Prohibitins and the functional compartmentalization of mitochondrial membranes

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Summary

Prohibitins constitute an evolutionarily conserved and ubiquitously expressed family of membrane proteins that are essential for cell proliferation and development in higher eukaryotes. Roles for prohibitins in cell signaling at the plasma membrane and in transcriptional regulation in the nucleus have been proposed, but pleiotropic defects associated with the loss of prohibitin genes can be largely attributed to a dysfunction of mitochondria. Two closely related proteins, prohibitin-1 (PHB1) and prohibitin-2 (PHB2), form large, multimeric ring complexes in the inner membrane of mitochondria. The absence of prohibitins leads to an increased generation of reactive oxygen species, disorganized mitochondrial nucleoids, abnormal cristae morphology and an increased sensitivity towards stimuli-elicited apoptosis. It has been found that the processing of the dynamin-like GTPase OPA1, which regulates mitochondrial fusion and cristae morphogenesis, is a key process regulated by prohibitins. Furthermore, genetic analyses in yeast have revealed an intimate functional link between prohibitin complexes and the membrane phospholipids cardiolipin and phosphatidylethanolamine. In light of these findings, it is emerging that prohibitin complexes can function as protein and lipid scaffolds that ensure the integrity and functionality of the mitochondrial inner membrane.

Key words: Membrane organization, Membrane scaffold, Mitochondria, Prohibitin

Introduction

Two decades have passed since the discovery of the first member of a remarkably conserved family of membrane proteins now known as prohibitins (McClung et al., 1989). Since then, members of this family have been identified in virtually all species, with two closely related proteins being expressed in each case (Nijtmans et al., 2002; Merkwirth and Langer, 2009). The absence of prohibitins leads to severe phenotypes and deficiencies in higher eukaryotes, indicating that these proteins have fundamentally important functions (Artal-Sanz et al., 2003; He et al., 2008; Merkwirth et al., 2008; Park et al., 2005). However, how prohibitins act at the molecular level has remained poorly understood, and even the subcellular localization of prohibitins has been controversial. Recent studies have now significantly advanced our understanding of the cellular role of prohibitins. Mitochondria have been identified as the predominant site of action of these proteins. In addition, high-molecular-weight complexes composed of multiple prohibitin subunits have been defined as the physiologically active structure in mitochondria, and the main downstream targets of prohibitin function have been characterized.

In this Commentary, we review the versatile cellular processes that have been linked to prohibitins, with a focus on their role in mitochondria. We also discuss a model in which prohibitins ensure mitochondrial membrane organization and functionality by serving as scaffolds for both proteins and lipids.

Prohibitins as multifunctional proteins?

Prohibitins comprise two evolutionarily conserved proteins, prohibitin-1 (PHB1) and prohibitin-2 (PHB2), which share more than 50% identical amino acid residues. Both proteins are present in organisms of all phylogenetic kingdoms. PHB1 was the first of the prohibitins to be discovered, and was identified on the basis that the expression of the gene was higher in normal cells compared with regenerating liver cells. Microinjection of *Phb1* mRNA into mouse embryonic fibroblasts (MEFs) prohibited cell-cycle progression, which gave rise to the name prohibitin (McClung et al., 1989). Although the antiproliferative activity of PHB1 was later attributed to the 3′ untranslated region of its mRNA, the name remained (Jupe et al., 1996a; Jupe et al., 1996b). PHB2 was isolated a few years later – together with PHB1 – as an interaction partner of the IgM receptor in mouse B cells, leading to the alternative names B-cell receptor-associated protein of 32 kDa (BAP32) and BAP37 for PHB1 and PHB2, respectively (Terashima et al., 1994).

Since these discoveries were made, a set of diverse functions has been attributed to both PHB1 and PHB2, opening up the possibility that prohibitins are multifunctional proteins. Prohibitins have been implicated in transcriptional regulation (Kurtev et al., 2004; Wang et al., 2002a) – giving rise to yet another name for PHB2, repressor of estrogen receptor activity (REA) (Montano et al., 1999) – as well as in the regulation of sister-chromatid cohesion (Takata et al., 2007), cellular signaling (Rajalingam et al., 2005), apoptosis (Fusaro et al., 2003), mitochondrial biogenesis and maintenance of mitochondrial DNA (Kasashima et al., 2008). It should be noted that these processes occur in different cellular compartments – the nucleus, the plasma membrane and mitochondria (Box 1). However, increasing evidence suggests that pleiotropic phenotypes that are observed in the absence of prohibitins reflect a dysfunction of mitochondria.

Mitochondrial functions of prohibitins

Prohibitins have been found to localize to mitochondria in all cell types examined to date. In vivo immunofluorescence studies, immunogold labeling and biochemical subcellular-fractionation experiments in various cell types and in different organisms have been used to identify prohibitins as integral membrane proteins of

Box 1. Functions of prohibitins in the nucleus and at the plasma membrane

Evidence for a non-mitochondrial function and localization of prohibitins to the nucleus or the plasma membrane has been provided in various mammalian cell lines. PHB1 has been proposed to act as a tumor suppressor protein. It was found to interact with the transcriptional regulators retinoblastoma-associated protein and E2F, and to inhibit transcription from E2F-responsive promoters and thereby suppress cell proliferation (Wang et al., 1999a; Wang et al., 1999b). PHB2 was shown to associate with members of the estrogen-receptor family and to inhibit transcription from target genes (Montano et al., 1999). The transcriptional inhibition by both prohibitins seems to be mediated by the recruitment of co-repressors (Kurtev et al., 2004; Wang et al., 2002b). A role for PHB1 in transcriptional activation from p53-responsive promoters, a process that involves a direct interaction between PHB1 and p53, has also been suggested (Fusaro et al., 2003). Another proposed function for PHB2 in the nucleus relates to sister-chromatid cohesion and is supported by the observation that RNAi-mediated depletion of PHB2 results in defects in chromosomal morphology and segregation that ultimately cause mitotic arrest (Takata et al., 2007).

Roles of prohibitins in signal transduction events at the plasma membrane have been suggested by the finding that PHB1 and PHB2 associate with the IgM receptor in B cells (Terashima et al., 1994). Furthermore, PHB1 was shown to interact with a capsular polysaccharide and with a synthetic peptide at the cell surface of human intestinal epithelial cells and white fat cells, respectively (Kolonin et al., 2004; Sharma and Qadri, 2004). How prohibitins act in these processes, however, remains unresolved. Finally, PHB1 was shown to directly interact with Raf at the plasma membrane and to mediate Ras-dependent displacement of the 14-3-3 protein from Raf (Rajalingam et al., 2005), which indicates that prohibitins are involved in modulating the Raf-MEK-ERK pathway. This pathway is crucial for epithelial-cell adhesion and migration.

the mitochondrial inner membrane (Artal-Sanz et al., 2003; Berger and Yaffe, 1998; Coates et al., 1997; Ikonen et al., 1995; Snedden and Fromm, 1997).

PHB1 and PHB2 are essential for cell proliferation and embryonic development in mice. Mouse embryos lacking either the *Phb1* or *Phb2* gene fail to develop beyond embryonic day 8.5 (He et al., 2008; Merkwirth et al., 2008; Park et al., 2005), and depletion of PHB1 or PHB2 impairs the proliferation of endothelial cells and MEFs (Merkwirth et al., 2008; Schleicher et al., 2008). These findings are in striking contrast to the previously proposed anti-proliferative role of prohibitins and their predicted function as a negative regulator of E2F-mediated transcription (as described in Box 1) (Wang et al., 1999a). The expression of PHB2 with mutations in its putative nuclear-localization sequences did not interfere with the proliferation of prohibitin-deficient MEFs (Merkwirth et al., 2008). By contrast, cells expressing PHB2 variants with mutations in residues that are essential for mitochondrial targeting were unable to maintain cell growth, indicating that the mitochondrial localization of PHB2 is essential for cell proliferation (Kasashima et al., 2006; Merkwirth et al., 2008). Whether the same holds true for PHB1 has yet to be determined.

