Organization of Open Complexes at *Escherichia coli* Promoters

LOCATION OF PROMOTER DNA SITES CLOSE TO REGION 2.5 OF THE σ70 SUBUNIT OF RNA POLYMERASE

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A cysteine-tethered DNA cleavage agent has been used to locate the position of region 2.5 of σ70 in transcriptionally competent complexes between *Escherichia coli* RNA polymerase and promoters. In this study we have engineered σ70 to introduce a unique cysteine residue at a number of positions in region 2.5. Mutant proteins were purified, and in each case, the single cysteine residue used as the target for covalent coupling of the DNA cleavage agent p-bromoacetamidobenzyl-EDTA-Fe (FeBABE). RNA polymerase core reconstituted with tagged σ derivatives was shown to be transcriptionally active. Hydroxyl radical-based DNA cleavage mediated by tethered FeBABE was observed for each derivative of RNA polymerase in the open complex. Our results show that region 2.5 is in close proximity to promoter DNA just upstream of the −10 hexamer. This positioning is independent of promoter sequence. A model for the interaction of this region of σ with promoter DNA is discussed.

Promoter recognition requires sequence-specific contacts by the transcriptional apparatus. At most promoters these contacts are made upstream from the transcription start point. Once the transcriptional apparatus has bound the promoter to form a closed complex, an isomerization event occurs to generate the open complex, forming the single-stranded template required for transcription. The bacterium *Escherichia coli* provides a good model for understanding protein-DNA interactions during transcription initiation. *E. coli* uses a single core RNA polymerase for transcription elongation with subunit composition α2ββ′. Promoter specificity is principally afforded by a separate subunit, σ, which associates with the core enzyme to give holoenzyme (RNAP) but dissociates once sequence-specific promoter DNA contacts are no longer required (1). The σ70 subunit, encoded by *rpoD*, is one of several σ subunits utilized by *E. coli* and is responsible for directing the transcription of most genes during vegetative growth. RNAP is capable of sequence-specific transcription initiation in the absence of other transcription factors. Factor-independent transcription is reliant on the ability of σ70 to make stable contacts with the promoter DNA (1, 2). *E. coli* promoters contain two very conserved motifs, the −10 and −35 hexamers (3), and several less-conserved sequences including the UP element (4) and the extended −10 motif (5). The extended −10 motif (5′-TGXTATAAT-3′) can drive factor-independent transcription at several bacterial promoters lacking homology to the consensus within the −35 region (6, 7). Therefore this TGX motif is able to compensate for a poor −35 hexamer. The TG motif has been shown to be important for promoter activity in several other bacterial species (8–11). Work from many laboratories has defined the regions within RNA polymerase that are responsible for sequence-specific contacts within promoter DNA. Regions 2.4 and 4.2 of σ70 contact the −10 and −35 hexamers, respectively (1, 2), whereas the C-terminal domain of the σ subunit (αCTD) contacts the UP element (4). Recent work from this laboratory has indicated that a newly defined region of σ70, region 2.5, is responsible for making sequence-specific contacts with the extended −10 motif (12). This region was identified by screening for altered or relaxed specificity mutants of σ70 capable of compensating for down-mutations within the extended −10 motif. One relaxed specificity mutant, σ70 E458G, was isolated (12). The E458G substitution partially suppressed the effect of changing the G-C base pair of the TG motif, suggesting a role for the side chain at position 458 in contacting the extended −10 motif.

The aim of the study presented in this paper is to complement the genetic study with biophysical data to support the suggested role of region 2.5. We wanted to show that, in open, transcriptionally competent complexes at *E. coli* promoters, region 2.5 of σ70 is located near to promoter DNA, just upstream of the −10 hexamer. To do this, we exploited a novel method that relies on tethering a DNA cleavage agent to a single specific amino acid side chain of a protein (13). The reagent p-bromoacetamidobenzyl-EDTA-Fe (FeBABE) has one reactive group facilitating covalent attachment to cysteine side chains, whereas a second group holds a single metal atom in a tight coordination complex (14). Under appropriate conditions, the divalent cation can participate in the generation of hydroxyl radicals, which attack deoxyribose units, resulting in DNA strand scission (15). Recently, this chemistry has been applied to the study of the interaction of αCTD of *E. coli* RNAP with promoter DNA. Hydroxyl radical DNA cleavage mediated by FeBABE showed that the two αCTD subunits are arranged asymmetrically, contacting different halves of the UP element and that activator contact patches are available on both subunits (16, 17). To identify sites on promoter DNA that are near to region 2.5 of σ70 in open complexes, amino acids in this region were replaced with cysteine for conjugation with the DNA cleavage agent FeBABE.

