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Genetics

Temperature-sensitive *Escherichia coli* mutant producing a temperature-sensitive σ subunit of DNA-dependent RNA polymerase

(tetracycline resistance/transposon-10)

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ABSTRACT **A gene affecting the σ subunit of DNA-dependent RNA polymerase is tightly linked to *dnaG* at 66 min on the *Escherichia coli* chromosome. In order to create an easily selectable marker in this region, we inserted transposon-10, which carries a gene determining resistance to tetracycline (*tet*) near 66 min, and the order *tolC-dnaG-σ-tet* was determined. We used frequency of cotransduction with *tet* as a criterion to screen a collection of spontaneous temperature-sensitive *Escherichia coli* mutants that might affect the σ subunit. One such mutant was found to map at the σ locus. The σ subunit isolated from this mutant is unstable at 46°C *in vitro* and has an altered electrophoretic mobility. The temperature sensitivity of RNA synthesis in this mutant indicates that most transcription in *E. coli* is σ dependent.**

The σ subunit of *Escherichia coli* DNA-dependent RNA polymerase is responsible for much of the specificity of *in vitro* transcription (1, 2). For example, RNA polymerase core enzyme (lacking σ subunit) binds weakly to many sites on T7 phage DNA *in vitro*, whereas RNA polymerase holoenzyme (containing σ subunit) binds strongly and only to those sites at which *in vivo* transcription begins (3).

Elucidation of the role of the σ protein in the specificity of gene expression has been delayed, however, by the lack of σ subunit mutants. Previous genetic studies on the electrophoretic differences among the σ proteins of enteric bacteria suggested that the structural gene for σ maps at about 66 min on the *E. coli* genome (4, 5). In order to rapidly screen potential σ mutants in this region of the genome, we have inserted the translocatable tetracycline-resistance (*tet*) element Tn10 near the σ gene. By using this readily selectable marker for rapid mapping of mutants, we have identified a spontaneous temperature-sensitive (ts) mutant that produces an altered σ protein and is deficient in the synthesis of RNA at nonpermissive temperatures.

MATERIALS AND METHODS

Media, Buffers, and Chromatographic Materials. LB broth (6) supplemented with 0.4% glucose was used; the NaCl concentration was adjusted to 0.1 M. L plates consist of LB broth and 2% Difco agar. LC plates contain, in addition, 5 mM $CaCl_2$ but 1.5% agar. Davis minimal medium is described by Calendar and Lindahl (7). Buffer P50 is described by Gonzalez *et al.* (8) and TGED buffer is described by Burgess and Jendrisak (9). TPG-CAA medium (10) enriched with 0.8% glucose, 0.01% arginine, 0.5 mM $CaCl_2$, and 20 amino acids and trace metals each at 1 μg/ml was used for radioactive labeling. Bio-Gel A-1.5m used for gel filtration and the ion-exchange material Bio-Rex 70 were purchased from Bio-Rad.

Bacterial and Phage Strains. Bacterial strains are listed in Table 1. The strains designated C- are derivatives of *E. coli* C (17), whereas the remaining strains are *E. coli* K-12 derivatives (16). Phage λ strain NK55, carrying the b221 deletion, the *CI857* and *Oam29* mutations, and the *tet* transposon Tn10 inserted in the CIII gene, was obtained from Nancy Kleckner (18). The P1 phage used for transduction was the high frequency transducing mutant 607H (19). Strain C-2359, carrying Tn10 near *dnaG*, was constructed by the following variant of the method described by Kleckner *et al.* (20): *E. coli* strain SK362 was infected with NK55 and was treated with tetracycline, so that *tet* transductants were selected (L. Csonka and A. J. Clark, unpublished results). P1 phage was grown by Brian Sauer on a mixed culture of *tet* transductants and used to transduce C-2309 by the methods described by Bowden *et al.* (11), selecting for tetracycline-resistance and the ability to grow at 42°C (*dnaG*$^+$). This procedure yielded transductants with a *tet* gene that is highly cotransducible with *dnaG*. C-2359 is one of these.

