DISSECTING ANNEXIN A11 IN ITS DOMAINS: STRUCTURAL AND FUNCTIONAL CHARACTERIZATION

P-32-031

G. Di Napoli[I], P. Olivieri[II], A. Fissore[I], E. Raccuia[I], V. Bincoletto[I], S.M.A. Arpicco[I], G. Catucci[III], G. Gilardi[III], M. Marengo[I], S. Oliaro-Bosso[I], S. Adinolfi[I]

[I]Department of Drug Science and Technology, University of Turin, Turin, Italy, [II]Biochemistry and Biology Institute, Potsdam University, Potsdam, Germany, [III]Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

Annexins are a family of Ca^{2+}-dependent phospholipid-binding proteins with several functions. Among these, there is Annexin A11, whose mutations have been found in patients with ALS, a neurodegenerative disease. Annexin A11 has the longest N-terminus domain within Annexins, formed by 197 amino acids of which most are glycine, proline, and tyrosine, and a very conserved C-terminus that hosts four calcium-binding motifs that has been suggested to be involved in the binding to phospholipids[1]. One of the functions recently attributed to this protein has been the ability to passively transport RNA granules, via its C-terminus that tethers to lysosomes, from the neuronal soma to the intrasynaptic space to enable protein translation in situ[2]. The aim of the present work has been the dissection of the Annexin A11 in its structural and functional domains, with a particular focus on measuring the affinity of the C-terminus for the calcium and the effect of the latter on the binding between the C-terminus and membrane phospholipids. A purification protocol has been set up to obtain the two domains soluble, on which circular dichroism has been performed showing that the C-terminus has mostly alpha-helices whereas the N-terminus is unstructured. This domain undergoes phase separation, which is why different concentrations of protein, salt and different temperature conditions have been tested to construct a phase diagram and control this phenomenon. The binding affinity of calcium to the C-terminus domain has been measured by fluorescence spectroscopy leading to a Kd of 0,6 µM. Lastly, pull down assays have been conducted in vitro with lysosomes-like liposomes, confirming that the purified C-terminus domain is able to interact with membrane phospholipids in a calcium-dependent manner, as suggested in vivo[2].

1) Previously published in: Gerke V. and Moss S.E (2002) Physiological Reviews 82, 331-371.
2) Previously published in: Liao Y. et al. (2019) Cell 179, 147-164.