Gene for acid-sensitive ion channel identified

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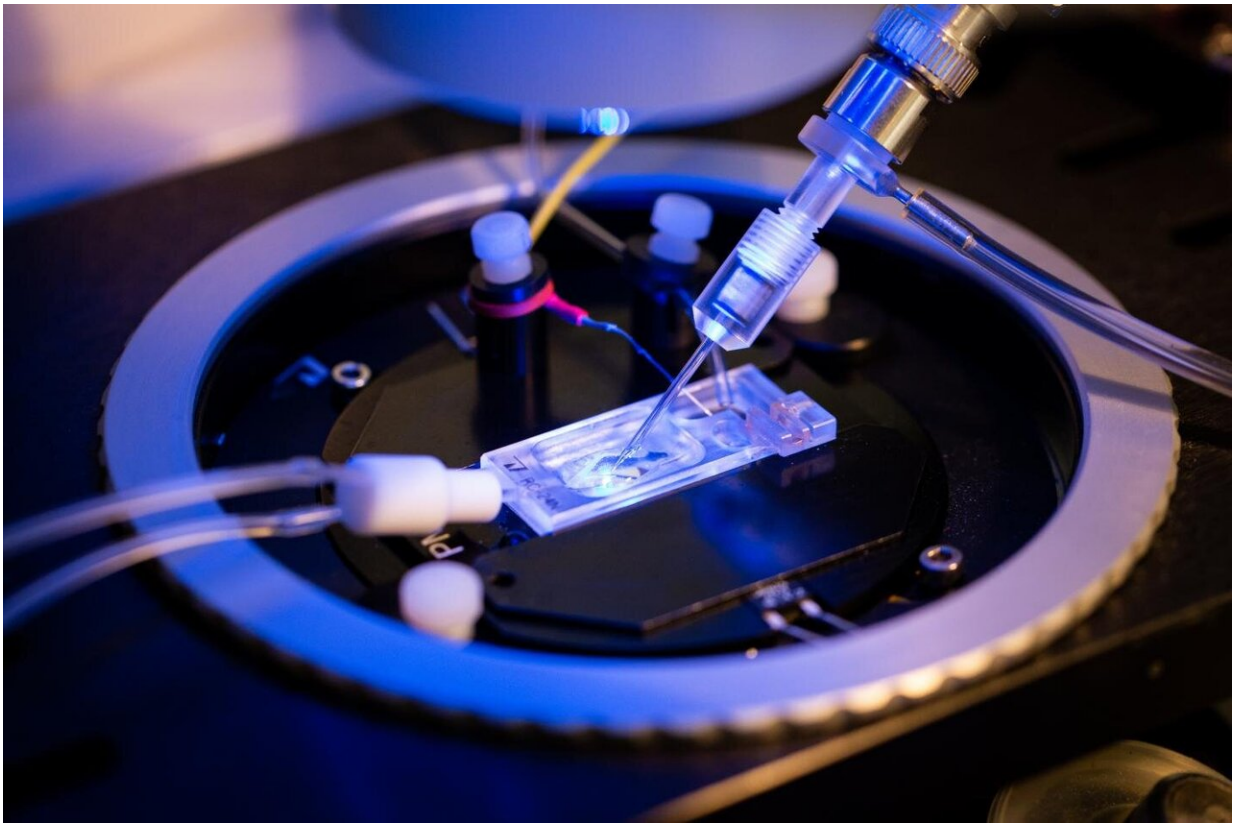

Patch clamp recording of acid activated chloride currents. Credit: Felix Petermann

In the human body the salt content of cells and their surrounding is regulated by sophisticated transport systems. Special channels in the cell membrane selectively permit salt ions to flow in and out of cells. A research team led by Professor Thomas Jentsch at the FMP and MDC

has now identified the molecular components of a previously unknown ion channel.

The membranes that encase cells and cell organelles are normally impermeable for charged particles such as salt ions. But there are loopholes: Transmembrane proteins can form channels that pass ions. In most cases, such ion channels open or close on receiving a particular signal transmitted, for example, voltage or a signaling molecule. The channels are often specialized to allow only specific ions—such as chloride, potassium, or sodium—to pass.

