BIOMEMBRANES

ARCHITECTURE, BIOGENESIS, BIOENERGETICS, AND DIFFERENTIATION

EDITED BY

Lester Packer

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PREFACE

The interface at which chemistry and biology meet is at the level of organization of the membrane. Until relatively recently, good methods for the study of biomembranes were unavailable. However, in recent years this has all changed. First there was electron microscopy, then techniques of cell fractionation in combination with biochemical analysis which are still being refined. This has given way to the fine details of analysis opened up by genetic studies and by the application of an avalanche of sophisticated physical and chemical techniques, including the use of external and internal probes to sense the organization and environment of membranes. Combination of these techniques has afforded a particularly powerful approach for the resolution of the unique specificity of biomembranes which lend directionality to metabolism. Indeed, reconstitution of the functions of natural membranes, a logical outgrowth of the present rapid rate of development, is presently witnessing amazing progress.

The present volume grew out of a desire to bring together some of the diverse contemporary approaches to the study of biomembranes. It was also inspired by a desire to bring talents together through the forum of an "International Conference on Biomembranes" held at the Department of Biological Sciences, Madurai University, Madurai, Tamilnadu, South India. Points of view represented are of ongoing research by Indian scientists and also of authorities in the four areas of biomembranes with which this volume deals: Biogenesis, Architecture, Bioenergetics, and Differentiation.

This unique opportunity came about as a result of the superb organization and determination of the convenors at the Department of Biological Sciences, Madurai University: Drs. J. Jayaraman, Kunthala Jayaraman, and A. Gnanam, who were aided in their efforts by Professor S. Krishnaswamy, officials of the University and the State of Tamilnadu, and national support from the University Grants Commission and the Indian Atomic Organization.

Support by scientific organizations in the countries of the contributing scientists also helped make this collection of papers possible.

L. Packer

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PART I

BIOGENESIS OF MEMBRANES

Considering our ignorance (until very recently) about the molecular basis of the structure, architecture and function of biomembranes, it is perhaps surprising that the past few years should also have witnessed an intense and expanding effort at studying the even more complicated problem of their biogenesis. This may have been foolhardy, or perhaps just reflects the unbounded optimism that motivates all of us. But whatever the reasons, the results obtained have rewarded the efforts beyond any reasonable expectation. As the chapters in this section will demonstrate, not only are we beginning to understand the limits and the schedule of the collaborative intra- and extra-organellar events that culminate in membrane synthesis, but in consequence of this understanding we have even obtained some novel insights into membrane structure and function itself.

REGULATION OF THE MITOCHONDRIAL GENETIC SYSTEM AND ITS EXPRESSION

Henry R. Mahler, Philip S. Perlman, Fred Feldman and Roberto Bastos

INTRODUCTION - NATURE OF THE SYSTEM

For the past ten years or so, our group at the Chemistry Department at Indiana University has been interested in the biogenesis of mitochondria. In trying to find out how these essential and ubiquitous organelles of eucaryotic cells are specified and assembled we - as have an ever increasing number of research groups throughout the world - have had recourse to a simple, unicellular eucaryote, with a high respiratory capacity, which occupies a favored place in the biochemical literature: Saccharomyces cerevisiae or baker's yeast. In addition to its many other advantages this proved a fortunate choice. For, as a facultative anaerobe, subject to strong catabolite repression and as a target of extensive and exhaustive genetic studies encompassing not only classical chromosomal, but also non-Mendelian, cytoplasmic (now identified as mitochondrial) systems of inheritance, this organism has provided us with possibly the most appropriate and certainly the most versatile experimental system imaginable (for recent review see 1-5).

Quite early on, Dr. Jayaraman, Dr. Tewari and we were able to obtain evidence for the presence in yeast mitochondria of a species of DNA separate and distinct from that found in the nucleus of the same organism (6-8). We also showed that the organelles appeared to contain what might constitute the basis of an autonomous system of gene expression, namely the capabilities for RNA and protein synthesis, the latter at least with properties quite different from those found in the cytosol of the same cell (6). Thanks to the work of a number

of investigators in our own group and particularly in other laboratories, we now know that we were dealing with a general phenomenon. "Normal" eucaryotic cells contain within their mitochondria a semi-autonomous genetic system: it consists of i) a unique DNA, ii) RNAs transcribed from the former, together with iii) all the enzymes and other proteins required for the replication and repair of the DNA, its transcription into RNA, and the utilization of the RNA species for purposes of protein synthesis. It is only semi-autonomous since it requires the co-operative participation of the classical nucleocytosol system not only for the specification but also the biosynthesis of the bulk - and perhaps the totality - of these various proteins (1-5). Similarly, the number and amount of polypeptides furnished the mitochondria by their own translational system is severly restricted. They are found first of all only among the enzyme complexes of the inner mitochondrial membrane to which they contribute 20% or less of its total mass. Furthermore, even among inner membrane functions only cytochrome c oxidase, the membrane integrated, oligomycin sensitive form of ATPase and to a lesser extent the ubiquinone-cytochrome c segment of NADH:cytochrome c reductase appear to require participation of the intramitochondrial system for their elaboration. More recent studies, with isolated highly purified enzyme complexes, particularly in the laboratories of Schatz (5,9,10), Tzagoloff (11,12), and Bücher, Sebald and Weiss (13, 14) summarized in Table I suggest the following: i) The biosynthesis of the complexes for which a mitochondrial participation has been inferred represents a co-operative venture that requires contributions by both systems of gene expression on the molecular level; ii) Since the complexes shown are estimated to contribute approximately 50% to the total mitochondrial mass the intrinsic contribution can account for virtually all of the anticipated mitochondrial products. As a corollary very few if any major products of mitochondrial translation remain to be identified. This inference is confirmed by the overlap of those

TABLE I
MITOCHONDRIAL SPECIFICATION OF THE MITOCHONDRIAL RESPIRATORY CHAIN

Complex	Description	Molecular weight $\times 10^{-6}$	Mitochondrial Component % Synthesis	Mitochondrial Component Mass $\times 10^{-6}$
I	NADH-ubiquinone reductase	0.7	0	
II	Succinate-ubiquinone reductase	0.2	0	
III	Ubiquinone-cytochrome c reductase	0.23	∿15 (1)	0.03
IV	Cytochrome c oxidase	0.20	∿50 (3)	0.10
V	Membrane bound, oligomycin sensitive ATPase complex	0.47	30 (3-4)	0.14
Totals		1.80		0.27

individual polypeptides of the inner membrane identified as synthesized by the particle with the corresponding entities isolated from highly purified complexes. In addition these and other studies (15-18) suggest that iii) at least for cytochrome oxidase and ATPase polypeptides of mitochondrial origin probably are not responsible for catalytic activity *per se* but instead fulfill an integrative or regulatory function. It is to problems of this sort, and in particular to possible intra- and extramitochondrial contributions in the regulation of the replication and differentiation of the organelle that we wish to address ourselves to in this chapter.

REGULATORY ASPECTS OF MITOCHONDRIOGENESIS

What controls the number of mitochondrial genomes per cell?

One of the questions that we hoped to settle was the relationship between nuclear ploidy (i.e., the number of nuclear chromosome sets) and the number of mitochondrial genomes per cell in a stable population. Although one might anticipate a coordinate response between the two numbers, and an early survey by Williamson (19)