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The Gene for Ribosomal Protein L13, *rplM*, Is Located Near *argR*, at About 70 Minutes on the *Escherichia coli* Chromosomal Linkage Map

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Mutants of *Escherichia coli* with alterations in the electrophoretic mobility of ribosomal protein L13 were used to locate *rplM*, the gene for this protein, on the chromosomal linkage map. *rplM* was situated between *gltB* and *argR*, at about 70 min.

The map position for most ribosomal protein genes has now been established for *Escherichia coli* (1). In addition, *rpmE*, the gene for protein L31, has recently been located on the chromosomal linkage map (4). *rplM*, the gene for ribosomal protein L13, has not yet been identified. The mapping of this gene could be done by using mutants with electrophoretically altered protein L13. Such mutants have been obtained among those isolated in a selection giving rise to strains with alterations in any of the ribosomal proteins (2, 5, unpublished data). These strains were VT12, VT385, VT523, and VT685.

The parental strain of these mutants, VT, was originally isolated not for mapping purposes, and VT and its derivatives have two disadvantages for mapping. They are derived from strain A19 and hence have only *metB* as a selective marker. Also, strain VT and its derivatives have lost the ability to transfer markers while retaining the surface exclusion properties of strain A19. The combination of very slow growth of the four VT mutants with altered protein L13, together with the retention of surface exclusion properties, made it impracticable to use these strains as recipients in crosses with Hfr strains.

Therefore, I decided to develop a selection giving rise to strains with mutational alterations in many different ribosomal proteins, using F^- strain TA10 (Table 1). I screened streptomycin-dependent mutants isolated from strain TA10 and chose mutant KM because it resembled strain VT in phenotype. Like strain VT, mutant KM gave rise to antibiotic-independent revertants with alterations in various ribosomal proteins. The ribosomal protein patterns of 40 revertants were analyzed on two-dimensional gels (3). Two revertants, KM25 and KM33, showed reproducible alterations in electrophoretic mobility of protein L13. The mutation in mutant KM25, which resulted in a protein that migrated faster toward the cathode in the first dimension than did the wild type and which was therefore more basic than the wild type (Fig. 1a and b), was mapped.

Hfr crosses indicated that the lesion was between the points of origin of strains KL14 (PO68) and KL228 (PO13), i.e., between 66 and 83 min on the *E. coli* chromosomal linkage map. An $argH^+$ recombinant of mutant KM25 was rendered *argG* by auxotroph enrichment with ampicillin and cycloserine as previously described (3). This strain was termed KM25-1. Selection of $argG^+$ or $aroE^+$ in crosses with Hfr strains showed that the mutation responsible for the alteration in protein L13 was probably between these two markers and nearer *argG*.

Transductions were made with phage P1*vir*. When strain TA10 was used as the donor of $argG^+$ into strain KM25-1, 5 of 40 transductants were $rplM^+$. An $argG^+rplM$ recombinant from this cross was used as the donor of $argG^+$ into strain JC411 (Table 1), and 2 of 20 transductants acquired the *rplM* mutation. Therefore, *argG* and *rplM* were about 10% cotransducible. Since the genes for ribosomal proteins S15, L21, and L27 are also near *argG* (7), albeit contransducing with the arginine marker at a frequency much higher than 10%, I decided to first exclude the possibility that *rplM* was near *rpsO*, *rplU*, or *rpmA*. Lysates of strain VT254 (*rpmA*) or VT449 (*rpsO*) (2) were used as donors of $argG^+$ into strain KM25-1. With VT254 as donor, 16 of 20 transductants were *rpmA* but only 2 were $rplM^+$. With strain VT449 as donor, 17 of 18 transductants were *rpsO*, whereas 2 were $rplM^+$. I concluded from these experiments that *rplM* was well separated from *rpsO*, *rplU*, and *rpmA*. In transductions with *uxaA* strain JR27, no linkage between *rplM* and *uxaA* was observed.

