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A Mutant σ^{32} with a Small Deletion in Conserved Region 3 of σ Has Reduced Affinity for Core RNA Polymerase

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σ^{70}, encoded by *rpoD*, is the major σ factor in *Escherichia coli*. *rpoD285* (*rpoD800*) is a small deletion mutation in *rpoD* that confers a temperature-sensitive growth phenotype because the mutant σ^{70} is rapidly degraded at high temperature. Extragenic mutations which reduce the rate of degradation of RpoD285 σ^{70} permit growth at high temperature. One class of such suppressors is located in *rpoH*, the gene encoding σ^{32}, an alternative σ factor required for transcription of the heat shock genes. One of these, *rpoH113*, is incompatible with *rpoD*$^+$. We determined the mechanism of incompatibility. Although RpoH113 σ^{32} continues to be made when wild-type σ^{70} is present, cells show reduced ability to express heat shock genes and to transcribe from heat shock promoters. Glycerol gradient fractionation of σ^{32} into the holoenzyme and free sigma suggests that RpoH113 σ^{32} has a lower binding affinity for core RNA polymerase than does wild-type σ^{32}. The presence of wild-type σ^{70} exacerbates this defect. We suggest that the reduced ability of RpoH113 σ^{32} to compete with wild-type σ^{70} for core RNA polymerase explains the incompatibility between *rpoH113* and *rpoD*$^+$. The *rpoH113* cells would have reduced amounts of σ^{32} holoenzyme and thus be unable to express sufficient amounts of the essential heat shock proteins to maintain viability.

Transcription in *Escherichia coli* is carried out by RNA polymerase, which exists in two forms: core RNA polymerase (α_2, β, β'; or E), which carries out elongation and termination, and holoenzyme (α_2, β, β', σ; or Eσ), which carries out specific initiation at promoter regions of the DNA (2). *E. coli* contains multiple sigma factors, and the specificity of promoter binding is determined by the particular σ subunit associated with the holoenzyme (for reviews, see references 19 and 37). One way to regulate global gene expression is to modulate the interaction of different σ factors with core RNA polymerase in response to physiological and environmental changes (19, 26, 37).

σ^{70}, the major σ factor in *E. coli*, is encoded by *rpoD* (12, 18, 31) and is essential for cell growth (17, 25, 35). The *rpoD285* mutation and the genetically identical *rpoD800* mutation confer a temperature-sensitive (Ts) growth phenotype (17, 20, 25, 30, 35) because this mutant sigma factor is unstable and rapidly degraded at high temperature (12, 13). Structural changes in the mutant sigma factor resulting from the 42-bp in-frame deletion in the *rpoD285* (*rpoD800*) allele presumably lead to its instability in vivo (13, 20) and in vitro (27). One class of extragenic suppressors of *rpoD285* is located in *rpoH* (*htpR*), the gene which encodes σ^{32} (14, 32, 33, 45). σ^{32} directs transcription initiation from heat shock promoters (5, 43, 45, 46) and is required for cell growth at temperatures above 20°C (47). Suppressors located in *rpoH* restore growth at high temperature by stabilizing the mutant σ^{70} (1, 16).

One of the *rpoH* alleles that suppresses the Ts growth phenotype of *rpoD285* is *rpoH113*, which results from an in-frame 72-bp deletion within *rpoH* (3). Interestingly, cells with this *rpoH* allele are unable to grow when wild-type σ^{70} is present (3, 16). We investigated the mechanism of incompatibility between *rpoH113* and *rpoD*$^+$ because we thought it likely that such a study could provide some insight into how various sigma factors interact in the cell to modulate transcription. Our results indicate that the mutant σ^{32} is present in cells expressing wild-type σ^{70} but is unable to promote transcription from heat shock promoters, probably because it is ineffective in competing with wild-type σ^{70} for binding to core RNA polymerase. This would lead to diminished expression of those heat shock genes essential for cell growth and the observed incompatibility between *rpoH113* and *rpoD*$^+$.

MATERIALS AND METHODS

Bacteria and plasmids. The *E. coli* K-12 strains and the plasmids used in this study are listed in Table 1. Plasmid pP_L-*rpoH113*, a gift of Richard Calendar, contains the *rpoH113* gene cloned into a pBR322 derivative that has a 3.0-kb *Eco*RI fragment from bacteriophage λ carrying the λ p_L promoter and λ *c*I857 repressor. pMRG7, a gift of Michael Gribskov and Richard R. Burgess, contains the *rpoD* gene on an *Hpa*I-*Pvu*II fragment inserted into pBR322 at the *Hin*dII site. Expression of *rpoD* is driven from the *lac*UV5 promoter carried on a 203-bp *Eco*RI fragment from pRZ4029 that is inserted in the *Eco*RI site of pBR322.

Plating efficiency. Overnight cultures grown in M9-glucose with ampicillin (50 μg/ml), supplemented with all the amino acids except proline, were diluted with 1× M9 salts and plated on M9-glucose-ampicillin (50 μg/ml) plates supplemented with all amino acids except proline. Where indicated, the plates contained 1.0 mM isopropyl-β-D-thiogalactopyranoside (IPTG) to induce expression of wild-type σ^{70}. The number of colonies was determined after incubating the plates either at 16 to 18°C for 10 days or at 30°C for 1 to 2 days.

Heat shock protein synthesis. For analysis of the rate of heat shock protein synthesis on one-dimensional gels, cells were grown in M9-glucose-ampicillin (50 μg/ml) with all the amino acids except methionine and proline. Then, 0.5 ml of exponentially growing cells was pulse-labeled with 10 μCi of

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TABLE 1. Bacterial strains and plasmid

Strain or plasmid	Relevant genotype	Origin or reference
Strains		
CAG9166	*thi lacZ4 argG75*/pMRG7	This lab; derivative of P90A5c (3)
CAG9214	*rpoD285 thi lacZ4 argG75*/pMRG7	This lab; derivative of 285 (3)
CAG9170	*rpoD285 rpoH113*/pMRG7	This lab; derivative of PM113 (3)
CAG11033	*rpoH113* λ cI857 λ p_L-*rpoH113*	R. Calendar
CAG11054	Δ(*lac pro*) *rpoH*$^+$ *supC*(Ts)/F' *lacI*^{q} Tn*5*::*lacZ*/P*tac-rpoH*	This lab; derivative of CSH26
Plasmid pMRG7	p*lacUV5-rpoD*$^+$	R. Burgess

[^{35}S]methionine (800 to 1,000 Ci/mmol) for 2 min and chased with an excess of cold methionine (3 mM) for 1.5 min either prior to or at various times following the addition of IPTG (1 mM) to induce the *rpoD*$^+$ gene. The labeled cells were precipitated on ice in 5% trichloroacetic acid, spun down, washed twice with 80% acetone, and dried under vacuum. The precipitates were resuspended in sodium dodecyl sulfate (SDS) sample buffer, and equal counts of samples were analyzed on SDS-polyacrylamide gels (22). The gels were then dried and subjected to autoradiography.