EXPERIMENTAL PROCEDURES

**Strains and Materials—** *E. coli* strain DH5α (supE44ΔlacU169Δ80 lacZAM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1) was used for all
The nucleotide sequence of three promoters used in this work; KAB-TG, KAB-TTcon, and galP1(1978A9A). The promoter consensus recognized by the E. coli σ70 holoenzyme is also shown. The –35 and extended –10 sequence elements are underlined.

Transcriptional activity of RNAP carrying σ70 derivatives. The figure shows run-off transcripts from promoters (i) KAB-TG and (ii) KAB-TTcon. The single cysteine derivative is indicated. Transcripts generated by RNAP containing σ70 tagged with FeBABE are indicated by a +.

Purified mutant subunits were dialyzed against storage buffer (10 mM Tris-HCl, pH 7.6, at 4 °C, 10 mM MgCl2, 0.1 mM EDTA, 100 mM KCl, and 50% glycerol) and stored at –20 °C. Conjugation of σ70 with FeBABE—FeBABE was synthesized and characterized as described previously (27). Single cysteine derivatives of σ70 were used for conjugation with FeBABE based on the method of Murakami et al. (16). Conjugation was initiated by mixing 1.2 mM of 6.67 μM protein solution in conjugation buffer (10 mM Hepes, 200 mM KCl, and 2 mM EDTA, pH of 8.0) with 9 μl of 18 mM FeBABE in Me2SO. After incubation at 37 °C for 4 h, excess unreacted FeBABE was removed by dialysis against storage buffer. The efficiency of conjugation was determined by estimating free side chains of both conjugated and un-conjugated proteins with the fluorescent reagent CPM (7-diethylamino-3-(4-maleimidylphenyl)-4-methylcoumarin) (Molecular Probes) (27).

Reconstitution of RNA Polymerase Holoenzyme—A 10-fold molar excess of σ70 was mixed with core RNAP and incubated at 20 °C for 30 min (26).

DNA Cleavage by FeBABE—RNAP holoenzyme (300 nM) was mixed with 25°F-end-labeled promoter fragment (0.4 nM) in a reaction volume of 35 μl (20 mM Hepes, pH 8.0, 5 mM MgCl2, 50 mM potassium glutamate, 50 μg/ml bovine serum albumin, 5 μg/ml poly(dI-dC)) and incubated at 37 °C for 20 min. Complexes were challenged with heparin (200 μg/ml at 37 °C for 5 min). DNA cleavage was initiated by the addition of sodium ascorbate (2 mM) followed by incubation at 37 °C for 20 min. Modified DNA was extracted with phenol/chloroform and precipitated with ethanol before analysis on a 6% polyacrylamide gel containing 6 M urea. Gels were calibrated with Maxam-Gilbert G + A sequence ladders and were processed and scanned using a PhosphorImager (Molecular Dynamics).