Although compelling evidence indicates that prohibitins have a predominantly mitochondrial function, they might serve functions outside mitochondria under certain conditions or in certain cell types (Box 1). For example, PHB2 was shown to translocate from mitochondria to the nucleus following the binding of estradiol by ERα and following exposure of HeLa cells to capsaicin, the active component of chilli peppers, which binds to PHB2 but not to PHB1 (Kasashima et al., 2006; Kuramori et al., 2009). These studies suggest that prohibitins have non-mitochondrial functions, depending on regulatory cues. However, further evidence to support the relocalization of prohibitins to other cellular compartments, and for the underlying molecular mechanism of this relocalization, is still needed, because protein export from mitochondria has not been observed previously under non-apoptotic conditions.

Prohibitins in mtDNA maintenance and respiratory-chain assembly

The function of prohibitins in mitochondria has been linked to respiration and the stability of mitochondrial DNA (mtDNA) and, additionally, to the morphology of mitochondria. Crosslinking studies identified PHB1 and PHB2 as peripheral components of mitochondrial nucleoids, which are nucleoprotein complexes that contain mtDNA (Bogenhagen et al., 2008; Bogenhagen et al., 2003; Wang and Bogenhagen, 2006). Proteins incorporated in these nucleoid complexes, such as mitochondrial transcription factor A (TFAM) and mitochondrial single-stranded DNA-binding protein (mtSSB), regulate the stability, packaging, replication, transcription and maintenance of mtDNA (Chen and Butow, 2005). Remarkably, downregulation of PHB1 expression in HeLa cells affects the organization of mitochondrial nucleoids and the steady-state level of TFAM (Kasashima et al., 2008). These findings suggest that PHB1 maintains the organization and copy number of mtDNA by regulating TFAM stability (Kasashima et al., 2008).

However, depletion of prohibitins in various cell lines does not significantly affect (or at least does not completely abolish) cellular respiration, indicating that prohibitins are not essential for the maintenance of the mitochondrial genome. Knockdown of *Phb1* expression in epithelial cells induces cellular senescence (Schleicher et al., 2008). This was shown to be due to an inhibition of complex I of the mitochondrial electron transport chain by a mechanism that is not currently understood at the molecular level but that leads to an increased production of reactive oxygen species (ROS) and the depolarization of mitochondrial membrane potential (Schleicher et al., 2008). Similarly, prohibitin-deficient yeast cells display a reduced replicative lifespan that is associated with age-dependent mitochondrial degeneration (Coates et al., 1997; Wang et al., 2008). This phenotype is suppressed if cytosolic protein expression is reduced, which might prevent the accumulation of non-assembled proteins in the mitochondrial inner membrane that, in turn, triggers proton leakage (Wang et al., 2008). Notably, respiration and the assembly of respiratory supercomplexes was not impaired in *Phb2*$^{-/-}$ MEFs (Merkwirth et al., 2008). It remains to be determined whether variable effects of the loss of prohibitins on respiratory activities reflect differences in experimental conditions, because different approaches have been used to deplete cells of prohibitins. Alternatively, cell-type-specific differences in how prohibitins affect the stability of mtDNA and the biogenesis of the respiratory chain might exist.

Prohibitins in the maintenance of mitochondrial morphology

Independent of their role in respiration, prohibitins regulate the morphology of mitochondria. Abnormal mitochondria accumulate in the nematode *Caenorhabditis elegans* after RNA-interference (RNAi)-mediated depletion of prohibitins or in prohibitin-deficient yeast cells (Artal-Sanz et al., 2003; Berger and Yaffe, 1998; Osman et al., 2009). These findings were corroborated by studies

in mammalian cells: RNAi-mediated knockdown of either *Phb1* or *Phb2* expression in HeLa cells caused fragmentation of the mitochondrial network (Kasashima et al., 2006), which is composed of highly interconnected tubules formed by balanced fusion and fission events (Hoppins et al., 2007). Similarly, fragmented mitochondria were observed in prohibitin-deficient MEFs, which suggests that the fusion of mitochondrial membranes is impaired in the absence of prohibitins (Merkwirth et al., 2008). In addition, prohibitins are required for cristae morphogenesis, as revealed by an ultrastructural analysis of mitochondria in prohibitin-deficient MEFs (Merkwirth et al., 2008). A disturbed cristae morphology might facilitate the release of cytochrome *c* from the intracristal space and thereby explain the increased sensitivity of prohibitin-deficient MEFs to apoptotic stimuli.

The aberrant mitochondrial morphology observed in the absence of prohibitins can be explained by an altered processing of OPA1 (Merkwirth et al., 2008), a large dynamin-like GTPase that is found in the mitochondrial intermembrane space and that regulates both mitochondrial fusion and cristae morphogenesis (Hoppins et al., 2007). Mutations in *OPA1* cause degeneration of retinal ganglion cells in autosomal dominant optic atrophy (Alexander et al., 2000; Delettre et al., 2000). Proteolytic processing of OPA1 splice variants, which are expressed in a tissue-specific manner (Akepati et al., 2008; Delettre et al., 2001), results in the accumulation of long and short OPA1 isoforms (Delettre et al., 2001; Ishihara et al., 2006; Satoh et al., 2003). Strikingly, long OPA1 isoforms are destabilized in prohibitin-deficient MEFs, indicating accelerated processing and degradation of OPA1 (Merkwirth et al., 2008). The importance of prohibitins for normal OPA1 processing explains the mitochondrial morphology defects observed in *Phb2*$^{-/-}$ cells, because mitochondrial fusion depends on both long and short OPA1 isoforms (Ishihara et al., 2006; Song et al., 2007). Indeed, the ectopic expression of a non-cleavable long OPA1 isoform restored a tubular mitochondrial network, cristae morphogenesis and apoptotic resistance of *Phb2*$^{-/-}$ MEFs (Merkwirth et al., 2008). These experiments thus identify OPA1 processing as the key process that is regulated by prohibitins in MEFs.

The mechanism by which prohibitins affect OPA1 processing remains to be determined. Prohibitins assemble with the ATP-dependent *m*-AAA protease in large supercomplexes in the inner membrane of yeast mitochondria and modulate the proteolysis of non-assembled membrane proteins by this protease (Steglich et al., 1999). Mammalian *m*-AAA proteases have been linked to OPA1 processing in cellular systems (Ishihara et al., 2006) and are indeed able to cleave OPA1 variants when they are expressed in yeast (Duvezin-Caubet et al., 2007). Accordingly, prohibitins might regulate proteolytic processing of OPA1 by the *m*-AAA protease.

Interestingly, a protein that is distantly related to prohibitins, known as stomatin-like protein 2 (SLP-2), has been identified in the mitochondrial inner membrane of mammalian cells (Da Cruz et al., 2008; Da Cruz et al., 2003). SLP-2 directly interacts with and stabilizes prohibitins (Da Cruz et al., 2008). Similarly to prohibitins, the function of SLP-2 has recently been linked to mitochondrial fusion and the processing of OPA1. Crosslinking studies led to the identification of the GTPase mitofusin-2 (MFN2), a central component of the fusion machinery in the mitochondrial outer membrane (Hoppins et al., 2007), as an interaction partner of SLP-2 (Hajek et al., 2007). SLP-2, but not PHB2, is required for mitochondrial hyperfusion, which is induced in response to stress conditions including UV irradiation or low concentrations of cycloheximide (Tondera et al., 2009). Depletion of SLP-2 under these stress conditions results in the destabilization of long OPA1 isoforms (Tondera et al., 2009), which resembles the result obtained when prohibitins are depleted under normal conditions. Therefore, this suggests that a complex network of related proteins regulates OPA1 processing and mitochondrial fusion.