For the experiment presented in Fig. 2 and Table 2, relative synthesis rates were measured by pulse-labeling followed by two-dimensional gel analysis. For these experiments, cells were grown in M9-glucose-ampicillin (50 μg/ml) medium lacking leucine, lysine, and proline, and 1-ml aliquots of exponentially growing cells were pulse-labeled with 35 μCi of [^{3}H]leucine (52 Ci/mmol) and 35 μCi of [^{3}H]lysine (46 Ci/mmol) for 2 min and chased with excess cold leucine and lysine for 1.5 min. Samples were prepared and analyzed by two-dimensional gel electrophoresis as described by O'Farrell (34). A constant amount of [^{35}S]methionine-labeled mutant or wild-type culture, obtained by labeling cells at 30°C for 20 min with [^{35}S]methionine and prepared by the method of O'Farrell (34), was added to each experimental sample labeled with [^{3}H]leucine and [^{3}H]lysine to permit normalization. The radioactive spots corresponding to the proteins to be analyzed were cut out from the gel and solubilized, and radioactivity was counted. The synthesis rate of these proteins was determined relative to total protein synthesis as described previously (11, 15).

S1 mapping. RNA was isolated by the method of Salser et al. (38). The S1 nuclease method used for mapping of *groE* transcripts has been described before (47). The labeled DNA probe was present in excess, and the amount of RNA used is indicated in the figure legends. Samples were hybridized overnight at 48°C, treated with S1 nuclease at 37°C for 60 min, and analyzed on a 6% polyacrylamide sequencing gel. Experiments were quantified by measuring the radioactivity in DNA fragments cut from gels.

Measuring the level of σ^{32}. The level of σ^{32} in the cell was examined by a Western immunoblot analysis described by Straus et al. (41), except that cells were grown in M9-glucose-ampicillin with all the amino acids minus proline. To control for sample loss, a constant volume of cell extract from CAG11033 (which overproduces the lower-molecular-weight form of σ^{32} encoded by *rpoH113*) was added to *rpoH*$^+$ samples before analysis. Similarly, a constant volume of cell extract from CAG11054 (which overproduces wild-type σ^{32}) was added to *rpoH113* strain samples before analysis.

Glycerol gradient analysis. Cell lysates were prepared by a modification of the method described by Fujita et al. (9, 40a). Cells were grown in M9-glucose-ampicillin (50 μg/ml) with all the amino acids except proline with or without IPTG (1 mM) to induce the *rpoD*$^+$ gene. Ten milliliters of culture was harvested by centrifugation and resuspended in 0.4 ml of 10 mM Tris-HCl buffer (pH 7.9) containing 25% sucrose and 0.1 M NaCl. Following addition of EDTA (1 mM) and lysozyme (0.5 mg/ml), cells were incubated on ice for 5 min, and Brij-58 (0.5%, vol/vol) and phenylmethylsulfonyl fluoride (1 mM) were added. Cells were sonicated for 10 s, and the cell lysate was incubated with DNase (20 mg/ml) and RNase (0.1 mg/ml) in the presence of Mg^{2+} (10 mM) for 1 h on ice. The entire sample (0.5 ml) was loaded on a 12-ml 15 to 30% (vol/vol) glycerol gradient in 10 mM Tris-HCl (pH 7.9)–10 mM $MgCl_2$–0.5 mM EDTA–0.1 mM dithiothreitol–0.2 M NaCl and centrifuged for 21 h at 37,000 rpm and 4°C in a Beckman SW40.1 rotor. Fractions (0.9 ml) were collected and subjected to Western blot analysis with anti-σ^{32} antiserum to determine the distribution of σ^{32}.

RESULTS

Experiment rationale. The *rpoH113* mutation was isolated as an extragenic suppressor of *rpoD285* (*rpoD800*) (16). In order to study the phenotype of the *rpoH113* single-mutant strain, we tried to move an *rpoD*$^+$ allele into the strain by P1 transduction with a linked Tn*10* marker. However, we were unable to construct this strain, indicating that *rpoD*$^+$ and *rpoH113* were incompatible (3, 16; data not shown). To understand the reason for this incompatibility, we have introduced a plasmid carrying the wild-type *rpoD*$^+$ gene under control of the inducible *lacUV5* promoter into the mutant and wild-type strains. Such strains exhibit conditional expression of wild-type σ^{70}. When grown in the presence of IPTG, wild-type σ^{70} is present in the cell. We have used these strains to systematically investigate the effect of wild-type σ^{70} on the expression and function of RpoH113 σ^{32}.

Incompatibility of *rpoH113* and *rpoD*$^+$ results from loss of σ^{32} function. The incompatibility of *rpoD*$^+$ and *rpoH113* could result from reduced function of RpoH113 σ^{32}, an idea suggested from the observation that increasing the amount of RpoH113 σ^{32} restores compatibility (3). If so, *rpoH113* should exhibit behavior similar to null alleles of *rpoH*. Such alleles are viable at temperatures only up to 20°C, indicating that σ^{32} function is required for viability above this temperature (47). To determine if this was the case, we examined colony-forming ability as a function of temperature when plasmid-encoded wild-type σ^{70} was induced by IPTG. Induction of wild-type σ^{70} reduced the efficiency of plating of the *rpoD285 rpoH113* cells more than 100-fold at 30°C (Table 2), which is consistent with our inability to construct an *rpoD*$^+$ *rpoH113* strain by transduction. However, induction of wild-type σ^{70} did not affect the efficiency of plating at 16°C. These data indicate that *rpoH113* and *rpoD*$^+$ are incompatible only when σ^{32} function is necessary for viability. When σ^{32} function is unnecessary for viability, the two are compatible. These results are consistent with the idea that the "incompatibility" of RpoH113 σ^{32} and wild-type σ^{70} reflects the loss or reduction of σ^{32} function in cells containing wild-type σ^{70}. Furthermore, it suggested that we could construct an *rpoD*$^+$ *rpoH113* strain by P1 transduction if we

TABLE 2. Effect of wild-type σ^{70} on viability[a]

Genotype	Temp (°C)	No. of colonies	
		−IPTG	+IPTG
rpoD285 rpoH113/(P*lac-rpoD*)	16	850	788
	30	952	4
rpoD285 rpoH$^+$/(P*lac-rpoD*)	30	210	165

[a] Equal volumes of cultures with the same A_{450} were plated on M9-glucose-ampicillin plates containing all the amino acids minus proline with and without IPTG to induce wild-type σ. Viable cells were counted after incubation at 16°C (5 to 10 days) or 30°C (1 day).

allowed the transductants to grow at temperatures of <20°C. This proved to be the case (data not shown).

