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Mutagenesis at the mRNA decoding site in the 16S ribosomal RNA using the specialized ribosome system in *Escherichia coli*

Anna S.Hui[1], Dennis H.Eaton[2] and Herman A.de Boer[3]

Genentech, Inc., 460 Point San Bruno Boulevard, San Francisco, CA 94080, USA

[1]Present address: Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, 231 Bethesda, Cincinnati, OH 45267, USA

[2]Present address: Pacific University, Forest Grove, OR, USA

[3]Present address: Gorlaeus Laboratoria, University of Leiden, Einsteinweg 5, 2300 RA Leiden, The Netherlands

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In the specialized ribosome system, a distinct pool of mutated ribosomes is dedicated to the translation of one particular mRNA species. This was accomplished by altering the Shine–Dalgarno sequence on the mRNA and its complementary anti-Shine–Dalgarno sequence on the plasmid-borne 16S rRNA gene. Here, using the specialized ribosome system, we were able to introduce mutations in key regions of the 16S rRNA and could study their effect on translation *in vivo*. The C1400 region has been implicated to play a role in the actual mRNA decoding process. Several ribosomal mutations were introduced in this region. We showed that substitution of the evolutionary highly conserved C1400 residue by a G- or an A-residue inhibits ribosomal activity by 80% and 50% respectively, whereas, a C to a U change at this conserved position does not affect overall ribosomal activity. The adjacent stem structure (1410– 1490) was also examined. Disruption of the stem by replacing either one of the arms of this stem, with a different sequence, inhibits ribosomal activity by ~80%. A small but significant restoration of translation could be achieved by recreating a complementary stem with a different sequence. We found that full reversion of activity could be obtained when such mutated ribosomes were made spectinomycin resistant by introducing a C to A substitution at position 1192 which is located far away in the secondary structure map of the 16S rRNA molecule. Based on these results we conclude that some, but not all, of the nucleotides in the conserved C1400 region play a key role in translation. Most importantly, we show that the specialized ribosome system provides a very powerful tool for structure and function studies of any region in the complex ribosomal RNA molecule.

Key words: mutagenesis/decoding site/rRNA/*E.coli*

Introduction

The information used to compose the complex secondary structure models for the 16S rRNA molecule has mostly been derived from chemical, enzymatic and phylogenetic data (reviewed in Woese *et al.*, 1983; Brimacombe, 1985; Ebel *et al.*, 1985; Lake, 1985). Mutational analyses have so far not contributed to the assembly and verification of this model because mutations in the 16S rRNA structural gene are relatively rare and limited to mutations conferring antibiotic resistance (Sigmund *et al.*, 1984; Moazed and Noller, 1986, 1987; and reviewed in Noller, 1984). The rarity of 16S rRNA mutations is caused by the fact that there are seven, essentially identical, ribosomal RNA operons per haploid genome; hence recessive mutations in one of the operons are masked by the function of the remaining operons. Also, no selection scheme for obtaining mutants that map in the structural ribosomal RNA gene is available other than selection schemes for antibiotic resistance (Noller, 1984; Sigmund *et al.*, 1984; Ettayebi *et al.*, 1985).

When plasmid borne *rrn*-operons were mutagenized semi-randomly or via site-directed mutagenesis techniques, it appeared that mutations in certain regions, e.g. the C1400 region (Jemiolo *et al.*, 1985), could not be obtained or mutants were isolated concomitantly with rearrangements in the plasmid indicating that such mutations are lethal to the cell (Gourse *et al.*, 1982; Zwieb and Dahlberg, 1984; and unpublished results from this laboratory). These mutations are presumably in essential regions of the rRNA molecule and hence of most interest to study. One plausible explanation for the dominant lethal effect of mutations in key parts of the 16S rRNA molecule might be that such molecules are able to assemble into 30S particles and are capable of binding to an mRNA, but are defective in other essential ribosomal functions, such as initiation or elongation. Such crippled ribosomes are not only translationally incapable but are also likely to prevent mRNA translation by ribosomes derived from the remaining wild-type operons.

