Generation of a molecular tool to manipulate levels of PtdIns(4,5)P2 in the nucleus of HeLa cells

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Introduction

PtdIns(4,5)*P*2 is a key lipid signalling molecule involved in many molecular processes. It is generated either via a phosphorylation by lipid kinases or de-phosphorylation by the 3-phosphatase PTEN (Fig 1).

PtdIns(4,5)P2 functions primarily in cytoplasmic membranes but has also been shown to be present in the nucleus where it interacts with a nuclear enzyme, DNA Topoisomerase IIα, to inactivate it. However, details of the nuclear mechanisms are unknown.

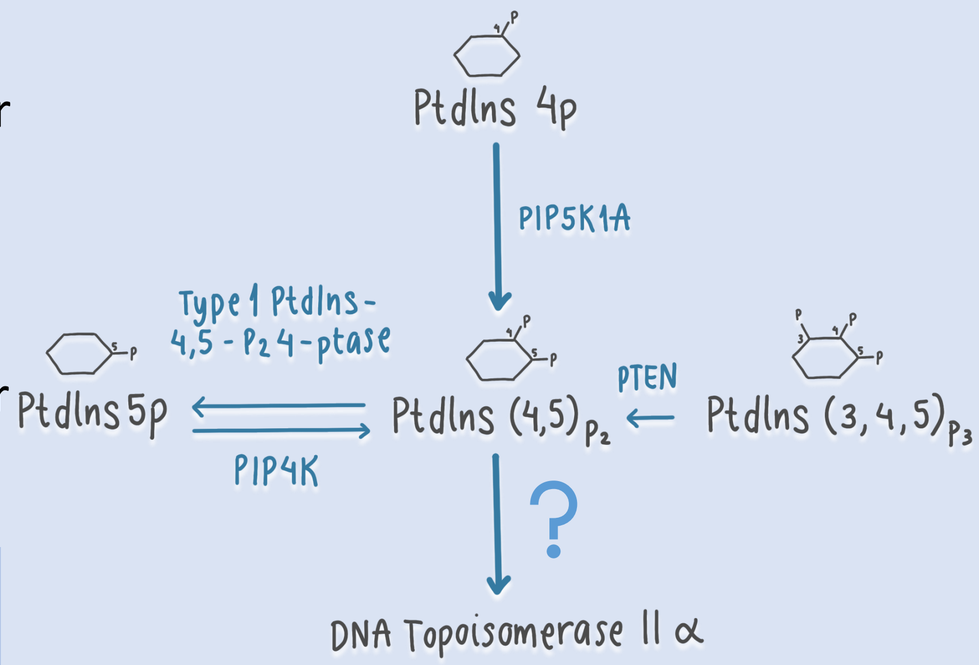


Figure 1: *Pathways regulating levels of PtdIns(4,5)P2*

Aim

To manipulate the levels of PtdIns(4,5)*P*2 in the nucleus by targeting the Type I PtdIns(4,5)P2-4Ptase to the nucleus.

Methods

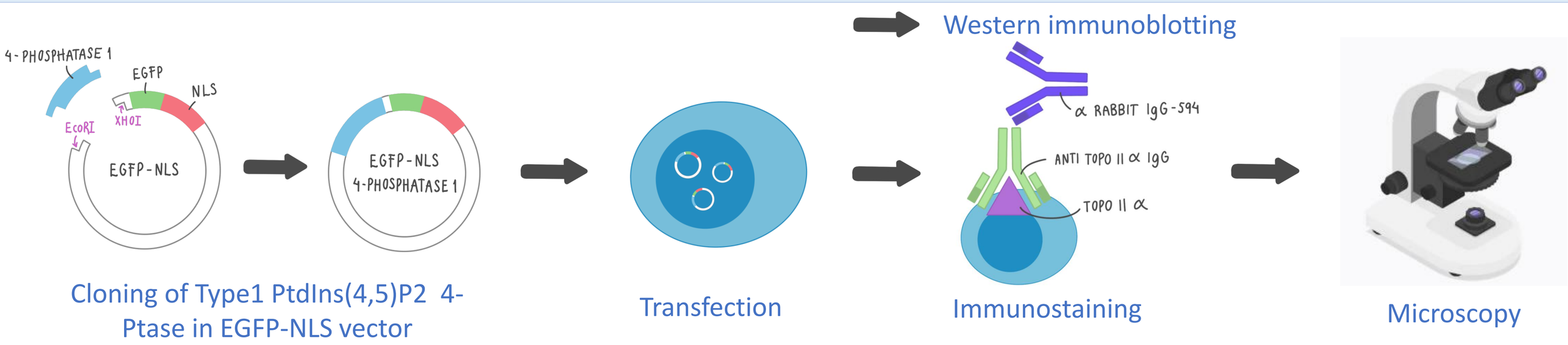


Figure 2: *Illustration of methodology from cloning the Type-1 PtdIns-4,5-P2 4-Ptase, transfecting into HeLa cells.*