RpoH113 σ^{32} appears to function normally with RpoD285 σ^{70} but not with RpoD$^+$ σ^{70}. The experiments described above suggested that the incompatibility of *rpoH113* and *rpoD*$^+$ results from reduced function of RpoH113 σ^{32} in the presence of wild-type σ^{70}. As a corollary, the compatibility of *rpoH113* and *rpoD285* suggests that RpoH113 σ^{32} functions normally with RpoD285 σ^{70}. One way to assess σ^{32} function is to examine the heat shock response, in which a transient increase in transcription of heat shock genes occurs in response to the transient accumulation of σ^{32} (24, 39, 41). We therefore examined the effect of wild-type σ^{70} on the heat shock response in mutant cells. The heat shock response in the *rpoH113 rpoD285* double-mutant strain is similar to that in wild-type cells, with a transient, dramatic increase in heat shock protein synthesis immediately upon upshift (Fig. 1, compare lanes 1 to 3 with lanes 13 to 15), suggesting that RpoH113 σ^{32} is probably functioning normally in the *rpoH113 rpoD285* strain. In contrast, expression of heat shock proteins in the *rpoH113 rpoD285* strain is dramatically reduced when wild-type σ^{70} is induced prior to temperature upshift (Fig. 1, compare lanes 13 to 15 with lanes 16 to 18), indicating that RpoH113 σ^{32} does exhibit reduced function in the presence of wild-type σ^{70}. Control experiments indicate that reduced expression of heat shock proteins following induction of σ^{70} is dependent on the *rpoH113* allele, as this procedure has only a small effect on the heat shock response in wild-type cells (Fig. 1, compare lanes 4 to 6 with lanes 1 to 3) or in the *rpoD285* strain (Fig. 1, compare lanes 10 to 12 with lanes 4 to 6).

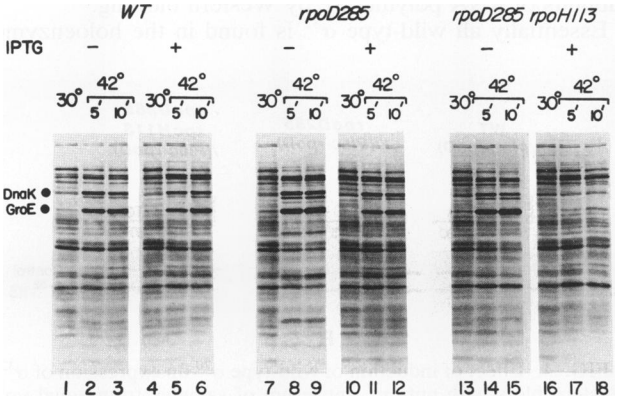

FIG. 1. Heat shock protein synthesis before and after induction of wild-type (wt) σ^{70}. Exponentially growing cells were divided into two cultures, and IPTG (1 mM) was added to one culture for 30 min to induce the expression of *rpoD*$^+$. Cells from both cultures were then pulse-labeled with [^{35}S]methionine at 30°C or at the indicated times at 42°C for 2 min, chased for 1.5 min, and analyzed on a 10% polyacrylamide–SDS gel as described in Materials and Methods. The gel was dried and subjected to autoradiography. The positions of the DnaK and GroEL heat shock proteins are indicated.

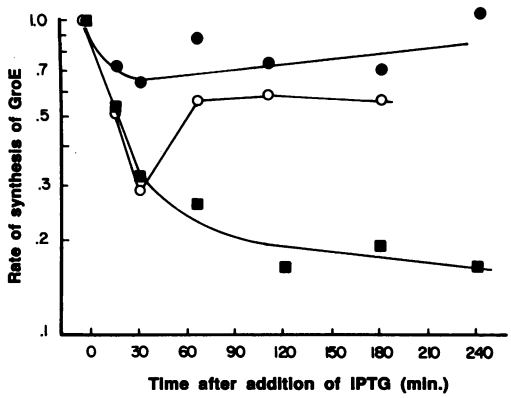

FIG. 2. Rate of synthesis of GroEL protein after induction of wild-type σ^{70} at 30°C. Cells from *rpoD*$^+$ *rpoH*$^+$/P*lac-rpoD*$^+$ (●), *rpoD285 rpoH*$^+$/P*lac-rpoD*$^+$ (○), and *rpoD285 rpoH113*/P*lac-rpoD*$^+$ (■) strains were pulse-labeled with [^{3}H]leucine and [^{3}H]lysine before and at the indicated times after the addition of IPTG, and proteins were quantitated on two-dimensional gels as described in Materials and Methods. The rate of synthesis of GroEL was normalized to the rate before addition of IPTG.

Induction of wild-type σ^{70} represses expression of heat shock proteins and transcription of heat shock genes in *rpoH113* strains. To quantitatively assess the effect of wild-type σ^{70} on expression of the heat shock proteins, we examined the effect of induction of σ^{70} on expression of the heat shock proteins during steady-state growth at 30°C by a quantitative two-dimensional gel analysis (see Materials and Methods). Data for the GroEL heat shock protein, presented in Fig. 2, indicated that the rate of GroEL synthesis decreased more than fivefold in the double-mutant strain following induction of σ^{70}. The rates of synthesis of the DnaK, C62.5, and F84.1 heat shock proteins were also decreased in the double-mutant strain (Table 3). In contrast, induction of σ^{70} had little effect (25%) on expression of heat shock proteins in wild-type cells (Fig. 2; Table 3). Repression in the double-mutant strain was specific for heat shock proteins, as several non-heat shock proteins (EF-G, Tu, and the β subunit of RNA polymerase) maintained normal rates of synthesis after induction of σ^{70} (Table 3). The transient repression of GroEL synthesis in the *rpoD285* single-mutant strain is discussed below.

