**Tuesday, February 5, 2008**

**Symposium 13: Voltage-dependent Proton Channels Come of Age**

## 1730-Symp Properties and Functions of Voltage-Gated Proton Channels

Thomas E. DeCoursey

*Rush University, Chicago, IL, USA.*

The elusive gene for the voltage-gated proton channel was identified in 2006 (Ramsey et al, 2006, *Nature 440*:1213; Sasaki et al, 2006, *Science 312*:589). Some predictions based on the electrophysiological behavior of native proton currents have been borne out, but there have been surprises. The protein has four transmembrane doamins that resemble S1–S4 of ordinary voltage-gated ion channels, but lacks S5–S6 that contain the aqueous pore. Thus, the channel evidently lacks a water-filled pore, consistent with the predictions of studies showing that the channel is perfectly selective for protons and the conductance has a large deuterium isotope effect and profound temperature sensitivity. Based on competition between $H^+$ and $Zn^{2+}$ (a potent inhibitor), the external binding site for $Zn^{2+}$ was predicted to comprise 2–3 His residues. Remarkably, the human proton channel protein does have two His residues facing the external solution, and mutating them to Ala greatly attenuates inhibition by $Zn^{2+}$ (Ramsey et al, 2006). However, most major features of proton channels await structural explanation. An archetypal characteristic of proton channels is the sensitivity of gating to pH, which suggests the existence of titratable sites. These groups have not been identified. The mechanism of proton selectivity is unknown. We are uncertain whether the channel is a monomer or multimer. "Activation" of the proton conductance in phagocytes involves phosphorylation by PKC of the channel or a distinct regulatory protein. The putative phosphorylation site remains hypothetical. Finally, the mechanism of voltage sensing has eluded structural identification. Despite the similarity of the proton channel to the voltage sensor of other channels, it is not clear that the mechanisms are identical. In summary, many key properties of the voltage-gated proton channel remain unexplained.

Supported by the NIH (HL61437) and by Philip Morris.

## 1731-Symp Proton Channels In Phagocytes: From Postulate To Reality

Nicolas Demaurex

*University of Geneva, Geneva, Switzerland.*

Phagocytic white blood cells kill microbes with superoxide radicals produced by a membrane enzyme, the phagocytic NADPH oxidase (phox). This process is essential for innate immunity, and patients with defective phox suffer from severe recurring bacterial and fungal infections. Voltage-gated proton channels provide a compensating charge for the electrons currents generated by the phox, and were postulated long ago to be required for bacterial killing by phagocytes. Electrophysiological recordings further established that, in phagocytes, proton and electron transport are closely associated: phox activation induces a −60 mV shift in the threshold of voltage-activation of proton currents, and the two activities co-segregate in excised patches. This close interaction led us to propose that the phox itself functions as proton channel. Consistent with this hypothesis, we showed that the expression of phox isoforms in HEK-293 cells is sufficient to generate proton currents that recapitulate the properties of native proton channels. However, proton currents persist in phagocytes lacking phox and are absent in COS-phox cells that express a functional oxidase, suggesting instead that the phox is not a proton channel but modulates the activity of a separate channel molecule. The cloning of the voltage-gated proton channel Hv1 will reveal whether Hv1 is the only channel that sustains the activity of the NADPH oxidase, and whether proton currents carried by Hv1 are also modulated by the activity of the phox. This will establish the physiological role of voltage-gated proton channels and open new avenues for the treatment of diseases linked to altered innate immunity.

## 1732-Symp Voltage-gated proton channels

Yasushi Okamura

*Okazaki Inst, Okazaki, Japan.*

Voltage-gated proton channels (Hv channels) were described in snail neurons, mammalian phagocytes, alveolar cells and microglia. They show high proton selectivity of ion permeation and voltage dependence that is pH-sensitive. We have identified a membrane protein, VSOP (also called Hv1) that consists of four transmembrane helices with homology to the voltage sensor of voltage-gated ion channels (Sasaki et al, 2006) and reported that this protein recapitulates most properties of Hv channels. The presence of VSOP/Hv1 protein on phagosome membranes supports the idea that VSOP/Hv1 regulates phagosomal pH and membrane potential. It has been proposed that Hv channels play roles in regulating NADPH oxidase activities through their involvement in membrane potentials and pH. Hv channels also potentially provide protons as substrate for conversion from superoxide anions to hydrogen peroxide, and further conversion by myeloperoxidase to hydrochlorite. Since pathogen or apoptic cells are often engulfed in closed membrane compartments, that are called phagosomes, it will be important to address whether voltage-gated proton channels exist on phagosome membranes. For this purpose, we purified phagosomes from mouse activated neutrophils following treatment with magnetic beads conjugated with IgG. Phagosomes were collected using the magnetic system and western blot was performed using anti-mouse-VSOP polyclonal antibodies. These antibodies were generated against the whole C-terminal cytoplasmic polypeptide of mVSOP. The phagosome membrane fraction was verified by the detection of p47phox, a subunit of NADPH oxidase complex, which is known to be exclusively present in phagosomes, but not on plasma membranes, using anti-p47phox antibody. Band of VSOP/Hv1 (31kd) was clearly detected in the membrane preparation positive for p47phox signal. To gain insights into physiological functions of VSOP/Hv1, we established a mouse line with null expression of VSOP/Hv1 protein, and are studying how phagocytosis function is impaired.

