Vladimir P. Skulachev

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Preface

This book is devoted to membrane bioenergetics, one of the most rapidly "growing points" of physico-chemical biology. In the last 2 decades, the developement of bioenergetic research has been so tempestuous and debates on crucial problems so uncompromising that we find it necessary to summarize, in a calm and orderly manner, the firmly established facts and separate them from what belongs to the realm of speculation. We will try to consider a great variety of described events within the framework of a single coherent concept using the same terminology.

Such is the aim of this book meant for a wide range of readers, from specialists working in this field, to university students taking an in-depth interest in biological energy transductions. In general, the monograph may serve as a textbook. My goal was to present an extensive analysis of the field and I hope that the majority of subjects related in some way to membrane bioenergetics are at least mentioned in the book and are included in the Subject Index. Certain sections are written in greater detail, particularly those dealing with novel and promising approaches (especially when the studies were carried out by our group: here, I would like to ask my reader for some leniency – in a way, I am in the shoes of the author of a chronicle dwelling on events he has witnessed at first hand).

The text is supplemented with a list of references. Albeit a long one, it includes but a small part of the membrane bioenergetic literature. While compiling this list, I gave preference to the pioneering publications on the subject matter and to the latest reviews or experimental papers containing the most important references. This may be helpful for finding further essential information, if necessary.

I am very grateful to Dr. A. A. Konstantinov and all the participants in the theoretical bioenergetics seminar at the A. N. Belozersky Laboratory of Moscow University for discussions and advice, to Drs. L. E. Bakeeva and D. B. Zorov for microphotographs and to Ms. O. O. Malakhovskaya, Mr. A. L. Drachev, Ms. T. N. Konstantinova, Mr. I. S. Kochubey and Ms. N. M. Goreyshina for their assistance in preparing the manuscript.

January, 1988 Vladimir P. Skulachev

Abbreviations

$\Delta\bar{\mu}H$	electrochemical H^+ potential difference
$\Delta\bar{\mu}I$	electrochemical difference in potentials of an ion I
$\Delta\bar{\mu}K$	electrochemical K^+ potential difference
$\Delta\bar{\mu}Na$	electrochemical Na^+ potential difference
$\Delta\Psi$	electric potential difference
Δp	proton-motive force
ΔpH	H^+ concentration difference
ΔpK	K^+ concentration difference
ΔpNa	Na^+ concentration difference
Δs	sodium ion-motive force
ADP	adenosine 5′-diphosphate
AMP	adenosine 5′-monophosphate
ATP	adenosine 5′-triphosphate
BAL	British anti-lewisite (2,3-dimercaptopropanol)
BChl	bacteriochlorophyll
$(BChl)_2$	bacteriochlorophyll dimer, or special pair
$(BChl)_2^*$	excited bacteriochlorophyll dimer
$(BChl)_2^+$	bacteriochlorophyll dimer cation radical
BPheo	bacteriopheophytin
cAMP	adenosine 3′,5′-cyclic monophosphate
cGMP	guanosine 3′,5′-cyclic monophosphate
CCCP	m-chlorocarbonylcyanide phenylhydrazone
Chl	chlorophyll
Chl*	excited chlorophyll
Chl^+	chlorophyll cation radical
CoA	coenzyme A
CoQ	coenzyme Q, or ubiquinone
CoQ^-	coenzyme Q anion radical
$CoQH_2$	reduced coenzyme Q, or ubiquinol
DBTQ	dibromotimoquinone
DCCD	N,N′-dicyclohexyl carbodiimide
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DNA	deoxiribonucleic acid
ESR	electron-spin resonance
FAD and $FADH_2$	flavin adenine dinucleotide oxidized and reduced respectively
FMN and $FMNH_2$	flavin mononucleotide oxidized and reduced respectively
FeS	non-heme iron-sulphur protein
GDP	guanosine 5′-diphosphate

