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# Prohibitin and mitochondrial biology

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## Abstract

**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 insight on the role of the prohibitin complex in mitochondrial biogenesis and metabolism. Here we review the recent studies and discuss their implications towards 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], hence named prohibitin. This activity was later attributed to the 3'-UTR of the prohibitin mRNA and found to be unrelated to the function of the protein itself [2]. The second prohibitin (PHB2) was isolated bound to the IgM antigen receptor, together with PHB1. 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 prohibitin proteins function mainly within mitochondria [5-8]; reviewed in [9, 10]. Nevertheless, a number of diverse cellular functions have also been attributed to both PHB1 and PHB2 in other cellular compartments. These include a role in cell cycle progression, regulation of transcription and cell surface signalling (Box 1; reviewed in [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.

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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## 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 [rho⁻] phenotype) [26]. Increased frequency of [rho⁻]-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 mitochondrially 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 disorganised 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

190    prohibitins in the mitochondrial inner membrane [42]. Stomatins contain an erythrocyte band-7

191    motif and belong to the SPFH family of proteins, which includes stomatins, prohibitins, flotillins

192    and HflK/C bacterial proteases [43]. Depleting HeLa cells of SLP-2 results in increased

193    proteolysis of PHB1, PHB2, and subunits of respiratory chain complexes I and IV. The stability

194    of prohibitins upon mitochondrial stress partially depends on SLP-2 [42], and PHB expression is

195    increased following mitochondrial stress [14, 15, 31, 44]. Recently, SLP-2/Stoml2 was shown to

196    interact with Mfn2 [45]. Apparently, only a small portion of Mfn2 is involved in forming Mfn2-

197    Stoml2 hetero-oligomers, and reduction of Stoml2 does not affect mitochondrial morphology in

198    HeLa cells [45]. Nevertheless, depletion of the *C. elegans* orthologue of SLP-2/Stoml2 (STL-1)

199    results in mitochondrial fragmentation [46]. Mfn1 and Mfn2 interact with Opa1 to mediate

200    mitochondrial fusion [47]. Because the major domains of Mfns are orientated towards the

201    cytoplasm, it is likely that the interaction between Mfns and OPA1 at the mitochondrial inner

202    membrane is not direct, but mediated by additional proteins, such as SLP-2/Stoml2 and/or

203    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 transmitochondrial

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

222   membranes. In this context, it is worth noting that mutations impairing the biosynthetic

223   machinery of PtdEtn and cardiolipin show synthetic lethality when combined with prohibitin

224   depletion in *S. cerevisiae* [19, 24] and a role for the PHB complex in lipid partitioning has been

225   suggested [24]. Taken together, the proposed similarity of the PHB complex with SNARE

226   proteins [17], its interaction with SLP-2/Stoml2 [42] and the interaction of SLP-2/Stoml2 with Mfn

227   [45], the Mfn-dependent role of MitoPLD in mitochondrial fusion [49] and the possible role of

228   prohibitins as lipid membrane organizers [24] suggest that the PHB complex might play a more

229   direct role in mitochondrial membrane fusion that suspected (Figure 1).

230

231        As mentioned above, prohibitins belong to the SPFH family of proteins [43, 51].

232   Members of the SPFH family have been found in lipid rafts [52] or directly interacting with lipids

233   [53]. They contain a conserved domain next to the predicted N-terminal transmembrane stretch

234   that has been called the PHB domain [52]. Although the function of this domain is not clear, it

235   has been proposed to bind lipids or lipid motifs [54]. In this context, the PHB complex could

236   have a role in keeping the mitochondrial outer and inner membrane in close proximity, or even

237   both mitochondrial inner membranes if located at mitochondrial cristae (Figure 1)

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239

240   **Prohibitin and oxidative phosphorylation**

241   Studies in *S. cerevisiae* suggest a role for prohibitin in the assembly of the oxidative

242   phosphorylation (OXPHOS) system. As mentioned previusly, the PHB complex might function

243   as a holdase/unfoldase type of chaperone and in membrane quality control in association with

244   the mitochondrial *m*-AAA proteases [15, 16]. Although there is no clear evidence for either an

245   association of the PHB complex with assembly intermediates or for an essential role in the

246   biogenesis of the OXPHOS system, experimental findings support a role for the PHB complex in

247   handling mitochondrial membrane proteins and in the stability of the OXPHOS system.

