CELL BIOLOGY

Sterol hindrance of Orai activation

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Orai channels at the plasma membrane mediate store-operated Ca^{2+} entry in response to Ca^{2+} depletion of the endoplasmic reticulum. Orai channels are gated by stromal-interacting molecule proteins, which act as Ca^{2+} sensors, and the association of these proteins is enhanced in cholesterol-rich lipid rafts. In research published in *Science Signaling*, Derler *et al.* report that cholesterol inhibits Orai function through direct association with the channel amino terminus.

During store-operated Ca^{2+} entry (SOCE), Ca^{2+} enters the cell through plasma membrane Orai channels to facilitate ER Ca^{2+} store refilling. Ca^{2+} is a ubiquitous signaling ion that regulates various physiological processes. Activation of plasma membrane (PM)–localized ligand-gated channels coupled to phospholipase C (PLC) generates the intracellular second messenger inositol 1,4,5-trisphosphate ($InsP_3$), which acts upon endoplasmic reticulum (ER)–resident $InsP_3$ receptors to release Ca^{2+} from the ER into the cytosol. As ER Ca^{2+} is depleted, stromal-interacting molecule (STIM) proteins located in the ER membrane undergo a conformational change owing to dissociation of Ca^{2+} from their luminal EF hands. This results in oligomerization and clustering of STIM at ER-PM junctions, facilitating coupling between the STIM protein and Orai channels and SOCE (*1*). In research published in *Science Signaling*, Derler *et al.* reveal a role for cholesterol as an inhibitor of Orai channels (*2*).

SOCE activation and the association of STIM and Orai in ER-PM junctions were previously thought to depend on the presence of lipid rafts, which may help to define the junctions. Lipid rafts are discrete domains within the plasma membrane, enriched in cholesterol, sphingolipids, and various proteins (*3*), including Orai1 and components of the SOCE machinery, along with other channels and pumps (TRPCs, PMCA, NCX, and CaV channels). Sequestration of membrane cholesterol from lipid rafts attenuates SOCE, decreasing the ability of a constitutively active STIM1 mutant (D76A) to form puncta (*4*). Depletion of cholesterol by methyl-β-cyclodextrin also decreases SOCE and inhibits the ability of STIM1 to coimmunoprecipitate with Orai1 (*5*).

In contrast, Derler *et al.* found that depletion of cholesterol did not affect the association of STIM and Orai (*2*). Further, depletion of free cholesterol in the membrane by cholesterol oxidase or filipin enhanced SOCE in both human embryonic kidney (HEK) 293 and rat basophilic leukemia 2H3 (RBL) cells. The differences observed between the groups may be due to the gentler approach chosen by Derler *et al.*, enabling them to specifically deplete cholesterol while keeping membrane integrity intact. It is possible that the use of filipin, for example, could promote SOCE at low concentrations by sequestering cholesterol, whereas at higher concentrations, nonspecific effects associated with compromised membrane integrity may lead to prevention of SOCE.

The group identified a putative cholesterol binding site in the extended transmembrane Orai1 N-terminal (ETON), which is integral to channel gating by interaction with STIM1 (*6*). This conserved ETON region comprises amino acids 73 to 90 of human Orai1, an α-helical extension of transmembrane region 1 (TM1) that emerges from the plasma membrane into the cytosol (Fig. 1). Cholesterol bound directly to a single site on an Orai1 N-terminal peptide (*2*), and mutation of this cholesterol interaction region potentiated SOCE in a manner similar to cholesterol depletion and indeed reduced direct cholesterol binding, but did not affect the interaction with STIM1. This suggests that STIM1-Orai1 interaction at the C terminus can still occur, with cholesterol only interfering with STIM1-dependent channel gating through Orai1 N-terminal interactions.

The crystal structure of *Drosophila* Orai indicates that the key cholesterol binding residues that correspond to Leu^{74} and Tyr^{80} in human Orai1 point in opposite directions, seemingly precluding the possibility that both side chains could simultaneously participate in the binding of cholesterol (*7*). One possibility proposed by Derler *et al.* is that the α-helix of the ETON region becomes "unstructured."

