FEBS Letters 583 (2009) 1391–1398

FEBS Letters

journal homepage: www.FEBSLetters.org

Minireview

The Miro GTPases: At the heart of the mitochondrial transport machinery

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ARTICLE INFO

Article history:
Received 2 March 2009
Revised 6 April 2009
Accepted 7 April 2009
Available online 18 April 2009

Edited by Peter Brzezinski

Keywords:
Miro
GTPase
Milton
Mitochondrion
Microtubule

ABSTRACT

Mitochondria are organelles of elaborate structure that in addition to supplying cellular energy, have significant roles in calcium homeostasis and apoptosis. Failure to maintain mitochondrial dynamics results in neurodegenerative diseases and neuromuscular pathologies. The Miro GTPases, which constitute a unique subgroup of the Ras superfamily, have emerged as essential regulators of mitochondrial morphogenesis and trafficking along microtubules. Miro GTPases function as calcium-dependent sensors in the control of mitochondrial motility. Increased awareness of the biological function of Miro GTPases can contribute to elucidate the molecular mechanisms underlying diseases caused by deregulated mitochondrial dynamics.

1. Introduction

Mitochondria are highly dynamic organelles that undergo constant changes in shape and distribution in order to perform the correct cellular assignment at the appropriate time and location. The classical view on mitochondria has stated that their prime role is to supply energy, in the form of ATP, to be utilized in cellular reactions. However, these organelles have turned out to play vital roles in calcium homeostasis, in formation of reactive oxygen species and in the initiation of apoptosis.

Mitochondria are transported along cytoskeletal tracks to areas in the cell where the energy demands are high and/or where calcium buffering is required. Rapid, long distance, transmission of mitochondria is accomplished via the microtubule network, whereas actin serves as tracks for short range transport of mitochondria to areas where the microtubules do not reach [1–3]. In addition, the integrity of intermediate filaments (IFs) is an important determinant for a correct localization of mitochondria as well as for the regulation of mitochondrial function (e.g. apoptosis, energy metabolism) [4]. When mitochondrial motility is inhibited, the organelle is suggested to dock to actin and microtubule-based cytoskeletal components [5,6]. However, the extra- and intracellular signals that control the motility of mitochondria are as yet elusive.

The mitochondrial morphology is controlled by precisely regulated cycles of fusion and fission. Fusion and fission are complex processes, due to the fact that the two mitochondrial membranes have to act in concert. In mammals, outer mitochondrial membrane fusion has been shown to depend on the activity of the Mitofusins1 and 2 (Mfn1 and 2), whereas OPA1, which is localized in the intermembrane space, is thought to be involved in the fusion of the inner membrane. Mammalian mitochondrial fission is, in turn, dependent on Drp1, a soluble, cytosolic protein that has to be recruited to the mitochondrial membrane. In yeast, the assembly of the fission machinery requires the presence of the outer mitochondrial membrane protein Fis1, however, in mammalian cells, this protein does not seem to be mandatory for Drp1 dependent assembly of the mitochondrial fission components. Activity of additional proteins, such as Endophilin B1 and MTP18, have also been ascribed roles in fission processes (reviewed in [7]). By enabling the exchange of mitochondrial content, mitochondrial fusion and fission prevent the accumulation of defective mitochondria and secure the availability of mitochondria in proliferating cells [8,9].

In eukaryotes, the dynamic properties of mitochondria are essential for embryonic development, proper neurological functions and the regulation of apoptosis (reviewed in [10]). Efficient mitochondrial trafficking is particularly important in neurons, where mitochondria are obligated to travel considerable distances along axons to supply synaptic endings with the energy needed for neurotransmitter release and recycling [11]. Furthermore, mitochondria play an important role in the regulation of neuronal

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0014-5793/$36.00 © 2009 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2009.04.015

calcium homeostasis [12]. Even though most mitochondrial proteins are encoded in the nuclear genome, recent data presented by Amiri and Hollenbeck indicate that mitochondria can replicate their DNA, divide and fuse locally within the axon, suggesting that the biogenesis of mitochondria is not limited to the cell body [13].

Even subtle perturbations of mitochondrial dynamics can, over time, give rise to severe effects in neurons. Inherited mutations in genes coding for mitochondrial dynamics proteins have been found to be responsible for a number of neurodegenerative diseases. For instance, mutations in *Mfn2* is linked to a dominant axonal form of the most common neuromuscular disorder, the Charcot-Marie-Tooth 2A (CMT2A) disease [14], and a majority of patients suffering from autosomal dominant optic atrophy harbour mutations in the *OPA1* gene [15]. Accumulating data indicate that aberrant mitochondrial dynamics can contribute to the pathogenesis of late-onset neurodegenerative conditions such as Amyotrophic Lateral Sclerosis (ALS), Huntington's, Parkinson's and Alzheimer's diseases [16].

In recent years, an effort has been made to identify the cellular components that govern mitochondrial dynamics. In this review article, we summarize the current knowledge of recently identified key components in mitochondrial dynamics; the Miro GTPases.

