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Translation Rates and Misreading Characteristics of *rpsD* Mutants in *Escherichia coli*

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Summary. Three ribosomal ambiguity (Ram) mutants, changed in ribosomal protein S4, have been examined with respect to elongation rate and misreading of translation in vivo and in vitro. Ram mutants increase misreading of nonsense codons in vivo, compared to wild type, between 2–50 times depending on the nature of the nonsense codon, its position, and which *rpsD* allele is present. Ram ribosomes also show an increased error frequency in vitro. The elongation rate of translation does not seem to be significantly changed, neither in vivo nor in vitro, irrespective of which *rpsD* allele is present.

We suggest that there exists no general relationship between the accuracy and the overall speed of translation in Ram strains.

Introduction

Mutations affecting the 30S ribosomal protein S4 of *Escherichia coli* are known to have at least four distinct effects: they cause phenotypic suppression of streptomycin dependence (Birge and Kurland 1970; Deusser et al. 1970; Donner and Kurland 1971); they are responsible for increased translation errors, referred to as the ribosome ambiguity (Ram) phenotype (Gorini 1971; Zimmerman et al. 1971); they lower the affinity of S4 for its binding site on 16S RNA, which leads to a defect in ribosome assembly (Green and Kurland 1971; Daya-Grosjean et al. 1972), and they disturb the regulation of the expression of S4's operon (Olsson and Isaksson 1980) at the level of translation (Olsson and Gausing 1980; Yates et al. 1980). That all four effects are simultaneously expressed to varying degrees in any randomly chosen S4 mutant is suggested by the survey of Olsson and his coworkers (Olsson et al. 1974; Olsson and Isaksson 1979a, 1979b; Olsson 1979; Olsson and Isaksson 1980). Here we focus attention on the Ram phenotype of S4 mutants, and we attempt to determine its relationship to a fifth parameter, the rate of polypeptide elongation by the mutant ribosome.

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Abbreviations: poly U, poly(uridylic acid); IPTG, isopropyl-B-D-thiogalactopyranoside; ATP, adenosine (5′) triphosphate; GTP, guanosine (5′) triphosphate; ONPG, *o*-nitrophenyl-B-D-galactoside; Phe, phenylalanine; Leu, leucine; EF-G, elongation factor G; EF-Tu, elongation factor Tu; EF-Ts, elongation factor Ts; Tet-R, tetracycline resistance

Our interest in a possible correlation between the speed and accuracy of translation is an outcome of the work of Gorini (1971). His studies revealed a complex pattern of interactions between mutant ribosomes and mutant tRNA's that influence the suppression of nonsense codons. Ninio (1974) showed that this pattern could be explained in part by assuming that certain mutational changes of ribosomes, for example those responsible for the S4 Ram phenotype, are expressed as characteristic changes in the relative rates with which aminoacyl-tRNA's are used in some unspecified step by the ribosomes for peptide bond formation. According to this analysis, speeding up this step could increase the rate of suppression, and slowing it down would lower the rate of suppression. Galas and Branscomb (1976) considered the possibility that such kinetic alterations of ribosomes might be expressed in the overall rate of polypeptide elongation. Accordingly, they studied a streptomycin-resistant mutant that restricts nonsense codon suppression and they found that it elongates β-galactosidase at a rate about 40% slower than does the wild-type. Such a reduction of translation rate is often (Bohman, unpublished results) but not always (Piepersberg et al. 1979) seen in the case of streptomycin-resistant mutants.

Here we study the converse situation: S4 mutants that have a higher than normal phenotypic suppression rate with nonsense mutations. We show that there is a rough correlation between the rate of nonsense suppression in vivo and the missense error rate in vitro in the case of three S4 mutants. Nevertheless, we confirm the observations of Piepersberg et al. (1979) that an S4 mutant with a Ram phenotype shows an overall translation rate which is indistinguishable from the wild type.

Indeed, wild-type ribosomes and the three S4 mutants function at nearly the same overall rate in vivo as well as in vitro. We conclude that there is no general, inverse relationship between the speed and accuracy of translation by mutant ribosomes.

