Novel mutants of 23S RNA: characterization of functional properties

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ABSTRACT

Single point mutations corresponding to the positions G2505 and G2583 have been constructed in the gene encoding *E.coli* 23S rRNA. These mutations were linked to the second mutation A1067 to T, known to confer resistance to thiostrepton (1). Mutant ribosomes were analyzed *in vitro* for their ability to direct poly(U) dependent translation, their missence error frequency and in addition their sensitivity to peptidyltransferase inhibitors. It was evident that the mutated ribosomes had an altered dependence on [Mg^{2+}] and an increased sensitivity to chloramphenicol during poly(U) directed poly(Phe) synthesis. In a transpeptidation assay mutated ribosomes were as sensitive to chloramphenicol as wild-type ribosomes. However, the mutant ribosomes exhibited an increased sensitivity to lincomycin. An increase in translational accuracy was attributed to the mutations at the position 2583: accuracy increased in the order G < A < U < C.

INTRODUCTION

A solid body of evidence has been published on the role of 23S RNA in the peptidyltransferase reaction, including crosslinking of photoreactive analogs of aa-tRNA, peptidyl-tRNA and puromycin (2,3), protection of rRNA against chemical modification by various ribosome ligands (4–6), inhibition of substrate binding by complementary oligodeoxynucleotides (7) and analysis of antibiotic resistant mutations in rRNA (for review 8). Based on these data, the central ring of domain V in the 23S RNA was defined as being part of peptidyltransferase centre. The importance of nucleotides within this region for cell viability and antibiotic resistance has been shown by site-directed mutagenesis (9–10). Protection of A2438, A2451 and A2602 are shown to be dependent on acyl moiety, U2506, U2584, U2585 on the 3' terminal A and G2505 is protected by tRNA, lacking 3' terminal CA (4, see fig.4).

In this paper the effects of mutations at the peptidyltransferase region of 23S RNA on the ribosomal functions, observed *in vitro*, are described. The mutant species of the gene for 23S RNA were expressed *in vivo*. In order to discriminate between the activities of plasmid encoded mutant ribosomes and wild-type ribosomes

a second mutation in the gene of 23S RNA, corresponding to the position A1067 of 23S RNA, was used. Transversion A to T confers a high level resistance of *in vitro* translation to thiostrepton (1). In the presence of the drug it is possible to selectively inhibit wild-type ribosomes. By comparing the activities of the mixed ribosomal populations in the presence and absence of thiostrepton, it was possible to detect alterations caused by mutations in 23S RNA.

MATERIALS AND METHODS

Plasmids and strains

Plasmid pLST1067T, containing the rrnB operon with single point mutation A to T at the position 1067 was a kind gift from Dr J. Thompson (1). Plasmid pNO2680 contains the *E.coli* rrnB operon under control of the lambda leftward promoter (11) and plasmid ptBsB contains the *E.coli* 23S RNA structural gene under control of the tac promoter (12). *E.coli* CJ236 (dut ung thi relA) was used for the preparation of single-stranded DNA template for site-directed mutagenesis, JM109 containing the lacI^q gene was the host for ptBsB based plasmids and pop2136, which contains the chromosomally located gene encoding for the temperature-sensitive lambda repressor cIts857, was used for expression of mutant rRNAs from the pNO2680 based plasmids. Plasmid pSP-65 (Pharmacia) containing the β-lactamase gene was used as a control during the cotransformation assays.

Construction of mutants

The single point mutations at the position 2505 (G to C) and at the position 2583 (G to A, U and C) of the 23S rRNA gene were constructed by oligonucleotide directed mutagenesis (13) using the SalI-BamHI fragment from rrnB cloned into m13 mp19. Following mutagenesis the Eco52I-BamHI fragments were cloned into expression vector ptBsB (12). XbaI-BamHI fragments from each ptBsB mutant were subcloned into pNO2680, carrying A1067 T transversion. The mutations were confirmed by DNA sequencing of the final expression vectors, as well as by sequencing the cellular 23S rRNA moiety, using the primer directed reverse transcriptase reaction (12). Manipulations with DNA were accordingly to (14). The resulting plasmids were designated as follows: single mutants A1067 to T—ptBsB1067T,

pNO1067T; in the case of the double mutants, all of which contained the 1067T mutation and the mutation at either position 2505 or 2583, these were indicated only by the latter mutation.

