Ingebritsen, T. S., Foulkes, J. G., & Cohen, P. (1983) *Eur. J. Biochem. 132*, 263-274.
Jacobs, S., Kull, F. C., Earp, H. S., Svoboda, M. E., Van Wyk, J. J., & Cuatrecasas, P. (1983) *J. Biol. Chem. 258*, 9581-9584.
Jessus, C., Goris, J., Cayla, X., Hermann, J., Hendrix, P., Ozon, R., & Merlevede, W. (1989) *Eur. J. Biochem. 180*, 15-22.
Jurgensen, S., Shacter-Noiman, E., Huang, C. Y., Chock, P. B., Vandenheede, J. R., & Merlevede, W. (1983) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 42*, 2026.
Jurgensen, S., Shacter, E., Huang, C. Y., Chock, P. B., Yang, S.-D., Vandenheede, J. R., & Merlevede, W. (1984) *J. Biol. Chem. 259*, 5864-5870.
Kasuga, M., Zick, Y., Blithe, D. L., Karlson, F. A., Haring, K. U., & Kahn, C. R. (1982) *J. Biol. Chem. 257*, 9891-9894.
Lau, K.-H. W., Farley, J. R., & Baylink, D. J. (1989) *Biochem. J. 257*, 23-36.
Li, H.-C. (1982) *Curr. Top. Cell. Regul. 21*, 129-174.
Martensen, T. S. (1984) *Methods Enzymol. 107*, 3-23.
Merlevede, W., Vandenheede, J. R., Goris, J., & Yang, S.-D. (1984) *Curr. Top. Cell. Regul. 23*, 177-215.
Okada, M., Owada, K., & Nakagawa, H. (1986) *Biochem. J. 239*, 155-162.
Petruzzelli, L. M., Ganguly, S., Smith, C. J., Cobb, M. H., Rubin, C. S., & Rosen, O. M. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6792-6796.
Ramachandran, C., Goris, J., Waelkens, E., Merlevede, W., & Walsh, D. A. (1987) *J. Biol. Chem. 262*, 3210-3218.
Resink, T. J., Hemmings, B. A., Tung, H.-Y. L., & Cohen, P. (1983) *Eur. J. Biochem. 133*, 455-461.
Roesler, W. J., & Khandelwal, R. L. (1986) *Arch. Biochem. Biophys. 244*, 397-407.
Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T., & Stanley, E. R. (1985) *Cell 41*, 665-676.
Shriner, C. L., & Brautigan, D. L. (1984) *J. Biol. Chem. 259*, 11383-11390.
Sols, A., & Marco, R. (1970) *Curr. Top. Cell. Regul. 2*, 227-273.
Stalmans, W., Bollen, M., & Mvumbi, L. (1987) *Diabetes Metab. Rev. 3*, 127-161.
Stern, D. F., Hefferman, P. A., & Weinberg, R. A. (1986) *Mol. Cell. Biol. 6*, 1729-1740.
Tonks, N. K., Diltz, D. C., & Fischer, E. H. (1988a) *J. Biol. Chem. 263*, 6722-6730.
Tonks, N. K., Diltz, D. C., & Fischer, E. H. (1988b) *J. Biol. Chem. 263*, 6731-6737.
Tonks, N. K., Charbonneau, H., Diltz, C. D., Fischer, E. H., & Walsh, K. A. (1988c) *Biochemistry 27*, 8695-8701.
Tung, H. Y. L., & Reed, L. J. (1987) *Anal. Biochem. 161*, 412-419.
Tung, H. Y. L., Alemany, S., & Cohen, P. (1985) *Eur. J. Biochem. 148*, 253-263.
Ushiro, H., & Cohen, S. (1980) *J. Biol. Chem. 255*, 8363-8365.
Waelkens, E., Goris, J., & Merlevede, W. (1987a) *J. Biol. Chem. 262*, 1049-1059.
Waelkens, E., Goris, J., & Merlevede, W. (1987b) *Adv. Enzyme Regul. 26*, 241-270.
Wuytack, F., Raeymaekers, L., Verbist, J., De Smedt, H., & Casteels, R. (1984) *Biochem. J. 224*, 445-451.
Yang, S.-D., Vandenheede, J. R., Goris, J., & Merlevede, W. (1980) *J. Biol. Chem. 255*, 11759-11767.