Materials and Methods

Chemicals: Poly U, ATP, GTP, phosphoenolpyruvate trisodium salt, putrescine, spermidine, myokinase (E.C. 2.7.4.3), pyruvate kinase (E.C. 2.7.1.40), ONPG and IPTG were purchased from Sigma Chemical Co., St Louis, Mo, USA. Radioactive amino acids were obtained from the Radiochemical Centre, Amersham, Bucks, England.

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Table 1. Strains of *Escherichia coli* K12 used in this study

Designation	Sex; extra chromosomal markers	Chromosomal markers	Relevant characters	Origin
O17	F^-	*rpsD*$^+$	Ts$^+$	Olsson et al. (1979a)
D12	F^-	*rpsD12*	Ts	Olsson et al. (1979a)
D14	F^-	*rpsD14*	Ts	Olsson et al. (1979a)
D16	F^-	*rpsD16*	Ts	Olsson et al. (1979a)
CSH23	F′ *lac*$^+$*proA*$^+$, *B*$^+$	*Δ* (*pro lac*) *supE rpsE thi*		Miller (1972)
UD121	F^-	*Δ* (*pro lac*) Ara *aroE* Tn10 *gyrA rpoB argE*(amber)		This paper
UD111	F^-	*Δ* (*pro lac*) Ara *gyrA rpoB argE*(amber)		Miller (1978)
UD132	F^-	*Δ* (*pro lac*) Ara *gyrA rpoB argE*(amber) *rpsD*$^+$	Ts$^+$	This paper
UD131	F^-	*Δ* (*pro lac*) Ara *gyrA rpoB argE*(amber) *rpsD12*	Ts	This paper
UD178	F^-	*Δ* (*pro lac*) Ara *gyrA rpoB argE*(amber) *rpsD14*	Ts	This paper
UD179	F^-	*Δ* (*pro lac*) Ara *gyrA rpoB argE*(amber) *rpsD16*	Ts	This paper
UD364	F′ *lacIZ* fusion *proA*$^+$, *B*$^+$	*Δ* (*pro lac*), *rpsL*(StrR)	carries ochre mutation in I part at position 189	From J. Miller
UD365	F′ *lacIZ* fusion *proA*$^+$, *B*$^+$	*Δ* (*pro lac*), *rpsL*(StR)	carries amber mutation in I part at position 189	From J Miller
UD366	F′ *lacIZ* fusion *proA*$^+$, *B*$^+$	*Δ* (*pro lac*), *rpsL*(StrR)	carries UGA mutation in I part at position 189	From J. Miller
UD367	F′ *lacIZ* fusion *proA*$^+$, *B*$^+$	*Δ* (*pro lac*), *rpsL*(StrR)	carries ochre mutation in I part at position 220	From J. Miller
UD368	F′ *lacIZ* fusion *proA*$^+$, *B*$^+$	*Δ* (*pro lac*), *rpsL*(StrR)	carries amber mutation in I part at position 220	From J. Miller
UD471	F′ *lacIZ* fusion *proA*$^+$, *B*$^+$	*Δ* (*pro lac*), *rpsL*(StrR)	carries UGA mutation in I part at position 220	From J. Miller
UD484	Hfr	*aroE* Tn10 Pro Lac *malA* Mtl Xyl *rpsL thi*	Aro Tn10 (Tet-R)	This paper
UD334	Hfr	as UB484, but lacking Tn10	Aro	This paper

Column material came from Pharmacia Fine Chemicals, Uppsala, Sweden.

Bacterial Strains and Genetic Methods: The genotypes and derivations of the *Escherichia coli* K12 strains used are listed in Table 1.

Transductions were performed as described by Miller (1972). To facilitate constructions of strains with various alleles of *rpsD*, a transposon (Tn10) was inserted next to *rpsD* in the following way: About 10^9 phages (0.1 ml) λNK 370 (*b*221, *c*I857, *c*I171, *O* (UGA261) were added to 1 ml of an overnight culture of strain 017 in TB medium containing 0.4% maltose and 10 mM Mg^{2+}. After 30 min incubation glucose was added to give 0.2% and incubation was continued for another 4 h at 33° C. Aliquots of 0.2 ml were spread on LB plates containing 20 μg/ml tetracycline and were incubated overnight at 33° C. About 1,000 tetracycline resistant colonies were pooled and resuspended in 5 ml LB and grown for 2 h at 37° C. A P1 lysate was made on this culture. This P1 lysate was then used to transduce into the strain containing an *rpsD* allele (which is temperature sensitive), and selection was made for Ts$^+$ (growth at 44.5° C) together with tetracycline resistance. Only one colony was saved. Starting from this clone, a P1 lysate was made and the inserted Tn10 was transduced into UD334 which is *aroE* and *rpsD*$^+$. One clone which maintained the *aroE* and *rpsD*$^+$ genes was saved. This strain, UB484, could then be used to construct *aroE* derivatives of other strains by cotransduction with Tet-R. Such an *aroE*, Tet-R strain (UD121) was finally used as a recipient in transductions in which various *aroE*$^+$, *rpsD* strains were used as donors. Only transductants in which the Tet-R marker had been lost by cotransduction were saved. In this manner strains UD132, UD131, UD178 and UD179 were made and they were used for the in vivo measurements of nonsense suppression and elongation rate.

