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Chromosomal Locations of the Genes for rRNA in *Escherichia coli* K-12†

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Chromosomal locations of the seven rRNA operons in *Escherichia coli* K-12 were studied by digesting DNA from various merodiploid strains with *SalI* restriction enzyme followed by Southern gel analysis with ^{32}P-labeled 23S rRNA as a probe. The seven unique *SalI* DNA fragments revealed in the autoradiograms were first correlated to the seven rRNA operons previously isolated as hybrid plasmids or transducing phages. The chromosomal locations of six (*rrnA*, *B*, *C*, *D*, *E*, and *G*) of the seven isolated operons were confirmed by increased gene dosage demonstrated in autoradiograms after Southern gel analysis of DNA from relevant merodiploid strains. The gene dosage analysis showed that the location of the remaining operon (now called *rrnH*) is between *metD* and *proA*. No evidence was obtained for the presence of *rrnF*, which was previously reported to map between *aroB* and *malA*. The chromosomal location of *rrnH* was confirmed by P1 transduction in the following way: a DNA fragment adjacent to *rrnH* was cloned into pBR322; the resulting hybrid plasmid was integrated at the homologous region of the chromosome of a *polA* mutant; and the ampicillin resistance marker originally carried by pBR322 was then used for mapping of the nearby *rrnH* by P1 transduction. A close linkage of *rrnH* to *metD* (about 60% cotransduction) was observed, and the data were consistent with the order *metD-rrnH-proA*. Thus, mapping of all seven rRNA operons has been completed. The present study has also determined the orientation of *rrnG* and *rrnH* and demonstrated that the direction of transcription of all the rRNA operons is identical to that of DNA replication.

Escherichia coli K-12 contains seven genes for rRNA which are dispersed on the chromosome (17, 19). The mapping and characterization of these genes are prerequisites to the understanding of their regulation. All of the rRNA genes (rDNA) have been isolated on transducing phages, plasmids, or both, and comprise similar transcription units, each giving rise to 16S, 23S, and 5S rRNA's and one or more tRNA's (for a review, see references 29 and 31). A collection of sixteen randomly cloned *E. coli* chromosome fragments contains representatives of six of the seven rRNA transcription units (17), here called "operons." Four groups (called groups II, III, IV and VI in references 14, 17, and 28) of these plasmids were assigned to previously known chromosomal locations (assigned to *rrnD*, *A*, *B* and *C*, respectively [14, 17]). Transducing phages bearing the *metA-purD* region of the chromosome (37) define a fifth location, *rrnE*, which is not represented in the plasmid bank. The remaining two groups (groups I and V) of the isolated rRNA operons were left unassigned in our previous studies.

In this paper, we report that one of the unassigned groups (group V) corresponds to *rrnG* (at 56 min), which was detected on transducing phages carrying *pheA* by Zurawaski and Brown (38). Another unassigned group (group I) was found to map near *metD* at 5 min, and not at the location of *rrnF* reported to be linked to *malA* (15, 16, 36). The presence of an rRNA operon at 5 min (called *rrnH*) was also shown by Hill and Harnish in the accompanying paper (13) and confirms the suggestion made by Anderson and Roth from their studies on chromosomal duplications in *Salmonella typhimurium* (1). The present study completes the mapping of all of the seven rRNA operons in *E. coli* K-12.

MATERIALS AND METHODS

Bacterial strains. The F′ merodiploids in Table 2 have been described previously (23, 30) and were obtained from the *E. coli* Genetic Stock Center, Yale University School of Medicine. Other strains are listed in Table 1.

Construction of pNO1530 and transformation. The 3-

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TABLE 1. Bacterial strains used

Strain	Genotype and comments	Source
P4X (= CGSC 261; NO2512)	Hfr (origin PO3; between *proB* and *lac*) *metB1 relA1 spoT1*	CGSC[a]
P804 (= CGSC 5053; NO2513)	Hfr (origin PO65; between *proB* and *lac*) Thi$^-$ λ^{d22}	CGSC
KL251 (= CGSC 4227; NO2496)	F$^-$ *thi-1 metE70 trpE38 purE42 proC32 leuB6 recA1 mtl1 xyl5 ara14 lacZ36 azi-6 strA109* (= *rpsL109*) *tonA23 tsx76 supE44*	CGSC
W4546 (= CGSC 5179; NO2497)	F$^-$ *nadC8 galT23*	CGSC
H5546 (= NO1500)	F$^-$ *dapD polC thr thy*	H. Shizuya
AB2072 (= NO1645)	F$^-$ *thr leu pro lac gal trpA58 thi his ilv metB tsx* Str^r	
CD4 (= CGSC 5096; NO2493)	Hfr (origin PO2A; between *lys* and *purE*) *metD1 proA3 relA1 metD88 lac3 malA36 tsx-76*	CGSC
NO2494	Constructed by crossing CD4 with AB2072, selecting Ilv$^+$ Leu$^+$ Thr$^+$ Str^r, and then introducing *recA* from Hfr KL16-99; F$^-$ *recA pro trpA his metB malA metD tonA*	This work
CH931 (= NO2383)	Hfr *polA lysA*; the same origin of chromosome transfer as HfrH (12)	C. Hill
NO2483	CH931 carrying pNO1530 integrated into the chromosome	This work
NO2488	*tonA* derivative of NO2483	This work

[a] Coli Genetic Stock Center, Yale University School of Medicine.

kilobase (kb) *Eco*RI fragment from pLC7-21 (28; see Fig. 7) was purified by agarose gel electrophoresis, eluted, and ligated with *Eco*RI-cleaved pBR322 which had been treated with heat and bacterial alkaline phosphatase (34). Transformation was carried out by using C600SF8 (3) as the recipient, and ampicillin-resistant colonies were selected. The transformants were then screened for the presence of a plasmid carrying the 3-kb *Eco*RI fragment. One hybrid plasmid, pNO1530, was selected for further studies, and its structure was determined by single and double digestion with *Eco*RI, *Sal*I, and *Bam*HI, the sites for which are shown in Fig. 7. Integration of this plasmid into the chromosome of CH931 was carried out by treating 2 ml of CH931 cells with 10 μg of the plasmid DNA and selecting ampicillin-resistant transformants.

