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REVIEW ARTICLE

Multienzyme complex of aminoacyl-tRNA synthetases: an essence of being eukaryotic

Chi V. DANG*‡ and Chuan V. DANG†
*Cancer Research Institute, Moffitt 1282, University of California, San Francisco, CA 94143, U.S.A., and
†Department of Biochemistry, University of California, Riverside, CA 92521, U.S.A.

Introduction

The aminoacyl-tRNA synthetases are intriguing enzymes which are capable of catalysing the formation of aminoacyl-tRNAs (Schimmel & Söll, 1979; Ofengand, 1982) and of the dinucleotide Ap_4A which appears to be involved in DNA synthesis (Zamecnik, 1983; Weinmann-Dorsch *et al.*, 1984). Initial studies on high-M_r synthetase complexes were reviewed (Dang *et al.*, 1982*a*; Dang & Yang, 1982). At that time, it was known that eukaryotic aminoacyl-tRNA synthetases occur in high-M_r complexes, in contrast with the prokaryotic counterparts. However, the nature of these complexes was not well understood. During the past several years, the efforts of several laboratories have yielded new insights into the characteristics of both high-M_r and 'free' aminoacyl-tRNA synthetases. An essence of being eukaryotic becomes apparent when the properties of the mammalian aminoacyl-tRNA synthetases are compared with those of yeast and prokaryotes.

Evolution of the aminoacyl-tRNA synthetase structure

The prokaryotic aminoacyl-tRNA synthetases occur with native M_r values of 50000–300000 and with subunit structures of α, α_2, α_4 and $\alpha_2\beta_2$ (Schimmel & Söll, 1979; Ofengand, 1982). The subunit structures of subclasses of synthetases such as those specific for Ile, Leu, Met, and Val (Wetzel, 1978) or Gln, Glu, and Arg (Lapointe, 1982) are similar and probably reflect common evolutionary origin. Enzymes of the same subclass such as *Escherichia coli* Ile-tRNA and Met-tRNA synthetases have remarkable sequence homology (Webster *et al.*, 1984).

The prokaryotic aminoacyl-tRNA synthetases display a striking size polymorphism of the polypeptides, which may vary from about 300 to 1000 amino acids (Schimmel *et al.*, 1984). Limited proteolysis of the *E. coli* dimeric Met-tRNA synthetase (Cassio & Waller, 1971) and constructions of gene deletions of the *E. coli* tetrameric Ala-tRNA synthetase (Jasin *et al.*, 1983) indicate that the *C*-terminal sequences are required for oligomerization. The yeast Met-tRNA synthetase, which lacks the corresponding *C*-terminal segment of the *E. coli* enzyme, occurs as a monomer (Fasiolo *et al.*, 1985). Tyr-tRNA synthetase from *Bacillus stearothermophilus* dimerizes via a hydrophobic patch not localized to the *C*-terminus (Bhat *et al.*, 1982; Jones *et al.*, 1985). Oligomerization of the *E. coli* Ala-tRNA synthetase is required for direct repression of the transcription *in vitro* of its own structural gene (Putney & Schimmel, 1981). A core *N*-terminal domain carries the catalytic functions of the aminoacyl-tRNA synthetase (Jasin *et al.*, 1983; Schimmel *et al.*, 1984).

The yeast and higher eukaryotic aminoacyl-tRNA synthetases have similar subunit structures to the corresponding prokaryotic enzymes, but in contrast, the subunit sizes are significantly larger than those of prokaryotes (Dang & Dang, 1983*a*). Several studies indicate that acquisition of larger aminoacyl-tRNA synthetase subunit sizes reflects the phenomenon of intracellular compartmentation. Crude mixtures of yeast aminoacyl-tRNA synthetases displayed affinity for anionic matrix (immobilized heparin), whereas the corresponding *E. coli* enzymes lacked this feature (Cirakoglu & Waller, 1985*c*). The purified yeast Lys-tRNA synthetase (M_r 2×73000) lost affinity for negatively charged liposomes or immobilized heparin upon proteolytic conversion to an active dimer (M_r 2×65500) which was similar in size to the *E. coli* enzyme (M_r 2×65000). Lower eukaryotic aminoacyl-tRNA synthetases appear to acquire a binding domain which promotes compartmentation within the cytoplasm (Fig. 1). The occurrence of yeast Lys-tRNA synthetase in high-M_r form was likely to result from interaction of the enzyme with microsomal particles (Dimitrijevic & Godefroy-Colburn, 1974).

