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Hyper-accurate ribososomes inhibit growth

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We have compared both *in vivo* and *in vitro* translation by ribosomes from wild-type bacteria with those from streptomycin-resistant (SmR), streptomycin-dependent (SmD) and streptomycin-pseudo-dependent (SmP) mutants. The three mutant bacteria translate more accurately and more slowly in the absence of streptomycin (Sm) than do wild-type bacteria. In particular, the SmP bacteria grow at roughly half the rate of the wild-type in the absence of Sm. The antibiotic stimulates both the growth rate and the translation rate of SmP bacteria by ~2-fold, but it simultaneously increases the nonsense suppression rate quite dramatically. Kinetic experiments *in vitro* show that the greater accuracy and slower translation rates of mutant ribosomes compared with wild-type ribosomes are associated with much more rigorous proofreading activities of SmR, SmD and SmP ribosomes. Sm reduces the proofreading flows of the mutant ribosomes and stimulates their elongation rates. The data suggest that these excessively accurate ribosomes are kinetically less efficient than wild-type ribosomes, and that this inhibits mutant growth rates. The stimulation of the growth of the mutants by Sm results from the enhanced translational efficiency due to the loss of proofreading, which more than offsets the loss of accuracy caused by the antibiotic.

Key words: streptomycin dependence/ribosomal mutants/proofreading/streptomycin/bacterial growth rate

Introduction

There are mutants of *Escherichia coli* that are intermediate to the classical streptomycin-resistant (SmR) and streptomycin-dependent (SmD) phenotypes which are associated with alterations of the ribosomal protein S12 (Birge and Kurland, 1967; Ozaki *et al.*, 1969). These intermediate forms were described by Zengel *et al.* (1977) as bacteria that, while not completely dependent on the antibiotic for growth, are strongly stimulated by the presence of Sm in the growth medium. We will refer to this as the pseudo-dependent (SmP) phenotype.

We have compared *in vivo* and *in vitro* the kinetics as well as the accuracy of translation for SmR, SmD and SmP ribosomes. Our data show that in the absence of antibiotic the SmP ribosomes are excessively aggressive proofreaders, which slows down their translation rates by the same degree to which their growth rate is inhibited. The addition of Sm selectively inhibits the proofreading flows and thereby speeds up the translation rates with a parallel stimulation of the growth rates. The stimulation of growth is accompanied by a dramatic increase of translational error frequencies. The data show that ribosomes that proofread excessively can inhibit the growth of bacteria. This is very likely the explanation for the SmD phenotype as well.

Results

The in vivo phenotypes

In order to have a firm, quantitative basis for the comparison of the protein synthetic activities of different mutants we have measured their rates of β-galactosidase synthesis using the method of Schleif *et al.* (1973) with minor modifications (Andersson *et al.*, 1982; Bohman *et al.*, 1984). These data are summarized in Table I. There we find that the wild-type ribosomes synthesize β-galactosidase at a rate of 15 amino acids per second; this may be compared with values of 9–15 per second for different, allelic SmR mutants (Bohman *et al.*, 1984). Since growth is not measurable for wild-type bacteria in 100 μg/ml Sm nor for SmD bacteria in the absence of antibiotic, it was not feasible to characterize these two strains under those particular conditions.

The data clearly confirm the suggestion of Zengel *et al.* (1977) that the addition of Sm stimulates the growth rate of the SmP mutant by increasing the efficiency of ribosome function. Thus, in the absence of antibiotic the SmP strain elongates polypeptide at a rate close to five amino acids per second, but at a concentration of 100 μg/ml of Sm the rate of elongation is 11 per second. The rate of protein synthesis by the drug-dependent mutant (SmD) in 100 μg/ml of antibiotic was likewise close to 10 amino acids per second.

We have also studied the influence of Sm on the accuracy of translation by the different mutants. Here, we have used the method of Miller (1972) to measure β-galactosidase activity produced by strains with nonsense mutations in a *lac* IZ fusion. The suppression of the nonsense codon UGA was studied when it was present at either position 189 or 220 in the I sequence of the fused gene; the appropriate strains and their analysis are described in detail elsewhere (Andersson *et al.*, 1982; Bohman *et al.*, 1984). The suppression frequencies obtained are summarized in Table I for the different mutants in

Table I.