Southern gel analysis. DNA was prepared as described previously (9), except that merodiploid strains were grown in minimal media under constant selection for the retention of F' factors and harvested at late log phase. Other experimental procedures were described previously (9).

RESULTS

Identification of the *rrn* operon carried by group V plasmid pLC23-30 as *rrnG*. As mentioned above, the chromosomal locations of two groups of the isolated *rrn* operons (group I and V *rrn* operons) were left undetermined in our previous studies (17). Their probable locations were 74 min for *rrnF* (15, 16, 36), and 56 min for *rrnG* (38). The transducing phage λphe415, which carries *rrnG* and *pheA* (38), was used to determine which of the two unassigned *rrn* operon groups corresponds to *rrnG*.

Electronmicrographic analysis of heteroduplexes formed between λphe415 and pLC28-21, a member of group I (17), showed that the homology between these DNAs was limited to the *rrn* operon. In addition, the characteristic "spacer bubble" (7, 17, 24) was observed at the spacer region, indicating that the group I *rrn* operon is not *rrnG* (data not shown). In contrast, heteroduplexes formed between λphe415 and pLC23-30 (a group V plasmid) demonstrated extensive homology which extends into flanking regions on both sides of the operon (data not shown; Fig. 1), although a 5.7-kb insertion loop was observed in the flanking region distal to the 23S rRNA gene, *rrl* (data not shown; see below and Fig. 1). No spacer bubble was observed. From these results we conclude that the *rrn* operon carried by group V plasmid pLC23-30 is *rrnG*. This conclusion was also supported by restriction enzyme analysis of λphe415 and pLC23-30 which is described below.

Figure 1 shows restriction enzyme maps of pLC23-30 and λphe415. A salient observation is that both pLC23-30 and λphe415 contain *Eco*RI, *Sal*I, and *Bam*HI sites at identical positions in the region proximal to the 16S rRNA gene, *rrs*, indicating the homology of DNA adjacent to *rrs*. In particular, the presence of a 1.5-kb *Sal*I fragment carrying the proximal portion of *rrs* is unique to *rrnG* (2; M. Ellwood, Ph.D. thesis, University of Wisconsin, Madison, 1981; see also below and Fig. 6 in this paper). Experiments shown in Fig. 2a and b demonstrate that the 1.5- and 2.3-kb *Sal*I fragments derived from λphe415 carry *rrs*, as visualized by the Southern gel technique with ^{32}P-labeled 16S rRNA as a probe, and that the identical fragments are also present in the *Sal*I digests of pLC23-30. It can also be seen in Fig. 2 that the 1.3- and 2.0-kb *Eco*RI fragments derived from λphe415 carry *rrs*, and that they are indistinguishable upon agarose gel elecrophoresis from those resulting from digestion of pLC23-30. The 2.3-kb *Sal*I and 2.0-kb *Eco*RI fragments also hybridize 23S rRNA (Fig. 2c), confirming the previous conclu-

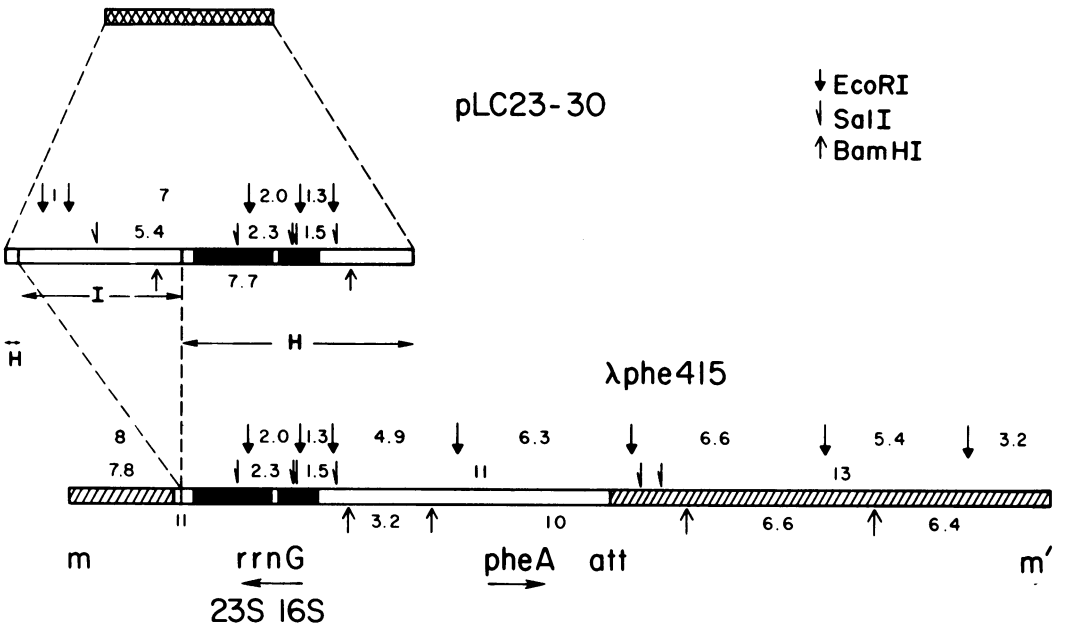

FIG. 1. Structures of pLC23-30 and λphe415. Symbols: ■, rDNA; □, chromosomal DNA; ▨ λ DNA; and ▨, ColE1 DNA. The region of the putative insertion in pLC23-30 is indicated as "I," and the regions of homology observed in heteroduplex analysis are indicated as "H" (see text for discussion). Numbers refer to sizes of restriction fragments in kb. m and m' are the left and right cohesive ends of λ DNA. Restriction maps of λphe415 and pLC23-30 were constructed by analyzing digests obtained after treatment of these DNAs with *Eco*RI, *Sal*I, and *Bam*HI, singly and in combination (data not shown), and using known restriction maps of λ and ColE1 DNA. The position and orientation of rDNA were inferred from the Southern gel analysis shown in Fig. 2 and by heteroduplex analyses (see text); the extent of λ DNA was determined by analysis of DNA heteroduplexes formed between λphe415 and λcI857S7 (data not shown). The restriction map of pLC23-30 agrees with that obtained by C. Squires (personal communication), and the map of λphe415 is consistent with that of K. Brown, L. Lace, and D. Killingly (personal communication).

