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Article Addendum

Building the Powerhouse

What are the Signals Involved in Plant Mitochondrial Biogenesis?

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ABSTRACT

With a central role in respiration and ATP production, regulation of mitochondrial form and function is essential for cell and organism survival. Our understanding of the molecular mechanisms and signaling events underlying plant mitochondrial biogenesis is limited. In a recent paper published in the Journal of Biological Chemistry we have demonstrated aspects of mitochondrial biogenesis that are dependent on an oxygen signal in the monocot model, rice. Specifically, we identified (1) a set of genes encoding mitochondrial components that are responsive to oxygen levels and (2) that a lack of oxygen represses the normal increase in the mitochondrial protein import capacity during germination, and that these changes culminate in a modified mitochondrial proteome and altered respiratory activity. These findings can be combined with an earlier study, which gave insights into the characteristics of promitochondrial structures found in dry seeds and how they change during the germination process. Together they provide evidence for regulation of mitochondrial biogenesis by developmental and environmental cues and transcriptional and post-transcriptional events. This information can be used to build a model of plant mitochondrial biogenesis within the context of seed germination and oxygen availability.

INTRODUCTION

The concept of organelle biogenesis incorporates changes in the number, volume and/or functionality of the organelle of interest. For semiautonomous organelles such as plastids and mitochondria, de novo synthesis is not possible as they contain their own genome. Thus, the process of mitochondrial and plastid biogenesis requires the coordination of two distinct genetic systems and involves multiple regulatory steps, from expression of organellar and nuclear encoded genes to protein targeting, import and assembly, as well as organellar fission and fusion. Plastid biogenesis is relatively well understood where an undifferentiated proplastid can give rise to chromoplasts, amyloplasts, leucoplasts, etioplasts and chloroplasts in response to developmental and environmental signals.[1] A similar model has been proposed for mitochondrial biogenesis where undifferentiated promitochondria give rise to fully functional mature mitochondria. Early events in plant seed germination offer an attractive system to study this process as the transition from a quiescent to an active state is associated with a rapid increase in respiration[2] and furthermore, there is evidence for the presence of promitochondria in dry seeds.[3-5]

THE ROLE OF PROMITOCHONDRIA AND OXYGEN SIGNALING IN PLANT MITOCHONDRIAL BIOGENESIS

We have utilised a rice germination system to investigate plant mitochondrial biogenesis. Rice offers the advantage that it can germinate and grow without oxygen,[6] allowing the process of mitochondrial biogenesis to also be examined in response to an oxygen signal. An initial study developed the rice system revealing that undifferentiated mitochondria are present in unimbibed rice embryo tissue and have a low respiratory activity,[7] in agreement with studies in other plant species.[4,5,8] However, the most striking feature of promitochondria was found to be the high abundance of mitochondrial protein import components, a property which makes sense in terms of subsequent maturation events requiring the rapid accumulation of nuclear encoded proteins. This study also provided evidence for the presence of a functional electron transport chain that is reliant on oxidation of external NADH, at early stages of development, when the TCA cycle is limited by low levels of its

