# Critical Review

# Prohibitin 2: At a Communications Crossroads

**Alberto Bavelloni**[1,2]
**Manuela Piazzi**[3]
**Mirco Raffini**[2]
**Irene Faenza**[3]
**William L. Blalock**[1,4*]

[1]*Laboratory of Musculoskeletal Cell Biology, Rizzoli Orthopedic Institute, Bologna, Italy*
[2]*Laboratory RAMSES, Rizzoli Orthopedic Institute, Bologna, Italy*
[3]*Department of Biomedical Sciences, University of Bologna, Bologna, Italy*
[4]*National Research Council of Italy, Institute of Molecular Genetics, Bologna, Italy*

## Abstract

Prohibitins (PHBs) are a highly conserved class of proteins first discovered as inhibitors of cellular proliferation. Since then PHBs have been found to have a significant role in transcription, nuclear signaling, mitochondrial structural integrity, cell division, and cellular membrane metabolism, placing these proteins among the key regulators of pathologies such as cancer, neuromuscular degeneration, and other metabolic diseases. The human genome encodes two PHB proteins, prohibitin 1 (PHB1) and prohibitin 2 (PHB2), which function not only as a heterodimeric complex, but also independently. While many previous reviews have focused on the better characterized prohibitin, PHB1, this review focuses on PHB2 and new data concerning its cellular functions both in complex with PHB1 and independent of PHB1. © 2015 IUBMB Life, 67(4):239–254, 2015

***Keywords:*** *prohibitin; nucleus; mitochondria; transcription; AKT; CaMK IV; cancer; differentiation; gene regulation; plasma membrane receptors; stress; inflammation; Alzheimer's; diabetes; myositis*

## Introduction

The prohibitin proteins are a ubiquitously expressed pair of proteins, prohibitin 1 (PHB, but for clarity we refer to PHB1 in this review) and prohibitin 2 [PHB2, also referred to as repressor of estrogen receptor activity (REA) or B-cell receptor associate protein (BAP)-37], belonging to the stomatin, prohibitin, flotillin, and HflK/C superfamily (1). It was originally observed that transfection of PHB1 cDNA resulted in cell cycle arrest; hence the designation "prohibitin" (1,2). Both PHB1 and PHB2 have been shown to be present in the nucleus, mitochondria and cytosol, as well as associated with certain cell membrane receptors (3). The PHBs are currently one of the best examples presenting clear and distinctive functions depending on intercellular localization. In the mitochondria, PHB1 and PHB2 form an alternating heterodimeric ring-like complex required for mitochondrial stability. In contrast, in the nucleus both PHBs result in the transcriptional suppression of target genes, but independently from one another (1,3). Although nuclear PHBs have been demonstrated to influence multiple transcription factors and the cell cycle, the majority of cellular effects observed following the loss of either prohibitin can be attributed to their function in the mitochondria (4).

Expression of the PHBs is correlated to the level of reactive oxygen species (ROS) and inflammation; therefore, diseases with an inflammatory component (cancer, diabetes, and neuromuscular degenerative disorders) likely present with alterations in PHB1/2 expression and/or localization. In fact, experimental alteration of PHB expression in diverse model systems mimics several inflammatory pathologies (5–9). While most of the knowledge to date concerning the PHBs has been gleaned from studies with PHB1, this review focuses on PHB2 and the recent advances in the field that implicate the PHBs as intercellular communicators between the nucleus and mitochondria.


*Volume 67, Number 4, April 2015, Pages 239–254*
*Address correspondence to: William L. Blalock, Institute of Molecular Genetics, National Research Council of Italy, c/o Laboratory of Musculoskeletal Cell Biology, Rizzoli Orthopedic Institute, via di Barbiano 1/10, Bologna, Italy 40136. Tel.: +39-0516366769. Fax: +39-051583593. E-mail: william.blalock@cnr.it*
*Received 19 December 2014; Accepted 6 February 2015*
*DOI 10.1002/iub.1366*
*Published online 21 April 2015 in Wiley Online Library (wileyonlinelibrary.com)*

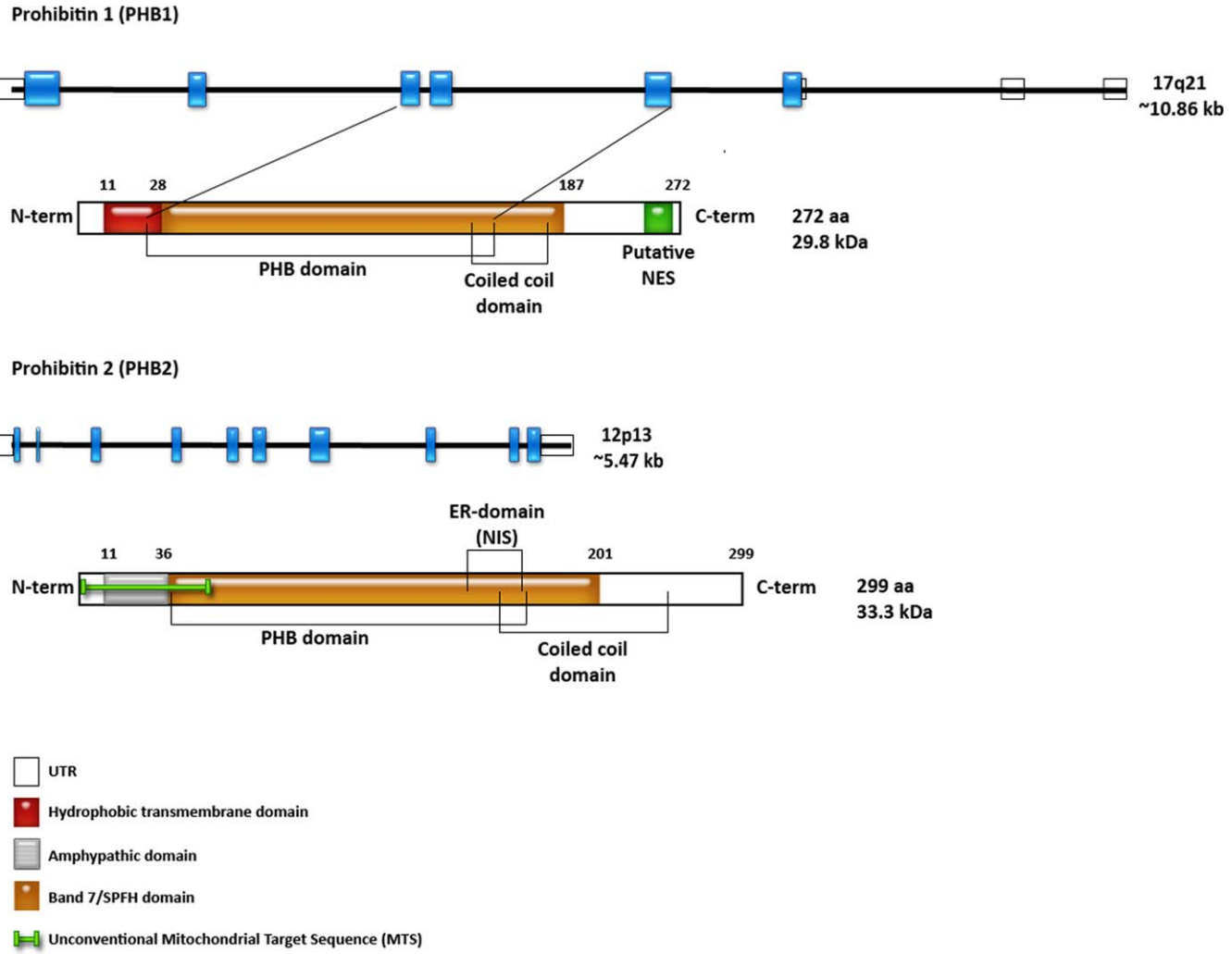


**FIG 1** *PHB1 and PHB2 gene structure and coding region and the resulting full-length protein. The gene structure including exons (boxes) and introns for PHB1 and PHB2 is shown. Shaded boxes indicated exon protein coding sequences while unshaded boxes indicate exon sequence that do not code for protein. The major domains of the resulting full-length PHB1 and PHB2 proteins and their approximate amino acid start and stop are indicated.*

## Gene Structure and Expression

The *phb2* gene, which was mapped to chromosome 12p13.31, covers 5.47 kb, encodes 10 exons, and is expressed in virtually all tested tissues (Fig. 1). As a gene highly conserved through evolution, the human *phb2* coding sequence maintains 91%, 69%, and 58% homology with *phb2* in mouse, fruit fly, and yeast, respectively. While transcriptive regulation of *phb2* is poorly understood, the *phb2* promoter is predicted to contain binding sites for approximately 130 diverse transcription factors, including GATA-1,-2,-3, Forkhead box protein (FoxO), CAAT-enhancer binding protein (C/EBP)-$\alpha$, signal transducer and activator of transcription (STAT)-1, -3, and -5, peroxisome proliferating-activated receptor (PPAR)-$\alpha$/-$\gamma$, nuclear factor (NF)-$\kappa$B, Sp1, and homeobox (HOX) factors (The Champion ChiP Transcription Factor Search Portal). Constitutive transcription factors such as Sp1 are likely responsible for the basal expression of *phb2*, and the presence of many of the other transcription factors may explain why PHB2 expression is tightly linked to metabolic tissues and inflammation. Interestingly, transcription factors known to be negatively regulated by PHB2 (ER$\alpha$, MyoD, and MEF-2) all have consensus binding sites within the *phb2* promoter, suggesting a feedback loop involving PHB2 expression.

