**Prof. Uri Ashery’s Research**

**Research Field**

Our current research interests are the molecular mechanisms of synaptic function under normal and pathological conditions.

**Research Activity**

The human brain is composed of more than a trillion nerve cells whose signal-carrying protrusions are interconnected at special points of contact called synapses. Neurotransmitter release at the synapse is a multi-step process that is coordinated by a large number of synaptic proteins and depends on proper protein-protein interactions. Modulation of these processes is believed to underlie the processes of learning and memory. Our main interest is to study the molecular mechanisms of these processes under normal conditions and during neurodegenerative diseases such as Huntington and Alzheimer diseases.

In the last years we have been investigating the function of key synaptic proteins in these processes using molecular biology, electrophysiology, biochemistry, Ca2+ imaging and computer simulation techniques. We took advantages of a unique experimental approach that allows efficient manipulations of the level and composition of specific proteins using overexpression and knock down approach. The approach allows studying structure-function relationships of synaptic proteins and their role in normal and pathological conditions. We perform detail electrophysiological and fluorescent measurements from chromaffin cells and neurons and compare the phenotype cells expressing the different mutated proteins. Specifically, we investigate the function of Munc13, Munc18, tomosyn, PLD and DOC2 in exocytosis. Our long-term goal is to correlate changes in protein expression to synaptic plasticity and learning and memory processes.

**Brief Summary**

We have found that tomosyn inhibits priming of vesicles and decreases exocytosis in chromaffin cells. In addition we have defined the N-terminal part of tomosyn and its linker as a minimal domain necessary for its inhibitory activity and found that the SNARE motif of tomosyn is involved in modulation of tomosyn activity.We are now investigating the role of tomosyn in synaptic plasticity processes (see below Boaz). In addition, we investigate tomosyn’s dynamic interactions with syntaxin and with other proteins and examine intramolecular interactions between different domains of tomosyn (see below Noa).In different projects we found that phosphorylation of Munc18 potentiates vesicle pool refilling. In addition, we found that PLD1 enhances priming of large dense core vesicles in chromaffin cells.
To elucidate the functions of calcium-dependent proteins in the synapse we investigated the effect of calcium and calmodulin on the priming activity of ubMunc13-2. We  propose that ubMunc13-2 is activated by two
Ca2+-dependent processes: a slow activation mode operating at low Ca2+ concentrations, where ubMunc13-2 acts as a priming switch, and a fast mode at high Ca2+ concentrations, in which ubMunc13-2 is activated in a Ca2+/CaM-dependent manner and accelerates vesicle recruitment and maturation during stimulation. In addition, we investigate the function of DOC2B, a double C2 domain protein in synaptic transmission. We found that the proteins DOC2A and DOC2B translocate to the plasma membrane in presence of [Ca2+]i below 0.5 µM, and we are currently investigating its function in exocytosis (see below Reut).

In a recent project we have started to elucidate the function of the active zone protein, Bassoon in secretion of large dense core vesicles from chromaffin cells (see below Anton, Merav, Reut). To investigate the role of synaptic proteins in synaptic plasticity we are investigating the changes in the expression levels and distribution of synaptic proteins during and following learning (see below Boaz) and the effects of manipulation of synaptic proteins on synaptic plasticity (see below Ayal). We have also several biophysical projects that aim to understand the dynamic of proteins diffusion, assembly and disassembly and signal transduction. We discovered the Ras-related particle, which we called rasosome. Rasosomes are small particles, containing multiple lipid-modified Ras proteins that diffuse randomly in the cytosol and interact with the plasma membrane.
We are now characterizing if rasosome sample the membrane at specific points, or hot spots, and if rasosome can transmit Ras-related signals (see below Merav). In a project related to Huntington disease, we investigate the role of the Huntingtin interacting protein 1 (HIP1) in endocytosis, and we show that HIP1 particles are collocalized with clathrin on the plasma membrane and appear and disappear concomitant with clathrin particles following treatments that influence pits formation (see below Irit).
These data suggest that HIP1 participates in early stages of clathrin-mediated endocytosis. In another project we investigate the kinetics of SNARE complex formation and disassembly by NSF. We found that SANRE complexes are assembled in two phases and that disassembly depends on the concentration of NSF and alpha SNAP. To learn more about the dynamics of the SANRE proteins on the PM, with or without syntaxin domains we are using a Monte Carlo-based 2-dimensional simulation (see below Dana).

In addition we have developed a novel simulation program that describes the process of exocytosis as dynamic interactions between synaptic proteins. This study provides an excellent platform to predict and quantify the effects of protein manipulations on exocytosis (see below Dana).