Media and Growth Conditions: For the preparation of ribosomes strains O17, D12, D14, and D16 were grown at 37° C with good aeration in a modified LB medium (Isaksson

et al. 1977) and 0.2% glucose to mid-exponential phase; then they were cooled on ice, harvested by centrifugation and frozen at −80° C.

When testing the nonsense suppression in vivo the strains UD132, UD131, UD178, and UD179, containing the fused *lacIZ* gene on an F′-factor, were grown with good aeration at 37° C in M9 minimal medium (Miller 1972) which contained 0.2% glucose and 0.4 mM arginine. The cells were grown 4–6 generations to mid-exponential phase, cooled on ice and kept there until tested.

Determination of Nonsense Suppression in vivo. Measurements of β-galactosidase activity were made as described by Miller (1972). The suppression values for the different nonsense codons were calculated as the amount of *o*-nitrophenol produced (measured as a change in the absorbance at 420 nm) normalized to the amount of cells and time of incubation in the assay, divided by the amount of *o*-nitrophenol produced in the corresponding strain but containing a nonmutated *lacIZ* fusion.

Determination of Elongation Rates in vivo. Determinations of translation rates were made essentially as described by Schleif et al. (1973). Strains UD132, UD131, UD178 and UD179 containing a normal inducible lactose operon on an F′-factor were grown in a shaking water bath at 37° C in M9 medium with 0.2% glycerol, supplemented with 0.4 mM arginine. After 4–6 generations of growth, (OD_{500} close to 0.5), 5 ml of each culture were quickly transferred to a sterile 30 ml Corex tube. This was surrounded by a water jacket and thus kept at 37° C. Stirring was by a magnetic stirring bar in the tube.

At time zero, 0.05 ml 0.1 M IPTG was added to the culture. Samples of 0.2 ml were then removed at short intervals and each immediately mixed with 0.3 ml ice-cold chloramphenicol solution (0.5 mg/ml in H_2O).

The samples were then processed as described by Miller (1972) with suitable dilution of the cell samples. After the addition of Na_2CO_3, and centrifugation, the formation of *o*-nitrophenol was determined by measuring the optical density at 420 nm (A_{420}) of each sample.

Values were corrected by subtracting the average absorbance of duplicate samples taken immediately after the addition of IPTG (i.e. less than 30 s after time zero) and by normalising cell densities at the start of the assay to an OD_{500} of 1.0. Finally, the data were transformed by normalizing ONPG incubation times to 1 h, although the actual incubations lasted around 15 h.

Finally, to obtain a straight line from which the time required for synthesis of the first β-galactosidase monomers may be extrapolated; the square root of the corrected absorbance values was plotted as a function of time (see Schleif et al. 1973). The β-galactosidase monomer was assumed to consist of 1170 amino acid residues (Zabin and Fowler 1970).

Preparation of Ribosomes: Ribosomes were prepared from strain O17, D12, D14, and D16 (Olsson and Isaksson 1979a) as described by Jelenc (1980) but an S-300 column was used instead of an S-200 column. The ribosomes were stored at −20° C in polymix buffer containing 30% w/v methanol.

