

Researchers discover key to body's ability to detect subtle temperature changes

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Scientists have long known the molecular mechanisms behind most of the body's sensing capabilities. Vision, for example, is made possible in part by rhodopsin, a pigment molecule that is extremely sensitive to light. It is involved in turning photons into electrical signals that can be decoded by the brain into visual information. But how the human body is able to sense a one-degree change in temperature has remained a mystery.

"For a long time, we didn't know how temperature sensing was being carried out in animals," said Jie Zheng, assistant professor in the Department of Physiology and Membrane Biology at the UC Davis School of Medicine. Huge progress was made in the last decade, Zheng said, when scientists discovered four ion channels sensitive to heat and two cold-sensitive ones.

"But, it was still unclear how only six temperature-sensor channels could cover wide ranges of temperature and still discriminate subtle differences," Zheng said.

Using a novel method based on a technique first used by physicists, Zheng and his colleagues now have shown that the subunits of one channel can come together with subunits from another channel or coassemble in laboratory cell cultures to form new functioning channels. Assuming this process also happens in normal cells, it suggests a likely mechanism for the thermosensitivity seen in all animal cells, Zheng explained.

"We found that, by reassembling subunits we potentially have a lot more than six channel types responsible for the sensing of temperature," he said.

The current findings are featured on the cover of the March issue of the *Journal of General Physiology* and were published online today.

Ion channels are pore-forming proteins found in the membranes of cells. They have the ability to open and close, regulating the flow of charged ions and controlling the voltage gradient found between the inside and outside of every living cell.

In the current study, Zheng and his colleagues focused on a group of ion channels called transient receptor potential (TRP) channels. In all, there are more than 20 TRP channels. Zheng's group studied four of the six channels that have been shown to be involved in sensing temperature.

Previous studies concluded that different thermosensitive TRP channel subunits did not coassemble, Zheng said. He realized, however, that there were some technical limitations to the previous work. So, he and his colleagues decided to use a technique they developed last year, called spectra FRET. Spectra FRET, or spectroscopy-based fluorescence resonance energy transfer, allows the researcher to observe interactions between different channel subunits under a microscope.

"This technique allows us to look at the channel subunit composition in real-time in live cells," Zheng said.

In the current experiments, cDNA coding for particular subunits is linked to cDNA coding for fluorescent proteins and then added to a culture of human embryonic kidney cells. The cells take up the DNA and then express the channel proteins, each now having a fluorescent protein tag. The researchers then observed the cells in the spectra FRET

apparatus.

"Using spectra FRET, we were able to focus on just the signal from the plasma membrane," Zheng explained. "What we found was that the subunits of one kind of heat-sensitive channel coassembled with subunits of other heat-sensitive channels to form new channels. This means that instead of four heat-sensitive channels we have a potential of 256 heat-sensitive channels with potentially different temperature sensitivity ranges."

Zheng and his colleagues then confirmed their results using a technique called patch clamping that allowed them to record the electrical current flowing through individual open channels.

"Using these single-molecule recordings, we see many different channel types," Zheng said. The next question we are trying to address is whether they really have different temperature sensitivity. We believe the answer is 'yes,' but we have to show that."

Zheng also believes it is likely that the channels responsible for sensing cold coassemble in the same way. The cold- and heat-sensing subunits, however, do not seem to coassemble, he said.

The findings by Zheng and his colleagues promise to help solve the mystery of temperature sensitivity in animals once and for all. And, because the cells with these ion channels in their membranes are also the cells that sense pain, the basic knowledge they have provided may one day prove useful to scientists looking for novel remedies for pain.

"We have to re-examine everything from how people acclimate to hot climates to how they respond to spicy food based on the understanding that there are many more kinds of channels involved," Zheng said.

Source: University of California, Davis

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