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The Gene for Ribosomal Protein L31, *rpmE*, Is Located at 88.5 Minutes on the *Escherichia coli* Chromosomal Linkage Map

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Two mutations resulting in an alteration in large-subunit ribosomal protein L31 were mapped at around 88.5 min on the *Escherichia coli* chromosomal linkage map. They were located between *metB* and *argH* and cotransduced over 90% with *metB*. These mutations were shown to define the structural gene of protein L31, *rpmE*.

The location on the *Escherichia coli* chromosomal linkage map of the genes for all but 5 of the 53 ribosomal proteins is now known (1, 8). A gene not yet identified is *rpmE*, the structural gene for ribosomal protein L31. One approach to the mapping of ribosomal protein genes has been to isolate mutants with alterations in proteins which can be detected on two-dimensional gels. The mutations responsible for these alterations can then be mapped. The application of this technique in the case of protein L31 has been hampered by the fact that this protein frequently cannot be detected on two-dimensional gels, which may explain why no mutants with alterations in this protein have been heretofore found.

I observed that, after ribosomes were washed in buffers containing 1 M ammonium chloride, protein L31 could always be detected on gels of ribosomal proteins. Therefore, ribosomes of 100 strains previously isolated by nitrosoguanidine mutagenesis in a selection producing mutants with alterations in many ribosomal proteins (4) were washed in buffer containing 1 M NH_4Cl (for details of buffers and techniques, see reference 6). Ribosomal proteins were extracted from ribosomes so treated and analyzed on two-dimensional gels. The gels of two mutants, VT587 and VT628, showed a reproducible alteration in protein L31. The type of alteration in each mutant was similar, although the two had independent mutational origins; the gel pattern of ribosomal proteins of mutant VT628 is shown in Fig. 1b. As can be seen, in comparison with the wild type (Fig. 1a), protein L31 of the mutant was less basic. Several other spots in the same region of the gel also appear to be shifted in the mutant strain in comparison with the wild type; the reason for this will be discussed later. Strain VT587 did not grow on minimal medium supplemented with methionine, in contrast to its parental strain, VT, and the additional auxotrophy could not be eliminated by transduction or selecting revertants. In contrast, strain VT628 grew well on minimal medium supplemented with methionine and was used for mapping the mutation responsible for the alteration in protein L31. The mutations in strains VT587 and VT628 were termed *rpmE1* and *rpmE2*.

Strain VT was isolated from A19 originally for nongenetic purposes. Therefore, it and its derivatives have the disadvantage of lacking auxotrophic markers useful for mapping. Such markers were introduced into mutant VT628, either by auxotroph enrichment techniques with a mixture of ampicillin and D-cycloserine (6) or by transduction. Strains used in this work are listed in Table 1. One of the derivatives of mutant VT628 obtained was VT628-1 (*rpmE2 metB thr ilv argH aroE nalA rpsE rpoB*), which was used in subsequent genetic work.

A19, as originally isolated (7), was an Hfr strain. However, mutant VT and its derivatives had lost the ability to transfer markers while retaining the surface exclusion properties of an Hfr strain. Therefore, when strain VT628-1 was to be used as the recipient in a mating, it was converted into a female phenocopy by growing to stationary phase and then transferring to buffer and shaking for a further 6 h at 37°C.

Approximate localization of the *rpmE* gene was made by crossing mutant VT628-1 with Hfr strains. Strains KL14 (PO68, at about 66 min and introducing markers clockwise) and KL209 (PO18, at about 91 min and introducing markers counterclockwise) were used. Matings with these strains indicated that *rpmE* was located between *ilv* at 84 min and *arg* at 89 min and was probably nearer the latter than the former. Transduction experiments were therefore made to pinpoint the gene ribosomal protein L31. A derivative of strain VT628-1, VT628-2, in which mutations deleterious for growth had been crossed out, was used in subsequent experiments.

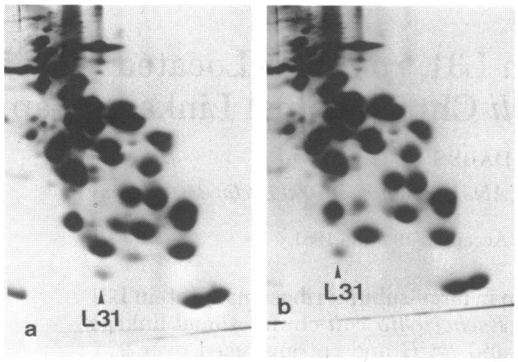

FIG. 1. *Two-dimensional electrophoregrams of 70S ribosomal proteins. (a) A19 (wild-type rpmE⁺); (b) VT628 (rpmE2). The gels are overloaded so that the normally faint spot of protein L31 (indicated by arrow) is clearly visualized.*