Purification of RNA Polymerase. RNA polymerase holoenzyme was purified according to the procedure of Burgess and Jendrisak (9). After the enzyme was eluted from the A-1.5m column, it was concentrated and loaded onto a phosphocellulose column as described by Gonzalez *et al.* (8). The RNA polymerase holoenzyme and core enzyme were then eluted as described (9). These fractions were diluted with TGED buffer (8) to a salt concentration of 0.1 M and concentrated on small DEAE-cellulose columns (1 ml of packed volume per 10 mg of protein). Each enzyme fraction was eluted from the DEAE-cellulose in TGED buffer containing 0.5 M NaCl, diluted, and further purified on a Bio-Rex 70 column (8). The core enzyme and σ subunit fractions were concentrated and stored as described by Burgess and Jendrisak (9).

Measurement of Nucleic Acid Synthesis by Pulse Labeling with Radioactive Nucleotides. Cells were grown at 30°C in enriched TPG medium to a density of approximately 10^8 cells per ml. Two 2-ml aliquots were then taken; one was incubated at 30°C and the other at 43.5°C. Timed samples were taken by transfer of 150 μl of the cell suspension to a tube containing 20 μl of a solution of labeled nucleotide at 0.05 mCi/ml (either [*methyl*-^{3}H]thymidine, 55 Ci/mmol, or [^{3}H]uridine, 22 Ci/mmol) in enriched TPG (1 Ci = 3.7×10^{10} becquerels). Each sample was incubated for 2 min, after which 100 μl was spotted onto Whatman 3 MM filter paper, allowed to dry 1 min, and then placed in cold 7.5% trichloroacetic acid. Filters were then batch washed and assayed for radioactivity by the procedure of Wolf (21).

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Abbreviations: *tet*-r, tetracycline-resistance; Tn10, a translocatable tetracycline-resistance element; ts, temperature sensitive.

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Table 1. Bacterial strains

Collection number	Relevant genotype	Origin or reference
C-2301	*uvrA thyA str tolC*	(11)
C-2309	*uvrA thyA str dnaG3*	(11)
C-2359	*uvrA thyA str* Tn10	This paper
C-2360	*uvrA thyA str* Tn10 *dnaG3*	From a transduction: C-2359 into C-2309, selecting *tet*-r
C-2367	*uvrA thyA str* Tn10 *tolC*	From a transduction: C-2359 into C-2301, selecting *tet*-r
K-1200	*endA, rna*	(12)
P90A5c	*argG lac thi*	(13)
285c	*argG lac thi* ts285	(13)
PM-90	*argG lac thi* ts285 Tn10	From a transduction: C-2359 into 285c, selecting *tet*-r
SK362	*argE his thi str lac*(ø80*lac*)	(14)
WZ35	*alt-1 cya metB str thyA*	(15)

Genetic nomenclature follows Bachmann *et al.* (16).

RESULTS

Insertion of a Transposon near *dnaG* and the σ Gene. In the 66-min region of the *E. coli* chromosome the gene order is *tolC-cca-*(*dnaG*, σ)-*uxaA* (5, 16, 22–25). The σ gene is about 90% cotransducible with *dnaG* (5, 23, 24), whereas *cca* and *dnaG* are only 70% cotransducible (J. Foulds and M. Deutscher, personal communication), suggesting that the *dnaG* gene is closer to σ than to *cca*.

Some of the mapping of the σ gene was done with two recently isolated σ mutants. One of these mutants produces a σ that is ts in promoting the transcription of T7 phage DNA but is not ts *in vivo* (23). The other σ mutation, *alt-1*, affects the specificity of σ action by allowing fermentation of arabinose

