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PH Domain Leucine-Rich Repeat Protein Phosphatase (PHLPP)

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Synonyms

SCN circadian oscillatory protein; SCOP

Historical Background

In 1999, Shimuzu et al. identified a novel transcript that oscillated in a circadian-dependent manner in the rat suprachiasmatic nucleus (SCN), a bundle of neurons in the brain responsible for controlling the mammalian circadian clock (Shimizu et al. 1999). Thus, they named this gene product SCN circadian oscillatory protein (SCOP), but the function of the protein encoded by this gene was shrouded in mystery. Six years later, Newton and colleagues identified this same gene in a rational search for a serine/threonine phosphatase responsible for dephosphorylating and inactivating the pro-survival kinase, Akt (Gao et al. 2005). Upon agonist stimulation, Akt is recruited to the plasma membrane by its phosphoinositide-sensing pleckstrin homology (PH) domain, where it becomes phosphorylated by another PH domain-containing kinase, the phosphoinositide-dependent kinase-1 (PDK-1). Hypothesizing that a phosphatase that regulates Akt may also have such a domain, they queried the human genome for a gene predicted to encode both a PH domain and a phosphatase domain: two such genes exist that encode two highly related proteins, which they named after the domain structure, hence PH domain leucine-rich repeat protein phosphatase, or PHLPP (pronounced "flip"). PHLPP1 (corresponding to the original SCOP) and PHLPP2 play central roles in suppressing survival signaling. In the decade since this discovery, PHLPP phosphatases have been implicated in the regulation of diverse signaling pathways, and deregulation of PHLPP homeostasis in the cell is associated with pathologies, most notably cancer.

PHLPP Domain Composition and Structure

The PHLPP family of serine/threonine phosphatases belongs to the PP2C branch of the metal-dependent protein phosphatase (PPM) family;

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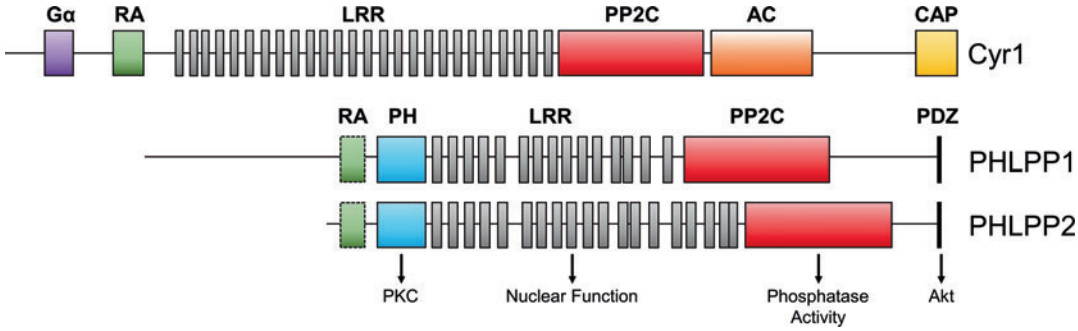
PP2C phosphatases rely on the presence of Mg^{2+} or Mn^{2+} for catalytic activity. The presence of four conserved Asp residues in the active site of PP2C phosphatases allows coordination of these metal ions. PHLPP1 and PHLPP2 have an unusual active site architecture in that only three of these four conserved acidic residues are present (Sierecki and Newton 2014). Similar to other PP2C phosphatases, PHLPP activity is insensitive to a number of common phosphatase inhibitors, such as microcystin and okadaic acid. Two small molecule inhibitors of the PHLPP phosphatases were discovered utilizing chemical and virtual screens of the National Cancer Institute (NCI) repository. These two compounds selectively inhibit PHLPP1 and PHLPP2 (in vitro IC_{50} values in 5 μM range) compared to the closely related PP2Cα (Sierecki et al. 2010).

