CODING PROPERTIES OF sRNA CONTAINING 8-AZAGUANINE*

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Received April 19, 1965

According to the adaptor theory, the translation of nucleotide sequences in RNA into amino acid sequences in protein is mediated via transfer RNA (Crick, 1958). Indeed, recent studies indicate that the anomalous attachment of an amino acid to sRNA will permit that amino acid to read codons not normally assigned to it (Chapeville et al., 1962; Epler et al., 1965). The adaptor theory also suggests that minor structural modifications in sRNA might alter specificity in the function of sRNA and thereby induce miscoding. It is known that following the addition of the base analog 8-azaguanine to a growing culture of Bacillus cereus, the analog replaces guanine in the newly synthesized sRNA (Levin, 1963). It was of interest, therefore, to determine the coding properties of this altered RNA by measuring its ability to participate in the synthesis of specific polypeptides.

The S-30 fraction was prepared from E. coli B essentially as described by Nirenberg and Matthaei (1961) and fractionated into ribosomes and S-122 by centrifugation at 122,000 x g for 90 minutes. In

*This investigation was supported in part by U. S. Public Health Service Research Grants from the National Cancer Institute.

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certain experiments, the S-122 fraction was freed of soluble RNA by fractionation on a DEAE cellulose column. The material was eluted with a linear gradient of 0 to 1 M NaCl in 0.05 M Tris-HCl buffer, pH 7.2. The fractions eluted in the range of 0.2 to 0.3 M NaCl contained the amino acid-activating enzymes ("DEAE enzymes") and were essentially free of soluble RNA. The latter was eluted in the range of 0.5 to 0.6 M sodium chloride. By using ribosomes plus "DEAE enzymes" or ribosomes plus a very small amount of S-122, a system was obtained which was dependent upon the addition of sRNA for maximal activity. This system was used to assess the function of various added sRNAs. The components of the assay system are described in detail in the legend to Table I.

Purified sRNAs were obtained by phenol extraction (Nirenberg and Matthaei, 1961) of the ribosomal supernatant fractions of normal B. cereus and B. cereus grown in the presence of 8-azaguanine. The procedures used for growing cells in the presence of 8-azaguanine have been described by Grünberger and Mandel (1965). It has been demonstrated that in this sRNA approximately twenty-five per cent of the guanine is replaced by azaguanine.

The addition of B. cereus sRNA containing 8-azaguanine ("B. cereus azaG") to the E. coli system results in a sixfold enhancement of poly U-directed phenylalanine incorporation, when compared to the incorporation obtained in the absence of supplementary sRNA (Table I). In three separate experiments, the response to B. cereus azaG sRNA was 75 to 100 per cent of the response obtained with an equal amount of E. coli B sRNA. The fact that low but significant incorporation occurs in the absence of supplementary sRNA presumably reflects the presence of small amounts of sRNA bound to the ribosomes. Previous studies have established that in addition to coding for phenylalanine, poly U can code for leucine and isoleucine (Nirenberg and Matthaei, 1961; Bretscher and Grunberg-Manago, 1962; Szer and Ochoa, 1964; Davies et al., 1964; Friedman and

TABLE I

CODING PROPERTIES OF AZAGUANINE CONTAINING sRNA

	Amino Acid	sRNA	Counts/min incorporated No poly U	(+) poly U
1)	C^{14} phenylalanine	none	174	2,432
		B. cereus azaG	56	12,779
2)	C^{14} leucine	none	17	206
		B. cereus azaG	105	662
3)	C^{14} isoleucine	B. cereus azaG	37	254
		" + Dihydrostreptomycin (7×10^{-4}M)	55	826
4)	C^{14} valine	B. cereus azaG	135	34
5)	C^{14} proline	none	11	11
		B. cereus azaG	5	21
6)	C^{14} alanine	B. cereus azaG	8	8
7)	C^{14} arginine	B. cereus azaG	35	43

Reaction mixtures contained the following components in a 0.4 ml volume (in μmoles unless otherwise specified): Tris-HCl buffer, pH 7.8, 4.0; potassium chloride, 24.0; Mg acetate, 4.0; β-mercaptoethanol, 2.4; ATP, 0.25; GTP, 0.01; CTP, 0.01; UTP, 0.01; phosphoenolpyruvate, 1.25; phosphoenolpyruvate kinase, 12 μgm; a mixture of C^{12}-L-amino acids (Nirenberg and Matthaei, 1961), excluding the radioactive amino acid, 0.0125 of each; the indicated C^{14}-L-amino acid, 0.5-1.2 mμmoles; E. coli B ribosomes, 10 D_{260} units; and DEAE enzymes, 250 μg protein. As indicated, assay systems contained either no additional sRNA, 250 μg of normal B. cereus sRNA, or 250 μg of B. cereus sRNA containing 8-azaguanine (azaG). The mixtures were incubated at 37°C for 45 minutes in the absence and presenceof 100 μg of polyuridylic acid. Reactions were stopped with TCA, heated at 85°C for 30 minutes, washed by membrane filtration, and assayed for radioactivity as previously described (Friedman and Weinstein, 1964). The specific activities of the C^{14}-L-amino acids, obtained from Schwarz BioResearch, Inc., were in the range of 850 to 1000 μc/mg.