Transcription of *phb2* results in the expression of an ~1,515 bp mRNA, encoding a 299-amino acid protein (Fig. 1). In addition to this transcript, 13 splice variants have been documented; 6 of these do not result in protein expression, while 7 transcripts code for either identified or putative PHB2 isoforms (see ENSEMBLE database: 78th release December 2014).

## PHB2 Protein and Post-translational Modification

Full-length PHB2 has a molecular weight of 33.3 kDa with an amino acid sequence similarity between human and mouse,

**TABLE 1** *High probability PHB2 phosphorylation sites*

| *Residue (aa)* | *Phosphorylation kinase* | *Characterization* | *References* |
|---|---|---|---|
| S91 | Akt, CaMK 4 | Biochemistry; mass spectrometry | 12, 13 |
| S105 | Aurora, Polo-like | Mass spectrometry | 15 |
| S151 | Aurora, Polo-like | Mass spectrometry | 15 |
| S176 | Akt | Biochemistry | 12 |
| S243 | Aurora, Polo-like | Mass spectrometry | 15 |
| Y248 | Akt-RSK-S6 (?); Tnk1 (?) | Mass spectrometry; mass spectrometry | 75 |
| S286 | Aurora, Polo-like | Mass spectrometry | 15 |

*Table 1 lists the highly probable phosphorylated sites in PHB2 and the kinase(s) responsible. Characterization of the phosphorylation site was either biochemical or targeted mass spectrometry.*

fruit fly, or yeast at 100%, 71%, and 56%, respectively (1). While the amino acid sequence similarity between PHB1 and PHB2 is only 54%, the PHB domains are 74% identical (1). Similar to PHB1, PHB2 contains a transmembrane domain (amino acids 1–36) required for mitochondrial localization, a central prohibitin domain (amino acids 36–201) followed by an overlapping coiled-coil domain (amino acids 188–264) (Fig. 1). In addition to these, PHB2 also possesses an ER-binding domain within the prohibitin domain. In contrast to PHB1, which contains a putative nuclear exclusion sequence, PHB2 contains a putative nuclear import sequence (NIS) located within the ER-binding domain, suggesting that post-translational modifications (PTMs) or association with other proteins dictates PHB2 sub-cellular localization (1,10) (Fig. 1).

A search of the PhosphoSite database reveals at least 32 potential post-translational modification sites (ubiquitylation, acetylation, and phosphorylation); the majority of which have not been validated (11). Table 1 shows the most noted PTMs and kinase(s) that likely phosphorylate the respective site. Approximately 15 Ser/Thr phosphorylation sites have been detected in a variety of tissues and cell lines (Fig. 2). Of these, only three (S91, S176, and S243) have been biochemically validated, while an additional three (S105, S151, and S286) are considered highly probable as they have been identified in a large number of independent studies (12–17). Similarly, six potential Tyr sites have been identified, but with the exception of Y248, none of these sites has been validated biochemically (14,18–21) (Fig. 2). Moreover, modification of 11 Lys residues has been identified using high-throughput proteomics (22–24) (Fig. 2). Many of these result from ubiquitylation, while others result from acetylation. It is evident by comparing mouse and human PHB2 data that both Lys modifications can occur at these sites, but the consequences of these modifications remain unknown (25).

Of the biochemically validated phosphorylation sites in PHB2, the best studied is S91. Serine 91 was demonstrated to be phosphorylated by both $Ca^{++}$/calmodulin-dependent kinase (CaMK) IV and AKT1/2 (12,13). Sun et al. demonstrated that CaMK IV associates with PHB2 and phosphorylates S91 during myocytic differentiation of C2C12 myoblasts. The introduction of a non-phosphorylated S91A mutant in this model system resulted in the inability of CaMK IV to relieve PHB2-mediated repression of MEF2-dependent transcription and myocyte differentiation (13). More recently, Bavelloni et al. demonstrated that nuclear PHB2 is phosphorylated by AKT1/2 during all-trans retinoic acid (ATRA)-mediated differentiation of promyelocytic leukemia cells (12). In addition to S91, S176 was also phosphorylated by AKT in this model system, although S176 phosphorylation was secondary to S91, suggesting a hierarchical phosphorylation. Interestingly, in this study, while exogenous expression of a S176A mutant had little effect on cell viability, exogenous expression of a S91A phospho-mutant resulted in a rapid and complete apoptosis of NB4 cells within 24 h after transfection (12). Such apoptosis is a hallmark of mitochondria catastrophe and this phenotype matched those observed in embryonic stem cells in which the PHBs were knocked-out and cell lines where PHBs were knocked-down using siRNA (4,8,14,26,27) (Fig. 3).

Using human derived T-cells and T-cell lines, Ross et al. found that PHB1 and PHB2 expression and phosphorylation increased following T-cell activation. PHB2 phosphorylation was subsequently mapped to S243 and Y248 (14). While the authors did not follow-up on the effects of S243 phosphorylation, they did demonstrate through the use of a Y248F PHB2 mutant, that phosphorylation of Y248 was not essential for cell survival or association with PHB1 (14).

## Protein-Protein Interactions and Complex Formation

The prohibitins have been identified in complex with proteins with diverse cellular functions. While some associations at the plasma membrane and in the mitochondria require both PHB1 and PHB2, the majority of protein-protein interactions are

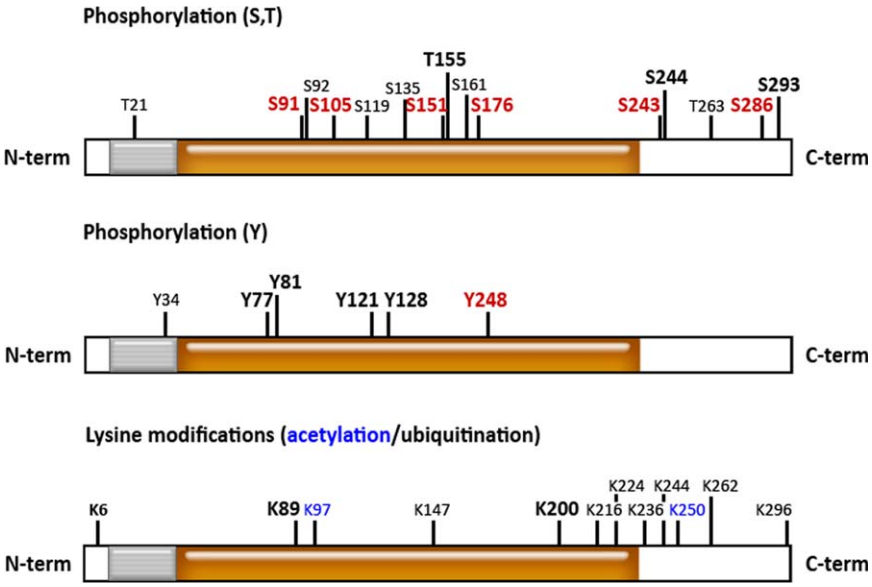


**FIG 2**

*PHB2 post-translational modification sites. Post-translational modification sites (PTMs) obtained from the PhosphositePlus database are subdivided into serine/threonine phosphorylation, tyrosine phosphorylation, and lysine modifications (acetylation/ubiquitylation). Those sites in bold red indicate that biochemical evidence has validated the modification at this site. Sites in bold black indicate those sites in which significant and specific evidence exists (e.g. targeted mass spectrometry) that the site is modified, but no biochemical validation has been conducted. Sites in small black indicate sites which have been identified by high-throughput techniques in a non-specific manner and no biochemical evidence exists.*

specific to each prohibitin. Table 2 lists those proteins known to interact with PHB2.

In the nucleus, many PHB2 interacting proteins are both global and specific transcription factors, including: the cAMP-dependent transcription factor (ATF)-2, $\beta$-catenin, COUP-TF1, COUP-TF2, the estrogen receptor (ER)-$\alpha$, interleukin enhancer binding factor (ILF)-3, MEF2A, MYOD1, Runt-related transcription factor (RUNX3), and transcription factor (TF)-E3 (28–33). Others are DNA modifying enzymes, including: the histone deacetylases (HDAC1, HDAC2, HDAC3, and HDAC5), breast-related carcinoma antigen (BRCA)-1, cyclin-dependent kinase (CDK)-2, polycomb-related proteins of the PRC2/EED-EZH2 complex, DNA repair associated enzymes, and cell cycle associated proteins (29,30,34–38). Several RNA-binding proteins required for RNA processing (ATP-dependent RNA helicase DDX20), stability [Epiplakin (EPPK1) and the basophile leukemia expressed protein (Bles03)] and transport (Staufen) also associate with nuclear PHB2 (29,39–41).

In the cytosol, most PHB2 interacting proteins are associated with the cytoskeleton and cytoskeletal transport [coatomer subunit gamma 1 (COPG1)], cellular signaling [MDM2 and receptor interacting S/T kinase (RIPK)-2], and ubiquitylation (22,23,29,37,42–44). Other cytoplasmic proteins that interact with PHB2 are associated with integral cell membrane proteins and cellular receptors, such as IGFR1 and integrins like VCAM1 (45,46).

