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4 Prohibitin and mitochondrial biology

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34 **Abstract**

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

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48 **The mitochondrial prohibitin complex**

49 The eukaryotic mitochondrial PHB complex comprises two highly homologous subunits, PHB1
50 and PHB2 (around 50% amino acid sequence identity and 60% similarity). The first mammalian
51 prohibitin (PHB1) was identified as a potential tumour suppressor with anti-proliferative activity
52 [1], hence named prohibitin. This activity was later attributed to the 3'-UTR of the prohibitin
53 mRNA and found to be unrelated to the function of the protein itself [2]. The second prohibitin
54 (PHB2) was isolated bound to the IgM antigen receptor, together with PHB1. Thus, both
55 proteins were also named B-cell-receptor complex-associated proteins (BAP32 and BAP37) [3].
56 In addition, PHB2 was identified as a repressor of nuclear estrogen receptor activity (termed
57 REA) [4]. Extensive and rapidly accumulating evidence suggests that both prohibitin proteins
58 function mainly within mitochondria [5-8]; reviewed in [9, 10]. Nevertheless, a number of diverse
59 cellular functions have also been attributed to both PHB1 and PHB2 in other cellular
60 compartments. These include a role in cell cycle progression, regulation of transcription and cell
61 surface signalling (Box 1; reviewed in [11-13]).

62

63 PHB1 and PHB2, with molecular weights of 32 and 34 kDa respectively, associate to
64 form a macromolecular structure of approximately 1 MDa at the mitochondrial inner membrane.

65 This high molecular weight complex has been identified in yeast, *C. elegans* and mammals [14-
66 16]. Prohibitin homodimers have not been detected [17, 18]. Instead, PHB1 and PHB2
67 associate with each other to form a heterodimeric building block [17]. About 12 to 16 PHB
68 heterodimers associate to form a ring-like structure at the mitochondrial inner membrane [17]
69 with a diameter of 20-25 nm [18]. The PHB complex is anchored in the mitochondrial inner
70 membrane through N-terminal hydrophobic regions, present in both PHB1 and PHB2. For yeast
71 PHB2, the transmembrane domain prediction algorithm TMHMM
72 (<http://www.cbs.dtu.dk/services/TMHMM/>) predicts a transmembrane helix at positions 37–59,
73 which leaves 36 amino acids at the matrix side with most of the protein facing the
74 intermembrane space. The homologous helical site at the N terminus of PHB1 is shorter and
75 may not fulfil the requirements for transmembrane spanning helices. For this reason, PHB1 is
76 considered to be membrane associated (Figure 1) [17]. Complex formation depends on both
77 PHB subunits. Depletion of either PHB1 or PHB2 results in the absence of the complex, while
78 the counterpart mRNA is still present. This indicates interdependence at the level of protein
79 complex formation [5, 14, 19, 20]. However, detailed structural data about this highly conserved
80 protein complex is still lacking.

81

82 Several roles have been suggested for mitochondrial prohibitins within mitochondria
83 (Figure 1). The PHB complex has been implicated in regulating membrane protein degradation
84 by the mitochondrial *m*-AAA protease [16], it has been proposed to function as a
85 holdase/unfoldase chaperone, which holds and stabilizes unassembled membrane proteins [10,
86 15] and potentially also plays a role in stabilizing the mitochondrial genome [21-23]. In addition,
87 the PHB complex has been implicated in mitochondrial morphogenesis [5], functioning as a
88 scaffold that recruits membrane proteins to a specific lipid environment [24]. Here we review the
89 mitochondrial functions attributed to the PHB complex and focus on their implications for ageing
90 and disease.

