Molec. Gen. Genetics 104, 351—359 (1969)

A Map of Four Genes Specifying Enzymes Involved in Catabolism of Nucleosides and Deoxynucleosides in *Escherichia coli*

S. I. Ahmad and R. H. Pritchard
Department of Genetics, University of Leicester, Leicester LEI 7RH

Received May 22, 1969

Summary. Four genes specifying the enzymes thymidine phosphorylase, purine nucleoside phosphorylase, deoxyribomutase and deoxyriboaldolase were mapped by transduction with phage P1. All pairs show greater than 90 per cent co-transduction. The gene order was found to be *dra-tpp-drm-pup*, and the gene cluster was shown to lie between the *hsp* and *ser*B loci on the chromosome map of *Escherichia coli*.

The existence of a cluster of four genes specifying enzymes [thymidine phosphorylase E.C. 2.4.2.4, purine nucleoside phosphorylase E.C. 2.4.2.1, deoxyriboaldolase E.C. 4.1.2.4 and deoxyribomutase (Manson and Lampen, 1951)] involved in the catabolism of deoxynucleosides and nucleosides located close to the left of the *thr* locus in both *Escherichia coli* and *Salmonella typhimurium* is now well documented (Alikhanian *et al.* 1966; Okada, 1966; Dale and Greenberg, 1967; Ahmad *et al.*, 1968; Eisenstark *et al.*, 1968; Lomax and Greenberg, 1968). A question of interest concerning this gene cluster is the biological role of the enzymes specified. One possibility might be that they are part of a larger system of enzymes whose function is the complete degradation of foreign DNA entering the cell either by conjugation or as a result of phage infection. This possibility was suggested to us by the fact that the gene loci responsible for the initial steps in breakdown of foreign DNA — the *hsp* genes (Arber, 1968; Meselson *et al.*, 1968) — are located in the same region of the linkage map of *E. coli* (see Taylor, and Trotter, 1967). Thus it was conceivable that all of these genes might be components of a single operon.

In order to examine this possibility and as part of a more detailed study of this gene cluster we have determined the relative locations of the four genes with respect to each other, to the *hsp* locus, and to the *ser*B locus, which also lies in the same region of the chromosome (Glover and Colson, 1969).

Materials and Methods

Bacterial Strains. These are listed in Table 1. It has been shown previously that the *dra*$^-$, *drm*$^-$ and *pup*$^-$ strains lack the enzymes specified by these genes (Ahmad *et al.*, 1968; Barth *et al.*, 1968). The *tpp*$^-$ mutants were isolated more recently. They have negligible thymidine phosphorylase activity after growth in presence or absence of thymidine as inducer.

Isolation of mutants. Azu^r (azauracil resistant) strains were isolated initially as colonies resistant to 5-fluorouracil (0.25 μg/ml). They were subsequently found to be resistant to high concentrations of azauracil (100 μg/ml) and to FU (2.5 μg/ml). We assume that these mutants lack uracil phosphoribosyltransferase (E.C. 2.4.2.9) activity, although direct evidence for this has not been obtained. They still possess thymidine phosphorylase and purine nucleoside

Table 1. *Genotype and derivation of strains used*

Strain	Mating type	*thr*	*leu*	*thy*	*met*	*thi*	*ser*	*lac*	*pup*	*tpp*	*dra*	*drm*	*azu*	*str*	*λ*	*hsp*	Origin
226	Hfr R4	+	+	+	−	+	+		+	+	+	+	S	S	+	+	see HAYES (1964)
P227	Hfr R4	+	+	+	−	+	+		+	+	+	+	R	S	+	+	from 226 (see Methods)
P228.2	Hfr R4	+	+	+	−	+	+		−	+	+	+	R	S	+	+	from P227 (see Methods)
SA6	Hfr R4	+	+	+	−	+	+		+	−	+	+	R	S	+	+	from P227 (see Methods)
P152	Hfr H	+	−	−	+	−	+	−	+	+	+	−	S	S	−		see HAYES (1964)
SA10	Hfr H	+	+	−	+	−	+	−	+	+	+	−	S	S	−		P(P228.2) × P152
CR34	F⁻	−	−	−	+	−	+	−	+	+	−	+	S	S	−		see OKADA *et al.* (1961)
SA48	F⁻	−	−	+	+	−	+	−	+	+	−	+	S	S	−		P(P228.2) × CR34
4K	F⁻	−	−	+	+	−	−		+	+	+	+	S	R	−	−	from Dr. S. GLOVER
SA49	F⁻	+	+	+	+	−	−		+	+	+	−	S	R	−	−	SA10 × 4K by conjugation
SA37	F⁻	+	+	+	+	−	−		+	+	+	−	R	R	−	−	from SA49
SA41	F⁻	+	+	+	+	−	+		−	+	+	−	R	R	−	−	P(P228.2) × SA37
SA46	F⁻	+	+	+	+	−	+		−	+	−	+	R	R	−	−	P(SA48) × SA41
SA47	F⁻	+	−	+	+	−	+		+	−	+	+	S	S	−		P(SA6) × SA48
SA48.1	F⁻	−	−	+	+	−	+	−	+	−	−	+	S	S	−		thymidine resistant derivative of SA48
SA48.2	F⁻	−	−	+	+	−	+	−	+	−	−	+	S	S	−		As SA48.1