The mammalian aminoacyl-tRNA synthetases, similar to the yeast enzymes, also bound to polyanionic carriers such as immobilized high-M_r RNA (Alzhanova *et al.*, 1980) or heparin (Hradec & Dusek, 1980). In contrast with the yeast aminoacyl-tRNA synthetases, some of the mammalian enzymes bound to ribosomes (Ussery *et al.*, 1977; Mirande *et al.*, 1983*b*; Pailliez & Waller, 1984) and occurred in a multienzyme complex (Fig. 1) (Cirakoglu & Waller, 1985*b*; Cirakoglu *et al.*, 1985; Dang *et al.*, 1982*a*,*b*; Dang & Dang, 1983*b*, 1984; Deutscher, 1984; Kellermann *et al.*, 1982; Mirande *et al.*, 1982*b*,*c*; Siddiqui & Yang, 1985). The high-M_r complex has been purified from a variety of cell types (Cirakoglu *et al.*, 1985) and consists of 11 major polypeptides, eight of which have been identified with aminoacyl-tRNA synthetases (Cirakoglu & Waller, 1985*b*).

The purified sheep Leu-tRNA, Lys-tRNA (Cirakoglu & Waller, 1985*a*) and Ile-tRNA (Lazard *et al.*, 1985) synthetases from the high-M_r complex displayed remarkable hydrophobic properties which were initially noted with the purified rat liver Lys-tRNA synthetase (Johnson *et al.*, 1980). Limited proteolysis of the sheep Leu-tRNA or Lys-tRNA synthetase yielded fully active enzymes which lacked the hydrophobic properties (Cirakoglu & Waller, 1985*a*). The rat liver high-M_r complex was

Abbreviation used: Ap_4A, P^1,P^4-bis-(5′-adenosyl) tetraphosphate.
‡ To whom correspondence and reprint requests should be addressed.

