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Learning How a Cell's Tiny Motor Powers its Mobility

Researchers have for the first time shown how the world's smallest moving machines generate the motion needed to transport their chemical cargo throughout cells. The discovery of how one tiny component of the motor protein kinesin powers its movement represents an important insight into one of the most fundamental aspects of biology.

"All cells are churning with internal motion, which involves transport of materials from one place in the cell to another," said Howard Hughes Medical Institute investigator Ronald Vale of the University of California, San Francisco. "It's like trafficking goods within a city. These goods include chromosomes during cell division, and transport of membranes or proteins within cells.

"An electron micrograph image of a microtubule track (cylindrical tube) with bound kinesin motors. The colored balls are gold particles that are chemically attached to specific locations on the kinesin motors. By following the positions of these gold particles, researchers can determine if a particular region of the kinesin protein changes its shape or location as the motor moves along its track."

"The kinesin motors responsible for this transport are the world's smallest moving machines, even the smallest in the protein world," he said. "So, besides their biological significance, it's exciting to understand how these very compact machines many orders of magnitude smaller than anything humans have produced have evolved that ability to generate motion."

Basically, the kinesin protein links with another kinesin to form a two-molecule ferry that moves cellular cargo along tram tracks composed of infinitesimal filaments called microtubules that criss-cross the cell's interior.

In the December 16, 1999, issue of the journal *Nature*, Vale and his colleagues describe how they analyzed the motion of individual kinesin molecules, ultimately pinpointing the portion of the kinesin protein responsible for generating movement.

The researchers' analyses showed that a tiny piece of the kinesin protein dubbed the "neck linker" abruptly stiffens like Velcro ' zipping up when the energy molecule ATP attaches to kinesin. This stiffening throws the neck linker forward and provides the mechanical force that puts the kinesin molecule in motion along the microtubule tracks. The discovery that motion is generated by the neck linker, which is composed of only 15 amino acids, also helped the scientists understand how two linked kinesin molecules coordinate their movement along the microtubule, said Vale.

"The kinesin motor walks along the microtubule much like a person walks along steppingstones across a pond," said Vale. "Just as a person has to step from stone to stone, there are only certain points where kinesin molecules can attach to a microtubule. Basically, the neck linker zippers up and throws its rearward partner forward to the next attachment site, like swinging the rear leg forward to the next steppingstone."

According to Vale, the linked kinesins take step after step along the microtubule by coordinating the cycling of ATP molecules, first onto one kinesin, then onto its partner with the ATPs alternately attaching, releasing their energy, and detaching as spent products.

Vale and his colleagues used several analytical techniques, each of which uncovered a different aspect of kinesin's motion mechanism. To begin their experiments, the scientists created kinesin molecules that included specific attachment points for various marker molecules that would help reveal how the neck linker moves. To obtain "snapshots" of the marker-carrying molecules at specific stages, the scientists treated the kinesins with altered versions of ATP, called analogues, that "froze" the kinesins at various stages of activity.

For example, in one experiment, the scientists attached a gold particle to the neck linker and used electron microscopy (performed by Ron Milligan at Scripps Research Institute) to obtain images of the kinesin at different stages. Those images revealed that in the absence of ATP analogues, the linker neck could pivot either forward or backward, but the binding of an ATP analogue locked the piece of protein in the forward position. After the kinesin released the ATP analogue, however, the neck linker again became mobile.

Another critical experiment using mutant kinesin molecules showed that neck linker motion was necessary for kinesin movement along the microtubule. "We studied two mutants that are both stuck at the ATP-binding step," said Vale. "However, one of these mutants can take a single step along the microtubule, and the other one can't. We predicted that if the neck linker motion was actually necessary for kinesin to take a step, then we should see such motion in the mutant that can take a step, but not in the one that can't. And that's what we saw very clearly."

According to Vale, basic understanding of how kinesin motors work could lead to medical therapies that either inhibit or stimulate kinesin activity.

"Humans have perhaps 50 different kinds of these kinesin motors, and if we understand how they work we might be able to selectively inhibit those involved in chromosome segregation in mitosis," said Vale. "Since cancer cells are constantly dividing, such inhibitors might have application as cancer chemotherapeutic agents.

"Also, there is some indication that certain neurodegenerative diseases might result from kinesin-related deficiencies in transport. In such cases, a therapy that stimulates the transport system might be effective in treatment."