248

249        Instability of mitochondrial-encoded subunits of the respiratory chain has been observed

250   in *phb*-null yeast cells [16, 27], and overexpression of the PHB complex in yeast results in

251   stabilization of newly synthesized mitochondrial encoded membrane subunits [15]. Prohibitins

252   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 [rho$^-$/rho$^0$] 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].

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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.

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## References

1. McClung, J.K., et al., *Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver.* Biochem Biophys Res Commun, 1989. **164**(3): p. 1316-22.
2. Jupe, E.R., et al., *Prohibitin in breast cancer cell lines: loss of antiproliferative activity is linked to 3' untranslated region mutations.* Cell Growth Differ, 1996. **7**(7): p. 871-8.
3. Terashima, M., et al., *The IgM antigen receptor of B lymphocytes is associated with prohibitin and a prohibitin-related protein.* EMBO J, 1994. **13**(16): p. 3782-92.
4. Montano, M.M., et al., *An estrogen receptor-selective coregulator that potentiates the effectiveness of antiestrogens and represses the activity of estrogens.* Proc Natl Acad Sci U S A, 1999. **96**(12): p. 6947-52.
5. Merkwirth, C., et al., *Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria.* Genes Dev, 2008. **22**(4): p. 476-88.
6. Ross, J.A., Z.S. Nagy, and R.A. Kirken, *The PHB1/2 phosphocomplex is required for mitochondrial homeostasis and survival of human T cells.* J Biol Chem, 2008. **283**(8): p. 4699-713.
7. Schleicher, M., et al., *Prohibitin-1 maintains the angiogenic capacity of endothelial cells by regulating mitochondrial function and senescence.* J Cell Biol, 2008. **180**(1): p. 101-12.
8. Wang, X., et al., *Reduced cytosolic protein synthesis suppresses mitochondrial degeneration.* Nat Cell Biol, 2008. **10**(9): p. 1090-7.
9. Merkwirth, C. and T. Langer, *Prohibitin function within mitochondria: Essential roles for cell proliferation and cristae morphogenesis.* Biochim Biophys Acta, 2009. **1793**(1): p. 27-32.
10. Nijtmans, L.G., et al., *The mitochondrial PHB complex: roles in mitochondrial respiratory complex assembly, ageing and degenerative disease.* Cell Mol Life Sci, 2002. **59**(1): p. 143-55.
11. Mishra, S., L.C. Murphy, and L.J. Murphy, *The Prohibitins: emerging roles in diverse functions.* J Cell Mol Med, 2006. **10**(2): p. 353-63.
12. Mishra, S., et al., *Prohibitin: a potential target for new therapeutics.* Trends Mol Med, 2005. **11**(4): p. 192-7.
13. Rajalingam, K. and T. Rudel, *Ras-Raf signaling needs prohibitin.* Cell Cycle, 2005. **4**(11): p. 1503-5.
14. Artal-Sanz, M., et al., *The mitochondrial prohibitin complex is essential for embryonic viability and germline function in Caenorhabditis elegans.* J Biol Chem, 2003. **278**(34): p. 32091-9.
15. Nijtmans, L.G., et al., *Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins.* EMBO J, 2000. **19**(11): p. 2444-51.
16. Steglich, G., W. Neupert, and T. Langer, *Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria.* Mol Cell Biol, 1999. **19**(5): p. 3435-42.
17. Back, J.W., et al., *A structure for the yeast prohibitin complex: Structure prediction and evidence from chemical crosslinking and mass spectrometry.* Protein Sci, 2002. **11**(10): p. 2471-8.
18. Tatsuta, T., K. Model, and T. Langer, *Formation of membrane-bound ring complexes by prohibitins in mitochondria.* Mol Biol Cell, 2005. **16**(1): p. 248-59.
19. Berger, K.H. and M.P. Yaffe, *Prohibitin family members interact genetically with mitochondrial inheritance components in Saccharomyces cerevisiae.* Mol Cell Biol, 1998. **18**(7): p. 4043-52.
20. He, B., et al., *A repressive role for prohibitin in estrogen signaling.* Mol Endocrinol, 2008. **22**(2): p. 344-60.
21. Bogenhagen, D.F., D. Rousseau, and S. Burke, *The layered structure of human mitochondrial DNA nucleoids.* J Biol Chem, 2008. **283**(6): p. 3665-75.
22. Kasashima, K., et al., *Human prohibitin 1 maintains the organization and stability of the mitochondrial nucleoids.* Exp Cell Res, 2008. **314**(5): p. 988-96.
23. Wang, Y. and D.F. Bogenhagen, *Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane.* J Biol Chem, 2006. **281**(35): p. 25791-802.
24. Osman, C., et al., *The genetic interactome of prohibitins: coordinated control of cardiolipin and phosphatidylethanolamine by conserved regulators in mitochondria.* J Cell Biol, 2009. **184**(4):583-96.
25. Coates, P.J., et al., *The prohibitin family of mitochondrial proteins regulate replicative lifespan.* Curr Biol, 1997. **7**(8): p. 607-10.

