*SELECTIVE RECOGNITION OF THE NATIVE CONFORMATION OF TRANSFER RIBONUCLEIC ACIDS BY ENZYMES**

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The recognition of a substrate by an enzyme is usually a highly specific process; and when the substrate is a macromolecule, as is sRNA,[1] the specificity may well depend not simply on its primary structure, but rather on the molecular architecture that is the expression of that primary structure under conditions of the physiological environment. Evidence that the macromolecular conformation of sRNA is an important feature in its recognition by specific aminoacyl-sRNA synthetases was recently provided by the discovery that certain sRNA's in yeast and *E. coli* can be trapped in a form in which they cannot serve as substrates for those enzymes.[2] Such *denatured* sRNA molecules could be completely *renatured* through brief heating in the presence of magnesium ions, with no deleterious effect on their primary structure, so that they completely recover their capacity to serve as substrates for enzymatic aminoacylation. In this respect, these renaturable sRNA molecules, which are usually present in the denatured state in conventional preparations of sRNA, were found upon renaturation to be indistinguishable from the biologically active form (*native*) of these molecules[2, 3] that is present in samples of sRNA prepared by very mild methods of isolation.[4]

The native and denatured forms of several of these sRNA's are readily interconvertible, and conditions have been determined under which each form can be maintained for a relatively long period of time. With the knowledge of such conditions, and the availability of one such sRNA in nearly homogeneous form, an $sRNA_{leu}$ from yeast, it has been possible to study the extent to which recognition of sRNA by several enzymes depends upon the integrity of the native conformation of the substrate. Thus, the enzymatic reactions catalyzed by yeast leucyl-sRNA synthetase, yeast adenylyltransferase, and the protein-synthesizing system of *E. coli* were investigated. In each case, the effectiveness of the native and denatured forms of yeast $sRNA_{leu}$ as a substrate or (after end-group oxidation) as an inhibitor was compared. The results indicate that recognition of that sRNA by the biosynthetic enzymes studied requires a unique native sRNA conformation which has an important tertiary structural component. A preliminary report of some of this work has recently been presented.[5]

Methods.—Polyribonucleotides: Poly UC (1:1), $s_{20,w} = 3.4$, was enzymatically synthesized in this laboratory. Poly UG (2:1), $s_{20,w} = 3.3$, was a product of Miles Chemical Co. Poly AC (1:1), poly CA (5:1), poly CAG (4:1:1), poly AUC (1:1:1), and poly CAU (4:1:1) were gifts of Dr. S. Ochoa.

$sRNA_{leu}$: An $sRNA_{leu}$ from yeast, earlier designated $sRNA_{leu}$III,[2] was isolated in a highly purified (80–90%, <2% of any other $sRNA_{leu}$) and nearly completely denatured state (>95 %) by means of countercurrent distribution and gel filtration on Sephadex G-100. The details of this procedure will be described subsequently.[7] The adenosine terminus, largely absent in sRNA obtained from stationary phase yeast,[8] was enzymatically added to some preparations after the first countercurrent distribution.

Stabilization of sRNA in native and denatured forms: Native $sRNA_{leu}$ was obtained by incubating denatured $sRNA_{leu}$ in a neutral buffer containing excess Mg^{++} (0.01–0.02 *M*) at 60° for 5

min. Charged or oxidized sRNA was stabilized in either form by dissolving the particular sRNA derivative in 0.01 *M* K-cacodylate + 0.001 *M* K_2-ethylenediaminetetraacetate (EDTA), pH 6.8, dialyzing against the same solvent for 48–72 hr, and then heating at 60° for 5 min. After cooling to 0°, $MgCl_2$ was added to a final concentration of 0.01 *M*. Half of the solution was kept at 0° until used (85–95% denatured sRNA), and half was heated at 60° for 2 min (>95% native sRNA). Denatured-charged or -oxidized $sRNA_{leu}$ samples contain a significantly larger amount of native $sRNA_{leu}$ than denatured-nonderivatized $sRNA_{leu}$ because the gel filtration step, which removed native material from the latter, was not repeated after charging or periodate oxidation.

