Oligonucleotide directed mutagenesis of *Escherichia coli* 5S ribosomal RNA: construction of mutant and structural analysis

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SUMMARY

The ribosomal 5S RNA gene from the *rrnB* operon of *E. coli* was mutagenised *in vitro* using a synthetic oligonucleotide hybridised to M13 ssDNA containing that gene. The oligonucleotide corresponded to the 5S RNA sequence positions 34 to 51 and changed the guanosine at position 41 to a cytidine. The DNA containing the desired mutation was identified by dot blot hybridisation and introduced back into the plasmid pKK 3535 which contains the total *rrnB* operon in pBR 322. Plasmid coded 5S rRNA was selectively labeled with ³²P using a modified maxicell system, and the replacement of guanosine G41 by cytidine was confirmed by RNA sequencing.

The growth of cells containing mutant 5S rRNA was not altered by the base change, and the 5S rRNA was processed and incorporated into 50S ribosomal subunits and 70S ribosomes. The structure of wildtype and mutant 5S rRNA was compared by chemical modification of accessible guanines with kethoxal and limited enzymatic digestion using RNase T₁ and nuclease S₁. These results showed that the wildtype and mutant 5S rRNA do not differ significantly in their structure. Furthermore, the formation, interconversion and stability of the two 5S rRNA A- and B-conformers are unchanged.

INTRODUCTION

The ribosomal 5S RNA of *E. coli* has been studied for many years and numerous structural details are known today. They have been used for the construction of secondary and tertiary structure models (1-3). The 5S RNA exists, however, in a number of different conformers (4-6), and the dynamic transition of these conformers seems to be of functional importance for the translating ribosome (6,7). Various methods have been employed to localise nucleotides in the 5S rRNA which are involved in structural transitions or which are functionally important (6,8-10). From these studies a number of nucleotides can be assumed to be

of special interest for the structural dynamic and the function of the 5S RNA.

With the development of in vitro mutagenesis techniques these nucleotides can now be changed and the effects of such alterations on the structure and function can be studied. We decided to replace the guanosine at the position 41 in 5S rRNA by a cytosine for the following reasons: 1. G41 is by far the most reactive nucleotide in the isolated 5S rRNA as well as in its ribosome bound form (8,11). 2. G41 shows a drastic change in the reactivity towards modifying reagents or nucleases during the transition from the A- to the B-form (6,10). 3. G41 is strongly protected against kethoxal modification when ribosomes are modified in the presence of bound tRNA (6). 4. G41 can be cross-linked to G72 in a high yield and will therefore be close to the central part of the 5S rRNA molecule where ribosomal protein L18 is known to interact (1,3).

In this paper we describe the first construction of a specific change of one base in rRNA using the oligonucleotide directed mutagenesis approach. We also took advantage of a modified maxicell system (12) which allows to specifically label the plasmid coded mutant rRNA to characterise its structure.

MATERIALS AND METHODS

Mutagenesis

The mutagenic oligonucleotide with the sequence ACCCCAUCCC-GAACUCAG was synthesised manually using the phosphite triester method (13). The 18mer was purified on a 20% polyacrylamide gel containing 7 M urea and its sequence was confirmed using a modified sequencing procedure according to (14). The plasmid pKK 3535 was completely digested with Bam H1 and Sal 1 and ligated to linearised M13 mp10 (PL Biochemicals) DNA obtained after digestion with the same enzymes. After transformation into competent JM101 cells (15) and the selection of white plaques using the X-Gal system (16) the DNA of the replicative form of 10 transformants was isolated on a small scale. The insertion of the 2661 base pair fragment and its right orientation was confirmed by restriction enzyme mapping using Bam H1, Sal 1 and Eco R1 as well as by the hybridisation of an endlabeled ³²P

