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MITOCHONDRIA CHLOROPLASTS AND BACTERIAL MEMBRANES

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Preface

Books on mitochondria and on photosynthesis abound. Thus the addition of yet another book on these subjects requires some justification. Most of the books on mitochondria fall into two categories, those which are research monographs, many of them being the proceedings of a scientific meeting, and those which form an introduction to the subject at about the first- or second-year University level. A not dissimilar situation applies to photosynthesis. The chloroplast as an organelle receives rather less emphasis. Although the cross-fertilisation of ideas between the fields of chloroplast and mitochondrial biochemistry is widely acknowledged, treatment of both subjects together is less common. In the present work, I have endeavoured to bridge the gap between the introductory and the advanced research-orientated works by attempting to provide a broad coverage of the main aspects of mitochondria and chloroplasts with a view to providing a jumping-off point for the extensive literature of reviews. Each chapter terminates with a short list of recent reviews.

The book is written as a textbook with the intention of conveying concepts and information and is aimed primarily at third-year undergraduate and graduate students. The background knowledge of students tends to be very variable, even among those of the same institution, and an attempt has been made to limit the knowledge required to understand the major concepts discussed. The more elementary material has been presented in a historical manner in order to demonstrate the development of the subject. The number of references has been kept to a minimum not only for economy of space, but also because references can create a forbidding distraction to the text. A major objective is to present a balanced account of the organelles although the notion of balance is inevitably subjective. Thus, the mitochondrion has been viewed both as the organelle responsible for oxidative phosphorylation and as a particle possessing a complex pattern of metabolic pathways and permeation systems some of which are not directly related to its energetic function. In recent years, the study of bacterial biochemistry has contributed significantly to an understanding of chloroplasts and mitochondria; two chapters summarising energetics of bacterial membranes have therefore been included.

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Abbreviations

$\Delta\psi$	membrane potential
Δp	proton motive force (PMF)
λ	wavelength
ADP	adenosine diphosphate
ALA	γ-aminolaevulinic acid
Ala	alanine
AMP	adenosine monophosphate
Asp	aspartic acid
ATP	adenosine triphosphate
CAM	crassulacean acid metabolism
CCCP	carbonylcyanide-*m*-chlorophenylhydrazone
Chl	chlorophyll
CoA	coenzyme A
CP	chlorophyll–protein complex
ctDNA	chloroplast DNA
cyt	cytochrome
DABS	*p*-(diazonium)-benzenesulphonate
DBTQ	dibromothymoquinone
DCCD	*N*,*N*-dicyclohexylcarbodiimide
DCMU	3(3,4-dichlorophenyl)-1,1-dimethylurea
DCPIP	dichlorophenol indophenol
DNA	deoxyribose nucleic acid
DNP	dinitrophenol
E_m	mid-point potential (pH included as subscript: $E_{m\,7.0}$)
EDTA	ethylenediaminetetra-acetic acid
EPR	electron paramagnetic resonance
F	Faraday
FAD	flavin adenine dinucleotide
FCCP	carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone
Fd	ferredoxin
Fe–S	iron–sulphur centre or protein
FMN	flavin mononucleotide
Fp	flavoprotein
GDP	guanosine diphosphate
GTP	guanosine triphosphate
His	histidine
Ile	isoleucine
kb	kilobase (= 1000 bases)
Lys	lysine
Met	methionine

MK	menaquinone
mRNA	messenger RNA
mtDNA	mitochondrial DNA
NAD, $NADH_2$	nicotinamide adenine dinucleotide (oxidised and reduced respectively)
NADP, $NADPH_2$	nicotinamide adenine dinucleotide phosphate (oxidised and reduced respectively)
OSCP	oligomycin sensitivity conferring protein
PGA	3-phosphoglyceric acid
Phe	phenylalanine
P*i*	inorganic phosphate
PP	pyrophosphate
PQ	plastoquinone
Pro	proline
PS I, PS II	pigment system or photosystem I and II respectively
Q	ubiquinone (also used for the primary acceptor of PS II)
RNA	ribonucleic acid
Ser	serine
SMP	submitochondrial particle
Thr	threonine
TMPD	tetramethylphenylenediamine
TPP	thiamin pyrophosphate
Try	tryptophan
Tyr	tyrosine
UDP	uridine diphosphate
UTP	uridine triphosphate
Val	valine

Contents

Chapter 1

Mitochondria and chloroplasts: basic concepts

1.1 The nature of the subcellular particles

(a) Introduction

The primary source of energy available for life on our planet is solar radiation. This may be converted to chemical energy by organisms possessing suitable photochemical pigments and associated systems. In green plants the process, photosynthesis, is concerned with the synthesis of organic compounds (e.g. hexose sugars) from carbon dioxide and water and may be represented as:

$$CO_2 + H_2O + \text{light energy} \longrightarrow C_6H_{12}O_6 + O_2$$

The fossil fuels, coal, oil and natural gas which are now being rapidly consumed, are ultimate products of this process and represent the photosynthetic activity of plants in earlier periods of geological time.

