

The Prohibitins: emerging roles in diverse functions

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Abstract

The prohibitins, Phb1 and Phb2 are highly conserved proteins in eukaryotic cells that are present in multiple cellular compartments. Initial investigations focused on the role of Phb1 as an inhibitor of cell proliferation hence the original name prohibitin. However both proteins appear to have a diverse range of functions and recent evidence suggests that the prohibitins have very similar but as yet only partially understood functions. In addition to their role as chaperone proteins in the mitochondria, and their ability to target to lipid rafts, there is now compelling evidence that both prohibitins are localized in the nucleus and can modulate transcriptional activity by interacting with various transcription factors, including the steroid hormone receptors, either directly or indirectly. In addition Phb1 and Phb2 are present in the circulation and can be internalized when added to cultured cells suggesting that the circulating prohibitins may have some regulatory role. This review presents some of the recent developments in prohibitin research and focuses on the similarities in the structure and function of these interesting proteins.

Keywords: prohibitins • repressor of estrogen receptor action • B-cell receptor associated protein • BAP32 • BAP37 • lipid rafts

Introduction

Prohibitin 1, (Phb1) has a molecular mass of ~30 kDa and is also known as B-cell receptor associated protein-32 (BAP32), whereas a related protein, prohibitin 2 (Phb2), sometimes referred to as prohibitone [1], B-cell receptor associated protein-37 (BAP37) [2] or repressor of estrogen receptor action (REA) [3] has a mass of ~37 kDa. For the purpose of this review we will use the nomenclature of Phb1 and Phb2. The prohibitin name is derived from an historical perspective and probably only relates to

one of the many physiological roles of these proteins. A Phb1 cDNA was first isolated by differential hybridization to RNA from normal versus regenerating rat liver [4] and consequently Phb1 was proposed to be an inhibitor of cellular proliferation, hence the name prohibitin. The corresponding mRNA when microinjected into normal human diploid fibroblasts attenuated DNA synthesis. However it was subsequently shown that this effect was attributable to the 3' untranslated region of the

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Table 1 Summary of sequence similarity between the human prohibitins and prohibitins from various other species

	Phb1			Phb2		
	Identities (%)	Positives (%)	Gaps (%)	Identities (%)	Positives (%)	Gaps (%)
M. musculus	99	99	0	100	100	0
R. norvegicus	96	96	1	99	100	0
D. melanogaster	74	87	0	70	87	1
S. cerevisiae	54	78	0	56	80	0

Phb1 mRNA rather than the coding region of the cDNA [5]. More recently Phb1 protein has been shown to be present in the nucleus [6, 7] and to interact with transcription factors important in cell cycle progression [6, 8]. Phb2 also interacts with nuclear transcription factors [3]. REA was studied for several years as an inhibitor of estrogen receptor action and when the cDNA was eventually cloned, it turned out to have a sequence identical to Phb2 [3].

The structure of the prohibitins

Both Phb1 and Phb2 are members of a superfamily of molecules, which includes in addition to the pro-

hibitins, stomatin, flotillin and HflKC [9]. These proteins share a structurally related domain, referred to as the SPFH (stomatin, prohibitin, flotillin and HflC and K) domain that is also known as the PHB domain. Overall there is ~53% amino acid sequence identity between Phb1 and Phb2 and allowing for conservative substitution, sequence similarity approaches 74% with the central region showing the most sequence similarity (Fig. 1 and Table 1).

The PHB domain which spans amino acid residues 26–187 of Phb1, is also conserved in a diverse variety of proteins from various species, including lower eukaryotes (Table 1). The exact function of this domain is unclear but may be important in facilitating partitioning into lipid microdomains and consequently facilitating membrane

