ISOLATION OF λ TRANSDUCING PHAGES CARRYING rRNA GENES AT THE *met*A–*pur*D REGION OF THE *ESCHERICHIA COLI* CHROMOSOME

Masayuki YAMAMOTO and Masayasu NOMURA

Institute for Enzyme Research, Departments of Genetics and Biochemistry, University of Wisconsin, Madison, Wisconsin 53706, USA

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1. Introduction

There are several sets of ribosomal RNA (rRNA) genes on the haploid chromosome of *Escherichia coli* (for a review, see [1]). Each set consists of 16 S rRNA, 'spacer' tRNA, 23 S rRNA and 5 S rRNA [2] and constitutes a unit of transcription [3–5]. Identification and isolation of all of these rRNA gene sets may be important for understanding the mechanism and regulation of rRNA synthesis. Several rRNA gene sets have already been identified and isolated in the form of transducing phage DNAs or episomal DNAs [6–10] and Kenerley and Nomura, in preparation; see also fig.4. In this paper, we describe isolation of several transducing phages carrying chromosomal DNA from the *met*A–*pur*D region at 89 min on the *E. coli* genetic map. Analysis of these transducing phage DNAs has demonstrated that there is one set of rRNA genes between *met*A and *pur*D.

2. Method

Bacteriophages λ*cI*857*S*7 (called 'λ' in this paper), λ*cI*857*S*7*xis*6*b*515*b*519 (called 'λ*b*Δ' in this paper) and λ*rif*^{d}18 [2,8,20] were used. Bacterial strains used are listed in table 1. NO1818 was constructed from CH440Su^{+} by deleting the λ attachment site according to the method described by Shimada et al. [11].

λ*pur*D and λ*met*A transducing phages were isolated using the method described by Schrenk and Weisberg [12]. λ*met*A2 and λ*pur*D8 were obtained using a mixed lysate prepared from KS302 with λ*b*Δ inserted randomly in the chromosome, and λ*met*A20 was obtained in the same way using a mixed lysate from NO1818. AB468 and AB2569 were used to select and screen *pur*D and *met*A transducing phages.

DNA–RNA hybridization was performed as described in the legend to table 1. DNA heteroduplex analysis was done as described by Davis et al. [13].

Table 1
Strains of *E. coli* used

Strain	Relevant genotype	Source
KS302	HfrH (*gal-uvr*B)$^{\Delta}$	K. Shimada
CH440Su^{+}	F^{-} *trp*A36 *arg*H *glyTsu*	C. Hill
NO1818	F^{-} *trp*A36 *arg*H *glyTsu* (*gal-uvr*B)$^{\Delta}$	This work
AB468	F^{-} *thi his pro*A *pur*D *mtl xyl gal*K *lac*Y	ECGSC[a]
AB2569	F^{-} *thi arg met*A *his pro*A *mtl xyl gal*K *lac*Y *tsx*	ECGSC[a]
NO1819	AB468(λ*cI*857*S*7*xis*6*b*515*b*519, λ*pur*D8)	This work
NO1820	AB2569(λ*cI*857*S*7*xis*6*b*515*b*519, λ*met*A2)	This work
NO1821	AB2569(λ*cI*857*S*7, λ*met*A20)	This work

[a]ECGSC = *E. coli* Genetic Stock Center, Yale University School of Medicine

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3. Results and discussion

Hill and Combriato reported that under certain conditions tandem duplications occur at high frequency in *E. coli* and that the duplicated chromosomal regions analyzed had frequently one end point between *met*A and *pur*D [14]. They suggested a crossover using a DNA sequence homology as a possible mechanism to generate duplications. Such crossovers could utilize the homology of two rRNA gene sets and suggest the presence of an rRNA gene set between *met*A and *pur*D. To test this possibility, we isolated several transducing phages carrying *met*A and/or *pur*D.

DNA was isolated from these transducing phages and analyzed for its ability to form DNA–RNA hybrids with radioactive rRNA. Positive hybridization results were observed with several transducing phages, indicating the presence of rRNA genes on these phage genomes. Table 2 shows the results of one such hybridization experiment using DNA from λ*met*A2, λ*pur*D8 and λ*met*A20. As a control, DNA from λ*rif*^{d}18 was used. λ*rif*^{d}18 carries one complete set of rRNA genes [2,8,15].