Unlike protein kinases that can phosphorylate specific consensus motifs, most phosphatases lack intrinsic substrate specificity. Rather, they rely on the presence of regulatory subunits and domains to dephosphorylate the correct substrate at the correct location and correct time. PHLPP phosphatases have their regulatory domains on the same polypeptide as the phosphatase domain, in contrast to other well-studied phosphatases, such as PP2A, whose regulatory regions are on separate protein subunits. PHLPP1 and PHLPP2 have a similar domain architecture, with a putative Ras association domain, a pleckstrin homology domain, a leucine-rich repeat (LRR) region, and a PDZ-binding ligand, in addition to a PP2C phosphatase domain (Fig. 1). The key difference between the two isozymes is that PHLPP1 contains a 56 kDa N-terminal extension which has yet to be fully characterized. These regulatory domains play an important role in docking PHLPP at specific scaffolds or to other proteins that allow PHLPP to be in close enough proximity to its substrates.

PHLPP is evolutionarily conserved from yeast to humans. The yeast homologue of PHLPP, called Cyr1, also contains a LRR region immediately followed by a PP2C phosphatase domain, a domain organization unique to this family of phosphatases (Fig. 1). However, the phosphatase domain of this yeast protein has yet to be characterized. Intriguingly, Cyr1 also contains a catalytic adenylate cyclase domain on its C-terminal end and is the only adenylate cyclase encoded for in the yeast genome, in contrast to the ten adenylate cyclases present in mammals. Cyr1 also has additional regulatory domains, such as a Gα-binding domain and a cyclase-associated protein 1 (CAP)-binding domain, which are important



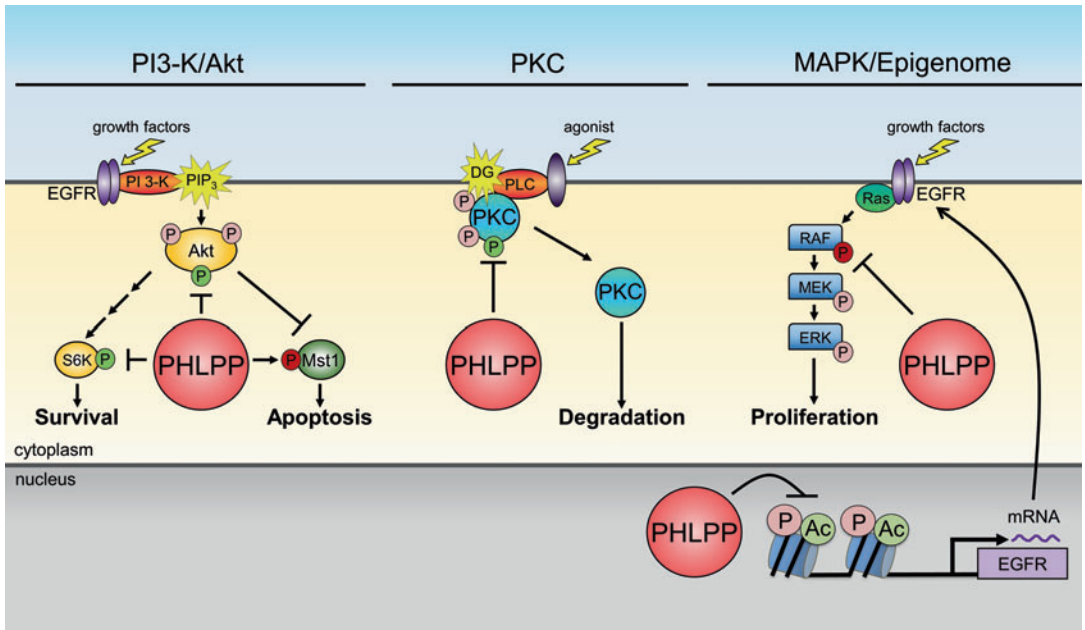
PH Domain Leucine-Rich Repeat Protein Phosphatase (PHLPP), Fig. 1 PHLPP domain structure: the PHLPP family of phosphatases is composed of two isozymes, PHLPP1 (1717 amino acids) and PHLPP2 (1323 amino acids). Both isozymes have similar domain structures: a predicted Ras association (RA) domain (*green*), a pleckstrin homology (PH) domain (*blue*), a leucine-rich repeat (LRR) region (*gray*), a PP2C phosphatase domain (*red*), and a PDZ-binding ligand (*black*). PHLPP1 has a large N-terminal extension approximately 50 kDa in size that is not present in PHLPP2. Cyr1 (2026 amino acids) is the yeast homologue of PHLPP. In addition to an RA domain, and a LRR region followed immediately by a PP2C phosphatase, it also contains a regulatory G-α-binding domain (*purple*), the only adenylate cyclase (AC) in yeast (*orange*), and a cyclase-associated protein 1 (CAP)-binding domain (*yellow*). *Dotted lines* denote predicted domains. Domains required for the cellular regulation of PKC, Akt, and the nuclear function of regulating EGFR transcription are indicated with an *arrow*