Phb1	1	M	A	A	K	V	F	E	S	I	G	K	F	G	L	A	L	A	V	A	G	G	V	V	N	S	A	L	Y	N	V	D	A	G	H	R	A	V	I	F	D	40		
Phb2	14	G	P	R	G	M	G	T	A	L	K	L	L	L	G	A	G	A	V	A	Y	G	V	R	E	S	-	V	F	T	V	E	G	G	H	R	A	I	F	F	N	52		
Phb1	41	R	F	R	G	V	Q	-	D	I	V	V	G	E	G	T	H	F	L	I	P	W	V	Q	K	P	I	I	F	D	C	R	S	R	P	R	N	V	P	V	I	T	80	
Phb2	53	R	I	G	G	V	Q	Q	D	T	I	L	A	E	G	L	H	F	R	I	P	W	F	Q	Y	P	I	I	Y	D	I	R	A	R	P	R	K	I	S	S	P	T	93	
Phb1	81	G	S	K	D	L	Q	N	V	N	I	T	L	R	I	L	F	R	P	V	A	S	Q	L	P	R	I	F	T	S	I	G	E	D	Y	D	E	R	V	L	P	120		
Phb2	94	G	S	K	D	L	Q	M	V	N	I	S	L	R	V	L	S	R	P	N	A	Q	E	L	P	S	M	Y	Q	R	L	G	L	D	Y	E	E	R	V	L	P	133		
Phb1	121	S	I	T	T	E	I	L	K	S	V	V	A	R	F	D	A	G	E	L	I	T	Q	R	E	L	V	S	R	Q	V	S	D	D	L	T	E	R	A	A	T	160		
Phb2	134	S	I	V	N	E	V	L	K	S	V	V	A	K	F	N	A	S	Q	L	I	T	Q	R	A	Q	V	S	L	L	I	R	R	E	L	T	E	R	A	K	D	173		
Phb1	161	F	G	L	I	L	D	D	V	S	L	T	H	L	T	F	G	K	E	F	T	E	A	V	E	A	K	Q	V	A	Q	Q	E	A	E	R	A	R	F	V	V	200		
Phb2	174	F	S	L	I	L	D	D	V	A	I	T	E	L	S	F	S	R	E	Y	T	A	A	V	E	A	K	Q	V	A	Q	Q	E	A	Q	R	A	Q	F	L	V	213		
Phb1	201	E	K	A	E	Q	Q	K	K	A	A	I	I	S	A	E	G	D	S	K	A	A	E	L	I	A	N	S	L	A	T	A	G	D	G	L	I	E	L	R	K	240		
Phb2	214	E	K	A	K	Q	E	Q	R	Q	K	I	V	Q	A	E	G	E	A	E	A	A	K	M	L	G	E	A	L	-	S	K	N	P	G	Y	I	K	L	R	K	252		
Phb1	241	L	E	A	A	E	D	I	A	Y	Q	L	S	R	S	R	N	I	T	Y	L	P	A	G	Q	S	V	L	L	Q	L	P	Q	272										
Phb2	253	I	R	A	A	Q	N	I	S	K	T	I	A	T	S	Q	N	R	I	Y	L	T	A	D	N	L	V	L	N	L	Q	D	E	284										

Fig. 1 Comparison of the amino acid sequence of the prohibitins. Identical residues are shown in blue.

Table 2 Some proteins containing a PHB domain

Protein	Species	References
Stomatin	Various species	10,31
Flotillins/Reggie proteins	Various species	10,57
HflC and K	<i>E. coli</i>	9
Vacuolin A and B	<i>Dictyostelium discoideum</i>	10
Podocin	Various species	56
Unc-1 and -24	<i>Caenorhabditis elegans</i>	10
Mec-2	<i>Caenorhabditis elegans</i>	10,31

association. That is, the PHB domain may constitute a lipid recognition motif [10]. In this regard, it is of note that both Phb1 and Phb2 have been identified on the plasma membrane [2, 11], in lipid droplets [12] and in lipid raft preparations [13]. Some proteins containing a PHB domain are listed in Table 2.

The protein sequences of mouse and rat Phb1 are virtually identical, and these differ from the human protein sequence by a single conserved amino acid. Phb1 orthologues have been identified in *Drosophila*, yeast and plants [14]. The *Drosophila* gene, *Cc* appears to be the orthologue of Phb1 and is vital for normal development [15]. Like Phb1, Phb2 is also highly conserved between human and rodents and shows a high degree of conservation in lower species. A comparison of the amino acid sequences of Phb1 and Phb2 from various species is shown in Fig. 2 and 3.

The genomic structure of Phb1 and -2 are somewhat dissimilar. The human PHB1 gene spans approximately 11 kb on chromosome 17q21 and has 7 exons (Fig. 4). The first and a small portion of the second exon comprise the 5' untranslated region whereas the largest exon, exon 7 contains ~700 bp of 3' untranslated RNA. Transcripts with varying lengths of 3' untranslated region have been identified [16]. Longer transcripts are present at higher levels in proliferating tissues and cells [17]. In general, the abundance of Phb1 mRNA has been reported to be inversely related to markers of cellular proliferation in a variety of cell and tissue types

[18–22]. As discussed above there is evidence that the 3' untranslated region of Phb1 may have a cell cycle regulatory function [5].

