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A Model of Membrane Biogenesis

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Fundamental questions dealing with the site of synthesis of membrane proteins and phospholipids, their integration into functional enzyme complexes and ultimately into membranes, remain largely unanswered at present. On the other hand, considerable progress has been made in elucidating the structure and organization of membranes and this new information has provided a conceptual framework for postulating possible mechanisms of membrane assembly. In this communication a model of membrane biogenesis is described which is based on studies of the structure and biosynthesis of constitutive enzymes of the mitochondrial inner membrane. Although the proposed mechanism will be discussed in terms of the biogenesis of this particular membrane, it could apply equally well to other biological membranes.

Because the biosynthetic processes involved in membrane biogenesis are still poorly understood, the model presented here is to a large extent an intuitive one. It must also be pointed out that a critical review of the existent literature in this area has not been attempted. Such a search would undoubtedly have uncovered plentiful evidence both pro and con. The model contains four essential points each of which has been intentionally specified in sufficient detail to allow experimental verification.

Organization of the Mitochondrial Inner Membrane

The mitochondrion is a highly specialized organelle—being concerned almost exclusively with the utilization of the energy of oxidation of NADH and succinate for the synthesis of ATP. The macromolecular assembly which carries out this function is localized in the inner membrane of the mitochondrion and is composed of four electron transfer complexes and of the oligomycin-sensitive ATPase complex. Although these five complexes account for the bulk of the membrane mass,[1] there are probably numerous additional enzymes

present in the membrane in lower concentrations. The complexes have been found in every instance to be high molecular weight enzymes (200,000 to 500,000)[2,3] consisting of multiple species of proteins and of phospholipids.

An important advance in understanding the structure of the membrane has emerged from the finding that the complexes, even in the most highly purified form, retain the capacity to spontaneously assemble into membranes.[4,5] This finding strongly implies that in addition to their specific catalytic roles, the enzyme complexes also function as the structural units of the membrane. According to this view, the membrane is a mosaic of its component enzymes. Although the mosaic model avoids the need of postulating any other organizational principle such as structural protein, it does not deny the possibility that there are structural proteins present within the domains of the enzyme complexes which are concerned with the protein-protein and protein-lipid interactions responsible for maintaining the structure of the membrane.

Site of Synthesis of the Membrane Proteins

Assuming the mosaic model of the membrane, the mechanism of biogenesis can be experimentally approached at the simpler level of the biosynthesis of the individual constitutive enzyme complexes of the membrane. This approach has been recently explored in a number of laboratories. The aim of these studies up to now has been primarily to define the site of synthesis of the various polypeptides of known constitutive enzymes of the inner membrane. From such studies it is now evident that both cytochrome oxidase[6-9] and the oligomycin-sensitive ATPase[10,11] contain some subunit proteins which are synthesized by the cytoribosomal system and some that are synthesized by the mitochondrial system of protein synthesis. The ATPase complex is so far the better documented example. This enzyme complex contains ten subunit proteins.[12] Of the ten subunits, four have been shown to be mitochondrial products[13] and six to be products of the cytoribosomal system of protein synthesis.[14,15]

Are there any characteristics which distinguish the mitochondrial and cytoplasmic products? Studies on the ATPase complex indicate that the more soluble proteins of this enzyme are made by the cytoribosomal system. The five subunit proteins of the oligomycin-insensitive ATPase, F_1, as well as the oligomycin sensitivity conferring protein (OSCP) are all synthesized by the cytoribosomal system.[10,14,15] Both F_1 and OSCP can be extracted under conditions which do not disrupt the basic structure of the membrane. Once separated from the membrane, these proteins behave as classical water soluble proteins. The mitochondrial products, however, are

highly insoluble species which are associated with phospholipid and can only be solubilized with surfactant reagents which cause a dissolution of the membrane.[13]

Green[16] has recently proposed that membranes are composed of two classes of proteins—*intrinsic* hydrophobic proteins which are bonded to phospholipids and form the basic structure of the membrane and *extrinsic* proteins which are water soluble species which are also tightly associated with the membrane but are not essential for the maintainance of the membrane structure.

