Review

Prohibitin and mitochondrial biology

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Prohibitins are ubiquitous, evolutionarily conserved proteins that are mainly localized in mitochondria. The mitochondrial prohibitin complex comprises two subunits, PHB1 and PHB2. These two proteins assemble into a ring-like macromolecular structure at the inner mitochondrial membrane and are implicated in diverse cellular processes: from mitochondrial biogenesis and function to cell death and replicative senescence. In humans, prohibitins have been associated with various types of cancer. While their biochemical function remains poorly understood, studies in organisms ranging from yeast to mammals have provided significant insights into the role of the prohibitin complex in mitochondrial biogenesis and metabolism. Here we review recent studies and discuss their implications for deciphering the function of prohibitins in mitochondria.

The mitochondrial prohibitin complex

The eukaryotic mitochondrial PHB complex comprises two highly homologous subunits, PHB1 and PHB2 (around 50% amino acid sequence identity and 60% similarity). The first mammalian prohibitin (PHB1) was identified as a potential tumour suppressor with anti-proliferative activity [1] and was hence named prohibitin. However, this activity was later attributed to the 3′-UTR of the prohibitin mRNA and was found to be unrelated to the function of the protein itself [2]. The second prohibitin (PHB2) was identified via its binding, together with PHB1, to the IgM antigen receptor. Thus, both proteins were also named B-cell-receptor complex-associated proteins (*BAP32* and *BAP37*) [3]. In addition, PHB2 was identified as a repressor of nuclear estrogen receptor activity (termed REA) [4]. Extensive and rapidly accumulating evidence suggests that both prohibitins function primarily within mitochondria [5–8] (reviewed in Refs. [9,10]). While not the focus of this review, a number of diverse cellular functions have also been attributed to both PHB1 and PHB2 in other cellular compartments. These include roles in cell cycle progression, the regulation of transcription, and in cell surface signalling (Box 1; reviewed in Refs. [11–13]).

PHB1 and PHB2, with molecular weights of 32 and 34 kDa respectively, associate to form a macromolecular structure of approximately 1 MDa at the mitochondrial inner membrane (IM). This high molecular weight complex has been identified in yeast, *C. elegans* and mammals [14–16]. Prohibitin homodimers have not been detected [17,18]. Instead, PHB1 and PHB2 bind to each other to form a heterodimeric building block [17]. About 12 to 16 PHB heterodimers then associate to form a ring-like structure at the mitochondrial IM [17] with a diameter of 20-25 nm [18]. The PHB complex is anchored in the mitochondrial IM through N-terminal hydrophobic regions present in both PHB1 and PHB2. For yeast PHB2, the transmembrane domain prediction algorithm TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) predicts a transmembrane helix at positions 37–59, leaving 36 amino acids on the matrix side with the bulk of the protein facing the intermembrane space. The homologous helical region at the N-terminus of PHB1 is shorter and may not fulfil the requirements for a membrane-spanning domain. For this reason, PHB1 is considered to be membrane-associated [17]. Complex formation depends on both PHB subunits. Depletion of either PHB1 or PHB2 results in the absence of the complex, indicating interdependence at the level of protein complex formation [5,14,19,20]. However, detailed

Glossary

Chronological lifespan: the time that non-dividing yeast cells remain viable in a stationary phase culture.
Diauxic shift: *S. cerevisiae* switch metabolism from fermentation to respiration when growing on glucose and in the presence of oxygen. During the first growth phase, when there is plenty of glucose and oxygen available, yeast cells prefer glucose fermentation to aerobic respiration. After glucose is depleted, yeast cells undergo a metabolic (or diauxic) shift, where the fermentative product ethanol is oxidised. This diauxic shift is accompanied by stimulation of mitochondrial function.
Hereditary spastic paraplegia (HSP): an inherited neurological disorder characterized by retrograde degeneration of cortical motor axons, progressive weakness (paraplegia), increased muscle tone and stiffness (spasticity) of the legs. Loss of function of paraplegin (encoded by the gene *SPG7*, a mitochondrial *m*-AAA-protease) causes HSP.
IgM: immunoglobulin M. An antibody that is present on B lymphocytes that are involved in the humoral immune response. IgM is the primary antibody against A and B antigens on red blood cells.
Leigh syndrome: a neurodegenerative disorder of infancy or childhood, generally due to mutations in nuclear or mitochondrial genes involved in mitochondrial energy metabolism.
***m*-AAA protease**: mitochondrial matrix-AAA protease. Member of membrane-bound ATP-dependent proteases that are present in eubacteria, mitochondria and chloroplasts and that can degrade membrane proteins.
Nucleoids: discrete protein-DNA complexes, organizing multiple mitochondrial DNA (mtDNA) molecules.
Petite mutants: the yeast *S. cerevisiae* can grow in the absence of mtDNA. Yeast strains that contain wild-type mtDNA, called [rho$^+$] cells, can respire and grow on non-fermentable carbon sources. Cells that contain deletions or mutations in mtDNA [rho$^-$] or have completely lost their mtDNA [rho^0] are called petite mutants. Petite mutants can grow by fermentation in glucose media. Petite-negative yeast species lose viability in fermentable carbon sources.
Replicative lifespan: yeast cells age chronologically but also undergo replicative senescence. The replicative lifespan reflects the number of buds generated by an individual mother cell.
Respiratory capacity: the ability of yeast cells to grow on non-fermentable carbon sources, where respiration (aerobic growth) is required.
[rho$^-$]: deletions of the mitochondrial genome that render yeast cells respiration-deficient (petite phenotype – see above).
Stationary phase: when nutrients are exhausted, yeast cells enter a stationary phase that is characterized by cell cycle arrest (G_0) and specific physiological, biochemical, and morphological changes.

