

to elicit an effect, as if the “apo” configuration required the loss of both side chains. Thus, the intrinsic ligand of the CNB homology domain (CNBhD) reduces the stability of the closed state relative to the open state. To determine whether the AA mutant phenotype represents a loss of function, we deleted the CNBhD. In contrast to the AA mutant, the deletion gave rise to faster activation kinetics but a more dramatic g-V shift of +40 mV. This finding indicates that, unlike HCN channel, in which the unliganded CNBD mimics the CNBD deletion, the AA mutant phenotype is not a loss of function of the CNBhD. Instead, both the intrinsically-liganded and apo conformations communicate with the gating machinery. The current properties can be recapitulated by a 7-state allosteric model, in which the AA mutant alters multiple transitions during channel gating whereas CNBhD deletion affects only the last opening step. We conclude that the EAG1 CNBhD serves a function mechanistically distinct from those of the corresponding domains in channels gated by cyclic nucleotides despite extensive sequence and structural homology.

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An Eag Domain Polypeptide Regulates the Deactivation Kinetics of the hERG 1A-3.1 Splice Variant Linked to Schizophrenia

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The Human Ether-à-go-go Related Gene (hERG) encodes a voltage-activated K⁺ channel. hERG contributes to the repolarization of the ventricular action potential as the primary component of the cardiac delayed rectifier K⁺ current (I_{Kr}) and has also been shown by many groups to modulate neuronal firing frequency. Disruption of channel function by inherited mutations in the gene encoding hERG has been shown to cause type 2 long QT syndrome (LQT2). hERG gating is characterized by rapid inactivation upon depolarization and rapid recovery from inactivation and slow closing (deactivation) upon repolarization. Unlike other K⁺ channels, hERG, ELK and EAG channels contain an N-terminal eag domain, composed of a PAS domain and a PAS-Cap domain in the N-terminal region. Inter-subunit interactions between the N-terminal eag domain and the C-terminal cyclic-nucleotide binding homology domain regulate slow deactivation kinetics in the original variant of hERG, hERG1a channels. A recently discovered splice variant, hERG 1a-3.1, has much faster deactivation and has been linked to schizophrenia. We demonstrate in HEK293 cells, that co-expression of hERG1a-3.1 with soluble eag domain peptide partially rescues deactivation kinetics, presumably by providing a fully functional eag domain to the eag-truncated hERG1a-3.1 to slow channel deactivation. Cells co-expressing hERG1a-3.1 + eag domain polypeptides had significantly slower fast and slow time constants of deactivation compared to hERG1a-3.1 channels alone (p<0.05). We conclude that eag domain polypeptides are able to partially restore deactivation gating. Current experiments are utilizing FRET to assess if the hERG1a-3.1 C-terminus interaction with the eag domain is physically disrupted.

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Eag K⁺ Channel Binding to CaMKII: Structural and Biochemical Characterization

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In *Drosophila*, the tetrameric *Ether-à-go-go* (EAG) voltage-gated K⁺ channel contains in its C-terminal cytoplasmic region a substrate-like binding domain for Ca²⁺/calmodulin-dependent protein kinase II (CaMKII-BD). This Ser/Thr kinase forms a dodecamer and each subunit includes an association domain mediating multimerization and a catalytic domain corresponding to the typical protein kinase fold. In between these domains, CaMKII contains a regulatory segment (RS) harboring a CaMKII-BD with homology to that of EAG followed by a Ca²⁺/CaM-binding region. This RS has a regulatory role, mediating the inhibition and activation of the kinase. CaMKII is activated by Ca²⁺/CaM-binding and can become Ca²⁺/CaM-independent upon phosphorylation of its CaMKII-BD. EAG is phosphorylated by CaMKII *in vivo*, and the two proteins form a stable complex *in vitro*. CaMKII-bound EAG keeps the kinase active in the absence of Ca²⁺/CaM and autophosphorylation.

The interaction of the constitutively active CaMKII kinase domain with the EAG CaMKII-BD has been characterized. The measurement of K_D values showed that the EAG CaMKII-BD is a high affinity CaMKII substrate, lying in the nanomolar range in comparison with the micromolar range of typical protein kinase substrates. Crystal structures were determined for the ternary complexes CaMKII-Mg²⁺/ATP-EAG and CaMKII-Mg²⁺/ADP-EAG^P corresponding to the initial and final states of the trans-phosphorylation mecha-

nism, respectively. These structures are globally very similar, with relative rearrangements at the catalytic site. An alanine scan of the EAG residues lying at the CaMKII-EAG interface revealed that practically every residue contributes significantly for the binding strength of the complex. The association of the two proteins in the cell might lie beyond the usual transient kinase-substrate association model, likely corresponding to a long-lasting robust complex.