Determination of Elongation Rate in vitro. Procedures and enzyme preparations were essentially as described by Wagner et al. (1981). *N*-acetyl-Phe-$tRNA^{Phe}$ was prepared as described by Ruusala et al. (1982). The standard protocol for a translation assay was as follows: The two mixtures, initiation mix (I) and factor mix (II), were prepared on ice. They both contained 5 mM Mg(OAc), 0.5 mM $CaCl_2$, 95 mM KCl, 5 mM NH_4Cl, 8 mM putrescine, 1 mM spermidine, 5 mM phosphate and 1 mM Dithioerythritol at pH 7.5, 1 mM ATP, 1 mM GTP and 6 mM Phosphoenolpyruvate. In addition mixture I contained in 50 µl: 10 µg poly(U), 20 pmol ribosomes and 20 pmol [^{3}H]-*N*-acetyl-Phe-$tRNA^{Phe}$ (460 cpm/pmol). Mixture II contained in 50 µl: 150 µM (^{14}C)-Phe (4 cpm/pmol), 300 µg total *E. coli* tRNA (Boehringer-Mannheim), 150 pmol EF-G, 150 pmol EF-Ts, 150–200 pmol EF-Tu, 5 µg pyruvate kinase, 0.3 µg myokinase and ~50–100 units of Phe tRNA synthetase.

Mixture I was preincubated in 50 µl aliquots for 10 min at 37° C. Mixture II was preincubated as a multiple of 50 µl aliquots in one tube for 10 min at 37° C. The elongation reaction was started by transferring 50 µl mixture II to a 50 µl aliquot of mixture I. The reaction was stopped after 10 s by the addition of 5 ml trichloroacetic acid containing 0.5% w/v of both Phe and Leu. Filtrations were done as described by Jelenc and Kurland (1979) For the calculation of Phe incorporation and the amount of *N*-acetyl-Phe incorporated the backgrounds were subtracted from the obtained values. The backgrounds were obtained by omission of poly(U) in control samples and ranged from 10–20 cpm in the (^{14}C) and 20–40 cpm in the (^{3}H)-channel.

Determination of Error Frequency in vitro: The procedure was the same as for elongation rate determination except for the following modifications: [^{3}H]-*N*-acetyl-Phe-$tRNA^{Phe}$ was omitted and insted 20 pmol [^{14}C]-*N*-acetyl-Phe-$tRNA^{Phe}$ (4 cpm/pmol) were added. 30 µM [^{3}H]-Leu (2,200 cpm/pmol) and ~5–10 units of Leu-tRNA synthetase were also included. The samples were incubated for 20 s. The background in the [^{3}H]-channel with [^{3}H]-Leu was about 120–140 cpm. The error rates were calculated by comparing the ^{3}H-Leu and ^{14}C-Phe incorporation in the same incubation mixtures (Wagner et al. 1982).

Results

a) Nonsense Suppression

The *rpsD* alleles analyzed here were classified earlier according to their ability to suppress phenotypically nonsense mutations in phage T4 (Olsson and Isaksson 1979a; Isaksson, unpublished data). These mutations were transduced into a strain (UD121) which was free of any nonsense supressor, was *rpsL*$^+$ and harbored a deletion for the entire *lac* operon. Since we desired more precise estimations of suppression frequencies, F′-factors containing a fused *lacI* and *lacZ* gene (Muller-Hill and Kania 1974; Miller et al. 1978), in which the *lacI* gene carries one of the three nonsense codons either at position 220 or 189, were introduced into the isogenic *rpsD* strains. Some of the F′-strains were constructed with the help of J. Miller (Geneva, Switzerland) to whom we are beholden. Suppression of the nonsense codons in the repressor part of the fused protein gives an active enzyme and the activity of this readthrough product can be measured by standard procedures (see Materials and Methods).

Table 2. Suppression of nonsense codons in vivo. Average of four independent measurements. Expressed as suppression $\times 10^4$. Assay and calculations were as described in Materials and Methods

Chromosomal background	Ribosomal genotype	F-prime factor from strain					
		Position 220			Position 189		
		UGA	*UAG*	*UAA*	*UGA*	*UAG*	*UAA*
		UD471	UD368	UD367	UD366	UD365	UD364
UD132	*rpsD*$^+$	99±9	21±5	12±5	50±9	2±1	1±0.5
UD179	*rpsD16*	527±122	33±9	27±4	612±164	12±3	8±2
UD131	*rpsD12*	930±369	52±23	54±15	1,750±278	30±15	15±6
UD178	*rpsD14*	1,550±500	34±6	28±4	2,575±625	23±3	17±5