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Box 1. Prohibitins outside of mitochondria

Despite accumulating evidence that PHB1 and PHB2 interdependently form a functional protein complex at the mitochondrial inner membrane, both proteins have been found either alone or together in other cellular compartments, including the nucleus and plasma membrane. A number of cellular functions have been proposed for these proteins outside mitochondria (reviewed in Refs. [11–13]).

Prohibitin (PHB1) was initially proposed to play a role in cell cycle progression [1,70]. PHB2 was later identified as a B-cell receptor-associated protein at the plasma membrane [3], and was named BAP37 (B-cell associated protein). In addition, both PHB1 and PHB2 were found at the plasma membrane of human intestinal epithelial cells where they function as a binding site for the Vi capsular polysaccharide of *Salmonella typhi* [71]. PHB1 has also been found to be the target for a proapoptotic peptide in adipose vasculature [72]. Furthermore, PHB1 has been implicated in mediating cellular Ras–Raf signalling at the membrane [13]. PHB1 has also been shown to modulate transcription in cell transfection experiments and to bind a wide range of proteins, including Retinoblastoma (Rb), E2-F, Brg1/Brm and p53 [11,12]. PHB2 was found to modulate muscle differentiation by binding to AKT [73,74] and was also found to repress estrogen receptor (ERα) [28].

Given the strong interdependence of PHB1 and PHB2 in mitochondria, it was puzzling how the separate proteins alone could be stable in other cellular compartments. Recently, PHB (PHB1) was also shown to repress ERα activity [20]. Interestingly, similarly to mitochondrial prohibitins, PHB and REA interact and stabilise each other *in vivo*. Reducing the amount of REA results in reduced PHB, and vice versa. Surprisingly, expressing both proteins together eliminates the transcriptional effects of the individual proteins. This suggests that heteromers of PHB and REA are inefficient as transcriptional co-repressors. Therefore, PHB and REA might only repress transcription when they are not paired [20]. How these evolutionarily conserved proteins can exert such a variety of functions within the cell is currently not understood.

structural data for this highly conserved protein complex are still lacking.

Several distinct roles have been put forward for mitochondrial prohibitins (Figure 1). The PHB complex has been implicated in regulating membrane protein degradation by the mitochondrial *m*-AAA protease [16] and it has been proposed to function as a holdase/unfoldase chaperone that holds and stabilizes unassembled membrane proteins [10,15]. The complex potentially also plays a role in stabilizing the mitochondrial genome [21–23]. In addition, the PHB complex has been implicated in mitochondrial morphogenesis [5], functioning as a scaffold that recruits membrane proteins to a specific lipid environment [24]. Here we review the mitochondrial functions attributed to the PHB complex and focus on their implications for ageing and disease.

Life without prohibitin

Disruption of the PHB complex in the yeast *Saccharomyces cerevisiae* decreases replicative lifespan of the cells but does not result in other observable growth phenotypes under laboratory conditions [19,25]. The shortening of replicative lifespan is accompanied by defects in mitochondrial membrane potential, extended cell division time, and other characteristic morphological changes of ageing cells [25]. Prohibitin depletion does not alter the chronological lifespan of non-dividing (G_0-arrested) cells, although *phb*-null mutants in stationary phase tend to lose respiratory capacity, a phenotype associated with deletions of the mitochondrial genome (the [rho$^-$] phenotype) [26]. Increased generation of [rho$^-$] cells can only be detected in old and non-dividing cells and not in young *phb*-null mother cells [19,27]. Similarly, only old *phb*-null mother cells at the end of their replicative lifespan show defective mitochondrial segregation and aberrant mitochondrial morphology [26]. By contrast, no mitochondrial morphology defects have been detected in younger *phb*-null cells [19,27]. This suggests that *phb*-null yeast cells undergo premature ageing, probably due to a slight but cumulative decline in cellular metabolic capacity.