Gene symbols as given by TAYLOR and TROTTER (1967) with the following additions and modifications: *azu*^{r}, azauracil resistance; *drm* = deoxyribomutase; *dra* = deoxyribaoldolase (*dra* and *drm* are synonymous with the locus *thy*R in TAYLOR and TROTTER, 1967); *pup* = purine nucleoside phosphorylase.

phosphorylase activities, and remain sensitive to high (10 μg/ml) FU concentrations, presumably because one or both of the following syntheses are possible:

$$\text{FU} + \text{Rib-1-P} \xrightarrow[\text{phosphorylase}]{\text{uridine}} \text{FU-Rib} + \text{P}_i \quad (1)$$

$$\text{FU} + \text{dRib-1-P} \xrightarrow[\text{phosphorylase}]{\text{thymidine}} \text{FU-dRib} + \text{P}_i \quad (2)$$

They can be sensitised to lower FU concentrations if deoxyadenosine (100 μg/ml) is added to the growth medium. This is presumably because deoxyadenosine provides a supply of dRib-1-P via purine nucleoside phosphorylase (BOYCE and SETLOW 1962) vis:

$$\text{deoxyadenosine} + \text{P}_i \xrightarrow[\text{phosphorylase}]{\text{purine nucleoside}} \text{dRib-1-P} + \text{adenine} \quad (3)$$

thus potentiating the synthesis of FUdRib via reaction (2). If this interpretation is correct then *pup*$^-$ and *tpp*$^-$ mutants should be found among resistant colonies appearing on plates containing a mixture of FU (2.5 μg/ml) and deoxyadenosine (100 μg/ml).

Resistant colonies were isolated in this way and classified nutritionally (see Table 2) and enzymologically. A proportion were found to be either *tpp*$^-$ or *pup*$^-$. The *pup*$^-$ mutant used in this work and the mutant *tpp-1*$^-$ (SA6) were isolated by this technique. The *tpp*$^-$ mutants SA48.1 and SA48.2 were obtained by making use of the fact that *dra*$^-$ strains are sensitive to thymidine (BREITMAN and BRADFORD, 1967). Double mutants *dra*$^-$ *tpp*$^-$ and *dra*$^-$ *drm*$^-$ will be resistant to thymidine, since sensitivity results, directly or indirectly, from the accumulation of dRib-5-P (BEACHAM *et al.*, 1968) which is not formed from thymidine in *tpp*$^-$ or *drm*$^-$ strains.

Table 2. *Growth properties of strains with different genotypes*

Genotypes					Sole carbon source			Inhibition by		
pup	*drm*	*tpp*	*dra*	*azu*	Thymidine	Adenosine	Deoxyadenosine	Thymidine	Deoxyadenosine	FU+deoxyadenosine
+	+	+	+	R	+	+	+	R	R	S
−	+	+	+	R	+	−	−	R	R	R
+	−	+	+	S	−	−	−	R	R	S
+	+	−	+	R	−	+	+	R	R	R
+	+	+	−	S	−	+	−	S	S	
+	−	+	+	R	−	−	−			S
−	−	+	+	R	−					R
+	−	−	+	R		−				R
+	+	−	−	S	−		−	R	S	
+	−	+	−	S	−		−	R	R	

Only those responses which have been confirmed experimentally are shown in the Table. H. O. KAMMEN (personal communication) and A. MUNCH-PETERSEN (personal communication) have found that deoxyribomutase is also responsible for the interconversion of Rib-1-P and Rib-5-P; hence the inability of *drm*$^-$ strains to grow on adenosine as sole carbon source.

