A gene from *Escherichia coli* affecting the sigma subunit of RNA polymerase

(transcription/cotransduction/*tolC*/*dnaG*)

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ABSTRACT The RNA polymerase sigma subunits of *Escherichia coli* K, *E. coli* C, and *Salmonella typhimurium* can be resolved by electrophoresis. Using this technique, we have analyzed *Salmonella* strains carrying F' plasmids from *E. coli* K in order to map the gene for the sigma factor. Partial diploid analyses show the location of the sigma gene at 62-66 min on the *E. coli* genetic map. This gene is cotransducible with *tolC* and *dnaG*, at 66 min.

The properties of homologous components of the transcription and translation apparatuses often vary between strains of enteric bacteria. Such natural differences provide a convenient tool for genetic mapping of these components when mutants are not available. In order to make use of such differences, investigators individually transfer small regions of the chromosome from one bacterial strain to another strain. The recipients are then screened for the desired phenotype, by using an appropriate assay. With this technique, it has been possible to map some of the genes coding for ribosomal proteins (1) as well as the gene coding for transcription termination factor rho (2). Until now, however, no genetic mapping has been reported for the RNA polymerase (RNA nucleotidyltransferase, nucleosidetriphosphate: RNA nucleotidyltransferase, EC 2.7.7.6) sigma subunit, which determines the selectivity of transcription *in vitro* (3). We have observed electrophoretic differences between the RNA polymerase sigma subunits of *Escherichia coli* strain K-12 (K), *E. coli* strain C, and *Salmonella typhimurium*, and have used these differences to map an *E. coli* K gene which causes synthesis of the sigma subunit characteristic of *E. coli* strain K.

MATERIALS AND METHODS

Chemicals and Buffers. Buffers and DNA-cellulose were prepared as described by Burgess and Jendrisak (4). TGED buffer is 10 mM Tris-HCl (pH 8), 5% (vol/vol) glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol. *N,N,N',N'*-Tetramethylethylenediamine (CH₂NMe₂)₂, Coomassie brilliant blue and ammonium persulfate were obtained from Bio-Rad Corp. Calf thymus DNA was obtained from Worthington Biochemical Co., [³H]CTP from ICN, and unlabeled nucleoside triphosphates, Trizma base, sodium deoxycholate, 2-mercaptoethanol, and lysozyme from Sigma Chemical Co. Glycerol was obtained from Mallinckrodt Chemical Co. and ammonium sulfate (enzyme grade) was from Schwarz/Mann.

Media. LB broth (5) and Davis minimal medium (6) supplemented with 0.5% glucose were used. Davis agar plates consist of Davis medium supplemented with 2% agar and L-amino acids (20 µg/ml) or adenine (20 µg/ml) as required.

Abbreviations: (CH₂NMe₂)₂, tetramethylethylenediamine; PhMeSO₃F, phenylmethylsulfonyl fluoride; NaDodSO₄, sodium dodecyl sulfate; TGED buffer, buffer containing 10 mM Tris-HCl, pH 8.0/5% (vol/vol) glycerol/0.1 mM EDTA/0.1 mM dithiothreitol.

Bacterial Strains. The bacterial strains used are described in Table 1. New merodiploid strains were constructed by transferring F' plasmids from *E. coli* K merodiploid strains into various *Salmonella typhimurium* and *E. coli* C strains, each containing nutritional mutations such that the presence of the F' plasmid would allow growth on minimal glucose medium. Transfer was accomplished by either of two methods. **Method 1:** a minimal glucose plate was spread with 0.1 ml (10⁷-10⁸ colony-forming units) of an overnight culture of the donor strain and 0.05 ml of the recipient strain, followed by incubation at 37° for 48 hr (13). **Method 2:** 0.5 ml samples of each overnight culture were inoculated into flasks containing 10 ml of minimal glucose medium supplemented with the nutritional requirements of each strain. These cultures were shaken at 37° until the optical density of the suspension was doubled. One milliliter of the recipient strain, 2 ml of the donor strain and 3 ml of LB broth were then added to a 250 ml Erlenmeyer flask and shaken very slowly at 37° for 60-90 min. The mating mixtures were centrifuged, concentrated to 10% of the initial volume, and 0.2 ml aliquots were spread on minimal glucose plates, followed by incubation at 37° for 48 hr. Colonies were grown in minimal glucose media and tested for ability to grow phages SP6 [specific for female *Salmonella* strains (14)], P22 [*Salmonella* specific (15)] and 186p [*E. coli* K specific (16)] with the parent strains as controls. Loss of ability to grow SP6 was considered additional evidence of the presence of the episome. From each *Salmonella* partial diploid strain constructed, F⁻ segregants were isolated and shown to regain both the nutritional marker and the ability to grow SP6. Large batches of all partial diploid strains were grown to a concentration of 10⁹ cells per ml in media selective for retention of the F' plasmid, in order to prepare the enzyme.

