

# Mooney and Landick Supplemental Material

## Materials and methods

### *Proteins*

Wild-type RNAP holoenzyme was purified from MRE600 *E. coli* cells as described previously (Hager et al. 1990).  $\beta'::\sigma^{70}$  RNAP was purified similarly from strain RL1094 (Supplementary Table 1), with modifications to remove  $\sigma^{70}$  or core RNAP, if present. Cells (3/4-log phase in 2X LB) were lysed and fractionated with polyethyleneimine (PEI; Acros Polymin P) as described (Burgess and Jendrisak 1975), with addition of protease inhibitors during lysis and addition of  $\text{ZnCl}_2$  to TGED buffer (TGEZD) as described by Polyakov *et al.* (1995). A fraction of  $\beta'::\sigma^{70}$  RNAP sometimes appeared in the 0.5 M NaCl TGEZD wash and was included in subsequent steps. After  $(\text{NH}_4)_2\text{SO}_4$  precipitation, crude  $\beta'::\sigma^{70}$  RNAP was adjusted to the conductivity of TGEZD + 0.3 M NaCl and fractionated on a 180 ml Heparin Sepharose 6 Fast Flow XK 26/40 column (Pharmacia; 5 ml/min) with a 300-ml gradient of TGEZD + 0.3 to 0.6 M NaCl after a 900 ml wash at 0.3 M NaCl.  $\beta'::\sigma^{70}$  RNAP eluting in a broad peak between 0.5 and 0.6 M NaCl was  $(\text{NH}_4)_2\text{SO}_4$ -precipitated, adjusted to the conductivity of TGEZD + 0.5 M NaCl, and fractionated on a Superdex HiLoad 26/60 column (Pharmacia; 2.5 ml/min) in TGEZD + 0.5 M NaCl. Pooled fractions containing  $\beta'::\sigma^{70}$  RNAP were adjusted to the conductivity of TGEZD + 0.15 M NaCl and fractionated on a 50 ml Bio-Rex 70 (Biorad) column (C 16/40, Pharmacia; equilibrated to TGEZD + 0.15 M NaCl; eluted with TGEZD + 0.6 M NaCl), as described by Burgess and Jendrisak (1975). Fractions containing  $\beta'::\sigma^{70}$  RNAP were pooled, adjusted to the conductivity of TGEZD + 0.25 M, and separated on a Mono-Q column (HR10/10, Pharmacia) as described (Hager et al. 1990). Pooled fractions containing  $\beta'::\sigma^{70}$  RNAP were concentrated with polysulfone centrifugal ultrafilters (Millipore Ultrafree) and dialyzed into storage buffer (0.01 M Tris, pH 7.9, 20% glycerol, 0.1 mM EDTA, 0.1 mM DTT, and 0.1 M NaCl).

Wild-type  $\sigma^{70}$  and NusA were purified as described (Gribskov and Burgess 1983; Chan and Landick 1989). HMK-tagged  $\sigma^{70}$  and His<sub>6</sub>-tagged  $\sigma^{32}$  were overexpressed as described by Arthur and Burgess (1998) and purified from the soluble fraction of cell lysates using Ni<sup>2+</sup>-NTA agarose (Qiagen) following the manufacturer's instructions.

### *Strain construction*

Plasmids and strains used in this study are listed in Supplementary Table 1. To construct the  $\beta'::\sigma^{70}$  fusion strain, pRM142 was transformed into RL324 and recombination was selected by growth on LB-Kan plates (Oden et al. 1990). Plasmid loss from single-colony isolates was confirmed by loss of ampicillin-resistance. Replacement of *rpoC* with the *rpoC::rpoD'*(9-603) fusion was confirmed by SDS-PAGE of cell lysates, which showed the presence of the  $\beta'::\sigma^{70}$  polypeptide and the absence of  $\beta'$ . P1vir lysate grown on this strain, RL1065, was used to transduce RL301, RL113, C600 K<sup>-</sup>, MC1060, and CAG20153 to give strains RL1374, RL1366, RL1390, RL1454, and RL1094, respectively.

