Proc. Natl. Acad. Sci. USA
Vol. 76, No. 12, pp. 6510–6514, December 1979
Genetics

Gene for the RNA polymerase σ subunit mapped in *Salmonella typhimurium* and *Escherichia coli* by cloning and deletion

(*dnaG*/*rpoD*/transcription)

JOHN G. SCAIFE*†, JOSEPH S. HEILIG*, LEE ROWEN‡, AND RICHARD CALENDAR*§

*Department of Molecular Biology, University of California, Berkeley, California 94720; and ‡Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Communicated by Donald A. Glaser, September 10, 1979

ABSTRACT **The genes for the RNA polymerase σ subunit (*rpoD*) and DNA primase (*dnaG*) of *Salmonella typhimurium* have been cloned into λ vectors. Combined restriction, deletion, and functional analysis of the cloned fragment allows us to map the genes precisely on the fragment, establishes the direction in which *rpoD* is transcribed, and reveals the existence of at least one new gene in the vicinity. A closely homologous, smaller fragment of *Escherichia coli* DNA, also cloned into λ, contains *rpoD* and at least part of *dnaG*.**

The genes coding for the σ subunit of RNA polymerase (*rpoD*) (1-6) and for primase (*dnaG*) (7, 8) play an important role in the regulation of bacterial growth. Much evidence suggests that *rpoD* is close to *dnaG* (2, 3). In this report we show that *rpoD* and *dnaG* are contained in a single 5-kilobase (kb) DNA sequence, implying that they are adjacent. To gain insight into their organization, we have cloned these genes in λ phage vectors and mapped them precisely by deletion and restriction endonuclease analysis. Studies on expression of the cloned genes in the presence and absence of λ repression allow us to establish the orientation of *rpoD* and the location of its promoter.

MATERIALS AND METHODS

Bacterial and Phage Strains. Bacterial strains and λ phage vectors are listed in Table 1. λgt7-(*dnaG*)St was isolated from a pool of hybrid phages made by M. Thomas by selecting phage carrying the *dnaG* gene. Phage (10^8 particles) were mixed with 10^9 BT308λ$^+$ cells and, after 15-min adsorption at room temperature, the mixture was spread on an agar plate and incubated at 42°C for 18 hr. Twenty-nine colonies were picked and restreaked. Cultures were grown and treated with $CHCl_3$. Plaques made by the released phage were picked and grown, and their DNA was analyzed by digestion with restriction enzymes. λNM540-(*rpoD dnaG*)St was constructed by ligating DNA obtained from *Hin*dIII digests of 500 ng each of λNM540 and λgt7-(*rpoD dnaG*)St in 10 μl containing 0.7 μg of T4 DNA ligase, 0.5 mM ATP, and 10 mM dithiothreitol. The mixture was incubated for 12 hr at 4°C. The DNA was packaged *in vitro* (22), and turbid plaques were picked and screened by plaque filter hybridization (23) to the *Salmonella typhimurium rpoD dnaG Eco*RI 10.8-kb insert fragment. Positive plaques were tested for transduction of BT308λ$^+$, selecting for growth at 42°C. The procedures and materials for growth of phage and bacteria have been described (20, 24).

Restriction Enzyme Analysis. Restriction nucleases were purchased from New England BioLabs or from Bethesda Research Laboratories and were used in accordance with the instructions of the supplier. Enzymes used were *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn* I, *Sal* I, *Sst* I, and *Xho* I. After they were digested, the DNA samples were analyzed by electrophoresis in 10-cm agarose gels containing 90 mM Tris·HCl at pH 8.3, 90 mM boric acid, 2.5 mM EDTA, and either 0.5% or 0.7% agarose. Electrophoresis was for 18 hr at 25 V or for 5 hr at 70 V. Restriction sites were mapped by comparing the DNA fragments from single-enzyme digests with fragments from double-enzyme digests.

RESULTS

Isolation of λ Phages Carrying *rpoD* and *dnaG*. In order to isolate a phage carrying the *S. typhimurium* genes for DNA primase and for the σ subunit of RNA polymerase, we screened a pool of λ phages containing *Eco*RI fragments of bacterial DNA for the ability to transduce *dnaG*. The phages, provided by M. Thomas and R. W. Davis, were made by ligating and packaging *Eco*RI-cleaved vector λgt7 (Table 1) and *S. typhimurium* DNA. They were used to infect BT308λ$^+$ [*Escherichia coli dnaG*308 (λ)], and colonies able to grow at 42°C were used as a source of the desired phage. Some of these, such as λgt7-(*rpoD dnaG*)St can transduce *rpoD*$^+$ as well as *dnaG*$^+$ (see below). They carry a 10.8-kb fragment of *S. typhimurium* DNA. Others cannot transduce to *rpoD*$^+$ and only contain a part of the 10.8-kb fragment. The whole fragment was purified, labeled with ^{32}P by nick translation, and used as a probe to identify a homologous DNA fragment from *E. coli* (Fig. 1). Fragments of *E. coli* DNA were separated electrophoretically, transferred onto a nitrocellulose sheet, and hybridized with the *S. typhimurium* probe. Autoradiography revealed that the *Salmonella* probe hybridized to a 9-kb *Hin*dIII fragment of *E. coli* DNA. For this reason, the 9-kb *Hin*dIII fragment was cloned into λNM762 (see Table 1). Selection for *dnaG*$^+$ transducing phages yielded a phage capable of transducing *rpoD*$^+$ as well as *dnaG*$^+$. DNA from this phage hybridized strongly with DNA from the *Salmonella* probe (Fig. 1).