5S RNA probe to the single stranded M13 DNA baked onto nitro-cellulose filters (17). 5 μ l of ssM13 DNA (0.8 pmol), 1 μ l 10X annealing buffer (0.2 M Tris-HCl, pH 7.5, 0.1 M MgCl₂, 0.5 M NaCl, 0.01 M DTT), 3 μ l of phosphorylated mutagenic oligonucleotide (20 pmol) and 1 μ l of M13 sequencing primer (17mer, PL Biochemicals, 20 pmol) were mixed, heated to 80 C for 5 minutes and incubated at 23 C for 5 minutes. 10 μ l enzyme/dNTP mix were added: 1 μ l 0.2 M Tris-HCl, pH 7.5, 0.1 M MgCl₂, 0.1 M DTT; 4 μ l 2.5 mM dNTP mix; 1 μ l 10 mM ATP, 1.5 μ l (30 U) T4 DNA ligase; 1.5 μ l water; 1 μ l (2.5 U) *E. coli* DNA polymerase (large fragment) and incubated at 15 C for 8 hours. Competent JM101 cells were transformed as described above, ssM13 DNA was isolated on a small scale from 48 white plaques and baked onto nitrocellulose filters (Schleicher & Schüll BA 85/20, 2 hours, 80 C). The mutagenic oligonucleotide was end-labeled with polynucleotide kinase and ³²P ATP and separated from unincorporated ³²P phosphate on NACS-column (BRL). The baked filters were pre-hybridised with buffer (3 ml 20 x SSC, 2 ml 50 x Denhardts, 0.2 ml 10% SDS in a total volume of 50 ml at 67 C for 1 hour), washed twice with the above buffer but containing the oligonucleotide probe and incubated for 2 hours at room temperature. The filters were washed six times with about 200 ml of 6 x SSC at increasing temperatures for 5 minutes and autoradiographed. Double stranded M13 DNA was isolated from cells giving a positive signal after washing at 54 C, and the Bam H1/Sal 1 fragment containing the presumably altered 5S RNA gene was isolated. pKK 3535 was partially digested with Bam H1, and the linear plasmid containing only one cut was isolated on a 0.8% agarose gel. The DNA was electroeluted and concentrated by binding and elution using BND cellulose. This linear DNA was partially digested with Sal 1, and fragments in the range of about 9000 base pairs were isolated as described above. The plasmid DNA was mixed with the mutagenised Bam H1/Sal 1 fragment obtained from the M13 replicative form and ligated according to (18). Competent HB101 cells were transformed and plated on agar plates containing LB and 50 μ g/ml ampicillin. The plasmid DNA of several transformants was prepared on a small scale and screened by restriction mapping using Eco R1 to confirm the insertion of the desired fragment.

Labeling of mutant 5S rRNA in maxicells

The modified labeling procedure was followed exactly as described (18). 5S rRNA was isolated after separation on a 10% polyacrylamide gel containing Tris-borate and EDTA, eluted in 10 mM Tris-HCl, 1% SDS and 2 mM EDTA and concentrated by ethanol precipitation. 5S RNA was purified on a 8.5% polyacrylamide gel containing 8 M urea. RNA was extracted and digested with 50 units of RNase T₁ (Sankyo) for 1 hour at 37 C in 10 mM Tris-HCl, pH 7.5, in the presence of 2.5 µg carrier tRNA. The resulting oligonucleotides were separated on PEI coated cellulose plates (Macherey & Nagel) in the two-dimensional system of Volckaert & Fiers (19). After autoradiography spots of interest were extracted and analysed using RNase A as described (19).

Isolation of the A- and B-conformers of 5S rRNA

The formation and isolation of the 5S A- and B-conformers were achieved as described previously (6).

