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Meeting abstract

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Combination of SILAC and *in situ* biotinylation to detect specific protein interacions

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Stable isotope labeling with amino acids in cell culture (SILAC) has emerged as a major technique for quantitative proteomics using cell culture. It has been applied to the investigation of many different biological processes such diverse as the characterization of signaling pathways and the determination of protein interactions.

For investigation of proteins, interacting with the Signal Transducer and activator of transcription 3 (STAT3), we combined SILAC with stringent precipitation of the biotinylated proteins and quantitative mass spectrometry analyses, to detect cellular interaction partners of STAT3 in mammalian cells.

STAT3 is a well-known protein, which plays crucial roles in different biological responses including early embryonic development as well as cell growth and apoptosis. Moreover, STAT3 is constitutively activated in oncogene-transformed cells and various primary tumors.

Although the JAK-STAT signaling pathway is one of the best known, there appears to be STAT3-specific regulatory proteins, which are not established until now. Hence, finding of novel STAT3-interacting proteins can encourage the knowledge of STAT3-protein network.

For metabolic labeling using SILAC, cells were grown in medium containing either only 'light' arginine or only 'heavy' 13C6-arginine, resulting in incorporation of labeled arginine in all sites. In mass spectrometry analyses the peptides containing the 13C6-arginine, has produced a 6 Da difference in mass, relative to the light form.

STAT3 fused with a tag for its biotinylation was expressed and biotinylated in HEK-293 cells, which were fully substituted with 13C-labeled arginine in place of the light arginine. In the parallel approach HEK cells, grown in normal medium, were transfected with STAT3 without the tag for biotinylation. After combining the cultured cells, STAT3 and its interacting proteins were specific precipitated, separated by SDS/PAGE, and analyzed by mass spectrometry. This method bears a strong potential to recover now protein interactions.