It can be seen from table 2 that λ*met*A2 includes most or all of the 23 S rRNA gene but not (or very little of) the 16S rRNA gene. λ*met*A2 does not carry the *pur*D gene. In contrast, λ*pur*D8, which does not carry the *met*A gene, appears to have only (most or all of) the 16 S rRNA gene, but not the 23 S rRNA gene. λ*met*A20, which carries both *met*A and *pur*D genes, appears to have a complete set of rRNA genes. The data is consistent with, but does not prove, the conclusion that one complete set of rRNA genes exists between *met*A and *pur*D. The following DNA heteroduplex analyses prove that this conclusion is correct: (i) The structures of heteroduplexes given in fig.1 (a) and (b), combined with the known location of the rRNA gene set on λ*rif*^{d}18 [2,15, cf. fig.3(b)], show that λ*met*A2 has bacterial DNA substitution in the left arm of λ*b*Δ, and carries only a distal part of a rRNA transcription unit which is in the same orientation (with respect to λ genes) as that carried by λ*rif*^{d}18. They also show that non-ribosomal bacterial DNA is located adjacent to the distal end of the rRNA transcription unit (fig.3(c)). (ii) The structures of heteroduplexes given in fig.1(c) and (d) (see also fig.2) show that λ*pur*D8 carries a bacterial DNA substitution in the right arm of λ*b*Δ, and that non-ribosomal bacterial

Table 2
Ability of various transducing phage DNAs to hybridize 16 S and 23 S rRNA

Source of DNA	Ability to transduce		^{3}H counts hybridized	
	*met*A	*pur*D	16 S	23 S
λ*b*Δ			177	169
λ*rif*^{d}18			891	1544
λ*met*A2	+	–	102	1697
λ*pur*D8	–	+	890	63
λ*met*A20	+	+	754	1383

Various phages were prepared by heat induction of lysogens (cf. table 1 and ref. [2]) and purified as described previously [2,17]. The purified phages were suspended in SM buffer (0.1 M NaCl, 0.02 M Tris, 1 mM $MgSO_4$, gelatin 0.01%, pH 7.5). 10 μl of the phage solutions (A_{260} = 8) were mixed with 60 μl of H_2O, 10 μl of 0.2 M EDTA (pH 8) and 10 μl of 1 N NaOH, and were left at room temperature for 25 min. 1 N HCl (10 μl) was then added together with 10 μl of 2 M Tris buffer (pH 7.2). To the resultant solutions containing denatured phage DNA, 0.4 ml of 2xSSC (0.3 M NaCl, 0.03 M Na-citrate) containing ^{3}H-labeled rRNA (4×10^4 cpm of 16 S rRNA or 6×10^4 cpm of 23 S rRNA; sp. act. of RNA, 5×10^5 cpm/μg; prepared according to ref. [18]) were added and hybridization was carried out at 66°C for 4 h. The reaction mixtures were then chilled and DNA–RNA hybrids were collected on nitrocellulose filters. The filters were processed as described before [19] and the radioactivity on the filters was determined.

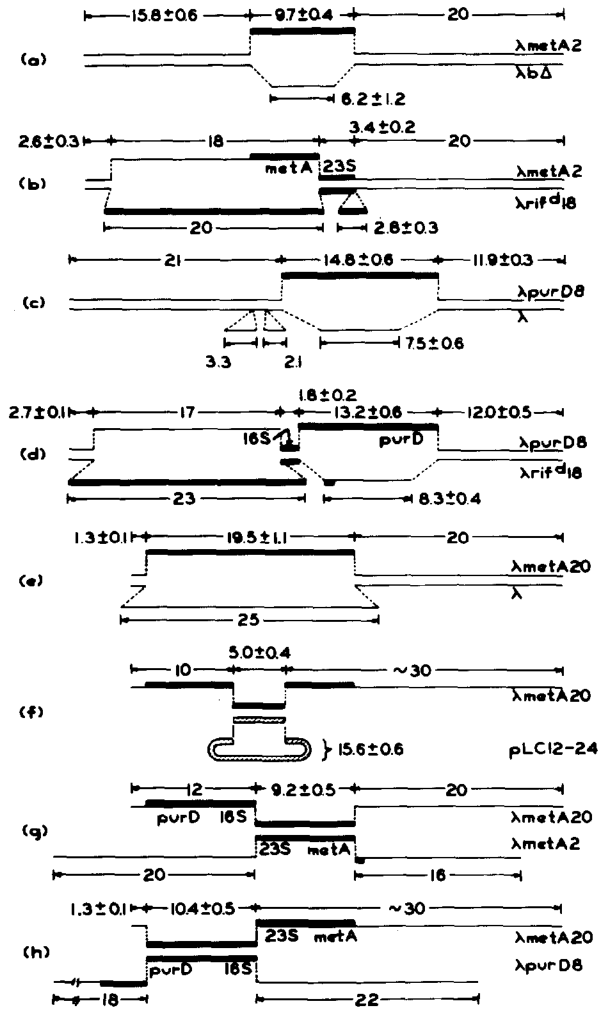

Fig.1. Schematic representation of the structure of heteroduplexes formed between the various phage DNAs. The thin solid lines represent λ (or λ*b*Δ) DNA and the heavy lines represent bacterial DNA. Plasmid DNA is hatched. Values given are in Kb (1 Kb is 1000 base pairs). For some pertinent distances, the standard deviations are included.