About 0.4 min away from *argG* toward *argR* is *gltB* (1). In strain PA340, which also has a *gdh* mutation mapping near *trp*, the *gltB* mutation

TABLE 1. Strains used

Strain	Phenotype and genotype	Source or reference
AT2472	Hfr *aroE24 thi-1 relA1*	L. Gorini
CP78	F⁻ *thr-1 leuB6 his-65 argH46 thi-1 gal-3 malA1 xyl-7 mtl-2 tonA2 supE44*	K. Isono
JC411	F⁻ *leu-6 his-1 argG6 metB1 lacY1 gal-6 xyl-7 mtl-2 malA1 rpsL104 tonA2 supE44*	K. Isono
JC5072	Hfr *thr-300 ilv-318 spc-300 recA67*	K. Isono
JR27	F⁻ *uxaA metC his thyA rpsL*	K. Isono
KL14	Hfr *thi-1 relA1*	K. Isono
KL228	Hfr *thi-1 leu-6 gal-6 lacY1 supE44*	K. Isono
MA1030	Hfr *argR64 thi-1 purF1*	B. Bachmann
PA340	F⁻ *thr-1 leuB6 gdh-1 hisG1 gltB31 argH1 thi-1 ara-14 lac-Y1 gal-6 malA1 xyl-7 mtl-2 rpsL9 tonA2*	B. Bachmann
TA10[a]	Same as CP78, *aroE24 rpsE nalA*	This work
KM[b]	Same as TA10, *rpsL*	This work
KM25	Same as KM, *rplM1*	This work
KM33	Same as KM, *rplM2*	This work
VT12[b]	Hfr[c] *metB1 relA1 rna-19 rpsL rplM3*	5
VT254	Hfr *metB1 relA1 rna-19 rpsL rpmA*	2
VT385	Hfr *metB1 relA1 rna-19 rpsL rplM4*	2
VT449	Hfr *metB1 relA1 rna-19 rpsL rpsO*	2
VT523	Hfr *metB1 relA1 rna-19 rpsL rplM5*	This work
VT685	Hfr *metB1 relA1 rna-19 rpsL rplM6*	This work

[a] *nalA* was obtained by spontaneous selection; *aroE24* was transduced in from a spectinomycin-resistant (*rpsE*) derivative of strain AT2472.

[b] The *rpsL* alleles in KM and VT strains are streptomycin-dependent.

[c] VT strains used in this work have lost the ability to transfer markers but have kept the surface exclusion properties characteristic of an Hfr strain.

causes a requirement for aspartic acid (8). An *argH*⁺*argG* derivative of strain PA340 was selected as previously described (3). Strain KM25 was used as the donor of *argG*⁺ into this derivative of strain PA340, and transductants were scored for the Asp phenotype and the presence of altered protein L13 (Table 2, cross 1). *rplM* was further from *argG* than was *gltB* (14 versus 38% cotransduction).

Strains with markers between *gltB* and *argR* were either unavailable or failed to show the expected phenotype, so further mapping was done by using *argR* strain MA1030 (Table 1). A P1 lysate of strain MA1030 was used to select *argG*⁺ into strain KM25-1, and transductants were tested for their ability to grow on 100 μg of canavanine per ml, as described previously (6), to score for *argR*. They were also checked for *rplM* (Table 2, cross 2). Of 48 *argG*⁺ recombinants, 3 were *argR* and 5 were *rplM*⁺. The three *argR* recombinants were also *rplM*⁺, which would have been very unlikely if these two loci were on opposite sides of *argG*. Cross 2 indicated that the gene order was *argG-rplM-argR*. Using this information with the data from cross 1, I determined the probable gene order to be *argG-gltB-rplM-argR*.

