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An Antibiotic Dependent Conditional Lethal Mutant with a Lesion Affecting Transcription and Translation

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Summary. A conditional lethal mutant of *E. coli* was isolated which required the presence of either the RNA polymerase targeted antibiotic, rifampicin, or the ribosomally targeted antibiotic, kasugamycin, for survival. This mutant was characterised. The locus of the mutation responsible for the antibiotic dependent phenotype, *ridA*, was mapped at about 70.5 min on the chromosomal linkage map, between *argR* and *fabE*. The mutant was investigated as a candidate for a strain with a lesion in some cellular component acting on both RNA polymerase and the ribosome. A close interaction with RNA polymerase was evident from the interplay arising from the combination of *ridA* and various *rpoB* mutations as manifested in the phenotype. The ability of kasugamycin, but not other ribosomally targeted aminoglycoside antibiotics, to relieve the lethality due to the *ridA* mutation was an indication of the specificity in the interaction of the *ridA* gene product with the ribosome.

Introduction

Linkage between the cellular processes or transcription and translation is poorly understood in *Escherichia coli*, as in other organisms. This is in spite of observations (e.g. Chakrabati and Gorini 1977) that quite a tight coupling may exist between the two processes. The nature of the moiety mediating the interplay of phenotypes in this case, if any, has not been determined. In vitro experiments to identify components which are candidates for such a mediator have been carried out, however. On the basis of their ability to alter the properties of purified RNA polymerase, ribosomal elongation factor EFTs (Biebricher and Druminski 1980) and initiator tRNA fmet $tRNA_f^{met}$ (Debenham et al. 1980) have been proposed. In another approach, (Friedman et al. 1981) it has been reported that mutations in ribosomal protein S10 have an effect on the transcription of phage lambda genes.

In this paper we describe an approach to the study of linkage in *E. coli* by using conditional lethal mutants for which the selection procedures are well established in this laboratory, viz., antibiotic dependent mutants. These have been found in most cases to be double mutants, with the phenotype being the product of an antibiotic resistance mutation together with a dependentizing mutation; usually the dependentizing mutation is specific for a particular antibiotic, indicating that the lesion is in a cellular component intimately involved in the reaction with which the antibiotic interferes. What we sought were mutants with a lesion that was lethal to the cell, but not lethal to cells when sub-inhibitory concentrations of either a ribosomally targeted or an RNA polymerase targeted antibiotic were present in the medium. Rifampicin (Rif) was the RNA polymerase targeted antibiotic that was chosen. Therefore, representative strains dependent on a variety of ribosomally targeted antibiotics were tested for their ability to grow instead on sub-inhibitory concentrations of rifampicin. When no candidates were detected in this screening, rifampicin-dependent (Rif-D) mutants (Dabbs 1979) were tested for their ability to grow on ribosomally targeted antibiotics. Two mutants exhibiting the desired phenotype were obtained, one of which (PB67) was selected for further study.

The growth characteristics of Rif-D mutants have not been investigated, and no locus responsible for the dependent phenotype has been mapped. Therefore, these were determined for mutant PB67 considering it as a Rif-D mutant per se as well as a candidate for a strain with a lesion in some cellular component shared by the transcriptional and translational machineries of the cell.

Materials and Methods

Bacterial strains used in this work are listed in Table 1. Media, growth conditions, and genetic techniques are as previously described (Dabbs 1980). Rifampicin stock solutions were made up in methanol, at 100× the final concentration needed. Kasugamycin stock solutions (also 100×) were made up in water. The *fabE* mutation was monitored by its temperature-sensitive (Ts) phenotype; the *argR* mutation was monitored by ability to grow on 100 μg/ml canavanine (Kadner and Maas 1971).

Results

Isolation of Mutants. Isolation of Rif-D mutants from strain CP78 by delayed antibiotic challenge through an underlay technique has been previously reported (Dabbs 1979). The same method was used with cells of strain TA10 mutagenised with ethylmethanesulphonate. Ten Rif-D mutants, PB61-PB70, were obtained. They exhibited a variety of phenotypes with respect to temperature and rifampicin concentration. Their ability to be dependent for growth on a variety of ribosomally targeted antibiotics instead of rifampicin was tested. Strain TA10 (Table 1) was spectinomycin resistant, streptomycin and kasugamycin sensitive, so the sub-inhibitory concentrations of these antibiotics used were 100 μg/ml, 2 μg/ml, and 50μg/ml respective-

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ly. (Sub-inhibitory, as used in this paper, was any concentration at which sustained growth was possible, albeit perhaps more slowly than in the absence of antibiotic.)

