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New Clues to How Cells Stay in Shape as Salinity Shifts

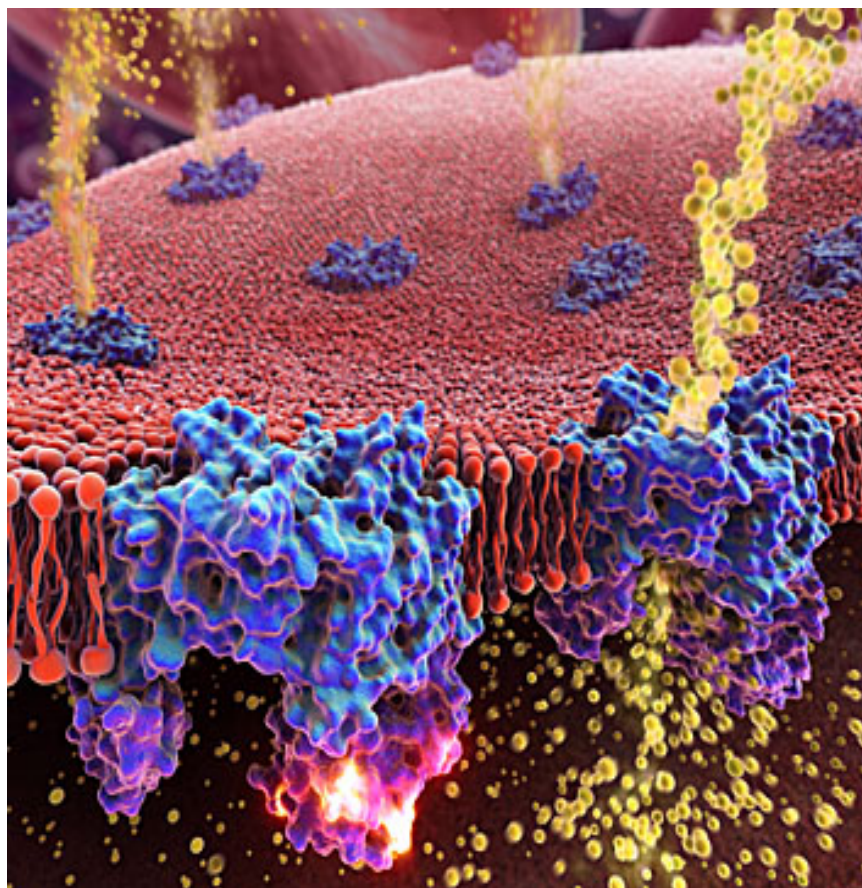


Image Title: Image shows ion cotransporter (blue) in the red blood cell membrane. When it is phosphorylated (white flash) it is inactive, but when it is dephosphorylated it is active, allowing potassium and chloride to leave the cell. - Xvivo

By deploying new proteomics technology, researchers have identified two molecular keys that unlock a crucial cellular door. This door releases salts from cells, helping cells maintain their size and shape and keeping them alive in the ever-changing salinity of their environment.

The finding, published in the August 7, 2009, issue of the journal *Cell*, could lead to new treatments for sickle cell disease, says Howard Hughes Medical Institute investigator Richard P. Lifton, who led the work.

Maintaining proper cell size is a basic, vital cellular function. For instance, if a person drinks a few liters of water, the salinity of his or her blood drops. If unopposed, this would push water into cells and swell them to bursting. Conversely, eating a bag of salty potato chips would pull water out of cells, shriveling them to death. “Cells have to be able to respond immediately to changes in salinity and solute in their environment,” says Lifton, also at the Yale University School of Medicine. “It’s critical for virtually every cell in the body.”

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To accomplish this, cells either release or take up chloride ions via cellular doors called cotransporters that move chloride – either into cells, together with sodium, or out of cells, along with potassium. These doors are embedded in the cell membrane. Potassium-chloride cotransporters open when the cell needs to reduce its chloride concentration, which in turn prevents water from entering and causing cell swelling. But exactly what opens these co-transporters has been unknown.

Lifton got onto the case in 2001, when he and his team discovered a new metabolic pathway that governs blood pressure. The key players in this pathway, Lifton found, were enzymes—specifically, protein kinases called WNKs (pronounced “winks”). “At the time we discovered them, their function was completely unknown,” says Lifton. “We knew that when the WNKs were mutated they caused high blood pressure in humans, but we knew nothing else about them.”

As his group investigated these proteins, it soon found that the WNKs helped regulate the flux of chloride ions into and out of cells. To learn more about how the WNKs were involved, Lifton focused on finding a link between WNKs and the potassium-chloride cotransporters, whose function as chloride chutes was well known. Because WNKs were protein kinases, which modify other proteins by attaching a phosphate group to them, Lifton reasoned that he should look for these modifications in the cotransporters. This atomic tag alters the function of the target protein in various ways, and over the past decade biologists have intensely studied phosphorylation and its role in myriad diseases.

Lifton wanted to see if the potassium-chloride co-transporters bore these marks. To do so, the team, led by lab member Jesse Rinehart, deployed a set of new technologies that can identify which segments of a protein carry a phosphate tag and precisely measure the fraction of proteins that carry this tag in different physiologic conditions. This technology, called quantitative phosphoproteomics, takes a cell, breaks all its proteins apart, then measures the mass of each protein fragment. A computer then sorts out which proteins these fragments are derived from and whether they carry a phosphate tag. “By the predictable change in mass that occurs due to phosphorylation, you can say, ‘Aha, I know that this particular site in this particular protein was phosphorylated,’” says Lifton.

The technique pinpointed two specific positions in the cotransporter where exposure to a low-salt environment rapidly altered the abundance of a phosphate tag. The team then made cells with mutant co-transporters that could not be phosphorylated at these sites and found that they had their doors swung wide open all the time, constantly releasing chloride. “You can watch these cells under a microscope shrivel up and turn into raisins,” Rinehart says.

Additional experiments with human red blood cells and mouse brain showed that phosphorylation of these sites correlated with the transporter’s activity in living cells. For example, in red blood cells that need to rid themselves of chloride, the number of cotransporters missing the phosphate tag rapidly increases. “Because the magnitude of these changes is relatively small, they would be very difficult to detect using conventional methods, but with the new quantitative techniques it was very clear,” says Lifton.

Further, when the researchers inhibited the activity of the WNK1 gene, this reduced the phosphorylation of the cotransporters’ regulatory sites. That means the WNKs are crucial to opening and closing the doors. More work is needed to determine the precise pathway that connects them, Lifton says.

This deeper understanding of how the cotransporters operate may pay dividends for people with sickle cell disease, a common and devastating hereditary blood disorder. A severe attack, known as a sickle cell crisis, triggers pain as sickle-shaped red blood cells block blood vessels and reduce circulation. The red blood cells are believed to kink into their characteristic sickle shape after their cotransporters become active and reduce cell volume.

“A goal in sickle cell research has been to try to figure out how to maintain the hydration of red blood cells so they don’t become sickle cells,” says Pat Gallagher, Professor of Pediatrics at Yale and a co-author of the new study. The new work points to two possible drug development strategies. “One way is to understand what is triggering the dephosphorylation of these sites on the cotransporter and then prevent that. Or, you could augment the phosphorylation of these sites, which would keep the cotransporters closed,” Lifton says.