for adenylate cyclase catalytic activity. Unlike human adenylate cyclase, yeast Cyr1 is not membrane bound and its activity is regulated by the small GTPase, Ras. In yeast, the cyclic AMP (cAMP)/protein kinase A (PKA) signaling pathway is vital for nutritional sensing and growth, with deletion of the gene encoding Cyr1 resulting in cell cycle arrest at the G1 phase (Matsumoto et al. 1982). Increases in the extracellular glucose concentration result in RAS-associated activation of adenylate cyclase and the production of cAMP, a critical cofactor for the enzyme PKA. Elevation of cAMP leads to robust changes in the transcriptome that support growth and fermentation (Russell et al. 1993). Whether mammalian PHLPP has retained any function in regulating cAMP/PKA signaling from its distant yeast homologue remains to be investigated.

PHLPP Signaling

PI3-K/Akt

The first identified substrate of PHLPP was the proto-oncogene AGC kinase, Akt (Fig. 2). Upon stimulation of cellular receptor tyrosine kinases by mitogenic signals, such as growth factors, the phosphoinositide 3-kinase (PI3-K) becomes activated and generates the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3) at the plasma membrane. The generation of PIP_3 results in Akt localization to the plasma membrane via its PH domain, where Akt is then phosphorylated on two key sites required for full catalytic activation: Thr308 on the activation loop and Ser473 at the hydrophobic motif. The phosphorylated enzyme is locked in an active conformation and phosphorylates substrates throughout the cell. Signaling by PI3-K/Akt is



PH Domain Leucine-Rich Repeat Protein Phosphatase (PHLPP), Fig. 2 PHLPP function: PHLPP suppresses cell signaling by diverse mechanisms. PHLPP directly dephosphorylates AGC kinases, such as Akt, S6K, and PKC, at their hydrophobic motifs (*green phosphate*). Dephosphorylation of Akt and S6K results in kinase inactivation. In the case of PKC, dephosphorylation results in its degradation, indirectly resulting in loss of kinase activity. PHLPP dephosphorylates non-AGC kinases as well, such as an inhibitory site (*red phosphate*) on the pro-apoptotic kinase, Mst1, leading to its activation. In the nucleus, PHLPP suppresses histone acetylation and phosphorylation to reduce gene expression of receptor tyrosine kinases, such as EGFR. Additionally, PHLPP1 inhibits RAF1 phosphorylation in vitro, which may provide another route to dampen MAPK (mitogen-activated protein kinase) signaling

terminated by dephosphorylation of PIP_3 by the lipid phosphatase PTEN and by dephosphorylation of Akt. Dephosphorylation of Thr308 was known to be catalyzed by the phosphatase PP2A but the identity of the Ser473 phosphatase awaited the discovery of PHLPP (Gao et al. 2005). There are three isozymes of Akt, and they are differentially regulated by PHLPP1 and PHLPP2 in cells: PHLPP1 selectively dephosphorylates Akt2, and PHLPP2 selectively dephosphorylates Akt1 (Brognard et al. 2007). Both isozymes dephosphorylate Akt3 in cells. The PDZ ligand on the C-terminal end of PHLPP1 (DPTL) is required for its cellular regulation of Akt, as deletion of this sequence suppressed the ability of PHLPP to dephosphorylate Akt in cells (Gao et al. 2005). As PHLPP1 and PHLPP2 have different PDZ ligand sequences (DTAL in PHLPP2), it is possible that these unique PDZ ligands drive selectivity for Akt by coordinating distinct signaling complexes. Interestingly, the PH domain of PHLPP is not involved in the cellular recognition of Akt; a construct lacking the PH domain is still able to dephosphorylate Akt. By dephosphorylating Akt, PHLPP suppresses the pro-survival pathways turned on by Akt activation. Thus, loss of PHLPP results in decreased apoptosis and increased cellular survival and proliferation, a hallmark of cancer.