A team led by Professor Thomas Jentsch from Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) and the Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC) has now identified a new transmembrane protein called TMEM206 as a new chloride ion channel. It is characterized by a so-far unique activation mechanism: When the pH level decreases in the cell environment, the channel opens and allows chloride, depending on the cell type, to flow out or into the cell. This type of ion channels may play a role in the development of heart attacks, strokes, and tumors, for these diseases are accompanied by an acidification in the affected tissue.

The starting point for the current research was an ion channel called ASOR (Acid-Sensitive Outwardly Rectifying anion channel). More than ten years ago, electrophysiological studies had detected and characterized pH-regulated chloride currents in cells of various vertebrates. "But the structure of the underlying channel had remained unknown. Back then, technology had not been advanced enough to perform a genome-wide screen to identify it. We have now discovered the gene encoding the protein that forms the ASOR channel," explains Professor Thomas Jentsch, head of the research group on Physiology and Pathology of Ion Transport at the MDC and FMP.

It took about four years to complete the study. Many methods had to be adapted or newly developed. For example, the team devised a special optical detection assay for the function of the ASOR channel that is compatible with high-throughput methods.

In order to search for DNA sequences relevant to the ASOR channel, the researchers performed a siRNA screen. This involves using small pieces of RNA (small interfering RNA, or siRNA) to systematically shut down genes one by one in cultured cells and then analyzing the functional consequences.

They also employed CRISPR-Cas9 technology and mutations changing channel properties to confirm that the thus identified gene was in fact coding for the channel. "For us, it was extremely helpful that the Screening Unit at the FMP, which has a facility specializing in these high-throughput methods, was our direct neighbor," says Jentsch. "There are numerous robots there that pipette the samples, and it is equipped with automated cell culture systems." The Screening Unit's siRNA library contains siRNAs for all 20,000 human genes, each of which must be evaluated separately. To be on the safe side, three runs were performed so that in the end a total of 60,000 individual results had to be analyzed bioinformatically.

"The identification of TMEM206 as central component of the ASOR channel is a major breakthrough. This opens the door to finally uncover the currently unknown physiological roles of the channel," summarizes Jentsch. Chloride ions are among the most important and prevalent electrolytes in the body. Their concentration can vary substantially between the extracellular space, the cytoplasm, and various intracellular organelles. The cell membrane forms a barrier for the negatively charged chloride, but special membrane proteins enable it to cross this barrier. Chloride ions either move along concentration gradients through channels or, by coupling to other ions, can be actively pumped across the

membrane by transporter proteins. Chloride channels carry out very diverse biological functions. The underlying proteins are also molecularly very diverse. They are regulated in a host of ways to govern the transport of chloride according to the need of the cell and the organism.

There is strong evidence that the ASOR channel plays a role in acid-induced cell death. The channel allows the passage of chloride ions only when the extracellular milieu is very acidic. Even though the channel is found in every mammalian cell, this occurs only in a few specialized cell types or under pathological conditions such as stroke or heart attack or within tumors. However, the fact that ASOR plays a harmful role in disease does not explain why it is found in all mammalian cells.

There are still many unanswered questions, says Jentsch. What is the significance of the strong pH-dependence of the channel? Why do all cells apparently have this ASOR channel? And where exactly is the channel located inside the cells? Is it also present in acidic organelles, such as lysosomes and endosomes? In order to further elucidate the structure and physiological functions of ASOR, the research group has developed antibodies against TMEM206 and are generating mice in which the gene for the channel is destroyed. They want to find out in which cell the channel protein is expressed and where exactly it is localized within the cells. In the future, they also hope to clarify the physiological function by using their mouse models.

More information: Florian Ullrich et al, Identification of TMEM206 proteins as pore of PAORAC/ASOR acid-sensitive chloride channels, *eLife* (2019). DOI: 10.7554/eLife.49187

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