The only ribosomally targeted antibiotic on which sustained growth was observed for any Rif-D mutant was kasugamycin: conditional lethal mutants PB67 and PB69 were able to grow when this aminoglycoside was in the medium instead of rifampicin. Mutant PB67 was selected for study since it grew well on defined media whereas mutant PB69 had requirements additional to those of parental strain TA10. These requirements could not be eliminated by transduction or reversion.

Rif Phenotype of Strain PB67. The minimum generation time of strain PB67 at 37° C was 70–75 min in rifampicin. Measurement of doubling time at 30° C and 42° C revealed that the mutant had a rather cold-sensitive phenotype (Table 2). The growth rate of the mutant in various concentrations of Rif was measured. Exponentially growing cells in medium containing an optimum concentration of antibiotic (determined to be about 10 μg/ml) were transfered to medium with any of a variety of Rif concentrations. The doubling time was measured after a lapse of 2 h to allow for adjustment of growth to the new conditions. The results are presented in Fig. 1. The curve shown is that for cells at 37° C; curves for 30° C and 42° C are qualitative-

Table 1. Strains used in this work

Strain	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
KL14	Hfr *thi-1 relA1*	K. Isono
KL228	Hfr *thi-1 leu-6 gal-6 lacY1 supE44*	K. Isono
L8	F⁻ *gltA5 fabE22 lct-1 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 strA20 tsx-57 tfr-5 supE44*	B. Bachmann
MA1030	Hfr *argR64 thi-1 purF1*	B. Bachmann
NF915	as strain CP78, but λ^s	N. Fiil
NF1803	Hfr *thi-1 recA1 srl*::tn10	S. Brown
TA10[a]	as strain CP78, *aroE24 rpsE nalA*	this work
VT26	Hfr *metB1 relA1 prmA*	Dabbs and Wittmann 1976
PB67	as strain TA10, *ridA1*	this work
PB67-1	as strain PB67, *aroE*⁺ *fabE22*	this work
PB67-2	as strain PB67-1, *argH*⁺	this work
PB67-3	as strain PB67, *argH*⁺	this work

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

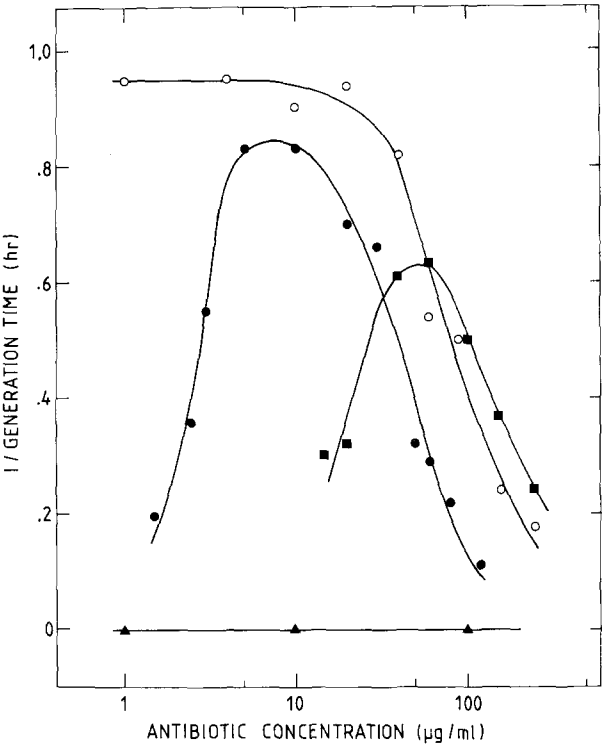

Fig. 1. Growth rate of mutant PB67 at various concentrations of antibiotic: (●) rifampicin; (■) kasugamycin; (▲) spectinomycin. (○) Response to rifampicin of a *ridA*⁺ transductant of mutant PB67. 1% methanol was also present in all rifampicin containing media; control experiments showed that mutant PB67 did not grow in medium supplemented with methanol alone. Determinations were made by measurement of optical absorption at 650 nm of cells growing in rich medium at 37° C