PHLPP regulates other nodes of the PI3-K/Akt signaling cascade as well, a first clue of its widening role in dampening cellular signaling output (Fig. 2). Gao and colleagues demonstrated that PHLPP dephosphorylates the hydrophobic motif of S6K1, an AGC kinase downstream of Akt (Liu et al. 2011). But, PHLPP is not just a "hydrophobic motif" phosphatase: Pardee and coworkers demonstrated that PHLPP can directly dephosphorylate the pro-apoptotic kinase Mst1, a member of the STE kinase family (Qiao et al. 2010). Mst1 is phosphorylated by Akt on Thr387, which results in inhibition of Mst1 catalytic activity, decreasing levels of apoptosis. Dephosphorylation of this site by PHLPP results in an increase in Mst1 activity and downstream signaling, resulting in increased levels of apoptosis. Thus, PHLPP suppresses pro-survival signaling not only by removing an activating phosphorylation on the pro-survival kinases Akt and S6K1 but also by removing an inactivating phosphorylation on the pro-apoptotic kinase Mst1.

Protein Kinase C

Similar to other AGC kinase family members such as Akt and S6K, protein kinase C (PKC) is also phosphorylated on its hydrophobic motif (Fig. 2). In contrast to Akt, where the phosphorylation of the hydrophobic motif is agonist-evoked and required for full catalytic activity, phosphorylation of the PKC hydrophobic motif is constitutive and required for protein stability. PHLPP dephosphorylates the hydrophobic motif of conventional and novel PKC family members (atypical PKC family members have a Glu at this position) (Gao et al. 2008). The PH domain of PHLPP is required for it to dephosphorylate PKC in the cell, most likely by playing a role in scaffolding PHLPP near PKC. Dephosphorylation of this site by PHLPP results in degradation of PKC (Gao et al. 2008). Thus, PHLPP activity controls the steady-state levels of conventional and novel PKC isozymes.

Regulation of Receptor Tyrosine Kinase Levels

To date, most studies have focused on the role of PHLPP in regulating cytoplasmic substrates. Recent evidence supports a novel role for PHLPP in the nucleus as a regulator of histone posttranslational modifications and gene transcription (Reyes et al. 2014). Specifically, both PHLPP1 and PHLPP2 have been shown to suppress the transcription of receptor tyrosine kinases (RTKs), such as the epidermal growth factor (EGF) receptor, reducing their steady-state levels and overall signaling output (Fig. 2). Studies with PHLPP1 reveal that this function depends on the LRR domain as constructs of PHLPP1 lacking the LRR, while retaining full activity toward cytosolic substrates such as Akt and PKC no longer regulate RTK transcription. This function is restored by addition of a nuclear localization signal to the LRR-deleted construct of PHLPP1, suggesting a role for the LRR in localizing PHLPP to the nucleus. Chromatin immunoprecipitation studies reveal that PHLPP1 associates with the promoters of genes that are sensitive to

PHLPP1 loss, including the EGF receptor. In addition to suppressing gene transcription, both PHLPP1 and PHLPP2 suppress acetylation and phosphorylation of specific histones on specific residues. Thus, for example, the double modification of acetylation on Lys 9 and phosphorylation on Ser 10 is significantly enhanced on histone H3 in embryonic fibroblasts from mice lacking PHLPP1. Whether PHLPP regulates the enzymes that catalyze histone posttranslational modifications, or whether it directly dephosphorylates histones remains to be determined.

