PROGETTO-1

Supervisore: Ivan de Curtis

Titolo/*Title*: **Structure-function characterization of TANC2, a novel postsynaptic candidate associated to neuropsychiatric disorders**

Curriculum: Biologia Cellulare e Molecolare

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: https://research.hsr.it/en/divisions/neuroscience/cell-adhesion.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Human TANC family includes 2 poorly defined scaffold proteins implicated in postsynaptic development, with several mutations associated to paediatric/adult neuropsychiatric disorders. TANC2 is a postsynaptic protein mutated in intellectual disability (ID) (Stucchi 2018). Liquid-liquid phase separation (LLPS) is relevant to synaptic function (de Curtis 2021; Ramallah 2022). Intrinsically disordered regions (IDRs) often drive LLPS (Banani 2017). Disorder prediction identifies IDRs in TANC2 as drivers of LLPS (Muskie 2020). We showed that the C-terminal IDR of TANC2 induces biomolecular condensates (BCs) in cells. The goal of this PhD project is to explore the hypothesis that TANC2 forms BCs important for synaptic organization. LLPS may recruit proteins "clients" by specific interactions regulated by phosphorylation (Garcia-Cabal & Cavatelli 2021).

Rationale and hypothesis

Based on prediction that TANC2 includes two IDRs at the N- and C-terminus, it is hypothesized that TANC2 undergoes LLPS with important functional consequences. This idea is supported by bioinformatic analysis that identify the tendency of TANC2 to form condensates, and by our preliminary data.

Objectives and specific aims

The PhD project has the following aims: (1) To show that postsynaptic TANC2 undergoes LLPS in cells, by reaching a critical concentration at specific intracellular sites (Astro & de Curtis 2015). Overexpression will be used to induce TANC2 BCs and to characterize their biophysical properties by FRAP (Sala, 2019). The ability to recruit "client proteins" will be determined by expression of TANC2 interactors. The PhD candidate will then switch to cultures of hippocampal neurons expressing endogenous TANC2, to analyse its role on synaptic organization. (2) The structure-function analysis will include: analysis of the IDRs that may be involved in LLPS (van der Lee 2014). Alpha Fold reveals both IDRs and structured domains in TANC2. We have shown that TANC2 IDR-C promotes BC-like condensates. Deletion of IDRs will be tested to support their requirement for LLPS and to recruit postsynaptic partners in hippocampal neurons (Aim 4). (3) Intriguingly, TANC2 has an N-terminal ATPase domain that is also mutated in ID patients; the function of this domain in synaptic organization is unknown and will start to be addressed. (4) Hippocampal neurons will be used to look at effects of IDRs, mutant IDRs, and/or ATPase mutants on post-synaptic organization.

Expected outcomes

The outcome from this project is expected to proof that TANC2 IDRs, and possibly the ATPase domain, are required for postsynaptic organization.

Competence Che dive acquihire lo student (Max 600 caratteri spazi inclusi):

The PhD student will have the chance to combine functional analysis with structural analysis to address molecular aspects relevant to TANC2 function in synaptic development. The student will acquire skills in molecular cell biology approaches, including analysis of synaptogenesis in hippocampal neuronal cultures; confocal, FRAP, TIRF imaging on fixed and live cells; cell biochemistry and protein structure-function analysis. Interestingly, the PhD candidate will have the chance to contribute to the analysis of structure and dynamics of TANC2 IDRs in collaboration with NMR experts.

Bibliografia (max. 15)

1. Stucchi R, Plucinska G, Hummel JJA, Zahavi EE, Guerra San Juan I, et al. Regulation of KIF1A-Driven Dense Core Vesicle Transport: Ca(2+)/CaM Controls DCV Binding and Liprin-alpha/TANC2 Recruits DCVs to Postsynaptic Sites. Cell Rep. (2018) 24:685-700.
2. de Curtis I. Biomolecular Condensates at the Front: Cell Migration Meets Phase Separation. Trends Cell Biol. (2021) 3:145-148.
3. Ramella M, Ribolla LM, de Curtis I. Liquid-Liquid Phase Separation at the Plasma Membrane-Cytosol Interface: Common Players in Adhesion, Motility, and Synaptic Function. J Mol Biol. (2022) 434:167228.
4. Banani SF, Lee HO, Hyman AA, Rosen MK Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol. (2017) 18:285-298.
5. Miskei M, Horvath A, Vendruscolo M, Fuxreiter M. Sequence-Based Prediction of Fuzzy Protein Interactions. J Mol Biol. (2020) 432:2289-2303.
6. Aguzzi, A. & Altmeyer, M. Phase Separation: Linking Cellular Compartmentalization to Disease. Trends Cell Biol. (2016) 26:547–558.
7. Garcia-Cabau C, Salvatella X. Regulation of biomolecular condensate dynamics by signaling. Curr Opin Cell Biol. (2021) 69:111-119.
8. Guo H, Bettella E, Marcogliese PC, Zhao R, Andrews JC, Nowakowski TJ, et al. Disruptive mutations in TANC2 define a neurodevelopmental syndrome associated with psychiatric disorders. Nat Commun. (2019) 10:4679.
9. Astro V, de Curtis I. Plasma membrane-associated platforms: dynamic scaffolds that organize membrane-associated events. Science Signal. (2015) 8:re1.
10. van der Lee R, Buljan M, Lang B, Weatheritt RJ, Daughdrill GW, et al. Classification of intrinsically disordered regions and proteins. Chem Rev. (2014) 114:6589-6631.
11. Sala K, Corbetta A, Minici C, Tonoli D, Murray DH, Cammarota E, Ribolla L, Ramella M, Fesce R, Mazza D, Degano M, de Curtis I. The ERC1 scaffold protein implicated in cell motility drives the assembly of a liquid phase. Sci Rep. (2019) 9:13530.