The Cloned *rpoD* and *dnaG* Genes Function *In Vivo*. The mutation *rpoD*285 results in thermolability of the σ subunit of the *E. coli* RNA polymerase. The evidence strongly suggests that *rpoD*285 is in the structural gene for σ (*rpoD*) (5). The *Eco*RI fragment of *S. typhimurium* cloned into phage λ efficiently transduced this mutant (PM101, Table 1) to temperature resistance. Moreover, transductants cured of the λ prophage with a heteroimmune λ phage, *imm* 434, regained temperature-sensitivity in 15 of 18 cases (data not shown). Thus, genetic evidence shows that the *Eco*RI fragment has an active gene able to compensate for *rpoD*285. The following evidence shows that this gene is *rpoD* from *S. typhimurium*.

Bacteria heavily irradiated with UV light virtually cease to make their own proteins but will transcribe and translate genes on an infecting transducing phage. A λ phage containing the *Eco*RI fragment directed UV-irradiated *E. coli* (159λ$^+$, in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: kb, kilobases; ts, temperature-sensitive; Ec, fragment from *Escherichia coli* DNA; St, a fragment from *Salmonella typhimurium*; $NaDodSO_4$, sodium dodecyl sulfate.

† On leave from Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland.

§ To whom reprint requests should be addressed.

Table 1. Bacterial and phage strains

Designation	Additional genotype	Origin or reference
	Bacteria	
159	*uvrA gal str*	9
159λ$^+$	*uvrA gal str* (λ*ind*$^-$)	9
159λimm^{21}	*uvrA gal str* (λ*imm*^{21})	From 159 by lysogenization
285c	F$^-$ *argG lac thi rpoD*285	5
594	*str*	10
BT308λ$^+$	*thy dnaG*308 *thi leu lac str* (λ)	7
K1200	*endA rna*	11
PC314	HfrC *dnaG*3	12
PM101	F$^-$ *argG lac thi rpoD*285(λ$^+$)	From 285c by lysogenization
PM102	F$^-$ *argG lac thi rpoD*285(λ$^+$) [λgt7-(*dnaG rpoD*)St]	From PM101 by transduction, selecting for growth at 42°C
PM106	HfrC *dnaG*3 (λ$^+$)	From PC314 by lysogenization
RW	HfrH with λ cryptic in *galT*; *supF* (from RW842)	13
W3102	λ^s	14
W3110	*thy*	15
Ymel	*supF* λ^s	15
	Phages	
λgt7	b522 *c*I(shnλ4-5)Δ *nin*5 (*ara*)*coli*6	Vector for *Eco*RI fragments derived from λgt4*
λgt7-(*rpoD dnaG*)St	b522 *c*I(shnλ4-5) *nin*5	†
λgt4S	*c*I857 *nin*5 *S*am100	*Eco*RI vector carrying the left arm of λgt4 (16) and the right arm of λgtWES·λB (17)†
λgt4S-(*rpoD dnaG*)St 1	*c*I857 (srIλ1-2)Δ *nin*5 Sam100	†
λgt4S-(*rpoD dnaG*)St 2	*c*I857 (srIλ1-2)Δ *nin*5 Sam100	†
λNM540	(srIλ1-2)Δ shnλ3$^+$ *imm*21 *nin*5 shnλ6°	Vector for *Hin*dIII fragments (18)
λNM540-(*rpoD dnaG*)St	(srIλ1-2)Δ shnλ3$^+$ *imm*21 *nin*5 shnλ6°	This paper
λNM762	(*att-red*)Δ *c*IΔ(KH54) *nin*5 (*supF*)	Vector for *Hin*dIII fragments (19)
λNM762-(*rpoD dnaG*)Ec	(*att-red*)Δ *c*IΔ(KH54) *nin*5	†
λ76-(*rpl rpoBC*)Ec	(*att-red*)Δ *c*IΔ(KH54) *nin*5	20

All bacterial strains are derivatives of *E. coli* K-12. Nomenclature follows that of Bachmann *et al.* (21). The mutations *rpoD*285, *dnaG*3, and *dnaG*308 are temperature-sensitive. Restriction targets are designated srIλ(*Eco*RI) and shnλ(*Hin*dIII) (19). St and Ec denote DNA derived from *S. typhimurium* and *E. coli*, respectively.