The repression of heat shock protein synthesis most likely resulted from decreased transcription of heat shock promoters by holoenzyme containing RpoH113 σ^{32} when wild-type σ^{70} is present. To determine if this is true, we measured *groE* mRNA levels at 30°C before and after σ^{70} induction with an S1 mapping protocol. The mRNA measurements were entirely consistent with the protein synthesis measurements for the wild-type and double-mutant strains. Upon induction of σ^{70}, transcription from the *groE* heat shock promoter is reduced only slightly (25%) in the wild-type cells but is reduced 5- to 10-fold in the double-mutant strain (Fig. 3). This reduction in transcription is sufficient to account for the observed reduction in GroEL synthesis. These results establish that the repression of heat shock protein synthesis

TABLE 3. Relative synthesis rates of heat shock and non-heat shock proteins after induction of *rpoD*$^+$ for 65 min at 30°C

Strain (genotype)	Synthesis rate ratio, +IPTG/−IPTG[a]						
	Heat shock proteins				Non-heat shock proteins		
	GroE	DnaK	F84.1	C62.5	EF-G	β[b]	EF-Tu
CAG9166 (*rpoD*$^+$ *rpoH*$^+$)	0.89	0.80	0.71	1.06	1.02	1.18	1.03
CAG9214 (*rpoD285 rpoH*$^+$)	0.54	0.51	0.95	0.86	1.01	1.03	1.03
CAG9170 (*rpoD285 rpoH113*)	0.27	0.28	0.46	0.35	0.96	1.16	0.97

[a] The synthesis rate ratio is calculated as: synthesis rate after IPTG addition/synthesis rate before IPTG addition.
[b] β polypeptide of RNA polymerase.

caused by the presence of wild-type σ^{70} in *rpoH113* strains results from reduced transcription of heat shock genes.

In the *rpoD285* single-mutant strain following induction of σ^{70}, synthesis of GroEL and other heat shock proteins is transiently repressed and then recovers (Fig. 2; Table 3). Interestingly, induction of σ^{70} does not significantly affect transcription from the *groEL* heat shock promoter in the *rpoD285* strain (Fig. 3), indicating that the temporary drop in GroEL synthesis observed in that strain results from a posttranscriptional event. In other work, we have shown that the *rpoD285* strain is altered in its translational capacity (46b). The temporary drop in GroEL synthesis may result from readjusting the translational efficiency of the strain to that characteristic of cells with wild-type σ^{70}.

RpoH113 σ^{32} is present in elevated amounts following induction of σ^{70}. The reduced function of RpoH113 σ^{32} in the presence of wild-type σ^{70} could result from a regulatory alteration leading to a reduced level of σ^{32}. σ^{32} is a very unstable molecule which is present in limiting amounts in the cell (41). If induction of σ^{70} resulted in the repression of RpoH113 σ^{32} synthesis, the level of σ^{32} would fall rapidly and the null phenotype associated with RpoH113 σ^{32} strains would be explained. We examined the amount of RpoH113 σ^{32} before and after induction of σ^{70} by a Western blotting protocol. To our surprise, the level of RpoH113 σ^{32} actually increases after induction (Fig. 4C), indicating that an undersupply of this sigma factor is not responsible for the loss-of-function phenotype exhibited by the mutant strain. Instead, it seems likely that RpoH113 σ^{32} itself exhibits reduced function in the presence of wild-type σ^{70}. Interestingly, the level of wild-type σ^{32} also increases after induction of σ^{70}, suggesting that a regulatory mechanism exists to adjust the relative amounts of these two sigma factors.

Less mutant σ^{32} is associated with core RNA polymerase in the presence of wild-type σ^{70}. If RpoH113 σ^{32} had a lower binding affinity for core RNA polymerase than wild-type σ^{32}, it might be unable to compete effectively with wild-type σ^{70}. Induction of σ^{70} would then decrease the amount of Eσ^{32} holoenzyme, leading to a decrease in transcription from heat shock promoters and hence to decreased expression of heat shock proteins. Since the presence of heat shock proteins is essential, the incompatibility of *rpoH113* and *rpoD*$^+$ would be explained. Note, however, that if this explanation is true, the compatibility of *rpoH113* with *rpoD285* would be most easily explained by assuming that *rpoD285* is also defective in binding core RNA polymerase, thus allowing the two sigmas to compete and coexist.

To compare the extent of association of mutant and wild-type σ^{32} with core RNA polymerase in vivo, we gently lysed cells and used glycerol gradient sedimentation to separate free σ^{32} from that bound to core RNA polymerase in the cell lysate. Each gradient fraction was then subjected to SDS-polyacrylamide gel electrophoresis to separate holoenzyme components and then analyzed for the presence of σ^{32} by Western blotting (Fig. 5). The location of RNA polymerase holoenzyme in the gradient was determined by analyzing each fraction for the presence of the β and β′ subunits of RNA polymerase by Western blotting.

Essentially all wild-type σ^{32} is found in the holoenzyme

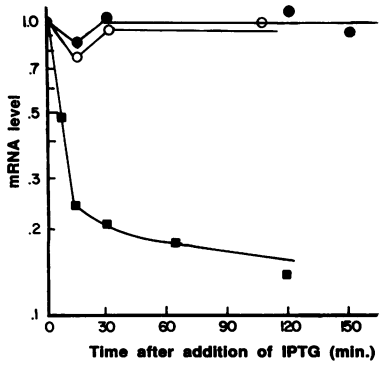

FIG. 3. Level of *groE* mRNA before and after induction of *rpoD*$^+$ at 30°C. Quantitation of *groE* transcripts from *rpoD*$^+$ *rpoH*$^+$/Plac-*rpoD*$^+$ (●), *rpoD285 rpoH*$^+$/Plac-*rpoD*$^+$ (○), and *rpoD285 rpoH113*/Plac-*rpoD*$^+$ (■) strains before or at the indicated times after IPTG addition was done by S1 mapping as described in Materials and Methods. The level of *groES* mRNA was normalized to that before the induction of *rpoD*$^+$.

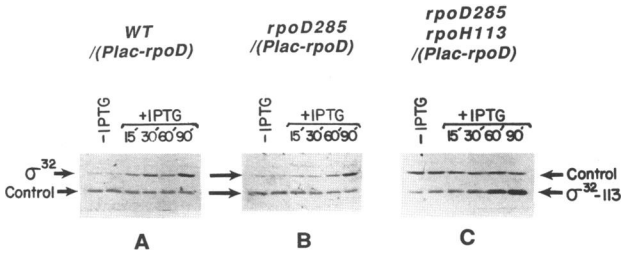

FIG. 4. Effect of induction of wild-type σ^{70} on expression of σ^{32}. Western blots with anti-σ^{32} antiserum of samples from equal volumes of various cells growing at 30°C in the absence or presence of IPTG were performed as described in Materials and Methods. The mutant σ^{32} encoded by *rpoH113* has a lower molecular weight due to an internal deletion in the structural gene. A constant amount of this mutant σ^{32} was added to each sample of the cells containing the *rpoH*$^+$ allele (panels A and B); conversely, a constant amount of wild-type σ^{32} was added to each sample of the cells containing the *rpoH113* mutation (panel C) to serve as a control for losses during the experimental analysis.

