at the docking site in association with the transport vesicle (*13*, *27*), renders the t-SNARE competent for v-SNARE interaction. The interaction of Ypt1p with Sed5p is transient, and after, or perhaps concomitant with, its dissociation the activated Sed5p engages the v-SNAREs, resulting in an assembled v/t-SNARE complex. Sec17p and Sec18p (SNAP and NSF) can then interact with the assembled complex and catalyze disassembly (*7*), allowing membrane fusion to occur.

REFERENCES AND NOTES

1. G. Palade, *Science* **189**, 347 (1975); J. E. Rothman, *Nature* **372**, 55 (1994).
2. T. Söllner *et al.*, *Nature* **362**, 318 (1993).
3. S. R. Pfeffer, *Annu. Rev. Cell. Dev. Biol.* **12**, 441 (1996).
4. I. C. Griff, R. Schekman, J. E. Rothman, C. A. Kaiser, *J. Biol. Chem.* **267**, 12106 (1992).
5. K. A. Eakle, M. Bernstein, S. D. Emr, *Mol. Cell. Biol.* **8**, 4098 (1988).
6. D. W. Wilson *et al.*, *Nature* **339**, 355 (1989).
7. T. Söllner, M. K. Bennett, S. W. Whiteheart, R. H. Scheller, J. E. Rothman, *Cell* **75**, 409 (1993).
8. H. R. Bourne, *ibid.* **53**, 669 (1988); M. Zerial and H. Stenmark, *Curr. Opin. Cell Biol.* **5**, 613 (1993).
9. A. P. Newman, J. Shim, S. Ferro-Novick, *Mol. Cell. Biol.* **10**, 3405 (1990).
10. M. Sogaard *et al.*, *Cell* **78**, 937 (1994).
11. K. G. Hardwick and H. R. Pelham, *J. Cell Biol.* **119**, 513 (1992).
12. N. Segev, J. Mulholland, D. Botstein, *Cell* **52**, 915 (1988); H. D. Schmitt, M. Puzicha, D. Gallwitz, *ibid.* **53**, 635 (1988); N. Segev, *Science* **252**, 1553 (1991); M. F. Rexach and R. W. Schekman, *J. Cell Biol.* **114**, 219 (1991).
13. J. P. Lian, S. Stone, Y. Jiang, P. Lyons, S. Ferro-Novick, *Nature* **372**, 698 (1994).
14. C. Dascher, R. Ossig, D. Gallwitz, H. D. Schmitt, *Mol. Cell. Biol.* **11**, 872 (1991).
15. R. Ossig, C. Dascher, H. H. Trepte, H. D. Schmitt, D. Gallwitz, *ibid.*, p. 2980.
16. V. V. Lupashin, S. Hamamoto, R. W. Schekman, *J. Cell Biol.* **132**, 277 (1996).
17. J. Pevsner *et al.*, *Neuron* **13**, 353 (1994); K. L. Schulze *et al.*, *ibid.*, p. 1099.
18. *Saccharomyces cerevisiae* RSY255 (*MAT***a** *leu2-3-112 ura3-52*), RSY271 (*MAT***a** *sec18-1 his4-619 ura3-52*), RSY272 (*MAT***a** *sec18-1 his4-619 ura3-52*), and RSY976 (*MAT***a** *ypt1-3 ura3-52*) were from R. Schekman. GFUI-6D (*MAT***a** P_{Gal}-*YPT1 ΔYPT1::HIS3 ura3-52 leu2-3-112 trp1 his3*) was from H. D. Schmitt. GWY141 (*MAT***a** *sec18-1 ypt1-3 ura3-52*) was obtained from a cross of RSY976 and RSY272 strains. GWY142 (*MAT***a** *sec18-1* P_{Gal}-*YPT1 ΔYPT1::HIS his 4-619 ura3-52*) was obtained from a cross of RSY272 and GFUI-6D. Genotypes were confirmed by complementation analysis. Genetic manipulations were performed as described [M. Rose, F. Winston, P. Hieter, *Methods in Yeast Genetics: A Laboratory Course Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1990)].
19. The plasmids used were as follows: pQE9-Ypt1 [full-length Bam HI–Eco RI *YPT1* polymerase chain reaction (PCR) product was inserted into pQE9 (QIAGEN)], pGST-Sed5 [Bam HI–Eco RI PCR product encoding the cytoplasmic domain of Sed5p was inserted into pGEX2T (Pharmacia)], pGEXSar1 (full-length GST-Sar1p fusion from R. Schekman), pGEX-Sec22 [Bam HI–Eco RI PCR product encoding the cytoplasmic domain of Sec22 was inserted into pGEX2T (Pharmacia) from R. Schekman]. pNB166 (*YPT1 URA3*, *CEN*) and pNB167 (*YPT1 URA3*, 2 μm) were from S. Ferro-Novick. pYCP50-*SLY1-20* (*SLY1-20*, *URA3*, *CEN*) was from H. D. Schmitt. pSLY1-20 (*SLY1-20*, *URA3*, 2 μm) was constructed (Acc I–Cla I fragment of *SLY1-20* inserted into pRS426 [R. S. Sikorski and P. Hieter, *Genetics* **122**, 19 (1989)]} in our laboratory by S. K. Sapperstein.
