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Genetics

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

(transcription/*rpoD1*/RNA nucleotidyltransferase)

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ABSTRACT **The *Escherichia coli* strain, ts-rnp5, originally described in 1975 by G. D. Burdick and H. Berger, is shown to possess an RNA polymerase (RNA nucleotidyltransferase) σ subunit with an activity 4–6 times less thermostable at 45° than σ from wild-type strains. This defect remains associated with the σ polypeptide through a variety of purification stages, including renaturation of σ after its elution from sodium dodecyl sulfate/polyacrylamide gels. The mutation responsible for decreased thermostability of σ, called *rpoD1*, cotransduces with *dnaG* and therefore is located at about 66 min on the *E. coli* genetic map.**

RNA polymerase (RNA nucleotidyltransferase) holoenzyme of *Escherichia coli* is a multisubunit enzyme of composition $\alpha_2\beta\beta'\sigma$ (1). The enzyme consists of core polymerase ($\alpha_2\beta\beta'$) and a dissociable initiation factor sigma (σ). The structural genes for β (*rpoB*) and β' (*rpoC*) form an operon (2) located at 88.5 min on the *E. coli* linkage map (3). The structural gene for α (*rpoA*) has been found within a cluster of ribosomal protein genes located at 72 min on the *E. coli* linkage map (4). Recently it has been shown that strains of *E. coli* containing episomes carrying the region around 66 min produce elevated levels of σ (5), that introduction of these episomes into *Salmonella typhimurium* results in the synthesis of σ characteristic of *E. coli* and distinguishable from *Salmonella* σ (5, 6), and that strains of *E. coli* C transduced with the *dnaG* marker at 66 min from *E. coli* K12 have obtained the σ characteristic of *E. coli* K12 (6).

One method for studying the role of RNA polymerase in transcription and regulation is to analyze mutations affecting the structural genes for RNA polymerase subunits. So far, mutations in the structural genes for α, β, and β' have been reported (see ref. 7 for a review; ref. 8). No mutants have been reported in the σ structural gene (*rpoD*).

We report here the characterization of a mutation that decreases the functional stability of the σ polypeptide *in vitro*. We show that the decreased thermostability of the altered σ remains associated with σ protein through all purification steps. The mutation is located close to *dnaG* at 66 min on the *E. coli* genetic map. We propose that the thermosensitive σ mutation described in this paper defines the σ structural gene and that it be called *rpoD1*.

MATERIALS AND METHODS

Media. Cells were grown in standard broth (1% bactotryptone/0.5% yeast extract/0.5% NaCl, wt/vol, adjusted to pH 7.0 with 1 M NaOH), except where indicated otherwise. Glucose was added to 0.2% for mating and transduction experiments; 1 mM $CaCl_2$ was added for P1 *vir* adsorption. Minimal medium for liquid and plate growth was M9 (9) with the addition of growth supplements to 20 μg/ml where required. Glucose (0.2%, wt/vol) was the standard carbon source. Buffer for resuspending bacteria was 10 mM Tris·HCl (pH 7.9)/1 mM $MgSO_4$.

Bacterial Strains. Those used in this work are described in Table 1.

Transduction. P1 *vir* phage was obtained from A. Wright. Transducing stocks were prepared as described by Miller (9). The transduction procedure was that of Miller (9) except that the multiplicity of infection was 0.5 and sodium citrate was added to 10 mM after absorption for 20 min at 37°.

Mating. All matings were at 37° on 0.45-μm Millipore filters placed on broth agar plates that had been warmed to 40°. Hfr matings were done at a ratio of 0.2 Hfr to 1 recipient. Nalidixic acid was used both to interrupt mating and to counterselect the Hfr cells (12).