No selectable markers beyond *argR* were available, so to confirm this gene order, I selected for canavanine resistance itself. This was possible with an *argH*⁺ derivative of strain KM25 as the recipient, when 200 μg of canavanine per ml was in selective plates and incubation was at 40°C. Under these conditions, *argR* transductants could be obtained at a much higher frequency than could spontaneous canavanine-resistant mutants. With strain KM25 Arg⁺ as the recipient and strain MA1030 as the donor of *argR*, five of eight transductants tested had acquired the wild-type form of protein L13. An *rplM argR* recombinant of this cross was the donor of *argR* into an Arg⁺ derivative of strain PA340. For conditions under which transductants greatly exceeded spontaneous mutants, I selected a slow-growing derivative of strain PA340 Arg⁺ by incubating an exponentially growing culture in rich medium with an ampicillin-cycloserine mixture (3). Many of the survivors of this treatment were slow growing. The rationale for isolating a slow-growing strain was that strain KM25-1, which was effective as a recipient in an *argR* selection, only grew at about 30% the rate of strain TA10 from which it was derived (probably owing to the presence of the suppressed streptomycin-dependent mutation). A slow-growing mutant of strain PA340

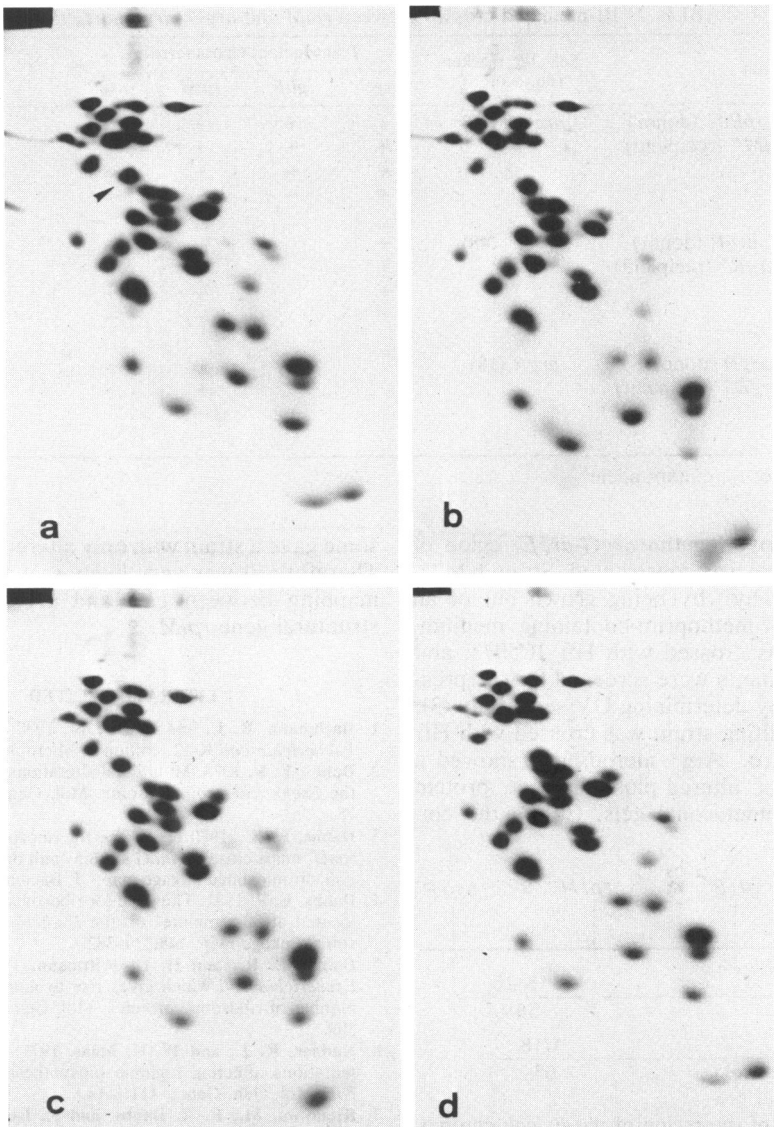

FIG. 1. Two-dimensional gel electropherograms of 70S ribosomal proteins from strains A19 (wild type, *rplM*$^+$) (a), KM25 (*rplM1*) (b), VT385 (*rplM4*) (c), and VT685 (*rplM6*) (d). The spot of protein L13 is indicated by an arrow (a). First-dimension runs were from left to right, with the cathode to the right. Second-dimension runs were from top to bottom, with the cathode on the bottom.

