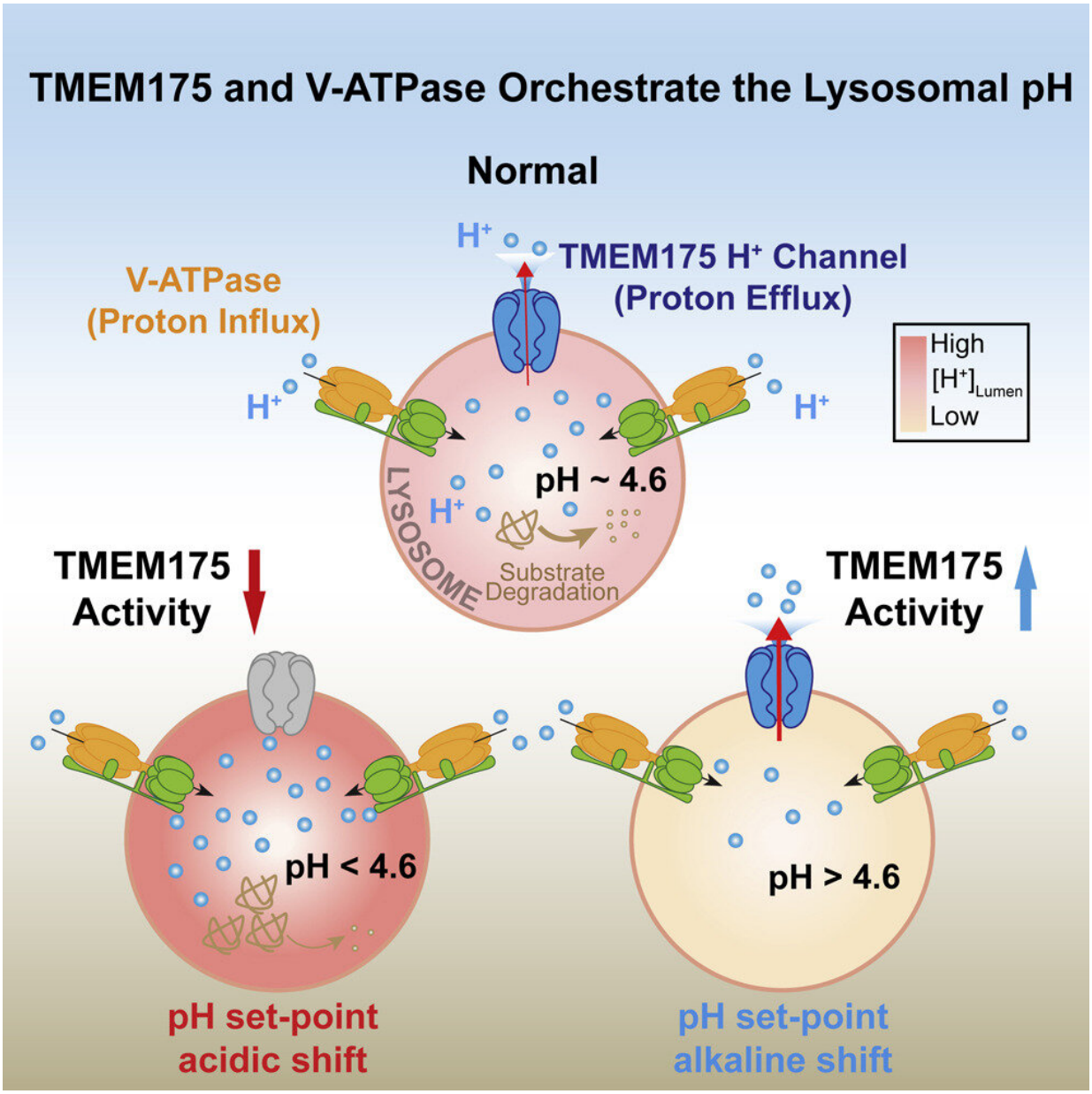
Researchers reveal new molecular mechanism for Parkinson's disease risk

June 23 2022



Graphical abstract. Credit: *Cell* (2022). DOI: 10.1016/j.cell.2022.05.021

In about a fifth of the cases of Parkinson's disease, look to a small, malfunctioning protein in the lysosome as a risk factor, say University of Michigan researchers.

Lysosomes are the garbage collectors of cells. These organelles are responsible for breaking down the "trash" in the cell—misfolded proteins, worn out organelles—that cells collect in a process called autophagy. Autophagy depends closely on lysosome function, and when lysosomes malfunction and this process is disrupted, causing cellular debris to build up, various disorders can occur. Many of these are degenerative disorders such as Alzheimer's disease, Duchenne muscular dystrophy and Tay-Sachs disease.