425    26.  Piper, P.W., et al., *The shortened replicative life span of prohibitin mutants of yeast appears*
426          *to be due to defective mitochondrial segregation in old mother cells.* Aging Cell, 2002. **1**(2):
427          p. 149-57.
428    27.  Birner, R., et al., *Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine*
429          *biosynthetic machinery with the prohibitin complex of Saccharomyces cerevisiae.* Mol Biol
430          Cell, 2003. **14**(2): p. 370-83.
431    28.  Park, S.E., et al., *Genetic deletion of the repressor of estrogen receptor activity (REA)*
432          *enhances the response to estrogen in target tissues in vivo.* Mol Cell Biol, 2005. **25**(5): p.
433          1989-99.
434    29.  Chen, J.C., C.Z. Jiang, and M.S. Reid, *Silencing a prohibitin alters plant development and*
435          *senescence.* Plant J, 2005. **44**(1): p. 16-24.
436    30.  Van Aken, O., et al., *Mitochondrial type-I prohibitins of Arabidopsis thaliana are required for*
437          *supporting proficient meristem development.* Plant J, 2007. **52**(5): p. 850-64.
438    31.  Coates, P.J., et al., *Mammalian prohibitin proteins respond to mitochondrial stress and*
439          *decrease during cellular senescence.* Exp Cell Res, 2001. **265**(2): p. 262-73.
440    32.  Wallace, D.C., *Mitochondrial diseases in man and mouse.* Science, 1999. **283**(5407): p.
441          1482-8.
442    33.  Czarnecka, A.M., et al., *Mitochondrial chaperones in cancer: from molecular biology to*
443          *clinical diagnostics.* Cancer Biol Ther, 2006. **5**(7): p. 714-20.
444    34.  Detmer, S.A. and D.C. Chan, *Functions and dysfunctions of mitochondrial dynamics.* Nat
445          Rev Mol Cell Biol, 2007. **8**(11): p. 870-9.
446    35.  Kasashima, K., et al., *Mitochondrial functions and estrogen receptor-dependent nuclear*
447          *translocation of pleiotropic human prohibitin 2.* J Biol Chem, 2006. **281**(47): p. 36401-10.
448    36.  Ahn, C.S., et al., *Prohibitin is involved in mitochondrial biogenesis in plants.* Plant J, 2006.
449          **46**(4): p. 658-67.
450    37.  Griparic, L., et al., *Loss of the intermembrane space protein Mgm1/OPA1 induces swelling*
451          *and localized constrictions along the lengths of mitochondria.* J Biol Chem, 2004. **279**(18):
452          p. 18792-8.
453    38.  Delettre, C., et al., *Mutation spectrum and splicing variants in the OPA1 gene.* Hum Genet,
454          2001. **109**(6): p. 584-91.
455    39.  Cipolat, S., et al., *Mitochondrial rhomboid PARL regulates cytochrome c release during*
456          *apoptosis via OPA1-dependent cristae remodeling.* Cell, 2006. **126**(1): p. 163-75.
457    40.  Duvezin-Caubet, S., et al., *OPA1 processing reconstituted in yeast depends on the subunit*
