



The PRP4K Kinase protein overexpression, production, purification and crystallization

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Abstract

The Pre-mRNA processing factor 4B Kinase is a serine/threonine kinase responsible for the phosphorylation of essential proteins of the tri-snRNP complex in the B Complex of the spliceosome in mammals¹, being that the overexpression of PRP4K has been associated with certain ovary cancer forms², Paclitaxel and Carboplatin resistance³. The understanding of the kinase domain is crucial for the ligand-based rational drug design, and our research group has been working with the obtainment of high resolution crystallographic structures of this protein domain. In this work we present some advances in the protein PRP4K kinase domain production and purification, and also the attempts of protein overexpression in mammalian cells.

Key words:

PRP4K, Crystallography, Kinase.

Introduction

The Pre-mRNA processing factor 4B Kinase is a serine/threonine kinase responsible for the phosphorylation of essential proteins of the tri-snRNP complex in the B Complex of the spliceosome in mammals¹, being that the overexpression of PRP4K has been associated with certain cancer forms². The uncover of the PRP4K protein interactome is important to the understanding of the PRP4K role in the cells, and the obtainment of high resolution crystallographic structures of the kinase domain is crucial for the ligand-based rational drug design. Using pcDNA-LIC cloning vectors, our group designed a series of protein constructions to achieve the protein overexpression in mammalian cells for phenotypic characterisation. In parallel, different constructions of the kinase domain with His-tag were designed and overexpressed in *Spodoptera frugiperda* SF9 insect cells. The protein was captured in nickel sepharose columns and the His-tag cleaved with TEV protease. The eluate was purified in Superdex gel column. The purified protein was analyzed by SDS-Page and mass spectrometer (micro-LC-MS/MS Q-ToF XEVO G2 XS – water, available in the Life Sciences Core Facility).

Results and Discussion

Different protein constructions in pcDNA-LIC cloning vectors for recombinant protein expression were designed and two of them presented promising results, being the overexpression confirmed by western-blot and RT-PCR analysis (see Image 1).

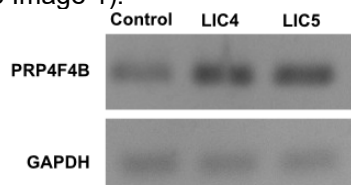


Image 1. Semi-quantitative RT-PCR showing PRPF4 overexpression (cDNA of HEK293 cells transfected with PRPF4-LIC4 and LIC5 to overexpress) in agarose gel.

With the overexpression confirmed, in the next experiments we will try to uncover the protein interactome by IP-MS (immunoprecipitation followed by mass spectrometry), an important experiment to help the understanding of the PRP4K role in the cancer resistance.

The kinase protein domain could be expressed, purified and crystallized as shown in Image 2.

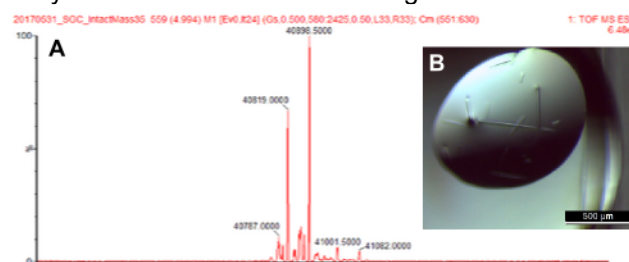


Image 2. A- Intact mass of the purified protein (Two major peaks of 40898.5000 Da and 40819.0000 Da were observed). B – PRP4K Crystal obtained.

Conclusions

From my contribution with the group we were able to establish the PRPF4 overexpression system, which allows phenotypic characterisation and will follow with the protein interactome. We also produced the recombinant kinase domain of PRP4K followed by purification and crystallography. The structure has been deposited in PDB and will be used to design selective chemical inhibitors.

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³ Corkery, D. P.; Le Page, C.; Meunier, L.; *et al.* Cell Cycle, **2014**, *14*, 1059–1069.