Biochimica et Biophysica Acta 1793 (2009) 27–32

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

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

Carsten Merkwirth, Thomas Langer *

Institute for Genetics, Centre for Molecular Medicine (CMMC) and Cologne Excellence Cluster on Cellular Stress Responses in Aging-associated Diseases (CECAD), University of Cologne, 50674 Cologne, Germany

ARTICLE INFO

Article history:
Received 4 April 2008
Received in revised form 14 May 2008
Accepted 16 May 2008
Available online 17 June 2008

Keywords:
Prohibitin
OPA1
Mitochondrial fusion
Cristae
Membrane microdomains
Apoptosis

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

* Corresponding author. Institut für Genetik, Universität zu Köln, Zülpicher Str. 47, 50674 Köln, Germany. Tel.: +49 221 470 4876; fax: +49 221 470 6749.
E-mail address: Thomas.Langer@uni-koeln.de (T. Langer).

0167-4889/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.05.013

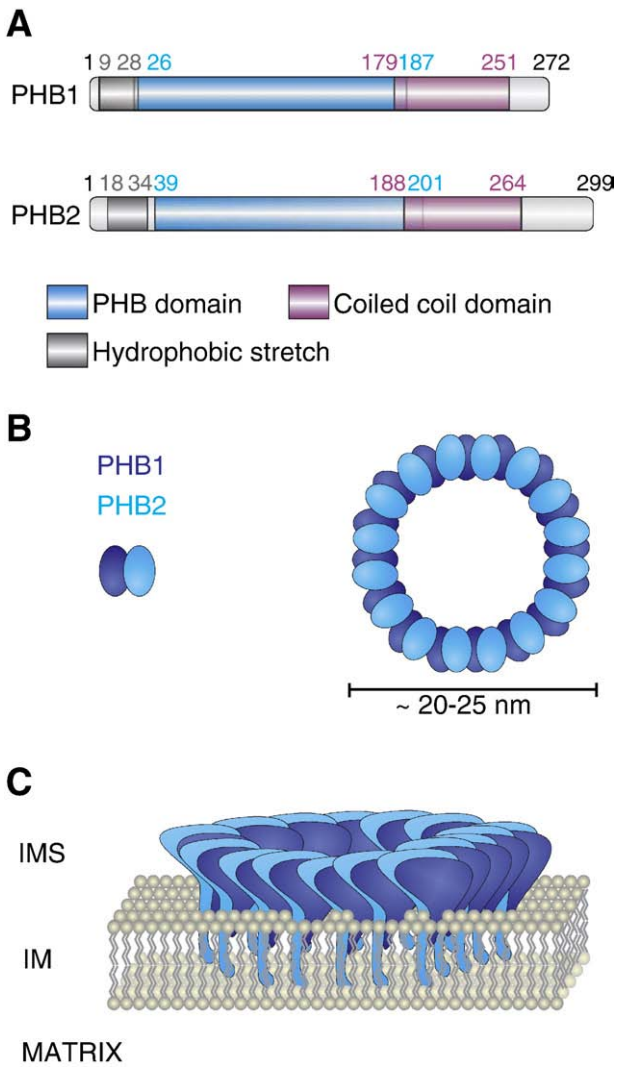

Fig. 1. Complex assembly of prohibitin subunits in mitochondria. Schematic representation of prohibitin subunits PHB1 and PHB2, the ring-shaped prohibitin complex and its topology in the mitochondrial inner membrane. (A) Domain structures of mammalian prohibitins. Gray boxes indicate hydrophobic stretches; 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

prohibitins [28]. Lamellar-shaped cristae were either almost completely absent or vesicular-shaped structures accumulated within prohibitin-deficient mitochondria. These structural alterations were attributed to the loss of long isoforms of OPA1 [28], which is not only required for mitochondrial fusion but also for cristae maintenance [43,44]. The activity of OPA1 depends on the balanced formation of long (L-OPA1) and short (S-OPA1) isoforms, the latter being derived from long isoforms by proteolytic processing [45,46,92]. The selective loss of long OPA1 isoforms in the absence of prohibitins suggests therefore an accelerated OPA1 processing and is sufficient to rationalize the aberrant mitochondrial ultrastructure in prohibitin-deficient cells. Consistently, expression of a non-cleavable OPA1 variant in these cells restored the tubular mitochondrial morphology demonstrating that prohibitins regulate mitochondrial morphology via OPA1 [28].

An impaired processing of OPA1 also explains the observed link of prohibitin function to apoptotic processes. Prohibitin-deficient MEFs did not undergo apoptosis, but exhibited an increased susceptibility towards various stimuli of apoptosis [28]. Notably, knock-down of individual prohibitin genes in HeLa or human T cells was observed to induce apoptosis [26,47] indicating cell-type specific differences. The induction of apoptosis requires restructuring of mitochondrial cristae at early stages of apoptosis to facilitate cytochrome c release from the intermembrane space [48,49], a process controlled by OPA1 [50]. A current model suggests that a complex containing L- and S-OPA1 controls mitochondrial cristae junctions and prevents the redistribution of cytochrome c from the cristal lumen to the peripheral intermembrane space [50]. Accordingly, the loss of L-OPA1 in prohibitin-deficient MEFs might facilitate cytochrome c release from intracristal compartments and allow the progression of the apoptotic programme. Consistently, expression of a non-cleavable L-OPA1 variant substitutes for the absence of prohibitins and protects prohibitin-deficient MEFs against apoptosis, demonstrating that prohibitins exert their anti-apoptotic function via OPA1 [28]. The processing of OPA1 thus appears to represent the key cellular process controlled by prohibitins. This notion is further substantiated by the observation that the growth of prohibitin-deficient MEFs is at least partially restored upon expression of L-OPA, suggesting a coupling of cell proliferation to mitochondrial morphogenesis [28].

