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Resistance to the Aminoglycoside Antibiotic Neamine in *Escherichia coli*

A NEW MUTANT WHOSE Nea^R PHENOTYPE RESULTS FROM THE CUMULATIVE EFFECTS OF TWO DISTINCT MUTATIONS

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A spontaneous mutant of *Escherichia coli* (strain AB2847), selected for resistance to the aminoglycoside antibiotic neamine, shows severe restriction of amber suppressors *in vivo*. Ribosomes isolated from the mutant exhibit only low misreading *in vitro* in the presence of the antibiotic. Genetic and biochemical analyses indicate that the neamine-resistant phenotype is the result of two distinct mutations. The first, *res3128*, appears to affect the gene (*strA*) coding for the ribosomal protein S12. Although it leads to a restrictive phenotype it does not, however, confer resistance to streptomycin. The second mutation, *X3128*, is located between the *strA* and *aroB* loci and is lethal when segregated from the *res3128* mutation. It may affect the ribosome at the level of a post-translational modification.

Many of the genes coding for ribosomal proteins are clustered near the *strA* locus, which maps at 72min on the genome of *Escherichia coli* (for reviews see Jaskunas *et al.*, 1974; DeWilde *et al.*, 1977). In this region of the genome several mutations leading to antibiotic resistance are associated with alterations in specific ribosomal proteins. Thus the *spcA* and *strA* loci, controlling respectively spectinomycin resistance and either dependence or resistance to streptomycin, specify the structural genes for ribosomal proteins S5 and S12 respectively (Bollen *et al.*, 1969; Ozaki *et al.*, 1969; Birge & Kurland, 1970), and the *eryA* and *eryB* genes (resistance to erythromycin) code for proteins L4 and L22 respectively (Brown & Apirion, 1974; Wittmann *et al.*, 1973).

Resistance to the aminoglycoside antibiotic neamine is associated with several types of ribosomal alterations. Thus some neamine-resistant mutants have an altered S17 protein coded for by the *neaA* gene (Bollen *et al.*, 1975; Yaguchi *et al.*, 1976), whereas other mutants owe their resistant phenotype to the cumulative effects of two distinct mutations, one in the gene coding for protein S5 and the other in the gene coding for protein S12 (DeWilde *et al.*, 1975; Yaguchi *et al.*, 1975). Furthermore, many streptomycin-resistant mutants exhibit a neamine-resistant phenotype (J. Davies, personal communication; A. Bollen, unpublished work).

In addition to these three classes of mutants a fourth type, with a locus designated *neaB*, has also been described (Cannon *et al.*, 1974), and in the present paper the properties of one such mutant (RH3128) are reported. We now find that the resistant phenotype results from the cumulative effects of two distinct mutations. The first one, here designated *res3128*, confers on the mutant a restricting phenotype as manifested by the inability to support the growth of certain amber mutants of the bacteriophage λh80 in the presence of an amber suppressor. This property is associated with an increase in the translational fidelity of the ribosomes of the mutant. The *res3128* mutation may well affect the structural gene for the ribosomal protein S12 (*strA* locus), but it does not, however, confer resistance to streptomycin. The second mutation, her edesignated *X3128*, maps between the loci for *strA* and *aroB*. This mutation, originally equated with the *neaB* locus (Cannon *et al.*, 1974) cannot be expressed independently from the *res3128* mutation and may affect the ribosome at the level of a post-transcriptional modification.

Materials and Methods

Bacterial strains

The various strains of *E. coli* used in the present study along with their derivation are described in Table 1.