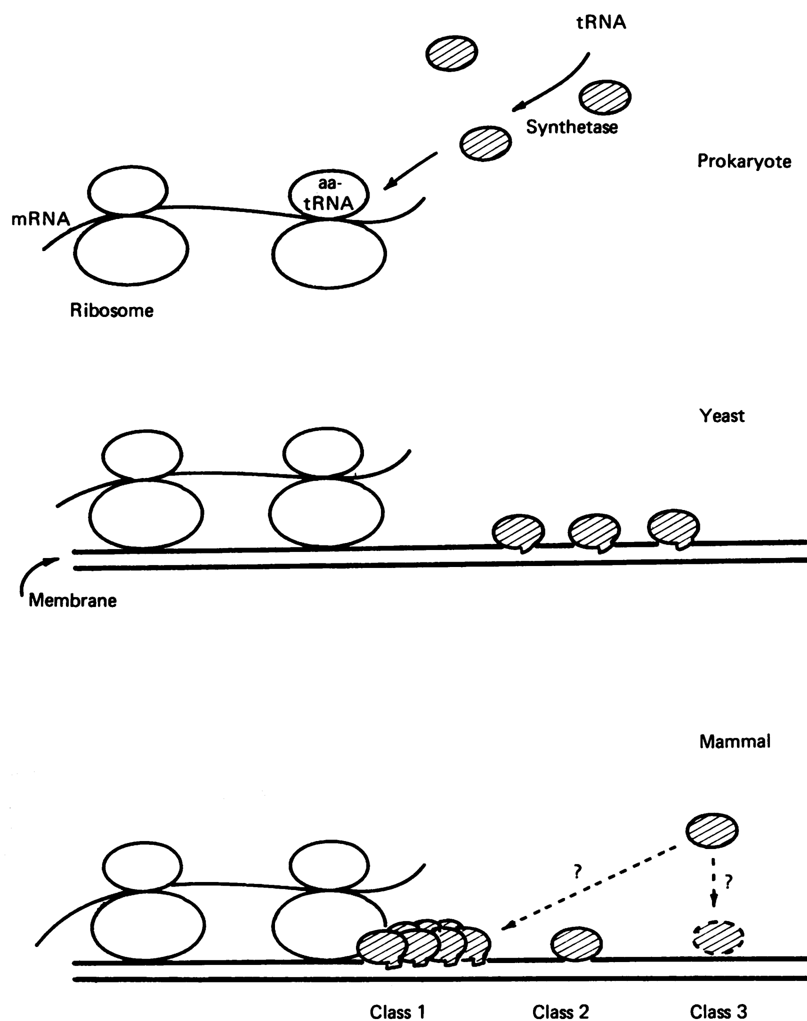

Fig. 1. Schematic comparison of the organization of prokaryotic and eukaryotic aminoacyl-tRNA synthetases

Top: the prokaryotic protein biosynthetic apparatus includes soluble ribosomes and aminoacyl-tRNA synthetases which catalyse the formation of aminoacyl-tRNA (aa-tRNA). Middle: some of the yeast synthetases acquire a domain which mediates membrane binding. Bottom: some mammalian aminoacyl-tRNA synthetases are found in membrane-bound multienzyme complexes (Class 1 enzymes) which are associated with ribosomes. Class 2 aminoacyl-tRNA synthetases may be membrane-bound or associated with the Class 1 enzymes. Class 3 enzymes are soluble *in vitro* and *in situ*, but they are occasionally associated with Class 1 enzymes. Whether Class 3 enzymes are membrane-bound is not known.

dissociated to smaller fragments by hydrophobic interaction chromatography (Dang & Yang, 1979) and by non-ionic detergents (Sihag & Deutscher, 1983) suggesting a role for hydrophobic interactions in holding the multienzyme complex together. The hydrophobic regions, which are likely to be the *C*-terminal domains, appear to be involved in the formation of high-M_r aminoacyl-tRNA synthetase multienzyme complexes.

Structure and composition of the multienzyme complex

The higher eukaryotic aminoacyl-tRNA synthetases may be classified into three classes (Table 1) according to size that is determined *in vitro*. Class 1 includes the enzymes found in the purified multienzyme high-M_r complex specific for Arg, Asp, Gln, Glu, Ile, Leu, Lys, Met, and Pro (Dang & Dang, 1983*a*; Cirakoglu *et al.*, 1985). Cys-tRNA and Val-tRNA synthetases, which may occur as homotypic complexes, belong to Class 2. Class 3 consists of enzymes specific for Ala, Asn, Gly, His, Phe, Ser, Thr, Trp, and Tyr which are frequently found unassociated with other aminoacyl-tRNA synthetases.

Although there appears to be some controversy over the number of aminoacyl-tRNA synthetases in the high-M_r complex (Berbec *et al.*, 1984; Dang & Dang, 1984; Deutscher, 1984; Walker *et al.*, 1983), direct evidence by immunoprecipitation gave the most convincing evidence. Using monospecific antibodies against Lys-tRNA or Met-tRNA synthetases, the Class 1 enzymes were coprecipitated from [^{35}S]methionine-labelled crude cell extracts or the purified multienzyme complex (Mirande *et al.*, 1982*a*, 1985*b*). Antibodies against Class 3 enzymes such as His-tRNA (Mathews & Bernstein, 1983; Tsui *et al.*, 1985) and Thr-tRNA (Mathews *et al.*, 1984) synthetases only precipitated the corresponding enzyme from crude cell extracts.

Multienzyme complexes larger than that containing nine synthetases have been postulated to occur *in vivo* (Berbec *et al.*, 1984; Walker *et al.*, 1983) based on

Table 1. Higher eukaryotic aminoacyl-tRNA synthetases

	Synthetase	10^{-3} × Subunit M_r	Subunit structure*	Source†	Reference
Class 1‡	Arg	74	2		Cirakoglu *et al.* (1985)
	Asp	57	2		
	Gln	95	1		
	Glu	158	1		
	Ile	139	1		
	Leu	129	1		
	Lys	77	2		
	Met	103	1		Dang *et al.* (1983)
	Pro	?	?		
Class 2§	Cys	120	α_2 or α_4		Pan *et al.* (1976)
	Val	110	α	Chick embryo	Bölöni *et al.* (1978)
Class 3‖	Ala	110	?	HeLa	Bunn *et al.* (1986)
	Asn	56	?	CHO	Cirullo & Wasmuth (1984)
	Gly	?	?		
	His	50	α_2		Yang *et al.* (1984)
	Phe	69,75	$\alpha_2\beta_2$		Tscherne *et al.* (1973)
	Ser	87	α_2	Bovine liver	Mizutani *et al.* (1984)
	Thr	85	α_2		Dignam *et al.* (1980)
	Trp	60	α_2	Bovine pancreas	Favorova *et al.* (1978)
	Tyr	65	α_2		Deak & Denes (1978)