Preparation of ribosomes

Transformants of *E.coli* JM109 carrying the plasmid ptBsB, were induced by 1 mM IPTG at $A_{600}=0.5$ and cells were harvested after 1 hour. *E.coli* pop2136 harboring pNO was grown at 30°C up until $A_{600}=0.2$ and subsequently induced for 2 hours at 42°C. The cells were lysed by lysozyme (0.5 mg/ml) in buffer A (60 mM NH_4Cl; 60 mM KCl; 12 mM $MgCl_2$; 20 mM Tris-HCl pH—8; 6 mM β-mercatoethanol) containing 16% (w/v) sucrose, by three freeze-thaw cycles. Cell debris was removed by centrifugation 16000 rpm, 15 min. Sorvall SS-34 rotor. Lysates were diluted three times with buffer A and loaded onto 1 ml sucrose cushion (20% sucrose in 0.5 M NH_4Cl; 12 mM $MgCl_2$; 20 mM Tris-HCl pH—7,6; 6 mM β-mercaptoethanol) in a Sw-60 (Beckman) rotor and the ribosomes were pelleted by centrifugation 33000 rpm, 12 hours. Ribosomes were redissolved in buffer A and stored in aliquots at —70°C. 70S ribosomes were prepared from the total ribosome fraction by sucrose gradient centrifugation (10-30% sucrose in buffer A) 20000 rpm 16 hours in a Beckman Sw-28 rotor. RNA was extracted from 70S fraction by phenol extraction and mutated regions were sequenced by dideoxy sequencing, using end-labeled primer (complementary to bases 2598—2617 in 23S RNA) (12).

Cell-free translation

Poly(U) translation was performed essentially as described in (15). 0.1 ml assay contained in buffer A 0.5 A_{260} units ribosomes, 0.01 mg bulk tRNA, 0.02 mg poly(U) (Reanal, Hungary), 2 mM ATP, 0.5 mM GTP, 8 mM phosphoenolpyruvate (PEP), 2 μg pyruvate kinase, 0.05 mM [^{3}H]Phe (40 cpm/pmol) (Amersham), 0.02 mM [^{14}C]Leu (700 cpm/pmol) (UVVVR, Czechoslovakia) and 0.2 mg S-100 enzyme. After 30 min. incubation at 30°C, reactions were stopped by addition 1 ml 5% trichloracetic acid (TCA), heated for 15 min. at 95°C. Precipitates were collected onto GF/A filters (Whatman) and counted for radioactivity in a Rackbeta 1219 (LKB) scincillation spectrometer. Thiostrepton (Calbiochem) was dissolved in 10% dimethylsulfoxide (DMSO) at concentration 0.05 mM. Therefore all assays in the presence of thiostrepton contained also 0.02—0.1% DMSO. Minus drug controls were made in the presence of DMSO.

Missence error frequency

The missence error frequency was determined by measuring the misreading of UUU codons by near-cognate $tRNA_4Leu$ competing with $tRNA^{Phe}$. The experiments were done in buffer A essentially as described by Ehrenberg *etal* (16). Source for elongation factors and synthetases was DEAE-fractionated S-100 supernatant (20 mg/ml protein). Two mixes were prepared: ribosome mix contained in 50 μl 0.5 A_{260} units of ribosomes, 0.2 mg/ml polyuridylic acid, 20pmoles $tRNA^{Phe}$ (1100 pmoles/A_{260}unit, Sigma) and 15 pmoles of thiostrepton if indicated; factor mix containing 60 pmoles $tRNA^{Phe}$, 4 mM ATP, 1 mM GTP, 16 mM phosphenolpyruvate, 7 μg pyruvate kinase, 0.12 mM [^{3}H]Phe (5.2 cpm/pmole), 0,06 mM [^{14}C] Leu (700 cpm/pmole), saturating amount of S-100 enzyme and between 0—150 pmoles $tRNA_4Leu$ (1520 pmoles/A_{260} unit, Subraiden). Ribosome mix was preincubated for 10 min. at 37°C, factor mix was preincubated for 5 min. at the same temperature.