5. Prohibitins and the respiratory chain

Loss of the prohibitin complex in MEFs or in yeast does not affect the mitochondrial membrane potential and respiratory activity [5,20,28] excluding an essential role of prohibitins for the biogenesis of the respiratory chain. However, cell-type specific differences are likely to exist. Recently, a crucial role of PHB1 for angiogenesis was revealed [33]. A reduced mitochondrial membrane potential and complex I activity was observed upon knock-down of PHB1 in endothelial cells, which was associated with a senescent-like phenotype [33]. Similarly, loss of prohibitins in yeast shortens replicative life span [20,6]. While this has been attributed to a defective mitochondrial segregation in old mother cells in yeast [51], the senescent phenotype of prohibitin-deficient endothelial cells was correlated with an increased production of reactive oxygen species (ROS) in these cells [33]. Overexpression of PHB1 in intestinal endothelial cells decreased the accumulation of ROS suggesting that prohibitins protect against oxidative stress [8]. As PHB1 but not PHB2 was overexpressed in these experiments, it will be of interest to examine the requirement of PHB2 for the apparently protective function of PHB1.

How prohibitins may affect complex I activity and ROS production in endothelial cells is currently not understood. Interestingly, an increase in ROS production and mitochondrial fragmentation was recently reported in a *Drosophila* model for optic atrophy caused by mutations in *Opa1* [52], raising the possibility that prohibitins act also in this process via OPA1. Moreover, it is noteworthy in this context that prohibitins have been co-purified with mitochondrial DNA nucleoids from *Xenopus* oocytes and HeLa cells [53,54]. Evidence for a reduced copy number and an altered status of mtDNA upon RNAi-mediated depletion of PHB1 from HeLa cells was provided, which was accompanied by reduced protein levels of transcription factor A (TFAM) [55], a DNA binding protein with essential functions for mtDNA metabolism [56]. Although a potentially suppressive effect of OPA1 has not been examined in these experiments, PHB1 appears to affect mtDNA organization in an OPA1-independent manner, as down-regulation of OPA1 did not recapitulate the effect of a PHB1 depletion on mtDNA [55].

6. Prohibitins as regulators of proteolytic processes in the inner membrane

While the regulation of mitochondrial dynamics and the processing of OPA1 have been identified as central processes controlled by prohibitins in mammalian cells, their activity remains poorly defined at a molecular level. The accelerated processing of OPA1 in the absence of prohibitins links their function to proteolytic processes in the inner membrane. This is reminiscent of earlier findings in yeast, which identified prohibitins in large assemblies with the *m*-AAA protease, a conserved ATP-dependent protease in the inner membrane (Fig. 2)

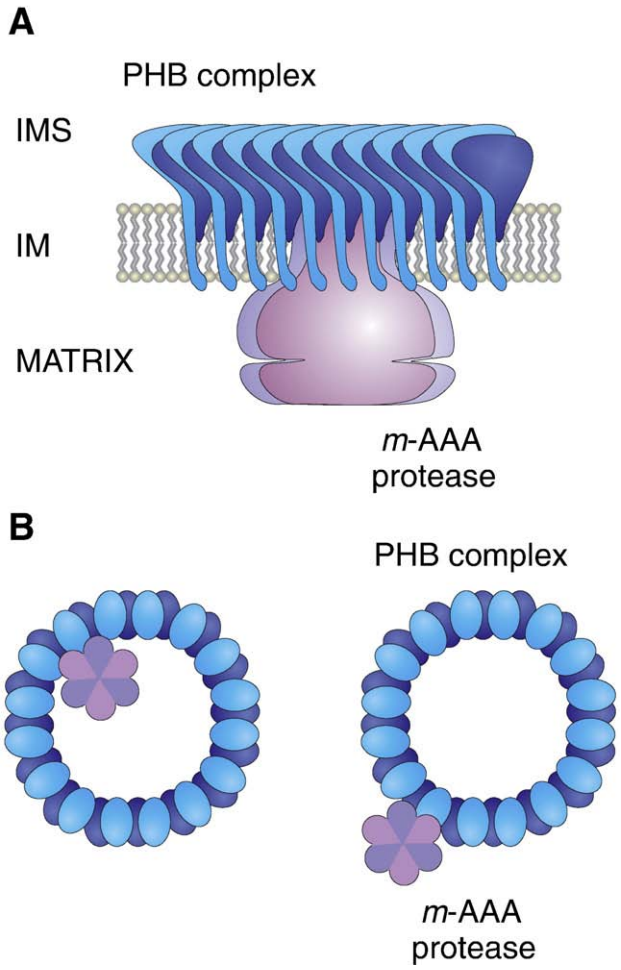

Fig. 2. Supercomplex of prohibitins with the ATP-dependent *m*-AAA protease. In contrast to prohibitins, *m*-AAA protease subunits expose their catalytic domains to the matrix space. The binding of the *m*-AAA protease to the inner or outer surface of ring-shaped prohibitin complexes remains to be established. (A) Side view of the assembled supercomplex. (B) Potential arrangement of prohibitins and *m*-AAA protease within the supercomplex. IMS = intermembrane space, IM = inner membrane.