Phenotype	Strain	Sm	Suppression frequency		Elongation rate (aa/s)
			UGA_{189} UD366	UGA_{220} UD471	CSH23
Wild-type	UD121	–	82	105	15
SmR	UK285	–	2.1	9.7	7.8[a]
SmR	UK285	+	5.5	16	9.4[a]
SmP	UK317	–	2.1	5.3	5
SmP	UK317	+	560	490	11
SmD	UD671	+	100	80	10

In vivo elongation rates and the suppression frequencies of the indicated nonsense codons were measured as described in the Materials and methods. When present, the streptomycin was at a concentration of 100 μg/ml. The strains used as donors for F-prime factor are indicated.
[a]See Bohman *et al. (1984).*

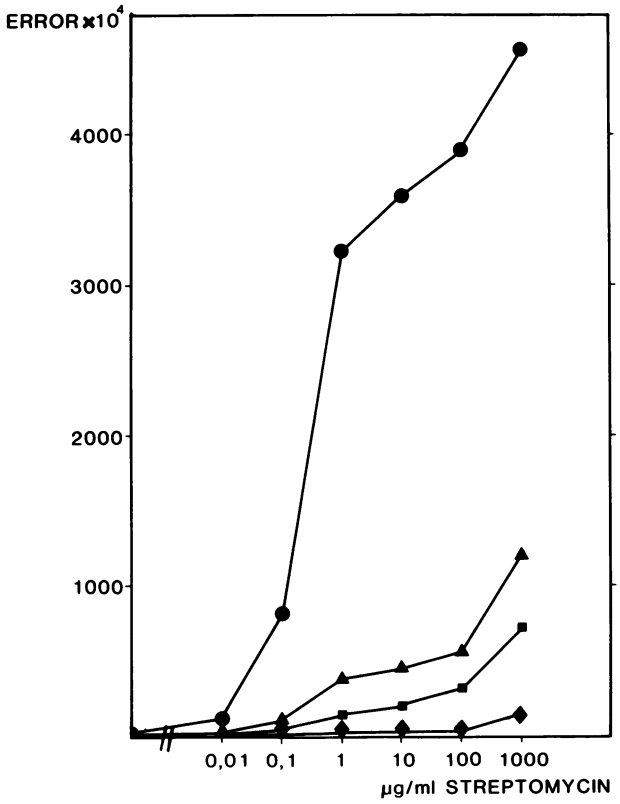

Fig. 1. Error frequency as a function of streptomycin for the four different ribosomal phenotypes (● 017, ▲ SmD, ■ SmP, ◆ SmR). The non-cognate tRNA species $tRNA_4^{Leu}$ was in 4-fold excess over the cognate one.

the presence and absence of Sm.

The greater accuracy of the restrictive SmR strain is reflected in the considerably lower suppression rate at both positions compared with the wild-type strain. Furthermore, the addition of 100 μg/ml of antibiotic has little effect on the accuracy of translation of the mutant *in vivo*. In contrast, the SmP strain, which in the absence of Sm has restriction of the suppression rates comparable with the SmR strain, is stimulated ~100-fold by antibiotic so that the suppression rates of the SmP mutant in 100 μg/ml Sm are 5- to 7-fold greater than the wild-type spontaneous level. The suppression rates of the SmD strain in 100 μg/ml Sm are strikingly close to the suppression rates of the wild-type in the absence of antibiotic.

We have previously shown that mutations and antibiotics that raise or lower the ribosome-dependent nonsense-suppression rates *in vivo* also have parallel effects on the missense frequencies expressed *in vitro* (Andersson *et al.*, 1982; Andersson and Kurland, 1983; Bohman *et al.*, 1983; Jelenc and Kurland, 1984; Ruusala and Kurland, 1984). Accordingly, the nonsense suppression data summarized in Table I indicate that the SmP bacteria are not resistant to the error-enhancing effects of Sm, a conclusion that is confirmed for the purified ribosomes below. Hence, the remarkable result is that the growth of SmP mutants is stimulated by an agent that simultaneously raises the translational error frequencies to much higher levels than those normally seen in wild-type bacteria.

Missense frequencies

Although they are qualitatively coupled to each other, Sm-induced changes in the absolute frequencies of the translation errors of mutant and wild-type ribosomes are different *in vivo* and *in vitro* (Andersson *et al.*, 1982; Bohman *et al.*, 1984; Ruusala and Kurland, 1984). This may be ascribed to the difference between the *in vivo* event responsible for nonsense suppression and that responsible for the *in vitro* missense event. The one involves a competition between tRNA and release factors while the other is the outcome of a competition between two tRNA isoacceptor species.