20. C. Barlowe *et al.*, *Cell* **77**, 895 (1994).
21. S. K. Sapperstein, V. V. Lupashin, H. D. Schmitt, M. G. Waters, *J. Cell Biol.* **132**, 755 (1996).
22. The *ypt1-3* allele was sequenced and had a C-to-A nucleotide change at position 284 of *YPT1* (GenBank number D50617, open reading frame YFL038C), which results in a serine-to-tyrosine change at residue 95 of Ypt1p.
23. V. V. Lupashin and M. G. Waters, data not shown.
24. N. Halachmi and Z. Lev, *J. Neurochem.* **66**, 889 (1996).
25. P. I. Hanson, H. Otta, N. Barton, R. Jahn, *J. Biol. Chem.* **270**, 16955 (1995).
26. E. P. Garcia, P. S. McPherson, T. J. Chilcote, K. Takei, P. DeCamilli, *J. Cell Biol.* **129**, 105 (1995).
27. J. P. Lian and S. Ferro-Novick, *Cell* **73**, 735 (1993).
28. Rabbit polyclonal antibodies were raised to recombinant Sed5p-His_6, GST-Sec22p, GST-Sly1p, or GST-Ypt1p and affinity-purified by standard techniques [E. Harlow and D. Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988)]. Antibodies to Bet1p were from R. Schekman.
29. Cells were grown to mid-logarithmic phase at 23°C in either synthetic complete (SC) or SC-Ura media supplemented with 1% casamino acids, harvested by centrifugation (5000*g*, 5 min), converted to spheroplasts (*10*), regenerated during a 20-min incubation in YP media (1% yeast extract, 2% peptone) with 1% glucose and 0.8 M sorbitol at 23°C, and lysed (*10*). The lysate was centrifuged at 15,000*g* for 15 min at 4°C. A 0.5-mg amount of supernatant protein (usually about 100 μl) was diluted to 1 ml in IP buffer [150 mM NaCl, 1% Triton X-100, 15 mM tris-HCl, (pH 7.5)] and mixed gently for 14 hours at 4°C with 15 μl of protein A–Sepharose beads bearing covalently attached affinity-purified antibodies (*28*) as indicated in the figure legends. The beads were washed four times with 1 ml of IP buffer at 23°C, and eluted with 40 μl of 2% SDS at 90°C. The eluates were resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (12% gel), electro-transferred onto polyvinylidene difluoride membrane, and probed with the indicated antibodies (*28*). Bound antibodies were visualized with chemiluminescent detection (Renaissance, DuPont NEN).
30. RSY255 and RSY271 spheroplasts (*29*) were incubated for 20 min at 23° or 38°C, sedimented, transferred to ice, and lysed in 20 mM Hepes-KOH (pH 7.0) and 150 mM potassium acetate with protease inhibitors (*10*) by gentle pipetting for 10 min. One-half volume of freshly prepared 10 mM 3,3′-dithiobis(sulfosuccinimidylpropionate) was added, and the reaction was incubated for 1 hour at 4°C with gentle mixing. The reaction was terminated with 1/10 volume of 1 M tris-HCl (pH 7.4) and incubation for 10 min at 4°C. The samples were adjusted to 1% SDS, heated to 90°C for 5 min, and diluted with nine volumes of IP buffer (*29*). The samples were centrifuged for 10 min at 15,000*g*, and 0.5 mg of soluble material was incubated for 4 hours at 23°C with 15 μl of protein A–Sepharose beads bearing covalently attached antibodies (*28*) to Sed5p. The samples were washed four times with IP buffer and eluted with 40 μl of 2% SDS at 90°C. The eluates were separated from the beads, 10 μl of 0.5 M dithiothreitol was added, and the samples were incubated for 20 min at 23°C. Samples (15 μl) were separated by SDS-PAGE (12% gel) and immunoblotted with antibodies (*28*) to Sed5p or Sly1p.