[21]. *m*-AAA proteases ensure protein quality control in the inner membrane and control crucial steps during mitochondrial biogenesis [57,58]. Loss of functionally conserved mammalian proteases results in neurodegeneration and impairs axonal development [59–61]. Deletion of prohibitins in yeast results in an accelerated proteolysis by the *m*-AAA protease suggesting a regulatory role of prohibitins during the degradation of membrane proteins [21]. Accordingly, the absence of prohibitins in mammalian cells may promote OPA1 processing by *m*-AAA proteases. Indeed, *m*-AAA proteases were proposed to mediate processing of OPA1 [45]. Reconstitution experiments in yeast revealed that various isoforms of the *m*-AAA protease differing in their subunit composition are able to cleave OPA1 [62]. However, direct evidence for a role of *m*-AAA proteases for OPA1 processing in mammalian cells still needs to be awaited. Notably, deletion of prohibitin genes in yeast does not affect the processing of the OPA1-homologue Mgm1, which is mediated by the rhomboid protease Pcp1 in the inner membrane [63–65]. Although functionally conserved and linked to OPA1 processing [64,66], the mammalian rhomboid protease PARL is not required for the cleavage of OPA1 [62,67]. It is therefore an attractive possibility that the role of prohibitins in this process is directly related to the peptidase involved in processing.

7. Prohibitins — organizers in the inner membrane of mitochondria?

Prohibitins have been proposed to exert chaperone activity [22]. However, in the absence of evidence for an association of prohibitin complexes with non-native polypeptides or assembly intermediates, alternative activities of prohibitins appear more likely. The size and the ring shape of prohibitin complexes suggest that they may act as scaffolds defining functional subcompartments, important for specific processes in the inner membrane. Such an activity of prohibitins may explain synthetic lethal interactions of prohibitins with a diverse set of genes in yeast [20,21,68,69]. These include *ATP10* and *ATP23*, which code for substrate-specific chaperones in the assembly of the F_1F_O-ATP synthase [69]. The genetic interaction of prohibitins with these assembly factors identifies the assembly of the F_O-particle as a process critically depending on prohibitin function and may reflect the hazardous effect of F_O-assembly intermediates in the absence of prohibitins [69]. Interestingly, processes in the outer membrane appear to be affected by prohibitins as well. Prohibitins are essential in cells lacking Mmm1, Mdm10 and Mdm12 [20], which were identified originally to affect mitochondrial morphology and inheritance, but recently were linked to the assembly of β-barrel proteins in the outer membrane [70].

A function of prohibitins as membrane scaffolds is further suggested by their sequence similarity to a group of distantly related membrane proteins found in prokaryotes and eukaryotes, termed the SPFH-family (for *s*tomatin/*p*rohibitin/*f*lotillin/*H*flK) (Fig. 3) [71–74]. Members of this widespread family form large assemblies in membranes and show an increased sequence similarity in predicted C-terminal coiled-coil regions. They are characterized by the presence of a PHB domain (also termed SPFH domain), which was proposed to have evolved independently in different proteins by convergent evolution [75]. The function of this domain is presently unclear but may be to facilitate partitioning into functional membrane domains [73,4,74]. Several members of this family have been found in association with lipid rafts [76–78] or to directly interact with lipids [79]. It should be noted, however, that such evidence does not exist for prohibitins nor has the existence of lipid microdomains been demonstrated in mitochondria. Nevertheless, it is conceivable that prohibitins may not only act as protein scaffolds but also affect the lateral partitioning of lipids in the inner membrane. This may explain the genetic interaction of prohibitin genes in yeast with *PSD1* coding for a mitochondrial phosphatidyl serine decarboxylase [68]. Moreover, increasing evidence points to an important role of lipids and lipid microdomains in various cellular fusion events [80–83] and apoptotic processes [84]. Surrounding membrane lipids may affect the vectorial membrane dislocation of OPA1 or the proteolytic activity of *m*-AAA proteases, hence providing a rationale for the control of mitochondrial morphology by prohibitins.