The data summarized in Figure 1 show that the Leu missense frequencies of wild-type ribosomes in our poly(U)-directed system (Wagner *et al.*, 1982) increase most dramatically in response to Sm, while the response of drug-dependent and SmP ribosomes is much more restrained. In order to standardize these results obtained *in vitro*, we have measured the characteristic error frequencies (E) of the ribosomes from different bacterial strains (Table IV). Here, E is the missense frequency supported by a given ribosome preparation when equal concentrations of the cognate aminoacyl-tRNA species and a non-cognate isoacceptor species are competing for the same codon-programmed ribosome (Ruusala *et al.*, 1982b). We observed that the error rates in the presence of Sm are according to this assay somewhat greater for SmD than for SmP ribosomes, which is the opposite rank order to that for nonsense suppression *in vivo* (Table II). It is worth emphasizing that all of these mutants (SmR, SmD and SmP) produce ribosomes that have a lower Leu missense frequency than wild-type in this assay, both in the presence and absence of antibiotic (Figure 1, Table IV).

Kinetic parameters

When we tried to duplicate *in vitro* the stimulation of the elongation rate of SmP ribosomes by Sm in a ribosome-limited system, we failed to detect the expected effects. This suggested that an increase of the k_{cat} of the mutant ribosomes was not responsible for the effect of Sm *in vivo*. Therefore in order to inspect the K_M values, we performed kinetic experiments in which the rate of elongation was measured as a function of the ternary complex concentration by varying the amounts of elongation factor Tu (EF-Tu) added to the incubation mixtures. We then analyzed the data by the Eadie-Hofstee method as described by Morris (1974) and adapted by us to the present system (Ruusala *et al.*, 1982b; Bohman *et al.*, 1984). The resulting values of k_{cat} and K_M for the ribosomes obtained from mutant strains in the presence and the absence of Sm are recorded in Table III.

In general, the k_{cat} values do not vary very much for the different sorts of ribosomes, and, most important, Sm has a small inhibitory effect in all cases (between 20 and 30%). Accordingly, the effect of the antibiotic that is expressed through its influence on the k_{cat} of the ribosomes will be to retard elongation slightly. In contrast, we observe a more complex influence of the antibiotic on the ribosomal K_M values for ternary complex.

In the case of wild-type ribosomes the antibiotic increases the value of K_M. This means that at a concentration of ternary complex that does not saturate the ribosome, this effect of Sm would decrease the rate of elongation. Indeed, the degree of inhibition at limiting concentrations of ternary complex is determined by the change in the ratio of k_{cat}/K_M (Fersht, 1977) which we refer to as the rate factor (R). For wild-type ribosomes, the rate factor decreases by more than a factor of two in excess Sm.

The K_M values of the mutant ribosomes respond in the opposite way: they decrease, which would tend to increase the rate of elongation in the presence of Sm and at rate-limiting

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

Designation	Sex; extrachromosomal markers	Chromosomal markers	Relevant characters	Origin
017	F^-	–	wt	Olsson *et al.*, 1979
UK186	F^-	*aroE*,Tn10	Aro^-,Tn10(Tet^R)	This laboratory
UK318	F^-	*rpsL*	SmP	This laboratory
NO1204	F^-	*thr,lacY,rpsL*	SmP	Zengel *et al.*, 1977
UK317	F^-	△*prolac,rpsL,argE*(amber),*rpoB,gyrA*,Ara	SmP	This laboratory
UK235	F^-	*rpsL*	SmR	Bohman *et al.*, 1984
UK285	F^-	△*prolac*,Ara,*gyrA,rpoB,argE,rpsL*999	SmR	Bohman *et al.*, 1984
UD666	F^-	*rpsL*	SmD	This laboratory
UD671	F^-	△*prolac,rpsL,argE*(amber),*rpoB,gyrA*,Ara	SmD	This laboratory
UD111	F^-	△*prolac,argE*(amber),*rpoB,gyrA*,Ara		Miller *et al.*, 1978
UD121	F^-	△*prolac,argE*(amber)*rpoB,gyrA*,Ara,*aroE*,Tn10	Aro,Tn10(Tet^R)	Andersson *et al.*, 1982
UD366	F'*lacIZ* fusion $proA^+B^+$	△*prolac,rpsL*,λ^R	Carries UGA mutation in I part at position 189	Andersson *et al.*, 1982
UD471	F'*lacIZ* fusion $proA^+B^+$	△*prolac,rpsL*,λ^R	Carries UGA mutation in I part at position 220	Andersson *et al.*, 1982
CSH23	F'$lac^+proA^+B^+$	△*lacpro,supE,rpsE,thi*	Lac^+	Miller, 1972