* Stoichiometry is given for the Class 1 enzymes.
† Unless otherwise indicated, the enzymes are from rat liver. CHO, Chinese hamster ovary cells.
‡ Class 1 enzymes occur in a multienzyme complex, $M_r \simeq 10^6$, $s_{20,w} \simeq 18$ S.
§ Class 2 enzymes may be homotypic complexes or loosely associated with Class 1 enzymes.
‖ Class 3 enzymes are usually found as unassociated forms *in vitro*.

observations of gel permeation chromatography of crude cell extract. Although the occurrence of larger complexes is plausible, immunoprecipitation experiments indicate that only nine synthetases occur in the high-M_r complex (Mirande *et al.*, 1985*b*). It cannot be ruled out that the antibodies used were unable to interact with larger complexes due to steric hindrance. Hence the Class 2 or Class 3 enzymes may possibly interact with the high-M_r Class 1 enzymes. Reconstitution experiments using the purified high-M_r complex and purified Class 2 and Class 3 enzymes may give insight into the higher organization of the aminoacyl-tRNA synthetases. Until additional evidence becomes available, the higher eukaryotic aminoacyl-tRNA synthetases can be reasonably classified according to Table 1.

The mammalian aminoacyl-tRNA synthetase multi-enzyme complex consists of 11 major polypeptides, eight of which have been identified with the Class 1 enzymes, except for Pro-tRNA synthetase (Cirakoglu *et al.*, 1985). Pro-tRNA synthetase may be loosely bound to the core complex (Fig. 2) (Dang & Yang, 1979). The aminoacyl-tRNA synthetases occur with simple stoichiometric ratios within the high-M_r complex (Fig. 2) (Johnson & Yang, 1981; Mirande *et al.*, 1982*b,c*). The purified complex has a native $M_r \simeq 10^6$ and $s_{20,w} \simeq 18$ S, suggesting a hydrodynamically open structure (Dang & Dang, 1984). Rat liver Lys-tRNA and Arg-tRNA synthetases contain carbohydrates (Dang *et al.*, 1982*b*). Other putative non-protein components of the complex such as lipids or non-tRNA polynucleotides remain to be studied with the purified high-M_r complex.

The ultrastructure of the high-M_r complex was misidentified with cylindrical structures (11.0 nm × 15.0 nm) which were most likely particles involved in repression of mRNA translation (Harris, 1983; Schmid *et al.*, 1984; Dang, 1984). The electron microscopic ultrastructure of the purified rabbit reticulocycte high-M_r complex is a globular mass consisting of about eight spherical subunits (8.0–10.0 nm) loosely held together (Gulik & Orsini, 1984). Treatment with crosslinking agents resulted in more compact ovoid structures with maximum dimension 35.0 nm.

Experiments *in situ* with cultured cells provided another approach to analyse the composition of the high-M_r complex. The aminoacyl-tRNA synthetase multienzyme complex was localized to the endoplasmic reticulum of cultured cells by immunofluorescent microscopy using anti-(Met-tRNA synthetase) antibody (Dang *et al.*, 1983). The complex was associated with the detergent-insoluble components of the rough endoplasmic reticulum. This observation was subsequently substantiated (Mirande *et al.*, 1985*a*). About 50% of Met-tRNA synthetase activity remained associated with the detergent-insoluble cytoskeletal framework. In contast, a Class 3 enzyme, Ser-tRNA synthetase, was totally soluble in the presence of Triton X-100. Similarly, rat hepatocyte His-tRNA synthetase was unassociated with the cytomatrix or the high-M_r complex *in situ* (Dang *et al.*, 1986). The anti-Jo1 (His-tRNA synthetase) autoantibody stained the hepatocyte cytoplasm in a diffuse and granular pattern. These studies indicate that the multienzyme amnoacyl-tRNA synthetase complex is partially associated with the cytomatrix via detergent-insoluble endoplasmic reticulum components. The Class