The genomic structure of human and rat PHB1 are similar with intron 2 and 3 being about 1 kb larger in the rat gene [23]. The PHB domain, which spans amino acid residues 26–187 is encoded by exon 3, 4 and 5 and it is these exons that are most highly conserved between Phb1 and Phb2. A coiled coil structure of alpha helices is present at the C-terminal end of the proteins, consisting of amino acids residues 177–211, and encoded largely by exon 6 [24]. A putative nuclear export sequence is present at the C-terminal, residues 257 to 270 [24, 25].

The human PHB2 gene is located on chromosome 12p13. It comprises 10 exons but has smaller introns than PHB1 and spans ~5.3 kb. The PHB domain is encoded by residues 39–201 [24]. Like Phb1, Phb2 also has a coiled coil structure at the C-terminal end of the protein. It also has both a putative signal peptide, residues 1–36 and a putative nuclear localization signal, residues 86–89 [24].

Both Phb1 and Phb2 probably exist as phosphorylated isoforms. In rat ovarian granulosa cells, Phb1 is present in a variety of isoforms, some of which appear to be phosphorylated derivatives [26]. Potential protein kinase C (PKC) and PKA sites have been identified in Phb2 [3]. However the functional significance of prohibitin phosphorylation and the identity of the kinases involved remain unclear. Phb1 can also undergo ubiquitination [27].

H. sapiens 1 M A A K V F E S I G K F G L A V A G G V V N S A L Y N V D A G H R A V I F D 40
M. musculus 1 M A A K V F E S I G K F G L A V A G G V V N S A L Y N V D A G H R A V I F D 40
R. norvegicus 1 M A A K V F G L I G K F G L A V A G G V V N S T L Y N V D A G H R A V I F D 40
D. melanogaster 1 M A A Q F F N R I G Q M G L G V A V L G G V V N S A L Y N V E G G H R A V I F D 40
S. cerevisiae 4 S A K L I D V I T K V A L P I G I A S G I Q Y S M Y D V K G G S R G V I F D 42

H. sapiens 41 R F R G V Q D I V V G E G T H F L I P W V Q K L I I F D C R S R P R N V P V I T 80
M. musculus 41 R F R G V Q D I V V G E G T H F L I P W V Q K P I I F D C R S R P R N V P V I T 80
R. norvegicus 41 R F Q G V Q D I V V G E G T H F L I P W V Q K P V I F D C R S Q P R N V P V V T 80
D. melanogaster 41 R F T G I K E N V V G E G T H F F I P W V Q R P I I F D I R S Q P R N V P V I T 80
S. cerevisiae 43 R I N G V K Q Q V V G E G T H F L V P W L Q K A I I Y D V R T K P K S I A T N T 82

H. sapiens 81 G S K D L Q N V N I T L R I L F R P V A S Q L P R I F T S I G E D Y D E R V L P 120
M. musculus 81 G S K D L Q N V N I T L R I L F R P V A S Q L P R I Y T S I G E D Y D E R V L P 120
R. norvegicus 81 G S K D L Q N V N I T L R I L F R P V T S Q L P R I Y T S I G L D Y A E R V L P 120
D. melanogaster 81 G S K D L Q N V N I T L R I L Y R P I P D Q L P K I Y T I L G Q D Y D E R V L P 120
S. cerevisiae 83 G T K D L Q M V S L T L R V L H R P E V L Q L P A I Y Q N L G L D Y D E R V L P 122

H. sapiens 121 S I T T E I L K S V V A R F D A G E L I T Q R E L V S R Q V S D D L T E R A A T 160
M. musculus 121 S I T T E I L K S V V A R F D A G E L I T Q R E L V S R Q V S D D L T E R A A T 160
R. norvegicus 121 S I T S E I L K S V V A R F N A E E L I T Q R E L V S R Q V S D D L T E R A A T 160
D. melanogaster 121 S I A P E V L K A V V A Q F D A G E L I T Q R E M V S Q R V S Q E L T V R A K Q 160
S. cerevisiae 123 S I G N E V L K S I V A Q F D A A E L I T Q R E I I S Q K I R K E L S T R A N E 162