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93 **Life without prohibitin**

94 Disruption of the PHB complex in *Saccharomyces cerevisiae* decreases replicative lifespan of
95 yeast cells but does not result in any other observable growth phenotype under laboratory

96 conditions [19, 25]. The shortening of yeast replicative lifespan is accompanied by defects in the
97 mitochondrial membrane potential, extended cell division time and other characteristic
98 morphological changes of ageing cells [25]. Prohibitin depletion does not alter the chronological
99 lifespan of non-dividing (G_0 -arrested) cells, although *phb*-null mutants in stationary phase tend
100 to lose respiratory capacity, which has been associated with deletions of the mitochondrial
101 genome (the [ρ^-] phenotype) [26]. Increased frequency of [ρ^-]-cell generation can only be
102 detected in old, non-dividing and not in young *phb*-null mother cells [19, 27]. Similarly, only old
103 *phb*-null mother cells at the end of their replicative lifespan show defective mitochondrial
104 segregation and aberrant mitochondrial morphology [26]. By contrast, no mitochondrial
105 morphology defects have been detected in younger *phb*-null cells [19, 27]. This suggests that
106 *phb*-null yeast cells undergo premature ageing, probably due to a slight, but cumulative decline
107 in cellular metabolic capacity.

108

109 In contrast to the relatively marginal observable effects in yeast, severe phenotypes are
110 associated with prohibitin deficiency in multicellular organisms (see also Box 2). In
111 *Caenorhabditis elegans* and in mice, prohibitins are required for embryonic development [5, 14,
112 20, 28]. Similarly, prohibitins are required for plant development [29, 30]. Post-embryonic
113 depletion of prohibitins in *C. elegans* results in pronounced germline defects such as diminished
114 oocyte production with smaller brood size [14]. Both embryonic and postembryonic effects
115 observed in *C. elegans* indicate that PHB proteins are specifically required in tissues that
116 undergo cellular proliferation. Extensive distortion of mitochondrial morphology is observed
117 following reduction of prohibitin expression in *C. elegans* body wall muscles [14]. During post-
118 embryonic development, nematode muscle cells do not proliferate but rather grow in size.
119 Normally, the number of mitochondria per cell increases to meet energy requirements of muscle
120 growth. The effects of prohibitin depletion suggest that the prohibitin complex plays an important
121 role in maintaining mitochondrial membrane integrity in these cells. Deletion of PHB2 in mouse
122 embryonic fibroblasts (MEFs) results in severely impaired cellular proliferation [5]. Accordingly, it
123 appears likely that tissues that rely heavily on mitochondrial function, i.e. proliferating cells, are
124 more susceptible to lack of prohibitin.

125

126 Changes in prohibitin expression levels further support a role for the PHB complex
127 during strong metabolic demands. For example, PHB expression increases in yeast cells during

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

138

139 Extensive studies on the expression patterns of both PHB proteins in mammalian
140 tissues and during murine development also support a role for prohibitins in regulating
141 mitochondrial metabolism. PHB proteins are highly expressed in cells that rely heavily on
142 mitochondrial function, including neurons, muscle, heart, liver, renal tubules, adrenal cortex,
143 brown adipocytes and pancreatic islet cells [31]. These tissues are often particularly susceptible
144 to mitochondrial dysfunction [32]. Plant prohibitins are predominantly expressed in proliferating
145 tissues [30]. Similarly, PHB proteins are expressed at higher levels in mammalian proliferating
146 cells, including neoplastic tissues (see also Box 2) [31, 33].

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149 **Prohibitin and mitochondrial dynamics**

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

157

158 Loss of prohibitins was first shown to severely affect mitochondrial morphology in *C.*
159 *elegans* body wall muscle cells [14]. In normal muscle cells, mitochondria appear tubular,
160 elongated, and well structured, running parallel to the body axis and often parallel to the
161 myofibrils. Upon prohibitin depletion, mitochondria appear fragmented and disorganized [14].
162 Similarly, loss of prohibitins results in the accumulation of fragmented mitochondria in MEFs and
163 HeLa cells [5, 35]. A possible mechanism for prohibitin-depletion mediated mitochondrial
164 fragmentation has been put forward after the discovery that PHB2 deletion results in OPA1
165 destabilization [5, 35]. OPA1 resides in the mitochondrial inner membrane and has a key role in
166 mitochondrial fusion and cristae morphology. Electron microscopy analysis of PHB2-depleted
167 MEFs revealed severe defects in lamellar cristae formation [5]. Similarly, lack of cristae has
168 been reported in plant mitochondrial depleted of prohibitin [36]. The mitochondrial fragmentation
169 and highly disorganized cristae of PHB2 depleted MEFs strikingly resembles the mitochondrial
170 morphology observed after OPA1 down-regulation [5, 37].