To overcome this dilemma and to facilitate future structure and function studies of any essential region in the 16S rRNA by mutational analysis, we developed the specialized ribosome system (de Boer *et al.*, 1985, 1986; Hui and de Boer, 1987; Hui *et al.*, 1987). In this system the anti Shine–Dalgarno (ASD) sequence near the 3′ end of the 16S rRNA molecule was altered. The Shine–Dalgarno (SD) sequence preceding the start codon of one particular mRNA (in this case that of the human growth hormone, hGH gene) was also changed into the complement of the altered ASD sequence. Previously, we showed that the resulting specialized ribosomal subpopulation translates predominantly the altered mRNA species (Hui and de Boer, 1987; Hui *et al.*, 1987). Since the specialized ribosomes represent a non-essential subpopulation of ribosomes in the cell and their presence along with the hGH does not effect cell viability, we expect that any mutation anywhere in the specialized 16S rRNA can now be made and that its effect on the translation of the specialized mRNA can be studied.

The C-residue at position 1400 (C1400) of the 16S rRNA molecule is conserved not only among prokaryotic ribosomes but also among eukaryotic ribosomes (Prince *et al.*, 1982)

with two exceptions known thus far (see Noller *et al.*, 1985). Ofengand and his colleagues (Ofengand *et al.*, 1979; Prince *et al.*, 1982) and Ehresmann *et al.* (1984) have shown that this C1400 can be photo cross-linked to the anticodon base in the wobble position (cmo5U) of $tRNA_1^{Val}$ bound in the ribosomal P-site thus forming a cyclobutane dimer. In addition, the C1400 is protected against certain chemical modifications by the $tRNA^{Phe}$ in an mRNA independent fashion (Moazed and Noller, 1986). Using DNA hybridization electron microscopy with a probe specific for the C1400 region it was shown that this region maps in the cleft of the 30S subunit (Keren-Zur *et al.*, 1979; Gornicki *et al.*, 1984; Oakes *et al.*, 1986). All these data indicate that the site of mRNA decoding is in very close proximity to the C1400 region. To gain insight into this putative role of the C1400 region we introduced several alterations in the C1400 area of the specialized ribosomes. We show that these mutations, some of which abolish specialized ribosomal activity completely, can be made without deleterious consequences to the cells and thus can be studied *in vivo*.

Results

The specialized ribosome system

The construction of the specialized ribosome system and its properties has been described in detail elsewhere (Hui and de Boer, 1987; Hui *et al.*, 1987). The plasmids encoding the systems are shown in Figure 1. In the wild-type system, the hGH gene is preceded by a consensus SD-sequence (5'GGAGG). Elsewhere on the same plasmid is the *rrnB* gene containing the wild-type ASD-sequence (5'CCTCC). In system X, the SD-sequence is 5'GTGTG and the ASD-sequence is its complement, i.e. 5'CACAC. In both systems the hGH mRNA is transcribed constitutively by a mutated *trp*-promoter (Hui *et al.*, 1987) whereas the plasmid borne *rrn*-operon is transcribed by the temperature-inducible λ P_L promoter. The *cI*857 containing host, *E.coli* K5637 (Hui *et al.*, 1987) is used throughout. Previously, we have shown that hGH synthesis from the specialized mRNA is almost completely dependent on the induction of the specialized *rrn*B operon (Figure 2; Hui and de Boer, 1987). Here, we describe the introduction of mutations at the putative decoding centre of the 16S rRNA and study their translation properties.

The mutations examined are shown in Figure 2A. They were introduced by oligonucleotide-directed mutagenesis procedures as described previously (Morinaga *et al.*, 1984; Hui *et al.*, 1987) using synthetic oligonucleotide primers with appropriate mismatches.

Mutations in the C1400 region

We introduced several different mutations into the C1400 region of 16S rRNA: (i) the C1400 residue was replaced by a U-, an A- or a G-residue; (ii) five base pairs (from C1400 to C1404) were deleted; (iii) the sequence from C1399 to C1404 was replaced by a completely different sequence; and (iv) the conserved methylated C1402 base was substituted by an A-residue. The results of these mutations on the efficiency of the translation of the hGH mRNA with system X are shown in Figure 2B and C. In the case where specialized ribosomes harbour the deletion, the C to G change at position 1400 or the C to A change at position 1402, the translational activity is reduced by 80–90%. However, in all these cases, the residual activity is