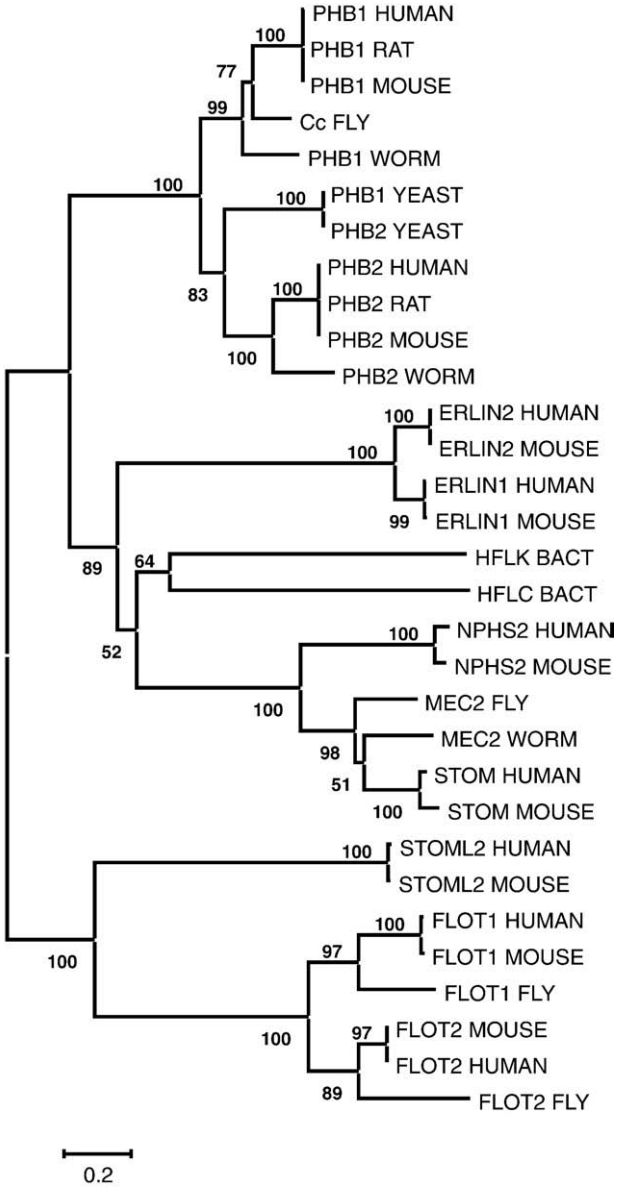

Fig. 3. Phylogenetic analysis of SPFH protein family members. Unrooted dendrogram depicting the relationship of 31 SPFH (PHB) domain containing proteins inferred from the neighbour-joining method. Supporting bootstrap values are indicated at node positions. Phylogenetic analyses were conducted with MEGA4 software [91].

8. Concluding remarks

Increasing evidence highlights the importance of high structural organization of the inner membrane for proper functioning of mitochondria. While protein import has been recognized early on to occur at contact sites between inner and outer membrane [85,86], a functional compartmentalization of other processes, like the fusion and fission of mitochondrial membranes, is just emerging. Respiratory supercomplexes are thought to increase the efficiency of oxidative phosphorylation by promoting substrate channelling [87,88]. Even more, higher oligomers may exert additional functions, as exemplified by higher oligomers of the F_1F_O-ATP synthase that contribute to the

shaping of cristae to sustain efficient ATP synthesis [89,90]. Prohibitins acting as protein or even lipid scaffolds may offer another means to ensure the functional integrity of the inner membrane. In view of their critical role for mitochondrial morphogenesis and functionality, a detailed characterization of their scaffolding function may broaden our understanding of how a functional compartmentalization of the inner membrane helps to maintain mitochondrial integrity.

Acknowledgements

We thank Takashi Tatsuta for critically reading of the manuscript and Sabine Jordan for her help with the figures. This work was supported by grants of the Deutsche Forschungsgemeinschaft, the European Union (6th Framework Programme), and the German-Israeli-Project (DIP grant F.5.1) to T.L.

