Mitochondrion 19 (2014) 214–221

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Mitochondrial biogenesis in plants during seed germination

Simon R. Law [a], Reena Narsai [b], James Whelan [b,*]

[a] Australian Research Council Centre of Excellence in Plant Energy Biology, University of Western Australia, 35 Stirling Highway, Crawley, Western Australia, 6009, Australia
[b] Department of Botany, Australian Research Council Centre of Excellence in Plant Energy Biology, School of Life Science, La Trobe University, Bundoora, Victoria, 3086, Australia

ARTICLE INFO

Available online 13 April 2014

Keywords:
Seed germination
Biogenesis
Promitochondrion
Signalling
Stress
Retrograde

ABSTRACT

Mitochondria occupy a central role in the eukaryotic cell. In addition to being major sources of cellular energy, mitochondria are also involved in a diverse range of functions including signalling, the synthesis of many essential organic compounds and a role in programmed cell death. The active proliferation and differentiation of mitochondria is termed mitochondrial biogenesis and necessitates the coordinated communication of mitochondrial status within an integrated cellular network. Two models of mitochondrial biogenesis have been defined previously, the growth and division model and the maturation model. The former describes the growth and division of pre-existing mature organelles through a form of binary fission, while the latter describes the propagation of mitochondria from structurally and biochemically simple promitochondrial structures that upon appropriate stimuli, mature into fully functional mitochondria. In the last decade, a number of studies have utilised seed germination in plants as a platform for the examination of the processes occurring during mitochondrial biogenesis. These studies have revealed many new aspects of the tightly regulated procession of events that define mitochondrial biogenesis during this period of rapid development. A model for mitochondrial biogenesis that supports the maturation of mitochondria from promitochondrial structures has emerged, where mitochondrial signalling plays a crucial role in the early steps of seed germination.

1. Introduction

The defining feature of the eukaryotic cell is the compartmental organisation of the cellular landscape compared to the relatively simpler structure of prokaryotic cells. While traditional hypotheses of the origin of mitochondria suggest an endosymbiotic event with a nucleated cell, no evidence for such a cellular organism exists. In fact, eukaryotic cells that lack 'true' mitochondria, such as Giardia and trichomonads that contain mitosomes and hydrogensomes, respectively, are now recognised to represent a subsequent loss of mitochondria, with these organelles derived from the same endosymbiotic ancestor (Henze and Martin, 2003). Recent observations suggest that it may have been the mitochondrial endosymbiotic event itself that prompted the formation of internal membrane structures of the cell, including the formation of the nucleus (Lane and Martin, 2010). In light of this, the central signalling and controlling role of the nucleus may not be as predominant as is widely believed, and recent studies reveal crucial roles for the mitochondrion in many processes, beyond that of the traditional metabolic organelle.

Mitochondria occupy many roles in the cellular landscape, including (1) the synthesis of vitamins such as ascorbic acid, folic acid and biotin and selected amino acids (Birke et al., 2012; Rebeille et al., 2007); (2) being a major site of ROS production and thus having a role in cellular signalling (Rhoads et al., 2006); (3) as active participants in various metabolic and physiological pathways such as nitrogen assimilation (Foyer et al., 2011), iron homeostasis (Jain and Connolly, 2013) and lipid metabolism (Baker et al., 2006); (4) playing a central role in programmed cell death (PCD) (Reape and McCabe, 2010); and (5) producing cellular energy in the form of ATP through oxidative phosphorylation (Lenaz and Genova, 2010). As a vestige of their endosymbiotic origin, mitochondria possess their own genome. Over time, significant portions of this genome have been lost or transferred to the host nucleus, where it was free to recombine with host DNA (Martin et al., 1993). Consequently, mitochondria are unable to arise *de novo* in the manner of other organelles such as peroxisomes, endoplasmic reticulum (ER) and Golgi apparatus. Instead, two models have been hypothesised to explain their proliferation and differentiation; termed mitochondrial biogenesis (Nisoli et al., 2004). The first proposes the growth and division of pre-existing mature organelles through a form of binary fission that betrays their bacterial ancestry, while the second posits that mature organelles develop from small and functionally simple precursor organelles, termed promitochondria (Nisoli et al., 2004).

