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Elusive Chloride Channel Corralled in Salamander Eggs

Tracking down new ion channels can be tedious, exhausting work. But sometimes a slight change in thinking can propel a stalled project, as it did for a couple of Howard Hughes Medical Institute researchers who had spent six years chasing down the genetic identity of the elusive calcium-activated chloride channel.

After the researchers settled on using eggs from the Mexican mole salamander—instead of the African tree frog—they were able to readily identify a gene from a new family of ion channels that control the flow of chloride ions into cells. These ion channels are vitally important, helping to regulate physiological processes, such as smell, taste sensing, smooth muscle contraction, and fluid secretion in glands and airways.

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— Lily Y. Jan

With the gene in hand, scientists now have an opportunity to begin testing new treatments for a variety of diseases influenced by the chloride concentration inside cells, including cystic fibrosis, asthma, bronchitis, hypertension, and perhaps even some aggressive cancers.

Howard Hughes Medical Institute investigators Lily Yeh Jan and Yuh Nung Jan published their findings in the September 19, 2008, issue of the journal *Cell*. The Jans and co-authors Björn Christian Schroeder and Tong Cheng are at the University of California, San Francisco.

The ebb and flow of chloride ions into and out of cells can alter cells' electrical properties, influencing nerve function, muscle contraction, and a variety of other processes. The channels that permit chloride to pass through the cell membrane are regulated by a variety of factors, such as voltage, pH, or specific molecules. In this study, the researchers were interested in calcium-activated chloride channels, which are common in epithelial cells that line the airway, smooth muscle cells that surround blood vessels, and in the membranes of neurons.

It has been difficult for researchers to isolate calcium-activated chloride channels because of their ubiquity—most notably in the very cells that are often used to clone channel proteins. Researchers hunting for new families of ion channels routinely turn to a technique called expression cloning to isolate the gene and protein that interest them. They start by introducing a large number of gene segments—one of which is suspected to code for the protein of interest—into cells, such as the eggs of the African frog, *Xenopus*. If an egg expresses the target protein, the researchers know that their quarry lies within the gene segment that they have inserted into that egg. They then subdivide that gene segment and repeat the process until they have narrowed down the region enough to locate the gene sequence in a database.

Xenopus eggs, however, are studded with calcium-activated chloride channels because those proteins are needed to allow only one sperm to enter and fertilize the eggs. Since the channels are abundant inside the eggs, researchers cannot use them as their test system. So the Jans and their colleagues decided to use eggs from Axolotl, the Mexican mole salamander, whose eggs allow multiple sperm to enter and, it turns out, do not produce calcium-activated chloride channels.

After a series of expression cloning screenings using Axolotl eggs, the researchers narrowed down the relevant genetic region enough to determine that a gene sequence called *TMEM16A* encoded the calcium-activated chloride channel.

The researchers tested *Xenopus* and mouse versions of the gene and confirmed that their protein acted like a calcium-activated chloride channel - that is, it let chloride flow through the cell membrane.

Lily Jan said the finding is likely to be very useful for testing potential drug therapy for cystic fibrosis. For example, drugs that activate the channel selectively might restore lung function that has been compromised by cystic fibrosis or other disorders. People with cystic fibrosis have a malfunction in one of two types of chloride channels in the lungs that control the flow of salt and water into the lumen, she said. The channel most often affected is the cystic fibrosis transmembrane conductance regulator, and the other is the calcium-activated chloride channel.

Current treatments for cystic fibrosis stimulate the calcium-activated chloride channel indirectly by activating the upstream receptor to release calcium from an internal store. Our hope is that new insight into the calcium-activated chloride channel will lead to drugs that directly modulate the channel, she said. The same strategy might be used to treat bronchitis and asthma. Conversely, drugs that block the channel in the smooth muscle that surrounds arteries might widen the arteries to reduce blood pressure in those with hypertension, Jan said.

Jan said that she and her colleagues were particularly surprised to learn that high levels of *TMEM16A* have been linked to aggressive cancers of the head, neck, and stomach. Other researchers had identified *TMEM16A* as part of an amplicon - a gene region overexpressed in some cancers and thought to be a

driving force behind malignancy. The action of this amplicon appears to correlate with a poor prognosis, she said. Certainly much more experimentation needs to be done to understand whether the *TMEM16A* gene is involved in driving cancers, said Jan. But if it is, it might make a useful drug target for treatment.