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Study of Rare Vision Disorder Leads to Discovery of New Family of Ion Channels

Efforts to understand the most common cause of vision loss in millions of elderly people have led to the discovery of an entirely new family of chloride ion channels that are found in animals from worms to humans.

In 1998, researchers showed that mutations in a gene that codes for the protein bestrophin were responsible for causing Best macular dystrophy, a hereditary disorder that strikes during childhood or early adolescence and causes impaired central vision. Until recently, the function of bestrophin has remained a mystery. Now, a collaborative team of Howard Hughes Medical Institute (HHMI) investigators at The Johns Hopkins University School of Medicine, has found that bestrophin is a chloride ion channel, a protein that forms a pore in cells through which charged ions can pass.

In addition to showing that bestrophin is an ion channel, the scientists searched the human genome and the DNA sequences of other organisms and found at least three other members of this chloride ion channel family in humans, four in the fruit fly and 24 in the worm *Caenorhabditis elegans*. None of the proteins they found had previously had a function assigned to it.

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- **Jeremy Nathans**

The discovery, published March 19, 2002, in the journal *Proceedings of the National Academy of Sciences*, is the first new chloride ion channel to be described since the cystic fibrosis transmembrane conductance regulator was discovered more than ten years ago. In addition to cystic fibrosis, mutations in chloride ion channels have been linked to several other human disorders, including diseases of the kidney and muscle.

“One of the reasons it has been so long since a new chloride ion channel was discovered is that chloride channels are structurally diverse, unlike, for

example, potassium channels, in which it is possible in many cases to recognize far flung family members through similarities [among the proteins],” said HHMI investigator Jeremy Nathans, who along with Hopkins colleague and HHMI investigator King-Wai Yau were senior authors of the study.

Two separate groups of scientists identified the bestrophin protein in 1998, but it was “not clear what it might do,” according to Nathans. “That’s where the field has been stuck.”

Nathans and HHMI research associate Hui Sun, who together had previously discovered the function of a protein responsible for a different form of hereditary macular dystrophy, decided to try to decipher the bestrophin mystery.

“Each time we study a hereditary macular dystrophy, we are looking at macular degeneration from another starting point in the disease mechanism,” said Nathans. By studying hereditary forms of the disease, scientists gain clues that can help them understand the more common age-related disorder, Nathans said. For example, ophthalmologists have noticed that macular degeneration seems to start by accumulation of material in the retinal pigment epithelium (RPE), the exact region where the bestrophin protein is located.

“The retina is like a Ferrari,” said Nathans. “It is a high performance, high maintenance tissue. The RPE is like the liver and kidney of the retina. It helps keep the retina running. Best disease teaches us that defects in the RPE can initiate macular degeneration.”

It was useless to try to guess the function of the bestrophin protein based on comparing it to other proteins of known function, said Sun, because it didn’t look like any other known protein. “This was a bioinformatics nightmare,” he said. “At the time we started, the future direction was pretty murky. But this puzzle intrigued us. We wondered what was happening here.”

By studying what was known about Best disease, the scientists concluded that the bestrophin protein might be an ion channel because patients with the disease have abnormal readings on electrooculograms, tests that measures ion movements in the eye. In addition, all people who inherit one copy of the mutated bestrophin gene and one normal copy of the gene have the disease. This fact led the scientists to believe that when the defective protein is present, it actively prevents the normal protein from functioning properly.

To study the protein, the scientists produced the human bestrophin protein and studied its ability to conduct chloride ions across a cell membrane. Yau and HHMI associate Takashi Tsunenari performed detailed studies of the electrical conductance of the bestrophin protein after it was expressed in an embryonic kidney cell.

“When an ion channel opens, a large flux of ions occurs,” said Yau. “It’s like a floodgate opening. With amplification, it is possible from even one cell to pick up the current. And from the behavior of the current we can derive a signature of each channel.”

This information provided evidence that the protein was indeed a chloride ion channel. But Nathans points out that many scientists had been fooled in the past into thinking they had found an ion channel when what they were actually seeing was the activity of their test cell, not the protein they were studying.

To prove they had found an ion channel, the scientists conducted several additional studies in which they tested bestrophin family members from the fruit fly and the worm. Each produced its own distinct ion channel “signature,” said Yau. In addition, when they tested the mutant bestrophin protein together with a normal bestrophin protein, they found that the mutant inhibited the electrical current produced by the normal protein.

Additional studies showed that the bestrophin protein assembles in a group of four or five units that form a pore through which the chloride ions pass. When mutant proteins assemble together with normal proteins, the result is a less effective channel. This explains why people with one copy of the mutant gene and one copy of the normal gene are still affected by the disease, said Yau.

The discovery that there are many members of the bestrophin protein family in diverse animals suggests that this newly discovered chloride ion channel family might play important roles in other cellular processes, said Nathans.