Hypothesis

A model for the structural organization of aminoacyl-tRNA synthetases in mammalian cells

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1. INTRODUCTION

The occurrence of high-M_r complexes of aminoacyl-tRNA synthetases from animal cells has been extensively documented over the past 15 years (reviews [1,2]). However, because of the disparate results reported concerning the composition and properties of these multienzyme complexes, their biological significance and structural organization have been difficult to comprehend.

A general model for the organization of these complexes was recently proposed by Deutscher [3]. The model supposes that the aminoacyl-tRNA synthetases from animal sources are amphipathic proteins composed of a hydrophilic catalytic domain and a hydrophobic domain that participates in complex formation. The suggestion is made that the enzymes are held together by a core composed of the hydrophobic extensions present on the individual proteins, with their catalytic centers exposed to the environment. Moreover, the possibility is considered that in vivo, the aminoacyl-tRNA synthetases may be associated with membranes [3]. Although this model may account for many of the findings reported in the literature, it fails to provide a satisfactory explanation for the general observation that several of the 20 aminoacyl-tRNA synthetases are, at best, only encountered as components of highly fragile supra-molecular assemblies, but never as part of stable complexes amenable to purification by conventional procedures.

The object of this paper is to propose an alternative model for the structural organization of aminoacyl-tRNA synthetases in mammalian cells, which accommodates the observed differences in behaviour among these 20 enzymes. Since the published data pertaining to these multienzyme complexes have been recently compiled [4] and reviewed [1,2], the model will be presented with reference to these reviews, citing only a few other relevant and recent reports.

2. CHARACTERISTICS OF AMINOACYL-tRNA SYNTHETASE COMPLEXES ISOLATED FROM ANIMAL CELLS

Several laboratories described large complexes comprising all or most of the 20 aminoacyl-tRNA synthetases, often containing other components as well, such as tRNA, lipids, elongation factors and tRNA modification enzymes (tabulation in [4]). Such fragile complexes were generally observed in extracts from cells lysed under gentle conditions, at

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low ionic strength. Attempts to purify these aggregates invariably led to breakdown into smaller 'core' complexes, with concomitant release of certain aminoacyl-tRNA synthetases as 'free' enzymes. It follows that there has been no experimental evidence to support the view that all 20 aminoacyl-tRNA synthetases may be physically associated within one large multienzyme complex. However, at the least, these findings indicate that all of the aminoacyl-tRNA synthetases possess the aptitude to associate with other components, albeit with different affinities.

Hardesty and co-workers [5] examined the subcellular distribution of 19 aminoacyl-tRNA synthetases in several eukaryotic cells (rabbit reticulocytes, mouse liver and embryo, chicken embryo, Friend leukemia cells and HeLa cells) under standard conditions of sample preparation. Sucrose gradient centrifugation of the post-ribosomal supernatant from each of these cell types showed that 10 aminoacyl-tRNA synthetases, specific for Arg, Asp, Glu, Gln, Ile, Leu, Lys, Met, Pro and Val, henceforth referred to as class I enzymes, sedimented mainly in the 18–25 S region of the gradient, while 9 others, specific for Ala, Asn, Cys, Gly, His, Phe, Ser, Thr and Tyr (class II) were present predominantly in the 4–9 S region of the gradient. Essentially similar sedimentation patterns were reported for the aminoacyl-tRNA synthetases from Chinese hamster ovary (CHO) cells [6] and rat skeletal muscle and liver [7].

Several laboratories have reported the purification of high-M_r complexes from different sources, using conventional fractionation procedures (tabulation in [1,3,4]). Although these complexes contained a variable number of aminoacyl-tRNA synthetases, it is noteworthy that the enzymes present invariably belonged to class I. Recent progress in purification procedures has allowed us to isolate an apparently ubiquitous multienzyme complex from CHO cells [8], rabbit liver and reticulocytes [9] as well as rat liver [10]. Each of these highly purified complexes contained the same 9 aminoacyl-tRNA synthetases corresponding to class I, specific for Arg, Asp, Glu, Gln, Ile, Leu, Lys, Met and Pro. Moreover, SDS-polyacrylamide gel electrophoresis revealed virtually identical polypeptide patterns composed of 12 components, 8 of which were assigned to constituent aminoacyl-tRNA synthetases (fig.1). A complex of very similar composition was also purified from sheep liver [9]. It differed from the above essentially by the absence of prolyl-tRNA synthetase [8], and by the presence of glutamyl-tRNA synthetase as a polypeptide of M_r 85000 instead of M_r 150000 [11], possibly a consequence of uncontrolled proteolysis. Furthermore, in the case of CHO cells, we showed that when the crude extract from metabolically [^{35}S]methionine-labeled cells was subjected to immunoprecipitation with antibodies directed to the purified complex from sheep liver,