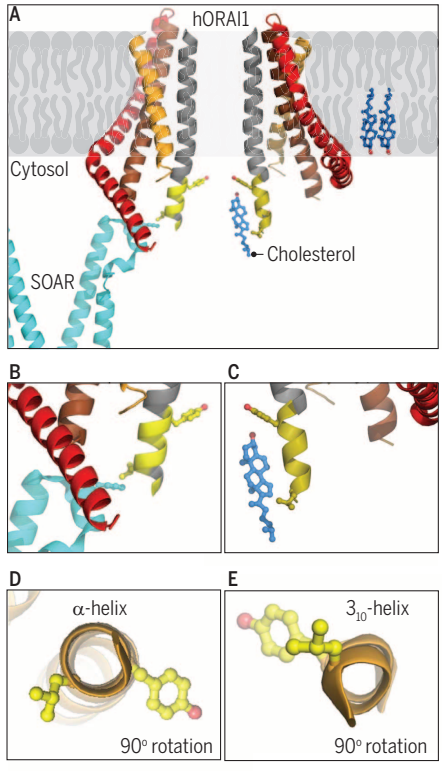

Fig. 1. Hypothetical model for cholesterol-Orai interactions. To illustrate the putative cholesterol-binding site, the *Drosophila* Orai structure [Protein Data Bank (PDB) accession number 4HKR] (*7*) was modified to match the sequence of human Orai1 by replacing Gln^{152} with Tyr. (**A**) (**left**) Human Orai1 is depicted in association with the STIM-Orai activating region (SOAR; PDB accession number 3TEQ) (*11*). (**right**) The N-terminal end of TM1 in human Orai1 was replaced with a 3_{10} helix, a conformation that might favor association with cholesterol at the expense of SOAR. (**B**) Detailed view of hypothetical SOAR-Orai1 interaction in cholesterol-free Orai1. (**C**) Detailed view of hypothetical cholesterol-Orai1 interaction. (**D**) End-on view of Orai1-TM1 as an α-helix, to illustrate the orientation of Leu^{74} and Tyr^{80} side chains in this conformation. (**E**) End-on view of Orai1-TM1 as a 3_{10}-helix to illustrate the orientation of the key cholesterol-binding residues in this conformation.

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For this to occur, the ETON domain would have to exist in an equilibrium between the α-helix observed in the crystal structure and random coil structures that may exist in the isolated Orai1 N-terminal peptide in solution (*2*). If so, the presence of cholesterol would presumably favor a random coil structure, making Orai1 unavailable for STIM interaction. Although hypothetically possible, it seems unlikely that an apparently stable α-helix would "unwind" under physiological conditions. Another interesting and perhaps more energetically favorable possibility would be a change in conformation between an α-helix and the less common 3_{10}-helical structure. Because 3_{10}-helices have three residues per turn, as opposed to the 3.6 residues per turn in a canonical α-helix (*8*), a conversion from α-helix to 3_{10} would enable Leu^{74} and Tyr^{80} to line up in the same direction, making cholesterol binding by these two residues possible (Fig. 1).

Orai1 is by no means the only channel that can bind cholesterol. As Derler *et al.* point out, TRPV1 channels are also inhibited by cholesterol, which binds to the S5 helix of the channel and decreases TRPV1 currents (*9*). Further, the large conductance potassium channel BK exhibits several cholesterol-binding domains (confusingly named "CRAC") in its cytosolic C-terminal tail (*10*). This is important because similar to Orai1, these sites are physically located outside of the PM. Hence, there is a precedent for cholesterol binding to the cytosolic sites of an ion channel.

There may also be as-yet-unappreciated pathophysiological implications for cholesterol regulation of Orai. Derler *et al.* draw the link between hypocholesteremia in patients with Smith-Lemli-Opitz syndrome exhibiting increased mast cell degranulation, a process dependent on Ca^{2+}. It remains to be seen whether other pathophysiological conditions are caused by the interaction of cholesterol and Orai, and how the more common scenario of hypercholesterolemia affects Orai. Questions remain about how dynamic and physiologically relevant the cholesterol-Orai interaction is. Do ER-PM junctions form exclusively at cholesterol-rich lipid rafts, in which case is cholesterol inhibition standard for "wild-type" SOCE responses, or is cholesterol binding to Orai turned on and off? Which conditions lead to the transport of cholesterol from the PM to its cytosolic binding site on Orai? How do cholesterol and STIM compete for the same region of Orai? Perhaps STIM can bind the C terminus of Orai upon store-depletion, leading to a conformational change that dissociates cholesterol from the N terminus and allows STIM to bind instead, facilitating channel opening. Derler *et al.* present an exciting concept of cholesterol-mediated SOCE regulation; however, the physiological implications and the mechanism for when and why cholesterol binds Orai remain to be elucidated.

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Acknowledgments: This work was supported by NIH grant GM097335 (to J.S.).

10.1126/scisignal.aaf2357

Citation: R. Hooper, B. S. Rothberg, J. Soboloff, Sterol hindrance of Orai activation. *Sci. Signal.* **9**, fs4 (2016).

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Sci. Signal. **9** (418), fs4.
DOI: 10.1126/scisignal.aaf2357