Table 1. *Bacterial strains*

Designation	Chromosomal markers	Source (reference or method)
RH3133 (AB2847)	*aroB,malA*,T6^R	Pittard & Wallace (1966)
RH1504	RH3113,*spcA*	Pittard & Wallace (1966)
RH3128	RH3113,Nea^R	Spontaneous neamine-resistant mutant, isolated on rich medium containing 50 μg of neamine/ml (Cannon *et al.*, 1974)
RH2557 (o*Rts4*)	*malA*,T6^R,*ts4*	DeWilde & Cabezón (1974)
RIB27	*malA*,T6^R,Nea^R	Obtained by transduction from K12S (phototroph) into RH3128 and selection for *aroB*$^+$ (G. Delcuve, in this work)
RH3134	*aroE353,spcA,tsx*$_{am}$,*ara*$_{am}$, *lac*$_{am}$,*galU*$_{K2am}$,*galE*	T. Cabezón (personal communication)
RH3056 (AT2455)	Hfr,*thi1,cysG44,mal18,rel1,strA*	Taylor & Trotter (1967)
RH3182	*aroE353,spcA,strA,cysG44,thi1*	Results from the cross between strains RH3056 and RH3134, selection for Spc^R and Str^R (T. Cabezón, in this work)
RIB41	*aroE353*,Nea^R	Obtained by transduction of strain RH3128 into RH3182 and selection for Cys$^+$, Str^S, Spc^S, AroE$^-$ and neamine-resistance (G. Delcuve, in this work)
RH3191	RH3113*strA*	Spontaneous streptomycin-resistant derivative of strain RH3113 (G. Delcuve, in this work)

Genetic methods

Bacteriophage-P1 transductions were performed as described previously by Cannon *et al.* (1974). To detect restricting phenotypes various stocks of the bacteriophage λh80*sus* were spotted at appropriate dilutions on a lawn of the relevant bacteria previously spread on nutrient agar plates. The bacteriophage λh80 mutants were obtained from the collection of the Laboratory of Genetics, University of Brussels. Bacteriophage λh80 is a hybrid between bacteriophages λ and φ80.

Biochemical methods

Procedures for the preparation of 70S ribosomes and 30S and 50S ribosomal subunits were as described by Held *et al.* (1974). Preparations of 'total' ribosomal proteins from isolated 50S and 30S ribosomal subunits (TP50 and TP30 respectively) were obtained by extraction of the particles with acetic acid (Hardy *et al.*, 1969).

Reconstitution of 30S ribosomal subunits was performed as described by Traub & Nomura (1968) and Traub *et al.* (1971). The protein fractions used in the reconstitution tests were previously separated by chromatography on CM-cellulose (CM-52; Whatman) as described by Bollen & Herzog (1970) and DeWilde *et al.* (1975). Individual proteins were identified by gel electrophoresis in both urea and sodium dodecyl sulphate (Traut *et al.*, 1973).

Misreading assays were performed essentially as described by Pestka *et al.* (1965) by using partially purified elongation factors EFT and EFG (Gordon *et al.*, 1971).

Total tRNA was charged with either [^{14}C]phenylalanine (424 mCi/mmol) or [^{14}C]isoleucine (300 mCi/mmol) obtained from New England Nuclear Corp. (Boston, MA, U.S.A.) and The Radiochemical Centre (Amersham, Bucks., U.K.) respectively, by the method of Ravel & Shorey (1971). Counting efficiency for ^{14}C was 80%. Radioactivity was measured by placing the Millipore filters in the scintillation liquid Omnifluor (New England Nuclear Corp.) (10 ml) and counting them in a Packard scintillation counter.

The nomenclature for the genes (*rps*) controlling the synthesis of individual ribosomal proteins follows the system described in Bachmann *et al.* (1976).

Neamine was kindly provided by Dr. G. B. Whitfield, Jr., of The Upjohn Co., Kalamazoo, MI, U.S.A.

Isolation and genetic analysis of the mutant RH3128

Spontaneous mutants resistant to 50 μg of neamine/ml were obtained from strain RH3113, and one of these (RH3128) was selected for further analysis. The mutant was sensitive to spectinomycin, streptomycin and neomycin.

Transductions were then performed to identify the neamine-resistant locus with respect to the *aroB*, *aroE* and *spcA* markers. The *strA* marker could not be used, as all Str^R strains so far tested are also resistant to neamine. Accordingly we used as a marker a thermosensitive mutation (*ts4*) located on the *aroE* side of gene *strA*, which co-transduces at 97% with this gene (DeWilde & Cabezón, 1974).

