

OCTOBER 17, 2002

## Ion Channels Enable Bacteria to Resist Stomach Acid

Researchers have found that a primitive type of ion channel similar to those found in mammalian nerve cells helps bacteria resist the blast of acid they encounter in the stomach of their hosts.

The discovery suggests a plausible mechanism whereby bacteria can fend off stomach acidity long enough to establish themselves in the intestine. More broadly, said the scientists, the finding represents the first insight into why bacteria have forms of the same ion channels — proteins that control the flow of ions through cell membranes — found in higher organisms.

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— Christopher Miller

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In an article published in the October 17, 2002, issue of the journal *Nature*, researchers led by Howard Hughes Medical Institute investigator Christopher Miller present evidence that the chloride ion channel is an integral part of the extreme acid resistance (XAR) response of the bacterium *E. coli*. Miller co-authored the paper with colleagues Ramkumar Iyer, Tina M. Iverson and Alessio Accardi, all of Brandeis University.

According to Miller, ion channels from bacteria have proven enormously useful to researchers studying the structure and function of ion channels because the bacteria enable the scientists to produce sufficient quantities of the proteins for their studies.

As ion channel researchers, we've been so happy at the boon of high-quality protein we've received from these bacterial genomes, that the whole question of why the channels are even there has been largely ignored, said Miller. Fortunately, however, Miller's postdoctoral fellow, Ramkumar Iyer, had the scientific intuition to explore whether the ion channels, known as CIC channels, might play a role in XAR.

We had identified two chloride channel genes in the bacteria, and we decided to go on a fishing expedition to explore their function, said Miller. When we first knocked them out, we saw no obvious changes in growth or behavior of the bacteria. Then, Ram decided to subject the altered bacteria to different stresses, reasoning that the channels might be involved in some kind of stress response. Otherwise, such channels in the membrane would prove deadly to [the bacteria].

Iyer struck pay dirt with his first experiments, which showed that the altered bacteria could not survive when they were exposed to high acidity. According to Miller, previous studies indicated that when bacteria are exposed to a very low pH of about 2, two kinds of XAR genes are activated to draw certain amino acids — glutamate or arginine — into the cells. Additional XAR enzymes then decarboxylate these amino acids to form gamma-amino butyrate or agmatine in chemical reactions that consume acid. These decarboxylation products are then transported out of the cell, the whole cycle acting as a virtual proton pump that keeps the cytoplasm from becoming too acidic in the acidic environment of the stomach. However, said, Miller, these proton pumps — because they move net positive charge outward — would grind to a halt unless there were some way to leak chloride out of the bacterial cells.

The chloride channel provides an electrical shunt or an electrical leak that allows the proton pump to keep turning over, said Miller. If there are no chloride channels — which is the case in our knockout *E. coli* — as the proton pump moves positive charge outward, it builds up a negative voltage on the inside of the cell, and this voltage imbalance across the membrane essentially turns that pump off. The chloride channel enables the proton pump to function because it allows a negative chloride ion to leak out with every positively-charged proton that gets pumped out.

The researchers next tested whether the channel is activated by acid shock. When they inserted the isolated ion channel proteins into artificial membrane bubbles called liposomes and exposed them to low pH, they found that the channels increased their rate of chloride uptake about 10-fold.

Our first guess is that like many channels, this one exists in an open and a closed state, said Miller. And what switches this bacterial chloride channel to an open state is not a neurotransmitter or voltage change, as is the case with their homologs in the mammalian nervous system, but a high extracellular acid concentration.

Miller and his colleagues also noted that pathogenic bacteria such as those that cause cholera or salmonellosis also have genes for CIC channels, and these bacteria might use the same mechanism to survive stomach acid and invade the intestine.

The discovery of the chloride channels role in bacteria could offer insights into the function of some of the mammalian homologs of these channels, said Miller. We have nine homologs of the CIC channels in our own genomes, and they are involved in numerous physiological functions, he said. Whats

striking is that researchers have developed evidence that some of these homologs appear to be involved in processes very similar to those we find in the XAR machinery of *E. coli*. For example, said Miller, researchers have evidence that the channels might play a role in the machinery that maintains necessary acidic conditions within the tiny sacs called endosomes that transport receptors from the cell surface into the cell interior. These functions, however, had their origins in the distant evolutionary past, he said.

It seems a strong implication from our work in bacterial ion channels over the past five years that these are ancient proteins, and not specialized machines for the specialized cells such as nerve cells in higher organisms, he said. Whats more, I would be very surprised if we didnt discover that bacteria other than the ones that go through the stomach hadnt developed uses other than the one we have found for these channels.