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A Gene Between *polA* and *glnA* Retards Growth of *Escherichia coli* When Present in Multiple Copies: Physiological Effects of the Gene for Spot 42 RNA

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We have isolated the single gene for spot 42 RNA of *Escherichia coli* on a 20-kilobase DNA fragment. Physical characterization of this cloned DNA fragment showed that it is homologous to a region at 86 min on the genetic map and extends from the 23S to 5S rRNA coding region of *rrnA* to the coding region of *glnA*, the gene for glutamine synthetase. Other genes included on this cloned DNA fragment are *polA*, *ntrC* (*glnG*), and *ntrB* (*glnL*). *E. coli* cells transformed with a multicopy plasmid clone of the gene for spot 42 RNA had about a 10-fold increase in the amount of spot 42 RNA they contained. The amount of 6S RNA in these cells was increased about twofold, although the gene for 6S RNA was not located on this plasmid or on the larger 20-kilobase fragment. Presence of this multicopy plasmid also affected the growth of cells. The generation time was increased under a variety of growth conditions, especially when cells were grown in medium with succinate as the carbon source. In addition, some strains of *E. coli* which have multicopy plasmids carrying the gene for spot 42 RNA were unable to respond normally to a shift into richer medium: upon upshift from minimal glucose to LB broth or minimal glucose plus 1% Casamino Acids, there was a 3- to 4-h lag before the culture adapted to the new medium. More than 90% of the cells in such cultures stopped dividing, although they remained viable. The plating efficiency of minimal-glucose-grown cells was 100-fold less on rich media than on minimal glucose medium. One revertant was isolated which regained the phenotype of pBR322-transformed cells. Analysis of this strain showed that the plasmid it contained had an insertion of an IS*1* element into the 5′ end of the coding region for the gene for spot 42 RNA.

Among the 4 to 6S RNAs of *Escherichia coli* is an unusual molecule which we call "spot 42 RNA" (18, 37). This RNA has an intermediate stability, with a half-life of one-third of a cell generation. When cells are labeled briefly with $^{32}PO_4{}^{-3}$, spot 42 RNA is the most highly labeled 4 to 6S RNA molecule, indicating a high rate of synthesis for this RNA. Production of spot 42 RNA is not under stringent control (42), and its accumulation is negatively regulated by cyclic AMP (9, 18, 37).

Spot 42 RNA is a 109-nucleotide-long primary transcript with a rho-independent terminator, and it contains no modified bases. Sequence analysis reveals many similarities between this molecule and mRNA in that it has a ribosome binding site, an A-U-G triplet followed by 14 triplets, and a U-G-A terminator (36; B. G. Sahagan, Ph.D. thesis, University of Wisconsin, Madison, 1977). Ten of the 15 triplets would code for hydrophobic amino acids. The chromosomal location of the gene for spot 42 RNA was recently shown to be 150 base pairs (bp) downstream from the gene for DNA polymerase I, *polA* (19, 20), located at 86 min on the *E. coli* map.

Even though much is known about the RNA sequence features and the production of spot 42 RNA, little is known about its function. The RNA itself or its product might occupy a regulatory role in cellular metabolism or be involved in some cellular structure. Another possibility is that there is no function for spot 42 RNA, although this seems unlikely in light of its sequence features (noted above) and physiological data demonstrating that accumulation of spot 42 RNA is modulated in response to overall cellular metabolism (37; Sahagan, Ph.D. thesis).

To learn more about the function of spot 42 RNA, we have isolated a 20-kilobase (kb) *Eco*RI fragment from *E. coli* DNA containing the single gene for this RNA and have constructed a restriction cleavage map of the fragment. We report here the results of our study on this gene

and neighboring ones which are located on this *Eco*RI fragment. We find that increasing the copy number of this gene has pleiotropic effects on cell growth.

MATERIALS AND METHODS

Strains. *E. coli* strains C600 (*recB recC hsdR hsdM lop-11 supE gal-96* Sm^r *leuB thi-1 thr*), HB101 (*recA hsdR hsdM supE lacZ leuB proA thi-1* Sm^r), and M7042 *recA* (*recA srl*::Tn*10*, *lacZ* Tc^r Sm^r; from A. Grossman) were used for transformation; K802 (*hsdR hsdM$^+$ galK lacY met supE*; from F. Blattner) was used for transfections. The DNA used for cloning was isolated from strain CP78 (*thi leu thr arg his rel$^+$*) (13). Strain UC317 containing the *glnA-glnG* plasmid (p811) was a gift from R. Haselkorn.

Media and growth of cells. LB broth contains (per liter) 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), and 5 g of NaCl. MOPS (morpholine-propanesulfonic acid) minimal medium (29) containing 1.27 mM phosphate was used for growth rate determinations and was supplemented with 30 μg each of threonine, leucine, proline, methionine, isoleucine, and valine per ml and 10 μg of thiamine per ml. Cells transformed with pBR322 or the recombinant plasmids described below were always grown in the presence of 100 μg of ampicillin per ml.

^{32}P labeling was done in MOPS minimal medium containing 0.25 mM phosphate, the supplements listed above, and 0.2% glucose. Carrier-free $^{32}P_i$ (New England Nuclear Corp.) was added to the cells at 0.5 mCi/ml when the cultures had reached an optical density at 450 nm (OD_{450}) of 0.7, and the cells were harvested at an OD_{450} of 1.0. At this cell density the phosphate supply had been depleted, although spot 42 RNA synthesis continued (37; Sahagan, Ph.D. thesis), thus enriching the RNA preparation for spot 42 RNA. RNA was isolated as described by Sahagan and Dahlberg (37).

Generation times were determined by following the OD_{450} of cultures grown at 37°C. Glucose was present at a final concentration of 0.2% except in the carbon source downshift experiments, when it was present at 0.02% and the succinate concentration was 0.8%.

Cloning and restriction mapping. Lambda CH10 DNA (5) and the enzyme *Mnl*I were gifts from D. Moore. Polynucleotide kinase was a gift from O. Uhlenbeck. T4 DNA ligase and restriction enzymes were purchased from Biotec, Inc., Bethesda Research Laboratories, Inc., or New England Biolabs, Inc., and were used as recommended by the manufacturers. The abbreviations for enzyme names used in the figures are as follows: Ba, *Bam*HI; Bg, *Bgl*II; Ha, *Hae*III; H2, *Hinc*II; H3, *Hin*dIII; Kp, *Kpn*I; Mn, *Mnl*I; Ps, *Pst*I; RI, *Eco*RI; Sa, *Sal*I; S3, *Sau*3A; Ss, *Sst*I; Xh, *Xho*I. Lambda phage libraries were screened as described before (3), using ^{32}P-labeled spot 42 RNA as a probe. pBR322 (6) and recombinant plasmid DNAs were prepared by the method of Clewell and Helinski (11). Restriction mapping of pRD1 DNA was accomplished by partial digestion of a 5'-end-labeled fragment (40).

Throughout this paper, we discuss fragment sizes as measured by their mobilities in gel electrophoresis. However, recent DNA sequence analysis by Joyce et al. (19, 20) shows that the true sizes of most of the fragments are actually 5 to 10% smaller than our data would imply.

Genomic DNA was digested with restriction enzymes and size fractionated in either a 5 to 20% sucrose gradient (25) or 5 to 20% NaCl gradients (10 mM Tris, 1 mM EDTA [pH 8.0]; after the method of K. Marcu [F. R. Blattner, Ph.D. thesis, Johns Hopkins University, Baltimore, Md., 1968]). Gradients were assayed for spot 42 RNA gene-containing fractions as shown in Fig. 1. DNA fragments of appropriate fractions were either ligated into the *Eco*RI sites of lambda CH10 to generate lambda CH10-42 or further digested with *Hae*III and subjected to another cycle of size fractionation. The fragments isolated by this latter procedure were then digested with *Sau*3A and ligated into the *Bam*HI site of pBR322 to generate pRD6A and pRD6B (see below).