171
172 OPA1 exists in various isoforms generated by alternative splicing [38] and proteolytic
173 processing involving m- and i-AAA mitochondrial proteases [39-41]. Five isoforms have been
174 described in MEFs and HeLa cells (two long isoforms, L-OPA1, and three short isoforms, S-
175 OPA1). Deletion of PHB2 in MEFs results in the specific loss of L-OPA1 isoforms and an altered
176 pattern in the accumulation of S-OPA1 isoforms [5]. In fact, defects in MEFs lacking PHB2
177 (mitochondrial fragmentation, aberrant cristae morphology, impaired cellular proliferation and
178 increased cytochrome c release after apoptotic stimulation), were all partially rescued by
179 overexpressing a non-cleavable L-OPA1 isoform [5], while expression of an S-OPA1 isoform
180 had no effect. These observations suggest that the central role of prohibitin in mitochondria is to
181 regulate OPA1 processing. If so, expression of L-OPA1 is expected to bypass PHB-depletion.
182 Because the activity of OPA1 depends on both L- and S-OPA1 isoforms, it would be interesting
183 to see if suppression of PHB deficiency is enhanced by the simultaneous expression of both
184 non-cleavable L-OPA1 and S-OPA1 isoforms. Further investigation of the mechanism by which
185 the PHB complex affects mitochondrial fusion and the proposed stabilisation of OPA1 will shed
186 light on these questions

187
188 Additional studies support the notion that prohibitins participate in mitochondrial
189 dynamics. A mitochondrial stomatin-like protein (SLP-2/Stoml2) has been shown to interact with

prohibitins in the mitochondrial inner membrane [42]. Stomatins contain an erythrocyte band-7 motif and belong to the SPFH family of proteins, which 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]. Recently, SLP-2/Stoml2 was 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]. Mfn1 and Mfn2 interact with Opa1 to mediate mitochondrial fusion [47]. Because the major domains of Mfns are orientated towards the cytoplasm, it is likely that the interaction between Mfns and OPA1 at the mitochondrial inner membrane is not direct, but mediated by additional proteins, such as SLP-2/Stoml2 and/or prohibitins.

204

205 A partial structure for the PHB complex was derived in an attempt to elucidate its
206 molecular mechanism of action [17]. The best-fit 3D structure was the four-helical bundle
207 structure of the t-SNAREs syntaxin 1A and the yeast Sso1p [17]. While one should be careful
208 when modelling unknown structures with less than 30% homology to known structures, it is
209 tempting to consider that proteins with a similar fold might share some functional properties.
210 SNARE proteins are key components of protein complexes that drive membrane fusion [48].
211 Mfns and OPA1 do not resemble SNARE proteins, which suggests, mitochondrial membrane
212 fusion occurs by a distinct mechanism. However, recent discoveries open the possibility that the
213 basic mechanism of mitochondrial fusion might be more similar to the fusion mechanisms used
214 by other organelles than previously believed [49]. Classical Phospholipase D (PLD) cleaves
215 phosphatidylcholine to produce phosphatidic acid; a fusogenic lipid important for SNARE
216 mediated membrane fusion [50]. A mitochondrial phospholipase D (MitoPLD) required for
217 mitochondrial fusion has recently been identified [49]. MitoPLD promotes transmtochondrial
218 membrane adherence in an Mfn-dependent manner by hydrolysing cardiolipin to generate
219 phosphatidic acid. MitoPLD IS located at the mitochondrial outer membrane with its catalytic
220 domain exposed to the cytosol. Because cardiolipin is synthesized in the inner membrane, it
221 needs to be transported to the outer membrane, 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] and 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 organizers [24] suggest that the PHB complex might play a more direct role in mitochondrial membrane fusion than suspected (Figure 1).