The first transduction (*a*) uses a bacteriophage-P1 stock previously grown on *E. coli* strain RiB27 (Nea^R) to infect strain RH1504 (AroB$^-$,Spc^R), whereas the second (*b*) uses strain RH2557 (ts) as the donor and strain RH3128 (AroB$^-$,Nea^R) as recipient. The results of both these transductions are presented in Table 2 and illustrated diagrammatically in Fig. 1. Results of the transduction (*a*) suggest that the Nea^R marker is located close to the *spcA* gene

Table 2. *Bacteriophage-P1 transduction mapping of the Nea^R character of strain RH3128*

The values listed for 'Non-selected markers' refer to the number of transductants having one or another phenotype. Thus in transduction (*b*) there are 220 transductants that fall into three classes. There are four transductants in class I (Nea^S,ts), 57 transductants in class 2 (Nea^S,ts$^+$) and 159 transductants in class 3 (Nea^R,ts$^+$). The concentration of neamine used in the assay to test the resistance or sensitivity to the drug was in all cases 100μg/ml. In transduction (*b*), the class of transductants Nea^S,ts$^+$ does not grow at 100μg of neamine/ml, but does show growth at 50μg/ml.

	Transduction (*a*)			Transduction (*b*)			Transduction (*c*)		
Donor	RIB27 (Nea^R)			RH2557 (ts)			RH2557 (ts)		
Recipient	RH1504 (AroB$^-$,Spc^R)			RH3128 (AroB$^-$,Nea^R)			RIB41 (AroE$^-$,Nea^R)		
Selected marker	AroB$^+$			AroB$^+$			AroE$^+$		
No. of transductants tested	322			220			328		
Non-selected markers		Spc^R	Spc^S		ts$^+$	ts		ts$^+$	ts
	Nea^S	321	0	Nea^R	159	0	Nea^R	322	0
	Nea^R	0	1	Nea^S	57*	4	Nea^S	0	6
		321	1		216	4		322	6
Co-transduction frequencies	*aroB–spcA* } 0.3%			*aroB–nea* 26%			*aroE–ts4* } 2%		
	aroB–nea }			*aroB–ts4* 2%			*aroE–nea* }		

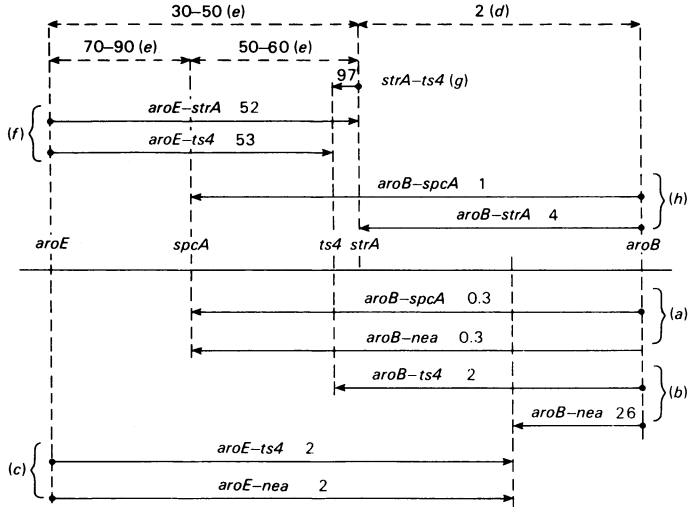


Fig. 1. *Linkage map of the aroE–aroB region showing results of transduction experiments involving the Nea^{R}3128 mutation together with data from the literature and control experiments*

References: (*a*), (*b*), (*c*), present study; (*d*) Marinus (1973); (*e*) Bollen *et al.* (1969); (*f*), (*g*) T. Cabezón, unpublished work; (*h*) DeWilde *et al.* (1975). In all cases the numbers show the frequency of P1 co-transduction expressed as a percentage.

(*aroB*–Nea^R, % co-transduction frequency = 0.3), which was unexpected in view of the observations of Cannon *et al.* (1974) that had placed a Nea^R marker at a different locus. However, in contrast with this result the second transduction (*b*) apparently places the Nea^R marker at a completely different locus between the *strA* and *aroB* loci (26% co-transduction with *aroB*), this site being closely similar to the *neaB* locus originally described by Cannon *et al.* (1974).