Leu-sRNA: This was prepared enzymatically under conditions of the assay for amino acid acceptor activity.[2] The reaction was stopped by adding 1 vol of water-saturated phenol, the mixture shaken for 10 min, and centrifuged to separate the phases. sRNA was precipitated from the aqueous phase with 2 vol of ethanol, washed with 80% and then 95% ethanol, dissolved in the cacodylate-EDTA buffer, and dialyzed. Dialysis for 72 hr, which inevitably led to some deacylation, was necessary to remove contaminating adenosine 5′-triphosphate (ATP) quantitatively.

$sRNA_{leu}$ with oxidized end group: This was made by incubating native $sRNA_{leu}$ containing a terminal adenosine 5′-phosphate (AMP) residue in 0.01 *M* $MgCl_2$ + 0.005 *M* NaH_2PO_4 + 0.01 *M* $NaIO_4$, pH 6.0, for 20 min at 35°.[9] Glucose was then added to a final concentration of 0.02 *M*, and after an additional 10 min of incubation, the solution was dialyzed for 24 hr against 1.5 *M* NaCl at 4°. The sRNA was precipitated with 2 vol ethanol, washed with 80% and 95% ethanol, and dried. Less than 1% of the amino acid acceptor activity remained after this periodate treatment.

Enzymes: Yeast leucyl-sRNA synthetase was purified according to Lagerkvist and Waldenström,[10] up to their Sephadex step. sRNA adenylyltransferase[11] was also obtained from this protein fraction. The leucyl-sRNA synthetase used in discharging experiments was further purified by gel filtration on Sephadex G-150 (2 × 90 cm) in 0.5 *M* KCl + 0.02 *M* Tris-HCl + 0.001 *M* dithiothreitol, pH 7.5. The most active fraction was used immediately after elution as the enzyme is unstable after this purification step. Attempts to discharge aminoacylated yeast sRNA with the same enzyme fraction used for aminoacylation failed, and further purification of the leucyl-sRNA synthetase proved necessary. Apparently an interfering enzyme, possibly an inorganic pyrophosphatase,[12] was present in the less purified enzyme preparation.

Crude arginyl-sRNA synthetase was prepared as described earlier.[2]

Assays: Leucyl and arginyl acceptor activities of sRNA were determined as described previously, but at 20°.[2]

Enzymatic discharging of sRNA[13] was followed by incubating 25 μmoles K-cacodylate, pH 6.8, 3 μmoles $MgCl_2$, 0.3 μmole Na_2-EDTA, 1.2 μmoles $Na_4P_2O_7$, 1.2 μmoles AMP, 120 μg enzyme, and 0.7 mμmole (7000 cpm) C^{14}-leu-sRNA (native or denatured) in a total volume of 1 ml at 25°. At different times, the sRNA in 0.1-ml aliquots was precipitated with 20 vol of cold 5% trichloroacetic acid (TCA). The precipitate was collected on membrane filters, washed with 100 vol of cold 5% TCA, dried, and counted at known efficiency in a Packard Tri-Carb scintillation counter.

The inhibition of aminoacylation of $sRNA_{leu}$ by the native and denatured forms of periodate-oxidized $sRNA_{leu}$ containing the terminal AMP residue was assessed by determining the initial velocity of aminoacylation at various substrate concentrations in the presence of a single concentration of oxidized sRNA in the native or denatured form. Thus, reaction mixtures containing 10 μmoles Tris-HCl, pH 7.5, 1.1 μmoles $MgCl_2$, 0.05 μmoles Na_2-EDTA, 0.5 μmole K-ATP, 6.4 mμM-C^{14}-leucine (sp. act. 100 μc/μM), 8.8, 17.8, 35.5, or 70.1 μμmoles $sRNA_{leu}$ (native), 68.3 μμmoles periodate-oxidized $sRNA_{leu}$ (native or denatured) when added, and 0.06 mg enzyme in a total volume of 0.1 ml were incubated at 25° for 30, 60, and 120 sec (and also 30 min to determine the saturation level of aminoacylation). The reaction was stopped by adding cold 5% TCA; sRNA was collected, washed, and its radioactivity determined as above.

