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Chapter 1

BIOBLASTS, CYTOMIKROSOMEN AND CHONDRIOSOMES: A SHORT INCOMPLETE HISTORY OF PLANT MITOCHONDRIAL RESEARCH

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1.1 Discovery

Advances in microscopy in the nineteenth century, spurred on by the new cell theory, enabled pioneering cell biologists to make the first descriptions of granular bodies within the eukaryotic cell (see Scott and Logan 2004 for a brief history of cell biology). While some of these granules were probably mitochondria, the various fixation and staining methods employed at the time made their unambiguous identification impossible (Cowdry, 1918; Hughes, 1959). Unambiguous identification of mitochondria and an absence of agreed defining features inevitably led to very complex terminology for what we now simply know as mitochondria. This lack of defining characteristics meant that many structures considered by some investigators to be mitochondria either were not or were composed of mitochondria as well as other uncharacterized organelles (e.g. endoplasmic reticulum, Golgi). As documented by Cowdry (1918), there were nearly 100 names in the literature for mitochondria, or structures confused with mitochondria, ranging from A (apparato reticulare interno, the early name given to Golgi and confused

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with mitochondria) to Z (zentralkapsel, which may also have been applied to structures that were in fact Golgi).

Mitochondria were first named 'cytomikrosomen' by La Valette St George in 1867 following observations of highly refractive bodies, forming the nebenkern, that could be stained in living insect sperm cells with Dahlia, which was routinely used as a stain for protein at the time (Douglas, 1935). Other investigators, such as Albert von Kölliker, who has been credited with the first isolation of mitochondria in 1888 (Lehninger, 1964), Hermann Henking, discoverer of the *x* chromosome, and Toyama, reported similar structures in insect cells before two sets of detailed studies were published independently around the turn of the century by Friedrich Meves and Carl Benda (Cavers, 1914). In 1898, Benda coined the term 'mitochondria', derived from the Greek *mitos*, a thread, and *chondros*, a grain, although this new name was not immediately universally accepted (Tribe and Whittaker, 1972).

1.2 Complexity of nomenclature

In his extensive 1918 review, Cowdry is clearly exasperated with the complexity of nomenclature, writing that the complications and confusion are due to 'hasty individual action in elaborating new names, often only to discard them in a new paper in favour of some other'. Some researchers sought to convey information about organelle morphology, others about physiology or chemistry. Indeed, Benda's term 'mitochondria' was not immediately accepted because observations had shown that mitochondria sometimes existed in forms other than grains or threads. New terms were therefore introduced, some by Benda, to subdivide mitochondria into different morphological forms, for example 'chondriokonts' for rod-like structures, 'mitochondries' for granules, 'chondriosphären' for spheres, 'chondriomites' for filaments of granules, 'chondriocontes' for straight or curved threads. Thankfully, the term 'mitochondria' won through and thus we are saved from having to learn and understand myriad names for what is the same structure, albeit in a different morphological state.

1.2.1 Discoveries of mitochondria in plants

The first recorded observation of mitochondria in plant cells (of *Equisetum* sp.) has been attributed (Wayne, 2010) to Wilhelm Hofmeister in 1851 (discoverer of the alternation of generations, amongst his many other pioneering contributions to plant biology) but a more detailed report of mitochondria in plant tissues was made by Meves in 1904 (Cavers, 1914; Millerd and Bonner, 1953), who found them in tapetum cells in the anthers of the white water lily, *Nymphaea alba.* Many further studies, often also using tapetum cells, followed up on Meves' work and inevitably led to new controversies, this time regarding the origin and function of mitochondria, just as a consensus was being

reached on their name. With regard to origin, some researchers believed that plant mitochondria were of nuclear origin, originating as protuberances of the nuclear membrane or from chromatin. However, in 1910, papers were published by Lewitsky and by Pensa who both concluded that mitochondria occurred neither in, nor did they arise from the nucleus, but that they instead underwent division (Cavers, 1914). So far so good, as it turned out, but both these researchers, along with Forenbacher in 1911 and Guillermond in 1911 and 1912, believed their results demonstrated that mitochondria gave rise to plastids, going against the prevailing Schimper–Meyer theory of the *sui generis* origin of chloroplasts (Cavers, 1914). This view was a red flag to Meves who, according to (Cavers, 1914), 'demanded more definite proofs that chondriomes can be distinguished from small chromatophores and that the actual transformation of the former into the latter can be actually seen directly in the living cell, as for instance in filamentous algae'. It was not long before other researchers re-examined the mitochondria-plastid link and concluded that there was no question of a morphological relationship between mitochondria and chloroplasts (Cavers, 1914).

