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A Pair of *Bacillus subtilis* Ribosomal Protein Genes Mapping Outside the Principal Ribosomal Protein Cluster

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Before now, the only ribosomal protein gene loci to be identified in *Bacillus subtilis* map within the principal ribosomal protein gene cluster at about 10° on the linkage map. Using mutants with alterations in large subunit ribosomal proteins L20 or L24, I mapped the corresponding genes near *leuA* at approximately 240°. The data were fully consistent with the fact that the genes for the two proteins were close together but not near any other ribosomal protein genes, as is also the case with the genes for the corresponding proteins of *Escherichia coli*.

Knowledge of the organization of the genes for ribosomal proteins in *Escherichia coli* is nearly complete (1). However, the reason for the organization which is observed remains unclear. There are four transcriptional units of these genes that are close together and that comprise the principal (Str-Spc) cluster. The rest of the genes are scattered around the chromosome, mostly in ones and twos, except for the Rif cluster. Within transcriptional units, genes for proteins of the large and small ribosomal subunits are often mixed together in a manner which also has no obvious explanation.

One way to increase our understanding of the arrangement found in *E. coli* is to adopt a comparative approach. The organism of choice in such a comparison is *Bacillus subtilis*, in which genetic analysis is readily undertaken by using transformation and one of several generalized transducing phages. Moreover, as a spore-forming, gram-positive bacterium, it is evolutionarily well separated from *E. coli*. The chromosome of *B. subtilis* is similar in size to that of *E. coli*, but the overall arrangement of loci on the linkage map is very different (6). Therefore, if similarities in gene organization were observed between the two organisms, this would strongly suggest that major constraints exist with respect to the arrangement of these genes.

In *B. subtilis*, as in *E. coli*, there is a principal cluster of ribosomal protein genes. This has been delineated on the basis of two sets of experiments, one involving the mapping of mutations for resistance to ribosomally targeted antibiotics (5), and the other involving interspecific transformation experiments between *Bacillus* species with electrophoretically distinguishable forms of ribosomal proteins (8). Beyond this, only a very limited amount of information is available concerning the arrangement of *B. subtilis* ribosomal protein genes. This has been summarized in a recent review (10). Before now, there has been no published report of a ribosomal protein gene locus that maps outside the principal cluster, although Chambliss and coworkers have mapped a mutation affecting the mobility of protein S4 near *aroG* (Mol. Gen. Genet., in press).

Recently, I have described a selection which readily gives rise to mutants with electrophoretic alterations in one or more ribosomal proteins of *B. subtilis*, as assayed on two-dimensional gels (3). The lesions causing the protein alterations result in no detectable phenotypes, but such strains provide convenient starting material for mapping experiments. Using these and other mutants, I have shown that the gene loci for an additional five ribosomal proteins are in the principal cluster. (4a; E. R. Dabbs, Mol. Gen. Genet., in press). The loci for 29 ribosomal protein genes have, therefore, now been identified as mapping within this cluster.

Although several mutations altering the cellular phenotype with respect to ribosomally targeted antibiotics have been mapped outside the principal cluster, the nature of the component which is altered is unknown. Using transducing phage PBS1, I screened mutants with alterations in any of a large number of ribosomal proteins for the presence or the absence of linkage of lesions causing protein alterations to the *cysA* marker, which is near the principal cluster. (Materials and methods have been summarized previously [3].) In a number of mutants, no linkage to *cysA* was demonstrable, although the expected segregation pattern was seen with respect to the spectinomycin-resistant *rpsE* allele

TABLE 1. Strains used in this work

Strain	Genotype	Source or reference
W168		Y. L. Pai
1A3	*trpC2 cysA14 purA16*	BGSC[a]
1A8	*trpC2 aroD120 lys-1*	BGSC
1A9	*trpC2 aroG932 leuA8 ald-1*	BGSC
CA302	As strain 1A3, *rpsE302*[b]	This work
DA32	As strain W168, *rplU1*[c]	This work
DB65	As strain 1A3, *rpmA1*	(3)

[a] *Bacillus* Genetic Stock Center, Ohio State University, Columbus, Ohio.

[b] Spontaneous mutant, selected by plating strain 1A3 on medium containing 400 μg of spectinomycin per ml.

[c] Selected from strain W168 in the same manner as described previously (3). Gene designation for ribosomal protein locus in the case of mutants DA32 and DB65 based on correspondence to *E. coli* proteins L21 and L27, as described in the text.

present in the recipient. It was likely that the ribosomal protein mutations of these strains mapped outside the principal cluster, and in this work, I present the results obtained with two strains, DA32 and DB65 (Table 1). Gels of ribosomal proteins from these mutants are shown in Fig. 1. Mutant DA32 had an alteration in large ribosomal subunit protein L20, and mutant DB65 had an alteration in large subunit protein L24 (numbering of proteins follows that described previously [8]). In both cases, the altered form of the protein was less basic than the wild type.