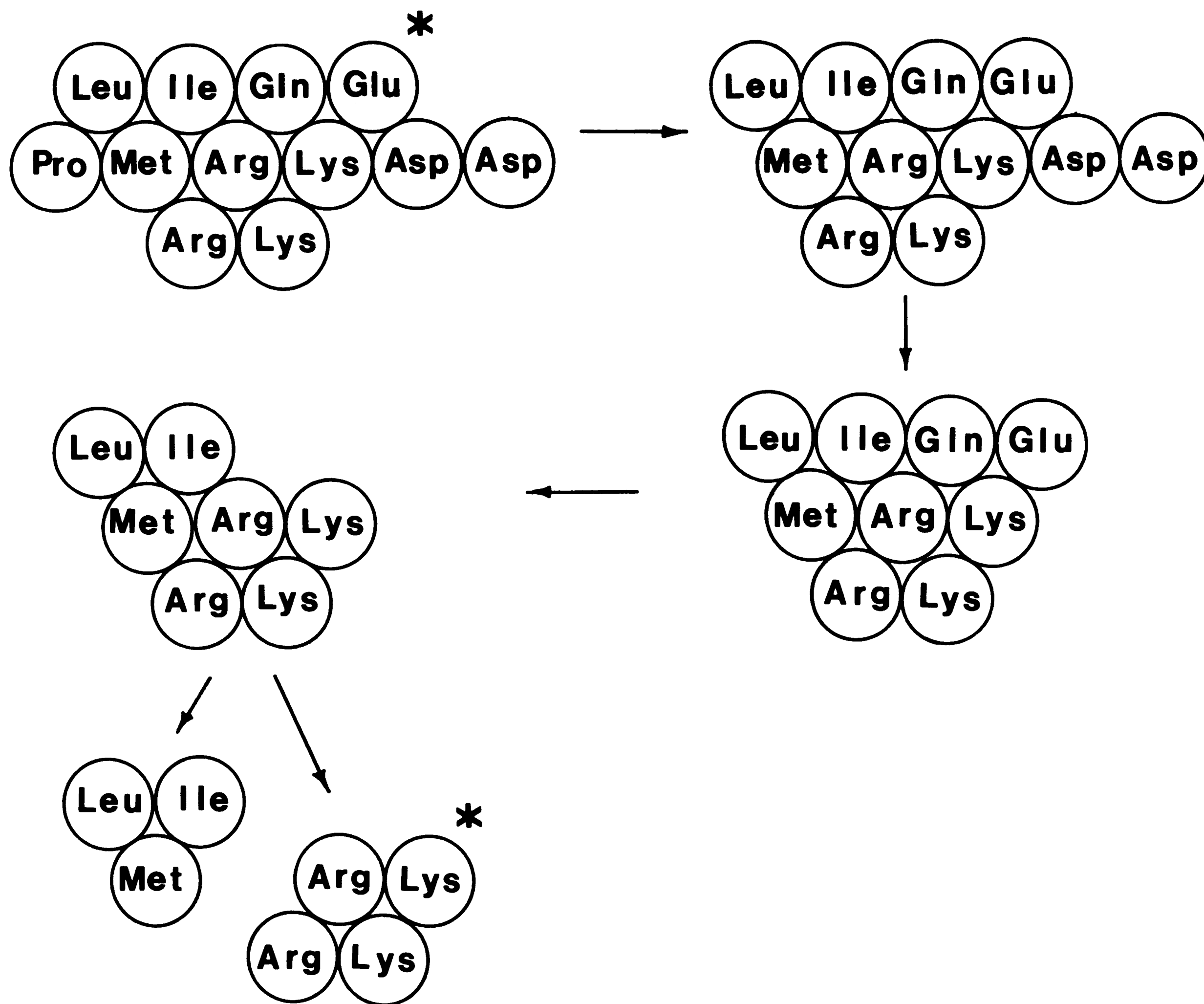

Fig. 2. Scheme of the structural organization of the aminoacyl-tRNA synthetase multienzyme complex

The associations between the aminoacyl-tRNA synthetases depicted are based upon the composition of complexes which have been either isolated or purified (*). The stoichiometry of the synthetase subunits is shown except for Pro-tRNA synthetase, the polypeptide of which has not been identified. The compositions of complexes which have not been purified are tentative. Other non-synthetase components of the complexes are not shown.