sion that these fragments are the spacer fragments which span the tRNA coding region between *rrs* and *rrl* (2, 24, 28). In addition, the data in Fig. 2c identify restriction enzyme fragments carrying the distal part of *rrl*. The results show that the structure of the distal part of pLC23-30 is different from that of λphe415. As mentioned above, the heteroduplex analysis showed that pLC23-30 contains a 5.7-kb DNA segment which is not present in λphe415. Since the 5.4-kb *Sal*I fragment carrying the distal *rrl* of pLC23-30 is not found in the genome of the original *E. coli* strain (see Fig. 4), it appears that pLC23-30 carries a 5.7-kb insertion in the region distal to *rrl*; the insertion must have occurred during the construction of the plasmid bank. Regardless of the uncertainty of the nature of this flanking region on pLC23-30, the locations of *rrs* and *rrl* on λphe415 are clearly established to be those shown in Fig. 1. Since the gene order on the chromosome, read counterclockwise, is *tyrA*, *pheA*, *rrnG* (38), and since the *pheA* gene is located between the right arm and *rrnG* on λphe415 (K. D. Brown, personal communication; Fig. 1), the gene order, read counterclockwise, is *tyrA*, *pheA*, *rrsG*, *rrlG*; that is, the orientation of *rrnG* is counterclockwise, as with *rrnD*.

Analysis of chromosomal locations of *rrn* operons by increased gene dosages in merodiploids. To identify the chromosomal location of the unassigned group I plasmid and to confirm chromosomal locations of other *rrn* operons, we examined various F' factors as to whether they carry *rrn* operons. As originally demonstrated by Venetianer and his co-workers (2, 19), digestion of total *E. coli* DNA with certain restriction enzymes produces a uniquely sized fragment for each operon which can be detected by hybridization with the radioactive rRNA after blotting onto nitrocellulose by the method of Southern (35). We used this technique to detect increases in rRNA gene dosage in merodiploid strains carrying additional rRNA genes on F' factors.

Figure 3 shows the map of the *E. coli* chromosome and the extent of the F' factors which were analyzed in this study. Total DNA was prepared from cells bearing an F' factor, digested with

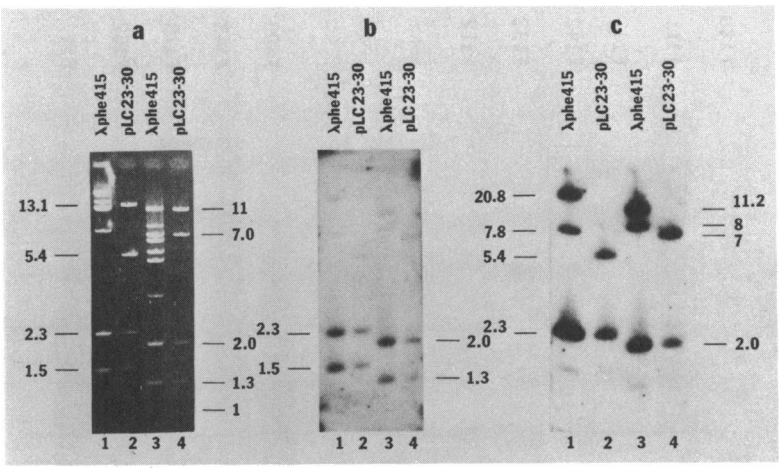

FIG. 2. Southern gel analysis of *Sal*I (lanes 1 and 2) or *Eco*RI (lanes 3 and 4) digested λphe415 and pLC23-30 DNAs. The ethidium bromide-stained gel shown in (a) was blotted onto a nitrocellulose filter. The filter was incubated with ^{32}P-labeled 16S rRNA for hybridization, and the autoradiogram indicating DNA fragments carrying *rrs* is shown in (b). An autoradiogram resulting from a hybridization experiment with a similar gel and ^{32}P-labeled 23S rRNA as a probe is shown in (c). Lanes 1 and 3 contain λphe415 DNA; lanes 2 and 4 contain pLC23-30 DNA. Sizes of pertinent DNA fragments are shown in kb. Faint bands seen below the 2.3-kb *Sal*I fragment or the 2.0-kb *Eco*RI fragment in (c) are due to the presence of small amounts of ^{32}P-labeled 16S rRNA in the ^{32}P-labeled 23S rRNA preparation used. It should be noted that, due to the presence of both melted and annealed λ cohesive ends, ^{32}P-labeled 23S rRNA hybridized to two *Sal*I fragments [20.8- and 7.8-kb fragments; lane 1 in (c)] in addition to the 2.3-kb spacer fragment. Similarly, ^{32}P-labeled 23S rRNA hybridized to both the 11.2- and 8-kb *Eco*RI fragments [lane 3 in (c)] in addition to the 2.0-kb spacer fragment.