RNA Polymerase Assay. The assay procedure for the various fractions generated during purification was as follows: 5 µl of each fraction was tested by addition to 45 µl of an assay mixture containing as a final concentration 20 mM Tris-HCl (pH 8), 20 mM MgCl₂, 20 mM 2-mercaptoethanol, 2 mM EDTA, 5 µg of T3 phage DNA, and 0.4 mM each of ATP, [³H]CTP (2 × 10⁵ cpm/nmol), GTP, and UTP. The assay mixture was incubated for 10 min at 37° and then the reaction was terminated with perchloric acid. The precipitates were collected on GF/C filters and assayed by liquid scintillation counting (17).

Polyacrylamide Gel Electrophoresis. Slab gel electrophoresis was performed by using a discontinuous buffer system (18) in the apparatus described by Studier (19) for 8 cm gels and a similar apparatus constructed to accommodate a gel 20 cm in length. The 20-cm apparatus is constructed so that the gel can be water-cooled during electrophoresis to eliminate heat buildup caused by the higher voltages needed for the longer gel. The gel ingredients were as described (19), except that the EDTA was omitted. The acrylamide concentration was either 7.5% or 10%: 7.5% gels gave better separation but more diffuse

Table 1. Bacterial strains

Strain	Relevant genotype	Origin/reference
<i>Salmonella typhimurium</i> :		
SB 3188	<i>proAB</i> 47, <i>argG</i> 459/F' 140	Gift of D. Beneski
TA 831	<i>hisF</i> 645 (deletion)	7
TA 2364	<i>lac</i> , <i>phoA</i> , <i>purE</i> , <i>purF</i> , <i>thi</i> /F' 254	Gift of G. Ames
<i>S. typhimurium</i>	<i>argA</i> 85	Gift of G. Ames
<i>S. typhimurium</i>	<i>argF</i> 88	Gift of G. Ames
<i>S. typhimurium</i>	<i>purA</i> 1	Gift of G. Ames
<i>S. typhimurium</i>	<i>purB</i> 12	Gift of G. Ames
<i>S. typhimurium</i>	<i>purF</i> 45	Gift of G. Ames
TM 1	<i>argF</i> /F' 111	This paper
TM 2	<i>purA</i> /F' 112	This paper
TM 4	<i>argA</i> /F' 143	This paper
TM 5	<i>purF</i> /F' 129	This paper
TM 6	<i>purB</i> /F' 126	This paper
TM 7	<i>hisF</i> 645/F' 150	This paper
TR 1175	<i>serA</i> 790, <i>lys</i> -554, <i>his</i> -3744, <i>hisO</i> 1242	Gift of J. Roth
TR 1359	TR 1175/F' 116	Gift of J. Roth
TR 1963	<i>purA</i> , <i>pro</i> /F' 117	Gift of J. Roth
TR 2096	<i>arg</i> , <i>pan</i> , <i>proAB</i> , <i>his</i> /F' (3'-6')	Gift of J. Roth
<i>E. coli</i> K-12:		
JC1553	<i>argG</i> 6, <i>metB</i> 1, <i>his</i> -1, <i>leu</i> -6, <i>recA</i> 1, <i>mtl</i> -2, <i>xyl</i> -2, <i>malA</i> 1, <i>lacY</i> 1, <i>gal</i> -6, <i>strA</i> 104, <i>tonA</i> 2, <i>tsx</i> -1, λ^R , λ^- , <i>supE</i> 44	8
KLF11/JC1553	JC1553/F' 111	8
KLF12/JC1553	JC1553/F' 112	8
KLF26/KL181	<i>thi</i> -1, <i>pyrD</i> 34, <i>his</i> -68, <i>trp</i> -45, <i>recA</i> 1, <i>mtl</i> -2, <i>xyl</i> -7, <i>malA</i> 1, <i>galK</i> 35, <i>strA</i> 118, λ^R , λ^- /F' 126	8
KLF29/JC1553	JC1553/F' 129	8
KLF43/KL259	<i>thi</i> -1, <i>tyrA</i> 2, <i>pyrD</i> 34, <i>his</i> -68, <i>trp</i> -45, <i>thyA</i> 33, <i>recA</i> 1, <i>mtl</i> -2, <i>xyl</i> -7, <i>malA</i> 1, <i>galK</i> 35, <i>strA</i> 118, λ^R , λ^- /F' 143	8
DFF1/JC1553	JC1553/F' 150	9
K1200	<i>endA</i> , <i>rna</i>	10
PC314	HfrC, <i>dnaG</i> 3	11
<i>E. coli</i> C:		
C-10	<i>endA</i>	10
C-2301	<i>uvrA</i> , <i>thy</i> , <i>str</i> , <i>tolC</i>	11
C-2309	<i>uvrA</i> , <i>thy</i> , <i>dnaG</i> 3; <i>str</i>	11