Mitochondrial biogenesis is a tightly regulated and multi-step procedure that requires the careful coordination of many processes, such as DNA replication, transcription, RNA modification (e.g., editing and splicing), translation and protein translocation, in addition to synchronising protein complex assembly with subunits from nuclear and mitochondrial origins (Chacinska et al., 2008; Giege et al., 2005; McCabe et al.,

* Corresponding author. Tel.: +61 3 90327488.
E-mail address: J.Whelan@LaTrobe.edu.au (J. Whelan).

http://dx.doi.org/10.1016/j.mito.2014.04.002
1567-7249/

2000; Menassa et al., 1999; Zmudjak et al., 2013). The literature describing the growth and division model of mitochondrial biogenesis has been established for many years from studies in *Saccharomyces cerevisiae* (yeast) and mammalian systems (Grivell, 1989; Nisoli et al., 2004; Tzagoloff and Myers, 1986). It has been shown that mitochondrial fission generally coincides with mitosis, with mitochondrial mass increasing during the period from S-phase to M-phase, ensuring that the resulting daughter cells receive a roughly equal complement of mitochondria upon cellular fission (Jahnke et al., 2009; Sanger et al., 2000). The second model of mitochondrial biogenesis, termed the maturation model, is not as well understood or studied. Initially observed in yeast systems, it describes the maturation of pre-existing populations of structurally and metabolically simple organelles, termed promitochondria, which develop into metabolically active 'mature' mitochondria. Specifically, populations of these promitochondria, with a deficient respiratory potential, were identified in yeast cells grown under anaerobic conditions, which were able to regain the ability to respire aerobically upon aeration (Plattner et al., 1970). A more recent study incorporated electron microscopy to define the ultrastructure of these precursor structures during the transition into mature mitochondria (Rosenfeld et al., 2004). The available evidence suggests that, unlike the growth and division model, which occurs far more frequently and under many different conditions, the maturation model is restricted to systems undergoing a fundamental phase transition, for example, yeast populations transitioning from anaerobic to aerobic conditions, or systems progressing from a state of quiescence to active metabolism, such as that seen during seed germination in plants.

2. Mitochondrial biogenesis during seed germination

2.1. Heterogeneity of mitochondrial populations in germinating seeds

Seed germination represents an attractive system to study mitochondrial biogenesis, as it is a unique stage in the plant life cycle that is characterised by an extended period of quiescence, followed by a switch to an energetically demanding state that necessitates the rapid and synchronised production of mitochondria. Accordingly, seed germination has been utilised in a number of studies examining mitochondrial biogenesis in both monocot and dicot plant species (Dai et al., 1998; Howell et al., 2006; Law et al., 2012; Logan et al., 2001). In maize and rice, electron microscopy and proteomic analysis in dry and imbibing seedlings characterised a population of promitochondria with poorly developed internal membranes lacking cristae significantly deficient in components of the electron transport chain. In maize, sucrose density gradients were used to fractionate crude homogenates of embryos dissected from dry seeds and seeds at four time points following imbibition, which identified the presence of two distinct subpopulations of mitochondria (referred to as *heavy* and *light* mitochondria) at every time point assayed (Logan et al., 2001). In dry seed, both subpopulations were composed of poorly developed mitochondria with *light* mitochondria exhibiting no significant changes in membrane morphology or protein content throughout the time course. However, upon continued imbibition, *heavy* mitochondria were observed to take on the characteristic appearance of mature mitochondria, with numerous cristae structures and an electron dense matrix, suggestive of a higher protein content (Logan et al., 2001). In rice, it was observed that promitochondria isolated from dry seeds were unable to import proteins, despite being relatively enriched in proteins with biogenesis functions such as the mitochondrial protein import components (Howell et al., 2006). However, just 30 min of imbibition was sufficient to restore import capacity, with significant protein import rates detected in isolated mitochondria (Howell et al., 2007). This suggests that all the necessary machinery for protein import is present in dry seeds and is rapidly activated upon imbibition. As germination progressed, the protein abundance of mitochondrial import components was observed to decrease, while proteins associated with primary metabolism increased in abundance (Howell et al., 2006). Paradoxically, protein import rates also increase over germination, while the abundance of the protein import machinery declines (Howell et al., 2006, 2007). This suggests that there may be some active degradation of the import machinery as the population shifts from promitochondria to mature mitochondria.