P1 Transduction. GLOVER's (1962) technique, with the following modifications was used. Cultures of the recipient strain were resuspended in nutrient broth supplemented with $CaCl_2$ (10^{-3} M). Phage was added (multiplicity of infection 0.02) and the mixture incubated at 37°C for 20 min. Transductants were selected by plating the mixture on M9 synthetic agar containing no $CaCl_2$ with sodium citrate (0.25%) and either glucose (20 mM) or an appropriate nucleoside or deoxynucleoside (5 mM) as sole carbon source.

Classification for hsp. Suspensions of cultures of strains to be classified were spotted on tryptone agar (Davis Japanese agar 11 g, Oxoid tryptone 10 g, NaCl 5 g, water 1 L) plates. After these had dried a drop of a suspension of a virulent mutant of bacteriophage λ grown on an *hsp*$^-$ host was placed on the centre of the spot using a phage concentration such that after overnight incubation at 37°C *hsp*$^-$ strains gave a clear zone and *hsp*$^+$ strains showed only isolated plaques.

Results

In two experiments (Table 3) the co-transduction frequency between *dra* and *thr* was found to be 29 per cent and 54 per cent, but that between *dra* and *tpp* was more than 95 per cent. Thus *dra* and *tpp* are more closely linked to each other than either of them is to *thr*. If we assume that transductants requiring a minimum of four exchanges between donor and recipient DNA will occur significantly less frequently than those requiring a minimum of two, then the discrepancy between the numbers of transductants in the reciprocal classes *dra*$^+$ *tpp*$^+$ and *dra*$^-$ *tpp*$^-$ indicates that the order of the three genes is *dra-tpp-thr*.

Table 3

Cross	Donor	Recipient	Selected transductants	Unselected markers			Total
					tpp$^+$	*tpp*$^-$	
1	SA6 *tpp*-1$^-$	SA48 *dra*$^-$ *thr*$^-$	*thr*$^+$	*dra*$^+$	0	503	
				dra$^-$	425	4	932
2	SA6 *tpp*-1$^-$	SA48 *dra*$^-$ *thr*$^-$	*dra*$^+$	*thr*$^+$	0	72	
				thr$^-$	8	165	245

Table 4

Cross	Donor	Recipient	Selected transductants	Unselected markers			Total
					thr$^+$	*thr*$^-$	
3	P228.2 *pup*$^-$	SA48.1 *tpp*-2$^-$ *dra*$^-$ *thr*$^-$	*dra*$^+$ *pup*$^+$	*tpp*$^+$	5	68	
				tpp$^-$	5	40	128
4	P228.2 *pup*$^-$	SA48.2 *tpp*-3$^-$ *dra*$^-$ *thr*$^-$	*dra*$^+$ *pup*$^+$	*tpp*$^+$	14	211	
				tpp$^-$	3	27	255
5	P228.2 *pup*$^-$	SA48.2 *tpp*-3$^-$ *dra*$^-$ *thr*$^-$	*dra*$^+$ *tpp*$^+$	*pup*$^+$	1	12	
				pup$^-$	59	46	128

The location of *pup* with respect to these three loci can be deduced from the data given in Table 4. In the four-factor crosses numbered 3 and 4 both *tpp* and *thr* were unselected markers. In both crosses the *tpp*$^+$:*tpp*$^-$ ratio among *thr*$^+$ transductants is not significantly different from the corresponding ratio among *thr*$^-$ transductants. This apparent absence of linkage implies that *tpp* does not lie between *thr* and *dra* or *thr* and *pup*. In cross 5, on the other hand, the ratio *pup*$^+$:*pup*$^-$ is significantly different among *thr*$^+$ and *thr*$^-$ transductants implying that *pup* does lie between *thr* and *dra* or *thr* and *tpp*. The data from these three experiments are therefore compatible either with the gene order *dra-tpp-pup-thr* or with the order *tpp-dra-pup-thr*. In addition, the fact that the proportion of *tpp*$^+$ transductants is higher in cross 4 than it is in cross 3 implies that *tpp*-3 is closer to *dra* than is *tpp*-2.