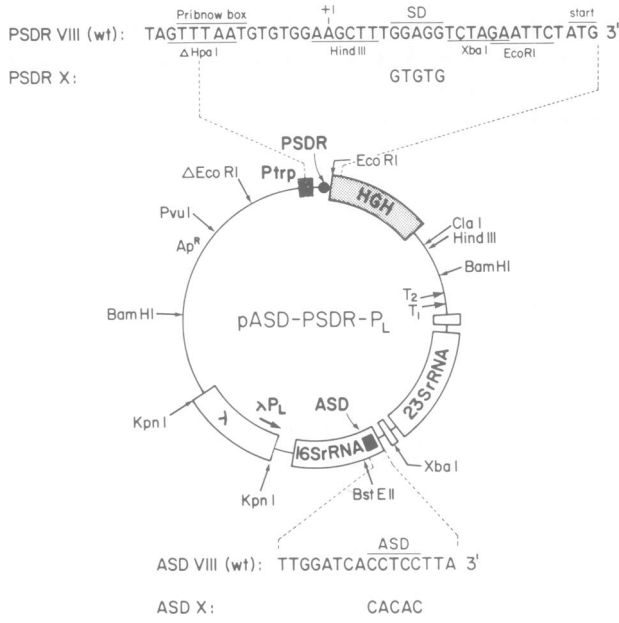

Fig. 1. Structure of the plasmids encoding the specialized ribosome system X and the control system VIII. The sequence of the portable Shine–Dalgarno region (PSDR) and that of the anti-Shine–Dalgarno (ASD) region is shown. Details of the system and its contruction have been described in Hui *et al.* (1987a,b).
Note that a modified *trp*-promoter transcribes the hGH gene in all systems constitutively. The specialized *rrn*B operon is under the transcriptional control of the temperature inducible λ P_L promoter.

significantly above that of the negative control system 207–10. The cells of this negative control contain a specialized mRNA but lack specialized ribosomes. The hGH that is synthesized in the negative control system is due to wild-type ribosomes translating the specialized mRNA at low efficiency (Hui and de Boer, 1987; Hui *et al.*, 1987).

Replacing the C1400 by an A-residue reduces the activity by ~50%. However, the C- to U- substitution does not have any significant effect on ribosomal activity (Figure 2B). This is quite remarkable since this C-residue is fully conserved in both prokaryote and eukaryote small rRNAs suggesting an essential role in protein synthesis. Since the U1400 and C1400 containing ribosomes have identical overall efficiencies, a functional difference, if any, must lie in another aspect of protein synthesis.

The rate of cell growth is the same in all cases studied here; i.e. only after about 3 h of induction of the mutated *rrn* operon, a reduction of the growth rate occurs. This is also observed for cells containing the specialized ribosome system X (data not shown) or the wild-type *rrn*B operon on a plasmid (Gourse *et al.*, 1985).

Replacement of the stem structure adjacent to the C1400 region

Many phylogenetically conserved stem structures are present in the 16S rRNA molecule (Woese *et al.*, 1983). To investigate the role of such a structure, we replaced the stem that is 3' adjacent to the C1400 region (Figure 2A) and modified its sequence into another stem (as found in nature in the 16S rRNA molecule of *Halobacterium volcanii*). In the first mutant (I), the C-residues at positions 1409 and 1411 were replaced by an A- and a G-residue respectively (see