Table 2. Generation time and reversion frequency of strains with various combinations of *rpoB* and *ridA* alleles

Temperature (°C)	Antibiotic[a]	Minimum doubling time (min) relevant genotype				Reversion frequency of mutant PB67[b]
		rpoB⁺ *ridA*⁺	*rpoB*⁺ *ridA1*	*rpoB67 ridA*⁺	*rpoB67 ridA1*	
30	Rif	41	120	110	450	1.6×10^{-4}
37	Rif	23	40	60	73	1.2×10^{-4}
42	Rif	22	36	45	50	1.0×10^{-2}
37	Ksg	24	33	68	95	1.5×10^{-4}

[a] Rif 10 μg/ml; Ksg 60 μg/ml

[b] Calculated from number of cells plated. Since there was an approximate two-fold increase in cell number after antibiotic deprivation (Fig. 2), the true reversion frequency would be about half the figures given

ly the same, differing only in size as a reflection of the temperature dependent growth rate.

Response of Strain PB67 to Other Antibiotics. The ability of strain PB67 to grow on kasugamycin (Ksg) had been detected by spotting of colonies of the strain on plates containing subinhibitory concentrations of this antibiotic. The requirement for Ksg was investigated systematically in the same way as for Rif. These data are presented in Fig. 1. They indicated that the optimal concentration was about 60 μg/ml Ksg, and strain PB67 then had a doubling time of about 95 min.

Growth curves of the mutant in Rif, and upon transfer to other antibiotics, are shown in Fig. 2. No sustained growth was possible at any concentration of streptomycin or spectinomycin (Fig. 2), nor paromomycin (not shown). Transfer of exponentially growing cells to medium lacking any antibiotic resulted in cessation of increase in total cell mass after about 150 min (i.e. 2 generations). Viable counts increased in parallel with OD_{650} for cells deprived of antibiotic (Fig. 2), for about 75 min (1 generation) then started to decrease. Therefore, removal of Rif was bacteriocidal to Rif-D mutant PB67. Cells of this strain which were depending for Ksg for growth showed similar behaviour upon removal of this antibiotic.

Streptomycin dependent strains, the best characterised dependent mutants, are generally also able to grow on paromomycin instead of streptomycin. However, for cells pre-grown in medium with one antibiotic, transfer to the other antibiotic or to a mixture of streptomycin and paromomycin is bacteriocidal (Gorini et al. 1967). The effect of transfer of cells of strain PB67 between Ksg and Rif-containing media was determined. Irrespective of which antibiotic the mutant was growing in before transfer, survival was about 95%.

The behaviour of strain PB67 in media containing both Rif and Ksg was studied. The optimal concentrations had been found to be 10 μg/ml and 60 μg/ml respectively (Fig. 1); the doubling times (at 37° C) were then about 72 min and 95 min. Cells growing in 60 μg/ml Ksg to which increasing amounts of Rif were added showed increments in growth rate up to the point when there was 60 μg/ml Ksg, 4 μg/ml Rif in the medium. At this point, the doubling time was similar to that for cells growing in 10 μg/ml Rif. With further increase in Rif concentration, the generation time lengthened. Cells growing in 4 μg/ml Rif showed faster growth if 20 μg/ml or 40 μg/ml Ksg was present, but once the aminoglycoside was in excess of 60 μg/ml growth slowed. Therefore, the two antibiotics Rif and Ksg acted additively to support growth of mutant PB67, although the fastest growth rate was never more than in optimal concentrations of Rif alone.

