Fluorine-19 nuclear magnetic resonance study of codon-anticodon interaction in 5-fluorouracil-substituted *E. coli* transfer RNAs*

Paul Gollnick, Charles C. Hardin⁺ and Jack Horowitz[§]

Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011, USA

Received 18 March 1986; Accepted 5 May 1986

ABSTRACT

Codon-anticodon interaction was investigated in fully active 5-fluorouracil-substituted *E. coli* tRNA^{Val} (anticodon FAC) by ¹⁹F NMR spectroscopy. Binding of the codon G_pU_pA results in the upfield shift of a ¹⁹F resonance at 3.9 ppm in the central region of the ¹⁹F NMR spectrum, whereas trinucleotides not complementary to the anticodon have no effect. The same ¹⁹F resonance shifts upfield upon formation of an anticodon-anticodon dimer between the ¹⁹F-labeled tRNA and *E. coli* tRNA^{Tyr} (anticodon QUA). These results permit assignment of the peak at 3.9 ppm to the 5-fluorouracil at position 34 in the anticodon of fluorouracil-substituted tRNA^{Val}. The methionine codon A_pU_pG also causes a sequence-specific upfield shift of a peak in the central part of the ¹⁹F NMR spectrum of fluorinated *E. coli* tRNA^{Met}. However, A_pU_pG has no effect on the ¹⁹F spectrum of ¹⁹F-labeled *E. coli* tRNA^{Met}, indicating possible conformational differences between the anticodon loop of initiator and chain-elongating methionine tRNAs. ¹⁹F NMR experiments detect no binding of C_pG_pA_pA to the complementary F_pF_pC_pG (replaces T_pψC_pG) in the T-loop of 5-fluorouracil-substituted tRNA^{Val}, in the presence or absence of codon, suggesting that the tertiary interactions between the T- and D-loops are not disrupted by codon-anticodon interactions.

INTRODUCTION

Recognition of a messenger RNA codon triplet by the appropriate transfer RNA is an essential step in the translation of the genetic code. A thorough understanding of this process requires detailed knowledge of the structure and dynamic properties of the codon-anticodon complex. Binding of complementary oligonucleotides to tRNA has been examined by means of equilibrium dialysis (1-5), fluorescence probes (6,7), chemical modification (8), and recently by ¹H NMR (9,10). These studies demonstrate flexibility in the anticodon loop and provide evidence for conformational changes on codon binding. The specific nature of these changes, however, remains unclear. Results of a number of investigations suggest that codon-induced conformational changes are transmitted to distant parts of the tRNA molecule, and may disrupt interactions between the T- and D-loops making the T_pψC_pG sequence in the T-loop available for binding to the complementary oligonucle-

otide $C_pG_pA_pA$ (11,12). Moreover, it has been established that trinucleotide codon binding promotes changes in tRNA conformation that lead to the association of tRNA molecules (10,13,14); this reaction may play an important role in efficient codon reading on the ribosome (15).

Recent results have shown that ^{19}F NMR spectroscopy of 5-fluorouracil (Fura)-substituted tRNAs is a sensitive method for probing the solution structure of tRNA (16-19). The ^{19}F -labeled tRNAs represent ideal subjects for investigation by ^{19}F NMR because they remain fully active in protein synthesis in vitro despite having essentially all their uracil and uracil-derived modified bases replaced by 5-fluorouracil (20-23). Fluorouracil is incorporated into all stems and loops of the tRNA, where each residue can serve as a site-specific reporter of structural changes in its vicinity. Advantage can then be taken of the extreme sensitivity of ^{19}F chemical shifts to the environment of the fluorine nucleus, to obtain conformational information on the loop regions of tRNA, which cannot be readily monitored by 1H NMR. Detailed structural interpretation will, however, be possible only when each of the ^{19}F resonances is assigned.