Table III. Kinetic constants of the interaction between ternary complex and four different ribosomes in the absence and presence of streptomycin (200 μg/ml).

Phenotype	Strain	Sm	$k_{cat}(s^{-1})$	K_M x 10^7(M)	R x $10^{-7}(M^{-1}s^{-1})$
Wild-type	017	–	14.9	6.5	2.3
		+	8.6	9.3	0.9
SmR	UK235	–	13.2	8.5	1.6
		+	11.4	5.4	2.1
SmP	UK318	–	9.6	10.8	0.9
		+	6.6	4.1	1.6
SmD	UD666	–	11.4	14.8	0.8
		+	9.0	4.1	2.2

The numbers were extracted from Eadie-Hofstee plots.

Table IV. Accuracy characteristics of the four ribosomal phenotypes in the absence and presence of streptomycin (200 μg/ml).

Phenotype	Sm	E x 10^4	f_c	f_w	F	I
Wild-type	–	6	1.1	45	41	41
	+	1027	1.0	1.6	1.6	6
SmR	–	2	1.4	160	110	45
	+	20	1.0	17	17	29
SmP	–	4	1.7	180	106	24
	+	100	1.0	3.5	3.5	29
SmD	–	4	2.4	100	42	60
	+	178	1.0	4.5	4.5	12

For definitions of E, f_c, f_w, F and I see Materials and methods and Results.

ternary complex concentrations. Accordingly, the predicted rate increases would correspond to factors of 1.3, 1.8 and 2.8 for SmR, SmP and SmD ribosomes, respectively.

In our previous study of the functions of SmR ribosomes, we observed a correlation betwen the amount of proofreading activity and the increased K_M of the ribosomes (Bohman *et al.*, 1984). Since we find a tendency for SmP and SmD ribosomes to have even greater K_M values than do SmR ribosomes, we next compared the proofreading activities of these ribosomes.

Proofreading activities

We recall that the excess dissipation of ternary complexes in the proofreading branches will influence the stoichiometric ratio of ternary complexes consumed per peptide bond for both cognate (f_c) and non-cognate (f_w) tRNA species (Ruusala *et al.*, 1982b). The ratio f_w/f_c is the proofreading factor (F), which describes the relative increase of accuracy in the proofreading branches of the system. This factor times the initial discrimination factor (I) makes up the accuracy of the system. The values corresponding to these characteristic parameters for wild-type, SmP, SmD and SmR ribosomes are listed in Table IV.

Since we are concerned here with mutant ribosomes that are relatively resistant to the antibiotic, we are obliged to work at high concentrations of Sm (200 μg/ml). Nevertheless, we observe relatively small effects of the antibiotic on the initial selectivity (I) and much larger inhibitions of the proofreading flows (F), as observed previously for other mutant ribosomes (Ruusala and Kurland, 1984). In addition, the data in Table IV show that the proofreading flows of SmP and SmD ribosomes are significantly more sensitive to Sm than is that of SmR ribosomes.

It is worth emphasizing that the proofreading flow that will have the greatest impact on the overall kinetic effect of the translation system *in vivo* is associated with the dissipation of cognate ternary complexes as summarized in f_c (Bohman *et al.*, 1984; Kurland and Ehrenberg, 1984). This follows from the fact that the error frequencies are normally so small. Accordingly, the effects of Sm on the f_c values of the mutants are of particular relevance to their phenotypes *in vivo*. We observe in all cases that the mutants have significantly higher f_c values than do wild-type ribosomes; the most extreme of these are the SmD ribosomes and the least extreme are the SmR ribosomes. In all cases Sm virtually abolishes the excess dissipation of cognate ternary complex. In other words, while Sm lowers the accuracy it also increases the efficiency of translation with respect to ternary complex consumption per peptide bond. For the case of SmD ribosomes the decrease in the ternary complex consumption per peptide is more than a factor of two.