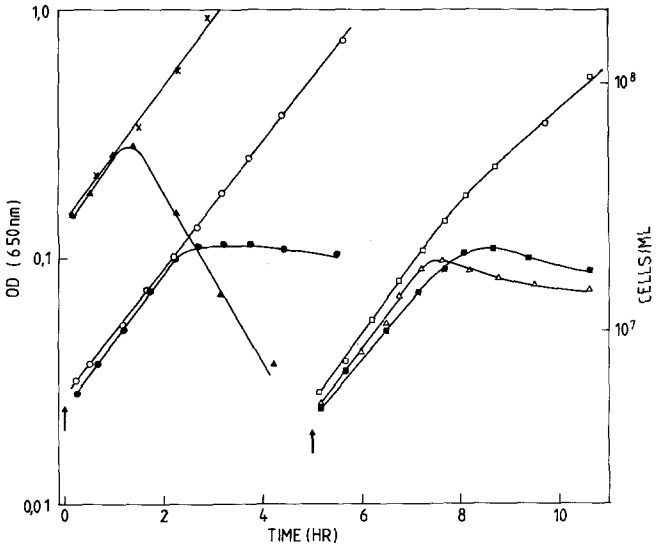

Fig. 2. Growth curves of mutant PB67 in various antibiotics, monitored at 650 nm. Cells were growing in: (○) rifampicin; (●) deprived of rifampicin, no other antibiotic present; (□) kasugamycin; (△) streptomycin; (■) spectinomycin. Viable counts were measured for the culture growing in rifampicin (X); and deprived of rifampicin (▲). Cells growing exponentially in medium containing 10 μg/ml rifampicin were transfered at the times indicated by arrows. Antibiotic concentrations: rifampicin, 10 μg/ml; kasugamycin, 60 μg/ml; streptomycin, 2 μg/ml; spectinomycin, 100 μg/ml

Cell Morphology. Viable counts versus OD_{650} were plotted for cells of strain PB67 growing in Rif or Ksg-containing medium. Combined results from several experiments were that at any given OD_{650} there were about 1.4 times the number of cells in Rif medium versus Ksg medium. The cells growing on the antibiotic less able to satisfy the conditional lethal lesion of mutant PB67 (as assayed by growth rate) were therefore larger. This was in accordance with the observation that *E. coli* cells sometimes become elongated when their physiology is stressed: mutants selected for a Ksg-D phenotype (Dabbs 1978) showed an elongated or filamentous morphology in the light microscope (Looman and Dabbs, unpublished results), and antibiotic deprivation exacerbated the effect. We inspected cells of strain PB67 grown in Rif or Ksg-containing media, or deprived of antibiotic, under the light microscope. No elongated or filamentous morphology was seen for any of the treatments.

Genetics. It was previously reported (Dabbs 1979) that the phenotype of Rif-D mutants was the product of at least two mutations, a Rif-R mutation at the *rpoB* locus plus a dependentizing mutation mapping away from *rpoB*. A lysate (phage P1*vir* was used for all transductions) of an $argH^+$ derivative of strain PB67 was used as donor of $argH^+$ into parental strain TA10. A Rif-R mutation was acquired by about 30% of transductants, a typical frequency for *argH-rpoB* cotransduction. Determination of the generation time of a Rif-R transductant revealed that the presence of this particular *rpoB* allele (termed *rpoB67*) slowed growth considerably and conferred a somewhat cold-sensitive phenotype.

Crosses between mutant PB67 and various Hfrs indicated that the dependentizing mutation (termed *ridA1; rid* from *r*ifampicin *d*ependent) was not near *rpoB*. Matings with Hfrs KL14 and KL228 pointed to *ridA* being near *aroE*, at about 71 min on the *E. coli* chromosomal linkage map. A lysate of strain CP78 was used to donate $aroE^+$ into mutant PB67, and 5 of 40 transductants checked had lost the Rif-D phenotype. Spectinomycin phenotype was also scored (since strain PB67 was Spc-R at the *rpsE* locus). The pattern of segregation of *ridA* and *rpsE* alleles gave evidence that these loci were on opposite sides of *aroE*. Strain CP78 was also used as donor of $ridA^+$ into mutant PB67 by selecting for ability to grow at 30° C in the absence of Rif on minimal medium (conditions that minimised the frequency at which spontaneous revertants arose). Only 5 of 112 $ridA^+$ transductants were $rpsE^+$. Therefore, *ridA* was not near the *str-spc* cluster of genes.