^{19}F NMR spectra of the three Fura-substituted E. coli tRNAs thus far purified, tRNA^{Val} (16,24), tRNA^{Met} (17), and tRNA^{Met} (25), show resolved ^{19}F resonances for virtually every incorporated fluorouracil. Each tRNA has two Fura residues in the anticodon loop. One, at position 33, replaces the conserved uracil located 5'-adjacent to the anticodon. The second 5-fluorouracil in (Fura)tRNA^{Val} is in the wobble position of the anticodon (position 34), normally occupied by uridine-5-oxyacetic acid. In both methionine tRNAs the second Fura is located at the 3'-end of the anticodon (position 36). These fluorouracils in the anticodon loop permit us to investigate codon-anticodon interaction by examining the effects of codon binding on the ^{19}F NMR spectra of these tRNAs.

MATERIALS AND METHODS

5-Fluorouracil-substituted tRNAs were isolated from E. coli B cells grown in 5-fluorouracil-containing medium (20), by slight modification of the method of Hills et al. (17). Amino acid accepting activity (20) was greater than 1.4 nmol per A_{260} unit for all tRNA preparations. E. coli tRNA^{Tyr} (anticodon QUA, where Q stands for queuosine), with an acceptor activity of 1.45 nmol per A_{260} unit, was obtained from Subriden RNA. G_pU_pA and $C_pG_pA_pA$ were synthesized from G_pU and C_pG (Sigma), respectively, using M. luteus polynucleotide phosphorylase (Boehringer-Mannheim) and ADP, by the equilib-

rium method (26). A_pU_pG was prepared by an RNase T_1 -assisted polynucleotide phosphorylase reaction, from A_pU (Sigma) and GDP (27). All oligonucleotides were purified by chromatography on DEAE-cellulose and DEAE-Sephadex A-25 (28), and were carefully desalted on a Bio-Gel P2 column. Nucleoside composition was verified by the tritium-derivative method of Randerath et al. (29).

For ^{19}F NMR spectroscopy, transfer RNA samples were dissolved in the buffer used in each experiment and dialyzed against this buffer overnight in a flow dialysis microcell (BRL Model 1200 MA). Ten percent D_2O was added to provide an internal deuterium lock. The sample (0.3 ml) was transferred to a Wilmad 529 A-10 spherical NMR microtube, which was suspended in a 10 mm NMR sample tube and surrounded with sample buffer containing 1 mM 5-fluorouracil as an external standard. Oligonucleotides were added as lyophilized solids. ^{19}F NMR spectra, unless otherwise noted, were obtained at room temperature on a Bruker WM300 pulsed FT NMR spectrometer operating at 282 MHz. Spectra were collected using 8K data points, no relaxation delay, and a pulse width sufficient to optimize the Ernst condition (30). ^{19}F chemical shifts are reported relative to free 5-fluorouracil; downfield shifts are indicated as positive.

RESULTS

Figure 1 shows the 282 MHz ^{19}F NMR spectra of 5-fluorouracil-substituted *E. coli* tRNA^{Val} recorded in the absence (Figure 1A) and presence (Figure 1B and 1C) of the codon G_pU_pA ; the convention adopted for labeling the resonances is indicated in Figure 1A. The only significant change that occurs in the spectrum on addition of increasing amounts of G_pU_pA is a progressive upfield shift of the ^{19}F resonance located at 3.90 ppm in the absence of codon (peak H). Such behavior is indicative of a fast exchange between two states of the Fura residue, free and complexed with codon, with the observed shift representing the weighted average of the chemical shifts of the two states. Changes in the chemical shift of peak H with increasing concentrations of G_pU_pA are plotted in Figure 2A. Linear regression analysis of a double reciprocal plot of these data indicates a maximum shift of 0.69 ppm at infinite G_pU_pA concentration, corresponding to the position of the peak when all the tRNA is complexed with codon. Scatchard analysis of these data (Figure 2B) was used to determine an association constant for the reaction:

Figure 1. Effect of the trinucleotide codon G_pU_pA on the 282 MHz ^{19}F NMR spectrum of 5-fluorouracil-substituted *E. coli* tRNA^{Val}. (A) Reference spectrum of the tRNA in the absence of oligonucleotide. (B) The ^{19}F spectrum after addition of 0.43 mM G_pU_pA . (C) Same with 2.23 mM G_pU_pA . The experimental conditions are: 0.33 mM (Fura)tRNA^{Val}, 50 mM sodium cacodylate, pH 6.0, 100 mM NaCl, 15 mM $MgCl_2$, 1 mM EDTA, and 10% D_2O .