References

[1] J.K. McClung, D.B. Danner, D.A. Stewart, J.R. Smith, E.L. Schneider, C.K. Lumpkin, R.T. Dell'Orco, M.J. Nuell, Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver, Biochem. Biophys. Res. Commun. 164 (1989) 1316–1322.
[2] E.R. Jupe, X.T. Liu, J.L. Kiehlbauch, J.K. McClung, R.T. Dell'Orco, Prohibitin in breast cancer cell lines: loss of antiproliferative activity is linked to 3′ untranslated region mutations, Cell Growth Differ. 7 (1996) 871–878.
[3] L.G.J. Nijtmans, M. Artal Sanz, L.A. Grivell, P.J. Coates, The mitochondrial PHB complex: roles in mitochondrial respiratory complex assembly, ageing and degenerative disease, Cell. Mol. Life Sci. 59 (2002) 143–155.
[4] S. Mishra, L.C. Murphy, L.J. Murphy, The prohibitins: emerging roles in diverse functions, J. Cell. Mol. Med. 10 (2006) 353–363.
[5] P.J. Coates, D.J. Jamieson, K. Smart, A.R. Prescott, P.A. Hall, The prohibitin family of mitochondrial proteins regulate replicative lifespan, Curr. Biol. 7 (1997) 607–610.
[6] P.W. Piper, D. Bringloe, Loss of prohibitins, though it shortens the replicative life span of yeast cells undergoing division, does not shorten the chronological life span of G0-arrested cells, Mech. Ageing Dev. 123 (2002) 287–295.
[7] A. Sharma, A. Qadri, Vi polysaccharide of Salmonella typhi targets the prohibitin family of molecules in intestinal epithelial cells and suppresses early inflammatory responses, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17492–17497.
[8] A.L. Theiss, R.D. Idell, S. Srinivasan, J.M. Klapproth, D.P. Jones, D. Merlin, S.V. Sitaraman, Prohibitin protects against oxidative stress in intestinal epithelial cells, FASEB J. 21 (2007) 197–206.
[9] M.G. Kolonin, P.K. Saha, L. Chan, R. Pasqualini, W. Arap, Reversal of obesity by targeted ablation of adipose tissue, Nat. Med. 10 (2004) 625–632.
[10] J. Mengwasser, A. Piau, P. Schlag, J.P. Sleeman, Differential immunization identifies PHB1/PHB2 as blood-borne tumor antigens, Oncogene 23 (2004) 7430–7435.
[11] S.C. Gamble, D. Chotai, M. Odontiadis, D.A. Dart, G.N. Brooke, S.M. Powell, V. Reebye, A. Varela-Carver, Y. Kawano, J. Waxman, C.L. Bevan, Prohibitin, a protein downregulated by androgens, represses androgen receptor activity, Oncogene 26 (2007) 1757–1768.
[12] M. Terashima, K.-M. Kim, T. Adachi, P.J. Nielsen, M. Reth, G. Köhler, M.C. Lamers, The IgM antigen receptor of B lymphocytes is associated with prohibitin and a prohibitin-related protein, EMBO J. 13 (1994) 3782–3792.
[13] M.M. Montano, K. Ekena, R. Delage-Mourroux, W. Chang, P. Martini, B.S. Katzenellenbogen, An estrogen receptor-selective coregulator that potentiates the effectiveness of antiestrogens and represses the activity of estrogens, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6947–6952.
[14] M.J. Nuell, D.A. Stewart, L. Walker, V. Friedman, C.M. Wood, G.A. Owens, J.R. Smith, E.L. Schneider, R. Dell'Arco, C.K. Lumpkin, D.B. Danner, J.K. McClung, Prohibitin, an evolutionary conserved intracellular protein that blocks DNA synthesis in normal fibroblasts and HeLa cells, Mol. Cell. Biol. 11 (1991) 1372–1381.
[15] L. Sun, L. Liu, X.J. Yang, Z. Wu, Akt binds prohibitin 2 and relieves its repression of MyoD and muscle differentiation, J. Cell Sci. 117 (2004) 3021–3029.
[16] H. Takata, S. Matsunaga, A. Morimoto, N. Ma, D. Kurihara, R. Ono-Maniwa, M. Nakagawa, T. Azuma, S. Uchiyama, K. Fukui, PHB2 protects sister-chromatid cohesion in mitosis, Curr. Biol. 17 (2007) 1356–1361.
[17] K. Rajalingam, C. Wunder, V. Brinkmann, Y. Churin, M. Hekman, C. Sievers, U.R. Rapp, T. Rudel, Prohibitin is required for Ras-induced Raf-MEK-ERK activation and epithelial cell migration, Nat. Cell Biol. 7 (2005) 837–843.
[18] M.G. Vander Heiden, J.S. Choy, D.J. VanderWeele, J.L. Brace, M.H. Harris, D.E. Bauer, B. Prange, S.J. Kron, C.B. Thompson, C.M. Rudin, Bcl-x(L) complements Saccharomyces cerevisiae genes that facilitate the switch from glycolytic to oxidative metabolism, J. Biol. Chem. 277 (2002) 44870–44876.
[19] G. Fusaro, P. Dasgupta, S. Rastogi, B. Joshi, S. Chellappan, Prohibitin induces the transcriptional activity of p53 and is exported from the nucleus upon apoptotic signaling, J. Biol. Chem. 278 (2003) 47853–47861.
[20] K.H. Berger, M.P. Yaffe, Prohibitin family members interact genetically with mitochondrial inheritance components in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 18 (1998) 4043–4052.
[21] G. Steglich, W. Neupert, T. Langer, Prohibitins regulate membrane protein degradation by the *m*-AAA protease in mitochondria, Mol. Cell. Biol. 19 (1999) 3435–3442.
[22] L.G.J. Nijtmans, L. de Jong, M.A. Sanz, P.J. Coates, J.A. Berden, J.W. Back, A.O. Muijsers, H. Van der Speck, L.A. Grivell, Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins, EMBO J. 19 (2000) 2444–2451.
[23] M. Artal-Sanz, W.Y. Tsang, E.M. Willems, L.A. Grivell, B.D. Lemire, H. van der Spek, L.G. Nijtmans, M.A. Sanz, The mitochondrial prohibitin complex is essential for embryonic viability and germline function in *Caenorhabditis elegans*, J. Biol. Chem. 278 (2003) 32091–32099.
[24] E. Ikonen, K. Fiedler, R.G. Parton, K. Simons, Prohibitin, an antiproliferative protein, is localized to mitochondria, FEBS Lett. 358 (1995) 273–277.