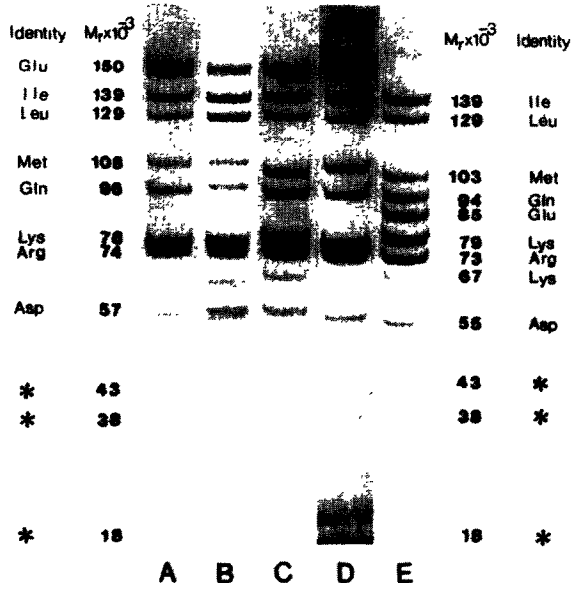

Fig.1. Polypeptide compositions of the purified aminoacyl-tRNA synthetase complexes from various animal species. The purified complexes from rabbit liver (A) and reticulocytes (B) [9], rat liver (C) [10], CHO cells (D) [8] and sheep liver (E) [9] were subjected to SDS-polyacrylamide gel electrophoresis, followed by staining with Coomassie brilliant blue R250. The apparent M_r and aminoacyl-tRNA synthetase assignment of the polypeptides from rabbit liver (left side) and sheep liver (right side) are from [11]. It was verified, by the protein blotting procedure, that the polypeptides from rabbit, rat and CHO cells which display identical or similar mobilities correspond to the same aminoacyl-tRNA synthetase [8,10]. Unidentified polypeptides are designated by an asterisk.

or to its homogeneous methionyl- or lysyl-tRNA synthetase components, the polypeptide compositions of the immunoprecipitates recovered were virtually indistinguishable from that of the complex purified by conventional procedures [8]. This not only demonstrates that the polypeptide components which characterize these purified complexes are associated within the same particle, but also constitutes the strongest evidence so far in support of the existence of this ubiquitous complex as a discrete, biologically relevant entity. In particular, this result seems difficult to reconcile with the often expressed view (e.g., [12]) that the purified stable complexes represent 'core' structures arising from dissociation of the more loosely bound aminoacyl-tRNA synthetases from a single large multienzyme complex comprising each of these enzymes. Further evidence for the distinction between class I and II enzymes came from studies in which the size distribution of each of the 20 aminoacyl-tRNA synthetases in the extract from CHO cells was examined by gel filtration on Bio-Gel A-5m equilibrated in 100 mM phosphate buffer [8]. Nine aminoacyl-tRNA synthetases, the same as those found in the purified complex, were co-eluted as a peak corresponding to an apparent M_r of $\sim 1.2 \times 10^6$. Seven of these enzymes occurred exclusively as high-M_r entities, only arginyl- and aspartyl-tRNA synthetase giving rise to an additional, low-M_r form representing about 25% of the total activity recovered. Of the 11 remaining aminoacyl-tRNA synthetases, all except valyl-tRNA synthetase were eluted as single peaks corresponding to apparent M_r values characteristic of 'free' enzymes, ranging from about 100000 for tryptophanyl-tRNA synthetase, to about 250000 for phenylalanyl- and cysteinyl-tRNA synthetases. Valyl-tRNA synthetase was the only other enzyme which occurred as a high-M_r entity, the size of which was distinctly lower than that of the other high-M_r enzymes. The latter enzyme has not been characterized to date, and will thus not be discussed further.

