Regulation of the membrane-associated tumor suppressor phosphatase PHLPP

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Abstract & Aims

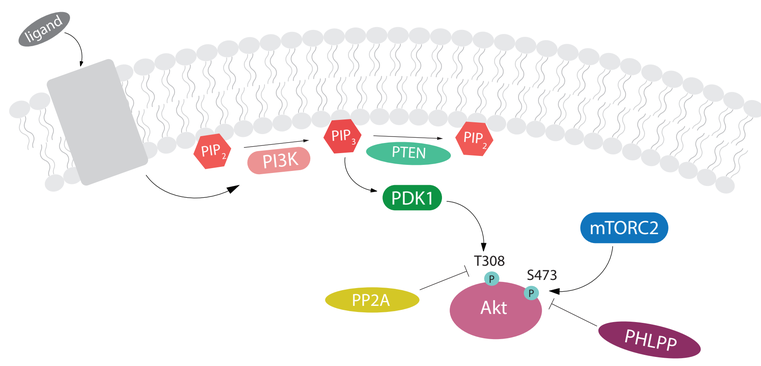
Regulation of substrate phosphorylation by kinases and their dephosphorylation by phosphatases is important for maintaining cellular homeostasis, as dysregulation of these processes can often lead to pathologies. While kinases phosphorylate their substrates only at residues in specific sequence motifs, phosphatases tend not to recognize these.

The PH-domain leucine-rich repeat phosphatase (PHLPP) was recently found to play an important role in negatively regulating substrates like Akt/Protein Kinase B and Protein Kinase C by dephosphorylating an important regulatory serine in their respective hydrophobic motifs [1], [2]. However, the precise mechanism of this reaction, and how substrate specificity is ultimately achieved, remains to be elucidated. By employing a combination of biochemical and structural biology approaches, we aim to understand this regulation by focusing on one of PHLPP's best-characterized substrates, Akt/PKB.

Aims

1. How is substrate specificity of PHLPP achieved?
2. Is PHLPP really an Akt hydrophobic motif phosphatase?
3. How and where does dephosphorylation occur in the cell?
4. How is PHLPP itself regulated?
5. What is the structure of PHLPP?

PI3K/Akt pathway

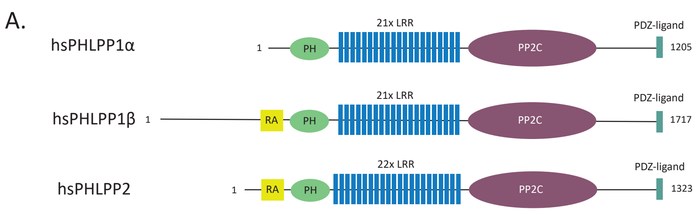


Akt/PI3K pathway

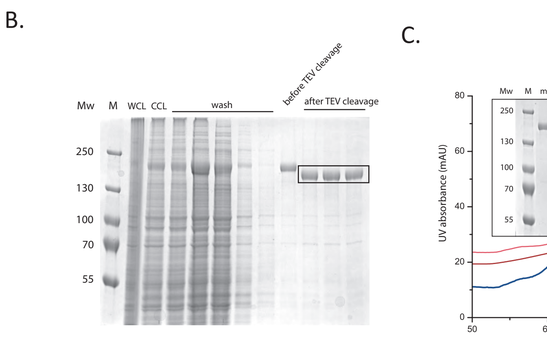
Akt is activated downstream of Phosphoinositide-3 kinase (PI3K) which phosphorylates Phosphatidylinositol-4,5-bisphosphate (PIP_2) to yield Phosphatidylinositol-3,4,5-trisphosphate (PIP_3). Once the lipid is available, Akt is recruited to the membrane and activated through phosphorylation of Thr308 at its activation loop (AL) by PDK1, and of S473 in its hydrophobic motif (HM) by mTORC2. Canonically, dephosphorylation is achieved by PP2A at the AL and by PHLPP at the HM of Akt.

