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Gating and calcium-sensing mechanisms of TRPA1 channels revealed. Commentary on “Irritant-evoked activation and calcium modulation of the TRPA1 receptor”

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Abstract

Novel structures of the human TRPA1 channel were determined in the presence of the agonist iodoacetamide and the antagonist A-967079, to reveal the open and closed states of the channel, respectively. The structures further revealed the location of Ca^{2+} modulatory site that is likely conserved among several TRP subgroups.

Graphical Abstract

The TRPA1 channel forms a unique clade of the transient receptor potential (TRP) superfamily, characterized structurally by an N-terminal domain of extended ankyrin-repeats, a transmembrane voltage-sensor like domain (VSLD), a central pore domain, and a C-terminal “TRP helix”. Functionally, TRPA1 is a calcium-permeable TRP channel expressed in cells that are part of our nociceptive pathways. These channels are activated by chemical irritants such as allyl isothiocyanate (AITC), which is responsible for the pungent taste of mustard and horseradish, as well as 2-chlorobenzalmononitrile, the active

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Author contributions

B.S.R. and S.E.B. wrote and edited the manuscript.

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ingredient in CS gas (“tear gas”). TRPA1 channel activity is also modulated by cytosolic Ca^{2+} in a bimodal fashion, with low concentrations facilitating activation, and higher $[\text{Ca}^{2+}]$ leading to desensitization (1, 2). Building on their previous work, the structural basis for activation and Ca^{2+} -modulation was recently addressed in a collaborative study from the Julius and Cheng groups at UCSF, by a combination of electrophysiology and cryo-electron microscopy (3, 4).

For structural studies, purified TRPA1 channels were incubated with either the irreversible agonist iodoacetamide (IA) to stabilize channels in the open state, or the antagonist A-967079 (A-96) to stabilize the closed state. These structures provide a clear picture of the conformational changes that underlying TRPA1 activation by nucleophiles like IA, which act primarily through covalent binding to the sulfhydryl side chains of C621 and C655 (located in the cytosolic ankyrin-repeat domain). Modification of the cysteines impacts the position of the TRP helix in a region the authors termed the “allosteric nexus”, where stimuli coming from the cytoplasmic region and/or VSLD integrates through the TRP helix, which is an extension of the pore-lining S6 helix. Thus movement of the nexus in turn leads to a rotation of the VSLD, which further facilitates a twist and upward translocation of the pore-lining helices, reshaping the ion permeation pathway (Figure 1A,B). The general idea that the TRP helix serves as a signal integrator in allosteric modulation of TRP channels has been around for more than 10 years (5). However, rigid body movement of the VSLD, which appears to transduce intracellular conformational changes to the selectivity filter of the channel, reveals a novel and unique mechanism for this domain, a structural homolog of the well-studied voltage-sensing domain (VSD) of voltage-gated Na, K, and Ca channels (6). Rather than moving in response to a change in transmembrane voltage, the VSLD in TRP channels transduces a chemical reaction by responding to ligand binding.

Owing to the high resolution of their agonist-bound structure (2.6 Å), the authors were further able to resolve a Ca^{2+} binding site within the TRPA1 cytosolic S2–S3 junction (within the VSLD), likely formed by the side chains of E788, E808, Q791 and N805 (Figure 1C). As it turns out, a Ca^{2+} binding site formed by homologous residues can be found in other TRP subgroups, specifically TRPM 2, 4, and 8 and TRPC5 (4, 7–9). Although the critical residues for the S2–S3 Ca^{2+} site are absent from TRPV1–4 channels, interestingly, some of the coordinating residues are present in TRPV5–6 where the S2–S3 linker has been related to Ca^{2+} -dependent inactivation (Figure 1D) (10). Thus the Ca^{2+} modulating role via the S2–S3 linker is emerging as a common theme among several TRP subgroups.

The Ca^{2+} site found in TRPA1, as well as TRPC5, likely has a high affinity for Ca^{2+} , as purification of the channels for these structural studies was performed in Ca^{2+} -free solutions (4, 7). The S2–S3 Ca^{2+} site was found to be important to TRPA1 channel function; previous work demonstrated that mutations of different combinations of residues forming this site can knock out either Ca^{2+} -mediated potentiation alone, or potentiation along with desensitization (1). The Chen and Julius groups show that in TRPA1-expressing HEK cells co-transfected with M1 muscarinic ACh receptor, whose activation leads to cytosolic Ca^{2+} release, activation of the M1 receptors with carbachol can consequently activate TRPA1 currents (4). Mutation of the Ca^{2+} -coordinating side chain of E788 to serine eliminates the carbachol-activated current, whereas activation of the channel by AITC remains intact. This

exciting new structural information will provide an important framework to elucidate these Ca^{2+} -dependent regulatory mechanisms, which may be critical not only to regulation of TRPA1, but other TRP channels involved in an array of sensory and signal transducing modalities.

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Highlights:

- Structures of the human TRPA1 channel were solved in the presence of the agonist iodoacetamide and the antagonist A-967079, to reveal open and closed states.
- A conserved Ca^{2+} modulatory site is found in a segment at the cytosolic end of the S2 and S3 transmembrane helices.
- We discuss insights gained from these structures toward gating and modulation in TRPA1 channels.

Figure 1. Structural basis for gating and Ca²⁺-sensing in TRPA1.

A, Structure of iodoacetamide (IA) bound, open TRPA1 (PDB ID 6V9X); two of the channel subunits have been removed for clarity. IA (indicated by red boxes) is covalently attached to C621 within the ankyrin domain (AD). The ligand interacts with residues in the allosteric nexus (AN) to elicit conformational changes in the membrane domain (MD). These, in turn, drive opening of both an upper gate (UG) and lower gate (LG), to open the ion permeation pathway, indicated by black lines. B, Structure of closed TRPA1 (PDB ID 6V9Y). In the absence of bound agonist, UG and LG constrict the pore to preclude ion permeation. C, Structure of the Ca^{2+} -bound S2–S3 linker region (PDB ID 6V9V). Key Ca^{2+} -coordinating side chains are shown. D, Partial sequence alignment of several TRP family orthologs in the S2–S3 linker region, showing strong conservation among TRPA1, TRPM2/4/8, TRPC5, and crTRP1.