Table 2. Transductional mapping of *tolC*, *dnaG*, *alt-1*, *ts*285, and Tn10

Donor (relevent markers)	Recipient	Selected marker	Distribution of unselected markers: Class	Number	Frequency, %	Frequency of cotransduction, %	
A.							
C-2367 (*tolC*$^-$, *dnaG*$^+$, *tet*-r)	C-2309 (*tolC*$^+$, *dnaG3*, *tet*-s)	*dnaG*$^+$	*tolC*$^+$, *tet*-r	98	49	*tet*-r/*dnaG*$^+$	84
			tolC$^+$, *tet*-s	13	7		
			tolC$^-$, *tet*-r	69	35	*tolC*$^-$/*dnaG*$^+$	44
			tolC$^-$, *tet*-s	19	9		
C-2367 (*tolC*$^-$, *dnaG*$^+$, *tet*-r)	C-2309 (*tolC*$^+$, *dnaG3*, *tet*-s)	*tet*-r	*tolC*$^+$, *dnaG*$^+$	140	47	*dnaG*$^+$/*tet*-r	84
			tolC$^+$, *dnaG3*	44	15		
			tolC$^-$, *dnaG*$^+$	110	37	*tolC*$^-$/*tet*-r	39
			tolC$^-$, *dnaG3*	6	2		
C-2360 (*tolC*$^+$, *dnaG3*, *tet*-r)	C-2301 (*tolC*$^-$, *dnaG*$^+$, *tet*-s)	*tolC*$^+$	*tet*-r, *dnaG*$^+$	1	2	*dnaG3*/*tolC*$^+$	53
			tet-r, *dnaG3*	29	48		
			tet-s, *dnaG*$^+$	27	45	*tet*-r/*tolC*$^+$	50
			tet-s, *dnaG3*	3	5		
B.							
C-2367 (*tolC*$^-$, *alt*$^+$, *tet*-r)	WZ-35 (*tolC*$^+$, *alt-1*, *tet*-s)	*tet*-r	*tolC*$^+$ *alt*$^+$	70	73	*alt*$^+$/*tet*-r	92
			tolC$^+$ *alt-1*	6	6		
			tolC$^-$ *alt*$^+$	18	19	*tolC*$^-$/*tet*-r	21
			tolC$^-$ *alt-1*	2	2		
C.							
PM-90 (*tolC*$^+$, ts-285, *tet*-r)	C-2301 (*tolC*$^-$, ts$^+$, *tet*-s)	*tolC*$^+$	ts$^+$ *tet*-r	2	2	ts-285/*tolC*$^+$	41
			ts$^+$ *tet*-s	56	57		
			ts-285 *tet*-r	34	35	*tet*-r/*tolC*$^+$	37
			ts-285 *tet*-s	6	6		
C-2367 (*tolC*$^-$, ts$^+$, *tet*-r)	285c (*tolC*$^+$, ts-285, *tet*-s)	*tet*-r	*tolC*$^+$, ts$^+$	134	67	ts-285/tet-r	73
			tolC$^+$ ts-285	51	26		
			tolC$^-$ ts$^+$	13	6	*tolC*$^-$/tet-r	7
			tolC$^-$ ts-285	1	1		
D.							
C-2360 (*dnaG3*, ts$^+$, *tet*-r)	285c (*dnaG*$^+$, ts-285, *tet*-s)	*tet*-r	ts$^+$	13	13		
			ts$^-$	87	87		
PM-90 (*dnaG*$^+$, ts-285, *tet*-r)	C-2309 (*dnaG3*, ts$^+$, *tet*-s)	*tet*-r	ts$^+$	1	1		
			ts$^-$	99	99		

tolC$^+$ strains are not sensitive to sodium deoxycholate (26) and *tolC*$^+$ transductants were selected on L plates upon which 0.25 ml of 20% sodium deoxycholate had been spread. Temperature-resistant transductants were selected on LC plates at 43.5°C. Tetracycline resistance was selected on L plates to which tetracycline at 15 μg/ml had been added. With the exception of the temperature resistance selections all plates were incubated at 30°C and scored after 48 hr. In order to score unselected markers we used the following procedures: Patch plates of recombinants were replica plated onto LC plates at 43.5°C to test for the ts$^+$ markers, onto LC plates seeded with colicin E1 to test for *tolC*$^-$, and onto L plates with deoxycholate to test for *tolC*$^+$. To screen for the acquisition of the tetracycline resistance element, patch plates were replica plated onto tetracycline-supplemented plates as described above.