Isolation of ts-rnp5. The original strain, ts-rnp5, was isolated by the penicillin selection method described by Miller (9) and a modification of the autoradiographic screening method used by Wechsler and Gross (13). Strain W3110 *thy*A$^-$ was mutagenized by treatment with 25 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich) per ml for 1 hr in 0.1 M citrate buffer, pH 5.5 (14). The mutagenized stocks were grown in Penassay broth (Difco) at 30° to a cell density of 1×10^8 cells per ml and shifted to 45° for 30 min. Penicillin was then added to a final concentration of 10,000 units/ml and incubation was continued at 45° for 1 hr. This constitutes the enrichment step for temperature-sensitive growth mutants. Cells were plated at 30°; individual colonies surviving the penicillin treatment were transferred to master plates and incubated at 30° until colonies were approximately 0.5 mm in diameter. Colonies were replica plated onto a sterile Millipore filter and the filter was placed on a minimal agar plate containing 40 μg of thymine and 20 μg of uracil per ml. These filters were incubated on the plate for 30 min at 45° and then transferred to fresh minimal agar plates as before except that 0.05 μCi of [^{14}C]uracil (29 Ci/mol) was added per ml in addition. Incorporation at 45° was terminated after 1 hr by placing the filters on agar with 0.1 M NaOH, followed by transfer to plates containing 100 μg of unlabeled uracil per ml. The filters were dried and subjected to autoradiography for 8–12 hr with Kodak No-Screen (NS54T) x-ray film. Colonies that did not incorporate labeled uracil at 45° did not exhibit an autoradiographic spot and were picked

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.
‡ Deceased.

Table 1. Bacterial strains

Strain	Relevant genotype	Origin (ref.)
W3110 *thyA*$^-$	F$^-$*thyA*$^-$	B. Bachmann (10)
ts-rnp5	F$^-$*thyA*$^-$*rpoD1*	This paper*
AB1157	F$^-$ *thi-1 leu-6 proA2 argE3 his-4 lac 1 galK2 ara-14 xyl-5 mtl-1 rpsL31 tsx-33 sup-37 supE44*	B. Bachmann (10)
AB1157 *nal*^{R}	As AB1157 except *nal*^{R}	This paper†
AB1157 *rpoD1 nal*^{R}	As AB1157 *nal*^{R} except *rpoD1*	This paper*
AB1157 *rpoD1 nal*^{R} *aroE*$^-$	As AB1157 *rpoD1 nal*^{R} except *aroE*$^-$ *rpsE*$^-$	This paper‡
AB1157 *rpoD1 nal*^{R} *tolC*$^-$	As AB1157 *rpoD1 nal*^{R} except *tolC*$^-$	This paper§
PC3	F$^-$*leu6 thyA47 dra 3 rpsL153 dnaG3*	P. Carl (11)
PC314	HfrC *dnaG3*	P. Carl (11)
NO1247	F$^-$*aroE*$^-$	M. Nomura
NO1247 *rpsE*$^-$	F$^-$ *aroE*$^-$ *rpsE*$^-$	This paper¶
LM5	F$^-$ *aspB*$^-$ *argG*$^-$ *rpsL*$^-$ λ^R	S. Baumberg
HfrH	Hfr *thi*$^-$	B. Bachmann (10)
KL14	Hfr *thi-1 relA1*	B. Bachmann
KLF2/JC1553	F'102 *argG6 metB1 his-1 leu6 recA1 mtl-2 xyl-7 malA1 gal-6 lac 1 rpsL104 tonA2 tsx-1 supE44* λ^R	B. Bachmann
KLF2/ts-rnp5	F'102 *thyA*$^-$ *rpoD1*	This paper*

* Construction described in *Materials and Methods*.
† Selected as resistant to 20 μg of nalidixic acid per ml on broth plates.
‡ The *aroE*$^-$ marker was transduced into the AB1157 strain by P1 grown on NO 1247 rpsE$^-$. Transductants were selected for rpsE$^-$ phenotype and screened for *aroE*$^-$ marker.
§ Selected as resistant to ColE1 and sensitive to 0.05% sodium deoxycholate on broth plates.
¶ Selected as resistant to 100 μg of spectinomycin per ml on broth plates.

and saved for further study. Strain ts-rnp5 was one of the colonies saved for further screening.