* Gift from R. W. Davis and M. Thomas.

† To be described elsewhere.

which phage genes are repressed) to make large quantities of a σ-sized protein (Fig. 2, lane a). The same fragment recloned in both possible orientations into another λ vector also made this protein (Fig. 2, lanes b and c; see also Fig. 4, lanes a and g). Phage λ itself did not make such a protein, nor did λ76(*rpl rpoBC*)Ec which does not have the *rpoD dnaG* fragment (Fig. 2, lane d). The σ subunit of *S. typhimurium* polymerase has a lesser mobility on $NaDodSO_4$/polyacrylamide gel electrophoresis than does that of *E. coli* (1, 2). This difference, detectable on 15% gels, is indicated (σ_s and σ_c) on Fig. 2. The heavily labeled band coded by the *Eco*RI fragment ran closer to the position of marker σ from *S. typhimurium*. This indication is strongly confirmed in the results reported below.

RNA polymerase was purified from a strain, PM101, containing *rpoD*285 and lysogenic for the transducing phage λgt7-(*rpoD dnaG*)St. If the fragment-coded protein is σ, then at least some of the enzyme from this lysogen should yield σ with the mobility characteristic of the *S. typhimurium* subunit. The greater part of the pure enzyme had this property (Fig. 3, lane a). It migrated behind the mutant σ from *E. coli rpoD*285 and wild-type *E. coli* σ (Fig. 3, lanes b and c) but at the same rate as σ purified from *S. typhimurium* (Fig. 3, lane d). Another polymerase preparation from this strain (not shown) contained both mutant-sized and *S. typhimurium*-sized σ, predominantly the latter.

Our combined observations show that the *Eco*RI fragment has a functional *rpoD* gene from *S. typhimurium* able to complement a σ mutation in *E. coli*.

To discover whether the *Eco*RI fragment has an active gene able to compensate *dnaG*3 we analyzed temperature-resistant transductants of the mutant PM106. Transductants containing λNM762-(*rpoD dnaG*)Ec, λgt7-(*rpoD dnaG*)St, or λgt4S-(*rpoD dnaG*)St 2 (Table 1) were each cured of the phage by heteroimmune superinfection. They become temperature-sensitive after curing. We infer that the *Eco*RI fragment has an intact and functional gene, probably *dnaG* from *S. typhimurium*, able to complement *dnaG*3. Interestingly, UV-irradiated cells infected with λgt7-(*rpoD dnaG*)St or λgt4S-(*rpoD dnaG*)St 2 did not synthesize detectable amounts of a protein migrating with primase marker from *E. coli* (Fig. 2).

Other Proteins Determined by the Cloned *Eco*RI Fragment. We observed two other proteins in UV-irradiated cells infected with λgt7-(*rpoD dnaG*)St, λgt4S-(*rpoD dnaG*)St 1, or λgt4S-(*rpoD dnaG*)St 2. In λ-lysogenic bacteria we detected a band, X1, at 58,000 daltons (Fig. 2). We are not yet convinced that this protein, which is not detected in cells labeled very soon after infection (Fig. 4), is the primary product of a gene on the *Eco*RI fragment. By contrast, we shall present evidence (see below) that the fragment does code for a 34,000-dalton protein, P-34, which can be seen when λgt4S-(*rpoD dnaG*)St 2 infects nonlysogenic cells (Fig. 4, lane h). Significantly, λgt4S-(*rpoD dnaG*)St 1, an identical phage with the *Eco*RI fragment in reverse orientation, did not make P-34 in nonlysogenic cells. Neither phage made the protein in λ-lysogenic bacteria. These combined observations suggest that the gene is using a phage promoter.

The *E. coli Hin*dIII Fragment Carries *rpoD*. The phage λNM762-(*rpoD dnaG*)Ec has a 9-kb *E. coli Hin*dIII fragment (see above). It transduced PM101 (*rpoD*285) to ts$^+$ at the same

