Researchers determine how ATP, molecule bearing 'the fuel of life,' is broken down in cells

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Researchers at the Louisiana State University Health Sciences Center have figured out how ATP is broken down in cells, providing for the first time a clear picture of the key reaction that allows cells in all living things to function and flourish.

Discovered some 80 years ago, adenosine triphosphate is said to be second in biological importance only to DNA. Each cell in the human body contains about a billion ATP molecules, and the power derived from the breakdown of them is used to deliver substances to their cellular homes, build needed complex molecules and even make muscles contract.

"ATP is the fuel of life. It's an energy currency molecule - the most important source of chemical and mechanical energy in living systems," explains Sunyoung Kim, the associate professor who oversaw the research published Feb. 19 in the Journal of Biological Chemistry.

Scientists for decades have worked to understand the critically important reaction but, until now, did not know how proteins in a cell extract and use the energy from ATP.

In its original form, an ATP molecule has three phosphate groups. While it has been known for some time that, for ATP breakdown to occur, the third phosphate group must be attacked by a hydroxide, or a water

molecule that has lost one of its protons, it was unknown what actually stripped away that proton, allowing the release of ATP's stores.

The team chose to investigate one particular family of protein machines that break down ATP - the kinesins.

Kinesins are tiny biological machines that work a lot like car engines, Kim says, travelling up and down cellular roadways in support of several functions, such as cellular division and cargo transport.

"We picked kinesins because they're the simplest known motor proteins. Usually, proteins that break down ATP are very large and have a lot of moving parts for mechanical work." Kim says. "The simpler and the smaller the system is, the more likely you can capture information about it in detail."

The team narrowed its study further to the human kinesin Eg5, which is essential for cell division - normal and abnormal - and is touted as an attractive target for next-generation cancer drugs. Inhibition of Eg5 kinesin, by disrupting its ability to break down ATP, may be able to block cancer progression, and a number of Eg5 inhibitors are in clinical trials.

To get a clear picture of how the kinesin and ATP interact, the team set out to use X-ray crystallography to develop a three-dimensional structure that would detail all the bonds and atomic contacts, explains assistant professor David Worthylake, one of the co-authors.

The challenge, though, was trapping the protein in the middle of the energy-releasing chain of events by coaxing it to hold onto a chemical mimic of ATP, in which the final phosphate cannot be removed as usual, and examining the "jammed" protein up close.

According to Courtney Parke, a graduate student and the first author of the team's paper, successfully trapping an ATP mimic is quite difficult. Before her team achieved it, only three other attempts had been successful. Still, all those successes were a bit unsatisfying, she says, because they didn't show how that first step in ATP breakdown occurred.

Further complicating matters, purified kinesin proteins typically are found bound to product of ATP breakdown, adenosine diphosphate, or ADP.

"We said, `You know what? We don't think that you can just insert the mimic of ATP into this purified protein with ADP already bound to it. We think ADP has to be taken out first. That's what the protein does naturally,'" Kim says. "So, instead of forcing the protein out of its normal sequence of steps in breaking down ATP, we pulled out the ADP first and then asked the Eg5 kinesin to bind the ATP mimic. And, lo and behold, we got the answer."

The surprising result was that the protein uses a string of water molecules to harness the energy of the reaction.

"Conventional wisdom pointed toward the reactive agent that starts the ATP breakdown process as being something in the protein, such as an amino acid," notes Edward Wojcik, an assistant professor and another co-author on the paper.

But, it wasn't an amino acid at all: It was a second water molecule that pulled the proton off the first water molecule.

"Each of these water molecules is attached to different part of the protein. And, normally, they hold tightly to each other as well, keeping two very distant parts of the protein connected by a molecular bridge,"

Kim explains. "Our data show, when the second water molecule takes the proton from the first one, the proton is transferred across this bridge. This causes the two different parts of the protein that the bridge holds together to unfurl, and you have motion in the protein."

That internal motion propels the nanomachine along its assigned roadway, allowing it to do its assigned duties.

"For such a relatively simple molecule, water still has some tricks to teach us, and I am still amazed that we found it to play such a pivotal role in the motor protein machinery," Wojcik says.

The team hopes that, with a clearer understanding of how these biological machines work, scientists will better understand how and why things are moved around inside cells, allowing them to figure out how to turn things on and off at will with novel drugs to help combat diseases.

"We believe many, if not all, proteins that use the energy from ATP breakdown may work the same way," Kim says.

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