The above data could be explained if the Nea^R phenotype of strain RH3128 were the result of two interdependent mutations. Indeed, since the Nea^R marker is present on the donor strain in transduction (*a*), but on the recipient strain in transduction (*b*), the co-transduction frequency (*aroB*–Nea^R) will depend in the first case (*a*) on the class of *aroB*$^+$–Nea^R transductants and in the second case (*b*) on the class of *aroB*$^+$–Nea^S transductants. Thus expression of the Nea^S phenotype, as seen in

Table 3. *Growth of various amber mutants of bacteriophage λh80 on strain RH3128*

Since the *malA* mutation deletes the λ-phage receptor site it was necessary to use the hybrid λh80 bacteriophage, which has a different host range. Key: +, growth (indicates an efficiency of plating approaching 1); −, no growth (indicates an efficiency of plating of less than 10^{-5}).

Bacteria	Phenotype	cI	P3am	R5am	Q203am	N7am	R216am
RH3113	AroB⁻,Sup⁺	+	+	+	+	+	+
RH3128	AroB⁻,Sup⁺,Nea^R	+	−	−	−	−	+
RH3191	AroB⁻,Sup⁺,Str^R	+	−	−	−	−	+

transduction (*b*), could result from the loss of one of the two postulated mutations, and in that case 26% co-transduction with gene *aroB* (on the *strA* side of this gene) would correspond to the map location of the lost mutation, originally identified as *neaB* (Cannon *et al.*, 1974) and now called here *X3128*. In contrast, the expression of the Nea^R phenotype as seen in transduction (*a*) would require the presence of both mutations, *X3128* plus an additional mutation (called here *res3128*) that maps close to gene *spcA*.

To confirm this hypothesis a third transduction was carried out by using bacteriophage P1, previously grown on strain RH2557, to infect strain RiB41 (AroE⁻,Nea^R). As indicated in Table 2(*c*) and also in Fig. 1, the co-transduction frequencies obtained for *aroE*–Nea^R and *aroE–ts4* (2%) were identical, although these values are not compatible with the co-transduction frequency for *aroE–ts4* obtained in other experiments (53%). It is possible, therefore, that recombination between the *ts4* mutation and the *X-3128* mutation leads to non-viable recombinants, which would be the case if the latter mutation becomes lethal when segregated from *res3128*.

Mutant strain RH3128 restricts amber suppressors

Strain RH3128 is derived from strain RH3113, which, as judged by its suppression pattern with amber mutants of λh80 bacteriophage, contains an amber suppressor similar to the *supE* locus (L. Desmet, personal communication). Both strains, in addition to RH3191, which was derived from strain RH3113, but is resistant to streptomycin, were tested for their ability to support the growth of several amber mutants of the hybrid bacteriophage λh80. The results of such a restriction assay are shown in Table 3 and indicate that the mutation(s) conferring resistance to neamine in strain RH3128 exerts a strong restricting effect on the expression of an amber suppressor. The restricting effect is also observed with the strain carrying the *strA* marker. A similar restriction has already been reported for several other bacterial strains resistant to streptomycin and neamine (Couturier *et al.*, 1964; Gorini, 1967; DeWilde *et al.*, 1975; Bollen *et al.*, 1975).

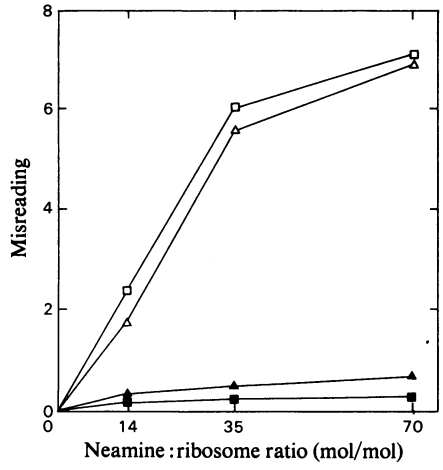


Fig. 2. *Neamine-induced misreading by 70S ribosomes reassociated from 30S and 50S ribosomal subunits*
Misreading is expressed as the number of pmol of [^{14}C]isoleucyl-tRNA incorporated in the presence of the drug/100 pmol of [^{14}C]phenylalanyl-tRNA incorporated in the absence of drug. A standard system contains 40 pmol of re-associated ribosomes and incorporates 323 pmol of [^{14}C]phenylalanyl-tRNA (34265 c.p.m.). Specific radioactivities of [^{14}C]phenylalanyl-tRNA and [^{14}C]isoleucyl-tRNA were 106 and 277 c.p.m./pmol respectively. □, 30S particles from strain RH3113 and 50S particles from strain RH3113; △, 30S particles from strain RH3113 and 50S particles from strain RH3128; ■, 30S particles from strain RH3128 and 50S particles from strain RH3128; ▲, 30S particles from strain RH3128 and 50S particles from strain RH3113.