Transformants were selected for colony growth on LB plates containing 100 μg of ampicillin per ml. Clones pRD1, pRD2, and pRD4 were all detected by screening plasmid DNAs isolated from small-scale plasmid preparations (4). The desired recombinants were identified by their sizes or characteristic restriction enzyme cleavage sites and hybridization to ^{32}P-labeled spot 42 RNA in a Southern blot analysis (41). pRD5A, pRD6A, and pRD6B were isolated by colony screening as described by Grunstein and Hogness (16) and modified by Loughney et al. (24), using ^{32}P-labeled spot 42 RNA as a probe.

pRD1 was made by subcloning the *Hin*dIII fragment containing the spot 42 RNA gene from lambda CH10-42 into the *Hin*dIII site of pBR322 (see Fig. 6 for the physical maps corresponding to all recombinant plasmids described). pRD2 was constructed by linearizing pRD1 DNA at the unique *Eco*RI site (of pBR322) and filling in the ends with avian myeloblastosis virus reverse transcriptase and deoxynucleotide triphosphates. After partial digestion of the linearized molecules with *Hinc*II, the mixture of fragments was ligated at low DNA concentrations (conditions favoring intramolecular ligation). pRD4 was generated by ligating the partial *Hinc*II digestion products of pRD2 DNA at low DNA concentrations as described above. The ligated DNA was digested with *Bam*HI to destroy any remaining parental pRD2 DNA.

pRD5A was constructed by ligation of a *Sau*3A digest of pRD2 DNA to *Bam*HI-digested pBR322 DNA. The ligated DNA was cut with *Bam*HI to destroy any parental pBR322 (the *Sau*3A fragment containing the spot 42 RNA gene does not regenerate a *Bam*HI site at either of its ends when it is ligated into a *Bam*HI site [19, 20]). pRD6A and pRD6B were made by ligating the size-selected *Sau*3A fragments of *E. coli* DNA (prepared as described above) into the *Bam*HI site of pBR322, as described for pRD5A.

A mutant plasmid, pRD7, was isolated from a culture which had been grown in MOPS medium for 15 to 20 generations. Cells were plated on LB-ampicillin plates, and large colonies were tested for the presence of plasmid DNA. Some of the large colonies contained plasmids which were about 800 bp larger than the starting plasmid, pRD6A.

Gel electrophoresis. DNA fragments were separated by electrophoresis in 0.8 to 1.2% agarose gels (39), localized by staining with ethidium bromide, and transferred to nitrocellulose filters (Schleicher & Schuell Ba85), using the method of Southern (41).

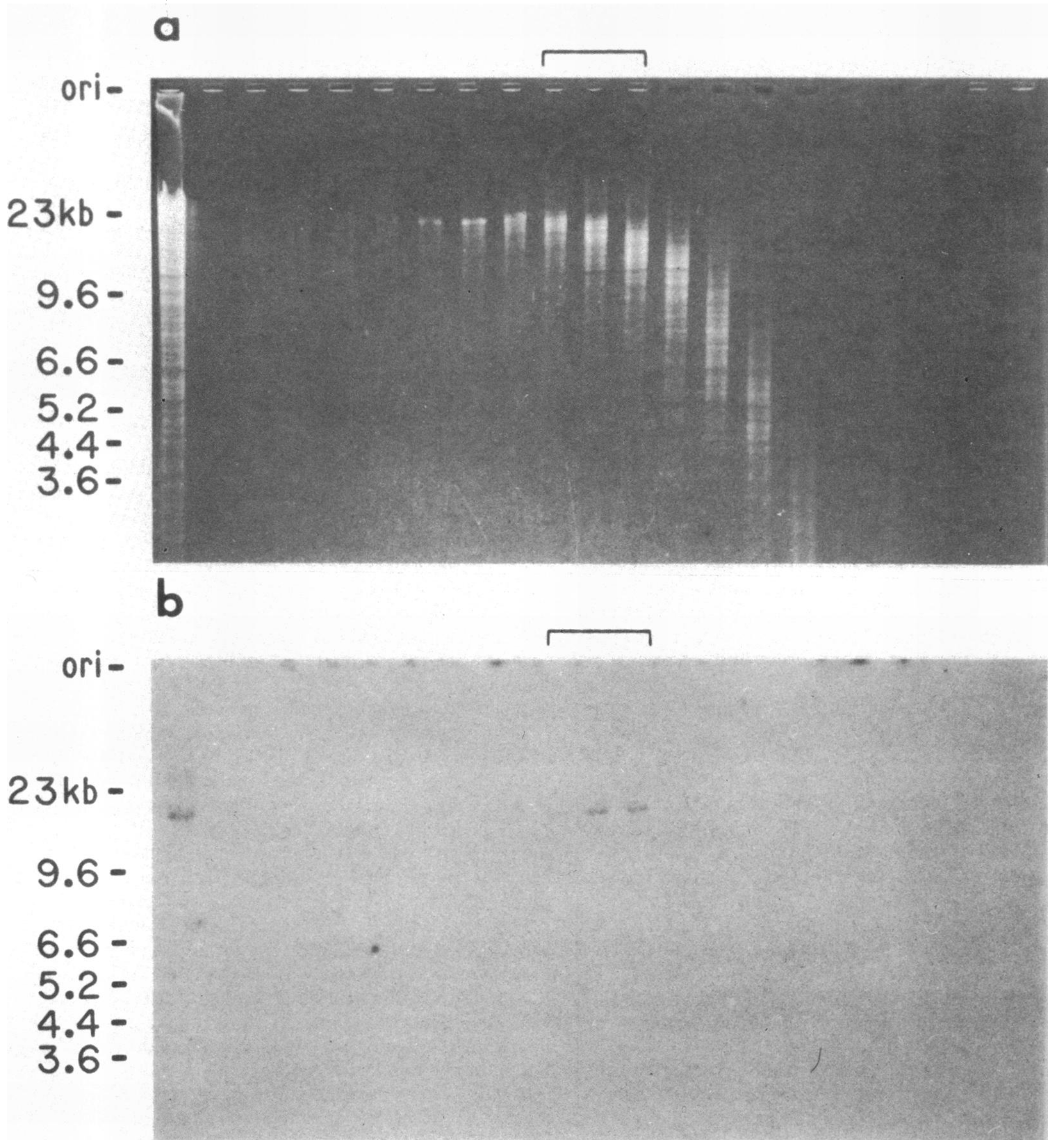

FIG. 1. Agarose gel analysis of gradient-fractionated *Eco*RI restriction fragments of *E. coli* DNA. (a) Ethidium-bromide stained agarose gel. (b) Autoradiogram of the nitrocellulose filter after hybridization to ^{32}P-labeled spot 42 RNA. *Eco*RI-digested CP78 DNA was separated on a 5 to 20% NaCl gradient, and a sample of each fraction was electrophoresed through a 0.8% agarose gel and transferred to a nitrocellulose filter (41). The filter was hybridized to a ^{32}P-labeled spot 42 RNA probe to locate those fractions which contained the spot 42 RNA gene. The leftmost lane is the unfractionated digest. The top of the gradient corresponds to the rightmost lane. The brackets indicate those fractions which were pooled, digested with *Hae*III, run on another gradient, and treated as above. The pooled fractions from the second gradient were digested with *Sau*3A and ligated to *Bam*HI-digested pBR322 DNA to construct pRD6A and pRD6B.

RNA was analyzed by one-dimensional electrophoresis in 10% (30:0.8) polyacrylamide gels containing 7.5 M urea (28 by 13.5 cm by 1.5 mm) for 18 h at 250 V. The buffer was 85 mM Tris-borate (pH 8.3)–2.8 mM EDTA. Two-dimensional gel electrophoresis was carried out as described by Ikemura and Dahlberg (17). In vivo ^{32}P-labeled 5S rRNA and spot 42 RNA isolated from two-dimensional gels were further purified by electrophoresis through a third-dimension gel (as in reference 36, except 16% polyacrylamide [19:1] and 7

M urea) and analyzed by RNase T_1 fingerprinting according to the method of Sanger et al. (38) before their use as hybridization probes.