As the PHBs also function in the mitochondria, many critical mitochondrial proteins associate with PHB2. These proteins consist of resident proteins belonging to the mitochondrial respiratory chain as well as mitochondrial transporters and membrane translocases (47). Some associated proteins are involved in cristae formation and maintenance of mitochondrial structure, while others are involved in mitochondrial-mediated translation. Prominent mitochondrial apoptosis and autophagy regulating proteins have also been found associated with PHB2; among these are the apoptotic related proteins SCaMC-1 (SLC25A24) and growth hormone-inducible transmembrane protein (GHITM), and the autophagy promoting E3-ligase RNF185 (47,48). As many of these PHB2:protein interactions are just coming to light, the functional outcome of PHB2 association with the majority of these proteins is still unclear.

## Signal Transduction

*Role of Nuclear PHB2.* In the nucleus, the prohibitin proteins have a significant role in regulating transcription factors either directly or indirectly, including estrogen receptor (PHB1/2), E2F family members (PHB1), p53 (PHB1), MEF2 (PHB2), MyoD (PHB2), STAT3 (PHB1), orphan nuclear hormone receptors (COUP-TF1 and II; PHB2) and PPAR$\gamma$ (PHB2) (3,8,30,33). In addition, both PHBs associate with and influence HDAC family members (3,30). Following estrogen receptor stimulation, PHB2 translocates from the cytosol/mitochondria to the nucleus in association with ER$\alpha$ and inhibits ER$\alpha$-responsive transcription (8). Kim et al. demonstrated that the brefeldin A-inhibited guanine nucleotide-exchange protein (BIG)-3, a protein often up-regulated in breast cancer, binds to and sequesters PHB2 in the cytosol, thus allowing activated

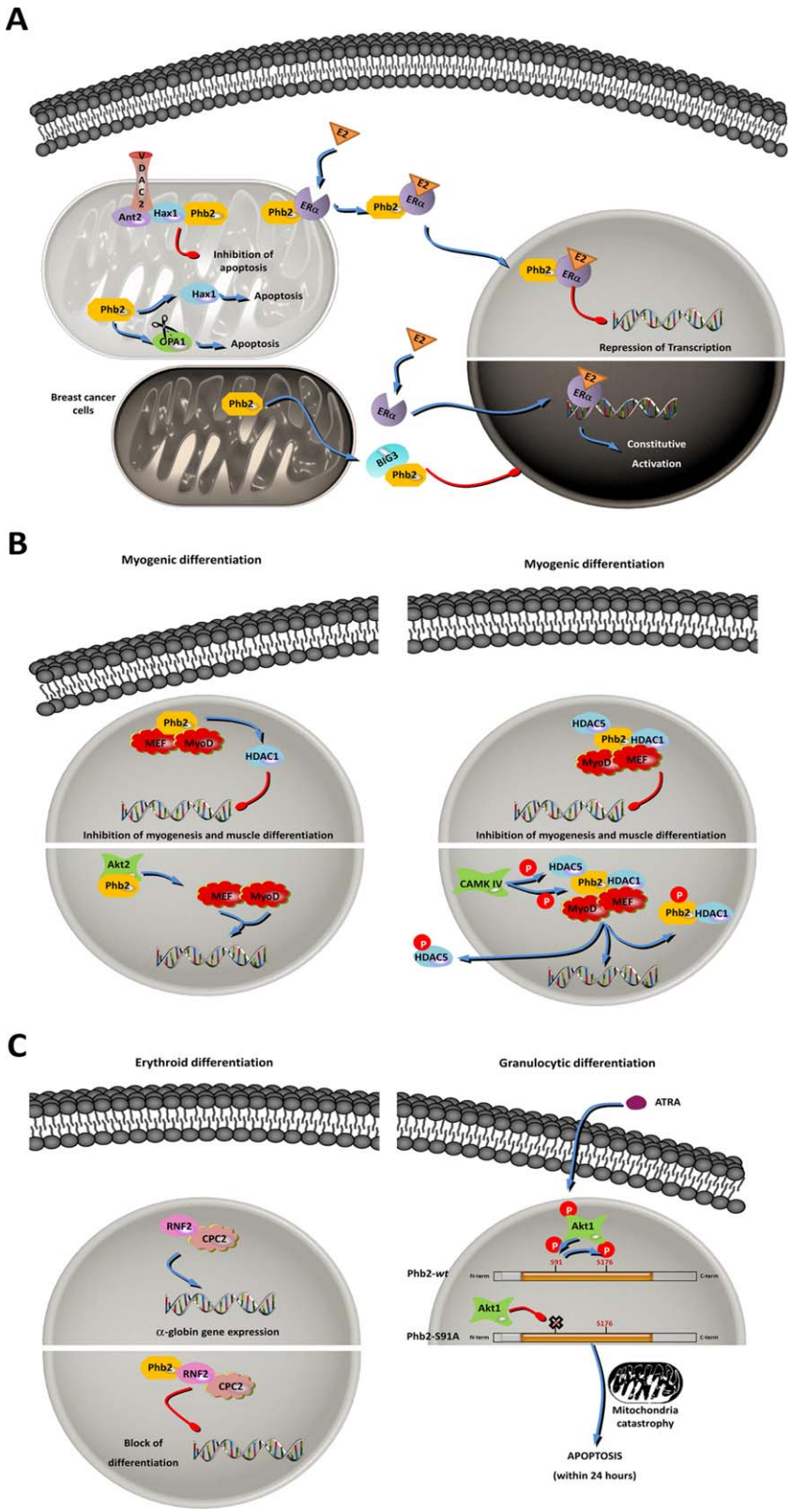


**FIG 3**

*Signal transduction mediated by PHB2. (A) Diagram of the canonical role PHB2 maintains in mitochondrial protection and in regulating estrogen receptor (ER)-α induced transcription. (B) The transcriptional regulating role of PHB2 in myogenic differentiation and its regulation by AKT2 binding and CaMK IV-dependent phosphorylation at S91. (C) The transcriptional regulating and anti-apoptotic role of PHB2 in myeloid differentiation. Left panel, erythroid differentiation involving the PHB2 binding of the E3 ligase RNF2. Right panel, the role of the hierarchical AKT-dependent phosphorylation of nuclear PHB2 during all-trans retinoic acid mediated differentiation of promyelocytic leukemia cells.*

ERα to freely translocate to the nucleus and induce estrogen-dependent transcription (38). When BIG3 was knocked-down, β-estradiol treatment led to increased PHB2 nuclear translocation and reduction of estrogen-dependent transcription (Fig. 3A).

In addition, PHB2 was found to have a suppressive effect on the orphan nuclear hormone receptors, COUP-TFI and II (30). As these receptors are intimately associated with early embryonic development and development of the immunological system, alterations in PHB2 expression would be predicted to have dire consequences in the early stages of organism development.

Importantly, the association of PHB2 with HDAC1 and/or HDAC5 is required for PHB2 to act as a transcriptional repressor of these nuclear receptors (30). This might explain why treatment with HDAC inhibitors reduces or blocks the transcriptional inhibitory effects mediated by PHB2. Thus, negative transcriptional effects of PHB2 result from both direct interaction with the nuclear receptor and the recruitment of HDACs to the site of transcription (Fig. 3A).

PHB2 was also demonstrated to inhibit the transcriptional activity of two myogenic regulatory factors, MyoD and MEF2, by forming a complex with them in the nucleus (33). Yeast-2 hybrid experiments indicated that PHB2 interacts directly with MyoD, but indirectly with MEF2. This interaction was inhibited by the association of AKT2 with PHB2 (Fig. 3B). Muscle differentiation is highly regulated by insulin/insulin-like growth factor receptor (IGFR) stimulation, which results in the activation of the PI3K-AKT-mTOR pathway. Nuclear translocation of activated AKT leads to the phosphorylation of diverse nuclear substrates and is known to be required for myocytic differentiation (49). While AKT2 was found associated with PHB2 in this study, PHB2 was not modified by AKT2, indicating the disruption of the PHB2:MyoD:MEF2 interaction was independent of AKT2 catalytic activity (33).

Insulin receptor signaling also regulates the process of adipogenesis. While activation of the PI3K-AKT-mTOR pathway is again observed, transcription of the majority of genes induced in adipogenesis are regulated by C/EBPα and PPARγ (50). During adipogenesis the levels of both PHB1 and PHB2 increase, and knock-down of either PHB leads to a loss of mitochondrial integrity, enhanced generation of ROS, and the inhibition of adipogenesis. Not surprisingly, PHB2 was recently shown to interact with PGC-1α, a co-transcriptional activator of PPARγ (3,51).

*Role of Mitochondrial PHB2.* In the mitochondrial environment, in contrast to the nucleus, the majority of PHB functions require both PHB1 and PHB2. Here, the PHBs have a role in maintaining the stability and health of mitochondria. Mitochondrial health is a factor of mitochondria DNA (mtDNA) content and the dynamic processes of (i) mitophagy, (ii) fission, and (iii) fusion. In the absence of these processes accumulation of damaged mtDNA, and/or damaged mitochondria, can surpass the threshold required for overt clinical manifestation of mitochondrial-related disease (52–55).

The process of mitophagy is separate from autophagy, as it results in the specific removal of damaged mitochondria (or, in the case of erythrocyte maturation, normal mitochondria) while maintaining other cellular organelles. Likewise, the scenario can be reversed, whereby general autophagy occurs in the absence of mitophagy. Mitophagy requires the association of parkin to the outer mitochondrial membrane (OMM), the ubiquitylation and proteosome-mediated degradation of OMM proteins, and the recruitment of beclin and LC3. As discussed below, proteosomal degradation of the OMM proteins is predicted to inhibit mitochondrial fusion and by default facilitate mitochondrial fission or fragmentation to aid in removal (52,54).

