Imaging reaction dynamics for cellular systems biology

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Our main objective is to elucidate how signaling networks process extracellular information thereby determining cellular phenotype. The cellular response to extracellular signals consists of the induction of specific gene expression patterns and the re-organization in space and time of stereo-specific macromolecular interactions that endow the cell with its specific morphology (cytoskeleton, membranes). We therefore develop quantitative experimental and theoretical approaches to derive and conceptualize physical principles that underlie the dynamics of signalling and cellular organization. We have an experimental emphasis on functional microscopic imaging approaches at multiple resolutions to study the localization of protein reactions/interactions, maintaining the inherent spatial organization of the cell. At the first level we investigate the cellular topography (spatial distribution) of activities that transmit signals from receptors at the cell surface. Here we ask, how spatial partitioning of intracellular signalling activities is achieved, and how this partitioning affects signal strength and duration of growth factor signals (1). At the second level, we investigate if specific reaction network logical topologies (patterns of connections) occur in signalling and how these give rise to specific responses to input signals (2). This entails the experimental elucidation of connections between reactions and the determination of enzyme kinetic parameters in living cells. For this, we use a reverse engineering approach in which network logical topology is derived by sampling reaction states of proteins in response to protein activity perturbations. Reaction network models are then populated with kinetic parameters obtained by quantitative imaging of enzyme kinetics *in cells* using new chemical biological approaches (3). The questions we are addressing with this strategy are whether there is a modular build-up of reaction networks as characterized by chemical connectivity, and if recurrent network topologies occur that give rise to specific dynamic responses. Here, we implement a strong recursion between modelisation of network dynamics and experiments that reveal dynamic properties of networks in living cells.

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A Systems View of the Cell Cycle by Parallel Quantitation of Proteome and Phosphoproteome

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Introduction

Eukaryotic cells replicate themselves by a controlled series of events known as the division cycle. Progression through the cell cycle is tightly regulated at defined stages by the assembly of specific protein complexes. This is regulated by changing expression via mRNA, protein degradation or protein activation through site-specific phosphorylation. We have quantified the proteome and phosphoproteome changes in synchronized HeLa cells from six different time points across the cell cycle and compared this to publically available microarray data [1] in an integrated systems biology approach.

Methods

SILAC labelled HeLa S3 cells were pre-synchronized by thymidine, released and collected at four different time points. Two sets of cells were further arrested by nocodazole, released and collected after 0.5 and 3 hrs. After lysis, cell populations were combined 1:1:1 using an asynchronized cell population as internal standard. The mixed lysates were digested with Lys-C and trypsin in-solution, fractionated by SCX and phosphopeptides were enriched using TiO_2, or separated by SDS-PAGE, excised and in-gel digested by trypsin. All LC-MS/MS experiments were performed on an LTQ-Orbitrap using real-time lock-mass calibration and multi-stage activation. Peptide MS/MS spectra were identified by Mascot, and automatically quantified and threshold-filtered for a final FDR<0.01 by using MaxQuant [2].

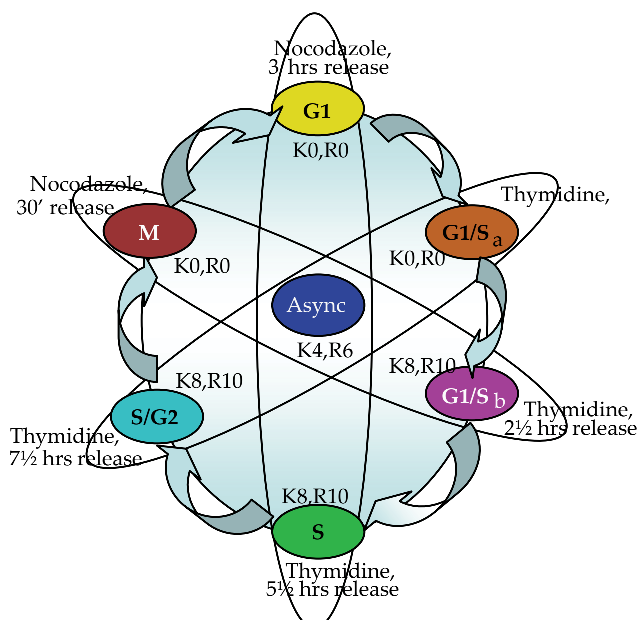

Figure Legend

Quantitative proteomics of the Cell cycle. HeLa S3 cells were SILAC labelled with three different isotopic forms of arginine and lysine, arrested in different cell cycle stages, lyzed and mixed 1:1:1 using an asynchronously growing cell population as the internal standard for quantification normalization between experiments.

