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Co-localization of PhK γ-181 and NA-14 in SH-SY5Y Cells

Brent Harney
brent.harney@uky.edu

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Brent Harney, Veronica Johnson, and Nancy A. Rice
Western Kentucky University, Department of Biology, Bowling Green, Kentucky

Abstract

Phosphorylase kinase (PhK) is a serine/threonine kinase that is the key enzyme in regulating the breakdown of glycogen to glucose. The catalytic subunit of PhK is γ, and is encoded by the PHKG1 gene. Previous *in silico* work in our lab identified an alternative polyadenylation signal in an intron in the human *PHKG1* gene that yields a truncated γ containing only the first 181 amino acids. RNA analysis showed this γ variant is found primarily in brain and heart, and when expressed recombinantly retains its ability to phosphorylate proteins. While no binding partners have been identified *in vivo*, NA-14 was identified as a potential partner through a yeast two-hybrid screen. NA-14 is a protein involved in microtubule dynamics. It localizes to centrioles and helps regulate spastin localization to them. Co-localization of γ-181 and NA-14 *in vivo* would suggest an alternative pathway of NA-14 regulation not sensitive to calcium. In this work, we use the neural cell line SH-SY5Y to investigate co-localization of γ-181 and NA-14 *in vivo* by immunofluorescent microscopy.

Materials and Methods

Cells

SH-SY5Y human neuroblastoma cells were used in this study. Cells were grown at 37°C, 5% CO_2 in a 1:1 mixture of Dulbelco's Minimal Essential Media (DMEM) growth media and F12 media plus 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were typically passaged 1:5 at ~80% confluency on a 100 mm tissue culture plate.

Immunostaining

SH-SY5Y cells were grown in normal growth media as described above on glass coverslips prior to immunostaining. For immunostaining, cells were fixed in a 70%:30% methanol:acetone solution for 5 min followed by blocking in 10% normal goat serum. Cells were incubated at room temperature with either a specific polyclonal antibody that recognized the truncated g181, a NA-14 polyclonal Ab (Proteintech, Inc), or E7 – a mAb that binds to tubulin monomers. Antibody complexes were detected using IgG secondary antibodies conjugated with either fluorescein isothiocyanate (FITC) or Texas Red (Southern Biotech) and visualized by fluorescence microscopy.

Immunofluorescence

The cells were visualized by immunofluorescence microscopy on the Zeiss Axioplan microscope housed in the WKU Biotechnology Center.

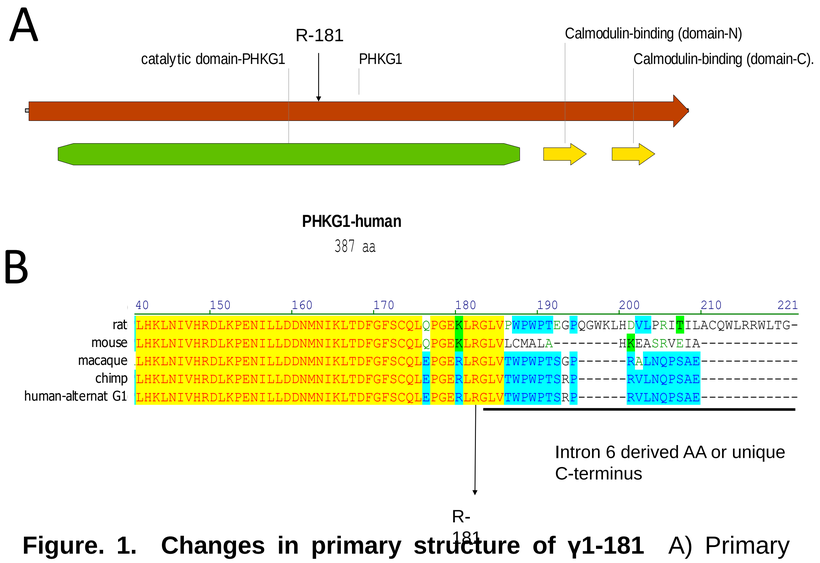

Figure. 1. Changes in primary structure of γ1-181 A) Primary structure of the full length human muscle PhK γ subunit and the location of R-181. B) ClustalW alignment of the protein sequences of γ1-181 in several species demonstrating the differences in the C-term.

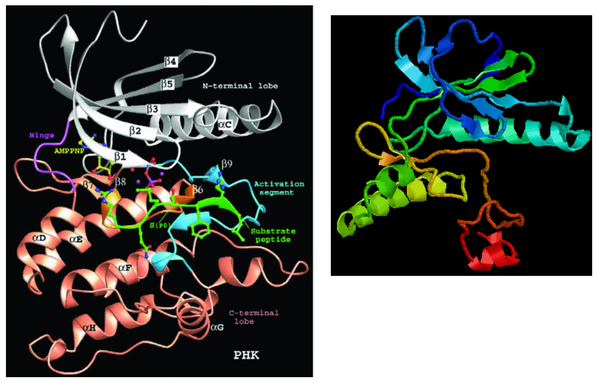

Figure 2. Modeled structure of PhK γ181 compared to the full length subunit. Left: PHK γ [L. Johnson, (2007) *Biochem. Soc. Trans*. 35, 7-11.] Right: Predicted PhK γ 181.

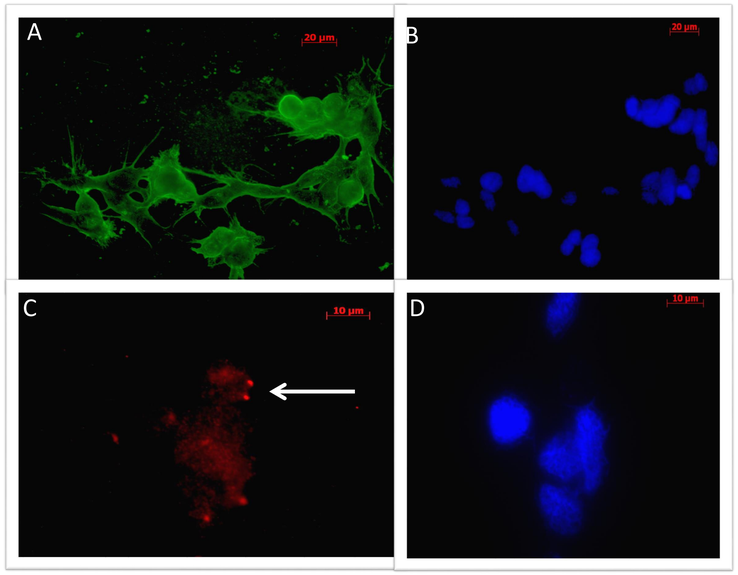

Figure 3. Localization of γ 181 and NA-14 in SH-SY5Y cells. A. γ181 appears to localize primarily to membranes compared to the localization of NA14 at centromeres (C). Panels B and D show nuclei staining for panels A and C respectively.

Conclusions

- Based on preliminary data in undifferentiated SH-SY5Y cells, γ-181 does not appear to co-localize with NA-14 at the centromere of dividing cells.
- Membrane association of γ181 in neural derived cells may implicate this kinase as a potential regulator of membrane or lipid dynamics.

Acknowledgements

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