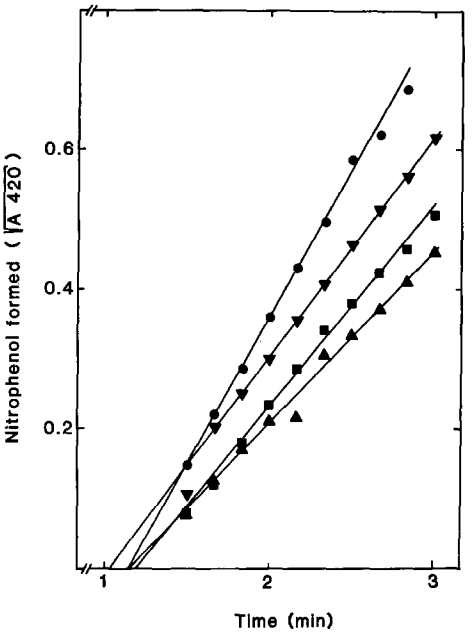

Fig. 1. Induction kinetics of β-galactosidase after addition of IPTG. Samples were assayed as described in Materials and Methods. Absorbance values are shown replotted as square root of actual values. Intercepts of the lines with the time axis give the time required for synthesis of the first β-galactosidase monomers. (●) *rpsD*$^+$, (▼) *rpsD16*, (■) *rpsD12*, (▲) *rpsD14*

The data obtained with the 24 relevant strains are summarized in Table 2. In wild-type background (*rpsD*$^+$) the spontaneous suppression frequencies for all three nonsense codons were somewhat higher when they were at position 220 than at position 189. This could be due to a context effect on the translational readthrough at these positions, or to the different relative frequencies of internal reinitiation of translation near these positions (Platt et al. 1972; Files et al. 1974; J. Miller, personal communication). When the different *rpsD* alleles were present, the suppression frequencies increased by a factor of 2–50 depending on the nonsense codon, its position, and which *rpsD* allele was present (see Table 2).

It is evident that the D16 mutant was the least effective phenotypic suppressor under all the conditions tested. The D12 mutant tends to be somewhat more effective than the D14 mutant with UAG and UAA nonsense codons. However, the suppression levels are low at these codons. In contrast, the suppression frequencies with the UGA codon

Table 3. Times required for synthesis of the β-galactosidase monomer (tβ-gal), average of three independent experiments; and overall rate of polypeptide synthesis. Assay and calculations were as described in Materials and Methods

Chromosomal background	F-prime factor from strain	Ribosomal genotype	tβ-gal (seconds)	Elongation rate (amino acids/second)
UD132	CSH23	*rpsD*$^+$	69.3±0.6	17
UD179	CSH23	*rpsD16*	62.3±0.7	19
UD131	CSH23	*rpsD12*	71.0±1	17
UD178	CSH23	*rpsD14*	69.3±0.9	17

are relatively high both at position 220 and 189. Interestingly, all three *rpsD* alleles are more effective with UGA at position 189 than at position 220; this is opposite to the order for spontaneous suppression. Most important, there is the same order of phenotypic suppression of the UGA codon at both positions: D14 > D12 > D16.

b) β-Galactosidase Elongation Rates

To determine the elongation rates of the different mutant ribosomes, the synthesis of β-galactosidase was measured after induction and the time required for the first active molecule to appear was estimated, (Schleif et al. 1973; Galas and Branscomb 1976; Piepersberg et al. 1979). The time course for expression of β-galactosidase activity in one representative experiment is shown in Fig. 1 and the data from several such experiments are summarized in Table 3.

The apparent elongation rate for β-galactosidase is very nearly the same for wild type ribosomes and those of D12 as well as D14. The rate of elongation by D16 ribosomes, the least error prone of the mutants, might be 10% faster; however, we are not convinced that this difference in rates is significant. Nevertheless, elongation rates close to 17 amino acids per second as measured here are in good agreement with previous estimates (Schleif et al. 1973; Piepersberg et al. 1979). Quite clearly, there is no systematic correlation between the overall speed of translation and the suppression frequencies characteristic of these strains.

c) Translation in vitro

Since the phenotypic suppression which is enhanced by the *rpsD* alleles is thought to involve a missense event at the

Table 4. Leucine misincorporation and elongation rate in a poly(U)-directed in vitro translation system. Assay and calculations were as described in Materials and Methods