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Promoter Recognition As Measured by Binding of Polymerase to Nontemplate Strand Oligonucleotide

M. T. Marr and J. W. Roberts

In transcription initiation, the DNA strands must be separated to expose the template to RNA polymerase. As the closed initiation complex is converted to an open one, specific protein-DNA interactions involving bases of the nontemplate strand form and stabilize the promoter complex in the region of unwinding. Specific interaction between RNA polymerase and the promoter in *Escherichia coli* was detected and quantified as the binding affinity of nontemplate oligonucleotide sequences. The RNA polymerase subunit sigma factor 70 contacted the bases of the nontemplate DNA strand through its conserved region 2; a mutation that affected promoter function altered the binding affinity of the oligonucleotide to the enzyme.

Escherichia coli RNA polymerase exists in two forms (*1*): a core enzyme (E) that consists of subunits $\beta\beta'\alpha_2$ and is sufficient for elongation, and a holoenzyme ($E\sigma^{70}$) that includes E and a sigma polypeptide required for specific initiation of transcription—usually the sigma factor 70 (σ^{70}). The holoenzyme recognizes primarily two hexameric sequences centered at −10 and −33, with +1 being the start of transcription; the −10 element is included in the region of initial promoter opening. The specificity for promoter recognition is carried by σ^{70}, which can bind to double-stranded promoter DNA (*2*). It is likely, however, that $E\sigma^{70}$ mediates promoter opening.

$E\sigma^{70}$-DNA interactions that form and stabilize the open-promoter complex in the region of unwinding involve primarily

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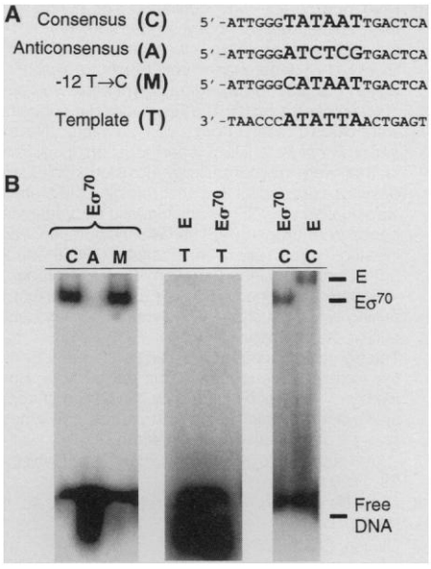

Fig. 1. EMSA for oligo binding. (**A**) Sequences of oligos C, A, M, and T are shown with the −10 element in larger letters. (**B**) RNA polymerase core (E) or holoenzyme (Eσ^{70}) was assayed for its ability to bind end-labeled oligos.