In Vitro Transcription—The activity of RNAP reconstituted with wild-type, un-conjugated, and conjugated σ70 derivatives was measured by in vitro transcription assays (28). Fragments used for cleavage analysis were also used as templates for in vitro transcription. The derivatives of the gal promoter were expected to generate run-off products of 51 nucleotides. DNA template (5 nm) and RNAP holoenzyme (100 nm) were preincubated in 12 μl of transcription buffer (10 mM Tris, pH 8.0, 50 mM NaCl, 2.5 mM MgCl2, 50 μM EDTA, 0.5 mM dithiothreitol, 25 μg/ml bovine serum albumin, 2.5% glycerol) for 5 min at 37 °C. Transcription was initiated by the addition of unlabeled nucleotide triphosphates ATP, CTP, GTP (200 μM), and UTP (10 μM), 0.5 μCi of [α-32P]UTP (800 Ci/mmol), 100 μg/ml heparin. Reactions were stopped by the addition of an equal volume of run-off stop mix (20 mM EDTA, 80% deionized formamide, 0.1% bromophenol blue, 0.1% xylene cyanol). Transcripts were analyzed on a 6% polyacrylamide gel containing 6 M urea and scanned using a PhosphorImager.
suggests that Glu-458 is part of a α-helix starting at Val-454. An octapeptide from Val-454 to Asn-461 was constructed using the molecular modeling package Quanta by Molecular Simulations, Inc. and energy minimized to place amino acid side chains in sterically favorable positions. This peptide was then manually docked into the major groove of a model of B-form DNA based on the sequence of KAB-TG from position 1 to 29 with the carboxyl group of Glu-458 making base-pair edge hydrogen bond interactions with N6 of adenine at position 15 and N4 of cytosine at position 14 (i.e. the template strand of the TG motif). In this position, no steric clashes were observed. The model was extended to include the helix of region 2.4 (helix 14) derived from the crystal structure of a tryptic fragment of σ70 (29). The coordinates obtained were used to dock helix 14 with residues 437 and 440 in hydrogen-bonding contact with the base pair edge at −12 (see Fig. 7). The minor groove at the center of the −10 element must be placed on the inside of a curvature for efficient promoter recognition by E. coli RNAp (32). In addition, many studies show that recognition of −10 and −35 elements is accompanied by promoter bending and suggest that the major groove of the −10 hexamer widens to accommodate σ (33). Such a promoter structure would allow favorable interactions between basic residues Arg-441 and Arg-446 and the phosphate backbone (T-A base steps of the −10 promoter consensus element distort double-strand DNA in solution) (34). The model in Fig. 7a places the carboxyl group of amino acid 448 1.0 nm from the amino group of residue 454. This would allow for a flexible unstructured 7-amino acid loop connecting the two helices shown. Methods of probing for single-stranded DNA assign position −12 as the upstream limit of the open complex (35, 36). The TG motif at position −14/15 would thus remain in a region of double-stranded DNA, whereas helix 14 is shown within the transcription bubble (Fig. 7b).

**RESULTS**

Construction and Conjugation of σ70 Mutants with FeBABE—In previous work, we used suppression genetics to identify a region of the α70 subunit of RNA polymerase, region 2.5, that interacts with the extended −10 motif of bacterial promoters (12). In this work, we have exploited a tethered DNA-cleaving agent to show that region 2.5 of α70 is in close proximity to the extended −10 motif. The reagent used was FeBABE, which is covalently attached to α70 by conjugation with a cysteine residue. Starting with an rpoD gene that had been mutated to remove all three native cysteine codons, single

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“FIG. 3. Quantification of the transcription activity of RNAP carrying σ70 derivatives. The activity of the σ70 derivatives relative to wild-type σ70 (100%) at KAB-TTcon (a) and KAB-TG (b). The black bars represent activity of derivatives before conjugation, and the gray bars show activity after FeBABE conjugation and correction for the presence of unconjugated σ70.”

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"http://www.pdb.bnl.gov."
In this study we analyzed the interaction of E. coli RNA polymerase-carrying FeBABE with different promoters. We chose promoters that were sufficiently strong such that, in our conditions, open complex formation would not be disrupted by the introduction of the bulky FeBABE probe into region 2.5 of the promoter. Thus, Figs. 2 and 3 show the transcription activity of RNAP holoenzyme preparations containing single-cysteine derivatives of the promoter before and after conjugation with FeBABE. With the exception of 422C, all derivatives retained at least 80% activity compared with wild-type before conjugation and at least 70% activity after conjugation with FeBABE.

Specificity of FeBABE Cleavage—In our first experiment, we examined the cleavage of a labeled Pet-HindIII fragment purified from pAA121 carrying the KAB-TG promoter (Fig. 1), using RNAP containing tagged with FeBABE positioned at 461. This DNA fragment contains the KAB-TG promoter as well as the pbla and pX (these promoters are located upstream from the EcoRI site in the pAA121 vector). The results in Fig. 4 show that cleavage is observed with holoenzyme reconstituted from FeBABE-conjugated 461C mutant. In contrast, no cleavage was observed with holoenzyme containing either wild-type or unconjugated 461C. The results (Fig. 4, lane 4) clearly show that cleavage is restricted to the three promoters. Further analysis revealed that similar patterns of FeBABE-mediated cleavage are observed at the KAB-TG, pbla, and pX promoters (data not shown).