Strain	Ribosomal genotype	$\frac{\text{Leu}}{\text{Leu}+\text{Phe}} \times 10^4$	Elongation rate (amino acids/second)
O17	*rpsD*$^+$	2.3	10
D16	*rpsD16*	12	9.7
D12	*rpsD12*	12	10
D14	*rpsD14*	23	9.6

nonsense codons (Gorini 1971) we attempted to measure the missense error frequencies expressed by the mutant ribosomes in vitro. A newly developed in vitro system for the translation of poly U during a 10 s elongation burst (Wagner et al. 1981) was used for this purpose. In this particular assay we monitor the leucine incorporation as well as the phenylalanine incorporation during the burst. The missense error frequency is calculated as the mole fraction of leucine incorporated into polypeptide.

As can be seen from the data in Table 4, the wild-type ribosomes (O17) translate poly U with a leucine error frequency close to 2×10^{-4}, while the Ram ribosomes do so with a five to ten times higher missense frequency. According to this assay the most error prone ribosomes are those from D14, which is in agreement with the rank ordering of the *rpsD* alleles with respect to their ability to suppress phenotypically UGA codons.

Naturally, we were also curious about the speeds at which these different ribosomes elongate polyphenylalanine. The data in Table 4 show that the rate of polypeptide synthesis is virtually the same for the wild-type ribosomes and the three mutant ribosomes. This rate is approximately two-thirds that of β-galactosidase elongation in vivo. This discrepancy could reflect some defect of our assay system. Alternatively, the rate of translating repetitive UUU codons may be slower than that for the average codon in the β-galactosidase messenger.

Discussion

There have been a large number of earlier studies of the effects of mutations in the *rpsD* gene on the phenotypic suppression of nonsense mutations (see Gorini 1971). Most of these studies have been performed with bacteria that also contained tRNA suppressor mutations and/or additional mutations that alter other ribosome components (e.g. the streptomycin resistance alleles of *rpsL*). Here, we have concentrated on the effects of the *rpsD* mutations in a wild-type background for the components of the translation apparatus. Nevertheless, the suppression patterns are not simple.

Thus, the extent of phenotypic suppression supported by three different Ram mutants varies depending on which nonsense codon is present. UGA is much more vigorously suppressed than UAG and UAA. Furthermore, the rank order of the Ram mutants with respect to the relative extent of suppression they support depends on whether the nonsense codon is UGA or one of the other two terminators. This pattern is consistent with there being a multiplicity of kinetic parameters that determine the outcome of the competition between a release factor and a nearly cognate aminoacyl-tRNA for the nonsense codons on the ribosome (Ninio 1974).

The interpretation (Gorini 1971) that the successful suppression of a nonsense codon in the absence of mutant suppressor tRNA's is due to a missense event is supported by the in vitro characteristics of the Ram ribosomes. Thus, we find that all three types of Ram ribosomes are characterized by a significantly greater leucine missense error frequency than wild-type ribosomes when translating poly U in vitro. Furthermore the Ram ribosome that most effectively suppresses UGA codons has the highest leucine missense error frequency in vitro.

Further analysis of the factors determining the suppression patterns obtained with different Ram mutants would be greatly facilitated by the identification of the particular wild-type tRNA's involved in the missense reading of nonsense codons. Here it is worth recalling that wild-type $tRNA^{Trp}$ has a surprisingly great propensity to suppress UGA codons (Hirsh 1971; Hirsh and Gold 1971). It may be that Ram ribosomes consistently suppress UGA more effectively than the other two nonsense codons because they enhance a codon ambiguity intrinsic to $tRNA^{Trp}$ which is not as pronounced in the tRNA species that are the wild-type suppressors of the other two nonsense codons. This suggestion is consistent with the finding that the suppression frequency with wild-type ribosomes is also greater for UGA codons than for UAG or UAA (Gorini 1974).

Finally, we have found no correlation between either the suppressor frequencies in vivo or the missense error frequencies in vitro and the rates of elongation by the different ribosome phenotypes. Indeed, the rates of elongation by wild type and Ram ribosomes are virtually indistinguishable under all conditions tested. These results together with those of Piepersberg et al. (1979) strongly suggest that there is no general relationship between the overall speed and accuracy of translation by different mutant ribosomes.

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