RESULTS

Characterization and isolation of the gene for spot 42 RNA. The number of regions in the *E. coli* genome complementary to spot 42 RNA was determined by hybridization of ^{32}P-labeled spot 42 RNA to Southern blots of *E. coli* DNA. When size-fractionated DNA restriction fragments were probed with ^{32}P-labeled spot 42 RNA, hybridization was observed to only one size class of fragments in each digest (Fig. 2). This indicated that DNA sequences complementary to spot 42 RNA were present at a single location in the *E. coli* genome; additional data (presented below) defined the direction of transcription of this gene and showed that it was present as a single copy.

The 20-kb *Eco*RI DNA fragment which contained the gene corresponding to spot 42 RNA (cf. Fig. 2b, arrow) was cloned to permit more detailed study. Appropriately sized fragments of *E. coli* DNA were prepared as described in the legend to Fig. 1 and were ligated into the *Eco*RI sites of lambda CH10 DNA to produce the clone, lambda CH10-42. The restriction enzyme cleavage map of the cloned fragment, determined by standard methods, is shown in Fig. 3. This map is consistent with the sizes of those genomic DNA fragments to which spot 42 RNA hybridized (Fig. 2b).

The DNA sequences corresponding to spot 42 RNA were localized to a 0.17-kb region of the cloned DNA. This was done by comparing the hybridization pattern of the RNA to Southern blots of pRD1 DNA digested by the enzyme *Mnl*I, *Sau*3A, or *Hae*III (data not shown). Spot 42 RNA hybridized to a 570-bp *Mnl*I fragment (asterisks, Fig. 3c), a 300-bp *Sau*3A fragment (circles, Fig. 3c), and a 1.5-kb *Hae*III fragment (triangles, Fig. 3c). These results placed the sequence as indicated by the arrow in Fig. 3c. The direction of transcription was determined by the observation that there is an *Mnl*I recognition site located within the spot 42 RNA coding region corresponding to positions 11 to 14 from the 5′ end of the RNA (36; Sahagan, Ph.D. thesis). These data also show that there is only one gene for spot 42 RNA in clone pRD1 and thus in the *E. coli* genome. These conclusions (Fig. 3) are confirmed by the sequence analysis of Joyce and Grindley (19) cited below.

Other genes encoded by the 20-kb *Eco*RI fragment. The location of the gene for spot 42 RNA (and its flanking sequences) in the *E. coli* genome was determined by the fortuitous observation of Joyce et al. (20) that a 5-kb *Hin*dIII fragment containing the gene for DNA polymerase I, *polA*, also contained the gene for spot 42 RNA. In fact, their sequence of the 5-kb *Hin*dIII fragment showed that the start site for spot 42 RNA was 146 nucleotides downstream from the translation stop codon of the *polA* gene; both genes were transcribed from the same DNA strand.

In an effort to determine the extent of the chromosomal DNA sequences contained on lambda CH10-42, we assayed for the presence of other genes known to map near *polA*. Closely linked genes included *rrnA* in the counterclockwise direction (43) and *glnA* in the clockwise direction (26).

We tested for the presence of the rRNA operon *rrnA* by hybridization of ^{32}P-labeled rRNA to size-fractionated DNA restriction fragments of lambda CH10-42 DNA. Hybridization of 5S and 23S, but not 16S, rRNA was observed to all fragments which originated from the left end of the cloned DNA (see Fig. 3a and b and 4b).

^{32}P-labeled lambda CH10-42 DNA hybridized to size-fractionated restriction digests of DNA from a clone which contained the *glnA* gene, p811 (cf. Fig. 3a). The pattern of hybridization observed (Fig. 5b) indicated that there was a 5.5-kb overlap between the left end of the insert in p811 and the insert of lambda CH10-42 (as indicated in Fig. 3a and b).

Thus, the 20-kb *Eco*RI insert fragment of lambda CH10-42 is homologous to a region of the *E. coli* chromosome which extends from the 23S to 5S coding region in the *rrnA* gene to the middle of the *glnA* gene. Included in this region are the sequences for the *ntrC* (27; called *glnG* in references 2 and 32), *ntrB* (27; called *glnL* in reference 10 and referred to as the "GlnC" phenotype in references 2 and 32), and *polA* (22) genes and the gene for spot 42 RNA. As discussed by Joyce and Grindley (19), their 5-kb *Hin*dIII clone (and thus the 20-kb clone) also contains the sequence for a 22-kilodalton protein located just downstream from the gene for spot 42 RNA; this gene is transcribed from the opposite DNA strand.

Effects of a multicopy plasmid containing the gene for spot 42 RNA. In an attempt to learn whether synthesis or accumulation of spot 42 RNA had an effect on cellular physiology, we subcloned the gene for this RNA into the multicopy plasmid pBR322. The subcloned DNA was obtained either from lambda CH10-42 DNA (clones pRD1 to pRD5A) or directly from *E. coli* genomic DNA (clones pRD6A and pRD6B) (as described in Materials and Methods). These subclones contained different amounts of the flanking sequences on one side or the other of the gene for spot 42 RNA (Fig. 6).