**Symposium 14: Mechanisms of Exo- and Endocytosis**

## 1733-Symp Dynamin Structure

Jenny Hinshaw

*NIDDK, NIH, Bethesda, MD, USA.*

The dynamin family of proteins are large GTPases involved in membrane remodeling events throughout eukaryotic cells. For example, dynamin is involved in the final stages of fission during endocytosis, and the dynamin-related protein (Drp1) is necessary for mitochondrial division. During endocytosis, dynamin is believed to wrap around the necks of coated pits and facilitate vesiculation. In support of this model, purified dynamin self-assembles into spirals (50 nm diameter) and readily form dynamin-lipid tubes, which constrict and fragment upon addition of GTP. Structural changes induced by GTP addition were characterized by solving the three-dimensional structure of dynamin in the constricted and non-constricted states using cryo-electron microscopy. Using a rigid-body Monte Carlo algorithm, the crystal structures of the GTPase and pleckstrin homology domains were fit to the cryo-EM densities. The GTPase domain is placed at the periphery of the helical array while the PH domain is in a position that allows for interactions with lipid headgroups. The placement of the crystal structures into the cryo-EM densities revealed a twisting motion of the GTPase, middle and GTPase-effector domains, which suggests a corkscrew model for dynamin constriction.

To determine if a common mechanism of action exists among the dynamin family members, we examined the structure and function of the Drp1 homologue in yeast, Dnm1. In collaboration with Dr. Jodi Nunnari (UC Davis) we have shown that Dnm1 assembles into large spirals with a diameter of 100 nm. Remarkably, the Dnm1 spirals have the same diameter as the observed mitochondrial constriction sites seen in vivo. Dnm1 also assembles onto liposomes and form Dnm1 decorated tubes that constrict significantly upon GTP addition. These results suggest that although dynamin family members share common characteristics, their structural properties are uniquely tailored to fit their function.

## 1734-Symp Pka Activation Bypasses The Requirement For Unc-31 In The Docking Of Dense Core Vesicles From *C.elegans* Neurons

Tao Xu[1], Ke-Ming Zhou[2], Yong-Ming Dong[2], Qian Ge[2], Dan Zhu[2], Wei Zhou[2], Xian-Guang Lin[2], Tao Liang[2], Zheng-xing Wu[2]

[1] *Institute of Biophysics, Beijing, China,*
[2] *Huazhong University of Science and Technology, Wuhan, China.*

The nematode *C. elegans* provides a powerful model system for exploring the molecular basis of synaptogenesis and neurotransmission. However, the lack of direct functional assays of release processes has largely prevented an in depth understanding of the mechanism of vesicular exocytosis and endocytosis in *C. elegans*. We address this technical limitation by developing direct electrophysiological assays, including membrane capacitance and amperometry measurements, in primary cultured *C. elegans* neurons. In addition, we have succeeded in monitoring the docking and fusion of singledense core vesicles (DCVs) employing total internal reflection fluorescence microscopy. With these approaches and mutant perturbation analysis, we provide direct evidence that UNC-31 is required for the docking of DCVs at the plasma membrane. Interestingly, the defect in DCV docking caused by-UNC-31 mutation can be fully rescued by PKA activation. We alsodemonstrate that UNC-31 is required for UNC-13-mediated augmentation of DCV exocytosis.

## 1735-Symp Intermediates in and Regulations of SNARE-mediated membrane fusion

Yeon-Kyun Shin

*Iowa State Univ, Ames, IA, USA.*

SNAREs are the central components of the intracellular membrane fusion machinery. Association of v- and t-SNAREs bridges two membranes, which facilitates the fusion. We use spin-labeling EPR to determine the structural transitions that SNAREs undergo during SNARE complex formation and membrane fusion. Additionally, we also use the newly developed single fusion assay based on wide field TIRF microscopy to dissect the individual steps along the fusion pathway. The SNARE assembly intermediates that are found with EPR and the fusion intermediates that are discovered with the single fusion assay will be discussed. We will also discuss the regulation of the SNARE-mediated membrane fusion by Ca2+, complexin, and synaptotagmin.

**Platform AG: Protein Conformation**

## 1736-Plat Probing the Cross-β Core Structure of Amyloid Fibrils by Hydrogen-Deuterium Exchange Deep UV Resonance Raman Spectroscopy

Igor K. Lednev, Ming Xu, Victor Shashilov

*University at Albany, SUNY, Albany, NY, USA.*

Studying the structure of amyloid fibrils is important for the detailed understanding of fibrillogenesis at a molecular level. Amyloid fibrils are non-crystalline and insoluble, and thus are not amenable to conventional X-ray crystallography and solution NMR. Several specialized techniques with less general capabilities have been developed and utilized for probing fibrillar structure. Transmission electron microscopy and scanning probe microscopy provide general information on fibril topology. The application of fiber X-ray diffraction and scattering has been limited to short peptides mimicking the core structure of the fibrils formed from amyloidogenic protein. Solid state NMR probes inter-atomic distances and torsion angles, which define local secondary structure and side-chain conformations. Deep UV resonance Raman (DUVRR) spectroscopy have been found to be a powerful tool for protein structural characterization at all stages of fibrillation (1, 2).

We report here on the first application of hydrogen-deuterium exchange DUVRR spectroscopy to probe the secondary structure of the fibril cross-β core (3). This method allowed for structural