Incorporation of the terminal AMP residue into sRNA was assayed at 20° in a mixture of 10 μmoles Tris-HCl, pH 7.5, 1 μmole $MgCl_2$, 0.05 μmole Na_2-EDTA, 2 μmoles KCl, 0.05 μmole C^{14}-ATP (sp. act. 8 μc/μM), 0.3 μmole phosphoenolpyruvate (PEP), 10 μg pyruvate kinase, 200 μg enzyme, and a limiting amount of native or denatured sRNA lacking the adenosine terminus in a total of 0.1 ml. Aliquots were taken at various times and the reaction stopped by adding cold 5% TCA. The precipitated sRNA was washed and counted as above.

Transfer of leucine from C^{14}-leu-sRNA to protein (hot TCA-insoluble material) was determined according to Nirenberg and Matthaei.[14] Yeast C^{14}-leu-sRNA (native or denatured) was added to a cell-free extract (30,000 × *g* supernatant, nonfrozen) from *E. coli* B containing sRNA, aminoacyl-sRNA synthetases, ribosomes, transfer enzymes, and messenger RNA. In experiments with synthetic polynucleotide messengers, an extract was used whose intrinsic messenger RNA had been destroyed through preincubation. Aliquots, incubated for various periods at 23°, contained 10 μmoles Tris-HCl, pH 7.8, 1 μmole Mg-acetate, 5 μmoles KCl, 0.6 μmole mercaptoethanol, 0.3 μmole ATP, 0.1 μmole guanosine 5′-triphosphate (GTP), 0.6 μmole PEP, 1 μg pyruvate kinase, 0.02 μmole of each of the 20 common amino acids, 12 μg polynucleotide messenger (when added), 1 μg C^{14}-leu-sRNA, and 10 μl *E. coli* extract in a total volume of 0.1 ml. The reaction was stopped by adding 10 vol of 10% TCA. After heating at 90° for 15 min, the residual precipitate was washed and counted as above.

Results.—Absolute recognition of native $sRNA_{leu}$ during enzymatic aminoacylation: The kinetics of aminoacylation of the "renaturable" $sRNA_{leu}$ (containing a terminal AMP residue) in the native and denatured forms are shown in Figure 1. The small amount of initial loading of the denatured form (<3%) is due to contaminating native $sRNA_{lew}$ in the sample, and the residual rate of charging is less than 0.2 per cent that of the native form. A similar result has been obtained with the native and denatured forms of the "renaturable" $sRNA_{arg}$[2] that has also been purified by countercurrent distribution. These results demonstrate that the native conformation of at least these sRNA's is an absolute requirement for enzymatic aminoacylation.

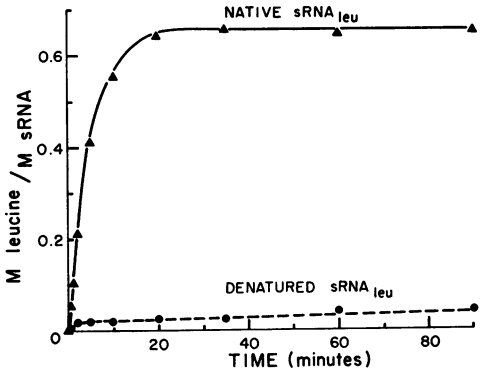

FIG. 1.—Kinetics of incorporation of C^{14}-leucine into native and denatured samples of highly purified yeast $sRNA_{leu}$.

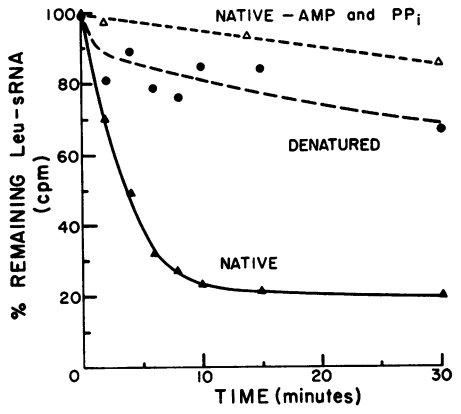

FIG. 2.—Kinetics of enzymatic discharging of native and denatured samples of highly purified yeast leu-sRNA. *Open triangles:* enzyme reaction mixture with native leu-sRNA sample, but lacking AMP and inorganic pyrophosphate (PP_i).