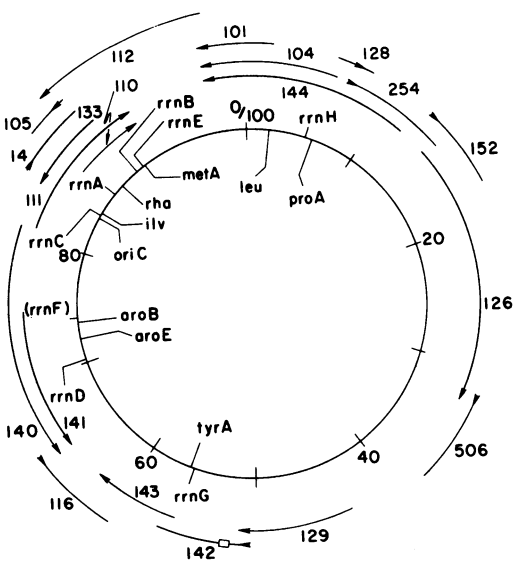

FIG. 3. Genetic map of *E. coli* K-12 showing locations of rRNA operons (*rrn*) and the extent of chromosomal regions duplicated by the F′ factors. Each F′ factor is represented by an arc encompassing the chromosomal region carried by the plasmid. Arrowheads represent the point of origin of the Hfr from which each plasmid was derived; the open rectangle in F142 represents a known deletion.

restriction enzyme *Sal*I, subjected to agarose gel electrophoresis, blotted onto nitrocellulose filters, and hybridized to ^{32}P-labeled 23S rRNA. *Sal*I digestion cleaves each operon twice, once in *rrs* and once in *rrl*, yielding a spacer fragment, which is close to the same size in all operons and hybridizes both 16S and 23S rRNA probes, and two unique fragments, one of which hybridizes only 16S rRNA and the other of which hybridizes only 23S rRNA (2, 19; see also above and Fig. 2). An autoradiogram of a typical experiment with ^{32}P-labeled 23S rRNA as a probe is shown in Fig. 4. Digestion of a control strain with *Sal*I (lane 8) gives rise to an intense 2.3-kb band representing the common spacer region and an array of seven single-copy bands, each representing a different *rrl* gene. An increase in dosage for one or more of the *rrl* genes can clearly be seen in several strains indicating that the F′ factors carried in these strains encompass the operons represented by the intensified bands. We first compared the sizes of these seven *Sal*I *rrl* fragments with those from the reference hybrid plasmids or transducing phages previously isolated and assigned each of the unique bands to an operon. We then determined which operons are duplicated by the F′ factors. The results of this analysis are summarized in Table 2 and will be discussed in detail below.

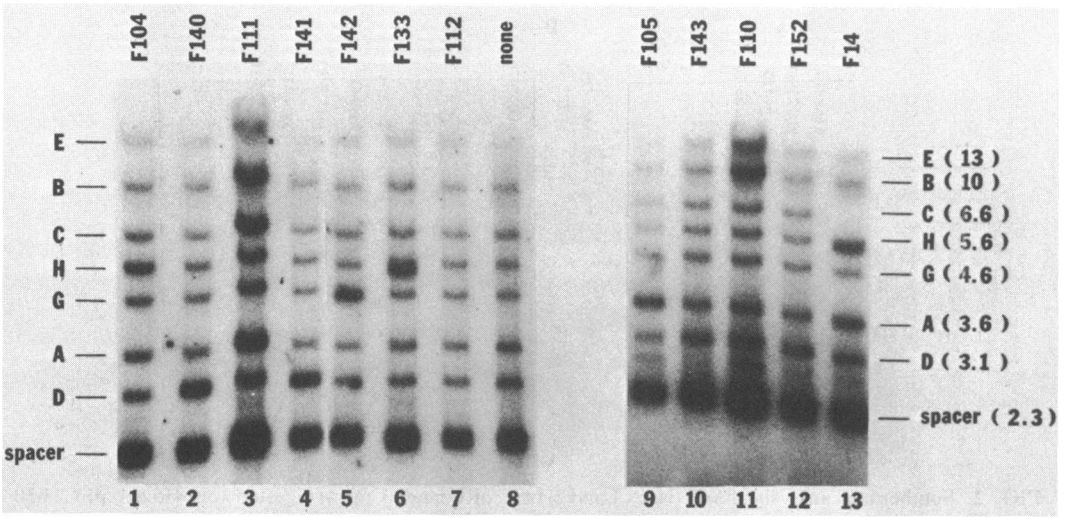

FIG. 4. Southern gel analysis of *SalI*-digested total DNA from various merodiploid strains using ^{32}P-labeled 23S rRNA as a probe. Autoradiograms are shown. Names of the *rrn* operons to which the unique fragments have been assigned are indicated; the sizes (in kb) of these fragments are shown in parenthesis. The spacer fragment is common to all operons and includes portions of both 16S and 23S genes. The merodiploid strains are indicated by the name of F′ factors; lane 8 contains DNA isolated from TX135 (8), which contains no plasmid. It can be seen that both F133 (lane 6) and F14 (lane 13) showed, in addition to the 5.6-kb *rrnH* fragment, a fragment which moved slightly faster than the 5.6-kb fragment. These fragments (about 5.4 kb) presumably reflect fusion of the standard 6.6-kb *rrnC* fragment with F factor DNA in these plasmids (see the footnotes of Table 2). The strain carrying F14, AB1206, does not have the 6.6-kb fragment corresponding to *rrnC* (see the footnotes of Table 2).

One of the hybrid plasmids, pLC19-3, was previously shown to carry *rrnA* (14), and the distal part of *rrlA* is present in a 3.6-kb *SalI* fragment of pLC19-3 (6). Thus, the 3.6-kb band in Fig. 4 corresponds to *rrnA*. This is consistent with the increase in the intensity of this band in strains carrying F′ factors F111 (Fig. 4, lane 3), F105 (lane 9), and F14 (lane 13; see the footnotes of Table 2), all of which cover the previously assigned location (86 min) for *rrnA*. (The increase was not observed with F133 [Fig. 4, lane 6], which is expected to include *rrnA*. Presumably the F133 plasmid or the host chromosome in the merodiploid strain used in the experiments carried a deletion covering this operon.)

The 10-kb band in Fig. 4 corresponds to *rrnB*, which maps at 89 min. In the previous studies on the structure of λ*rif*^{d}18, which carries *rrnB* (21), the size of the *SalI* fragment containing the distal 23S rRNA gene was estimated to be about 10 kb (2). This was also confirmed by direct comparison of *SalI* digests of λ*rif* ^{d}18 DNA with chromosomal digests in the Southern gel analysis with ^{32}P-labeled 23S rRNA as a probe (data not shown). The expected increase in the intensity of this band was observed with F110 (Fig. 4, lane 11) and F111 (lane 3), but not with F112 (lane 7). The F112 plasmid (or the host chromosome) in the strain we used may carry a deletion of *rrnB*.

