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Mechanism of TRPM2 channel gating revealed by cryo-EM

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Abstract

Transient receptor potential melastatin 2 (TRPM2) is a non-selective cation channel that allows Ca^{2+} influx across the plasma membrane and efflux from lysosomes upon opening. TRPM2 is best known as a biosensor of reactive oxygen species (ROS), which mediates some of the body's responses to oxidative stress. As such, TRPM2 is involved in a plethora of biological processes including immune response, insulin secretion, body temperature control and neuronal cell death, and represents an emerging therapeutic target for many human diseases, from diabetes to inflammatory and neurodegenerative diseases. A direct ligand of TRPM2 is ADP-ribose (ADPR), which accumulates in cells at high levels of ROS, and activates TRPM2 synergistically with intracellular calcium (Ca^{2+}). Here, we describe recent cryo-electron microscopy (cryo-EM) structures of TRPM2 and summarize the insights they provided into the gating mechanism of the channel.

Graphical Abstract

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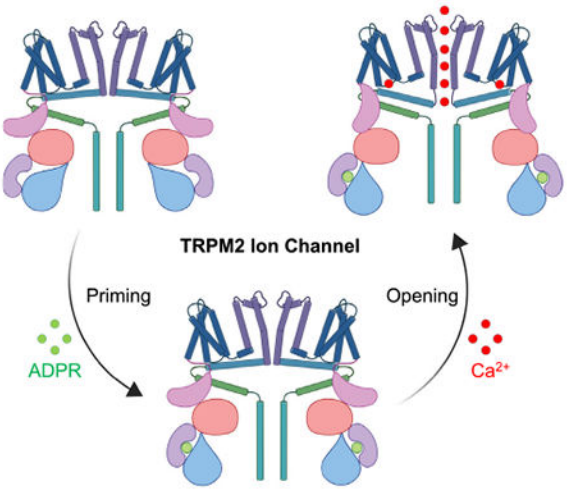
Author contributions

All authors contributed to the text and figures of the manuscript.

Conflict of interest

The authors declare no conflict of interest.



Transient receptor potential melastatin 2 (TRPM2) is a non-selective cation channel gated by ADP-ribose (ADPR) and calcium (Ca2+). A recent structural study revealed the gating mechanism of human TRPM2, with priming by ADPR as the first step followed by Ca2+-induced channel opening. The human TRPM2 structures, together with the structures of its zebrafish and sea anemone homologs, provided an in-depth mechanistic picture of TRPM2 channel gating across species.

Keywords

ADPR; calcium; cryo-EM; gating; ion channel; structure; TRP channel; TRPM2

Introduction

The transient receptor potential melastatin (TRPM) channels belong to the TRP channel superfamily of non-selective cation channels. TRP channels carry out diverse physiological functions and are known to respond to noxious stimuli [1,2]. For instance, TRPV1 responds to capsaicin and TRPM8 senses cold. Our knowledge of the gating mechanisms of TRP channels has been greatly advanced by recent structural studies, especially on TRPV channels [3,4]. Compared to the extensively studied TRPV channels, TRPMs have larger molecular weights and more complex folds, which pose greater challenges to their structural investigations. In human, there are eight TRPMs, among which TRPM2, TRPM6, and TRPM7 contain additional C-terminal enzyme or enzyme-like domains that regulate channel opening. More specifically, TRPM6 and TRPM7 have a kinase domain, and TRPM2 contains a NUDT9 homology (NUDT9H) domain [5–8].

Transient receptor potential melastatin 2 is widely expressed in the nervous and immune system and plays vital roles in numerous physiological responses to reactive oxygen species (ROS), including insulin secretion, inflammatory cytokine production, and body temperature maintenance [9–11]. Dysregulation in TRPM2 activation has been associated with many diseases such as diabetes, cancer, inflammatory and neurodegenerative diseases, and cardiac or renal ischemia–reperfusion injury [12–16]. Oxidative stress results in an increased

cellular level of ADP-ribose (ADPR), which, together with intracellular calcium (Ca^{2+}), can synergistically activate the channel. How TRPM2 is gated by ADPR and Ca^{2+} at the molecular level has been a long-standing question in the field.