We used the nomenclature recommended by Bachmann *et al.* (12). The genetic markers listed are on the host chromosome; the plasmids cover the regions indicated in Fig. 2 as described in detail by Low (8).

bands than the 10% gels. Samples were prepared by adding 50 μ g of protein in 10–100 μ l of storage buffer to 200 μ l of sample buffer (20), and then were placed in a boiling-water bath for 2 min; 5–20 μ l of sample was run in each gel slot. The gels were run at 35 mA, stained, and then destained as described (20).

RNA Polymerase Purification. The procedure of Burgess and Jendrisak (4), modified for use on a smaller scale, was employed. All procedural steps were carried out at 4°. Ten grams of frozen cells in 30 ml of grinding buffer without phenylmethylsulfonyl fluoride was suspended by stirring in a beaker for 30 min, and then left standing for 20 min. Sodium deoxycholate (4%, 0.5 ml) was added with stirring and the suspension was allowed to stand for 20 min. The material was transferred to 50 ml centrifuge tubes, and 40 ml containing 0.2 M NaCl was added and the mixture was homogenized in a Dounce homogenizer with a tight-fitting glass pestle. The mixture was centrifuged in the SS-34 Sorvall rotor at 15,000 rpm

for 45 min and the pellet was discarded. Three milliliters of 10% Polymix P was added dropwise while the solution was stirred, and the stirring was continued for 20 min. The material was transferred to 50-ml tubes, homogenized to disperse any large clumps, and centrifuged at 7000 rpm for 20 min in the same rotor. The supernatant was discarded, the pellet was completely resuspended in 30 ml TGED containing 0.5 M NaCl, and then centrifuged as before. Again the supernatant was discarded, the pellet was resuspended in 20 ml of TGED + 0.0 M NaCl, centrifuged again, and the pellet discarded. To the supernatant, we added 7.5 g of ammonium sulfate, which was dissolved by stirring for 20 min. Then the solution was centrifuged at 10,000 rpm for 20 min in the SS-34 rotor. The supernatant was discarded and the pellet was dissolved in enough TGED buffer to give a conductivity lower than that of 0.15 M NaCl. The total volume varied between 25 and 40 ml. The solution was passed over a 15 ml (1.2 $\times$ 14 cm) calf-thymus DNA-cellulose column

FIG. 1. NaDodSO₄/polyacrylamide gel electrophoresis of RNA polymerase purified from various strains of enteric bacteria. The procedure is described in *Materials and Methods*. The gel contained 7.5% acrylamide and was 20 cm long. The positions of the known RNA polymerase subunits are indicated on the left. The sigma subunits denoted S and K came from *Salmonella typhimurium* and *E. coli* K, respectively. The strains used were (a) *E. coli* C-10, (b) *E. coli* K1200 (K) (c) *S. typhimurium* TR 1175 (S), (d, f, h) S + K, (e, g) *S. typhimurium* SB3188, containing *E. coli* K F'140, (i, j) *S. typhimurium* TR 1359, containing *E. coli* K F'116.

equilibrated with TGED at 0.15 M NaCl. The column was washed with 15 ml of TGED containing 0.15 M NaCl and eluted with a 100-ml linear salt gradient (0.15–1.0 M NaCl), and 1-ml fractions were collected. The 5–10 fractions containing the peak material, and the contents were precipitated with 2–3.5 g of ammonium sulfate. The pelleted protein was dissolved in 0.2–0.4 ml of TGED buffer and passed through a Bio-Gel A-1.5m column (1.2 × 25 cm) in TGED containing 0.5 M NaCl, and 0.5-ml fractions were collected. The material from the peak fractions was dialyzed overnight against storage buffer (4). The entire procedure took 12–14 hr, and two to five preparations were handled at the same time.