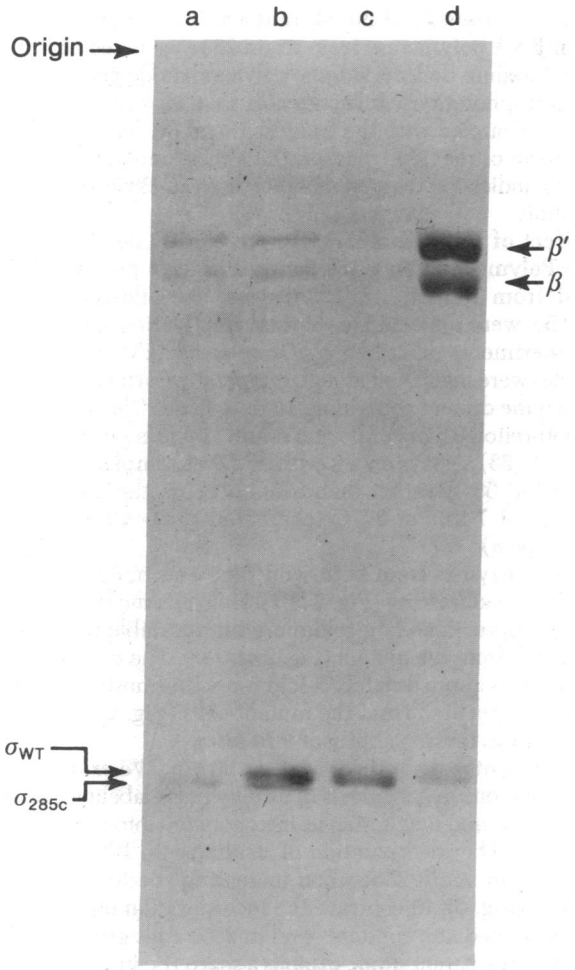

FIG. 1. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of RNA polymerase holoenzyme and σ subunit purified from *E. coli* K strain P90A5c and its spontaneous mutant derivative 285c. Gels (20-cm) were prepared and run as described by Harris *et al.* (5). The positions of RNA polymerase subunits β, β', and σ are indicated. Slots: a, σ from strain 285c; b, mixture of σ preparations from 285c and P90A5c; c, σ from P90A5c; d, reference marker (RNA polymerase holoenzyme from *E. coli* strain K-1200). The σ preparation from strain P90A5c contains 25 mol of σ for every mol of β or β'. The 285c preparation contains 5 mol of σ per mol of β or β'.

in vivo in the absence of either cyclic AMP or the catabolite activator protein and exhibits temperature-sensitivity of growth on rich medium (24). Holoenzyme containing σ from an *alt-1* strain transcribes the lactose operon *in vitro* without the normal requirement for cyclic AMP and catabolite activator protein (24). However, holoenzyme containing *alt-1* σ is not ts *in vitro* for initiating transcription of most templates (A. A. Travers, personal communication).

A readily selectable marker in the 66-min region would be extremely useful for the genetic identification of possible σ mutants that are ts for growth. Mutants of *dnaG* cannot be used because they are also ts; there is no good selection for *cca*$^+$, and *tolC* and *uxaA* are not conveniently close to the σ gene. Insertion of a drug-resistance transposon near *dnaG* and the σ gene provided a solution to this problem. We introduced Tn10 near 66 min by using cotransduction of *dnaG*$^+$ and *tet*-r from a pool of strains with random insertions of the transposon (ref. 20; a gift of L. Csonka). The *tet*-r marker is 90% cotransducible with *dnaG* and must also lie close to the σ gene. Table 2 part A summarizes the transductional crosses that were done to establish the order of the *tolC*, *dnaG*, and *tet*-r genes. In the first cross, in which *dnaG*$^+$ is selected, the inheritance of the donor