DNA is adjacent to the proximal end of a rRNA transcription unit which lacks the distal part (fig.3(d)). The orientation of the rRNA gene set is the same as that carried by λ*rif*^{d}18. (iii) λ*met*A20 has a bacterial DNA substitution in the left arm of λ*b*Δ (fig.1(e)) and carries a complete rRNA gene set in the middle of the bacterial DNA (fig.1(f)). DNA from a plasmid pLC12-24 carrying one complete set of rRNA genes from the *aro*E region on the *E. coli* genetic map (Kenerley and Nomura, in preparation) was used to locate the rRNA gene set in the λ*met*A20 genome. (iv) The structures of heteroduplexes given in fig.1 (g,h) show that the orientation of bacterial DNA in λ*met*A20 with respect to λ genes is opposite to that in λ*met*A2 and λ*pur*D8, and that λ*met*A20 has a region homologous to a major part of the bacterial DNA (the distal part of the rRNA gene set and non-ribosomal bacterial DNA) carried by λ*met*A2. Similarly, λ*met*A20 has homology to a major part of the bacterial DNA (the proximal part of the rRNA gene set and non-ribosomal bacterial DNA) carried by λ*pur*D8. Therefore, the order of the bacterial genes carried by λ*met*A20 is *pur*D-16 S RNA-23 S RNA-*met*A. (v) The structure of the heteroduplex formed between λ*met*A2 and λ*pur*D8 (not shown) failed to indicate any homology between the bacterial DNAs carried by these phages. In addition, the size of the bacterial DNA carried by λ*met*A20 (19.5 ± 1.1 Kb, see fig.1(e), 1 Kb = 1000 base pairs) is approximately equal to the sum of the size of the part of λ*met*A20 homologous to λ*met*A2 DNA (9.2 ± 0.5 Kb, see fig.1(g)) and that homologous to λ*pur*D8 (10.4 ± 0.5 Kb, see fig.1(h)). This suggests that the right end (in fig.3(c)) of rDNA carried by λ*met*A2 may be the same as the left end (in fig.3(d)) of rDNA carried by λ*pur*D8 and that this site represents the 'pseudo-attachment site' (cf. ref. [11]) where λ*b*Δ had been inserted in the original lysogen(s) which

Fig.2. Electron micrographs of a DNA heteroduplex between λ*pur*D8 and λ*rif*^{d}18. Schematic representation of the structure is shown in fig.1(d). m and m' are the left and the right ends of the λ molecule, respectively. The double strand between A and B represents the region containing the 16 S RNA gene. The length of 1 Kb of DNA duplex is indicated by a bar.

Fig.3. The structures of λ (a), λ*rif*^{d}18 (b), λ*met*A2 (c), λ*pur*D8 (d), and λ*met*A20 (e). The regions representing λ DNA are hatched. The regions representing rRNA genes are stippled. The arrows indicate orientation of the rRNA gene set (the direction is 16 S to 23 S RNA gene). The locations of *b*519 and *b*515 deletions carried by λ*b*Δ are also indicated in (a). The exact position of the right end of the rRNA gene set carried by λ*rif*^{d}18 is not known, but is very close to the junction of bacterial and λ DNA [15].