The molecular structure and function of prohibitins

Prohibitins form large, multimeric ring complexes

The finding that a plethora of functions are associated with prohibitins raises the intriguing issue of how prohibitins mediate these diverse cellular processes at the molecular level. The analysis of the native structure of PHB1 and PHB2 in yeast, nematodes and mammals has revealed that both proteins are present in a high-molecular-weight complex (~1.2 MDa) in the inner membrane of mitochondria (Artal-Sanz et al., 2003; Nijtmans et al., 2000; Steglich et al., 1999; Tatsuta et al., 2005). Co-immunoprecipitation experiments in both yeast and mammalian cells provide support that PHB1 and PHB2 physically interact (He et al., 2008; Ross et al., 2008; Steglich et al., 1999). The function of both prohibitin subunits is interdependent in various organisms (Artal-Sanz et al., 2003; Berger and Yaffe, 1998; Kasashima et al., 2006; Merkwirth et al., 2008). The absence of either prohibitin does not affect the expression of the other, but results in its degradation. These observations substantiate immunodepletion experiments that show that all PHB1 and PHB2 subunits are present in a complex with the respective other subunit, never as free proteins (Coates et al., 2001). Thus, the mitochondrial prohibitin complex (PHB complex) represents the physiologically active structure and, because the stabilities of PHB1 and PHB2 depend on one another, similar phenotypes are expected in the absence of either protein. In the light of these findings, previous reports that have proposed distinct functions of PHB1 or PHB2 in the nucleus or at the plasma membrane (Box 1) should be revisited.

The assembly of PHB complexes in the mitochondrial inner membrane has been investigated in detail in the yeast *Saccharomyces cerevisiae*. The analysis of purified yeast PHB complexes by single-particle electron microscopy revealed a ring-like structure with an outer diameter of about 200-250 Å (Tatsuta et al., 2005) (Fig. 1A). Crosslinking experiments detected interactions exclusively between Phb1 and Phb2 subunits and did not detect any homodimeric crosslink adducts, suggesting that the ring complexes are built up of multiple Phb1 and Phb2 subunits that alternate with each other (Back et al., 2002) (Fig. 1B). Phb1 and Phb2 are targeted to mitochondria by means of unconventional N-terminal targeting sequences, which are not cleaved upon mitochondrial import (Fig. 1C). Insertion of N-terminal hydrophobic sequences into the inner membrane is mediated by the Tim23 translocase and facilitated by the Tim8-Tim13 complex, a chaperone complex in the mitochondrial intermembrane space. The C-terminal coiled-coil domains of Phb1 and Phb2 (Fig. 1C), both of which expose large domains to the intermembrane space, are required for the subsequent oligomerization into large complexes. Initially, intermediate complexes of ~120 kDa that probably represent hetero-oligomeric tetramers of Phb1 and Phb2 subunits are formed. These intermediates act as building blocks for the formation of large ring-like assemblies (Back et al., 2002).

A chaperone function of prohibitins?

The fact that PHB complexes have large dimensions and form ring-like structures stimulated different proposals on the molecular function of prohibitins. The formation of ring complexes is

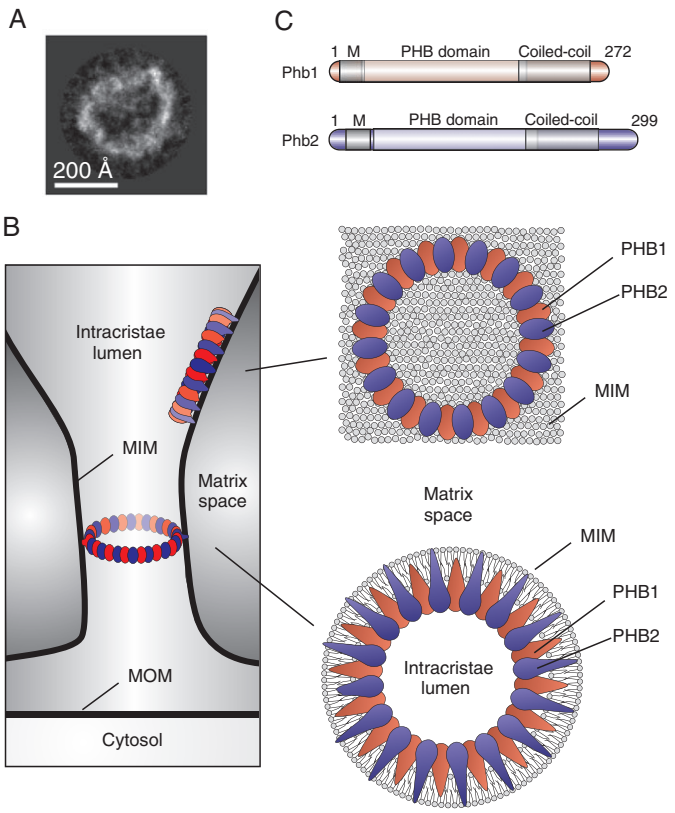

Fig. 1. The PHB complex in the mitochondrial inner membrane. (A) Single-particle electron micrograph of a purified yeast PHB complex (Tatsuta et al., 2005). (B) Multimeric PHB ring complexes in the inner membrane of mitochondria. Crosslinking studies suggest an alternative arrangement of PHB1 and PHB2 subunits in ring complexes (Back et al., 2002). PHB ring complexes integrated into the inner membrane might define local membrane domains (upper right). In view of the similar diameter of PHB complexes and cristae tubules, an assembly of PHB subunits perpendicular to the axis of cristae tubules is also conceivable (lower right). According to this scenario, PHB complexes would form a diffusion barrier within cristae membranes and could contribute to the stabilization of cristae tubules. (C) Domain structure of yeast Phb1 and Phb2. M, membrane domain; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane.

reminiscent of chaperonins, which are molecular-chaperone proteins that mediate protein folding. On the basis of the finding that prohibitins interact with unassembled respiratory-chain subunits, and that there is limited sequence similarity between prohibitins and chaperonins (Nijtmans et al., 2002; Nijtmans et al., 2000), a chaperone or holdase function for prohibitins has been proposed. Although the absence of prohibitins has not been found to cause general defects in the assembly of respiratory-chain complexes in various organisms, it has been observed that non-assembled subunits of the respiratory-chain complex are more rapidly degraded by the *m*-AAA protease in the absence of prohibitins (Nijtmans et al., 2002; Steglich et al., 1999). It should be noted, however, that this might also be due to the fact that *m*-AAA proteases have altered proteolytic activity in the absence of prohibitins. Moreover, the dimensions of PHB ring complexes substantially exceed those of chaperonins, and the sequence similarity between prohibitins and chaperonins is limited and is confined to a hinge region located between the ATPase and substrate-binding domains – that is, to a region that is not directly involved in substrate interaction. Therefore, a function of prohibitins as chaperones or holdases remains speculative.

Box 2. The SPFH protein family

The middle region of prohibitins contains a domain composed of ~160 amino acid residues. This domain has been identified in several other proteins and was termed the SPFH domain after the stomatin, prohibitin, flotillin and HflK/C founding proteins (and has also been referred to as the PHB domain) (Tavernarakis et al., 1999). Additional members include erlins, podocin and MEC-2. SPFH proteins localize to different membranes but share common characteristics, such as a similar domain structure. N-terminal sequences are required for correct subcellular localization and membrane attachment, whereas coiled-coil regions located C-terminally to the SPFH domain mediate assembly into high-molecular-weight complexes of greater than 1 MDa (Browman et al., 2007; Morrow and Parton, 2005). These complexes are often composed of multiple copies of two closely related SPFH proteins, which, in the case of prohibitins and flotillins, have been shown to be functionally interdependent – that is, one subunit is degraded in the absence of the other. Single-particle electron-microscopy analysis of PHB and erlin complexes identified ring assemblies with a diameter of 200-250 Å (Pearce et al., 2009; Tatsuta et al., 2005). Interestingly, many SPFH proteins cofractionate with detergent-resistant membranes, and stomatin-like proteins directly bind to cholesterol (Browman et al., 2007; Morrow and Parton, 2005). Accordingly, SPFH proteins have been linked to the establishment of distinct functional domains in different cellular membranes that play roles in diverse cellular processes: stomatin-like proteins regulate ion channels and mechanosensation; flotillins affect signaling across the plasma membrane and regulate membrane curvature and vesicle budding during clathrin-independent endocytosis; erlins target inositol (1,4,5)-trisphosphate receptors in the ER membrane for degradation; and prohibitins and bacterial HflK/C proteins associate with AAA proteases and are involved in proteolytic processes (Glebov et al., 2006; Huber et al., 2006; Kihara et al., 1996; Pearce et al., 2007; Wetzel et al., 2007).

A scaffold function of prohibitins?