Absolute recognition of native leu-sRNA during enzymatic deacylation: Figure 2 describes the kinetics of enzymatic deacylation of native and denatured leu-sRNA along with the results of a control. Whereas the native form is readily discharged by the leucyl-sRNA synthetase, less than 15 per cent of the denatured sample is discharged at a similar rate, this amount corresponding to the incompleteness of the denaturation process. The further slow rate of deacylation of the denatured form corresponds to that seen with the control for native leu-sRNA that contains enzyme,

but lacks the other reactants required for reversal of the acylation reaction. These results, which are completely analogous to the findings for the acylation reaction, are in agreement with the observation of Gartland and Sueoka[15] that only one of the two alternative forms of *E. coli* tryptophanyl-sRNA identified by them could be enzymatically discharged.

Relative recognition of native and denatured forms of $sRNA_{leu}$ with oxidized nucleoside terminus: While the foregoing results demonstrate an absolute qualitative requirement for the native conformation of the sRNA substrate of the aminoacyl-sRNA synthetase, they do not measure the relative recognition by the enzyme of the native and denatured forms of the sRNA in terms of binding of the sRNA by the enzyme. In the selective interaction between a particular sRNA and its aminoacyl-sRNA synthetase, only the sRNA's specific for the corresponding amino acid serve as competitive inhibitors after periodate oxidation.[16] Hence, to assess the dependence on sRNA conformation of the interaction of the $sRNA_{leu}$ with its aminoacyl-sRNA synthetase even in the absence of catalysis, the ability of the native and denatured forms of periodate-oxidized $sRNA_{leu}$ to inhibit the aminoacylation of native $sRNA_{leu}$ was determined.

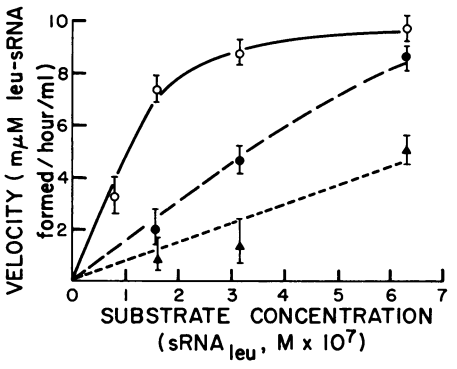

FIG. 3.—Inhibition of aminoacylation of highly purified native $sRNA_{leu}$ by periodate-oxidized $sRNA_{leu}$. *Open circles:* no inhibitor added; *filled circles:* inhibitor in denatured conformation; *triangles:* inhibitor in native conformation.

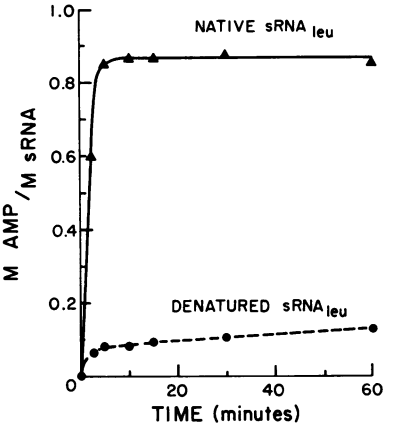

FIG. 4. Kinetics of incorporation of terminal C^{14}-AMP into native and denatured samples of highly purified yeast $sRNA_{leu}$—pCpC.

Michaelis plots of the kinetics of loading in the presence and absence of a constant amount of the native or denatured "inhibitor" are shown in Figure 3. The K_m for $sRNA_{leu}$ determined therefrom is 1.0×10^{-7} M, which is similar to the value of 1.6×10^{-7} M obtained for a purified yeast $sRNA_{ser}$ with its homologous aminoacyl-sRNA synthetase.[17] Despite the small number of data points and their apparent inaccuracy, it appears from Figure 3 that the denatured form of the oxidized $sRNA_{leu}$ is a potent inhibitor of the reaction, though less so than the oxidized native form. Since the denatured-oxidized $sRNA_{leu}$ contained less than 10 per cent of its native counterpart,[18] its substantial inhibitory capability indicates that it is recognized by the leucyl-sRNA synthetase. *Assuming* that the observed inhibitions are strictly competitive, inhibition constants, K_i, of approximately 1×10^{-7} M

and 2×10^{-7} M have been calculated for the native and denatured conformations, respectively, of oxidized $sRNA_{leu}$.

