## Symposium: EAG-Family Channels: Mechanisms of Disease

**1068-Symp**
**hERG Subunit-Specific Contributions to Gating and Disease**
**Gail A. Robertson, Ph.D**.
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From the identification of leg-shaking Drosophila mutants to the development of drug safety tests and therapeutic treatments for cancer, the Ether-à-go-go family of ion channels has emerged as a rich collection of important targets for translational research - applications of basic science to problems affecting the human population. Critical to these developments has been the biophysical characterization of channel behavior as it relates to their physiological and pathological roles. We have focused on hERG subunits as components of the repolarizing current cardiac $I_{Kr}$, which is a target for inherited and acquired long QT syndrome (LQTS). In association with the originally-identified hERG 1a isoform, hERG 1b modifies channel gating kinetics, increases current amplitude and alters pharmacological sensitivity to a range of drugs. Computational models and the identification of a LQTS patient with a 1b-specific mutation support a role for 1b in normal repolarization, a conclusion bolstered by new findings of 1b abundance in human donor ventricle. These and other findings regarding the composition and macromolecular context of channels producing cardiac $I_{Kr}$ will be discussed.

**1069-Symp**
**Role of the N-Terminal PAS Domain of hERG K Channels in Gating, Disease and Rescue**
**Matt Trudeau**.
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Human ERG (Kv11 or KCNH2) channels are voltage-activated K channels in the eag family of channels. hERG is similar to other eag family member as they are homologous to voltage- activated K channels but also share homology with cyclic nucleotide-gated (CNG) channels. hERG shares important structural features with other members of the eag family including an N-terminal PAS domain (Per-Arnt-Sim) and C-terminal cyclic nucleotide-binding homology domain that is similar to the CNB domain of CNG, however eag family channels are not regulated by cyclic nucleotide. We have recently shown several new properties for the hERG PAS domain and CNBHD. Recently, we showed that the PAS domain made a direct interaction with the rest of the channel and markedly regulated slow deactivation, which is a characteristic of wild-type hERG. The CNBHD also plays a role in deactivation gating; it is necessary for regulation of deactivation as channels with the CNBHD deleted lost slow deactivation. Biochemical pull-down interactions and electrophysiology experiments suggest that the PAS domain makes a direct interaction with the CNBHD and that this interaction underlies slow deactivation. Mutations in hERG-PAS that are linked to LQTS disrupt the interaction of the PAS domain with the rest of the channel, indicating that disruption of this interaction may be the mechanism behind some instances of LQTS. The PAS domain itself can be used to fix aberrant gating in channels with PAS domain mutations, as if the recombinant PAS domain substitutes for the covalently attached, but mutated PAS domain. Here we will also discuss new data regarding the regulatory effect of the PAS domain on other gating transitions besides deactivation and the role of the region that connects the PAS domain to the transmembrane domain of the channel.

**1070-Symp**
**Cytoplasmic Domains of EAG Channels**
**Joao Morais-Cabral**.
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The EAG channels (or more correctly, KCNH channels) are voltage-gated potassium channels that play important roles in cardiac repolarization, neuronal excitability, cellular proliferation and tumor growth. These channels are tetrameric potassium channels with 6 trans-membrane (TM) helices per subunit and characteristic large N- and C-terminal cytoplasmic regions. The function of the cytoplasmic regions remains unclear; it is thought that they are involved in channel regulation since they can be phosphorylated and can interact with kinases, integrins and calmodulin. Interestingly, these cytoplasmic regions contain well defined structural domains, a PAS (Per-Arnt-Sim) domain in the N-terminus and a CNB (cyclic nucleotide binding) homology domain in the C-terminus. The exact role of these domains is still unknown but recent structural and functional data is revealing interesting features.

**1071-Symp**
**EAG1: A Potassium Channel Involved in Cancer**
**Walter Stuhmer**.
Max Planck Institute for Experimental Medicine, Gottingen, Germany.
There is increasing evidence for the causal involvement of ion channels in cancer. Being extracellularly accessible, they constitute a novel and promising targets to treat cancer. Kv10.1 is among the best-characterized ion channels implicated in cancer. Kv10.1 is practically not detected in normal tissues outside the central nervous system, but is aberrantly expressed with very high frequency (>70%) in tumor cells from diverse origin. Its potential to favor tumor progression is maintained to a large extent even if it is unable to permeate ions. We have therefore searched for other mechanisms that mediate and/or favor its malignancy and found that: a) siRNA against Kv10.1, inhibition of channel gating by astemizol and imipramine (both unspecific Kv10.1 blockers) or by a monoclonal blocking antibody are all able to reduce proliferation rates in cell lines expressing the channel. b) Kv10.1 has a high turnover rate at the membrane and is rapidly ubiquitinated and degraded. c) it's expression greatly increases the secretion of VEGF under hypoxia, favoring angiogenesis in a manner similar to the Hypoxia Inducible Factor (HIF). d) Kv10.1 can be detected in the inner nuclear membrane and even potassium currents can be recorded from these membranes, indicating that it is functional. e) miRNA 34 regulates Kv10.1 and p53 in a reciprocal manner.
In retrospective studies it could be shown that Kv10.1 expression in hemaotopetic cells correlates with poor prognosis in a specific leukemia (AML M4). Linking a Fab-fragment specific against Kv10.1 to TNF-related apoptosis inducing ligand (TRAIL), it was possible to selectively induce apoptosis in cancer cells expressing Kv10.1 by an autocrine, and adjacent non-Kv10.1 bearing cells by a paracrine mechanism.

## Symposium: Cargo Transport by Coupled Molecular Motors

**1072-Symp**
**On the Cooperativity of Microtubule Motors during Cargo Transport and Directional Filament Sliding**
**Stefan Diez**[1,2].
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The stepping behavior of single microtubule-based motor proteins has been studied in great detail. However, in cells, these motors often do not work alone but rather function in groups when they transport cellular cargo or slide microtubules against each other. Until now, the cooperative interactions between motors in such groups are poorly understood. We will report on in vitro experiments specifically designed to study the activity of coupled motors. With respect to cargo transport by rigidly-coupled kinesin-1 motors, we investigated (i) the step-size distribution of small groups of motors, (ii) the motility behavior in the presence of obstacles, and (iii) the tug-of-war between antagonistically acting force generators. With respect to the activity of diffusively-coupled kinesin-14 motors during cell division, we studied (i) the directional sliding of microtubules, (ii) the adaptive braking by passive crosslinkers, and (iii) the generation of torsional motion. All observed behaviors result from the collaborative action of multiple motors - none of the described properties is found in the activity of single motors.

**1073-Symp**
**Collective Dynamics of Multiple Microtubule Motors: Connecting *In Vitro* Observations to *In Vivo* Functions**
**Michael R. Diehl**.
Bioengineering, Rice University, Houston, TX, USA.
Despite the efficiencies of many microtubule motors, sub-cellular cargos are often transported by motor teams composed of multiple processive molecular motors. Furthermore, mutant motors associated with diseases can be coupled to cargos that are also outfitted with copies of wild-type motors. Together, these observations raise important questions regarding the role of motor cooperation in intracellular transport. Although abilities to characterize the transport behaviors of multiple motor systems have improved substantially, many aspects of multiple-motor dynamics remain poorly understood. Here, I will describe our efforts to create structurally-defined complexes composed of multiple kinesin motors and examine their dynamics at the 'single-motor' level. I will also describe a transition rate model that predicts the load-dependent transport behaviors of these complexes from detailed measurements of a single motor's elastic