To map *ridA* more precisely, a three point cross was made with *fabE* strain L8 (Table 1) as donor of $aroE^+$ (for markers in this region of the chromosomal linkage map, see Bachmann and Low 1980). The results are shown in Table 3 (cross 1). They

Table 3. P1 mediated crosses between *ridA* and *argR*, *fabE*, and *aroE*

	Cross	Selected marker (no. scored)	Transductant characteristics[a]			Number of transductants (% of total)
			aroE	*fabE*	*ridA argR*	
1	*aroE*$^+$*fabE ridA*$^+$(donor)	*aroE*$^+$	+	+	+	1 (1)
	aroE fabE$^+$*ridA* (recipient)	(80)	+	+	−	63 (79)
			+	−	+	5 (6)
			+	−	−	11 (14)
2	*aroE fabE*$^+$*ridA*$^+$(donor)	*ridA*$^+$	+	+	+	22 (55)
	aroE$^+$*fabE ridA* (recipient)	(40)	−	+	+	(2 (5)
			+	−	+	16 (40)
			−	−	+	0 (0)
3	*fabE*$^+$*ridA*$^+$*argR* (donor)	*fabE*$^+$	+	+	+	17 (15)
	fabE ridA argR$^+$(recipient)	(110)	+	+	−	77 (70)
			+	−	+	14 (13)
			+	−	−	2 (2)

[a] + indicates wild type, − indicates mutant allele

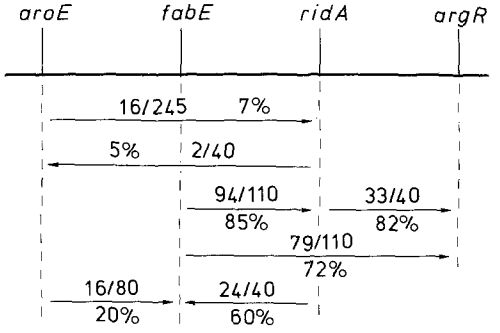

Fig. 3. The region of the *E. coli* chromosomal linkage map around *ridA*. The figures summarise the data from all transduction experiments made involving *aroE*, *fabE*, *ridA*, and *argR* alleles. Arrow points to the marker being scored

indicated a gene order *aroE-fabE-ridA*; 16 of 80 *aroE*$^+$ transductants were *fabE* whilst 6 of 80 were *ridA*$^+$. Strain PB67-1 (an *aroE*$^+$*fabE ridA* transductant from cross 1) was used as recipient and strain TA10 (*aroE fabE*$^+$*ridA*$^+$) was used as donor of *ridA*$^+$. Table 3 (cross 2) shows results which confirm that *ridA* is further from *aroE* than *fabE*.

Three point crosses were made between *fabE*, *argR*, and *ridA* alleles. Strain PB67-1 was transduced to *argH*$^+$, since testing for the *argR* phenotype using canavanine resistance (Kadner and Maas 1971) required the absence of arginine from media. This *argH*$^+$ strain (PB67-2) was transduced to *fabE*$^+$ with a lysate of strain MA1030 (Table 1), and colonies were checked for *ridA* and *argR* alleles. Segregation of markers (Table 3, cross 3) indicated that *ridA* was nearer *fabE* than *argR* (85% and 72% cotransduction with *fabE* respectively). The data from this and previous crosses are also summarised in Fig. 3. The same strains as above were also used in a selection for *ridA*$^+$ but a complete determination of phenotype could not be made for all transductants since the *fabE* mutation was a Ts mutation; *ridA*$^+$*fabE* recombinants therefore had to be checked for *argR* phenotype at 30° C, but at this temperature the canavanine resistant phenotype due to the *argR* mutation was not clear.

The order of loci *aroE-fabE-ridA-argR* was however supported by results of an earlier transduction (see above). In this, 7% of *aroE*$^+$ transductants had become *ridA*$^+$. When strain MA1030 was donor of *aroE*$^+$ to *argH*$^+$ derivatives of either strain PB67 (PB67-3) or parental strain TA10, no cotransduction between *aroE* and *argR* was found (0 of 334). Strain MA1030 was also used as donor of *ridA*$^+$ to strain PB67-3, and the segregation of markers pointed to *ridA* being situated between *aroE* and *argR*. About 1.5% of transductants from this cross were *aroE*$^+$*argR*, which contrasted with the failure to detect this class of recombinant when *aroE*$^+$ was selected for.