Now, U-M researchers have discovered how a mutated protein called TMEM175 acts as a risk factor in about 20% of cases of Parkinson's disease. In Parkinson's, nerve cells in the area of the brain that controls movement begin to fail and die. According to the National Institute on Aging, researchers think Parkinson's is a result of a combination of genetic and environmental factors.

The U-M researchers found that if mutated, TMEM 175 does not properly regulate the acidity of the environment within the lysosome. If the acidity in that environment is not correct, enzymes within lysosomes stop working effectively, and the organelles cannot perform their roles correctly. Their study results are published in the journal *Cell*.

"The lysosome actually needs an optimal pH. Here is an analogy: body temperature has to be 37 degrees Celsius. Thirty-eight is too high and 36 is too low," said lead author Haoxing Xu, professor of molecular, cellular

and developmental biology. "The enzymes in lysosomes require a pH optimum of roughly 4.6. Anything outside of 4.6 pH may cause metabolic dysfunction, and therefore accumulation of cellular garbage, which will eventually cause neurodegeneration and metabolic diseases."

To keep the lysosome in acidic equilibrium, Xu knew there must be an ion channel that regulates the acidity of that environment by regulating the concentration of hydrogen ions (called protons in this context—the nucleus of a hydrogen atom with the atom stripped of its electron) within the lysosome.

Xu, aided by co-authors including Richard Hume, U-M professor of molecular, cellular and developmental biology, first determined the exact acidity of the lysosome's lumen. Then, the researchers combed through a list of proteins associated with the lysosome but that had not yet been well described.

What was very well understood was how protons get into lysosomes: there's a specific membrane transporter called the V-ATPase that loads protons, but what was unclear was how to get to homeostasis, or how get the pH to 4.6 and not let it keep going more acidic, according to Hume. Some indirect evidence suggested there was probably a proton permeable channel in lysosomes whose molecular basis was unknown that was somehow involved with the pH homeostasis.

"We were interested that if there was the V-ATPase to pump a proton in, lysosomes must have an ion channel protein to release the proton when the proton level was too high inside the lysosome," Xu said.

To study ion channels in the lysosome, the Xu lab previously developed a specialized technique called the lysosome patch-clamp. The patch-clamp allows the researchers to selectively "turn on" certain ion channels in the lysosome in order to better understand their function. The researchers

have used this technique to find channels for other ions: calcium, sodium, potassium, chloride and iron, and now protons.

U-M postdoctoral researchers and co-authors Meiqin Hu and Ping Li made a list of all the genes that encode membrane proteins known to be present in lysosomes, then did what's called an overexpression screening assay. This means the researchers tested several dozen membrane proteins one at a time to see if the lysosome's membrane became more permeable to protons. This would suggest that one of the candidates that they screened was responsible for proton efflux in the lysosome.

The researchers zeroed in on the trans-membrane TMEM 175, which gave dramatically enhanced proton permeability in the screening assay, Hume said.

"That got Meiqin, Haoxing and the rest of the lab really excited, because that protein was not totally unknown," Hume said. "Importantly, it is one of the highest vulnerability genes for mutations that cause Parkinson's disease."

Previous guesses about how the protein was a risk factor for Parkinson's centered around examining its function as a potassium channel.

"Quite honestly, those explanations didn't make sense because it is hard to rationalize a mechanism such as changing potassium flux across the lysosome should have led to Parkinson's-like deficits," Hume said. "But as soon as one realized that TMEM175 was probably a proton channel, then the rationale for how a mutation of that protein could cause Parkinson's seemed pretty obvious."

The researchers cross-checked their work in two ways. First, Hu and doctoral student Ce Wang used proton imaging, using protein fluorescent indicators, to measure the acidity of the lysosomes when they knocked

out TMEM175. Second, they used mice whose ability to produce the protein TMEM175 was knocked out. This means they weren't making that transporter protein channel in their lysosomes. The team showed alpha-synuclein accumulation in the cells of the knockout mice. Alpha-synuclein accumulation is known to be toxic in patients with Parkinson's disease.

In both cases, cells showed a decrease in the enzymatic activity that breaks down cellular junk, including alpha-synuclein aggregates, indicating that TMEM175 was responsible for regulating cellular acidity and degradation.

"In the end, we are very confident that this is the protein that's controlling the proton leak in the lysosome," Xu said. "This paper is exciting because mutations in this protein happen to be high risk for Parkinson's disease."

More information: Meiqin Hu et al, Parkinson's disease-risk protein TMEM175 is a proton-activated proton channel in lysosomes, *Cell* (2022). DOI: 10.1016/j.cell.2022.05.021

Provided by University of Michigan