

Analysis of the human phosphatase system PP2A

Application Note 1: CaptiVate™ technology

■ Summary

- PP2A subunits PPP2CB, PPP2R1A and PPP2R2A were stably integrated in HEK293 cells by the FRT (Flippase recombination target) system
- SH-double affinity purification was performed
- Samples were analysed by LC-MS/MS using an LTQ-Orbitrap mass spectrometer
- The list of protein ID's were filtered against a background database generated in-house
- A protein network was assembled and proteins were grouped into classes relevant to PP2A function
- The application of CaptiVate™ technology revealed new interactors of the PP2A system

■ Introduction

The serine/threonine phosphatase PP2A is a highly conserved phosphatase complex with important biological function and has been linked to regulate a variety of vital cellular processes including apoptosis, transcription and cell proliferation and cellular transformation [1].

The majority of PP2A complexes exist as a heterotrimeric assembly between a catalytic, a scaffolding and a regulatory B subunit. The human genome encodes a large number of

B-type regulatory subunits (>15) and thereby exceeds the number of catalytic and scaffolding subunits (n=2) within the human system. This in turn provides a molecular basis for the assembly of PP2A into a multitude of possible heterotrimeric phosphatase complexes. It is assumed that the B-type regulatory subunits provide substrate binding and specificity to individual heterotrimers and thereby allowing the control of diverse biological processes by combinatorial assembly of the phosphatase complex.

■ Methods – CaptiVate™ technology



Generation of isogenic cell lines expressing the bait protein using the FRT system

Highly efficient Strep/HA-double affinity purification

Sensitive protein identification using nano-LC-Orbitrap-MS

Filtering vs. background database to get specific interactors

Results

Here, we present the analysis of PP2A phosphatase specific subunits to monitor the performance and applicability of the CaptiVate™ technology. First, we monitored the efficiency of the Strep/HA-double affinity purification by using Western blotting (Figure 1). We routinely obtain a purification yield of 30-40% of the starting material, which is among the highest yields reported for double affinity purification protocols [2].

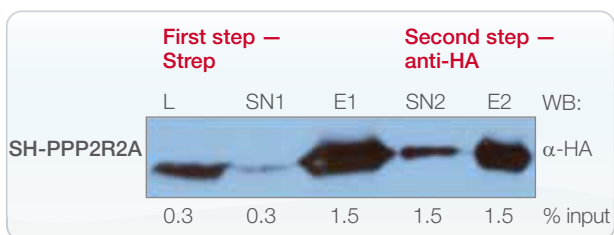


Figure 1: Monitoring of SH-double affinity purification.

Cells expressing the SH-tagged version of PPP2R2A were lysed. The lysate (L) was applied to StrepTactin beads and eluted with lysis buffer containing biotin (E1). The second purification step was performed using anti-HA agarose. The final eluate was obtained by pH shift elution (E2). SN1: supernatant after first purification; SN2: supernatant after second purification.

Cell lines expressing the PP2A subunits PPP2RCB (catalytic subunit), PPP2R1A (scaffolding subunit) and PPP2R2A (regulatory B subunit) were used to generate a phosphatase interaction network of PP2A. Following SH-purification and LC-MS/MS analysis using an LTQ-Orbitrap mass spectrometer the list of protein ID's was filtered against a background database generated in-house.

From the resulting list of proteins a protein interaction network was assembled and the protein interaction database Biogrid was used as a reference database to detect new protein interactions (Figure 2).

In total we identified 69 proteins, which correspond to 103 protein interactions associated with the bait proteins. Among the 103 interactions, 36 interactions were previously known according to Biogrid information (Figure 2, blue lines), whereas 67 protein interactions did not occur in the database (Figure 2, red lines). In order to annotate the identified proteins, GO annotations were used and proteins were grouped into classes relevant to PP2A function (cell signalling, apoptosis, cell proliferation, protein folding, transcriptional control).

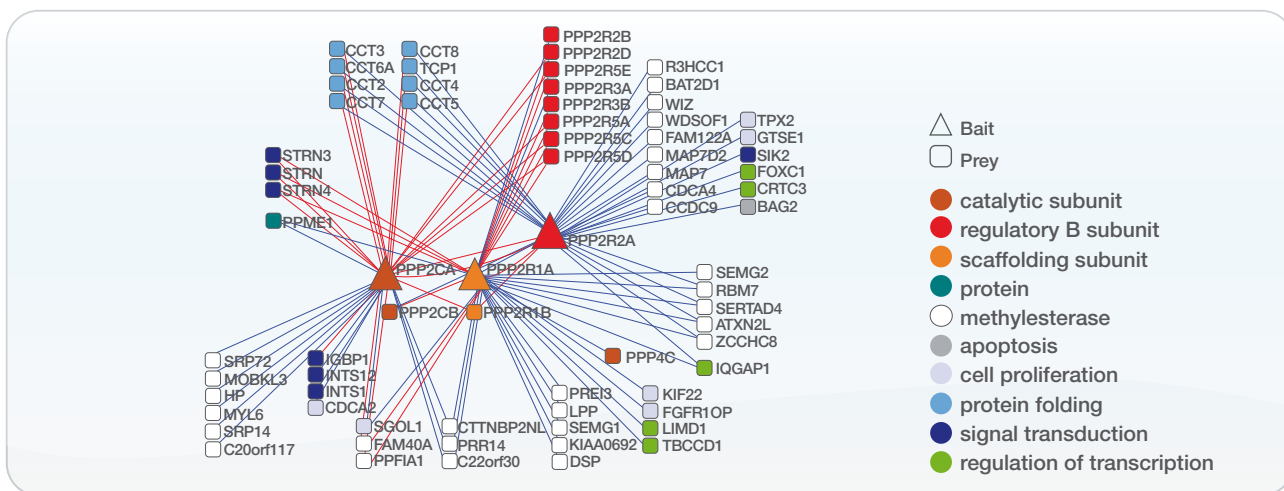


Figure 2: PP2A interaction network.

Protein-protein interactions and different classes of protein complexes within the human PP2A interaction network are shown. Blue lines indicate previously known interactions; red lines indicate interactions not occurring in the Biogrid database.

Dualsystems Biotech AG
Grabenstrasse 11a
CH-8952 Schlieren/Switzerland

Phone +41 44 738 50 00
Fax +41 44 738 50 05

www.dualsystems.com

Dualsystems Biotech Inc.
P.O. Box 717
San Carlos, CA 94070 / USA

Phone +1 650 678 53 24
Fax +1 866 434 99 33

www.dualsystems.com