Reaction was started by mixing 50 μl of ribosome mix by the same amount of factor mix. Incubation was at 37°C for 1 min. Reaction was stopped by addition 1 ml of 5% TCA, heated at 95°C for 15 min. and filtrated through GF/A filter (Whatman). Filters were processed as described in previous section.

Puromycin assay

Dipeptide synthesis was studied essentially as described (15). Ribosomes (0.5 A_{260} units per 50 μl assay) were preincubated with poly(U) (0.01 mg) and bulk tRNA (0.017 mg) in buffer A for 10 min. at 37°C in the presence of thiostrepton (0.37 μM). After addition 3 pmoles of N-ac[^{3}H]Phe-tRNA^{Phe} (1.5×10^5 cpm/pmol, charging efficiency 1000 pmol/A_{260} unit) incubation was continued for 20 min. at 37°C. Trasnslocation was carried out at 0°C for 30 min. by adding EF-G (3 μg) and GTP (0.2 mM) followed by puromycin reaction (0.5 mM puromycin) 30 min. at 0°C. Reactions were stopped by 200 μl 1 M potassium phosphate (pH—7.0); 10mM EDTA. N-Ac[^{3}H]Phe-puromycin was extracted by 1 ml ethylacetate, 0.5 ml of organic layer was counted in 10 ml of scincillation cocktail (High-Safe, LKB). Background values were obtained from minus puromycin experiments.

RESULTS

The effect of mutations on the cell growth

The mutation A1067 to T in the 23S RNA is known to reduce bacterial growth rate 1,5 fold (1). The effect of double mutations on the cell viability was studied using derivatives of the pNO vector transformed into the *E.coli* strain pop2136. At 30°C the lambda promoter is repressed and mutant RNAs are not significantly expressed. At 42°C the temperature-sensitive repressor cI_{857} is inactive and rRNA genes are transcribed from the plasmid. The plasmid pSP-65 was used as a control in the cotransformation assay. The number of colonies were the same at 30°C and 42°C for both pSP-65 alone and in combination with pNO 1067T, indicating that transformed bacteria could grow equally well at both temperatures. When derivatives of the pNO containing the double mutant 23S RNA gene were used for transformation, no colonies were found at 42°C. In a cotransformation assay at 30°C there were 10—20 times more colonies than at 42°C. Four colonies from each temperature group were analyzed for plasmid content and it was found that at 42°C the transformants exclusively contained plasmids pSP-65. From these data we concluded that mutations C2505 and all base substitutions at position 2583 were lethal at 42°C.

Cell-free translation by mutant ribosomes

For *in vitro* assays the ribosomes were isolated from *E.coli* JM109 carrying one of ptBsB plasmids and pop2136 transformed with one of pNO plasmids. RNA from 70S fraction was sequenced using end-labeled primers directed towards the 2500—2600 region and showed that ribosomes encoded by the pNO plasmids constituted 40—50% of the total 70S population, following 2 hours induction. By contrast, ribosomes encoded by ptBsB plasmids represented approximately 30% following 1 hour induction (see also 12). Further induction did not yield a higher percentage of mutant ribosomes.

In the presence of 5-fold molar excess of thiostrepton poly(U) translation on the ribosomes from uninduced cells (wild-type) were inhibited 97—99%. The ribosomes derived from pNO1067U were inhibited 50%, while the ribosomes from

Table I. Poly(U) dependent protein synthesis in the presence and absence of thiostrepton. Phe incorporation into peptides is in pmoles. Ratio of protein synthesis in the presence and absence of the drug is given as resistance. A-ribosomes were derived from ptBsB plasmids in *E.coli* JM109, B-ribosomes encoded by pNO plasmids in pop2136.

Ribosomes	10 mM MgCl$_2$			25 mM MgCl$_2$		
	−thio	+thio	resistance	−thio	+thio	resistance
A						
wild-type	54	1.62	0.03	26	0.26	0.01
U1067	52	9.36	0.18	27	4.86	0.18
C2505	33	4.29	0.12	38	8.36	0.23
C2583	29	3.48	0.12	39	9.36	0.24
B						
wild-type	52	0.78	0.015	32	0.32	0.01
U1067	49	20.6	0.42	31	13.7	0.44
C2505	41	13.5	0.33	32	16.9	0.51
C2583	36	10.4	0.29	35	18.9	0.54

Table II. Poly(U) dependent incorporation of Phe and Leu into peptides in the presence of thiostrepton. Ribosomes were isolated from *E.coli* pop2136 carrying one of the pNO plasmids.