The 6.6-kb band corresponds to *rrnC*, because our hybrid plasmid pLC22-36, which carries both *rrnC* and *ilvE*$^{+}$ (17), yielded a *SalI* fragment carrying the distal portion of the *rrl* gene which comigrates with the 6.6-kb chromosomal band (data not shown). In the experiment shown in Fig. 4, this conclusion was supported by the increased intensity of this band in F111. (Concerning the absence of this band in F14, and appearance of a novel 5.4-kb band in F14 and F133, see the footnotes of Table 2).

The *rrnD* operon and a nearby chromosome marker *aroE* were previously shown to be carried by the hybrid plasmid pLC22-11 (17). Comparison of *SalI* digests of this plasmid DNA with the chromosomal digests showed that the 3.1-kb fragment in Fig. 4 is the *SalI rrl* fragment derived from *rrnD* (data not shown). As expected, an increase in the intensity of the 3.1-kb band was observed with F140 (Fig. 4, lane 2) and F141 (lane 4), both of which cover the *rrnD* locus at 71 min.

The largest *SalI* fragment (13 kb) in Fig. 4 corresponds to *rrnE*, because deletion of *rrnE* results in the disappearance of this band (9). The increase in the intensity of this band in F110 is consistent with this assignment. (The increase in F111 is not clear in Fig. 2 because of inefficient transfer of the 13-kb fragment to nitrocellulose. However, the presence of *rrnE* on the F111 plasmid was clearly demonstrated by the South-

TABLE 2. *rrn* operons detected on F′ factors by Southern gel analysis[a]

F′ factor	min	Merodiploid strain	Operons detected
F101	98–2	KLF1/AB2463	
F104	98–6	KLF4/AB2463	*rrnH*
F144	98–12	KLF44/KL251	*rrnH*
F128	6–8	E5014	
F254	8–14	ORF4/KL251	
F152	14–17	F152/KL253	
F126	16–30	KLF26-KL181	
F506	35–37	F506/JE5519	
F129	44–51	KLF29/JC1553	
F142	51–56	KLF42/KL253	*rrnG*
F143	56–61	KLF43/KL259	
F716	60–65	KLF16/KL110	
F140	68–80	MAF1/JC1553	*rrnD*
F141	68–75	KLF41/JC1553	*rrnD*
F111	81–91	KLF11/JC1553	*rrnA*, *B*, *C*, and *E*[b]
F14	84–89	AB1206	*rrnA* + novel fragment[c]
F133	84–88	KLF33/JC1553	Novel fragment[c]
F105	86–89	KLF5/AB2463	*rrnA*
F110	86–91	KLF10/JC1553	*rrnB* and *E*
F112	88–98	KLF12/JC1553	

[a] Examples of the Southern gel analysis are shown in Fig. 4. A blank in the last column means that no *rrn* operon was detected by the analysis. The chromosomal regions covered by the F′ factors are indicated in minutes and are shown in Fig. 3.

[b] Concerning the detection of *rrnE* on this plasmid, see the text.

[c] It is known that in the strain carrying F14, AB1206, the chromosomal region corresponding to the F′ factor is deleted (10, 11, 33). The work by Davidson and his co-workers (7, 32), combined with the physical structure of the *rrnC-ilvA*, *D*, and *E* region (26), indicates that the *Sal*I fragment containing the distal *rrlC* is fused to F-factor DNA on F14 (note that *rrnC* corresponds to *rrnB2* in reference 7; see Fig. 5 of reference 7); hence, *rrlC* is presumably deleted in the AB1206 chromosome. This inference is supported by the absence of the 6.6-kb *rrnC* band in the strain carrying F14, AB1206, and the presence of a new fragment which migrates slightly faster than the 5.6-kb *rrnH* fragment (Fig. 4, lane 13). A similar novel fragment seen in F133 (Fig. 4, lane 6) may also represent the standard *rrnC* fragment fused to F-factor DNA. Unlike AB1206, the merodiploid strain carrying F133 does not carry a chromosomal deletion; hence, the standard 6.6-kb *rrnC* fragment is present in a single copy. We also note that a definite increase in the intensity of the 3.6-kb band was seen in the strain carrying F14, AB1206 (Fig. 4, lane 13; unpublished data). If the chromosome region corresponding to the F′ factor is in fact completely deleted in this strain (see above), and if one F14 molecule per chromosome carries *rrnA*, no increase in the dosage of *rrnA* would be expected. It is possible that F14 exists in more than one copy per chromosome. From the degree of increase in the intensity of *Sal*I fragments in some other merodiploids shown in Fig. 4 (e.g., the 4.6-kb band in lane 5), it appears that other F′ factors may also exist in more than one copy.

ern gel analysis after digestion of DNA with a mixture of *Sal*I, *Eco*RI, and *Bam*HI [data not shown], as was done in our previous work [9]. The *rrnE* operon, like *rrnB*, appears to be absent in the F112 plasmid used in the present analysis.)

The assignment described above leaves two *Sal*I bands, the 4.6- and 5.6-kb bands, uncharacterized. We first found that the 5.6-kb band corresponds to our group I rRNA operon, because pLC28-21, a plasmid which belongs to this group (17), revealed a *Sal*I fragment with the same size which hybridizes ^{32}P-labeled 23S rRNA in Southern gels. Therefore, the 4.6-kb band must correspond to our group V rRNA operon, which is *rrnG*. Although *Sal*I digestion of pLC23-30, the only available member of group V, did not produce the 4.6 kb band (because of the presence of the presumptive insertion; see above), the conclusion that the 4.6-kb band corresponds to *rrnG* at 56 min was supported by the observed increase in the intensity of this band in F142 (Fig. 4, lane 5), which encompasses min 51 through 56.