In contrast to the relatively marginal observable effects in yeast, severe phenotypes are associated with prohibitin deficiency in multicellular organisms (Box 2). Prohibitins are required for embryonic development in both *Caenorhabditis elegans* and in mice, [5,14,20,28]. Similarly, prohibitins are required for plant development

Box 2. Prohibitin in ageing and disease

Mammalian cell senescence is accompanied by reduced expression of PHB proteins. This decrease correlates with a heterogeneous decline in mitochondrial membrane potential during ageing [31]. Studies in yeast provide direct support for the involvement of the PHB complex in the ageing process. Deletion of either or both of the PHB genes shortens the replicative lifespan of yeast by about one third [19,25]. Cells deleted for PHB1 and PHB2 show a roughened cell surface and prolonged cell cycle after fewer divisions compared to wild type, indicating that the normal ageing process is accelerated in cells lacking the PHB complex [25]. Similarly, depletion of prohibitin shortens the lifespan of petunia flowers [29].

Although the mechanism by which prohibitin influences ageing remains elusive, clear evidence links the PHB complex to mitochondrial function. Thus, it is likely that prohibitin influences longevity by affecting mitochondrial metabolism. Lack of the PHB complex results in increased ROS production [7] and sensitivity to free radicals [14,36]. Accumulation of cellular damage as a consequence of free radical production has been suggested to drive the ageing process [75] and may be responsible for the reduced lifespan produced by PHB depletion. However, recent reports demonstrating lack of correlation between oxidative damage and longevity challenge the free radical theory of ageing [76].

Mitochondrial dysfunction underlies the pathology of a broad spectrum of diseases. Myopathies and neuropathies are among the most common types of disorders associated with mitochondrial defects. Other disorders including diabetes, hearing loss, and kidney failure can also be caused by mitochondrial dysfunction [32,77]. To date, no mutations in PHB genes have been found to cause human disease. Nevertheless, mutations in the mitochondrial *m*-AAA-metalloprotease, that interacts with the PHB complex [16], cause hereditary spastic paraplegia in humans [78]. In addition, high levels of prohibitin expression in tumours indicate a potential role in carcinogenesis [33]. Expression of PHB proteins is also elevated in yeast cells devoid of *SHY1*, the orthologue of SURF1, which is implicated in the pathogenesis of the Leigh syndrome [44]. Altered expression of PHB1 correlates with loss of mitochondrial function in the liver of knockout mice deficient in S-adenosylmethionine synthesis and in obese patients at risk for nonalcoholic steatohepatitis [79]. In addition, abnormal prohibitin levels have been reported in Parkinson disease [80] and schizophrenia [81]. These findings emphasise the importance of the PHB complex in maintaining mitochondrial homeostasis that is crucial for human health.