An alternative but not mutually exclusive model suggests that prohibitins have a scaffolding function in the mitochondrial inner membrane. Accordingly, prohibitins might be required for inner-membrane organization and, for instance, for the recruitment of *m*-AAA proteases to specific functional sites. Such a view is supported by the similarities between prohibitins and a large family of distantly related membrane proteins, the SPFH family (Tavernarakis et al., 1999), which also includes mitochondrial SLP-2 (Box 2). A number of SPFH proteins have been found in defined membrane domains, suggesting that they act as scaffold proteins. The finding that SPFH-family members form high-molecular-weight complexes with a similar structural organization to those formed by prohibitins suggests that these proteins serve similar functions in distinct cellular membranes (Browman et al., 2007; Morrow and Parton, 2005). A role of prohibitins as scaffold proteins is also consistent with recent genetic evidence that links their function to mitochondrial phospholipid metabolism in yeast.

Prohibitins and the homeostasis of mitochondrial phospholipids

Although prohibitins are essential in higher eukaryotes (He et al., 2008; Park et al., 2005), yeast cells deficient for prohibitins are

viable but show a decreased replicative lifespan (Coates et al., 1997). However, a number of genes have been identified as essential for the survival of prohibitin-deficient yeast cells in various studies (Berger and Yaffe, 1998; Birner et al., 2003; Osman et al., 2009; Osman et al., 2007; Steglich et al., 1999). A systematic genetic survey of ~4,600 yeast strains lacking non-essential genes identified 35 genes that were essential in the absence of prohibitins and were therefore termed genetic interactors of prohibitins (GEP) genes (Osman et al., 2009). The majority of these genes encode mitochondrial proteins, highlighting the major role of prohibitins in mitochondria. Strikingly, GEP genes fall into distinct functional groups, including genes that control respiratory-chain assembly and those required for mitochondrial morphology and the assembly of β-barrel proteins. *ATP10*, *ATP23* and *OXA1* – three genes that are specifically required for the assembly of the membrane-embedded F_o-part of the mitochondrial ATP synthase (Jia et al., 2007; Tzagoloff et al., 2004; Zeng et al., 2007) – are among the synthetic lethal interactors of prohibitins, indicating that an impaired assembly of the F_o-part is deleterious in the absence of prohibitins (Osman et al., 2007). The enzymes that mediate the terminal steps in phosphatidylethanolamine (PE) and cardiolipin (CL) biosynthesis, Psd1 and Crd1, are essential for the survival of yeast cells in the absence of prohibitins (Birner et al., 2003; Osman et al., 2009).

The analysis of two GEP genes, *GEP1* and *UPS1*, provided further insight into the molecular function of prohibitins. Gep1 and Ups1 are members of the conserved but largely uncharacterized MSF1′/PRELI protein family (Dee and Moffat, 2005; Sesaki et al., 2006). Whereas Ups1 has been linked to processing of the OPA1 homolog Mgm1 in yeast (Sesaki et al., 2006), the function of Gep1 is unknown. Strikingly, overexpression of the phosphatidylserine (PS) synthase Cho1 restores the growth of *Δgep1Δphb1* cells, suggesting that increased levels of PS, the precursor for PE, were beneficial in these cells (Osman et al., 2009). Further experiments unraveled an essential role for Gep1 and Ups1 in promoting normal PE and CL levels within mitochondria, respectively, corroborating the functional link of prohibitins to the metabolism of mitochondrial phospholipids. Indeed, the levels of mitochondrial PE and/or CL are reduced in the majority of yeast strains lacking GEP genes (Osman et al., 2009) (Fig. 2). Downregulation of prohibitins in cells with low PE levels causes severe disturbances of the mitochondrial inner membrane and ultimately results in the dissipation of the mitochondrial membrane potential and cell death (Osman et al., 2009). It can therefore be concluded that prohibitins are essential for the integrity of the mitochondrial inner membrane if the levels of PE and CL are reduced.

A model for prohibitins as protein and lipid scaffolds

The characterization of the genetic interactome of prohibitins in yeast suggests that prohibitins and the membrane lipids CL and PE contribute to similar processes within the mitochondrial inner membrane that are essential for cell survival. Alterations in CL levels or in the composition of the fatty-acid chain of CL are associated with many pathophysiological states (Chicco and Sparagna, 2007; Joshi et al., 2008). CL ensures the optimal activity of various inner-membrane proteins, the stability of respiratory-chain complexes (Schlame, 2008; Wenz et al., 2009) and modulates apoptosis (Choi et al., 2007; Gonzalvez et al., 2008). Interestingly, PE and CL are related phospholipids and share the tendency to form non-bilayer, hexagonal phases; these play roles in membrane contact zones during fusion and fission processes and in the transmembrane movement of proteins (Cullis and de Kruijff, 1979; Dowhan, 1997; Schlame et al., 2000; van den Brink-van der Laan et al., 2004). The physiological relevance of these biophysical similarities is highlighted by the observation that yeast or *Escherichia coli* cells with dramatically decreased levels of PE and CL are inviable (Gohil et al., 2005; Rietveld et al., 1993). Both PE and CL tend to aggregate and form defined lipid clusters in the plasma membrane of bacteria (Kawai et al., 2004; Matsumoto et al., 2006; Nishibori et al., 2005). These clusters might play important roles in bacterial cell division and sporulation, and might serve as platforms for the recruitment of soluble proteins that bind to lipid head groups or to proteins with transmembrane segments that preferentially localize in these clusters (Matsumoto et al., 2006). It seems likely that this holds true also for the mitochondrial inner membrane, and that a lateral segregation of PE and CL in the inner membrane is crucial for mitochondrial processes.

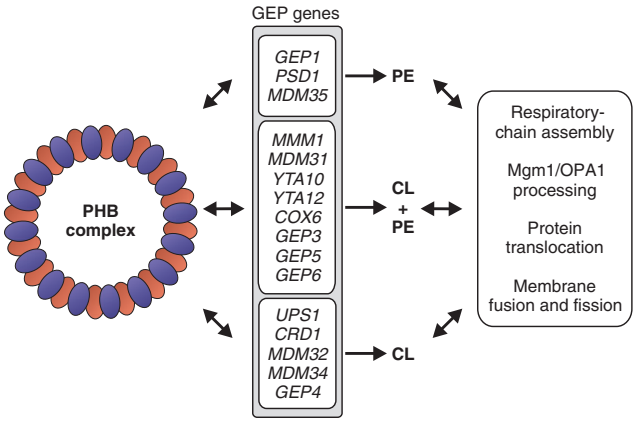

Fig. 2. GEP genes as novel regulators of mitochondrial CL and PE. Many genes essential for the survival of prohibitin-deficient yeast cells (GEP genes) are required to maintain mitochondrial PE and/or CL levels, and are associated functionally with diverse membrane-associated processes. The primary role of several GEP genes, either in regulating mitochondrial phospholipid metabolism or in other membrane processes, remains to be determined.

We propose that the scaffold function of PHB complexes supports the formation of such functional microdomains within the mitochondrial inner membrane (Fig. 3A). When levels of PE and CL are not limiting, the function of prohibitins is dispensable in yeast, and an asymmetric lipid distribution can be maintained in the membrane owing to the distinct biophysical properties of different phospholipids. However, prohibitin function becomes essential in yeast if PE or CL levels decrease. PHB complexes might support the formation of PE or CL patches under these conditions and locally enrich these phospholipids within the inner membrane. Clearly, further experimental support for a function of prohibitins as lipid scaffolds is needed, including evidence for a direct interaction between phospholipids and PHB complexes, or for an asymmetric lipid distribution in the inner membrane. It should be noted, however, that members of the SPFH family of proteins (Box 2) associate with defined membrane domains and interact directly with specific phospholipids (Browman et al., 2007; Huber et al., 2006). For example, flotillins, stomatins and podocin in other membranes cofractionate with detergent-resistant lipid rafts composed of cholesterol and sphingolipids (Bickel et al., 1997; Schwarz et al., 2001; Snyers et al., 1999). However, mitochondrial membranes contain only low amounts of cholesterol and sphingolipids, meaning that a similar lipid composition of prohibitin-associated membrane