Ribosomes	10 mM MgCl$_2$			15 mM MgCl$_2$		
	pm Phe	pm Leu	Leu/Phe+Leu	pm Phe	pm Leu	Leu/Phe+Leu
U1067	17.7	0.24	0.013	20.9	3.03	0.127
A2583	16.9	0.16	0.0094	23	2.52	0.099
C2583	11.5	0.056	0.0048	15.4	0.73	0.045
U2583	10.4	0.096	0.0091	12.2	0.99	0.075

ptBsB1067U were inhibited 82% (Table I). Drug resistance on double mutant ribosomes U1067/C2505 and U1067/C2583 was in both strains dependent on Mg^{2+} concentration: at 25 mM the resistance was nearly 2 times higher than at 10 mM (Table I). It is clear from table I that in all cases the resistance to thiostrepton was higher on the ribosomes derived from *E.coli* pop2136 strain. Thus, the data from poly(U) translation in the presence and absence of thiostrepton are in good correlation with the RNA sequencing data. It should be noted that the ribosomes containing the U1067 mutation are also inhibited in the presence of excess of thiostrepton 10−20%.

The effect of magnesium ions concentration on the translational activity was tested during poly(U) dependent poly(Phe) synthesis on the ribosomes derived from pNO plasmids in the presence of thiostrepton. The data are presented on the fig. 1. It was evident that the ribosomes containing the single mutation U1067 had an optimum at 12 mM MgCl$_2$ and at higher concentration of Mg^{2+}, processivity of poly(Phe) synthesis decreased. Ribosomes containing the double mutation clearly had a different dependence on [Mg^{2+}]. At 10−12 mM MgCl$_2$ the processivity was lower for the double mutants as compared with the ribosomes containing the U1067 mutation alone. In contrast, at 25 mM MgCl$_2$ all the double mutants exhibited higher activity during poly(U) translation than single mutant ribosomes. It is interesting to note that the double mutants C2505 and C2583 had virtually the same dependence on [Mg^{2+}]. The double mutant with U2583 showed the best processivity at high magnesium concentration and the U1067/A2583 mutation reflected the smallest difference on the Mg^{2+} concentration as compared with ribosomes containing the single mutation U1067.
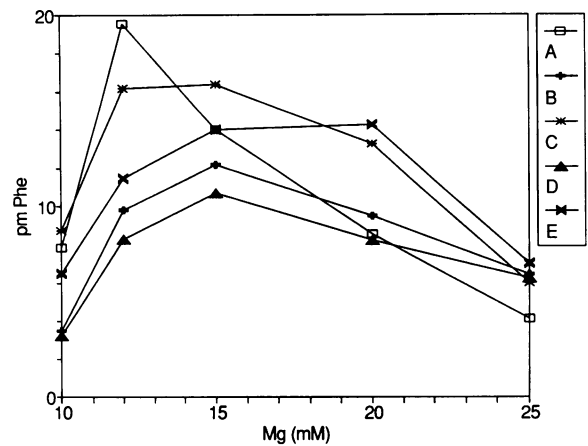

Fig. 1. Poly(U) directed translation by mutant ribosomes. Dependence on Mg^{2+} concentration. 0.5 A$_{260}$ units of ribosomes were tested for activity in poly(Phe) synthesis in the presence of thiostrepton (1 μM) at different magnesium concentrations: A-single mutant U1067; B−E double mutants: C2505 (B); A2583 (C); C2583 (D); U2583(E).

The effect of mutations on the translational accuracy

The effect of mutations in 23S rRNA on the fidelity of translation was analyzed in conventional poly(U) translation assay and accordingly to the method of Ehrenberg (16). Table II shows the incorporation of Phe (correct) and Leu (incorrect) by mutant ribosomes at two magnesium ion concentrations in a conventional poly(U) translation system. In order to reduce the activity of wild-type ribosomes, the assays were carried out in the presence of thiostrepton. Incorporation of both amino acids into peptides was affected by increasing Mg^{2+} concentration, the effect on Leu incorporation was more dramatic. As it can be seen on table II the incorporation of Leu is lower on double mutant ribosomes as compared to single mutant ones at both concentrations of MgCl$_2$.