[25] T. Tatsuta, K. Model, T. Langer, Formation of membrane-bound ring complexes by prohibitins in mitochondria, Mol. Biol. Cell 16 (2005) 248–259.
[26] K. Kasashima, E. Ohta, Y. Kagawa, H. Endo, Mitochondrial functions and estrogen receptor-dependent nuclear translocation of pleiotropic human prohibitin 2, J. Biol. Chem. 281 (2006) 36401–36410.
[27] B. He, Q. Feng, A. Mukherjee, D.M. Lonard, F.J. DeMayo, B.S. Katzenellenbogen, J.P. Lydon, B.W. O'Malley, A repressive role for prohibitin in estrogen signaling, Mol. Endocrinol. 22 (2008) 344–360.
[28] C. Merkwirth, S. Dargazanli, T. Tatsuta, S. Geimer, B. Lower, F.T. Wunderlich, J.C. von Kleist-Retzow, A. Waisman, B. Westermann, T. Langer, Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria, Genes Dev. 22 (2008) 476–488.
[29] P.J. Coates, R. Nenutil, A. McGregor, S.M. Picksley, D.H. Crouch, P.A. Hall, E.G. Wright, Mammalian prohibitin proteins respond to mitochondrial stress and decrease during cellular senescence, Exp. Cell Res. 265 (2001) 262–273.
[30] J.W. Back, M.A. Sanz, L. De Jong, L.J. De Koning, L.G. Nijtmans, C.G. De Koster, L.A. Grivell, H. Van Der Spek, A.O. Muijsers, A structure for the yeast prohibitin complex: Structure prediction and evidence from chemical crosslinking and mass spectrometry, Protein Sci. 11 (2002) 2471–2478.
[31] C.M. Koehler, The small Tim proteins and the twin Cx3C motif, Trends Biochem. Sci. 29 (2004) 1–4.
[32] S.E. Park, J. Xu, A. Frolova, L. Liao, B.W. O'Malley, B.S. Katzenellenbogen, Genetic deletion of the repressor of estrogen receptor activity (REA) enhances the response to estrogen in target tissues in vivo, Mol. Cell. Biol. 25 (2005) 1989–1999.
[33] M. Schleicher, B.R. Shepherd, Y. Suarez, C. Fernandez-Hernando, J. Yu, Y. Pan, L.M. Acevedo, G.S. Shadel, W.C. Sessa, Prohibitin-1 maintains the angiogenic capacity of endothelial cells by regulating mitochondrial function and senescence, J. Cell Biol. 180 (2008) 101–112.
[34] S. Wang, N. Nath, M. Adlam, S. Chellappan, Prohibitin, a potential tumor suppressor, interacts with RB and regulates E2F function, Oncogene 18 (1999) 3501–3510.
[35] S. Wang, G. Fusaro, J. Padmanabhan, S.P. Chellappan, Prohibitin co-localizes with Rb in the nucleus and recruits N-CoR and HDAC1 for transcriptional repression, Oncogene 21 (2002) 8388–8396.
[36] D. Choi, S.J. Lee, S. Hong, I.H. Kim, S. Kang, Prohibitin interacts with RNF2 and regulates E2F1 function via dual pathways, Oncogene 27 (2008) 1716–1725.
[37] V. Kurtev, R. Margueron, K. Kroboth, E. Ogris, V. Cavailles, C. Seiser, Transcriptional regulation by the repressor of estrogen receptor activity via recruitment of histone deacetylases, J. Biol. Chem. 279 (2004) 24834–24843.
[38] D.C. Chan, Mitochondrial fusion and fission in mammals, Ann. Rev. Cell Dev. Biol. 22 (2006) 79–99.
[39] H.M. McBride, M. Neuspiel, S. Wasiak, Mitochondria: more than just a powerhouse, Curr. Biol. 16 (2006) R551–560.
[40] K.L. Cerveny, Y. Tamura, Z. Zhang, R.E. Jensen, H. Sesaki, Regulation of mitochondrial fusion and division, Trends Cell. Biol. 17 (2007) 563–569.
[41] S.A. Detmer, D.C. Chan, Functions and dysfunctions of mitochondrial dynamics, Nat. Rev., Mol. Cell Biol. 8 (2007) 870–879.
[42] S. Hoppins, L. Lackner, J. Nunnari, The machines that divide and fuse mitochondria, Annu. Rev. Biochem. 76 (2007) 751–780.
[43] A. Olichon, L. Baricault, N. Gas, E. Guillou, A. Valette, P. Belenguer, G. Lenaers, Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome *c* release and apoptosis, J. Biol. Chem. 278 (2003) 7743–7746.
[44] L. Griparic, N.N. van der Wel, I.J. Orozco, P.J. Peters, A.M. van der Bliek, Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria, J. Biol. Chem. 279 (2004) 18792–18798.
[45] N. Ishihara, Y. Fujita, T. Oka, K. Mihara, Regulation of mitochondrial morphology through proteolytic cleavage of OPA1, EMBO J. 25 (2006) 2966–2977.
[46] Z. Song, H. Chen, M. Fiket, C. Alexander, D.C. Chan, OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L, J. Cell Biol. 178 (2007) 749–755.
[47] J.A. Ross, Z.S. Nagy, R.A. Kirken, The PHB1/2 phosphocomplex is required for mitochondrial homeostasis and survival of human T cells, J. Biol. Chem. 283 (2008) 4699–4713.
[48] L. Scorrano, M. Ashiya, K. Buttle, S. Weiler, S.A. Oakes, C.A. Mannella, S.J. Korsmeyer, A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis, Dev. Cell 2 (2002) 55–67.
[49] M.G. Sun, J. Williams, C. Munoz-Pinedo, G.A. Perkins, J.M. Brown, M.H. Ellisman, D.R. Green, T.G. Frey, Correlated three-dimensional light and electron microscopy reveals transformation of mitochondria during apoptosis, Nat. Cell. Biol. 9 (2007) 1057–1065.
[50] C. Frezza, S. Cipolat, O. Martins de Brito, M. Micaroni, G.V. Beznoussenko, T. Rudka, D. Bartoli, R.S. Polishuck, N.N. Danial, B. De Strooper, L. Scorrano, OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion, Cell 126 (2006) 177–189.
[51] P.W. Piper, G.W. Jones, D. Bringloe, N. Harris, M. MacLean, M. Mollapour, The shortened replicative life span of prohibitin mutants of yeast appears to be due to defective mitochondrial segregation in old mother cells, Aging Cell 1 (2002) 149–157.