GMP	guanosine 5′-monophosphate
GTP	guanosine 5′-triphosphate
HQNO	2-heptyl-4-hydroxyquinoline N-oxide
MQ	menaquinone
NAD^+ and NADH	nicotinamide adenine dinucleotide oxidized and reduced respectively
$NADP^+$ and NADPH	nicotinanide adenine dinucleotide phosphate, oxidized and reduced respectively
PMS	phenasine methosulphate
PQ	plastoquinone
$PQ^{\cdot-}$	plastoquinone anion radical
PQH_2	plastoquinol
P_i	inorganic phosphate
PP_i	inorganic pyrophosphate
TMPD	tetramethyl-*p*-phenylenediamine
TPB^-	tetraphenylborate
TPP^+	tetraphenyl phosphonium

Contents

1 Introduction

1.1 A "Biology Building" and the Place of Bioenergetics

To classify biological sciences, one may use at least three different criteria:

1. The level of complexity of the subject matter;
2. The functional aspects;
3. Methodology.

In Fig. 1, we tried to construct a "biology building" by using these criteria as three spatial dimensions. The building is eight storeys high, assuming that each level of complexity occupies one storey. The top storey is for biosphere studies. Ecology comes next down the ladder. It deals with the communities of different living species. The next storey is occupied by a group of biological sciences investigating individual species of animals, plants, bacteria and their taxonomy. In fact, here we have the classical aspects of zoology, botany, bacteriology and virology. All these studies may be called "biology of species" to distinguish them from those occupying the higher and the lower storeys. The sciences studying the structure and functioning of individual organisms and their organs belong to the realm of anatomy and physiology. Next comes the living cell. A corresponding science

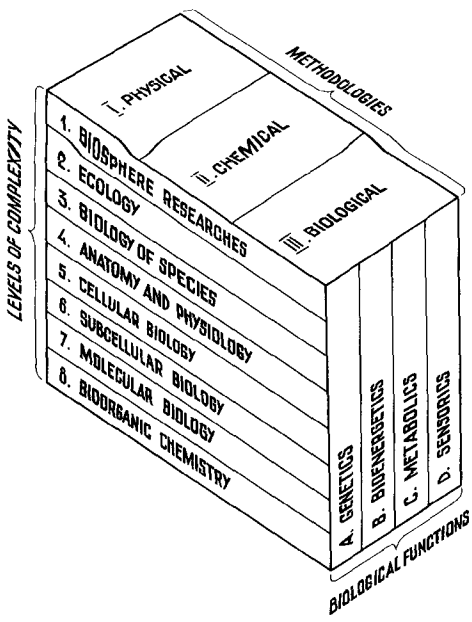

Fig. 1. The "Biology Building": interrelationships of the biological sciences

is cell biology, or cytology. The study of intracellular organelles and their fragments, homogenates, cell ghosts and other cell-free supramolecular systems may be regarded as subcellular biology. Investigations at the level of functioning biological macromolecules or their complexes are the domain of molecular biology. This is the last and simplest level where the biological function is still present. The structure and physico-chemical properties of pure substances composing the living organism are studied at the ground floor level, so to speak. Since most of them are organic compounds, the science is defined as bioorganic chemistry. Sometimes biochemists deal with inorganic substances; in these cases one may speak of bioinorganic chemistry. However, it makes little sense to consider it as a special science, for the number of its objects is rather small.

This seems about all there is to the "horizontal" sciences. Let us now consider "vertical" sciences, proceeding from the functional principle. The four most important functions are inherent in all living organisms, namely (1) self-reproduction, (2) the ability to obtain energy from external sources, (3) the ability to utilize chemical substances of the environment for synthesizing the components of the body and (4) the ability to perceive and process signals from the outer as well as from the inner medium of the organism. Each of these functions can be studied at different "horizontal" levels of organization.

Genetics is the science dealing with self-reproduction. It is bioenergetics that in keeping with the "vertical" principle should deal with the function of energy supply. Accordingly, we may define *bioenergetics as a branch of of functional biology studying (1) transduction of the energy of external sources into utilizable forms and (2) the use of this energy when various types of work are carried out by the living systems.*

In conformity with this definition, one may distinguish, e.g. molecular bioenergetics, cellular bioenergetics, as well as the bioenergetics of the organism, of biocenosis and of the biosphere.

