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STRUCTURAL BIOLOGY

Pain-sensing TRPA1 channel resolved

The TRPA1 ion channel activates pain pathways in response to noxious compounds. The structure of TRPA1 has now been solved, providing insight into how it functions.

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Rooted in place, many plants resort to chemical warfare to survive predation by bacteria, fungi, insects and mammalian herbivores. They produce pungent natural chemicals, such as capsaicin, which makes chilli peppers 'hot', and the thiosulfinates that make onion chopping a tear-jerker. Reactive chemicals in onions, wasabi and related spices activate the ion channel TRPA1, a relative of the capsaicin receptor TRPV1. In a paper published on *Nature*'s website today, Paulsen *et al.*[1] follow up their description of TRPV1 (refs 2, 3). Using electron cryo-microscopy techniques, they define the full-length, single-particle structure of TRPA1 to around 4 ångströms, a level of resolution that reveals its general features.

TRP channels are found in almost all cell types in eukaryotes (organisms that include plants, animals and fungi). There are 27 human TRP-channel genes, which mostly encode weakly selective cation channels that enable ion flux across membranes in response to the binding of extracellular or intracellular ligands, or to changes in temperature or membrane voltage. On opening, TRP channels reduce the voltage across membranes and enable cations such as calcium ions (Ca^{2+}) to flow into the cytoplasm[4].

TRPA1 is found in the plasma membranes of pain-detecting sensory nerves[4]. It activates pain pathways that trigger avoidance behaviours and pathways that promote longer-lasting biological responses, such as inflammation[5]. Blocking TRPA1 function is therefore a promising strategy for treating pain. Pungent agents from wasabi, and other TRPA1 triggers, are known to be electrophiles, activating the channel by forming covalent bonds with specific cysteine or lysine amino-acid residues. However, the overall organization of the channel has been a mystery, frustrating efforts to understand exactly how ligand binding activates TRPA1.

Ion channels that open in response to ligand binding or voltage changes typically share the same primary architecture, in which each subunit of the channel contains six membrane-spanning α-helical domains, S1–S6 (ref. 6). Paulsen *et al.* report that, like archetypal voltage-gated potassium channels[6], four TRPA1 subunits come together to form a homotetrameric ion channel, with domains S5 and S6 of each subunit contributing to a shared ion-conducting pore. In highly voltage-sensitive channels, the S1–S4 domain is a charged adaptor, which enables voltage changes across the membrane to open and close (gate) the pore[6]. TRP channels are less voltage-sensitive than some other channels, and their S1–S4 domain probably translates the energy of ligand binding into this gating movement.

Paulsen and co-workers show that TRPA1 channels, like other ion channels, have two primary constrictions, one at either end of the channel's ion-conducting pathway (Fig. 1). Similar to bacterial voltage-gated sodium channels[7,8], each subunit of the channel's outer pore domain contains two short 'pore helices', which point into the ion-conduction pathway — a difference from the typical solitary pore helix found in other channels. These helices slope steeply down to the first constriction and ion-selectivity filter, which is big enough to allow partially hydrated Ca^{2+} ions to pass through. The authors find that one type of TRPA1 antagonist binds to the channel close to S5, S6 and the first pore helix, which could explain its ability to inhibit gating.

The second constriction is formed by two hydrophobic amino-acid residues from each subunit. It is lower down in the membrane, close to the cytoplasmic face of