The process of fission results from activation of the dynamin-related protein (DRP)-1, which in conjunction with several OMM binding proteins, including FIS1, result in the pinching-off and division of mitochondria. The activation of DRP1 is controlled by diverse PTMs and/or de-modifications, including phosphorylation, sumoylation, ubiquitylation and nitrosylation. Phosphorylation/dephosphorylation of DRP1 is probably the best understood regulatory mechanism involved in DRP1 activation. Dephosphorylation of DRP1 at S637 by the calcium-dependent phosphatase calcineurin leads to the translocation of DRP1 from the cytosol to the mitochondria, while phosphorylation of this site by protein kinase A (PKA) inhibits DRP1 activity. In addition, phosphorylation at S616 by cdk/cyclin B complex during mitosis results in "mitochondrial replication-like" fission in preparation for cell division. Interestingly, the process of fission involves the close association of the endoplasmic reticulum, thereby physically linking the major sites of the unfolded protein response (UPR), cellular respiration, apoptosis, and $Ca^{++}$ homeostasis. Inactivation of DRP1 or alteration of its binding partners leads to mitochondrial elongation, which apparently has an anti-apoptotic effect under conditions of cellular stress, but would be predicted to have detrimental effects under conditions of cellular division, as elongation would greatly interfere with mitochondrial segregation during mitosis (52,54,55).

Fusion in contrast, results from the association and interaction of the mitofusins (MFN)-1 and −2, dynamin GTPase proteins associated with the OMM, and the Optic Atrophy (OPA)-1 (and likely OPA-3A) protein, a dynamin GTPase associated with the inner mitochondrial membrane (IMM). The process produces larger mitochondria as a result of the fusion of smaller mitochondria. Phosphorylation of MFN2 during stress results in its ubiquitylation and proteosomal degradation which favors fission and thus, mitochondrial fragmentation. In contrast, phosphorylation by the PTEN-induced putative kinase, PINK1, actually assists in mitophagy by converting MFN2 into a mitochondrial outer membrane recruiter/receptor for parkin. OPA1, on the other hand, is regulated by proteolytic cleavage through the activity of multiple *m*-AAA protease family members. Proteolytic cleavage generates both short and

**TABLE 2** ***PHB2:protein interactions***

| *Transcription factors* | *Acc. #* | *Gene name* | *Common name* | *Localization* | *Reference* |
|---|---|---|---|---|---|
| | P15336 | ATF2 | cAMP-dependent transcription factor 2 | N | NCBI |
| | P35222 | CTNNB1 | Beta-catenin | N/C | NCBI |
| | Q99814 | EPAS1 | Endothelial PAS domain containing protein 1 | N | NCBI |
| | P03372 | ESR1 | Estrogen Receptor-alpha | N/M/C | NCBI |
| | Q14192 | FHL2 | Four and a half LIM domains | N | NCBI |
| | Q12906 | ILF3 | Interleukin enhancer binding factor | N/C | NCBI |
| | Q02078 | MEF2A | Myocyte enhancing factor 2A | N | NCBI |
| | P15172 | MYOD1 | Myoblast determination protein 1 | N | NCBI |
| | Q8IXH7 | NELFCD | Negative elongation factor C/D | N | NCBI |
| | P10589 | NR2F1 | COUP-TF1 | N | NCBI |
| | P24468 | NR2F2 | COUP-TF2 | N | NCBI |
| | Q13761 | RUNX3 | Runt-related transcription factor | N/C | NCBI |
| | Q99594 | TEAD3 | Transcriptional enhancer factor 5 | N | NCBI |
| | P19532 | TFE3 | E-box binding transcription factor-E3 | N | NCBI |
| DNA modifying proteins | | | | | |
| | P38398 | BRCA1 | Breast cancer type-1 susceptibility protein | N/C | NCBI |
| | O75530 | EED | Polycomb protein EED | N | NCBI |
| | Q15910 | EZH2 | Histone-lysine *N*-methyltransferase EZH2 | N | NCBI |
| | Q13547 | HDAC1 | Histone deacetylase-1 | N | NCBI |
| | Q92769 | HDAC2 | Histone deacetylase-2 | N | NCBI |
| | O15379 | HDAC3 | Histone deacetylase-3 | N | NCBI |
| | Q9UQL6 | HDAC5 | Histone deacetylase-5 | N | NCBI |

**TABLE 2** *(Continued)*

| *Transcription factors* | *Acc. #* | *Gene name* | *Common name* | *Localization* | *Reference* |
|---|---|---|---|---|---|
| | Q14676 | MDC1 | Mediator of DNA checkpoint-1 | N | NCBI |
| | Q96T76 | MMS19 | MMS19 nucleotide excision repair protein homolog | N/C | NCBI |
| | Q6ZW49 | PAXIP1 | PAX interacting protein-1 | N | NCBI |
| | Q99496 | RNF2 | E3-ubiquitin ligase RING2 | N | NCBI |
| | Q8IXJ6 | SIRT2 | NAD-dependent protein deacetylase sirtuin 2 | N/C | NCBI |
| | Q15022 | SUZ12 | Polycomb protein SUZ12 | N | NCBI |
| RNA binding/ processing | | | | | |
| | Q9H9G7 | AGO3 | Argonaute-3 | C/P-bodies | NCBI |
| | Q9H3H3 | AI837181 | Basophilic leukemia-expressed protein Bles03 | C | NCBI |
| | Q9UHI6 | DDX20 | ATP-dependent RNA helicase DDX20 | N/C | NCBI |
| | P58107 | EPPK1 | Epiplakin | C | NCBI |
| | O95793 | STAU1 | Double-stranded RNA-binding protein Staufen homolog 1 | C/rER | NCBI |
| Cell cycle | | | | | |
| | P30305 | CDC25B | M-phase inducer phosphatase 2 | C | NCBI |
| | P24941 | CDK2 | Cyclin dependent kinase 2 | N/C | NCBI |
| | Q9H8V3 | ECT2 | Protein ECT2 | N/C | NCBI |
| | Q02241 | KIF23 | Kinesin-like protein KIF23 | N/C | NCBI |
| Cytoskeleton/structural proteins | | | | | |
| | Q9Y678 | COPG1 | Coatomer subunit gamma-1 | C | NCBI |
| | O95466 | FMNL1 | Formin-like protein-1 | C | NCBI |
| | P02751 | FN1 | Fibronectin | ECM | NCBI |

**TABLE 2** *(Continued)*

| *Transcription factors* | *Acc. #* | *Gene name* | *Common name* | *Localization* | *Reference* |
|---|---|---|---|---|---|
| | P14923 | JUP | Junction plakoglobin | C/PM | NCBI |
| | Q8N1F7 | NUP93 | Nucleoporin complex protein 93 | NM | NCBI |
| | Q92621 | NUP205 | Nucleoporin complex protein 205 | NM | NCBI |
| | Q9UJZ1 | SLP2 | Stomatin-like protein 2 | M | 47 |
| Signal transduction | | | | | |
| | P07550 | ADRB2 | Beta-andrenergic receptor | PM | NCBI |
| | P31749 | AKT1 | RAC-alpha serine/ threonine-protein kinase | N/C/PM | NCBI |
| | P08069 | IGF1R | Insulin-like growth factor-1 receptor | PM | NCBI |
| | P13612 | ITGA4 | Integrin alpha-4 | PM | NCBI |
| | Q5TH69 | KIAA1244 | Brefeldin A inhibited guanine nucleotide-exchange protein-3 | C/M | NCBI |
| | Q00987 | MDM2 | E3 ubiquitin-protein ligase Mdm2 | N/C | NCBI |
| | P35232 | PHB1 | Prohibitin | N/C/M/PM | NCBI |
| | O43353 | RIPK2 | Receptor interacting S/T protein kinase-2 | C | NCBI |
| | Q9Y4E8 | USP15 | Ubiquitin carboxyl-terminal hydrolase 15 | N/C | NCBI |
| | P19320 | VCAM1 | Vascular cell adhesion protein 1 | PM | NCBI |
| Cellular respiration | | | | | |
| | P06576 | ATP5B | ATP synthase subunit beta | M | 47 |
| | P13073 | COX4I1 | Cytochrome c oxidase subunit 4 isoform 1 | M | 47 |
| | P14854 | COX6B1 | Cytochrome c oxidase subunit 6B1 | M | 47 |
| | P09669 | COX6C | Cytochrome c oxidase subunit 6C | M | 47 |
| | O95167 | NDUFA3 | NADH dehydrogenase 1 alpha, subcomplex 3 | M | 47 |