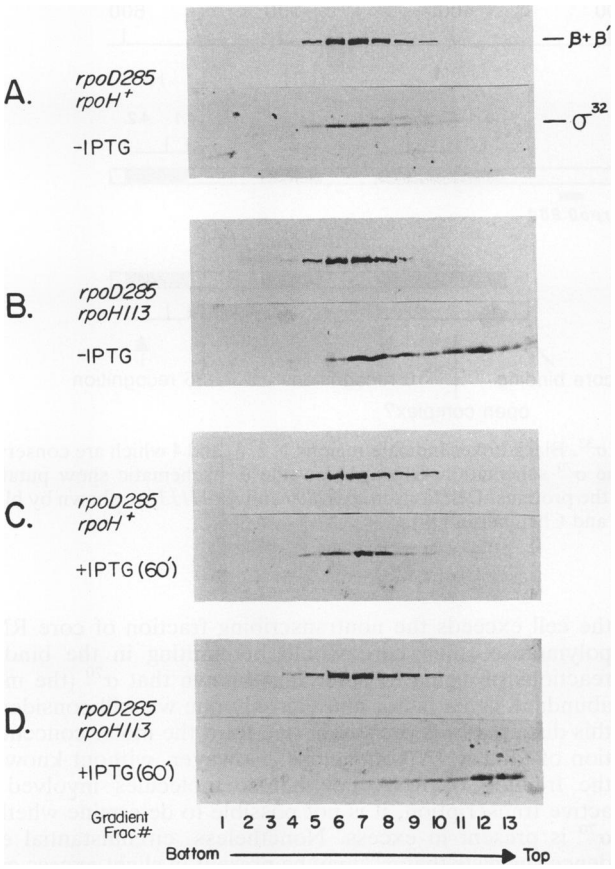

FIG. 5. Analysis of σ^{32} distribution between holoenzyme form and free form by sedimentation. Cell extracts from both *rpoD285 rpoH*$^+$/P*lac-rpoD*$^+$ (panels A and C) and *rpoD285 rpoH113*/P*lac-rpoD*$^+$ (panels B and D) before (panels A and B) and 60 min after (panels C and D) induction of wild-type σ^{70} were sedimented in a 15 to 30% glycerol gradient. Fractions were collected and analyzed by Western blotting with anti-σ^{32} antiserum to determine the distribution of σ^{32}. The positions for holoenzyme were determined by analyzing the same Western blot with monoclonal antibodies against the β and β′ subunits of *E. coli* RNA polymerase as described in Materials and Methods. The samples after induction (panels C and D) have about 1.8-fold more σ^{70} than those without induction (panels A and B), as determined by quantitative Western blotting of parallel samples.

peak of the gradient (Fig. 5A) while only about 50% of the mutant σ^{32} is bound to RNA polymerase (Fig. 5B). Moreover, the mutant σ^{32} is not cleanly separated into a bound and free peak, suggesting that the bound σ^{32} is continually dissociating from core RNA polymerase during the sedimentation run. These results strongly suggest that RpoH113 σ^{32} has a weakened affinity for core RNA polymerase. This binding defect is manifested more strongly when wild-type σ^{70} is present. Wild-type σ^{32} remains bound following induction of σ^{70}, indicating that it can compete effectively with σ^{70} for core RNA polymerase (Fig. 5C). In strong contrast, almost all RpoH113 σ^{32} is found in the free peak following this treatment, indicating that wild-type σ^{70} is an effective competitor for the mutant enzyme (Fig. 5D). These results support the idea that RpoH113 σ^{32} is defective in binding to core RNA polymerase and that this defect is enhanced by the presence of wild-type σ^{70}.

DISCUSSION

We have investigated the mechanism of incompatibility between *rpoD*$^+$ and *rpoH113*. Our results indicate that incompatibility is manifested only when σ^{32} function is required for viability (above 20°C) and results from reduced function of the mutant σ^{32} holoenzyme in the presence of wild-type σ^{70}. Transcription of heat shock genes by holoenzyme containing the mutant σ^{32} [Eσ^{32}(RpoH113)] is inhibited by induction of wild-type σ^{70}, leading to a deficit of heat shock proteins. An insufficient amount of those heat shock proteins essential for viability probably accounts for the incompatibility of *rpoH113* and *rpoD*$^+$.

Our experiments suggest that the altered affinity of RpoH113 σ^{32} for core RNA polymerase underlies the incompatibility phenotype. Glycerol gradient sedimentation experiments indicated that while essentially all wild-type σ^{32} is associated with core RNA polymerase, only 50% of the RpoH113 σ^{32} is in the holoenzyme peak. Hence, mutant σ^{32} appears to have reduced core binding. Moreover, induction of wild-type σ^{70} in the *rpoH113 rpoD285* strain enhances this binding defect. When wild-type σ^{70} is present, almost no RpoH113 σ^{32} is associated with core RNA polymerase. A decreased amount of Eσ^{32} would account for the reduced expression of the heat shock genes and explain the incompatibility of *rpoH113* and *rpoD*$^+$. Overproducing RpoH113 σ^{32} should restore compatibility between *rpoH113* and *rpoD*$^+$ by permitting sufficient production of Eσ^{32}. This in fact proves to be true. When *rpoH113* is present on a multicopy plasmid, it is compatible with *rpoD*$^+$ (3), suggesting that increasing the amount of RpoH113 σ^{32} compensates for its weakened affinity for core RNA polymerase.

Although the argument is indirect, our experiments also suggest that RpoD285 σ^{70} may have a lower affinity for core RNA polymerase than does wild-type σ^{70}. RpoH113 σ^{32} functions normally in strains containing RpoD285 σ^{70}, as judged by the fact that *rpoD285 rpoH113* strains are viable and that expression of heat shock proteins is normal both during steady-state growth and after temperature upshift. These results would be explained if RpoH113 σ^{32} were able to compete with RpoD285 σ^{70}, although it is unable to compete with wild-type σ^{70}. If so, the mutant σ^{70} as well as the mutant σ^{32} could be defective in binding. A direct measure of the binding affinity is required to determine whether this is true.