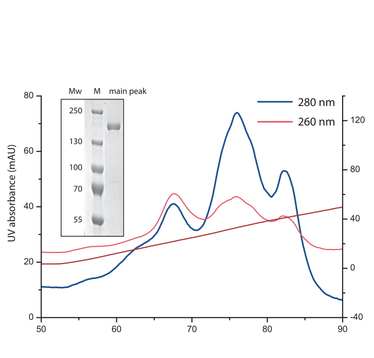
Results

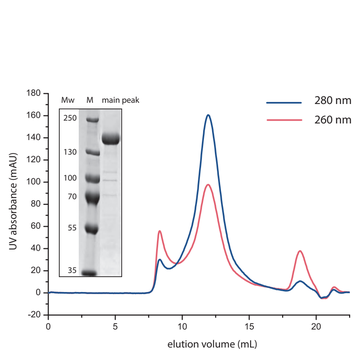
a) Purification of full-length PHLPP2



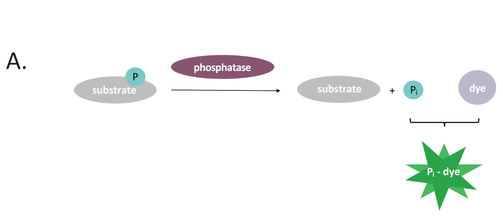
A. Overview of the different isoforms of PHLPP. $PHLPP2^{1-1323}$ was used for purification.
B. Affinity purification on GST-beads. N-terminal GST-tag was subsequently removed by TEV-cleavage.
C. Ion-exchange chromatogram (Inlet: SDS-PAGE gel with PHLPP2 at ca. 150 kDa, main peak fraction).
D. Size-exclusion chromatogram (Inlet: SDS-PAGE gel with PHLPP2 at ca. 150 kDa, main peak fraction)





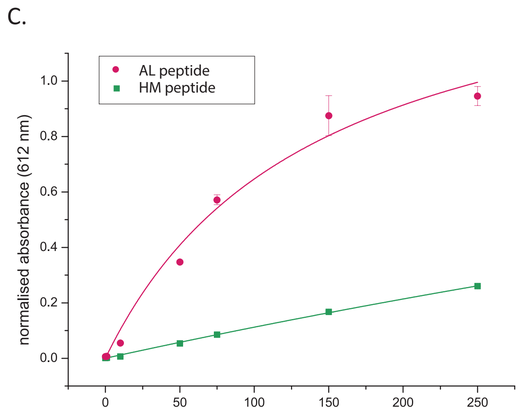


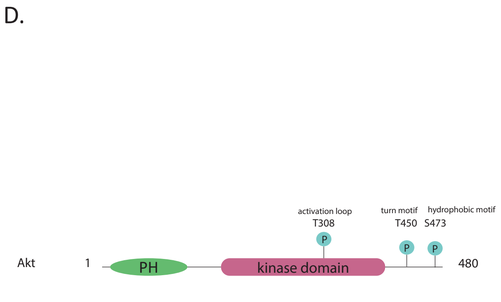
b) Biochemical characterization of purified PHLPP2: dephosphorylation of synthetic Akt phospho-peptides and in vitro purified full-length Akt



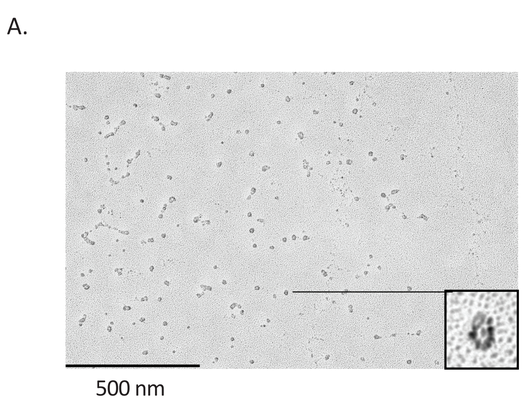
B.