### *Plating efficiencies and strain phenotypes*

In  $\beta'::\sigma^{70}$  strain RL1094, expression of chromosomal, non-tethered  $\sigma^{70}$  is under the control of the *trp* repressor and can be shut-off by withholding indole acrylic acid (IAA). To test function of the  $\sigma^{70}$  tethered to RNAP (Fig. 1), we compared the plating efficiency

of this strain and the same strain lacking  $\beta'::\sigma^{70}$  in the presence and absence of IAA. Equal volumes (3  $\mu$ l) of serially diluted early-log-phase cultures were plated on LB or LB-IAA plates. The efficiency of plating (EOP) is the number of colonies formed in the absence of IAA divided by the number formed in its presence.

To test the *in vivo* ability of  $\beta'::\sigma^{70}$  RNAP to protect against  $\sigma^{70}$ RC584 (Fig. 5), we evaluated the toxicity of increasing expression of  $\sigma^{70}$ RC584 in either a strain with only the wild-type level of  $\sigma^{70}$  or one that additionally contained  $\beta'::\sigma^{70}$  RNAP. The mutant  $\sigma^{70}$ RC584 was expressed from pRM389 in  $\Delta lacY$  strains (wild-type, RL113 and  $\beta'::\sigma^{70}$ , RL1366). Equal volumes of early log phase cultures were grown in LB-Amp (without induction of the mutant  $\sigma^{70}$ RC584), serially diluted and spotted onto plates containing increasing [IPTG] or no IPTG. EOP was determined at each [IPTG] as the number of colonies with IPTG divided by the number of colonies without IPTG and is the average of at least three measurements. Additional  $\sigma^{70}$  was produced in strains carrying pBAD $\sigma^{70}$  by inclusion of arabinose (0.2%) in the plates. We estimated that pBAD $\sigma^{70}$  overexpressed  $\sigma^{70}$   $\geq 5$ -fold (by quantitative immunoblot of cultures grown in liquid medium; data not shown).

To test *nus* factor function (Table 2), strains RL301 (wild-type  $\sigma^{70}$  level), RL1094 ( $\beta'::\sigma^{70}$ , no additional  $\sigma^{70}$ ), and RL1374 ( $\beta'::\sigma^{70}$  plus  $\sigma^{70}$ ) were grown overnight in lambda broth (10 g tryptone, 2.5 g NaCl per liter) supplemented with 0.2% maltose and 10 mM MgSO<sub>4</sub>. A portion of this culture (0.3 ml) was mixed with 2.5 ml lambda top agar (10 g tryptone, 2.5 g NaCl, and 7 g agar per liter) at 45 °C and plated onto lambda plates (10 g tryptone, 2.5 g NaCl, and 10 g agar per liter) at room temperature.  $\lambda$ C1c17 phage (requires *E. coli nus* functions; Friedman *et al.* 1976) was serially diluted into  $\lambda$  dilution media (5.8 g NaCl, 2 g MgSO<sub>4</sub> per liter of 50 mM Tris-HCl, pH 7.5); dilutions (3  $\mu$ l) were spotted onto bacterial lawns. EOP reported is the number of plaques formed on each strain divided by number of plaques formed on the wild-type strain and is the average of six measurements. No plaque formation was seen for a *nusA* mutant strain.

To test  $\sigma^{32}$  and  $\sigma^E$  function (Table 2), strains C600 K<sup>-</sup> (wild-type  $\sigma^{70}$  level), RL1094 ( $\beta'::\sigma^{70}$ , no additional  $\sigma^{70}$ ), and RL1390 ( $\beta'::\sigma^{70}$  plus  $\sigma^{70}$ ) in early log phase were serially diluted into LB and plated (3  $\mu$ l) onto LB plates. Plates were incubated at either 37 °C or 45 °C. EOP reported for each strain is the number of colonies formed at 45 °C divided by the number formed at 37 °C and is the average of six measurements.

