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Location of the Genes for 16S and 23S Ribosomal RNA in the Genetic Map of *Escherichia coli**

M. T. Yu†, C. W. Vermeulen‡, and K. C. Atwood†

DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF ILLINOIS, URBANA, ILL. 61801

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Abstract. By means of RNA–DNA hybridization with DNA from different merodiploids of *Escherichia coli*, the genes for 16S and 23S ribosomal RNA were found near minute 74 of the genetic map.

Introduction. Approximately 0.3% of the DNA of normal strains of *E. coli* forms an enzyme-resistant complex with the combined 16S and 23S ribosomal RNA (rRNA).[1] This quantity of DNA (the rDNA) is sufficient for about five copies of each gene, a multiplicity necessary to satisfy the requirement for rRNA synthesis. If these genes are included in the diploid region of a partial diploid strain, the rRNA/DNA saturation plateau will be elevated, whereas if the genes are not within the diploid region the plateau will be normal or depressed. Thus the genes can be mapped by the use of different partial diploids. We have employed the DNA of merodiploids covering, in the aggregate, the entire genetic map. The results indicate a close grouping of the rRNA genes near minute 74, between *rbs* and *ilv*.

Materials and Methods. Preparation of labeled rRNA: Cells grown in minimal medium containing 0.2 mM phosphate were inoculated into 200 ml of the same medium containing 40 mCi of $Na_2H^{32}PO_4$. At the final cell concentration of 3×10^8 per ml, nonradioactive phosphate was added to a concentration of 0.05 M. After 1.5 generations, the cells were harvested and RNA was extracted by the method of Midgely,[2] which was modified by the omission of the Hughes press step and by the addition of lysozyme (50 μg/ml) and three cycles of freezing and thawing. The final ethanol precipitate was dissolved in NaCl, 0.15 M $MgCl_2$, 0.01 M Tris buffer (pH 7.2) 0.01 M, treated with 20 μg/ml of purified DNase (Worthington Biochemicals), loaded on a methylated albumin–kieselguhr (MAK) column, and eluted with a linear NaCl gradient from 0.2 to 1.1 M in the same buffer. The ribosomal fractions were pooled and dialyzed against double strength saline–sodium citrate (2× SSC).

Preparation of labeled DNA: Cultures were grown in minimal medium plus required supplements with 200 μCi of [^{3}H]adenosine per liter, and harvested in stationary phase. DNA was extracted by Marmur's procedure.[3] The final preparation of ethanol-precipitated fibers was dissolved in $^1/_{100}$ SSC, brought to pH 13 for 10 min by addition of $^1/_{10}$ vol of 1 M NaOH for denaturation, and neutralized with 1 N HCl. When unstable merodiploids were used, heterozygosity for markers was tested at the time of harvest. The DNA was rejected if less than 90% of the cells sampled proved to be heterozygous for the available markers.

rRNA–DNA hybridization: The filter method of Gillespie and Spiegelman[4] was used. The ratio of RNase-resistant [^{32}P]rRNA to [^{3}H]DNA was obtained from the counts and predetermined specific activities. The saturation value was taken as the average of the last three points on a reasonably horizontal plateau, ordinarily achieved at

concentrations of 3, 4, and 5 μg of RNA per ml, and rounded to the nearest 0.01%. Control filters bearing DNA of related F⁻ strains were incubated in the same reaction mixtures as those bearing merodiploid DNA. In principle, this provides a correction for errors in specific activity of [^{32}P]rRNA and secular variations in annealing conditions.