(i) Slow growth. Presence of the 5-kb sub-

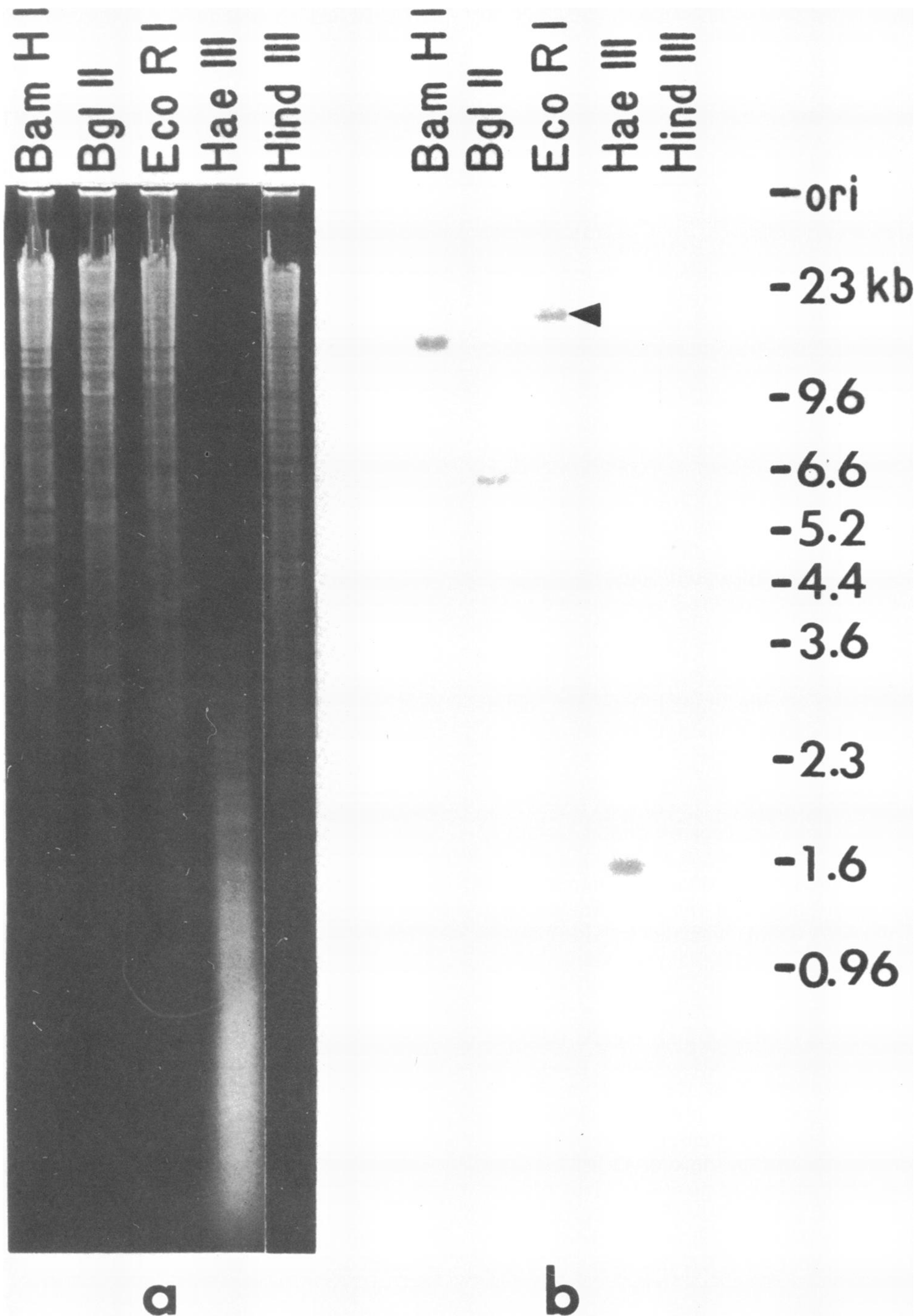

FIG. 2. Hybridization analysis of the *E. coli* spot 42 RNA gene. (a) Ethidium bromide-stained agarose gel. (b) Autoradiogram of the nitrocellulose filter after hybridization. *E. coli* DNA from strain CP78 was digested with the restriction enzyme indicated above each lane, electrophoresed through a 1% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized to purified ^{32}P-labeled spot 42 RNA. The arrow points to the 20-kb *Eco*RI fragment corresponding to the insert fragment in lambda CH10-42. The sizes of the hybridizing bands were measured by comparison with lambda DNA size markers. The spot 42 RNA-hybridizing fragment sizes are as follows: *Bam*HI, 15.1 kb; *Bgl*II, 6.45 kb; *Eco*RI, 19.6 kb; *Hae*III, 1.55 kb; *Hin*dIII, 5.1 kb.

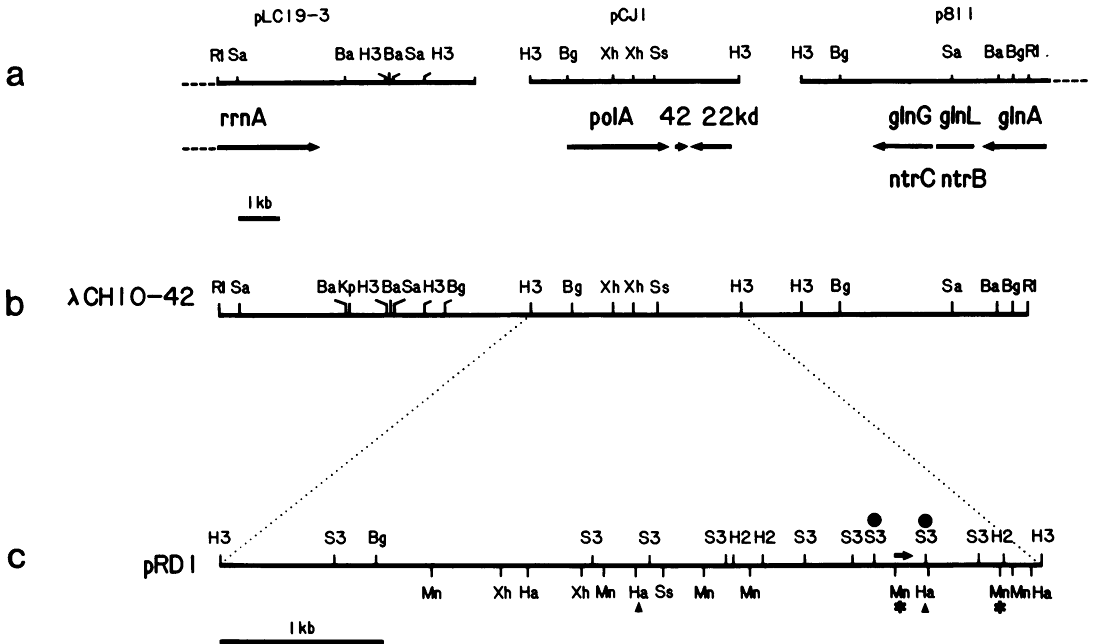

FIG. 3. Restriction enzyme cleavage maps of lambda CH10-42, pRD1, and related cloned fragments. Restriction maps of other cloned fragments which show homology to the lambda CH10-42 insert fragment are presented at the top (a) and are aligned over the lambda CH10-42 restriction map (b), which contains the cloned 20-kb *Eco*RI fragment to which spot 42 RNA hybridizes. Clones pLC19-3 (23) and pCJ1 (20) are included for comparison since they have DNA from the region defined by lambda CH10-42. The restriction map of the 5-kb *Hin*dIII fragment from pRD1 is shown in (c). For clarity, only the cloned *E. coli* DNA sequences are shown. The directions of transcription, approximate lengths of transcripts, and locations of genes included on these clones are indicated by arrows and are taken from references 2, 10, 12, 19, 27, Ellwood (Ph.D. thesis), and Joyce and Grindley (personal communication). The direction of transcription and location of the spot 42 RNA gene are indicated by an arrow above the pRD1 insert map. Dashed lines indicate that the cloned fragment extends further. The restriction map of the 20-kb *Eco*RI insert from lambda CH10-42 is oriented so that rightward on the restriction map corresponds to the clockwise direction on the *E. coli* genetic map. Thus, the left arm of lambda is adjacent to the "*glnA* end" of the insert and the right arm is adjacent to the "*rrnA* end" of the insert. Restriction sites marked by special symbols (triangles, circles, and asterisks) are explained in the text (see Results).

cloned fragment (pRD1) which contained the *polA* gene was inhibitory to cell growth (as also noted by Joyce et al. [19, 20]) since its removal resulted in an increased growth rate. Removal of other sequences flanking the gene for spot 42 RNA (clones pRD4, pRD5A, and pRD6A; cf. Fig. 3 and 6) had no additional effect on the generation time of these transformed HB101 cells (Table 1). HB101 cells transformed with plasmids containing the gene for spot 42 RNA consistently had generation times longer than did HB101(pBR322) cells when grown in a variety of media. The difference in generation times was most striking in minimal medium containing succinate as the carbon source (three times as long; in LB-glucose medium the difference was only 1.5-fold [Table 2]). This effect on growth rate was seen even with pRD6A, which contained only 161 bp upstream and 15 bp downstream of the spot 42 RNA coding region.

(ii) Adaptation. The ability of HB101(pRD6A) cells to respond to changes in media was tested (Table 3). As expected, shifts from rich to minimal media resulted in a lag period. The lag for cells containing pRD6A was not significantly increased over that found for cells containing pBR322 (Table 3, downshift).

