CELL BIOLOGY

# How to STIMulate Calcium Channels

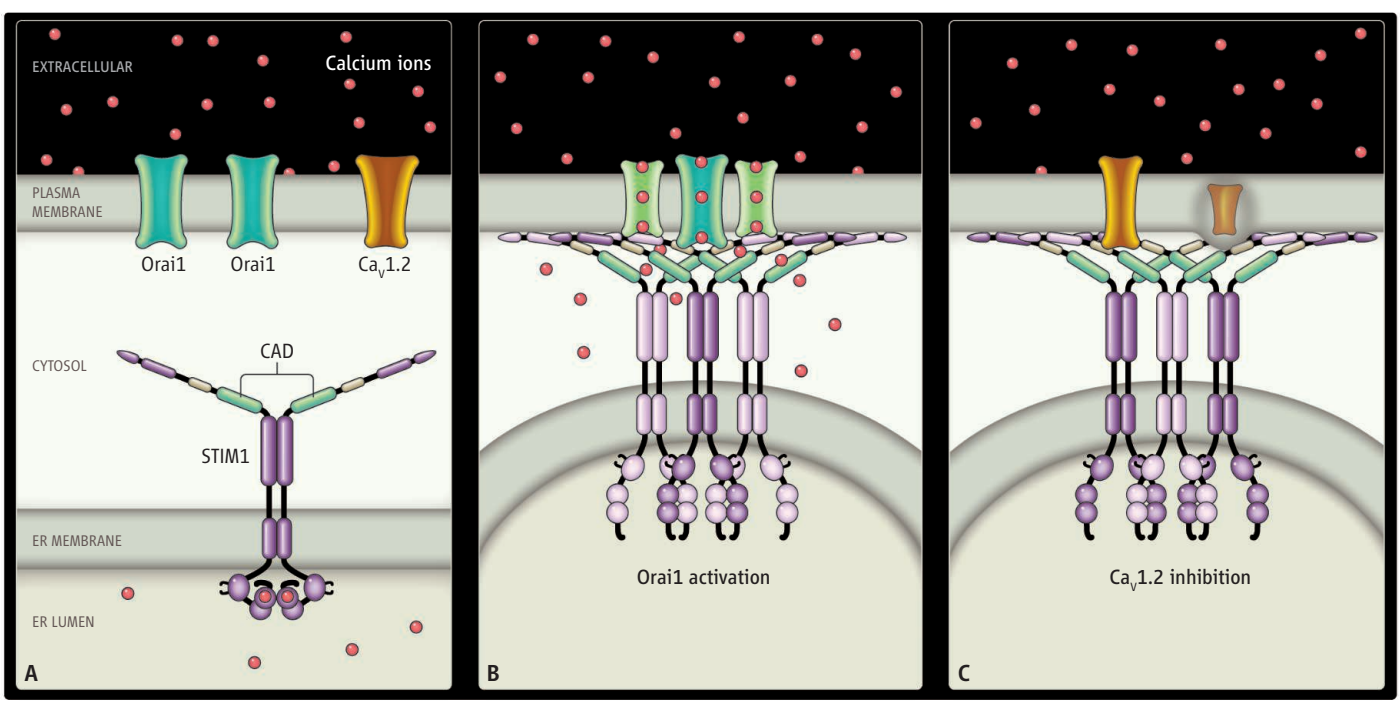
Two studies reveal the reciprocal regulation of two different calcium channels.

**Michael D. Cahalan**

The movement of calcium across cell membranes regulates many important physiological processes in vertebrates, as various as the contraction of heart muscle, the firing of brain cells, the expression of genes by immune cells, and the secretion of hormones. Cells have two main types of calcium channels that enable calcium ions ($Ca^{2+}$) to flow into the cell. Voltage-gated $Ca^{2+}$ channels typically function only in electrically excitable cells, such as neurons, heart muscle cells, and insulin-producing cells in the pancreas. By contrast, store-operated $Ca^{2+}$ channels work only in electrically inexcitable cells, such as lymphocytes and other cells of the immune system. Researchers have long puzzled over this pattern, in part because biochemical evidence suggested that both channel types are present in both excitable and inexcitable cells. On pages 101 and 105 of this issue, reports by Park *et al*. and Wang *et al*. (*1*, *2*) help solve the puzzle by presenting a new mechanism that determines which type of $Ca^{2+}$ channel predominates in a particular cell type. These two groups show that the protein STIM1, which was already known to activate store-operated $Ca^{2+}$ channels, inhibits voltage-gated $Ca^{2+}$ channels. Together, the reports help to illuminate a reciprocal calcium control mechanism that, if it goes wrong, can have life-threatening consequences.