458          *composition of the m-AAA protease in mitochondria.* Mol Biol Cell, 2007. **18**(9): p. 3582-90.
459    41.  Griparic, L., T. Kanazawa, and A.M. van der Bliek, *Regulation of the mitochondrial dynamin-*
460          *like protein Opa1 by proteolytic cleavage.* J Cell Biol, 2007. **178**(5): p. 757-64.
461    42.  Da Cruz, S., et al., *SLP-2 interacts with prohibitins in the mitochondrial inner membrane and*
462          *contributes to their stability.* Biochim Biophys Acta, 2008. **1783**(5): p. 904-11.
463    43.  Tavernarakis, N., M. Driscoll, and N.C. Kyrpides, *The SPFH domain: implicated in*
464          *regulating targeted protein turnover in stomatins and other membrane-associated proteins.*
465          Trends Biochem Sci, 1999. **24**(11): p. 425-7.
466    44.  Nijtmans, L.G., et al., *Shy1p occurs in a high molecular weight complex and is required for*
467          *efficient assembly of cytochrome c oxidase in yeast.* FEBS Lett, 2001. **498**(1): p. 46-51.
468    45.  Hajek, P., A. Chomyn, and G. Attardi, *Identification of a novel mitochondrial complex*
469          *containing mitofusin 2 and stomatin-like protein 2.* J Biol Chem, 2007. **282**(8): p. 5670-81.
470    46.  Ichishita, R., et al., *An RNAi screen for mitochondrial proteins required to maintain the*
471          *morphology of the organelle in Caenorhabditis elegans.* J Biochem, 2008. **143**(4): p. 449-
472          54.
473    47.  Guillery, O., et al., *Metalloprotease-mediated OPA1 processing is modulated by the*
474          *mitochondrial membrane potential.* Biol Cell, 2008. **100**(5): p. 315-25.
475    48.  Jahn, R. and R.H. Scheller, *SNAREs--engines for membrane fusion.* Nat Rev Mol Cell Biol,
476          2006. **7**(9): p. 631-43.
477    49.  Choi, S.Y., et al., *A common lipid links Mfn-mediated mitochondrial fusion and SNARE-*
478          *regulated exocytosis.* Nat Cell Biol, 2006. **8**(11): p. 1255-62.
479    50.  Davletov, B., E. Connell, and F. Darios, *Regulation of SNARE fusion machinery by fatty*
480          *acids.* Cell Mol Life Sci, 2007. **64**(13): p. 1597-608.
481    51.  Browman, D.T., M.B. Hoegg, and S.M. Robbins, *The SPFH domain-containing proteins:*
482          *more than lipid raft markers.* Trends Cell Biol, 2007. **17**(8): p. 394-402.
483    52.  Rivera-Milla, E., C.A. Stuermer, and E. Malaga-Trillo, *Ancient origin of reggie (flotillin),*
484          *reggie-like, and other lipid-raft proteins: convergent evolution of the SPFH domain.* Cell Mol
485          Life Sci, 2006. **63**(3): p. 343-57.
486    53.  Huber, T.B., et al., *Podocin and MEC-2 bind cholesterol to regulate the activity of*
487          *associated ion channels.* Proc Natl Acad Sci U S A, 2006. **103**(46): p. 17079-86.