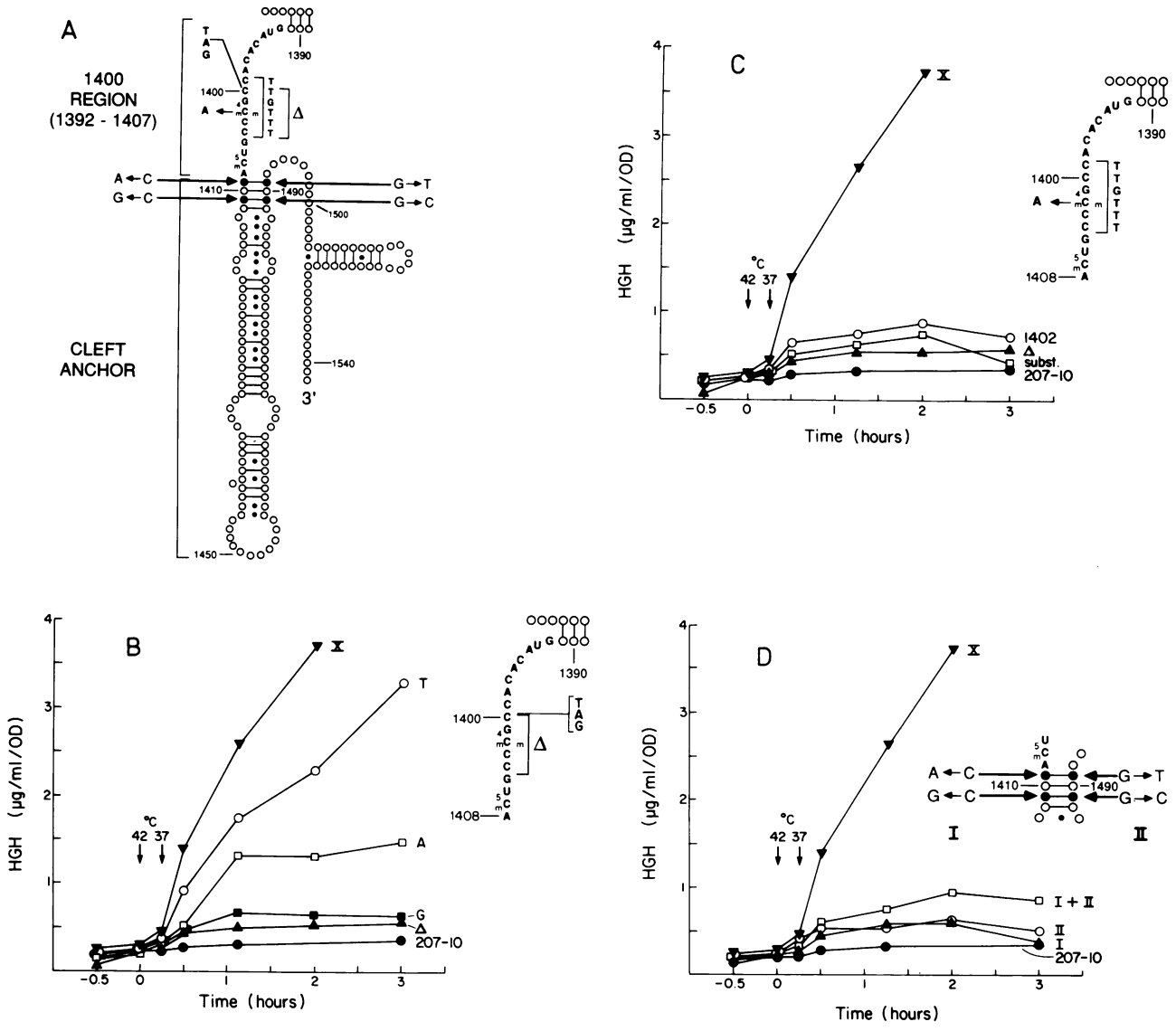

Fig. 2. (**A**) Schematic diagram of the C1400 region of the 16S rRNA molecule and the adjacent 1410−1490 stem structure. All mutations described in this paper are indicated. The cytidine residue at 1400 is marked as well as the extent of the substitution (to 5′ TTGTTT) and the deletion (△). The replacement of the left arm (mutant I) of the 1410−1490 stem; the replacement of the right arm (mutant II) of the 1410−1490 stem and the replacement of both arms (mutant I+II) are also shown. This figure has been adapted from Oakes *et al.* (1986). (**B**) hGH accumulation in *E.coli* cells containing the specialized system X shown in Figure 1 and the various mutant derivatives. ▼---▼: specialized system X and ●---●: control for system X (207−10). This control reflects hGH levels in cells that make a specialized hGH mRNA constitutively but lack a specialized *rrn*B operon. At $t = 0$ the temperature was raised from 30°C to 42°C for 15 min after which incubation was continued at 37°C for the indicated times. Details of the procedure have been described elsewhere (Hui and de Boer, 1987). The hGH accumulation in cells containing a mutation at position C1400 of the specialized rRNA is shown as indicated with T, A or G. The effect of the deletion in the C1400 region is also shown. The sequence of all the mutations is shown on the right. (**C**) The effect of a substitution in the C1400 region and the effect of the C1402 to A substitution in the specialized rRNA on hGH levels. The positive and negative controls are as in **B**. (**D**) hGH accumulation in cells containing the specialized system X with two mutations in the left arm (I): the right arm (II) or in both arms (I+II) of the 1410−1490 stem, structure. The controls are as described in **B**.