H. sapiens 161 F G L I L D D V S L T H L T F F G K E F T E A V E A K Q V A Q Q E A E R A R F V V 200
M. musculus 161 F G L I L D D V S L T H L T F F G K E F T E A V E A K Q V A Q Q E A E R A R F V V 200
R. norvegicus 161 F G L I L D D V S L T H L T F F G K E F T E A V E A K Q V A Q Q E A E T A R F V V 200
D. melanogaster 161 F G F I L D D I S L T H L T F F G R E F T L A V E M K Q V A Q Q E A E K A R F V V 200
S. cerevisiae 163 F G I K L E D V S I T H M T F G P E F T K A V E Q K Q I A Q Q D A E R A K F L V 202

H. sapiens 201 E K A E Q K K A A I I S A E G D S K A A E L I A N S L A T A G D G L I E L R K 240
M. musculus 201 E K A E Q K K A A I I S A E G D S K A A E L I A N S L A T A G D G L I E L R K 240
R. norvegicus 201 E K A E H Q K K A A I I S A E G D A K A A E L I A N S L A T A G D G L I E L R K 240
D. melanogaster 201 E K A E Q Q K L A S I I S A E G D A E A G L L A K S F G E A G D G L V E L R R 240
S. cerevisiae 203 E K A E Q E R Q A S V I R A E G E A E S A E F I S K A L A K V G D G L L I R R R 242

H. sapiens 241 L E A A E D I A Y Q L S R S R N I T Y L P A G Q S V L L Q L P Q 272
M. musculus 241 L E A A E D I A Y Q L S S S Q N I T Y L P A G Q S V L L Q L P Q 272
R. norvegicus 241 L E A A E D I A Y Q L S S S Q N I T Y L P V G P S S V G P S V R Q S M F L R L P Q 281
D. melanogaster 241 I E A A E D I A Y Q L S R S R G V A Y L P S G Q S T L L N L P 271
S. cerevisiae 243 L E A S K D I A Q T L A N S S N V Y L P S Q 267

Fig. 2 Comparison of the amino acid sequence of the Phb1 from various species. Residues that are identical in all five species are shown in blue whereas residues identical in all three mammalian species are shown in green. Residues shown in red are identical in all species shown with the exception of yeast.

Cellular localization of the Prohibitins

Both Phb1 and Phb2 have been detected in the human circulation by a differential immunization technique [28]. Levels are reported to be higher in cancer patients than normal subjects, however the exact concentrations of Phb1 and Phb2 in plasma have not been determined [28]. Although Phb2 has a putative signal peptide [24], both Phb1 and Phb2 more likely find their way into the circulation *via* release from cells in lipid droplets [12].

The nature and function of the circulating prohibitins remain unclear. Recent studies in our laboratory indicate that Phb1 rapidly associates with high molecular weight molecules when incubated with human plasma and that Phb1 binds strongly to complement component C3 and may enhance complement activation (Moulik *et al.*, unpublished observations). Further studies are underway to determine whether this interaction is functionally important. Whether Phb2 also binds to complement C3 has still to be determined.

Immunohistochemical analysis demonstrated that Phb1 is present in high abundance in the vasculature of white adipose tissue of mice and human subjects [11]. However it is not clear where the Phb1 that is present on endothelium in white adipose tissue is actually expressed. Lipoprotein lipase is also present on endothelial cells in adipose tissue but *in situ* hybridization suggests that these endothelial cells do not express lipoprotein lipase at detectable levels [29]. Rather adipocyte-derived lipoprotein lipase may find its way to the endothelium *via* lipid droplets [29]. A similar mechanism may account for the relatively higher abundance of Phb1 on adipose tissue endothelium compared to other tissues [11]. Both Phb1 and Phb2 have been found on the plasma membrane of B lymphocytes in association with the IgM receptor [2]. While it is likely that Phb1 and Phb2 are present on the plasma membrane of other cell types, this has not been demonstrated nor has there been a systematic study of the abundance of Phb1 and Phb2 in various cell and tissue types. Other members of this gene family such as the stomatins and flotillins, which share a PHB domain, are also localized to plasma membranes [10, 30, 31].

Although the prohibitins are present in the circulation and on the plasma membrane, the majority of Phb1 and Phb2 appears to be localized with the cell.

Immunohistochemistry and subcellular fractionation experiments localize both Phb1 and Phb2 predominantly to the mitochondria in most cells and tissues [32–35]. Phb1 and Phb2 exist as a large molecular weight ring complex of multiple copies of each protein at the mitochondrial inner membrane [4, 36].