STIM proteins first came to light in the $Ca^{2+}$ signaling field 5 years ago; the name comes from their initial identification as a stromal-interacting molecule. Studies revealed that STIM proteins play an essential role in the function of store-operated $Ca^{2+}$ channels in *Drosophila* (Stim) (*3*) and in human cells (STIM1 and STIM2) (*4*). Later, researchers screening the *Drosophila* genome identified another family of proteins involved in the function of store-operated $Ca^{2+}$ channels: the Orai proteins (Orai1, Orai2, and Orai3 in humans) (*5*–*7*). By subtly altering the structure of Orai proteins, researchers showed that they play a key role in calcium "selectivity," or a channel's ability to recognize $Ca^{2+}$ ions and allow them to pass through a pore in the membrane (*8*–*10*). Together, these studies showed that STIM and Orai proteins work closely together to mediate the activity of store-operated $Ca^{2+}$ channels. In general, STIM proteins function as a signaling relay; when $Ca^{2+}$ stores are depleted at the endoplasmic reticulum (ER) within the cell, for instance, they can help to activate and open Orai channels in the outer plasma membrane (PM) (see the figure). Experiments have demonstrated

Department of Physiology and Biophysics, University of California, Irvine, CA 92697, USA. E-mail: mcahalan@uci.edu

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**STIMulating.** STIM1 in the ER membrane activates Orai1 (green) and inhibits $Ca_V1.2$ (orange) in the plasma membrane. (**A**) The cell at rest. Calcium ions (red) are abundant in the extracellular space and within the ER lumen but are rare in the cytosol. Functional domains of STIM1 include the EF hand (with calcium ions bound), and the CRAC-activating domain (CAD, green). (**B** and **C**) ER-PM junctions when the cell is activated and $Ca^{2+}$ is depleted from the ER lumen. Calcium ions unbinding from the EF hand of STIM1 trigger translocation of STIM1 to ER-PM junctions. The cytosolic junctional gap between ER and PM is sufficiently narrow (10 to 20 nm) to allow for direct molecular interaction of STIM1 with Orai1 and $Ca_V1.2$. The CAD of STIM1 recruits Orai1 to ER-PM junctions and opens Orai1 channels by direct binding to STIMulate $Ca^{2+}$ influx (B). $Ca_V1.2$ channel proteins are also recruited to ER-PM junctions but are inhibited by the CAD interacting with the C terminus of $Ca_V1.2$ (C).





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several functions of STIM proteins in activating store-operated $Ca^{2+}$ channels (*11*).

In their studies, Wang *et al*. and Park *et al*. examined the role that STIM proteins might also play in regulating voltage-gated $Ca^{2+}$ channels. Specifically, they examined one medically important member of this diverse family, known as the $Ca_V1.2$ subunit ($\alpha_{1C}$). $Ca_V1.2$ forms the so-called L-type $Ca^{2+}$ channels found in a wide array of cells, including those in the cortex, hippocampus, cerebellum, neuroendocrine system, heart, and arterial smooth muscle. Mutations in the $Ca_V1.2$ gene *CACNA1C* cause Timothy and Brugada syndromes, which are associated with life-threatening cardiac arrhythmias. A number of treatments, including the drugs verapamil, diltiazem, and various dihydropyridines, specifically target $Ca_V1.2$ channels.

