Frameshift suppression at tandem AGA and AGG codons by cloned tRNA genes: assigning a codon to *argU* tRNA and T4 tRNA^{Arg}

R.A.Spanjaard⁺, K.Chen¹, J.R.Walker¹ and J.van Duin^{*}

Department of Biochemistry, Gorlaeus Laboratoria, University of Leiden, PO Box 9502, 2333 CC Leiden, The Netherlands and ¹Department of Microbiology, University of Texas, Austin, TX 78712-1095, USA

Received June 20, 1990; Revised and Accepted August 6, 1990

ABSTRACT

Arginine is coded for by CGN (N = G, A, U, C), AGA and AGG. In *Escherichia coli* there is little tRNA for AGA and AGG and the use of these codons is strongly avoided in virtually all genes. Recently, we demonstrated that the presence of tandem AGA or AGG codons in mRNA causes frameshifts with high frequency. Here, we show that phaseshifts can be suppressed when cells are transformed with the gene for tRNA^{Arg}_{T4} or *E. coli* tRNA^{Arg}_{*argU*}, demonstrating that such errors are the result of tRNA depletion. Bacteriophage T4 encoded tRNA^{Arg} (anticodon UCU) corrects shifts at AGA-AGA but not at AGG-AGG, suggesting that this tRNA can only read AGA. Similarly, comparison of the translational efficiencies in an *argU* (Ts) mutant and in its isogenic wild type parent indicates that *argU* tRNA (anticodon UCU) reads AGA but not AGG. An *argU* (Ts) mutant barely reads through AGA-AGA at 42°C but translation of AGG-AGG is hardly, if at all, affected. Overexpression of *argU*⁺ relaxes the codon specificity. The thermosensitive mutant in *argU*, previously called *dnaY* because it is defective in DNA replication, can be complemented for growth by the gene for tRNA^{Arg}_{T4}. This implies that the sole function of the *argU* gene product is to sustain protein synthesis and that its role in replication is probably indirect.

INTRODUCTION

Recently, we noticed that translation of the tandem arginine codons AGG caused about 50% of the ribosomes to shift to the +1 phase. The same error, albeit to a lower extent, was observed when translation was challenged with double AGA codons. Shifts did not occur at single AGG or AGA codons. These high frequency shifts were dependent on the tandem rare codons being expressed at a high level from a multicopy plasmid. Preliminary evidence suggested that the error arises by tRNA^{Arg} shortage

since it could be corrected by decreasing the mRNA concentration or increasing the tRNA concentration (1). Varenne and Lazdunski (2) had already predicted on theoretical grounds that the depletion of a tRNA species is much more severe when the corresponding codons appear in tandem rather than scattered. Our account of the events is therefore that ribosomes read the first arginine codon but stall when the second one appears in the ribosomal A-site. At this point, about half of the ribosomes escape by moving to the +1 reading mode, whereas the other half eventually find a cognate tRNA and proceed normally (1). The precise mechanism is poorly understood but the similarity with frameshifting in yeast at the TYA-TYB boundary may be interesting. Here, too, the ribosome is supposed to pause over an AGG codon occupying the A-site and suppression of the shift is achieved by introducing extra tRNA recognizing this AGG codon (3 and P. Farabaugh, personal communication).

In this report we extend our previous studies by examining frameshift suppression in response to expression of plasmid borne tRNA^{Arg} genes. Whether or not a cloned tRNA can correct a frameshift error at AGG-AGG or AGA-AGA is used as an assay to determine whether the tRNA can read the AGA or AGG codon.

One of the tRNAs tested is derived from bacteriophage T4 and has the anticodon UCU (4); the other one is *E. coli argU* tRNA (5). The *argU* locus was previously called *dnaY* because a mutation in this gene resulted in thermosensitive DNA replication (6). Later, the gene product was shown to be a tRNA^{Arg} with anticodon UCU (7,8).