Construction of AB1157 *rpoD1*. This mutant strain (ts-rnp5) was unable to grow on broth media at 42° as a result of a lesion located at 14 min on the *E. coli* map (15). However, this phenotype is not a consequence of thermolabile σ since recombinants between HfrH and ts-rnp5, selected for the ability to grow on broth plates at 42°, retain the thermolabile σ activity characteristic of the ts-rnp5 parent. The lesion at 14 min has not been characterized further. In order to study the mutation affecting σ stability, we transferred it from the original heavily mutagenized strain into AB1157 by the procedure detailed below.

F′ episomes mediate chromosome transfer from the Hfr origin present on the F′ episome with an efficiency approximately 1% that of the cognate Hfr. This low efficiency of transfer may reflect the fraction of the cells in which the F′ episome is integrated at any time. A derivative of ts-rnp5 carrying F′ episome KLF2 was identified as a galactose-fermenting colony sensitive to the male-specific phage MS2 after a mating between KLF2/JC1553, which is *gal*$^-$, and ts-rnp5. KLF2/ts-rnp5 was then mated with the *argE*$^-$ strain, AB1157 *nal*^{R}, and *argE*$^+$*nal*^{R} recombinants selected. Recombinants were screened for thermolabile σ. Those recombinants possessing AB1157 markers and thermolabile σ were termed AB1157 *rpoD1*.

Purification of RNA Polymerase. RNA polymerase holoenzyme, core polymerase, and σ subunit were prepared from wild-type cells (W3110 *thyA*$^-$) and mutant cells (ts-rnp5) by the procedure of Burgess and Jendrisak (16) except that the final purification of σ was done by gel filtration chromatography on Ultrogel AcA 44 (LKB). These components will be referred to as highly purified.

Gel Electrophoresis. Purity of the RNA polymerase preparations was routinely monitored by electrophoresis on 8.75% polyacrylamide/sodium dodecyl sulfate ($NaDodSO_4$) stacking gels prepared by the procedure of Laemmli and Favre (17) as modified by Burgess and Jendrisak (16). σ subunit was eluted and renatured from $NaDodSO_4$ gels by the following modification of the method of Weber and Kuter (18). Ten micrograms of highly purified σ and 20 μg of carrier β-lactoglobulin (Schwarz/Mann) were subjected to electrophoresis on cylindrical gels and the region containing σ was visualized by soaking the gel in 0.5 M KCl/1 mM dithiothreitol. The $NaDodSO_4$ bound to the protein band is precipitated by the KCl and a white band is observed. The region of the gel containing σ was cut out, rinsed in water, and crushed, and the protein was eluted for 6 hr at 4° with 1 ml of 50 mM Tris·HCl (pH 7.9)/50 μg of bovine serum albumin per ml (Pentex)/0.1 mM EDTA/1 mM dithiothreitol/0.1% $NaDodSO_4$. Solid urea was added to 6 M; 30 min later, 0.1 ml of Dowex 1-X2 resin added. After 30 min of occasional mixing, the mixture was centrifuged to remove the gel and resin and the supernatant was removed and dialyzed at 4° against 10 mM Tris·HCl (pH 7.9)/0.1 mM EDTA/5% (wt/vol) glycerol/1 mM dithiothreitol/0.2 M NaCl to remove the urea and to allow renaturation of the σ protein.

DNA. T4 DNA was extracted from CsCl-banded T4 phage by lysis of the phage in 0.5% $NaDodSO_4$ at 65° followed by precipitation of $NaDodSO_4$ and protein by 0.5 M KCl. Extracted DNA was dialyzed against several changes of 1 mM EDTA/10 mM Tris (pH 7.9) and stored at 4°.

Rapid Partial Purification of RNA Polymerase. The RNA polymerase micropurification procedure of Gross *et al.* (19) was followed through the step *iv* extract except that 100 ml of cells were grown at 30°. and harvested at an absorbance at 590 nm of 0.6. The volumes of solutions A, B, C, and D were doubled. The step *iv* extract, which contained about 100 μg of RNA polymerase per ml, approximately 10% pure, was used to assay the thermostability of σ protein in the mapping experiments reported here.

