PHYSIOLOGY

CaCl-ing Channels Get the Last Laugh

A long-sought ion-channel gene with intriguing links to cancer and development has been identified.

H. Criss Hartzell

Typically, ion-channel biophysicists and developmental and cancer biologists rarely attend the same journal clubs. But two studies might change this, one by Caputo *et al*. on page 590 in this issue (*1*) and the other by Yang *et al*. (*2*). Both studies show that a transmembrane protein, TMEM16A [also called anoctamin-1 (ANO1)], whose expression increases in many tumors, is a calcium (Ca^{2+})-activated, chloride (Cl^-) channel.

The Ca^{2+}-activated Cl^- channels were first described in the 1980s as mediating the fast block to polyspermy in amphibian oocytes (*3*). These channels, activated by increases in the concentration of intracellular Ca^{2+} ions that occur upon fertilization, conduct Cl^- ions across the plasma membrane, causing the cell to depolarize and prevent additional sperm entry. Similar channels in many cell types, including mammalian, play roles as diverse as epithelial fluid secretion, amplification of the olfactory receptor potential, and regulation of vascular tone (*3*). Yet, Ca^{2+}-activated Cl^- channels have been "a function in search of a molecule" for more than a decade. Previous claims that other molecules (CLCAs, ClC-3, and tweety) function as Ca^{2+}-activated Cl^- channels have been contentious because the ionic currents (electric currents carried by ions) conducted by these proteins do not exhibit the appropriate pharmacology, kinetics, voltage dependence, or Ca^{2+} sensitivity (*3*). Bestrophins, which can function as Cl^- channels and as regulators of voltage-gated Ca^{2+} channels, fit the bill more closely, but not exactly (*4*).

Why has the Ca^{2+}-activated Cl^- channel been so hard to find? One reason is that every cell expresses Cl^- channels, and the pharmacological tools needed to identify Cl^- channels are not very selective. Furthermore, because overexpressing some membrane proteins paradoxically increases the expression of endogenous Cl^- channels, heterologous expression of putative Cl^- channels can produce false-positives. These obstacles have made it hard to identify candidate Ca^{2+}-activated Cl^- channels.

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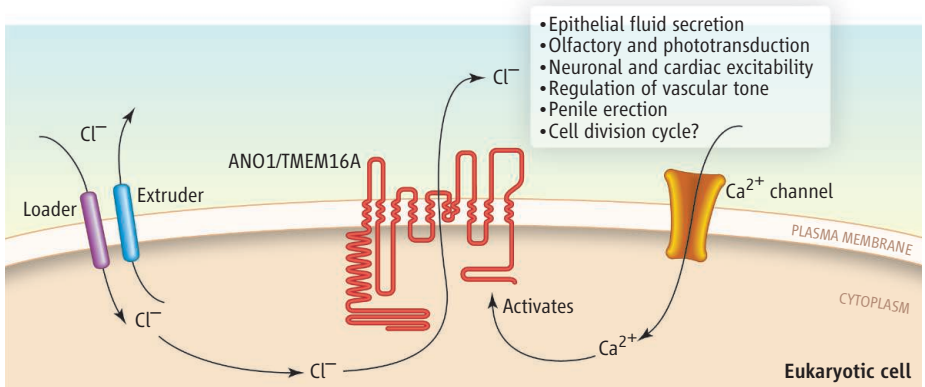

Channel identity. ANO1, an eight–transmembrane domain–containing protein, mediates Ca^{2+}-activated Cl^- ionic currents involved in many physiological processes, possibly including cell proliferation.