In the past few years, cryo-electron microscopy (cryo-EM) structures of TRPM4 and TRPM8 shed light on the general architecture of the TRPM family [17–20]. However, the structures of these channels were obtained in closed, inactive states, leaving their gating mechanisms unclear. Recently, structures of human (*hs*), zebrafish (*dr*), and sea anemone (*nv*) TRPM2 in different conformational states were solved, from which gating mechanisms of TRPM2 have been proposed [21–24]. Together, these TRPM2 structures not only provide an understanding on species-specific contributions of the NUDT9H domain to channel activation, but also highlight an allosteric propagation mechanism that integrates cytoplasmic signals and local conformational changes at the transmembrane (TM) region to enlarge the ion permeation pore.

Overall architecture of human TRPM2

The assembled human TRPM2 (*hs*TRPM2) channel is a tetramer of the multi-domain subunit with dimensions of approximately 100 Å by 100 Å by 150 Å (Fig. 1A,B) [21]. Each monomer consists of an N-terminal TRPM-homology region (MHR) arm formed by MHR1/2, MHR3, and MHR4 domains, followed by a transmembrane (TM) region composed of the S1-S4 voltage sensor-like domain (VSLD) and the S5–S6 pore domain. Immediately after the TM region are the TRP helices H1 and H2, a rib helix, and a pole helix. These are then followed by the unique NUDT9H domain at the very C-terminus (Fig. 1A,C). The whole structure features a three-tier domain organization, with MHR1/2/3, the pole helix, and NUDT9H forming the bottom or most intracellular tier. The middle tier contains MHR4 and the rib helix. The top tier consists of pre-S1, the TM domain, and the TRP helices (Fig. 1A,B).

Characteristic of the TM region of TRP channels, the VSLD of one subunit interacts with the S5–S6 pore domain of a neighboring subunit in a domain-swapped manner. The rib and pole helices form a scaffold that supports the channel, with the pole helices intertwined into a coiled-coil that resembles a central spine (Fig. 1D). Besides these conserved features, a notable characteristic of apo *hs*TRPM2 is that the C-terminal NUDT9H domain, rather than hanging flexibly at the bottom tier, forms extensive interactions with the N-terminal MHR arm both of its own subunit (in cis) and of a neighboring subunit (in trans) (Fig. 1E). The trans-interaction, mediated by the P-loop of NUDT9H and MHR1/2 of the neighboring subunit, appears to lock the channel in an inactive state by restraining subunit movements in the absence of ligands.

Molecular mechanism of human TRPM2 opening

Although NUDT9H directly binds ADPR and is required for human TRPM2 co-activation by ADPR and Ca^{2+}, it is the S5–S6 pore domain that controls the pore size and the ion permeation pathway. Considering the spatial separation between NUDT9H and the pore domain, there likely exists an allosteric signal propagation mechanism. To uncover the

gating mechanism of *hs*TRPM2, Wang *et al.* obtained the cryo-EM structures of TRPM2 in complex with ADPR (primed state) and with both ADPR and Ca^{2+} (open state) in addition to the apo-state structure [21]. Viewing from the intracellular side, MHR1/2 and NUDT9H undergo a dramatic 27° counterclockwise rotation from the apo state to the primed state (Fig. 2). A consequence of this large rotation at the bottom tier is that the trans-interaction mediated by the P-loop of NUDT9H is abolished to allow further conformational changes upon Ca^{2+} binding, hence the name 'primed state'.

With ADPR binding to prime the channel, the Ca^{2+} ion bound at the top tier of the channel is not only coordinated by S2 and S3 of VSLD, but also by TRP helix H1, leading to a tilt at H1 in comparison to the apo and primed states. TRP H1 was here defined as an allosteric center due to its interaction with VSLD, close association with MHR4, and direct connection to the pore gating helix S6 via a bent junction. This strategic location of TRP H1 links the cytosolic and TM domains of the channel and propagates conformational changes initiated at the Ca^{2+}-binding site. Therefore, in addition to inducing a tilt at TRP H1 to drag S6 to enlarge the pore for ion flux, Ca^{2+} binding allosterically elicits a 15° clockwise rotation in the cytosolic region by TRP H1-MHR coupling (Fig. 2).