Our frameshift suppression studies using *argU* tRNA and its thermosensitive counterpart suggest that this tRNA reads AGA but not AGG. Similarly tRNA^{Arg}_{T4} only prevents shifts at AGA but not at AGG and this tRNA is therefore proposed to recognize AGA only. Our *in vivo* codon assignment for tRNA^{Arg}_{T4} and *E. coli* tRNA^{Arg}_{UCU} is consistent with reported modifications at the U wobble base of their anticodon (4,8). The results of our *in vivo* experiments confirm the pattern that eubacteria and eukaryotes tend to use two separate tRNAs to decode NNA and NNG in a mixed codon family.

* To whom correspondence should be addressed

⁺ Present address: Division of Genetics, Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

MATERIALS AND METHODS

Plasmids, bacterial strains and growth conditions

Strain M5219 contains a defective λ prophage carrying the thermosensitive repressor *cI857*, and the transcription antitermination factor *N* (9). Strain GM10 and the isogenic parent GM241 have been described (6). Growth conditions and induction of the p_L promoter were as before (1). The $tRNA^{Arg}$ gene from phage T4 is present on a 640 base pairs *EcoRI* fragment (10) which was cloned in the *EcoRI* site of the *cat* gene of pACYC184 (11) in two different orientations with respect to the *cat* gene promoter. Wild type and mutant *argU* genes which are, together with their own promoter, present on a *HindIII* fragment of 4.5 kilobases (7), were cloned in the *HindIII* site of pACYC184. The orientation of the fragment did not alter the phenotype of the clone. All other plasmids in this study are derivatives of the compatible plasmid pBR322 carrying the p_L promoter (1). When strains GM10 and GM241 are challenged with the plasmid borne frameshift sequence shown in Fig. 1 they are also transformed with plasmid *pcI857* (12). This plasmid encodes the thermosensitive λ repressor, necessary to suppress the p_L promoter that controls transcription of the frameshift messenger.

RESULTS

Frameshifting at AGG-AGG and AGA-AGA sequences

The essential features of the plasmid that contains the frameshift sequence are displayed in Fig. 1. The efficiently expressed coat gene of phage MS2 is fused to a test sequence that facilitates measurement of frameshifting by the presence of an out-of-phase stop codon. Normal error-free translation starting at the coat gene yields a protein of 147 amino acids, whereas +1 shifts occurring in the test sequence cause premature termination and result in the synthesis of a 106 amino acids long polypeptide. Both products can be easily visualized by immunoblotting using a serum raised against the coat protein. In lanes 2 and 3 of Fig. 2 we recapitulate our finding that the sequences AGG-AGG and AGA-AGA elicit high frequency +1 shifts. The lower band marked '106 aa' shows the frameshift product (1).

$tRNA^{Arg}$ encoded in bacteriophage T4 suppresses shifts at AGA-AGA but not at AGG-AGG

We have cloned the $tRNA^{Arg}$ gene from bacteriophage T4 in the *cat* gene of pACYC184 to assay the effect of this tRNA on frameshifting in strain M5219. As shown in lane 6 of Fig. 2 phaseshifts on tandem AGA codons disappear when this gene is inserted codirectionally to the *cat* gene promoter. Cloning the tRNA gene in the other orientation does not suppress the shift (lane 7). This result demonstrates that frameshifting is caused by tRNA depletion at the tandem arginine codons.

Surprisingly, $tRNA_{T4}^{Arg}$ is not able to suppress shifts at double AGG codons (lane 8) indicating that it can not read AGG. Its anticodon is UCU and the apparent specificity is consistent with the presence of a modification of the U in the wobble position (4,13).

AGA and AGG codons are read by different tRNAs in *E. coli*

As a first approach to deduce the specificity of endogenous *E. coli* $tRNA^{Arg}$ for the two minor arginine codons we tested whether frameshifts occur on the sequence AGA-AGG and AGG-AGA. If the two codons are read by the same tRNA the frameshift is predicted to persist because the demand for this tRNA is not altered by the codon change. If, however, each codon has its