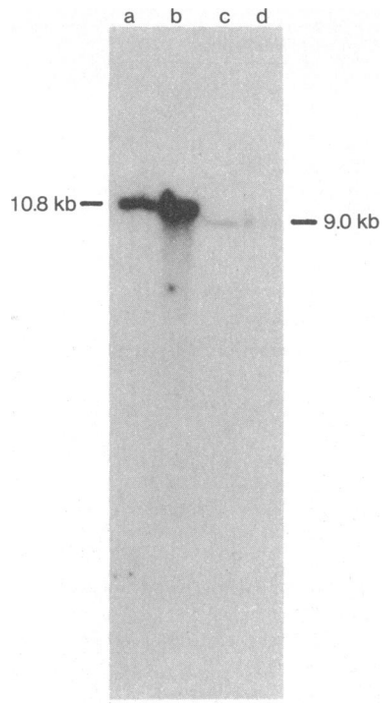

FIG. 1. Hybridization analysis of restriction fragments from *E. coli* and *S. typhimurium*. ^{32}P-Labeled probe was prepared by treating λgt7-(*rpoD dnaG*)St with *Eco*RI and electrophoretic purification of the cloned *S. typhimurium rpoD-dnaG* fragment, which was labeled by nick translation (25). DNA samples were electrophoresed in 0.5% agarose gel for 16 hr at 25 V and transferred to Schleicher & Schuell nitrocellulose sheet (25). The DNA samples on nitrocellulose were then hybridized with the probe for 18 hr at 44°C in 10 ml of 50% formamide/60 mM NaCl/40 mM NaH_2PO_4, pH 7/4 mM EDTA/0.3% sodium dodecyl sulfate ($NaDodSO_4$)/calf thymus DNA (0.5 mg/ml) containing 10 ng of probe per ml (10^5 cpm/ml). Hybridized bands were visualized by autoradiography (2-day exposure). Lanes: a, λgt7-(*rpoD dnaG*)St DNA (5 ng) cleaved with *Eco*RI; b, *S. typhimurium* LT2 DNA (1 μg) cleaved with *Eco*RI; c, λNM762-(*rpoD dnaG*)Ec DNA (5 ng) cleaved with *Hin*dIII; d, *E. coli* DNA (1 μg) cleaved with *Hin*dIII. Complete genotype of phages carrying *rpoD* and *dnaG* genes is given in Table 1.

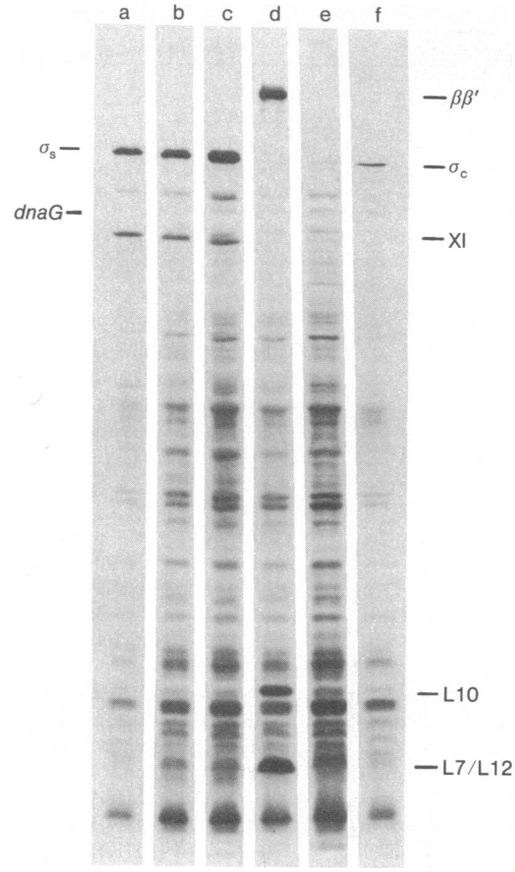

FIG. 2. Long-term labeling of cloned gene products in UV-irradiated lysogenic bacteria. L-broth-grown bacteria (2×10^8/ml) were prepared, irradiated, infected, labeled with [^{35}S]methionine, and lysed according to Messer *et al.* (26). Irradiated bacteria were infected at a multiplicity of 5–10, grown at 37°C for 20 min, labeled with [^{35}S]-methionine (10 μCi/ml; approximately 750 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) for 40 min and chased (4 min) by 1:40 dilution with unlabeled methionine. The extracts (5–50 μl, providing approximately 10^5 cpm per sample) were electrophoresed on a 12% $NaDodSO_4$/polyacrylamide gel (27) for 4 hr at 35 mA after the stacking process had been completed. The stained gel was dried and autoradiographed (20) for 2 days. Lanes: a, λgt7-(*rpoD dnaG*)St; b, λgt4S-(*rpoD dnaG*)St 1; c, λgt4S-(*rpoD dnaG*)St2; d, λ76; e, uninfected cells; f, λNM762-(*rpoD dnaG*)Ec. Stained markers [σ_s from *S. typhimurium* and σ_c and primase (*dnaG*) from *E. coli*] were traced onto the autoradiogram after processing. A long exposure (9 days) still showed only σ_c in lane f.

frequency (one to five transductants per 10^3 plaque-forming particles) as did λgt7-(*rpoD dnaG*)St, containing the *S. typhimurium Eco*RI fragment. It also transduced BT308λ$^+$ (*dnaG*308) to ts$^+$ (at a somewhat lower frequency), leading to the conclusion that λNM762-(*rpoD dnaG*)Ec carries the *dnaG rpoD* segment of the *E. coli* chromosome. Our conclusion is strongly supported by the fact that λNM762-(*rpoD dnaG*)Ec directs UV-irradiated cells to make a protein with the mobility of marker σ from *Escherichia coli* (Fig. 2, lane f). This technique has not revealed any other *E. coli* protein determined by the *Hin*dIII fragment. Because we do not find a protein of the mobility of DNA primase in polyacrylamide gels, we cannot be sure that this fragment includes the entire *dnaG* gene.