Relative recognition of native and denatured forms of $sRNA_{leu}$ by sRNA adenylyltransferase: This enzyme catalyzes incorporation of the terminal AMP residue into all sRNA's without regard for their amino acid specificity; but it is generally inactive with other types of RNA.[19] The kinetics described in Figure 4 of incorporation of AMP into native and denatured samples of $sRNA_{leu}$ (lacking the adenosine terminus) show a clear preference by the enzyme for the native form of the substrate. While the small amount of initial rapid incorporation of C^{14}-AMP into the denatured sample must be due to the 6–10 per cent contamination of the $sRNA_{leu}$ with other sRNA's, mainly $sRNA_{ser}$, the subsequent slow rate of incorporation ($\sim$0.4% that of native sRNA) does indicate that the denatured $sRNA_{leu}$ is a poor but definitely recognized substrate. The same result was obtained at 4° as at 20°, and when a more purified preparation of the enzyme was employed. Consequently, it seems unlikely that this slow incorporation of AMP into denatured $sRNA_{leu}$ reflects renaturation of this sRNA or some other experimental artifact.

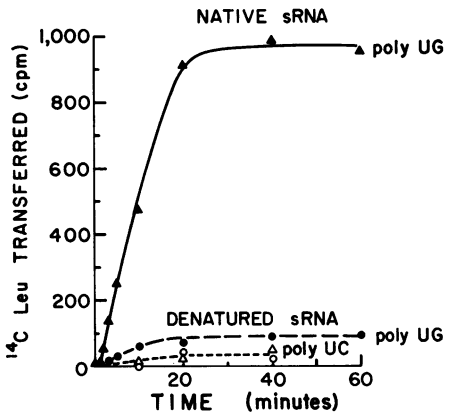

FIG. 5.—Kinetics of messenger-dependent transfer of C^{14}-leucine from native and denatured samples of highly purified yeast leu-sRNA into polypeptides by an *E. coli* protein-synthesizing system. Leu-sRNA (2000 cpm) was added in each experiment. Note that the only "blank" values subtracted were those obtained from "zero time" samples containing the complete reaction mixture. *Triangles:* values obtained with native leu-sRNA; *circles:* values obtained with denatured leu-sRNA.

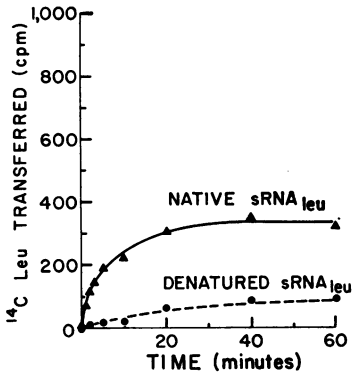

FIG. 6.—Same as Fig. 5, except that natural "endogenous" messenger was employed. Thus, the *E. coli* extract was not preincubated and no polynucleotide messenger was added.

Absolute recognition of native leu-sRNA by a protein-synthesizing system: Since it was known[20] that a protein-synthesizing system from *E. coli* can transfer leucine from yeast leu-sRNA into polypeptides in response to the polynucleotide messengers poly UC and poly UG, the ability of the system to distinguish between the native and denatured forms of the "renaturable" yeast leu-sRNA was investigated. The kinetics of transfer into polypeptides of C^{14}-leucine from this yeast leu-sRNA in its native and denatured conformations in response to these two messengers is shown in Figure 5. It can be seen that transfer occurs only in response to poly UG, the amount of apparent incorporation with poly UC as messenger being less than 2 per