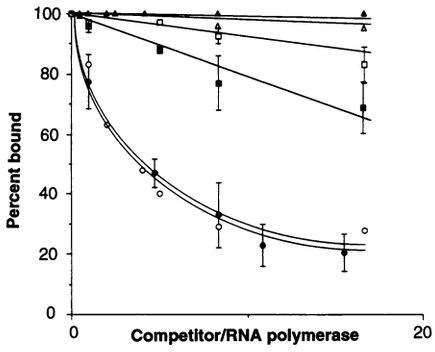

Fig. 2. Equilibrium competition between labeled oligo C and unlabeled oligo A (triangles), M (squares), or C (circles) for binding to RNA polymerase holoenzyme. Percentage bound is plotted as a function of competitor oligo per RNA polymerase. Open symbols designate Eσ^{70} and filled symbols designate Eσ$^{70}_{Q437H}$.

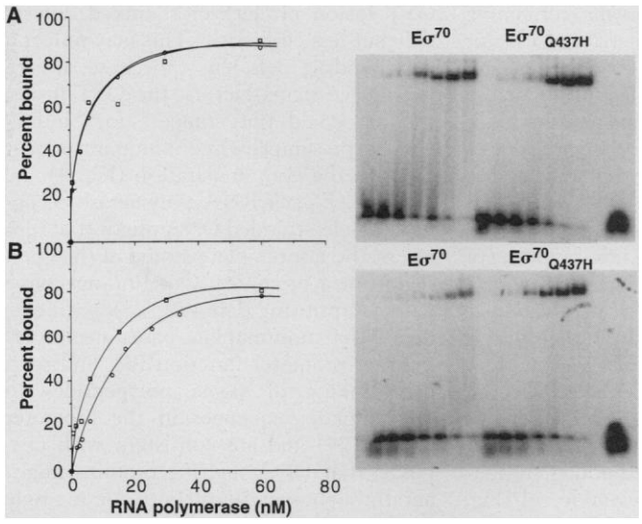

Fig. 3. Dependence of oligo binding on RNA polymerase concentration. Percentage of bound oligo C (**A**) or oligo M (**B**) is plotted against concentration of RNA polymerase. Circles denote Eσ^{70} and squares denote Eσ$^{70}_{Q437H}$. On the right of each graph is an image of a typical (but different and independent) EMSA.

the bases of the nontemplate DNA strand and not those of the template strand (*3*, *4*). We used an electrophoretic mobility-shift assay (EMSA) (*5*) to investigate the specific binding of holoenzyme (*2*, *6*–*9*) to small segments of single-stranded DNA that contain the −10 nontemplate strand promoter sequence. A 19-base oligonucleotide (oligo) (*10*, *11*) containing variants of the −10 element of the λ late gene promoter $P_{R'}$ was used as the single-stranded binding species (Fig. 1A). The λ sequences were chosen as a naturally occurring context for the different −10 hexamers. The nontemplate sequence of $P_{R'}$ from −18 to +1 was modified in the −10 region in three ways (Fig. 1A). Oligo C contained the nontemplate consensus TATAAT hexamer, whereas oligo A contained nucleotides rarely found at each position in the −10 hexamer (*12*); oligo M contained a T to C mutation at the −12 position, a strong down mutation in many promoters (*13*). Oligo T, the complement of oligo C (Fig. 1A), was used to determine possible interaction of template strand with RNA polymerase.

Eσ^{70} bound oligo C and oligo M but failed to shift oligo A or T (Fig. 1B). Because the surrounding sequences of the three nontemplate oligos are identical, this result implies that the holoenzyme recognizes the single-stranded nontemplate sequence in the −10 region. Core enzyme bound all three nontemplate oligos nonspecifically (Fig. 1B) (*14*), as would be expected from the existence of numerous specific and nonspecific binding sites in the ββ′ and the α subunits (*15*, *16*). The failure to bind the template oligo could be significant, or it might be an artifact of this particular sequence.

