NUCLEAR / NUCLEOLAR LOCALISATION AND TRANSPORT MECHANISM OF PHOSPHATIDYLINOSITOL 4-KINASE ISOFORM PI4K230

Annamaria Kakuk

Supervisor: György Vereb

UNIVERSITY OF DEBRECEN
MEDICAL AND HEALTH SCIENCE CENTER
DEPARTMENT OF MEDICAL CHEMISTRY

Summary

We set as an aim to investigate the possible nuclear occurrence of PI4K230, to find its interaction partners, and to uncover molecular mechanisms by which nuclear and nucleolar transport of PI4K230 occurs. Our results are summarized as follows:

The presence of PI4K230 in the nucleoli of ethanol fixed neuronal and non-neuronal cells with indirect immunofluorescence using antibodies to four of its distinct epitopes. Based on our colocalisation results, nucleolar PI4K230 exhibits prominent staining in dense fibrillar components.

PFA treatment had no effect on immunoreactivity of PI4K230 in cell lysate or immunoblot but masked reversibly the immunoreactive epitopes of PI4IK230 in the nucleolus by crosslinking PI4K230 with tightly associated nucleolar components.

In experiments with siRNA interfering with the expression of PI4K230, elimination of PI4K230 immunoreactivity from the cytoplasm and nucleoli was observed, while the remaining PI4K230 accumulated in the nucleus.

The PI4K230 exists in detergent-resistant proteolipid complexes of the nucleolus interacting with nucleolar DNA and/or RNA, either directly or through associated nucleic acid-bound constituents.

Though recombinant PI4K230 expressed in COS-7 cells does not accumulate in the nucleolus even after 2 days but in transport assays PI4K230 expressed in Sf9 cells enriches in the nucleoplasm of digitonin permeabilized HeLa cells.

Nuclear import directed by the monopartite NLS (NLS1) of PI4K230 is a WGA-sensitive and energy-dependent process mediated by importin α1/β and importin α3/β complexes that interact directly with NLS1.

An expressed 506 amino aid fragment of PI4K230 containing its bipartite NLS is effectively transported to the nucleolus with importin α1/β and importin α3/β heterodimers in a WGA-sensitive and energy-dependent manner. Although the bipartite NLS sequence (NLS2) itself linked to BSA does not translocate to the nucleus, but when linked to trypsin inhibitor that small enough to pass freely the NPC it directs the cargo to the nucleolus. In this capacity, the complete NLS2 is more potent than either of its two fragments.

Kulcsszavak: Foszfatidilinozitol 4-kináz PI4K230 izoforma, sejtmagvacska, sejtmagi transzport

Key words: Phosphatidylinositol 4-kinase isoform PI4K230, nucleolus, nuclear import