Fig. 1. Basic features of our plasmid borne frameshift assay system. The coat gene fragment of the MS2 phage directs translation as indicated by the straight arrowheaded line. Shifts to the +1 direction that occur at the $AGG_{X}AGG_{X}$ sequence will yield premature translation termination at the overlined stop codon and a product of 106 amino acids (aa). The full sequence of the *EcoRI*-*BamHI* fragment is given. If in the sequence $AGG_{X}AGG_{X}$, AGG is the zero frame, then GGU is the +1 frame. p_L is the main leftward promoter of phage λ . Dots indicate the reading frame and the DNA shown is inserted in pPLc236 which is a derivative of pBR322 (9).

own tRNA, frameshifts will be absent. As shown in lanes 4 and 5 of Fig. 2 these mixed codon sequences do not elicit phase errors, strongly suggesting that separate tRNAs are present in *E. coli*; one kind for AGA and another for AGG. This interpretation is consistent with the sequence of two minor $tRNA^{Arg}$ species in *E. coli* (8). One has the anticodon 5' CCU and this tRNA is therefore likely to be the AGG reading species. The other tRNA is identical to the *argU* gene product. It contains the anticodon UCU in which the wobble base is mnm^5s^2U (5-methylaminomethyl-2-thiouridine) (Yokoyama, personal communication and reference 8). This tRNA is, therefore, the candidate for the AGA reading species.

argU tRNA is the AGA reading species

The *dnaY* (*argU*) gene of *E. coli* fulfills an important function in the cell since a mutant in this allele was isolated that is thermosensitive in DNA replication (6). This mutant, GM10, does not grow at 42°C (Fig. 3c and reference 6) but growth can be restored by introducing the *argU^{ts}* gene on a multicopy plasmid (Fig. 3b). This transformation simply increases the concentration of *argU^{ts}* tRNA in GM10. Supposedly, the affinity of the mutant tRNA for one of its reaction partners is lowered and such a defect can be compensated for by the higher concentration of the tRNA when produced from a multicopy plasmid. Whatever the mechanism, the important point we wish to make is that the *argU^{ts}* gene produces a tRNA that, albeit with reduced efficiency, fulfills its authentic function.

We have transformed M5219 with *argU^{ts}*, cloned in pACYC184, and compared frameshift suppression over AGA-AGA and AGG-AGG in this construct. We find that shifts over AGA-AGA are now suppressed but those over AGG-AGG are not (Fig. 2, lanes 15 and 13). Apparently, *argU^{ts}* tRNA can read AGA but not AGG. Since, as we showed above, *argU^{ts}* can replace the wild type tRNA we presume that our codon assignment also holds for wild type $tRNA_{UCU}^{Arg}$.

Translation of AGA but not AGG is crippled in an *argU^{ts}* mutant at 42°C

It is possible to confirm our codon assignment for *argU* tRNA in an independent way by challenging the GM10 mutant, in the absence of an exogenous tRNA supply and at the restrictive

Fig. 2. Immunoblot analysis of frameshifting in several clones described in the text. Induction was for 30 min at 42°C except lane 16 where induction was at 44°C. In all lanes M5219 is used as host.

lane 1, control pBR322;
lane 2, (AGG)₂;
lane 3, (AGA)₂;
lane 4, AGG-AGA;
lane 5, AGA-AGG;
lane 6, (AGA)₂ + tRNA_{T4}(+);
lane 7, (AGA)₂ + tRNA_{T4}(-);
lane 8, (AGG)₂ + tRNA_{T4}(+);

lane 9, (AGG)₂ + tRNA_{T4}(-);
lane 10, control pBR322;
lane 11, (AGG)₂ + pACYC184;
lane 12, (AGG)₂ + *argU*;
lane 13, (AGG)₂ + *argU*^{ts};
lane 14, (AGA)₂ + *argU*;
lane 15, (AGA)₂ + *argU*^{ts};
lane 16, (AGA)₂ + *argU*^{ts} (44°C).

tRNA_{T4}(+) means that the tRNA^{Arg}_{T4} gene is cloned in the cat gene of pACYC184 in the same orientation as the cat gene promoter; tRNA_{T4}(-), against the orientation of the cat gene promoter. The *argU* gene is cloned in pACYC184. (AGG)₂, (AGA)₂ is AGG-AGG, AGA-AGA, respectively. The mobility difference between the frameshift proteins in lanes 2 and 3 is consistent with the incorporation of different amino acids at the site of the shift (glycine (GGU) vs asparagine (GAU)). The uppermost band in the gel is the result of readthrough at the UGA stop codon in the vector (1).