**TABLE 2** *(Continued)*

| *Transcription factors* | *Acc. #* | *Gene name* | *Common name* | *Localization* | *Reference* |
|---|---|---|---|---|---|
| | O75489 | NDUFS3 | NADH dehydrogenase Fe-S protein 3 | M | 47 |
| | O95298 | NDUFC2 | NADH dehydrogenase 1, subunit C2 | M | 47 |
| | O43920 | NDUFS5 | NADH dehydrogenase 1 alpha, subcomplex 5 | M | 47 |
| | Q9P0J0 | NDUFA13 | NADH dehydrogenase 1alpha, subcomplex 13 | M | 47 |
| | P31930 | UQCRC1 | Cytochrome b-c1 complex subunit 1 | M | NCBI |
| | P22695 | UQCRC2 | Cytochrome b-c1 complex, subunit 2 | M | 47 |
| Proteases | | | | | |
| | Q9Y4W6 | AFG3L2 | AFG3-like protein 2 | M | 47 |
| | Q9UQ90 | SPG7 | Paraplegin | M | 47 |
| | Q96TA2 | YME1L1 | ATP-dependent $Z^{++}$ metalloprotease | M | 47 |
| Mitochondrial transport/translation | | | | | |
| | Q8WXX5 | DNAJC9 | DnaJ homolog subfamily C member 9 | M | 47 |
| | Q14197 | ICT1 | Peptidyl tRNA hydrolase ICT1 | M | 47 |
| | Q9Y3D7 | PAM16 | Mitochondrial inner membrane translocase subunit TIM16 | M | 47 |
| | Q9Y584 | TIM22 | Mitochondrial import inner membrane translocase subunit Tim22 | M | 47 |
| | O14925 | TIMM23 | Mitochondrial import inner membrane translocase subunit Tim23 | M | 47 |
| | Q3ZCQ8 | TIMM50 | Mitochondrial import inner membrane translocase subunit TIM50 | N/M | 47 |

**TABLE 2** *(Continued)*

| *Transcription factors* | *Acc. #* | *Gene name* | *Common name* | *Localization* | *Reference* |
|---|---|---|---|---|---|
| | P49411 | TUFM | Mitochondrial translation elongation factor Tu | M | NCBI |
| Miscellaneous | | | | | |
| | P02771 | AFP | Alpha-fetoprotein | C/ECM | NCBI |
| | A6NK59 | ASB14 | Ankyrin repeat and SOCS box protein 14 | ND | NCBI |
| | Q8WXJ9 | ASB17 | Ankyrin repeat and SOCS box protein 17 | ND | NCBI |
| | Q8NBU5 | ATAD1 | ATPase AAA domain containing protein 1 | M/PM/Px | 47 |
| | Q2TAZ0 | ATG2A | Autophagy-related protein 2, isoform alpha | APh | NCBI |
| | Q07021 | C1QBP | Compement component 1Q subcomponent binding protein | N/C/M | NCBI |
| | Q8WWC4 | C2ORF47 | Uncharacterized protein C2orf47, mitochondrial | M | 47 |
| | Q9Y2C4 | EXOG | Nuclease ExoG | M | 47 |
| | Q9H3K2 | GHITM | Growth hormone-inducible transmembrane protein | M | 47 |
| | Q08380 | LGALS3BP | Galectin-3 binding protein | ECM | NCBI |
| | Q9Y6C9 | MTCH2 | Mitochondrial carrier homolog 2 | M | 47 |
| | Q9NX40 | OCIAD1 | OCIA domain containing protein | M/Endo | 47 |
| | Q6P996 | PDXDC1 | Pyriodoxal-dependent decarboxylase domain containing protein 1 | G | NCBI |
| | P06454 | PTMA | Prothymosin alpha | N/Ex | NCBI |
| | | PTPH2 | Peptidyl tRNA hydrolase 2 | C/M | 47 |
| | Q96GF1 | RNF185 | E3 ubiquitin-protein ligase RNF185 | M | NCBI |

**TABLE 2** *(Continued)*

| *Transcription factors* | *Acc. #* | *Gene name* | *Common name* | *Localization* | *Reference* |
|---|---|---|---|---|---|
| | Q13530 | SERINC3 | Serine incorporation 3 | PM | NCBI |
| | Q6NUK1 | SLC25A24 | Calcium-binding mitochondrial carrier protein SCaMC-1 | M | 47 |
| | P61956 | SUMO2 | Small ubiquitin-related modifier 2 | N | NCBI |
| | Q9H061 | TMEM126A | Transmembrane protein 126A | M | 47 |
| | P0CG48 | UBC | Polyubiquitin-C | N/C | NCBI |
| | P11441 | UBL4A | Ubiquitin-like protein 4A | C | NCBI |

*Proteins indentified in complex with PHB2 by co-immunoprecipitation and western blotting, yeast two-hybrid, or affinity capture-mass spectrometry are listed by the protein accession number in UniProtKB (Acc #), gene name, common name, and subcellular compartment of localization. The proteins have been grouped according to function. For subcellular localization: N = nucleus, NM= nuclear membrane, M = mitochondria, C = cytoplasm, PM = plasma membrane, ECM = extracellular matrix, Ex = exosome, Endo = endosome, Px = peroxisome, rER = rough endoplasmic reticulum, G = golgi apparatus, APh = autophagesome, ND = not determined. References: NCBI is referred to for those proteins with PubMed links that are listed as PHB2 interactors on the NCBI Gene site. Proteins not indexed on this site are reference to their direct source. While the human homologues are listed, proteins referenced to Richter-Dennerlein et al.[47] refer to proteins discovered in a mouse model.*

long forms of OPA1, which regulate IMM (cristae) fusion, as well as a soluble form of OPA1, which regulates cristae morphology and stability. The loss of OPA1 or OPA3A leads to altered mitochondrial fragmentation. Moreover, it is appropriately in the cristae where much of the cellular machinery for oxidative phosphorylation, the TCA cycle and $\beta$-oxidation of fatty acids resides. As OPA1 is more closely associated with cristae maintenance during IMM remodeling, loss of OPA1 causes severe distortions in the cristae, which result in the loss of mitochondrial membrane potential and $Ca^{++}$ homeostasis, altered glucose and fatty acid metabolism, and the generation of ROS; thus leading to severe cellular damage/disease or apoptosis (52,54–56).

The prohibitins represent integral IMM proteins that serve to maintain mitochondria structure and function (57). Tetrameric complexes of alternating PHB1 and PHB2 insert into the IMM with the coiled-coil domains exposed to the inner membrane space and form the 120 kDa precursor subunits of a large PHB1:PHB2 ring structure (~1.2 MDa) (57). Loss of either PHB-1 or-2 results in alterations in the cristae morphology and a phenotype very similar to the loss of OPA1 (3,57). The PHBs function as mitochondrial scaffolding/chaperone proteins that protect newly synthesized and noncomplexed mitochondrial proteins from being degraded by the ATP-dependent *m*-AAA proteases associated with the large PHB1:PHB2 complex (47,58). Thus, the PHBs assist in the transport and stability of diverse mitochondrial proteins (3,57).

OPA1 represents the canonical protein affected by the PHB1/2:*m*-AAA complex (4,59) (Fig. 3A). In PHB2−/− mouse embryo fibroblasts (MEFs), the longer isoform of OPA1 was observed to be extremely unstable and rapidly cleaved, leading to arrest of cellular proliferation and an increased susceptibility to apoptotic stimuli (4). Overexpression of a noncleavable long isoform of OPA1 in PHB2−/− MEFs completely compensated for PHB2 deficiency, suggesting that the mitochondrial phenotype of PHB2-null MEFs was strictly associated with the proteolytic processing of long OPA1 isoforms (4) (Fig. 3A).

In addition to OPA1, the stability of several respiratory complexes has been linked to the presence of PHBs in the mitochondria. While loss of PHB1 leads to the proteolytic degradation of Complex I, PHB2 influences the stability of Complex IV via a mechanism involving sphingosine-1-phosphate (S1P) (60,61). The PHB2:S1P complex associates with cytochrome c oxidase through an interaction between PHB2 and cytochrome c oxidase, subunit 4 and is responsible for the correct processing and assembly of the cytochrome c oxidase complex (60).

Likewise, PHB2 also modulates the stability of the VDAC2/ANT2/HAX1 complex. Reduced expression of PHB2 was reported to result in decreased mitochondrial HAX1 and loss of mitochondrial integrity, caspase 9/caspase 3 activation and subsequent apoptosis. Like OPA1, the loss of PHB2 likely leads to enhanced proteolytic degradation of HAX1 (8) (Fig. 3A). Thus, it is evident that alterations in PHB2 would be expected

to both directly and indirectly lead to decreased mitochondrial fusion, uncoupling of oxidative phosphorylation, loss of mitochondrial membrane potential, $Ca^{++}$ release, an increase in ROS generation and cell damage/death.

The PHBs also loosely associate with nucleoids, small nucleoprotein complexes, which regulate the stability/maintenance, replication, transcription, and assembly/condensation of mtDNA; thus, it has been proposed that the PHBs may stabilize the mitochondrial genome (62,63). But as loss of either PHB has little direct effect on the stability of the mitochondrial genome, the involvement of either PHB1 or PHB2 is likely not mandatory for this aspect of mitochondrial physiology (57).

*Role of Cytosolic/Membrane Receptor-Associated PHB2.* Other than nuclear or mitochondrial localization, the prohibitins are also associated with diverse cell surface receptors at the plasma membrane. Fu et al. demonstrated that PHB2 is required for insulin-like growth factor binding protein (IGFBP)-6 induced cell migration of rhabdomyosarcoma cells, and that IGFBP6 and PHB2 directly interact. The authors also demonstrated that IGFBP6 enhances PHB2 tyrosine phosphorylation in an indirect manner, thus establishing an additional link between the PHBs and insulin/insulin-like growth factor signaling (64).