Figure 2D). In another mutant (II), the G-residues at 1489 and 1491 were replaced by a C- and a T-residue respectively. In the third mutant (I+II), all four replacements were introduced in order to restore a stem structure with a different nucleotide composition. The effect of these mutations on the overall translational efficiency of the specialized ribosomes as measured by the translation of the specialized hGH mRNA is shown in Figure 2D. The mutations I and II diminish ribosomal activity severely (by ~80%) but, again, the residual activity is above the background level as compared to cells containing the hGH gene but lacking specialized ribosomes. In the double mutant (I+II), where a stem structure is reconstructed, some restoration of ribosomal activity does occur. Here, the HGH level is about twice that in the cells containing the mutations I or II alone. Clearly, recreation of a stem structure does not completely restore ribosomal activity implying that the sequence may play a more important role than merely providing a stem structure at this particular place.

Mutations in the C1400 region do not affect the specificity of mRNA selection

Previously we showed (Hui and de Boer, 1987) that altering the ASD-sequence severely affects the relative specificity

of the specialized ribosomes with respect to their low translational activity of the various endogenous wild-type mRNAs. Here, we examine the question whether the mutations around the mRNA decoding site affect this relative specificity towards wild-type mRNAs. To analyse the translation products of the wild-type endogenous mRNAs, synthesized by specialized ribosomes, the wild-type ribosomes must be inactivated while leaving the specialized ribosome subpopulation undisturbed. We introduced the spectinomycin mutation into the 16S rRNA of the specialized ribosomes (Hui and de Boer, 1987; Hui *et al.*, 1987) by substituting the C-residue at position 1192 for a T residue. This single base mutation is sufficient to render the ribosome spectinomycin resistant (Sigmund *et al.*, 1984).

Production of specialized ribosomes was induced at the non-permissive temperature for 2 h. After spectinomycin treatment, the proteins were metabolically labelled with [^{35}S]methionine. Under these conditions only the *de novo* synthesized proteins, derived from the spectinomycin resistant specialized ribosomes, are labelled. These proteins are the translation products of the specialized hGH mRNA and of a number of endogenous wild-type mRNAs that are recognized, with a relatively low efficiency, by specialized ribosomes (see also Hui and de Boer, 1987).

The results of these experiments are shown in Figure 3. The relative intensities of the hGH bands corresponding to cells harbouring the various mutants are in good agreement with the hGH levels in cells containing the same mutations in the *spc*^{S} system (Figure 2B–D) implying that the additional *spc*^{R} mutation does not affect the overall translational efficiency. However, there is one exception (lane 9) which will be discussed below.

The efficiency with which the mutant specialized ribosomes translate the non-specialized mRNAs is proportional to their translational efficiency of the specialized hGH mRNA itself. For example, the C to T substitution at position 1400 has no effect (cf. lane 3 with lane 10), whereas a C to G substitution (cf. lane 4 with lane 10) has a strong negative effect on translation of the endogenous mRNAs. The C1400 to A1400 substitution has an intermediate effect on translation of these mRNAs (cf. lane 2 with lane 10). None of these mutations affect the relative intensities of the bands of the background profile, i.e. none of these mutations affects the relative ribosomal specificity towards the various wild-type mRNAs. Therefore, we conclude that the C1400 region and the 1410–1490 stem is not involved in the mRNA selection process.

The spc^R *mutation at position 1192 restores the activity of ribosomes with an altered 1410–1490 stem structure*

Figure 2D showed that only partial restoration of the specialized ribosome activity occurred when the mutations I and II were combined. This experimental result was obtained with spectinomycin-sensitive ribosomes. Surprisingly, when the ribosomes harbouring mutation I together with mutation II were made spectinomycin-resistant by the substitution at position 1192 described earlier, complete restoration of ribosomal activity occurred. This is shown in lane 9 of Figure 3. Restoration of ribosomal activity also occurs with regard to translation of the non-specialized mRNAs (i.e. the background profile in lane 9 is equally as strong as that of system X in lane 10).