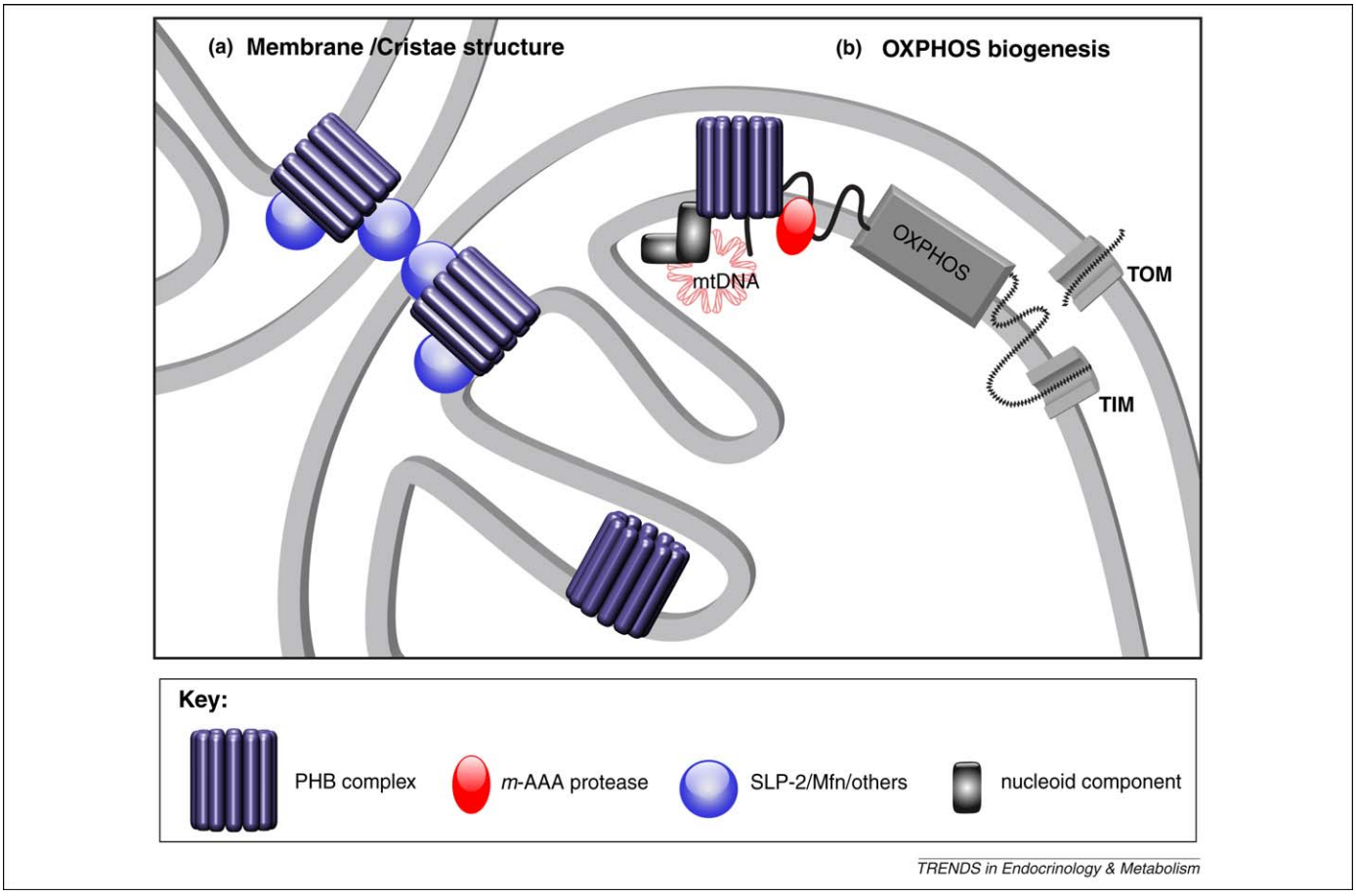

Figure 1. Possible roles of the PHB complex in mitochondria. **(a)** Maintenance of mitochondrial membrane and cristae structure. The PHB complex is shown, interacting with mitochondrial inner and outer membrane proteins as part of a complex that might facilitate mitochondrial fusion (e.g. Stomatin, Stoml2/SLP-2; Ref. [42]; Mitofusin (Mfn), Ref. [45], and others). The PHB complex might also participate in the formation and/or maintenance of cristae junctions [5]. Additionally, the PHB complex could have a role in keeping the two membranes of a crista in close proximity. **(b)** Biogenesis of OXPHOS complexes. The PHB complex may assist with protein folding and assembly in cooperation with the m-AAA protease [15,16]. Association with mitochondrial nucleoids [21–23] may ensure protection of highly hydrophobic mitochondrial-encoded OXPHOS subunits until they are assembled into functional complexes with nuclear-encoded subunits. The mitochondrial translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM) that mediate import of nuclear encoded mitochondrial proteins are also depicted. The oxidative phosphorylation system (OXPHOS) is schematically shown. The PHB complex is represented as 12 heterodimers, each containing one PHB-1 and one PHB-2 (note that given the predicted size of the complex, 12 to 16 PHB-1/PHB-2 heterodimers have been proposed to assembled into the PHB complex) [17].

[29,30]. Post-embryonic depletion of prohibitins in *C. elegans* results in pronounced germline defects such as diminished oocyte production with smaller brood size [14]. Both embryonic and postembryonic effects observed in *C. elegans* indicate that PHB proteins are specifically required in tissues that undergo cellular proliferation. Extensive distortion of mitochondrial morphology is observed following reduced prohibitin expression in *C. elegans* bodywall muscles [14]. During post-embryonic development, nematode muscle cells do not proliferate but rather grow in size. The number of mitochondria per cell normally increases to meet the energy requirements of muscle growth. The effects of prohibitin depletion suggest that the prohibitin complex plays an important role in maintaining mitochondrial membrane integrity in these cells. Inactivation of PHB2 in mouse embryonic fibroblasts (MEFs) results in severely impaired cellular proliferation [5]. Accordingly, it appears likely that tissues that rely heavily on mitochondrial function, notably proliferating cells, are more susceptible to lack of prohibitin.

Changes in prohibitin expression levels further support a role for the PHB complex during periods of strong metabolic demand. For example, PHB expression increases in yeast cells during diauxic shift when cells switch from non-oxidative to oxidative metabolism. Furthermore, yeast mutants defective in the synthesis of the mitochondrial-encoded Cox1p subunit show increased levels of the PHB complex [10]. Finally, inhibition of mitochondrial translation results in increased PHB expression in human cells and in *C. elegans* [14,31]. Mitochondrial- and nuclear-encoded subunits of the respiratory chain need to assemble stoichiometrically in the mitochondrial membrane, and imbalances between subunits represent a threat to membrane integrity and mitochondrial function. For example, production of reactive oxygen species (ROS) might increase, as well as leakage of H^+ and ROS. Therefore, these findings support a role for the PHB complex as a holdase type of chaperone specifically required in situations of mitochondrial stress [10].