Figure 2. (A) Plot of the change in chemical shift position (in ppm) of peak H in the ^{19}F NMR spectrum of (Fura)tRNA^{Val} as a function of added G_pU_pA . Experimental conditions are those of Figure 1, except that the concentration of (Fura)tRNA^{Val} is 0.22 mM. (B) Scatchard plot of G_pU_pA binding to 5-fluorouracil-substituted tRNA^{Val}. The ratio of bound to free G_pU_pA was determined from the fraction of the maximal upfield shift of peak H in the ^{19}F spectrum of (Fura)tRNA^{Val}.

Figure 3. The 282 MHz ^{19}F NMR spectrum of Fura-substituted tRNA^{Val} before (A) and after (B) addition of 1.13 mM ApUpG , which is not complementary to the anticodon of the tRNA. Experimental conditions are those of Figure 1, except that the concentration of tRNA is 0.38 mM.

The value found, 2500 M^{-1} at 20°C and 0.1 M NaCl , agrees well with the published value of 3000 M^{-1} for the association of GpUpA with normal tRNA^{Val} , determined by equilibrium dialysis at -2°C and 1.0 M NaCl (31). Normal tRNA^{Val} has a modified nucleoside, uridine-5-oxyacetic acid, at position 34 in the anticodon; in $(\text{Fura})\text{tRNA}^{\text{Val}}$ this is replaced by 5-fluorouridine (20). It is interesting to note that this substitution does not significantly affect the affinity for codon.

Addition of trinucleotides not complementary to the anticodon of tRNA^{Val} has no effect on the chemical shift of peak H, as shown in Figure 3 for the trinucleotide ApUpG . Similar results were obtained with ApApA and UpApA (data not shown), indicating that the shift observed with GpUpA is codon specific and not due to nonspecific effects of added oligonucleotides. Care was exercised in desalting the oligonucleotides because several ^{19}F resonances, especially peak A, shift upfield as ionic strength increases (24).

The anticodon of *E. coli* $\text{tRNA}_2^{\text{Tyr}}$ (QUA) is complementary to that of $(\text{Fura})\text{tRNA}^{\text{Val}}$ (FAC) and the two tRNAs form a stable complex through hydrogen bond interactions between their respective anticodons (32,33). Association constants for the formation of such complexes are three orders of

Figure 4. The 282 MHz ^{19}F NMR spectrum of the $[(\text{Fura})\text{tRNA}^{\text{Val}}\cdot\text{tRNA}^{\text{Tyr}}]$ complex. (A) Control spectrum of $(\text{Fura})\text{tRNA}^{\text{Tyr}}$ (0.36 mM). (B) Spectrum after addition of *E. coli* tRNA^{Tyr} (0.32 mM) to $(\text{Fura})\text{tRNA}^{\text{Val}}$ (0.32 mM), (C) Spectrum after the addition of 4.0 mM $\text{C}_p\text{G}_p\text{A}_p\text{A}$ to the tRNA dimer in (B). Spectra were recorded at 32°C.

magnitude larger than those for nucleotide codon binding to the anticodon (reviewed in 34). Addition of an equimolar amount of *E. coli* tRNA^{Tyr} to $(\text{Fura})\text{tRNA}^{\text{Val}}$ produces an upfield shift of peak H in the ^{19}F spectrum of the fluorinated tRNA, from 3.9 ppm to 3.2 ppm (compare Figure 4A and B); no other significant changes in the spectrum are discernible. The magnitude of the observed shift of peak H is in good agreement with the extrapolated maximum upfield shift found on binding of the trinucleotide codon $\text{G}_p\text{U}_p\text{A}$ (Figure 2), and is the result expected for a tight complex between the two anticodons. Significant broadening of the ^{19}F resonances in the spectrum of the complex is also observed (Figure 4), and is even more evident in spectra taken at somewhat lower temperatures (22°C). This broadening can be attributed to the longer rotational correlation time of the tRNA-tRNA dimer.