The protein components of the ATPase complex can be operationally separated into these two categories of proteins. The cytoplasmic products exhibit properties of extrinsic proteins while the mitochondrial products behave as intrinsic proteins. The first idea of the model proposed here is that all intrinsic proteins of the membrane are synthesized by the mitochondrion and all extrinsic proteins are synthesized by the cytoribosomal system.

Chemical Nature of the Mitochondrial Products

It has been known for some time that mitochondria contain substantial amounts of proteolipids,[17] i.e. proteins which are soluble in mixtures of chloroform and methanol. The function of these proteins is still not clear. Some recent studies suggest that the complexes of the mitochondrial inner membrane contain low molecular weight proteins which exhibit solubility properties characteristic of proteolipids. The ATPase complex of yeast and of beef mitochondria contain subunit proteins which are soluble in chloroform:methanol.[18] Cattell *et al.*[19] have recently reported that a protein which binds dicyclohexylcarbodiimide, a potent inhibitor of the mitochondrial ATPase, has the properties of a proteolipid. Studies on cytochrome oxidase have also revealed that several subunit proteins of this complex are soluble in acidic chloroform:methanol.[18] That proteolipids may be integral components of many membrane complexes is also supported by the finding that a highly purified preparation of the Ca^{2+} ATPase of sarcoplasmic reticulum contains a low molecular weight protein which is soluble in acidic chloroform:methanol.[20]

Although the chemistry of proteolipids is still obscure, some work on a brain proteolipid isolated by Stoffyn and Folch-Pi[21] suggests that the hydrophobic properties of this protein may be due to the presence of esterified fatty acids. Thus, the purified brain proteolipid has been shown to contain esterified fatty acids which could not be accounted for by contaminating phospholipids. Stoffyn and Folch-Pi[21] have suggested that the fatty acids of the brain proteolipid may be esterified directly to some amino acids of the protein moiety. The proteolipid purified from the ATPase of sarcoplasmic reticulum has also been

found to contain saponifiable fatty acids which are in excess to the phospholipid content of the preparation.[20]

A question which may be raised is whether there is any relationship of the mitochondrially synthesized products (intrinsic proteins) to the membrane proteolipids. Here again the evidence at present is only fragmentary, but there is some indication from studies on the ATPase complex of yeast mitochondria that at least two of the mitochondrially synthesized subunits of this complex are soluble in chloroform: methanol.[18] Recently Kadenbach[22] has reported that a low molecular weight peptide synthesized by rat liver mitochondria has the properties of a proteolipid. The second idea of the model is that the mitochondrial products or intrinsic proteins are proteolipids with esterified fatty acids.

An extension of this notion is that (1) all membrane complexes contain as integral components one or more subunits which are proteolipids, (2) the hydrophobic properties of this class of proteins is due in part if not entirely to the presence of protein esterified fatty acids, (3) the proteolipids can combine with phospholipids through hydrophobic interactions of the fatty acids of the proteins and of phospholipids, and (4) the ability of the proteolipids to combine with phospholipids determines the capacity of membrane enzyme complexes to spontaneously assemble into membrane structures.

Cytoplasmic Control of Mitochondrial Protein Synthesis

Studies with yeast have shown that the synthesis of certain extrinsic membrane proteins by the cytoplasmic system is independent of mitochondrial protein synthesis. Thus, the synthesis of inner membrane proteins such as cytochrome *c*,[23] F_1,[14] and OSCP[15] proceeds normally even when mitochondrial protein synthesis is inhibited by chloramphenicol. An analogous situation is found in "petite" mutants of yeast which have a defective mitochondrial protein synthesizing system. Such mutants have been shown to synthesize cytochrome *c*[24] and F_1.[10, 25] The converse does not hold—at least not in the case of the ATPase complex. Very little synthesis of the four intrinsic proteins of the ATPase complex occurs when the cytoribosomal system is inhibited by cycloheximide.[11] These mitochondrial products of the ATPase, however, are synthesized in the presence of cycloheximide, provided the cells are first incubated in chloramphenicol.[11] Moreover, it was found that the chloramphenicol preincubation caused a marked stimulation of overall mitochondrial protein synthesis during the subsequent incubation in cycloheximide. These results indicate that during the initial incubation in chloramphenicol some cytoplasmic products accumulate which are necessary for mitochondrial protein synthesis. In a normally growing culture

of cells, presumably the synthesis of both mitochondrial and cytoplasmic products is a synchronized process and the latter do not accumulate.