Procedure for Heat Inactivation. The material to be thermally inactivated was diluted to the protein concentration indicated in the legend to Fig. 2 in 10 mM Tris·HCl (pH 7.9)/1 mM dithiothreitol/500 μg of bovine serum albumin per ml/ enough NaCl to yield a final NaCl concentration of 0.5 M. A 250-μl sample was placed in a sterile, siliconized glass tube,

heated at 37° for 1 min, and then placed in a water bath at the inactivation temperature. At intervals, 20-μl samples were removed to siliconized glass tubes on ice and subsequently assayed at 37° for σ activity. The temperatures used for inactivation are indicated in the legend to Fig. 2 and were chosen to give half-times of inactivation of between 1 and 4 min for the most labile component.

Assay for σ Activity. The activity of σ was measured by its ability to stimulate core polymerase to transcribe T4 DNA (20). Transcription by core polymerase in the absence of σ was subtracted from each point. When saturating amounts of σ were added, transcription was 40–60 times greater than with core polymerase alone. In all assays reported in this paper, excess core was present so that σ was limiting and the amount of transcription observed was directly proportional to the amount of σ added.

Each assay for σ activity contained (in 100 μl) 25 mM Tris·HCl (pH 7.9), 200 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 0.2 mM CTP, GTP, and ATP, 0.05 mM UTP, 10 μCi of [^{3}H]UTP per ml, 35 μg of T4 DNA per ml, 1 mM K_2HPO_4, 1 mM dithiothreitol, and 500 μg of bovine serum albumin per ml. Core polymerase was added at 1 μg per assay except when temperature inactivation of core polymerase or holoenzyme was studied. Samples were incubated at 37° for 10 min, precipitated with 3 ml of cold 5% trichloroacetic acid on ice, filtered onto Whatman GF/C filters, washed, and dried; radioactivity was measured in a scintillation counter (16).

RESULTS

Thermal inactivation studies of RNA polymerase from mutant and wild-type cells

Thermal inactivation studies were carried out on σ and core polymerase prepared from holoenzyme. The purity of the σ and core polymerase used in these studies was analyzed by electrophoresis on $NaDodSO_4$/polyacrylamide gels and is shown in Fig. 1 (lanes A–F). We find that the stimulatory activity of σ isolated from cells containing the mutation *rpoD1* is 4–6 times less thermostable than that from wild-type cells. This difference is observed whether σ is assayed on core purified from mutant (Fig. 2*A*) or wild-type (Fig. 2*B*) cells. The decrease in thermostability of σ from mutant cells is not a consequence of a destabilizing impurity such as a protease since a 1:1 mixture of σ from mutant and wild-type cells shows a biphasic decay curve with half-lives characteristic of each σ alone (Fig. 2*A*). The differential thermostability of each σ is maintained when heat inactivation is carried out in the presence of homologous core (Fig. 2*C*) or heterologous core (data not shown). Core polymerase from mutant and wild-type cells shows identical thermostability (Fig. 2*D*).

The decreased thermostability of σ from mutant cells compared to that from wild-type cells might result from damage to σ during the purification procedure. However, when equal weights of mutant and wild-type cells are mixed and RNA polymerase partially purified from the mixture, we find that the decay of σ activity after heating was biphasic with the half-lives expected from the sum of the individual decay curves (Fig. 2*E*). We conclude that the difference in σ thermostability cannot be due to an artifact occurring during purification.

Analysis of our σ preparations from mutant and wild-type cells on $NaDodSO_4$/polyacrylamide gels indicates that some impurities are still present (Fig. 1, lanes E and F). The difference in thermostability might be due to impurities that remain noncovalently associated with the σ protein. We therefore further purified our σ preparations by preparative electrophoresis on $NaDodSO_4$/polyacrylamide gels followed by elution from the gel and renaturation. Gel analysis of this $NaDodSO_4$ gel-purified material indicates that the major visible contaminants in our σ preparation are removed by this procedure (Fig. 1, lanes G and H). Only the carrier bovine serum albumin and σ are visible. We find that renatured σ from mutant cells is substantially less thermostable than that from wild-type cells (Fig. 2*F*). Thus, the differential thermostability results from a difference in the σ polypeptide itself or from a covalently bound constituent.

