

A Tail of Multiple Calcium-sensing Domains

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Why does she do the things that she does? In the process of evolution Mother Nature has come up with some very complicated, seemingly illogical solutions. Probing the structure–function relationships of ion channel proteins by site-directed mutagenesis has led to many marvelous insights, but more commonly we find that molecular evolution is devilishly difficult to understand and predict; for every published success using the techniques of site-directed mutagenesis, there are many other attempts by hardworking talented researchers that don't work out. Rather than logical “intelligent design” molecular evolution is opportunistic and desultory; trying to figure out what Mother Nature had in mind in designing these complex proteins often puts researchers in the same frame of mind as, legend tells us, Sigmund Freud, was in after a particularly exasperating session with a female patient, when he exclaimed “What do women want? Dear God, what do they want?”

Site-directed mutagenesis of voltage-gated potassium channels started out crisply after the cloning and heterologous expression of the first voltage-gated potassium channels, with a lucid analysis of N-type inactivation (Hoshi et al., 1990). However, analysis of the mechanism of voltage gating of potassium channels has been frustrating, producing an ongoing debate that even crystal structures have not resolved (Long et al., 2005). More quietly, the analysis of calcium sensing in the voltage- and calcium-dependent BK channels has progressed in a collegial manner, with a series of laboratories each making significant contributions and passing the baton to the next. Cloning of the mammalian BK channel revealed a “core” structure similar to voltage-dependent potassium channels but with an extensive “tail” carboxyl extension containing highly conserved structural elements unlike those seen in voltage-dependent channels (Butler, et al., 1993; Pallanck and Ganetzky, 1994; Tseng-Crank et al., 1994). Further studies identified a high-affinity calcium-sensing site on the tail termed the “calcium bowl” (Schreiber and Salkoff, 1997) and showed that the channel was constructed along modular lines; the distal tail region containing the calcium bowl could be removed from the core of the channel and be coexpressed as a separate peptide,

thereby reconstituting all normal channel properties (Wei et al., 1994; Schreiber et al., 1999). Two other laboratories demonstrated calcium binding to the tail domain containing the calcium bowl (Bian et al., 2001; Bao et al., 2004). Yet other laboratories identified a second high-affinity calcium sensor on the proximal tail region, embedded within the upstream RCK domain (Bao et al., 2002; Xia et al., 2002). Additional research further extended the concept of modularity by substituting pH sensors from a related channel for the BK calcium sensors (Xia et al., 2004), and other studies identified a third lower-affinity cation-sensing site on the tail that can sense magnesium as well as calcium (Shi et al., 2002; Xia et al., 2002). Now, the baton has returned to the Magleby group, which has extended these studies. Previous results from Magleby's lab showed that BK channels can open with zero, one, two, three, or four functional calcium bowls, and that each subunit with a functional calcium bowl contributes a stepwise increase to both the cooperativity of activation and the apparent Ca^{2+} sensitivity as the number of functional subunits increases (Niu and Magleby, 2002). The present article now examines how the structurally distinct calcium sensing sites in the RCK1 domain might work in concert with the calcium bowls (see Qian et al. on p. 389 of this issue). A hallmark of the BK channel is its ability to function over a very broad range of calcium concentrations. One way of achieving this might be to incorporate several independent calcium-sensing sites that differ in their sensitivities and which successively “kick in” as ambient calcium concentrations are raised. In contrast, Qian et al., found that the two high-affinity sites are roughly equivalent in their effects. Why are the two distinct sites equivalent? This question becomes even more puzzling when considering that the two sites bear no resemblance to each other in their primary structure. Furthermore, each can function independently of the other. By shuffling wild-type and mutant calcium sensing sites around on different subunits, the authors examined channels with eight different possible configurations of high-affinity Ca^{2+} sensors on the subunits. The results show that the RCK1 sensor and Ca bowl contribute about equally to Ca^{2+} activation of the channel when there is only one high-affinity Ca^{2+} sensor per subunit. However, an RCK1 sensor and a Ca bowl on the same subunit are much more effective than

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when they are on different subunits, indicating positive intrasubunit cooperativity. This complicated system of cooperativity involving calcium-sensing sites on different, as well as the same subunits, may underlie the responsiveness of BK channels to such a broad range of calcium concentrations.

Why do the calcium-sensing sites in BK channels, though functionally very similar, bear no resemblance to each other, at least as far as primary structure is concerned? Some calcium-binding proteins with more than one calcium-sensing site have evolved by duplication of the calcium-sensing domain. Calmodulin is one example, and “intelligent design” might lead that way, especially if the multiple calcium-sensing sites have similar sensitivities. Perhaps some unknown structural complementarity underlies the complex design of BK channels and is necessary to achieve the desired cooperativity. Whatever the reason and route to the present design, this arrangement seems to be optimized; BK channel structure is highly conserved among invertebrates as well as vertebrates and is apparently ideal for various functional roles not only in different animals but also in a plethora of different cell types and organs in a single animal.

Structurally, it remains to be determined how cooperativity between the sites is achieved. The authors offer an allosteric model incorporating intrasubunit cooperativity nested within intersubunit cooperativity, which approximates their results (the P_o vs. Ca^{2+} response) for eight possible subunit configurations of the high-affinity Ca^{2+} sensors. Thus, for now, lacking a detailed structural model, we must be content with the mysterious W , the empirical intrasubunit cooperativity factor, introduced by Magleby to make the model of cooperativity work (one wonders if the symbol “ W ” was borrowed from the W used to describe a hypothetical dark energy component of Einstein’s cosmological constant). A final picture of cooperativity between sites may only be settled after crystal structure of the tail is achieved showing how the different sites interact. In any event, this new and elegant contribution from the Magleby lab will help us left brain–biased researchers appreciate what Mother Nature had in mind in her unintelligent design of a marvelously useful, and very

complicated, protein for sensing calcium over a broad concentration range.

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