Extensive studies on the expression patterns of both PHB proteins in mammalian tissues and during murine development support a role for prohibitins in regulating mitochondrial metabolism. PHB proteins are highly expressed in cells that rely heavily on mitochondrial function, including neurons, muscle, heart, liver, renal tubules, adrenal cortex, brown adipocytes and pancreatic islet cells

[31]. These tissues are often particularly susceptible to mitochondrial dysfunction [32]. Plant prohibitins are also predominantly expressed in proliferating tissues [30]. Similarly, PHB proteins are expressed at higher levels in mammalian proliferating cells, including neoplastic tissues (Box 2) [31,33].

Prohibitin and mitochondrial dynamics

Mitochondria are highly dynamic structures that fuse continuously and divide to adjust the shape and distribution of the mitochondrial network depending on cell type and energy demands, therefore playing critical role(s) in cell physiology. Conserved protein machineries located in the outer and inner membrane of mitochondria regulate fusion and fission events and include several dynamin-like GTPases [34]. Among these, mitofusins (Mfn1, Mfn2) and optic atrophy 1 protein (OPA1) are required for mitochondrial fusion, and dynamin-related protein (DRP1) is required for mitochondrial fission.

Loss of prohibitins was first shown to severely affect mitochondrial morphology in *C. elegans* bodywall muscle cells [14]. In normal muscle cells, mitochondria appear tubular, elongated, and well-structured, running parallel to the body axis and often to the myofibrils. Upon prohibitin depletion, mitochondria appear fragmented and disorganized [14]. Similarly, loss of prohibitins results in the accumulation of fragmented mitochondria in MEFs and HeLa cells [5,35]. A possible mechanism for mitochondrial fragmentation mediated by depletion of prohibitin was put forward following the discovery that PHB2 deletion results in OPA1 destabilization [5,35]. OPA1 resides in the mitochondrial IM and plays a key role in mitochondrial fusion and in determining the morphology of cristae. Electron microscopic analysis of PHB2-depleted MEFs revealed severe defects in the formation of lamellar cristae [5]. Similarly, lack of cristae has been reported in plant mitochondrial depleted of prohibitin [36]. The mitochondrial fragmentation and highly disorganised cristae of PHB2-depleted MEFs strikingly resembles the mitochondrial morphology observed after OPA1 down-regulation [5,37].

OPA1 exists in various isoforms generated by alternative splicing [38] and proteolytic processing involving m- and i-AAA mitochondrial proteases [39–41]. Five isoforms have been described in MEFs and HeLa cells (two long isoforms, L-OPA1; and three short isoforms, S-OPA1). Deletion of PHB2 in MEFs results in the specific loss of L-OPA1 isoforms and an altered pattern in the accumulation of S-OPA1 isoforms [5]. In fact, defects in MEFs lacking PHB2 (mitochondrial fragmentation, aberrant cristae morphology, impaired cellular proliferation, and increased cytochrome *c* release after apoptotic stimulation) were all partially rescued by the overexpression of a non-cleavable L-OPA1 isoform [5], while expression of an S-OPA1 isoform had no effect. These observations suggest that the central role of prohibitin in mitochondria is to regulate OPA1 processing. If so, expression of L-OPA1 would bypass PHB-depletion. Because the activity of OPA1 depends on both L- and S-OPA1 isoforms, it would be interesting to see if suppression of PHB deficiency is enhanced by simultaneous expression of both non-cleavable L-OPA1 and S-OPA1 isoforms. Further investigation of the mechanism by which the PHB complex affects mitochondrial fusion and the proposed stabilisation of OPA1 will shed light on these questions.

Additional studies support the notion that prohibitins participate in mitochondrial dynamics. A mitochondrial stomatin-like protein (SLP-2/Stoml2) has been shown to interact with prohibitins in the mitochondrial IM [42]. Stomatins contain an erythrocyte band-7 motif and belong to the SPFH family of proteins that includes stomatins, prohibitins, flotillins and HflK/C bacterial proteases [43]. Depleting HeLa cells of SLP-2 results in increased proteolysis of PHB1, PHB2, and subunits of respiratory chain complexes I and IV. The stability of prohibitins upon mitochondrial stress partially depends on SLP-2 [42], and PHB expression is increased following mitochondrial stress [14,15,31,44]. SLP-2/Stoml2 was recently shown to interact with Mfn2 [45]. Apparently only a small portion of Mfn2 is involved in forming Mfn2-Stoml2 hetero-oligomers, and reduction of Stoml2 does not affect mitochondrial morphology in HeLa cells [45]. Nevertheless, depletion of the *C. elegans* orthologue of SLP-2/Stoml2 (STL-1) results in mitochondrial fragmentation [46]. Both Mfn1 and Mfn2 interact with Opa1 to mediate mitochondrial fusion [47]. Because the major domains of the Mfns are orientated towards the cytoplasm, it is likely that the interaction between Mfns and OPA1 at the mitochondrial IM is not direct but is instead mediated by additional proteins, possibly SLP-2/Stoml2 and/or prohibitins.