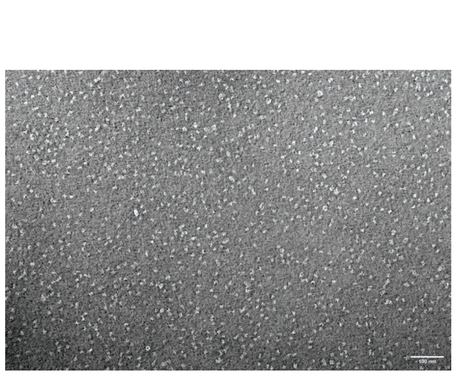
Hydrophobic motif	HFPQFSYSAS (P)
Activation loop	GATMKTFCGT (P)



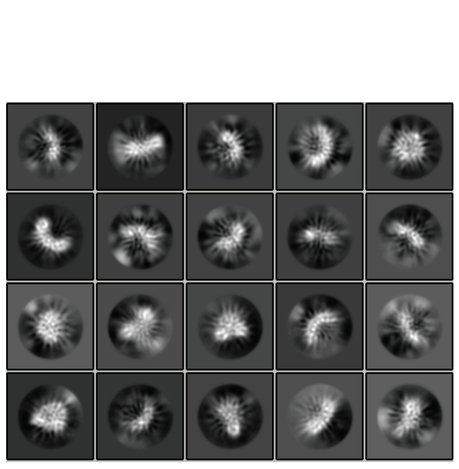


c) Electron microscopy data of PHLPP2

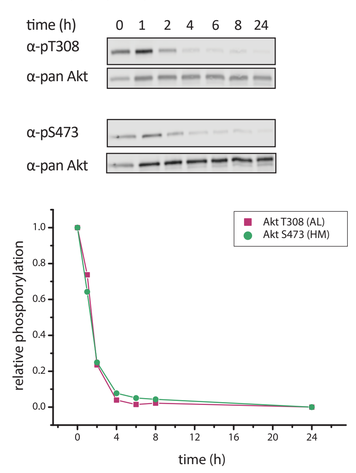




C.



A. Rotary shadowing electron microscopy with full-length PHLPP2. Scale bar represents a length of 500 nm.
B. Negative-stain electron microscopy of full-length PHLPP2. The scale bar represents a length of 100 nm.
C. First 2D classifications of the dataset in B. Data was processed in RELION [3].



A. Dephosphorylation assay mechanism.
B. Phosphopeptides of the activation loop (AL), or the hydrophobic motif (HM) of Akt that were used as substrates for PHLPP in the dephosphorylation assay.
C. Dephosphorylation of full-length PHLPP incubated with increasing concentrations of AL or HM peptides plotted against the absorbance at 612 nm.
D. wt Akt construct used in dephosphorylation assay in E
E. Western blot of the dephosphorylation time-course of 0.375 uM wt full-length Akt with 1 uM PHLPP.

Discussion & Future Objectives

The biochemical characterization of the recombinantly purified protein showed that PHLPP2 favours the amino acid sequence of Akt's activation loop over the hydrophobic motif which is in contrast to previously published data. Also, there was no preference for either residue when dephosphorylating in vitro purified full-length Akt. For these reasons we'd now like to employ a phospho-proteomics approach in order to look for a PHLPP substrate in an unbiased manner.

Furthermore, since there is no structural data available for PHLPP and crystallization failed so far, we would like to determine the structure of full-length PHLPP2 by cryoEM. A structure would provide us with information about the nature of its catalytic center, residues that are important for catalytic activity and metal-ion coordination, and the arrangement of PHLPP's domains with respect to each other.

References

[1] Gao et al., Molecular Cell, Vol. 18, 13-24, 2005

[2] Gao et al., JBC, Vol. 283, NO. 10, 6300-6311, 2008

[3] Scheres, Journal of Structural Biology, 180(3), 519-530, 2012

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