Continuing the functional classification, we come to the science investigating the supply and conversion of substances rather than of energy. This field is usually regarded as a part of biochemistry, enzymology, etc. However, taking into account the great significance of functional biology which, in fact, integrates the knowledge furnished by the "horizontal" biological sciences, we think that a discipline studying each of the four main biological functions should be qualified as a separate science having a name of its own. By way of analogy with genetics and bioenergetics, the science investigating the metabolism of substances may be called "metabolics". As to a future science which will encompass all the biochemical, physiological, etc., studies on the reception, transmission and the processing of signals of different sensors, it may be defined as "sensorics".

Yet another way of classifying biological sciences may be based on the methodology employed. In this context, three principal methods should be taken into account: the physical, chemical and biological ones. In the first case, the corresponding science is biophysics and in the second, biochemistry. As to biological methodology, it may be exemplified by evolutionary, philo- and ontogenetic approaches, the natural selection doctrine, etc.

More "storeys" and sections can be added to the "biology building" shown in Fig. 1, if necessary. For instance, the development of mathematical approaches

may generate some day a need of having four, rather than three methodological sections, etc.

The scheme shown in Fig. 1 considers only the pure (fundamental) aspects of biology. A similar "building" may be constructed for applied biology (biotechnology).

Any pure biology research may find a "room" in the given scheme. In particular, the aim of this book is to consider the problems located in "rooms" B, I–III, 5–8. These coordinates denote the physical, chemical and biological aspects of bioenergetics at the levels descending from the living cell down to the organic and inorganic molecules of biological origin. We shall concentrate on the *membrane-linked bioenergetic systems* of key significance for obtaining the biologically convertible energy. Priority will be given to biological methodology: after all, the author is a biologist himself.

1.2 Essential Definitions

1.2.1 Energy-Transducing Membranes

Biological membranes may be defined as natural films of 5–7 nm thickness consisting of proteins and lipids. The lipid constituent is more or less standard in various biomembranes. It is usually represented by phospholipids or, much less frequently, by glyco- or sulpholipids. It is its protein composition that determines the biomembranès specific nature, its "face". Among the membrane proteins, one may find many enzymes, porters, receptors and pigments.

The most important function of many types of biomembranes consists in the transduction of energy from one form to another. This function is carried out by specific proteins plugged through the hydrophobic layer of the membrane impermeable to the majority of solutes which are present in the membrane-washing solutions.

Any energy-transducing membrane is competent in the interconversion of (1) the chemical energy of respiratory substrates or ATP, or light energy and (2) the electric energy of the transmembrane potential difference ($\Delta\Psi$) or the osmotic energy of transmembrane gradients of solutes. Besides, in some membranes, the transduction of electric or osmotic energy to a mechanical one has been shown to occur. These systems are responsible for the motility of prokaryotes. In certain tissues of warm-blooded animals and some plants, production of heat due to the discharge of the membrane potential is of functional significance and can be therefore regarded as a special type of membrane-linked energy transduction.

Among membranes which clearly fall into the energy-transducing category, the following ones are most significant: the inner mitochondrial membrane, the inner (cytoplasmic) bacterial membrane, the outer membrane of eukaryotic cells (plasmalemma), the membrane of bacterial chromatophores, the thylakoid membrane of chloroplasts and cyanobacteria and the vacuolar membrane (tonoplast) of plants and fungi.

