New insights into molecular function of large- conductance voltage- and calcium- activated potassium channels (BK) and calcium nanodomains

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In neurons, sites of Ca^{2+} influx and Ca^{2+} sensors are located within 20-50 nm, in subcellular "Ca^{2+} nanodomains". Such tight coupling is key for the functional properties of synapses and neuronal excitability. Two main players act together in nanodomains, coupling Ca^{2+} signal to membrane potential: the voltage-dependent Ca^{2+} channels (Cav) and the large conductance Ca^{2+} and voltage-gated K^+ channels (BK). BK channels are characterized by synergistic activation by Ca^{2+} and membrane depolarization, but the molecular mechanism underlying channel function is not completely understood. Information about isolated moieties of the channel has been obtained using different approaches. Nevertheless, the specialized behavior of this channel must be studied in the whole protein complex at the membrane to determine the complete range of structures and movements critical to its *in vivo* function.

In our laboratory we combine genetics, biochemistry, electrophysiology and spectroscopy, which we correlate with protein structural analysis, to investigate the real time structural dynamics underlying the molecular coupling of Ca^{2+}, voltage and activation of BK channels at the membrane as well as their structural organization and clustering with Cav complexes in reconstituted nanodomains taking advantage of imaging techniques. In addition, BK subcellular localization and role in Ca^{2+} neuronal nanodomains make these channels perfect candidates as reporters of local changes in [Ca^{2+}] restricted to specific subcellular regions close to the membrane. We have created fluorescent variants of the channel that report BK activity induced by Ca^{2+} binding, or Ca^{2+} binding and voltage. We aim to optimize and deploy these novel optoelectrical reporters to study relevant Ca^{2+}-induced processes both in cellular and animal models. Overall, optically-active BK channels with spectrally-separate photoactivation and FRET modules offer many possibilities for the study of activation in mammalian cells.