Although previous workers reported the presence of an *rrn* operon (*rrnF*) at 74 min between *aroB* and *malA* (15, 16, 36), neither F140 (Fig. 4, lane 2) nor F141 (lane 4) showed an increase in the intensity of the 5.6-kb band, the band which corresponds to our group I *rrn* operon, even though both plasmids revealed the presence of *rrnD* in the Southern gel analysis mentioned above. We confirmed the presence of the *aroB*$^+$ and *malA*$^+$ genes on these plasmids used in the experiments; therefore, deletion of *rrnF* in the plasmids appears unlikely, although the possibility can not be excluded rigorously by these negative experiments. Stronger evidence against the presence of *rrnF* is the finding that a strain carrying F104 does show the 5.6-kb band of

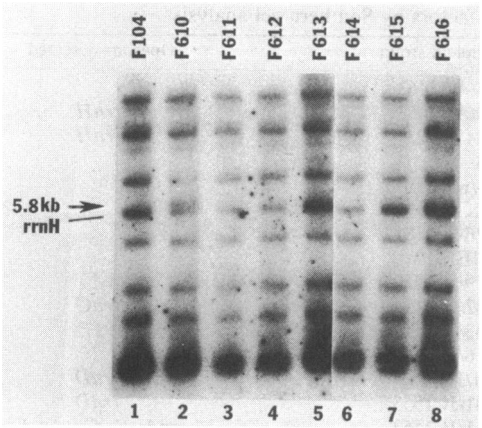

FIG. 5. Southern gel analysis of *SalI*-digested DNA from strains carrying F' factors which cover the *leu-proA* region. ^{32}P-labeled 23S rRNA was used as a probe, and autoradiograms are shown. F104 is a positive control carrying *rrnH* on the plasmid (Table 2). Other F' factors were present in merodiploid strains derived from KL251 (see Table 3). The arrow indicates the position of a novel band in lane 2.

increased intensity (Fig. 4, lane 1). The plasmid F104 extends from 98 min through 6 min, but does not cover the chromosome location proposed for *rrnF*. A similar increase in the intensity of the band was observed with F144, but not with F128, F254, and F101 (Table 2), suggesting that the map location of the newly found *rrn* operon is between *leu* and *proA*. Thus, all seven *rrn* operons revealed by the Southern gel analysis and by the isolated plasmid and transducing phages have been accounted for by *rrn* operons at chromosomal locations different from the location proposed for *rrnF*. We conclude that the *E. coli* K-12 strains we analyzed do not carry an *rrn* operon corresponding to *rrnF*, and that the group I *rrn* operon isolated and characterized in our previous studies is located in the *leu-proA* region. We proposed to call this newly localized *rrn* operon *rrnH*.

Further analysis of *rrnH* by using newly constructed merodiploids and the orientation of *rrnH*. Various F' merodiploids were constructed by mating an Hfr strain with *recA* mutant by the method of Low (22). The Hfr strains used transfer *proA* and *B* at 6 min as a leading marker, followed by other markers counterclockwise. Several new merodiploid strains were then analyzed for genetic markers and the presence of *rrnH* on the F' factors. As shown in Fig. 5 and Table 3, the plasmids F615 and F616 (Fig. 5, lanes 7 and 8), which do not extend to *nadC* (or *leuB*), do carry *rrnH*. This indicates that *rrnH* maps clockwise to *nadC*. Additional useful information on *rrnH* was obtained with F610, which carried *lacZ* and *proA*, but not *metD*. In this case, a novel *SalI* fragment (5.8 kb) slightly larger than the standard 5.6-kb *rrlH* fragment was observed in the Southern gel analysis (Fig. 5, lane 2). Our interpretation is that the chromosomal DNA in F610 terminated within the 5.6-kb *SalI rrl* fragment, resulting in the fusion of that fragment to F factor DNA which does not normally flank it. If the orientation of *rrnH* is the same as that of *rrnC*, *A*, *B*, and *E* (clockwise orientation), as suggested by the analysis of chromosomal duplications using this locus (1), that is, if *rrlH* is clockwise to *rrsH*, the above interpretation would predict that the 16S rRNA gene is absent on the F610 plasmid. This prediction was tested by analyzing unique *SalI* fragments by using the Southern gel technique with ^{32}P-labeled 16S rRNA as a probe. As shown in Fig. 6, comparison of the F615 sample (lane 3) with a control plasmid without *rrnH* (F611, lane 1) indicates that the 2.6-kb fragment is the unique *SalI* fragment carrying the proximal por-

TABLE 3. *rrnH* and other genetic markers carried by F' factors in *leu-pro* region[a]

F' factor	Genes carried by plasmids							*rrnH* fragment
	leuB	*nadC*	*tonA*	*dapD*	*metD*	*proA*	*lacZ*	
F610	−	−	−	−	−	+	+	Novel fragment
F611	−	−	−	−	−	+	+	−
F612	−	−	−	−	−	+	+	−
F613	+	+	+	+	+	+	+	+
F614	−	−	−	−	−	+	+	−
F615	−	−	+	+	+	+	+	+
F616	−	−	+	+	+	+	+	+

[a] F' factors were constructed by interrupted mating of the Hfr P4X (or P804 for F614) with the *recA* mutant KL251 which is also *leuB*$^-$ *tonA*$^-$ *lacZ*$^-$ *proC*$^-$, and selecting for recipients which received *proC*$^+$. The resulting merodiploids were screened for the presence of the following markers on the plasmids: *leuB*, *nadC*, *tonA*, *dapD*, *proA*, and *lacZ* (these markers map in this order). *leuB*, *tonA*, and *lacZ* were screened directly. The presence of *nadC*$^+$ was determined by mating the merodiploids with W4546, which is *nadC*$^-$; the presence of *dapD*$^+$ was determined by mating with H5546, which is *dapD*$^-$; and the presence of *metD*$^+$ and *proA*$^+$ was determined by mating with NO2494, which is *metD*$^-$ *proA*$^-$. Screening for *rrnH* is shown in Fig. 5.