Nuclear localization of Phb1 has also been consistently demonstrated by a number of investigators [6–8, 37]. Export of Phb1 from the nucleus to the cytoplasm of breast cancer cells in response to the apoptotic stimuli has been reported [8]. Consistent with these observations, a putative nuclear export site has been identified in Phb1 [25]. Nuclear localization of Phb2 has also been reported [38, 39].

As is apparent from the above discussion, Phb1 and Phb2 colocalize in multiple cellular and extracellular compartments. In yeast, there is evidence of interdependence of prohibitin protein levels, that is, nullification of either prohibitin, results in diminished abundance of the other [40]. This is presumed to be due to stabilization by heterodimerization [9]. In yeast, homodimerization does not appear to be prevalent since such dimers are not detected in crosslinking experiments [36, 41]. However it is not clear whether this interdependence is operative in higher organisms. Genetic nullification of Phb1 in the mouse has not been investigated, however Phb2^{-/-} mice demonstrate embryonic lethality, while Phb2^{+/-} mice demonstrate minimal phenotype with the exception of enhanced estrogen responsiveness [42]. Phb1 levels were not reported in these Phb2^{+/-} mice so it remains unclear whether there are compensatory changes in Phb1 abundance in Phb2^{+/-} mice.

Prohibitins and mitochondrial function

Many investigators, particularly those working with lower organisms, have considered that the sole or major function of the prohibitins is that of chaperones for imported proteins in mitochondria. The prohibitins are essential for normal mitochondrial development and while null mutants of either Phb1 or Phb2 in *C. elegans* have minimal phenotype alone, mutations in these genes associated with other mitochondrial proteins such as the mitochondrial inheritance components, Mdm12p, Mdm10p, and Mmm1p [40] or components of the phosphatidylethanolamine biosynthetic pathway [43] are lethal. In the mito-