To test  $\sigma^N$  function (Table 2), strains RL301 (wild-type  $\sigma^{70}$  level), RL1094 ( $\beta'::\sigma^{70}$ , no additional  $\sigma^{70}$ ), and RL1374 ( $\beta'::\sigma^{70}$  plus  $\sigma^{70}$ ) in early log phase were spun, washed and resuspended in W minimal media (per 500 ml: 5.25 g K<sub>2</sub>HPO<sub>4</sub>, 2.25 g KH<sub>2</sub>PO<sub>4</sub>, 10 ml 20% glucose, 10 mg thiamine, 0.215 ml of 1M MgSO<sub>4</sub>), serially diluted, and plated (3  $\mu$ l) onto either minimal W medium (as above, plus 7.5 g agar) containing Arg (0.1 mg/ml) or Gln (2 mg/ml). Growth on W-Arg plates requires  $\sigma^N$ -directed transcription of *glnA*, growth on Gln-containing media is  $\sigma^N$ -independent (Tintut and Gralla 1995). EOP is the number of colonies on W-Arg plates divided by the number on W-Gln plates and is the average of three measurements. No growth was evident on W-Arg plates for a strain deleted for *rpoN* ( $\sigma^N$ ); this strain grew normally on W-Gln.

To test  $\sigma^F$  function (Table 2), strains C600 K<sup>-</sup> (wild-type  $\sigma^{70}$  level), RL1094 ( $\beta'::\sigma^{70}$ , no additional  $\sigma^{70}$ ), and RL1390 ( $\beta'::\sigma^{70}$  plus  $\sigma^{70}$ ) in early log phase were serially diluted and stabbed with a glass pipette into motility agar plates (15 g peptone, 5 g NaCl, and 2.5 g agar per liter, Togashi *et al.*, 1997). Flagella synthesis requires  $\sigma^F$  function; control strains lacking  $\sigma^F$  (RL301, RL1374; see Jishage and Ishihama 1997) were non-

motile under the testing conditions. The swarm size was measured after 12 hours incubation at 37 °C; swarm size (diameter in mm) was divided by the swarm size of the wild-type strain and is the average of six measurements.

To test  $\sigma^S$  function (Table 2), strains C600 K<sup>-</sup> (wild-type  $\sigma^{70}$  level), RL1094 ( $\beta'::\sigma^{70}$ , no additional  $\sigma^{70}$ ), and RL1390 ( $\beta'::\sigma^{70}$  plus  $\sigma^{70}$ ) were grown overnight to stationary phase. 10  $\mu$ l of 3% H<sub>2</sub>O<sub>2</sub> was added to 50  $\mu$ l of liquid culture spotted onto a petri dish (Farewell et al., 1998). O<sub>2</sub> evolution was observed as strong bubbling, indicating  $\sigma^S$ -dependent expression of catalase from *kat*. No such strong bubbling was observed for a strain lacking *rpoS* (encoding  $\sigma^S$ ).

**Supplementary Table 1.** Strains, plasmids, oligonucleotides, and transcription templates

Name	Description	Source or note
<u>Strains</u>		
C600K <sup>-</sup>	C600 <i>galK</i>	<i>E. coli</i> genetic stock center
CAG20153	C600 <i>galK</i> , $\Omega$ (Cam)-P <sub>trp</sub> - <i>rpoD</i>	(Lonetto et al. 1998)
MC1060	$\Delta$ <i>lacX74 galU galK hsr<sup>-</sup> hsm<sup>+</sup> strA</i>	<i>E. coli</i> genetic stock center
RL301	CY15001; W3110 <i>trpR tnaA2</i>	(Yanofsky and Horn 1981)
RL113	RL301 $\Delta$ <i>lacU169</i>	This work
RL324	JC7623; $\lambda$ F <sup>-</sup> <i>recB21 recC22 sbcB15 thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his4 argE3 rpsL31(Str) tsx-33 supE44</i>	(Horii and Clark 1973)
RL585	<i>leu</i> (Am) <i>trp</i> (Am) <i>lacZ</i> 2110(Am) <i>galK</i> (Am) <i>galE rpsL tsx supD43,74</i> (Ts) <i>sueA rpoBcI</i> (Am) $\Delta$ ( <i>recA-srl</i> )306 <i>srl-301::Tn10-84</i>	(Landick et al. 1990)
RL602	<i>leu</i> (Am) <i>trp</i> (Am) <i>lacZ</i> 2110(Am) <i>galK</i> (Am) <i>galE rpsL tsx sueB sueC supD43,74</i> (Ts) <i>rpoC325</i> (Am) $\Delta$ ( <i>recA-srl</i> )306 <i>srl-301::Tn10-84</i>	(Weilbaecher et al. 1994)
RL1065	pRM142 recombined with RL324; <i>rpoC::rpoD zja::kan</i>	This work
RL1094	C600K <sup>-</sup> , $\Omega$ (Cam)-P <sub>trp</sub> - <i>rpoD rpoC::rpoD zja::kan</i>	This work
RL1366	RL301 $\Delta$ <i>lacU169 rpoC::rpoD zja::kan</i>	This work
RL1374	W3110 <i>trpR tnaA2 rpoC::rpoD zja::kan</i>	This work
RL1454	MC1060 <i>rpoC::rpoD zja::kan</i>	This work
<u>Plasmids</u>		
pBAD $\sigma^{70}$	P15 <i>ori spc P<sub>araB</sub>-rpoD</i> derived from pBAD33.	(Ko et al. 1998)
pCL102b	T7 A1 promoter - A29 - <i>his</i> pause	(Chan and Landick 1989)
pCL391	<i>bla ColEIori lacI<sup>q</sup> P<sub>trc</sub> rpoD</i>	(Sharp et al. 1999)