In summary, the conformational changes that enable the *hs*TRPM2 channel opening likely take place by allosteric propagation, from ADPR-triggered disruption of the trans-interaction mediated by NUDT9H, to the Ca^{2+}-induced tilt at TRP H1, and finally to the movement of the S6 pore gating helix. It is worth noting that although this mechanistic model depicts ADPR and Ca^{2+} binding as sequential events for simplicity, they may occur simultaneously and prime each other to share the energetic cost required for the large conformational changes during channel opening.

Structures and gating mechanisms of TRPM2 in other species

Other than *hs*TRPM2, structures of zebrafish TRPM2 (*dr*TRPM2) and sea anemone TRPM2 (*nv*TRPM2) have been solved, revealing a gating mechanism similar to *hs*TRPM2 with some important differences [22–24]. The structure of *nv*TRPM2 was determined in complex with Ca^{2+}, while the structures of *dr*TRPM2 contain states – the apo state, in complex with Ca^{2+}, and in complex with Ca^{2+} and ADPR. Many mechanistic details of channel opening are shared between *hs*TRPM2 and *dr*TRPM2, including the rotation of the cytosolic MHR arm, the movement of the TM region, and Ca^{2+} coordination by the TRP helix H1. On the other hand, comparison of these TRPM2 structures reveals several species-specific features.

Calcium-binding sites

Structures of *dr*TRPM2 and *nv*TRPM2 in complex with Ca^{2+} show that when ADPR is absent, the channels are in closed conformations. In both structures, Ca^{2+} is coordinated by residues in VSLD helices S2 and S3 (Fig. 3A) [23,24]. However, symmetry differs between the two Ca^{2+}-bound structures, with *nv*TRPM2 adopting a fourfold symmetric architecture whereas *dr*TRPM2 a twofold symmetric intermediate state obvious in the MHR1/2 and MHR3 domains. Strikingly, upon the engagement of ADPR, the Ca^{2+}-binding site moves toward the TRP helix H1, shown by the open-state structures of *hs*TRPM2 and *dr*TRPM2 (Fig. 3B) [21,22]. The consequence is that Ca^{2+} is not only coordinated by the S2 and S3

helices, but also by the TRP H1. This interaction with TRP H1 turns out to be a critical step toward channel opening.

ADPR-binding sites

The relatively low-resolution structures of *hs*TRPM2 in primed and open states did not resolve the bound ADPR. However, binding measurements confirmed that the NUDT9H domain in *hs*TRPM2 directly associates with ADPR with an affinity of around 15 μM, and the putative binding site locates at the cleft between the N-terminal domain (NTD) and C-terminal domain (CTD) of NUDT9H (Fig. 3C) [21]. In contrast, the MHR1/2 domain, not the NUDT9H domain, was shown to bind ADPR in *dr*TRPM2 [22]. Two conserved Arg residues were identified to be critical for ADPR engagement and channel gating in zebrafish TRPM2. However, mutation of *hs*TRPM2 residues equivalent to the ADPR-binding residues in *dr*TRPM2 did not substantially compromise channel opening, suggesting a notable difference in ADPR sensing between the two species [21,22].

Comparison of NUDT9H

The positioning of and interactions formed by NUDT9H also exhibit evolutionary divergence. In *hs*TRPM2, the P-loop of NUDT9H is responsible for contacting MHR1/2 in trans to lock adjacent subunits in place and restrict rotational movement in the absence of ADPR and Ca^{2+}. Comparatively, the P-loop is deleted in *dr*TRPM2 NUDT9H, hence the lack of trans-interaction in the apo-state structure (Fig. 3C, D). In addition, it has been shown that the enzymatic activity of NUDT9H in TRPM2 varies across species [25,26]. While the NUDT9H domain of *nv*TRPM2 degrades ADPR, *hs*TRPM2 NUDT9H binds ADPR but lacks the hydrolase activity, putatively due to the loss of two glutamate residues that catalyze the reaction. While the physiological relevance of these species-specific NUDT9H features is unclear, they reflect mechanistic complexity in TRPM2 gating and merit further study.