488    54. Morrow, I.C. and R.G. Parton, *Flotillins and the PHB domain protein family: rafts, worms*
489        *and anaesthetics.* Traffic, 2005. **6**(9): p. 725-40.
490    55. Bourges, I., et al., *Structural organization of mitochondrial human complex I: role of the ND4*
491        *and ND5 mitochondria-encoded subunits and interaction with prohibitin.* Biochem J, 2004.
492        **383**(Pt. 3): p. 491-9.
493    56. Taylor, S.W., et al., *Characterization of the human heart mitochondrial proteome.* Nat
494        Biotechnol, 2003. **21**(3): p. 281-6.
495    57. Benedetti, C., et al., *Ubiquitin-like protein 5 positively regulates chaperone gene expression*
496        *in the mitochondrial unfolded protein response.* Genetics, 2006. **174**(1): p. 229-39.
497    58. Yoneda, T., et al., *Compartment-specific perturbation of protein handling activates genes*
498        *encoding mitochondrial chaperones.* J Cell Sci, 2004. **117**(Pt 18): p. 4055-66.
499    59. Dunn, C.D., et al., *A genomewide screen for petite-negative yeast strains yields a new*
500        *subunit of the i-AAA protease complex.* Mol Biol Cell, 2006. **17**(1): p. 213-26.
501    60. Chen, X.J. and R.A. Butow, *The organization and inheritance of the mitochondrial genome.*
502        Nat Rev Genet, 2005. **6**(11): p. 815-25.
503    61. Kim, J.Y., et al., *Mitochondrial DNA content is decreased in autosomal dominant optic*
504        *atrophy.* Neurology, 2005. **64**(6): p. 966-72.
505    62. Jones, B.A. and W.L. Fangman, *Mitochondrial DNA maintenance in yeast requires a protein*
506        *containing a region related to the GTP-binding domain of dynamin.* Genes Dev, 1992. **6**(3):
507        p. 380-9.
508    63. Yarosh, W., et al., *The molecular mechanisms of OPA1-mediated optic atrophy in*
509        *Drosophila model and prospects for antioxidant treatment.* PLoS Genet, 2008. **4**(1): p. e6.
510    64. Gilkerson, R.W., J.M. Selker, and R.A. Capaldi, *The cristal membrane of mitochondria is the*
511        *principal site of oxidative phosphorylation.* FEBS Lett, 2003. **546**(2-3): p. 355-8.
512    65. Reichert, A.S. and W. Neupert, *Contact sites between the outer and inner membrane of*
513        *mitochondria-role in protein transport.* Biochim Biophys Acta, 2002. **1592**(1): p. 41-9.
514    66. Mandal, S., et al., *Mitochondrial regulation of cell cycle progression during development as*
515        *revealed by the tenured mutation in Drosophila.* Dev Cell, 2005. **9**(6): p. 843-54.
516    67. Dinkelmann, M.V., et al., *SPD-3 is required for spindle alignment in Caenorhabditis elegans*
517        *embryos and localizes to mitochondria.* Genetics, 2007. **177**(3): p. 1609-20.
518    68. Kruger, N. and I.M. Tolic-Norrelykke, *Association of mitochondria with spindle poles*
519        *facilitates spindle alignment.* Curr Biol, 2008. **18**(15): p. R646-R647.
520    69. Takata, H., et al., *PHB2 protects sister-chromatid cohesion in mitosis.* Curr Biol, 2007.
521        **17**(15): p. 1356-61.
522    70. Nuell, M.J., et al., *Prohibitin, an evolutionarily conserved intracellular protein that blocks*
523        *DNA synthesis in normal fibroblasts and HeLa cells.* Mol Cell Biol, 1991. **11**(3): p. 1372-81.
524    71. Sharma, A. and A. Qadri, *Vi polysaccharide of Salmonella typhi targets the prohibitin family*
525        *of molecules in intestinal epithelial cells and suppresses early inflammatory responses.* Proc
526        Natl Acad Sci U S A, 2004. **101**(50): p. 17492-7.
527    72. Kolonin, M.G., et al., *Reversal of obesity by targeted ablation of adipose tissue.* Nat Med,
528        2004. **10**(6): p. 625-32.
529    73. Heron-Milhavet, L., et al., *Akt2 is implicated in skeletal muscle differentiation and*
530        *specifically binds Prohibitin2/REA.* J Cell Physiol, 2008. **214**(1): p. 158-65.
531    74. Sun, L., et al., *Akt binds prohibitin 2 and relieves its repression of MyoD and muscle*
532        *differentiation.* J Cell Sci, 2004. **117**(Pt 14): p. 3021-9.
533    75. Beckman, K.B. and B.N. Ames, *The free radical theory of aging matures.* Physiol Rev,
534        1998. **78**(2): p. 547-81.
535    76. Doonan, R., et al., *Against the oxidative damage theory of aging: superoxide dismutases*
536        *protect against oxidative stress but have little or no effect on life span in Caenorhabditis*
537        *elegans.* Genes Dev, 2008. **22**(23): p. 3236-41.
538    77. Inoue, K., et al., *Generation of mice with mitochondrial dysfunction by introducing mouse*
539        *mtDNA carrying a deletion into zygotes.* Nat Genet, 2000. **26**(2): p. 176-81.
540    78. Casari, G., et al., *Spastic paraplegia and OXPHOS impairment caused by mutations in*
541        *paraplegin, a nuclear-encoded mitochondrial metalloprotease.* Cell, 1998. **93**(6): p. 973-83.
542    79. Santamaria, E., et al., *Functional proteomics of nonalcoholic steatohepatitis: mitochondrial*
543        *proteins as targets of S-adenosylmethionine.* Proc Natl Acad Sci U S A, 2003. **100**(6): p.
544        3065-70.
545    80. Ferrer, I., et al., *Abnormal levels of prohibitin and ATP synthase in the substantia nigra and*
546        *frontal cortex in Parkinson's disease.* Neurosci Lett, 2007. **415**(3): p. 205-9.
547    81. Smalla, K.H., et al., *A comparison of the synaptic proteome in human chronic schizophrenia*
548        *and rat ketamine psychosis suggest that prohibitin is involved in the synaptic pathology of*
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$\alpha$)