Strains: Large episomes were produced through the intermediate of double males,[5] after the method employed by Maas and Clark.[6] PD-1 and PD-2 were obtained from A. R. Kaney, and JC537 from A. J. Clark. Smaller episomes were found by screening for early transfer of late markers. Reduced derivative episomes were isolated by means of screening for simultaneous loss of episomal markers in a *recA* background. KLF-11, F-14, and the F-14 chromosome deficiency strain, AB1206, were obtained from Brooks Low, many Hfr strains from E. A. Adelberg, the anomalous partial diploid, X137Ex2, from Roy Curtiss, III, and *recA* strains from P. Howard-Flanders. The strains are described in Tables 1, 2, and 3. Marker symbols are those of Taylor and Trotter.[7]

TABLE 1. *rRNA-DNA hybridizations with DNA of strains having the unit genome.*

Strain	Description	rRNA/DNA, per cent
AB2271	Hfr *ilv thr lac str*	0.24
AB2297	Hfr *pur ilv* λ^- *xyl*	0.25
3300	Hfr *lacI*	0.27
AB750	Hfr *his*-transposition *malB lac gal*	0.28
AB257	Hfr *met*	0.28
		0.29
AB2462	F⁻ *thr leu pro his recA str argE*	0.28
		0.30
		0.31
		0.31
		0.31
		0.32
		0.33
		0.34
F⁻ segregant from JC537		0.29
JC1553	F⁻ *leu lac recA str argG mal xyl mtl metB*	0.29
AB492	Hfr *lac str mal xyl mtl arg thi*	0.29
361	F⁻ *ara lacY str xyl mtl fdp*	0.30
F⁻ segregant from F301-22		0.30
2311	F⁻ *ara pro lac his str xyl mtl ilv fdp*	0.31
23-5	F⁻ *ara lac* λ^- *try his recA str xyl mtl ilv fdp*	0.31
		0.31
AB674	Hfr +	0.32
AB301	Hfr *met*	0.33
AB1206	F-14/deletion, *thi his pro lac gal str*	0.33
		0.33

Results and Discussion: To provide a basis for comparison, all of the results with single genome strains are shown in Table 1, although some of these were controls in Tables 2 and 3. The differences between merodiploid and control values in Table 2, representing the relative rDNA contributions of the merogenotes, are distributed around zero, while those in Table 3 are all positive. Outside the observed normal range the strains display a broad distribution of rDNA contents (Table 3), only a few being twice normal. No correction has been made for merogenote size; it is negligible for short regions, and does not bring order to the data. Fortunately the correction is not essential to the mapping problem. We could not anticipate the quantitative effect of a given mero-

TABLE 2. *rRNA-DNA hybridizations with DNA of merodiploid strains in which the diploid region does not include minute 74.*

Strain	Description	Diploid map region	rRNA/DNA, per cent Mero-diploids	F^- controls	Difference
X137Ex2	F^- (*ara valS leu azi tfrA pro*)$^{+/-}$ *tsx str thi*	88–09	0.24	0.29	−0.05
PD-3	F′ (*pur xyl mtl*)$^{+/-}$ *gua*$^{-/+}$ *arg met pro*	40–73.8	0.24	0.29	−0.05
F301-46T	*fdp*$^{+/-}$ F′ *ilv try his str*	<84–86	0.27	0.31	−0.04
F750	F′ *fdp*$^{+/-}$ *ilv try his str*	79.3–>84	0.28	0.31	−0.03
F48-1	F′ (*thr leu pro*)$^{+/-}$ *his recA str argE*	79.3–15	0.29	0.30	−0.01
F48-4	Like F48-1		0.29	0.30	−0.01
F301-22	*fdp*$^{+/-}$ F′ *ara leu stry xyl*	<84–86	0.30	0.30	0.00
PD-1	F′ (*try his*)$^{+/-}$ *recA str xyl mtl met arg mal leu lac*	15–40	0.31	0.29	0.02
F301-33T	Like F301-46T		0.31	0.31	0.00
F111-Y	*argE*$^{+/-}$ (*argB* or *C*)$^{+/-}$ *F′ his recA str thr leu pro*	<77–86	0.33	0.31	0.02
F301-29	Like F301-46T		0.33	0.31	0.02
F111-Y2	Like F111-Y		0.54	0.31	0.03
KJS-1	F^+ Deletion of all markers in KLF-11, with JC1553 chromosome	<72–72*	0.34	0.28	0.06

Note: Merogenotes derived from double males extend between the sex factor integration sites of the parent Hfr strains. These are taken to be: AB257, 15; AB492, 40; AB674, 73; AB2271 and AB313, 73.8; AB750, 79.3; and AB301, 86.