Data from four three-factor crosses are given in Table 5. They provide evidence that *drm* lies between *dra* and *pup* but do not define the position of *tpp* with

Table 5

Cross	Donor	Recipient	Selected transductants	Unselected markers				Total	Compatible gene order[a]
				dra$^+$	*dra*$^-$	*pup*$^+$	*pup*$^-$		
6	P152 *drm*$^-$	SA46 *pup*$^-$ *dra*$^-$	*drm*$^+$ *pup*$^+$	11	99			110	*drm-dra-pup* or *dra-drm-pup*
7	SA48 *dra*$^-$	SA41 *pup*$^-$ *drm*$^-$	*dra*$^+$ *drm*$^+$			107	20	127	*drm-pup-dra* or *dra-drm-pup*
8	SA6 *tpp*$^-$	SA46 *pup*$^-$ *dra*$^-$	*tpp*$^+$ *dra*$^+$			24	135	159	*dra-pup-tpp* or *dra-tpp-pup*
9	SA6 *tpp*$^-$	SA41 *pup*$^-$ *drm*$^-$	*tpp*$^+$ *drm*$^+$			52	8	60	*tpp-pup-drm* or *tpp-drm-pup*

[a] The inverse of each gene order shown is also compatible with the data.

Table 6

Cross	Donor	Recipient	Selected transductants	Unselected markers				
					drm$^+$ *hsp*$^+$	*drm*$^-$ *hsp*$^+$	*drm*$^+$ *hsp*$^-$	*drm*$^-$ *hsp*$^-$	Total
10	P228.2 *pup*$^-$	SA37 *ser*$^-$ *drm*$^-$ *hsp*$^-$	*ser*$^+$	*pup*$^+$	0	3	0	21	
				pup$^-$	17	0	274	4	319
11	SA6 *tpp*-1$^-$	SA37*ser*$^-$ *drm*$^-$ *hsp*$^-$	*ser*$^+$	*tpp*$^+$	1	1	5	12	
				tpp$^-$	16	0	219	1	255
						thr$^+$	*thr*$^-$		
12	SA48 *dra*$^-$ *thr*$^-$	SA37 *ser*$^-$ *drm*$^-$	*ser*$^+$			*drm*$^+$	137	78	
						drm$^-$	5	2	222

[a] The selected transductants were not scored with respect to *dra*.

respect to the other three loci unambiguously. The gene orders given in the last column of this table are those compatible with the data if we are correct in assuming that transductants requiring four exchanges will be significantly less frequent than those requiring two. Comparison of these possible orders shows that two gene arrangements are consistent with the data. They are *dra-tpp-drm-pup* and *dra-drm-pup-tpp* (or the inverse of these orders). If we now compare these gene orders with those compatible with the data in Tables 3 and 4, we find that only one gene order is consistent with all the data reported. It is *dra-tpp-drm-pup-thr*.

The data in Table 6 provide evidence bearing on the location of *ser*B. Among *ser*$^+$ transductants in cross 10 there were only four recombinants between *drm* and *pup*, all of which had the genotype *drm*$^-$ *pup*$^-$. Transductants of this type require two exchanges and the absent reciprocal recombinant class four exchanges if the gene order is *drm-pup-ser*. Similarly in cross 11 there were seven recombinants between *tpp* and *drm*, six being *tpp*$^+$ *drm*$^+$ and one *tpp*$^-$ *drm*$^-$. By an analogous argument the indicated gene order is *tpp-drm-ser*.

Finally, in cross 12 it was found that the *drm*$^+$:*drm*$^-$ ratio was not significantly different among *thr*$^+$ and *thr*$^-$ transductants, implying that *drm* is not located between *ser* and *thr*. The data also show that *drm* is closer to *ser* than it is to *thr* and the indicated gene order is therefore *drm-ser-thr*.

The only gene order compatible with the data from all twelve crosses is: *dra-tpp-drm-pup-ser-thr*.

If this order has been correctly determined we should expect that the *hsp* locus should lie to the left of *dra* since GLOVER and COLSON (1969) have shown that this locus lies to the left of *ser* and gives only about 20 per cent co-transduction with it. The data in Table 6 seem to be incompatible with this order. Among *ser*$^+$ transductants the proportion which carry the donor allele *drm*$^+$ is smaller among those which are *hsp*$^+$ than it is among those which are *hsp*$^-$ in both crosses. This result by itself would indicate that the gene order was *hsp-ser-drm*. In neither case is the difference in the proportion of *drm*$^+$ transductants among *hsp*$^+$ and *hsp*$^-$ classes statistically significant however. In addition, the co-transduction frequency between *hsp* and *ser* found in crosses 10 and 11 (6.3 and 7.2 per cent respectively) is lower than that found by GLOVER and COLSON (1969). They observed about 20 per cent co-transduction although the *hsp*$^-$ *ser*$^-$ strain used by them was the parent of the one used here. It is therefore possible that the presence of the additional mutations being mapped by us in these strains interfered with the expression of the *hsp* phenotype as determined under the conditions described under Methods.