230

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 have a role in keeping the mitochondrial outer and inner membrane in close proximity, or even both mitochondrial inner membranes if located at mitochondrial cristae (Figure 1)

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239

240 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.

248

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 stabilization of newly synthesized mitochondrial encoded membrane subunits [15]. Prohibitins have been shown to associate with two subunits of complex IV in yeast [15] and with subunits of

253 complex I in mammals [55, 56]. Expression of prohibitins increases in situations of imbalance
254 between nuclear- and mitochondrial-encoded OXPHOS proteins in yeast, *C. elegans* and
255 mammals [14, 15, 31, 42, 44]. In addition, depletion of PHB-2 in *C. elegans* signals the
256 mitochondrial unfolded protein response and strongly activates mitochondrial chaperones [57,
257 58]. Moreover, reduced cytosolic protein synthesis, which results in reduced load of cytosolic
258 proteins onto the mitochondrial inner membrane, suppresses mitochondrial degeneration of
259 *phb*-null yeast cells [8].

260

261 Yeast cells depleted of PHB genes have reduced mitochondrial membrane potential
262 [24, 25]. Similarly, low prohibitin levels in plants result in reduced membrane potential and
263 oxygen consumption [36]. Furthermore, knockdown of both *phb-1* and *phb-2* genes in *C.*
264 *elegans* results in slightly reduced oxygen consumption [14]. Endothelial, PHB1-depleted cells
265 have depolarized mitochondria and show reduced complex I activity. The activities of
266 complexes II and III were normal, while complex IV was not measured in these experiments.
267 Oxygen consumption was maintained apparently by a compensatory mechanism that allowed
268 electron flow through complexes II and III [7]. However, in MEFs depleted of PHB2, membrane
269 potential, ATP levels, oxygen consumption and electron transport chain activities were normal
270 [5], suggesting cell-type specific differences in the requirement of the PHB complex for
271 appropriate OXPHOS function.

272

273 Prohibitins have also been functionally and physically associated to mitochondrial DNA
274 (mtDNA). Yeast cells lacking mtDNA [ρ^0/ρ^0] become petite-negative after Phb1p depletion
275 [59]. This phenotype is genetic background-dependent since strains deleted for PHB1 in a
276 different background are viable after mtDNA loss [19]. Physical association of prohibitins to
277 mtDNA nucleoids has been reported in *Xenopus* oocytes and in HeLa cells [21-23]. RNAi-
278 mediated down-regulation of PHB1 in HeLa cells results in altered organization and reduced
279 copy number of mtDNA, attributed to the destabilisation of the mitochondrial transcription factor
280 A (TFAM) [22], which is essential for mtDNA maintenance [60]. How do prohibitins affect mtDNA
281 when most of the prohibitin complex faces the intermembrane space? It has been suggested
282 that the PHB complex might interact with mtDNA via protein components of mitochondrial
283 nucleoids. Alternative explanations also exist. Mitochondrial nucleoids are attached to the
284 mitochondrial inner membrane. It is therefore, possible that alterations of protein and/or lipid

285 composition in the mitochondrial inner membrane after PHB depletion, as well as the
286 pronounced defect in cristae morphology observed, may affect the attachment of nucleoids to
287 the inner membrane. In agreement with this, loss of mtDNA is also observed in patients with
288 OPA1 mutations [61] and in yeast cells depleted of the OPA1 homologue Mgm1 [62]. Because
289 mtDNA encodes for essential subunits of the OXPHOS system, regardless whether prohibitins
290 depletion affects directly or indirectly mtDNA, the net result will likely be defects in OXPHOS.

291

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

302

303

304 **Conclusions and challenges**

305 Despite decades of investigation, the function of the PHB complex still remains a mystery. Is the
306 PHB complex a holdase/unfoldase chaperone that protects the membrane from unfolded and
307 unassembled proteins, assisting their degradation? Or, do prohibitins act as protein and/or DNA
308 scaffolds? Does the PHB complex have a direct role in mitochondrial membrane
309 morphogenesis? Much still remains to be understood about this highly evolutionarily conserved
310 inner mitochondrial membrane complex. New ideas have been put forward, including a putative
311 role in mitochondrial genome stability, mitochondrial membrane morphology or in mitochondrial
312 membrane fusion (Figure 2).