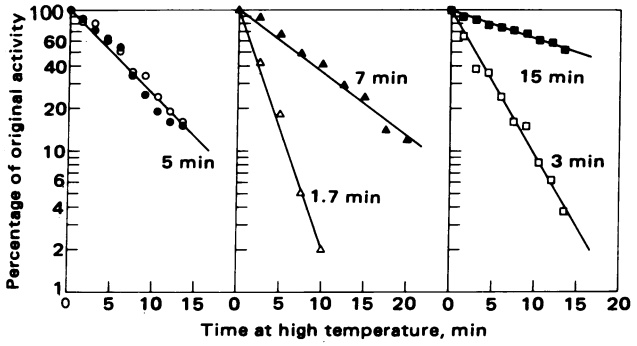

FIG. 2. Thermal inactivation of RNA polymerase components purified from strains P90A5c (wild type) and 285c (mutant). The half-life of the RNA polymerase activity is indicated on the figure. The various RNA polymerase preparations were diluted into enzyme diluent (23) to a final volume of 0.25 ml, and at each time point 20 μl was withdrawn and assayed as described by Gross *et al.* (23), using 2.5 μg of phage T3 DNA per assay rather than 3.5 μg of phage T4 DNA. The triphosphate concentrations were 0.4 mM and the NaCl concentration was 0.1 M. Each assay contained 5 μCi [^{3}H]UTP. (*Left*) Incubation at 50°C of core enzymes at 30 μg/ml from P90A5c (●) (100% = 93 pmol or 51,800 cpm) and 285c (○) (100% = 147 pmol or 80,300 cpm) and subsequent assay in the presence of wild-type σ at 4 μg/ml. The activity of σ alone (1.0 pmol or 500 cpm) accounts for less than 1% of the activity in the presence of unheated core. (*Center*) Incubation at 46.5°C of holoenzymes at 30 μg/ml from P90A5c (▲) (100% = 101 pmol or 55,000 cpm) and 285c (△) (100% = 110 pmol or 60,165 cpm). (*Right*) Incubation at 46.5°C of σ subunits from P90A5c (■) and 285c (□) and subsequent assay in the presence of wild type core enzyme at 30 μg/ml. The activity of the σ preparations alone (0.2 pmol or 100 cpm) accounts for less than 1% of the activity of σ in the presence of added core (66 pmol or 36,000 cpm). The activity of core enzyme alone (11 pmol or 6000 cpm) is responsible for approximately 15% of the total initial activity. This background value was subtracted from the total value measured at each time, and these corrected values were used to generate Fig. 2 (*Right*). This, therefore, represents the activity resulting from the stimulation of core enzyme by σ subunit. The concentration of wild-type σ is 3 μg/ml; the concentration of mutant σ was tested in the range 3–9 μg/ml due to its higher sensitivity to dilution. When small aliquots of RNA polymerase in 50% glycerol are diluted into relatively larger volumes of enzyme diluent, large losses of activity can occur (3, 9). Unfortunately, the 285c σ subunit is more sensitive to dilution than the wild-type σ subunit. When the two σ preparations (from 50% glycerol) are assayed directly, they have approximately equal specific activities. However, when the two σ preparations are diluted into enzyme diluent prior to being assayed, the mutant specific activity is usually only 40–70% of the wild-type specific activity. Thus, to achieve an initial activity level that is equal for both wild-type and mutant σ dilutions that are to be thermally inactivated, the mutant σ protein concentration must be about 2-fold greater than the wild-type σ protein concentration. Varying the mutant σ concentration affects the initial activity level but has little effect on the half-life (data not shown).

tet-r allele disfavors the inheritance of the *tolC* allele and vice versa, suggesting that *dnaG* is the middle marker. The other two crosses of Table 2 part A confirm this conclusion. In both cases in which the donor *tet*-r and *tolC* alleles are coinherited, inheritance of the recipient *dnaG* allele is very rare. These considerations, along with an analysis of the minority classes of transductants, suggest that *tolC-dnaG-tet*-r is the most likely gene order.

Part B of Table 2 shows that *alt-1* is very tightly linked to *tet*-r, probably more so than is *dnaG*, and demonstrates the usefulness of the *tet*-r marker in recognizing σ mutants.

Mapping of a σ Mutant That Is ts Both *In Vivo* and *In Vitro*. In order to find a σ mutant with temperature-sensitivity both *in vivo* and *in vitro*, we performed a genetic screening on 55 spontaneous ts *E. coli* mutants. These mutants were selected by their ability to survive temperature induction of a λ pro-

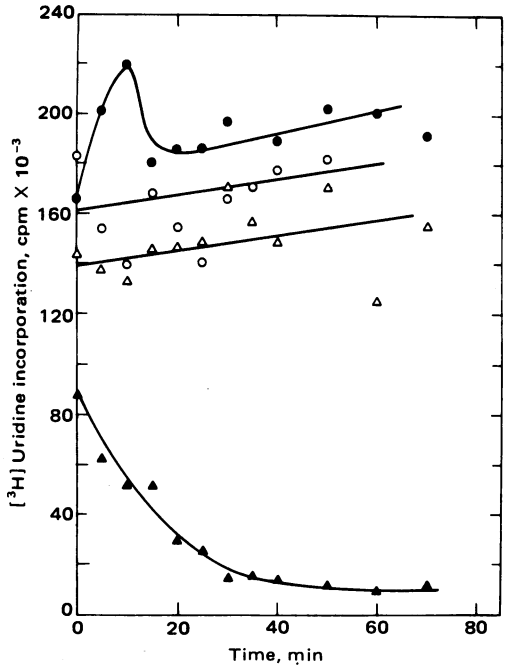

FIG. 3. RNA synthesis in P90A5c and ts mutant 285c at high and low temperatures. Incorporation of [^{3}H]uridine was performed as described in *Materials and Methods*. P90A5c: ●, 43.5°C and ○, 30°C; 285c: ▲, 43.5°C and △, 30°C.