Biochemical analysis of the mutant strain RH3128

It has been observed by other workers that misreading in protein-synthesizing systems *in vitro* (Davies *et al.*, 1964; DeWilde *et al.*, 1975; Bollen *et al.*, 1975) is decreased in some mutants resistant to streptomycin and neamine, and this low misreading has been correlated with restriction *in vivo* (Strigini & Brickman, 1973). It was therefore decided to study the translational fidelity of ribosomes isolated from the

strain RH3128 by measuring the incorporation of [^{14}C]isoleucyl-tRNA as directed by poly(U) *in vitro* in the presence of various amounts of neamine. The 70S ribosomes from the mutant strain (RH3128) were found to misread significantly less than those isolated from the parent strain (RH3113) (results not shown). Subsequent assays, with 70S ribosomes re-associated from 30S and 50S ribosomal subunits derived from both mutant and parent strains, indicated that the 30S ribosomes from the mutant are responsible for the increased fidelity of the translocation process (see Fig. 2). Further experiments were therefore carried out involving reconstitution of 30S ribosomal subunits from 16S rRNA and total ribosomal proteins (TP30) prepared from either the mutant or the parent strain. The resultant particles were assayed for misreading, and, as illustrated in Fig. 3, it is clear that the low misreading property is associated with a protein moiety derived from the 30S ribosomal particle of the mutant strain RH3128. Fig. 3 also indicates that the extent of misreading observed with reconstituted ribosomes containing the TP30 fraction from strain RH3128 is always higher with particles containing 16S rRNA from the parent strain than with those containing 16S rRNA from the mutant.

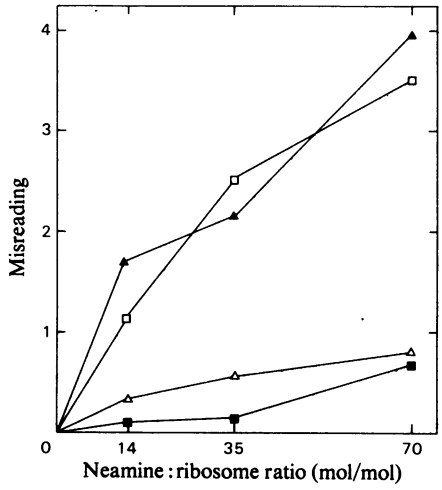


Fig. 3. *Neamine-induced misreading by 30S ribosomal subunits reconstituted from 16S rRNA and total proteins from either wild-type or mutant strains*

Misreading is defined in the legend to Fig. 2. Specific radioactivities of [^{14}C]phenylalanyl-tRNA and [^{14}C]isoleucyl-tRNA were 84 and 315c.p.m./mol respectively. A standard reconstitution system contained 40pmol of ribosomes and incorporated 244pmol of [^{14}C]phenylalanyl-tRNA (20536c.p.m.). The 50S ribosomal subunits used for re-association of 70S ribosomes were of parental origin in all experiments, and other components were as follows: □, 16S rRNA from strain RH3113 and TP30 from strain RH3113; △, 16S rRNA from strain RH3113 and TP30 from strain RH3128; ■, 16S rRNA from strain RH3128 and TP30 from strain RH3128; ▲, 16S rRNA from strain RH3128 and TP30 from strain RH3113.