Concluding remarks and future perspectives

Recent cryo-EM structures of TRPM2 provide insights into the gating mechanism of the channel. First, in *hs*TRPM2, the unique C-terminal NUDT9H domain mediates trans-interaction with a neighboring subunit to lock the channel in a closed conformation in the absence of ADPR and Ca^{2+}. Second, the binding of ADPR and Ca^{2+} triggers the dramatic conformational changes that lead to channel opening, including large rotations at the MHR arm and local movements in the TM region. Third, the TRP H1 helix serves as an allosteric center responsible for integrating structural signals in the TM region and the cytosolic domains. The *hs*TRPM2 structures, together with the structures of TRPM2 in other species, have broadened our understanding of this functionally important channel and allow us to make new hypotheses for how TRPM2 is gated by other factors. For example, the opening of TRPM2 is also regulated by PIP2, temperature, and calmodulin [7,10,27]. We speculate that these factors may also modulate the opening of TRPM2 via conformational changes that are similar to what we have observed during the channel opening by ADPR and Ca^{2+}. Further study to address these questions will surely advance our understanding on the gating mechanisms of TRP channels.

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Abbreviations

ADPR	ADP-ribose
Ca^{2+}	calcium
cryo-EM	cryo-electron microscopy
CTD	C-terminal domain
dr	zebrafish
hs	human
NTD	N-terminal domain
NUDT9H	NUDT9 homology
nv	sea anemone
ROS	reactive oxygen species
TRPM	transient receptor potential melastatin

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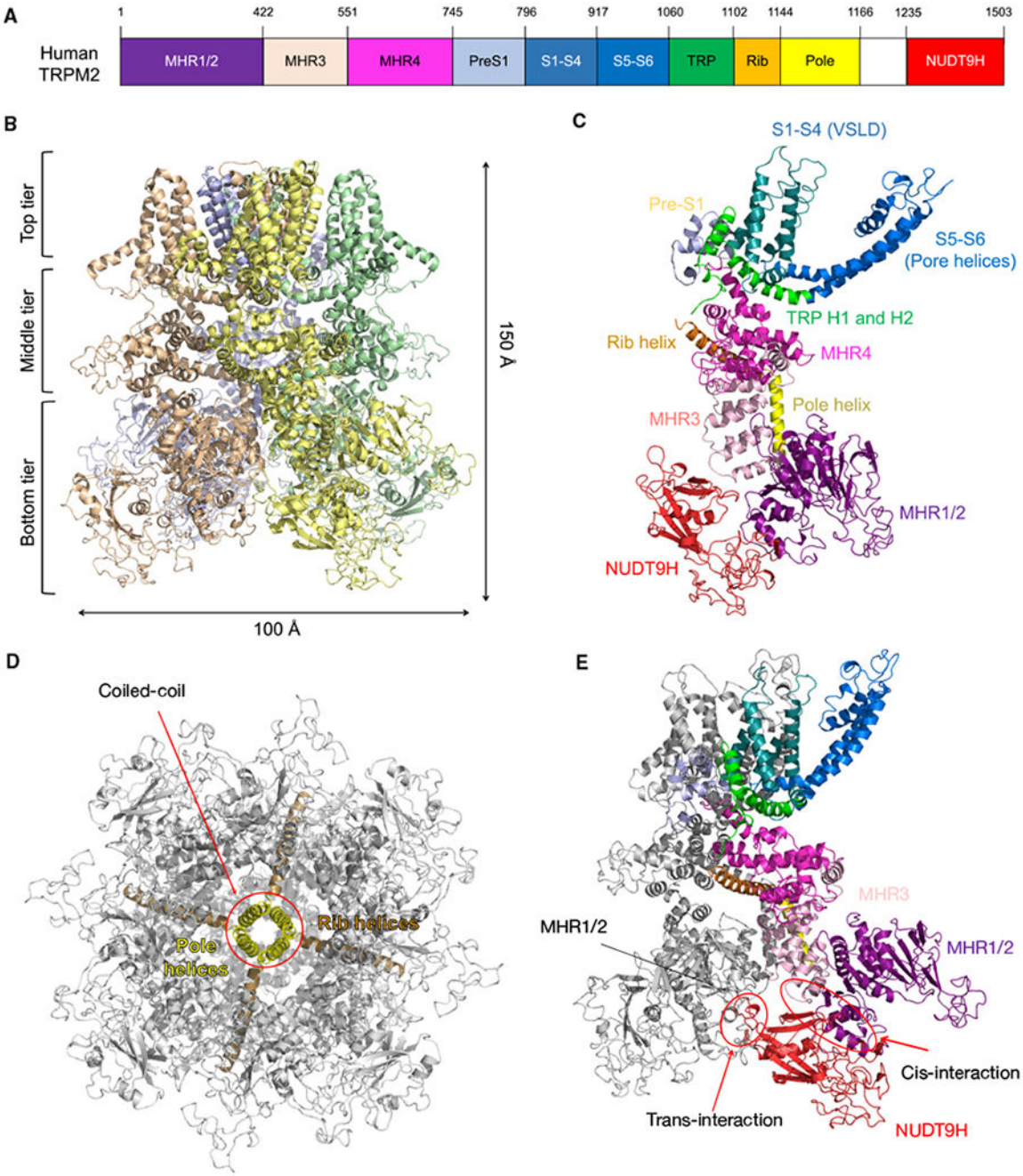