1.3 Mitochondria are dynamic

In the early 1900s, a time when some researchers refused to accept that mitochondria were specific, non-artefactual, independent, heritable constituents of the cytoplasm, others were convinced that mitochondria were a structure of considerable importance given their ubiquity across the animal and plant kingdoms. Among them was a husband and wife team of embryologists, Warren and Margaret Lewis (Margaret was also probably the first person to culture mammalian cells), who are credited with being the first to focus on the remarkable dynamics of the intriguing new organelle (Lewis and Lewis, 1914).

In their 1914 paper, Lewis and Lewis wrote of mitochondria in living tissue:

> [they] are almost never at rest, but are continually changing their position and also their shape. The changes in shape are truly remarkable not only in the great variety of forms, but also in the rapidity with which they change from one form to another.

Furthermore, the Lewises were able to witness mitochondria fusion and division:

> granules can be seen to fuse together into rods or chains, and these to elongate into threads, which in turn anastomose with each other and may unite into a complicated network, which in turn may again break down into threads, rods, loops and rings.

The Lewises are clearly enthralled by the dynamism they witness and end their paper with questions about mitochondrial biogenesis and function that, just as authors claim now, must wait for a more extensive study.

That extensive study was published the next year and runs to 62 pages (Lewis and Lewis, 1915). At the end of this remarkable piece of work, which describes the morphology and dynamics of mitochondria, their staining properties and their relation to other cell structures, the Lewises return to the question of the origin and function of mitochondria. A logical process then follows: they note that mitochondria have been found in almost every kind of cell, in plants, animals and protozoa. They remind readers that mitochondria have been claimed to form fibrillae in a variety of tissues, and to form secretory granules in the salivary, gastric and mammary glands, and to aid formation of the retina cells, and that they form the external shell of Foraminifera protists. We are further reminded of claims of direct or indirect roles in fat generation, and in the biogenesis of leucoplasts, chloroplasts and chromoplasts. The Lewises find all these claims difficult to reconcile. They believed instead that the mitochondria 'are too universal in all kinds of cells' to function in such specific ways, and, given what is known of biochemistry, considered it 'practically impossible' for mitochondria to form all these different structures. They conclude succinctly: 'They [mitochondria] are, in all probability, bodies connected with the metabolic activity of the cell' (Lewis and Lewis, 1915).

Despite the Lewises' detailed description of fusion and division of mitochondria in 1915, 82 years passed before identification of the first genetic mediator of mitochondrial fusion (the *Drosophila melanogaster fzo* gene) (Hales and Fuller, 1997) and a further 2 years before publication of the first mitochondrial division gene, *DNM1* (Sesaki and Jensen, 1999) (see Chapter 4). In the intervening years, researchers, having finally generally agreed on the name 'mitochondria', and that they were true organelles, instead focused their efforts on discovering mitochondrial function.

1.4 Mitochondrial function and outputs

The view held by the Lewises, that mitochondria were the sites of cellular oxidation, had been first proposed by Kingsbury (1912). Earlier, Altman had proposed his 'bioblasts' as the elementary particle of life, a view at least partially shared with Meves and Benda who, based on their observations of transfer of mitochondria from sperm to egg at fertilisation, were both of the view that mitochondria transported heritable characteristics. Indeed, Meves was careful to declare that his belief in a genetic role for mitochondria was in addition to the nuclear chromosomes – a view well ahead of its time. Despite Meves' standing, and this extensive hypothesis about a role in inheritance, most researchers believed plant mitochondria, as with animal mitochondria, were involved in nutrition. Kingsbury commented that although much morphological work had been performed using fixation and staining, there was

'too little cognizance of what kind of substances such a technique would be likely to preserve and bring out'. Kingsbury suggested that reducing power and protoplasmic activity were linked and that the mitochondria were the structures responsible for the consumption of oxygen in respiration. However, as noted by Cowdry (1924), determination of function required a greater knowledge of mitochondrial chemistry.

1.4.1 Vital staining of mitochondria with Janus green B and identification of mitochondria as sites of redox

A key event in the determination of mitochondrial function can be traced back to the demonstration by Leonor Michaelis in 1900 that mitochondria were capable of producing an oxidation-reduction change in the vital stain Janus green B (Tribe and Whittaker, 1972). Indeed, Lehninger (1964) stated that one of the most significant steps in our understanding of the function of mitochondria came from the development first of crystal violet as a mitochondrial stain by Benda in 1898 and then the vital staining of mitochondria with Janus green B. In 1913, Warburg demonstrated that the oxidation of metabolites was associated with insoluble, granular elements of the cell (Kennedy and Lehninger, 1949; Tribe and Whittaker, 1972), although he did not link these observations to mitochondria. This link was provided by Albert Claude who purified the 'respiratory particles' from rat liver by differential centrifugation and showed that they stained with Janus green, thereby identifying them as mitochondria as seen by light microscopy.