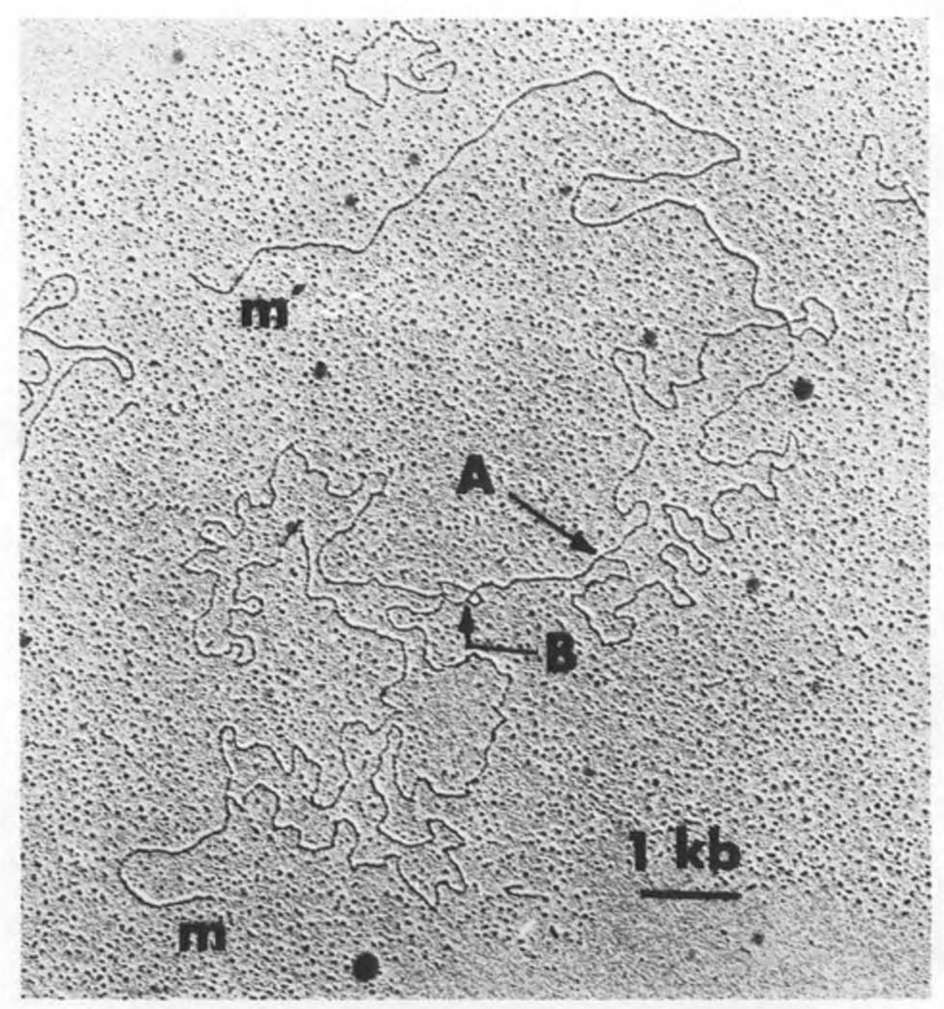
m'
A
B
1 kb
m

Fig. 2

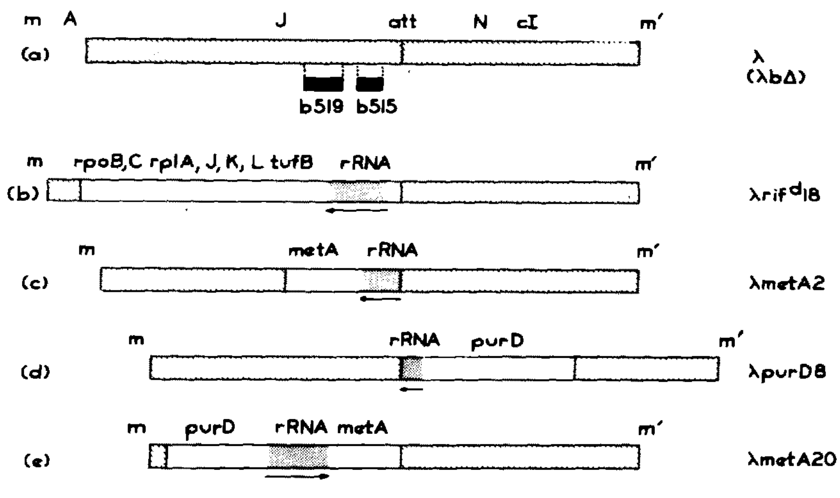
m A J att N cI m'
(a)
λ
(λbΔ)
b519 b515
m rpoB,C rplA, J, K, L tufB rRNA m'
(b)
λrif^d18
m metA rRNA m'
(c)
λmetA2
m rRNA purD m'
(d)
λpurD8
m purD rRNA metA m'
(e)
λmetA20