The increased steady-state levels of RTKs upon loss of PHLPP result in amplified signaling through the MEK/ERK cascade, enhancing proliferation (Fig. 2). Gao and colleagues also reported that knockdown of either PHLPP1 or PHLPP2 in colorectal cancer cells increased the amplitude of ERK signaling, resulting in increased cell migration and invasion (Li et al. 2014). However, they suggested that PHLPP may directly dephosphorylate the kinase RAF1 (c-RAF), an enzyme downstream of EGFR and Ras, as both isozymes can dephosphorylate an activating site, phospho-Ser338 in vitro and both interact with c-RAF in cells (Li et al. 2014). These data suggest a complex role for PHLPP in the regulation of MAPK signaling, in both a direct and indirect manner.

PHLPP and Disease

Given its role in regulating key survival and growth pathways, it comes as no surprise that deregulation of PHLPP expression or activity has been correlated with a number of disease states, most notably cancer. Both PHLPP1 and PHLP2 have been well established as tumor suppressors. Both genes are located on chromosomal regions that are frequently deleted in diverse cancers (as reviewed in O'Neill et al. 2013). A multitude of cellular studies have confirmed that loss of PHLPP expression results in increased cellular proliferation and growth. Furthermore, PHLPP1 knockout mice develop prostate neoplasias, which progress to carcinomas when combined with partial loss of the tumor suppressor PTEN (Chen et al. 2011).

Heart disease and diabetes are two diseases in which Akt activity is reduced rather than activated, and increased PHLPP activity has been implicated in both of these pathologies. Notably, PHLPP1, but not PHLPP2, protein expression is elevated in skeletal muscle and adipose tissue in diabetic patients compared to healthy counterparts (Andreozzi et al. 2011; Cozzone et al. 2008). Since Akt activity is important for cellular glucose uptake following insulin stimulation, increased PHLPP dephosphorylation of Akt would result in a dampened response following insulin stimulation. This could be a potential mechanism contributing to insulin insensitivity in type II diabetic patients. In the context of the heart, Akt activity is vital in regulating cardiac cell survival. Brown and colleagues demonstrated that PHLPP1 knockout mice subjected to cardiac stress display a cardioprotective phenotype (Miyamoto et al. 2010). Following ischemia and reperfusion, PHLPP1 knockout mice have increased levels of phosphorylated Akt and decreased infarct size compared to wild-type controls. These results demonstrate that decreased PHLPP activity may provide a therapeutic benefit in the context of heart disease.

PHLPP has been implicated in a number of other physiological processes that could contribute to disease, but a clear link has yet to be defined. For example, Sassone-Corsi and colleagues have demonstrated that PHLPP1 is required for proper maintenance of the circadian rhythm in mice (Masubuchi et al. 2010); mice lacking PHLPP1 have deficiencies in light-induced resetting of the circadian rhythm. Although the mechanism of this pathway has yet to be elucidated, these results illustrate that changes in PHLPP expression or activity could result in circadian rhythm disorders. Recent evidence suggests a role for PHLPP in the regulation of bone development (Bradley et al. 2013, 2015). Westendorf and colleagues demonstrated that PHLPP1 expression is downregulated to allow increased signaling through Akt during chondrocyte proliferation. Further work from Westendorf's group demonstrated that expression of PHLPP1 was elevated in human patients with osteoarthritis and that mice lacking PHLPP1 have decreased susceptibility to cartilage deterioration in a surgically induced osteoarthritis model.

Summary

PHLPP has emerged as a key player in the suppression of survival signaling pathways. Its best characterized function to date is its dampening of growth factor signaling by directly dephosphorylating the hydrophobic motif of Akt. However, it also suppresses survival signaling by removing an activating phosphorylation on the pro-apoptotic kinase, Mst1. Most recently, it has been shown to generally suppress growth factor signaling by setting the amplitude of receptor tyrosine kinase signaling output: specifically, it suppresses the transcription, and thus steady-state levels, of RTKs such as the EGF receptor. PHLPP also regulates the steady-state levels of PKC isozymes by dephosphorylating their hydrophobic motif to promote their degradation. Because PKC isozymes are generally tumor suppressors, it is intriguing to speculate whether PHLPP may have oncogenic roles in some contexts. What is clear is that maintaining proper balance of PHLPP expression and activity in the cell is key for preventing pathologies. By further expanding our understanding of how PHLPP functions in the cell, we can gain a greater understanding of how to target it for therapeutic benefits.

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