PBS1 lysates of the two strains were prototrophic donors into a kit of strains with markers distributed around the *B. subtilis* chromosome. Pairs of markers were selected for simultaneously, and ribosomal proteins of five transductants from each selection were resolved on two-dimensional gels. When strain 1A9 was the recipient and selection was for Leu^+, together with Aro^+, then three of five transductants acquired the mutation causing altered protein mobility with either strain DA32 or DB65 as the donor. No other strain of the kit gave any transductant with the altered form of ribosomal protein of donors DA32 and DB65, and no other ribosomal

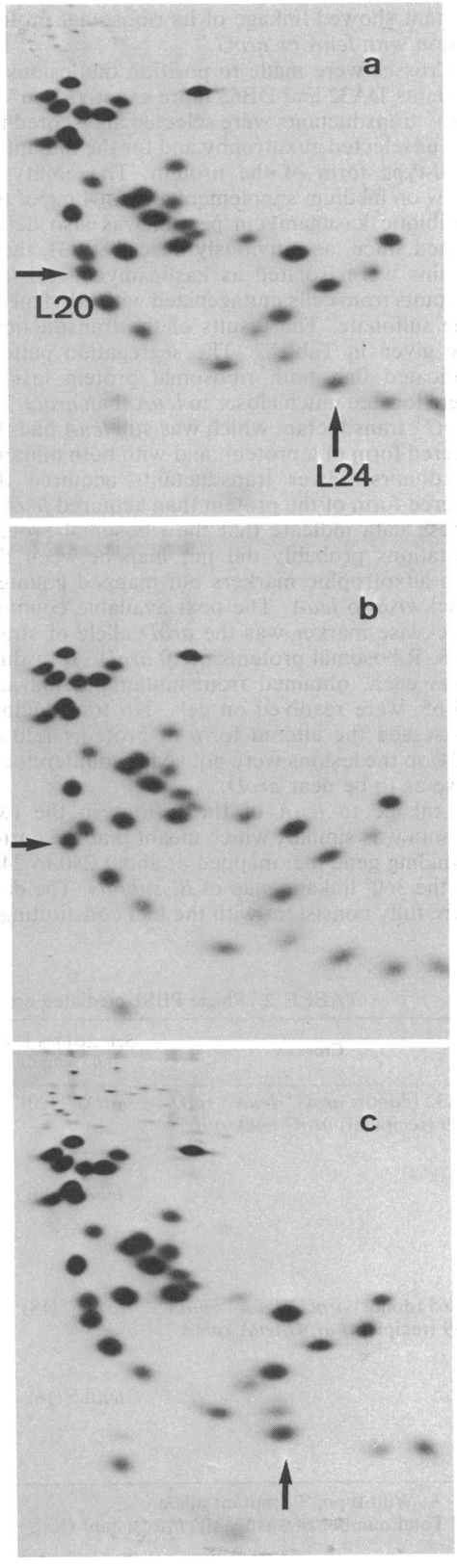

FIG. 1. Two-dimensional gel electropherograms of 70S ribosomal proteins of strains used in this work. The spots of *B. subtilis* proteins L20 and L24 are indicated. (a) Strain W168 (wild type); (b) strain DA32; (c) strain DB65. First-dimension runs were from left to right, with the cathode on the right. Second-dimension runs were from top to bottom, with the cathode on the bottom. About 300 μg of protein was applied to the gel.

mutant showed linkage of its ribosomal protein lesion with *leuA* or *aroG*.

Crosses were made to position the lesions of mutants DA32 and DB65 more exactly. Leu$^+$ or Aro$^+$ transductants were selected and scored for the unselected auxotrophy and for the mutant or wild-type form of the protein. The ability to grow on medium supplemented with 4 mg of the antibiotic kasugamycin per ml was also determined since, as previously described (3), these strains were isolated as kasugamycin-resistant mutants from cells mutagenized with ethyl methane sulfonate. The results of the transductions are given in Table 2. The segregation pattern indicated that both ribosomal protein lesions were located much closer to *leuA* than *aroG*. No *aroG*$^+$ transductant which was still *leuA* had the altered form of a protein, and with both mutants as donors, fewer transductants acquired the altered form of the protein than acquired *leuA*$^+$. These data indicate that the ribosomal protein mutations probably did not map between the two auxotrophic markers but mapped counterclockwise to *leuA*. The next available counterclockwise marker was the *aroD* allele of strain 1A8. Ribosomal proteins of 10 *aroD*$^+$ transductants each, obtained from mutants DA32 and DB65, were resolved on gels. No transductant possessed the altered form of proteins L20 or L24, so the lesions were not so far counterclockwise as to be near *aroD*.