**Table S1 continued.**

pET-H <sub>6</sub> HMKσ <sup>70</sup>	T7p- His <sub>6</sub> -tagged <i>rpoD</i>	(Arthur and Burgess 1998)
pET-H <sub>6</sub> σ <sup>32</sup>	T7p- His <sub>6</sub> -tagged <i>rpoH</i>	T. Arthur, unpubl.
pRL648	<i>bla</i> ColEI <sub>ori</sub> M13 <sub>ori</sub> <i>lacI</i> <sup>q</sup> (QK78) P <sub>trc</sub> <i>rpoC</i> (BCCP) <i>zja::kan</i>	R. Landick, unpubl.
pRL663	<i>bla</i> ColEI <sub>ori</sub> M13 <sub>ori</sub> <i>lacI</i> <sup>q</sup> (QK78) P <sub>trc</sub> <i>rpoC</i> (H <sub>6</sub> )	(Severinov et al. 1997)
<u>Plasmids, continued</u>		
pRL706	<i>bla</i> ColEI <sub>ori</sub> M13 <sub>ori</sub> <i>lacI</i> <sup>q</sup> (QK78) P <sub>trc</sub> <i>rpoB</i> (H <sub>6</sub> )	(Severinov et al. 1997)
pRLG770- <i>UP full con</i>	full consensus promoter with strong UP element derived from pRLG3749 ( <i>full con</i> promoter)	T. Gaal, unpubl.
pRLG3749	pRLG770 with <i>full con</i> promoter (-54 to +16)	(Gaal et al. 2001)
pRM103	pRL648 with <i>HindII</i> site downstream of <i>kan</i> knocked out by partial digestion and Klenow treatment	This work
pRM142	<i>bla</i> ColEI <sub>ori</sub> M13 <sub>ori</sub> <i>lacI</i> <sup>q</sup> (QK78) P <sub>trc</sub> <i>rpoC::rpoD'</i> (9-603) <i>zja::kan</i> . <i>Sall</i> - <i>HindIII</i> from pRM314 into similarly cut pRM103	This work
pRM314	<i>bla</i> ColEI <sub>ori</sub> M13 <sub>ori</sub> <i>lacI</i> <sup>q</sup> (QK78) P <sub>trc</sub> <i>rpoC::rpoD'</i> (9-603). <i>EcoRI</i> (Klenow treated)- <i>HindIII</i> fragment from pCL391 into <i>XhoI</i> (Klenow-treated) - <i>HindIII</i> of pRL663	This work
pRM317	<i>bla</i> ColEI <sub>ori</sub> M13 <sub>ori</sub> <i>lacI</i> <sup>q</sup> (QK78) P <sub>trc</sub> <i>rpoC::rpoD'</i> (9-529):: <i>rpoH</i> ( <i>C-term.82aa</i> ). <i>XhoI</i> - <i>HindIII</i> of pSAK273 into pRM314 <i>XhoI</i> - <i>HindIII</i>	This work
pRM328	<i>bla</i> ColEI <sub>ori</sub> M13 <sub>ori</sub> <i>lacI</i> <sup>q</sup> (QK78) P <sub>trc</sub> <i>rpoB::rpoD'</i> (9-603). <i>EcoRI</i> (Klenow-treated)- <i>HindIII</i> of pCL391 into pRL706 <i>XhoI</i> (Klenow-treated)- <i>HindIII</i>	This work
pRM389	<i>bla</i> ColEI <sub>ori</sub> <i>lacI</i> <sup>q</sup> P <sub>trc</sub> <i>rpoD</i> (RC584); pCL391 <i>rpoD</i> (RC584)	(Siegele et al. 1989); this work.
pRM444	T7 A1 promoter, consensus pause at 462	this work
pSAK273	P <sub>lac</sub> <i>rpoD</i> (1-529):: <i>rpoH</i> ( <i>C-term.82aa</i> )	(Kumar et al. 1995)
pX20con	λP <sub>R</sub> promoter DNA with 20 bp insertion, consensus pause at +37	(Ring et al. 1996)