In order to determine whether or not the effect on Leu incorporation on mutant ribosomes is due to reduced mistranslation, we have used the method of Ehrenberg (16) for determination of missence error frequencies. This method is based on titration of ribosomes by miscognate tRNA$_4$Leu in the presence of a constant amount of tRNA^{Phe}. The missence error frequencies were detected on mutant ribosomes in the presence and absence of thiostrepton; wild-type ribosomes were analyzed in the absence of drug. In this assay, equimolar concentrations of the drug were sufficient to almost completely inhibit wild-type ribosomes. The active fraction of ribosomes in the presence of thiostrepton was 1−5 pmoles per one A$_{260}$ unit, as determined by Nac-Phe incorporation into poly(Phe) during 20 seconds (data not shown). This value did not differ significantly for the different mutants at optimal magnesium ion concentrations, but activity was preparation dependent. The missence error frequencies are presented on table III. Leucine misincorporation by single mutant U1067 ribosomes was nearly the same as that observed in the presence or absence of the drug. The same value was obtained on the wild-type ribosomes in the absence of the drug, indicating that under conditions used the A1067 to T transversion had no effect on the accuracy of translation. The same was true for the G2505 to C transversion (table III). On the other hand it implied that the presence of thiostrepton did not affect the selection aa-tRNA by the resistant ribosomes. By contrast it was evident that

Table III. Missence error frequencies of poly(U) translation in the presence and absence of thiostrepton. The wild-type ribosomes were isolated from *E.coli* pop2136 without plasmid, mutant ribosomes were isolated from the same strain carrying the pNO plasmid with mutated 23S RNA gene.

Ribosomes	−thiostrepton	+thiostrepton
wild-type	0.0119	0.0122
U1067	0.0121	0.0122
C2505	0.0120	0.0118
A2583	0.0109	0.0101
C2583	0.0082	0.0039
U2583	0.0098	0.0057

Table IV. The effect of lincomycin on transpeptidation with mutant ribosomes in the presence of thiostrepton. Backgrounds were detected in the absence of puromycin and subtracted.

Ribosomes: Lincomycin (mM)	[³H]NacPhe-puromycin (cpm).				
	U1067	C2505	A2583	U2583	C2583
0	10121	6578	7459	8387	6121
0.05	8418	3649	4885	5187	3830
0.1	7637	3032	4319	4212	3162
0.3	6297	2305	3243	3335	2263
0.5	4887	1735	2366	2408	1756
1	3099	1068	1620	1603	1101
5	446	239	301	287	

both in the presence and absence of thiostrepton, the misincorporation of Leu was lower on ribosomes mutated at the position 2583 (table III). In the absence of the drug both, wild-type and mutant ribosomes participated in the polymerization reaction. In the presence of thiostrepton, wild-type ribosomes contributed less than 5% and nearly all amino acids incorporated into hot TCA insoluble material were due to the mutant ribosomes (see table I). As compared the missence error frequencies in the presence and absence of the drug, the single mutant U1067 and the double mutant U1067/C2505 ribosomes exhibited the same translational accuracy, while the ribosomes mutated at the position G2583 were more accurate in the presence of the drug. Taking into account that in the absence of thiostrepton mutated ribosomes contributed 40−50% in translation (percentage of mutant 23S RNA in the 70S fraction) it is evident from table III that mutant ribosomes translated poly(U) with the same accuracy both, in the presence and absence of the drug. The effect on the translational accuracy was biggest on C, intermediate on U and smallest on A at the position 2583.