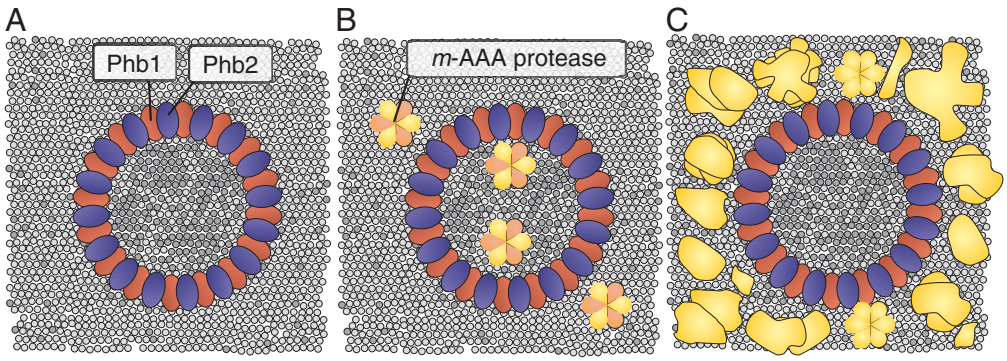

Fig. 3. PHB complexes as putative membrane scaffolds. PHB complexes might contribute to the spatial organization of the mitochondrial inner membrane in various non-mutually exclusive ways. (A) PHB complexes as lipid scaffolds. PHB complexes might support an asymmetrical distribution of phospholipids in the mitochondrial inner membrane. CL and/or PE (represented by dark-gray circles) might be enriched in the interior of PHB ring complexes. (B) PHB complexes as protein scaffolds. PHB complexes might recruit specific proteins, such as *m*-AAA proteases, to distinct functional sites. By acting both as protein and lipid scaffolds, PHB complexes might define functional microdomains within the inner membrane. (C) Fence-like function of PHB complexes. PHB complexes might exclude membrane proteins (indicated in yellow) from specific areas of the membrane bilayer and thereby generate protein-free lipid patches with functional relevance.

is unlikely. Moreover, a recent study did not provide any evidence for the existence of detergent-resistant lipid rafts in mitochondrial membranes (Zheng et al., 2009). It is therefore an attractive possibility that different SPFH proteins stabilize membrane domains that differ in their phospholipid compositions. The functional link between prohibitins and CL and PE suggests that prohibitins assist in the formation of membrane domains that are specifically enriched in CL and PE.

The interaction between PHB complexes and *m*-AAA proteases indicates that prohibitins can also act as protein scaffolds (Steglich et al., 1999) (Fig. 3B). *m*-AAA proteases might be recruited to distinct membrane domains whose lipid composition facilitates the degradation or processing of specific substrates or activates the proteolytic activity. The sequestration of *m*-AAA proteases by PHB complexes could modulate the access of proteolytic substrates to these membrane domains. In agreement with this scenario, loss of prohibitins leads to an increased turnover of non-assembled membrane proteins by the *m*-AAA protease in yeast (Steglich et al., 1999). Conversely, a function for PHB complexes as protein scaffolds could exclude proteins from specific areas of the membrane and thereby allow the formation of protein-free areas in the mitochondrial inner membrane, which is considered to be the most protein-rich membrane in the cell (Fig. 3C). This fence-like function of prohibitins might be of particular importance for membrane fusion events, which are known to depend on non-bilayer lipids (Dowhan, 1997).

Disruption of membrane organization and mitochondrial dysfunction

A role of prohibitins as membrane scaffolds suggests that a defined spatial membrane organization is required to maintain various mitochondrial activities. The assembly of *m*-AAA proteases with PHB complexes suggests that distinct membrane domains have a role in proteolytic processes. Indeed, the processing of Mgm1 and the morphogenesis of cristae were identified as processes that require CL and PE within mitochondria (Osman et al., 2009; Sesaki et al., 2006). Consistently, Mgm1 binds anionic phospholipids including CL in vitro (Meglei and McQuibban, 2009). PHB complexes acting as membrane scaffolds might recruit proteases, or ensure a specific lipid environment that facilitates Mgm1 processing, and thereby prevent mitochondrial fragmentation under conditions of limited CL or PE. The accelerated processing of OPA1 in *Phb2*$^{-/-}$ MEFs might result from a similar lipid dependence and impaired inner-membrane organization (Merkwirth et al., 2008). Similarly, the importance of CL for OPA1 cleavage could explain the disturbed mitochondrial morphology in cells lacking CL synthase or tafazzin, an enzyme involved in cardiolipin remodeling that is mutated in Barth syndrome (Choi et al., 2007; Claypool et al., 2008; Schlame, 2008). Differences in PE and CL levels in the mitochondrial membranes of different species might also provide an explanation for the puzzling observation that prohibitins are dispensable in yeast but essential in vertebrates. Alternatively, prohibitins might have acquired additional functions in higher eukaryotes. It is of interest in this context that the processing of OPA1, which has been identified as the key activity regulated by prohibitins in MEFs, is probably mediated by the *m*-AAA protease in mammalian cells (Ishihara et al., 2006). By contrast, the OPA1 homolog Mgm1 in yeast is cleaved by the rhomboid protease Pcp1 (Freeman, 2008; McQuibban et al., 2003).

Mitochondria-dependent apoptosis is another process that is influenced by the phospholipid composition of mitochondrial membranes and might depend on the scaffolding function of PHB complexes. CL provides an anchor and activating platform for caspase-8 at the mitochondrial surface (Gonzalvez et al., 2008), and affects Bax insertion and activation in liposomes in vitro (Lucken-Ardjomande et al., 2008). Depletion of CL releases cytochrome *c* from the mitochondrial inner membrane and accelerates stimuli-elicited apoptosis (Choi et al., 2007). It is therefore possible that a disturbed inner-membrane organization in the absence of PHB complexes facilitates cytochrome-*c* release and increases the sensitivity towards apoptotic stimuli in *Phb2*$^{-/-}$ MEFs (Merkwirth et al., 2008).

The assembly of the respiratory chain or of the F_1F_o-ATP synthase might be other processes that rely at least partially on the formation of prohibitin-assisted membrane domains. This is supported by the synthetic lethal interaction of yeast prohibitins with a number of known assembly factors of the respiratory chain (Osman et al., 2009). The assembly of the F_o-part of ATP synthase seems to be particularly sensitive to the loss of prohibitins (Osman et al., 2007). Assembly intermediates that accumulate in prohibitin-deficient yeast cells might disrupt the integrity of the inner membrane, resulting in proton leakage and cell death. Similarly, the increased generation of ROS and the aging phenotype in endothelial cells or in yeast (Coates et al., 1997; Schleicher et al., 2008; Wang et al., 2008) could be explained

by a compromised assembly of respiratory complexes in the absence of both prohibitins and a defined membrane organization.

Conclusions and perspectives

The emerging role of prohibitins as protein and lipid scaffolds highlights the importance of spatial membrane organization for normal mitochondrial function. The inner membrane, which is probably the most protein-rich membrane of the cell, has a complex ultrastructure, and increasing evidence suggests a functional compartmentalization by macromolecular complexes. Interactions between components of protein translocases in the outer and inner membrane (TOM and TIM complexes, respectively) allow the coordinated transport of nuclear-encoded proteins across both mitochondrial membranes (Bolender et al., 2008; Reichert and Neupert, 2002). Supercomplexes between respiratory-chain complexes are thought to increase the efficiency of oxidative phosphorylation by promoting substrate channeling (Acin-Perez et al., 2008; Schägger and Pfeiffer, 2000; Strauss et al., 2008). Prohibitin ring complexes might provide yet another means for inner-membrane organization and define functional membrane domains by acting as a scaffold for both proteins and lipids. The finding that distantly related membrane proteins of the SPFH family are present in other cellular membranes indicates that protein-assisted formation of functional membrane domains might be a novel organizing principle of general relevance. However, a challenging task for future studies will be to provide direct evidence for the existence of prohibitin-dependent membrane domains. It has been difficult thus far to unambiguously prove the existence of lipid microdomains in any membrane, because microdomains are believed to be extremely small and short-lived, and therefore be near the limits of detection using currently available methods. New, more powerful microscopic methods might allow visualization of an asymmetric lipid distribution in mitochondrial membranes in the future and open up new possibilities for further defining the role of prohibitins in organizing the mitochondrial inner membrane.