Analysis of reactive guanosines using kethoxal

The kethoxal reaction was performed with 10 µl of the A- or B-form 5S RNA samples after adding 40 µl of a saturated kethoxal solution in 50 mM Na-cacodylate, pH 7.2, 2 mM MgCl₂ and 10% ethanol for the A-form and the same buffer with 2 mM EDTA instead of the MgCl₂ for the B-form 5S RNA. Samples were incubated for 30 minutes at 30 C. The diagonal fingerprint was performed as described by Noller (20) with some modifications. PEI coated cellulose plates (20 cm x 20 cm) were used instead of DE-paper. Oligonucleotides were separated chromatographically using 1 M formic acid-pyridine, pH 4.3, 4 M urea for both dimensions. The RNase T₁ digestion before separation in the first dimension was performed in 10 µl of 100 mM NH₄-acetate, pH 5.5, 20 mM Na-borate and 5 units of RNase T₁ for 1 hour at 37 C. Digestion before separating in the second dimension was performed by spotting 2.5 units of RNase T₁ in 250 µl on the PEI plate and incubating for 2 hours at 37 C.

Limited RNase T₁ and nuclease S₁ digestions

Limited RNase T₁ and nuclease S₁ digestions and the separation of the hydrolysis products were performed exactly as described (10).

Figure 1: Scheme for the construction of the C41-mutation in 5S rRNA by oligonucleotide directed mutagenesis. (For details see text.)

RESULTS

Oligonucleotide directed mutagenesis of 5S rRNA

For the construction of the base change an oligodeoxynucleotide with the sequence ACCCCAUCCCGAACUCAG was synthesised as described in Material and Methods. This sequence corresponds to the 5S rRNA sequence of bases at the positions 34 to 51 with G41 being replaced by a C. Figure 1 outlines the strategy of mutagenesis and the selection of the mutation. We isolated a Sal I/Bam HI fragment containing the 5S rRNA gene from the plasmid pKK 3535 which contains the complete *rrnB* operon (21,22). This fragment was cloned into M13 mp10 DNA which had been cut with Sal I and Bam HI prior to ligation. Single stranded DNA was isolated from white plaques, and the correct insertion and orientation were confirmed by restriction enzyme digestion and by hybridisation with ^{32}P endlabeled 5S rRNA using dot blots. The actual mutagenesis was accomplished using the two primer method described by Norris et al. (23). The phosphorylated muta-

Figure 2: Dot blot hybridisation of ssDNA after the construction of the mutation using the mutagenic oligonucleotide. Single stranded DNA from individual plaques were bound to nitrocellulose filters and hybridised to the 5'-³²P labeled oligodeoxynucleotide as described in Methods. The filters were washed with 6 x SSC buffer at 20, 30, 35, 40, 45, 49 and 54 C. Arrows indicate samples that showed strong hybridisation signals after washing at 54 C.

genic oligonucleotide together with the universal sequencing primer (17mer) was hybridised to the ssDNA of M13 containing the 5S DNA insertion. Double stranded DNA was synthesised by adding the four deoxynucleotide triphosphates and DNA polymerase I (Klenow fragment). After ligation with T4 DNA ligase this DNA was used to transform competent JM 101 cells. Plaques containing the mutagenised 5S rRNA gene could be clearly distinguished from those with unaltered DNA after dot blot hybridisation with the ³²P end labeled mutagenic oligonucleotide and washing at various temperatures as seen in Figure 2. Three out of 48 plaques tested gave still a positive hybridisation signal after a wash of 54 C. The replicative form of M13 DNA of two positive clones was isolated and digested again with Sal I and Bam HI to obtain the DNA fragment containing the potential mutation in the 5S rRNA gene. This DNA fragment was introduced back into pKK 3535 at the right position and orientation by ligating it to a pKK 3535 DNA fragment of 9203 base pairs which was obtained after partial digestion with Sal I and Bam HI (see Figure 1). After transformation plasmid DNA from transformed cells were screened by restriction mapping using Eco RI to confirm that a complete plasmid had