phage, presumably because they do not allow expression of λ phage early genes at high temperature (13). Several of these ts mutations were linked to *argG* at 68 min in crosses with Hfr strains (L. Isaksson, unpublished). These mutants were further mapped by using phage P1 and showed cotransduction of the corresponding wild-type allele with our Tn10 insertion. One mutation, ts285, mapped near *tet*-r on the *tolC* side (Table 2 part C) and was therefore expected to be a σ conditional-lethal mutant. To order the *dnaG3* and ts285 markers, we performed the P1 transductional crosses shown in Table 2 part D. These crosses select for the transfer of *tet*-r from the donor and score for temperature-sensitivity. Because both donor and recipient are ts and considering that the ts markers are very close to *tet*-r, the production of ts$^+$ transductants must be the result of a crossover that occurs between the *dnaG3* and ts285 markers. When the ts marker which is closest to *tet*-r is in the donor strain, an additional crossover is required to produce ts$^+$ progeny. In fact, when the donor carries ts285 and *tet*-r, only 1% of the transductants are ts$^+$, whereas 13% of the transductants are ts$^+$ when the donor carries *dnaG3* and *tet*-r. Therefore, the gene order must be *dnaG3*-ts285-*tet*-r.

In order to map *alt-1* more exactly, we repeated the crosses in part D of Table 2, using *alt-1* in place of ts285. The results for *alt-1* and ts285 are similar, placing ts285 and *alt-1* very close together between *dnaG* and *tet*-r, as might be expected for two σ mutants.

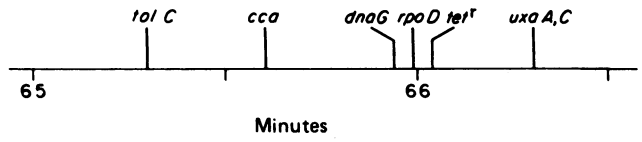

FIG. 4. Fine structure map of the *E. coli* chromosome around 66 min. Placement of *rpoD* and *tet*-r(Tn10) comes from the results described in Table 2 as well as the results of Gross *et al.* (23) for *rpoD1*. The *alt-1* mutant maps at the *rpoD* locus according to the results of Travers *et al.* (24) and Table 2 part B.

Altered Mobility of the Mutant σ Subunit. Upon purification of RNA polymerase from strain 285c we noted that the σ subunit sodium dodecyl sulfate/polyacrylamide gels exhibited an electrophoretic mobility greater than that of wild-type σ (Fig. 1). Coupled with the transductional evidence of the genetic locus of the ts285 marker, this altered mobility provided a strong indication that the mutation in strain 285c affected the σ subunit.

Effect of High Temperature on Wild-Type and Mutant RNA Polymerases *In Vitro*. Samples of RNA polymerase purified from mutant strain 285c and its wild-type parent, P90A5c, were subjected to thermal inactivation studies based on experiments described by Gross *et al.* (23) (Fig. 2). The samples were incubated at high temperature after dilution into an enzyme diluent containing 10 mM Tris·HCl (pH 8), 1 mM dithiothreitol, 0.5 mg of bovine serum albumin per ml, and 0.5 M NaCl (23). Assays on a T3 phase DNA template were performed at 33°C rather than 37°C because the 285c σ has a half-life of 7 min at 37°C (compared to 30–40 min for the wild-type σ).

Core enzymes from both wild type and mutant have the same thermal stability (Fig. 2 *left*). Holoenzyme from the wild type is approximately 4-fold more thermostable than the holoenzyme from the mutant (Fig. 2 *center*). The σ subunit from wild type is approximately 5-fold more thermostable than the σ subunit purified from the mutant cells (Fig. 2 *right*). Thus ts285 causes thermolability of σ *in vitro*.