In B lymphocytes, both PHB1 and PHB2 were found associated with B7.2/CD86, the antigen presenting cell receptor that associates with the T-cell co-activating receptor CD28. This interaction was increased following co-stimulation of CD40 [Tumor necrosis factor receptor super family member (TNFRSF)-5] and the IL-4R with CD40L (TRAP) and IL-4, respectively (65). In primed mouse B lymphocytes, stimulation of B7.2/CD86 results in the activation of NF-$\kappa$B and a subsequent increase in the Oct-1 transcription factor culminating in a clinically significant increase in the expression of IgG1 on the B-cell surface (65). Lucas et al. demonstrated that in the absence of PHB1 and PHB2, the CD86-mediated induction of IgG1 was significantly reduced similar to that observed when either the CD86 cytoplasmic domain was truncated or the PKC phosphorylation sites of the CD86 cytoplasmic domain were mutated to alanine residues. Both the CD86 cytoplasmic domain and the PHBs were required for CD86-induced phosphorylation of I$\kappa$B-$\alpha$. In contrast, only the PHBs were required for CD86-induced phosphorylation of PLC$\gamma$ and protein kinase C$\alpha/\beta$, which phosphorylated NF-$\kappa$B p65 (65). These data indicate a specific role for the PHBs in inflammatory signaling and immunity.

PHB1 and PHB2 are also present on the surface of platelets and lipid rafts where they associate with proteinase-activated receptor (PAR)-1. Disruption of the PHB1:PHB2:PAR-1 association using inhibitory antibody or siRNA mediated knock-down caused a dramatic loss in granular secretion, calcium mobilization, $\alpha$IIb$\beta$3 activation, and platelet aggregation in response to low dose thrombin or PAR-1 activating peptide, indicating a receptor specific role for the PHB complex in platelet aggregation (66).

Finally, the association of PHBs with the plasma membrane is implicated in infectious disease. The PHB1:PHB2 complex was found to associate with amino acid residues 790 to 800 in the carboxyl terminus of the HIV glycoprotein (67). Inhibition of this interaction did not block virus replication in permissive cells, although the time frame of infection was delayed, but it did abolish viral replication and spread in non-permissive cells. Likewise, *Salmonella typhi* uses the PHBs as virulence factors to enhance infection. The binding of the *S. typhi* Vi protein was demonstrated to suppress both the innate immune response and T-cell activation (68,69). Similarly interaction of the PHB1:PHB2 complex with the SARS-CoA non-structural protein-2 results in suppression of the host immune response (70). In mosquito cell lines, data suggest that prohibitin may serve as a cellular receptor for Dengue virus infection (71).

*Gene Structure and Expression.* The role of the PHBs in the nucleus, cytoplasm, and mitochondria place them at the crossroads of most metabolic and inflammatory pathologies. With the exception of increased breast cancer susceptibility in women harboring a single nucleotide polymorphism in PHB1 (C729T), no human diseases have been clinically reported to result from or be associated with an alteration in either prohibitin, but it is evident that altered expression, mutation, or loss of the PHBs are potentially involved in diverse diseases (72). As nuclear PHBs have a role in the regulation of multiple transcription factors, alteration in PHB1 or 2 localization and protein binding could have varied effects depending on the transcription factor and the tissue involved. The characteristics of associated pathologies involving PHB2 might range from developmental abnormalities during embryogenesis and spontaneous abortion to more specific characteristics such as lipodystrophy, diabetes, immunodeficiencies, and muscular dystrophy/wasting (52,53).

From what is currently known about cytoplasmic/plasma membrane associated PHB2, the most pronounced effects of altered PHB2 expression, modification and protein:protein interaction at these cellular sites would be expected to be mostly immunological in nature, with both the T-cell response to infections pathogens as well as the susceptibility to certain infectious agents being altered (65,67,68,70). What may be a more important influence of the PHBs in disease development is the role of the PHBs in mitochondrial stability.

The importance of both hereditary and spontaneous (age-related) mitochondrial alterations to the development of diverse diseases is becoming more evident. To date mitochondrial-related diseases include, Charcot-Marie-Tooth syndrome, autosomal dominant optic atrophy, Leber optic atrophy, Leigh syndrome, Kearns-Sayre syndrome, amyotrophic lateral sclerosis (ALS), and Parkinson's disease, to name a few; and likely also include other metabolic diseases such as cancer, diabetes and Alzheimer's disease (52,54). With most of these diseases, alteration in a specific mitochondrial associated gene(s) has been demonstrated as the cause of disease.

The alteration of these genes has been shown to cause disease by having a more global effect on the proper functioning of a particular mitochondrial process, such as oxidative-phosphorylation (Leber optic atrophy), fusion (Charcot-Marie-Tooth syndrome and optic atrophy), or mitophagy (Parkinson's disease) (52). In addition, mitochondrial diseases do not display an "all or none" pattern and are governed instead by a certain penetrance of the alteration (53). In other words, a certain threshold of faulty mitochondria must be reached before an overt disease phenotype is observed. Added to this, cells having a higher metabolic role or requirement (energy demand) are more sensitive to the presence of defective mitochondria. Thus, mitochondrial diseases may present with somewhat overlapping phenotypes of diverse severity involving multiple tissue to varying degrees. The critical role of the PHBs in maintaining mitochondrial stability suggests that loss or alteration of either PHB could have pleiotropic effects, and thus the resulting phenotypes could be potentially endless. To date, while no mutation in PHB2 has been documented as the causative agent in disease, PHB2 expression, localization and post-translational modification have been associated with several pathologies.

PHB2 expression is often up-regulated in cancer and phosphorylation of diverse Ser, Thr, and Tyr residues of PHB2, including Y248, has been observed in multiple cancer cell lines and tissues (3,11,45). One explanation for the involvement of the PHBs in cancer is the increased metabolic dependency on mitochondrial respiration. Cancer cells have an increased energy demand fulfilled through enhanced glycolysis and cellular respiration. The consequences of these processes result in intercellular imbalances of ROS and $Ca^{++}$ stores. Under these conditions, the mitochondrial demands of the cell are at their maximum and thus, the mitochondria are highly susceptible to oxidative damage. The increased presence of the PHBs in the mitochondria in this scenario, likely results in enhanced mitochondrial stability, although the data to support this conclusion are conflicting. In diverse cell lines loss of PHB1 or PHB2 was shown to result in reduced cellular division with no loss in mitochondrial integrity, while another study demonstrated that siRNA mediated knock-down of PHB1 or PHB2 results in loss of cancer cell proliferation with significant loss of mitochondrial integrity (4,8,61).

In breast cancer, the role of the PHBs has been established outside the mitochondria. In fact, the PHBs are mostly found localized to the mitochondria in normal breast epithelium, but are almost exclusively nuclear in breast cancer cells (38,45). While PHB1 was shown in breast carcinoma cells to co-localize with the E2F1 transcription factor and retinoblastoma (Rb) protein in the nucleus leading to the disruption of the E2F1/Rb interaction, cell cycle dependent transcription and cell division, PHB2 was shown to specifically associate with ER$\alpha$ (3,8,38). It might be interpreted that nuclear PHBs are required for tumorgenesis, but the presence of the PHBs in the nucleus of breast cancer cells may actually represent the cells attempt at a normalizing response (45).

As the PHBs are also required for the survival of tissues with elevated metabolic demands, such as the liver, heart, $\beta$-cell islets of the pancreas, retinal epithelium and neuronal tissue, the expression of the PHBs in these tissues is highly protective against metabolic and oxidative stress (3,73). In a $\beta$-cell specific PHB2 knock-out mouse model, Supale et al. demonstrated that loss of PHB2 in $\beta$-cell islets results in an increase glucose intolerance, $\beta$-cell death, and subsequent severe diabetes. Death of the $\beta$-cells resulted from the proteolytic cleavage of OPA1 and other respiratory chain complex proteins, mitochondrial dysfunction and eventually fragmentation/degradation of the mitochondria (6). Similarly, loss of PHB2 expression in a conditional, neuronal-specific knock-out mouse model resulted in instability of OPA1, alteration of the mitochondrial ultrastructure, altered localization of the mitochondria, the appearance of hyperphosphorylated Tau protein and neurodegeneration, suggesting a role for the loss of mitochondrial integrity in the inflammatory pathology associated with Alzheimer's disease (5).

## Conclusions

Cellular division and metabolism as well as cell death are processes that require communication and coordination between diverse sub-cellular compartments. Evidence indicates that the PHBs represent two of these communicator/coordinator proteins. Many unique functions of the PHBs are highly dependent on localization. Of particular interest are the activities conducted by the PHBs in the nucleus and mitochondria. Data examining the phosphorylation of both PHB1 and PHB2 indicated that these proteins are phosphorylated by AKT1/2 and CaMK IV (12,13). What is interesting is that these proteins become phosphorylated under differentiation conditions, involving the activation of the PI3K-AKT-mTOR axis or the increase in intracellular $Ca^{++}$, which require strict coordination between nuclear and mitochondrial signaling. It was previously demonstrated that AKT phosphorylates PHB1 on T258, resulting in dissociation of PHB1 from phosphatidylinositol-3,4,5-phosphate (PIP3) (74). In the case of PHB2, Sun et al. indicated that phosphorylation of S91 by CaMK IV in myocytes in response to differentiating stimuli promotes myogenesis by disrupting the association of PHB2 with MyoD and MEF2 (13) (Fig. 3B). On the other hand, the data presented by Bavelloni et al. indicate that AKT1/2 also phosphorylates S91 during differentiation, and more importantly phosphorylation of S91 is required for survival in this cell model. The cellular death reported by Bavelloni et al. following overexpression of a PHB2 (S91A) mutant was highly indicative of mitochondrial dysfunction and very similar to findings reported following the expression of PHB mutants lacking or containing alterations in the mitochondrial transmembrane domain (MTS), critical for mitochondrial localization (4,8,12) (Fig. 3C). While neither study by Sun et al. nor by Bavelloni et al. examined whether phosphorylation of S91 increased the presence of PHB2 in the mitochondria, the reported findings

from these two studies might actually indicate this to be the case. Under these conditions, phosphorylation of nuclear PHB2 on S91 might promote nuclear export of PHB2 and mitochondrial re-localization. This remains to be seen, but it is clear that the prohibitins represent a novel class of proteins that shuttle between diverse compartments where they assume completely unrelated functions to coordinate a single response, making them viable targets for the treatment of diverse pathologies.