We have studied the variation of ternary complex utiliz-

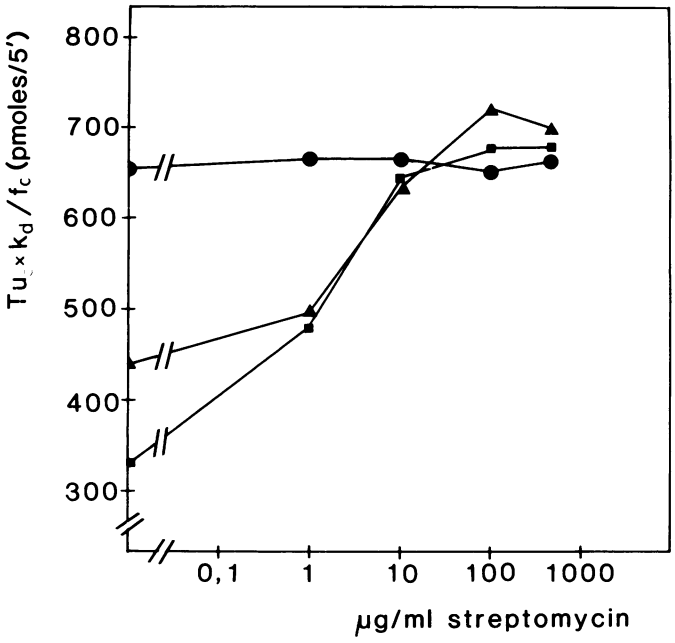

Fig. 2. Phenylalanine incorporation into polypeptide in the absence of EF-Ts as a function of streptomycin concentration. Three different ribosomal phenotypes are shown (● 017, ■ SmD, ▲ SmP).

ation as a function of antibiotic concentration by the mutant ribosomes in more detail. Thus, we limit the rate of translation by adding very small amounts of EF-Tu to the system, and we titrate the system with antibiotic. We then measure the value for the ratio $Tu_o \cdot k_d/f_c$, which has been described previously (Ruusala *et al.*, 1982b). Here, Tu_o is the total amount of EF-Tu added, k_d is the rate constant for the dissociation of GDP from the binary complex with EF-Tu, and f_c is the stoichiometric ratio of ternary complex consumed per peptide bond. Since we fix Tu_o experimentally and k_d is a constant, the measured ratio provides an index of the number of peptide bonds formed per dissipated ternary complex.

The data summarized in Figure 2 show that the efficiency of peptide bond formation by wild-type ribosomes is virtually unaffected by increasing concentrations of Sm. Elsewhere we have reported very small effects of the antibiotic on restrictive SmR ribosomes (Bohman *et al.*, 1984). In contrast, the SmP ribosomes and to a somewhat greater degree the SmD ribosomes utilize ternary complex more efficiently to synthesize peptide bonds at the higher concentrations of antibiotic than in its absence. Indeed, the stoichiometric ratio of peptide bonds formed per ternary complex consumed more than doubles when SmD ribosomes are assayed in saturating concentrations of Sm.

Discussion

The existence of mutants that produce proteins at error frequencies significantly lower than those of wild-type shows that gene expression is normally not carried out at an accurcy that is near a physical maximum (Kurland and Ehrenberg, 1984). Rather, the accuracy of gene expression seems to be adjusted to a biologically determined optimum. Here, the errors of gene expression will occur at those frequencies for which the deleterious effects of the errors themselves are just balanced by the additional costs entailed in reducing them to still lower frequencies.

The notion of an accuracy cost is not simply a convenient metaphor. Rather it is a concept that has been described rigorously for the particular kinetic framework of exponential bacterial growth (Ehrenberg and Kurland, 1984). Here, the critical boundary condition is that the rate of bacterial growth is assumed to approach a maximum. Accordingly, the costs of accuracy are expressed in parameters such as the reduction in the kinetic efficiency of biosynthesis devices attending an increase in their accuracy of function. Similarly, the costs can be calculated as the fractions of the metabolic flows invested in the suppression of errors, a parameter that is particularly useful for describing proofreading systems. In order to explore this cost analysis experimentally, it is necessary to identify the principal costs of accuracy as well as the major error sources in gene expression.