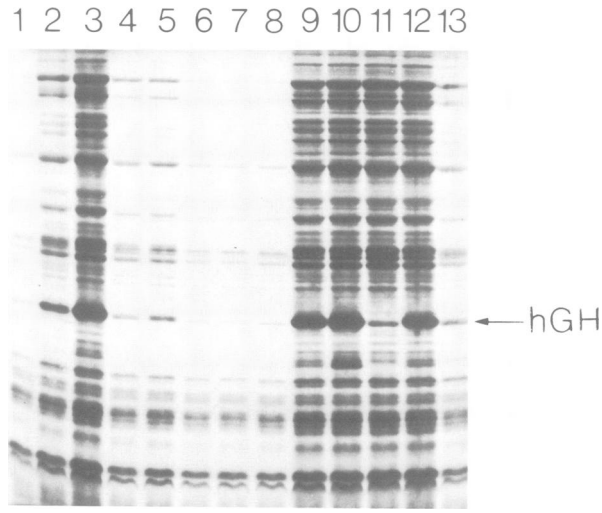

Fig. 3. Profile of the proteins produced *in vivo* by spectinomycin-resistant specialized ribosomes in the presence of spectinomycin. The experiment was carried out as described previously (Hui and de Boer, 1987; Hui *et al.*, 1987). The protein profiles of cells of *E.coli* K5637 (*cI*857) containing the various plasmids are shown. These cells were grown at 30°C for 2 h in M9 medium without casamino acids. After 2 h of induction at 37°C, spectinomycin (GIBCO) was added (0.5 mg/ml final conc.); 5 min later, [^{35}S]methionine (760 μCi/μM) was added. After 30 min of incubation at 37°C, proteins were extracted and separated by $NaDodSO_4$ polyacrylamide (12.5%) gel electrophoresis. An autoradiogram is shown. **Lane 1**, C1400 region deleted; **lane 2**, C1400 to A; **lane 3**, C1400 to T; **lane 4**, C1400 to G; **lane 5**, C1402 to A; **lane 6**, substitution of C1400 region to 5′TGTTTT; **lane 7**, mutation in left arm of the 1410–1490 stem (mutant I); **lane 8**, mutation in right arm of the 1410–1490 stem (mutant II); **lane 9**, mutation in both arms of the 1410–1490 stem (mutant I+II); **lane 10**, original specialized ribosome system X (Hui and de Boer, 1987; Hui *et al.*, 1987); **lane 11**, a negative control. This system is the same as system X but lacks the hGH gene (note that an unknown protein co-migrates with hGH); **lane 12**, mutation in both arms (1410–1490) as in lane 9. This mutant was constructed independently from that used in lane 9 (see text); **lane 13**, spectinomycin-sensitive system X in the presence of spectinomycin. The position of the human growth hormone band is indicated.

In Table I results of a similar experiment are shown. The experiment was carried out (i) to quantify the extent of reversion described above and (ii) to compare directly the hGH levels produced in the various mutants with or without the *spc*^{R} mutation. The hGH levels were measured without addition of spectinomycin to the medium in order to eliminate any possible artifactual effects on translation caused by the antibiotic itself. Clearly, the *spc*^{R} mutation has no effect on the hGH levels in any system except in case of the mutant with the altered 1410–1490 stem. Here, a 4-fold increase of ribosomal activity can be attributed to the 1192 base change. No increase is seen in the mutants of stem I or stem II alone. To establish that only this single base mutation is responsible for restoration of translational capacity and not due to any fortuitous unknown mutation elsewhere in 16S rRNA gene, the plasmids with mutation I+II (*spc*^{S}) were reconstructed and made spectinomycin-resistant by either replacing the *Sma*I fragment spanning the *spc* locus or by mutagenesis directly at this site. The resultant plasmids are I+II′ and I+II″ respectively. The hGH levels in cells containing these plasmids are the same as those in cells containing system X or mutant I+II *spc*^{R}. Therefore, the specialized ribosomes with an altered 1410–1490 stem struc-

Table I. Quantitation of the hGH levels in cells containing the spc^R or the spc^S specialized ribosome system X and its mutant derivatives