Just as technology harnesses the energy of organic compounds by their combustion in the presence of oxygen, so living cells are able to use the energy in organic compounds for life processes such as the synthesis of fresh cellular material and muscular movement. For hexoses, this oxidative process may be represented:

$$C_6H_{12}O_6 + O_2 \longrightarrow CO_2 + H_2O + \text{energy}$$

The two processes above are thermodynamically but not mechanistically the reverse of one another. However, as we shall see, they have similarities, for example in regard to ATP synthesis. Except in simple organisms (prokaryotes) the major part of both processes takes place in discrete organelles within the cell. The photosynthetic system is housed within the chloroplast, while the essential oxidative systems are the property of the mitochondrion. Both organelles are surrounded by a double membrane which is selectively permeable to cell constituents, so that the organelles constitute metabolic compartments separate from the rest of the cell but connected to it in a manner controlled by the permeation systems.

The reason for discussing both the chloroplast and the mitochondrion within the compass of a single book lies in the fact that both possess similar chains of oxidation – reduction reactions which are linked to ATP synthesis. In addition, the oxidation – reduction systems are also linked to complex metabolic cycles. Further, both are semi-autonomous in possessing a partial hereditary system, a small 'chromosome' coding for some of the particle's proteins which can be totally synthesised within the particles.

(b) The mitochondrion – a metabolic system for ATP synthesis

The major functions of the mitochondrion can be conveniently listed as:

(i) the oxidation of pyruvate to acetyl coenzyme A
(ii) the oxidation of fatty acids to acetyl coenzyme A
(iii) the oxidation of acetyl coenzyme A to CO_2 and reduced cofactors (e.g. $NADH_2$)
(iv) the oxidation of reduced cofactors ($NADH_2$) by oxygen forming water
(v) synthesis of ATP coupled to $NADH_2$ oxidation, oxidative phosphorylation.

All five of these processes are more or less intimately linked (see Fig. 1.1) and may be considered as part of the oxidative system for ATP synthesis. Thus the *raison d'être* of the mitochondrion is to be the major supplier of ATP for the cell. However, the mitochondrion also has a significant role in other processes such as nitrogen metabolism and various biosynthetic systems, for example in porphyrin synthesis and in steroid hormone synthesis in the adrenal cortex.

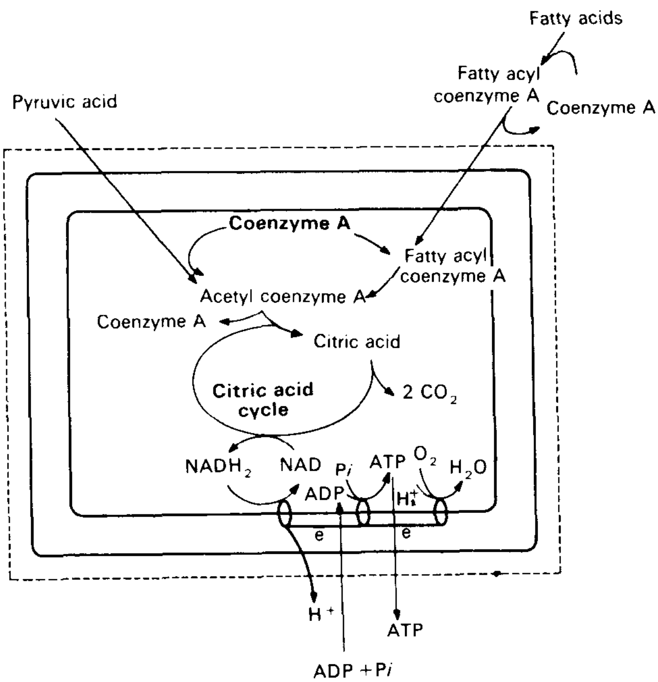

Fig. 1.1 Outline of the major metabolic pathways of the mitochondrion.

The study of the biochemistry of the mitochondrion has proceeded by several almost separate lines which have slowly converged on the particle as the main focus of investigation. Workers such as Szent-Györgyi who were interested in the chemistry of respiration demonstrated the catalytic effect of four-carbon dicarboxylic acids on oxygen uptake. This work was complemented by earlier studies on dehydrogenases by Wieland and others and was brought to fruition by Krebs with the formulation of the citric acid cycle. Later Lehninger showed the cycle to be a property of the mitochondrion.

A second line of investigation was initiated by Warburg, who showed, in his studies on carbon monoxide inhibition of respiration, that an iron porphyrin was involved in oxygen metabolism. Subsequently Keilin rediscovered the cytochromes and isolated cytochrome *c*. From observations of the absorption spectra of tissues, he was able to construct a scheme for the respiratory chain.