Our results on the effect of wild-type σ^{70} on the activity of RpoH113 σ^{32} are most easily understood if these two sigma factors compete for binding to core RNA polymerase. Competition among sigmas would be most severe when the concentration of free sigmas in the cell exceeds that of core RNA polymerase. In this case, the amount of any particular holoenzyme is determined by the binding affinity of that σ factor for core polymerase as well as by the amount of that sigma. Competition between σ^{32} and σ^{70} would explain why the presence of wild-type σ^{70} preferentially affects the ability of RpoH113 σ^{32} to associate with core RNA polymerase. The reduced binding affinity of RpoH113 σ^{32} for core RNA polymerase means that it is less able than wild-type σ^{32} to compete with σ^{70} for binding to core RNA polymerase. Hence, induction of σ^{70} would preferentially reduce the amount of Eσ^{32}(RpoH113) compared with Eσ^{32}(RpoH$^+$). Likewise, σ competition could explain why *rpoH113* is compatible with *rpoD285* but not with *rpoD*$^+$. If RpoD285 σ^{70} has a reduced binding affinity, it would be less effective than wild-type σ^{70} in competing with RpoH113 σ^{32} for core RNA polymerase. The net result of the competition would

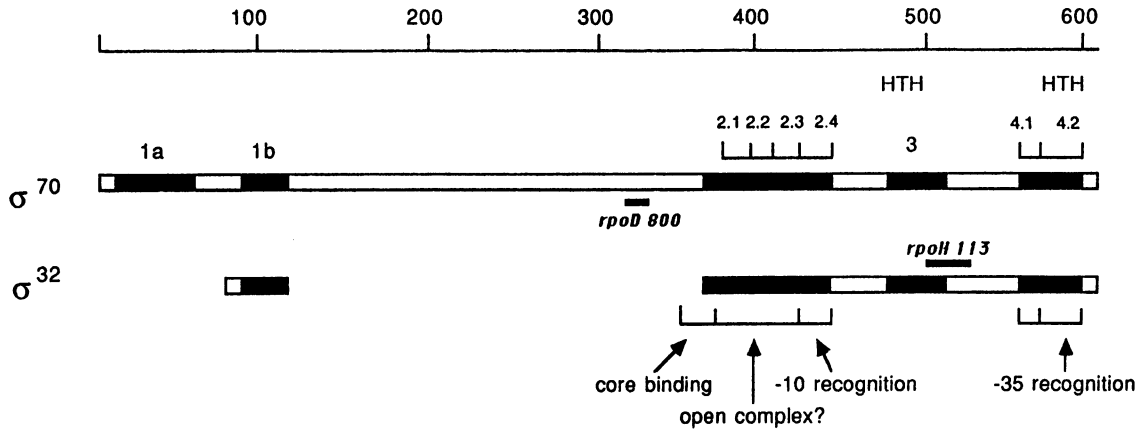

FIG. 6. Schematic representation of areas conserved between σ^{70} and σ^{32}. Black boxes indicate regions 1, 2, 3, and 4 which are conserved in the family of bacterial σ factors. Subregions are indicated above the σ^{70} schematic. Arrows below the σ^{32} schematic show putative functional regions. HTH indicates the putative helix-turn-helix regions of the proteins. Deletions in *rpoD800* and *rpoH113* are shown by black bars. The alignments are from Gribskov and Burgess (10) and Helmann and Chamberlin (19).

be sufficient $E\sigma^{32}$(RpoH113) to permit viability in cells with RpoD285 σ^{70} but not in cells with wild-type σ^{70}.

Models other than sigma factor competition could be invoked to explain why a σ^{32} mutant with reduced binding affinity would be functional in *rpoD285* cells but not in *rpoD*$^+$ cells. For example, *rpoD285* cells could have some alteration in gene expression which enhanced the ability of RpoH113 σ^{32} to bind to core RNA polymerase. However, additional evidence supports the idea that σ^{32} and σ^{70} compete for limiting core RNA polymerase. A prediction of this model is that decreasing the amount of σ^{70} would restore compatibility. This proves to be the case. The *rpoH113* allele is compatible with a strain which has only 30% of the normal level of σ^{70} because it carries *rpoD40*, a nonsense mutation in the σ^{70} structural gene, and *supF*, a suppressor tRNA (46a). Compatibility with *rpoD40* fulfills the prediction of the sigma competition model that decreasing the amount of σ^{70} will enhance the function of RpoH113 σ^{32}. Further evidence comes from the observation that decreasing the amount of σ^{70} to 5 to 10% of its normal level increases expression of the heat shock proteins fivefold after temperature upshift (35). Taken together, these experiments support the proposition that core RNA polymerase is limiting for the binding of sigma factors in the cell. In this regard, it was initially surprising that overproducing σ^{70} did not significantly diminish the expression of heat shock proteins in wild-type cells. However, our data suggest that the cell has a regulatory mechanism to deal with this possible imbalance in gene expression. As shown in Fig. 4, upon overexpression of σ^{70}, the cell overproduces σ^{32}, which presumably readjusts the ratio of sigma factors so that expression of $E\sigma^{70}$ and $E\sigma^{32}$-transcribed genes remains in balance. It is interesting that both *rpoD* and *rpoH* have promoters for more than one kind of holoenzyme (6–8, 29, 43). This genetic organization may facilitate maintaining the appropriate ratio of sigma factors in the cell.

There are no definitive data in the literature addressing the question of whether core RNA polymerase is limiting for the binding of sigma factors. Total RNA polymerase in the cell is composed of transcribing and nontranscribing RNA polymerase. This latter fraction would include RNA polymerase free in the cell as well as that bound to promoter or nonpromoter DNA. If the concentration of sigma factors in the cell exceeds the nontranscribing fraction of core RNA polymerase, then core would be limiting in the binding reactions of sigma to core. It is known that σ^{70} (the most abundant sigma factor and the only one we will consider in this discussion) is present at one-third the molar concentration of total RNA polymerase. However, without knowing the fraction of RNA polymerase molecules involved in active transcription, it is not possible to determine whether σ^{70} is present in excess. Nonetheless, circumstantial evidence suggests that σ^{70} may be present in slight excess over "nontranscribing" or "free" core RNA polymerase. The strongest evidence for this proposition comes from studies on the RNA polymerase content of the bacterial nucleoid. Since the nucleoid must be isolated under high salt (1 M) conditions, only actively transcribing RNA polymerases remain associated with this structure, permitting an independent estimate of this fraction. While the experiments are not completely definitive, it is reported that the vast majority of RNA polymerase in the cell sediments with the nucleoid (36, 40). In addition, based on the transcription rate of RNA polymerase in vivo and the total number of RNA polymerases, the fraction of active polymerase has been calculated. This calculation indicates that about 70% of the RNA polymerases are actively transcribing in rapidly growing cells and suggests that σ^{70} is approximately equivalent to or in slight excess over the nontranscribing fraction (21).