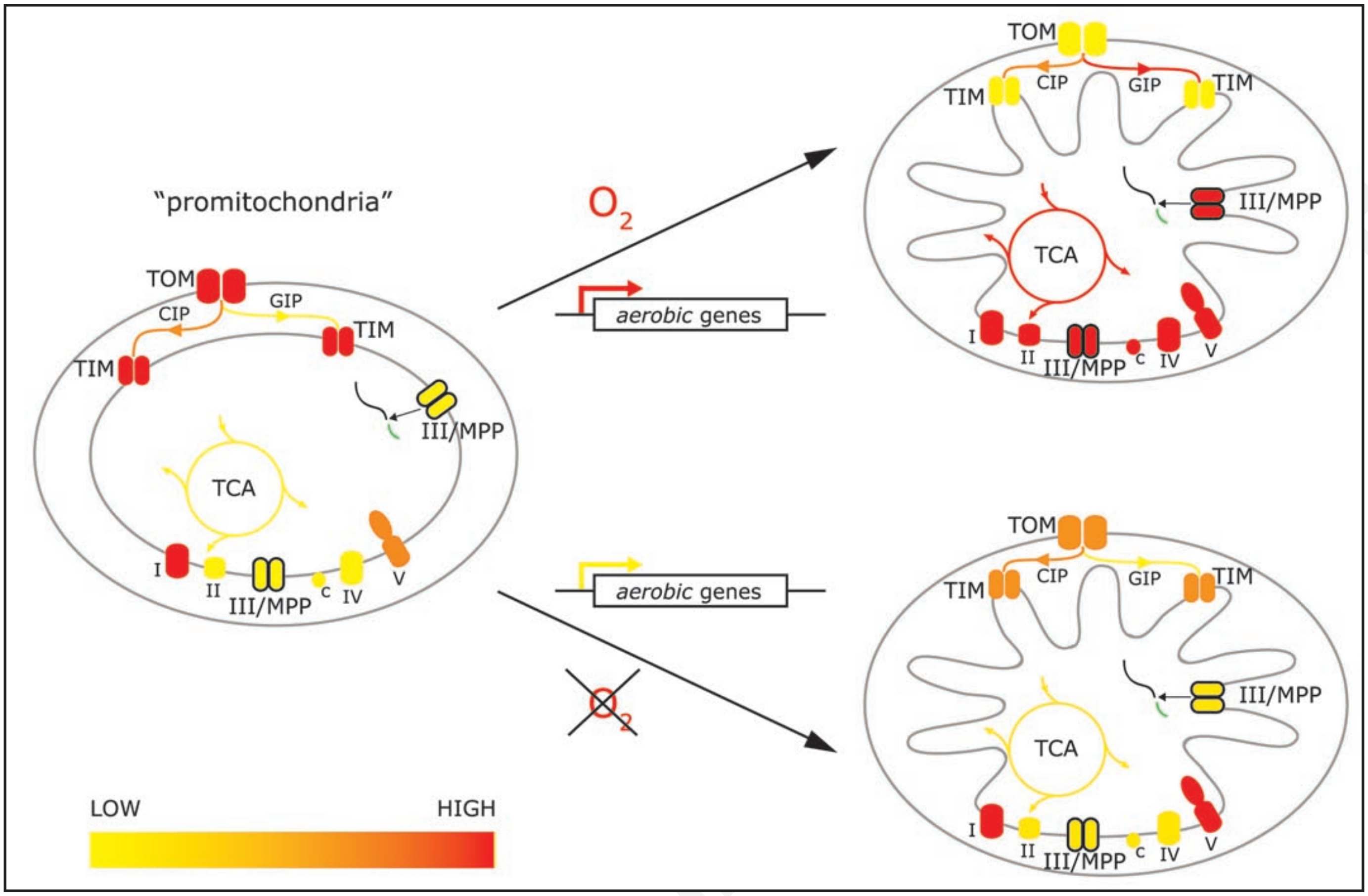

Figure 1. A model for plant mitochondrial biogenesis. Levels of mitochondrial components and functions are indicated by heat map colouration, with yellow indicating low levels and red indicating high levels. During germination promitochondria present in dry seeds, that have a simple ultrastructure, undergo morphological differentiation, such as cristae development, in both the presence and absence of oxygen. Components of the mitochondrial protein import apparatus (TOM and TIM) are at high levels in promitochondria, decrease to low levels in the presence of oxygen but decrease to a lesser extent under anaerobic conditions. Paradoxically, the capacity of the general import pathway (GIP) increases during germination under aerobic conditions but does not increase under anaerobic conditions. The capacity of the carrier import pathway (CIP) remains at similar levels regardless of developmental stage or oxygen availability. Levels of TCA cycle enzymes and some components of the respiratory chain (complexes I–V and cytochrome c) are at very low levels in promitochondria, increase substantially under aerobic conditions, and to a lesser extent in the absence of oxygen, driven by the transcriptional response of their respective nuclear encoded genes (*aerobic* genes). In plants, the mitochondrial processing peptidase (MPP) is integrated into complex III (bc_1 complex) of the respiratory chain and the increase in its abundance during germination is dependent on oxygen. This correlates with the capacity of the general import pathway, suggesting a mechanistic link between respiration and mitochondrial protein import.

constituent enzymes. Therefore, this investigation demonstrated that promitochondria are not simply empty, double-membrane bound cellular compartments, but contain components that facilitate their rapid differentiation into fully functional respiring mitochondria.

