The Ministry of Silly Walks in each of your cells

November 5 2015

The chemical Ministry of Silly Walks. Kinesin walks along microtubules because its free 'leg' moves erratically as a result of interaction with the environment and sometimes it comes across a 'pavement slab' to which it can cling. The kinesin walk being generously presented by Dr. Krzysztof Soza?ski from the Institute of Physical Chemistry of the Polish Academy of Sciences in Warsaw, Poland. Credit: IPC PAS, Grzegorz Krzyzewski

Inside mammalian cells, kinesin plays the same role as do trucks and locomotives within our countries: It is the main driving force behind the transport of manufactured goods. No wheels are involved, but there are 'legs' - two moving heads, which are used to walk on the fibres of the cytoskeleton. Recent studies have revealed the mechanism of this 'walk,' and it turns out that kinesin is... funny.

In a famous Monty Python sketch, John Cleese plays a bureaucrat performing bizarre steps in the Ministry of Silly Walks. But odd steps appear to be quite common in nature, and inside every one our cells. Research on intracellular transport, carried out by the Institute of Physical Chemistry of the Polish Academy of Sciences (IPC PAS) in Warsaw, Poland, in cooperation with the Dresden University of Technology, explains the movement of kinesin, the protein responsible for the transport of large molecules inside the cells of mammals. Quite unexpectedly, an additional conclusion has arisen from the research: In the Ministry of Silly Walks, kinesin would certainly be in the running for a ministerial post.

Intracellular transport takes place along the fibres of the cytoskeleton, a structure developed by eukaryotic cells (possessing a cell nucleus). The fibres forming the network - microtubules - are made of polymers of the protein tubulin twisted spirally into long tubes. Since each 'brick' of the polymer, i.e., each monomer, consists of a pair of alpha-tubulin and beta-tubulin. Along the microtubule, the alpha and beta domains are arranged alternately, like the black-and-white squares along the length of a chessboard if it were rolled up into a tube.

The microtubules are the roads along which the intracellular tractors, the kinesin molecules, move. One part of kinesin is equipped with fragments that bind to molecules in order to transport them, while the driving part

consists of a flexible connector, the so-called linker, fastening together the two 'legs', i.e. the movable domains that step along the 'chess fields' of the microtubules. In addition, the legs are so large that kinesin can only step on every second monomer (that is, fields of the same colour).

"Kinesin walks along the microtubules. But how? In order to understand the problem, one needs only to realize that kinesin does not wander along the microtubules like a man does along the pavement. Its movements are more reminiscent of what a mountain climber does when scaling a vertical wall without any safeguards: one mistake, and he falls off," says Prof. Robert Holyst (IPC PAS). "How does kinesin know it can free one leg without risking detachment from the microtubules? It isn't an animal equipped with eyes and a brain, it's just a simple molecule. Where does it get the energy to take the step?"

So far in scientific publications, there have been several descriptions of the mechanism responsible for the movement of kinesin, but none has been clearly confirmed experimentally. Although experiments have shown the theoretically predicted slowing of the movement of kinesin, this has only been when it was transporting very large cargo—and only when the viscosity of its surroundings has been increased, using long polymers, to thousands of times greater than the viscosity of water.

"What has been tested so far can be illustratively compared to investigating how the speed of a juggernaut loaded with cargo decreases with the velocity of the wind into which it is travelling. We wanted to do something else. The driving mechanism itself was what interested us. So we removed the cargo... and poured sand into the engine," says Dr. Krzysztof Sozański (IPC PAS).

According to the theory, which the researchers have developed over several years, the viscosity experienced by particles depends partly on the size of the obstacles in their environment. The situation is similar to

that of a crowded bus: It is difficult for people to squeeze through to the exit, but in the same environment, an insect flies about quite freely. The obstacles (people) are, in fact, too large to cause it problems; the bug simply bypasses them. So it was clear that since the polymers used by other researchers to slow down kinesin were of a considerable size, they increased the viscosity felt not by kinesin itself, but by its large cargo.

Experiments, in which small molecules that could collide directly with kinesin's legs were used to increase the viscosity of its environment, were carried out in the laboratories of Prof. Stefan Diez in the B CUBE Centre for Molecular Bioengineering at the Dresden University of Technology. Without its cargo, kinesin slowed down, even at an ambient viscosity that was five times the viscosity of water. With this method of controlling the movement of kinesin, researchers from the IPC PAS performed successive experiments that provided data proving one of the earlier proposals for the mechanism of movement of kinesin.

The cycle of movement begins when one leg of kinesin is attached to the microtubule and the other, with an attached ADP (a product of the hydrolysis of ATP molecules) remains free. In this configuration, the freed leg, interacting with the environment, performs random movements. However, its range is insufficient to reach the next domain on the microtubule and the kinesin remains in its place. Everything changes when an ATP molecule joins the leg that is attached. Kinesin becomes more flexible and the movements of the free leg have increased range. Tossing and turning in all directions, the leg pulls kinesin until it reaches the next domain on the microtubule. Then it binds to the substrate, releasing ADP, after which it becomes still until there is hydrolysis of ATP on the leg behind it. ATP is converted into ADP, releasing energy, detaching the leg—and the cycle loops around again.

"Thus ATP, the major energy source in cells, is not the energy source in the movement of kinesin," says Dr. Sozański. "The hydrolysis of ATP

only releases the leg. The leg moves around chaotically, as a result of accidental interactions with the environment, until it hits the next chess square on the microtubule. In fact, it is the environment that is driving the steps of kinesin."

The time of diffusible movement of the kinesin leg is approximately two milliseconds, while the time of connection-disconnection of ATP is approx. 10 milliseconds. By skillfully increasing the viscosity, researchers from the IPC PAS extended the former to approx. 10 milliseconds, effectively destroying the synchronization between the two processes. Kinesin froze.

Research on the movement of kinesin is not only of great significance for biologists, but also for engineers and chemists dealing with molecular motors. Kinesin is, in fact, structurally very similar to myosin, a protein in which, thanks to the energy released by ATP, there is a change in the structure of the molecule and power is generated (this is the mechanism that is responsible for the contraction of our muscles). Meanwhile, kinesin movement is of a completely different nature—its source is the phenomenon of diffusion of the legs.

"Such different sources of movement in such similar molecules should encourage caution in designers of molecular motors. Encourage caution—but also inspire," concludes Prof. Holyst.

More information: "Small Crowders Slow Down Kinesin-1 Stepping by Hindering Motor Domain Diffusion"; K. Sozański, F. Ruhnow, A. Wiśniewska, M. Tabaka, S. Diez, R. Holyst; *Physical Review Letters* (in press), 2015.

Provided by Polish Academy of Sciences