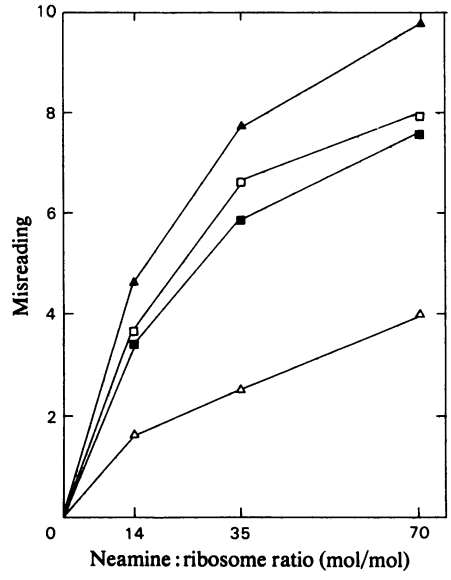


Fig. 4. *Neamine-induced misreading by 30S ribosomal subunits reconstituted from wild-type 16S rRNA and total proteins from either wild-type or mutant strains in the presence or absence of a 5-fold molar excess of pure wild-type S12 protein*

Misreading is defined in the legend to Fig. 2. Specific radioactivities of [^{14}C]phenylalanyl-tRNA and [^{14}C]-isoleucyl-tRNA were 84 and 315c.p.m./pmol respectively. A standard reconstituted system containing 40pmol of ribosomes incorporated 212pmol of [^{14}C]phenylalanyl-tRNA (17784c.p.m.). The 50S ribosomes used to carry out the experiment were of parental origin in all cases. □, TP30 fraction from wild-type ribosomes; △, TP30 fraction from strain RH3128; ■, TP30 fraction from wild-type ribosomes plus a 5-fold molar excess of S12 protein purified from wild-type ribosomes; ▲, TP30 fraction from strain RH3128 plus a 5-fold molar excess of S12 protein purified from wild-type ribosomes.

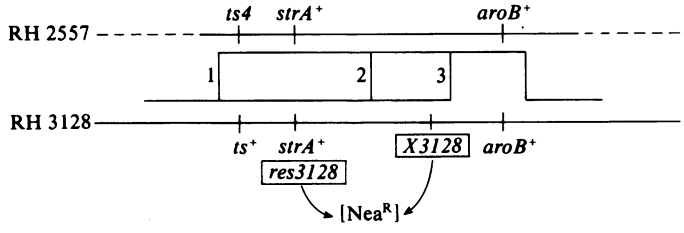
Ribosomal proteins from 30S particles isolated from both strains were then analysed on CM-cellulose columns and also on two-dimensional polyacrylamide gels. No differences in chromatographic behaviour or mobility were observed. Accordingly 30S ribosomal subunits were reconstituted starting with core particles (CP30) and split proteins (SP30) isolated from both parent and mutant ribosomes by treatment with 1M-LiCl (Homann & Nierhaus, 1971). Mis-

reading assays performed on the various reconstituted particles revealed that the low misreading property was associated with one of the few proteins removed incompletely from 30S ribosomal subunits by treatment with 1 M-LiCl. These proteins are identified, on two-dimensional polyacrylamide gels, as S6, S9, S12 and S16, and of these proteins S12 seems the most likely candidate for producing the low-misreading property, since it is involved in the control of translational fidelity (Ozaki *et al.*, 1969; Gorini, 1974).

Because of the above results 30S ribosomal subunits were reconstituted from wild-type 16S rRNA and total proteins isolated from either parent or mutant strains, further reconstitutions also being carried out in the presence of a 5-fold molar excess of pure wild-type S12 protein. Misreading assays were then performed in the presence of 50S ribosomal subunits from the parent strain, and as shown in Fig. 4 the wild-type protein S12 was found to restore normal misreading capacity to reconstituted ribosomes. This experiment demonstrates that strain RH3128 has an altered S12 protein, which we presume is responsible for the increased translational fidelity of its ribosomes assayed *in vitro*. The above data are in agreement with the hypothesis that one of the two mutations present in strain RH3128 could affect the *strA* gene.

A more detailed analysis was then carried out on the three classes of transductants described in Table 2(*b*). For each of them we carried out restriction tests *in vivo*, determined their resistance to both neamine and streptomycin and checked their ability to induce misreading *in vitro*. As illustrated in Fig. 5, transductants of class 2 are less resistant to neamine than is the double mutant (class 3), and they are also sensitive to streptomycin. Nevertheless they show restriction of amber suppressors, although this restriction is lower than that observed for the double mutant. Furthermore ribosomes from class-2 transductants exhibit increased translational fidelity. It seems likely therefore that class-2 transductants carry only the restrictive mutation, *res3128*, and have lost the second mutation, *X3128*. Both mutations together lead to high resistance to neamine and severe restriction of amber suppressors.