3 enzymes are either unassociated or loosely associated with the high-M_r aminoacyl-tRNA synthetase complex and/or the cytomatrix.

Functional significance of aminoacyl-tRNA synthetase complex

The functional significance of the high-M_r aminoacyl-tRNA synthetase complex remains poorly understood, even though the structural organization is now much better defined. The enzymes in the multienzyme complex specific for Arg, Asp, Gln, Glu, Ile, Leu, Lys, Met, and Pro do not appear to share any common underlying biochemical principles. There is no correlation with essential amino acids or chemical classes of amino acids.

Six of the nine aminoacyl-tRNA synthetases, excluding the Asp, Met, and Pro enzymes, may be involved in assuring translation fidelity through complex formation (Ryazanov, 1984). These enzymes recognize amino acids which are coded by codons in shared boxes of the genetic code. Misrecognition of the third codon nucleotide will result in amino acid substitutions. Hence, the association of aminoacyl-tRNA synthetases with tRNA modification enzymes (Agris *et al.*, 1983) is proposed to assure modification of the first nucleotide of the tRNA anticodon prior to participation of tRNAs in translation. The evidence for the association between aminoacyl-tRNA synthetases and tRNA sulphurtransferase or tRNA methyltransferase is indirect; thus further studies are necessary to substantiate this hypothesis.

Complex formation reduced the thermal lability of Leu-tRNA (Hampel *et al.*, 1984) and Lys-tRNA (Dang, 1982) synthetases. However, a small (60 kDa) form of rat liver Arg-tRNA synthetase was less labile than the complexed enzyme (Deutscher & Ni, 1982; Vellekamp *et al.*, 1985). This 60 kDa enzyme was a proteolytic product of the 72 kDa complexed Arg-tRNA synthetase. Thermal lability of the latter form isolated from the multienzyme complex was not examined. Changes in thermal lability by complex formation is not a function in itself, but rather it is a manifestation of protein–protein interaction within the multienzyme complex.

The temperature-sensitive Chinese hamster ovary (CHO) cells mutant tsH1 was shown to lack the high-M_r Leu-tRNA synthetase activity which is thermolabile (Hampel *et al.*, 1978, 1984). Studies with other thermolabile aminoacyl-tRNA synthetase mutants (Gln-2, His-1, and Lys-101) suggested that high levels of extracellular amino acids were needed to protect mutants which lacked the high-M_r complex against thermal death (Hampel *et al.*, 1984; Pahuski *et al.*, 1983). These results must be cautiously interpreted, since the tsH1 Leu-tRNA synthetase CHO mutant was recently shown to contain the Leu-tRNA synthetase polypeptide in the high-M_r form. This was demonstrated by immunoprecipitates of the complex from crude cell extract and purification of the mutant CHO high-M_r complex (Mirande *et al.*, 1985*b*).

The complexed and free aminoacyl-tRNA synthetases appeared to have similar substrate affinities as determined by steady-state kinetics in most instances (Dang *et al.*, 1985; Mirande *et al.*, 1983*a*; Vellekamp *et al.*, 1985). Simultaneous aminoacylation reactions catalysed by the complex occurred independently (Dang *et al.*, 1985; Mirande *et al.*, 1983*a*). The aminoacyl-tRNA synthetase multienzyme complex is unique in that it catalyses parallel reactions that do not appear to affect each other.

The catalytic constant for aminoacylation by the free rat liver Lys-tRNA synthetase was 2-fold higher than for the complexed enzyme (Wahab & Yang, 1985*a*). In addition, Ap_4A production by the free enzyme was 6-fold higher than for the high-M_r Lys-tRNA synthetase (Wahab & Yang, 1985*b*). The kinetic mechanism for Ap_4A synthesis by the free Lys-tRNA synthetase was via random substrate binding, whereas the complexed enzyme bound substrates in an ordered fashion (Wahab & Yang, 1985*b*). Perturbation of the *E. coli* Ala-tRNA synthetase polypeptide at a site distal to the catalytic site by gene deletion led to altered catalytic function (Jasin *et al.*, 1984). Thus synthetase catalytic functions may be modulated by complex formation, but these changes are significant only if there is physiological interconversion between complexed and free enzymes. Regenerating rat liver Pro-tRNA synthetase displayed a shift to the high-M_r form as compared with normal rat liver (Del Monte *et al.*, 1986). More heterogeneous size distribution of aminoacyl-tRNA synthetases have been reported in hepatoma cells as compared with normal hepatocytes, but the significance of this difference is unknown (Perego *et al.*, 1982).