* Maximum left limit of deletion.

TABLE 3. *rRNA-DNA hybridizations with DNA of merodiploid strains in which the diploid region includes minute 74.*

Strain	Description	Diploid map region	rRNA/DNA, per cent Mero-diploids	F^- controls	Difference
ARI-AM	F′ *ilv*$^{+/-}$ Deletion in F-14 *metE argE argH pyrE**	73.8–74.5†	0.38	0.29	0.09
ARI-A	F′ (*ilv metE*)$^{+/-}$ *pyrE** Deletion in F-14	73.8–76.8†	0.40	0.29	0.11
F-34	(*ilv metE argE argH*)$^{+/-}$ F′ *his**	73–86	0.40	0.32	0.08
KRI-3A	F′ (*pyrE ilv metE*)$^{+/-}$ *argE argH** Deletion in KLF-11	<72–74.5†	0.41	0.31	0.10
Kb24-IV	F′ *ilv*$^{+/-}$ *metF argE his** Deletion in KLF-11	<72–74.5†	0.41	0.31	0.10
F-33	Like F-34		0.43	0.32	0.11
ARI-2	F′-14 (*ilv metE argE argH*)$^{+/-}$*	73.8–>77.3	0.41	0.28	0.13
ARI-1	Like ARI-2		0.44	0.28	0.16
			0.46	0.34	0.12
KLF-11/JC1553	Colony isolate	<72–79.3	0.45	0.32	0.13
KLF-11/JC1553	As received		0.51	0.32	0.19
F-35	Like F-34		0.51	0.32	0.19
KRI	F′ KLF-11		0.63	0.34	0.29
	(*pyrE ilv metE argE argH*)$^{+/-}$*	<72–79.3	0.60	0.28	0.32
Kb24	F′ KLF-11 (*ilv metF argE*)$^{+/-}$ *his**		0.65	0.34	0.31
			0.57	0.28	0.29
JC537	(*str malA met arg*)$^{+/-}$ F′ *thr leu pro*	59–86	0.67	0.29	0.38
PD-2	Hfr with terminal duplication (*ilv arg pro*)$^{+/-}$ *thr*$^{-/+}$ *leu*$^{-/-}$ *his recA*	73.8–15	0.72	0.29	0.43

* *thr leu pro recA str*

† Minimum left limit of deletion.

genote on the hybridization plateau because that effect depends not only on merogenote length and rDNA content, but also on the stages in which the episomal and chromosomal replication cycles are arrested in stationary phase. The data are compatible with episome to chromosome ratios close to unity, but give no reason to presume that the episomal map regions are in general precisely diploid, or that all chromosomal regions are haploid. Finally, since we suspect that deviations from normal rDNA content are unstable, we surmise that a reliable estimate of the rDNA content of a merogenote can be made only if it has been recently transferred to a normal F^- strain.

Figure 1 shows the merogenotes in relation to the mapping convention of Taylor and Trotter.[7] Without attempting to speculate further for the moment on the sources of variability, or to employ a statistical treatment, we perceive