Mutation in system X	hGH levels (μg/ml/OD)		
	spc^S	spc^R	Ratio spc^R/spc^S
none	3.9	2.7	0.7
C1400 region	0.6	0.7	1.2
C1400 to A	1.4	1.0	0.7
C1400 to T	2.7	2.5	0.9
C1400 to G	0.8	0.9	1.1
C1402 to A	0.9	0.9	1.0
Substitution C1400 region	0.6	0.8	1.3
Mutant I	0.5	0.7	1.4
Mutant II	0.6	0.7	1.2
Mutant I+II	0.7	2.7	3.9
I+II′	n.d.	2.5	–
I+II″	n.d.	2.6	–

The cells containing the specialized ribosome system X with the indicated mutations were grown at 30°C in Mg and harvested after 2 h of induction at 37°C (in the absence of spectinomycin). The hGH levels were measured using a radio-immunoassay as described before (Hui and de Boer, 1987; Hui *et al.*, 1987). The average values of two different dilutions of the same sample are shown. Similar ratios were obtained when hGH levels were assayed after 3 h of induction (data not shown). The experiment was repeated three times with similar results. The mutants I+II′ and I+II″ spc^R were derived from the mutant I+II spc^S by replacing the *Sma*I fragment spanning the *spc* locus (position 1192 of *rrn*B) by the corresponding *Sma*I fragment, from a spc^R *rrn*B operon.

ture regain their full translational capacity by a base change in the 16S rRNA molecule located far away in the primary and secondary structure map.

Discussion

The data presented here and in a previous paper (Hui and de Boer, 1987) demonstrate that ribosomes can be directed to a single mRNA species by making the appropriate mutations in the SD- and ASD-region. We show that additional mutations in essential regions of the 16S rRNA molecule can be introduced and that, in this system, their effects on translation can be analysed without encountering complications due to killing of the host.

Thus far, no mutations in the 16S rRNA operon have been obtained by genetic means (with one exception; see Murgola *et al.*, 1988), other than those conferring antibiotic resistance, because of the presence of seven essentially identical operons, and the fact that mutations in key regions are dominantly lethal. We have reduced this complex genetic system to a relatively simple one; a non-essential ribosomal species translates a non-essential mRNA species *in vivo*. Thus, this system can be used to complement genetic approaches. For example, when the SD of the mRNA for a selectable marker would be changed into SDX, secondary site revertants of inactivating mutations within the 16S rRNA (with ASD X) might be obtained using appropriate selection schemes.

We do not know whether the observed reduction of the translational activity of the ribosomes mutated in the C1400 region is due to a reduction in translational initiation, reduction of the elongation process, a reduced rate of ribosome assembly or a combination of the above mentioned defects. The possibility of ineffective ribosome assembly should be considered since Krzyzosiak *et al.* (1987) noted that *in vitro*-synthesized G1400 containing rRNA is inefficient in the assembly process. However, another publication from the same group (Ofengand *et al.*, 1987) showed that such G1400 containing rRNA could be assembled efficiently provided that higher rRNA concentrations were used in the assembly reaction.

Here, it is shown that a C-residue at position 1400 is not an absolute requirement for overall ribosome function although this region is highly conserved in evolution. Full activity is maintained when this C-residue is replaced by a U-residue. Apparently, the presence of an amino instead of a ketoxy group on these pyrimidines is not critical for overall ribosomal activity. However, the U1400 ribosomes may differ from the C1400 ribosomes in another function of the ribosome, for example, in translational fidelity. Interestingly, in the two natural exceptions, referred to earlier, position 1400 is occupied by a U-residue.

Figure 2B shows a 50% reduction of protein synthesis by A1400 containing ribosomes *in vivo*. This result is in quantitative agreement with the observation of Ofengand *et al.* (1987). They find that the *in vitro*-produced A1400 ribosomes had only a modest effect on non-enzymatic binding of amino acyl-tRNA to the P-site. Their G1400 mutant showed a much stronger reduction in P-site binding. The residual polypeptide synthesis rate using a synthetic mRNA and A1400 or G1400 ribosomes was 60% and 25%, respectively, of that of unmutated ribosomes (Ofengand *et al.*, 1987).