Some eukaryotic aminoacyl-tRNA synthetases, like their prokaryotic counterparts, are capable of synthesizing Ap_4A through the following mechanism (Blanquet *et al.*, 1983; Zamecnik, 1983):

$$E + Xaa + ATP \rightarrow E \cdot Xaa\text{–}AMP + PP_i$$
$$E \cdot Xaa\text{–}AMP + ATP \rightarrow E + Xaa + Ap_4A$$

where E is the enzyme, Xaa is amino acid and PP_i is pyrophosphate. The sheep Phe-tRNA and Lys-tRNA synthetases are capable of Ap_4A synthesis which was significantly stimulated by Zn^{2+} (Brevet *et al.*, 1982). The aminoacylation activities were concurrently inhibited by Zn^{2+}. The free Lys-tRNA synthetase from rat liver was 6-fold more active than the complexed enzyme in Ap_4A synthesis, which was in part due to a higher sensitivity to Zn^{2+} (Wahab & Yang, 1985*a*). An alternative mechanism of Ap_4A synthesis by rat liver Lys-tRNA synthetase has been proposed (Hilderman, 1983) which does not require the presence of amino acid. This has not been reproducible (Wahab & Yang, 1985*b*).

Ap_4A appears to play a role in DNA synthesis and cell proliferation (Weinmann-Dorsch *et al.*, 1984; Zamecnik, 1983). It may also be involved in cellular stress response such as with heat-shock (Brevet *et al.*, 1985; Guedon *et al.*, 1985; Varshavsky, 1983). The control of Ap_4A production by the aminoacyl-tRNA synthetases is not yet understood and the intracellular Ap_4A level is also affected by Ap_4A hydrolases. Elucidation of the control of intracellular Ap_4A levels will require physiological studies using cultured cells to measure Zn^{2+} fluxes, aminoacylation, and Ap_4A synthetase and hydrolase activities (Grummt *et al.*, 1986). Such studies suggested that Zn^{2+} enhanced cellular Ap_4A synthesis and DNA synthesis, but inhibited Ap_4A hydrolase activity.

Indirect evidence from investigations with rat liver suggested that the high-M_r aminoacyl-tRNA synthetase complex was phosphorylated *in vivo* (Damuni *et al.*, 1982). This was associated with a decrease in aminoacyl-tRNA synthetase activities. The study was not reproducible (Mirande *et al.*, 1985*a*). In addition, attempts to examine phosphorylation directly by immunoprecipitation, gel electrophoresis, and autoradiography were unsuccessful. Phosphorylation of Thr-tRNA (Gerken & Arfin, 1984*b*) and His-tRNA (Mathews & Bernstein, 1983; Gerken *et al.*, 1986) synthetases occurred in cultured cells, but the significance is unknown.

The purified rabbit reticulocyte aminoacyl-tRNA synthetase complex was phosphorylated by casein kinase I *in vitro* as demonstrated by gel electrophoresis and autoradiography (Pendergast & Traugh, 1985). Phosphorylation of the polypeptides corresponding to enzymes specific for Glu, Ile, Lys, and Met was accompanied by a reduction in the aminoacylation activities of these enzymes. It was suggested that casein kinase I may be a component of the high-M_r complex. Additional studies are needed to demonstrate the physiological phosphorylation of the aminoacyl-tRNA synthetase multienzyme complex, since there are clearly controversies over whether phosphorylation of the high-M_r complex actually occurs *in vivo*.