Quite unexpectedly, HB101(pRD6A) cells were unable to respond in a normal way to upshift conditions. Only a small percentage of the cells in a culture of HB101(pRD6A) grown in minimal glucose medium were able to continue growing after upshift to rich media. This was true for an upshift either into minimal glucose medium supplemented with 1% Casamino Acids or into LB-glucose broth. This was evident both in liquid culture and after transfer to plates. For example, there was a long lag period after transfer to either of the rich media, and the increase in OD_{450} of the culture at the end of that period was nonlogarithmic for one to two generations (Table 3, upshift; Fig. 7). This observation was

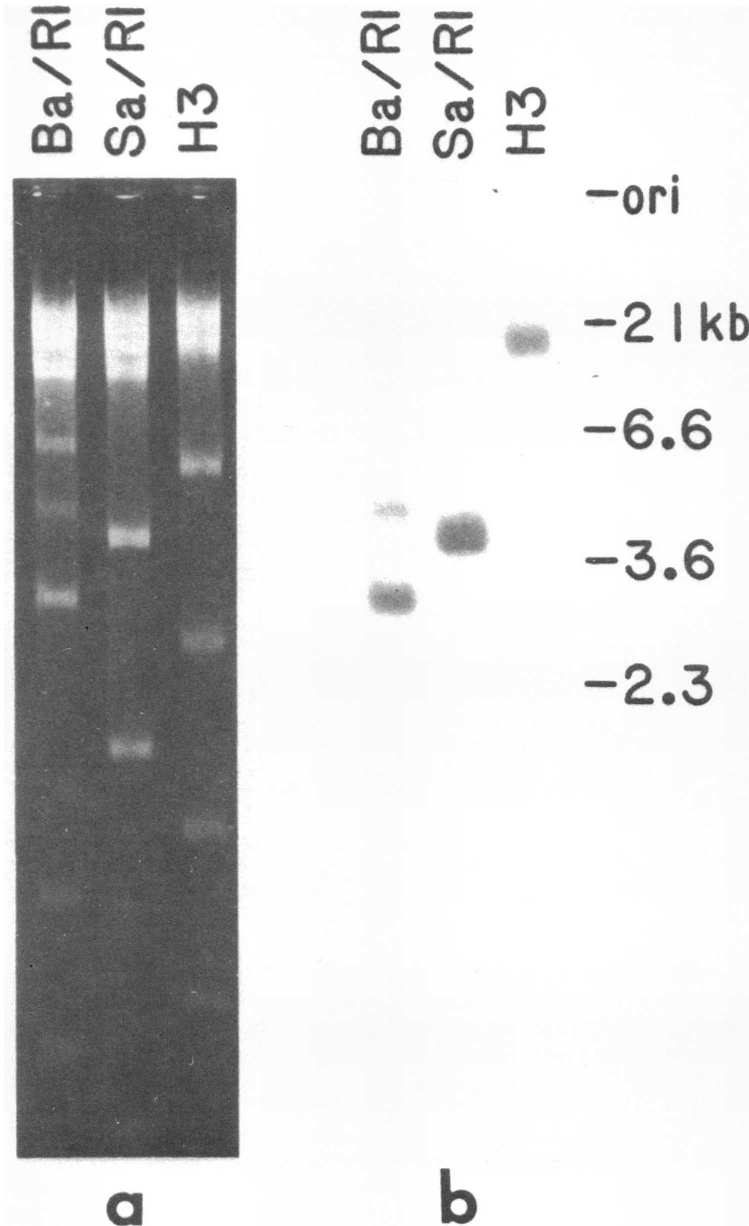

FIG. 4. Hybridization analysis of lambda CH10-42 DNA, using ^{32}P-labeled 5S rRNA as a hybridization probe. (a) Ethidium bromide-stained agarose gel. (b) Autoradiogram of the nitrocellulose filter after hybridization. Lambda CH10-42 DNA was digested with the enzymes indicated above each lane, electrophoresed through a 1.2% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized to a ^{32}P-labeled 5S rRNA probe. Hybridization was to a 3.1-kb *Bam*HI/*Eco*RI fragment, a 3.7-kb *Sal*I fragment, and a 14.1-kb *Hin*dIII fragment. (There are no other *Hin*dIII sites leftward in the lambda CH10 sequences.) This pattern of hybridization localizes the 5S rRNA coding region of *rrnA* to the leftmost *Sal*I/*Bam*HI fragment on the 20-kb fragment from lambda CH10-42 (cf. Fig. 3b). The 4.1-kb band in the *Bam*HI + *Eco*RI digest is due to partial *Bam*HI digestion of the DNA.

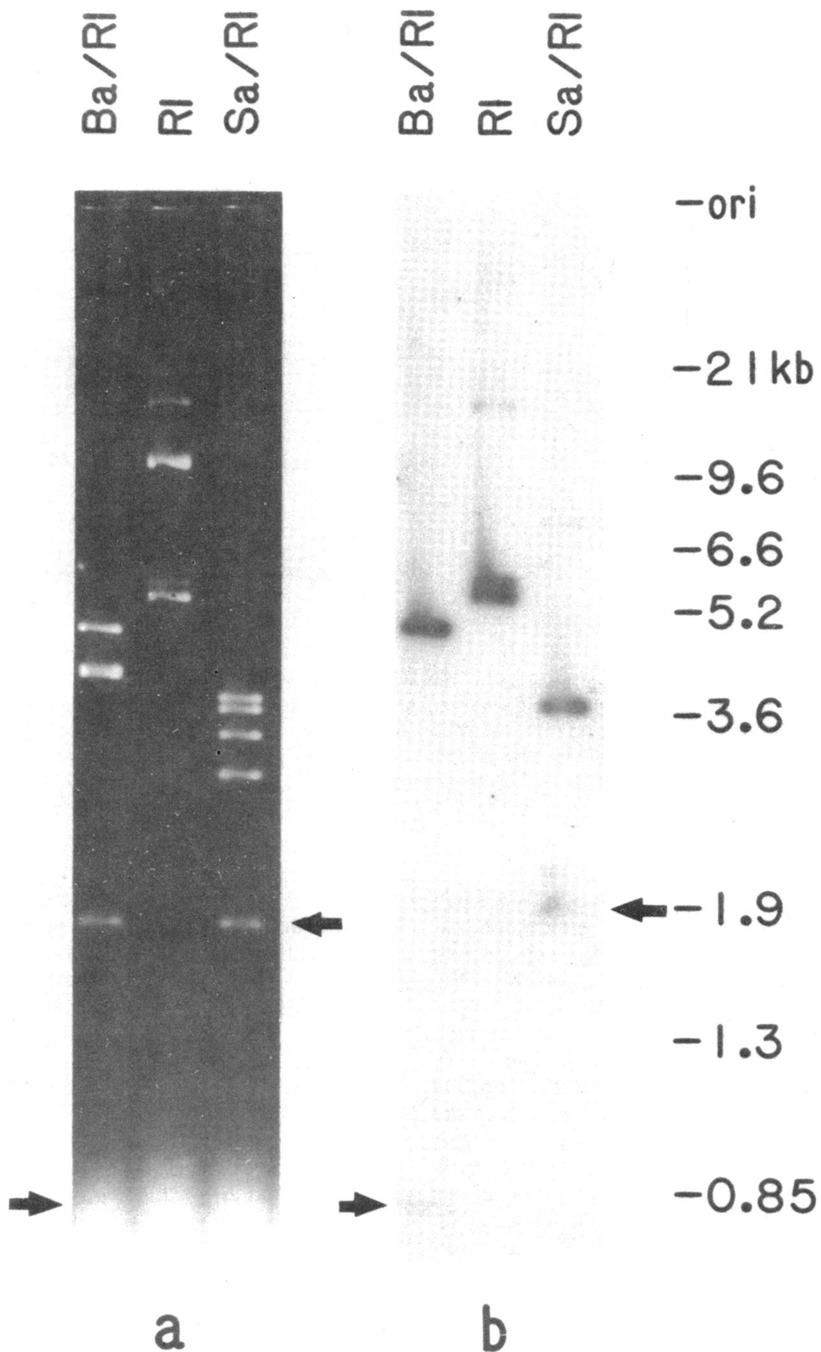

FIG. 5. Comparison of the *glnA-glnG* clone, p811, and lambda CH10-42. (a) Ethidium bromide-stained agarose gel. (b) Autoradiogram of the nitrocellulose filter after hybridization. p811 DNA was digested with the enzyme(s) indicated above each lane, electrophoresed through a 1% agarose gel, and transferred to a nitrocellulose filter as described previously. The filter was hybridized to a 5′-end ^{32}P-labeled *Hin*dIII/*Eco*RI digest of lambda CH10-42 DNA. p811 contains an 11-kb *Hin*dIII fragment from *E. coli* DNA in the *Hin*dIII site of pBR322. An *Eco*RI site in the pBR322 vector portion of the p811 clone (not shown), which is 29 bp to the left of the *Hin*dIII site indicated in Fig. 3a, defines one end of the hybridizing *Eco*RI fragment in all of the digests. Hybridization was to 4.7- and 0.76-kb fragments in the Ba/RI lane, a 5.4-kb fragment in the RI lane, and 3.6- and

