4 Plant Mitochondrial Cytochromes

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1 Introduction

This chapter on plant mitochondrial cytochromes will report mainly on higher plants. Cytochromes from fungus, especially from yeast and from Neurospora, have been more extensively studied, as they are easier to prepare than those from higher plants. The starting material is homogenous, can be grown in large quantities and genetically manipulated, and thus provides a good tool for studies on biosynthesis. However, mitochondria from higher plants are now relatively easy to prepare in a high state of purity, by density gradient centrifugation in sucrose or Percoll, and they remain functional in oxidative and phosphorylative activities (see Chap. 1, this Vol.).

Higher plants contain cytochromes not only in mitochondria but also in chloroplasts and other membrane systems. In chloroplasts cytochromes are involved in electron transfer, even if the exact details of their functioning is not completely known. In other membranous systems, studies are scarce, but indicate the presence of b type cytochromes in microsomes and also in cellular fractions enriched in plasmalemma and perhaps in tonoplast membranes. Whether these b type cytochromes are involved in particular electron transport is not known, but they appear to be linked to special metabolism at certain steps of plant development: for example, these systems are inducible, much like those found in animal tissues, for detoxification. They are probably linked to the NAD or NADP cytochrome c reductases in these membranes, but their roles in metabolism are unknown; cytochrome c is certainly not the only physiological acceptor.

The presence of cytochromes in higher plants was reported in the first paper published by Keilin (1925) relating the presence in animal tissues and yeast of a new kind of pigment, the cytochrome. These discoveries were made possible by the use of the ocular microspectroscope and the Hartridge reversion spectroscope owing to their low dispersion. If MacMunn was in fact the first to have observed spectroscopically the cytochromes, which he named histoheatin, his observations were only done on animal tissues and Keilin was the first to show their presence in higher plants.

Among the Japanese workers who, in the 1930’s, developed numerous studies on cytochromes, Yakushiji (1934) did spectroscopic investigations on the presence of cytochromes in higher plants and algae. In soybean cotyledons he observed four bands characteristic of cytochromes, one of which, at 630–640 nm, he attributed to cytochrome a₂ (at that time found in some bacteria), in addition to the a, b, c components; moreover, he found that seed respiration was inhib-
ited by carbon monoxide. Other observations were made on either nonchlorophyllous tissues or acetone powder from green leaves (where he could see a sharp band at 550 nm, which eventually shifted to between 550 and 565 nm).

In the same period, LUNDEGÅRDH and BURSTRÖM (1933, 1935) associated anion uptake in roots with the part of respiration sensitive to cyanide or carbon monoxide: they postulated an anionic respiration giving rise to an active transport of anions bound to a flux of electrons through an organized respiratory system, including cytochromes (see LUNDEGÅRDH 1960b, for details). This concept was retained by MITCHELL (1961) when formulating the chemi-osmotic theory and the proton pumping redox loops.

The basic ideas of anionic respiration bound to the cytochrome system were initially in favor of a localization of the system near the outer surface of the cytoplasm. When it was shown that the cytochrome system is localized in mitochondria, LUNDEGÅRDH proposed that the organelles must manage at times to come into close contact with the outer plasma membrane, and then move to the tonoplast (LUNDEGÅRDH 1960b).

However, the views of LUNDEGÅRDH were critized by SMITH and CHANCE (1958) on the basis of slow kinetic responses of the cytochromes in relation to anion uptake. They suggested that the main response in LUNDEGÅRDH'S experiments was the one of a broad "cytochrome b" band, and that perhaps this band could be related to a nonrespiratory hemoprotein system involved in anion transport.

Knowledge of mitochondrial cytochromes rests on mitochondrial purity. Higher plant mitochondria were isolated earlier by differential centrifugation (MILLERD et al. 1951, GODDARD and BONNER 1960, BONNER 1967) and later by density gradient centrifugation. DOUCE et al. (1972) developed a method which is generally used now for the preparation of purified mitochondria which have retained their outer membrane. LOOMIS (1974) has reviewed the many pitfalls which must be bypassed for establishing grinding and assay media adapted to the preparation of mitochondria from various plant materials. Mitochondria from leaves were more difficult to prepare than from nonchlorophyllous tissues, due to chloroplast brittleness: chloroplast fragments heavily contaminate mitochondrial fractions. However, in density gradient centrifugation of pea and tobacco leaf homogenates, DUCET (1960) could show a band specifically stained by Janus green, which spectroscopically exhibited cytochrome bands and had a high cytochrome oxidase activity.

A number of reviews have been published on plant cytochromes including a chapter by LUNDEGÅRDH in the first Encyclopedia of Plant Physiology (1960a). The book, *Cytochromes*, by LEMBERG and BARRETT (1973) contains many references devoted to plant cytochromes. This chapter will focus mainly on more recent developments rather than seeking to present a complete survey of the subject.