and the transcription factor FIS to expression from the seven *rrn* P1 promoters in *Escherichia coli*. *J Bacteriol* **183**: 6305–6314
- Koch C, Kahmann R (1986) Purification and properties of the *Escherichia coli* host factor required for the inversion of the G segment in bacteriophage Mu. *J Biol Chem* **261**: 15673–15678
- Lamond AI (1985) Supercoiling response of a bacterial tRNA gene. *EMBO J* **4**: 501–507
- Lamond AI, Travers AA (1983) Requirement for an upstream element for optimal transcription of a bacterial tRNA gene. *Nature* **305**: 248–250
- Meng W, Belyaeva T, Savery NJ, Busby SJ, Ross WE, Gaal T, Gourse RL, Thomas MS (2001) UP element-dependent transcription at the *Escherichia coli* *rrnB* P1 promoter: positional requirements and role of the RNA polymerase alpha subunit linker. *Nucleic Acids Res* **29**: 4166–4178
- Murakami K, Owens JT, Belyaeva TA, Meares CF, Busby SJ, Ishihama A (1997) Positioning of two α subunit carboxy-terminal domains of RNA polymerase at promoters by two transcription factors. *Proc Natl Acad Sci USA* **94**: 11274–11278
- Muskhelishvili G, Buckle M, Heumann H, Kahmann R, Travers AA (1997) FIS activates sequential steps during transcription initiation at a stable RNA promoter. *EMBO J* **16**: 3655–3665
- Muskhelishvili G, Travers AA (1997) Stabilisation of DNA micro-loops by FIS—a mechanism for torsional transmission in transcription activation and DNA inversion. *Nucl Acids Mol Biol* **11**: 179–190
- Muskhelishvili G, Travers AA, Heumann H, Kahmann R (1995) FIS and RNA polymerase holoenzyme form a specific nucleoprotein complex at a stable RNA promoter. *EMBO J* **14**: 1446–1452
- Pemberton IK, Muskhelishvili G, Travers AA, Buckle M (2002) FIS modulates the kinetics of successive interactions of RNA polymerase with the core and upstream regions of the *tyrT* promoter. *J Mol Biol* **318**: 651–663
- Rippe K, Guthold M, von Hippel PH, Bustamante C (1997) Transcriptional activation via DNA-looping: visualization of intermediates in the activation pathway of *E. coli* RNA polymerase- σ^{54} holoenzyme by scanning force microscopy. *J Mol Biol* **270**: 125–138
- Rivetti C, Guthold M, Bustamante C (1999) Wrapping of DNA around the *E. coli* RNA polymerase open promoter complex. *EMBO J* **18**: 4464–4475
- Shin M, Song M, Rhee JH, Hong Y, Kim YJ, Seok YJ, Ha KS, Jung SH, Choy HE (2005) DNA looping-mediated repression by histone-like protein H-NS: specific requirement of $E\sigma^{70}$ as a cofactor for looping. *Genes Dev* **19**: 2388–2398
- Rochman M, Aviv M, Glaser G, Muskhelishvili G (2002) Promoter protection by a transcription factor acting as a local topological homeostat. *EMBO Rep* **3**: 355–360
- Ross W, Gourse RL (2005) Sequence-independent upstream DNA- α CTD interactions strongly stimulate *Escherichia coli* RNA polymerase-*lacUV5* promoter association. *Proc Natl Acad Sci USA* **102**: 291–296
- Ross W, Salomon J, Holmes WM, Gourse RL (1999) Activation of *Escherichia coli* *leuV* transcription by FIS. *J Bacteriol* **181**: 3864–3868
- Travers AA, Lamond AI, Mace HAF, Berman ML (1983) RNA polymerase interactions with the upstream region of the *E. coli* *tyrT* promoter. *Cell* **35**: 265–273