Linkage to *leuA* of the lesions in the two donors was similar, which meant that the corresponding gene loci mapped at about 240 to 245° on the 360° linkage map of *B. subtilis*. The data were fully consistent with the loci constituting a pair, as is true in a number of instances in *E. coli*. Table 2 also shows that both donor strains harbored a kasugamycin resistance mutation mapping in the *leuA-aroG* region. With both donors, Leu$^+$ and Aro$^+$ transductants acquired the resistance mutation at a similar frequency. These lesions showed no cosegregation with ribosomal protein alterations and probably mapped between the two auxotrophic markers. Previously (9), we mapped in the same region a kasugamycin resistance mutation which contributed to the phenotype of a kasugamycin-dependent strain.

There were two additional points concerning the mutations which resulted in changed electrophoretic behavior of ribosomal proteins L20 and L24. The lesions could be in the structural genes for these proteins or in genes involved in processing or modifying these proteins. In *B. subtilis*, the absence of a mating system meant there was no ready method for constructing merodiploids, which would allow one to distinguish between the former and latter alternative. Several arguments favored the likelihood of structural gene mutations. In *E. coli*, a set of mutants similar to those described here have proven overwhelmingly to be strains with lesions in ribosomal protein structural genes; with those mutants in which the mutation was in a modifier gene, the altered migration in gels was slight or—with regard to the first dimension—nonexistent. Also, the corresponding proteins of *E. coli* (see below) are not known to undergo any modification or processing.

To determine the *E. coli* protein to which *B. subtilis* protein L24 corresponded, I took advan-

TABLE 2. Phage PBS1-mediated crosses between *rplU*, *rpmA*, *leuA*, and *aroG* loci

Cross	Selected marker (no. scored)	Transductant characteristics[a]				No. of transductants	No. of Ksg^r transductants[b]
		aroG	*leuA*	*rplU*	*rpmA*		
DA32 (donor) *aroG*$^+$ *leuA*$^+$ *rplU*	*aroG*$^+$ (20)	+	+	+		1	8
1A9 (recipient) *aroG leuA rplU*$^+$		+	+	−		1	
		+	−	+		18	
		+	−	−		0	
	leuA$^+$ (20)	+	+	+		2	10
		+	+	−		2	
		−	+	+		6	
		−	+	−		10	
DB65 (donor) *aroG*$^+$ *leuA*$^+$ *rpmA*	*aroG*$^+$ (18)	+	+		+	1	8
1A9 (recipient) *aroG leuA rpmA*$^+$		+	+		−	2	
		+	−		+	15	
		+	−		−	0	
	leuA$^+$ (16)	+	+		+	2	9
		+	+		−	3	
		−	+		+	4	
		−	+		−	7	

[a] +, Wild type; −, mutant allele.

[b] Total number of kasugamycin-resistant (Ksg^r) transductants obtained in each selection.

tage of a series of *E. coli* mutants whose ribosomes lacked one of a number of ribosomal proteins (2; unpublished data). The ribosomes of these mutants have been used in specific binding experiments to identify a single protein of *B. subtilis* (and other organisms) corresponding to the missing *E. coli* protein (manuscript in preparation). An *E. coli* mutant lacking ribosomal protein L27 showed specific binding of *B. subtilis* protein L24 to its ribosomes. I concluded that *B. subtilis* protein L24 very probably corresponded to *E. coli* protein L27. *B. subtilis* protein L20 is the homolog of *E. coli* protein L21 based on amino acid sequence data (S. Osawa, personal communication).

Determination of protein correspondence allowed a genetic comparison to be made between *B. subtilis* and *E. coli*. In *E. coli*, the genes for proteins L21 and L27 are a tightly linked pair which is separated from any other ribosomal protein genes (7). This work has shown that in *B. subtilis*, the corresponding two protein genes were near one another and were well separated from any other ribosomal protein genes. This parallel reinforced findings from other work (Dabbs, in press) that the arrangement of ribosomal protein genes with respect to one another is strikingly similar in the two procaryotes; the composition of and the order within transcriptional units is apparently conserved, the main difference being the degree to which these transcriptional units are clustered together or dispersed over the chromosome.

Thus, in *B. subtilis*, the Rif cluster of ribosomal protein genes is close to the Str-Spc cluster (6), whereas in *E. coli*, it is far away. I have shown that, whereas in *E. coli* the ribosomal protein S9 gene is about 2.5 min from the main cluster (4), in *B. subtilis* the gene for the homologous protein is immediately adjacent to genes of the Str-Spc cluster (4a). In *E. coli*, the gene loci for proteins L21 and L27 are less than 1 min from the protein S9 gene, but in *B. subtilis*, the distance is about 40% of the chromosome. The main reason for this is that in *B. subtilis*, the Str-Spc and Rif clusters, together with the protein S9 gene, are much closer to the origin of chromosomal replication than in *E. coli*. In *B. subtilis*, the genes for proteins L20 and L24 are about 60% of the way between the origin and termination points of chromosome replication, whereas in *E. coli* the corresponding genes are about 30% of the way between the two points.

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