In our second set of experiments, both strands of the consensus extended –10 promoter tagged with FeBABE at different positions. The first promoter, galP1(19T8A9A), is a derivative of galP1, which has been changed to introduce a consensus –10 element. It is an extended –10 promoter containing a UP element but has a –35 hexamer with no homology with the consensus. To investigate the effects of a –10 extension on the pattern of cleavage, we also compared the KAB-TG and KAB-TTcon promoters. These promoters, which are also derived from the galP1 sequence, have similar activities. KAB-TG carries an extended –10 motif and canonical –10 (4/6 fit to consensus) and –35 (5/6 fit to consensus) hexamer sequences. KAB-TTcon lacks the extended –10 motif but carries an improved –35 (6/6 fit to consensus) hexamer.

Nontemplate Strand Cleavage—Fig. 5a shows the patterns of cleavage on the nontemplate strand of the galP1(19T8A9A), KAB-TG, and KAB-TTcon promoters in open complexes with RNAP holoenzymes carrying tagged with FeBABE at positions 581, 422, 454, 458, 459, and 461. For each holoenzyme, the pattern of cleavage is similar at all three promoters, with some small variations in the positions and intensities of cleavage. For example, some upstream cleavages at the galP1 (19T8A9A) promoter are reduced. This is consistent with this promoter’s lack of homology to the consensus within the –35 hexamer, leading to weaker interactions with upstream sequences. However, the overall similarity in cleavage patterns suggests that the organization of the different parts of the promoter is the same, irrespective of the precise promoter sequence. In contrast, changes in the position of FeBABE in the template result in marked variations in the positions of cleavage. Cleavage by FeBABE tethered at positions 422, 454, and 458 in open complexes at the lacUV5 promoter (37). The data here are in agreement with those previously published. FeBABE attached to 581C cleaved promoter DNA at positions –44 to –45, –34 to –37, and –24 to –26. FeBABE positioned on 422C cleaved promoter DNA with very low efficiency at position –13 to –12. The FeBABE positioned within region 2.5 (at residues 454, 458, 459, and 461) resulted in cleavage around –20 for all the different derivatives, with additional cleavages at other positions being dependent on which derivative was studied. The predominant position of cleavage resulting from FeBABE tethered at 458 is –20 for all three promoters; however, additional upstream cleavage around –36 is seen for complexes at the KAB-TG promoter. Cleavage by FeBABE attached to 459C is limited to DNA around position –20. FeBABE located at 454 cleaves the nontemplate strand at position –20 but also at –17 to –16. Cleavage at –17 to –16 is not observed for other different derivatives. FeBABE positioned at 461 again cleaves at –20, but additionally, there is cleavage from –13 to –1.

Template Strand Cleavage—Fig. 5b shows cleavage on the template strand of the different promoters by FeBABE, located at different positions in the promoter. There were marked variations in the positions and intensities of cleavage. Differences in the positions and intensities of DNA cleavage were observed compared with the nontemplate strand. A single region of promoter DNA at positions –13 and –14 is sensitive to hydroxyl radical attack by FeBABE when attached to all four positions in region 2.5. In addition FeBABE tethered at 458 and 459 cleaves at positions –21 to –22, and 458C generates more distal cleavage at –38 to –40. FeBABE at 454C also gives unique cleavage at –18 to –19 and, in common with 461C, cleaves downstream at –7 to –8.
FIG. 5. Detailed FeBABE-mediated DNA cleavage at three promoters nontemplate strand (a) and template strand (b). The positions of FeBABE attachment to $\sigma^{70}$ are indicated. The promoters studied are: 1, KAB-TTcon; 2, KAB-TG; 3, galP1(19T8A9A); M, Maxam-Gilbert G+A tracks (shown as calibration markers).