Noller *et al.* (1985) have formulated an elegant model describing the possible molecular basis of the role of the C1400 region in the decoding process. Their model postulates stabilization of a short duplex (formed by the bases C1404 and G1405 with C1496 and G1497) by a correctly paired codon–anticodon duplex through coaxial stacking of both. In this extended duplex, the base in the wobble position of the tRNA is directly adjacent to the C1400 residue (Noller *et al.*, 1985). Substitution of the C1400 by any other nucleotide as described in this paper would not necessarily disturb the stacking of these duplexes. In the model of Noller *et al.*, the C1400 residue itself is not involved in the helical structure. Substituting this C by a G-residue as described in this paper might promote undesired stabilization of the stacked duplex involving pairing of G1400 with C1501. Both the deletion of the C1400 region and the substitution of the C1400 region do not directly affect the bases involved in the presumed pairing of 1404–1405 with 1496–1497. The low level of protein synthesis catalysed by these mutated ribosomes is likely due to other constraints imposed on the local RNA structure.

Disruption of the 1410–1490 stem greatly reduces ribosomal activity. Noller *et al.* (1985) postulated that this stem might assist in bringing the base pairs 1404–1405 and 1496–1497 in close proximity to facilitate the subsequent stacking with the codon–anticodon helix. Disruption of the 1410–1490 stem might impede the formation of the adjacent stacked structure. Since recovery of protein synthetic capacity is only partial upon restoration of the stem (with a different composition) this explanation may not be sufficient.

The position at 1402 is occupied by a C-residue which is methylated on the base and on the ribose moiety. We find that replacement of this C-residue by an A-residue reduces the translational yield by ~85%. Nothing is known about

the role of this methylated C-residue at 1402. van Knippenberg (1985) has postulated that this modified base, together with several other methylated bases in the rRNA molecule, forms part of a hydrophobic pocket lining the decoding site. It is unlikely that the A at 1402 is modified by any of the methyltransferases. Reduced hydrophobicity in this part of the decoding site might explain the reduced translational capacity of the A1402 ribosomes. The structural change caused by the substitution itself can equally well explain the observations. Jemiolo *et al.* (1985) generated a C1402 to a U1402 substitution using (random) bisulphite mutagenesis on a single stranded region spanning this area. Their observation is that cells bearing such a mutation in a plasmid borne rRNA operon grew with a 20% reduced rate. This group did not find other mutations in this area which was interpreted to mean that mutations outside position 1402 are not tolerated by the cell, i.e. they are presumably dominantly lethal. Given our finding that the C1400 to U1400 substitution does not affect ribosomal activity one would expect that bisulphite mutagenesis of this region would have yielded the same substitution. We do not have an explanation for this discrepancy.

Fortuitously, and to our surprise, we found full reversion of the double mutant in the 1410–1490 stem upon introduction of the C1192 to U substitution. Apparently, a long range interaction exists between the base-paired 1192 region and the 1410–1490 stem structure. To our knowledge, no such interaction involving these areas has been reported (see also Spitnik-Elson and Elson, 1985).

In conclusion we believe that the specialized ribosome system combined with suitable mutagenesis and selection procedures provides a powerful system that is highly suitable for elucidating novel, and confirming other, interactions between all molecules that make up the complex 30S particle.

Materials and methods

A detailed account of all the methods used is given previously (Hui *et al.*, 1987) and is summarized in the legends to the figures. Plasmid amplification was done in *Escherichia coli* 294 (F^-, *sup*E44, *end*A1, *thi*-1, *hsd*R4). Transformation with plasmids containing the P_L promoter of bacteriophage λ was done in *E.coli* K5716(F′, Δ(*lac*–*pro*), *sup*E, *tra*D36, *pro*AB, *lacI*^{Q}ZΔM15), which is a λ lysogen derived from JM101. Temperature induction of the specialized ribosome with λ P_L was done in *E.coli* K5637 (*c*I857,Δ *Bam cro*^{27}, *O*am^{29}). Both K5716 and K5637 were constructed by H.I.Miller (Genentech, Inc.).

Plasmids used here were constructed as described (de Boer *et al.*, 1985). The structure of the hGH gene and the hGH immunoassay are described by Goeddel *et al.* (1979).

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