The Genetic Organization of the *rpoD-dnaG* Segment. In order to provide points of reference in the *rpoD-dnaG* region of the genome, a restriction nuclease cleavage map was constructed by digesting the DNA of the transducing phages with several restriction enzymes and comparing the products of single and double digestions. The results of these experiments are presented in Fig. 5. The alignment of the *E. coli* and *S. typhimurium* genomes is based on the analysis of heteroduplex molecules formed between λNM540-(*rpoD dnaG*)Ec and λgt4S-(*rpoD dnaG*)St 1, which show homology of 6 kb to the left of the rightward *Hin*dIII site and further homology for 1.5 kb to the right of the leftward *Hin*dIII site (S. Scherer, personal communication). The location of *rpoD* and *dnaG* on the *S. typhimurium* genome was determined by analysis of spontaneous deletions of the transducing phages. The deletions were selected from two phage stocks. One, λgt4S-(*rpoD dnaG*)St 2, has its fragment inserted into λ in the orientation shown. The fragment is reversed in the other phage, λgt4S-(*rpoD dnaG*)St 1, which is otherwise identical. Deletion phages, selected as survivors of heat-shock treatment (31), were analyzed for restriction sites and transducing ability. Four types of deletion pinpoint the *rpoD* and *dnaG* genes on the fragment. Deletions of λgt4S-(*rpoD dnaG*)St 1—Δ1-1, Δ1-3, and Δ1-6—show that the genes are in the right half of the fragment. Two of them (Δ1-3 and Δ1-6) cut into *rpoD*. These deletion phages make no σ in UV-irradiated bacteria (Fig. 4, lanes e and f) but instead make a new protein 9/10th the size of σ, which could be a large NH_2-terminal fragment of the subunit.

Deletions of λgt4S-(*rpoD dnaG*)St 2 (Δ2-5 and Δ2-7) order *rpoD* and *dnaG*. In addition, Δ2-7 behaves unusually in UV-irradiated bacteria (Fig. 4, lanes k and l). It makes about one-fifth the amount of σ (according to densitometer tracings) than

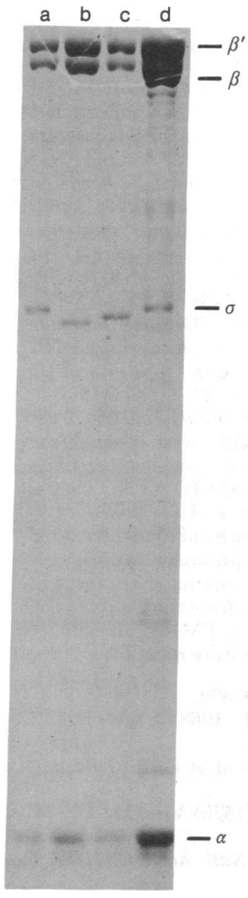

FIG. 3. NaDodSO$_4$/polyacrylamide gel electrophoresis of RNA polymerase performed as described by Harris *et al.* (2). RNA polymerase was purified by the procedure of Burgess and Jendrisak (28) with modifications for smaller quantities of cells (2). The gel contained 7.5% acrylamide and was 20 cm long. The positions of the RNA polymerase subunits are indicated at the right. Lanes: a, RNA polymerase isolated from the *E. coli* 285c derivative strain PM102, which carries the *rpoD* gene of *S. typhimurium;* b, RNA polymerase from the parent strain 285c; c, RNA polymerase from *E. coli* strain K1200; d, RNA polymerase from *S. typhimurium* (gift of M. Chamberlin).