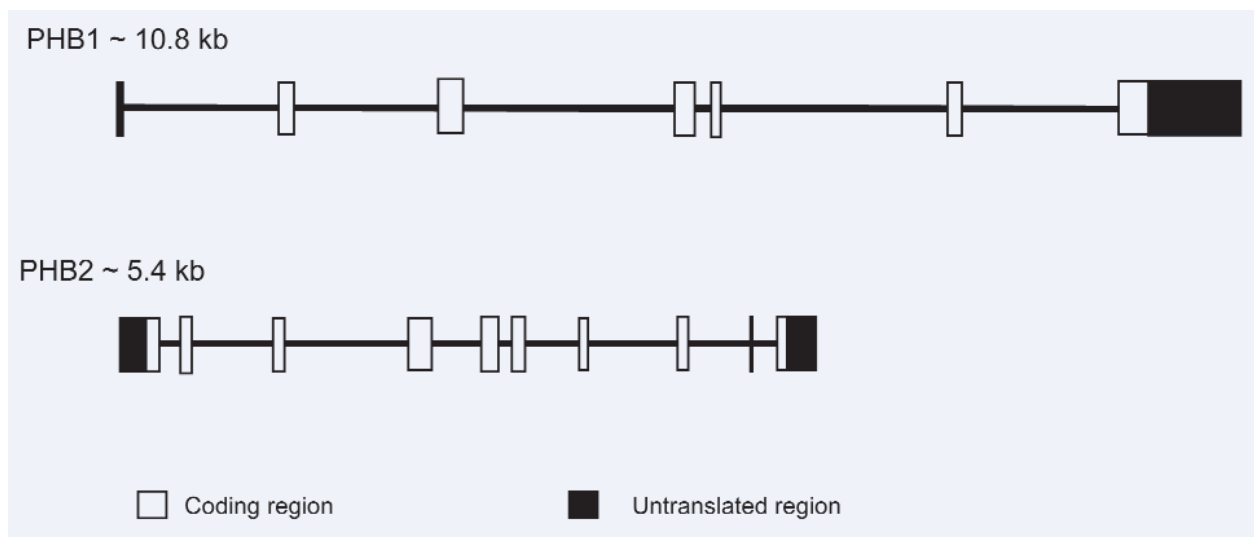


Fig. 4 The genomic structure of human PHB1 and PHB2 genes

chondria, the prohibitins are assembled into a ring-like structure with 16–20 alternating Phb1 and Phb2 subunits [36]. Here it is thought that the prohibitins acts as chaperones, protecting newly imported proteins from degradation by the mAAA protease [32], until the remaining members of mitochondrial respiratory chain complexes have been imported and assembled. This is likely an over simplification of the functional role of the prohibitins in the mitochondria. We recently reported that Phb1 is a potent inhibitor of pyruvate carboxylase [44], a mitochondrial enzyme involved in replenishing tricarboxylic acid cycle intermediates [45]. When added to murine adipocytes, Phb1 can be translocated to the mitochondria and can inhibit anaplerosis [44]. It is not clear whether Phb2 shares this activity, nor is it clear whether Phb1 inhibition of pyruvate carboxylase of physiological significance.

The prohibitins may have a role modulating and maintaining mitochondrial function. Changes in Phb1 abundance, and Phb1 isoforms, have been documented during cellular senescence in cultured human fibroblasts [46]. Similarly, a marked reduction in mitochondrial oxidative and phosphorylation activity has been reported in elderly subjects compared to younger adults [47]. Changes in Phb1 isoforms that occur with cellular senescence may be important in this age-related decline in mitochondrial function or possibly are a consequence of it. Changes in Phb2 during cellular senescence have not been reported.

Prohibitins as modulators of gene expression

There is now compelling evidence that the prohibitins are not just localized in the nucleus but also play an active role in modulating gene transcription. Both Phb1 and Phb2 interact with a variety of nuclear proteins involved in gene transcription (Table 3), and have been implicated in the modulation of steroid hormone regulated transcription [3, 37, 38, 42].

In breast cancer cell lines, Phb1 colocalizes in the nucleus with E2F1, retinoblastoma protein (pRb) and p53, and can modulate E2F1 and p53 mediated transcriptional activity [6, 8, 48]. The interaction of Phb1 with E2F1 and pRb appears to be complex. The coiled-coil domain of the Phb1 appears to be important in the interaction of this protein with both E2F and HDAC1 [49]. Interestingly this region of Phb1 protein alone appears to be sufficient to disrupt the pRb/E2F pathway to induce apoptosis when transfected into cell lines [49]. Phb1 interacts with a different region of E2F1 than pRb, since Phb1-mediated repression of E2F can be mediated by signals that do not involve pRb [50]. Amino acid residues 304 to 357 which represent a highly conserved domain within the so called “marked-box” of E2F is specifically required for binding to Phb1 but is not involved in repression of E2F by pRb [50, 51]. Furthermore, Phb1-mediated transcriptional repression may require histone deacetylase activity in many circumstances, but unlike pRb, additional co-repressors like NCoR

Table 3 Putative interactions between the prohibitins and nuclear proteins

Prohibitin	Nuclear factor	References
Phb1	Estrogen Receptor	51
	HDAC1	49, 51
	Retinoblastoma protein	6, 48
	p53	8
	E2F	48, 50
	BRG1/BRM	51
	Androgen receptor	37
	N-CoR	6
Phb2	Estrogen Receptor	3, 42
	SRC-1	3
	Akt	39

are also involved [51]. Phb1 appears to repress E2F mediated transcription using different molecular mediators and facilitates channeling of specific signaling pathways other than the pRb pathway since E1A, p38 kinase and cyclins D and E can reverse pRb function but have no effect on Phb1 mediated repression of E2F1 in certain cell lines [50].

Recent evidence suggests that recruitment of Brahma-related gene 1 (Brg-1) and the related gene Brm, to E2F responsive promoters is required for repression of E2F mediated transcription by Phb1 and involves the JNK1 pathway [51]. Brg-1 and Brm, encode ATP-dependent enzymes belonging to SWI-SNF complex, are involved in chromatin remodeling and in mediating hormone-dependent transcriptional activation by nuclear receptors. The Brg1/Brm complex has also been implicated in pRb-independent modulation of E2F1-dependent repression of gene activation [52] and transcriptional activation by the estrogen receptor (ER) [53]. Ligand activation of the ER recruits Brg-1/Brm to estrogen-responsive genes and promotes activities that alter the acetylation state of chromatin [51], and consequently results in activation of genes that allow for entry into the cell cycle and cell proliferation. Depletion of Phb1 by antisense or siRNA strategies inhibits the growth inhibitory effect of the anti-estrogens, 4-hydroxytamoxifen and ICI

182780 in MCF-7 and ZR-75 breast cancer cells [51]. Similarly, transfection of a construct expressing residues 304-357 of E2F that squelch Phb1-E2F interaction also blocked the effects of the anti-estrogens leading to the suggestion that Phb1 may be involved in mediating the anti-proliferative actions of estrogen antagonists [51]. Recently, Gamble *et al.*, reported that Phb1 can block the androgen-stimulated growth stimulation in prostate cells suggesting that Phb1 may have a role in modulating androgen regulated gene expression [37].