Arg$^+$, PA340-1, was chosen. The nature of the additional mutation was not determined, except that it was not in the *argG-argR* region of the chromosome. When *argR* was selected from an *argR rplM* strain into PA340-1, then 10 of 18 transductants were *rplM* but only 1 transductant was *gltB*$^+$ (Table 2, cross 3). The results of this cross supported the gene order *gltB-rplM-argR*. All of the above transduction data pertaining to *rplM* are summarized in Fig. 2.

Genetic experiments were also done with other mutants having alterations in protein L13, such as VT385, in which L13 was less basic than it is in the wild type (Fig. 1c), and VT685, in which L13 was even more basic than it is in strain KM25 (Fig. 1d). Strains VT12 and VT523 (data not shown) were also used. In all cases, the mutation responsible for the change in electrophoretic mobility was about 10% cotransducible with *argG*.

For confirming that the mutations defined the structural gene for ribosomal protein L13, a

TABLE 2. P1-mediated crosses between *rplM* and *argG*, *argR*, or *gltB*

Cross	Selected marker (no. scored)	Transductant characteristic[a]				No. of transductants (% total)
		argG	*gltB*	*rplM*	*argR*	
1. *argG*$^+$ *gltB*$^+$ *rplM1* (donor)	*argG*$^+$ (29)	+	+	+		7 (24)
argG gltB rplM$^+$ (recipient)		+	+	−		4 (14)
		+	−	+		18 (62)
		+	−	−		0 (0)
2. *argG*$^+$ *rplM*$^+$ *argR* (donor)	*argG*$^+$ (48)	+		+	+	2 (4)
argG rplM1 argR$^+$ (recipient)		+		+	−	3 (6)
		+		−	+	43 (90)
		+		−	−	0 (0)
3. *gltB*$^+$ *rplM1 argR* (donor)	*argR* (18)		+	+	−	0 (0)
gltB rplM$^+$ *argR*$^+$ (recipient)			−	+	−	8 (44)
			+	−	−	1 (6)
			−	−	−	9 (50)

[a] +, Wild type; −, mutant allele.

merodiploid covering the *argG-aroE* region of the chromosome was constructed. Strain KM25-1 was made Thy$^-$ by being grown out of an inoculum in trimethoprim-containing medium. This strain was crossed with Hfr JC5072, and Thy$^+$ recombinants were screened for the presence of *recA* by determining UV sensitivity (3). When the resulting strain was crossed with Hfr KL228, the Aro$^+$ Arg$^+$ merodiploid showed a double spot of altered-plus-wild-type protein L13 on two-dimensional gels. Curing the episome gave a strain with only altered protein L13. Therefore, it was very likely that the mutations mapping between *gltB* and *argR* defined the structural gene *rplM*.

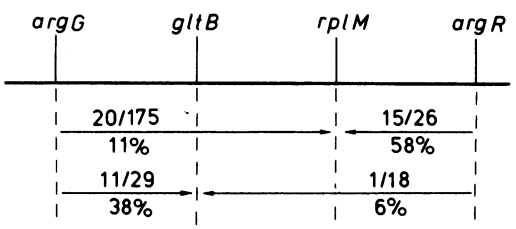

FIG. 2. Map of the region of the *E. coli* chromosomal linkage map around *rplM*. Data are summarized from all P1 transduction experiments involving the *rplM1* allele of the *rplM* locus. Arrows point to the marker being scored.

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