313

314 The recent observation that lack of the PHB complex results in a dramatic
315 destabilisation of OPA1 [5, 35] provides new insight into the effect of PHB depletion on

316 mitochondrial ultrastructure and cristae morphology. Altered cristae morphology caused by lack
317 of prohibitin [5, 36], may underlie the destabilisation of mitochondrial transcription factor A
318 (TFAM) and mtDNA, which will ultimately result in defective OXPHOS and other mitochondrial
319 metabolic pathways. However, additional evidence suggests that the PHB complex has
320 functions beyond OPA1 stabilisation. Deletion of the *C. elegans* orthologue of OPA1 (EAT-3)
321 results in viable animals, whereas prohibitin deficiency is lethal [14]; see also
322 <http://www.wormbase.org/>). This dramatic difference suggests that prohibitins have additional
323 functions, independent of OPA1 stabilisation and consequent maintenance of cristae
324 morphology.

325

326 Immuno-electron microscopy on mammalian cells shows that the OPA1 protein is
327 mostly distributed throughout cristae, with only a small portion localized to the boundary space
328 between mitochondrial inner and outer membranes [37]. In this context, it would be interesting
329 to determine the specific mitochondrial sub-localisation of the PHB complex. Localisation at the
330 inner boundary membrane would indicate a role in cristae formation or in mediating connections
331 between the inner and the outer mitochondrial membranes, and would also be in agreement
332 with its proposed role as holdase in the process of OXPHOS complex assembly (Figure 1).

333 Assembly of the respiratory chain and ATP synthase requires both proteins imported from the
334 cytosol and mitochondrially synthesised subunits. While cristal membranes seem to be the
335 principal site of oxidative phosphorylation [64], OXPHOS complexes are more likely to assemble
336 in the inner boundary membrane where mitochondrial- and nuclear-encoded subunits first
337 encounter each other [65]. Additionally, localisation at cristae would suggest a role in cristae
338 morphology or even in maintaining cristae membranes in close proximity. These are testable
339 alternative hypotheses in the quest of further investigating the role of the PHB complex.

340

341 Ample evidence indicates that the PHB complex ensures the functional integrity of
342 mitochondrial membranes and is essential for cellular proliferation. Nevertheless, how lack of
343 prohibitin affects mitochondrial morphology and how mitochondrial morphology defects impair
344 cellular proliferation remain unknown. Mutations in OXPHOS components have been reported
345 to inhibit cell division through AMP kinase and cyclin E [66]. Additionally, mitochondria associate
346 with spindle poles and have a role in spindle positioning and alignment in eukaryotic cells and in
347 *C. elegans* [67, 68]. Knock down of PHB2 in HeLa cells affects sister chromatid cohesion and

348 spindle formation during mitosis [69]. It would be interesting to determine whether impaired
349 proliferation of cells lacking prohibitins is due to defects on mitochondrial energy metabolism,
350 due to defective mitochondrial morphology, or both.

351

352 Despite the considerable recent progress towards deciphering the function of
353 prohibitins, the above seemingly disparate observations underline our poor overall
354 understanding of the PHB complex. Resolving the three-dimensional structure of the PHB
355 complex will certainly help in defining its function at the molecular level. In addition, the genetic
356 dissection of prohibitin in animal models holds promise for unravelling novel mechanisms by
357 which mitochondrial biogenesis and function influence fundamental cellular processes including
358 pathogenesis and ageing.

