Hypothesis

Does the complex of aminoacyl-tRNA synthetases and tRNA-modifying enzymes prevent miscoding?

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Several aminoacyl-tRNA synthetases of higher eukaryotes have always been found as multienzyme complexes. There are indications that these complexes can be associated with some tRNA-modifying enzymes. The function of such complexes is unclear. I have noticed that 6 out of 7 aminoacyl-tRNA synthetases most commonly occurring in complexes correspond to a group of tRNAs which must always contain a modified U in the first position of their anticodons. A hypothesis is proposed according to which association of 6 aminoacyl-tRNA synthetases with U-modifying enzymes can protect a cell from miscoding.

Aminoacyl-tRNA synthetase *Multienzyme complex* *tRNA modification* *Wobble position* *Miscoding*

1. INTRODUCTION

Several aminoacyl-tRNA synthetases of higher eukaryotes, in contrast to those of prokaryotes, have been found as multienzyme complexes with M_r values of more than 10^6 (for reviews see [1,2]). There have been communications that besides aminoacyl-tRNA synthetases these complexes contain some tRNA-modifying enzymes such as tRNA methyltransferase [3–5] and tRNA sulfurtransferase [5,6]. Multienzyme complexes most commonly contain 7 out of 20 aminoacyl-tRNA synthetases, namely those specific for leucine, isoleucine, methionine, arginine, lysine, glutamine and glutamic acid [1,2]. Moreover, the only homogeneous complex which was purified in Waller's laboratory from different tissues of sheep and rabbit contained only the 7 mentioned aminoacyl-tRNA synthetases [7,8].

Two questions arise: what is the role of the multienzyme complex of aminoacyl-tRNA synthetases and tRNA-modifying enzymes and why are only the same 7 out of 20 aminoacyl-tRNA synthetases implicated?

2. HYPOTHESIS

From the table of the genetic code (fig.1) it can be seen that all 7 aminoacyl-tRNA synthetases of the complex have a common feature: they are specific for amino acids encoded by codons of the mixed families. (The group of 4 codons that have their first two nucleotides in common will be referred to as a codon family (for example, UUN or GUN, etc.; N is any nucleotide). The family will be referred to as 'mixed' if its codons code for different amino acids (or amino acid and terminator) and 'non-mixed' if all 4 codons code for the same amino acid.) Moreover, 6 out of the 7 aminoacyl-tRNA synthetases are specific for amino acids encoded by codons of the mixed families ending in A. tRNAs reading these codons must have U in the first position of the anticodon (wobble position).

On the other hand, in all known tRNAs reading the mixed families U in the wobble position is always modified [9]. This seems necessary to prevent miscoding. It has been shown for mitochondria [10–12] that the tRNA having an unmodified U in the wobble position can recognize 4 codons

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	U	C	A	G	
U	UUU Phe UUC UUA Leu UUG	UCU UCC UCA UCG Ser	UAU Tyr UAC UAA Ochre UAG Amber	UGU Cys UGC UGA Opal UGG Trp	U C A G
C	CUU CUC CUA CUG Leu	CCU CCC CCA CCG Pro	CAU His CAC CAA CAG Gln	CGU CGC CGA CGG Arg	U C A G
A	AUU AUC Ile AUA AUG Met	ACU ACC ACA ACG Thr	AAU Asn AAC AAA AAG Lys	AGU Ser AGC AGA AGG Arg	U C A G
G	GUU GUC GUA Val GUG	GCU GCC GCA GCG Ala	GAU Asp GAC GAA GAG Glu	GGU GGC GGA Gly GGG	U C A G

Fig.1. The genetic code. Amino acids corresponding to mixed family codons terminating in A are enframed. These are Leu, Ile, Glu, Gln, Lys, Arg, i.e., 6 out of 7 amino acids, whose aminoacyl-tRNA synthetases are most often encountered within the complex. The corresponding tRNAs must always contain a modified U in the wobble position.

(XXU, XXC, XXA, XXG) instead of two (XXA and XXG) as predicted by the wobble hypothesis. If U modified in a certain way is in the first anticodon position, this tRNA can recognize only two codons (XXA and XXG) or even only XXA. As a result, if the tRNA having U in the wobble position corresponds to the non-mixed family codons then, no matter whether the U is modified or not, this tRNA will recognize only the codons for its amino acid. If the tRNA having U in the wobble position corresponds to the mixed family codons, then in the case when U is not modified this tRNA apparently can recognize not only the codons for its amino acid (XXA or XXG) but also the codons for another amino acid (XXU or XXC), which results in miscoding. Therefore, the tRNAs corresponding to codons of the mixed families can be grouped as tRNAs in which U must be modified to prevent miscoding. It could not be a chance coincidence that all 6 aminoacyl-tRNA synthetases necessary for aminoacylation of such tRNAs are usually encountered in the form of multienzyme complexes.

