One signal elicits thousands of answers

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Cell signaling mechanisms often transmit information via protein modifications, most importantly the reversible attachment of phosphate, the so-called protein phosphorylation. Researchers at the Max Planck Institute of Biochemistry in Martinsried have now developed a technology to identify and quantify the specific sites in proteins that get phosphorylated in answer to certain stimuli in living cells.

Under the lead of Matthias Mann, the scientists found 6,600 phosphorylation sites - 90 percent of which were unknown - in 2,244 proteins and observed their temporal dynamics. All these phosphorylation sites are now listed in the newly created Phosida database to make them available for efficient use by scientists working in different areas, among them tumour researchers: Defects in cellular signaling often occur in many types of cancer (*Cell*, November 2, 2006).

The mammalian cell constantly receives signals from its surroundings to which it has to respond appropriately. Growth factors, for example, can lead to growth of a cell, its differentiation or proliferation. Defects in these tightly regulated and controlled processes can cause cancer and other human diseases. In recent decades, knowledge of the important players in signal transduction has been painstakingly accumulated, mainly through the study of individual molecules in specific pathways. This approach may fall short though, because the cellular answer to environmental stimuli often doesn't show on the level of production but the modification of proteins after their synthesis. "Phosphorylation is the most important and most thoroughly researched modification," says Mann. "An estimated one-third of all cellular proteins are affected.

Therefore, the dynamic phosphoproteome provides a missing link in a global, integrative view of cellular regulation."

Mann and his team improved and extended a previously developed technology, which enabled them to identify for the first time all phosphorylations of all proteins in living cells - and in their temporal dynamic. For this approach cell cultures were stimulated by EGF for different lengths of time. The "Epidermal Growth Factor" is known for causing the phosphorylation of a multitude of enzymes and proteins along a signal transduction pathway. In the following step all proteins were isolated from the cells, divided into different fractions and analysed via mass spectrometry. This technology allows the precise identification of structure and composition of unknown compounds, here the cellular proteins. In total, 6,600 specific phosphorylation sites in 2,244 proteins were detected. "Comparing our results with the listings in existing databases showed that more than 90 percent of our sites were novel. This suggests that the majority of cellular phosphorylation sites still await identification."

Equally surprising was the discovery that about half of all cellular proteins harbour more than one phosphorylation sites, which in many cases behave differently. "This makes more than one way of phosphorylation possible where proteins serve as integrating platforms for a variety of incoming stimuli", says Mann. "This integration of signals could be independent, with phosphorylation of each site occurring separately from the others. It could also be dependent so that a 'priming site' has to be phosphorylated first for the subsequent modification of all other sites in the protein. In any case, the degree of phosphorylation should always be measured site specifically rather than for the protein as a whole". For the efficient use of their results the research team created the Phosida database (www.phosida.com), where all the phosphorylation sites are listed with additional information and connections to respective listings in other databases. An interesting

service for scientists with widely varying expertise, not the least for tumour researchers because they have to investigate defects in cellular signaling which often occurs in progressed forms of cancer. The new technology will allow the search for new data - which might not be necessary too soon. "Our study revealed more phosphorylation sites than all previous studies combined," says Mann.

Citation: Jesper V. Olsen, Blagoy Blagoev, Florian Gnad, Boris Macek, Chanchal Kumar, Peter Mortensen, and Matthias Mann, Global, In Vivo, and Site-Specific Phosphorylation Dynamics in Signaling Networks, *Cell*, November 2, 2006

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