Figure 3: RNase T₁ fingerprints of wildtype and mutant 5S RNA. Wildtype (pKK 3535) and mutant (pC41-5) 5S RNA were digested with RNase T₁ and separated in two dimensions (19). Both fingerprints differ by one oligonucleotide. The sequences as established by secondary analysis are indicated by an arrow.

been formed. Out of 18 transformants investigated 7 contained the 5S rRNA gene in the right orientation on a full size plasmid. Characterisation of the mutation in 5S rRNA by labeling in maxicells and RNA-fingerprinting

The introduction of the desired mutation could not be proven directly by restriction enzyme analysis because a restriction site was not destroyed by the base change and no new restriction site was created. Therefore we tested the 5S rRNA directly by fingerprinting with RNase T₁. Using this approach a complication arose because seven ribosomal operons exist in the host cell itself which would not carry the mutation. We solved this problem by specifically labeling the plasmid coded rRNA using the modified maxicell procedure worked out by Stark et al. (12). Plasmid coded rRNA was labeled, the RNA was extracted and the 5S rRNA was separated by gel electrophoresis (see Material and Methods). The RNA was digested with RNase T₁ and the resulting oligonucleotides were separated using the two-dimensional fingerprint system of Volckaert & Fiers (19). Figure 3 shows the fingerprint of wildtype and mutant C41 5S rRNA after RNase T₁ digestion.

As expected the mutation of G41 to a C results in the for-

mation of the new RNase T₁ oligonucleotide ACCCCAUCCCG while the wildtype oligonucleotide ACCCCAUG disappears in the mutant RNA. The new oligonucleotide can clearly be identified in the fingerprint. The correct sequence was further conformed for the pC41-5 mutant by secondary analysis using RNase A digestion of the corresponding T₁-oligonucleotides. No other difference in the primary structure of the wildtype and the C41 mutant could be detected. The results shown here prove that the desired base-change was successfully introduced and that mutant RNA with this particular base change was expressed by cells containing the mutant plasmid pC41-5. Four transformants contained the mutation while three showed a T₁-fingerprint identical to the unmutagenised 5S rRNA.

The growth rates of cells containing the mutant plasmid was not changed significantly when compared with pKK 3535. Mutant 5S RNA was incorporated into 50S ribosomal subunits and 70S ribosomes as was shown by two-dimensional gel electrophoresis of ribosomes containing ³²P labeled plasmid coded RNA (data not shown).

Structural characterisation of the mutant 5S rRNA

One of the structural characteristics of 5S rRNA from *E. coli* is the formation of A- and B-conformers which can be separated by gel electrophoresis. G41 is highly reactive and accessible in the A-form but becomes completely unreactive and protected in the B-form of unmutated 5S rRNA. It was of special interest how the mutant 5S RNA we constructed would influence the formation of the A- and B-conformer. Under the standard conditions of re- and denaturation (6) both the wildtype and C41 5S RNA will form the A- and B-conformer to the same extent.

The formation of the A-form is a magnesium ion requiring process (24). We therefore tested whether the magnesium ion dependence for the formation of the A-form is the same in both 5S RNAs. Again, as can be seen in Figure 4, the requirement for the magnesium ion concentration to form the A-conformer is the same for both types of RNAs. In the presence of 100 mmolar monovalent cations a 0.7 mmolar magnesium ion concentration is required for the complete renaturation of both wildtype and mutant 5S rRNA.

Figure 4: Magnesium ion dependence of the B- to A-form inter-conversion of wildtype and mutant 5S RNA. 5S RNA samples were heated to 70 C and slowly cooled down after the addition of defined Mg^{2+} concentrations. The samples were separated on 8% polyacrylamide gels, and the ratio of A- and B-conformers was determined by densitometry.