359

360

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366

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 549 *schizophrenia*. *Mol Psychiatry*, 2008. **13**(9): p. 878-96.
- 550

550 **Text Boxes**

551

552 **Box 1. Prohibitins outside of mitochondria**

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

558

559 Initially, prohibitin (PHB1) was proposed to play a role in cell cycle progression [1, 70].
560 Later PHB2 was identified as a B-cell receptor associated protein at the plasma membrane [3],
561 and named BAP37 for B-cell associated protein. In addition, both PHB1 and PHB2 were found
562 at the plasma membrane of human intestinal epithelial cells, functioning as a binding site for the
563 Vi capsular polysaccharide of *Salmonella typhi* [71]. PHB1 has also been found to be the target
564 of a proapoptotic peptide in adipose vasculature [72]. Furthermore, PHB1 has been implicated
565 in mediating cellular Ras-Raf signalling at the membrane [13]. PHB1 has also been shown to
566 modulate transcription in cell-transfection experiments and to bind to a wide range of proteins,
567 including Retinoblastoma (Rb), E2-F, Brg1/Brm and p53 [11, 12]. PHB2 was found to modulate
568 muscle differentiation by binding to AKT [73, 74] and also to represses estrogen receptor (ER α)
569 activity, hence termed REA [28].

570

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

580

581

582 **Box 2. Prohibitin in ageing and disease**

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

591

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

600

601 Mitochondrial dysfunction underlies the pathology of a broad spectrum of diseases.
602 Myopathies and neuropathies are among the most common types of disorders associated with
603 mitochondrial defects. Other disorders such as diabetes, hearing loss and kidney failure are
604 also caused by mitochondrial dysfunction [32, 77]. To date, no mutations in the PHB genes
605 have been found to cause human disease. Nevertheless, mutations in the mitochondrial *m*-
606 AAA-metallocprotease, which interacts with the PHB complex [16] cause hereditary spastic
607 paraplegia in humans [78]. In addition, high levels of prohibitin expression in tumours indicate
608 their potential role in carcinogenesis [33]. Expression of PHB proteins is also elevated in yeast
609 cells devoid of *SHY1*, the orthologue of *SURF1*, which is associated with Leigh syndrome [44].
610 Altered expression of PHB1 correlates with loss of mitochondrial function in the liver of knockout
611 mice deficient in S-adenosylmethionine synthesis and in obese patients who are at risk for
612 nonalcoholic steatohepatitis [79]. In addition, abnormal levels of prohibitin have been reported in

613 Parkinson's disease [80] and in schizophrenia [81]. These findings emphasise the importance
614 of the PHB complex in maintaining mitochondrial homeostasis, which is critical for human
615 health.

616

617

618 **Box 3. Glossary**

619 **IgM:** Immunoglobulin M. An antibody that is present on B lymphocytes, which are involved in
620 the humoral immune response. IgM is the primary antibody against A and B antigens on red
621 blood cells.

622 **m-AAA protease:** Mitochondrial matrix-AAA protease. Member of membrane-bound ATP-
623 dependent proteases that are present in eubacteria, mitochondria and chloroplasts and that can
624 degrade membrane proteins.

625 **Relicative lifespan:** Yeast cells age chronologically, but also undergo replicative senescence.
626 The replicative lifespan reflects the number of buds generated by an individual mother cell.

627 **Chronological lifespan:** Time that non-dividing yeast cells in a stationary phase culture remain
628 viable.

629 **Stationary phase:** When nutrients are exhausted yeast cells enter a stationary phase that is
630 characterized by cell cycle arrest (G_0) and specific physiological, biochemical, and
631 morphological changes.

632 **Respiratory capacity:** Ability of yeast cells to grow on non-fermentable carbon sources, where
633 respiration (aerobic growth) is required.

634 **[rho]:** Deletions of the mitochondrial genome that render yeast cells respiratory-deficient (petite
635 phenotype).

636 **Petite mutants:** The yeast *Saccharomyces cerevisiae* can grow in the absence of mtDNA.
637 Yeast strains that contain wild-type mtDNA, called [rho⁺] cells, can respire and grow on non-
638 fermentable carbon sources. Cells that contain deletions or mutations in mtDNA [rho] or have
639 completely lost their mtDNA [rho⁰] are called petite mutants. Petite mutants can grow by
640 fermentation in glucose media. Petite-negative cells lose viability in fermentable carbon sources.