Weinstein, 1964). Table I indicates that the B. cereus azaG sRNA can also participate in these reactions. In the case of isoleucine, dihydrostreptomycin was added to enhance this effect (Davies et al., 1964; Friedman and Weinstein, 1964). The valine codon, like those of phenylalanine, leucine, and isoleucine, contains two uracil residues. Nevertheless, in the E. coli system, poly U does not code for valine. In the

presence of B. cereus azaG sRNA, poly U also failed to code for valine.

According to current codon assignments, most of the anticodon regions in the sRNAs for phenylalanine, leucine, isoleucine, and valine should not contain guanine and, therefore, the function of these sRNAs might not be influenced by the substitution of azaguanine for guanine. On the other hand, it seemed possible that the occurrence of this substitution in sRNAs whose anticodons are rich in guanine might result in miscoding. It is known that 8-azaguanine has a pK of 7.0 as compared to the pK of 9.3 for guanine. This suggests that, at neutral pH, 8-azaguanine would be more anionic than guanine and therefore might "base-pair" with uracil rather than cytosine (Grünberger and Sorm, 1963). Anticodons, therefore, which contain 8-azaguanine rather than guanine might fit codons containing uracil rather than cytosine. This possibility was examined by testing whether, in the presence of B. cereus azaG sRNA, poly U could be made to code for proline (CCC), alanine (CCG), or arginine (GCC). No miscoding of this type was observed (Table I).

Consistent with this negative result are subsequent studies which indicate that the coding properties of a copolymer containing uridylic and 8-azaguanylic acid are similar to that of a UG copolymer (Grünberger et al., 1965). This suggests that in messenger RNA, 8-azaguanine behaves like guanine with respect to anticodon interaction. This copolymer did not code for tyrosine (UUA), which it should have if 8-azaguanine behaved like adenine.

The above experiments indicate that B. cereus azaG sRNA can function in amino acid incorporation, and no evidence of miscoding was observed. On the other hand, when compared to normal B. cereus sRNA, a consistent, quantitative difference in the ability of the substituted sRNA to participate in the poly U-leucine reaction has been observed. This is illustrated in Table II.

TABLE II

EFFECT OF AZAGUANINE SUBSTITUTION IN sRNA ON LEUCINE-PHENYLALANINE AMBIGUITY

Amino Acid	sRNA	Counts/min incorporated			Ambiguity Ratio**
		No poly U	(+) poly U	Net*	
C^{14} phenyl-alanine	B. cereus	1093	13,399	12,306	
C^{14} leucine	B. cereus	1560	5,180	3,620	29.4 per cent
C^{14} phenyl-alanine	B. cereus azaG	888	15,892	15,004	
C^{14} leucine	B. cereus azaG	641	1,970	1,329	8.9 per cent

With the exception that the S-122 fraction (approximately 50 μg protein) was used in place of the DEAE enzymes, the assay system is the same as that described in Table I.

*Net = Counts/min incorporated in the presence of poly U minus counts/min incorporated in the absence of poly U.

**Ambiguity ratio = Net leucine incorporation X 100/net phenylalanine incorporation.

Normal B. cereus sRNA and B. cereus azaG sRNA gave fairly equivalent poly U-phenylalanine reactions. However, the poly U-leucine reaction obtained with the azaG sRNA is less than 50 per cent that obtained with normal B. cereus sRNA. Thus the ambiguity ratio obtained with normal B. cereus sRNA is 29.4 per cent, whereas with azaG sRNA, this ratio is only 8.9 per cent. Qualitatively similar results were obtained when the two sRNAs were compared over a range of 5 to 120 μg of sRNA per assay system.

Interpretation of the present studies is complicated by the fact that in the preparation of B. cereus azaG sRNA approximately 50 per cent of the material obtained probably represents normal sRNA synthesized prior to the addition of analog to the culture medium. It is therefore possible that only those molecules which do not contain 8-azaguanine participate in amino acid incorporation. For this reason, the present experiments were done in the range of sRNA limitation in an attempt to "force" the utiliza-

tion of all the added sRNA. The fact that, in the poly U-phenylalanine reaction, quantitatively similar results were obtained, with equal amounts of E. coli B sRNA, normal B. cereus sRNA, and B. cereus azaG sRNA, suggests that the bulk of the azaG sRNA did participate in the reaction. Further evidence for this is provided by the differences noted in the leucine-phenylalanine ratios obtained with normal B. cereus and azaG sRNA. Nevertheless, more direct evidence, of the type obtained with methyl-deficient sRNA (Peterkofsky et al., 1964; Starr, 1963; Littauer et al., 1963), is required to be certain that the azaG sRNA actually participates in accepting and transferring amino acids. If this proves to be the case, then it would appear that the substitution of 8-azaguanine in sRNA does not inactivate its function in protein synthesis. Consistent with these observations are recent studies by Levin (1965) indicating that azaG sRNA will accept amino acids. Levin and Litt (1964) obtained evidence, however, of an altered secondary structure in azaG sRNA. It is possible that this change in secondary structure relates, in an as yet unknown manner, to its lower activity in the poly U-leucine reaction. It is not excluded, therefore, that in vivo, where protein synthesis must occur with high precision, the toxic effects of this analog (Grünberger and Sorm, 1963) are due to a small change in the coding function of sRNA and/or mRNA.

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