Figure 5: Effect of the kethoxal modification of wildtype and mutant 5S RNA on the interconversion of the A- and B-conformers. An autoradiogram after separation of wildtype (pKK 3535) and mutant (pC41-5) 5S RNA samples on an 8% polyacrylamide gel is shown. A^K and B^K are 5S RNA conformers after reaction with kethoxal for 30 minutes. $A^{K,D}$ are kethoxylated A-conformers after denaturation and $B^{K,R}$ are kethoxylated B-conformers after renaturation. XC denotes the position of the xylene cyanol marker dye.

Figure 6: Analysis of kethoxal reactive guanosines of the A- and B-conformers of wildtype and mutant 5S RNA. The autoradiograms of 4 diagonal fingerprints are shown. A, B are wildtype (pKK 3535) and C, D are mutant pC41-5 5S RNA samples of the A- or B-forms as indicated. In A the 5S RNA oligonucleotides on the diagonal are numbered according to (25). Some of the off diagonal spots characteristic for modified guanosines are indicated, and the corresponding guanosine positions are given by numbers.

The interconversion of the 5S RNA A- and B-forms can normally be affected after modification of the 5S RNA with kethoxal. The kethoxylated A-form can be easily converted to the B-form but the kethoxylated B-form will not yield the A-conformer upon renaturation (6). Figure 5 shows that the pC41-5 5S rRNA can be converted from the A- to the B-form after kethoxylation but not from the B- to the A-form exactly as shown for the unaltered wildtype 5S rRNA. We conclude from this experiment that a guanosine at position 41 is not required for the inhibited interconversion from the B- to the A-form.

Kethoxal which reacts specifically with single stranded guanosines is a very sensitive probe for RNA secondary structure

Figure 7: Limited RNase T₁ and nuclease S₁ digestion patterns of the A- and B-forms of wildtype and mutant 5S RNA. Samples were digested in either the A- or B-form as indicated by the letters. K is an undigested control sample. In L an alkaline hydrolysate has been separated to obtain a random ladder. Arrows indicate hydrolysis fragments resulting from cleavage at position 41. Note that the corresponding fragments can only be seen in the RNase T₁ track of the wildtype pKK 3535. Numbers at the margin indicate sequence positions of the corresponding fragments. XC denotes the xylene cyanol marker dye.

and has therefore been used to characterize the A- and B-form of 5S rRNA (8). Figure 6 shows the analysis of the kethoxylated guanosines in the two forms in both mutant C41 and wildtype 5S rRNA. The difference in the kethoxal reactivity between A- and B-form is very clear for both types of RNA. However, no difference in the reactivity of the wildtype and the mutant 5S RNA can be detected, of course with the exception of position 41 which has no modifiable G in the mutant in this place. The analysis of the kethoxal reactive guanosines is in very good agree-

ment with the results of Noller and Garrett (8). We conclude from these experiments that there is no major change in the 5S rRNA structure when G41 is replaced by a C in both A- and B-form.

The secondary and tertiary structure of 5S RNA has also been analysed systematically by limited enzymatic digestion using base and structure specific nucleases (10). Here we used RNase T₁ and nuclease S₁ to detect possible structural differences of mutant and wildtype 5S RNA in the A- and B-form, respectively. These results are shown in Figure 7. Although clear differences are apparent in the hydrolysis pattern of the A- and B-form, only minor changes can be detected for the mutant and wildtype 5S rRNA, respectively. Again we conclude that only very little structural change if any is caused by this particular mutation.

DISCUSSION

The 5S rRNA of Escherichia coli has been shown to be essential for the function of a translating ribosome (26). Chemical modification of ribosomes in the presence and absence of bound tRNA support this idea of a functional implication of 5S rRNA in tRNA binding. These studies show that G41 is strongly protected from kethoxal modification when tRNA is bound to the ribosome. Furthermore the accessibility and reactivity of this nucleotide is changed drastically in different conformers of the 5S rRNA. The G41 is therefore supposed to be localised in a flexible domain within the structure of the molecule. Not only in the isolated but also in the ribosome associated state is G41 highly reactive and accessible. It is therefore suggested that G41 is very important and may be involved in some of the functions of the ribosome.