A partial structure for the PHB complex was derived in an attempt to elucidate its molecular mechanism of action [17]. The best-fit 3D structure was the four-helical bundle structure of the t-SNAREs syntaxin 1A and the yeast Sso1p [17]. While one should be careful when modelling unknown structures with less than 30% homology to known structures, it is tempting to consider that proteins with a similar fold might share some functional properties. SNARE proteins are key components of protein complexes that drive membrane fusion [48]. Mfns and OPA1 do not resemble SNARE proteins, suggesting that mitochondrial membrane fusion occurs by a distinct mechanism. However, recent discoveries open the possibility that the basic mechanism of mitochondrial fusion might be more similar than previously supposed to the fusion mechanisms used by other organelles [49]. For example, classical phospholipase D (PLD) cleaves phosphatidylcholine to produce phosphatidic acid, a fusogenic lipid important for SNARE mediated membrane fusion [50]. A mitochondrial phospholipase D (MitoPLD) required for mitochondrial fusion has been identified recently [49]. MitoPLD promotes transmitochondrial membrane adherence in an Mfn-dependent manner by hydrolysing cardiolipin to generate phosphatidic acid. MitoPLD is located at the mitochondrial OM with its catalytic domain exposed to the cytosol. Because cardiolipin is synthesized in the IM, it needs to be transported to the OM, probably through contact sites between the two membranes. In this context it is worth noting that mutations impairing the biosynthetic machinery of PtdEtn and cardiolipin show synthetic lethality when combined with prohibitin depletion in *S. cerevisiae* [19,24]; a role for the PHB complex in lipid partitioning has been suggested [24]. Taken together, the proposed

similarity of the PHB complex with SNARE proteins [17], its interaction with SLP-2/Stoml2 [42] and the interaction of SLP-2/Stoml2 with Mfn [45], the Mfn-dependent role of MitoPLD in mitochondrial fusion [49], and the possible role of prohibitins as lipid membrane organisers [24], all suggest that the PHB complex might play a more direct role in mitochondrial membrane fusion than suspected (Figure 1).

As mentioned above, prohibitins belong to the SPFH family of proteins [43,51]. Members of the SPFH family have been found in lipid rafts [52] or directly interacting with lipids [53]. They contain a conserved domain next to the predicted N-terminal transmembrane stretch that has been called the PHB domain [52]. Although the function of this domain is not clear, it has been proposed to bind lipids or lipid motifs [54]. In this context, the PHB complex could play a role in keeping the mitochondrial outer and inner membrane in close proximity, or even in maintaining the proximity of adjacent mitochondrial inner membranes if located at mitochondrial cristae (Figure 1).

Prohibitin and oxidative phosphorylation

Studies in *S. cerevisiae* suggest a role for prohibitin in the assembly of the oxidative phosphorylation (OXPHOS) system. As mentioned previously, the PHB complex might function as a holdase/unfoldase type of chaperone and in membrane quality control in association with the mitochondrial *m*-AAA proteases [15,16]. Although there is no clear evidence for either an association of the PHB complex with assembly intermediates, or for an essential role in the biogenesis of the OXPHOS system, experimental findings support a role for the PHB complex in handling mitochondrial membrane proteins and in the stability of the OXPHOS system.

Instability of mitochondrial-encoded subunits of the respiratory chain has been observed in *phb*-null yeast cells [16,27], and overexpression of the PHB complex in yeast results in the stabilisation of newly synthesised membrane subunits encoded by the mitochondrion [15]. Prohibitins have been shown to associate with two subunits of complex IV in yeast [15] and with subunits of complex I in mammals [55,56]. Prohibitin expression increases in situations of imbalance between nuclear- and mitochondrial-encoded OXPHOS proteins in yeast, *C. elegans* and mammals [14,15,31,42,44]. In addition, depletion of PHB-2 in *C. elegans* signals the mitochondrial unfolded-protein response and strongly activates mitochondrial chaperones [57,58]. Moreover, reduced cytosolic protein synthesis, that results in reduced loading of cytosolic proteins onto the mitochondrial IM, suppresses mitochondrial degeneration in *phb*-null yeast cells [8].