Further confirmation was provided by pioneering work in Albert Claude's laboratory by George E. Palade, that combined subcellular fractionation and subsequent biochemistry with electron microscopy, not only to confirm the isolated particles as mitochondria but also to subsequently define the structures of the mitochondria. By combining structure and functional studies in this way, Palade did much to invent the field of cell biology. In 1953, Palade and Fritiof S. Sjöstrand published their results on mitochondrial ultrastructure (Palade, 1953; Sjostrand, 1953). The two models were slightly different, with Palade proposing the existence of the cristae mitochondriales which form invaginations from an inner membrane, while Sjöstrand believed the inner membrane was not continuous with the outer and that the matrix, proposed by Palade, was a fixation artefact. Sjöstrand was, however, correct about the organelle having a double membrane, which was more clearly presented in his thinner ultramicrotome sections, although even on this point Palade had not been adamant since he had stated that 'in favourable electron micrographs the mitochondrial membrane appears to be double' (Palade, 1953).

Even before the contributions of Palade, Claude and their co-workers that were vital to linking biochemistry and cytology, Albert Lehninger was convinced that mitochondria were the sites of oxidative energy transduction (Kennedy, 1992). One of Lehninger's key discoveries was the inhibition of

fatty acid oxidation and oxidative phosphorylation in particulate cell extracts by exposure to hypotonic buffers. This observation was the subject of subsequent graduate studies by Eugene L. Kennedy which allowed Kennedy and Lehninger (1948) to conclude that fatty acid oxidation, oxidative phosphorylation and the reactions of the Krebs cycle took place in a single organelle bounded by a semi-permeable membrane. Next, using the newly described Palade method of differential sucrose density gradient centrifugation to purify mitochondria, Kennedy and Lehninger (1948, 1949) were able to present convincing evidence that the active organelle was the mitochondrion. In 1953, after over 50 years of use, the Janus green B reaction was formally linked to the reoxidation of the reduced dye by mitochondrial localized cytochrome oxidase (Lazarow and Cooperstein, 1953). The identification of cytochromes themselves as respiratory pigments was made by Keilin in 1925, who stated that they were a common biochemical feature of higher plants, animals and yeasts (Keilin, 1925). Despite Otto Warburg's refusal to accept their role (Slater, 2003), Keilin correctly identified cytochromes *a*, *b* and *c* as being major constituents of the respiratory chain, and they were later confirmed as being localized to mitochondria by Chance and Williams (1955).

While the studies just described paved the way for elucidation of individual reactions, their substrates, enzymes and products, and the association of these reactions into pathways, they did not complete the line-up of respiratory pathways open to plants. Not long after the discovery of cytochromes, Genevois in 1929 described a respiratory pathway in sweet pea (*Lathyrus odoratus*) that was resistant to cyanide, and hence independent of cytochromes (reviewed in Rogov *et al*., 2014). This alternative oxidation pathway was later associated with mitochondria in cellular preparations from *Arum maculatum* spadix by James and Elliot (1955), and found also to exist in other kingdoms, including yeast. The multiple roles of the alternative oxidase (AOX) have been debated for some time (including thermogenesis, energy overflow, resistance to cytotoxic compounds and antioxidant properties), but at its core this terminal oxidase provides plant mitochondria with a non-ATP-generating pathway in the electron transport chain that aids in cellular homeostasis (Vanlerberghe, 2013).

1.5 Mitochondrial DNA

By the 1960s, evidence was starting to grow that mitochondria contained their own nucleic acids (Nass and Nass, 1963a,b) (see Chapters 2 and 3), and were capable of producing proteins independently of cytoplasmic ribosomes (Haldar *et al*., 1967). The extension of mtDNA studies to plants (Suyama and Bonner, 1966) led to increasing interest in the transcriptional and translational machinery contained within these organelles (see Chapter 6 for a review of RNA metabolism). The ribosomal component of plant mitochondria was characterized in a series of biochemical experiments by Leaver and Harmey

(1972, 1973, 1976), who demonstrated that these ribosomes contained a 5S rRNA subunit, which is absent in animals and yeast. The mitochondrion is thus viewed as semi-autonomous. The mitochondrial genome encodes a few proteins, but they are vital, and these proteins are synthesized on mitochondrial ribosomes, from mRNA transcripts encoded in the mtDNA, transcribed and edited within the mitochondrion (see Chapter 6 for a review of RNA editing). But semi-autonomy is best reserved to thinking about the provision of the mitochondrion with the protein complement necessary for function. But that function is not autonomous – the mitochondrion is part of the cell.