TABLE 1. *Strains used in this work*

Strain	Relevant phenotype/genotype	Source/reference
VT587[a]	Hfr *rpmE1 metB1 rna-19*	4
VT628	Hfr *rpmE2 metB1 rna-19*	4
VT628-1[b]	Hfr *rpmE2 rpsE metB1 aroE24 argH46 ilv thr rpoB nalA rna-19*	This work
AM10	Hfr *rpmD10 metB1 rna-19*	5
AT2472	Hfr *aroE24 thi-1 relA1*	L. Gorini
CP78	F⁻ *thr-1 leuB6 his-65 argH46 thi-1*	K. Isono
JC411	F⁻ *leu-6 his-1 argG6 metB1*	K. Isono
JC5072	Hfr *thr-300 ilv-318 spc-300 recA67*	K. Isono
JG108	F⁻ *rha metE thy*	W. Kelley
KL14	Hfr *thi-1 relA1*	K. Isono
KL25	Hfr	B. Bachmann
KL209	Hfr *malB16*	K. Isono

[a] The derivatives of strain A19 used in this work have kept the exclusion properties characteristic of an Hfr strain but have lost the ability to transfer markers.

[b] *aroE24* and *argH46* were introduced by transduction from AT2472 and CP78, respectively, using nearby spectinomycin resistance or rifampin resistance markers; *ilv* and *thr* were introduced by auxotroph enrichment techniques, as described in reference 6; and *nalA* was introduced by spontaneous selection for resistance to 50 μg of nalidixic acid per ml.

I transduced the *metB* mutation present in strain A19 and its derivatives. When a lysate of strain KL209 was used as the donor of *metB*⁺, 20 of 22 transductants also acquired the wild-type form of protein L31. Therefore, the *rpmE* gene was close to *metB*. This was confirmed by using a *metB*⁺ *rpmE* transductant as the donor of *metB*⁺ to *metB rpmE*⁺ strain JC411; 32 of 33 *metB*⁺ transductants had acquired the *rpmE* mutation of strain VT628-2. Overall, 52 of 55 *metB*⁺ transductants (95%) acquired the form of protein L31 of the donor strain (see Fig. 2).

Three-point crosses were made to determine on which side of *metB* the *rpmE* gene was located. An *rpmE argH metB*⁺ derivative of strain VT628-2 was used as the recipient, with a P1 lysate of strain JC411 used as the donor of *argH*⁺; transductants were scored for the methionine phenotype, and two-dimensional gels of ribosomal proteins were analyzed (Table 2).

This experiment confirmed that *rpmE* was close to *metB*. It also indicated that *rpmE* was nearer the *argECBH* cluster than was *metB*; 17 of 79 (22%) *argH*⁺ transductants were *metB*, and 18 of 79 (23%) were *rpmE*⁺. The same conclusion was reached when VT628-2 (*rpmE argH metB*) was used as the recipient and KL209 was the donor of *argH*⁺ (Table 2). Of 80 transductants, 32 (40%) were *metB*⁺ and 33 (41%) were *rpmE*⁺ (Fig. 2). A P1 lysate of strain JG108 (*metB*⁺ *argH*⁺ *rha*) was also used as the donor of *metB*⁺ into strain VT628-2. In agreement with the order *rha-metB-rpmE-argH*, all *metB*⁺ *argH*⁺ transductants were *rpmE*⁺ (20 of 20), but 4 of 20 *rha metB*⁺ transductants were still *rpmE*. A rifampin-resistant mutant of strain CP78 was used as the donor of *rpoB* into strain VT628. Of 11 rifampin-resistant transductants, 2 (18%) were *rpmE*⁺ (Fig. 2).

TABLE 2. *P1-mediated crosses between rpmE, metB, and argH loci*

Cross	Selected marker (no. scored)	Transductant characteristics of: *metB*	*rpmE*	*argH*	No. of transductants (% of total)
metB1 rpmE⁺	*argH*⁺	+	+	+	1 (1)
argH⁺ (donor) ×	(79)	−	+	+	17 (22)
metB⁺ *rpmE2*		+	−	+	61 (77)
argH46 (recipient)		−	−	+	0 (0)
metB⁺ *rpmE*⁺	*argH*⁺	+	+	+	32 (40)
argH⁺ (donor) ×	(80)	−	+	+	1 (1)
metB1 rpmE2		+	−	+	0 (0)
argH46 (recipient)		−	−	+	47 (59)

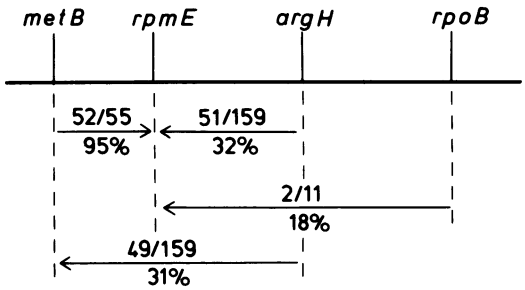

FIG. 2. *Map of region of E. coli chromosomal linkage map around rpmE as determined by P1 transduction experiments described in this work. Arrow indicates marker being scored.*

Once the *rpmE* locus was identified, transductions were also done with mutant VT587. A lysate of this strain was used as the donor of *argH*$^+$ into strain CP78. The pattern of segregation of *metB* and the mutation responsible for the alteration in protein L31 pointed to a very similar location for the mutation in both strains with altered L31.

