# Researchers clarify catalysis mechanism of cell growth protein Ras

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Proteins accelerate certain chemical reactions in cells by several orders of magnitude. The molecular mechanism by which the Ras protein accelerates the cleavage of the molecule GTP and thus slows cell growth is described by biophysicists at the Ruhr-Universität Bochum led by Prof. Dr. Klaus Gerwert in the Online Early Edition of the journal *PNAS*.

Using a combination of infrared spectroscopy and computer simulations, they showed that Ras puts a phosphate chain under tension to such an extent that a phosphate group can very easily detach - the brake for cell growth. Mutated Ras is involved in tumour formation, because this reaction slows down and the brake for cell growth fails. "Our findings could help to develop small molecules that restore the Ras proteins to the right speed", says Prof. Gerwert. "Such molecules would then be interesting for molecular cancer therapy."

The Ras protein switches the cell growth off by detaching a phosphate group from the small bound guanosine triphosphate, GTP for short. GTP has three interlinked phosphate groups. If it is present in water, the third phosphate group can split off spontaneously - even without the help of the protein Ras. This process is very slow though. Ras accelerates the splitting by a magnitude of five, a second protein, called GAP, by a further magnitude of five. What causes this acceleration has now been found out by the Bochum team.

Ras brings the chain of three phosphate groups at the GTP into a certain

shape. It turns the third and second phosphate group to each other so that the chain is tensioned. "Like winding up a spring in a toy car by turning a screw", explains Prof. Gerwert. "Ras is the screw, the phosphate groups form the spring." The protein GAP tensions the spring further by also turning the first phosphate group against the second. In this way, the GTP gets into such a high-energy state that the third phosphate group can easily detach from the chain - like when the toy car drives off spontaneously after winding up the spring.

The results were obtained by the Bochum researchers using the time-resolved fourier transform infrared spectroscopy (FTIR) developed at the Institute of Biophysics. With this technique, the scientists track reactions and interactions of proteins with high spatial and temporal resolution; much more precisely than using a microscope. "However, the spectroscopy does not deliver such nice pictures as a microscope, but only very complex infrared spectra", explains PD Dr. Carsten Kötting. "Like a secret code that has to be deciphered."

To this end, Till Rudack simulated the protein responses on modern computing clusters and calculated the corresponding infrared spectra. Due to the enormous computational effort, large molecules such as a complete protein cannot currently be reliably described using these so-called quantum mechanical simulations. Therefore, the researchers limited their analysis to GTP and the part of the Ras or GAP protein that interacts directly with GTP. They described the rest of the proteins with a less elaborate molecular dynamics simulation. "When bringing together all the different simulations, it is easy to be led astray", says Till Rudack. "Therefore you have to check the quality of the results by comparing the simulated with the measured infrared spectra." If the spectra obtained with both techniques match, the structure of proteins can be determined to an accuracy of a millionth of a micrometre. This was the case in the Bochum study.

Molecular cancer therapy is already used successfully with diseases such as chronic myeloid leukaemia (CLM) in the form of the drug Gleevec. Molecules with a similar effect against the mutated Ras protein have not yet been found. "Since we are now able to investigate the reactions of the Ras protein with significantly better resolution, new hope is forming that it will be possible to defuse the mutated molecule using drugs such as Gleevec and restore the rhythm of the cell" says Gerwert.

**More information:** T. Rudack, F. Xia, J. Schlitter, C. Kötting, K. Gerwert (2012): Ras and GTPase-activating protein (GAP) drive GTP into a precatalytic state as revealed by combining FTIR and biomolecular simulations, *PNAS*, doi: 10.1073/pnas.1204333109

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