Effect of Nucleotide Substitution on the Peptidyltransferase Activity of 2′(3′)-*O*-(Aminoacyl) Oligonucleotides†

Masakatsu Tezuka‡ and Stanislav Chládek*
Michigan Cancer Foundation, Detroit, Michigan 48201
Received June 26, 1989; Revised Manuscript Received September 8, 1989

ABSTRACT: Seven 2′(3′)-*O*-(aminoacyl) trinucleotides with structures derived from the 3′-terminal C-C-A sequence of aa-tRNA via nucleotide substitutions were investigated as acceptor substrates in the peptidyltransferase reaction and as inhibitors of substrate binding to the peptidyltransferase A site. It was found that all tested compounds were active in both systems, although substitution in the first and second nucleotide position results in some decrease of acceptor activity. Remarkably, replacement of natural cytidylic acid residues in C-C-A-Phe with guanylic acid moieties resulted only in a small decrease of acceptor or binding activity. The results indicate that the acceptor sequence of aa-tRNA is not probably engaged in base pairing with a sequence of 23S RNA during its interaction with the peptidyltransferase A site.

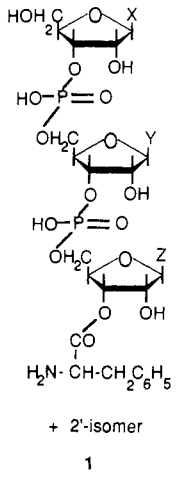
The common 3′-terminal C-C-A[1] sequence of aa-tRNA[1] and peptidyl-tRNA specifically interacts with acceptor and donor sites of peptidyltransferase during the peptide chain elongation process on the ribosome. Simple analogues of the 3′-terminus of aa- and peptidyl-tRNA, such as 2′(3′)-*O*-(aminoacyl) or peptidyl nucleosides and oligonucleotides, are capable of interacting with peptidyltransferase A and P sites and participate

†This investigation was supported in part by U.S. Public Health Service Grant GM 19111 from the National Institute of General Medical Sciences, an Institutional Grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit, and a grant from the Ministry of Education, Science and Culture, Japan. This is paper 47 in the series "Aminoacyl Derivatives of Nucleosides, Nucleotides and Polynucleotides". For paper 46, see Hagen and Chládek (1989).
‡Present address: Department of Radiobiochemistry, School of Pharmaceutical Science, University of Shizuoka, Shizuoka-Shi, Japan.

[1] Abbreviations: aa-tRNA, aminoacyl transfer ribonucleic acid; A-Gly, 2′(3′)-*O*-glycyladenosine (similar abbreviations are used for oligonucleotide derivatives); A_{260} unit, quantity of material contained in 1 mL of solution that has an absorbance of 1.00 at 260 nm when measured in a 1-cm path-length cell; poly(U), poly(uridylic acid); $tRNA^{Phe}_{yeast}$, transfer ribonucleic acid from yeast, specific for phenylalanine. Standard abbreviations for nucleosides and oligonucleotides are according to CBN recommendations (Sober, 1970).

0006-2960/90/0429-667$02.50/0

Chart I



1a; X = Y = Cyt; Z = Ade
1b; X = Ura; Y = Cyt; Z = Ade
1c; X = Cyt; Y = Ura; Z = Ade
1d; X = Y = Cyt; Z = Ura
1e; X = Cyt; Y = Z = Ade
1f; X = Cyt; Y = Gua; Z = Ade
1g; X = Gua; Y = Cyt; Z = Ade
1h; X = Y = Gua; Z = Ade

+ 2'-isomer

1

in peptide bond formation as acceptors and donors of the peptidyl residue, thereby mimicking the presence of charged tRNAs. Thus, low molecular weight models such as 2'-(3')-*O*-(aminoacyl) oligoribonucleotides become important tools for an investigation of substrate specificity of peptidyltransferase (Chládek & Sprinzl, 1985). The structure of chemically synthesized 2'(3')-*O*-(aminoacyl)oligonucleotides may be altered relatively easily, and the effect of such modification on the peptidyltransferase activity can readily be evaluated.

