SMP: Protein

Project: Isolation and Functional Characterisation of Protein Complexes

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Introduction

Transcriptional regulation in higher eukaryotic cells is required for all aspects of cellular proliferation and cell growth. As a step towards understanding these processes at a molecular level, this project aims at elucidating the molecular make-up of the transcriptional and signal transduction machinery, both under normal physiological conditions as well as in states of disease or stress. This will be achieved by systematic isolation and mass spectrometric analysis of signalling factors and complexes from human cell cultures. The obtained results will be important for building a comprehensive interaction map of native transcription- and signal transduction-factors. Hence, this project aims to provide insight into some of the major pathways that regulate cell proliferation, growth and stress signalling in higher eukaryotic cells (Fig. 1). Moreover, this work will complement interaction data from high-throughput yeast-2-hybrid screening and will expand our knowledge of signalling events occurring in both healthy and disease tissue. This is an essential prerequisite for the development of diagnostic tools and ultimately for the treatment of complex genetic diseases such as cancer.

Key Question

Consequently, this project addresses the question: How do molecular interactions regulate or mediate biological processes and how do changes in interactions lead to diseases?

Approach

We have established an efficient platform to isolate cell organelles and large protein complexes using magnetic bead-based affinity purification protocols, which offers the opportunity to isolate microgram quantities of native protein complexes (1). Using this technology in parallel with tandem-affinity purification (TAP) (2), protein complexes will be isolated and then characterised by mass spectrometry. Characterising isolated native protein complexes has to date typically relied on SDS-PAGE to detect protein bands that are unique in comparison to one or several control experiments. This approach is, however, limited to the study of rather strong interactions, for which protein ligands can be distinguished from non-biospecific binders. To overcome this limitation, we have developed a new technique based on enzyme-catalysed differential stable isotope incorporation of pull-down protein mixtures followed by nano-LC MALDI mass spectrometry analysis (3). A second objective of this project is to analyse a subset of the isolated protein complexes by cryo-electron microscopy in order to determine their three-dimensional structure. The data obtained by mass spectrometry and cryo-EM will be highly complementary, with the former establishing the composition of a complex, and the latter the structure and interactions between the components.

Results

We applied large-quantity-cell extracts (5 x 10^9 cells) for TAP and immunoprecipitation experiments for the isolation of native protein complexes from human cells. So far, over 30 bait proteins were expressed and are now in the immediate pipeline targeted for protein-protein interaction determination. In addition, an immuno-affinity purification method for endogenous protein complex was improved for subsequent MS analysis and was applied to isolate key regulatory signalling molecules from exponentially growing as well as from synchronised mitotic cell cultures. Protein complex samples are analysed by MALDI TOF MS or enzyme-catalysed differential stable isotope incorporation of pull-down protein mixtures followed by nano-LC MALDI mass spectrometry analysis. The suitability of the isolated complexes for cryo-electromicroscopic characterisation is systematically determined by electron microscope negative staining.

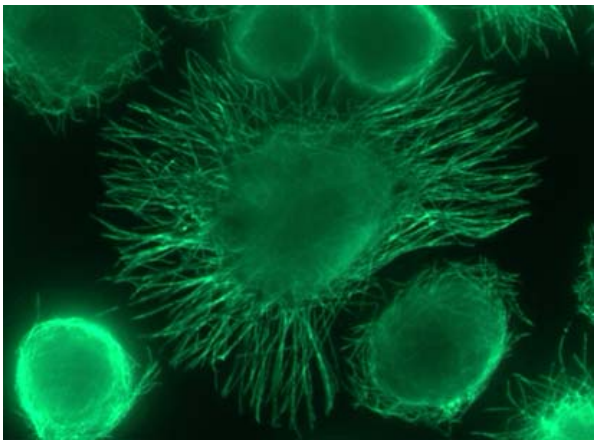

Fig 1: *Immunofluorescence microscopy image of tissue culture cells immunolabelled for microtubule filaments that are important for maintaining cell shape and mediating cell division. One aim of this project is to identify and characterise protein complexes that interlink microtubule organisation and function to signalling and cell cycle regulatory pathways (4). (Image taken by Hannah Müller)*

Outlook

In collaboration with the other partners of the SMP protein consortium we are currently undertaking a characterisation of some of the central signalling pathways in the cell that are relevant for human diseases. Hence, the application of high-throughput techniques (5) and bioinformatics modelling will allow us to uncover protein-protein interactions, functional and structural relationships that operate in the molecular networks of the human body.

Lit.: ***1.*** *Lange et al. Hsp90 is a core centrosomal component and is required at different stages of the centrosome cycle in Drosophila and vertebrates. EMBO Journal. 2000 19(6), 1252-1262.* ***2.*** *Rigaut et al., A generic protein purification method for protein complex characterization and proteome exploration. Nat. Biotechnol., 1999 17, 1030-1032* ***3.*** *Gustavsson N, Greber B, Kreitler T, Himmelbauer H, Lehrach H, Gobom J. A proteomic method for the analysis of changes in protein concentrations in response to systemic perturbations using metabolic incorporation of stable isotopes and mass spectrometry. Proteomics. 2005 Aug 1;* ***4.*** *Lange B.M.H. Integration of the centrosome in cell cycle control, stress response and signal transduction pathways. Current Opinion in Cell Biology, 2002 14, 35-43* ***5.*** *Sauer, S. et al . Miniaturization in functional genomics and proteomics. Nature Genetics Review. 2005, 6(6):465-476.*

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