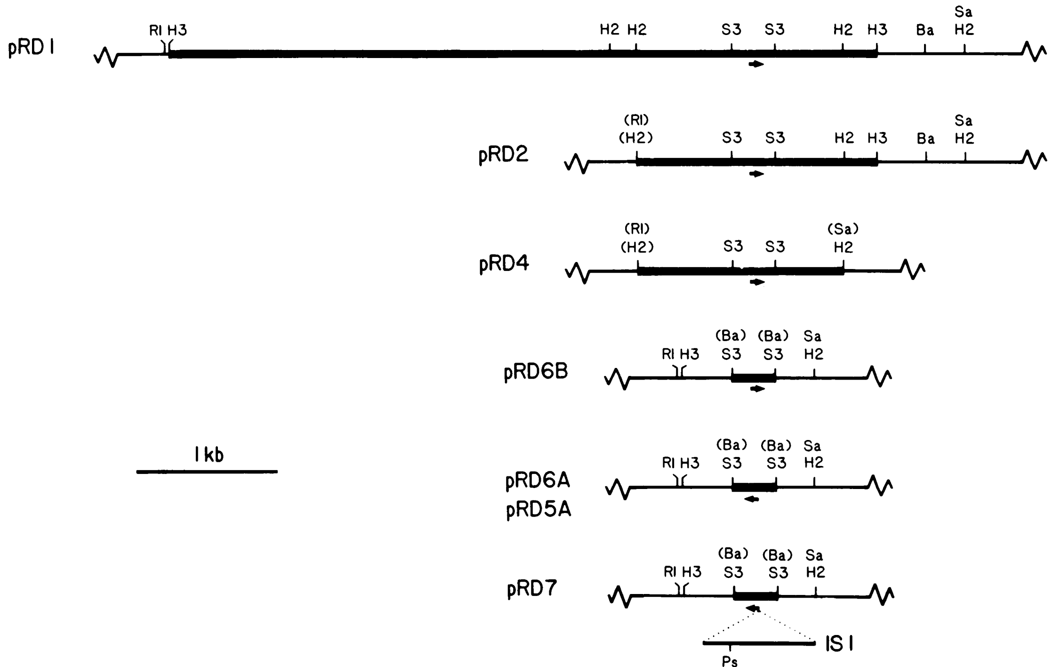

FIG. 6. Plasmid clones containing the gene for spot 42 RNA. The plasmid clones and subclones used in this work are shown aligned with respect to the gene for spot 42 RNA; their derivations are outlined in the text. The gene for spot 42 RNA and the direction of transcription are indicated by arrows. Cloned *E. coli* DNA sequences are represented by heavy lines, and the pBR322 vector sequences are shown as thin lines. Restriction sites destroyed upon cloning are in parentheses. As noted in the text, pRD1 to pRD5A are derived from lambda CH10-42, whereas pRD6A and pRD6B are derived directly from genomic DNA. Other genes contained on these clones are indicated in Fig. 3a.

consistent with either the growth of only a small percentage of the population or a slow adaptation of the entire population to the new growth conditions. The growth of only a portion of the culture probably accounted for at least part of the outgrowth since cells grown in minimal glucose medium (as described above) had a very low plating efficiency on rich plates but a normal one after the onset of logarithmic growth in the upshift medium (Table 4). This effect of pRD6A was observed only during an upshift from minimal glucose medium to LB or minimal plates supplemented with 1 or 2% Casamino Acids. Normal plating efficiencies were observed in all other cases, i.e., rich to rich or rich to poor media. pBR322 had no effect on the plating efficiency of HB101 under any of these conditions.

(iii) Other effects. Colonies containing the gene for spot 42 RNA were significantly smaller than the colonies of HB101(pBR322) cells (for example, cf. Fig. 8a and b). This observation was true regardless of the history of the culture and was true for any medium used in the plates.

An additional phenotype of cells containing pRD6A was an increase in the amount of 6S RNA. This is an abundant RNA of unknown function (7). Its level was increased about two-fold in cells containing the pRD6A plasmid (cf. Fig. 9), regardless of the strain tested (HB101 and M7042 *recA*; CSR603 [data not shown]). The reason for the change in 6S RNA accumulation remains obscure since it does not hybridize to the cloned 20-kb *Eco*RI DNA fragment in a Southern blot analysis (data not shown).

Effect of a mutation in the gene for spot 42 RNA. HB101(pRD6A) cells contained multiple copies of the pRD6A plasmid, comparable in amount to the number of copies of pBR322 in HB101(pBR322) cells (as determined by the yield of plasmid DNA obtained from several plasmid preparations from these strains). As expected, the amount of spot 42 RNA was significantly higher in cells containing pRD6A

1.85-kb fragments in the Sa/RI lane. This pattern can only be generated by the overlap of the two maps indicated in Fig. 3. The positions of the 0.76-kb Ba/RI and 1.85-kb Sa/RI fragments are indicated by arrows. The faint bands in the RI and Sa/RI lanes are due to partial digestion by *Eco*RI.

TABLE 1. Effects of different plasmids containing the gene for spot 42 RNA on the generation time of HB101 cells

Plasmid	Generation time (min) at 37°C	
	LB broth	MOPS + 0.2% glucose[a]
pRD1	210	NG
pRD2	50	140
pRD4	45	144
pRD6A	45	138
pBR322	30	65

[a] Described in the text. NG, No detectable change in OD_{450} after at least 36 h.

than in cells containing pBR322 (Fig. 9). Thus, the slow growth and the responses to medium changes were caused by either the elevated number of the genes for spot 42 RNA or the elevated level of the gene product(s). To distinguish between these two alternatives, we isolated a mutation in the gene carried on plasmid pRD6A.

We used the slow growth of HB101(pRD6A) cells in minimal glucose medium as a selection for faster-growing revertant strains. Analysis of the plasmid DNA isolated from one of these strains showed that it was about 770 bp larger than pRD6A; we called this altered plasmid

TABLE 2. Effects of plasmid pRD6A on growth of HB101 and M7042 *recA* cells in different media

Cells	Plasmid	Generation time (min)			
		LB broth	MOPS		
			0.2% glucose	0.8% succinate–0.02% glucose[a]	
				Before depletion	After depletion
HB101	pRD6A	45	138	200	≥480
	pBR322	30	65	90	180
		32	57	ND	ND
M7042 *recA*	pRD6A	26	70	86	≥480
	pBR322	26	50	50	118
		24	46	ND	ND

[a] Two growth rates are presented for this condition. The first column represents the generation time of the culture before glucose depletion (at OD_{450} of 0.4), and the second column is the generation time after glucose depletion and diauxie when the culture is growing again in log phase, utilizing succinate as the carbon source. The longer generation time of these strains before glucose depletion (compared with the same strains growing in 0.2% glucose) is due to the presence of succinate, has been noted for another strain (37; Sahagan, Ph.D. thesis), and is not a result of the reduced glucose concentration in this particular experiment, ND, Not determined.