It is established that the peptidyltransferase activity increases in the order A–Gly < C-A-Gly < C-C-A-Gly, whereas activities of U-C-C-A-Gly and C-U-C-C-A-Gly are roughly similar to that of C-C-A-Gly (Bhuta et al., 1982; Scalfi-Happ et al., 1987). Similarly, binding activities to the peptidyltransferase A site are on the order of C-A-Phe < C-C-A-Phe = C-A-C-C-A-Phe (Bhuta et al., 1982). This seems to indicate an important and specific role for both cytidine residues in a molecule such as C-C-A-Gly (or by implications in positions 75 and 74 of tRNA) in the peptidyltransferase reaction. On the other hand, it appears that the bases adjoining the C-C-A sequence of tRNA (bases 72 and 73) probably do not bind directly to the peptidyltransferase A site, although they contribute to the formation of the orderly structure of the tRNA 3'-terminus.

In order to study the involvement of aglycons of the aa-tRNA 3'-terminus in the peptidyltransferase reaction in greater detail, we have synthesized eight 2'(3')-*O*-L-phenylalanyl trinucleotides with different base sequences (see Chart I). We report herein the effect of "base mutations" of the natural C-C-A-Phe sequence on the acceptor activity and binding to the peptidyltransferase A site of these model compounds. It was found that replacement of "natural" cytosine with the guanine moiety has a surprisingly small effect on the biological activity of 2'(3')-*O*-L-phenylalanyl trinucleotides. Accordingly, it would appear that the peptidyltransferase A site probably does not specifically bind the cytosine residues of the acceptor molecules.

Materials and Methods

2'(3')-*O*-(Aminoacyl) oligoribonucleotides were prepared by chemical synthesis as described previously (Happ et al., 1987; Hagen et al., 1988; Hagen & Chládek, 1989). All synthetic compounds were purified by chromatography on a C_{18} SPE Baker column, which separates the aminoacylated products from small amounts of deacylated materials formed during deblocking. The pentanucleotide fragment C-A-C-C-A-[^{3}H]Phe was prepared from [^{3}H]Phe-tRNA^{Phe}yeast (sp act. of [^{3}H]Phe = 500 mCi/mmol) by RNase T_1 cleavage as described by Pestka (1971) and Bhuta et al. (1982).

General methods, sources of reagents, and biochemicals are the same as described in previous papers of this series (Chládek et al., 1974; Bhuta et al., 1982; Scalfi-Happ et al., 1987). Preparations of 70S ribosomes and Ac[^{14}C]Phe-tRNA (0.83 nmol of phenylalanine/mg of tRNA; obtained from unfractionated *Escherichia coli* MRE 600 tRNA) have also been reported previously (Chládek et al., 1974; Bhuta et al., 1982).

Assay of the Peptidyltransferase Activity (System A). Peptidyltransferase activity was measured essentially as described previously (Chládek et al., 1974; Bhuta et al., 1982). Each reaction mixture contained in 0.10 mL 50 mM Tris-HCl (pH 7.4), 100 mM NH_4Cl, 100 mM $MgCl_2$, 4.0 A_{260} units of NH_4Cl-washed 70S ribosomes, 10 μg of poly(U), Ac-[^{14}C]Phe-tRNA (0.2 A_{260} unit; 13000 cpm/A_{260} unit; ca. 0.14 μM), and the acceptors in the given concentrations. The reaction mixtures were incubated at 37 °C for 30 min and processed by precipitation with 2.5% trichloroacetic acid. The percent release is calculated from the amount of Ac[^{14}C]-Phe-tRNA retained on filters after incubation in the presence and absence of acceptor substrates. In the absence of acceptor, the total radioactivity retained on the filter was 2200 cpm. The peptidyltransferase reaction products were identified after hydrolysis with 6 N KOH by paper electrophoresis as described by Chlαcuadek et al. (1974) as Ac[^{14}C]Phe-Phe by a comparison with authentic samples (results not shown).