Fig. 3. Growth of GM10 at 42°C after transformation with genes for tRNA^{Arg} cloned in pACYC184. a, + *argU*; b, + *argU*^{ts}; c, + vector pACYC184; d, + tRNA^{Arg}_{T4} (inactive gene orientation); e, + tRNA^{Arg}_{T4} (active gene orientation); f, as e but with mutated anticodon (5' TCT → 5' CCT); g, GM241 + tRNA^{Arg}_{T4} (inactive gene orientation). Growth medium did not contain antibiotics.

temperature, with the tandem AGA and AGG codons. If AGA is indeed only read by *argU* tRNA one expects that at 42°C the mutant will not or hardly be able to read through double AGA codons. In Fig. 4 lanes 5 and 6 we compare the ability of mutant and wild type to translate AGA tandems. Clearly, GM10 makes very little of the 147aa protein as compared to wild type. In addition, substantial frameshifting accompanies the little that is produced. Apparently, the decoding of the AGA codon depends on the presence an active *argU* tRNA.

If, as we suggest, *argU* tRNA is not involved in reading AGG codons we expect that double AGG codons are translated equally well in mutant and wild type, *i.e.* the yield of the 147aa protein should approximately be the same in GM10 and GM241 when

challenged with AGG-AGG. The results of this test, presented in Fig. 4 (lanes 1 and 2 *versus* lane 3), show indeed that tandem AGG codons are decoded with comparable efficiency in mutant and wild type. In other words, reading of AGG seems not dependent on an intact *argU* gene product. These results strongly suggest that *argU* reads AGA but not AGG.

Two unexpected observations in Fig. 4 need further comment. First, in contrast to what was found for M5219, ribosomes in GM241 do not shift on AGA-AGA. A plausible explanation is that there is less frameshift messenger in GM241 than in M5219 resulting in a more balanced ratio of tRNA and AGA codons. In contrast to M5219, GM241 lacks the λ antitermination factor *N*, and transcription from the *p_L* promoter is, therefore, more

Fig. 4. Frameshifting over tandem AGG or AGA codons in GM10 (*argU*^{ts}) and GM241 (wild type) at 42°C as revealed by immunoblots. Induction is as in Fig. 2. Lane 1, AGG-AGG; GM10. Lane 2, as lane 1 but half-size sample. Lane 3, AGG-AGG; GM241. Lane 4, AGA-AGA; GM10. Lane 5 as lane 4 but half-size sample. Lane 6, AGA-AGA; GM241. All clones carry a compatible plasmid encoding the thermosensitive λ repressor clts857. The band marked x is an *E. coli* protein crossreacting with our serum. It can be used to compare the amounts of cell extract applied to the gel. For instance lane 5, but not lane 4 carries about an equal quantity of cell mass as lane 6. The cell mass applied in lane 3 is estimated to be intermediate to that present in lanes 1 and 2.

prone to premature termination. It is known that the *N*-derivative of M5219 shows 2 to 3 fold reduced expression from the *p_L* promoter at the induction times used by us (9). In accordance, we have noted that shifts over AGG-AGG are also reduced in GM241 as compared to M5219.

The second unexpected finding of Fig. 4 is that when GM241 and GM10 are challenged with AGG-AGG at the restrictive temperature, there is, in GM10, less instead of the expected equal level of phase shifting. It seems as if the AGG decoding tRNA functions better in the absence of an active *argU* tRNA. This would be so if somewhere in their reaction cycle both tRNAs compete for the same factor (e.g. arginyl-tRNA synthetase). Although there may be other explanations for the somewhat unexpected findings they seem unlikely to affect the conclusions reached. The fact that strain GM241 shifts at AGG-AGG but not at AGA-AGA argues in itself for the involvement of different sets of tRNA at the two different codons.