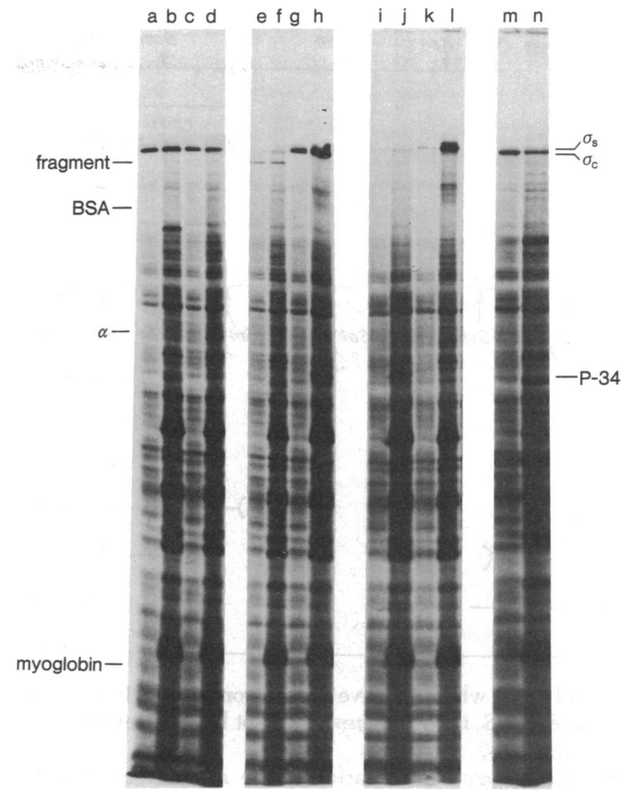

FIG. 4. Short-term labeling of UV-irradiated lysogenic and nonlysogenic bacteria with λ(*rpoD dnaG*) phages and deletion mutants. Maltose-induced, broth-grown bacteria (159 and 159λ$^+$) were prepared by the method of C. Epp and M. Pearson (personal communication). Bacteria at about 10^8 per ml were concentrated 10-fold in minimal medium (26), UV-irradiated (5000 ergs/mm^2), and infected with phage at 4°C for 30 min (multiplicity, 5–10) before dilution 1:5 into fresh minimal medium containing [^{35}S]methionine (50 μCi/ml). After 10-min labeling, we added NaN_3 (final concentration, 50 mM) and poured the mixture over ice. The cells were pelleted at 4°C and treated as described in the legend to Fig. 2. Extracts from lysogenic (159λ$^+$) and nonlysogenic (159) bacteria are in adjacent lanes. The positions at which bovine serum albumin (BSA), the α subunit of *E. coli* RNA polymerase, and myoglobin appeared on the destained gel are indicated. Lanes: a, λgt4S(*rpoD dnaG*)St 1, 159λ$^+$; b, λgt4S(*rpoD dnaG*)St 1, 159; c, Δ1-1, 159λ$^+$; d, Δ1-1, 159; e, Δ1-3, 159λ$^+$; f, Δ1-3, 159; g, λgt4S(*rpoD dnaG*)St 2, 159λ$^+$; h, λgt4S(*rpoD dnaG*)St 2, 159; i, Δ2-5, 159λ$^+$; j, Δ2-5, 159; k, Δ2-7, 159λ$^+$; l, Δ2-7, 159; m, λNM762(*rpoD dnaG*)Ec, 159λ$^+$; n, λNM762(*rpoD dnaG*)Ec, 159.

does the parent phage in lysogenic bacteria. This suggests that it has lost an element necessary for its expression, probably the promoter. However, in nonlysogenic bacteria it makes at least as much σ as does the parent (Fig. 4, lanes l and h). In this orientation, deletion past the *Eco*RI junction connects the bacterial DNA to the strong leftward promoter, P_L, of phage λ (32), which could permit read through to *rpoD*.

Thus, the properties of these deletions strongly suggest that *rpoD* is transcribed leftward (i.e., clockwise) on the map of *S. typhimurium*. Moreover, the fact that Δ2-7 does make some σ-sized protein in lysogenic bacteria suggests that its promoter is only partly deleted, arguing that this element lies between *rpoD* and *dnaG*. Alternatively, Δ2-7 could end within *dnaG*, eliminating a major upstream promoter for *rpoD* but leaving intact a minor promoter between the two structural genes.

Although the genetic evidence shows that the cloned *S. typhimurium dnaG* gene is complete and active in *E. coli* we have not seen its product in UV-irradiated bacteria. P-34 cannot be the *dnaG* product because it is not made by a phage, λNM540-(*rpoD dnaG*)St 1, that contains the *Hin*dIII subfragment oriented leftward (data not shown). This phage can complement a *dnaG*3 mutant. For this reason we have placed the gene for P-34 at the extreme right of the cloned *Eco*RI fragment.

DISCUSSION

Cloning and Expression of *rpoD*. Previous evidence for the location of *rpoD* was based on electrophoretic differences between σ proteins from different strains of enteric bacteria (1, 2) as well as the mapping of temperature-sensitivity and specificity mutations (3–6). Although these results could be due to modification of σ by a gene mapping at 66 min, the cloning results described here show unequivocally that the structural gene coding for *S. typhimurium* σ protein maps at 66 min.