cent and the same for either form. With poly UG there is a very marked difference in both the initial rate and final level of transfer of C^{14}-leucine into polypeptide from the two forms of leu-sRNA. Thus, the initial rate of transfer from native leu-sRNA is $\sim$ 8 times greater than from the denatured form; and whereas 47 per cent of the C^{14}-leucine from the native sample is incorporated into polypeptides, only 5–7 per cent is incorporated from the denatured one.[21] The small amount of apparent transfer from the denatured form of the sRNA is of doubtful significance. The incorporation observed with the denatured $sRNA_{leu}$ can be explained by the residual native leu-sRNA in the preparation of denatured leu-sRNA employed. The diminished rate of that incorporation compared to what occurs with the native sample is probably due to competition from the *E. coli* leu-sRNA. This low level of apparent transfer from denatured leu-sRNA was observed at two different Mg^{++} concentrations, 0.01 *M* and 0.003 *M*, suggesting that the amount incorporated is not due to infidelity deriving from an overabundance of Mg^{++}. The very small amount of incorporation observed in response to poly UC (and other copolymers containing U and C residues, Table 1), which is independent of the conformation of the sRNA, is most likely due to a small contamination of the sRNA preparation by another leu-sRNA.

TABLE 1
MESSENGER-DEPENDENCE OF TRANSFER OF C^{14}-LEUCINE FROM NATIVE AND DENATURED LEU-sRNA INTO POLYPEPTIDES

Messenger	Native Leu-sRNA			Denatured Leu-sRNA		
	Cpm	Cpm − bgd.	Incorp. (%)	Cpm	Cpm − bgd.	Incorp. (%)
—	66	—	—	50	—	—
Poly UG (2:1)	1886	1820	54	301	251	7.4
Poly UC (1:1)	180	114	3.4	—	—	—
Poly AC (1:1)	49	0	0	43	0	0
Poly CA (5:1)	58	0	0	50	0	0
Poly AUC (1:1:1)	141	75	2.2	65	15	0.4
Poly CAU (4:1:1)	46	0	0	42	0	0
Poly CAG (4:1:1)	45	0	0	42	0	0

Four μg sRNA (3384 cpm) was added to each reaction mixture, which was incubated for 30 min.

While the results to this point suggest absolute discrimination by the protein-synthesizing system in favor of the native conformation of yeast leu-sRNA in the presence of the normal messenger, they do not rule out the possibility that the conformational change attending the denaturation of yeast leu-sRNA leads to the presentation of a new anticodon. Such a possibility has recently been suggested[22] to explain the results of experiments with the active and inactive forms of *E. coli* try-sRNA in which the *binding* of the two forms to ribosomes in response to a variety of polynucleotides was tested. In that case the "inactive" try-sRNA was found to bind to ribosomes, but only in response to polynucleotides that do not contain the normal code words for tryptophan. It was concluded that the denaturation process changes the fidelity of codon recognition of sRNA in protein synthesis. To test this possibility with the added specificity deriving from sRNA-enzyme interactions leading to peptide bond synthesis, the ability of native and denatured leu-sRNA to have their amino acid transferred to polypeptides in response to a variety of synthetic polynucleotide messengers was studied. *The results* (Table 1) *do not give any indication of selective transfer of leucine from the denatured leu-sRNA*. Of the 64 possible coding triplets, 45 would seem to have been adequately provided for by the polynucleotides used, and another 7 may have been. The remaining 12 triplets

all contain U and G residues in their code words. Consequently, the incorporation that they might stimulate would have to be detected against a "background incorporation" like that seen with poly UG itself; this was not technically feasible.

To test those remaining coding triplets, a protein-synthesizing system programed with intrinsic messenger RNA was used (Fig. 6). In this experiment as well, native leu-sRNA was the preferred donor. It showed a higher rate (~20-fold) and greater final level (5–6-fold) of incorporation than the denatured leu-sRNA. The small amount of apparent transfer from the denatured sRNA sample is again at least partly due to contaminating native sRNA. While these results rule out the possibility that the denatured sRNA recognizes a *commonly* occurring codon, it cannot be rigorously excluded that it can respond to some *rarely* occurring codon.

Discussion.—The major focus of the present work has been to investigate the role of sRNA conformation in its recognition by biosynthetic enzymes. Virtually absolute specificity for the native conformation of the sRNA substrate has been observed in all three enzymatic processes investigated, of which one is necessary for the completion of sRNA synthesis, and the other two are associated with protein synthesis.