Fig. 1.

Apo-state structure of human TRPM2. (A) Domain arrangement of human TRPM2. (B) Ribbon diagram, dimensions, and three-tier architecture of human TRPM2. The four subunits are colored differently. (C) One subunit from the apo-state TRPM2 with domains colored individually. (D) The rib and pole helices form a central scaffold to support the TRPM2 channel. (E) The NUDT9H domain mediates extensive interactions with MHR domains both in cis and in trans.

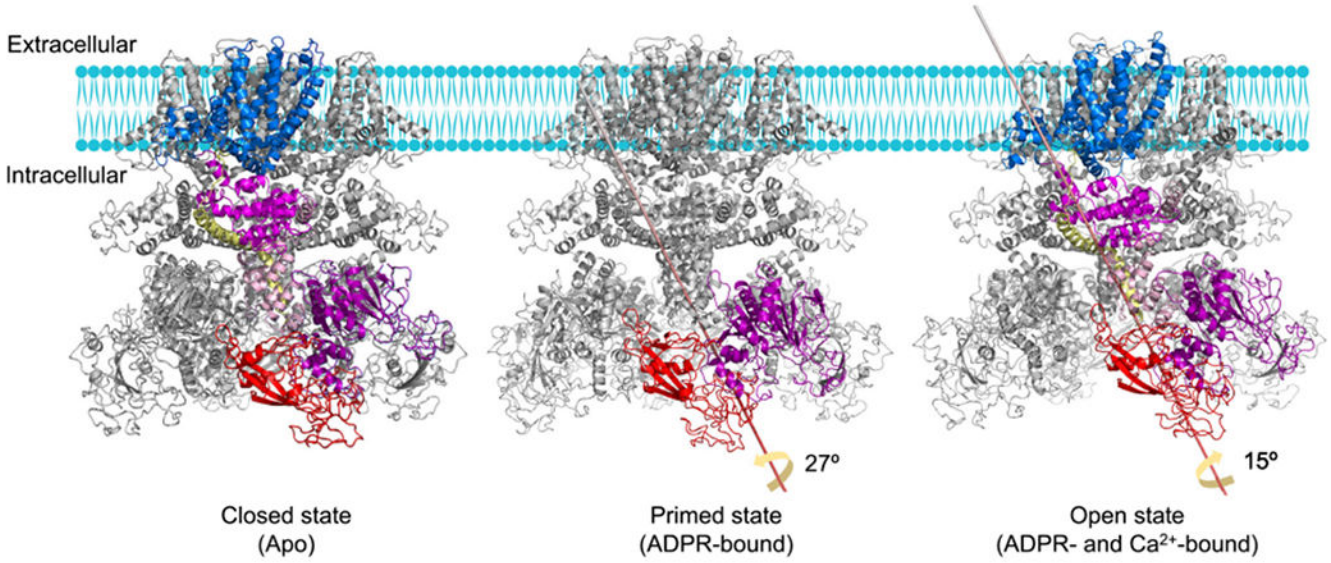

Fig. 2.

Molecular mechanism of human TRPM2 gating. In the apo state, NUDT9H mediates trans-interactions with the MHR1/2 domain, which may lock the channel in a closed conformation by limiting the rotation of the MHR arm. Upon ADPR engagement, MHR1/2 and NUDT9H undergo a large rotation, thereby disrupting the trans-interaction to prime the channel for opening. When both Ca^{2+} and ADPR bind, rotation of the cytosolic domains and movement of the TM region are integrated by TRP helix and then propagated to the S6 helix, leading to the enlargement of lower gate to increase the opening probability of the channel.