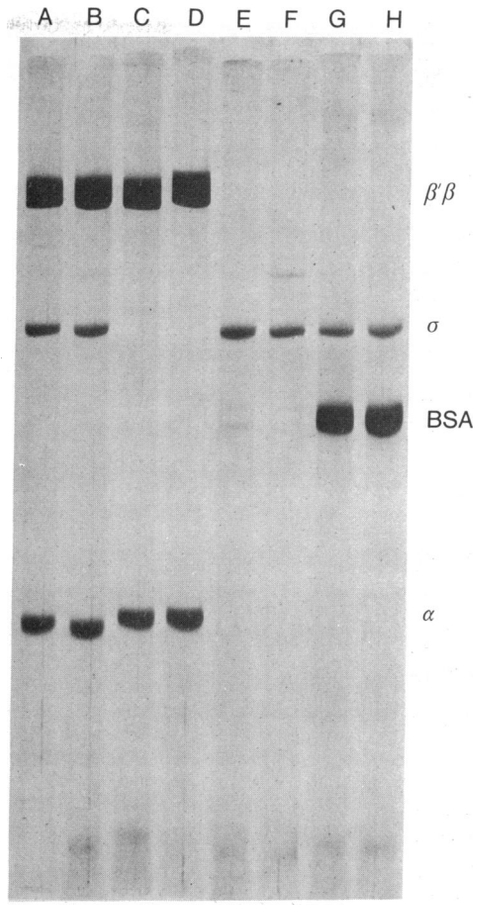

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis of protein fractions used for heat inactivation studies. (A) Mutant holoenzyme; (B) wild-type holoenzyme; (C) mutant core polymerase; (D) wild-type core polymerase; (E) mutant σ; (F) wild-type σ; (G) mutant σ after elution and renaturation from $NaDodSO_4$ gels; (H) wild-type σ after elution and renaturation from $NaDodSO_4$ gels. Subunits are indicated on the right. Bovine serum albumin (BSA) in gels G and H is present as a carrier during elution and renaturation.

Mapping the lesion resulting in decreased thermostability of σ

All genetic mapping experiments used the AB1157 *rpoD1* strain described in *Materials and Methods*. Our initial studies indicated that the growth of AB1157 *rpoD1* in broth was indistinguishable from that of the parental strain at all temperatures tested. Since we were unable to find an *in vivo* phenotype to score the type of σ present in the cell, all further analyses of genetic crosses relied on the strong *in vitro* phenotype described above (Fig. 2*E*). RNA polymerase from selected recombinant classes was partially purified and the rate of thermal inactivation of sigma activity was determined in order to score recombinants for their σ phenotype.