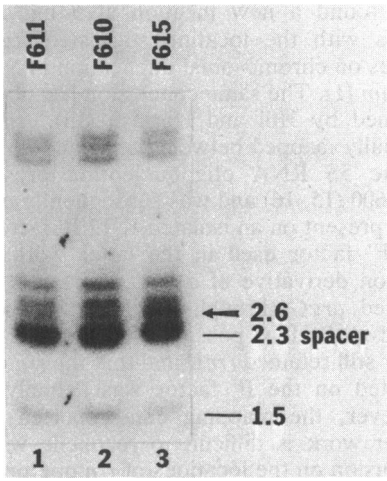

FIG. 6. Southern gel analysis of *Sal*I-digested DNA isolated from merodiploids by using ^{32}P-labeled 16S rRNA as a probe. An autoradiogram is shown. Numbers on the right represent fragment sizes in kb.

tion of the *rrs* gene from *rrnH*. F610 did not manifest any increase in intensity of this or any other band (Fig. 6; lane 2), indicating the absence of the *rrs* gene on this plasmid. We conclude that the orientation of *rrnH* is clockwise. In addition, since the F610 plasmid does not carry *metD* and other genes counterclockwise to *metD*, *rrnH* must be located between *metD* and *proA*.

Mapping of *rrnH* by P1 transduction. We first inserted the ampicillin-resistant gene (Ap^r) near the chromosomal *rrnH* locus by the method of Greener and Hill (12) and then mapped the Ap^r gene by P1 transduction to infer the location of *rrnH* on the chromosome. It is known that in a *polA* host, colE1-derived plasmids cannot be maintained, since they are dependent upon DNA polymerase I for their replication (18). However, if a colE1-derived plasmid contains DNA homologous to any portion of the genome, it can integrate into the chromosome by homologous recombination and thus circumvent its requirement for the *polA* gene product (12). We purified the 3-kb *Eco*RI fragment (labeled B in Fig. 7) from pLC7-21, a colE1 hybrid plasmid which belongs to group I (17) and hence carries *rrnH*. The fragment was cloned (fortuitously together with fragment A containing a part of *rrnH*; Fig. 7) into the *Eco*RI site of pBR322, a colE1-derived vector which carries the Ap^r gene. A *polA* mutant, CH931, was then transformed with the resulting hybrid plasmid (pNO1530), and Ap^r transformants were selected. Seven transformants were obtained in this way, whereas transformation with pBR322 yielded no Ap^r transformants, suggesting that the cloned DNA provided a means for integration of pNO1530 into the host chromosome. Preliminary mapping of the Ap^r in the transformants by interrupted matings indicated that the Ap^r was near *proA* in all cases and therefore that the integration of pNO1530 into the chromosome presumably took place at or near *rrnH* (Fig. 7).

P1 lysates were prepared from each of the transformants, and transduction was carried out by using CD4 (*metD proA*) as a recipient. *metD*$^+$ transductants were selected, and cotransduction of Ap^r was analyzed. Cotransduction was observed in every case, and its frequency was on the average 60% (ranging from 42 to 87%; data not shown). In the same experiments, *proA* was cotransducible with *metD* at only about 20%, indicating that the site of Ap^r lies counterclockwise to *proA* and near *metD* on the standard *E. coli* map.

To determine upon which side of *metD* the Ap^r marker lies, three-factor transduction experiments were performed (Table 4). NO2488, a

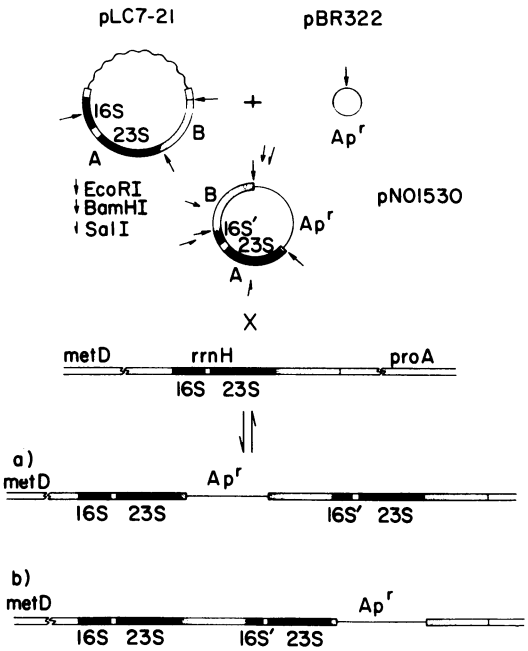

FIG. 7. Construction of strains bearing an ampicillin resistance (Ap^r) marker linked to *rrnH*. Symbols: ■, *rrn* operons; □, non-ribosomal *E. coli* DNA; —, pBR322; and ∿, ColE1 DNA in pLC7-21. Stippling indicates the natural orientation of the 3-kb *Eco*RI fragment immediately distal to *rrnH* (fragment B), as it exists in pLC7-21. Cloning of this purified fragment into pBR322 yielded a clone (pNO1530) which also contains the distal *Eco*RI fragment from *rrnH* (fragment A). Integration of pNO1530 into CH931, the *polA* recipient, could result in either of two configurations, depending upon whether the recombination event took place within fragment A (a) or fragment B (b).

TABLE 4. Mapping of the ampicillin resistant gene (Ap^r) integrated at *rrnH* by transduction (P1 donor, *tonA metD*$^+$ Ap^r; recipient, *tonA*$^+$ *metD* Ap^s)[a]

Expt no.	Selected marker	No.	Unselected marker	No.	%
1	Ap^r	100	*tonA*$^+$ *metD*	23	23
			tonA metD	1	1
			tonA$^+$ *metD*$^+$	48	48
			tonA metD$^+$	28	28
2	*metD*$^+$	89	*tonA*$^+$ Ap^s	38	42
			tonA$^+$ Ap^r	18	20
			tonA Ap^s	14	15
			tonA Ap^r	21	23