Finally, we should note that the gene order *dra-tpp-thr* given by our data differs from that deduced by LOMAX and GREENBERG (1968) who concluded that the order was *tpp-dra-thr*. This latter order was based on the presence of only two transductant colonies in one recombinant class and none in the reciprocal class, however, and the data do not therefore provide unequivocal evidence for a gene order different from that proposed here.

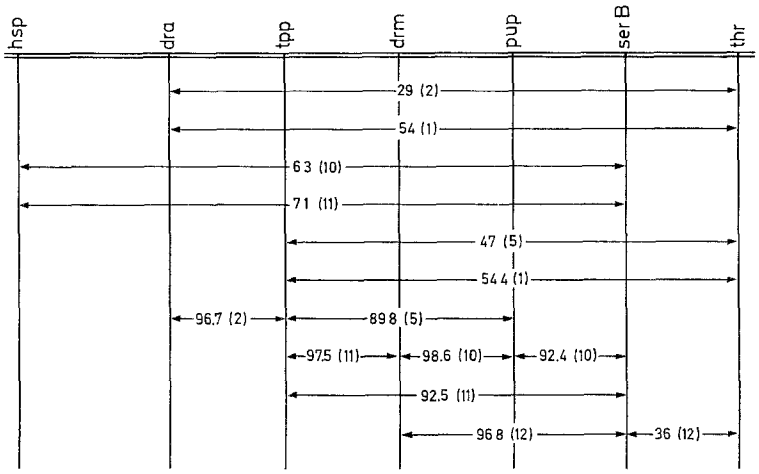

Fig. 1. Linkage Map. The gene order given is based on the criteria described in the text. The numbers in brackets after each of the given co-transduction frequencies refer to the cross number from which the frequencies are calculated. Co-transduction frequencies were not calculated from crosses in which the selected transductants required an exchange between a donor and recipient marker to avoid possible complication due to negative interference (Gross and Englesberg, 1959)

Discussion

The very close linkage of the four genes specifying enzymes involved in the catabolism of nucleosides suggests that they may be components of a single unit of transcription. Consistent with this possibility, these four enzymes are inducible and share the same inducer, deoxyribose-5-phosphate (Rachmeler, Gerhardt and Rosner, 1961; Breitman and Bradford, 1967, 1968; Barth *et al.*, 1968). On the other hand, although the aldolase and thymidine phosphorylase are probably coordinately induced, and the mutase and purine nucleoside phosphorylase likewise, the induction ratio of the two pairs seems to be different. In addition, Kammen (personal communication) finds that the mutase is optimally induced by ribosides whereas thymidine phosphorylase and aldolase are not. It therefore seems more likely on present evidence that the two pairs of enzymes are components of two adjacent operons, the gene order (Fig. 1) being consistent with such a possibility. The properties of an interesting mutant described by Munch-Petersen (1968) also appear to be more easily explainable in terms of a two-operon model. This mutant lacks detectable mutase activity, but unlike the majority of *drm*$^-$ strains (which have low levels of the other three enzymes, and are not inducible by deoxyribosides since their catabolism to dRib-5-P is blocked) this mutant is apparently constitutive for both thymidine phosphorylase and aldolase. The level of these enzymes is less than that normally found under optimal inducing conditions however. This mutant thus has properties which are similar to some operator constitutive (o^c) mutations of the *lac* operon (Jacob *et al.*, 1964), but on a single operon model can hardly be a mutant of this type since the *drm* gene lies between *pup* and *tpp*. On a two operon model, a mutant

with these properties might arise as a result of a deletion encompassing the operator region of the *dra-tpp* operon and extending into the *drm* gene, thus fusing the two operons together and bringing the *dra-tpp* operon under control of the operator of the *pup-drm* operon. Such an interpretation would also imply that both operons are transcribed in a counter-clockwise direction with respect to the standard linkage map (Taylor and Trotter, 1967). It seems unlikely that very close linkage between these four genes is fortuitous, and if they belong to more than one operon other reasons for this close linkage will need to be sought. The possibility that the linkage to the *hsp* loci also is not fortuitous seems less likely since the co-transduction frequency is less than 20 per cent.

This work is supported by Grant No. B/SR/3113 from the Science Research Council.

References

Ahmad, S. I., P. T. Barth, and R. H. Pritchard: Properties of a mutant of *Escherichia coli* lacking purine nucleoside phosphorylase. Biochim. biophys. Acta (Amst.) **161**, 581 (1968).