FIG. 6. Summary of cleavage pattern at KAB-TG. Boxes above each horizontal line represent nontemplate strand cleavage. Boxes below the line indicate template strand DNA cleavage. The “gray scale” reflects the observed cleavage efficiency of DNA, black being more efficient. The position of single cysteine replacement in $\sigma^{70}$ for FeBABE tagging is shown next to each line. Numbers below the tick marks provide a reference for the positions of strand cleavage along the DNA template.
There is increased cleavage at \(-7/-8\) by FeBABE positioned at 461. Faint upstream cleavage for FeBABE attached at 461 was seen at \(-21/22\) (common to 458C and 459C). Promoter-specific differences were seen in the cleavage pattern from FeBABE at 454, with an increase in the intensity of bands at \(-8\) corresponding to an alteration of promoter bias toward the \(-10\) element. As previously observed, FeBABE positioned at 581 in conserved region 4.2 cleaves promoter DNA from \(-38\) to \(-41\) and at \(-28/-29\), whereas 422C in conserved region 2.3 gives weak cleavage at \(-16/-17\). Fig. 6 shows the cleavage pattern from both strands of promoter KAB-TG in schematic form.

**DISCUSSION**

This study and previous work (37) has shown that the cysteine residues present in the wild-type \(\sigma^{70}\) subunit of RNA polymerase are not essential for transcription initiation. Thus, it is possible to introduce cysteine residues in \(\sigma^{70}\) and to attach the DNA cleavage agent FeBABE and still retain transcriptional competence. In this work, FeBABE attached to the \(\sigma^{70}\) subunit of RNA polymerase was used to probe open complexes at a set of related promoters. The first important point to note is that the overall structure of RNA polymerase bound at promoters carrying different sequence elements appears to be very...
similar; the cleavage data was similar for all three variants of the galP1 promoter (Fig. 1) as well as the pX and plba promoters. FeBABE attached to positions 422 in region 2.3 and 581 in region 4.2 were used as controls in our experiments, and the data are similar to those observed in a previous study (37). This is consistent with the view that RNA polymerase uses a variety of protein-DNA interactions to form essentially the same open complex at different classes of promoters. Note that the principal information gained from this technique concerns location, and the relative intensities of different bands cannot be interpreted to give detailed information about binding mechanisms. For this reason, we chose to work with strong promoters, where open complex formation would not be hindered by the bulky substitution of FeBABE.

The major conclusion from this study is that, in open complexes, region 2.5 of $\sigma^{70}$ is close to promoter DNA sequences just upstream of the −10 hexamer. One aim of this work was to propose a model for the interaction of region 2.5 of $\sigma^{70}$ with promoter DNA. Interpretation of the data is complicated by the fact that the holoenzymes carrying FeBABE-modified $\sigma^{70}$ proteins are interacting with DNA that is known to be both bent and unwound. However, Fig. 7 shows possible models of how regions 2.4 and 2.5 of $\sigma^{70}$ may interact with promoter DNA, based on the data obtained from this study and previous genetic work as discussed below. The similarity of cleavage pattern observed for FeBABE tethered at 454 and 461 and for FeBABE tethered at 458 and 459 is consistent with region 2.5 being $\alpha$-helical. The cleavage pattern seen for FeBABE at 459 indicates that the reagent contacts the double-stranded promoter DNA around position −20. In the model, consistent with the length of the FeBABE spacer arm, the $\beta$ carbon of Thr-459 is approximately 1.2 nm away from the proposed position of radical release. The FeBABE attached to 459C may be constrained, allowing it to sit in only one position relative to the DNA. We propose that position 459 is buried by the helix of region 2.5. FeBABE positioned at 458 results in the same cleavage pattern seen for 459C, but with additional weaker upstream DNA cleavage around the −35 hexamer, which may be because of bending and wrapping of upstream DNA sequences (33). The cleavage patterns observed with FeBABE positioned at 454 and 461 are more complex. We suggest that this complexity arises from the location of these side chains in an exposed position in the region 2.5 helix and the fact that promoter DNA is melted downstream from position −12 (12, 38).