[a] NO2488, used as a P1 donor, is a *tonA* derivative of NO2483, which carries pNO1530 integrated near or at *rrnH* and hence is ampicillin resistant. The recipient was CD4 which is *tonA*$^+$ *metD*$^-$ and does not carry pNO1530. We note that in the transduction experiments described above, recipients used were *polA*$^+$. Gel electrophoretic analysis of DNAs prepared from Ap^r transductants revealed that they each contained a plasmid indistinguishable from pNO1530, whereas transductants selected by nutritional markers which were Ap^s contained no plasmid (data not shown). This observation indicates that the entire plasmid was in fact integrated into the chromosome in the original *polA* mutant at a site near *metD*, presumably as in Fig. 7, and supports the validity of mapping of *rrnH* by using the Ap^r marker.

tonA derivative of one of the transformants, was used as a donor. It can be seen that the data are consistent with the order of *tonA-metD*-Ap^r, but not with the order of *tonA*-Ap^r-*metD*. Thus, the conclusion obtained from P1 transduction experiments agrees with that obtained from the Southern gel analysis of the F610 merodiploid strain described above, namely, that *rrnH* is located between *metD* and *proA*.

DISCUSSION

We have failed to find an *rrn* operon at the location of *rrnF*, which was reported to map between *aroB* and *malA* (15, 16, 36). Instead, we have found a new location at 5 min, which agrees with the location suggested from the studies on chromosomal duplication in *S. typhimurium* (1). The same conclusion has now been obtained by Hill and Harnish (13). *rrnF* was originally mapped between *malA* and *aroB* as a unique 5S RNA oligonucleotide present in MRE600 (15, 16) and was subsequently claimed to be present on an isolated K-12 F′ factor (36). The F′ factor used in the latter work was a deletion derivative of a larger F′ factor which covered *argG* through *malA* and hence contained *rrnD*. It is possible that the deletion F′ factor still retained *rrnD* and that the *rrn* operon detected on the F′ factor was actually *rrnD*. However, the mapping data reported in the former work is difficult to reconcile with our conclusion on the locations of *rrn* operons in *E. coli* K-12. We cannot exclude the possibility that the strain MRE600 used by Jarry and Rosset (15, 16) actually carried *rrnF* at the location reported.

The present study completes mapping of all of the seven *rrn* operons in *E. coli* K-12. Table 5 is a summary of our current knowledge on the seven *rrn* operons. Genes for spacer tRNA's and distal tRNA's were previously identified and studied on isolated *rrn* operons, but chromosomal locations for some of these genes remained undetermined (14, 27, 28). Completion of the mapping of *rrn* operons allows us to locate all of these tRNA genes on the chromosome. For example, a gene for $tRNA_1^{Asp}$ identified on the group I plasmid (14, 28) can now be placed at 5 min.

The present study has also clarified the orientation of *rrnG* and *rrnH* and demonstrates that the direction of transcription of all the *rrn* operons is identical to that of DNA replication. It is possible, as previously speculated (31), that this striking chromosomal organization has evolved to prevent collisions of the DNA replication machinery with actively transcribing RNA polymerase molecules on the *rrn* operons.

TABLE 5. Chromosomal locations and encoded tRNA genes of rRNA operons in *E. coli* K-12

rRNA operon	Chromosomal location (min)	Orientation[a]	Spacer tRNA gene[b]	Distal tRNA gene[b]	Isolated plasmids[b]
rrnA	86	+	*ile, ala*		Group III
rrnB	89	+	*glu*		Group IV
rrnC	84	+	*glu*	*asp, trp*	Group VI
rrnD	71	−	*ile, ala*	*thr*	Group II
rrnE	90	+	*glu*		(λ*metA*20)
rrnG	56	−	*glu*		Group V
rrnH	5	+	*ile, ala*	*asp*	Group I

[a] +, Clockwise; −, counterclockwise.

[b] Hybrid plasmids carrying *rrn* operons were characterized and classified into six groups in the previous studies (17). Spacer tRNA genes and distal tRNA genes listed are based on the studies on these isolated hybrid plasmids and λ*metA*20 (8, 14, 24, 27, 28). *ile*, *ala*, *glu*, *asp*, *trp*, and *thr* refer to the genes for $tRNA_1^{Ile}$, $tRNA_{1B}^{Ala}$, $tRNA_2^{Glu}$, $tRNA_1^{Asp}$, $tRNA^{Trp}$, and $tRNA^{Thr}$, respectively.

Since all the *rrn* operons are now mapped, one can calculate growth rate-dependent changes in the copy number of each operon in exponentially growing cells by using equations developed by previous workers (4, 5). We first note that the total number of rRNA genes per unit amount of DNA (or protein) in the population increases only slightly with growth rates. Assuming that 40 min is required to complete one round of chromosomal replication (5), one can calculate that the total rRNA gene dosage increases only about 20% from $\mu = 0.9$ to $\mu = 2.7$ (doublings per hour) when the rRNA synthesis rate per unit amount of DNA increases about 10-fold over the same range of μ value (20, 25). Clearly, the change in the relative gene dosage is insufficient to account for the growth rate dependent increase in rRNA synthesis rate. On the other hand, the ratio of the number of replication origin-proximal operons (such as *rrnC*) to that of replication origin-distal operons (such as *rrnG* or *H*) changes significantly. Calculation shows that the ratio of *rrnC* to *rrnG* will increase about 60% from $\mu = 0.9$ to $\mu = 2.7$ (doublings per hour). Thus, the copy number of the gene for $tRNA^{Trp}$ which is unique to *rrnC* is expected to be relatively high in cells growing in rich media. Questions about whether such variation in the relative frequency of each operon is in fact reflected in the relative abundance of unique tRNA's (such as $tRNA^{Trp}$) and, if so, whether the variation has any physiological significance are presently unanswered. Equally unknown are the effects on cellular physiology of altered chromosomal organization as a result of unequal crossing-over at the *rrn* operons (C. W. Hill and B. W. Harnish, Proc. Natl. Acad. Sci. U.S.A., in press). Completion of mapping of the *rrn* operons does not give answers to the regulation of rRNA synthesis, but it does provide a basis for designing and interpreting experiments related to the *rrn* operons such as those mentioned above.

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