To explore the possibility of codon-induced conformational changes affecting the interaction between the T- and D-loops, we have examined the binding to $(\text{Fura})\text{tRNA}^{\text{Val}}$ of $\text{C}_p\text{G}_p\text{A}_p\text{A}$, which is complementary to the $\text{T}_p\text{T}_p\text{C}_p\text{G}$

Figure 5. The 282 MHz ^{19}F NMR spectrum of (Fura)tRNA^{Val} (0.66 mM) recorded: (A) before the addition of oligonucleotides; (B) after addition of 2 mM CpGpApA ; and (C) after addition of 2 mM CpGpApA plus 3.5 mM of the trinucleotide codon GpUpA . Experimental conditions are those of Figure 1.

sequence found in the T-loop of most tRNAs. In the fluorinated tRNAs the $\text{T}_p\psi\text{C}_p\text{G}$ sequence is replaced by $\text{F}_p\text{F}_p\text{C}_p\text{G}$ (17,20), and binding of CpGpApA should perturb the ^{19}F resonances of fluorouracil residues 54 and 55. Figure 5 shows the ^{19}F NMR spectrum of (Fura)tRNA^{Val} before (A) and after (B) the addition of a 3-fold molar excess of CpGpApA . Only a minor upfield shift of peak I is observed. This is not specific to addition of the tetranucleotide, NH_4Cl and spermine produce a similar effect (results not shown). Identical results were obtained at 32°C at a higher concentration of CpGpApA (10-fold molar excess over tRNA). Addition of CpGpApA to (Fura)tRNA^{Val} together with the codon triplet GpUpA results only in the upfield shift of peak H (3.9 ppm), which also shifts when GpUpA is added alone (compare Figure 5C with Figures 1B and C). CpGpApA does induce changes in the ^{19}F spectrum of (Fura)tRNA^{Val} when the tRNA is part of a tight anticodon-anticodon complex with tRNA^{Tyr}. Under these conditions several ^{19}F resonances in the 2.7 to 3.4-ppm range (peaks H to L) shift on addition of CpGpApA (Figure 4C); these peaks, however, do not correspond to Fura residues 54 and 55 in the T-loop (see Discussion).

Figure 6. Effect of the trinucleotide codon ApUpG on the 282 MHz ^{19}F NMR spectrum of 5-fluorouracil-substituted *E. coli* $\text{tRNA}_{\text{Met}}^{\text{Met}}$. (A) Reference spectrum of the tRNA in the absence of oligonucleotide. (B-D) ^{19}F spectra after addition of: (B) 0.8 mM, (C) 1.6 mM, and (D) 2.4 mM ApUpG . The experimental conditions are those of Figure 1, with a tRNA concentration of 0.43 mM.

The effects of codon binding on the ^{19}F spectra of the two fluorine-labeled *E. coli* methionine tRNAs are shown in Figures 6 and 7. Addition of ApUpG to $(\text{Fura})\text{tRNA}_{\text{Met}}^{\text{Met}}$ has a similar effect to that observed when GpUpA associates with valine tRNA. One resonance, in the central region of the ^{19}F spectrum of the fluorinated methionine tRNA, peak I (originally at 4.2 ppm), shifts progressively upfield with increasing concentration of ApUpG (Figure 6). Scatchard analysis of these data yields a binding constant of 1200 M^{-1} at 20°C and 0.1 M NaCl, which agrees with the previously reported value of 1700 M^{-1} determined by equilibrium dialysis at 2° in 0.2 M NaCl (35). Oligonucleotides not complementary to the anticodon of $(\text{Fura})\text{tRNA}_{\text{Met}}^{\text{Met}}$ (e.g., GpUpApA) do not affect the chemical shift position of peak I (results not shown).