Fig. 3

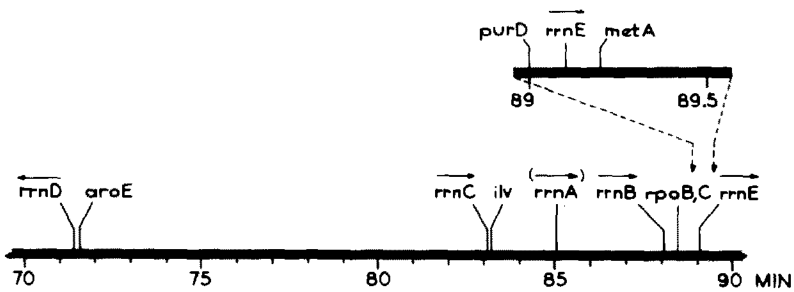

Fig.4. The locations of rRNA genes on the *E. coli* genetic map. The arrows show the orientation of the rRNA gene sets. The figure is based on previous [2,6,10,15,16] as well as the present work. The orientation of *rrn*A has not been directly studied, but is inferred as indicated in parenthesis (cf. [6,16]).

produced these transducing phages. Furthermore, this size comparison shows that there is only one set of rRNA genes between *pur*D and *met*A on the λ*met*A20 genome (fig.3(e)).

Since λ*met*A20 was isolated from a strain which was different from the strain used for the isolation of λ*met*A2 and λ*pur*D8, the above results strongly indicate that the arrangement of bacterial genes in the λ*met*A20 genome is identical to that on the *E. coli* chromosome. We conclude that there is one set of rRNA genes between *pur*D and *met*A at 89 min of the *E. coli* chromosome and we suggest the name *rrn*E. Figure 4 summarizes the known locations of rRNA gene sets and their orientations on the *E. coli* genetic map.

Recently Hill and his co-workers obtained evidence which is consistent with the conclusion that the duplications they studied previously ([14] see above) take place by crossing over between two rRNA gene sets [16]. They inferred the presence of a rRNA gene set between *met*A and *pur*D. The present work gives direct evidence for the location and the orientation of the suspected rRNA gene set.

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References

[1] Jaskunas, R., Nomura, M. and Davies, J. (1974) in: Ribosomes (Nomura, M., Tissières, A. and Lengyel, P., eds) pp. 333–368, Cold Spring Harbor Laboratory, New York.
[2] Lund, E., Dahlberg, J. E., Lindahl, L., Jaskunas, S. R., Dennis, P. P. and Nomura, M. (1976) Cell 7, 165–177.
[3] Dunn, J. J. and Studier, F. W. (1973) Proc. Natl. Acad. Sci. USA 70, 3296–3300.
[4] Nikolaev, N., Silengo, R. and Schlessinger, D. (1973) J. Biol. Chem. 248, 7967–7969.
[5] Ginsburg, D. and Steitz, J. A. (1975) J. Biol. Chem. 250, 5647–5654.
[6] Deonier, R. C., Ohtsubo, E., Lee, H. J. and Davidson, N. (1974) J. Mol. Biol. 89, 619–629.
[7] Ohtsubo, E., Soll, L., Deonier, R. C., Lee, H. J. and Davidson, N. (1974) J. Mol. Biol. 89, 631–646.
[8] Lindahl, L., Jaskunas, S. R., Dennis, P. P. and Nomura, M. (1975) Proc. Natl. Acad. Sci. USA 72, 2743–2747.
[9] Jørgensen, P. and Fiil, N. P. (1976) in: Alfred Benzon Symposium IX; Control of Ribosome Synthesis (Kjeldgaard, N. O. and Maaløe, O., eds) pp. 370–382, Academic Press, New York.
[10] Jørgensen, P. (1976) Molec. Gen. Genet. 146, 303–307.
[11] Shimada, K., Weisberg, R. A. and Gottesman, M. E. (1972) J. Mol. Biol. 63, 483–503.
[12] Schrenk, W. J. and Weisberg, R. A. (1974) Molec. Gen. Genet. 137, 101–107.
[13] Davis, R. W., Simon, M. and Davidson, N. (1971) in: Methods in Enzymology (Grossman, L. and Moldave, K., eds) Vol. 21, pp. 413–428, Academic Press, New York.
[14] Hill, C. W. and Combriato, G. (1973) Molec. Gen. Genet. 127, 197–214.
[15] Yamamoto, M., Lindahl, L. and Nomura, M. (1976) Cell 7, 179–190.
[16] Hill, C. W. (1976) in: DNA Insertion Elements, Plasmids and Episomes (Bukhari, A., Shapiro, J. and Adhya, S., eds) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, in press.

[17] Miller, J. H. (1972) in: Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[18] Morgan, E. A. and Kaplan, S. (1976) Molec. Gen. Genet. 147, 179–188.
[19] Pedersen, S. and Kjeldgaard, N. (1972) Molec. Gen. Genet. 118, 85–91.
[20] Kirschbaum, J. B. and Konrad, E. B. (1973) J. Bacteriol. 116, 193–201.