It is probably premature to speculate about the nature of the binding defect of the mutant sigmas in the absence of a direct determination of their binding affinities. However, we would like to point out that a consideration of the mutational alterations in these sigmas is revealing. Both RpoD285 σ^{70} and RpoH113 σ^{32} mutant polypeptides result from in-frame deletions in the sigma structural gene. While these deletions could result in conformational changes which affect core binding indirectly, they may identify regions of sigma which are directly involved in binding to core RNA polymerase. There is some support for the latter idea, although the fact that the mutant sigmas are functional argues that the regions they identify do not constitute the major contacts between sigma and core. A recent analysis of deletion derivatives of σ^{70} has identified amino acids 361 to 390, located near the very N terminus of conserved region 2 of sigmas (Fig. 6), as important for binding to core RNA polymerase (23). The

rpoD285 deletion removes amino acids 330 to 343 (20), which are located sufficiently close to those identified by Lesley et al. (23) to make it possible that some of them could contact core RNA polymerase. The *rpoH113* deletion removes amino acids 178 to 201 (3), which are located in the C terminus of σ^{32}, in a region which is homologous to conserved region 3 of sigmas (Fig. 6). The fact that a σ^{70} monoclonal antibody, IS4, interacts with region 3 in free σ^{70} but not in holoenzyme (43) suggests that this region of sigma may be in proximity to core RNA polymerase. Thus, both the *rpoD285* and the *rpoH113* mutations may identify amino acids with direct but ancillary roles in core RNA polymerase binding.

In summary, our investigations indicate that the incompatibility of *rpoH113* and *rpoD*$^+$ results from the reduced function of RpoH113 σ^{32} in the presence of wild-type σ^{70}. We present preliminary evidence that the reduced function of the mutant sigma results from a decrease in its binding affinity for core RNA polymerase. Our experiments are most easily interpreted if free sigma is in excess over nontranscribing RNA polymerase. Competition between these two sigma factors for limiting core RNA polymerase would provide the cell with an additional way to regulate gene expression. Thus far, the only studies done to directly address the relative binding constants of alternative sigmas and primary sigmas for core RNA polymerase were done with bacteriophage sigmas from T4 (σ^{gp55}) or from SPO1 (s^{gp28}). In these two cases, the primary sigma factor has considerably higher affinity for core RNA polymerase than does the phage sigma (4, 28, 44), and additional mechanisms must be invoked to explain the massive switch to transcription of the phage genome. Determining the binding affinities of alternative bacterial sigma factors for core RNA polymerase is required for assessing the contribution of sigma competition to global gene expression.

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REFERENCES

1. **Baker, T. A., A. D. Grossman, and C. Gross.** 1984. A gene regulating the heat shock response in *Escherichia coli* also affects proteolysis. Proc. Natl. Acad. Sci. USA **81**:6779–6783.
2. **Burgess, R. R., A. A. Travers, J. J. Dunn, and E. K. F. Bautz.** 1969. Factor stimulating transcription by RNA polymerase. Nature (London) **221**:43–46.
3. **Calendar, R., J. W. Erickson, C. Halling, and A. Nolte.** 1988. Deletion and insertion mutations in the *rpoH* gene of *Escherichia coli* that produce functional σ^{32}. J. Bacteriol. **170**:3479–3484.
4. **Chelm, B. K., J. J. Duffy, and E. P. Geiduschek.** 1982. Interaction of Bacillus subtilis RNA polymerase core with two specificity-determining subunits. Competition between sigma and the SPO1 gene 28 protein. J. Biol. Chem. **257**:6501–6508.
5. **Cowing, D. W., J. C. Bardwell, E. A. Craig, C. Woolford, R. W. Hendrix, and C. A. Gross.** 1985. Consensus sequence for *Escherichia coli* heat-shock gene promoters. Proc. Natl. Acad. Sci. USA **82**:2679–2683.
6. **Erickson, J. W., and C. A. Gross.** 1989. Identification of the σ^E subunit of *Escherichia coli* RNA polymerase: a second alternate sigma factor involved in high-temperature gene expression. Genes Dev. **3**:1462–1471.
7. **Erickson, J. W., V. Vaughn, W. A. Walter, F. C. Neidhardt, and C. A. Gross.** 1987. Regulation of the promoters and transcripts of *rpoH*, the *Escherichia coli* heat-shock regulatory gene. Genes Dev. **1**:419–432.
8. **Fujita, N., and A. Ishihama.** 1987. Heat-shock induction of RNA polymerase σ^{32} synthesis in *Escherichia coli*: transcriptional control and a multiple promoter system. Mol. Gen. Genet. **210**:10–15.
9. **Fujita, N., A. Ishihama, Y. Nagasawa, and S. Ueda.** 1987. RNA polymerase sigma-related proteins in *Escherichia coli*: detection by antibodies against a synthetic peptide. Mol. Gen. Genet. **210**:5–9.
10. **Gribskov, M., and R. R. Burgess.** 1986. Sigma factors from *E. coli*, *B. subtilis*, phage SPO1 and phage T4 are homologous proteins. Nucleic Acids Res. **14**:6745–6763.
11. **Gross, C. A., A. D. Grossman, H. Liebke, W. Walter, and R. R. Burgess.** 1984. Effects of the mutant sigma allele *rpoD800* on the synthesis of specific macromolecular components of the *Escherichia coli* K12 cell. J. Mol. Biol. **172**:283–300.
12. **Gross, C. A., J. Hoffman, C. Ward, D. Hager, G. Burdick, H. Berger, and R. Burgess.** 1978. Mutation affecting thermostability of sigma subunit of *Escherichia coli* RNA polymerase lies near the *dnaG* locus at about 66 min on the *E. coli* genetic map. Proc. Natl. Acad. Sci. USA **75**:427–431.
13. **Grossman, A. D., R. R. Burgess, W. Walter, and C. A. Gross.** 1983. Mutations in the *lon* gene of *E. coli* K12 phenotypically suppress a mutation in the sigma subunit of RNA polymerase. Cell **32**:151–159.
14. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat-shock promoters. Cell **38**:383–390.
15. **Grossman, A. D., D. B. Straus, W. A. Walter, and C. A. Gross.** 1987. σ^{32} synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*. Genes Dev. **1**:179–184.
16. **Grossman, A. D., Y. N. Zhou, C. Gross, J. Heilig, G. E. Christie, and R. Calendar.** 1985. Mutations in the *rpoH* (*htpR*) gene of *Escherichia coli* K-12 phenotypically suppress a temperature-sensitive mutant defective in the σ^{70} subunit of RNA polymerase. J. Bacteriol. **161**:939–943.
17. **Harris, J. D., J. S. Heilig, I. I. Martinez, R. Calendar, and L. A. Isakssen.** 1978. Temperature-sensitive *Escherichia coli* mutant producing a temperature-sensitive σ subunit of DNA-dependent RNA polymerase. Proc. Natl. Acad. Sci. USA **75**:6177–6181.
18. **Harris, J. D., I. I. Martinez, and R. Calendar.** 1977. A gene from *Escherichia coli* affecting the sigma subunit of RNA polymerase. Proc. Natl. Acad. Sci. USA **74**:1836–1840.
19. **Helmann, J. D., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57**: 839–872.
20. **Hu, J. C., and C. A. Gross.** 1983. Marker rescue with plasmids bearing deletions in *rpoD* identifies a dispensable part of *E. coli* sigma factor. Mol. Gen. Genet. **191**:492–498.
21. **Ingraham, J. L., O. Maaloe, and F. C. Neidhardt.** 1983. Growth of the bacterial cell. Sinauer Associates Inc., Sunderland, Mass.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
23. **Lesley, S. A., and R. R. Burgess.** 1989. Characterization of the *Escherichia coli* transcription factor σ^{70}: localization of a region involved in the interaction with core RNA polymerase. Biochemistry **28**:7728–7734.
24. **Lesley, S. A., N. E. Thompson, and R. R. Burgess.** 1987. Studies of the role of the *Escherichia coli* heat shock regulatory protein σ^{32} by the use of monoclonal antibodies. J. Biol. Chem. **262**: 5404–5407.
25. **Liebke, H., C. Gross, W. Walter, and R. Burgess.** 1980. A new mutation, *rpoD800*, affecting the sigma subunit of *E. coli* RNA polymerase is allelic to two other sigma mutants. Mol. Gen. Genet. **177**:277–282.
26. **Losick, R., and J. Pero.** 1981. Cascades of sigma factors. Cell **25**:582–584.
27. **Lowe, P. A., U. Aebi, C. Gross, and R. R. Burgess.** 1981. *In vitro* thermal inactivation of a temperature-sensitive σ subunit mutant (*rpoD800*) of *Escherichia coli* RNA polymerase proceeds by aggregation. J. Biol. Chem. **256**:2010–2015.