Caputo *et al.* used microarray gene analysis to identify ANO1 as the channel responsible for increasing the Ca^{2+}-activated Cl^- current in human bronchial epithelial cells exposed to the cytokine interleukin-4. Yang *et al.* selected ANO1 from a bioinformatic search of potential Cl^- channel genes. Each group then used RNA interference (RNAi) to reduce the expression of ANO1 in various mammalian cells and tissues. In each case, the processes disrupted by RNAi treatment—secretion, ion fluxes across the plasma membrane, or whole-cell ionic currents—were exactly what one would expect following reduced expression of Ca^{2+}-activated Cl^- channels. Furthermore, in cell types that do not normally express this type of channel, overexpression of ANO1 induced an ionic current with the properties expected of a Ca^{2+}-activated Cl^- channel. Finally, mutations at critical sites in the protein altered channel function. Yang *et al.* provide the most convincing demonstration: A ~30-fold increase in relative cation permeability caused by substituting a negative charge (glutamic acid) for a positive charge (arginine) at position 621, which lies within a segment that probably inserts into the membrane from the extracellular side but does not cross it (the so-called reentrant loop) (see the figure) (*5*). This structure appears in the pore of a number of ion channels. Also, Yang *et al.* show that ANO1 is the first candidate Ca^{2+}-activated Cl^- channel that can be activated in cells by receptors at the cell surface that, when activated themselves, cause an increase in the concentration of intracellular Ca^{2+}.

Members of the ANO channel family are found in all eukaryotic kingdoms. With 10 mammalian members and multiple splice variants, ANO is the second largest of the five known Cl^- channel families (GABA/glycine receptors, ANOs, ClCs, bestrophins, and the cystic fibrosis transmembrane conductance regulator) (*4*, *6*). Interestingly, ANO8 and ANO10, the most divergent subfamilies, lack part of the reentrant loop that includes arginine 621. Moreover, one of the ANO7 RNA transcripts encodes a 179–amino acid cytosolic protein (*5*), suggesting that ANO channels could have additional nonchannel functions.

ANO channels have attracted the interest of cancer biologists as targets for therapeutic antibodies and as biomarkers because they are highly expressed in tumors and are accessible cell-surface proteins (*5*, *7*). The idea that ion channels play a role in cancer is not new (*8*, *9*), but the addition of this well-studied channel family to the list of proteins associated with cancer heralds new mechanistic insights. Although it does not appear that mutations in ANO1 are linked to carcinogenesis (*10*), ANO channels may participate in cell proliferation. The activities of several anion channels correlate with the cell division cycle (*11*, *12*). And, intriguingly, a mutant of an ANO channel [called Axs (aberrant x-segregation)] in the fly *Drosophila melanogaster* is linked to aberrant chromosomal segregation (nondisjunction) and progression of meiosis (*13*). Axs is ~35% identical to ANO8 and ANO10, and like them, it lacks the reentrant loop. Whether this means

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that Axs is not a Cl^- channel or that this part of the protein is not essential for Cl^- channel function remains to be seen.

Although the exact functions of ANO channels in cancer are speculative, ANO1 and ANO5 have clear roles in development. Mutations in a conserved amino acid (cysteine 356) in the first extracellular loop of ANO5 produce gnathodiaphyseal dysplasia, a bone fragility syndrome that is caused by chondrocyte and osteoblast dysfunction. Similarly, ANO1 dysfunction causes an endoskeletal defect in mice, but by a different mechanism. Mice engineered to lack ANO1 die after birth, apparently because of tracheal cartilage malformation (*14*). ANO1 is expressed in the mouse tracheal epithelium and, intriguingly, the authors speculate that ANO1 may play a role in asymmetric cell divisions that are necessary for tracheal epithelial stratification.

An immediate question is whether all ANO proteins are Cl^- channels. It is also unclear how ANO channels are regulated by Ca^{2+} given that they do not have obvious Ca^{2+}-binding sites. Perhaps there are other regulators. Now that one of the last holdouts of a major channel family has been identified, we can begin exploring the links between its function and cancer and development.