More recently we have examined which aspects of mitochondrial biogenesis are dependent on an oxygen signal.[9] Historically, changes in mitochondrial properties in response to oxygen availability have been examined in yeast[10] and a role for mitochondria in cellular oxygen sensing has been proposed.[11] Our investigation using rice revealed that as in yeast, some nuclear genes encoding mitochondrial components can be classified as *aerobic* i.e., are transcribed optimally in the presence of oxygen. We also demonstrated that a lack of oxygen represses the normal increase in the capacity of the general import pathway during germination. It was found that both transcript and protein levels of subunits of the mitochondrial processing peptidase (MPP), which cleaves presequences from imported proteins, were reduced under anaerobic conditions while the expression of other mitochondrial protein import components was not affected by oxygen levels. This difference was reconciled by the fact that, in plants, MPP is integrated into the cytochrome bc_1 complex of the respiratory chain,[12-15] whose levels are repressed under anaerobic conditions. In contrast, MPP in yeast is a soluble mitochondrial enzyme with no direct connection to the respiratory chain.[16]

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL BIOGENESIS

Both studies provide evidence that the regulation of mitochondrial biogenesis involves transcriptional and post-transcriptional regulatory events. We have observed dramatic increases in protein abundance of TCA cycle components and the heme requiring complexes of the respiratory chain (complexes III and IV and the mobile electron carrier cytochrome *c*) which correlate with increases in transcript levels suggesting that levels of some mitochondrial components are controlled at the level of transcription. The clearest example of post-transcriptional regulation relates to the changes seen in protein levels of components of the mitochondrial protein import machinery, which decline markedly, both over germination and in response to oxygen availability, while their transcript levels remain relatively stable. The dramatic decline in the protein abundance of import components indicates that active breakdown of these components is occurring. Whilst the mechanisms behind this degradative process are unknown, it clearly requires the coordinated action of both cytosolic factors, for the breakdown of the outer membrane-located TOM20, and the mitochondrial proteolysis machinery, for the breakdown of components of the inner membrane-located TIM complexes. Furthermore, this degradation appears to be regulated in response to oxygen as mitochondria isolated from anaerobically grown rice embryo tissue maintain protein import components at higher levels of abundance, presumably in an attempt to compensate for the reduced capacity of the general import pathway outlined previously.

A MODEL FOR PLANT MITOCHONDRIAL BIOGENESIS

By combining the findings of both of the studies discussed[7,9] a model for mitochondrial biogenesis in rice can be proposed (Fig. 1). The maturation of promitochondria, rich in components of the protein import apparatus, occurs in both the absence and presence of oxygen but results in the generation of mitochondria with different characteristics. While both show morphological differentiation, the "aerobic" mitochondria show reduced levels of components of the mitochondrial protein import apparatus, higher levels of TCA cycle components and the heme requiring complexes of the electron transport chain, and a higher capacity of the general import pathway, compared to "anaerobic" mitochondria. Thus, some aspects of mitochondrial biogenesis, such as cristae formation and carrier import pathway function, appear to be developmentally controlled and occur regardless of oxygen availability, while others, such as the expression of nuclear encoded *aerobic* genes and the general import pathway, are regulated by an oxygen signal. This demonstrates that promitochondria present in the dry seed can give rise to different "types" of mitochondria, dependent on environmental cues.

PERSPECTIVES

The identification of the key regulatory components involved in these processes is at an early stage. Research is focused on elucidation of the signaling pathways and identification of the transcription factors involved in the control of plant mitochondrial biogenesis and on defining the mechanisms modifying plant mitochondrial function in response to environmental cues, such as oxygen availability. An understanding of which mitochondrial components are coregulated will facilitate future investigations. By determining the subsets of genes that respond to particular developmental and environmental signals, such as germination and oxygen availability, further dissection of the molecular mechanisms (regulatory factors and signalling pathways) underlying the process of plant mitochondrial biogenesis will be possible. We are hopeful that the system we have established will be useful in this endeavor.

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