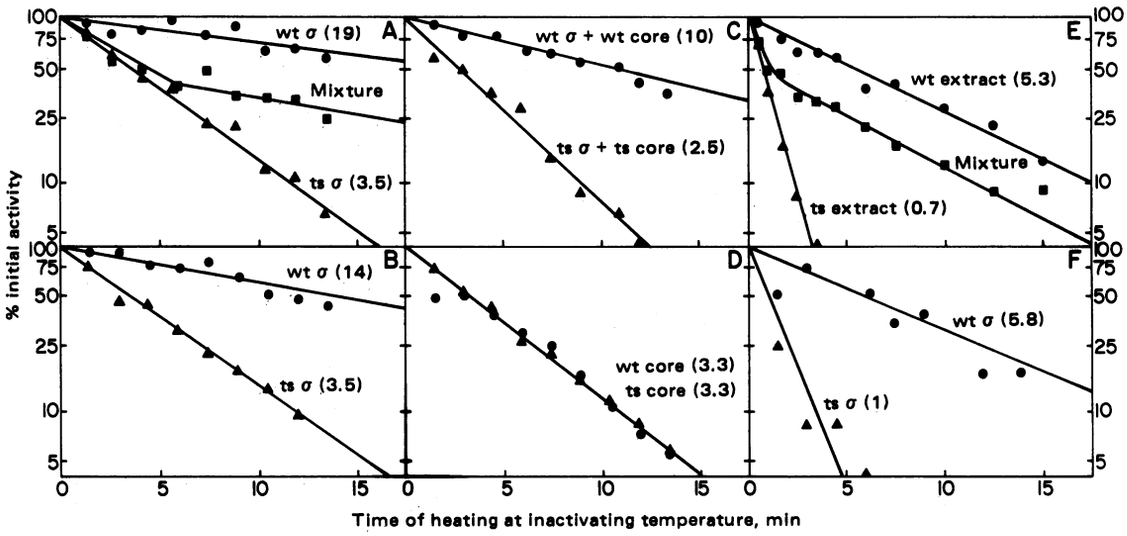

FIG. 2. Kinetics of heat inactivation of wild-type and temperature-sensitive RNA polymerase components. The ordinate represents the logarithm of the percent initial activity. In all cases 100% activity is about 60,000 cpm of [^{3}H]UTP incorporated. A background of 1000–2000 cpm, representing transcription due to core polymerase alone, has been subtracted from each point. The half-lives (in min) are indicated in parentheses on the figures. Symbols are: ▲, component purified from ts-rnp5 mutant cells; ●, component purified from wild-type cells; ■, mixture of temperature-sensitive and wild-type components. (*A*) Inactivation at 46.5° of purified mutant σ, wild-type σ, and a mixture of the two at 3 μg/ml; assayed by the ability to stimulate transcription of T4 DNA by mutant core polymerase. (*B*) Inactivation at 46.5° of mutant and wild-type σ at 3 μg/ml; assayed by the ability to stimulate wild-type core polymerase. (*C*) Inactivation at 46.5° of mutant and wild-type σ at 3 μg/ml in the presence of 50 μg of homologous core polymerase per ml; assayed for the ability to transcribe T4 DNA with no additional added core polymerase. The ratio of σ to core in the assay was the same as in *A* and *B* but core was added before, rather than after, heat inactivation. (*D*) Inactivation at 52.6° of mutant and wild-type core polymerase at 30 μg/ml; assayed by the ability to transcribe T4 DNA in the presence of saturating amounts of σ added just before the assay. (*E*) Inactivation at 45° of extracts, containing 15 μg of RNA polymerase per ml, made from mutant, wild-type, and a 1:1 mixture of mutant and wild-type cells; assayed as in *B*. (*F*) Inactivation at 46.5° of mutant and wild-type σ after elution from Na-DodSO$_4$/polyacrylamide gels and renaturation; assayed as in *B*.

The location of the *rpoD1* mutation was roughly determined by asking which Hfr strains transfer wild-type σ as an early marker into AB1157 *rpoD1*. The Hfr strain KL14 transfers its genetic material clockwise from an origin located at 65.5 min on the *E. coli* map. When KL14 is mated with AB1157 *rpoD1* *aroE*$^-$ *nal*^{R} for a short time (5 min) and recipient cells recombinant for the *aroE* marker at 72 min are selected, 75% of the recombinants have the wild-type σ phenotype of KL14. Therefore, the σ mutation is transferred early by KL14 and most probably maps between the KL14 origin at 65.5 min and the *aroE* marker at 72 min on the *E. coli* map.

The *rpoD1* mutation was localized more precisely by transductional analysis. We prepared a P1 *vir* phage stock on AB1157 *rpoD1* and used it to transduce strain NO 1247 with an *aroE*$^-$ marker at 72 min and strain LM5 with an *aspB*$^-$ marker at 68.5 min and an *argG*$^-$ marker at 68 min. We found no cotransduction between *rpoD1* and these markers.

However, when we used this P1 phage stock to transduce strain PC3, containing a *dnaG*ts marker at 66 min, out of 73 *dnaG*$^+$ transductants, 59 (or 81%) were also recombinant for σ. Similar results (89%) were obtained with PC314, another *dnaG*ts strain. This 81–89% cotransduction with *dnaG* indicates that *rpoD1* is located about 0.1 min from *dnaG*, very close to 66 min.

We have asked whether *rpoD1* is located clockwise or counterclockwise from the *dnaG* marker. As a third marker in the cross, we used *tolC*, located at about 65.5 min. This marker cotransduces about 15% with *dnaG* (11). We prepared a P1 stock on a *tolC*$^-$ derivative of AB1157 *rpoD1* and transduced PC314 to *dnaG*$^+$. Results are shown in Table 2, where it can be seen that the frequency of σ recombinants among transductants recombinant for both *tolC* and *dnaG* is about the same as the frequency among those recombinant only for *dnaG*. This result would not be expected if σ were located between *tolC* and the *dnaG*3 marker.