Newly developed in vitro mutagenesis techniques enabled us to change the nucleotide at position G41 into any desired base. We chose cytosine to replace the guanosine for two reasons: First, the exchange should not be semiconservative, because too small a change would be introduced into the 5S molecule to create a conformational change. Therefore a purine had to be exchanged by a pyrimidine. Second, there is a proposed secondary interaction in 5S rRNA between nucleotides at the positions 37

to 40 and 73 to 77 (3). Replacement of G41 by a C could add another G-C base pair to this interaction and might therefore stabilise the A-form of 5S rRNA. This would provide an answer whether this structure exists or not.

In the mutagenesis approach we used M13 single stranded DNA and the two primer method of Norris et al. (23). The successful construction of the point mutation could in principle be confirmed by sequencing either on the DNA- or the RNA level. In our case RNA sequence determination offers an advantage because the mutation can be detected easily in a simple two-dimensional RNase T₁ fingerprint (see Results). Furthermore, the analysis of the RNA provides direct information whether the mutated RNA is expressed and processed accurately. Plasmid coded ribosomal RNA could be selectively labeled with ³²P using a modified maxicell labeling procedure (12).

This analysis clearly demonstrated the G41 to C41 change in the 5S rRNA mutants and also indicated that the mutant 5S rRNA was expressed and processed normally. No other additional base changes at other positions were found. The presence of mutant 5S rRNA in 50S subunits and 70S ribosomes as well as the unaltered growth rate of cells containing the mutant plasmid pC41-5 suggests that the mutant 5S RNA functions quite normally. Further studies in vitro and in vivo will be necessary to prove the functional activity of the mutant RNA. 5S rRNA isolated from cells containing the mutant plasmid is a mixture of mutant and wildtype RNA close to equimolar amounts (12). Reasonable reconstitution experiments can therefore only be performed after separation of mutant and wildtype RNA. In our study we used specifically ³²P labeled mutant 5S rRNA from maxicell preparations to compare the structure of wildtype and C41 5S rRNA. The structures of the pure A- and B-forms were compared respectively in order to avoid artefacts due to conformational heterogeneity. From the structural analysis the following can be summarised: 1. The interconversion of the 5S rRNA A- and B-conformers is not inhibited in the C41 mutant, and the Mg²⁺ ion dependence for the renaturation is the same as for the unaltered 5S RNA. This result makes an additional base pairing between C41 and G72 in the A-form unlikely and is in agreement with results

obtained by (10). 2. G41 clearly is not responsible for the inhibition of the renaturation of the B-form after kethoxal modification. 3. From the kethoxal reactivity of the single stranded guanosines and the limited enzyme digestion studies we conclude that neither the A- nor the B-form of the mutant 5S RNA shows a significant structural change when compared to the wild-type RNA.

These results are very surprising after all the effects that can be attributed to the guanosine at position 41. Nevertheless they are in agreement with the data of Zagorska et al. (27) who have shown that very likely the CCGAACUCAG sequence at positions 42 to 51 is not obligatory for protein synthesis.

G41 seems to be a very good indicator group for detecting changes in the overall structure, but is very likely not directly involved in the maintenance of the structure itself. Our findings confirm that the method of site directed mutagenesis provides useful information which supplements the results of chemical modification studies in an unexpected way.

Attempts to construct deletions in the 5S rRNA have been unsuccessful so far although deletions at various positions in the 23S and 16S rRNA gene could be obtained in the same experiment (28). It seems as if cells containing pKK 3535 with deletions in the 5S RNA gene cannot survive. However, the point mutation we constructed does not affect the structure of 5S RNA and probably conserves the function. The mutation will therefore be very helpful for future studies, because it can easily be recognised by the loss of the hyperreactive guanosine at position 41 and by the change in the RNase T₁ sensitivity. The base change of G41 to a C should provide a way for separating the wildtype and mutant 5S rRNA because the unmutagenised 5S rRNA can be almost quantitatively cut at the G41 position by RNase T₁, while the mutant RNA will not be digested under those conditions and can be separated in its uncleaved form. Such properties will be of great help for in vitro studies and are currently under way. Also the investigation of the in vivo rRNA processing and ribosome assembly is facilitated by the use of our construction.

