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Why is the Precursor of an Apoptosis-specific Inflammatory Cytokine in the Center of Eukaryotic Multisynthetase Complexes?

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There is now strong evidence that complicated biological processes, such as protein biosynthesis, are highly organized *in vivo*. However, most details of the molecular interactions involved are unknown. Within this context, a subset of the eukaryotic aminoacyl-tRNA synthetases, the enzymes that covalently link amino acids with the proper tRNAs, are isolated as a multiprotein complex. This particle is unusual among multienzyme assemblies in that catalysis is of parallel, rather than consecutive, metabolic reactions. Another striking fact is that the precursor form of an inflammatory apoptosis-specific cytokine is located at a central position in the structure of the multisynthetase complex. [1]. That is, the carboxyl-terminus of one of the auxiliary proteins within the particle, p43, is endothelial-monocyte activating polypeptide II (EMAP II) [2]. This cytokine, originally identified in chemically induced tumors, has significant roles in such diverse biological responses as inflammation, apoptosis and angiogenesis [reviewed in 3]. What is the basis of this incongruous association of the precursor to EMAP II with a complex of proteins that is part of the essential protein biosynthesis machinery?

Our focus for some time has been determination of three-dimensional structures of aminoacyl-tRNA synthetase complexes and analysis of their interior protein topographies using computational microscopy as a major analytical tool. For example, we have performed the first purification to homogeneity of nuclear multisynthetase complex for comparison with that from cell cytosol. As isolated from human K562 cells, the particles exhibit the characteristic polypeptide compositions and component enzyme activities with the exception of a reduced amount of glutaminyl-tRNA synthetase. Characterization of their ultrastructures shows that at approximately 30 Å resolution, no major differences are apparent between the two samples nor with respect to the particle from rabbit (**Figure 1**). As an example of our study of polypeptide placement, we have located the region of one of the active sites of the bifunctional glutamyl-/prolyl-tRNA synthetase via purified $tRNA^{pro}$ bound to the particle isolated from rabbit reticulocytes. As shown in **Figure 2,** two areas of new density are evident in the labeled sample. Site 1 has been assigned as that of the tRNA covalently linked to the active site by attachment through the periodate-oxidized 3' end. The density at site 2 appears to be from non-specific association of the anticodon region with a second multisynthetase particle. These conclusions are based on previous data and the prevalence of dimers in the micrographs.

We are also investigating biological role(s) of p43/EMAP II, especially in regard to its association with the protein biosynthesis machinery. HPLC and immunoblot analyses indicate that essentially all p43 in fresh cell lysate is associated with high molecular mass material. Final samples of high molecular mass multisynthetase complex contain only full-length p43. However, a substantial amount of other forms of the polypeptide are seen throughout the purification in column fractions that do not contain intact complex. These data combined with the central location of p43 within the multisynthetase complex suggest that this polypeptide is poised to have significant influence on particle structure and function during normal metabolism, as well as in pathological states.

Our current hypothesis is that cleavage of p43 into EMAP II destabilizes the multisynthetase complex and leads to not only release of active cytokine, but disruption of the essential process of protein biosynthesis. As outlined in **Figure 3**, one possible relation of this to pathological processes is exacerbation of cell death after hypoxic events due to induction of inflammation and apoptosis. It is noteworthy that redistribution of aminoacyl-tRNA synthetases into smaller particles occurs after experimental myocardial ischemia [4] and high levels of EMAP II are released from tumor cells in response to hypoxia [5]. A body of evidence is accumulating that integrity of the multisynthetase complex may be of importance in persistent damage after heart attack and stroke, as well as in inflammatory diseases such as arthritis and even endometriosis.

References

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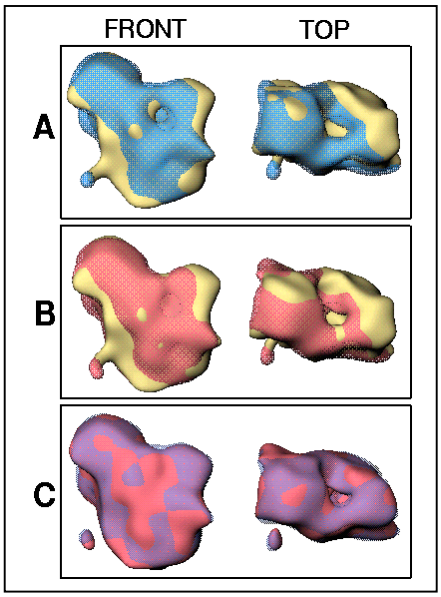

Fig. 1. Comparison of three-dimensional volumes of negatively stained samples of multisynthetase complex from rabbit (yellow), human cytosol (blue) and human nucleus (pink). A, B and C present overlays of pairs of the three volumes from two views.

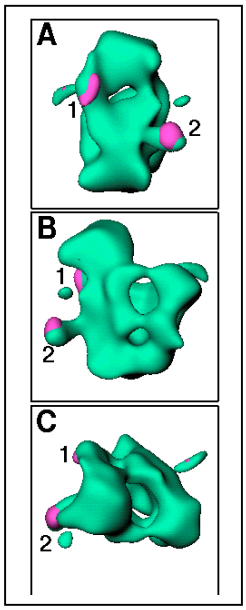

Fig. 2. Localization of bound $tRNA^{pro}$ within the rabbit multisynthetase complex. Densities specific to the labeled complex are shown in pink.

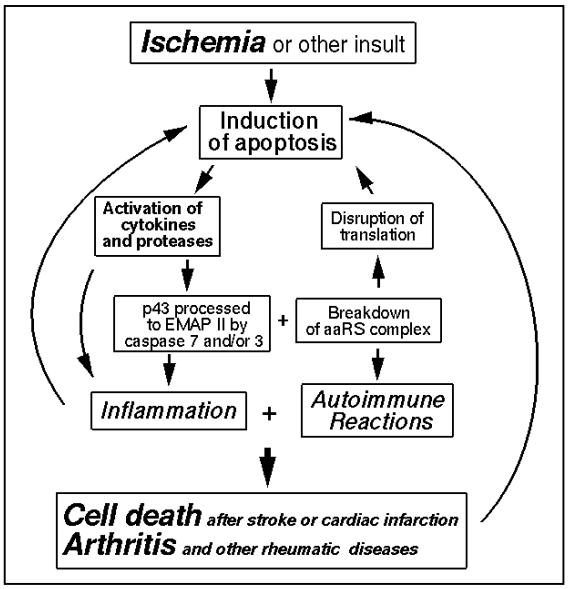

Fig. 3. Scheme illustrating possible role of the multisynthetase complex in cellular pathology.

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