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Examining the Role of Direct cAMP-Binding Versus PKA-Mediated Effects on Interactions between the Cardiac Potassium Channel α -Subunit Proteins Herg and KvLQT1

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KvLQT1 and hERG are the voltage-gated K⁺ channel α -subunits of the cardiac repolarizing currents I_{Ks} and I_{Kr}, respectively. These currents function *in vivo* with some redundancy to maintain appropriate action potential durations (APDs) in cardiomyocytes. As such, protein-protein interactions between hERG and KvLQT1 may be important in normal cardiac electrophysiology, as well as in arrhythmia and sudden cardiac death. Previous phenomenological observations of functional, mutual downregulation between these complementary repolarizing currents in transgenic rabbit models have motivated our investigations into interactions between hERG and KvLQT1. Moreover, reports of interplay between hERG and KvLQT1 have been described both in the literature and anecdotally, and our recent work demonstrated that the interaction is mediated by the C-termini of both α -subunits and is modulated by cAMP. We hypothesize that direct binding of cAMP to the putative cyclic nucleotide binding domain (CNBD) in the hERG C-terminus abrogates hERG-KvLQT1 interactions, as assessed by FRET analyses. We have developed ion channel fusions to GFP variants, which include hERG CNBD mutants as well as phospho-null and phospho-mimetic mutants. cAMP levels are altered in heterologous cells expressing ion channel constructs through membrane-permeable cAMP analogs or forskolin along with IBMX. Through classic biochemical assays and quantitative FRET approaches, we aim to delineate the direct role of cAMP from that of downstream, PKA-mediated effects in regulating interactions between KvLQT1 and hERG in model systems. This work potentially furthers our understanding of hERG-KvLQT1 interactions and may elucidate mechanisms that underlie many types of arrhythmia as well as characterize novel interactions between two distinct potassium channel families.

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The Subproteome of mitoBKCa from Cardiomyocytes Reveals Novel Insights into BK Channel Function and Pathology

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The mitochondrial large-conductance Ca²⁺-activated K⁺ channel (mitoBKCa) of adult cardiomyocytes contains a C-terminal 50 amino acid insert (DEC) as essential for mitochondrial targeting. The activation of mitochondrial BK channels (mitoBKCa) protects the heart from ischemia and reperfusion injury, highlighting its significant role in cardiac function. Here, we characterized the subproteome of mitoBKCa in cardiomyocytes in the quest to better understand its physiological and pathological relevance in the heart. To this end, we used GST-DEC and GST proteins to pull down proteins interacting with DEC using cardiomyocyte lysates and mitochondrial lysates. We also used monoclonal BK antibody and IgG to immunoprecipitate proteins interacting with mitoBKCa using Percoll purified mitochondrial lysates from whole heart. In all, we identified 212 cellular proteins and 157 mitochondrial proteins interacting with DEC; and 60 mitochondrial proteins interacting with mitoBKCa. Gene ontology analysis revealed mitoBKCa potential contribution to multiple cellular functions. It could contribute to oxidative respiration through cytochrome c oxidase (5 subunits) and ADP/ATP translocase (AAC), including AAC1 (10 peptides, n=2) and AAC2 (13 peptides, n=2), and regulate mitochondrial metabolism through acetyl-Coenzyme transferase 2 (7 peptides, n=2). In addition, our data revealed that mitoBKCa has a broader impact. It might facilitate the pathological process of Parkinson's disease and multiple types of cancer by interacting with critical enzymes, such as NADH dehydrogenase (7 subunits, 3-7 peptides each) and integrin linked kinase (2 peptides, n=1). Excitingly, mitoBKCa interacted with 15 different subunits of the ATP synthase, which forms the permeability transition pore, underscoring a new mechanism for mitoBKCa to protect the heart. In summary, proteomic analysis identified a wide variety of cellular and mitochondrial proteins forming complex with mitoBKCa channel, revealing new paradigms for BK channel function.