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Novel Protein is Both Ion Channel and Enzyme

Researchers have discovered a new protein component of cell-signaling pathways that does double duty acting as both an ion channel that controls calcium entry into cells and as an enzyme that activates itself and perhaps other proteins. While they do not yet know how the protein is activated, they have found that it is present in many tissues, including brain, kidney and heart. The researchers speculate that the protein might be involved in cell proliferation or cell death, making it a potential new drug target.

Howard Hughes Medical Institute investigator David E. Clapham and colleagues Loren W. Runnels and Lixia Yue at Children's Hospital in Boston and Harvard Medical School reported on the new protein in an article published online on January 19, 2001, on *Scienceexpress*, the Web-based counterpart of *Science* magazine.

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The new protein, called transient receptor protein-phospholipase C-interacting kinase (TRP-PLIK), is a member of a family of ion channel proteins that controls the entry of calcium into the cell, said Clapham. "The TRP channels are important to cell regulation because calcium is the most tightly controlled ion in biology," he said. "The concentration of calcium outside the cell is about twenty-thousand-fold that inside the cell. Its entry is controlled so precisely because it triggers many cell processes, from muscle contraction to the firing of neurons." Despite their importance, though, little is known about how TRP channels are activated, he said.

Furthermore, it appears that TRP-PLIK is unique among TRP channels, said Clapham, because it is both a calcium ion channel and a kinase, an enzyme that activates other proteins by phosphorylating or adding a phosphate to them. "The most exciting part of this finding for us was that TRP-PLIK is bi-functional," he said. "It contains a domain whose opening and closing acts as a gate for calcium and a domain that acts as a kinase."

"The finding is also important because, although there are channels known to have various enzymes in them, it's generally not known what they do. However, in this case, we know that the enzyme phosphorylates the channel itself and that this is important for channel function. There is also good evidence that the kinase can phosphorylate other proteins in the cell."

According to Clapham, the discovery of TRP-PLIK came as first author Loren Runnels was searching for kinases that interacted with phospholipase C-beta, an enzyme that is a key to release of calcium from stores within the cell.

"Loren pulled out this kinase on a yeast two-hybrid screen that appeared to be quite unusual, and at first he thought that he had isolated the whole protein," said Clapham. "In searching genome databases, he discovered that it turned out to be only a segment of a larger protein."

Additional experiments revealed that the rest of the segment was an ion channel, and a member of the TRP family, said Clapham. "It was a stroke of luck because we worked on TRP channels anyway, and Loren didn't come at it from that angle, but from the kinase angle," he said. The scientists named the protein TRP-PLIK to reflect its dual function. A search of tissues for RNA that codes for TRP-PLIK revealed some presence in brain and skeletal muscle, with a stronger signal in kidney, heart, liver and spleen, said Clapham.

"When we began experiments to express the channel in cells to determine its properties, the most important feature was that it passed a small amount of current into the cell, also admitting calcium," said Clapham.

To explore the role of the kinase segment of TRP-PLIK, the scientists produced mutant versions of the kinase to cripple its action, finding that the mutations eliminated the activity of the channel. The kinase itself is a member of a unique class called alpha kinases because they phosphorylate alpha helices in proteins.

While the mechanism of the action of TRP-PLIK is known, its biological function in the cell remains a mystery, said Clapham. "However, we do know that this TRP channel belongs to a class that seems to be involved in cancer and apoptosis, or programmed cell death," he said. "For example, an enzyme that is most closely related to this TRP is melastatin, whose action seems to block the progression of the skin cancer melanoma."

A possible involvement in cell proliferation, along with the fact that the PLIK kinase interacts with phospholipase C, leads Clapham and his colleagues to believe that they might have found a promising metabolic control point, thus a potential drug target.

He speculated that TRP-PLIK might be an element of a control pathway in which cell-surface receptors called G-protein-coupled receptors connect to phospholipase C, which activates the PLIK kinase, in turn activating the TRP channel.

"Ion channels are targets for perhaps a third of all drugs, either directly or indirectly including anti-hypertensives, anti-depressants and anti-arrhythmic heart drugs," said Clapham. "The bi-functional TRP-PLIK protein could offer a new approach to drugs that turn cell division on or off, or cause cells to go into apoptosis and die."