[52] W. Yarosh, J. Monserrate, J.J. Tong, S. Tse, P.K. Le, K. Nguyen, C.B. Brachmann, D.C. Wallace, T. Huang, The molecular mechanisms of OPA1-mediated optic atrophy in Drosophila model and prospects for antioxidant treatment, PLoS Genet. 4 (2008) e6.
[53] D.F. Bogenhagen, Y. Wang, E.L. Shen, R. Kobayashi, Protein components of mitochondrial DNA nucleoids in higher eukaryotes, Mol. Cell Proteomics. 2 (2003) 1205–1216.
[54] D.F. Bogenhagen, D. Rousseau, S. Burke, The layered structure of human mitochondrial DNA nucleoids, J. Biol. Chem. 283 (2008) 3665–3675.
[55] K. Kasashima, M. Sumitani, M. Satoh, H. Endo, Human prohibitin 1 maintains the organization and stability of the mitochondrial nucleoids, Exp. Cell Res. 314 (2008) 988–996.
[56] X.J. Chen, R.A. Butow, The organization and inheritance of the mitochondrial genome, Nat. Rev., Genet. 6 (2005) 815–825.
[57] M. Koppen, T. Langer, Protein degradation within mitochondria: versatile activities of AAA proteases and other peptidases, Crit. Rev. Biochem. Mol. Biol. 42 (2007) 221–242.
[58] T. Tatsuta, T. Langer, Quality control of mitochondria: protection against neurodegeneration and ageing, EMBO J. 27 (2008) 306–314.
[59] G. Casari, M. De-Fusco, S. Ciarmatori, M. Zeviani, M. Mora, P. Fernandez, G. DeMichele, A. Filla, S. Cocozza, R. Marconi, A. Durr, B. Fontaine, A. Ballabio, Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease, Cell 93 (1998) 973–983.
[60] F. Ferreirinha, A. Quattrini, M. Priozzi, V. Valsecchi, G. Dina, V. Broccoli, A. Auricchio, F. Piemonte, G. Tozzi, L. Gaeta, G. Casari, A. Ballabio, E.I. Rugarli, Axonal degeneration in paraplegin-deficient mice is associated with abnormal mitochondria and impairment of axonal transport, J. Clin. Invest. 113 (2004) 231–242.
[61] F. Maltecca, A. Aghaie, D.G. Schroeder, L. Cassina, B.A. Taylor, S.J. Phillips, M. Malaguti, S. Previtali, J.L. Guenet, A. Quattrini, G.A. Cox, G. Casari, The mitochondrial protease AFG3L2 is essential for axonal development, J. Neurosci. 28 (2008) 2827–2836.
[62] S. Duvezin-Caubet, M. Koppen, J. Wagener, M. Zick, L. Israel, A. Bernacchia, R. Jagasia, E.I. Rugarli, A. Imhof, W. Neupert, T. Langer, A.S. Reichert, OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria, Mol. Biol. Cell 18 (2007) 3582–3590.
[63] M. Herlan, F. Vogel, C. Bornhövd, W. Neupert, A.S. Reichert, Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA, J. Biol. Chem. 278 (2003) 27781–27788.
[64] G.A. McQuibban, S. Saurya, M. Freeman, Mitochondrial membrane remodelling regulated by a conserved rhomboid protease, Nature 423 (2003) 537–541.
[65] H. Sesaki, S.M. Southard, A.E. Hobbs, R.E. Jensen, Cells lacking Pcp1p/Ugo2p, a rhomboid-like protease required for Mgm1p processing, lose mtDNA and mitochondrial structure in a Dnm1p-dependent manner, but remain competent for mitochondrial fusion, Biochem. Biophys. Res. Commun. 308 (2003) 276–283.
[66] S. Cipolat, T. Rudka, D. Hartmann, V. Costa, L. Serneels, K. Craessaerts, K. Metzger, C. Frezza, W. Annaert, L. D'Adamio, C. Derks, T. Dejaegere, L. Pellegrini, R. D'Hooge, L. Scorrano, B. De Strooper, Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling, Cell 126 (2006) 163–175.
[67] L. Griparic, T. Kanazawa, A.M. van der Bliek, Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage, J. Cell Biol. 178 (2007) 757–764.
[68] R. Birner, R. Nebauer, R. Schneiter, G. Daum, Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine biosynthetic machinery with the prohibitin complex of *Saccharomyces cerevisiae*, Mol. Biol. Cell 14 (2003) 370–383.
[69] C. Osman, C. Wilmes, T. Tatsuta, T. Langer, Prohibitins Interact Genetically with Atp23, a Novel Processing Peptidase and Chaperone for the F_1F_O-ATP Synthase, Mol. Biol. Cell 18 (2007) 627–635.
[70] C. Meisinger, S. Pfannschmidt, M. Rissler, D. Milenkovic, T. Becker, D. Stojanovski, M.J. Youngman, R.E. Jensen, A. Chacinska, B. Guiard, N. Pfanner, N. Wiedemann, The morphology proteins Mdm12/Mmm1 function in the major beta-barrel assembly pathway of mitochondria, EMBO J. 26 (2007) 2229–2239.
[71] N. Tavernarakis, M. Driscoll, N.C. Kyrpides, The SPFH domain: implicated in regulating targeted protein turnover in stomatins and other membrane-associated proteins, Trends Biochem. Sci. 24 (1999) 425–427.