References

Acin-Perez, R., Fernandez-Silva, P., Peleato, M. L., Perez-Martos, A. and Enriquez, J. A. (2008). Respiratory active mitochondrial supercomplexes. *Mol. Cell* **32**, 529-539.

Akepati, V. R., Muller, E. C., Otto, A., Strauss, H. M., Portwich, M. and Alexander, C. (2008). Characterization of OPA1 isoforms isolated from mouse tissues. *J. Neurochem.* **106**, 372-383.

Alexander, C., Votruba, M., Pesch, U. E., Thiselton, D. L., Mayer, S., Moore, A., Rodriguez, M., Kellner, U., Leo-Kottler, B., Auburger, G. et al. (2000). *OPA1*, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat. Genet.* **26**, 211-215.

Artal-Sanz, M., Tsang, W. Y., Willems, E. M., Grivell, L. A., Lemire, B. D., van der Spek, H., Nijtmans, L. G. and Sanz, M. A. (2003). The mitochondrial prohibitin complex is essential for embryonic viability and germline function in Caenorhabditis elegans. *J. Biol. Chem.* **278**, 32091-32099.

Back, J. W., Sanz, M. A., De Jong, L., De Koning, L. J., Nijtmans, L. G., De Koster, C. G., Grivell, L. A., Van Der Spek, H. and Muijsers, A. O. (2002). A structure for the yeast prohibitin complex: structure prediction and evidence from chemical crosslinking and mass spectrometry. *Protein Sci.* **11**, 2471-2478.

Berger, K. H. and Yaffe, M. P. (1998). Prohibitin family members interact genetically with mitochondrial inheritance components in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **18**, 4043-4052.

Bickel, P. E., Scherer, P. E., Schnitzer, J. E., Oh, P., Lisanti, M. P. and Lodish, H. F. (1997). Flotillin and epidermal surface antigen define a new family of caveolae-associated integral membrane proteins. *J. Biol. Chem.* **272**, 13793-13802.

Birner, R., Nebauer, R., Schneiter, R. and Daum, G. (2003). Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine biosynthetic machinery with the prohibitin complex of Saccharomyces cerevisiae. *Mol. Biol. Cell* **14**, 370-383.

Bogenhagen, D. F., Wang, Y., Shen, E. L. and Kobayashi, R. (2003). Protein components of mitochondrial DNA nucleoids in higher eukaryotes. *Mol. Cell Proteomics* **2**, 1205-1216.

Bogenhagen, D. F., Rousseau, D. and Burke, S. (2008). The layered structure of human mitochondrial DNA nucleoids. *J. Biol. Chem.* **283**, 3665-7365.

Bolender, N., Sickmann, A., Wagner, R., Meisinger, C. and Pfanner, N. (2008). Multiple pathways for sorting mitochondrial precursor proteins. *EMBO Rep.* **9**, 42-49.

Browman, D. T., Hoegg, M. B. and Robbins, S. M. (2007). The SPFH domain-containing proteins: more than lipid raft markers. *Trends Cell Biol.* **17**, 394-402.

Chen, X. J. and Butow, R. A. (2005). The organization and inheritance of the mitochondrial genome. *Nat. Rev. Genet.* **6**, 815-825.

Chicco, A. J. and Sparagna, G. C. (2007). Role of cardiolipin alterations in mitochondrial dysfunction and disease. *Am. J. Physiol. Cell Physiol.* **292**, C33-C44.

Choi, S. Y., Gonzalvez, F., Jenkins, G. M., Slomianny, C., Chretien, D., Arnoult, D., Petit, P. X. and Frohman, M. A. (2007). Cardiolipin deficiency releases cytochrome c from the inner mitochondrial membrane and accelerates stimuli-elicited apoptosis. *Cell Death Differ.* **14**, 597-606.

Claypool, S. M., Boontheung, P., McCaffery, J. M., Loo, J. A. and Koehler, C. M. (2008). The cardiolipin transacylase, tafazzin, associates with two distinct respiratory components providing insight into Barth syndrome. *Mol. Biol. Cell* **19**, 5143-5155.

Coates, P. J., Jamieson, D. J., Smart, K., Prescott, A. R. and Hall, P. A. (1997). The prohibitin family of mitochondrial proteins regulate replicative lifespan. *Curr. Biol.* **7**, 607-610.

Coates, P. J., Nenutil, R., McGregor, A., Picksley, S. M., Crouch, D. H., Hall, P. A. and Wright, E. G. (2001). Mammalian prohibitin proteins respond to mitochondrial stress and decrease during cellular senescence. *Exp. Cell Res.* **265**, 262-273.

Cullis, P. R. and de Kruijff, B. (1979). Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochim. Biophys. Acta* **559**, 399-420.

Da Cruz, S., Xenarios, I., Langridge, J., Vilbois, F., Parone, P. A. and Martinou, J. C. (2003). Proteomic analysis of the mouse liver mitochondrial inner membrane. *J. Biol. Chem.* **278**, 41566-41571.

Da Cruz, S., Parone, P. A., Gonzalo, P., Bienvenut, W. V., Tondera, D., Jourdain, A., Quadroni, M. and Martinou, J. C. (2008). SLP-2 interacts with prohibitins in the mitochondrial inner membrane and contributes to their stability. *Biochim. Biophys. Acta* **1783**, 904-911.

Dee, C. T. and Moffat, K. G. (2005). A novel family of mitochondrial proteins is represented by the Drosophila genes slmo, preli-like and real-time. *Dev. Genes Evol.* **215**, 248-254.

Delettre, C., Lenaers, G., Griffoin, J. M., Gigarel, N., Lorenzo, C., Belenguer, P., Pelloquin, L., Grosgeorge, J., Turc-Carel, C., Perret, E. et al. (2000). Nuclear gene *OPA1*, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat. Genet.* **26**, 207-210.

Delettre, C., Griffoin, J. M., Kaplan, J., Dollfus, H., Lorenz, B., Faivre, L., Lenaers, G., Belenguer, P. and Hamel, C. P. (2001). Mutation spectrum and splicing variants in the *OPA1* gene. *Hum. Genet.* **109**, 584-591.

Dowhan, W. (1997). Molecular basis for membrane phospholipid diversity: why are there so many lipids? *Annu. Rev. Biochem.* **66**, 199-232.

Duvezin-Caubet, S., Koppen, M., Wagener, J., Zick, M., Israel, L., Bernacchia, A., Jagasia, R., Rugarli, E. I., Imhof, A., Neupert, W. et al. (2007). OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria. *Mol. Biol. Cell* **18**, 3582-3590.

Freeman, M. (2008). Rhomboid proteases and their biological functions. *Annu. Rev. Genet.* **42**, 191-210.

Fusaro, G., Dasgupta, P., Rastogi, S., Joshi, B. and Chellappan, S. (2003). Prohibitin induces the transcriptional activity of p53 and is exported from the nucleus upon apoptotic signaling. *J. Biol. Chem.* **278**, 47853-47861.

Glebov, O. O., Bright, N. A. and Nichols, B. J. (2006). Flotillin-1 defines a clathrin-independent endocytic pathway in mammalian cells. *Nat. Cell Biol.* **8**, 46-54.

Gohil, V. M., Thompson, M. N. and Greenberg, M. L. (2005). Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine and cardiolipin biosynthetic pathways in Saccharomyces cerevisiae. *J. Biol. Chem.* **280**, 35410-35416.

Gonzalvez, F., Schug, Z. T., Houtkooper, R. H., MacKenzie, E. D., Brooks, D. G., Wanders, R. J., Petit, P. X., Vaz, F. M. and Gottlieb, E. (2008). Cardiolipin provides an essential activating platform for caspase-8 on mitochondria. *J. Cell Biol.* **183**, 681-696.

Hajek, P., Chomyn, A. and Attardi, G. (2007). Identification of a novel mitochondrial complex containing mitofusin 2 and stomatin-like protein 2. *J. Biol. Chem.* **282**, 5670-5681.