28. **Malik, S., K. Zalenskaya, and A. Goldfarb.** 1987. Competition between sigma factors for core RNA polymerase. Nucleic Acids Res. **15:**8521–8530.
29. **Nagai, H., R. Yano, J. W. Erickson, and T. Yura.** 1990. Transcriptional regulation of the heat shock regulatory gene *rpoH* in *Escherichia coli*: involvement of a novel catabolite-sensitive promoter. J. Bacteriol. **172:**2710–2715.
30. **Nakamura, Y.** 1978. RNA polymerase mutant with altered sigma factor in *Escherichia coli*. Mol. Gen. Genet. **165:**1–6.
31. **Nakamura, Y., T. Osawa, and T. Yura.** 1977. Chromosomal location of a structural gene for the RNA polymerase σ factor in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **74:**1831–1835.
32. **Neidhardt, F. C., and R. A. VanBogelen.** 1981. Positive regulatory gene for temperature-controlled proteins in *Escherichia coli*. Biochem. Biophys. Res. Commun. **100:**894–900.
33. **Neidhardt, F. C., R. A. VanBogelen, and V. A. Vaughn.** 1984. The genetics and regulation of heat-shock proteins. Annu. Rev. Genet. **18:**295–329.
34. **O'Farrell, P. H.** 1975. High-resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250:**4007–4021.
35. **Osawa, T., and T. Yura.** 1981. Effects of reduced amount of RNA polymerase sigma factor on gene expression and growth of *Escherichia coli*: studies of the *rpoD40* (amber) mutation. Mol. Gen. Genet. **184:**166–173.
36. **Pettijohn, D., K. Clarkson, C. Kossman, and J. Stonington.** 1970. Synthesis of ribosomal RNA on a protein-DNA complex isolated from bacteria: a comparison of ribosomal RNA synthesis *in vitro* and *in vivo*. J. Mol. Biol. **52:**281–300.
37. **Reznikoff, W. S., D. A. Siegele, D. W. Cowing, and C. A. Gross.** 1985. The regulation of transcription initiation in bacteria. Annu. Rev. Genet. **19:**355–387.
38. **Salser, W., R. Gesteland, and A. Bolle.** 1967. *In vitro* synthesis of bacteriophage lysozyme. Nature (London) **215:**588–591.
39. **Skelly, S., T. Coleman, C. F. Fu, N. Brot, and H. Weissbach.** 1987. Correlation between the 32-kDa sigma factor levels and *in vitro* expression of *Escherichia coli* heat shock genes. Proc. Natl. Acad. Sci. USA **84:**8365–8369.
40. **Stonington, O. G., and D. E. Pettijohn.** 1971. The folded genome of *Escherichia coli* isolated in a protein-DNA-RNA complex. Proc. Natl. Acad. Sci. USA **68:**6–9.
40a. **Straus, D.** Personal communication.
41. **Straus, D. B., W. A. Walter, and C. A. Gross.** 1987. The heat-shock response of *E. coli* is regulated by changes in the concentration of σ^{32}. Nature (London) **329:**348–351.
42. **Strickland, M. S., N. E. Thompson, and R. R. Burgess.** 1988. Structure and function of the σ^{70} subunit of *Escherichia coli* RNA polymerase. Monoclonal antibodies: localization of epitopes by peptide mapping and effects on transcription. Biochemistry **27:**5755–5762.
43. **Taylor, W. E., D. B. Straus, A. D. Grossman, Z. F. Burton, C. A. Gross, and R. R. Burgess.** 1984. Transcription from a heat-inducible promoter causes heat shock regulation of the sigma subunit of *E. coli* RNA polymerase. Cell **38:**371–381.
44. **Williams, K. P., R. Muller, W. Ruger, and E. P. Geiduschek.** 1989. Overproduced bacteriophage T4 gene 33 protein binds RNA polymerase. J. Bacteriol. **171:**3579–3582.
45. **Yamamori, T., and T. Yura.** 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal regulation in *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **79:**860–864.
46. **Yano, R., M. Imai, and T. Yura.** 1987. The use of operon fusions in studies of the heat-shock response: effects of altered σ^{32} on heat-shock promoter function in *Escherichia coli*. Mol. Gen. Genet. **207:**24–28.
46a. **Zhou, Y. N.** Unpublished data.
46b. **Zhou, Y. N., and C. A. Gross.** Submitted for publication.
47. **Zhou, Y. N., N. Kusukawa, J. W. Erickson, C. A. Gross, and T. Yura.** 1988. Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor σ^{32}. J. Bacteriol. **170:**3640–3649.