Article Addendum

Phylogenetic profiles reveal structural/functional determinants of TRPC3 signal-sensing antennae

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Biochemical assessment of channel structure/function is incredibly challenging. Developing computational tools that provide these data would enable translational research, accelerating mechanistic experimentation for the bench scientist studying ion channels. Starting with the premise that protein sequence encodes information about structure, function and evolution (SF&E), we developed a unified framework for inferring SF&E from sequence information using a knowledge-based approach. The Gestalt Domain Detection Algorithm-Basic Local Alignment Tool (GDDA-BLAST) provides phylogenetic profiles that can model, ab initio, SF&E relationships of biological sequences at the whole protein, single domain and single-amino acid level.1,2 In our recent paper,3 we have applied GDDA-BLAST analysis to study canonical TRP (TRPC) channels1 and empirically validated predicted lipid-binding and trafficking activities contained within the TRPC3 TRP_2 domain of unknown function. Overall, our in silico, in vitro, and in vivo experiments support a model in which TRPC3 has signal-sensing antennae which are adorned with lipid-binding, trafficking and calmodulin regulatory domains. In this Addendum, we correlate our functional domain analysis with the cryo-EM structure of TRPC3.3 In addition, we synthesize recent studies with our new findings to provide a refined model on the mechanism(s) of TRPC3 activation/deactivation.

Correlating Computational Models to Experimental Evidence

In our recent theoretical paper,1 we have proposed that phylogenetic profiles built using ontologically related functional domain profiles provide a unified framework for deriving SF&E information from protein sequences. We applied this approach to study transient receptor potential channels (TRP) as a benchmark set of proteins.4 Overall, functional annotation for TRP channels is lacking. For example, formal queries of TRPC protein sequences with popular domain detection algorithms (Pfam, SMART, NCBI-CDD, Interproscan) predict that TRPC channels contain only one functional domain (ion channel), and two domains whose function is unknown (ankyrin, TRP_2), thus leaving the majority of the protein without functional annotation. This is in stark contrast to the literature in which multiple protein and lipid interactions,5-12 as well as sites for covalent modification have been identified.13-17

In Figure 1A, we present our computational analysis of human TRPC3 with multiple structural/functional profiles related by ontology (i.e., keywords) for peripheral lipid-binding, integral lipid-binding, calmodulin-binding and ankyrin repeats. The results identify multiple lipid/calmodulin-binding modules along the cytosolic N and C termini. Indeed, 4/5 regions have been experimentally validated to bind lipids in TRP channels.9,11,18,19 In our recent manuscript,4 we validated GDDA-BLAST predictions in the TRP_2 domain of TRPC3 for lipid-binding/trafficking activities. Indeed, TRP_2 has relatively non-specific lipid-binding activity, which upon mutation of S209/S213 to mirror TRPC1, increases its lipid-binding affinity. This is associated with defective channels unable to insert into the plasma membrane in response to diacylglycerol (DAG)-mediated membrane destabilization. Furthermore, based on (i) our analysis of trafficking motifs in TRPC3 (which are comprised primarily of SNARE domain profiles),1 and (ii) the role of TRPC3 TRP_2 in its own vesicle fusion,4 we suggest that TRPC3 contains “SNARE-like” activity, similar to that observed for TRPM7.20 Taken together, our recent findings provide a mechanistic and functional difference between DAG-sensitive TRPCs (3/6/7) and DAG-insensitive TRPCs (1/4/5), which has been a controversial question in the TRPC field for almost a decade.21,22

A recent study by Mio et al. obtained a cryo-EM structure of Transient Receptor Potential Channel 3 (TRPC3) and modeled the six transmembrane helices with the atomic structure of the potassium channels KcsA and Kv1.2,3 (Fig. 1B). Interestingly, these authors also determined that TRPC3 contains a globular, and presumably hydrophobic, inner-shell surrounded by signal sensing antenna derived from the cytosolic N and C-termini. Indeed, GDDA-BLAST...
predicts the inner-shell in TRPC3/6/7 as well as signal sensing antennae with the capacity to make multiple lipid contacts with the plasma membrane (i.e., swelling). Based on our computational functional domain predictions we have provided a rough annotation on the cryo-EM structure. Although speculative, it is intriguing how GDDA-BLAST results correlate to known structural elements.3,23 A number of implications can be derived from such a model. For example, the N-terminus forms a “thumb” on the ankyrin repeat “hand”. Further, the C-terminus forms an “arm” perpendicular to the plasma membrane that could be parallel when bound to PIP2 (TRP-box) and/or PIP3 (C-terminal arm).11,19 Two regions between the ankyrin repeats and the inner-shell are not obvious as to their relative amino acid positions. Nevertheless, we propose that the linker (green) comprises the TRP_2 domain, and that the “knob-like projection” (turquoise) near the plasma membrane is a lipid-binding domain.