He, B., Feng, Q., Mukherjee, A., Lonard, D. M., DeMayo, F. J., Katzenellenbogen, B. S., Lydon, J. P. and O'Malley, B. W. (2008). A repressive role for prohibitin in estrogen signaling. *Mol. Endocrinol.* **22**, 344-360.

Hoppins, S., Lackner, L. and Nunnari, J. (2007). The machines that divide and fuse mitochondria. *Annu. Rev. Biochem.* **76**, 751-780.

Huber, T. B., Schermer, B., Muller, R. U., Hohne, M., Bartram, M., Calixto, A., Hagmann, H., Reinhardt, C., Koos, F., Kunzelmann, K. et al. (2006). Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels. *Proc. Natl. Acad. Sci. USA* **103**, 17079-17086.

Ikonen, E., Fiedler, K., Parton, R. G. and Simons, K. (1995). Prohibitin, an antiproliferative protein, is localized to mitochondria. *FEBS Lett.* **358**, 273-277.

Ishihara, N., Fujita, Y., Oka, T. and Mihara, K. (2006). Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. *EMBO J.* **25**, 2966-2977.

Jia, L., Dienhart, M. K. and Stuart, R. A. (2007). Oxa1 directly interacts with Atp9 and mediates its assembly into the mitochondrial F1Fo-ATP synthase complex. *Mol. Biol. Cell* **18**, 1897-1908.

Joshi, A. S., Zhou, J., Gohil, V. M., Chen, S. and Greenberg, M. L. (2008). Cellular functions of cardiolipin in yeast. *Biochim. Biophys. Acta* **1793**, 212-218.

Jupe, E. R., Liu, X. T., Kiehlbauch, J. L., McClung, J. K. and Dell'Orco, R. T. (1996a). The 3′ untranslated region of prohibitin and cellular immortalization. *Exp. Cell Res.* **224**, 128-135.

Jupe, E. R., Liu, X. T., Kiehlbauch, J. L., McClung, J. K. and Dell'Orco, R. T. (1996b). Prohibitin in breast cancer cell lines: loss of antiproliferative activity is linked to 3′ untranslated region mutations. *Cell Growth Differ.* **7**, 871-878.

