EXPRESSION, PURIFICATION AND INITIAL CRYSTALLIZATION SCREENINGS OF A NOVEL PHOSPHATASE TERNARY COMPLEX FORMED BY α4, TIPRL AND PP2Ac.

Bezerra, G.A.[1], Navarro, M.V.A.S.[1], Smetana, J.H.C.[1], Zanchin, N.I.T.[1] and Guimarães, B.G.[1]

[1] Laboratório Nacional de Luz Síncrotron - Campinas SP Brazil

[1]Departamento de Bioquímica - Instituto de Biologia - Unicamp

Protein phosphatase-2A (PP2A) is involved in the regulation of cell homeostasis through the negative regulation of signaling pathways initiated by protein kinases. As several cancers are characterized by the aberrant activity of oncogenic kinases, PP2A has progressively been considered as a potential tumor suppressor. The α4 protein interacts directly with the catalytic subunits of 2A phosphatases. We had previously shown that TIPRL, the human orthologue of yeast Tip41, also interacts directly with the catalytic subunits of PP2A, PP4 and PP6, forming a rapamycin-insensitive complex with Alpha-4 in human cells. To better understand this new protein complex, we started a project aiming at the 3D structure determination of the proteins TIPRL and α4 and their complex with PP2Ac. The N-terminal domain of α4 (α4?222) and PP2Aca were co-expressed in *E. coli* BL21(DE3) transformed with the plasmids pET28a-α4?222 and pProEx-HtB-PP2Ac. TIPRL was expressed separately as a GST fusion in the *E. coli* BL21(DE3) strain transformed with the plasmid pET-GST-TEV-TIPRL. In both cases, expression was induced by the addition of 0.5 mM IPTG at $O.D._{600}$=1.0 after dropping the temperature from 37°C to 25°C, and cells were grown for additional 4 hours. The cell extracts were then collected by centrifugation and the soluble fraction was applied into a glutathione S-transferase affinity. The complex was eluted by incubation with TEV protease at 4°C overnight, then concentrated and applied to a Superdex 200 size-exclusion column. Quality of the sample was assessed by Dynamic Light Scattering and the purified complex was submitted to initial crystallization screens, which produced polycrystals in several conditions.

Acknowledgements: LNLS, FAPESP, CBME/CEPID/FAPESP