Assay of Binding of C-A-C-C-A-[^{3}H]Phe to 70S Ribosomes (System B). The inhibition of the C-A-C-C-A-[^{3}H]Phe binding to 70S ribosomes by 2'(3')-*O*-(aminoacyl) oligonucleotides was carried out essentially as described previously (Bhuta et al., 1982). In a final volume of 0.10 mL, the following additions were made: 50 mM Tris-HCl (pH 7.4; 24 °C), 50 mM NH_4Cl, 400 mM KCl, 40 mM $MgCl_2$, 4.0 units of NH_4Cl-washed 70S ribosomes, 10 μg of poly(U), 50 μg of tRNA^{Phe}yeast, 20 pmol of C-A-C-C-A-[^{3}H]Phe (5500 cpm), and the test compounds at vaious concentrations. The reactions were stopped by addition of cold buffer (3 mL containing 50 mM Tris-HCl, 50 mM NH_4Cl, 400 mM KCl, and 40 mM $MgCl_2$, pH 7.4). The amount of C-A-C-C-A-[^{3}H]Phe bound to ribosomes was determined by Millipore filtration as described by Pestka (1971). The percent of inhibition was calculated from the ratio of C-A-C-C-A-[^{3}H]Phe bound to the ribosome in absence of inhibitors (3.54 pmol) and in presence of inhibitors.

Results

The activity of synthetic 2'(3')-*O*-L-phenylalanyl trinucleotides (**1**) was examined in two ribosomal systems. In system A, the transfer of the AcPhe residue from donor Ac-Phe-tRNA to an acceptor was measured, whereas inhibition of C-A-C-C-A-Phe binding to the peptidyltransferase A site was examined in system B. In general, system A would be expected to be more specific, since some compounds might inhibit binding to the peptidyltransferase A site without actually being acceptors (Ringer et al., 1975). As it may be seen from Table I and Figures 1 and 2, the results from both systems are quite similar; all oligonucleotides (**1**) are observed to bind to the A site, since they compete with C-A-C-C-A-Phe for binding to the peptidyltransferase A site [in the presence of an excess of deacylated tRNA^{Phe}yeast and poly(U)]. Moreover, all tested compounds are functional analogues of the aa-tRNA as well, since they can react as acceptors of the AcPhe residue from the AcPhe-tRNA donor in system A. It may be seen from Table I and Figures 1 and 2 that variation of the nucleotide sequence in compounds **1** influences, to some

Table I: K^{app}_M and K^{app}_i of Various Substrates and Inhibitors in the Peptidyltransferase Reaction[a]

compd	K^{app}_M (M)[b]	K^{app}_i (M)[c]
C-C-A-Phe (**1a**)	3.0×10^{-7}	3.6×10^{-7}
U-C-A-Phe (**1b**)	3.5×10^{-7}	6.0×10^{-7}
C-U-A-Phe (**1c**)	4.2×10^{-7}	7.0×10^{-6}
C-C-U-Phe (**1d**)	7.2×10^{-6}	$\sim 10^{-4}$
C-A-A-Phe (**1e**)	4.6×10^{-6}	4.0×10^{-6}
C-G-A-Phe (**1f**)	9.5×10^{-7}	9.0×10^{-7}
G-C-A-Phe (**1g**)	1.1×10^{-7}	9.5×10^{-7}
G-G-A-Phe (**1h**)	7.5×10^{-7}	2.0×10^{-6}

[a] K^{app}_M and K^{app}_i are defined as the concentration of substrate at half of the maximum activity (Glick et al., 1979). [b] System A. [c] System B.