Kasashima, K., Ohta, E., Kagawa, Y. and Endo, H. (2006). Mitochondrial functions and estrogen receptor-dependent nuclear translocation of pleiotropic human prohibitin 2. *J. Biol. Chem.* **281**, 36401-36410.
Kasashima, K., Sumitani, M., Satoh, M. and Endo, H. (2008). Human prohibitin 1 maintains the organization and stability of the mitochondrial nucleoids. *Exp. Cell Res.* **314**, 988-996.
Kawai, F., Shoda, M., Harashima, R., Sadaie, Y., Hara, H. and Matsumoto, K. (2004). Cardiolipin domains in Bacillus subtilis marburg membranes. *J. Bacteriol.* **186**, 1475-1483.
Kihara, A., Akiyama, Y. and Ito, K. (1996). A protease complex in the Escherichia coli plasma membrane: HflKC (HflA) forms a complex with FtsH (HflB), regulating its proteolytic activity against SecY. *EMBO J.* **15**, 6122-6131.
Kolonin, M. G., Saha, P. K., Chan, L., Pasqualini, R. and Arap, W. (2004). Reversal of obesity by targeted ablation of adipose tissue. *Nat. Med.* **10**, 625-632.
Kuramori, C., Azuma, M., Kume, K., Kaneko, Y., Inoue, A., Yamaguchi, Y., Kabe, Y., Hosoya, T., Kizaki, M., Suematsu, M. et al. (2009). Capsaicin binds to prohibitin 2 and displaces it from the mitochondria to the nucleus. *Biochem. Biophys. Res. Commun.* **379**, 519-525.
Kurtev, V., Margueron, R., Kroboth, K., Ogris, E., Cavailles, V. and Seiser, C. (2004). Transcriptional regulation by the repressor of estrogen receptor activity via recruitment of histone deacetylases. *J. Biol. Chem.* **279**, 24834-24843.
Lucken-Ardjomande, S., Montessuit, S. and Martinou, J. C. (2008). Contributions to Bax insertion and oligomerization of lipids of the mitochondrial outer membrane. *Cell Death Differ.* **15**, 929-937.
Matsumoto, K., Kusaka, J., Nishibori, A. and Hara, H. (2006). Lipid domains in bacterial membranes. *Mol. Microbiol.* **61**, 1110-1117.
McClung, J. K., Danner, D. B., Stewart, D. A., Smith, J. R., Schneider, E. L., Lumpkin, C. K., Dell'Orco, R. T. and Nuell, M. J. (1989). Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver. *Biochem. Biophys. Res. Commun.* **164**, 1316-1322.
McQuibban, G. A., Saurya, S. and Freeman, M. (2003). Mitochondrial membrane remodelling regulated by a conserved rhomboid protease. *Nature* **423**, 537-541.
Meglei, G. and McQuibban, G. A. (2009). The dynamin-related protein Mgm1p assembles into oligomers and hydrolyzes GTP to function in mitochondrial membrane fusion. *Biochemistry* **48**, 1774-1784.
Merkwirth, C. and Langer, T. (2009). Prohibitin function within mitochondria: essential roles for cell proliferation and cristae morphogenesis. *Biochim. Biophys. Acta* **1793**, 27-32.
Merkwirth, C., Dargazanli, S., Tatsuta, T., Geimer, S., Lower, B., Wunderlich, F. T., von Kleist-Retzow, J. C., Waisman, A., Westermann, B. and Langer, T. (2008). Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria. *Genes Dev.* **22**, 476-488.
Montano, M. M., Ekena, K., Delage-Mourroux, R., Chang, W., Martini, P. and Katzenellenbogen, B. S. (1999). An estrogen receptor-selective coregulator that potentiates the effectiveness of antiestrogens and represses the activity of estrogens. *Proc. Natl. Acad. Sci. USA* **96**, 6947-6952.
Morrow, I. C. and Parton, R. G. (2005). Flotillins and the PHB domain protein family: rafts, worms and anaesthetics. *Traffic* **6**, 725-740.
Nijtmans, L. G., de Jong, L., Artal Sanz, M., Coates, P. J., Berden, J. A., Back, J. W., Muijsers, A. O., van der Spek, H. and Grivell, L. A. (2000). Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. *EMBO J.* **19**, 2444-2451.
Nijtmans, L. G., Artal, S. M., Grivell, L. A. and Coates, P. J. (2002). The mitochondrial PHB complex: roles in mitochondrial respiratory complex assembly, ageing and degenerative disease. *Cell Mol. Life Sci.* **59**, 143-155.
Nishibori, A., Kusaka, J., Hara, H., Umeda, M. and Matsumoto, K. (2005). Phosphatidylethanolamine domains and localization of phospholipid synthases in Bacillus subtilis membranes. *J. Bacteriol.* **187**, 2163-2174.
Osman, C., Wilmes, C., Tatsuta, T. and Langer, T. (2007). Prohibitins interact genetically with Atp23, a novel processing peptidase and chaperone for the F1Fo-ATP synthase. *Mol. Biol. Cell* **18**, 627-635.
Osman, C., Haag, M., Potting, C., Rodenfels, J., Dip, P. V., Wieland, F. T., Brugger, B., Westermann, B. and Langer, T. (2009). The genetic interactome of prohibitins: coordinated control of cardiolipin and phosphatidylethanolamine by conserved regulators in mitochondria. *J. Cell Biol.* **184**, 583-596.
Park, S. E., Xu, J., Frolova, A., Liao, L., O'Malley, B. W. and Katzenellenbogen, B. S. (2005). Genetic deletion of the repressor of estrogen receptor activity (REA) enhances the response to estrogen in target tissues in vivo. *Mol. Cell. Biol.* **25**, 1989-1999.
Pearce, M. M., Wang, Y., Kelley, G. G. and Wojcikiewicz, R. J. (2007). SPFH2 mediates the endoplasmic reticulum-associated degradation of inositol 1,4,5-trisphosphate receptors and other substrates in mammalian cells. *J. Biol. Chem.* **282**, 20104-20115.
Pearce, M. M., Wormer, D. B., Wilkens, S. and Wojcikiewicz, R. J. (2009). An Endoplasmic Reticulum (ER) Membrane Complex composed of SPFH1 and SPFH2 mediates the ER-associated degradation of Inositol 1,4,5-trisphosphate receptors. *J. Biol. Chem.* **284**, 10433-10445.
Rajalingam, K., Wunder, C., Brinkmann, V., Churin, Y., Hekman, M., Sievers, C., Rapp, U. R. and Rudel, T. (2005). Prohibitin is required for Ras-induced Raf-MEK-ERK activation and epithelial cell migration. *Nat. Cell Biol.* **7**, 837-843.
Reichert, A. S. and Neupert, W. (2002). Contact sites between the outer and inner membrane of mitochondria-role in protein transport. *Biochim. Biophys. Acta* **1592**, 41-49.
Rietveld, A. G., Killian, J. A., Dowhan, W. and de Kruijff, B. (1993). Polymorphic regulation of membrane phospholipid composition in Escherichia coli. *J. Biol. Chem.* **268**, 12427-12433.
Ross, J. A., Nagy, Z. S. and Kirken, R. A. (2008). The PHB1/2 phosphocomplex is required for mitochondrial homeostasis and survival of human T cells. *J. Biol. Chem.* **283**, 4699-4713.
Satoh, M., Hamamoto, T., Seo, N., Kagawa, Y. and Endo, H. (2003). Differential sublocalization of the dynamin-related protein OPA1 isoforms in mitochondria. *Biochem. Biophys. Res. Commun.* **300**, 482-493.
Schägger, H. and Pfeiffer, K. (2000). Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. *EMBO J.* **19**, 1777-1783.
Schlame, M. (2008). Cardiolipin synthesis for the assembly of bacterial and mitochondrial membranes. *J. Lipid Res.* **49**, 1607-1620.
Schlame, M., Rua, D. and Greenberg, M. L. (2000). The biosynthesis and functional role of cardiolipin. *Prog. Lipid Res.* **39**, 257-288.
Schleicher, M., Shepherd, B. R., Suarez, Y., Fernandez-Hernando, C., Yu, J., Pan, Y., Acevedo, L. M., Shadel, G. S. and Sessa, W. C. (2008). Prohibitin-1 maintains the angiogenic capacity of endothelial cells by regulating mitochondrial function and senescence. *J. Cell Biol.* **180**, 101-112.
Schwarz, K., Simons, M., Reiser, J., Saleem, M. A., Faul, C., Kriz, W., Shaw, A. S., Holzman, L. B. and Mundel, P. (2001). Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J. Clin. Invest.* **108**, 1621-1629.
Sesaki, H., Dunn, C. D., Iijima, M., Shepard, K. A., Yaffe, M. P., Machamer, C. E. and Jensen, R. E. (2006). Ups1p, a conserved intermembrane space protein, regulates mitochondrial shape and alternative topogenesis of Mgm1p. *J. Cell Biol.* **173**, 651-658.
Sharma, A. and Qadri, A. (2004). Vi polysaccharide of Salmonella typhi targets the prohibitin family of molecules in intestinal epithelial cells and suppresses early inflammatory responses. *Proc. Natl. Acad. Sci. USA* **101**, 17492-17497.
Snedden, W. A. and Fromm, H. (1997). Characterization of the plant homologue of prohibitin, a gene associated with antiproliferative activity in mammalian cells. *Plant Mol. Biol.* **33**, 753-756.
Snyers, L., Umlauf, E. and Prohaska, R. (1999). Association of stomatin with lipid-protein complexes in the plasma membrane and the endocytic compartment. *Eur. J. Cell Biol.* **78**, 802-812.
Song, Z., Chen, H., Fiket, M., Alexander, C. and Chan, D. C. (2007). OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. *J. Cell Biol.* **178**, 749-755.
Steglich, G., Neupert, W. and Langer, T. (1999). Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. *Mol. Cell. Biol.* **19**, 3435-3442.
Strauss, M., Hofhaus, G., Schroder, R. R. and Kuhlbrandt, W. (2008). Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. *EMBO J.* **27**, 1154-1160.
Takata, H., Matsunaga, S., Morimoto, A., Ma, N., Kurihara, D., Ono-Maniwa, R., Nakagawa, M., Azuma, T., Uchiyama, S. and Fukui, K. (2007). PHB2 protects sister-chromatid cohesion in mitosis. *Curr. Biol.* **17**, 1356-1361.
Tatsuta, T., Model, K. and Langer, T. (2005). Formation of membrane-bound ring complexes by prohibitins in mitochondria. *Mol. Biol. Cell* **16**, 248-259.
Tavernarakis, N., Driscoll, M. and Kyrpides, N. C. (1999). The SPFH domain: implicated in regulating targeted protein turnover in stomatins and other membrane-associated proteins. *Trends Biochem. Sci.* **24**, 425-427.
Terashima, M., Kim, K. M., Adachi, T., Nielsen, P. J., Reth, M., Kohler, G. and Lamers, M. C. (1994). The IgM antigen receptor of B lymphocytes is associated with prohibitin and a prohibitin-related protein. *EMBO J.* **13**, 3782-3792.
Tondera, D., Grandemange, S., Jourdain, A., Karbowski, M., Mattenberger, Y., Herzig, S., Da Cruz, S., Clerc, P., Raschke, I., Merkwirth, C. et al. (2009). SLP-2 is required for stress-induced mitochondrial hyperfusion. *EMBO J.* **28**, 1589-1600.
Tzagoloff, A., Barrientos, A., Neupert, W. and Herrmann, J. M. (2004). Atp10p assists assembly of Atp6p into the F0 unit of the yeast mitochondrial ATPase. *J. Biol. Chem.* **279**, 19775-19780.
van den Brink-van der Laan, E., Killian, J. A. and de Kruijff, B. (2004). Nonbilayer lipids affect peripheral and integral membrane proteins via changes in the lateral pressure profile. *Biochim. Biophys. Acta* **1666**, 275-288.
Wang, S., Nath, N., Adlam, M. and Chellappan, S. (1999a). Prohibitin, a potential tumor suppressor, interacts with RB and regulates E2F function. *Oncogene* **18**, 3501-3510.
Wang, S., Nath, N., Fusaro, G. and Chellappan, S. (1999b). Rb and prohibitin target distinct regions of E2F1 for repression and respond to different upstream signals. *Mol. Cell. Biol.* **19**, 7447-7460.
Wang, S., Fusaro, G., Padmanabhan, J. and Chellappan, S. P. (2002a). Prohibitin co-localizes with Rb in the nucleus and recruits N-CoR and HDAC1 for transcriptional repression. *Oncogene* **21**, 8388-8396.
Wang, S., Zhang, B. and Faller, D. V. (2002b). Prohibitin requires Brg-1 and Brm for the repression of E2F and cell growth. *EMBO J.* **21**, 3019-3028.
Wang, X., Zuo, X., Kucejova, B. and Chen, X. J. (2008). Reduced cytosolic protein synthesis suppresses mitochondrial degeneration. *Nat. Cell Biol.* **10**, 1090-1097.
Wang, Y. and Bogenhagen, D. F. (2006). Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane. *J. Biol. Chem.* **281**, 25791-25802.
Wenz, T., Hielscher, R., Hellwig, P., Schagger, H., Richers, S. and Hunte, C. (2009). Role of phospholipids in respiratory cytochrome bc(1) complex catalysis and supercomplex formation. *Biochim. Biophys. Acta* **1787**, 609-616.
Wetzel, C., Hu, J., Riethmacher, D., Benckendorff, A., Harder, L., Eilers, A., Moshourab, R., Kozlenkov, A., Labuz, D., Caspani, O. et al. (2007). A stomatin-domain protein essential for touch sensation in the mouse. *Nature* **445**, 206-209.
Zeng, X., Neupert, W. and Tzagoloff, A. (2007). The metalloprotease encoded by ATP23 has a dual function in processing and assembly of subunit 6 of mitochondrial ATPase. *Mol. Biol. Cell* **18**, 617-626.
Zheng, Y. Z., Berg, K. B. and Foster, L. J. (2009). Mitochondria do not contain lipid rafts and lipid rafts do not contain mitochondrial proteins. *J. Lipid Res.* **50**, 988-998.