Hypothesis

CONTROL OVER ASSEMBLY OF THE MITOCHONDRIAL INNER MEMBRANE: SELECTION BY A PERFORMANCE CRITERION

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1. Introduction

Elucidation of principles underlying the assembly of various intracellular membranes and organelles is an indispensable prerequisite for understanding numerous aspects of cell function. Successful reconstitution of tobacco mosaic virus [1], large and small subunits of *Escherichia coli* ribosome [2], and structural elements of bacteriophages [3] suggested that their assembly in vivo is accomplished solely through recognition and spontaneous binding of component parts owing to their high mutual affinity. The next question to be considered is whether the same principle can suffice for the more complex structures, in particular the mitochondrial inner membrane. The gist of this paper is an hypothesis that membrane assembly is supervised according to the principle of section by a performance criterion.

2. How mitochondrial inner membrane is designed

The mitochondrial inner membrane is known to contain some 40 types of polypeptides [4,5] embedded in an elaborate lipid milieu and highly organized in a unique fashion to form the energy-transformation machinery with related enzymic and transport systems [6,7]. The fine structure of the membrane is marked by:

(i) Asymmetric location of individual proteins with respect to the sides of the membrane; and
(ii) Sub-integration of proteins into various enzymic complexes [5,8].

Abbreviations: complex II, succinate:ubiquinone reductase; complex III, ubiquinol: cytochrome *c* reductase; complex IV, cytochrome *c* oxidase

According to the data available, 20–40% of the inner membrane proteins are mitochondrially made, while the others are synthesized on cytoplasmic ribosomes (see references in [5,9]). Several enzymic complexes, such as complexes III and IV of the respiratory chain and H^+-ATPase, are built of both cytoplasmic and mitochondrial translation products [5,9]. It is interesting to note that some cytoplasmically made proteins (e.g., succinate dehydrogenase, NADH dehydrogenase, F_1-ATPase) face the mitochondrial matrix, whereas certain proteins of intramitochondrial origin (e.g., subunits II and III of the cytochrome *c* oxidase, cytochrome *b*) face the intermembrane space (see references in [5]). That is, membrane asymmetry is determined not only by the site of synthesis of its proteins.

3. How mitochondrial inner membrane is assembled: hypotheses and facts

A key role in membrane assembly has been ascribed [10] to mitochondrially made proteins. These were presumed to:

(i) Specifically interact so as to form the 'basic structure' of the membrane; and
(ii) Selectively bind a defined set of cytoplasmic translation products.

While the latter assumption has received some experimental support [11–16], the former is purely speculative. Moreover, there are grounds to think that the binding of cytoplasmic partners is not very specific since it takes place even when mitochondrial protein synthesis is blocked [17–19].

A number of works have discussed the role of heme in the binding of cytochromal proteins to mitochondria. In particular, heme attachment was found

to be essential for the insertion of apocytochrome c_1 [20] and apocytochrome c [21]. However, the heme appears to be a poor candidate for the part of an assembly-controlling factor, since it is not obligatory even for apocytochrome binding [22], to say nothing of non-cytochrome proteins.

It has been shown in [23] that oxygen is necessary for the formation of cytochrome c oxidase from presynthesized subunits in yeast. Unfortunately, no plausable explanation has been offered for this phenomenon.

The possibility of spontaneous self-assembly of the mitochondrial inner membrane could only be directly shown by reconstituting it from individual proteins and phospholipids. No such data are available, though a certain set of inner membrane functions can be reconstituted from oligoenzyme complexes in proteoliposomes (see [24–27]).

Thus at present we have no clear ideas on the mechanisms of inner membrane assembly. It can only be said that it is a multi-stage process involving the proteolytic processing [24–34] of some precursor proteins (which possibly takes place in the membrane itself [32]), attachment of heme to apocytochromes, and lateral and transmembrane movements of proteins in the search for their partners in complexes. As the specificity of the interaction between the molecular constituents of the inner membrane is unknown, a reasonable assumption is that membrane assembly must be subject to errors. It follows therefrom that this process should be somehow supervised.

4. Control over assembly of the mitochondrial inner membrane

Ways and means of control over the assembly of the inner membrane can be envisaged from the data on its degradation and stabilization.