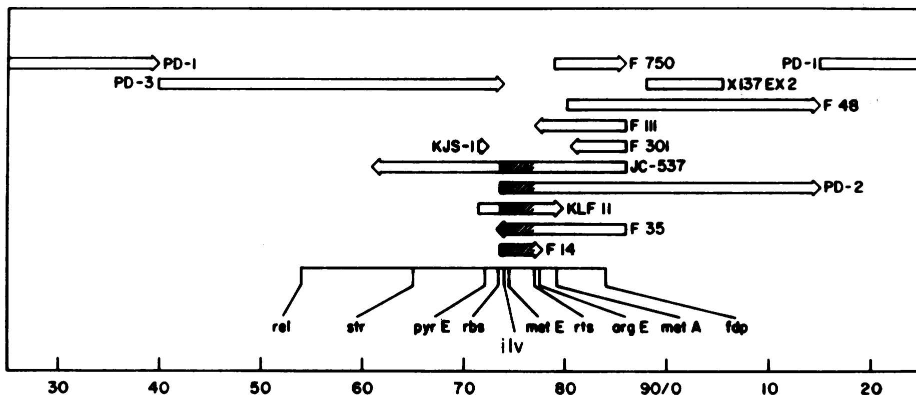

FIG. 1.—Extent of the merogenotes in the genetic map. Cross-hatching indicates the region overlapped only in strains with augmented rDNA. The solid region is the locus of the rRNA genes inferred from these data combined with the partitioning of the rDNA between the episome and chromosome of strain AB1206. (F111 is F111Y in text.)

that all strains in which the merogenote does not overlap the interval between the origins of PD-3 and F111Y have saturation plateaus in the normal range (Table 2), while the others have elevated plateaus (Table 3). Thus the data are consistent with a close grouping of the rRNA genes if the strains in Table 2 are considered to have no rDNA in the diploid region, and those in Table 3 to have some rDNA in the region. Less direct experimental approaches to mapping the rRNA genes have suggested that they occupy two widely separate positions.[8,9] The foregoing evidence does not support that hypothesis.

Although the genes occupy a restricted chromosomal segment, we cannot prove that they are contiguous. It seems likely, however, that their multiplicity evolved through a mechanism that creates tandem duplications; the assumption that some of these have been translocated is gratuitous. Hence we believe a single tandem array to be the most likely configuration, and that assumption is implicit in the discussion that follows. A somewhat more precise location of the genes can be deduced from the quantitative relations among rDNA contents in certain groups of strains.

F-14, KLF-11, PD-2, and F111Y: The results with KLF-11/JC1553 are in

doubt because the opportunity had existed for diminution of rDNA in the initially normal JC553 chromosome. The full complement of rDNA is apparently contained in PD-2 and in the KLF-11 merogenotes in strains KRI and Kb24, whereas the F-14 merogenotes in strains ARI-1 and ARI-2 contain about half the normal amount. To account for this difference it may first be noted that the total map distance of some 4 min in F-14, from just to the left of *ilv* to just to the right of *arg*, is inside KLF-11; hence it could be argued that the part of the rDNA in KLF-11 that is not in F-14 must be either in the right end of KLF-11, or in the left end. The right ends—including *arg*—of both KLF-11 and F-14, however, are overlapped by F-111Y, which is devoid of rDNA, suggesting that the differential segment in KLF-11 is in the left end. On the other hand, the F-termini of PD-2 and F-14 are considered to be the same, since both are ultimately derived from the same Hfr strain, AB313. If the F site is an invariable limit to the extent of an F-merogenote, then the difference between F-14 and PD-2 could be attributed to persistent doubling of the F-terminus of PD-2 at stationary phase arrest, or to secondary diminution of the rDNA of F-14.

F-33, F-34, and F-35: These ostensibly identical merogenotes are derived from double male segments with origin between *ilv* and *rbs*, and F between *pil* and *pyrB*. The rDNA content of F-33 and F-34 resembles that of F-14, whereas F-35 appears to contain substantially more than F-14, but less than KLF-11. It has been noted in other contexts that where the region between an origin and the first selected marker is superfluous, it may be spontaneously lost by gradual stages.[10] Thus the variable and diminished rDNA contents of these isolates may be interpreted as secondary partial deficiencies, more likely to the left of *ilv* than to the right.