An equilibrium competition assay (*17*) was used to quantify the relative affinity of Eσ^{70} for the different oligos. Holoenzyme was incubated with labeled oligo C and increasing concentrations of unlabeled oligo competitor. After electrophoresis, the amount of radioactivity bound to the polymerase was measured (Fig. 2). Competition by unlabeled oligo C reflects a stoichiometry of binding of about one oligo per enzyme. Oligo A did not compete detectably even in 20-fold excess over polymerase (200-fold molar excess over oligo C). Oligo M competed poorly but detectably, by a factor of 15 to 20 less than oligo C. This result confirmed that Eσ^{70} binds specifically to the nontemplate sequence of the −10 element.

Several mutations in region 2.4 of σ^{70} have been isolated as suppressors of mutations in the −10 consensus (*13*, *18*, *19*). The σ^{70} mutation Gln^{437} → His (σ$^{70}_{Q437H}$) suppresses a T to C mutation at the −12 position (*13*), causing mutant promoter function to be about eight times greater, but not affecting wild-type promoter activity. To confirm that the detected binding reflects interactions important for promoter function, we used EMSA to examine the mutant sigma factor and the oligo containing the −12 T to C mutation (oligo M). σ$^{70}_{Q437H}$ was constructed with a histidine tag to facilitate purification; the histidine tag does not interfere with transcriptional activity or EMSA when σ^{70} is complexed with E (*14*). In vitro transcription with purified polymerase shows that the mutant sigma factor has wild-type activity on wild-type $P_{R'}$ and partially suppresses a T to C mutation in $P_{R'}$ (*14*). This is similar to the results of in vivo analysis (*13*).

In addition to Eσ^{70}, we tested Eσ$^{70}_{Q437H}$ in equilibrium competition against labeled oligo C (Fig. 2). As expected, oligo A failed to compete, and competition by oligo C showed identical stoichiometry for Eσ^{70} and Eσ$^{70}_{Q437H}$. However, oligo M competed with oligo C about threefold greater for binding to Eσ$^{70}_{Q437H}$ than to Eσ^{70}. The implication that Eσ$^{70}_{Q437H}$ binds oligo M with greater affinity than Eσ^{70} was verified by use of EMSA to measure an apparent binding constant (K_d) for oligos C and M with both mutant and wild-type polymerase (Fig. 3). Both Eσ^{70} and Eσ$^{70}_{Q437H}$ bound oligo C with a K_d of ~3 nM. However, Eσ$^{70}_{Q437H}$ bound oligo M with twofold greater affinity than Eσ^{70}, with K_d values of ~7 and ~15 nM, respectively. The difference was subtle but reproducible. Binding was also qualita-

tively different for holoenzyme containing the two sigma factors; mutant sigma factor displayed less smearing between the free and bound DNA, possibly reflecting a smaller dissociation rate. We presume that this binding preference accounts for suppression of the −12 T to C promoter mutation by the σ^{70}_{Q437H} mutation.

The difference in apparent K_d with the two sigma factors indicated that binding of the nontemplate oligos is directed by σ^{70}. To confirm this, we used an ultraviolet cross-linking assay (*20*); ultraviolet light is expected to cross-link only polypeptides that are in intimate contact with the DNA. Binding reactions containing labeled oligo C and various forms of RNA polymerase were irradiated and then analyzed on an SDS-polyacrylamide gel (Fig. 4). Although the β and β′ subunits cross-linked slightly, the predominant species had the mobility of the σ subunit. To verify that σ was cross-linked, we used holoenzyme reconstituted with three sigma polypeptides of different molecular mass that recognize the same −10 region, and also a σ^{70} truncation: *Bacillus subtilis* SigA ($E\sigma^A$; 43 kD), *E. coli* RpoS ($E\sigma^S$; 41 kD), domain 2 of sigma factor 70 ($E\sigma^{70}_{104-448}$; 39 kD), and σ^{70} amino acids 360 to 528 fused to glutathione-S-transferase (GST) ($E\sigma^{70}_{GST\ 360-528}$; 40 kD). Both the σ^{70} fragment, defining domain 2 (*21*), and the GST fusion product (*14*) were sufficient to bind oligo C in the context of holoenzyme. Each of the three sigma polypeptides cross-linked efficiently to oligo C (Fig. 4), shifting the mobility of the major cross-linked species according to the molecular mass of the sigma polypeptide used; SigA and RpoS ran larger than expected because full-length sigma polypeptides run anomalously in SDS–polyacrylamide gel electrophoresis. The GST fusion product cross-linked detectably but less efficiently. This may reflect a core binding deficiency, because, unlike the native sigma factors, the GST fusion product could not compete for binding with a (presumptive) contaminant σ fragment in the core preparation (Fig. 4).