A third line of study was developed by Kalckar and by Belitzer and Tsybakova leading to the concept of oxidative phosphorylation in which ATP synthesis is coupled to respiratory activity.

Starting with the observations of Knoop, the system of fatty acid oxidation was investigated and this also was eventually seen as a property of the mitochondrion.

Cytologists in the late nineteenth century had become aware of the mitochondrial particles in cells. A number of attempts were made to study these particles either *in situ* with dyes or by isolating them. However, it was not until 1940, when Claude began a systematic investigation of large granules (mitochondria) and small granules (microsomal fraction) isolated from liver homogenates by differential centrifugation, that a biochemical understanding of mitochondria became possible. By 1950, it had become clear that the fatty acid oxidising system, the citric acid cycle and the respiratory chain with its associated phosphorylation system were all located in the mitochondrion. With the advent of the electron microscope, the detailed structure of the mitochondrion was formulated, particularly by Palade. This led to a study of enzyme distribution within the structure and to the problem of transport of metabolites across the membranes. A further area of investigation developed as workers became aware of the questions associated with the biogenesis of the mitochondrion leading to studies of the synthesis of mitochondrial protein.

Although bacteria are too small to possess mitochondria (*Escherichia coli*, a short rod $0{\cdot}8 \times 1{\cdot}2\ \mu m$ has dimensions similar to mitochondria) many are capable of similar oxidative metabolism and possess comparable systems of respiration and phosphorylation. Investigations of these systems in bacteria have augmented the studies on the mitochondrion.

(c) The chloroplast – an energy-transducing system

The prime function of the chloroplast is photosynthesis. The earliest ideas on this subject were developed in the eighteenth century by a colourful group of amateur naturalists. Stephen Hales (1677–1761) was the first of several workers to study photosynthesis as a process of gas exchange. In his essay *Vegetable Staticks*, he wrote 'Plants very probably draw through their leaves some part of their nourishment from the air . . . may not light also, by freely entering surfaces of leaves and flowers, contribute much to the ennobling principles of vegetables?'

The eighteenth century was also the era of development of ideas on the chemistry of gases associated with the names of Black, Scheele, Priestley, Cavendish, Lavoisier and others. The new insights had an impact on the understanding of respiration and particularly of photosynthesis. Joseph Priestley demonstrated the purification of air in the light in the presence of a photosynthesising system – a piece of mint. Air in a glass jar in which a candle had burned out, was restored so that the candle would again burn in it. The full significance of these experiments was not realised by Priestley who interpreted them in terms of the current phlogiston theory. However, later work showed convincingly the evolution of oxygen from photosynthesising algae. Priestley's experiments, published in the Proceedings of the Royal Society in 1772, attracted the attention of a Dutch physician, Ingen-Housz, who became physician to the Empress Maria Theresa. The experiments of Ingen-Housz convinced him of the importance of light in the air-restoration process and the active production of oxygen in the light. In his book *Food of Plants and Renovation of Soil*, he claimed that plants obtained their carbon by decomposition of the carbonic acid of air and that they evolved oxygen. Thus towards the end of the eighteenth century a coherent concept of photosynthesis began to develop, namely the synthesis of organic matter from carbon dioxide in the light with the evolution of oxygen.

These views were supported by the careful weighing experiments on plants by de Saussure (1767–1845). He showed that plants obtain most of their elements from the soil, but all the carbon from the CO_2 of the atmosphere. He also found that the increase in dry weight of plants was considerably greater than the increase in carbon and concluded that water was also assimilated. His data showed that the assimilation of water was linked to the photosynthetic process although he did not realise this. An understanding of the role of water in photosynthesis had to wait until later.

The later part of the nineteenth century saw many developments in ideas on photosynthesis. Three only will be mentioned here. First, Robert Mayer, who formulated the law of conservation of energy, drew attention to the energetic aspects of photosynthesis as a process in which light energy is converted into chemical energy. Secondly, J. Sachs observed the development of starch grains in photosynthesising chloroplasts. Thirdly, Engelmann carried out the first action spectrum for photosynthesis. Engelmann illuminated a filamentous alga with light passed through a prism. Motile bacteria sensitive to the level of dissolved oxygen moved to those parts of the filament where the greatest oxygen evolution occurred. This was in the parts illuminated with the blue and red regions of the spectrum, providing confirmation for the role of chlorophyll in photosynthesis.

Thus at the beginning of the twentieth century it was possible to write a simple equation for photosynthesis:

$$\text{Carbon dioxide} + \text{water} + \text{light energy} \xrightarrow{\text{chlorophyll}} \text{carbohydrate} + \text{oxygen}$$

The first step in analysis of this equation was the demonstration that the photosynthetic process consisted of separable light and dark reactions. A variety