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A novel bioluminescence-based assay for functional evaluation of the Nav channelosome

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Protein:protein interactions are critical molecular determinants of ion channel function and emerging targets for pharmacological interventions. Yet, current methodologies for the rapid detection of ion channel macromolecular complexes are still lacking. Here, we adapted the split-luciferase complementation assay, LCA, to detect the assembly of the voltage-gated Na^+ (Nav) channel C-tail and the intracellular fibroblast growth factor 14 (FGF14), a functionally relevant component of the Nav channelosome that controls gating and targeting of Nav channels through direct interaction with the channel C-tail. In the LCA, two complementary N-terminus and C-terminus fragments of the Firefly luciferase were fused, respectively, to a chimera of the CD4 transmembrane segment and the C-tail of Nav1.6 channel or FGF14. Co-expression of the two constructs in live cells led to a robust assembly of the FGF14:Nav1.6 C-tail complex, which was attenuated by introducing single-point mutations at the predicted FGF14:Nav channel interface. To evaluate the dynamic regulation of the FGF14:Nav1.6 C-tail complex by signaling pathways, we screened a library of kinase inhibitors, searching for potential modulators that could dynamically regulate the FGF14:Nav channel complex assembly. Through a platform of counter screenings, we show that the p38/MAPK inhibitor, PD169316, and the IκB kinase inhibitor, BAY 11-7082, reduce the FGF14:Nav1.6 C-tail complementation, highlighting a potential role of the p38MAPK and the IκB/NFκB pathways in controlling neuronal excitability through protein:protein interactions. We envision the methodology presented here as an innovative tool to allow functional evaluations of protein:channel complexes toward probe development and drug discovery targeting ion channels implicated in human disorders.

Biography

Currently at the University of Texas Medical Branch as Assistant Professor in the Department of Pharmacology and Toxicology, Fernanda Laezza has completed her M.D. in Italy, her Ph.D. at Emory University and her post-doctoral studies at Washington University. Her research focuses on developing new approaches to studying macromolecular complexes within ion channels and leveraging this innovative source of protein–protein interaction interfaces toward discovery of new intracellular pathways that regulate neuronal excitability and therapeutic agents that target human channelopathies. She has published in high-profile journals, including Science, Journal of Neuroscience and Journal of Physiology and received a number of Awards.

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