**Table S1 continued.**

pXY306	$\lambda P_{R'}$ promoter DNA, wild-type pause at +16	(Ring et al. 1996)
<u>Primers</u>		
213	5'-CAGCTTCACCCACGTTC	<i>rpoB</i> reverse
645	5'-CAGTTCCTACTCTCGCATG	T7 A1 DS
947	GGAGAGACA ACTTAAAGAG	T7A1 US
3148	TTTGAATGCGGTCACACGTTAGCA	$\lambda P_{R'}$ US
3638	GTCAGAGGATTCCGCCAGAATTC	$\lambda P_{R'}$ DS
<u>Primers, continued</u>		
3260	GACGGCGATGAAGAAATTGCG	GroE US
3261	CCGTATCTGTTATGGTGACGCC	GroE DS
4371	CCGCGGATCGTATCACGAGGCCCTTTCG	$P_{UPFullcon}$ US
4372	GCGCTACGGCGTTTCACTTCTGAGTTC	$P_{UPFullcon}$ DS
<u>Transcription Templates</u>		
T7 A1 (A29; <i>his</i> pause)	PCR of pCL102b with 947 and 645	
$P_{UPFullcon}$	PCR of pRLG770- <i>UP full con</i> with 4371 and 4372	
$\lambda P_{R'}$ pause +16 and +37	PCR of pXJ306 or pX20con with 3148 and 3638	
T7 A1(+462 pause)	PCR of pRM444 with 947 and 213	

**References**

- Arthur, T.M. and Burgess, R.R. 1998. Localization of a  $\sigma^{70}$  binding site on the N terminus of the *Escherichia coli* RNA polymerase  $\beta'$  subunit. *J. Biol. Chem.* **273**: 31381-31387.
- Burgess, R.R. and Jendrisak, J.J. 1975. A procedure for the rapid, large-scale purification of *Escherichia coli* DNA-dependent RNA polymerase involving polymin P precipitation and DNA-cellulose chromatography. *Biochemistry* **14**: 4634-4638.
- Chan, C. and Landick, R. 1989. The *Salmonella typhimurium his* operon leader region contains an RNA hairpin-dependent transcription pause site. *J. Biol. Chem.* **264**: 20796-20804.