In contrast to the results with $\text{tRNA}_{\text{Met}}^{\text{Met}}$, addition of the codon ApUpG to 5-fluorouracil-substituted initiator methionine tRNA, has no effect on the ^{19}F NMR spectrum (Figure 7) even when the oligonucleotide is present in a

Figure 7. The 282 MHz ^{19}F NMR spectrum of 5-fluorouracil-substituted *E. coli* tRNA^{Met} (0.48 mM) in the absence of added ApUpG (A), and after addition of 0.9 mM (B), and 1.8 mM (C), ApUpG . Experimental conditions are those of Figure 1.

6-fold molar excess. The codon-containing tetranucleotide ApUpGpA also does not perturb the ^{19}F spectrum of $(\text{Fura})\text{tRNA}^{\text{Met}}$ (results not shown). The anticodon loop of the $(\text{Fura})\text{tRNA}^{\text{Met}}$ used in these experiments is intact, as judged by polyacrylamide gel electrophoresis under denaturing conditions (7 M urea); no evidence of RNA degradation is found. Moreover, methionyl $(\text{Fura})\text{tRNA}^{\text{Met}}$ is active in poly(AUG)-dependent binding to *E. coli* ribosomes (Ira S. Schwartz, New York Med. Col., personal communication).

DISCUSSION

Addition of increasing amounts of the triplet codon GpUpA to $(\text{Fura})\text{tRNA}^{\text{Val}}$ results in a progressive upfield shift of peak H (at 3.9 ppm) in the ^{19}F NMR spectrum of the tRNA (Figures 1 and 2). The sequence complementary to GpUpA occurs only once in tRNA^{Val} , at the anticodon. This consideration, together with the finding that trinucleotides not complementary to the anticodon do not shift the ^{19}F resonance at 3.90 ppm (Figure 3), and the close agreement of the value for the binding constant determined in these studies (Figure 2) with the value in the literature, determined by

equilibrium dialysis, permits us to conclude with some confidence that the observed effect is due to G_pU_pA binding to the anticodon of $(F\text{Ura})tRNA^{Val}$. Further evidence to support this conclusion comes from the observation that formation of an anticodon-anticodon complex (32,33) between $(F\text{Ura})tRNA^{Val}$ (anticodon FAC) and *E. coli* $tRNA^{Tyr}$ (anticodon QUA) produces a similar upfield shift of peak H (Figure 4). Moreover, we have found that binding of G_pU_pA to $(F\text{Ura})tRNA^{Val}$ causes an upfield shift in the methyl proton resonance at 2.50 ppm (downfield from DSS) in the 1H NMR spectrum of the tRNA (results not shown). Kastrup and Schmidt (36) have assigned this resonance to the methyl group of m^6A , which is located adjacent to the anticodon. Geerdes et al. (9) noted a similar upfield shift of the C_{11} methyl resonance of the Y base adjacent to the codon of yeast $tRNA^{Phe}$, as a result of codon binding to the anticodon.