DISCUSSION

The σ subunit of RNA polymerase plays an important role in the selectivity of RNA chain initiation *in vitro*. Until now, a genetic analysis of the σ protein requisite for definition of its role *in vivo* as well as that *in vitro* has not been possible. We report here a mutation known to affect σ activity. This mutation, *rpoD1*, renders the σ protein 4–6 times less thermostable than σ isolated from wild-type cells.

We have thoroughly investigated the *in vitro* behavior of σ

Table 2. Mapping of *rpoD1* by P1 transduction

Markers*		No. in class†		
Scored	Selected	*rpoD*$^+$	*rpoD1*	Total‡
tolC$^+$	*dnaG*$^+$	4(10)	38(90)	42(100)
tolC$^-$	*dnaG*$^+$	6(14)	36(86)	42(100)

Recombinants were formed by P1 transduction. The donor (P1 stock grown on AB1157 *tolC*$^-$ *rpoD1*) was *tolC*$^-$ *dnaG*$^+$ *rpoD1*. The recipient (PC314) was *tolC*$^+$ *dnaG*ts *rpoD1*$^+$. Marker *tolC* is located at about 65.5 min on the *E. coli* genetic map; marker *dnaG* is at about 66 min.

* After transduction, *dnaG*$^+$ cells were selected by growth on broth plates at 42° and scored *tolC*$^-$ if they were unable to grow on broth plates containing 0.05% sodium deoxycholate at 37° (11).

† Numbers in parentheses indicate % of the total tested recombinants in this class.

‡ All *tolC*$^-$ *dnaG*$^+$ recombinants, but only random subset of the *tolC*$^+$ *dnaG*$^+$ recombinants, were grown up and assayed. The frequency of *tolC*$^-$ *dnaG*$^+$ recombinants was 16% of the total *dnaG*$^+$ recombinants.

from the mutant strain. We find that the thermolability remains associated with the σ protein through a variety of purification steps, including column chromatography and renaturation from gels. Furthermore, copurification of σ from wild-type cells with that from mutant cells does not increase the thermolability of wild-type σ. We conclude that σ thermolability in the mutant cells is not caused by a substance present only in mutant extracts during purification.

We have used linked transduction by bacteriophage P1 to localize the *rpoD1* mutation on the *E. coli* genetic map. We find that the mutational lesion is about 81–89% cotransducible with *dnaG* and is therefore located at about 66 min on the *E. coli* map. We can select that class of transductants recombinant not only for *dnaG* but also for *tolC*, another marker located at 65.5 min. If the σ gene were located between *tolC* and *dnaG*, we would expect 100% of the cells recombinant for both *tolC* and *dnaG* to be recombinant for σ as well. We find instead that the fraction recombinant for σ is the same whether transductants are recombinant for *dnaG* only or for *tolC* and *dnaG*. This result is consistent with the mutational lesion affecting σ thermostability being located clockwise from the *dnaG 3* marker. Our conclusions are consistent with the map position recently reported by two groups for the σ gene based on differences in the electrophoretic mobilities of σ subunits from *E. coli* C and K and from *Salmonella*. Nakamura *et al.* (5) and Harris *et al.* (6) have found by F′ mapping that the σ gene is located close to *dnaG*. In addition, the transductional data of Harris *et al.* (6) indicate that this gene is very close to *dnaG*.

We have asked what effect this mutation in the σ gene has on the physiology of the *E. coli* cell. We find that both mutant and wild-type cells show a similar cutoff in permissible growth temperature at about 43° on broth plates. However, we have recently found that a PC314 *dnaG*$^+$ transductant possessing thermolabile σ grows significantly more slowly at 43° in M9 medium supplemented with amino acids than a similar transductant with wild-type σ. The nature of the *in vivo* defect must be clarified and that of the alteration in the σ polypeptide chain must be determined.

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