A PPR protein involved in regulating nuclear genes encoding mitochondrial proteins?

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The novel pentatricopeptide repeat protein PNM1 has recently been shown to be dual localized to the nucleus and mitochondria. In the nucleus it binds proteins involved in regulating gene expression, especially a TCP transcription factor. This class of proteins was recently shown to control the expression of nuclear genes encoding mitochondrial proteins that contain cis-acting “site II” regulatory elements in their promoter regions. The analysis of mutant plants showed that some genes with site II elements have increased expression levels when PNM1 is not present in the nucleus. This suggests that PNM1 might act as a negative regulator for the expression of an unknown number of genes with site II elements. Altogether, PNM1 might act as a nuclear regulator and/or could be a retrograde messenger molecule from mitochondria to the nucleus for the fine-tuning of nuclear gene expression required for mitochondrial biogenesis.

Mitochondria are semi-autonomous organelles that have arisen from an endosymbiotic event. They have retained a genome and harbor a complete gene expression machinery. However, the vast majority of mitochondrial proteins are encoded in the nucleus and have to be imported into mitochondria from the cytosol.^{1,2} Thus, mitochondrial biogenesis relies heavily on the coordinated expression of nuclear encoded genes. The mechanisms underlying this coordination and their regulation remain poorly understood. We have recently identified PNM1, a pentatricopeptide repeat (PPR) protein, dual localized to both mitochondria and

the nucleus, which could be involved in this regulation³ (Fig. 1). PPR proteins are ubiquitous in eukaryotes but particularly prevalent in higher plants.⁴ Most of them are predicted to localize to organelles and to be involved in post-transcriptional processes,⁵ many of which are plant-specific.^{6,7} These proteins are the most numerous family of regulatory proteins involved in gene expression in organelles.

How is the Dual Localization of PNM1 Achieved?

The dual localization of PNM1 to the nucleus and mitochondria has been established by several complementary approaches, i.e., in vivo by confocal microscopy, with specific antibodies used on purified cell fractions, by immunohistochemistry and electron microscopy. The mechanism by which this dual localization is achieved is unknown. The presence of two localization signals, an N-terminal mitochondrial targeting signal (MTS) as well as a C-terminal nuclear localization signal (NLS) could mean that PNM1 location is achieved by dual targeting as proposed and observed for a fast growing number of proteins in the plant cells.^{8,9} However, results of GFP fusion experiments show that the full length PNM1 is preferentially localized to mitochondria and that nuclear localization is only achieved when the MTS is masked or absent. In the same line of evidence, results show that the version of PNM1 detected in nuclear fractions seems to correspond to the mature (ΔMTS) PNM1. Taken together these results suggest that PNM1 could first be

Key words: pentatricopeptide repeat protein, plant mitochondria, crosstalk, retrograde regulation, TCP transcription factor

Submitted: 02/14/11

Accepted: 02/14/11

DOI: 10.4161/psb.6.5.15148

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Addendum to: Hammani K, Gobert A, Hleibieh K, Choulter L, Small I, Giegé P. An Arabidopsis dual-localized pentatricopeptide repeat protein interacts with nuclear proteins involved in gene expression regulation. *Plant Cell* 2011; 23:730-40; PMID: 21297037; DOI: 10.1105/tpc.110.081638.

Figure 1. Model for the distribution and function of PNM1 in both mitochondria and the nucleus of plant cells. In mitochondria, PNM1 is associated with ribosomes in an RNA-dependent manner. In the nucleus, it can interact with the nucleosome assembly protein NAP1 and the transcription factor TCP8. The analysis of mutants where PNM1 is unable to localize to the nucleus suggests that PNM1 is a negative regulator of nuclear genes encoding mitochondrial proteins. It is unclear whether PNM1 is dual targeted to both mitochondria and the nucleus in vivo or if it is re-exported from mitochondria for its nuclear localization.

Figure 2. Hypothesis for possible retrograde regulation between mitochondria and the nucleus involving PNM1. In this model, PNM1 would accumulate in the nucleus in response to external and/or developmental signals perceived by plant mitochondria. In the nucleus it would interact with TCP8. Transcription factors of the TCP family are known to bind cis-acting regulatory sequences called site II elements (SII), which have been shown to be involved in the coordinated expression of nuclear genes encoding mitochondrial proteins. Thus, the interaction of PNM1 with TCP8 might result in negative regulation of an unknown number of genes encoding mitochondrial proteins harboring site II elements in their promoter regions.

translocated to mitochondria and subsequently released from mitochondria for nuclear localization. The mechanism by which this mitochondrial export would take place is totally unknown. It could be an active transport involving an unidentified translocation system or alternatively a passive process where PNM1 would e.g., be released from disrupted mitochondria.

A Proposed Mitochondria to Nucleus Regulatory Pathway Involving PNM1

After its transfer to the nucleus, PNM1 is able to interact with the nucleosome assembly protein NAP1 and the transcription factor TCP8. Both proteins are involved in the control of gene expression in plant nuclei.^{10,11} TCP transcription factors were proposed to be involved in the coordinated expression of genes encoding mitochondrial proteins because they bind DNA sequences called site II elements that are prevalent in the promoter region of genes encoding components of the mitochondrial oxidative phosphorylation machinery. In contrast genes encoding components of alternative respiratory pathways do not show a particular prevalence for site II elements.^{12,13} The hypothesis of the involvement of site II elements and TCP transcription factors in the expression of mitochondrial proteins has been confirmed recently,^{14,15} in particular through the finding that TCP transcription factors mediate the coordinated expression of mitochondrial proteins during the circadian cycle.¹⁶ In this light, the results presented by Hammani et al. suggest that PNM1 might participate in the fine-tuning of this TCP-mediated coordinated expression. To achieve this, PNM1 might compete with site II elements for binding to TCP transcription factors. Alternatively, PNM1 might have a direct inhibitory effect on transcription activation itself (Fig. 2). The investigation of the precise distribution of PNM1 between mitochondria and the nucleus at different developmental stages, in different organs and/or in response to environmental stimuli will help to understand whether PNM1 is constantly present in the nucleus at equal levels or whether it is elicited in response to particular situations and might thus serve as a retrograde signaling molecule toward the nucleus.

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