Fluorine-19 chemical shifts are extremely sensitive to the environment of the fluorine nucleus. In other experiments (18,19,24,25) it was shown that changes in the 2° (helical) and 3° structure of ^{19}F -labeled tRNAs lead to major changes in the ^{19}F NMR spectrum. We, therefore, expect that base-pairing between the FUrA residue in the anticodon and an adenine in the bound trinucleotide codon, will cause a shift of the corresponding ^{19}F resonance. Accordingly, we assign peak H to FUrA 34 at the 5' (wobble) position of the anticodon of $(F\text{Ura})tRNA^{Val}$. It is unlikely that the shift of peak H is an indirect effect of codon-induced gross conformational changes in the tRNA, because only one resonance in the ^{19}F spectrum is affected by the binding of G_pU_pA . Assignment of peak H to FUrA 34 is consistent with results (24) showing that FUrA H is in a single-stranded region of the tRNA. This FUrA residue is accessible to pH titration, having a pK_a of 7.6, similar to that of free 5-fluorouridine (37). Determination of the degree of solvent exposure by measurement of the solvent isotope shift (SIS) on transferring the tRNA from H_2O to 2H_2O (24), indicates that FUrA H has a SIS value close to that of free 5-fluorouridine and is, by this criterion, completely exposed. Furthermore, FUrA H readily forms an adduct with bisulfite (24), a reagent that preferentially reacts with pyrimidines in single-stranded regions of tRNA (38,39).

The trinucleotide methionine codon A_pU_pG induces the upfield shift of one resonance, peak I, in the central (4 to 5 ppm) region of the ^{19}F NMR spectrum of $(F\text{Ura})tRNA_m^{Met}$ (Figure 6). Because the shift is codon-specific, and the calculated binding constant agrees well with the literature value, we

make a preliminary assignment of peak I to Fura 36 at the 3' position of the anticodon of (Fura)tRNA_m^{Met}.

Interestingly, no change in the ¹⁹F NMR spectrum of fluorine-substituted *E. coli* initiator methionine tRNA is observed upon addition of the codon triplet A_pU_pG (Figure 7) or of A_pU_pG_pA (data not shown). This is surprising in view of an earlier report which showed that the affinity of *E. coli* tRNA_m^{Met} for A_pU_pG is three times greater than that of tRNA_m^{Met} (35). Since the anticodon of (Fura)tRNA_m^{Met} is intact, two explanations of this result are possible: a low binding constant for the codon, possibly because incorporation of 5-fluorouracil has changed the conformation of the anticodon loop to one unfavorable for codon binding; or, in contrast to the results with tRNA_m^{Met}, codon binding to tRNA_m^{Met} does not affect the magnetic environment of Fura 36 in the anticodon. Subtle conformational differences between the anticodon loop of initiator tRNAs and chain-elongating tRNAs have been detected by X-ray diffraction analysis of tRNA crystals (40) and by the enzymatic probe, nuclease S1 (41).

Trinucleotide codon binding to (Fura)tRNA_m^{Val} and (Fura)tRNA_m^{Met} results in the shift of only one peak in the ¹⁹F NMR spectra of these tRNAs, which we assign to the Fura residue in the anticodon. No other significant changes in the spectra occur, even though another 5-fluorouracil is present at position 33, directly adjacent to the anticodon of each tRNA. We also see no evidence for a trinucleotide codon-induced aggregation of fluorouracil-substituted tRNAs, as is observed with other tRNAs (10,13,14); the resonances in the ¹⁹F NMR spectra are not broadened on codon binding, as occurs upon formation of the anticodon-anticodon dimer (Figure 4).

There are no indications of a codon-induced disruption of D-loop/T-loop tertiary interactions. Several recent experiments suggest that the two farthest downfield peaks in the ¹⁹F spectrum of (Fura)tRNA_m^{Val} represent Fura 54 and 55, in the T-loop (16,19,24,25). For example, ¹⁹F {¹⁹F} nuclear Overhauser (NOE) studies show a reciprocal NOE between peaks A and B (19,25). If the fluorine-labeled tRNA has a structure similar to the crystal structure of yeast tRNA^{Phe}, only Fura 54 and 55 are close enough to each other (4 to 5 Å) to give an appreciable ¹⁹F homonuclear NOE. (Distance measurements were carried out with the program DISPLA written by P. Briley, Department of Biochemistry, University of Iowa, Iowa City, IA). Codon binding, either oligonucleotide codon or the complementary anticodon of *E. coli* tRNA_m^{Tyr}, has no effect on the chemical shift of peaks A or B in the ¹⁹F spectrum of (Fura)tRNA_m^{Val} (Figures 1 and 4).

