What makes plants electrically excitable

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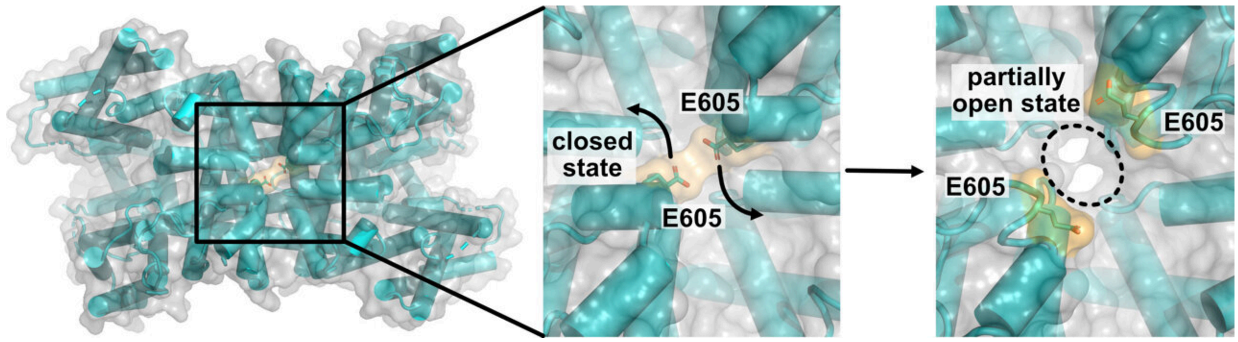

3D sketch of the TPC1 channel protein looking at the vacuolar pore entrance from above. Positional changes of amino acid residues such as E605 during the transition from a closed channel state to a partially open pore state. Credit: Thomas Mueller

Plant cells use electrical signals to process and transmit information. In 1987, as a postdoc of Erwin Neher in Göttingen, biophysicist Rainer Hedrich discovered an ion channel in the central vacuole of the plant cell, which is activated by calcium and electrical voltage, using the patch-clamp technique (Nobel Prize for Neher and Sakmann 1991).

In 2019, Hedrich's team at Julius-Maximilians-Universität Würzburg (JMU) identified this TPC1 channel as an important element for electrical communication in plants. If the channel fails, signal transmission is slowed down. If it is hyperactive, i.e. open for too long, the plant is highly stressed and has problems growing.

These reactions make it clear: plants must strictly control the opening time of the ion channel TPC1 so that the electrical communication between their cells runs smoothly.

The structure explains how the channel is switched on

A publication in the journal *PNAS* now provides new insights into the molecular function and regulation of the TPC1 channel. This was achieved by combining two areas of expertise: A JMU team led by Rainer Hedrich and Irene Marten was responsible for plant biophysics, and a group led by Robert M. Stroud and Sasha Dickinson from the University of California in San Francisco was responsible for structural biology.

Using high-resolution cryo-electron microscopic imaging, the U.S. team clearly demonstrates that massive conformational changes occur in several protein domains before the channel opens. An electrical stimulus initiates a rotating movement of the voltage sensor domain. This flaps away amino acid residues that serve as binding sites for inhibitory calcium ions in the vacuolar entry region of the channel—clearing the way for ion flow.

The vacuolar calcium content keeps the voltage sensor in check

Hedrich and Marten's team was able to show that the TPC1 channel is switched on when the calcium level in the cell plasma rises in response to external stimuli. An increase in the calcium level in the vacuole, on the other hand, slows down an excessive ion flux through the channel and virtually renders the vacuolar membrane insensitive to Calcium-dependent electrical stimuli.

The newly discovered binding site for vacuolar calcium ions in the ion-conducting pore of the channel plays a crucial role in this process.

"We were able to elucidate the function of this channel domain with patch clamp measurements," says JMU professor Irene Marten. "When calcium binds to the vacuolar pore-binding site, negative feedback with the voltage sensor occurs, which means that the movement of the voltage sensor domain is strongly impaired. As a result, the channel remains closed and there is no electrical excitation of the vacuole. If, on the other hand, the pore amino acid residues are removed from the ion transport pathway, no vacuolar calcium binding occurs and the channel opening is strongly facilitated."

Question about the evolution of TPC1 channels

The publication in *PNAS* further contributes to the fact that the plant ion channel TPC1 is now one of the best understood voltage-dependent ion channels. This knowledge can help to better understand TPC1-dependent processes in animal cells as well.

What will the scientists do next? "We are looking into the question of whether the TPC1 channels of different plant species differ in terms of regulation and also in other properties, and whether this opens up new possibilities for adaptation to the environment," says Rainer Hedrich. "In doing so, we are also taking into account regulators that play a role in animal TPC1 channels. The studies should also give us an insight into the evolution of TPC1 channels."

More information: Miles Sasha Dickinson et al, Molecular basis of multistep voltage activation in plant two-pore channel 1, *Proceedings of the National Academy of Sciences* (2022). DOI: 10.1073/pnas.2110936119