Although we have been unable to demonstrate an alteration in the primary structure of protein S12 in ribosomes from strain RH3128, the phenotype of strains carrying only the *res3128* mutation is similar to that of other *res* strains described by Garvin & Gorini (1975) with respect to sensitivity to streptomycin, restriction of amber suppressors and increased translational fidelity of ribosomes *in vitro*. The second mutation, *X3128*, appears to be lethal when segregated from the *res3128* mutation, but we do not know which gene is altered by the *X3128* mutation. However, we have been unable to detect an altered ribosomal protein associated with the *X3128* mutation, and its map location is some distance away from the known loci for the structural



Class of transductants	Phenotype	Supposed genotype	(*a*) Growth on: Sm_{200}	Nea_{50}	Nea_{100}	(*b*) Restriction of amber λh80 bacteriophage: cI	P3am	R5am	Q203am	N7am	R216am	(*c*) Misreading
Class 1	$AroB^+,Nea^S,ts$	res^+,X^+	−	−	−	+	+	+	+	+	+	40
Class 2	$AroB^+,Nea^S,ts^+$	$res3128,X^+$	−	±	−	+	−	−	+	+	+	6
Class 3	$AroB^+,Nea^R,ts^+$	$res3128,X3128$	−	+	+	+	−	−	−	−	+	3

Fig. 5. *Genetic and biochemical analysis of transductants derived from the cross between strain RH*2557 *and strain RH*3128 (*a*) Sm_{200}, Nea_{50} and Nea_{100} indicate the concentrations of streptomycin or neamine used in the test (μg/ml). (*b*) Explanations for the restriction assay are given in the legend of Table 3. (*c*) The misreading extent is defined in the legend to Fig. 2. Specific radioactivities of [^{14}C]phenylalanyl-tRNA and [^{14}C]isoleucyl-tRNA were 80 and 254 c.p.m./pmol respectively. The system contained 40 pmol of 70S ribosomes and incorporated 236 pmol of [^{14}C]phenylalanyl-tRNA (18957 c.p.m.). The molar ratio of neamine to ribosomes was approx. 70:1. The numbers 1, 2 and 3 on the diagram refer to the transductant classes defined in the legend to Table 2. The lines drawn between the two strains indicate where crossing over takes place to produce the three different classes of transductants.

Table 4. *Neamine-resistant mutants derived from various parental strains of E. coli*

Strain	Genotype	Mutant	Mutation	Reference
KMBL-146	*argG,ilv,his,thr,leu,pyrF,thyA,supE,lac*	*nea314,nea319*	*spcA*(S5) and *strA*(S12)	DeWilde *et al.* (1975)
K12S	Prototroph	*nea301,nea302*	*neaA*(S17)	Bollen *et al.* (1975)
AB2847	*aroB,malA,T6*^{R}*,supE*(?)	*nea3128*	*res-3128* and *X-3128*	This study

genes of many ribosomal proteins. The possibility remains, therefore, that the *X3128* mutation affects ribosomes at the level of a post-transcriptional modification. Indeed reconstitution of 30S ribosomal subunits from mutant ribosomal proteins and 16S rRNA from either the parent or the mutant strains produces particles differing in degree of translational fidelity *in vitro* (see Fig. 3), since the presence of parental 16S RNA allows a higher extent of misreading *in vitro*. This effect can be correlated with the less severe restriction of amber suppressors observed in strains carrying only the *res3128* mutation.

Spontaneous double mutants leading to altered ribosomes have been characterized by other workers (Garvin & Gorini, 1975; DeWilde *et al.*, 1975), although the molecular mechanism(s) leading to retention of such mutations, at a relatively high frequency (10^{-6}–10^{-8}), is unknown. Clearly, however, it is possible that the different types of neamine-resistant mutants so far isolated arise from parent strains whose genetic backgrounds predispose them towards a given mutation. Thus each neamine-resistant mutant so far known, and listed in Table 4, arose spontaneously and preferentially from distinct strains of *E. coli*.