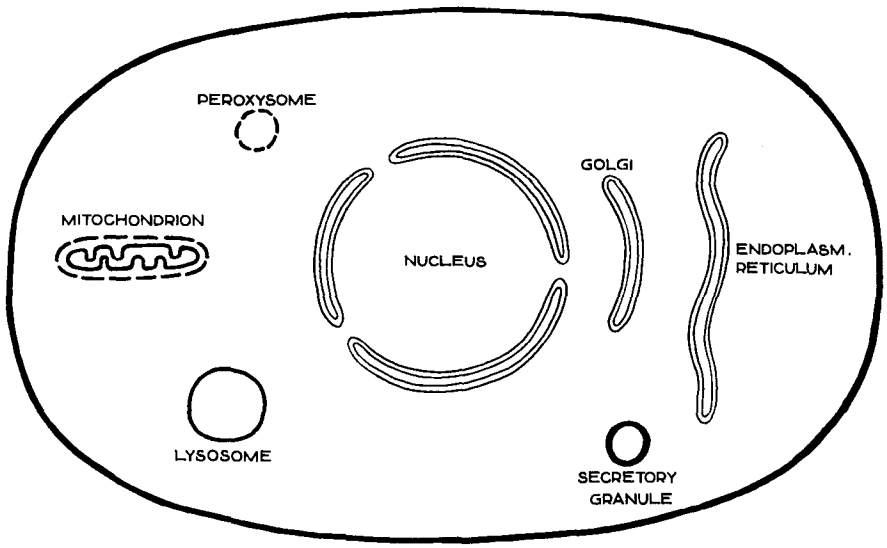

Fig. 2. Membrane structures of the animal cell. *Filled solid contours*: energy transduction processes are firmly established. *Filled dashed contours*: energy transduction is impossible. *Empty contours*: energy transduction is possible, but is yet not directly proved

The energy transduction ability is not a common propoerty of all the biomembranes. At least in two cases it is quite clear that the membrane cannot be energized. We mean here, the outer membranes of mitochondria and of Gram-negative bacteria containing special proteins, the porins, which form rather large pores permeable to low-molecular mass compounds [1046, 279, 864]. The role of these membranes is largely confined to that of the barriers for proteins localized in the periplasm of bacteria or in the intermembrane space of mitochondria. Besides, certain receptor-like proteins have also been found here. Sometimes these receptors are identical to porins. In animal cells, the outer mitochondrial membrane proves to be the only porin-containing structure [864]. Nevertheless, at least one more membrane resembles the outer membrane of mitochondria in that it is permeable to small hydrophilic solutes. This is the peroxisome membrane. Features similar to outer mitochondrial and bacterial membrane seem to be inherent in the outer membrane of the chloroplast envelope [732].

Traditionally, membranes of the endoplasmic reticulum and the cell nucleus are regarded as incompetent in energy transductions. However, recently indications were obtained [1635, 89] that membranes of the Golgi apparatus can transduce ATP energy to the pH gradient, and so the question of the bioenergetic functions of the related reticular and nuclear membranes needs further investigation. The present state of the problem with respect to the animal cells is illustrated in Fig. 2.

1.2.2 Coupling Ions

In the great majority of cases, the membrane-linked energy transductions include the following chain of events:

$$\text{energy source} \rightarrow \bar{\mu}I \rightarrow \text{the work}, \tag{1}$$

where $\Delta\bar{\mu}I$ is the transmembrane difference in the electrochemical potentials of an ion (I). (The physical nature of $\Delta\bar{\mu}I$ will be considered in Sect. 1.3, conformably to $\Delta\bar{\mu}H$.)

Equation (1) means that the energy is first utilized to transport an ion against the electric field and/or in the direction of its concentration increase. This process is often defined as energization of the membrane. Then, the obtained $\Delta\bar{\mu}I$ is used a a driving force to perform various kinds of work. These processes of utilization of the external energy and performance of work appear to be coupled to formation and use of $\Delta\bar{\mu}I$ so that ion I can be called a "coupling ion".

Until recently, it was generally accepted that in energy-transducing membranes, H^+ plays the role of a coupling ion, as it was first suggested by P. Mitchell in 1961 [973]. The only exception was assumed to be the animal cell outer membrane employing Na^+ instead of H^+ as the ion which couples ATP hydrolysis to the accumulation of various solutes inside the cell. However, according to recent

Table 1. Bioenergetic classification of membranes

A. Energy-transducing membranes using H^+ as the coupling ions
   1. Inner mitochondrial membrane
   2. Thylakoid membrane of chloroplasts and cyanobacteria
   3. Inner envelope membrane of chloroplasts
   4. Inner (cytoplasmic) membrane of many bacteria
   5. Membrane of bacterial chromatophores
   6. Outer cell membrane of plants and fungi
   7. Vacuolar membrane of plants and fungi (tonoplast)
   8. Membrane of chromaffin and some other secretory granules in animal cells

B. Energy-transducing membranes using Na^+ as the coupling ion
   1. Outer membrane of animal cells
   2. Inner (cytoplasmic) membrane of some marine alkalotolerant aerobic bacteria or marine anaerobic bacteria