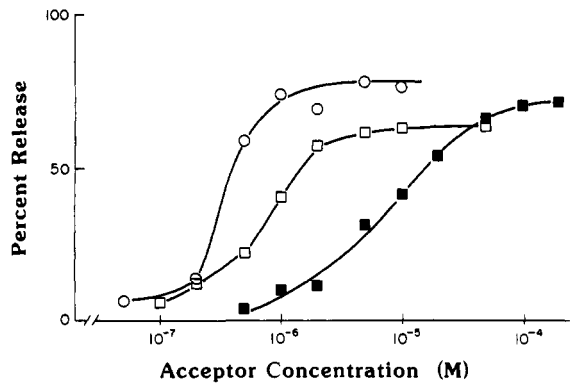


FIGURE 1: 2′(3′)-O-L-Phenylalanyl trinucleotide dependent release of the Ac[^{14}C]Phe residue from the Ac[^{14}C]Phe-tRNA–70S ribosome–poly(U) complex (system A). Percent release represents the acceptor-dependent decrease in CCl_3COOH-precipitated counts trapped by Millipore membranes. For other details, see Materials and Methods, Chládek et al. (1974), and Bhuta et al. (1982). (○) C-C-A-Phe (**1a**); (■) C-C-U-Phe (**1d**); (□) G-G-A-Phe (**1h**).

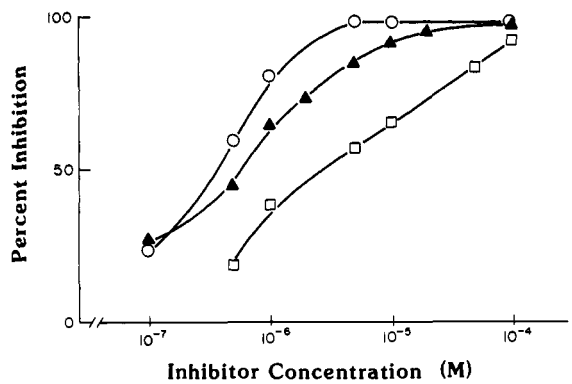


FIGURE 2: Inhibition of C-A-C-C-A-[^{3}H]Phe binding to ribosomes by 2′(3′)-O-L-phenylalanyl trinucleotides (system B). Percent inhibition is the difference in ribosome-C-A-C-C-A-[^{3}H]Phe complex retained on the Millipore membrane in the absence and in the presence of an inhibitor, relative to the amount bound in the absence of inhibitor. Zero percent inhibition equals 3.54 pmol of C-A-C-A-[^{3}H]Phe bound to ribosome. (○) C-C-A-Phe (**1a**); (▲) U-C-A-Phe (**1b**); (□) G-G-A-Phe (**1h**).

degree, the inhibitory and acceptor properties of **1** but does not render any of the investigated 2′(3′)-O-L-phenylalanyl trinucleotides inactive in either assay system.

Evidently, the joining of a third nucleotide residue, especially Cp, to an inactive 2′(3′)-O-L-phenylalanyl dinucleotide, e.g., C-U-Phe (Ringer & Chládek, 1974), renders it reasonably active in system A (e.g., C-C-U-Phe). It may also be seen that replacement of the Cp moiety in the second position of "natural" C-C-A-Phe with other nucleotides has a more profound negative effect than that of an analogous replacement in the third position. Thus, U-C-A-Phe is more active than C-U-A-Phe, or analogously, G-C-A-Phe is a better acceptor than C-G-A-Phe or G-G-A-Phe (Table I and Figure 1). It has been noted previously that joining a Cp residue to 2′(3′)-O-(aminoacyl)adenosines significantly increases the latter's acceptor activities. Moreover, this effect cannot be achieved by joining other nucleotides (Rychlík et al., 1967; Eckermann et al., 1974; Quiggle & Chládek, 1980). This indicates that the ribosomal nucleotide loci for binding the second and third nucleotides at the acceptor site of peptidyltransferase do not contribute equally to the acceptor activity of substrates.