TABLE 3. Growth lag after shift between minimal glucose and rich media[a]

Shift	Cells	Growth lag (min)
Downshift from LB-glucose to minimal glucose	HB101(pRD6A)	60
	HB101(pRD7)	45
	HB101(pBR322)	45
Upshift from minimal glucose to LB-glucose	HB101(pRD6A)	200
	HB101(pRD7)	20
	HB101(pBR322)	20
	M7042 *recA*(pRD6A)	10
	M7042 *recA*(pBR322)	<5

[a] Cultures growing in mid-log phase were collected by filtering through nitrocellulose. Cells were suspended in the appropriate media (LB-grown cells were rinsed with minimal medium first), and the OD_{450} was monitored until the culture had attained logarithmic growth. The growth lag was defined as the time between dilution of the culture and the beginning of logarithmic growth. The growth lag of a control culture (transferred to the same kind of medium in which it was originally growing) was subtracted from the experimental values. All media were maintained at 37°C.

pRD7. The extra DNA was identified as the insertion element IS*1* by restriction enzyme analysis of pRD7 DNA, using the enzymes *Rsa*I, *Pst*I, *Eco*RI, *Hin*dIII, and *Hin*fI (30). Restriction mapping revealed that the insertion caused a duplication of an *Rsa*I site (8, 14). Since this site

Cells containing plasmids with the IS*1* insertion in the gene for spot 42 RNA [HB101(pRD7)] were indistinguishable from HB101(pBR322) cells with respect to colony size, generation times, plating efficiency, and behavior during upshift and downshift conditions (Table 3). The steady-state level of spot 42 RNA had also returned to the levels found in HB101(pBR322). Therefore, it was likely that the alterations in these phenotypes were caused by the presence of excessive spot 42 RNA gene product(s) rather than by an excess of the gene itself. The only remaining anomalous characteristic in HB101(pRD7) cells was an elevated level of 6S RNA.

Strain dependence. Some of the effects of multiple copies of the gene for spot 42 RNA depended on the particular strain of *E. coli* used. We tested the phenotypes associated with pRD6A in another strain, M7042 *recA*. Although these cells grew more slowly than M7042 *recA*(pBR322) cells in minimal glucose medium, the difference in generation time was not twofold as it was for HB101 transformants (Table 2). The steady-state levels of spot 42 RNA and 6S RNA were elevated comparably in both pRD6A-containing strains (cf. Fig. 9). M7042 *recA*(pRD6A) behaved the same as M7042 *recA*(pBR322) with

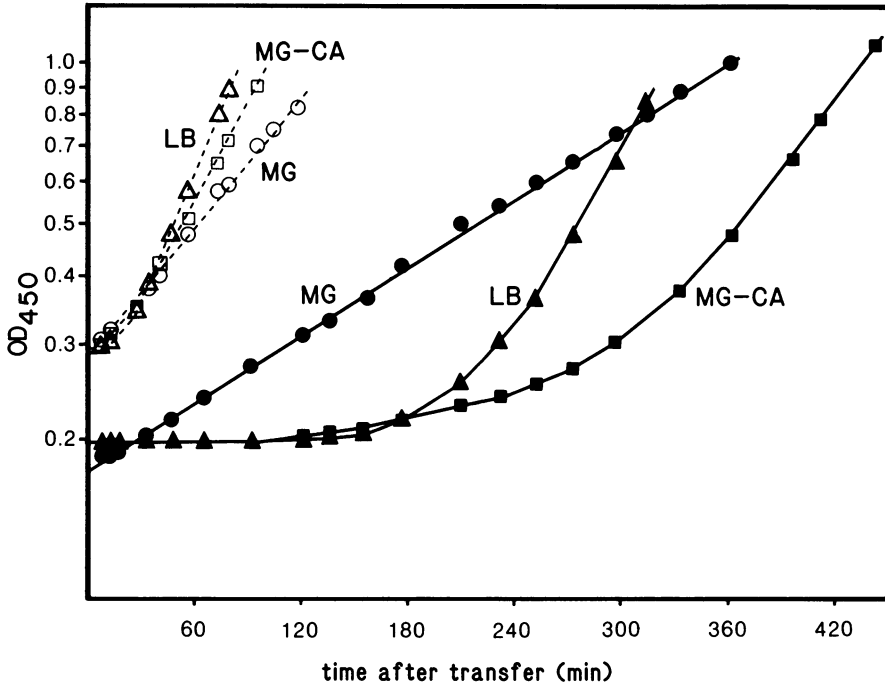

FIG. 7. Effect of pRD6A on ability of HB101 to respond to medium upshifts. Cultures of HB101(pBR322) (open symbols and dashed lines) and HB101(pRD6A) (closed symbols and solid lines) were grown to mid-log phase in minimal glucose medium, filtered, and suspended in prewarmed (37°) minimal glucose (MG; circles), minimal-glucose + 1% Casamino Acids (MG-CA; squares), and LB-glucose (LB; triangles) media, and the OD_{450} was monitored until the cultures had adapted to the new medium.

regard to ability to tolerate shifts between minimal glucose and rich media, colony size, and plating efficiency of minimal-glucose-grown cells on LB agar. The only growth phenotype observed in M7042 *recA*(pRD6A) was an extensive lag and slowed growth in response to a carbon source downshift from glucose to succinate (Table 2).

DISCUSSION

Physical analysis of lambda CH10-42 DNA. We report the isolation and characterization of a recombinant phage clone, lambda CH10-42, which contains the gene for spot 42 RNA on a 20-kb *Eco*RI fragment of *E. coli* DNA. From hybridization analysis and restriction enzyme

TABLE 4. Plating efficiency of HB101 transformants after growth in minimal glucose medium or upshift to minimal glucose medium plus Casamino Acids

Condition	Plasmid	Growth medium[a]	Colonies/ml per OD_{450} in given plating medium		Plating efficiency (LB/MG ratio)
			MG	LB	
Growth	pRD6A	MG	4.2×10^8	6.1×10^6	1.5×10^{-2}
	pBR322	MG	2.9×10^8	1.8×10^8	0.62
Upshift[b]	pRD6A	MG shifted to MG + CA	2.5×10^8	4.7×10^5	1.9×10^{-3}
		After outgrowth	2.0×10^8	1.4×10^8	0.70
	pBR322	MG shift to MG + CA	2.6×10^8	2.1×10^8	0.81
		After outgrowth	2.1×10^8	0.7×10^8	0.33

[a] MG, Minimal glucose medium; CA, Casamino Acids.

[b] The two values presented are derived from plating immediately after the upshift (first value) and after the culture had adapted to logarithmic growth in the upshift medium (second value).

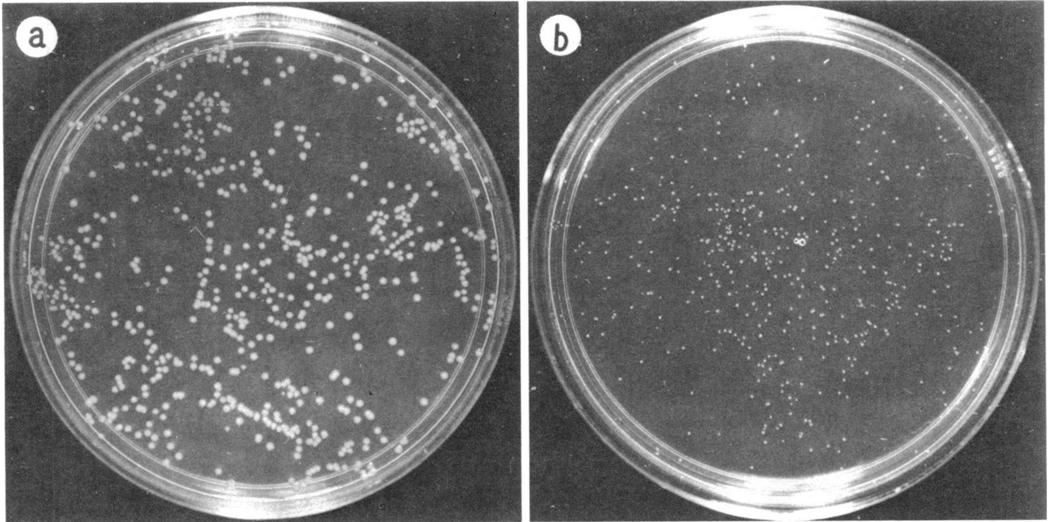

FIG. 8. Small-colony phenotype of HB101(pRD6A) cells. (a) HB101(pBR322); (b) HB101(pRD6A). LB agar plates were incubated at 37°C for 24 h.

mapping, we conclude that the gene for spot 42 RNA is a single-copy gene. The cloned *Eco*RI fragment is homologous to a region of the *E. coli* genome which begins within the 23S rRNA coding region of *rrnA* and extends into the coding region of the *glnA* gene. Both of these genes are located at 86 min on the genetic map (1, 26, 43). Other genes included on lambda CH10-42 are *ntrC* (*glnG*), *ntrB* (*glnL*), the gene for spot 42 RNA, *polA*, and a region coding for a 22-kilodalton protein.