2. The Miro GTPases

The Miro GTPases constitute one of the six major branches in the Ras superfamily together with Ras, Rab, Ran, Arf and Rho. Members of the Ras superfamily typically act as regulators of diverse cellular processes by cycling between biologically active GTP- and inactive GDP-bound conformations [17]. The Miro proteins harbour two GTPase domains that flank a pair of calcium-binding EF-hands. The EF hands are functional, since they were shown to bind $^{45}Ca^{2+}$ in a dot blot overlay assay [18]. Two genes encoding Miro GTPases exist in human: *Miro1* and *Miro2* (also known as *RhoT1* and *RhoT2*). Human Miro1 and Miro2 both consist of 618 amino acid residues and share 60% sequence identity. The N-terminal GTPase domain displays similarities with the Rho GTPases, and therefore the Miro proteins were initially classified as Rho GTPases [19]. However, because of their distinct structural and functional properties, the Miro GTPases are now considered to constitute a separate subfamily of the Ras superfamily [20,21]. The C-terminal GTPase domain of the Miro proteins is only distantly related to other Ras superfamily members. Additionally, the Miro proteins lack the insert domain, a unique surface-exposed alpha-helical region that distinguishes Rho proteins from other Ras-related proteins. The insert domain has been ascribed roles in the activation of some effectors [22,23]. An additional and significant difference between the Miro proteins and Rho GTPases is the absence of CAAX-box in Miro. The CAAX-motif, which is typically present in Rho GTPases, is subject to post-translational isoprenylation that confers membrane targeting of the protein [24]. Instead of a CAAX-domain, the Miro proteins express a C-terminal transmembrane domain that is responsible for the insertion of the proteins into the outer mitochondrial membrane, resulting in exposure of the additional Miro domains into the cytosol [25,26] (Fig. 1a). The Rho GTPases are known to be potent regulators of the cytoskeleton [27]. However, the fact that transient expression of human Miro (hMiro)1 wild-type, or hMiro1 with a constitutive active mutation in its N-terminal GTPase domain (P13V), in COS-7 cells did not induce any obvious divergence in the organization of actin or microtubule structures, further supports the notion that the activity and roles of the Miro proteins are different from the Rho GTPases [19]. Hence, rather than visibly affecting cytoskeletal structures, the Miro GTPases are regulators of mitochondrial dynamics.

Members of the Miro subfamily are conserved throughout the eukaryotic phyla (Fig. 1b). The levels of Miro expression however, vary between different tissues and most likely correlate with mitochondria density and energy demand in a specific type of cell. In humans, both Miro1 and Miro2 appear to be universally expressed, demonstrating particularly high expression levels in heart and skeletal muscle tissues [19]. The expression profile analysis of the mouse Miro2 ortholog, ARHT2, showed that ARHT2 is ubiquitously expressed throughout adult tissues, with high expression in heart, testis, skeletal muscle and brain, and lower expression in liver, lung and spleen [28]. The fact that the Miro GTPases are conserved from yeast to human, and are expressed in most cell types, suggest that these proteins have an essential role in organism development and cell survival.

3. The role of Miro GTPases in mitochondrial dynamics

Ectopic expression of mutated Miro in cell culture models and in vivo models, have shown that deregulation of Miro causes dramatic effects on the mitochondrial distribution and morphology in a variety of cell types [18,19,25,29–32].

In COS-7 cells, ectopic expression of hMiro1 resulted in the formation of interconnected thread-like mitochondrial networks, an effect that was abolished in cells expressing hMiro1 carrying a dominant negative N-terminal GTPase domain or non-functional EF-hand domain [26]. Strikingly, hMiro1 or hMiro2 constructs carrying constitutively active mutations in their N-terminal GTPase domain, caused perinuclear aggregations of mitochondria in a high percentage of the cells. Furthermore, expression of the constitutively active variant of hMiro1 was found to result in an increased apoptotic rate. Notably, cells over-expressing the dominant negative hMiro1 variant displayed collapsed mitochondrial network to a higher degree than cells over-expressing hMiro1 wild-type [19]. These findings demonstrate that both an activating and a negative mutation in the N-terminal GTPase domain of hMiro disturb mitochondrial dynamics in a manner that gives rise to similar, but not identical, phenotypes.

In contrast to hMiro1, over-expression of hMiro2 was not found to give rise to interconnected mitochondria [26]. However, similar to what was seen in cells expressing hMiro1, expression of hMiro2 resulted in the formation of clustered mitochondria. Despite the fact that the two human isoforms of Miro are 60% identical and are likely to be co-expressed in most tissues, the finding that hMiro2 does not induce thread-like mitochondria in COS-7 cells could suggest that a functional distinction between hMiro1 and hMiro2 may wait to be revealed.

In summary, the data from the cell culture studies showed that the N-terminal GTPase and EF-hand domain are essential for the function of hMiro, whereas the GTP-binding capacity of the C-terminal GTPase domain had no obvious influence on mitochondrial morphology [26]. Evidently, the question arises; what are the functional properties of the C-terminal GTPase domain? Is it merely an evolutionary relic or is it an active domain with properties contributing to the overall function of the protein? In yeast, both the N-terminal and C-terminal GTPase domains have been shown to be important for the function of the Miro ortholog Gem1p. The introduction of a plasmid encoding Gem1p with a dominant negative mutation in GTPase domain 2, was unable to restore the tubular mitochondrial structures in budding yeast Gem1p null cells, suggesting that the C-terminal GTPase domain is indeed important for Miro function [25]. However, the potential biological function(s) of the Miro C-terminal GTPase in higher eukaryotic organisms remains elusive.

In contrast to animal cells, which mainly depend on the microtubule network for mitochondrial motility, plant cells and yeast