641 **Diauxic shift:** *S. cerevisiae* switches metabolism from fermentation to respiration when growing
642 on glucose and in the presence of oxygen. During the first growth phase, when there is plenty of
643 glucose and oxygen available, yeast cells prefer glucose fermentation to aerobic respiration.

644 After glucose is depleted yeast cells undergo a metabolic (or diauxic) shift, where the

645 fermentative product ethanol is oxidised. Diauxic shift is accompanied by stimulation of
646 mitochondrial function.

647 **Nucleoids:** Discrete protein-DNA complexes, organizing multiple mitochondrial DNA (mtDNA)
648 molecules.

649 **Hereditary spastic paraplegia (HSP):** Inherited neurological disorder characterized by
650 retrograde degeneration of cortical motor axons, progressive weakness (paraplegia), increased
651 muscle tone and stiffness (spasticity) of the legs. Loss of function of paraplegin (encoded by the
652 gene SPG7, a mitochondrial *m*-AAA-protease) causes HSP.

653 **Leigh syndrome:** Neurodegenerative disorder of infancy or childhood, generally due to
654 mutations in nuclear or mitochondrial genes involved in mitochondrial energy metabolism.

655

655 **Figure legends**

656

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

673

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

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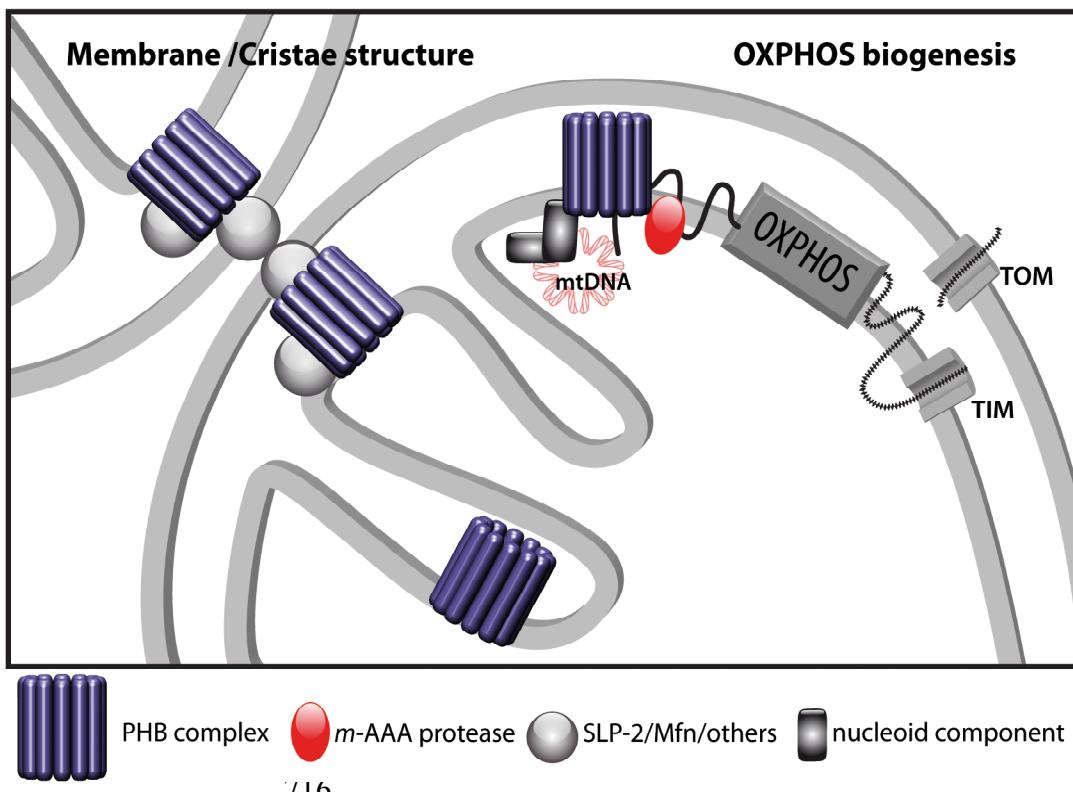
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686 Figure 1
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