Mol231:Interaction mapping of the phosphoinositide pathway with the alternative splicing factor PTBP1

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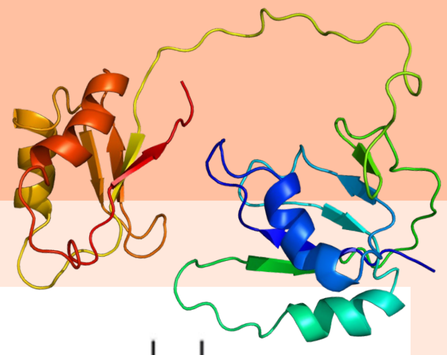


Introduction

In higher eukaryotes, mRNA undergoes alternative splicing, creating several different mRNA variants. Deregulation of this process is linked to several diseases, including cancer (1). While the molecular mechanisms of splicing are well known, the regulation of alternative splicing remains largely unknown.

Using mass spectrometry-based interactomics, an interaction was identified between the alternative splicing factor, polypyrimidine tract-binding protein 1 (PTBP1), and the signalling lipids polyphosphoinositides (PPIns), PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 (2,3). The exact interaction site involving two lysine residues, K339A and K440L, has been found (not published). **The specificity of interaction amongst all PPIns is however still unclear.**

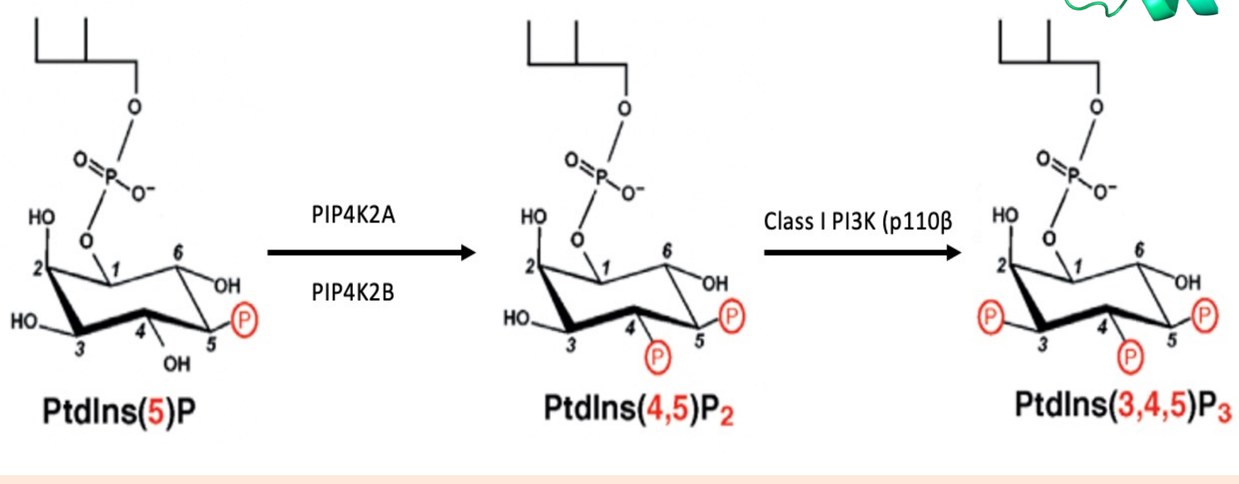


Figure 1: Metabolic pathway of phosphatidylinositol(4,5)P_2 in the nucleus

Aim

To determine the specificity of interaction of phosphoinositides and metabolizing enzymes with PTBP1.