The conclusion that T-loop/D-loop tertiary interactions are not greatly perturbed on codon binding is supported by our inability to detect binding of $C_pG_pA_pA$ to (Fura)tRNA^{Val}. Addition of the tetranucleotide has no effect on the ^{19}F NMR spectrum of either the free tRNA or the tRNA complexed with the triplet codon G_pU_pA (Figure 5). Binding of $C_pG_pA_pA$ to the $F_pF_pC_pG$ sequence which replaces $T_p\psi_pC_pG$ in the T-loop, would certainly result in the shift of one or more ^{19}F resonances, and the absence of such shifts indicates that $F_pF_pC_pG$ is not available for oligonucleotide binding, even when a codon is bound to the anticodon. This conclusion is consistent with the results of proton NMR studies (9,42,43). We cannot, however, rule out the possibility of small conformational changes that do not alter the magnetic environment of any incorporated fluorines in the tRNA.

A high concentration of $C_pG_pA_pA$ does induce the shift of several resonances in the 2.5-3.5 ppm region of the ^{19}F spectrum of (Fura)tRNA^{Val}, when the anticodon of the tRNA is bound in a tight complex to the complementary anticodon of *E. coli* tRNA^{Tyr} (Figure 4C). The resonances affected, however, do not correspond to the Fura residues at positions 54 and 55 in the T-loop (peaks A and B). Further experiments will be necessary to determine the nature of this structural change and whether it represents a specific effect of $C_pG_pA_pA$.

ACKNOWLEDGEMENTS

We are grateful to Jennifer Morris, Michele Smart and Michael Strayer for expert technical assistance. Thanks are also due to Dr. P. E. Sorter of Hoffmann-LaRoche, Nutley, N.J., for a generous supply of 5-fluorouracil. This investigation was supported by a grant from the U.S. Public Health Service, GM 32838.

*Journal Paper No. J-11939 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. 2566.

+Present address: Department of Chemistry, University of California-Berkeley, Berkeley, CA 94720, USA.

§To whom correspondence should be addressed

REFERENCES

1. Uhlenbeck, O.C., Baller, J. and Doty, P. (1970) *Nature* 225, 508-510.
2. Uhlenbeck, O.C. (1972) *J. Mol. Biol.* 65, 25-41.
3. Eisinger, J. and Spahr, P.F. (1973) *J. Mol. Biol.* 73, 131-137.
4. Pongs, O., Bald, R. and Reinwald, E. (1972) *Eur. J. Biochem.* 32, 117-125.