There are two obvious mechanisms which could explain these observations. The first is that products of the cytoribosomal system act as activators of the mitochondrial protein synthesizing system. In the case of the ATPase complex, these activators could be F_1, OSCP or even some other protein(s) which are not intrinsic components of the ATPase. An alternative explanation of this phenomenon is that the intrinsic proteins of the membrane are made jointly by the mitochondrial and cytoribosomal systems. According to this mechanism, the cytoplasmically derived part of the protein is a water soluble peptide which is then converted to an intrinsic membrane protein by the addition of a hydrophobic peptide (handle) which is synthesized by the mitochondrion. This mechanism could explain the observed cytoplasmic control of mitochondrial protein synthesis if the synthesis of handles were dependent on the availability of the cytoplasmically made peptides. The third idea of the model is that the mitochondrial system of protein synthesis makes only part of the intrinsic proteins of the membrane. The handles of the intrinsic proteins could be small peptides with esterified fatty acids which confer upon the cytoplasmic peptides proteolipid properties.

Can such a mechanism be reconciled with what is known about the mitochondrial machinery of protein synthesis? It has become evident from recent work that the mitochondrion is responsible for the synthesis of a large number of membrane proteins.[26–28] These observations are difficult to explain in view of the small amount of DNA in the mitochondrion which in addition to coding for messenger RNA also codes for transfer RNA (see ref. 29 for a review of this topic). The mechanism proposed here could help to explain how a large number of distinct proteins may be coded for by mitochondrial DNA if one assumes that only a limited number of different handles are made by the mitochondrion. In fact it is only necessary to postulate a single species of handle since the enzymatic and functional specificity of the intrinsic proteins could reside in that portion of the polypeptides which are coded for by the nuclear DNA.

Synthesis and Assembly of Membrane Enzymes

A question which is paramount to understanding membrane biogenesis is whether the constitutive enzymes are synthesized outside of the membrane and are subsequently incorporated as preassembled units or whether the synthesis is an intramembrane process. There is no evidence at present that membrane enzymes can be found in free form. The exception to this are proteins such as cytochrome *c* or

enzymes such as F_1 which are composed entirely of extrinsic proteins that are synthesized independently of the mitochondrial system.[14] A reasonable guess at present is that the central events in the assembly of membrane complexes occur within the membrane itself. The fourth idea of the model proposed here is that the membrane contains growing points which consist of the whole protein synthesizing machinery of the mitochondrion. This machinery synthesizes the hydrophobic handles—low molecular weight peptides which combine with phospholipids to form the basic substructure of the complexes and also of the membrane mosaic. The cytoplasmic counterparts of the intrinsic proteins are then enzymatically condensed to the handles possibly through peptide bond formation.

Although some membrane complexes could be composed exclusively of intrinsic proteins, most, probably contain extrinsic protein components as well, e.g. the ATPase complex. The incorporation of the extrinsic protein components into the membrane complexes could be a self-assembly process. There are currently a number of examples of extrinsic proteins of the mitochondrial inner membrane which can spontaneously interact with their intrinsic protein counterparts to reconstitute functional enzyme complexes.[30, 31]

Biogenesis of Other Cellular Membranes

With the exception of mitochondria and chloroplasts, no other cellular membranes have been shown to synthesize their own proteins. It must be borne in mind, however, that *in vitro* protein synthesis by mitochondria and chloroplasts is very low when compared to that of the classical cytoribosomal system.[29] The absence of a detectable protein synthesizing activity in an isolated membrane fraction could merely reflect a stringent control by the cytoplasmic system for that particular membrane. In principle, therefore, all membranes could have their own protein synthesizing machinery designed to make the hydrophobic handles of their intrinsic proteins.

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