Methods

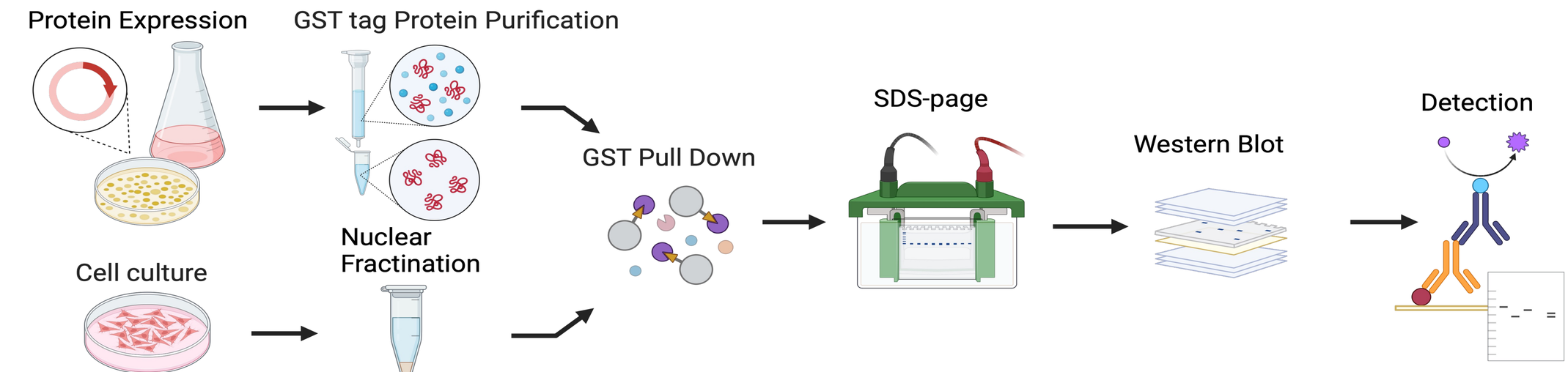
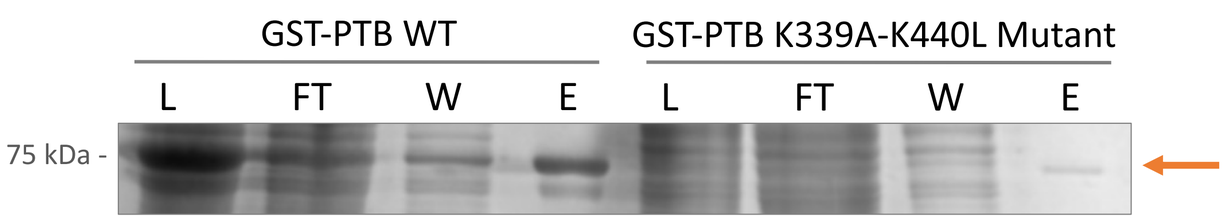


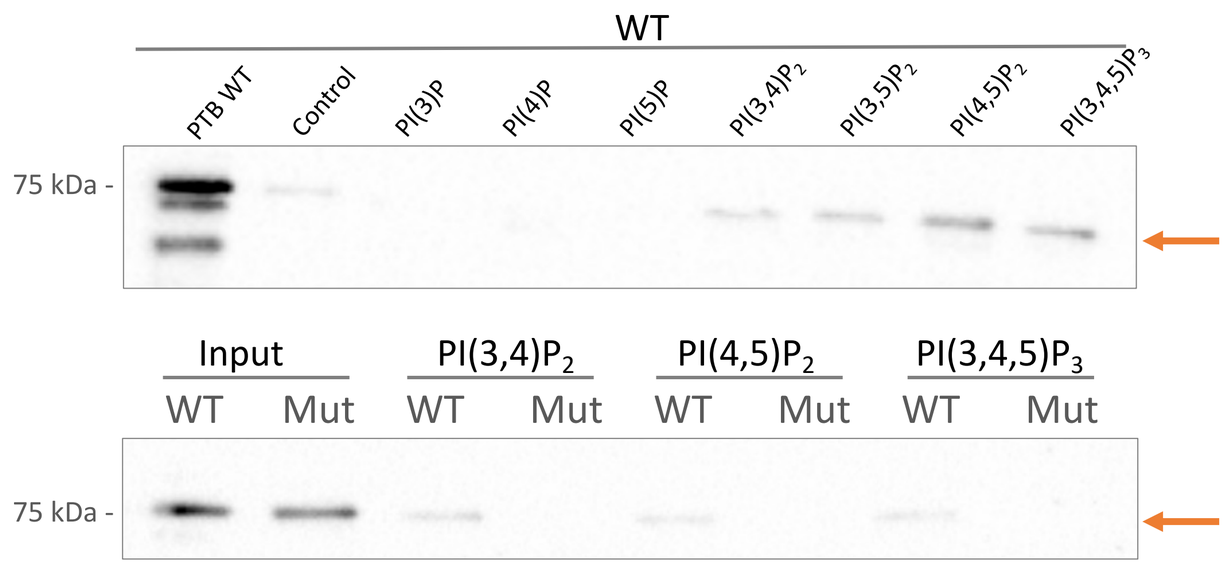
Figure 2: Illustration of main methods. Figure created using Biorender.com