The only relevant direct measurements of missense errors in bacteria indicate that most of these are introduced at the level of the ribosome. Thus, the missense frequencies at two different codons are reduced 70–80% by the introduction of a ribosomal protein allele that raises the accuracy of translation (Bouadloun *et al.*, 1983). Indeed, estimates of the error frequencies for RNA polymerase function (Rosenberg and Foskett, 1981) as well as for aminoacyl-tRNA synthetase suggest that these are on average lower than those of the ribosome. In addition, the metabolic investment in the ribosome-mediated functions seems to be quite a bit greater than for aminoacyl-tRNA synthetase and RNA polymerase. We recall that the natural substrates for polypeptide synthesis are GTP as well as aminoacyl-tRNA bound to EF-Tu and GTP bound to EF-G. Thus, four pyrophosphate equivalents are consumed per peptide bond, without taking into accont proofreading dissipations; to these must be added the metabolic investment in the macromolecular devices that mediate these substrate flows during peptide bond formation. It is not obvious why the metabolically most expensive step in gene expression should also be the noisiest step in the process.

The results of the present study illustrate the notion of a trade-off between the costs and benefits of translational accuracy. We suggest that the pseudo-dependent strain of bacteria grows more rapidly in the presence of Sm because, even though the accuracy of translation has been reduced, the increased kinetic efficiency of the mutant ribosomes more than compensates for this. Thus, we find that the error rate in the absence of antibiotic is much lower for the pseudo-dependent mutant than for the wild-type. Although the antibiotic dramatically increases the error frequency of the pseudo-dependent ribosomes *in vivo*, even at saturating concentrations of Sm the error rate of the mutant is only several-fold greater than that of wild-type in the absence of Sm. Most important, the rate of polypeptide synthesis by the mutant is more than doubled by the antibiotic *in vivo*. The kinetic characterization of the influences of Sm *in vitro* provides a simple explanation for the diverse effects of the antibiotic on the different bacterial strains.

We have previously shown in a study of wild-type and Ram mutants that Sm is a relatively specific inhibitor of the ribosomal proofreading flow, which means that it will simultaneously increase the errors of translation as well as improve the kinetic efficiency of the translation apparatus (Ruusala and Kurland, 1984). Therefore, the effect of the antibiotic on the growth of the bacteria will depend on the particular kinetic characteristics of its ribosomes. Here, we have described mutants (SmD and SmP) that produce ribosomes that are

very aggressive proofreaders. Therefore these mutant ribosomes translate slowly at an unnecessarily high accuracy level in the absence of antibiotic. As we have shown here, Sm reduces the proofreading flow of the SmP ribosomes and thereby increases their kinetic efficiency to a sufficient degree to stimulate their rates of elongation at least 2-fold.

There are three kinetic efficiency parameters associated with proofreading that are identifiable in the present study and that are relevant to growth limitations. One of these is the number of GTP molecules that must be generated to produce a peptide bond. Increasing the accuracy by increasing this stoichiometric ratio could lead to a situation *in vivo* in which the availability of GTP limits the rate of translation in a mutant that is an excessive proofreader. A second related parameter is the regeneration rate of ternary complex, which might be limited by the concentration of EF-Ts in the bacterium (Ruusala *et al.*, 1982a). Accordingly, an increase in the number of ternary complex cycles per peptide bond could lead to a situation in which the rate of translation is limited by the functions of EF-Ts. Finally, vigorous proofreading can lead to a kinetic situation in which the degree of saturation of ribosomes with ternary complex is lowered, and this would be reflected in an increase in the effective K_M of the ribosomes for ternary complex (Bohman *et al.*, 1984). This effect could lead to a situation in which the rate of polypeptide synthesis is retarded because the saturation level of ribosomes at a given ternary complex concentration is lowered as was observed for the pseudo-dependent ribosomes *in vitro*. We cannot be certain about the extent to which these different kinetic parameters influence the pseudo-dependent mutant *in vivo*. Any one, two or all three could account for slow growth in the absence of drug as well as for the increased rates of protein synthesis in response to Sm, which would stimulate the growth rates in the presence of antibiotic.