Production of Antisera. Antiserum specific for RNA polymerase holoenzyme was prepared as described by Calendar and Berg (21). Antiserum specific for sigma subunit was a gift of Wolfram Zillig and was prepared according to the procedure of Linn *et al.* (22), by injecting goats subcutaneously instead of injecting rabbits in the footpads, and by adjusting the amount of material injected proportionally to the weight of the animal.

RESULTS

While examining preparations of RNA polymerase from *E. coli* strains K and C, we noted that the sigma subunit of *E. coli* C RNA polymerase migrates more rapidly than the corresponding component of the *E. coli* K enzyme (Fig. 1, slots a and b) in sodium dodecyl sulfate/acrylamide gel electrophoresis. In an effort to locate the gene responsible for this altered property of the sigma protein, we began to construct partial K/C strain diploids by introducing F' plasmids from *E. coli* K into *recA*[−]

FIG. 2. Circular map of *E. coli* genes, indicating the position of genes carried by F' plasmids. Each arc carries the number of the F' plasmid (from Tables 1 and 2) as well as an arrow indicating the direction and point of origin of ancestral Hfr strain, where known.

strains of *E. coli* C. Unfortunately, the resulting strains grew extremely poorly and were difficult to maintain. By using *recA*⁺ recipients we eliminated this problem, but introduced another: the high frequency of recombination between the *E. coli* C chromosome and the *E. coli* K genes on the plasmid followed by segregation of recombinants made negative results meaningless. We could not interpret the presence of only one type of sigma subunit in these diploids to mean that the plasmid in question was devoid of the gene for sigma.

In order to circumvent these problems, we introduced the same F' plasmids into *Salmonella typhimurium*. The sigma subunit of *S. typhimurium* is readily resolved from K sigma by electrophoresis (Fig. 1, slots b–d, f, and h), and its genome does not recombine with that of *E. coli* K (8) even though the general order of genes is similar (23). Thus, we obtained (as gifts or through construction in our own lab, see Table 1) a collection of *Salmonella typhimurium* strains carrying F' plasmids with *E. coli* K genes. We purified RNA polymerase from these strains and examined the sigma subunits by electrophoresis (Fig. 1, slots e, g, i, and j). The 11 F' plasmids tested in this way are diagrammed in Fig. 2. Among them they carry 85% of the *E. coli* genetic map. *Salmonella* strains carrying ten of these F' plasmids synthesize only the characteristic *Salmonella* sigma (Table 2). However, TR 1359, carrying F'116 which covers 59–66 min of the *E. coli* genetic map, synthesizes sigma subunit with the electrophoretic mobility expected from *E. coli* K, as well as a smaller amount of *Salmonella typhimurium* sigma protein (Fig. 1, slots i and j). TM 4 which contains F'143, which overlaps part of F'116, has only the *Salmonella*-sized sigma protein.

The *E. coli* K-like sigma subunit has been copurified with *Salmonella* RNA polymerase core from strain TR1359 through Polymin P fractionation, ammonium sulfate precipitation, DNA-cellulose affinity chromatography, and a gel filtration step that selects for uncommonly large protein aggregates. This

Table 2. Electrophoretic properties of sigma subunits from partial diploid strains

Partial diploid strain	F'
TR 2096	3'-6'
TA 2364	254
TM 6	126
TM 7	150
TM 5	129
TM 4	143
TR 1359	116
SB 3188	140
TM 1	111
TM 2	112
TR 1963	117

Strain TR 1175, the female parent of TR 1359, has only the *Salmonella*-sized sigma (see Fig. 1). All sigma subunits were *Salmonella*-sized except TR 1359 that had sigma subunits characteristic of both *Salmonella* and *E. coli*.

protein also shows the immunological properties of *E. coli* K sigma protein. Antibodies prepared against *E. coli* K sigma protein were incubated with an extract from TR 1359 and the precipitate was examined electrophoretically (Fig. 3). A protein

FIG. 3. NaDodSO₄/polyacrylamide gel electrophoresis of RNA polymerase and of proteins precipitated from an extract of *Salmonella*/F'116 strain TR 1359 by specific antisera. The gel contained 75% acrylamide and was 20 cm long. Slots b and d were derived by adding the indicated antiserum to a crude extract of TR 1359 prepared by using the lysis procedure and immune precipitation described (24). Slots a, c, and e are purified enzyme preparations that were mixed and used as markers (K = *E. coli* K; S = *S. typhimurium*).