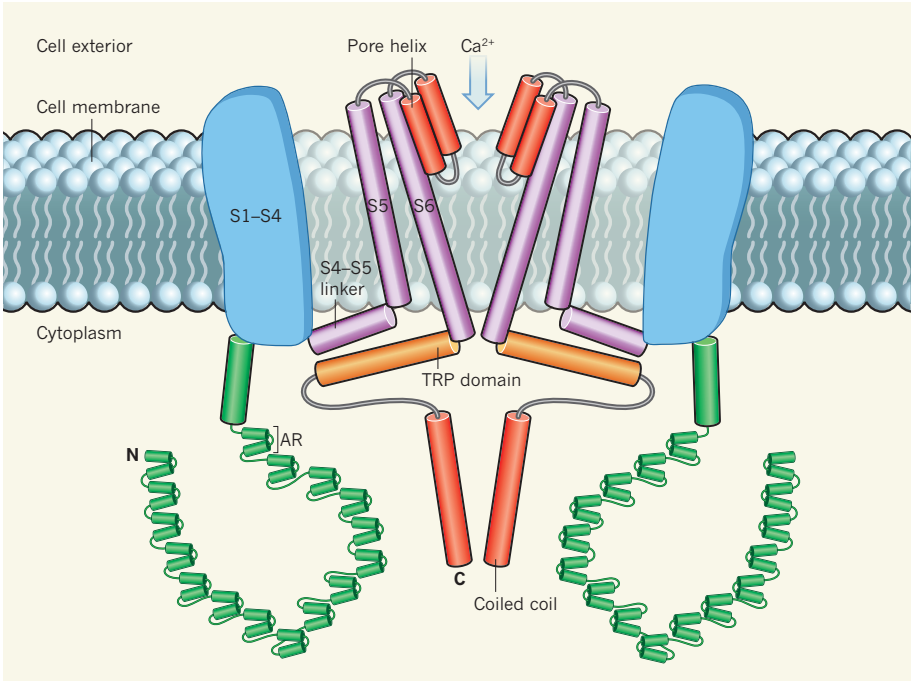

Figure 1 | The structure of TRPA1. A schematic of key features of the ion channel TRPA1, which mediates the cellular influx of cations such as calcium ions (Ca^{2+}). Two TRPA1 subunits are shown, although the channel is comprised of four. Paulsen *et al.*[1] report that each subunit contains six membrane-spanning α-helical domains, S1–S6. Two pore helices link S5 and S6 at the extracellular surface, where a constriction regulates influx of Ca^{2+}. The helical TRP domain is part of a second, lower constriction. Sixteen ankyrin repeats (ARs) at the amino-terminal end (N) of the subunit cover a carboxy-terminal (C) coiled-coil structure, providing a large cytoplasmic surface for interactions with noxious agents. It is probable that the molecular interactions of ligands with ARs lead to conformational changes, conveyed through the S4–S5 linker structure, that open the channel. The lower constriction is closed to ions in the authors' structure, and may be in either a closed or desensitized state.

the channel, and the authors seem to have captured it in a closed conformation.

For channel aficionados, this is a familiar story, so what is different about the TRPA1 structure? Paulsen and colleagues find that around 80% of TRPA1's mass is outside the channel's core, in the amino- and carboxy-terminal domains. A coiled-coil domain in the C terminus forms the cytoplasmic stalk of the channel, and is surrounded by 'ankyrin repeats', which it is speculated[9] contain the cysteine residues targeted by electrophilic TRPA1 activators. An ankyrin repeat is a motif of 33 amino acids that forms two α-helices separated by loops. They are one of the most common, evolutionarily conserved structural motifs, and generally form protein-interaction domains[10].

Paulsen and co-workers' three-dimensional reconstruction and modelling experiments indicate that each of the 4 subunits of TRPA1 has at least 16 ankyrin repeats, 5 of which are well resolved in the authors' structure and 11 of which their models suggest form a propeller-like structure, resembling the backs of four armadillos, suspended below the membrane (see Fig. 1c of the paper[1]). The researchers propose that this structure regulates gating of the pore.

All TRP channels contain a TRP domain — a helix at the base of S6. In Paulsen and co-workers' structure, this helix runs perpendicular to the pore helices, parallel to the membrane and suspended above the ankyrin repeats, where it might contribute to regulation of the lower pore. Perhaps most interesting is the coiled-coil stalk, which seems to mediate bundling of the four subunits through interactions between predicted α-helices at the base of the channel. This is quite different from TRPV1, which has a splayed base formed by six ankyrin repeats, one of which mediates subunit interactions by contacting a three-stranded β-sheet structure on an adjacent subunit[2,3].

Negatively charged phosphatidylinositol lipids such as PtdIns(4,5)P_2 are stationed at membrane surfaces, and are involved in signal-transduction cascades that regulate the activity of many ion channels and transporters[11]. Soluble polyphosphate molecules, such as inositol hexakisphosphate ($InsP_6$), are less well known as channel regulators. Paulsen *et al.* found that the presence of $InsP_6$ was necessary for channel formation, and provide evidence that it binds directly to the channel, close to the coiled coil. $InsP_6$ is negatively charged, and the authors report charge–charge interactions with four adjacent, positively charged amino acids in the coiled coil. Thus, $InsP_6$ seems to be a helper molecule, required for successful channel formation or stabilization.

Defining the structure of TRPA1, like the previous achievement with TRPV1, is a milestone in TRP-channel biology. These two structures suggest that the basic architecture of TRPs is broadly similar to that of voltage-gated potassium channels — they assemble as tetramers surrounding variably sized and charged gates. Although the latest study does not tell us how electrophiles and other noxious compounds open the TRPA1 channel, it suggests that they evoke interactions through the large surface area of the ankyrin repeats. In the future, more-detailed structures of TRPA1 in different conformations will reveal regulatory features, such as why the channel becomes sensitized and desensitized to calcium, and, perhaps more importantly, how channel function can be blocked to treat asthma, inflammation and pain. ■

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