Yeast cells depleted of PHB genes have reduced mitochondrial membrane potential [24,25]. Similarly, low prohibitin levels in plants result in reduced membrane potential and oxygen consumption [36]. Furthermore, knockdown of both *phb-1* and *phb-2* genes in *C. elegans* results in slightly reduced oxygen consumption [14]. PHB1-depleted endothelial cells have depolarised mitochondria and show reduced complex I activity with normal activity of complexes II and III; complex IV activity was not measured in these experiments. Oxygen consumption was maintained apparently by a compensatory mechanism that allows electron flow through complexes II and III [7]. However, membrane potential, ATP levels, oxygen consumption and electron transport chain activities were normal in MEFs depleted of PHB2 [5], perhaps suggestive of cell type-specific differences in the requirement of the PHB complex for appropriate OXPHOS function.

Prohibitins have also been functionally and physically associated to mitochondrial DNA (mtDNA). Yeast cells lacking mtDNA [rho^-/rho^0] become petite-negative after Phb1p depletion [59]. This phenotype is dependent on the genetic background because strains lacking PHB1 on a different background are viable after mtDNA loss [19]. Physical association of prohibitins with mtDNA nucleoids has been reported both in *Xenopus* oocytes and in HeLa cells [21–23]. RNAi-mediated down-regulation of PHB1 in HeLa cells results in altered organization and reduced mtDNA copy number; this was attributed to destabilisation of the mitochondrial transcription factor A (TFAM) [22], which is essential for mtDNA maintenance [60]. How do prohibitins affect mtDNA when most of the prohibitin complex faces the intermembrane space? It has been suggested that the PHB complex might interact with mtDNA via protein components of mitochondrial nucleoids. Alternative explanations also exist. Mitochondrial nucleoids are attached to the mitochondrial IM. It is therefore possible that alterations to the protein and/or lipid composition of the mitochondrial IM after PHB depletion, as well as the pronounced defect in cristae morphology observed, may affect the attachment of nucleoids to the IM. In agreement, mtDNA loss is also observed in patients with OPA1 mutations [61] and in yeast cells depleted of the OPA1 homologue Mgm1 [62]. Because mtDNA encodes essential subunits of the OXPHOS system, and prohibitins play a key role in mitochondrial membrane integrity, prohibitin depletion is likely to result in OXPHOS deficits regardless of whether prohibitin directly or indirectly interacts with mtDNA.

Altered morphology of cristae and loss of mtDNA within fragmented mitochondria may increase the production of free radicals by disrupting OXPHOS. Indeed, lack of PHB1 in endothelial cells results in increased levels of reactive oxygen species (ROS), often associated with a phenotype resembling senescence [7]. Prohibitin depletion in *C. elegans* and in plants causes increased sensitivity to oxidative stress [14,36], indicating elevated endogenous ROS formation. Similarly, nematodes deleted for *eat-3*, the orthologue of OPA1, are sensitive to free radical-induced damage. Expression of the mitochondrial matrix Fe/Mn-superoxide dismutase SOD-2 is increased in *eat-3* mutants, and disruption of the *sod-2* gene severely compromises the survival of *eat-3* mutant nematodes. Interestingly, increased ROS production and mitochondrial fragmentation has also been reported in *Drosophila* OPA1 mutants [63].

Conclusions and challenges

The function of the PHB complex still remains a mystery despite decades of investigation. Is the PHB complex a holdase/unfoldase chaperone that protects the membrane from unfolded and unassembled proteins, so assisting their degradation? Or do prohibitins act as protein and/or DNA