The effect of mutations on antibiotic sensitivity

The sensitivity of mutant ribosomes to antibiotics which inhibit the peptidyltransferase reaction, were analyzed during poly(U) translation and during a 'puromycin assay'. No differences were detected between wild-type, single mutant U1067 and double mutant ribosomes with respect to their sensitivity to erythromycin (data not shown). Interestingly, notable differences were found with chloramphenicol. Data is presented on poly(U) translation as a function of chloramphenicol concentration, in the presence of thiostrepton (fig.2). The single mutant U1067 and double mutant U1067/A2583 were only slightly inhibited by chloramphenicol, using concentrations up to 3 mM. By contrast, the double mutants containing C2505, U2583 and C2583 were more sensitive than the wild-type ribosomes.
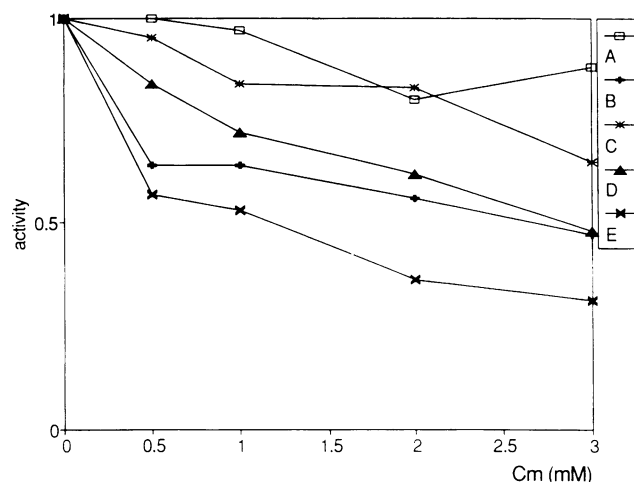

Fig. 2. Poly(U) directed translation by mutant ribosomes. Dependence on chloramphenicol (Cm) concentration in the presence of thiostrepton (1 μM). Activities of mutant ribosomes are expressed as ratio to 0 concentration of chloramphenicol. A-single mutant U1067; B−E double mutants: C2505 (B); A2583 (C); C2583 (D); U2583(E).
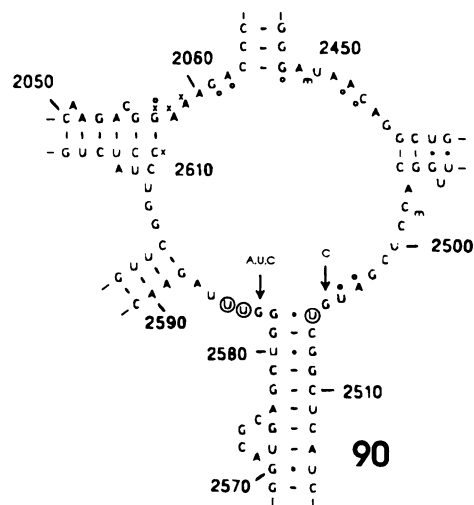

Fig. 3. Central ring of domain V of *E.coli* 23S RNA. Mutations described in this study are indicated by arrows. Protection of encircled bases are dependent upon the presence of the 3′ terminal A (4). Nucleotides whose alteration can confer resistance to chloramphenicol (o) and to macrolides or lincomycin (x) (8).

In order to study the effects of antibiotics that interfere with peptidyltransferase on the mutant ribosomes, the ability of thiostrepton to block translocation on wild-type ribosomes was employed. NacPhe-tRNA was bound to the ribosomal A-site following occupation of the P-site by deacylated tRNA. After EF-G promoted translocation, peptidyl-tRNA becomes competent to puromycin. When this reaction is carried out in the presence of thiostrepton translocation occurs only on the U1067 containing ribosomes. For the peptide bond synthesis assay, 4 μM chloramphenicol inhibited the transfer reaction by 50% with both wild-type and mutant ribosomes indicating that the mutations in 23S RNA had no effect during this particular assay. Small differences between mutant ribosomes were detected in the inhibition pattern by lincomycin (see table IV). 50% inhibition

of the reaction on the mutant ribosomes was obtained with the following concentrations of lincomycin: single mutant U1067 0,35 mM and double mutants: C2505 0,07 mM; A2583 0,15 mM; U2583 and C2583 at 0,1 mM. Thus mutations C2505, U2583 and C2583 led to increased sensitivity to lincomycin during peptidyltransferase.

DISCUSSION

The effects of mutations at position G2505 (to C) and G2583, (all three substitutions) have been studied *in vitro*. These universally conserved nucleotides are located in the central ring of domain V (see fig.3). Substitution of one of these nucleotides by C could lead to an extension of helix 90 (17) by one base pair. The mutant genes were expressed under the control of two different inducible promoters: lambda p_L and tac. In these systems high levels of expression of plasmid encoded rRNA is possible.