5. Freier, S.M. and Tinoco, I. (1975) *Biochemistry* 14, 3310-3314.
6. Yoon, K., Turner, D.H. and Tinoco, I. (1975) *J. Mol. Biol.* 99, 507-518.
7. Labuda, D., Striker, G. and Pörschke, D. (1984) *J. Mol. Biol.* 174, 587-604.
8. Wagner, R. and Garrett, R.A. (1979) *Eur. J. Biochem.* 97, 615-621.
9. Geerdes, H.A.M., Van Boom, J.H. and Hilbers, C.W. (1980) *J. Mol. Biol.* 142, 195-217.
10. Clore, M.G., Gronenborn, A.M. and McLaughlin, L.W. (1984) *J. Mol. Biol.* 174, 163-173.
11. Schwarz, U., Menzel, H.M. and Gassen, H.G. (1976) *Biochemistry* 15, 2484-2490.
12. Gassen, H.G. (1980) *Prog. Nucleic Acid Res. Mol. Biol.* 24, 57-86.
13. Geerdes, H.A.M., Van Boom, J.H. and Hilbers, C.W. (1980) *J. Mol. Biol.* 142, 219-230.
14. Pörschke, D. and Labuda, D. (1982) *Biochemistry* 21, 53-56.
15. Labuda, D. and Pörschke, D. (1983) *J. Mol. Biol.* 167, 205-209.
16. Horowitz, J., Ofengand, J., Daniel, W.E., Jr. and Cohn, M. (1977) *J. Biol. Chem.* 252, 4418-4421.
17. Hills, D.C., Cotten, M.L. and Horowitz, J. (1983) *Biochemistry* 22, 1113-1122.
18. Gollnick, P., Hardin, C.C. and Horowitz, J. (1984) *Fed. Proc., Fed. Am. Soc. Exp. Biol.* 43, 1885.
19. Gollnick, P., Hardin, C.C. and Horowitz, J. (1985) *Fed. Proc., Fed. Am. Soc. Exp. Biol.* 44, 1616.
20. Horowitz, J., Ou, C.-N., Ishaq, M., Ofengand, J. and Bierbaum, J. (1974) *J. Mol. Biol.* 88, 301-312.
21. Ofengand, J., Bierbaum, J., Horowitz, J., Ou, C.-N. and Ishaq, M. (1974) *J. Mol. Biol.* 88, 313-325.
22. Ramberg, E.S., Ishaq, M., Rulf, S., Moeller, B. and Horowitz, J. (1978) *Biochemistry* 17, 3978-3985.
23. Chinalli, O., Horowitz, J. and Ofengand, J. (1978) *Biochemistry* 17, 2755-2760.
24. Hardin, C.C., Gollnick, P., Kallenbach, N.R., Cohn, M. and Horowitz, J., submitted for publication.
25. Hardin, C.C., Gollnick, P. and Horowitz, J., in preparation.
26. Thach, R.E. and Doty, P. (1965) *Science* 148, 632-634.
27. Mohr, S.C. and Thach, R.E. (1969) *J. Biol. Chem.* 244, 6566-6576.
28. Schetters, H., Gassen, H.G. and Matthaai, H. (1972) *Biochem. Biophys. Acta* 272, 549-559.
29. Randerath, K., Gupta, R. and Randerath E. (1980) *Methods Enzymol.* 65, 639-680.
30. Shaw, D. (1976) in *Fourier Transform NMR Spectroscopy*, p. 179, Elsevier, New York.
31. Pongs, O. and Griese, K. (1972) *FEBS Letters* 26, 297-300.
32. Eisinger, J. and Gross, N. (1974) *J. Mol. Biol.* 88, 165-174.
33. Grosjean, H., Soll, D. G. and Crothers, D.M. (1976) *J. Mol. Biol.* 103, 499-519.
34. Grosjean, H. and Chantrenne, H. (1980) in *Chemical Recognition in Biology*, Chapeville, F. and Haenni, A.L., Eds., pp. 347-368, Springer Verlag, Berlin.
35. Högenauer, G., Turnowsky, F. and Unger, F.M. (1972) *Biochem. Biophys. Res. Commun.* 46, 2100-2106.
36. Kastrup, R.V. and Schmidt, P.G. (1978) *Nucleic Acids Res.* 13, 6283-6298.
37. Wempen, I., Duschinsky, L., Kaplan, L. and Fox, J.J. (1961) *J. Am. Chem. Soc.* 83, 4755-4766.

38. Furuichi, Y., Wataya, Y., Hayatsu, H. and Ukita, T. (1970) *Biochem. Biophys. Res. Commun.* 41, 1185-1191.
39. Shapiro, R., Servis, R.E. and Welcher, M. (1970) *J. Am. Chem. Soc.* 92, 422-424.
40. Woo, N.H., Roe, B.A. and Rich, A. (1980) *Nature* 286, 346-351.
41. Wrede, P., Woo, N.H. and Rich, A. (1979) *Proc. Natl. Acad. Sci. USA* 76, 3289-3293.
42. Davanloo, P., Sprinzl, M. and Cramer, F. (1979) *Biochemistry* 18, 3189-3199.
43. Geerdes, H.A.M., Van Boom, J.H. and Hilbers, C.W. (1978) *FEBS Letters* 88, 27-32.