To determine whether the *rpoB67* and *ridA1* mutations present in mutant PB67 were not only necessary but also sufficient for the observed phenotype, the *rpoB* mutation was transduced into strain TA10. An *aroE*$^+$ derivative of strain PB67 was then used to introduce the *ridA1* allele into the *rpoB67* recipient, in the presence of 20 μg/ml Rif. Of 71, 3 (about 4%) transductants were Rif-D. Therefore, the two mutations identified in mutant PB67 were sufficient to give the phenotype.

In order to find out if the Rif-D phenotype was present when the *ridA1* mutation was in an *rpoB*$^+$ (Rif-S) context, a lysate of the same donor as in the previous experiment was used to obtain *aroE*$^+$ transductants of strain TA10. When 3 μg/ml rifampicin (a sub-inhibitory concentration for Rif-S strains) was present in selective plates, 2 of 54 transductants were Rif-D. Therefore, the *rpoB67* mutation only served to broaden the range of antibiotic concentration at which the strain would grow; its presence was not necessary for the dependent phenotype to be expressed. The *ridA1* mutation was also transduced into an *aroE* derivative of strain JC411. In this quite different strain of *E. coli* the Rif-D phenotype was also expressed; therefore, a particular strain background was not necessary for the phenotype to be in evidence.

Other rpoB Alleles and ridA1. As was stated above, it was proposed that in dependent mutants the dependentizing mutation interacted with, or at a point close to, the cellular component or reaction that the antibiotic exerted its effect on. In the case of rifampicin, this was RNA polymerase. Interaction of the *ridA1* allele with mutant alleles of the *rpoB* gene (coding for the β subunit of RNA polymerase) was apparent from two experiments. Exposure of an exponentially growing culture of an *rpoB*$^+$ *ridA1* strain (in 4 μg/ml Rif, when the phenotype was stable) to 100 μg/ml Rif led to the selection of *rpoB* Rif-R mutants. In many of these, the Rif-D phenotype was reverted or sup-

pressed. To ensure that we were not observing reversion just due to instability of the dependent phenotype, we performed another experiment. Strain JC411 was used to isolate Rif-R mutants with a variety of phenotypes. These *rpoB* mutations were transduced into a *ridA1 aroE*$^+$ derivative of strain TA10. In some of the resulting *ridA1 rpoB* strains the Rif-D phenotype was maintained, in some the minimal antibiotic concentration necessary for growth was increased, and in some the Rif-D phenotype was no longer present. We did not test whether this was because particular *rpoB* alleles suppressed the Rif-D phenotype arising from the *ridA1* mutation, or whether the presence of the two mutant alleles in the same strain was not possible and the *ridA* mutation reverted. The interaction between *ridA1* and *rpoB* alleles was often different even for *rpoB* mutations that were phenotypically indistinguishable on their own. These observations suggested a quite intimate interaction between the product of the *ridA* locus and RNA polymerase.

The absence of heterogeneity in mutant alleles at the *ksgA* locus (confering high level Ksg resistance) precluded a comparable investigation with this ribosomally targeted antibiotic. However, addition of Ksg to *ridA* strains growing in 60 μg/ml Ksg to give a final concentration of 240 μg/ml resulted in mutants with a heterogeneity of phenotypes, similar to that which was observed when Rif was added to cells growing in medium with low concentrations of that antibiotic. (Strains of *E. coli* used in this work grew quite well in the presence of Ksg below 100 μg/ml, but not significantly above 150 μg/ml.) These kasugamycin mutants were not analysed further, but were taken as perhaps indicating an interaction between the *ridA* gene product and the ribosome, the target of Ksg action.

ridA1 was Recessive. With a view to the future cloning of the *ridA* gene, *ridA1* was transduced into strain NF915 (a lambda-sensitive derivative of strain CP78). The resulting strain was made *argH*$^+$ then *argG* as previously described (Dabbs 1980), and *aroE* was transduced from strain AT2472 using the nearby Spc-R (*rpsE*) marker. *recA* was introduced from strain NF1803 using the tetracycline-resistant Tn10 transposon inserted nearby. The resulting strain was crossed with a methionine auxotroph of strain KL14, obtained by auxotroph enrichment. Selection on plates lacking arginine, methionine, and aromatic amino-acids gave strains merodiploid in the *argG-aroE* portion of the chromosome. These merodiploids no longer possessed a Rif-D phenotype. Therefore, the phenotype arising from the *ridA1* mutation was recessive to that of *ridA*$^+$.

