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Review

Prohibitin function within mitochondria: Essential roles for cell proliferation and cristae morphogenesis

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ABSTRACT

Prohibitins comprise an evolutionary conserved and ubiquitously expressed family of membrane proteins. Various roles in different cellular compartments have been proposed for prohibitin proteins. Recent experiments, however, identify large assemblies of two homologous prohibitin subunits, PHB1 and PHB2, in the inner membrane of mitochondria as the physiologically active structure. Mitochondrial prohibitin complexes control cell proliferation, cristae morphogenesis and the functional integrity of mitochondria. The processing of the dynamin-like GTPase OPA1, a core component of the mitochondrial fusion machinery, has been defined as a key process affected by prohibitins. The molecular mechanism of prohibitin function, however, remained elusive. The ring-like assembly of prohibitins and their sequence similarity with lipid raft-associated SPFH-family members suggests a scaffolding function of prohibitins, which may lead to functional compartmentalization in the inner membrane of mitochondria.

1. Introduction

A screen for potential regulators of cell proliferation led to the identification of a gene with apparently anti-proliferative activity which hence was termed prohibitin [1]. Although this activity was later attributed to the 3′ untranslated region of the gene [2], prohibitin became the founding member of a conserved protein family, with two highly homologous members, termed prohibitin 1 (PHB1) and prohibitin 2 (PHB2), ubiquitously expressed in eukaryotic cells [3,4]. A diverse array of cellular roles have been attributed to prohibitins since then, linking their function to aging [5,6] and a variety of disease states, like inflammation [7,8], obesity [9] and cancer [10,11]. Their molecular activity, however, remained largely elusive. PHB2 was identified as a binding partner of the IgM isotype of the B-cell receptor in the plasma membrane (and termed BAP37) [12] and, independently of PHB1, as a repressor of nuclear estrogen receptor activity (and termed REA) [13]. Besides the initially proposed role in cell cycle progression [1,14], prohibitins have also been implicated in transcriptional regulation [13,15], the regulation of sister chromatid cohesion [16], cellular signaling [12,17], apoptosis [18,19] and mitochondrial biogenesis [20–23]. How such a diverse range of functions can be exerted by evolutionary conserved proteins remained poorly understood and is controversially discussed, even more as prohibitins were localized to different cellular compartments, the plasma membrane, the nucleus and mitochondria in different studies. Recent functional studies, however, emphasize the role of mitochondria-localized prohibitins for cellular homeostasis. Here, we will review mitochondrial functions of prohibitins and the emerging evidence that the majority of cellular functions, if not all, can be attributed to prohibitin complexes localized in the inner membrane of mitochondria.

2. Functional prohibitin complexes in the inner membrane of mitochondria

Two members of the prohibitin family, PHB1 and PHB2, which are highly homologous to each other and share more than 50% identical amino acid residues, are expressed in eukaryotic cells and were localized to the mitochondrial inner membrane in various organisms [24,5,20,23]. Hydrophobic stretches at the amino terminal end anchor PHB1 and PHB2 to the membrane, while large carboxy terminal domains of ~30 kDa are exposed to the intermembrane space. These domains consist of a so-called PHB domain, characteristic of the SPFH-family of membrane proteins (see below), and a predicted coiled-coil region at the carboxy terminal end, which is crucial for the assembly of prohibitin complexes in yeast [25] (Fig. 1A).

Large membrane-bound complexes of PHB1 and PHB2 have been identified in various organisms. These structures are composed of multiple copies of PHB1 and PHB2 subunits and possess a native molecular mass of >1 MDa [21–23]. As first noted in yeast and later confirmed in *Caenorhabditis elegans* and mammalian cells, deletion of one prohibitin gene leads to the loss of both prohibitin proteins [20,23,26–28]. This does not reflect transcriptional co-regulation of

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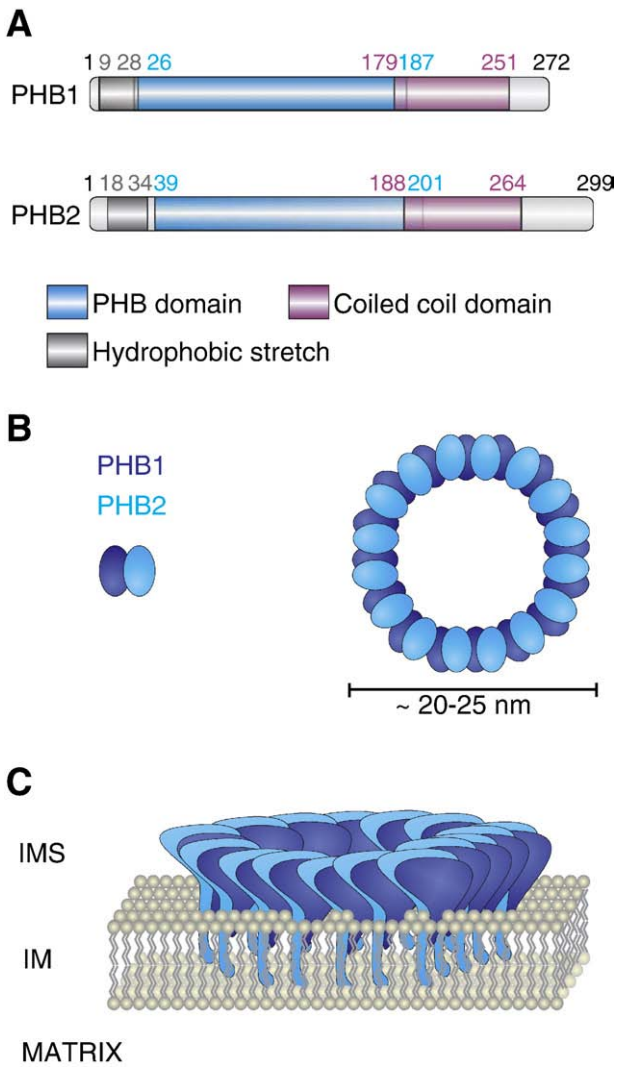