Deletion mapping shows that the *dnaG* and *rpoD* genes are adjacent. The *rpoD* gene is transcribed clockwise on the *Salmonella* chromosome (33) from a promoter that probably lies between *dnaG* and *rpoD*. The evidence suggests that P-34 is also transcribed clockwise. However, because we do not see a protein of the size expected for primase, we are unable to determine the direction of transcription of *dnaG*.

Synthesis of σ subunit in *E. coli* is markedly derepressed by the *N* gene product of phage λ. This protein reverses some termination signals for transcription and thus allows readthrough into adjacent genes (34). Perhaps σ synthesis is regulated by an anti-termination mechanism (35).

The λ(*rpoD*) phages reported here have the dual capacity to synthesize N protein and σ from the cloned DNA. Furthermore, σ synthesis can be observed in the presence (in nonlysogenic bacteria) and absence (in lysogenic bacteria) of the N protein. We have not found derepression of σ labeling after infection of UV-irradiated bacteria (see Fig. 4). It will be interesting to discover whether this difference is due to the absence of a promoter and N-reversible termination site from the

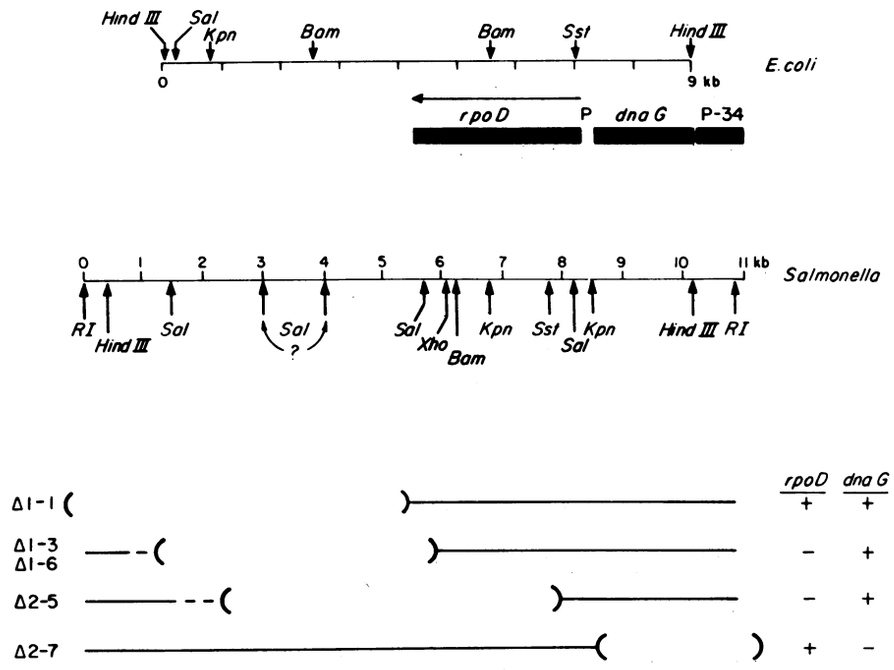

FIG. 5. Map of the *S. typhimurium* and *E. coli* genomes in the *dnaG rpoD* region. The restriction enzyme cleavage sites were determined with λgt7-(*rpoD dnaG*)St and λNM762-(*rpoD dnaG*)Ec. We indicate two possible locations for one of the *Sal* cleavage sites. The alignment of the *E. coli* and *S. typhimurium* genome fragments is based on heteroduplex analysis of λgt4S-(*rpoD dnaG*)St1 and λNM540-(*rpoD dnaG*)Ec, which show homology of 6 kb extending from the right *Hin*dIII site and 1.5 kb extending from the left *Hin*dIII site (S. Scherer, personal communication). The parentheses enclose the deleted *S. typhimurium* DNA. These deletions locate the positions of the genes. Deletions Δ1-1, Δ1-3, and Δ1-6 were selected from λgt4S-(*dnaG rpoD*)St 1 and deletions Δ2-5 and Δ2-7 were selected from λgt4S(*dnaG rpoD*)St 2 by heating phage suspensions in 20 mM EDTA (29). They all leave the phage *int* and *xis* genes intact. Some delete the righthand *Eco*RI bacterial-phage joint and are presumed to arise at *att* (30). The others are internal deletions of the cloned DNA. We indicate the ability of each phage to transduce PM101 and PM106 (Table 1) to temperature resistance.

piece of DNA which we have cloned from *E. coli*. It is of course possible that *S. typhimurium* does not have N-reversible termination sites.