The model presented in Fig. 7b shows a conformation for open complex promoter DNA consistent with the cleavages observed from FeBABE at positions 454, 458, and 459, and 461. The modeling of region 2.5 as an $\alpha$-helix is consistent with the data where FeBABE at positions 458 and 459 (100° apart on the $\alpha$-helix) only cleave promoter DNA upstream on the TG motif, and FeBABE cleavages from positions 454 and 461 span the TG motif. The FeBABE cleavage upstream of the TG is best modeled with double-stranded DNA. In contrast, the complex pattern downstream of the TG motif cannot be modeled on double-stranded DNA, because the DNA was probed in the open complex. Modeling of region 2.5 as an $\alpha$-helix in contact with the TG motif has important consequences for the orientation of $\sigma^{70}$ and, in particular, region 2.4. Thus, in addition to the results presented here, we used previous genetic data to orientate both regions 2.4 and 2.5 with respect to the promoter (12, 30, 31). The aromatic residues Tyr-430 and Trp-433 are implicated in DNA melting and believed to interact at the −10/−11 positions on promoter DNA. The Gln-437 and Thr-440 residues interact with the base pair at −12. The Gln-458 residue is involved in the binding of the extended −10 motif at −14/−15 by region 2.5 of $\sigma^{70}$. Hence the structures shown in Fig. 7, a and b, represent proposed orientations of regions 2.4 (helix 14) and 2.5 that can account for both the genetic and the biophysical data. Note that the orientation of helix 14 proposed in Fig. 7 is different to that suggested by Owens et al. (37) on the basis of cleavage patterns generated by FeBABE, located at positions 132, 376, 396, and 422 in $\sigma^{70}$. Our present data is insufficient to prove either proposal (because of flexibility both in the DNA and in $\sigma^{70}$ just downstream of helix 14). For example, increased DNA distortion could fit the data presented by Owens et al. to the models shown in Fig. 7. Similarly our data could be fitted to the Owens et al. model if a sharp kink is introduced into $\sigma^{70}$ between helix 14 and region 2.5.

According to the model presented here (Fig. 7b), the seven amino acids immediately downstream of helix 14 form a junction between separate domains of $\sigma^{70}$ (39). Flexibility of the loop would allow movement of region 2.4 relative to 2.5 during open complex formation. In the closed complex, this loop constrains the helix of region 2.4 relative to promoter DNA in the orientation shown (Fig. 7b). This model shows how region 2.5 can serve as an anchor, providing a scaffold on which the open complex may be built. These observations support the idea that the TG motif sets a limit on the conformational fluctuation of the −10 region (34, 40, 41). This is consistent with analysis of the temperature dependence of promoter opening at galP1 and supports a mechanism of open complex formation whereby melting nucleates around −10 (42, 43). Such a feature would be of particular importance at extended −10 promoters that lack an identifiable −35 hexamer.