Overproduction of *argU* tRNA leads to loss of specificity

tRNAs that read NNA but not NNG and have a U in the wobble position of their anticodon derive their specificity from modification of this U (13,15,16,22). When such tRNAs are overproduced, the modification system is not always able to keep pace with the supply which may result in undermodified tRNA which could lead to loss of specificity (14,17-20).

We have cloned the wild type *argU* gene under control of its own promoter on pACYC184 in strain M5219. A similar

construct using pBR322 in stead of pACYC184 as vector was previously shown to increase the *argU* tRNA concentration by a factor of 50-100 (7). A recent analysis showed an undermodified fraction in overproduced *argU* tRNA. This fraction lacked the t⁶A modification at position 37 (S. Yokoyama, personal communication).

When plasmid borne *argU* is introduced in M5219, frameshifts over tandem AGA codons disappear as expected (Fig. 2, lane 14). However, errors arising at AGG-AGG are almost fully suppressed also, suggesting that specificity is indeed lost upon overproduction (lane 12). It is not clear whether this is due to the hypomodification. It is simply possible that the high concentration of *argU* tRNA causes misreading of AGG codons which are normally not recognized by this tRNA, thereby suppressing the frameshift. This explanation is supported by the finding that the introduction of an excess of one tRNA species dramatically increases decoding errors (23).

The defect in strain GM10 is caused by the inability of the *argU*^{ts} gene product to sustain an adequate level of protein synthesis

Although this paper provides substantial evidence that the *argU* (*dnaY*) gene product can function as a tRNA in protein synthesis it still seemed conceivable that it fulfills some other function needed for DNA replication. There is one strong argument that this is probably not the case. When the T4 gene is introduced into GM10 growth at 42°C is restored (Fig. 3e). Since this T4 tRNA is different in sequence and in details of the secondary structure it is unlikely that it can substitute for *argU* tRNA in a function other than decoding mRNA. We propose, therefore, that the defect of GM10 is a deficiency to produce a (class of) protein(s) at the required rate (see discussion).

DISCUSSION

Differential frameshift suppression reveals the codon specificity of tRNA^{Arg}_{T4} and *argU* tRNA

In this paper we show that +1 ribosomal frameshifts arising during the translation of tandem AGG or AGA codons can be suppressed by introducing more tRNA^{Arg}. This result identifies tRNA shortage as the cause of the translation error. Since frameshifting at the different templates responded differently to an exogenous supply of tRNA, this offered the possibility to determine the codon specificity of the tRNA used. For instance, tRNA^{Arg}_{T4} prevents shifts over AGA but not over AGG. We therefore conclude that this tRNA only recognizes AGA. Using the same approach, *E. coli* tRNA^{Arg}_{UCU} was found to read AGA and not AGG. This result was in fact obtained using *argU*^{ts} tRNA. Our inability to obtain the same specificity for overexpressed wild type *argU* tRNA could be caused by hypomodification, but could also be the result of illegal reading of AGG due to an excessive supply of the tRNA^{Arg}. Our codon assignment is consistent with the existence of two arginine decoding tRNAs in *E. coli*. One with anticodon 5' CCU and presumably reading AGG, the other the *argU* gene product, carrying a 5' substituted 2-thiouridine in the wobble position (8). Our proposal is also consistent with the observation that frameshifts do not take place over the sequences AGG-AGA and AGA-AGG, a finding that indicates that the two codons are read by two different RNAs. In addition, a mutant (GM10) containing *argU*^{ts} tRNA barely reads through AGA at 42°C whereas it does read through AGG.

Our attempts to relax the specificity of tRNA^{Arg}_{T4} have failed. When the gene was cloned behind the *lac* promoter only a marginal decrease in frameshifting over the sequence AGG-AGG was noticed (not shown). We have also mutated the DNA sequence of its anticodon from 5' TCT to 5' CCT. Although this change destroyed both the property to suppress AGA-AGA induced frameshifts and to sustain growth of strain GM10 at 42°C (Fig. 3f), it did not lead to frameshift suppression at AGG codons. It is possible that the substitution interferes with tRNA maturation or aminoacylation. A UGA suppressor tRNA derived from tRNA^{Arg}_{T4} was also shown to be inefficient (21).