Alikhanian, S. E. I., T. S. Iljina, E. S. Kalioeva, S. V. Kameneva, and U. U. Sukhodolec: A genetic study of thymineless mutants of *E. coli* K 12. Genet. Res. 8, 83 (1966).

Arber, W.: Host-controlled restriction and modification of bacteriophage. 18th Symp. Soc. Gen. Microbiol., eds. L. V. Crawford and M. G. P. Stoker, p. 295. Cambridge: Cambridge University Press 1968.

Barth, P. T., I. R. Beacham, S. I. Ahmad, and R. H. Pritchard: The inducer of the deoxynucleoside phosphorylases and deoxyriboaldolase in *Escherichia coli*. Biochim. biophys. Acta (Amst.) **161**, 554 (1968).

Beacham, I. R., A. Eisenstark, P. T. Barth, and R. H. Pritchard: Deoxynucleoside-sensitive mutants of *Salmonella typhimurium*. Molec. Gen. Genetics **102**, 112 (1968).

Boyce, R. P., and R. B. Setlow: A simple method of increasing the incorporation of thymidine into the deoxyribonucleic acid of *Escherichia coli*. Biochim. biophys. Acta (Amst.) **61**, 618 (1962).

Breitman, T. R., and R. M. Bradford: The absence of deoxyriboaldolase activity in a thymineless mutant of *Escherichia coli* strain 15: A possible explanation for the low thymine requirement of some thymineless strains. Biochim. biophys. Acta (Amst.) **138**, 217 (1967).

— — Inability of low thymine-requiring mutants of *Escherichia coli* lacking phosphodeoxyribomutase to be induced for deoxythymidine phosphorylase and deoxyriboaldolase. J. Bact. **95**, 2434 (1968).

Dale, B., and G. R. Greenberg: Genetic mapping of a mutation in *Escherichia coli* showing reduced activity of thymidine phosphorylase. J. Bact. **94**, 778 (1967).

Eisenstark, A., R. Eisenstark, and S. Cunningham: Genetic analysis of thymineless mutants of *S. typhimurium*. Genetics **58**, 493 (1968).

Glover, S. W.: Valine-resistant mutants of *Escherichia coli* K-12. Genet. Res. **3**, 448 (1962).

—, and C. Colson: Genetics of host-controlled restriction and modification in *Escherichia coli*. Genet. Res. **13**, 227 (1969).

Gross, J., and E. Englesberg: Determination of the order of mutational site governing L-arabinose utilization in *Escherichia coli* B/r by transduction with phage P1 bt. Virology **9**, 314 (1959).

Hayes, W.: The Genetics of Bacteria and their Viruses, 2nd ed. Oxford: Blackwell Scientific Publications 1964.

Jacob, F., A. Ullman et J. Monod: Le promoteur, élément génétique nécessaire à l'expression d'un opéron. C. R. Acad. Sci. (Paris) **258**, 3125 (1964).

Lomax, M. S., and G. R. Greenberg: Characteristics of the *deo* operon: Role in thymine utilization and sensitivity to deoxyribonucleosides. J. Bact. **96**, 501 (1968).

Manson, L. A., and J. O. Lampen: The metabolism of hypoxanthine desoxyriboside in animal tissues. J. biol. Chem. **191**, 95 (1951).

MESELSON, M., and R. YUAN: DNA restriction enzyme from *E. coli*. Nature (Lond.) **217**, 1110 (1968).

MUNCH-PETERSEN, A.: Thymineless mutant of *E. coli* with deficiencies in deoxyribomutase and deoxyriboaldolase. Biochim. biophys. Acta (Amst.) **161**, 279 (1968).

OKADA, T.: Mutational site of the gene controlling quantitative thymine requirement in *Escherichia coli* K12. Genetics **54**, 1329 (1966).

— K. YANAGISAWA, and F. J. RYAN: A method for securing thymineless mutants from strains of *E. coli*. Z. Vererbungsl. **92**, 403 (1961).

RACHMELER, M., J. GERHARDT, and J. ROSNER: Limited thymidine uptake in *Escherichia coli* due to an inducible thymidine phosphorylase. Biochim. biophys. Acta (Amst.) **49**, 222 (1961).

TAYLOR, A. L., and C. D. TROTTER: Revised linkage map of *Escherichia coli*. Bact. Rev. **31**, 332 (1967).

Communicated by P. STARLINGER

Prof. Dr. R. PRITCHARD
Dept. of Genetics
Univ. of Leicester
Leicester, LEI 7RH, England