Discussion

A conditional lethal mutant of *E. coli* requiring the presence in the medium of either the RNA polymerase targeted antibiotic, rifampicin, or the ribosomally targeted antibiotic, kasugamycin, for survival was isolated and characterised. It was studied as a candidate for a mutant with the lesion in a cellular component linking transcription and translation. In this mutant, PB67, the locus of the mutation responsible for the conditional lethal phenotype, *ridA*, was located at about 70.5 min on the chromosomal linkage map, between *argR* and *fabE*. The *ridA1* mutation was both necessary and sufficient for the conditional lethal phenotype.

In this region of the map (Bachmann and Low 1980), *prmA*, *grpD*, and *envB* are the only other loci associated with a property that could be tested. By virtue of the inability of *ridA* strains to grow in the absence of antibiotic, the *ridA1* mutation was clearly different from any mutations previously located in this region of the linkage map. Since *prmA* gene product acts on a component of the ribosome (Colson et al. 1979), transduction experiments were carried out to see if *ridA* and *prmA* were close. Strain VT26 (Dabbs and Wittmann 1976) possessed a *prmA* mutation (Colson and Dabbs, unpublished results). This mutant allele caused an alteration in the electrophoretic migration of ribosomal protein L11 in gels (Dabbs and Colson, unpublished results). Using this alteration to monitor the status of *prmA*, crosses were made between *prmA* and *ridA* alleles. The two loci cotransduced only at the level of about 25%. On the basis of the published map position (Saito and Uchida 1977), the *grpD* locus may be close to *ridA*. Moreover, mutant alleles at this locus affect initiation of DNA replication of phage lambda, so this locus has a product which is probably also involved in macromolecular synthesis.

In this work, we have tentatively identified the *ridA1* lesion as being in some cellular component involved closely in both transcription and translation. However, alternative explanations were possible. For example, it might be that the lesion caused some general metabolic imbalance which could be remedied by slowing cell growth; one way to do this would be to add antibiotic to the medium to hinder but not prevent growth. Such an explanation could apply to some other Rif-D mutants; these mutants (unpublished results) manifested a Rif-D phenotype at 37° C in rich medium, but were not dependent at 30° C in rich medium or at 37° C in minimal medium. These were both conditions which, like the addition of low concentrations of antibiotic, slowed growth.

In contrast to the generality of conditions suppressing the conditional lethal phenotype in such mutants, there was a specificity in the case of mutant PB67. The specificity with respect to RNA polymerase was observed in the interaction of different *rpoB* alleles with *ridA1* leading to a variety of phenotypes. With respect to the ribosome, there was specificity in that only one ribosomally targeted antibiotic, kasugamycin, could prevent the lethality of the *ridA1* mutation. If it were only a matter of Ksg slowing growth, then some concentration of spectinomycin or streptomycin should also suffice. This was not the case.

An advantage of using antibiotics in the study of the ribosome is that they act at different stages in the process of translation of mRNA into polypeptide (for example, see Nierhaus and Wittmann 1980, for a recent summary). Kasugamycin acts early in the initiation process, interfering with the binding of fmet $tRNA_f^{met}$ to the ribosome (Okuyama and Tanaka 1972). Streptomycin and spectinomycin also affect initiation, but at a point subsequent to that of kasugamycin. The ability of kasugamycin, but not the other aminoglycoside antibiotics tested, to reverse the lethality due to *ridA1* mutation might be because the *ridA* gene product was involved in an early stage of the ribosomal initiation process; it was tempting to tie this to the report (Debenham et al. 1980) that fmet $tRNA_f^{met}$ can modulate the properties of RNA polymerase.

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