Mixed codon families ending in a purine use as a rule two tRNAs

In the mixed codon families (where NNPU and NNPy have different meanings) the triplets NNA and NNG could basically be read by a single tRNA carrying a U in the wobble position of the anticodon. This decoding pattern is indeed found in mitochondria but does not hold for eubacteria or for the eucaryotic cytoplasm. In yeast, for instance, the mixed codon families CAPu, AAPu and AGPu are read by two tRNAs and the same is true in *E. coli* for the codons CAPu, UUPu and AGPu. Recent experiments suggest, however, that in *E. coli* AAPu and GAPu are read by a single tRNA (30). In yeast, there is no example yet where a single tRNA reads both codons in the NNPU mixed family. The reason for this redundancy, if any, is unknown.

GM10 is probably only defect at the translational level

GM10 was isolated as a ts mutant defective in DNA replication. It has been reported that the *dnaY* gene encodes a tRNA^{Arg} gene (6) and this is fully confirmed by the present report. There has always remained a possibility that the gene product would have a function outside protein synthesis, but our finding that GM10 can be complemented for growth by tRNA^{Arg}_{T4} renders this option unlikely. Perhaps, GM10 is unable to produce certain proteins that function in DNA replication at a sufficient rate (see below).

Usage of AGG-AGG and AGA-AGA in *E. coli* coding regions

In *E. coli* CGU and CGC are used with high preference but AGA and AGG are avoided even in low expressed genes. The discrimination against AGA or AGG tandems is almost absolute. A survey of all sequenced *E. coli* and phage coding regions (GenBank tape release 56) shows that AGGAGG and AGAAGA occur 21 and 246 times, respectively, but only five times is the reading frame Arg-Arg. AGG-AGG is found in the *hlyA* gene (24), and AGA-AGA in *recF* (25), *trmD* (26) and two times in the *old* gene of phage P2 (27). As far as we know, no specific regulatory function has yet been assigned to their presence.

Translation at hungry AGA codons

We have challenged strain GM10 with AGA-AGA at 42°C. Under these circumstances the ribosome barely passes through the AGA codons (Fig. 4, lane 5). At the same time about 50% +1 phase shifting occurs. The extremely low yields of correct and frameshift protein indicate that the ribosome must already be stalled at the first AGA codon. Strongly reduced translation rates of AGA containing messengers have also been found by others working with *argU* mutants. The *old* gene of phage P2 carries 2 AGG codons and 6 AGA codons, two of which occur in tandem. The presence of this gene in an *E. coli* *pin* 3 strain (the *pin* 3 mutation also maps in the *argU* gene) results in an *old*⁻ phenotype. The *old* phenotype is restored by transformation

with the wild type *argU* gene. Apparently, the Old protein cannot be made in sufficient amounts in the *pin* 3 mutant (27). Similarly, it has been found that in strain GM10 the cloned cI gene of phage λ which contains 5 AGA codons, is very poorly expressed at 42°C (28).

We want to stress that the presence of single AGA and AGG codons probably does not seriously affect product formation in wild type strains. The natural concentration of the corresponding tRNAs, though low, is sufficient. For example, the rat interferon-α₁ gene contains nine AGA and three AGG codons scattered through the sequence. Overproducing *argU* tRNA in an *E. coli* strain transformed with the rat interferon gene under *p*_L control, increased interferon production less than twofold (29).

ACKNOWLEDGEMENTS

We are very grateful to Dr. McClain for the tRNA^{Arg} clone of bacteriophage T4 and to Dr. Fiers and coworkers for the MS2 cDNA and the expression vectors. Drs. Grosjean, Lagerkvist, Björk and Yokoyama are thanked for valuable discussions and comments. Drs. Yokoyama and Farabaugh have contributed to this paper by making their unpublished results available to us. This research was supported by Stichting Technische Wetenschappen Grant LBI37.0496 to R. A. S. Work at the University of Texas was supported by Public Health Service grant GM 34471 from the National Institute of Health, by Welch Foundation grant F 949, and, in part, by the American Cancer Society grant MV-429.