We are persuaded by the data obtained with the SmD ribosomes *in vitro* that their excessive proofreading and its suppression by antibiotic will account at least in part for this bacterial phenotype. The excess ternary complex dissipation of these ribosomes *in vitro* is 14 times greater than that of wild-type ribosomes and twice that of the pseudo-dependent ribosomes. This, taken together with a putative ribosome assembly defect (Hummel and Böck, 1984), might account for the complete growth dependence of these mutants on the presence of antibiotic. Finally, it appears that the only reason that the growth of the restrictive SmR mutants is not stimulated more dramatically by Sm is that they simply do not accumulate the antibiotic at sufficiently high internal concentrations *in vivo* (Table II). We have shown both here and elsewhere (Bohman *et al.*, 1984) that *in vitro* the kinetic efficiency of translation by restrictive SmR ribosomes is improved at very high concentrations of antibiotic.

Materials and methods

Chemicals

Poly(U), GTP, ATP, phosphoenolpyruvate (PEP) trisodium salt, putrescine, spermidine, L-phenylalanine, L-leucine, chloramphenicol, streptomycin sulphate, ONPG, IPTG and myokinase (EC 2.7.4.3) were purchased from Sigma Chemical Co., St. Louis, MO. Radioactive phenylalanine and dihydrostreptomycin were obtained from Amersham International, Bucks, UK, and radioactive leucine from New England Nuclear, Boston, MA. *Escherichia coli* total tRNA and pyruvate kinase (EC 2.7.1.40) were obtained from Boehringer Mannheim, Mannheim, FRG.

Purifications and preparations

Ribosomes were prepared from strain 017, UK318, UK235 and UD666 as described by Jelenc (1980) except that the ribosomes were stored at −80°C in polymix buffer without methanol. Control experiments where [^{3}H]dihydrostreptomycin was added during growth showed that <3% of the ribosomes still retained dihydrostreptomycin after purification. The purification of EF-G (Wagner and Kurland, 1980) and EF-Tu (Lebermann *et al.*, 1980; Wagner and Kurland, 1980), EF-Ts (Arai *et al.*, 1972), Phe tRNA synthetase (Wagner *et al.*, 1982) and Leu tRNA synthetase (Wagner *et al.*, 1982) have been described earlier. Preparation of isoaccepting tRNAs (tRNA^{Phe} and tRNA^{Leu}) and N-acetyl-Phe tRNA^{Phe} were done according to Ruusala *et al.* (1982b).

Strains

The genotypes and derivations of the *E. coli* K12 strain used are listed in Table II. UD666 (SmD) was isolated as described by Momose and Gorini (1970). Transductions were performed as described by Miller (1972). The *rpsL* alleles from strains N01204 and UD666 were transduced into UK186 and UD121 as described in Andersson *et al.* (1982). F′ factors from strain UD366, UD471 and CSH23 were introduced to UD111, UK317, UK285 and UD671 by cross-streaking on plates selecting for Pro$^+$ and using rifampicin as counter-selection.

In vivo assays

Determination of elongation rate and nonsense suppression were done as described in Andersson *et al.* (1982).

In vitro assays

Error and elongation rates. Assays were performed as described in Andersson *et al.* (1982). Streptomycin was added together with the ribosomes during pre-incubation.

Determination of kinetic constants. Assays were performed as described in Bohman *et al.* (1984). When present the streptomycin was at a concentration of 200 μg/ml.

f_c Determination

Two mixtures containing polymix buffer were prepared on ice. Mixture I contains in 50 μl: 80 pmol of ribosomes, 100 pmol [^{3}H]N-acetyl-Phe-tRNA^{Phe} (150 c.p.m./pmol), 20 μg poly(U), 0 or 20 μg of streptomycin and 50–200 pmol of EF-Tu. Mixture II contains in 50 μl: 2 mM ATP, 2 mM GTP, 12 mM PEP, 5 μg pyruvate kinase, 0.3 μg myokinase, 50 pmol EF-G 30 nmol [^{14}C]phenylalanine (1 c.p.m./pmol), 100 units Phe-tRNA synthetase, 0 or 100 pmol EF-Ts, 250 pmol tRNA^{Phe}. After 10 min pre-incubation at 37°C the reaction was started by transferring 50 μl mixture II to 50 μl mixture I and stopped after 5 min incubation at 37°C by the addition of 5 ml trichloroacetic acid containing 0.5% w/v of Phe and Leu. Processing of samples was as described by Jelenc and Kurland (1979).