Evolutionary Conservation of the *rpoD* Gene. Wiggs *et al.* (36) have shown that RNA polymerases from a wide variety of unrelated bacterial species all recognize the same promoters on coliphage T7 DNA. These authors argue that a great divergence of promoter specificity would hinder the evolutionary process. Consistent with such conservation of promoter specificity *in vitro*, we have found that the σ subunit of *S. typhimurium* can substitute for *E. coli* σ in PM101 (*rpoD*285) and remains associated with *E. coli* core enzyme through many steps of purification (Fig. 3). These observations are borne out by the fact that the *rpoD* genes from these two enteric bacteria show considerable homology in nucleotide sequence (Fig. 1; S. Scherer, personal communication), although in general the bacterial DNA sequences show significant diversity (37). Labeled restriction fragments of *rpoD* may provide an effective probe to identify the *rpoD* genes of less-related bacterial species.

The excellent technical assistance of Audrey Nolte, the advice of Walt Ream and Dan Schindler, and the encouragement of Arthur Kornberg are greatly appreciated. This research was supported by National Institutes of Health Research Grant AI 08722 from the National Institute of Allergy and Infectious Diseases, by Research Grant VC-188 from the American Cancer Society, and by National Science Foundation Research Grant PCM 76-19935.

1. Nakamura, Y., Osawa, T. & Yura, T. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1831–1835.
2. Harris, J. D., Martinez, I. I. & Calendar, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1836–1840.
3. Gross, C., Hoffman, J., Ward, C., Hager, D., Burdick, G., Berger, H. & Burgess, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 427–431.
4. Travers, A. A., Buckland, R., Goman, M., Le Grice, S. S. G. & Scaife, J. G. (1978) *Nature (London)* **273**, 354–358.
5. Harris, J. D., Heilig, J. S., Martinez, I. I., Calendar, R. & Isaksson, L. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6177–6181.
6. Nakamura, Y. (1978) *Mol. Gen. Genet.* **165**, 1–6.
7. Wechsler, J. A. & Gross, J. D. (1971) *Mol. Gen. Genet.* **113**, 273–284.
8. Rowen, L. & Kornberg, A. (1978) *J. Biol. Chem.* **253**, 758–764.
9. Ptashne, M. (1967) *Proc. Natl. Acad. Sci. USA* **57**, 306–313.
10. Campbell, A. (1965) *Virology* **27**, 329–339.
11. Durwald, H. & Hoffmann-Berling, H. (1968) *J. Mol. Biol.* **31**, 334–346.
12. Bowden, D. W., Twersky, R. S. & Calendar, R. (1975) *J. Bacteriol.* **124**, 167–175.
13. Enquist, L. W. & Weisberg, R. A. (1976) *Virology* **72**, 147–153.
14. Echols, H. & Green, L. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2190–2194.
15. Bachmann, B. J. (1972) *Bacteriol. Rev.* **36**, 525–557.
16. Panasenko, S. M., Cameron, J. R., Davis, R. W. & Lehman, I. R. (1977) *Science* **196**, 188–189.
17. Leder, P., Tiemer, D. & Enquist, L. (1977) *Science* **196**, 175–177.
18. Borck, K., Beggs, J. D., Brammar, W. J., Hopkins, A. S. & Murray, N. (1976) *Mol. Gen. Genet.* **146**, 199–207.
19. Murray, N. E., Brammar, W. J. & Murray, K. (1977) *Mol. Gen. Genet.* **150**, 53–61.
20. Linn, T. & Scaife, J. G. (1978) *Nature (London)* **276**, 33–37.
21. Bachmann, B. J., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40**, 116–167.
22. Hohn, B. & Murray, K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3259–3263.
23. Benton, W. D. & Davis, R. W. (1977) *Science* **186**, 180–182.
24. Harris, J. D. & Calendar, R. (1978) *Virology* **85**, 343–358.
25. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
26. Messer, W., Bergmans, H., Meijer, M., Womack, J. E., Hansen, F. G. & von Meyenberg, K. (1978) *Mol. Gen Genet.* **162**, 269–275.
27. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–689.
28. Burgess, R. R. & Jendrisak, J. J. (1975) *Biochemistry* **14**, 4634–4638.
29. Shigeru, S. & Uchida, H. (1977) *Virology* **83**, 277–286.
30. Davis, R. W. & Parkinson, J. S. (1971) *J. Mol. Biol.* **56**, 403–423.
31. Parkinson, J. S. & Huskey, R. J. (1971) *J. Mol. Biol.* **56**, 369–384.
32. Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. (1978) in *The Operon*, eds. Miller, J. & Reznikoff, W. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 345–371.
33. Sanderson, K. E. & Hartman, P. E. (1978) *Microbiol. Rev.* **42**, 471–519.
34. Roberts, J. W. (1976) in *RNA Polymerase*, eds. Losick, R. & Chamberlin, M. J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 247–272.
35. Nakamura, Y. & Yura, T. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4405–4409.
36. Wiggs, J., Bush, J. & Chamberlin, M. (1979) *Cell* **16**, 97–109.
37. Brenner, D. & Falkow, S. (1971) *Adv. Gen.* **16**, 81–118.