matters arising

Substitution of guanine for a specific base in tRNA by extracts of Ehrlich ascites tumour cells

WE previously reported the partial purification of an enzyme from Ehrlich ascites tumour that catalysed the incorporation of GMP from GTP into specific tRNA molecules[1]. The active primer tRNAs for this reaction were found from *Escherichia coli*, Ehrlich ascites tumour, salcoma 180, and tissues of mice infected with Friend virus, but not from tissues of normal mice. Among the ten purified tRNAs of *E. coli*, only $tRNA_{II}^{Leu}$, $tRNA_{III}^{Ser}$, $tRNA_{I}^{Tyr}$ and $tRNA_{II}^{Tyr}$ were active as primers. These results suggested that, in mouse, primer RNAs are specific to tumours or tissues infected with tumour viruses. We thought that guanosine triphosphate was the actual substrate, because the reaction was neither inhibited by creatine phosphate and creatine phosphokinase, nor by inorganic phosphate, but inhibited by inorganic pyrophosphate.

More recently, however, we have found that the incorporation of radioisotope from ^{3}H-GTP into an acid insoluble fraction was not due to GTP but due to guanine contamination in the reaction mixture. It was shown by thin-layer chromatography on an Avicel SF cellulose plate that about 6% of radioactivity was accounted by ^{3}H-guanine. We have found that α-^{32}P-GTP was not incorporated in this reaction, and ^{14}C-GTP was not incorporated. It is very likely that ^{3}H-labelled GTP apparently decomposed to yield ^{3}H-guanine by self-decomposition due to radiation[2]. In fact, the enzymatic incorporation of guanine showed an absolute requirement for acceptor tRNA (Table 1), but no energy source, for example ATP, was required.

Purified *E. coli* tRNAs were tested as acceptors for guanylation by the enzyme (Table 1). $tRNA^{Tyr}$ and $tRNA^{Asp}$ were very active. Slightly active tRNAs, $tRNA^{Asn}$, $tRNA^{His}$, $tRNA_{III}^{Ser}$ $tRNA_{II}^{Leu}$ and $tRNA_{II}^{Val}$, were determinated with the activities of charging of the specific amino acid. $tRNA^{Asn}$ and $tRNA^{His}$ were not contaminated with $tRNA^{Asp}$ or $tRNA^{Tyr}$, but $tRNA_{III}^{Ser}$, $tRNA_{II}^{Leu}$, and $tRNA_{II}^{Val}$ were found to be appreciably contaminated with $tRNA^{Asp}$ or $tRNA^{Tyr}$. Thus probably only $tRNA^{Tyr}$, $tRNA^{Asp}$, $tRNA^{Asn}$, and $tRNA^{His}$ act as acceptors. It can be concluded that the primer activity of $tRNA_{III}^{Ser}$ and $tRNA_{II}^{Leu}$ in the preceding paper[1] is due to such contaminating tRNAs. Four tRNAs that were were active in guanylation contained in common the modified base Q in the first position of anticodon[3,4].

Table 1. Incorporation of ^{14}C-guanine into amino acid-specific *E. coli* tRNAs

E. coli tRNAs	(A_{260})	^{14}C-Gua incorporated (pmol)
$tRNA_{II}^{Tyr}$	0.006	2.0
	0.05	4.3
$tRNA^{Asp}$	0.05	4.7
	0.5	9.8
$tRNA^{Asn}$	0.05	0.3
	0.5	1.0
$tRNA^{His}$	0.05	0.5
	0.5	1.9
$tRNA_{III}^{Ser}$	0.05	0.5
	0.5	1.7
$tRNA_{II}^{Leu}$	0.05	0.2
$tRNA_{II}^{Val}$	0.5	0.7
$tRNA^{Phe}$	0.5	<0.1
$tRNA^{Glu}$	0.5	<0.1
$tRNA^{fMet}$	0.5	<0.1
-RNA		0

The enzyme was partially purified by the same procedure described by Itoh *et al*[1]. The standard reaction mixture contained in a total volume of 0.25 ml, 22 μmol of Tris–HCl, *p*H 8.0, 3.2 μmol of $MgCl_2$, 0.02 μmol of 2-mercaptoethanol, 10 nCi of ^{14}C-guanine (55 mCi mmol^{-1}), 30 μg of enzyme protein and each of the indicated tRNAs were added. After incubation at 36 °C for 20 min, the cold 5% trichloroacetic acid-insoluble materials were collected on membrane filters, and the radioactivity of the dried filters was counted by liquid scintilation counter. *E. coli* tRNAs were supplied by Dr S. Nishimura (National Cancer Research Institute).

After $tRNA_{II}^{Tyr}$ labelled with ^{14}C-guanine by the enzymatic reaction was isolated and treated with RNase T_2, it was fractionated by two dimensional cellulose thin layer chromatography. The radioactive product obtained by the treatment of ^{14}C-guanine incorporated $tRNA_{II}^{Tyr}$ with RNase T_1 was fractionated by two-dimensional PEI (polyethylenimine)-cellulose chromatography. The results obtained from these chromatograms and acceptor activities of tRNAs suggested that the enzyme catalysed the reaction to exchange the Q base for guanine at the first position of anticodon.

Farkas and his co-workers previously reported guanylation of tRNA in rabbit reticulocytes[6,7]. They showed that the guanylated tRNA of rabbit reticulocytes were $tRNA^{His}$ and $tRNA^{Asn}$ (ref. 8). Okada *et al.* recently studied this guanylation reaction in order to identify the exact position of guanylation and the guanylated species of tRNA. They showed that the Q base in the first position of the anticodon of *E. coli* tRNAs ($tRNA^{Tyr}$, $tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{His}$) was replaced by guanine by the guanylating enzyme from rabbit reticulocytes[9]. It was found that tRNAs isolated from Ehrlich ascites tumour and AH7974 rat ascites hepatoma were active as acceptor for guanylation with the enzyme from Ehrlich ascites tumour, but normal mouse and rat liver tRNAs were not active (ref. 1 and unpublished data). Kasai *et al.* reported that the Q* (a derivative of Q) content of liver tRNA of AH7974 rat ascites hepatoma was twofold greater than that of normal rat[10]. One of the major factor of a difference of guanylation activity among these tRNAs is probably the modification of nucleoside such as Q and Q*. Furthermore, a specific structure or sequence common to these tRNAs may be recognised by the enzyme. It will be interesting to examine the structure of tRNAs from tumour cells concerning the guanylation activity.

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