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REFERENCES

1. Gross, C. A., Chan, C. L., and Lonetto, M. A. (1996) Philos. Trans. R. Soc. Lond.-Biol. Sci. 351, 475–482
2. Gross, C. A., Lonetto, M., and Losick, R. (1992) in Transcriptional Regulation (McKnight, S. L., and Yamamoto, K. R., eds) pp. 129–176, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Hawley, D. K., and McClure, W. R. (1983) Nucleic Acids Res. 11, 2237–2255
4. Ross, W., Gocinski, K. K., Salomon, J., Igarashi, K., Zou, C., Ishihama, A., Severinov, K., and Gourse, R. L. (1993) Science 262, 1407–1413
5. Bown, J. A., Barne, K. A., Minchin, S. D., and Busby, S. J. W. (1997) Nucleic Acids Mol. Biol. 11, 41–52
6. Kumar, A., Malloch, B. R., Fujita, N., Smillie, D. A., Ishihama, A., and Hayward, R. S. (1993) J. Mol. Biol. 232, 406–418
7. Minchin, S. D., and Busby, S. J. W. (1993) Biochem. J. 299, 771–775
8. Helmann, J. D. (1986) Nucleic Acids Res. 23, 2351–2360
9. Bashyam, M. D., and Tyagi, A. K. (1998) J. Bacteriol. 180, 2568–2573
10. Sabelnikov, A. G., Greenberg, B., and Lacks, S. A. (1989) J. Mol. Biol. 250, 144–155
11. Gross, C. A., and Robinowitz, J. C. (1986) J. Biol. Chem. 261, 11409–11415
12. Barne, K. A., Bown, J. A., Busby, S. J. W., and Minchin, S. D. (1997) EMBO J. 16, 4034–4040
13. Heilge, G. M., Marusak, R., Meares, C. F., and Noller, H. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1113–1116
14. Sundberg, M. W., Meares, C. F., Goodwin, D. A., and Diamanti, C. I. (1974) Nature 250, 587–588
15. Tullius, T. D., and Domonowski, B. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5469–5473
16. Murakami, K., Kimura, M., Owens, J. T., Meares, C. F., and Ishihama, A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1709–1714
17. Murakami, K., Owens, J. T., Belyaeva, T. A., Meares, C. F., Busby, S. J. W., and Ishihama, A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11274–11278
18. Hanahan, D. (1983) J. Mol. Biol. 166, 557–580
19. Perrin, S., and Gilliland, G. D. (1996) Nucleic Acids Res. 18, 7433–7438
20. Igarashi, K., and Ishihama, A. (1991) Cell 65, 1015–1022
21. Owens, J. T., Miyake, K., Murakami, K., Chmura, A. J., Fujita, N., Ishihama, A., and Meares, C. F. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6021–6026
22. Busby, S. J. W. (1997) Isolation and Characterisation of an Altered Specificity Mutant of the Escherichia coli RNA Polymerase $\sigma^{70}$ Subunit. Ph.D thesis, The University of Birmingham, UK
23. Birnbaum, A. H. A., Ponnambalam, S., Chan, B., and Busby, S. (1986) Gene 41, 67–74
24. Chan, B., and Busby, S. (1989) Gene 84, 227–236
25. Burns, H. D., Belyaeva, T. A., Busby, S. J. W., and Minchin, S. D. (1996) Biochem. J. 317, 305–311
Region 2.5 of RNA Polymerase $\sigma^{70}$ Subunit

26. Fujita, N., and Ishihama, A. (1996) *Methods Enzymol.* 273, 121–130
27. Greiner, D. P., Miyake, R., Moran, J. K., Jones, A. D., Negishi, T., Ishihama, A., and Meares, C. F. (1997) *Bioconjugate Chem.* 8, 44–48
28. Busby, S., Kolb, A., and Minchin, S. D. (1994) in *Protein-DNA Interactions: Principles and Protocols* (Neale, G. G., ed) Vol. 30, pp. 397–411, Humana Press Inc., Totowa, NJ
29. Malhotra, A., Severinova, E., and Darst, S. A. (1996) *Cell* 87, 127–136
30. Waldburger, C., Gardella, T., Wang, R., and Susskind, M. M. (1990) *J. Mol. Biol.* 206, 591–603
31. deHaseth, P. L., and Helmann, J. D. (1995) *Mol. Microbiol.* 16, 817–824
32. Pérez-Martín, J., Rojo, F., and de Lorenzo, V. (1994) *Microbiol. Rev.* 58, 268–290
33. Spassky, A., Rimsky, S., Busby, H., and Busby, S. (1998) *EMBO J.* 7, 1871–1879
34. Sasse-Dwight, S., and Gralla, J. D. (1989) *J. Biol. Chem.* 264, 8074–8081
35. Buckle, M., and Busby, S. D. (1994) in *Transcription: Mechanisms and Regulation* (Conaway, R. C., and Conaway, J. W., eds) pp. 207–225, Raven Press, Ltd., New York
36. Owens, J. T., Chmura, A. J., Murakami, K., Fujita, N., Ishihama, A., and Meares, C. (1998) *Biochemistry* 37, 7670–7675
37. Chan, R., Minchin, S., and Busby, S. (1990) *FEBS Lett.* 267, 46–50
38. Chen, Y. F., and Helmann, J. D. (1997) *J. Mol. Biol.* 267, 47–59
39. Severova, E., Severinov, K., Fenyö, D., Marr, M., Brody, E. N., Roberts, J. W., Chait, B. T., and Darst, S. A. (1996) *J. Mol. Biol.* 263, 637–647
40. Jiang, Y. L., and Helmann, J. D. (1995) *Biochemistry* 34, 8465–8473
41. Burns, H., and Minchin, S. (1994) *Nucleic Acids Res.* 22, 3840–3845
42. Grimes, E., Busby, S., and Minchin, S. (1991) *Nucleic Acids Res.* 19, 6113–6118
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