While we can purify mitochondria, obtain snapshot information on their component materials and measure their activities, we must remain fully aware that we have ripped the mitochondria from their natural habitat and are no more likely to see natural behaviour from them than from a polar bear in Edinburgh Zoo. The signalling between mitochondria and nucleus, and indeed between mitochondria and other organelles, that is known to be important for function (see Chapter 7) has been lost. Isolated mitochondria will be stressed (see Chapter 8 for a review of mitochondria biochemistry and stress), and any 'recovery' probably more hopeful than actual. Luckily, technology allows more and more investigations to be performed *in vivo*; advances in imaging technology and sensors provide physiological readouts at incredible resolution. And development of synthetic biology, fuelled by knowledge gleaned from studies on isolated organelles, will allow experimenters to determine the extent to which the complex 3D ultrastructure of the plant cell, and its dynamism, is necessary for function.

1.6 Mitochondria, photosynthesis and carbon cycling

Plant mitochondria were shown to be a central part of maintaining efficient photosynthesis in the late 1970s, when they were identified as being the site for glycine oxidation (see Chapter 10). In C_3 plants, around 25% of photosynthetic output can be lost through the oxygenation reaction of Rubisco, which leads to the production of phosphoglycolate. After processing by chloroplasts and peroxisomes to glycine, this metabolite is shuttled to the mitochondria where it is oxidized, allowing further processing by peroxisomes to glycerate where it can re-enter the photosynthetic pathway. Studies by Kisaki *et al*. (1971), Woo and Osmond (1977) and Moore *et al*. (1977) showed that the enzyme activity responsible for glycine decarboxylation was localized to the mitochondria.

1.7 A trigger for death

The living-giving role of mitochondria in eukaryotes was well established by the middle of the twentieth century, but the role of mitochondria in programmed cell death took longer to become established in plants

than in metazoans. However, there is now a good deal of evidence to suggest that this organelle is a central part of the response (see Chapter 11). In animals, the induction of apoptosis (cf. programmed cell death) leads to several mitochondrial processes, including the translocation of Bax from the cytosol to the outer mitochondrial membrane, and the release of cytochrome *c* from the inter membrane space to the cytoplasm. Cytochrome *c* interacts with cytosolic factors that lead to the induction of caspase activity, a group of cysteine proteases that degrade cellular components in an orderly fashion (for review, see Desagher and Martinou, 2000, and Martinou and Youle, 2011). While there are no caspase homologues in higher plants, there is clear evidence for the early release of cytochrome *c* in plant programmed cell death (Balk *et al*., 1999). In addition, a family of proteins dubbed 'metacaspases' act in a similar manner to mammalian caspases (Lam and Zhang, 2012), indicating that the cell death pathway is relatively conserved (see Chapter 11).

1.8 Known knowns, known unknowns and unknown unknowns of mitochondrial biology

This introductory chapter has provided a brief historical overview of the key early discoveries in plant mitochondrial research. Inevitably, there are huge gaps; for example, there was no mention of Fe-S metabolism, arguably more important than aerobic respiration to some organisms. But the beauty of this book is that you can simply flick to Chapter 5 and fill that gap.

As the mass of research published on plant mitochondria grows, it becomes increasingly difficult to keep abreast of the subject. The amount of published research 'lost' to history increases. There is an increasing amount of information that is known but that we, as individuals, do not know. At least we know we do not know some details. Indeed, if we were being honest with ourselves, we may admit to not knowing more than just the details about some aspects of the subject of our research. So, we are comfortable in our ignorance of the known unknown. In that regard, review articles and books like this one, with chapters written by experts, are extremely important in reminding us all about those personal known unknowns.

Famously, in 2002, the serving US Secretary of State, Donald Rumsfeld, said during a press briefing:

> There are known knowns. There are things we know that we know. There are known unknowns. That is to say, there are things that we now know we don't know. But there are also unknown unknowns. There are things we do not know we don't know.

Many thought this statement nonsensical, but the concept of the unknown unknown, that is, the existence of things we do not know, as a species, we do not know, meaning even their existence is beyond our current conceptual

framework, probably arose with the dawn of consciousness. This book provides you with a selection of chapters reviewing the known knowns of the wonderful world of mitochondria, and the authors comment often on the known unknowns. However, as experts and not soothsayers, we cannot comment on the unknown unknowns, but it is exciting, and realistic, to think that some novel and unexpected mitochondrial function may yet be discovered.

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