The two groups found that the sequence of events involving STIM1 begins in the same way for both store-dependent and voltage-regulated channels, but the end result is very different. After $Ca^{2+}$ store depletion in the ER, a structure known as the EF hand of STIM1 unbinds $Ca^{2+}$, and STIM1 oligomers translocate to form clusters immediately adjacent to the plasma membrane at ER-PM junctions. If voltage-gated $Ca^{2+}$ channel proteins are expressed, they, like Orai subunit proteins, accumulate adjacent to STIM1. There, STIM1 and $Ca_V1.2$ interact via specific domains that protrude into the cytosol: a CRAC-activating domain (CAD) in STIM1 and the C terminus of $Ca_V1.2$. CAD opens Orai1 channels, but—as the two new papers show—it inhibits $Ca_V1.2$ channel activity in two ways: acutely (immediately) by physical interaction, and more slowly by causing internalization, a process that inhibits the channel and results in subsequent degradation of the channel protein. The two reports differ with respect to the relative importance of the acute and internalization phases (Wang *et al*. find that the acute phase is strong, Park *et al*. less so), but the outcome is the same: STIM1 regulates Orai1 and $Ca_V1.2$ reciprocally. In inexcitable lymphocytes, where STIM1 is relatively abundant, voltage-gated $Ca^{2+}$ channels are inhibited and Orai1 is activated. In neurons and presumably other excitable cells, STIM1 is not so abundant and voltage-gated $Ca^{2+}$ channel activity predominates.

As the activator for one $Ca^{2+}$ channel (Orai1) and an inhibitor for another ($Ca_V1.2$), STIM1 assumes a new functional importance. In excitable cells, membrane depolarization (a change in electrical charge) activates $Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels, but in lymphocytes depolarization actually inhibits $Ca^{2+}$ influx through Orai channels, because the electrical driving force for $Ca^{2+}$ to enter is reduced. This potentially resolves a long-standing controversy regarding the type of $Ca^{2+}$ channel in lymphocytes (*12*). Even if subunits of voltage-gated $Ca^{2+}$ channels are expressed, they are not functional because of inhibition by STIM1. More important, STIM1's reciprocal functional interaction guarantees that only one type of $Ca^{2+}$ channel or the other will be active.

Many questions remain concerning the generality of the findings. Does STIM1 block other voltage-gated $Ca^{2+}$ channel family members? In native tissue, $Ca_V1.2$ subunits interact with numerous other cellular proteins that help with subcellular localization and modulation. Do they physically or functionally protect the channels from being inhibited by STIM1? When STIM1 is knocked out in mice or in rare patients with mutations of STIM1, what are the consequences for voltage-dependent $Ca^{2+}$ channel function in vivo? Answers to these questions should further improve our understanding of how $Ca^{2+}$ signaling is regulated.


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10.1126/science.1196348

CHEMISTRY

# A Balancing Act for Taxol Precursor Pathways in *E. coli*

**Tiangang Liu and Chaitan Khosla**

A fermentation process that balances the activities of several bacterial and plant enzymes makes a precursor to an anticancer drug in substantial quantities.

Taxol (paclitaxel) is a widely used cancer drug that was first isolated from the bark of the Pacific yew tree, *Taxus brevifolia*. In 1991, during early stages of its clinical use, 130 kg of Taxol were extracted from 1000 tons of bark, which required cutting down more than 500,000 mature Pacific yew trees (*1*). Fortunately, Taxol is presently made by less destructive methods, either through chemical conversion of a related molecule derived from needles of the more prevalent European yew, *T. baccata* (*2*), or from cultured plant cells (*3*). Nonetheless, both these plant-based processes are difficult, and this valuable drug remains expensive. On page 70 of this issue, Ajikumar *et al*. (*4*) have made progress toward making Taxol in *Escherichia coli*, biotechnology's workhorse bacterium, in substantial quantities by balancing several enzymatic pathways to make its complex multicyclic core (see the figure).

Taxol belongs to a large family of natural products called terpenes, which also includes artemisinin (a malaria drug), carvone (a flavoring agent), and pinene (the main constituent of turpentine). The carbon skeletons of all terpene molecules (taxadiene, in the case of Taxol) are made from two closely related five-carbon precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are made in virtually all cells from substrates such as glucose. Each terpene skeleton then undergoes a series of further modifications by a set of enzymes that execute a sequence of reactions that leads to the complex natural product. The conversion of IPP and DMAPP into Taxol is a more complex process that so far has been seen only in cells from the Pacific yew bark.

Nature has two chemical processes for converting glucose into IPP and DMAPP. The "mevalonate pathway" operates in animal cells, as well as in microbes such as yeast. It was the target of a related recent effort aimed at reducing the cost of artemisinin manufacture (*5*). An unrelated process, the "nonmevalonate" pathway, synthesizes IPP and DMAPP in bacteria and plant cells.

Departments of Chemistry and Chemical Engineering, Stanford University, Stanford, CA 94305, USA. E-mail: liutg@stanford.edu; khosla@stanford.edu