Mutations at the positions 2505 and 2583 did not prevent processing and assembly of the 23S RNA into 50S particles. The resulting subunits were able to associate with 30S subunits in amount comparable to the wild-type 50S subunits. The mutated ribosomes were able to translate poly(U) *in vitro*. However, *in vivo* expression of these mutations were shown to be lethal at 42°C.

The mutated regions were linked to the mutation U1067, which is known to confer resistance to thiostrepton *in vitro* (1). By comparing the results plus and minus thiostrepton, no differences were detected with single mutant U1067 ribosomes during poly(U) translation, in the ribosomal accuracy and in the sensitivity to peptidyltransferase inhibitors.

The magnesium ion concentration has multiple effects on ribosomal structure and function. It is known to stimulate tRNA binding (18) and inhibit translocation (over 15 mM) (19). At higher magnesium concentrations translational accuracy is decreased (16). The mutations C2505 and C2583, which favor base-pairing with juxtaposed G reduces significantly reduced the processivity of poly(U) translation at low Mg^{2+} concentration. In contrast at higher concentration of magnesium ions, all double mutants exhibited higher processivity as compared to the single mutant U1067 ribosomes. Both the reduced processivity at low Mg^{2+} concentration and the resistance to the inhibitory effect of high Mg^{2+} concentrations indicated that tRNA binding was affected on the ribosomes containing double mutations.

The sensitivity of mutant ribosomes to chloramphenicol and lincomycin was increased, the response to erythromycin was not affected as compared with wild-type ribosomes. Lincomycin does not inhibit poly(U) dependent poly(Phe) synthesis and erythromycin is inactive in the NacPhe-tRNA puromycin system, whereas chloramphenicol can block both steps (20). Chloramphenicol and lincomycin are known to inhibit acceptor substrate binding, erythromycin destabilizes the peptidyl-tRNA complex with ribosomes (20). In addition, erythromycin and chloramphenicol have been shown to induce oligopeptidyl-tRNA release during poly(U) translation *in vitro* (20, 15). Hypersensitivity of mutated ribosomes to chloramphenicol and lincomycin can be caused by a reduced binding affinity of tRNA 3' end to the ribosomal peptidyltransferase centre. Several mutations in the central ring of domain V of 23S RNA are known to confer resistance to chloramphenicol, among them are 2503 in *E.coli* (10) and 2504 in mammalian mitochondria (8). G2505 is protected against chemical modification by the drug (5). In

this context it is interesting to note that the G2505 to C transversion led to increased sensitivity of *in vitro* translation to chloramphenicol. Observation, that mutations at positions 2505 and 2583 conferred hypersensitivity to chloramphenicol during poly(U) directed translation but not in the puromycin reaction indicated that tRNA binding to the ribosomal A site was the likely step to be affected by these mutations. Mutant ribosomes were slightly more sensitive to lincomycin during peptidyltransferase assay, as compared to wild-type ribosomes. Obviously, chloramphenicol and lincomycin both inhibit acceptor substrate binding but each in a different manner.

It is known that mutations in both small and large ribosomal subunit proteins can affect translational accuracy (21, 22). The mutations at the position 2583 are the first examples for fidelity mutations in 23S ribosomal RNA. One possible way to explain this phenomenon is the assumption that binding of the aa-tRNA to the peptidyltransferase centre is weaker (or alternatively transfer reaction is slower) by the 2583 mutant. In this way, ribosomes would have a longer time for aa-tRNA dissociation during proofreading. Accordingly, the peptidyltransferase reaction must be limiting and an irreversible step for aa-tRNA selection, as has been suggested earlier (23). This model predicts that mutant ribosomes need more GTP's to hydrolyze to aa-tRNA binding. Moazed and Noller have demonstrated that protection against chemical modification of bases A2439, A2451 and A2602 in 23S RNA are dependent upon the acyl moiety on tRNA, protection of bases U2506, U2584 and U2585 depend on the presence of 3' terminal A (4, see also fig.3). U2584 and U2585 were shown to cross-link with photo-reactive Phe-tRNA at the ribosomal A site. All these data are in agreement with our conclusion that the region around helix 90 in 23S RNA is involved in aa-tRNA binding to the ribosomal A site.

ACKNOWLEDGEMENTS

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