## Acknowledgements

This research was supported by a Leukemia Research Foundation Investigator Grant (to WLB) and the "5X1000" Fund to the Laboratory of Musculoskeletal Cell Biology, Rizzoli Orthopedic Institute.

### References

[1] Mishra, S., Murphy, L. C., and Murphy, L. J. (2006) The Prohibitins: emerging roles in diverse functions. J. Cell Mol. Med. 10, 353–363.

[2] McClung, J. K., Danner, D. B., Stewart, D. A., Smith, J. R., Schneider, E. L., et al. (1989) Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver. Biochem. Biophys. Res. Commun. 164, 1316–1322.

[3] Thuaud, F., Ribeiro, N., Nebigil, C. G., and Desaubry, L. (2013) Prohibitin ligands in cell death and survival: mode of action and therapeutic potential. Chem. Biol. 20, 316–331.

[4] Merkwirth, C., Dargazanli, S., Tatsuta, T., Geimer, S., Lower, B., et al. (2008) Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria. Genes Dev. 22, 476–488.

[5] Merkwirth, C., Martinelli, P., Korwitz, A., Morbin, M., Bronneke, H. S., et al. (2012) Loss of prohibitin membrane scaffolds impairs mitochondrial architecture and leads to tau hyperphosphorylation and neurodegeneration. PLoS Genet. 8, e1003021.

[6] Supale, S., Thorel, F., Merkwirth, C., Gjinovci, A., Herrera, P. L., et al. (2013) Loss of prohibitin induces mitochondrial damages altering beta-cell function and survival and is responsible for gradual diabetes development. Diabetes 62, 3488–3499.

[7] Theiss, A. L., Idell, R. D., Srinivasan, S., Klapproth, J. M., Jones, D. P., et al. (2007) Prohibitin protects against oxidative stress in intestinal epithelial cells. FASEB J. 21, 197–206.

[8] Kasashima, K., Ohta, E., Kagawa, Y., and Endo, H. (2006) Mitochondrial functions and estrogen receptor-dependent nuclear translocation of pleiotropic human prohibitin 2. J. Biol. Chem. 281, 36401–36410.

[9] Liu, X., Ren, Z., Zhan, R., Wang, X., Zhang, Z., et al. (2009) Prohibitin protects against oxidative stress-induced cell injury in cultured neonatal cardiomyocyte. Cell Stress Chaperones 14, 311–319.

[10] Theiss, A. L., and Sitaraman, S. V. (2011) The role and therapeutic potential of prohibitin in disease. Biochim. Biophys. Acta 1813, 1137–1143.

[11] Hornbeck, P. V., Kornhauser, J. M., Tkachev, S., Zhang, B., Skrzypek, E., et al. (2012) PhosphoSitePlus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse. Nucleic Acids Res. 40, D261–D270.

[12] Bavelloni, A., Piazzi, M., Faenza, I., Raffini, M., D'Angelo, A., et al. (2014) Prohibitin 2 represents a novel nuclear AKT substrate during all-trans retinoic acid-induced differentiation of acute promyelocytic leukemia cells. FASEB J. 28, 2009–2019.

[13] Sun, L., Cao, X., Liu, B., Huang, H., Wang, X., et al. (2011) CaMK IV phosphorylates prohibitin 2 and regulates prohibitin 2-mediated repression of MEF2 transcription. Cell Signal 23, 1686–1690.

[14] Ross, J. A., Nagy, Z. S., and Kirken, R. A. (2008) The PHB1/2 phosphocomplex is required for mitochondrial homeostasis and survival of human T cells. J. Biol. Chem. 283, 4699–4713.

[15] Kettenbach, A. N., Schweppe, D. K., Faherty, B. K., Pechenick, D., Pletnev, A. A., et al. (2011) Quantitative phosphoproteomics identifies substrates and functional modules of Aurora and Polo-like kinase activities in mitotic cells. Sci. Signal 4, rs5.

[16] Zhao, X., Leon, I. R., Bak, S., Mogensen, M., Wrzesinski, K., et al. (2011) Phosphoproteome analysis of functional mitochondria isolated from resting human muscle reveals extensive phosphorylation of inner membrane protein complexes and enzymes. Mol. Cell Proteomics 10, M110 000299.

[17] Zhou, H., Di Palma, S., Preisinger, C., Peng, M., Polat, A. N., et al. (2013) Toward a comprehensive characterization of a human cancer cell phosphoproteome. J. Proteome Res. 12, 260–271.

[18] Gu, T. L., Goss, V. L., Reeves, C., Popova, L., Nardone, J., et al. (2006) Phosphotyrosine profiling identifies the KG-1 cell line as a model for the study of FGFR1 fusions in acute myeloid leukemia. Blood 108, 4202–4204.

[19] Rikova, K., Guo, A., Zeng, Q., Possemato, A., Yu, J., et al. (2007) Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 131, 1190–1203.

[20] Ding, V. M., Boersema, P. J., Foong, L. Y., Preisinger, C., Koh, G., et al. (2011) Tyrosine phosphorylation profiling in FGF-2 stimulated human embryonic stem cells. PLoS One 6, e17538.

[21] Knowlton, M. L., Selfors, L. M., Wrobel, C. N., Gu, T. L., Ballif, B. A., et al. (2010) Profiling Y561-dependent and -independent substrates of CSF-1R in epithelial cells. PLoS One 5, e13587.

[22] Kim, W., Bennett, E. J., Huttlin, E. L., Guo, A., Li, J., et al. (2011) Systematic and quantitative assessment of the ubiquitin-modified proteome. Mol. Cell 44, 325–340.

[23] Wagner, S. A., Beli, P., Weinert, B. T., Nielsen, M. L., Cox, J., et al. (2011) A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. Mol. Cell Proteomics 10, M111013284.

[24] Weinert, B. T., Scholz, C., Wagner, S. A., Iesmantavicius, V., Su, D., et al. (2013) Lysine succinylation is a frequently occurring modification in prokaryotes and eukaryotes and extensively overlaps with acetylation. Cell Rep. 4, 842–851.

[25] Wagner, S. A., Beli, P., Weinert, B. T., Scholz, C., Kelstrup, C. D., et al. (2012) Proteomic analyses reveal divergent ubiquitylation site patterns in murine tissues. Mol. Cell Proteomics 11, 1578–1585.

[26] Kowno, M., Watanabe-Susaki, K., Ishimine, H., Komazaki, S., Enomoto, K., et al. (2014) Prohibitin 2 regulates the proliferation and lineage-specific differentiation of mouse embryonic stem cells in mitochondria. PLoS One 9, e81552.

[27] Liu, D., Lin, Y., Kang, T., Huang, B., Xu, W., et al. (2012) Mitochondrial dysfunction and adipogenic reduction by prohibitin silencing in 3T3-L1 cells. PLoS One 7, e34315.

[28] Lau, E., Kluger, H., Varsano, T., Lee, K., Scheffler, I., et al. (2012) PKCepsilon promotes oncogenic functions of ATF2 in the nucleus while blocking its apoptotic function at mitochondria. Cell 148, 543–555.

[29] Ewing, R. M., Chu, P., Elisma, F., Li, H., Taylor, P., et al. (2007) Large-scale mapping of human protein-protein interactions by mass spectrometry. Mol. Syst. Biol. 3, 89.

[30] Kurtev, V., Margueron, R., Kroboth, K., Ogris, E., Cavailles, V., et al. (2004) Transcriptional regulation by the repressor of estrogen receptor activity via recruitment of histone deacetylases. J. Biol. Chem. 279, 24834–24843.

[31] Martini, P. G., Delage-Mourroux, R., Kraichely, D. M., and Katzenellenbogen, B. S. (2000) Prothymosin alpha selectively enhances estrogen receptor transcriptional activity by interacting with a repressor of estrogen receptor activity. Mol. Cell Biol. 20, 6224–6232.

[32] Havugimana, P. C., Hart, G. T., Nepusz, T., Yang, H., Turinsky, A. L., et al. (2012) A census of human soluble protein complexes. Cell 150, 1068–1081.

[33] Sun, L., Liu, L., Yang, X. J., and Wu, Z. (2004) Akt binds prohibitin 2 and relieves its repression of MyoD and muscle differentiation. J. Cell Sci. 117, 3021–3029.

[34] Woods, N. T., Mesquita, R. D., Sweet, M., Carvalho, M. A., Li, X., et al. (2012) Charting the landscape of tandem BRCT domain-mediated protein interactions. Sci. Signal 5, rs6.