Isolated mitochondria have been long known to degrade rapidly. Most often degradation is ascribed to the cleavage of membrane phospholipids under the action of endogenous phospholipase A_2, oxidative destruction of phospholipids and breakdown of mitochondrial proteins (reviewed in [5]). As follows from [35–38], in all cases degradation of the oxidative phosphorylation system (which is a major membrane component) is prevented or retarded in conditions when the system performs its energy-transformation functions. This means of stabilization is physiological and quite universal [5]. The role of energy transformation in the maintenance of the integrity of the oxidative phosphorylation system in vivo has been demonstrated for the facultatively anaerobic yeast, *Saccharomyces cerevisiae* [39,40].

Obviously, the most general criterion of the correctness of inner membrane assembly is a functional one, which implies the ability of the membrane to generate the transmembrane electrochemical potential of H^+, to carry out ADP-phosphorylation and metabolite transport. Taking into account the dependence of the stability of the oxidative phosphorylation system on its functional state, one can suggest that improperly assembled membrane elements that fail to carry out their potential functions are eliminated in the course of membrane differentiation. This principle (a principle of selection by a functional criterion) had been formulated in [35]. To test the idea, it was first of all necessary to find out:

(i) Whether anomalous (non-functioning) elements can occur; and, if so,
(ii) Whether they are subject to elimination.

Data along this line were obtained in studies on the development of the mitochondrial respiratory system of *S. cerevisiae*. The results [41,42] indicate that yeast growth in a galactose medium is accompanied by formation of a portion of respiratory chain components (complexes II–IV) that do not contribute to the respiratory activity of the mitochondrial population and are proteolytically eliminated during mitochondrial differentiation. As follows from general considerations, the content of non-functioning components is determined by the relation of their formation and elimination rates. Accordingly, situations are conceivable in which such components are accumulated to a detectable extent only in the presence of proteinase inhibitors. In particular, this refers to yeast growing in a glucose medium [42].

According to the data available [5,42], the non-functioning components possess potential catalytic activity and do not participate in mitochondrial respiration only because of improper alignment within the membrane which hinders interaction with adjacent electron carriers. There are also grounds to think that the elimination of non-functioning complexes is selective [5,42].

5. Conclusion

Thus, some experimental evidence is already available for the formation of non-functioning respiratory

chain components and their selective elimination in vivo. This strongly argues in favour of the inner membrane assembly being controlled by a performance criterion so that the only viable structures are those capable of energy transformation.

There are a number of points to be clarified:

(i) More detailed information is needed on the non-functioning respiratory chain components: how they are oriented towards the sides of the membrane; how they are distributed within a membrane (at random or in clusters) and in the mitochondrial population (whether they are present in every organelle or only in some);

(ii) One has to know the set of proteinases (mitochondrial or cytoplasmic) involved in elimination and the actual sequence of events (i.e., whether defective elements are destroyed directly in the membrane or are first 'shed' into the cytoplasm by endogenous proteinases or phospholipases).

Obviously, there are no trivial approaches as yet to solve these problems. Still, efforts along this line appear to be necessary since this is a matter of elucidating the fundamental principles underlying the proliferation and differentiation of mitochondrial as well as probably other intracellular membranes.