- Friedman, D.I., Baumann, M., Baron, L.S. 1976. Cooperative effects of bacterial mutations affecting  $\lambda$  N gene expression. I. Isolation and characterization of a nusB mutant *Virology* **73**:119-127
- Gaal, T., Ross, W., Estrem, S.T., Nguyen, L.H., Burgess, R.R., and Gourse, R.L. 2001. Promoter recognition and discrimination by E $\sigma$ S RNA polymerase. *Mol. Microbiol.* **42**: 939-954.
- Gribskov, M. and Burgess, R.R. 1983. Overexpression and purification of the  $\sigma$  subunit of *Escherichia coli* RNA polymerase. *Gene* **26**: 109-118.
- Hager, D.A., Jin, D.J., and Burgess, R.R. 1990. Use of Mono Q high-resolution ion-exchange chromatography to obtain highly pure and active *Escherichia coli* RNA polymerase. *Biochemistry* **29**: 7890-7894.
- Horii, Z. and Clark, A.J. 1973. Genetic analysis of the recF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. *J. Mol. Biol.* **80**: 327-344.
- Jishage, M., Ishihama, A. 1997. Variation in RNA polymerase  $\sigma$  subunit composition within different stocks of *Escherichia coli* W3110. *J. Bacteriol.* **179**: 959-963.
- Ko, D.C., Marr, M.T., Guo, J., and Roberts, J.W. 1998. A surface of *Escherichia coli*  $\sigma^{70}$  required for promoter function and antitermination by phage lambda Q protein. *Genes & Dev.* **12**: 3276-3285.
- Kumar, A., Grimes, B., Logan, M., Wedgwood, S., Williamson, H., and Hayward, R.S. 1995. A hybrid sigma subunit directs RNA polymerase to a hybrid promoter in *Escherichia coli*. *J. Mol. Biol.* **246**: 563-571.
- Landick, R., Stewart, J., and Lee, D.N. 1990. Amino acid changes in conserved regions of the  $\beta$ -subunit of *Escherichia coli* RNA polymerase alter transcription pausing and termination. *Genes & Dev.* **4**: 1623-1636.
- Lonetto, M.A., Rhodius, V., Lamberg, K., Kiley, P., Busby, S., and Gross, C. 1998. Identification of a contact site for different transcription activators in region 4 of the *Escherichia coli* RNA polymerase  $\sigma^{70}$  subunit. *J. Mol. Biol.* **284**: 1353-1365.
- Oden, K., DeVaux, L., Vibat, C., Cronan Jr., J., and Gennis, R. 1990. Genomic replacement in *Escherichia coli* K-12 using covalently closed circular plasmid DNA. *Gene* **96**: 29-36.
- Polyakov, A., Severinova, E., and Darst, S. 1995. Three-dimensional structure of *E. coli* core RNA polymerase: promoter binding and elongation conformations of the enzyme. *Cell* **83**: 365-373.
- Ring, B., Yarnell, W., and Roberts, J. 1996. Function of *E. coli* RNA polymerase  $\sigma$  factor  $\sigma^{70}$  in promoter-proximal pausing. *Cell* **86**: 485-493.
- Severinov, K., Mooney, R., Darst, S., and Landick, R. 1997. Tethering of the large subunits of *Escherichia coli* RNA polymerase. *J. Biol. Chem.* **272**: 24137-24140.
- Sharp, M.M., Chan, C.L., Lu, C.Z., Marr, M.T., Nechaev, S., Merritt, E.W., Severinov, K., Roberts, J.W., and Gross, C.A. 1999. The interface of  $\sigma$  with core RNA polymerase is extensive, conserved, and functionally specialized. *Genes & Dev.* **13**: 3015-3026.

- Siegele, D.A., Hu, J.C., Walter, W.A., and Gross, C.A.. 1989. Altered promoter recognition by mutant forms of the  $\sigma^{70}$  subunit of *Escherichia coli* RNA polymerase. *J. Mol. Biol.* **206**: 591-603.
- Tintut, Y. and Gralla, J.D. 1995. PCR mutagenesis identifies a polymerase-binding sequence of  $\sigma^{54}$  that includes a  $\sigma^{70}$  homology region. *J. Bacteriol.* **177**: 5818-5825.
- Togashi, F., Yamaguchi, S., Kihara, M., Aizawa, S.I., and Macnab, R.M. 1997. An extreme clockwise switch bias mutation in fliG of *Salmonella typhimurium* and its suppression by slow-motile mutations in motA and motB. *J Bacteriol* **179**: 2994-3003.
- Weilbaecher, R., Hebron, C., Feng, G., and Landick, R. 1994. Termination-altering amino acid substitutions in the  $\beta'$  subunit of *Escherichia coli* RNA polymerase identify regions involved in RNA chain elongation. *Genes & Dev.* **8**: 2913-2917.
- Yanofsky, C. and Horn, V. 1981. Rifampicin resistance mutations that alter the efficiency of transcription termination at the tryptophan operon attenuator. *J. Bacteriol.* **145**: 1334-1341.