During the preparation of the manuscript we learned that the group of H.F. Noller has constructed a 5S RNA mutant with a

base change at position C36. Apparently this mutant has some phenotypic changes in the stability of the A- and B-conformers.

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REFERENCES

1. Garrett, R.A., Douthwaite, S. and Noller, H.F. (1981) Trends in Biochemical Sciences 6, 137-139.
2. Pieler, T. and Erdmann, V.A. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 4599-4603.
3. Hancock, J. and Wagner, R. (1982) Nucleic Acids Res. 10, 1257-1269.
4. Aubert, M., Bellemare, G. and Monier, R. (1973) Biochimie 55, 135-142.
5. Kao, T.H. and Crothers, D.M. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 3360-3364.
6. Göringer, H.U., Bertram, S. and Wagner, R. (1984) J. Biol. Chem. 259, 491-496.
7. Kime, M.J. and Moore, P.B. (1982) Nucleic Acids Res. 10, 4973-4983.
8. Noller, H.F. and Garrett, R.A. (1979) J. Mol. Biol. 132, 621-636.
9. Rabin, D., Kao, T.H. and Crothers, D.M. (1983) J. Biol. Chem. 258, 10813-10816.
10. Göringer, H.U., Szymkowiak, C. and Wagner, R. (1984) Eur. J. Biochem., in press.
11. Delihias, N., Dunn, J.J. and Erdmann, V.A. (1975) FEBS Lett. 58, 76-80.
12. Stark, M.J.R., Gourse, R.L. & Dahlberg, A.E. (1982) J. Mol. Biol. 159, 417-439.
13. Matteucci, M.D. and Caruthers, M.H. (1981) J. Am. Chem. Soc. 103, 3185-3191.
14. Maxam, A.M. and Gilbert, W. (1980) Methods in Enzymology 65, 499-560.
15. Dagert, M. and Ehrlich, S.D. (1979) Gene 6, 23-26.
16. Gronenborn, B. and Messing, J. (1978) Nature 272, 375-377.
17. Zoller, M.J. and Smith, M. (1982) Nucleic Acids Res. 10, 6487-6500.
18. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory.

19. Volckaert, G. and Fiers, W. (1977) *Analyt. Biochem.* 83, 228-239.
20. Noller, H.F. (1974) *Biochemistry* 13, 4694-4703.
21. Brosius, J., Dull, T.J., Sleeter, D.D. and Noller, H.F. (1981) *J. Mol. Biol.* 148, 107-127.
22. Brosius, J., Ullrich, A., Raker, M.A., Gray, A., Dull, T.J., Gutell, R.R. and Noller, H.F. (1981) *Plasmid* 6, 112-118.
23. Norris, K., Norris, F., Christiansen, L. and Fiil, N. (1983) *Nucleic Acids Res.* 11, 5103-5112.
24. Weidner, H. and Crothers, D.M. (1977) *Nucleic Acids Res.* 4, 3401-3414.
25. Brownlee, G.G., Sanger, F. and Barrell, B.G. (1968) *J. Mol. Biol.* 34, 379-412.
26. Dohme, F. and Nierhaus, K.H. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 2221-2225.
27. Zagorska, L., Van Duin, J., Noller, H.F., Pace, B., Johnson, K.D. and Pace, R.N. (1984) *J. Biol. Chem.* 259, 2798-2802.
28. Zwieb, C., Jemiolo, D.K., Jacob, W., Wagner, R. and Dahlberg, A.E. (1984) submitted.