REFERENCES

- Spanjaard, R.A. and van Duin, J. (1988) Proc. Natl. Acad. Sci. USA 85:7967-7971.
- Varenne, S. and Lazdunski, C. (1985) J. Theor. Biol. 120:99-110.
- Clare, J.J., Belcourt, M. and Farabaugh, P.J. (1988) Proc. Natl. Acad. Sci. USA 85:616-6820.
- Mazzara, G.P., Seidman, J.G., McClain, W.H., Yesian, H., Abelson, J. and Guthrie, C. (1977) J. Biol. Chem. 252:8245-8253.
- Lindsey, D.F., Mullin, D.A. and Walker, J.R. (1989) J. Bact. 171:6197-6205.
- Henson, J.M., Chu, H., Irwin, C.A. and Walker, J.R. (1979) Genetics 92:1041-1059.
- Garcia, G.M., Mar, P.K., Mullin, D.A., Walker, J.R. and Prather, N.E. (1986) Cell 45:453-459.
- Kiesewetter, S., Fischer, W. and Sprinzl, M. (1987) Nucl. Acids Res. 15:3184.
- Remaut, E., Stanssens, P. and Fiers, W. (1981) Gene 15:81-93.
- Mazzara, G.P., Plunkett III, G. and McClain, W.H. (1981) Proc. Natl. Acad. Sci. USA 78:889-892.
- Chang, A.C.Y. and Cohen, S.N. (1978) J. Bacteriol. 134:1141-1156.
- Remaut, E., Tsao, H. and Fiers, W. (1983) Gene 22:103-113.
- Sekiya, T., Takeishi, K. and Ukita, T. (1969) Biochim. Biophys. Acta 182:411-426.
- Wilson, R.K. and Roe, B.A. (1989) Proc. Natl. Acad. Sci. USA 86:409-413.
- Björk, G.R., Ericson, J.U., Gustafsson, C.E.D., Hagervall, T.G., Jönsson, J.H. and Wikström, P.M. (1987) Ann. Rev. Biochem. 56:263-287.
- Muramatsu, T., Nishikawa, K., Nemoto, F., Kuchino, Y., Nishimura, S., Miyazawa, T. and Yokoyama, S. (1988) Nature (London) 336:179-181.
- Bienz, M. and Kubli, E. (1981) Nature (London) 294:188-190.
- Beier, H., Barciszewska, M. and Sickinger, H.D. (1984) EMBO J. 3:1091-1096.
- Yanofsky, C. and Soll, L. (1977) J. Mol. Biol. 113:663-667.
- Zurawsky, G., Elsevier, D., Stauffer, G.V. and Yanofsky, C. (1978) Proc. Natl. Acad. Sci. USA 75:5988-5992.
- Kao, S. and McClain, W.H. (1977) J. Biol. Chem. 252:8254-8257.
- Buckingham, R.H. and Grosjean, H. (1986) Accuracy in Molecular Processes: its control and relevance to living systems, eds. Galas D.J., Kirkwood T.B.L., and Rosenberger R.F. pp. 83-126. Chapman and Hall, London.

23. Atkins, J.F., Gesteland, R.F., Reid, B.R. and Anderson, C.W. (1979) *Cell* 18:1119–1131.
24. Felmlee, T., Pellet, S. and Welch, R.A. (1985) *J. Bacteriol.* 163:94–105.
25. Blonar, M.A., Sandler, S.J., Armengod, M.-E., Ream, L.W. and Clark, A.J. (1984) *Proc. Natl. Acad. Sci. USA* 81:4622–4626.
26. Byström, A.S., Hjalmarsson, K.J., Wikström, P.M. and Björk, G.R. (1983) *EMBO J.* 2:899–905.
27. Haggard-Ljungquist, E., Barreiro, V., Calendar, R., Kurnit, D.M. and Cheng, H. (1990), *Gene*, in press.
28. Chen, K.S., Peters, T.C. and Walker J.R. (1990) *J. Bact.* 172:2504–2510.
29. Spanjaard, R.A. (1989) Ph.D. Thesis, University of Leiden.
30. Komine, Y. (1990) *J. Mol. Biol.* 221, in press.