Results

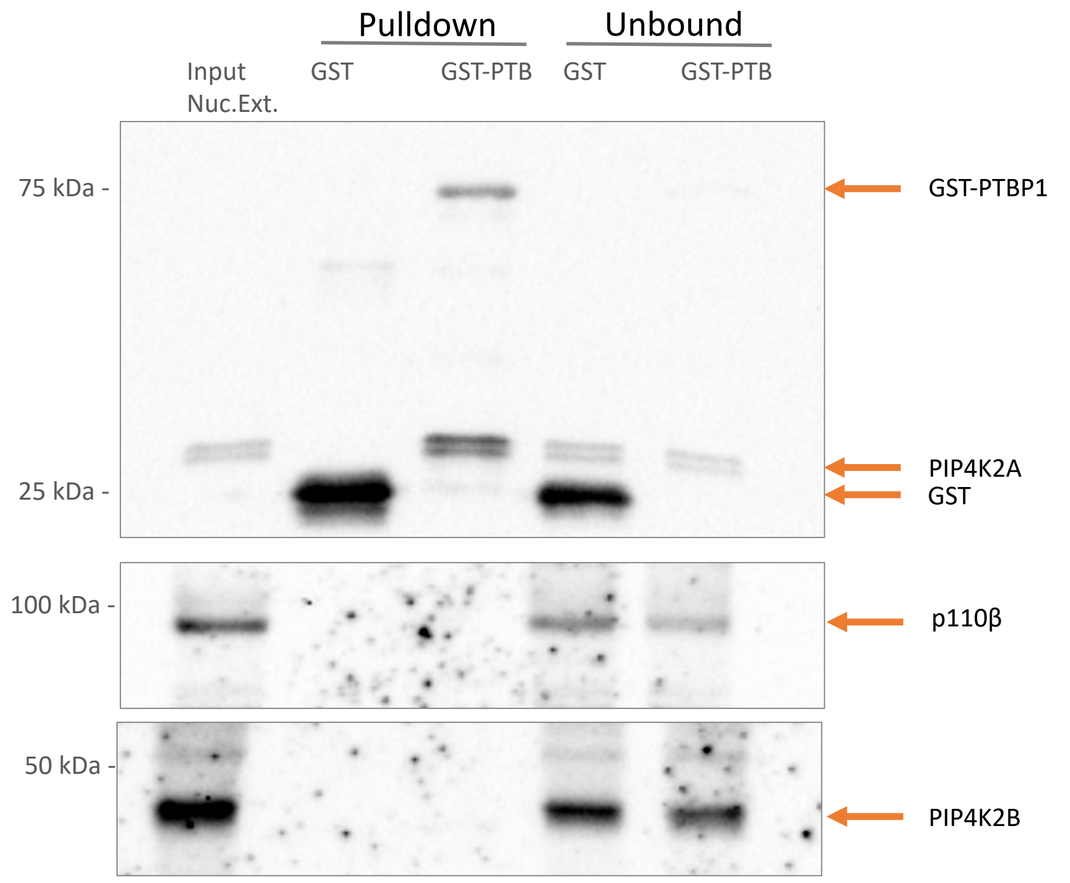
Protein Purification



Interaction of polyphosphoinositides with GST-PTB WT via K339-K440



Interaction between GST-PTB and the PI(4,5)P_2 generating enzyme



Conclusion

From the PIP-Pulldown, GST-PTBP1 WT was found to interact with PI(4,5)P_2 and PI(3,4,5)P_3. The GST-PTBP1 Mutant showed no interactions with the polyphosphoinositides.
GST-PTBP1 showed no direct interaction with p110β and the PI(4,5)P_2 generating enzyme PIP4K2B.
However, a direct interaction between GST-PTBP1 and the PI(4,5)P_2 generating enzyme PIP4K2A was showed.

Refrences:
1. Baralle FE, Giudice J. Alternative splicing as a regulator of development and tissue identity. Nat Rev Mol Cell Biol. 2017;18(7):437-51.
2. Lewis AE, Sommer L, Arntzen MO, Strahm Y, Morrice NA, Divecha N, et al. Identification of nuclear phosphatidylinositol 4,5-bisphosphate-interacting proteins by neomycin extraction. Mol Cell Proteomics. 2011;10(2):M110 003376.
3. F Mazloumi Gavgani, M Skuseth Slinning, A Papdiné Morovicz, V Smith Arnesen, D C Turcu, S Ninzima, CS D'Santos and AE Lewis (2021). Nuclear phosphatidylinositol 3,4,5-trisphosphate interactome uncovers an enrichment in nucleolar proteins. Molecular & Cellular Proteomics. 20:100102. doi: 10.1016/j.mcpro.2021.100102.
4. Dai S, Wang C, Zhang C, Feng L, Zhang W, Zhou X, He Y, Xia X, Chen B, Song W. PTB: Not just a polypyrimidine tract-binding protein. J Cell Physiol. 2022 May;237(5):2357-2373. doi: 10.1002/jcp.30716. Epub 2022 Mar 14. PMID: 35288937.