Fig. 1. Complex assembly of prohibitin subunits in mitochondria. Schematic representation of prohibitin subunits PHB1 and PHB2, the ring-shaped prohibitin complex and its topology in the mitochondrial inner membrane. (A) Domain structures of mammalian prohibitins. Gray boxes indicate hydrophobic stretches; blue, PHB domains (also termed SPFH domains); violet, coiled-coil domains. Numbers in corresponding colours refer to the respective amino acid residues in murine PHB1 and PHB2. (B) Dimers of PHB1 and PHB2 as building blocks of prohibitin complexes. Heterodimers assemble into ring-like prohibitin complexes with alternating subunit composition. The average stoichiometry of the complex is speculative. The average diameter of ring complexes is ~20–25 nm. (C) The prohibitin complex is anchored to the mitochondrial inner membrane via N-terminal hydrophobic stretches. Carboxy terminal PHB (SPFH) and coiled-coil domains are exposed to the intermembrane space (IMS). IM = inner membrane.

both genes, but rather degradation of prohibitin subunits in the absence of the respective assembly partner. Hence, complexes formed by PHB1 and PHB2 subunits represent the physiologically active structure and functional defects observed upon deletion or inactivation of individual prohibitin genes must be attributed to the loss of these complexes. This is also in agreement with coimmunoprecipitation experiments in human fibroblasts which revealed a quantitative assembly of PHB1 and PHB2 subunits [29].

Although detailed structural information is still lacking, studies in yeast provided first insight into the subunit arrangement of prohibitin complexes in the inner membrane of mitochondria (Fig. 1). Single particle electron microscopic images of purified yeast prohibitin complexes revealed a ring-like shape with a diameter of ~20–25 nm [25]. This is consistent with earlier crosslinking studies which detected only heteromeric crosslink adducts and therefore pointed to a ring-like assembly of alternating PHB1 and PHB2 subunits [30]. Heterodimers of PHB1 and PHB2 appear to represent building blocks for larger ring assemblies. PHB1 newly imported into yeast mitochondria associates first with Tim8/13 complexes in the intermembrane space, which function as molecular chaperones during the biogenesis of inner and outer membrane proteins [31]. The subsequent insertion into the inner membrane is mediated by the TIM23 translocase and accompanied by the assembly with PHB2 subunits into ~120 kDa complexes, before large ring complexes are formed [25]. Evidence for homomeric interactions between prohibitin subunits were not obtained in these studies further corroborating the notion that prohibitin subunits are active only in heterooligomeric assemblies.

3. Mitochondria-localized prohibitin complexes and cell proliferation

Severe phenotypes are associated with the loss of prohibitin subunits in multicellular organisms. Prohibitins are required for the embryonic development of *C. elegans* [23] and mice [32,27,28], hampering further functional studies on mammalian prohibitins on the organismal level. Knock-down experiments on a cellular level, however, revealed essential functions of PHB1 and PHB2 for cell proliferation [28,33]. Deletion of *Phb2* leads to the loss of both PHB1 and PHB2 proteins and impairs cell proliferation of mouse embryonic fibroblasts (MEFs) [28]. These findings are in striking contrast to the previously proposed anti-proliferative role of PHB1 [1,14] and the predicted function as a negative regulator of E2F-mediated transcription [34–36].

Despite compelling evidence for a mitochondrial localization of prohibitins, PHB1 and PHB2 have also been localized to the nucleus and the plasma membrane in certain cell types [19,9,37,7,17,26]. This raises the possibility that the requirement of prohibitins for cell proliferation reflects non-mitochondrial activities. Therefore, a functional complementation assay was developed to assess the dependence of cell proliferation on mitochondrial targeting of PHB2 [28]. Unconventional non-cleavable presequences at the amino terminal end of yeast prohibitins as well as murine PHB2 ensure mitochondrial sorting and insertion into the inner membrane [25,26]. Replacement of arginine residues by alanine within the sorting signal of murine PHB2 impairs targeting to mitochondria [28]. Expression of various mutant PHB2 variants in *Phb2*-deficient MEFs revealed a striking correlation between cell growth and mitochondrial targeting of PHB2: only those PHB2 variants that were correctly targeted to mitochondria were capable of maintaining cell proliferation [28]. At the same time, the growth of MEFs was not affected by mutations in predicted nuclear localization signals in PHB2. These findings suggest strongly that cell proliferation depends on prohibitin functions within mitochondria.

4. Prohibitin and the morphogenesis of mitochondrial cristae

Mitochondria constitute a reticulated network of interconnected tubules which is constantly remodelled by balanced fusion and fission events [38–40]. This dynamic behaviour depends on conserved protein machineries in the outer and inner membrane, including mitofusins and OPA1, dynamin-like GTPases in the outer and inner membrane of mitochondria, respectively [41,42]. The loss of prohibitins in MEFs or HeLa cells has severe consequences for the reticular mitochondrial network and leads to the accumulation of fragmented mitochondria [26,28]. Similarly, an abnormal mitochondrial morphology was observed in body wall muscle cells of *C. elegans* upon down-regulation of prohibitins [23]. These phenotypic alterations are most easily explained by an impaired fusion of mitochondrial membranes and concomitantly ongoing fission events and hence suggest that the prohibitins are essential components of the mitochondrial fusion machinery.

A detailed ultrastructural analysis in prohibitin-deficient MEFs revealed a defective morphogenesis of cristae in the absence of