Like Phb1, Phb2 also has an important role in modulating estrogen-induced gene transcription. Phb2 was identified in a yeast two-hybrid screening assay where a dominant negative ER was used as bait [3]. The clone identified in this fashion was named REA for “repressor of estrogen receptor activity,” and encoded Phb2. It potentiated the inhibitory activities of dominant negative ERs and antiestrogen-liganded ER when transfected into CHO cells with ER expression vectors (either ER α or ER β) and an ERE-CAT reporter construct but had no effect on transcription mediated by several other members of the steroid hormone receptor superfamily, including the androgen receptor [3]. Furthermore, it suppressed in a dose-dependent manner SRC-1-mediated enhancement of ER transcriptional activity [3]. The ER-interacting

Phb1 153 DLTERAATFGLILDDVSLTHLTFGKEFTEAVEAKQVAQQE
Phb2 167 ELTERAKDFSLILDDVAITELSF SREYTA AVEAKQVAQQE

Fig. 5 Amino acid sequence of the putative region of Phb2 that interacts with the estrogen receptor and the comparative region in Phb1

region of Phb2 maps to amino acids residues 175–198 [54], a region within the coiled coil domain. This region shows good homology with Phb1 (Fig.4), however the exact region of Phb1 that is involved in mediating the growth inhibitory effects of antiestrogen is not clear.

In human breast cancer biopsies Phb2 expression positively correlates with ER levels and inversely with tumor grade [55]. Genetic nullification of Phb2 in mice is embryonically lethal, however hemizygous mice are viable [42]. Interestingly hemizygous female, Phb2^{-/+} mice, but not male mice of the same genotype demonstrate an increased body weight relative to age-matched wild-type animals beginning after puberty. Furthermore hemizygous animals revealed a greater uterine weight gain, epithelial hyperproliferation, a substantially greater up-regulation of estrogen responsive genes and a loss of down regulation of genes in the uterus in response to estrogen stimulation compared to wild-type mice [42]. These studies suggest that Phb2 has a physiologically relevant role in modulating estrogen responsiveness *in vivo* and this may be important in hormone responsive breast cancer. As discussed above there are no reports of genetic nullification of Phb1, but it is likely from the discussion above that a similar phenomenon may be observed in hemizygous Phb1 mutant mice as that reported in hemizygous Phb2.

Concluding remarks and future directions

There is now compelling evidence that the prohibitins have additional functions in addition to their role as chaperones for imported proteins in mitochondria and inhibitors of cell proliferation. They are present in the circulation, as well as many cellular compartments and generally tend to co-localize. Genetic deletion in both the mouse and lower species suggests that deletion of

either of these genes is likely to be lethal at an early embryonic age. However studies in yeast suggest that there is an interdependence of expression and suggest that neither of the prohibitins are found in isolation. It is not clear whether this is the case in higher species. Phb1 and Phb2 may exist as homodimers or in protein complexes without the other prohibitin present in certain subcellular compartments. Presumably “free” prohibitin, Phb1 or Phb2 is likely to have functionally different effects to Phb1/Phb2 heterodimers. This is particularly relevant because recent evidence suggests that the nuclear prohibitins may have distinct but possibly overlapping functions in modulating gene expression, particularly steroid hormone mediated gene expression.

There is evidence that the prohibitins can undergo post-translational modification, such as phosphorylation and ubiquitination, but the role of post-translational modification in modulating the various functions of the prohibitins has yet to be investigated. The function of the extracellular and circulating prohibitin also remains to be determined. There is emerging evidence that Phb1 binds complement C3 and may be involved in its activation. Further exploration in this area may provide some insight into the very early observation that the prohibitins are associated with the IgM receptor. Clearly we are still at a very early stage in understanding the biology of the prohibitins. Conditional knock-out experiments, where very specific questions are addressed, are likely to be important methods of determining the physiological significance of the various interactions of the prohibitins that have been demonstrated *in vitro*.

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