Perhaps tRNA methyltransferase and tRNA sulfurtransferase associated with the aminoacyl-tRNA synthetase complex also deal with this group of tRNAs and modify U in the wobble position. Among the identified U derivatives in eukaryotic tRNAs corresponding to the mixed family codons the following can be encountered in the wobble position: 5-methoxycarbonylmethyl-2-thiouridine in $tRNA^{Lys}$ and $tRNA^{Glu}$, 2-thiouridine in $tRNA^{Glu}$ and 5-methoxycarbonylmethyluridine in $tRNA^{Arg}$ [9].

Can the tRNA-modifying enzymes associated with aminoacyl-tRNA synthetases carry out these modifications? It is noteworthy that incubation with tRNA of tRNA sulfurtransferase associated with aminoacyl-tRNA synthetases yielded only 2-thiouridine among the reaction products [5]. In tRNAs sequenced to date this modification is encountered only in the first position of the anticodon. The mechanism of 5-methoxycarbonylmethyluridine formation is unknown at present; it presumably includes several consecutive reactions which can require the tRNA methyltransferase activity.

Thus, the complex of aminoacyl-tRNA synthetases and tRNA-modifying enzymes contains all 6 aminoacyl-tRNA synthetases specific for tRNAs reading mixed family codons terminating in adenine and having a modified U in the wobble position; and, on the other hand, it contains the tRNA-modifying enzymes capable of carrying out relevant modification of the U.

This calls up an idea that the modification of the U in the first position of the anticodon in tRNAs reading the mixed family codons and the aminoacylation of such tRNAs are coupled in a cell. Coupling of modification and aminoacylation of definite tRNAs can protect a cell from miscoding caused by the ability of tRNAs containing an unmodified U in the wobble position to recognize 4 codons. If the tRNA interacts with a complex of aminoacyl-tRNA synthetases and tRNA-modifying enzymes it will be simultaneously aminoacylated and modified. This serves to prevent the appearance of an aminoacylated tRNA with an unmodified U in the first position of the anticodon; upon binding with the ribosome such a tRNA could cause incorrect incorporation of an amino acid into the protein. Thus, the complex of aminoacyl-tRNA synthetases and tRNA-modifying enzymes can act as a 'filter' for the ribosome rejecting aminoacyl-tRNAs containing an unmodified U in the wobble position.

3. DISCUSSION

The presented hypothesis explains why among 20 aminoacyl-tRNA synthetases only some are usually encountered within the complex: the complex should contain only those aminoacyl-tRNA synthetases whose tRNA must always have a modified U in the wobble position. As seen from the table of the genetic code (fig.1), these are aminoacyl-tRNA synthetases for leucine, isoleucine, lysine, arginine, glutamine and glutamic acid. Aminoacyl-tRNA synthetases for these amino acids are encountered more often than others within the complex [1,2] and are present in a homogeneous complex of 7 aminoacyl-tRNA synthetases isolated in Waller's laboratory [7,8].

The hypothesis also explains why tRNA-modifying enzymes are present in the complex with aminoacyl-tRNA synthetases. This is due to the need of coupling between aminoacylation and modification of tRNAs reading the codons of mixed families ending in purine. To prevent miscoding caused by unrestricted wobbling of the U in the wobble position of the corresponding tRNAs it must be modified. This modification must occur prior to or at least simultaneously with aminoacylation in order to preclude the entering of tRNA with unmodified U into the ribosome.

In prokaryotes this seems to be achieved due to large concentrations of tRNA-modifying enzymes. In eukaryotes the cell volume is 1000-times larger than in prokaryotes and the proteins of the translation apparatus have a tendency to compartmentation and complex formation [13]. Therefore, in the case of eukaryotes perhaps the assembling of tRNA-modifying enzymes in a complex with aminoacyl-tRNA synthetases whose tRNAs must have a modified U in the first position of the anticodon is more favourable than the increase of the concentration of tRNA-modifying enzymes.

The presence of a modified U in the first position of the anticodon has been demonstrated for the majority of cytoplasmic tRNAs corresponding to mixed family codons terminating in A, namely for $tRNA^{Leu}$, $tRNA^{Glu}$, $tRNA^{Gln}$, $tRNA^{Lys}$ and $tRNA^{Arg}$ [9]. In isoleucine tRNAs sequenced up to date there have been found no U derivatives in the first position of the anticodon but in all organisms studied, including mammals [14], there is a $tRNA^{Ile}$ reading only the AUA codon. Though this tRNA has not yet been studied, it is quite possible that it contains a modified U in the first position of the anticodon.

The presence of methionyl-tRNA synthetase within the complex can also be connected with the necessity of $tRNA_m^{Met}$ modification. In eukaryotic $tRNAs_m^{Met}$ (but not in $tRNA_i^{Met}$) cytosine in the first position of the anticodon is often methylated at ribose moiety. Though the role of this modification is unknown, perhaps just the necessity of cytosine methylation in the first position of the anticodon of $tRNA_m^{Met}$ somehow explains the presence of methionyl-tRNA synthetase in the complex with tRNA-modifying enzymes.

The suggested hypothesis does not contradict the data that the complex can contain more than 7 aminoacyl-tRNA synthetases [15] as well as other enzymes, e.g., specific ribonucleases [5] or elongation factors [16], as the aminoacyl-tRNA synthetase complex can also have other functions besides protection from miscoding.

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