That hydrophobic interactions may play a prominent role in the assembly of the stable multienzyme complexes was strongly suggested by indirect evidence, such as the partial destabilization of the multienzyme complex from rat liver by hydrophobic interaction chromatography [13] or by the synergistic action of neutral detergents and chaotropic salts [14]. Direct evidence was recently obtained by showing that leucyl- and lysyl-tRNA synthetases [15], as well as isoleucyl-tRNA synthetase (Lazard, Mirande and Waller, submitted), derived from the complex from sheep liver and purified to homogeneity, displayed markedly hydrophobic properties. Moreover, in the case of lysyl-tRNA synthetase, it was shown that a fully active proteolytically modified form, similar in size to the corresponding prokaryotic enzyme, had lost the hydrophobic property [15], establishing the existence of a hydrophobic domain distinct from the catalytic domain, as was proposed in [3]. Furthermore, hydrophobic interaction chromatography of the purified complex from sheep liver on hexyl-agarose showed that leucyl- and lysyl-tRNA synthetases were the least hydrophobic components of this complex [15].

3. MODEL FOR THE STRUCTURAL ORGANIZATION OF MAMMALIAN AMINOACYL-tRNA SYNTHETASES IN VIVO

In the light of the foregoing considerations, we propose that the class I aminoacyl-tRNA synthetases from animal cells have acquired hydrophobic properties, leading to the integration of 9 of them into an ubiquitous, structurally stable multienzyme complex. We further assume that the class II enzymes have not acquired hydrophobic properties and thus are not implicated in the formation of stable complexes. Several arguments support the latter contention. Firstly, class II enzymes have never been found as components of purified, stable complexes (tabulation in [1,3,4]). Secondly, several of these enzymes which have been purified to homogeneity from various animal cells (seryl- [16], threonyl- [17], tryptophanyl- [18] and histidyl-tRNA synthetases [19]) display properties characteristic of hydrophilic enzymes. Thirdly, homogeneous tryptophanyl-tRNA synthetase from beef pancreas, which is not a constituent of stable complexes, does not bind to phenyl-Sepharose in conditions in which leucyl-tRNA synthetase, a component of the stable multienzyme complex from sheep liver, displays strong affinity for this hydrophobic support [15].

It remains that class II enzymes do possess the capacity to associate with other components, albeit

weakly, as demonstrated by the isolation of fragile supra-molecular aggregates containing all or most of the aminoacyl-tRNA synthetases [4]. Moreover, while the functional significance of these assemblies is not understood in any detail, it has been generally considered [1–4] that they may be representative of a supra-molecular organization of the whole protein synthetic machinery in vivo. This implicitly assumes that each of the 20 aminoacyl-tRNA synthetases must be involved in this organization. Our model for the structural organization of aminoacyl-tRNA synthetases within the cytoplasm of animal cells proposes that all aminoacyl-tRNA synthetases, including the class I enzymes pre-assembled as a stable multienzyme complex through hydrophobic interactions, are associated with cellular components at or near the site of protein synthesis, by electrostatic interactions.

