Fast-acting enzymes with two fingers: Protein structurally and dynamically explained

December 19 2012

Researchers at the RUB and from the MPI Dortmund have uncovered the mechanism that switches off the cell transport regulating proteins. They were able to resolve in detail how the central switch protein Rab is down-regulated with two "protein fingers" by its interaction partners. The structural and dynamic data is reported by the researchers led by Prof. Dr. Klaus Gerwert (Chair of Biophysics, RUB) and Prof. Dr. Roger S. Goody (Max Planck Institute for Molecular Physiology, Dortmund, Germany) in the Online Early Edition of the journal *PNAS*.

"Unlike in the cell growth protein Ras, which is regulated with only one 'finger', we have surprisingly found a two-finger switch-off mechanism in Rab. This throws a completely new light on the functioning of certain enzymes, the small GTPases, to which Rab belongs", Klaus Gerwert explains.

Switch proteins associated with various diseases

Unlike Ras proteins that regulate cell growth, Rab GTPases (also called Rab proteins) control various transport operations between different areas of a cell. If the transport system is disrupted, diseases such as obesity can occur. The Rab proteins work as a switch, just like the Ras proteins. In the "on" state, the high-energy molecule GTP is bound, in the "off" state, the lower-energy GDP. The cleavage of GTP to GDP is catalysed by the so-termed RabGAP proteins. In so doing, GTP is split

into GDP and phosphate. The research team observed the underlying reaction in time and space for the first time with the highest possible atomic resolution.

First a snapshot, then a whole film

Using X-ray structure analysis, the researchers first determined the spatial structure of the protein complex. The data showed a finger of the amino acid arginine, and a second finger of glutamine. The arginine finger was already known from Ras. The glutamine finger is new and surprising. RabGAP penetrates into the GTP-binding pocket of Rab with both fingers and accelerates the GTP cleavage over five orders of magnitude. The biophysicists observed this dynamic process in real time using FTIR spectroscopy. "In contrast to X-ray structure analysis, FTIR spectroscopy not only gives us a snapshot of the reaction, but an entire film", says PD Dr. Carsten Kötting. The result: both catalytic fingers penetrate simultaneously into the GTP-binding pocket and leave it with the phosphate cleaved from the GTP.

Medically interesting mechanism

In their experiment, the researchers examined the protein Rab1b and the RabGAP TBC1D20. Other Rab proteins and RabGAPs are similar to these two representatives. "Thus, we assume that they also interact via a two-finger mechanism", Konstantin Gavriljuk speculates. The ability of the two-finger system to also switch off mutated Rab proteins, i.e. mutated GTPases, could also be medically very interesting. It would be conceivable to develop small molecules that mimic the two-finger mechanism, and thus switch off other mutant GTPases, such as Ras, which emit uncontrolled growth signals and thus are involved in tumour formation.

More information: K. Gavriljuk, E.-M. Gazdag, A. Itzen, C. Kötting, R.S. Goody, K. Gerwert (2012): Catalytic mechanism of a mammalian Rab•RabGAP complex in atomic detail, *PNAS*, DOI: 10.1073/pnas.1214431110