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References

[1] Butler, P. G. J. and Durham, A. C. H. (1977) Adv. Prot. Chem. 31, 187–251.
[2] Garrett, R. A. (1979) Int. Rev. Biochem. 25, 121–177.
[3] Casjens, S. and King, J. (1975) Annu. Rev. Biochem. 44, 555–611.
[4] Harmon, H. J., Hall, J. D. and Crane, F. L. (1974) Biochim. Biophys. Acta 344, 119–155.
[5] Luzikov, V. N. (1980) Regulation of Mitochondrial Assembly, Nauka, Moscow (in Russian).
[6] Mitchell, P. (1972) FEBS Symp. 28, 353–370.
[7] Skulachev, V. P. (1972) Energy Transformation in Biomembranes, Nauka, Moscow (in Russian).
[8] De Pierre, J. W. and Ernster, L. (1977) Annu. Rev. Biochem. 46, 201–262.
[9] Schatz, G. and Mason, T. L. (1974) Annu. Rev. Biochem. 43, 51–87.
[10] Tzagoloff, A. (1972) J. Bioenerget. 3, 39–45.
[11] Henson, C. P., Perlman, P., Weber, C. N. and Mahler, H. R. (1968) 7, 4445–4454.
[12] Tzagoloff, A., Akai, A. and Sierra, M. F. (1972) J. Biol. Chem. 247, 6511–6516.
[13] Tzagoloff, A. (1970) J. Biol. Chem. 245, 1545–1551.
[14] Tzagoloff, A. (1971) J. Biol. Chem. 246, 3050–3056.
[15] Ebner, E., Mason, T. L. and Schatz, G. (1973) J. Biol. Chem. 248, 5369–5378.
[16] Cabral, F. and Schatz, G. (1978) J. Biol. Chem. 253, 4396–4401.
[17] Katan, M. B., Pool, L. and Groot, G. S. P. (1976) Eur. J. Biochem. 65, 95–105.
[18] Sebald, W., Weiss, H. and Jackl, G. (1972) Eur. J. Biochem. 30, 413–417.
[19] Mason, T. L. and Schatz, G. (1973) J. Biol. Chem. 248, 1355–1360.
[20] Ross, E. and Schatz, G. (1976) J. Biol. Chem. 251, 1997–2004.
[21] Korb, H. and Neupert, W. (1978) Eur. J. Biochem. 91, 609–620.
[22] Clejan, L., Beattie, D. S., Gollub, E. G., Liu, K.-P. and Sprinson, D. B. (1980) J. Biol. Chem. 255, 1312–1316.
[23] Woodrow, G. and Schatz, G. (1979) J. Biol. Chem. 254, 6088–6093.
[24] Ragan, C. I. and Hinkle, P. C. (1975) J. Biol. Chem. 250, 8472–8476.
[25] Leung, K. H. and Hinkle, P. C. (1975) J. Biol. Chem. 250, 8467–8471.
[26] Eytan, G. D., Matheson, M. J. and Racker, E. (1976) J. Biol. Chem. 251, 6831–6837.
[27] Höjeberg, B. and Rydström, J. (1977) Biochem. Biophys. Res. Commun. 78, 1183–1190.
[28] Schatz, G. (1979) FEBS Lett. 103, 203–211.
[29] Maccecchini, M.-L., Rudin, Y., Blobel, G. and Schatz, G. (1979) Proc. Natl. Acad. Sci. USA 76, 343–349.
[30] Côté, G., Solioz, M. and Schatz, G. (1979) J. Biol. Chem. 254, 1437–1439.
[31] Poyton, R. O. and McKemmie, E. (1979) J. Biol. Chem. 254, 6763–6771.
[32] Poyton, R. O. and McKemmie, E. (1979) J. Biol. Chem. 254, 6772–6780.
[33] Ries, G., Hundt, E. and Kadenbach, E. (1978) Eur. J. Biochem. 91, 179–191.
[34] Michel, R., Wachter, E. and Sebald, W. (1979) FEBS Lett. 101, 373–376.
[35] Luzikov, V. N. (1973) Sub-Cell. Biochem. 2, 1–31.
[36] Romani, R. J., Tuskes, S. E. and Özelkök, S. (1974) Arch. Biochem. Biophys. 164, 743–751.
[37] Goldblatt, M. J. and Romani, R. J. (1977) Arch. Biochem. Biophys. 183, 149–155.
[38] Parce, J. W., Cunningham, C. C. and Waite, M. (1978) Biochemistry 17, 1634–1639.
[39] Luzikov, V. N., Zubatov, A. S. and Rainina, E. I. (1970) FEBS Lett. 11, 233–236.
[40] Luzikov, V. N., Zubatov, A. S. and Rainina, E. I. (1973) J. Bioenerget. 5, 129–149.
[41] Luzikov, V. N., Makhlis, T. A. and Galkin, A. V. (1976) FEBS Lett. 69, 108–110.
[42] Galkin, A. V., Tsoi, T. V. and Luzikov, V. N. (1980) Biochem. J. 190, 145–156.