Shortened derivatives of F-14: Simultaneous loss of arg^+ and $metF^+$ from F-14 in ARI-1 gave rise to the merogenote in ARI-A, apparently a deletion of a minute or more of map length near the right end. In strain ARI-AM, *arg*, *metF*, $^+$ and $metE^+$ are lost, a deletion of about 3 min. In both of these the rDNA content of unmodified F-14 is essentially unchanged, which indicates that the rDNA of F-14 is in the left end, certainly to the left of *metE* and possibly to the left of *ilv*.

Shortened derivatives of KLF-11: Two deletions were obtained of markers to the right of *metE*, and one of all genetic markers. The latter, as expected, has lost its rDNA, but the first two have also lost some rDNA, the remaining amount of which falls within the range of F-14 and its derivatives. Although this clearly represents a secondary change, it cannot be related in any simple way to the deletions with the hypothesis that the rDNA is to the left of *metE*. It recalls the possibility, however, that the rDNA in F-14 had been similarly diminished prior to our experiments. If F-14 had originated with a full complement, some of which was subsequently lost, then no part of the rDNA in KLF-11 need necessarily lie outside its region of overlap with F-14. Although this question cannot be resolved with certainty at present, the manner in which F-14 originated has a decisive bearing on the location of the rRNA genes.

F-14 and AB1206: The F-14 merogenote was derived from a mating of Hfr AB313 with AB1171F$^-$ through a rearrangement in which, to a first ap-

proximation, a unit genome was conserved; that is, the product of the rearrangement comprises the F-merogenote and a chromosome with a corresponding deletion.[11] This product is strain AB1206. If F-14 contained all of the rDNA at the time of its origination, then the chromosome with the corresponding deletion should contain none. The hybridization plateau for AB1206 is, however, normal (Table 1). At the same time F-14, freshly transferred from AB1206 to normal F^- strains, carries approximately half the normal complement of rDNA. We conclude that the remaining portion is in the chromosome of AB1206.

The presence of rDNA in the deletion chromosome can have two explanations: the left boundary of the deleted segment does not exactly coincide with that of the merogenote, or the rearrangement involved a break within the rDNA. In either case the result facilitates a refinement of the map location, since the chromosomal rDNA cannot be within the deleted segment. Pittard and Ramakrishnan[11] demonstrated that the deletion includes both *arg* and *ilv;* they concluded that it extends over most, if not all, of the F-14 region. Compelling evidence for this conclusion is that the frequency of transfer of chromosomal markers by AB1206 is from 10^{-3} to 10^{-4} of the corresponding frequency when F-14 is in a strain with a normal chromosome.

Since the preceding experiments have eliminated all regions to the right of *arg,* we conclude that the rDNA is to the left of *ilv;* that is, between *rbs* and *ilv.* This conclusion encounters the difficulty that the cotransduction frequency of rbs^+ and ilv^+ with P1 bacteriophage, 70–87%,[7] suggests that the interval may be too small to contain all of the genes. We have no sufficient basis on which to judge the seriousness of this discrepancy. The integration site of the sex factor in Hfr AB313 is also between *rbs* and *ilv.*[7] The question remains open whether the rDNA occupies the still more restricted region between the F-terminus of AB313 and *ilv,* or was divided into two segments by the integration of the sex factor that gave rise to AB313. The latter interpretation would require a less complex rearrangement to produce AB1206, but it would also require an *ad hoc* explanation for the absence of rDNA in PD-3.

Finally, it is noteworthy that the locus is not adjacent to its regulatory gene. *rel,* or to the *ery, lin, spc, str,* and *rts* loci associated with ribosomal proteins.

Abbreviations: rRNA, ribosomal RNA; rDNA, DNA that anneals with rRNA.

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† Present address: Department of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, N.Y. 10032.

‡ Present address: Department of Biology, College of William and Mary, Williamsburg, Va. 23185.

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