Thus, *E. coli* RNA polymerase recognizes single-stranded DNA oligos that represent the nontemplate strand of the open region of a promoter. Our findings agree with footprinting data (*22*, *23*), with evidence that nontemplate bases are important for promoter function (*3*), and with cross-linking of sigma polypeptides to nontemplate sequences in the promoter region (*24*) and are consistent with proposals that DNA melting proteins might act through specific affinity for a single strand of DNA (*25*, *26*). Furthermore, we have shown that the agent of binding is the σ subunit. By the use of overlapping truncations in the cross-linking assay and a mutation in region 2.4 of σ^{70} that suppresses a promoter mutant, as well as by homology alignment with *B. subtilis SigA* (*27*), we have localized the region of interaction to amino acids 374 to 448 of σ^{70}, encompassing region 2. An atomic structure of region 2 has shown that Gln^{437} and other residues involved in recognizing the −10 segment and melting the DNA are arrayed on one face of an α helix, where they might contact the bases of the nontemplate DNA strand (*28*). Whether $E\sigma^{70}$ recognizes TATAAT specifically as double-stranded DNA and later transforms this binding to the single-strand interaction we describe, or whether single-strand binding is the only base-specific interaction in the region of melting, arising after other forces (for example, superhelical coiling energy) (*29*, *30*) initiate the opening process, remains unknown.

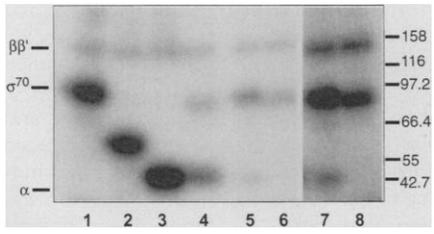

Fig. 4. UV cross-linking of RNA polymerase subunits to oligo C. Autoradiogram of SDS gel analysis of a UV cross-linking experiment is shown. Lanes 1 to 5, different forms of holoenzyme: $E\sigma^{70}$, $E\sigma^A$, $E\sigma^S$, $E\sigma^{70}_{104-448}$, and $E\sigma^{70}_{GST\ 360-528}$; lane 6, core (E) alone; lanes 7 and 8, overexposure of lanes 5 and 6 to show weakly cross-linking GST fusion sigma polypeptide. Positions of proteins were verified by silver staining and are shown on the left for $E\sigma^{70}$; molecular sizes of protein markers (in kilodaltons) are indicated on the right.