[72] M.F. Langhorst, A. Reuter, C.A. Stuermer, Scaffolding microdomains and beyond: the function of reggie/flotillin proteins, Cell. Mol. Life Sci. 62 (2005) 2228–2240.
[73] I.C. Morrow, R.G. Parton, Flotillins and the PHB domain protein family: rafts, worms and anaesthetics, Traffic 6 (2005) 725–740.
[74] D.T. Browman, M.B. Hoegg, S.M. Robbins, The SPFH domain-containing proteins: more than lipid raft markers, Trends Cell Biol. 17 (2007) 394–402.
[75] E. Rivera-Milla, C.A. Stuermer, E. Malaga-Trillo, Ancient origin of reggie (flotillin), reggie-like, and other lipid-raft proteins: convergent evolution of the SPFH domain, Cell. Mol. Life Sci. 63 (2006) 343–357.
[76] U. Salzer, R. Prohaska, Stomatin, flotillin-1, and flotillin-2 are major integral proteins of erythrocyte lipid rafts, Blood 97 (2001) 1141–1143.
[77] C.A. Stuermer, D.M. Lang, F. Kirsch, M. Wiechers, S.O. Deininger, H. Plattner, Glycosylphosphatidyl inositol-anchored proteins and fyn kinase assemble in noncaveolar plasma membrane microdomains defined by reggie-1 and -2, Mol. Biol. Cell 12 (2001) 3031–3045.
[78] D.T. Browman, M.E. Resek, L.D. Zajchowski, S.M. Robbins, Erlin-1 and erlin-2 are novel members of the prohibitin family of proteins that define lipid-raft-like domains of the ER, J. Cell Sci. 19 (2006) 3149–3160.
[79] T.B. Huber, B. Schermer, R.U. Muller, M. Hohne, M. Bartram, A. Calixto, H. Hagmann, C. Reinhardt, F. Koos, K. Kunzelmann, E. Shirokova, D. Krautwurst, C. Harteneck, M. Simons, H. Pavenstadt, D. Kerjaschki, C. Thiele, G. Walz, M. Chalfie, T. Benzing, Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 17079–17086.
[80] R.A. Fratti, Y. Jun, A.J. Merz, N. Margolis, W. Wickner, Interdependent assembly of specific regulatory lipids and membrane fusion proteins into the vertex ring domain of docked vacuoles, J. Cell Biol. 167 (2004) 1087–1098.
[81] K. Altmann, B. Westermann, Role of essential genes in mitochondrial morphogenesis in Saccharomyces cerevisiae, Mol. Biol. Cell 16 (2005) 5410–5417.
[82] T. Boukh-Viner, T. Guo, A. Alexandrian, A. Cerracchio, C. Gregg, S. Haile, R. Kyskan, S. Milijevic, D. Oren, J. Solomon, V. Wong, J.M. Nicaud, R.A. Rachubinski, A.M. English, V.I. Titorenko, Dynamic ergosterol- and ceramide-rich domains in the peroxisomal membrane serve as an organizing platform for peroxisome fusion, J. Cell Biol. 168 (2005) 761–773.
[83] S.Y. Choi, P. Huang, G.M. Jenkins, D.C. Chan, J. Schiller, M.A. Frohman, A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis, Nat. Cell Biol. 8 (2006) 1255–1262.
[84] S.Y. Choi, F. Gonzalvez, G.M. Jenkins, C. Slomianny, D. Chretien, D. Arnoult, P.X. Petit, M.A. Frohman, Cardiolipin deficiency releases cytochrome c from the inner mitochondrial membrane and accelerates stimuli-elicited apoptosis, Cell Death Differ. 14 (2007) 597–606.
[85] A.S. Reichert, W. Neupert, Contact sites between the outer and inner membrane of mitochondria-role in protein transport, Biochim. Biophys. Acta 1592 (2002) 41–49.
[86] A. Chacinska, P. Rehling, B. Guiard, A.E. Frazier, A. Schulze-Specking, N. Pfanner, W. Voos, C. Meisinger, Mitochondrial translocation contact sites: separation of dynamic and stabilizing elements in formation of a TOM-TIM-preprotein supercomplex, EMBO J. 22 (2003) 5370–5381.
[87] C.M. Cruciat, S. Brunner, F. Baumann, W. Neupert, R.A. Stuart, The cytochrome bc1 and cytochrome c oxidase complexes associate to form a single supracomplex in yeast mitochondria, J. Biol. Chem. 275 (2000) 18093–18098.
[88] H. Schagger, K. Pfeiffer, Supercomplexes in the respiratory chains of yeast and mammalian mitochondria, EMBO J. 19 (2000) 1777–1783.
[89] P. Paumard, J. Vaillier, B. Coulary, J. Schaeffer, V. Soubannier, D.M. Mueller, D. Brethes, J.P. di Rago, J. Velours, The ATP synthase is involved in generating mitochondrial cristae morphology, EMBO J. 21 (2002) 221–230.
[90] M. Strauss, G. Hofhaus, R.R. Schroder, W. Kuhlbrandt, Dimer ribbons of ATP synthase shape the inner mitochondrial membrane, EMBO J. (2008).
[91] K. Tamura, J. Dudley, M. Nei, S. Kumar, MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0, Mol. Biol. Evol. 24 (2007) 1596–1599.
[92] S. Duvezin-Caubet, R. Jagasia, J. Wagener, S. Hofmann, A. Trifunovic, A. Hansson, A. Chomyn, M.F. Bauer, G. Attardi, N.G. Larsson, W. Neupert, A.S. Reichert, Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology, J. Biol. Chem. 281 (2006) 37972–37979.