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Nuclear phospholipase Cβ1 interactome: a morphological and proteomic approach

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Inositide-dependent phospholipase Cβ1 (PI-PLCβ1b) has two isoforms generated by alternative splicing (PI-PLCβ1a and PI-PLCβ1b). In murine erythroleukemia (MEL) cells both the isoforms are present within the nucleus, but PI-PLCβ1b is exclusively nuclear. Our group has demonstrated that PI-PLCβ1 nuclear localisation is crucial for its function, although the mechanism by which PI-PLCβ1 is imported into the nucleus has never been carefully investigated.

The purpose of the present study was to get more insights on the protein interactome of PI-PLCβ1b, namely the proteins present in the nucleus. Immuno-affinity purification coupled with tandem mass spectrometry analysis have been used to purify and identify PI-PLCβ1b interaction binding partners from Friend's erythroleukemia isolated nuclei. Gene ontology and protein-protein interaction network were performed to analyze data. Some interactions were already characterized, such as the binding with the splicing factor SRp20 and the lamin B. Among the proteins identified, the binding of eEF1A and prohibitin 2 with PI-PLCβ1b was confirmed by western blot analysis. Of particular interest was the identification of importin a, importin b1 and Ran, which interact with PI-PLCβ1b. These proteins are believed to be involved in the import mechanism from the cytoplasm to the nucleus. Further analysis by overexpressing both wild type and cytoplasmatic mutant of PI-PLCβ1, suggests that importin b1 is responsible for the localisation of PI-PLCβ1b in the nucleus, giving new insight into the mechanism of trafficking of this signaling molecule.

Keywords: Nucleus, proteomics, signalling, phospholipase Cβ1.