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References

Bachmann, B. J., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40**, 116–167

Birge, E. & Kurland, C. G. (1970) *Mol. Gen. Genet.* **109**, 356–369

Bollen, A. & Herzog, A. (1970) *FEBS Lett.* **6**, 69–72

Bollen, A., Helser, T., Yamada, T. & Davies, J. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 95–100

Bollen, A., Cabezón, T., DeWilde, M., Villarroel, R. & Herzog, A. (1975) *J. Mol. Biol.* **99**, 795–806

Brown, M. E. & Apirion, D. (1974) *Mol. Gen. Genet.* **133**, 317–322

Cannon, M., Cabezón, T. & Bollen, A. (1974) *Mol. Gen. Genet.* **130**, 321–326

Couturier, M., Desmet, L. & Thomas, R. (1964) *Biochem. Biophys. Res. Commun.* **16**, 244–248

Davies, J., Gilbert, W. & Gorini, L. (1964) *Proc. Natl. Acad. Sci. U.S.A.* **51**, 883

DeWilde, M. & Cabezón, T. (1974) *Arch. Int. Physiol. Biochim.* **82**, 181

DeWilde, M., Cabezón, T., Villarroel, R., Herzog, A. & Bollen, A. (1975) *Mol. Gen. Genet.* **142**, 19–33

DeWilde, M., Cabezón, T., Herzog, A. & Bollen, A. (1977) *Biochimie* **59**, 125–140

Garvin, R. & Gorini, L. (1975) *Mol. Gen. Genet.* **137**, 73–78

Gordon, J., Lucas-Lenard, A. L. & Lipmann, F. (1971) *Methods Enzymol.* **20**, 281–291

Gorini, L. (1967) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **26**, 5–8

Gorini, L. (1974) in *Ribosomes* (Nomura, M., Tissières, A. & Lengyel, P., eds.), pp. 791–803, Cold Spring Harbor Laboratory, Cold Spring Harbor

Hardy, S. J. S., Kurland, C. G., Voynow, G. & Mora, G. (1969) *Biochemistry* **8**, 2897–2905

Held, W. A., Ballov, B., Mizushima, S. & Nomura, M. (1974) *J. Biol. Chem.* **249**, 3103–3110

Homann, H. E. & Nierhaus, K. H. (1971) *Eur. J. Biochem.* **20**, 249–257

Jaskunas, S. R., Nomura, M. & Davies, J. (1974) in *Ribosomes* (Nomura, M., Tissières, A. & Lengyel, P., eds.), pp. 333–368, Cold Spring Harbor Laboratory, Cold Spring Harbor

Marinus, M. G. (1973) *Mol. Gen. Genet.* **127**, 47–55

Ozaki, M., Mizushima, S. & Nomura, M. (1969) *Nature (London)* **222**, 333–339

Pestka, S., Marchal, R. & Nirenberg, M. W. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **53**, 639–646

Pittard, J. & Wallace, B. J. (1966) *J. Bacteriol.* **91**, 1494–1508

Ravel, J. & Shorey, R. A. L. (1971) *Methods Enzymol.* **20**, 306–316

Strigini, P. & Brickman, D. (1973) *J. Mol. Biol.* **75**, 659–672

Taylor, A. L. & Trotter, C. D. (1967) *Bacteriol. Rev.* **31**, 332–353

Traub, P. & Nomura, M. (1968) *J. Mol. Biol.* **34**, 575–593

Traub, P., Mizushima, S., Lowry, C. V. & Nomura, M. (1971) *Methods Enzymol.* **20**, 391–407

Traut, R. R., Bollen, A., Sun, T. T., Hershey, J. W. B., Sundberg, G. J. & Pierce, R. L. (1973) *Biochemistry* **12**, 3266–3273

Wittmann, H. G., Stöffler, G., Apirion, D., Rosen, L., Tanaka, K., Tamaki, M., Takata, R., Dekio, S., Otaka, E. & Osawa, S. (1973) *Mol. Gen. Genet.* **127**, 175–189

Yaguchi, M., Wittmann, H. G., Cabezón, T., DeWilde, M., Villarroel, R., Herzog, A. & Bollen, A. (1975) *Mol. Gen. Genet.* **142**, 35–43

Yaguchi, M., Wittmann, H. G., Cabezón, T., DeWilde, M., Villarroel, R., Herzog, A. & Bollen, A. (1976) *J. Mol. Biol.* **104**, 617–620