REFERENCES AND NOTES

1. R. Burgess *et al.*, in *RNA Polymerase and the Regulation of Transcription*, W. Reznikoff, Ed. (Elsevier, New York, 1987), p. 3.
2. A. J. Dombroski, W. A. Walter, M. T. J. Record, D. A. Siegele, C. A. Gross, *Cell* **70**, 501 (1992).
3. C. W. Roberts and J. W. Roberts, *ibid.* **86**, 495 (1996).
4. B. Z. Ring, W. S. Yarnell, J. W. Roberts, *ibid.*, p. 485.
5. For the EMSA, 60 nM polymerase and 6 nM oligo were incubated for 30 min at room temperature in binding buffer containing 20 mM tris-HCl (pH 8.0), 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, and bovine serum albumin (100 μg/ml). The complexes were run on a 5% 0.5× tris-borate EDTA native polyacrylamide gel at 4°C. The gel was imaged and radioactivity was measured on a Fuji BAS2000 or a PhosphorImager (Molecular Dynamics). Bound oligo was defined as any radioactivity of less mobility than the free DNA, including the defined band at the protein position and radioactivity released during electrophoresis. The positions of core and holoenzyme were determined by silver staining of standards.
6. RNA polymerase was purified (*7*) or purchased from Epicentre Technologies (Madison, WI). RNA polymerase core enzyme was further purified on Biorex70 (*8*) or purchased from Epicentre Technologies. The GST fusion sigma polypeptide was purified as described (*2*). The His_6-labeled σ subunits were overexpressed from a pET-28 vector (Novagen) in *E. coli* BL21(DE3). After induction, the sigma factors were renatured from inclusion bodies as described (*2*), except that after refolding the protein was purified with Ni^{2+}–nitrilotriacetic acid agarose beads from Qiagen (*9*). The Q437H mutation was created by polymerase chain reaction (PCR) with a primer containing the mutation. The PCR product was then subcloned into the pET-28 vector containing wild-type sigma polypeptide. Clones containing the mutation were verified by sequencing. The sigma polypeptide was then purified exactly as the wild-type His-tagged sigma polypeptide. Holoenzyme was reconstituted by incubation of core enzyme for 30 min on ice with excess (five times greater) of the desired sigma factor.
7. D. A. Hager, D. J. Jin, R. R. Burgess, *Biochemistry* **29**, 7890 (1990).
8. R. R. Burgess and J. J. Jendrisak, *ibid.* **14**, 4634 (1975).
9. Qiagen, Chatsworth, CA.
10. Oligos were synthesized at the Cornell DNA synthesis facility, radiolabeled as described (*11*) except that the reaction time was extended to 12 hours, and purified on an 8% polyacrylamide gel by a modification of the crush-and-soak method (*11*).
11. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989).
12. D. K. Hawley and W. R. McClure, *Nucleic Acids Res.* **11**, 2237 (1983).
13. C. Waldburger, T. Gardella, R. Wong, M. M. Susskind, *J. Mol. Biol.* **215**, 267 (1990).
14. M. T. Marr and J. W. Roberts, data not shown.
15. E. Nudler, E. Avetissova, V. Markovtsov, A. Goldfarb, *Science* **273**, 211 (1996).
16. W. Ross *et al.*, *ibid.* **262**, 1407 (1993).
17. EMSA was performed as described above, except that the oligo concentration was 0.05 nM, and the polymerase concentration varied from 0 to 60 nM.
18. D. A. Siegele, J. C. Hu, W. A. Walter, C. A. Gross, *J. Mol. Biol.* **206**, 591 (1989).
19. P. Zuber *et al.*, *ibid.*, p. 605.
20. For cross-linking, 20-μl binding reaction mixtures containing 6 nM oligo C and 60 nM RNA polymerase were exposed on ice for 10 min to 256-nm light from an ultraviolet transilluminator with the filter removed and analyzed on an SDS–8% polyacrylamide gel. The cross-linked species were visualized by autoradiography, and the proteins were localized by silver staining.
21. E. Severinova *et al.*, *J. Mol. Biol.* **263**, 637 (1996).
22. P. Schickor, W. Metzger, W. Werel, H. Lederer, H. Heumann, *EMBO J.* **9**, 2215 (1990).
23. U. Siebenlist, R. B. Simpson, W. Gilbert, *Cell* **20**, 269 (1980).
24. M. Buckle, J. Geiselmann, A. Kolb, H. Buc, *Nucleic Acids Res.* **19**, 833 (1991).
25. M. J. Chamberlin, *Annu. Rev. Biochem.* **43**, 721 (1974).
26. P. H. von Hippel and J. D. McGhee, *ibid.* **41**, 231 (1972).
27. M. Lonetto, M. Gribskov, C. A. Gross, *J. Bacteriol.* **174**, 3843 (1992).
28. A. Malhotra, E. Severinova, S. A. Darst, *Cell* **87**, 127 (1996).
29. J. E. Stefano and J. D. Gralla, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1069 (1982).
30. P. L. deHaseth and J. D. Helmann, *Mol. Microbiol.* **16**, 817 (1995).

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