f_w Assay

Two mixtures, initiation mix (I) and factor mix (II) both containing the polymix buffer components were prepared on ice. Mixture I in addition contains in 50 μl: 180 pmol ribosomes, 200 pmol [^{3}H]N-acetyl-Phe-tRNA^{Phe} (150 c.p.m./pmol), 50 μg poly(U), 200 pmol EF-G, 50–250 pmol EF-Tu and 0 or 20 μg of streptomycin. Mixture II contains in 50 μl: 2 mM ATP, 2 mM GTP, 12 mM PEP, 5 μg pyruvate kinase, 0.3 μg myokinase, 100 units of Phe-tRNA synthetase, 50 units of Leu-tRNA synthetase, 380 pmol unlabelled phenylalanine, 15 nmol [^{14}C]leucine (20 c.p.m./pmol), 600 pmol tRNA^{Phe}, 600 pmol tRNA$^{Leu}_4$, 50 pmol EF-Tu and 0 or 150 pmol of EF-Ts.

After 10 min pre-incubation at 37°C, 50 μl mixture II was added to 50 μl mixture I and incubated for 15 min at 37°C. The reaction was then stopped by adding 5 ml trichloroacetic acid containing 0.5% w/v of Phe and Leu. Sample processing was then as described by Jelenc and Kurland (1979).

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References

Andersson,D.J. and Kurland,C.G. (1983) *Mol. Gen. Genet.*, **191**, 378-381.
Andersson, D.I., Bohman,K., Isaksson,L.A. and Kurland,C.G. (1982) *Mol. Gen. Genet.*, **187**, 467-472.
Arai,K.J., Kawakita,M. and Kaziro,Y. (1972) *J. Biol. Chem.*, **247**, 7029-7037.
Birge,E.A. and Kurland,C.G. (1970) *Mol. Gen. Genet.*, **109**, 356-369.
Bohman,K., Ruusala,T., Jelenc,P.C. and Kurland,C.G. (1984) *Mol. Gen. Genet.*, in press.
Bouadloun,F., Donner,D. and Kurland,C.G. (1983) *EMBO J.*, **2**, 1351-1356.

Ehrenberg,M. and Kurland,C.G. (1984) *Q. Rev. Biophys.*, **17**, 45-82.
Fersht,A. (1977) in *Enzyme Structure and Mechanism*, W.H.Freeman and Co., Reading, San Francisco, p. 20.
Hummel,H. and Böck,A. (1983) *Mol. Gen. Genet.*, **191**, 167-175.
Jelenc,P.C. and Kurland,C.G. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3174-3178.
Jelenc,P.C. (1980) *Anal. Biochem.*, **105**, 369-374.
Jelenc,P.C. and Kurland,C.G. (1984) *Mol. Gen. Genet.*, **194**, 195-199.
Kurland,C.G. and Ehrenberg,M. (1984) *Prog. Nucleic Acid Res. Mol. Biol.*, in press.
Lebermann,R., Antonsson,B., Giovanelli,R., Guariguata,R., Schumann,R. and Wittinghofer,A. (1980) *Anal. Biochem.*, **104**, 29-36.
Miller,J.H. (1972) *Experiments in Molecular Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
Momose,H. and Gorini,L. (1971) *Genetics*, **67**, 19-38.
Morris,J.G. (1974) *A Biologist's Physical Chemistry*, published by Edward Arnold Limited, London, p. 287.
Ozaki,M., Mizushima,S. and Nomura,M. (1969) *Nature*, **222**, 333-339.
Rosenberg,R.F. and Foskett,G. (1981) *Mol. Gen. Genet.*, **183**, 561-563.
Ruusala,T., Ehrenberg,M. and Kurland,C.G. (1982a) *EMBO J.*, **1**, 75-78.
Ruusala,T., Ehrenberg,M. and Kurland,C.G. (1982b) *EMBO J.*, **1**, 741-745.
Ruusala,T. and Kurland,C.G. (1984) *Mol. Gen. Genet.*, in press.
Schleif,R., Hess,W., Finkelstein,S. and Ellis,D. (1973) *J. Bacteriol.*, **115**, 9-14.
Wagner,E.G.H. and Kurland,C.G. (1980) *Biochemistry (Wash.)*, **19**, 1234-1240.
Wagner,E.G.H., Jelenc,P.C., Ehrenberg,M. and Kurland,C.G. (1982) *Eur. J. Biochem.*, **122**, 193-197.
Zengel,J.M., Young,R., Dennis,P.P. and Nomura,M. (1977) *J. Bacteriol.*, **129**, 1320-1329.

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