A combination of restriction enzyme mapping data (cf. Fig. 3), Southern hybridization data (Fig. 4 and 5), and the restriction maps of the *glnA* (2) and *rrnA* (12; M. S. Ellwood, Ph.D. thesis, University of Wisconsin, Madison, 1981) regions permit us to determine the direction of transcription and location of the genes known to be on lambda CH10-42. Transcription of the *rrnA* and *polA* genes has been shown genetically to proceed in the clockwise direction on the *E. coli* genetic map (22). *glnA* and *glnG* are transcribed in the counterclockwise direction (2, 35). Our data confirm these directions (cf. Fig. 3). The direction of transcription of a gene for a 36-kilodalton polypeptide involved in regulation of nitrogen metabolism (10, 27), the *ntrB* (*glnL*) gene, has not been determined. The clockwise direction of transcription of spot 42 RNA was determined from our restriction mapping and hybridization data and was confirmed by the sequence data (19, 20).

Unassigned regions on the 20-kb *Eco*RI fragment. About half of the 20-kb *Eco*RI fragment has been assigned to known genes. There are two regions of about 5 and 3 kb which remain unassigned. These are found between *rrnA* and *polA* and between the 22-kilodalton protein gene (19) and *ntrC* (*glnG*), respectively (cf. Fig. 3a). Part of the region between *rrnA* and *polA* (about 900 bp) has been sequenced, but no evidence has been found which would indicate the presence of any gene there (C. M. Joyce and N. D. F. Grindley, personal communication). Nakamura and Yura (28) described a gene in this region which had a conditionally lethal allele, *am100*. Since the *am100* mutation maps between the *polA* and *rha* loci (28) (the *rha* locus is at 87 min; 1) and since deletion of the region between the *rha* and *glnA* genes was not lethal (31), it is likely that the gene carrying the *am100* mutation is located between *glnA* and *polA*. Possibly, this is the gene for the 22-kilodalton protein or for spot 42 RNA.

Phenotypes of cells containing multiple copies of the gene for spot 42 RNA. Previous studies of the spot 42 RNA transcript produced considerable information concerning its accumulation during various growth conditions, its intracellular locations, and its sequence (17, 37; Sahagan, Ph.D. thesis). However, they did not provide direct information about any phenotype associated with this gene. The experiments presented in this paper demonstrated that when an increased number of the genes for spot 42 RNA were present in HB101 or M7042 *recA* cells, several changes in cellular phenotypes occurred.

Two phenotypes were unique to HB101(pRD6A). One was the small colony size. The other was an inability to respond normally to an upshift from minimal glucose medium to rich media (LB broth or minimal glucose + 1%

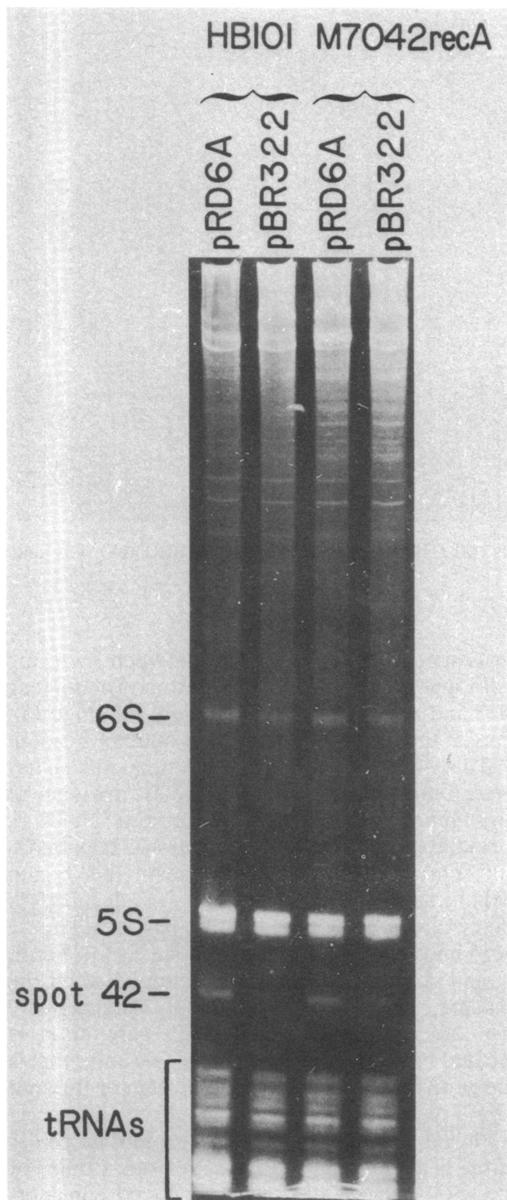

FIG. 9. Comparison of total RNA from cells transformed by either pRD6A or pBR322 DNA. RNA was isolated from HB101 cells and M7042 *recA* cells containing the indicated plasmid and electrophoresed through a 10% polyacrylamide gel containing 7.5 M urea. RNAs were detected by ethidium bromide staining. The mobilities of 6S RNA, 5S rRNA, spot 42 RNA, and tRNAs are indicated.

Casamino Acids) whether this upshift occurred in liquid medium or on plates.

Several properties or phenotypes or both were common to both M7042 *recA*(pRD6A) and HB101(pRD6A); these were a 10-fold increase in the steady-state level of spot 42 RNA, a 2-fold increase in the steady-state level of 6S RNA, and a long lag and slow growth rate when shifted from a medium containing glucose to one containing succinate. Whereas pRD6A caused an increase in the generation times of both M7042 *recA* and HB101 (relative to the effects seen with pBR322), this increase was greater in HB101 than in M7042 *recA* (cf. Table 2).

A spontaneously derived mutant version of pRD6A, called pRD7, was isolated. This plasmid contained the insertion sequence IS*1* located within the coding region for spot 42 RNA, near the 5′ end of the gene. The behavior of HB101 (pRD7) no longer resembled that of HB101(pRD6A). Significantly, its behavior had become indistinguishable from that of HB101(pBR322). HB101 cells transformed with pRD7 had normal levels of spot 42 RNA (i.e., the level found in cells which do not contain a plasmid or contain only pBR322 plasmid). Thus, excessive amounts of the product(s) of the gene for spot 42 RNA had caused the phenotypes observed before the insertion of IS*1*. The only differences between pRD7 and pBR322 transformants was the increased amount of 6S RNA found in HB101(pRD7); the level of 6S RNA was comparable to the amount seen in HB101(pRD6A).

It has been noted previously (36; Sahagan, Ph.D. thesis) that the spot 42 RNA sequence contained features found in mRNAs such as a ribosome binding site, an A-U-G (followed by 14 codons), and a U-G-A terminator codon. If this RNA were used as a messenger, the protein product would be a short, hydrophobic oligopeptide with the sequence fMet-Phe-Tyr-Leu-Ser-Asp-Leu-Leu-Leu-His-Val-Ile-Gly-Phe-Gly (36). Such a molecule could interact either with other proteins, perhaps modifying their activities, or with cell membranes. A putative spot 42 peptide located in the membrane could be a good ionophore, allowing for the free passage of H^+ ions associated, for example, with a protonated histidyl residue. In addition, this peptide might be expected to alter membrane fluidity. It is not clear whether such functions might be related to the normal physiological role of the spot 42 RNA gene product. Nevertheless, overproduction of such a peptide could account for at least some of the pleiotropic effects observed in cells containing pRD6A.

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