Autoantibodies to aminoacyl-tRNA synthetases

Autoantibodies to aminoacyl-tRNA synthetases appear to be the hallmark of polymyositis, an autoimmune inflammatory muscle disease of unknown cause. The anti-Jo1 antibody, which occurs in 30% of polymyositis patients, inhibited His-tRNA synthetase from various mammalian sources (Mathews & Bernstein, 1983; Yang *et al.*, 1984; Tsui *et al.*, 1985; Dang *et al.*, 1986). The autoantibody precipitated a 50 kDa polypeptide from [^{35}S]methionine-labelled cell extracts (Mathews & Bernstein, 1983; Tsui *et al.*, 1985). Immunoaffinity purification using the anti-Jo1 antibody yielded a rat liver His-tRNA synthetase with a subunit M_r of 50000 (erroneously determined to be 64000; Yang *et al.*, 1984) and native $M_r \simeq 120000$.

In contrast with previous reports of nuclear localization, the Jo1 antigen or His-tRNA synthetase appeared to be exclusively cytoplasmic and unassociated with the detergent-insoluble cytomatrix (Dang *et al.*, 1986). The cDNA for CHO His-tRNA synthetase was molecularly cloned from histidinol-resistant mutants, in which the His-tRNA synthetase gene was amplified (Tsui *et al.*,

1985). This development will be of great importance in elucidating the pathogenesis of the anti-Jo1 antibody.

Autoantibodies against Thr-tRNA and Ala-tRNA synthetases also occurred at lower frequencies than the anti-Jo1 antibody in polymyositis patients (Mathews *et al.*, 1984; Bunn *et al.*, 1986). It may be possible to clone the Thr-tRNA synthetase gene from a borrelidin-resistant CHO mutant which overproduces the enzyme (Gerken & Arfin, 1984*a*).

Viruses have been implicated as the causative agents in polymyositis. Certain animal and viral RNAs bind to aminoacyl-tRNA synthetases through tRNA-like 3′-end conformations (Florentz *et al.*, 1984; Joshi *et al.*, 1983). In fact, these RNAs are aminoacylatable. Evidence for such viral RNAs in polymyositis tissue is lacking, but it is suggested that the autoantibodies result from an altered immunogenicity of the aminoacyl-tRNA synthetase bound to a viral RNA which is released from damaged cells (Mathews & Bernstein, 1983). It is noteworthy that the myositis autoantibodies, heretofore discovered, are directed at the Class 3 aminoacyl-tRNA synthetases. Perhaps the soluble nature of these enzymes allows for their release from damaged cells, in contrast with the bound Class 1 enzymes. This may be sufficient in the absence of viral RNA to induce autoantibody formation.

Conclusion

In higher eukaryotes, the aminoacyl-tRNA synthetases specific for Arg, Asp, Gln, Glu, Ile, Leu, Lys, Met, and Pro form a multienzyme complex which can be purified from different sources to homogeneity. The other synthetases may be loosely bound to the core aminoacyl-tRNA synthetase complex, but solid evidence for this is still lacking. These other 'free' enzymes may be compartmentalized in the cytoplasm by binding to intracellular membranes. These features are clearly distinct from the corresponding enzymes from prokaryotes such as *E. coli*. The high-M_r complex is not found in yeast, but the yeast synthetases appear to be membrane-bound.

Complex formation of the aminoacyl-tRNA synthetases appears to modulate certain catalytic functions as studied *in vitro* by comparing the free and complexed enzymes. The physiological significance of these differences is not understood. Evidence for the interconversion of free to complexed enzymes as a mode of metabolic control is lacking. Although the physiological significance of the aminoacyl-tRNA synthetase multienzyme complex is still elusive, it seems reasonable to speculate that metabolic compartmentation by complex formation is the function for these enzyme clusters.

Autoantibodies to aminoacyl-tRNA synthetases appear to be the hallmark of polymyositis, an autoimmune muscle disease. Whether these autoantibodies play a role in the pathogenesis of the disease or are epiphenomena is unknown. The antigens to the autoantibodies appear to belong only to the class of enzymes which are not in the high-M_r complex. Perhaps the soluble nature of these enzymes plays a role in the generation of the autoantibodies.

The aminoacyl-tRNA synthetases are intriguing enzymes that are more versatile than expected, as they are capable of aminoacylation activities and of production of Ap_4A, a dinucleotide that is proposed to be the intracellular signal that links the events of protein synthesis with those of DNA synthesis.

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