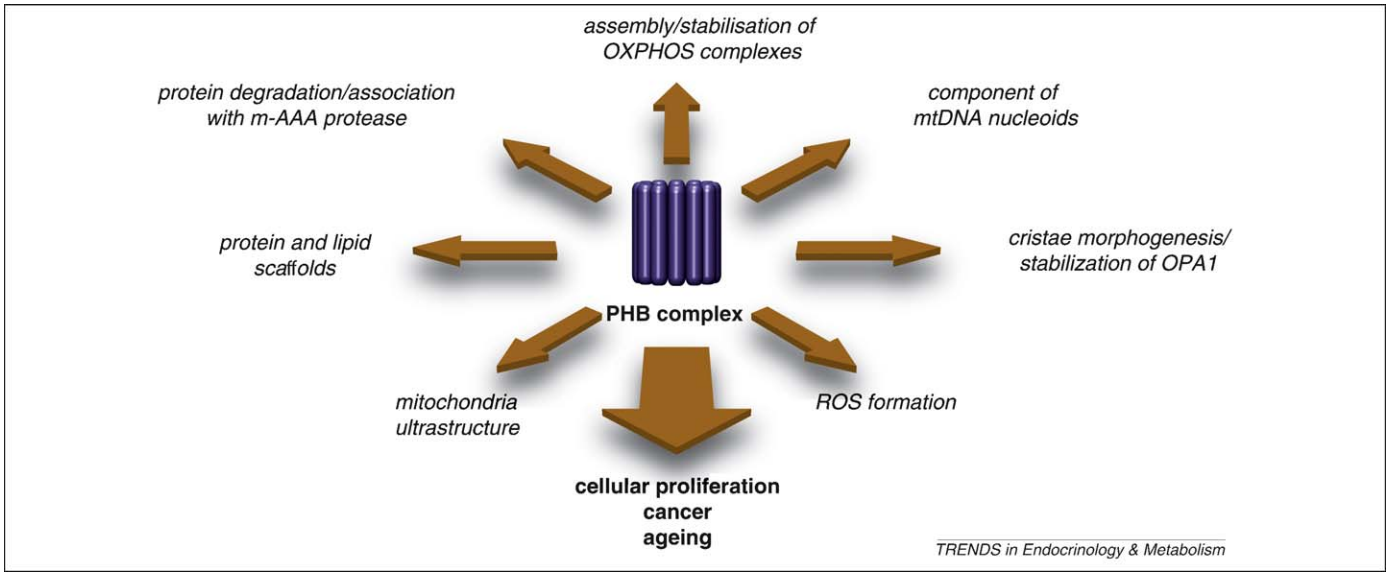

Figure 2. Involvement of the PHB complex in mitochondrial biology and cellular function. The PHB complex has been proposed to play diverse roles within mitochondria (indicated by arrows). Although the exact mechanism of action of prohibitins remains unknown, the pronounced effects of prohibitin depletion in various organisms highlight the importance of this evolutionarily conserved PHB protein complex.

scaffolds? Does the PHB complex play a direct role in mitochondrial membrane morphogenesis? Much still remains to be understood about this highly evolutionarily-conserved inner mitochondrial membrane complex. New ideas have been put forward, including putative roles in mitochondrial genome stability, mitochondrial membrane morphology, and in mitochondrial membrane fusion (Figure 2).

The recent observation that lack of a PHB complex results in a dramatic destabilisation of OPA1 [5,35] provides new insight into the effects of PHB on mitochondrial ultrastructure and the morphology of cristae. Altered cristae morphology caused by lack of prohibitin [5,36] may underlie the destabilisation of mitochondrial transcription factor A (TFAM) and mtDNA, ultimately resulting in defects in OXPHOS and in other mitochondrial metabolic pathways. However, additional evidence suggests that the PHB complex has functions beyond OPA1 stabilisation. Deletion of EAT-3, the *C. elegans* OPA1 orthologue, results in viable animals, whereas prohibitin deficiency is lethal (Ref. [14]; see also http://www.wormbase.org/). This dramatic difference suggests that prohibitins have additional functions independent of OPA1 stabilisation and the maintenance of crista morphology.

Immuno-electron microscopy of mammalian cells shows that the OPA1 protein is mostly distributed throughout cristae, with only a small portion localised within the boundary space between mitochondrial inner and outer membranes [37]. In this context it would be interesting to determine the specific mitochondrial sub-localisation of the PHB complex. Localisation at the inner boundary membrane would indicate a role in the formation of crista or in mediating connections between the IM and OM, and would also be in agreement with its proposed role as a holdase in the process of OXPHOS complex assembly (Figure 1). Assembly of the respiratory chain and ATP synthase requires both proteins imported from the cytosol and mitochondrially synthesised subunits. While the membranes of cristae seem to be the principal site of oxidative phosphorylation [64], OXPHOS complexes are more likely to assemble in the inner boundary membrane where mitochondrial- and nuclear-encoded subunits first encounter each other [65]. Additionally, localisation to cristae would suggest a role in crista morphology or even in maintaining the membranes of cristae in close proximity. These are testable alternative hypotheses in the quest to understand the role of the PHB complex.

Ample evidence indicates that the PHB complex ensures the functional integrity of mitochondrial membranes and is essential for cellular proliferation. Nevertheless, how lack of prohibitin affects mitochondrial morphology and how mitochondrial morphological defects impair cell proliferation remain unknown. Mutations in OXPHOS components have been reported to inhibit cell division through AMP kinase and cyclin E [66]. Additionally, mitochondria associate with spindle poles and have a role in spindle positioning and alignment in eukaryotic cells including *C. elegans* [67,68]. Knockdown of PHB2 in HeLa cells affects sister chromatid cohesion and spindle formation during mitosis [69]. It will be interesting to determine whether impaired proliferation of cells lacking prohibitins is due to defects in mitochondrial energy metabolism, defective mitochondrial morphology, or both.

Despite considerable recent progress in deciphering the function of prohibitins, the above seemingly disparate observations underlie our poor overall understanding of the PHB complex. Resolving the 3D structure of the PHB complex will certainly help define its function at the molecular level. In addition, genetic dissection of prohibitin in animal models holds promise for the unravelling of novel mechanisms by which mitochondrial biogenesis and function influence fundamental cellular processes including pathogenesis and ageing.

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