2.2. Which comes first, the mitochondrion or the promitochondrion?

Little is known about the formation of promitochondrial structures. There is some speculation that they derive from the selective degeneration of mature, metabolically active mitochondria, akin to the dedifferentiation of chloroplasts into non-photosynthesising proplastids, observed at the end of the seed maturation phase (Allorent et al., 2013). It is during these final stages of maturation that the seed can lose as much as 95% of its water content, transforming the seed into an incredibly resilient structure capable of tolerating extended periods of debilitating conditions. During this time, cells become a highly hostile environment for membrane bound structures such as mitochondria (Manfre et al., 2009). To circumvent cellular damage that can result from an anhydrobiotic lifestyle, such as protein denaturation and membrane fusion, a number of mechanisms have arisen to provide desiccation tolerance in the seeds of higher plants (for a review, see Macherel et al., 2007). An example of such an adaptation is the accumulation of members of a diverse group of proteins, known as late embryogenesis abundant (LEA) proteins, during the final stages of seed desiccation (Galau et al., 1986). The identification and characterisation of an LEA protein localised in the matrix space of mitochondria (LEAM) in pea (*Pisum sativum*) led to the findings that during desiccation, LEAM undergoes a conformational change that facilitates its insertion into the inner membrane, conferring desiccation protection to this vital site of cellular energy metabolism (Grelet et al., 2005; Tolleter et al., 2010). This could be one of the essential steps in the degeneration of mature mitochondria into promitochondrial structures; however, an intensive examination of mitochondria during embryogenesis and seed maturation is required to validate this hypothesis.

As mitochondria cannot arise *de novo*, the promitochondrial structure must have all the components necessary to facilitate differentiation into a fully functional mature mitochondrion. Thus, despite their differences in morphology, promitochondria must contain the mitochondrial genome and the cognate machinery required to express this genome, despite much of this machinery being synthesised in the cytosol and imported along with some species of tRNAs. The import of these mitochondrial proteins requires energy, in the form of both ATP and a membrane potential, so promitochondrial structures must be capable of supporting both. In terms of a membrane potential, it should be noted that as little as 30 mV is sufficient to support protein import, compared to the several-fold higher levels that drive ATP production via oxidative phosphorylation (Pfanner and Neupert, 1985). Additionally, it has been shown in plant mitochondria that oxidation of external NADH (which have been demonstrated to be active in imbibed seeds) by alternative oxidase (AOX) is capable of supporting protein import, as this results in the generation of a sufficient membrane potential (Whelan et al., 1995).

In terms of ATP, almost immediately upon imbibition, low levels of oxygen consumption and substrate level phosphorylation are likely to be sufficient to support import. In fact, the amount of ATP required for protein import into mitochondria is likely small in comparison to that required for protein synthesis (Piques et al., 2009). Thus, while promitochondrial structures differ to mitochondria with high metabolic activities seen in various organs or tissues, they cannot be viewed as being devoid of any metabolic activity. Rather, it appears that the proteins required for this activity are present in dry seeds and upon imbibition are activated to prime mitochondrial biogenesis (Ehrenshaft and Brambl, 1990; Howell et al., 2006; Logan et al., 2001). Likewise, mature mitochondria, which display high levels of metabolic activity, still retain a biogenesis capacity. Thus, promitochondria and mitochondria represent an organelle where both biogenesis and