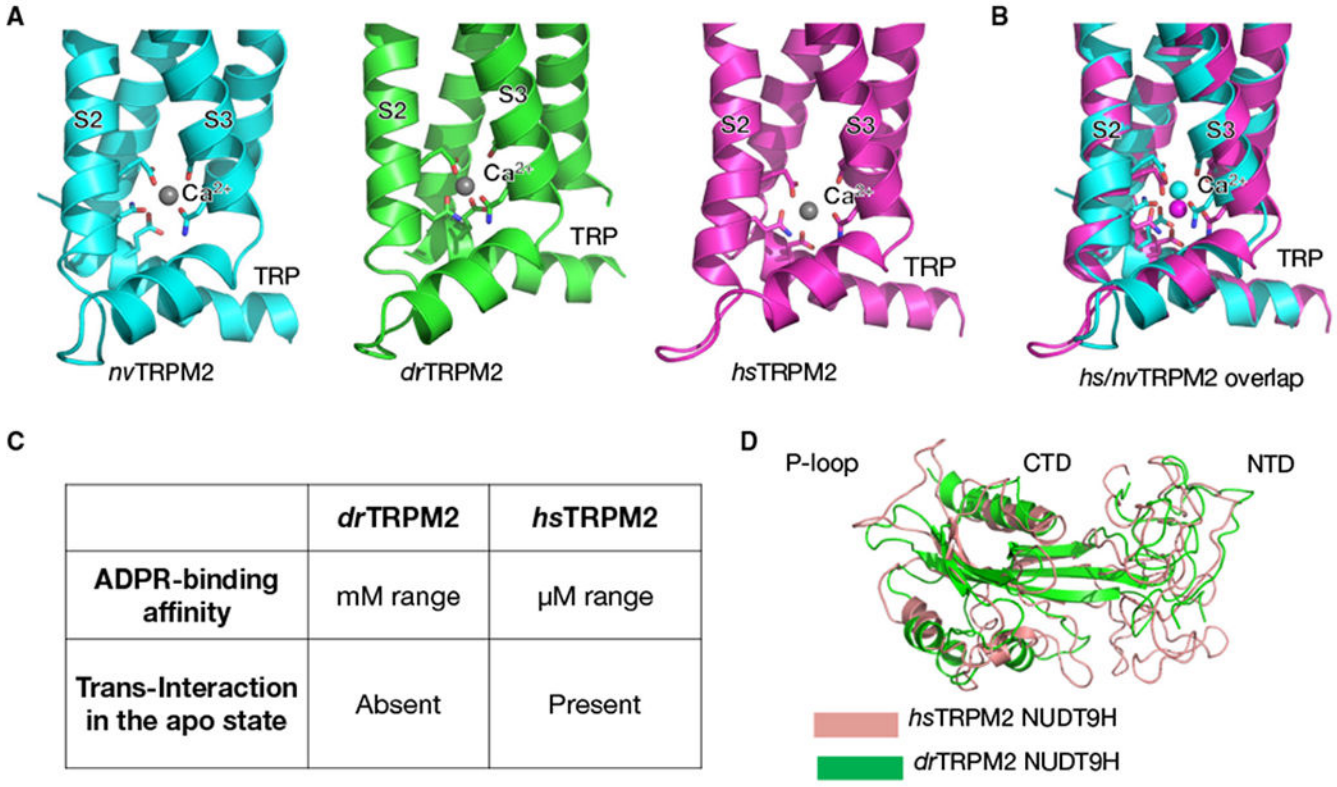

Fig. 3.

Comparison of TRPM2 gating across species. (A) Ca^{2+}-binding sites of sea anemone (*nv*), zebrafish (*dr*), and human (*hs*) TRPM2. (B) Overlay of the Ca^{2+}-binding sites of *hs*TRPM2 and *nv*TRPM2. (C) Comparison of the NUDT9H domains of *dr*TRPM2 and *hs*TRPM2. (D) Overlap of the NUDT9H domains of *dr*TRPM2 and *hs*TRPM2.