While the absolute requirement for the native conformation of sRNA in the reactions catalyzed by the aminoacyl-sRNA synthetases shows that the structural modification resulting in the denatured form is enough to prevent the enzymes from performing their catalytic functions, the ability of the periodate-oxidized $sRNA_{leu}$ to act as an inhibitor in its denatured conformation indicates that the enzyme recognition site on the sRNA must be sufficiently intact to permit some association between the denatured sRNA and its aminoacyl-sRNA synthetase.[23] In view of the high degree of specificity of an aminoacyl-sRNA synthetase and its sRNA substrates, this finding cannot yet be generalized for the "denatured" forms of other sRNA's, although it does seem likely. In the other two reactions, those involving terminal addition of AMP on to sRNA and peptide bond formation, the relevant enzymes do not normally discriminate between different sRNA's. Consequently, these enzymes must recognize conformational features common to all sRNA's. Therefore, the high degree of specificity displayed in these reactions in favor of the native conformation has two important implications. One is that some structural feature(s) common to *all* native sRNA's has been modified in the denatured form. The other is that there is great similarity in conformation of all sRNA's; otherwise, much poorer discrimination between the native and denatured conformations would be expected in these reactions. This conclusion is consistent with the notion that some of the discrimination in the peptide bond synthesis reaction occurs at the level of sRNA interaction with the ribosomes.[22]

It seems likely that the major difference between the native and denatured states is at the level of tertiary structure, as there is little difference in the amount and type of secondary structure in the two conformations, both of which are monomer forms.[5,7]

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[1] Abbreviations: sRNA = transfer ribonucleic acid; $sRNA_x$ = uncharged x-specific sRNA; x-sRNA = aminoacylated x-specific sRNA; poly ACGU = random copolyribonucleotide containing adenylate, cytidylate, guanylate, and uridylate residues (other copolymers designated in analogous manner); TCA = trichloroacetic acid; K_m = Michaelis constant; K_i = inhibition constant.

[2] Lindahl, T., A. Adams, and J. R. Fresco, these PROCEEDINGS, **55**, 941 (1966).

[3] It was originally thought[2] that sRNA isolated in the native state from yeast[4] is more resistant to the denaturing effect of chelating agents than the Mg^{++}-renatured material. Such a distinction is no longer warranted since the observed difference has now been found to be due to the stabilizing effect of some contaminant in the preparations of native sRNA. The contaminant, presumably some protein, can be removed by means of gel filtration; its effect can also be minimized simply by diluting the native sRNA preparations. Hence, it appears that native and renatured $sRNA_{leu}$ are the same; the terms are thus synonymous.

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[14] Nirenberg, M. W., and J. H. Matthaei, these PROCEEDINGS, **47**, 1588 (1961).

[15] Gartland, W., and N. Sueoka, these PROCEEDINGS, **55**, 948 (1966).

[16] Torres-Gallardo, J., and M. Kern, these PROCEEDINGS, **53**, 91 (1965). In the present work, too, periodate-oxidized $sRNA_{ala}$ did not inhibit ($K_i > 10^{-5}$ M) the aminoacylation of native $sRNA_{leu}$.

[17] Makman, M. H., and G. L. Cantoni, *Biochemistry*, **5**, 2246 (1966).

[18] As judged from its spectral properties.[7] It should be noted in this connection that nonoxidized denatured $sRNA_{leu}$ also inhibits the aminoacylation of native $sRNA_{leu}$.

[19] Daniel, V., and U. Z. Littauer, *J. Biol. Chem.*, **238**, 2102 (1963).

[20] Bennett, T. P., J. Goldstein, and F. Lipmann, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 233.

[21] As *E. coli* leucyl-sRNA synthetase does not charge any of the major yeast $sRNA_{leu}$, the observed incorporations cannot be due to a shunting of C^{14}-leucine from the yeast leu-sRNA to *E. coli* $sRNA_{leu}$. Cf. Yamane, T., and N. Sueoka, these PROCEEDINGS, **51**, 1178 (1964).

[22] Sueoka, N., T. Kano-Sueoka, and W. Gartland, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 31, in press (1966).

[23] This finding is consistent with the observation that an aminoacyl-sRNA synthetase catalyzes the aminoacylation of sRNA's for one amino acid from different species at greatly different rates, even when the Michaelis constants are quite similar. Cf. Loftfield, R. B., and E. A. Eigner, *Acta Chem. Scand.*, **17**, s117 (1963).