RNA Synthesis in the σ Mutant Strain. We examined the effect of σ on RNA synthesis *in vivo* by pulse-labeling cells with [^{3}H]uridine and monitoring its incorporation into acid-insoluble material. The incorporation of uridine into RNA decreases abruptly in strain 285c upon increasing the temperature to 43.5°C (Fig. 3). In contrast, the incorporation of [^{3}H]uridine is maintained at a constant level in 285c cells grown at 30°C. P90A5c, the parent strain, exhibits a sharp rise in RNA synthesis as expected upon being placed at 43.5°C, and uridine incorporation soon levels off to a rate comparable to that of P90A5c grown at 30°C.

DNA synthesis in 285c, as indicated by the incorporation of [*methyl*-^{3}H]thymidine, declines much more slowly than uridine incorporation at the nonpermissive temperature (data not shown), indicating a primary lesion in RNA synthesis.

DISCUSSION

Studies on natural variants of the protein indicate that the structural gene for the RNA polymerase sigma subunit, *rpoD*, is located near *dnaG* at 66 min on the *E. coli* chromosome (4, 5). Two mutants mapping at 66 min have recently been shown to affect σ. One of these mutants produces a ts σ protein, but displays a wild-type phenotype (23). The other mutant (*alt-1*) has a ts phenotype, but produces a σ that is not ts *in vitro* (ref. 24; A. A. Travers, personal communication). We report here a mutant that is ts both *in vivo* and *in vitro*, showing that σ is essential for *E. coli* growth. This mutant increases the electrophoretic mobility of the σ protein on sodium dodecyl sulfate/polyacrylamide gels. The alteration of all of the cell's σ protein by this single mutation strengthens the idea that a single gene at 66 min codes for the synthesis of σ protein. The alteration in electrophoretic mobility may reflect a reduction in the size of the σ protein, for instance, by introduction of a nonsense mutation near the carboxyl terminus. Alternatively, the mutant σ protein may be reduced in size by proteolysis. Additional genetic and chemical studies of this mutant will be needed to distinguish between these and other alternatives.

The *rpoD* ts mutant reported here was identified from a large collection of spontaneous mutants (13) by the use of a transposon

(Tn10) inserted near *dnaG*. The fine structure map of the 66-min region, which is derived from the crosses described in Table 2 and in refs. 5, 16, 22–25, is shown in Fig. 4. This map includes the transposon, which is not present in most *E. coli* strains.

The association of the Tn10 transposon with the *E. coli* genes at 66 min may facilitate efforts to introduce this region into a plasmid that could be used to induce synthesis of the σ protein *in vivo* or *in vitro* (27). This would provide definitive proof that the *rpoD* gene is indeed at 66 min.

The shutoff of RNA synthesis in strain 285c at nonpermissive temperature (Fig. 3) is sufficiently rapid and complete to suggest that the σ subunit may be required for all transcription initiations in *E. coli*. However, a more careful analysis of the kinds of transcripts synthesized after the temperature shift might reveal a special class of transcripts that are initiated in the absence of σ.

Some classes of middle and late phage genes can be transcribed in the absence of σ (28, 29). It should be possible to confirm that coliphage T4 late genes are expressed without σ (29) by using strain 285c and shifting to nonpermissive temperature after T4 early genes have been expressed. The same procedure could be used to test whether expression of other coliphage late genes is σ dependent.

Another σ ts mutant, similar to the one reported here, has been isolated by the technique of localized mutagenesis (30).

Note Added in Proof. Fifteen independent ts^+ revertants of strain 285c have been isolated. σ from all 15 revertants exhibits the mobility characteristic of 285c σ during sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Thus these reversion events must have occurred at sites distinct from the original ts285 mutation, which may be a short deletion. Mapping analysis of seven of these ts^+ revertants supports the idea that they occur at secondary sites.

We appreciate the advice and encouragement of Laszlo Csonka, who provided the pool of Tn10 insertion strains, and we wish to thank Brian Sauer for phage and bacterial strains, for the P1 stock used in preparing C-2359, and for many useful discussions concerning the handling of these strains. We thank Barbara Small for assistance in some of the transductions. We are grateful to Murray Deutscher, Yoshikazu Nakamura, and Andrew Travers for providing us with unpublished data. This work was supported in part by National Institutes of Health Grant AI 08722 from the National Institute of Allergy and Infectious Diseases and CA 14097 from the National Cancer Institute; Research Grant PCM 76-19935 from the National Science Foundation; Grant VC-188 from the American Cancer Society; University of California President's Undergraduate Fellowships to J.S.H. and I.M.; and a grant to L.I. and C.G. Kurland from the Swedish National Science Foundation.

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