Nevertheless, there does not appear to be a strict requirement for a C-C-A sequence in 2′(3′)-O-(aminoacyl) oligonucleotides either for binding to the peptidyltransferase A site or even for acceptor activity. These findings argue against the proposal that Watson–Crick pairing of the 3′-terminal nucleotides in aa-tRNA with a complementary sequence of 23S RNA is required for function of ribosomes.

Discussion

The interaction of the 3′-terminal sequence of both aa- and peptidyl-tRNAs with ribosomal A and P sites is an integral part of the ribosomal mechanism of synthesis of peptide bonds. It is well-known that only 3′-terminal C-C-A sequences of both tRNAs are sufficient for binding to peptidyltransferase; therefore, the peptidyltransferase reaction can be carried out between two fragments (Chládek & Sprinzl, 1985).

It has become increasingly apparent that rRNAs play a crucial role in the function of ribosome and it is now believed that 23S RNA is involved, perhaps fundamentally, in peptidyltransferase activity (Noller, 1984; Moore, 1988). One of the plausible functions of 23S RNA in peptidyltransferase activity could be to provide binding sites for the 3′-termini of both aa- and peptidyl-tRNA (Peattie & Herr, 1981; Noller et al., 1981; Chládek & Sprinzl, 1985). Indeed, peptidyltransferase A and P sites have been located in the central loop of domain V of 23S RNA (Hall et al., 1988; Steiner et al., 1988). This region of 23S RNA also encompasses several nucleotides that are sites of resistance of antibiotics inhibiting the peptidyltransferase reaction (Moazed & Noller, 1987; Vester & Garrett, 1988). It has been proposed that binding of the 3′-termini of both aa- and peptidyl-tRNAs involves Watson–Crick pairing which is stabilized via coaxial stacking interactions. This model requires the existence in 23S RNA of single-stranded and genetically conserved G-G (A site) and U-G-G (P site) sequences or possibly G-G-U and U-G-G-U sequences, respectively (Noller et al., 1981; Chládek & Sprinzl, 1985). In fact, no other single-stranded genetically preserved G-G sequence can be found in the 23S RNA molecule other than the G-G^{2608} sequence located in the central loop of domain V (Noller et al., 1981).

It is already obvious that the 3′-terminal adenosine of aa-tRNA cannot be involved in Watson–Crick pairing with an uridine residue of 23S RNA, since many 2′(3′)-O-(aminoacyl) nucleoside models that cannot form Watson–Crick pairs are fully active as acceptors in the peptidyltransferase reaction (Chládek & Sprinzl, 1985). These analogues include, e.g., puromycin, whose acceptor activity is indistinguishable from that of 2′(3′)-O-L-phenylalanyladenosine (Rychlík et al., 1969).

The results reported in this paper argue against a role for Watson–Crick base pairing between the universal C-C sequence of the 3′-terminus of aa-tRNA with a G-G sequence of 23S RNA. If G-C-A-Phe or C-G-A-Phe were to pair with, e.g., a G-G sequence of 23S RNA, it would be necessary to postulate the existence of highly unusual G-G pairs (Topal & Fresco, 1976). However, these results should not be construed to deny a role of 23S RNA in the formation of the peptidyltransferase acceptor site. Rather, it seems likely that a

C-C-A binding pocket could be constructed out of 23S RNA tertiary structure *without* invoking Watson–Crick bonding.[2] Indeed, it is known that guanosine nucleosides and nucleotides bind into a pocket in self-splicing introns (Bass & Cech, 1984). It should be also noted that our results do confirm some sequence specificity for the C-C-A terminus–ribosome interaction, namely, at the second nucleotide position (equivalent to position 75 of tRNA). A large decrease of activity of C-C-U-Phe relative to C-C-A-Phe also indicates that the uridine moiety is only poorly accommodated by the adenosine[76] binding locus at the peptidyltransferase site [see also Rychlík et al. (1969)].