The position of the spot corresponding to ribosomal protein L31 on two-dimensional gels is indicated in Fig. 1a and b. Enlargements of the pertinent regions are shown in Fig. 3a and b. Also arrowed in Fig. 3a is another spot to the right of the spot of protein L30 which was invariably observed in electropherograms of ribosomal proteins from transductants with wild-type protein L31 and invariably not observed in electropherograms of ribosomal proteins from transductants with altered protein L31. If this spot also represented protein L31, then, were it changed in migration to the same degree as the spot definitely established as protein L31, it would coincide with the spot of protein L30.

A mutant apparently lacking protein L30, AM10, has been isolated (5). The lesion responsible for this absence probably maps in the gene for L30, *rpmD* (unpublished data). Derivatives of strain CP78 were constructed which possessed this mutation, *rpmD10*, together with either wild-type or mutant loci of *rpmE*. Electropherograms of ribosomal proteins of these two strains are shown in Fig. 3c and d; from these, it was observed that the second spot was also shifted to the degree corresponding with the spot previously identified as protein L31. Since the shift of the two spots went in tandem in all of the several hundred transductants analyzed by two-dimensional gels, it was very likely that both represented protein L31.

Furthermore, inspection of these and other gels revealed that a spot located to the right of and below the spot of protein L29 (Fig. 3c) in strains with wild-type protein L31 was located to the left of and below the L29 spot in strains with altered protein L31 (Fig. 3d). A fourth spot, located to the left of the spot of protein L27 (Fig. 3c), was shifted further to the left in all gels of strains with altered protein L31 (Fig. 3d). Since no gels of transductants were observed with only some of the four spots (which together produced a lozenge configuration listing slightly to the right) shifted, it was very likely that all spots represented forms of protein L31.

This unusual behavior can be explained in terms of the amino acid sequence of protein L31 (2): although the protein has only 62 amino acid residues, it has two cystine bridges. With both bridges intact, the molecule would be very compact. Breakage of one or both bridges would result in a much less compact structure under the denaturing environment prevailing in the gels used and, hence, a significantly different behavior under the sieving conditions of polyacrylamide.

To demonstrate that the mutations causing alterations in protein L31 were in the structural gene *rpmE* rather than in the modifier or processing gene, merodiploids covering the putative *rpmE* gene were constructed. *rpmE rpmD10 metB* strains were made *recA* as previously described (6) and crossed with Hfr KL209. Nalidixic acid was included in plates to counterselect against the Hfr strain. *metB*$^+$ merodiploids were grown on minimal medium containing the necessary supplements, and two-dimensional gels of ribosomal proteins were made. Merodiploids showed double spots of mutant plus the wild-type form of protein L31 (cf. Fig. 3e and Fig. 3c and d). Curing of the episome resulted in strains with only an altered form of protein L31. Therefore, it was very likely that the mutations which had been mapped defined the structural gene for protein L31, *rpmE*.

The *rpmE* gene is about 0.5 min counterclockwise from *argECBH* on the *E. coli* chromosomal linkage map, about the same distance as is (clockwise) the Rif cluster of genes coding for elements of the transcriptional and translational

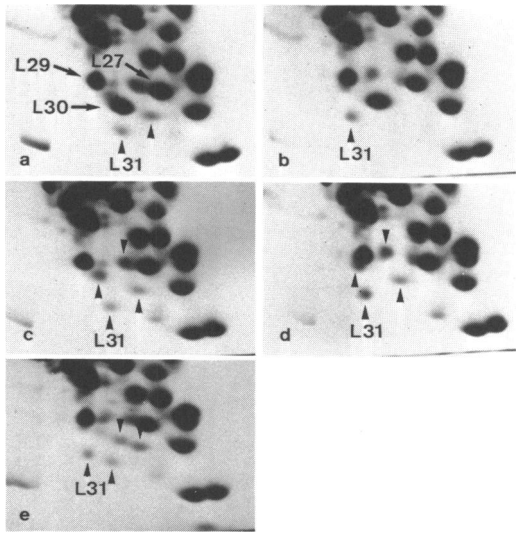

FIG. 3. *Portions of two-dimensional electropherograms of 70S ribosomal proteins of (a) A19 (rpmE*$^+$*), (b) VT628 (rpmE2), (c) CP78 rpmD10 rpmE*$^+$*, (d) CP78 rpmD10 rmpE2, and (e) rpmE2 rpmE*$^+$ *merodiploid of strain CP78 rpmD10 recA67. The spot corresponding to protein L31 is indicated, and other spots identified in this work as being L31 are shown by arrows. The spots of other proteins referred to in this work are also identified.*

machinery. There is evidence that the gene coding for protein L31 may likewise be one of a group coding for ribosomal functions, since a mutation affecting the thermolability of the 50S subunit, *rit*, has been mapped between *metB* and *argH* (9). Also, a mutation affecting maturation of the 50S subunit, *rimD*, has been localized in this region (3).

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