C. Energy-transducing membranes specialized in ion sequestration
   1. Lysosomal and probably Golgi apparatus membranes (ATP $\rightarrow \Delta\bar{\mu}H$)
   2. Outer cell membranes of some animal cells, e.g. of gastic mucosa cells (ATP $\rightarrow \Delta\bar{\mu}H$)
   3. Sarcoplasmic reticulum and other Ca^{2+}-storing vesicles (ATP $\rightarrow \Delta\bar{\mu}Ca$)

D. Membranes that are unable to transduce energy
   1. Outer mitochondrial membrane
   2. Outer envelope membrane of chloroplasts
   3. Outer bacterial membrane
   4. Peroxysomal membrane

E. Relation to energy-linked functions remains obsure
   1. Endoplasmic reticulum (microsomes)
   2. Membrane of the cell nucleus

observations in certain bacteria, (1) Na^+ substitutes for H^+ in the process of membrane energization and (2) $\Delta\bar{\mu}Na$ is then utilized to support all kinds of membrane-linked work. This means that H^+ is not unique as the coupling ion (see Chap. 7).

There are some other ions besides H^+ and Na^+ which can also be transported across the membrane against their electrochemical potential. For instance, in the endoplasmic reticulum of muscle and some other tissues, and in the outer membrane of many cells, there is Ca^{2+}-ATPase, transporting Ca^{2+} at the expense of the ATP energy. However, the role of this process is simply to sequester Ca^{2+} from the cytosol and $\Delta\bar{\mu}Ca$ is never used to support the work. Similar reasoning seems to be true for K^+ ions pumped by Na^+/K^+- or H^+/K^+ATPases (see Sects. 4.5.3 and 7.1.3.2 respectively), and for Cl^- ions pumped by halorhodopsin (Sect. 3.5.8.1).

The classification of biological membranes is given in Table 1. Coupling membranes are listed in groups A and B. These energy-transducing membrane structures couple energy-releasing and energy-consuming processes via circulation of an ion (H^+ in group A, or Na^+ in group B). Group C includes membranes in which $\Delta\bar{\mu}I$ formation is the final event of the energy-transducing process. This is the case when the function of the membrane consists in ion sequestration. Group D indicates membranes definitely incapable of energy transduction. Some cases, when the existence of energy-linked functions is an open question, are listed in group E.

1.2.3 Convertible Energy Currencies of the Living Cell

Energy transduction can occur (1) in membranes or (2) in non-membranous components of the cell, i.e. in cytosol, myofibrils, microfilaments, microtubules or the nucleus. In these two groups of processes, two different forms of convertible energy currency are used. These are $\Delta\bar{\mu}I$ and ATP in (1) and (2) respectively. $\Delta\bar{\mu}I$ can be reversibly converted to ATP. These processes are catalyzed by H^+-ATP synthases (H^+-ATPases) in "protonic" membranes bearing $\Delta\bar{\mu}H$ and by Na^+- or Na^+/K^+-ATPases in "sodium" membranes which bear $\Delta\bar{\mu}Na$.

In Fig. 3 a scheme is shown which describes the energetics of living cells using $\Delta\bar{\mu}H$ as the convertible membrane-linked energy currency. According to the scheme, the energy of the light or respiratory substrates can be utilized by enzymes of the photosynthetic or respiratory redox chains or (in halobacteria) by bacteriorhodopsin to form $\Delta\bar{\mu}H$. The latter can support various types of work in the "protonic" membrane, with ATP synthesis being the most important. Substrate-level phosphorylations serve as an alterantive mechanism of ATP formation that operates with no $\Delta\bar{\mu}H$ involved. Such phosphorylations occur in the glycolytic chain (glyceraldehyde phosphate dehydrogenase and enolase reactions) and in oxidative decarboxylation of α-ketoglutarate [1377, 1380]. $\Delta\bar{\mu}H$-linked ATP formation is a major but not the only process of $\Delta\bar{\mu}H \rightarrow$ chemical work energy transduction. $\Delta\bar{\mu}H$-supported synthesis of inorganic pyrophosphate and the transfer of reducing equivalents in the direction of more negative redox potentials (e.g. reverse electron transfer in respiratory chain and transhydrogenase reaction) belong to the same type of energy transduction. The $\Delta\bar{\mu}H$-driven uphill transport