It is also probable that base stacking, which certainly plays an important role in recognition of the aa-tRNA 3′-terminus by ribosomal sites or by other enzymes, is not the only feature of the recognition mechanism: C-A-A-Phe has low activity in both testing systems, even though its capacity for base stacking should be better than that of, e.g., "natural" C-C-A-Phe (Warshaw & Tinoco, 1966).

It would now appear important to perform similar experiments with oligonucleotide models mimicking peptidyl-tRNA and modified tRNA species themselves, and in fact, these experiments are in progress in our laboratory.

ACKNOWLEDGMENTS

We are indebted to Drs. J. K. Christman and J. P. Horwitz (Michigan Cancer Foundation) for a critical reading of the manuscript.

REFERENCES

Bass, B. L., & Cech, T. R. (1984) *Nature 308*, 820–826.
Bhuta, P., Kumar, G., & Chládek, S. (1982) *Biochim. Biophys. Acta 696*, 208–211.
Chládek, S., & Sprinzl, M. (1985) *Angew. Chem., Int. Ed. Engl. 24*, 371–391.
Chládek, S., Ringer, D., & Quiggle, K. (1974) *Biochemistry 13*, 2727–2735.
Eckermann, D. J., Greenwell, P., & Symons, R. H. (1974) *Eur. J. Biochem. 41*, 547–554.
Glick, B. R., Chládek, S., & Ganoza, M. C. (1979) *Eur. J. Biochem. 97*, 23–28.
Hagen, M. D., & Chládek, S. (1989) *J. Org. Chem. 54*, 3189–3195.
Hagen, M. D., Scalfi-Happ, C., Happ, E., & Chládek, S. (1988) *J. Org. Chem. 53*, 5040–5045.
Hall, C. C., Johnson, D., & Cooperman, B. S. (1988) *Biochemistry 27*, 3983–3990.
Happ, E., Scalfi-Happ, C., & Chládek, S. (1987) *J. Org. Chem. 52*, 5387–5391.
Moazed, D., & Noller, H. F. (1987) *Biochimie 69*, 879–884.
Moore, P. B. (1988) *Nature 331*, 223–227.
Noller, H. F. (1984) *Annu. Rev. Biochem. 53*, 119–162.
Noller, H. F., Kop, J.-A., Wheaton, V., Brossius, J., Gutell, R. R., Kopylov, A. M., Dohme, F., Herr, W., Stahl, D. A., Gupta, R., & Woese, C. R. (1981) *Nucleic Acids Res. 9*, 6167–6189.
Peattie, D. A., & Herr, W. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2273–2277.
Pestka, S. (1971) *Methods Enzymol. 20*, 502–508.
Quiggle, K., & Chládek, S. (1980) *FEBS Lett. 118*, 172–175.
Ringer, D., & Chládek, S. (1974) *FEBS Lett. 39*, 75–78.
Ringer, D., Quiggle, K., & Chládek, S. (1975) *Biochemistry 14*, 514–520.
Rychlík, I., Chládek, S., & Žemlička, J. (1967) *Biochim. Biophys. Acta 138*, 640–642.
Rychlík, I., Cerná, J., Chládek, S., Žemlička, J., & Haladová, Z. (1969) *J. Mol. Biol. 43*, 13–24.
Scalfi-Happ, C., Happ, E., Ghag, S., & Chládek, S. (1987) *Biochemistry 26*, 4682–4688.
Sober, H. A. (1970) *Handbook of Biochemistry*, CRC Press, Cleveland, OH.
Steiner, G., Kuechler, E., & Barta, A. (1988) *EMBO J. 7*, 3949–3955.
Topal, M. D., & Fresco, J. R. (1976) *Nature 263*, 285–289.
Vester, B., & Garrett, R. A. (1988) *EMBO J. 7*, 3577–3587.
Warshaw, M. W., & Tinoco, I. (1966) *J. Mol. Biol. 20*, 29–38.

[2] We are indebted to a reviewer for suggesting this interpretation.