[35] Neganova, I., Vilella, F., Atkinson, S. P., Lloret, M., Passos, J. F., et al. (2011) An important role for CDK2 in G1 to S checkpoint activation and DNA damage response in human embryonic stem cells. Stem Cells 29, 651–659.
[36] Hwang, C., Giri, V. N., Wilkinson, J. C., Wright, C. W., Wilkinson, A. S., et al. (2008) EZH2 regulates the transcription of estrogen-responsive genes through association with REA, an estrogen receptor corepressor. Breast Cancer Res. Treat. 107, 235–242.
[37] Cao, Q., Wang, X., Zhao, M., Yang, R., Malik, R., et al. (2014) The central role of EED in the orchestration of polycomb group complexes. Nat. Commun. 5, 3127.
[38] Kim, J. W., Akiyama, M., Park, J. H., Lin, M. L., Shimo, A., et al. (2009) Activation of an estrogen/estrogen receptor signaling by BIG3 through its inhibitory effect on nuclear transport of PHB2/REA in breast cancer. Cancer Sci. 100, 1468–1478.
[39] Weinmann, L., Hock, J., Ivacevic, T., Ohrt, T., Mutze, J., et al. (2009) Importin 8 is a gene silencing factor that targets argonaute proteins to distinct mRNAs. Cell 136, 496–507.
[40] Hutchins, J. R., Toyoda, Y., Hegemann, B., Poser, I., Heriche, J. K., et al. (2010) Systematic analysis of human protein complexes identifies chromosome segregation proteins. Science 328, 593–599.
[41] Milev, M. P., Ravichandran, M., Khan, M. F., Schriemer, D. C., and Mouland, A. J. (2012) Characterization of staufen1 ribonucleoproteins by mass spectrometry and biochemical analyses reveal the presence of diverse host proteins associated with human immunodeficiency virus type 1. Front. Microbiol. 3, 367.
[42] Nicholson, J., Scherl, A., Way, L., Blackburn, E. A., Walkinshaw, M. D., et al. (2014) A systems wide mass spectrometric based linear motif screen to identify dominant in-vivo interacting proteins for the ubiquitin ligase MDM2. Cell Signal 26, 1243–1257.
[43] Xu, Y., Cai, M., Yang, Y., Huang, L., and Ye, Y. (2012) SGTA recognizes a noncanonical ubiquitin-like domain in the Bag6-Ubl4A-Trc35 complex to promote endoplasmic reticulum-associated degradation. Cell Rep. 2, 1633–1644.
[44] Lee, S. J., Choi, D., Rhim, H., Choo, H. J., Ko, Y. G., et al. (2008) PHB2 interacts with RNF2 and represses CP2c-stimulated transcription. Mol. Cell Biochem. 319, 69–77.
[45] Yoshimaru, T., Komatsu, M., Matsuo, T., Chen, Y. A., Murakami, Y., et al. (2013) Targeting BIG3-PHB2 interaction to overcome tamoxifen resistance in breast cancer cells. Nat. Commun. 4, 2443.
[46] Humphries, J. D., Byron, A., Bass, M. D., Craig, S. E., Pinney, J. W., et al. (2009) Proteomic analysis of integrin-associated complexes identifies RCC2 as a dual regulator of Rac1 and Arf6. Sci. Signal 2, ra51.
[47] Richter-Dennerlein, R., Korwitz, A., Haag, M., Tatsuta, T., Dargazanli, S., et al. (2014) DNAJC19, a mitochondrial cochaperone associated with cardiomyopathy, forms a complex with prohibitins to regulate cardiolipin remodeling. Cell Metab. 20, 158–171.
[48] Iioka, H., Iemura, S., Natsume, T., and Kinoshita, N. (2007) Wnt signalling regulates paxillin ubiquitination essential for mesodermal cell motility. Nat. Cell Biol. 9, 813–821.
[49] Noguchi, S. (2005) The biological function of insulin-like growth factor-I in myogenesis and its therapeutic effect on muscular dystrophy. Acta Myol. 24, 115–118.
[50] Kawai, M. and Rosen, C. J. (2010) The IGF-I regulatory system and its impact on skeletal and energy homeostasis. J. Cell Biochem. 111, 14–19.
[51] Endo, H. (2012) Mitochondrial function of prohibitin 2 (PHB2). US Pat. 8,153,362.
[52] Scheibye-Knudsen, M., Fang, E. F., Croteau, D. L., Wilson, D. M., III, and Bohr, V. A. (2014) Protecting the mitochondrial powerhouse. Trends Cell. Biol, in press. doi: 10.1016/j.tcb.2014.11.002.
[53] Mishra, P. and Chan, D. C. (2014) Mitochondrial dynamics and inheritance during cell division, development and disease. Nat. Rev. Mol. Cell Biol. 15, 634–646.
[54] Burte, F., Carelli, V., Chinnery, P. F., and Yu-Wai-Man, P. (2015) Disturbed mitochondrial dynamics and neurodegenerative disorders. Nat. Rev. Neurol. 11, 11–24.
[55] Kasahara, A. and Scorrano, L. (2014) Mitochondria: from cell death executioners to regulatorsof cell differentiation. Trends Cell Biol. 24, 761–770.
[56] Montgomery, M. K. and Turner, N. (2015) Mitochondrial dysfunction and insulin resistance: an update. Endocr. Connect. 4, R1–R15.
[57] Osman, C., Merkwirth, C., and Langer, T. (2009) Prohibitins and the functional compartmentalization of mitochondrial membranes. J. Cell Sci. 122, 3823–3830.
[58] Steglich, G., Neupert, W., and Langer, T. (1999) Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. Mol. Cell Biol. 19, 3435–3442.
[59] Ishihara, N., Fujita, Y., Oka, T., and Mihara, K. (2006) Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. EMBO J. 25, 2966–2977.
[60] Strub, G. M., Paillard, M., Liang, J., Gomez, L., Allegood, J. C., et al. (2011) Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration. FASEB J. 25, 600–612.
[61] Schleicher, M., Shepherd, B. R., Suarez, Y., Fernandez-Hernando, C., Yu, J., et al. (2008) Prohibitin-1 maintains the angiogenic capacity of endothelial cells by regulating mitochondrial function and senescence. J. Cell Biol. 180, 101–112.
[62] Bogenhagen, D. F., Wang, Y., Shen, E. L., and Kobayashi, R. (2003) Protein components of mitochondrial DNA nucleoids in higher eukaryotes. Mol. Cell Proteomics 2, 1205–1216.
[63] Wang, Y. and Bogenhagen, D. F. (2006) Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane. J. Biol. Chem. 281, 25791–25802.
[64] Fu, P., Yang, Z., and Bach, L. A. (2013) Prohibitin-2 binding modulates insulin-like growth factor-binding protein-6 (IGFBP-6)-induced rhabdomyosarcoma cell migration. J. Biol. Chem. 288, 29890–29900.
[65] Lucas, C. R., Cordero-Nieves, H. M., Erbe, R. S., McAlees, J. W., Bhatia, S., et al. (2013) Prohibitins and the cytoplasmic domain of CD86 cooperate to mediate CD86 signaling in B lymphocytes. J. Immunol. 190, 723–736.
[66] Zhang, Y., Wang, Y., Xiang, Y., and Lee, W. (2012) Prohibitins are involved in protease-activated receptor 1-mediated platelet aggregation. J. Thromb. Haemost. 10, 411–418.
[67] Emerson, V., Holtkotte, D., Pfeiffer, T., Wang, I. H., Schnolzer, M., et al. (2010) Identification of the cellular prohibitin 1/prohibitin 2 heterodimer as an interaction partner of the C-terminal cytoplasmic domain of the HIV-1 glycoprotein. J. Virol. 84, 1355–1365.
[68] Santhanam, S. K., Dutta, D., Parween, F., and Qadri, A. (2014) The virulence polysaccharide Vi released by Salmonella Typhi targets membrane prohibitin to inhibit T-cell activation. J. Infect. Dis. 210, 79–88.
[69] Sharma, A. and Qadri, A. (2004) Vi polysaccharide of Salmonella typhi targets the prohibitin family of molecules in intestinal epithelial cells and suppresses early inflammatory responses. Proc. Natl. Acad. Sci. USA 101, 17492–17497.
[70] Cornillez-Ty, C. T., Liao, L., Yates, J. R., 3rd, Kuhn, P., and Buchmeier, M. J. (2009) Severe acute respiratory syndrome coronavirus nonstructural protein 2 interacts with a host protein complex involved in mitochondrial biogenesis and intracellular signaling. J. Virol. 83, 10314–10318.
[71] Kuadkitkan, A., Wikan, N., Fongsaran, C., and Smith, D. R. (2010) Identification and characterization of prohibitin as a receptor protein mediating DENV-2 entry into insect cells. Virology 406, 149–161.
[72] Jupe, E. R., Badgett, A. A., Neas, B. R., Craft, M. A., Mitchell, D. S., et al. (2001) Single nucleotide polymorphism in prohibitin 3′ untranslated region and breast-cancer susceptibility. Lancet 357, 1588–1589.
[73] (2014) GeneCards:The Human Gene Compendium: PHB2.
[74] Ande, S. R. and Mishra, S. (2009) Prohibitin interacts with phosphatidylinositol 3,4,5-triphosphate (PIP3) and modulates insulin signaling. Biochem. Biophys. Res. Commun. 390, 1023–1028.
[75] Moritz, A., Li, Y., Guo, A., Villén, J., Wang, Y., et al. (2010) Akt-RSK-S6 kinase signaling networks activated by oncogenic receptor tyrosine kinases. Sci Signal 3, ra64.