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ASTRONOMY

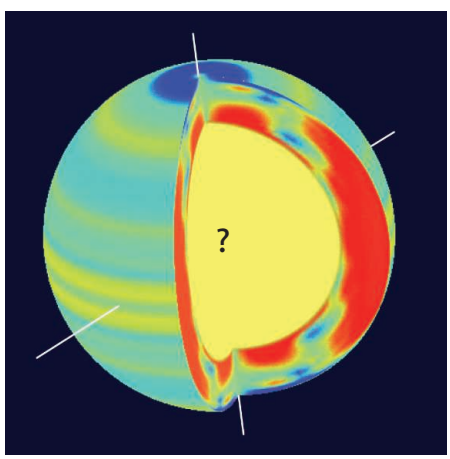
Aspects of Our Sun

Accurate determination of the shape of the Sun sheds light on its inner workings.

Gary A. Chapman

The shape of the Sun touches on several issues in cosmology and solar physics, including whether Einstein's General Theory of Relativity is the correct theory of gravity, and to what extent the solar interior rotates. On page 560 of this issue, Fivian *et al.* (*1*) present compelling satellite-based observations showing that the Sun's shape is in agreement with what is expected from the rotation of its visible surface. These latest observations eliminate the possibility of a rapidly rotating core and remove one of the last remaining challenges to the validity of General Relativity from solar system studies.

Gravity is still a bit mysterious. Of the four fundamental forces, it defies unification with the other three (electromagnetism, weak nuclear, and strong nuclear). Einstein's General Theory of Relativity, the currently accepted theory of gravity, has passed all tests that have been devised for it with flying colors. Early on, the only tests involved the solar system and the Sun's gravity (*2*, *3*). The two most famous tests were the deflection of starlight and the precession of Mercury's orbit (the motion of its perihelion). Both of these effects are caused by the warping of spacetime in the vicinity of the Sun. General Relativity was able to explain the precession of 43 arc sec per century that was not accounted for by the Newtonian gravitational effects of the other planets. However, another theory of gravity, the scalar-tensor theory of Brans and Dicke (*4*), suggested a different value of the precession that depended on a proposed "coupling" of a mass with the mass of the universe. For a modestly small value of this coupling constant, the scalar-tensor theory predicted a smaller value of the precession than was observed. Then, in order for the scalar-tensor theory to agree with the observed precession of Mercury's orbit, the Sun needed to have an excess oblateness (i.e., the equatorial radius minus the polar radius) over and above what is expected from its observed surface rotation (with a rotation period of about 28 days). This proposed excess oblateness supplied the small additional precession needed to bring the sum of the two into agreement with observations.



Measuring up. A suite of sensitive aspect sensors onboard the spacecraft RHESSI is used to determine the oblateness of the Sun.

IMAGE CREDIT: SOHO/NASA AND ESA

Observations with a specialized telescope at Princeton (*5*) showed that the Sun had an oblateness of 50 parts per million of the mean radius and that it was most likely caused by a rapidly rotating core (with a rotation period of 1 to 2 days). Helioseismologists have looked for this rapidly rotating core; the results have been inconclusive but have tended toward the negative. The oblateness due to the known surface rotation is about 8 ppm, or about one-sixth of that measured in (*5*). The finding of an excess solar oblateness led to years of controversy (*6*–*10*). An improved telescope derived from the earlier Princeton one found that facular contrast (the intensity of a facular feature divided by the quiet Sun intensity at the same disk position minus 1) rose toward the solar limb, the apparent edge of the solar disk (*11*), contradicting earlier results (*12*). The increase in facular contrast toward the limb of the Sun means that faculae most likely play a dominant role in the Sun's oblateness, which is the main point made by Fivian *et al.*

The spacecraft RHESSI (Reuven Ramaty High Energy Solar Spectroscopic Imager) is designed to image solar sources of high-energy radiation. Fivian *et al.* used the set of sensitive aspect sensors onboard the spacecraft to obtain a highly accurate measurement of the solar oblateness. They did find an excess oblateness but were able to show that it is due to magnetic features.

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