Spirin and co-workers (review [20]) observed that in higher eukaryotes, a variety of proteins involved in protein biosynthesis, including initiation and elongation factors [21,22] as well as varying proportions of each of the 20 aminoacyl-tRNA synthetases [23,24], displayed the property of binding to immobilized ribosomal RNA or synthetic polynucleotides in vitro. The finding that in the same conditions, the corresponding proteins from prokaryotes did not display this property led to the postulate that the RNA-binding property was an evolutionary acquisition of those eukaryotic proteins, to ensure their confinement at the site of utilization, in response to the increased cellular volume [25]. Later studies showed that immobilized heparin was equally effective in selectively retaining those proteins [26,27], suggesting that the polyanionic character alone was responsible for binding to these carriers. We recently showed [28] that the aminoacyl-tRNA synthetases from the lower eukaryote yeast, like the corresponding enzymes from animal cells but unlike those of prokaryotic origin, bind to polyanionic carriers in vitro, through ionic interactions. The general observation that lower eukaryotic aminoacyl-tRNA synthetases invariably possess longer polypeptide chains than do their prokaryotic homologues [29] suggested a possible relationship between chain elongation and the capacity to bind to polyanionic supports. This correlation was clearly established in the case of yeast lysyl-tRNA synthetase, by showing that a proteolytically derived fully active form of the enzyme, similar in size to the corresponding prokaryotic enzyme, had lost the ability to bind to polyanionic supports [28]. Arguing that the evolutionary acquisition of this property by all lower eukaryotic aminoacyl-tRNA synthetases was unlikely to be fortuitous and thus probably served an important function in vivo unrelated to catalysis, we suggested that it promotes the compartmentalization of these enzymes within the cytoplasm, through electrostatic associations with as yet undefined, negatively charged cellular components, at or near the site of protein synthesis [28]. The observation that all of the class II enzymes from animal cells which have been purified to date display structural parameters nearly identical to those of the corresponding enzymes from lower eukaryotes (tabulation in [11]), as well as the finding that the aminoacyl-tRNA synthetases from animal cells display the property of binding to polyanionic carriers [23,24], support the view that the above considerations may well apply to aminoacyl-tRNA synthetases from animal cells.

The present model proposes that class II aminoacyl-tRNA synthetases associate with subcellular structures individually, while the class I enzymes bind as a pre-assembled complex. This would account for the observed fragility of the supra-molecular complexes, which are readily dissociated into 'free' enzymes and stable 'core' complexes, notably by more drastic extraction conditions or by elevating the ionic strength of the extraction medium. Moreover, the finding that other components of the protein biosynthesis machinery from higher eukaryotes also display the ability to bind to polyanionic carriers in conditions where their prokaryotic counterparts do not [21,22] may account for the fact that non-synthetase components, such as elongation factors or tRNA modifying enzymes, were occasionally reported as components of these fragile supra-molecular assemblies [4].

It is known that the purified high-M_r complexes from sheep and rabbit bind strongly to the polyanionic carriers heparin-Ultrogel or tRNA-Sepharose [9]. Suggestions concerning the mode of electrostatic association of these complexes with negatively charged carriers were presented in [15]. We recently showed that when normal rat kidney

(NRK) cells were extracted with Triton X-100, a substantial proportion of the high-M_r complex remained bound to the remnant cytoskeletal framework fraction, through ionic interactions [30]. Furthermore, immunocytological studies by double indirect immunofluorescence microscopy revealed an association of the complex with as yet unidentified components of the endoplasmic reticulum [30], in agreement with earlier results by Dang et al. [31]. Whatever the nature of the cellular components implicated in this association, our model supposes that the class II enzymes would bind to these components individually, through ionic interactions.

Although our model explicitly differentiates the class I and II aminoacyl-tRNA synthetases with regard to their mode of association with subcellular structures, the reason why only certain mammalian aminoacyl-tRNA synthetases would have acquired hydrophobic properties allowing their pre-assembly as a stable multienzyme complex remains obscure. No underlying physiological or biochemical principle has yet been recognized to account for the nature of the aminoacyl-tRNA synthetases implicated. Moreover, evidence was presented that no catalytic advantage was accrued from their integration into a complex, at least in vitro [32].

Our comparative studies on leucyl- and lysyl-tRNA synthetases from yeast and animal cells clearly suggest that the formation of stable multienzyme complexes is conditioned by the evolutionary acquisition of hydrophobic properties [15]. Indeed, those lower eukaryotic enzymes which do not form stable complexes [33,34] also do not bind to hydrophobic supports in conditions where their mammalian counterparts, derived from the multienzyme complex, are strongly bound. If the selective pressure for acquisition of hydrophobic properties were related to stabilizing of the 'loose' network of aminoacyl-tRNA synthetases electrostatically bound to cellular components, by establishing interconnections between these enzymes through hydrophobic interactions, the possibility may be envisaged that at the stage of evolution representative of mammals, certain aminoacyl-tRNA synthetases have yet to acquire this property. This, in turn, would suggest that in more distantly related vertebrate species, such as fish or birds, the structural organization of aminoacyl-tRNA synthetases may differ from that of mammals.

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