FIG. 4. NaDodSO₄/polyacrylamide gel electrophoresis of RNA polymerase purified from *E. coli* strains C and K, and from a K/C hybrid strain. The methods are described in Fig. 1. The strains employed were (a) *E. coli* K1200; (b) *E. coli* C-10; (c, f) *E. coli* C-10 plus *E. coli* K1200; (d) *E. coli* C-2309 that contains the *tolC-dnaG* region of *E. coli* K; (e) *E. coli* C-2309 plus *E. coli* C-10.

with the mobility of *E. coli* K sigma subunit was one of the major proteins precipitated. Thus, F'116 causes synthesis of a protein which behaves as the sigma subunit of *E. coli* K by the criteria of copurification with RNA polymerase, electrophoretic mobility, and interaction with antiserum against sigma.

Because the gene affecting sigma is found on F'116 but not on F'143 or F' 140, its genetic locus must lie between 62 and 66 min on the *E. coli* genetic map (Fig. 2). Two *E. coli* K genes in this region have been transduced into *E. coli* C: *tolC* and *dnaG* (11). Thus, we tested the *E. coli* C strain (C-2309) containing these two *E. coli* K genes, in order to see whether the sigma gene had been cotransduced. Fig. 4 (slots d and e) shows that this was the case, and that the gene affecting sigma must map at 66 min. *E. coli* C-2301, the strain which served as a recipient for the *E. coli* K *tolC* and *dnaG* genes, produces sigma characteristic of *E. coli* strain C.

DISCUSSION

The sigma subunit of *E. coli* RNA polymerase is responsible for much of the specificity of transcription *in vitro* (25, 26). For example, RNA polymerase containing a sigma subunit copies only that strand of T7 phage DNA that is copied *in vivo*, while RNA polymerase lacking sigma copies both strands of T7 DNA (27). Thus, it is likely that sigma is essential for the viability of *E. coli*. Mutations affecting sigma are needed in order to test this proposition and also to elucidate the role of sigma in the

specificity of coliphage gene expression. Sigma may not be necessary for all stages of phage gene expression: *B. subtilis* phage SP01 replaces the sigma subunit with a phage-coded protein after the early stages of phage growth are complete (28). The mapping of the structural gene for sigma will facilitate the isolation of sigma mutants through use of localized mutagenesis (29, 30). We believe we have determined the general map position for the sigma subunit structural gene to be at 66 min.

We cannot eliminate the possibility that we have mapped an enzyme which modifies the sigma protein and we considered this possibility. So far, we have not been able to detect modification: *Salmonella typhimurium* or *E. coli* K labeled with ^{35}S has been mixed with nonradioactive *E. coli* K or *E. coli* C, respectively, then lysed, and incubated at 20° for 10 min before the RNA polymerase was purified. This treatment did not modify the electrophoretic mobility of the labeled sigma protein. Friesen *et al.* (31) have reported that a transducing phage carrying the gene for *E. coli* protein synthesis elongation factor Ts (4 min on the *E. coli* map) also codes for the synthesis of a sigma-like protein. Thus, it is possible that the *E. coli* carries more than one structural gene for sigma protein. A strain which is partially diploid for the 4 min region, however, does not produce altered sigma subunit. Thus, the phenomenon that we observe is different from that of Friesen *et al.* (31). If the phenomenon we observe is due to a structural gene for sigma protein and if a structural gene for sigma exists at 4 min on the *E. coli* map, then that structural gene is either deleted from our F' plasmid or is not expressed in *Salmonella*.

Y. Nakamura, T. Osawa and T. Yura (accompanying paper) have found a gene, affecting the properties of sigma factor, in *E. coli* F' plasmids which carry the *metC-argG* region (around 66 min) introduced into *Proteus* and *Salmonella* strains. These independent results are in agreement with ours.

Definitive proof for a structural gene for sigma at 66 min could be obtained by construction of specialized transducing phages carrying genes between *tolC* and *dnaG*. One such phage should cause synthesis of sigma subunit in cells pretreated with ultraviolet light (31, 32) or in transcription-translation system *in vitro* (32).

Note Added in Proof: Results of P1 transduction with donor C-2301 and recipient C-2309 indicate that the sigma gene is >90% cotransducible with *dnaG* and is much closer to *dnaG* than to *tolC*.

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