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11:00 a.m.

LSU Health Sciences Center

Lion's Bldg.
2020 Gravier Street
8th Floor Large
Conference Room

Neuroscience Center of Excellence

**LSU Health Sciences Center School of Medicine,
New Orleans**

Faculty Candidate

"Identification of Protein-interactors/ functional regulators of the ABC transporter Ycf1p from Sacharomyces cerevisiae and its human homologue, MRP1"

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The multidrug resistance-associated (MRP) subfamily of ATP-binding cassette (ABC) transporters play a key role in protecting cells from environmental and endogenous toxins. The MRPs are characterized by their ability to transport substrates as glutathione conjugates. I am studying Ycf1p, a prototypical representative of the six-member MRP subfamily in yeast. Ycf1p resides in the vacuolar membrane and mediates glutathione-dependent resistance to heavy metals (*e.g.* cadmium) and production of red pigment in an *ade2* yeast strain. To better understand cellular components involved in Ycf1p function we have embarked on a search for Ycf1p protein interactors. Identification of membrane protein interactors is challenging because their hydrophobic nature precludes the use of standard approaches. Instead, we used the integrated membrane protein yeast two hybrid (*i*MYTH) system, in collaboration with Dr. Igor Stagljar (U. of Toronto), which is specifically designed circumvent this problem. An *i*MYTH screen identified six potential Ycf1p interactors. Strains deleted for several of these genes result in cadmium sensitivity and *ade2* pigmentation defects similar to an *ycf1*Δ strain. We have focused further analysis on one of these, Tus1p, a guanine nucleotide exchange factor (GEF) for Rho1p. In the absence of Tus1p, neither the expression nor trafficking of Ycf1p is altered. Strikingly, in an *in vitro* transport assay using purified Ycf1p-containing vacuoles, I found that addition of WT (*TUS1*) cytosolic extract and not Δ*tus1* cytosolic extract stimulates transport. Thus, Tus1p plays a novel and unanticipated role in regulating the activity of Ycf1p. Interestingly, our first screen (with C-terminally tagged prey) has identified a number of proteins that are involved in the synthesis of lipid signaling molecules derived from phophotidylinositol-3-phosphate (PIP), suggesting a role for Ycf1p in PIP signaling or a PIP requirement for proper function. A second screen *i*MYTH (with N-terminally tagged prey) has revealed a number of kinases which also interact with Ycf1p and regulate its function in vivo. My current research is focused on examining how both Ycf1p and human MRP1 function is regulated via phosphorylation. In the future I plan to use the *i*MYTH technology and current yeast genetic methods to identify new and interesting roles for the MRPs in lipid signaling and cellular cytoprotection and to identify regulators of MRP function which could potentially be the target of new therapeutics used to treat MRP-associated diseases. I ultimately will examine the role of homologous mammalian proteins and their role in the regulation of human MRPs via cell culture. In conclusion, my studies suggest that *i*MYTH represents a robust methodology to identify key physiological regulators for all classes of ABC protein and will be a tremendous advantage to my work, defining the role of the MRPs in human neurological diseases such as Alzheimer's and epilepsy, and the role of MRPs in neuronal integrity and maintenance.