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$\alpha$ 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-metalloprotease, 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 **Replicative 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 [rho$^0$] 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

**Figure legends**

**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) [42], Mitofusin (Mfn) [45], or 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 with nuclear-encoded subunits into functional complexes. The mitochondrial translocase of the outer membrane (TOM) and translocase of the inner membrane (TIM) that mediate import of nuclear encoded mitochondrial proteins are also depicted. The oxidative phosphorylation system (OXPHS) 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].

**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.

686    Figure 1
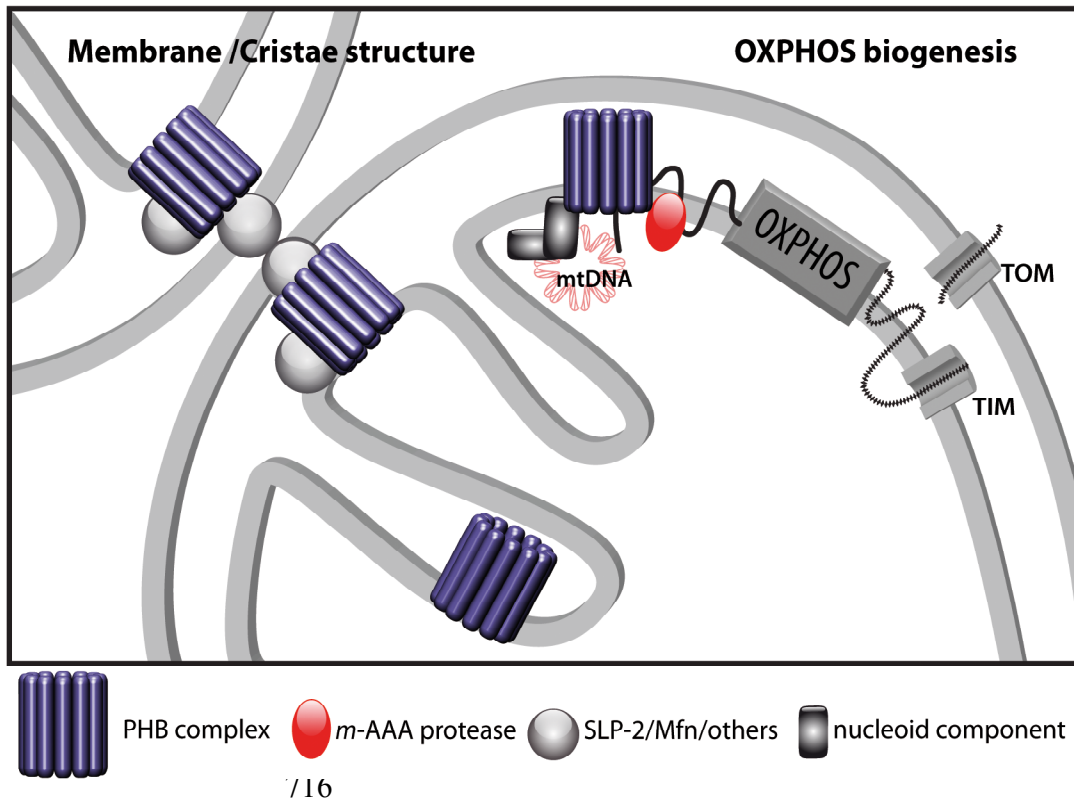687



Membrane /Cristae structure                    OXPHOS biogenesis

OXPHOS

mtDNA

TOM

TIM

PHB complex    *m*-AAA protease    SLP-2/Mfn/others    nucleoid component

717
718
719    Figure 2
720

722
724
726



assembly/stabilisation of
OXPHOS complexes

protein degradation/association
with *m*-AAA protease

component of
mtDNA nucleoids

protein and lipid
scaffolds

PHB complex

cristae morphogenesis/
stabilization of OPA1

mitochondria
ultrastructure

ROS formation

cellular proliferation

cancer

ageing