Results

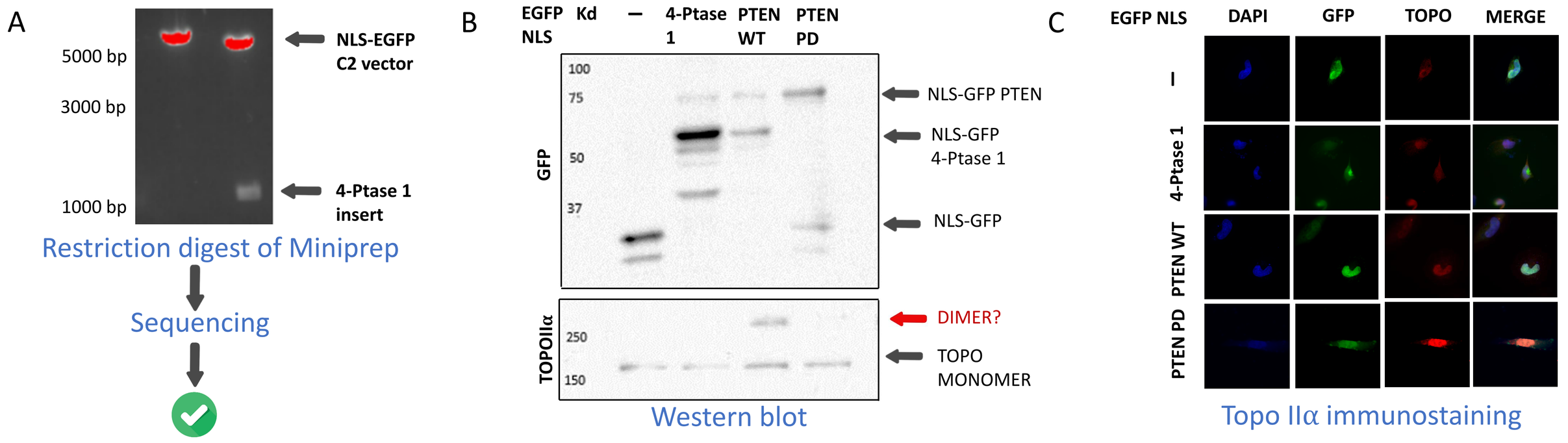


Figure 3: *NLS-EGFP-4Ptase 1 cloning, protein expression and subcellular localisation. A. Restriction digest of miniprep and sequencing, B, western blot and C. immunofluorescent imaging results of HeLa cells transfected with the indicated constructs*.

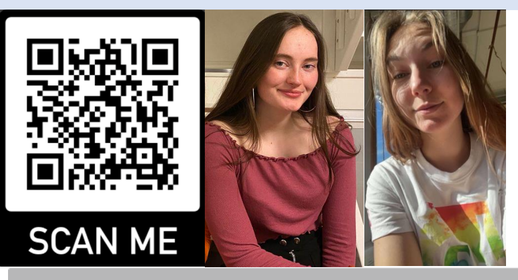
Conclusions

Type1 PtdIns(4,5)P2 4-Ptase was successfully cloned in the vector and is expressed well following transfection. However, it can still be observed in the cytoplasm, likely in the Golgi, suggesting that the NLS is not strong enough for anchoring it to the nucleus.

Type 1 PtdIns(4,5)P2 4-Ptase does not seem to affect the levels of DNA Topoisomerase IIα whereas the 3-fosfatase PTEN appears to increase a potential dimer.

Further analysis

- Clone 4-Ptase into a 3xNLS to increase its anchoring in the nucleus
- Compare with a 4,5-P2 antibody
- Generate a C133S phosphatase dead mutant 4-Ptase-1



Sources:
WIS, A. E., SOMMER, L., ARNTZEN, M., STRAHM, Y., MORRICE, N. A., DIVECHA, N. & D'SANTOS, C. S. 2011. Identification of nuclear phosphatidylinositol 4,5-bisphosphate-interacting proteins by neomycin extraction. Mol Cell Proteomics, 10, M110.003376.
UNGEWICKELL, A., HUGGE, C., KISSELEVA, M., CHANG, S. C., ZOU, J., FENG, Y., GALYOV, E. E., WILSON, M. & MAJERUS, P. W. 2005. The identification and characterization of two phosphatidylinositol-4,5-bisphosphate 4-phosphatases. Proc Natl Acad Sci U S A, 102, 18854-9

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