Figure 1. Structure/Function Modeling of TRPC3. (A) GDDA-BLAST histogram depicting phylogenetic profile signals for peripheral lipid-binding (magenta, 131 profiles), integral lipid-binding (yellow, 98 profiles), ankyrin repeats (turquoise, 54 profiles) and calmodulin-binding (green, 166 profiles). We observe multiple calmodulin-binding domains that overlap with lipid-binding domains in the N-terminal ankyrin repeats, as well as a major site in the C-terminus. These results predict at least 5 lipid-binding domains and 4 calmodulin-binding sites in TRPC3. (Functional phylogenetic profiles for TRP channels available upon request) (B) left: Cryo EM structure of TRPC3 from Mio et al.3 where they suggest the N-terminal and C-terminal tails of TRPC3 form signal-sensing antennae. right: Artistic representation of the domain architecture for TRPC3. The N-terminal and C-terminal regions, channel, inner-shell and ankyrin repeats are approximated from the Mio et al. study.3 The other regions are based on the overall topology when compared with our GDDA-BLAST results. Based on this model, each region of the channel predicted to interact with lipid/calmodulin has a solvent-accessible surface that would allow for protein/lipid-interactions, particularly if these regions moved to contact the plasma membrane.
Figure 2. For figure legend, see page 136.
Figure 2. Theoretical Model for the TRPC3 life cycle. Components: (left) Graphical representation of the lipid-binding (magenta) and calmodulin-binding (green) domain predictions from GDDA-BLAST. Inset within the lipid-binding domains, lipid-binding specificity of each region is depicted. Below these predictions are known and predicted (S209/S213) PKC and PKG phosphorylation sites (red), and the binding regions for known TRPC3-interacting proteins.5,6,9,12,25,27 We observe that the phosphorylation and protein interactions sites correlate with our lipid/calmodulin interaction sites, suggesting multiple regulation mechanisms for these interactions, which has been observed for TRP channels.9 (right) Graphical representation of the lipid-binding, calmodulin binding and phosphorylation sites for a TRPC3 homo-tetramer. (PL = phospholipids, S1P = sphingosine-1-phosphate) Insertion: Receptor activation (GPCR, RTK, etc.) signals for the activation of PLC, generating DAG and IP3, as well as signaling for VAMP2-dependent trafficking of TRPC3 to the plasma membrane, where the vesicle docks.5 Inset: Docking occurs through VAMP2 interactions with SNARE complexes, as well as TRPC3 TRP_2 lipid-binding.4 Following, increased DAG levels destabilize the plasma membrane, allowing TRPC3 TRP_2 domain lipid-binding, facilitating vesicle fusion.4 Activation: We propose that once inserted, the TRP_2 domain associates with phospholipids, stabilizing the ion-channel in the membrane. Following, PLCγ binds to the N-terminus of TRPC3, where the channel can then “search” for PIP_2.9 Concomitantly, the C-terminus of TRPC3 can search for PI3P,11 spatially localizing the channel, as has been observed for other membrane-associated proteins such as PhospholipaseA2.28 Once properly localized, interactions with both lipids and proteins (IP_3R, RACK1, Homer, PLCγ) control the overall activity of the channel. We propose that these interactions swell the ion-channel by placing the N- and C-terminal tails of TRPC3 parallel to the plasma membrane. Deactivation: Increased Ca^2+ and DAG levels allow PKC to phosphorylate TRPC3 within lipid/protein binding domains, altering these interactions.8 Increased Ca^2+ also activates calmodulin, which associates with phosphorylated TRPC3, blocking the protein/lipid-interactions. Losses of these interactions allow the N- and C-terminus of TRPC3 to dissociate from the plasma membrane, closing the channel. Following, the channel can be endocytosed through a Homer-dependent pathway.26 Upon endocytosis and decreases in intracellular Ca^2+, calmodulin is released from TRPC3, allowing the channel to be “reset” for recycling to the plasma membrane, or targeted for degradation.

Conclusions

Our functional models of TRPC3 are far from “high-definition”. For instance, we have not yet modeled other known regulatory elements for these channels including homers, IP_3R and nitrosylation. Further, our current models cannot predict, de novo, the functional consequences of such interactions (e.g., what does calmodulin do when bound to the N-terminus? Which PIP_2-binding sites are stimulatory vs. inhibitory?). Added complexity stems from the ability of TRPC channels to form homo- and hetero-multimers; our models cannot predict how these domains control function within a specific tetramer. That being stated, even in this nascent stage, GDDA-BLAST analysis can speed the discovery process for the bench scientist by providing a rationale for directed experimentation including mutagenesis. Results from such experiments can be iterated back onto our computational models, towards improving the resolution of our computational measures. In closing, we propose that additional development of GDDA-BLAST will lead to refined phylogenetic profiles which can accurately predict protein domain function as well as the specificity of their interactions.

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