Results

We have developed a technology that combines SILAC for quantitation with phosphopeptide enrichment strategies and high-performance mass spectrometry [3] and utilized this to determine the dynamic profiles for more than six thousand proteins and fifteen thousand unique phosphosites over the cell cycle at a very low false-discovery rate Surprisingly, the majority of the detected phosphosites were regulated, mainly in mitosis. Likewise, half of the proteins were regulated over the cell cycle, whereas only 10% of the mRNA is significantly changing. Bioinformatics analysis by gene ontology reveals that proteins for which both mRNA and protein abundance levels change are highly enriched in known cell cycle players, whereas proteins with non-changing mRNA and protein levels are mainly involved in metabolism and biosynthesis functions. Remarkably, proteins that change in abundance but not at the mRNA level have important functions in the transcriptional machinery. Our quantitative proteomics strategy provides a powerful tool for global studies of in vivo proteome and phosphoproteome dynamics on a systems biology level to an unprecedented depth.

Innovative aspects

First global proteome and phosphoproteome quantified over the cell cycle by combining SILAC, high-performance mass spectrometry and software developments.

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Exploring Different Enzymes to Allow Facile Peptide Sequencing by ETD

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Introduction

Trypsin and collision activated dissociated (CAD) is the method of choice for protein identification and characterization. However, in many cases, it does not provide easily interpretable spectra and it cannot allow elucidation of full protein sequences on its own. In this work we evaluate multiple enzymes for the emerging peptide sequencing technique electron transfer dissociation (ETD) (1) and compare it to CAD. We evaluate a number of parameters for successful protein coverage and peptide sequencing.

Methods

In-solution digests of cell lysates, purified protein complexes and standard proteins were performed using a number of common and not so common enzymes. The resulting proteolytic peptides were analyzed using nanoLC MS/MS coupled to either an LTQ-FT with CAD or an LTQ ion trap which can perform CAD, ETD and collision activated ETD (CA-ETD or ETcaD).

Results

A bioinformatics pipeline was established to allow correlation of all experiments. The analysis also allowed the establishment of key criteria for successful ETcaD/ETD sequencing such as peptide composition and length. All results were compared to equivalent experiments performed using CAD.

It was found that a certain enzyme produces peptides with specific criteria allowing production of clear and simple spectra which are easily interpretable; ideal for *de novo* sequencing applications.

When using the Pol II and III complexes as a model protein mixture (2) near complete coverage of all proteins present could be achieved. Additionally, we were able to map several new and earlier established N-acetylation and phosphorylation sites. Our experiments also revealed which combination of proteases and MS/MS activation methods can be best used to obtain full proteomic coverage of known and unknown proteins.

Innovative aspects

- Establishment of criteria for successful ETD peptide sequencing.
- Deduction of optimal combination tandem mass spectrometric techniques and enzymes for maximal protein coverage.

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New Targets of Snake Venom Metalloproteinases Revealed by Proteomic Approaches

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Introduction

Proteomics has become an indispensable tool to the discovery of novel target substrates for snake venom metalloproteinases (SVMPs) involved with local hemorrhage (1). The SVMPs have been shown to participate in this process by proteolytically degrading extracellular matrix (2, 3) and by inhibiting platelet aggregation. Over the past several years our laboratories have reported the *in vitro* effects of SVMPs on tissues and cultured cells. Recently we have focused on the application of proteomic approaches to assess the effects of SVMPs at the level of a tissue in a living organism.

Methods

HF3, a P-III class SVMP, is the most potent hemorrhagic toxin from *Bothrops jararaca* venom. The target proteins for proteolysis by HF3 *in vivo* were evaluated by 2D electrophoresis (2-DE), in gel trypsin digestion and LC/MS/MS analysis of differential spots. Mice were injected with HF3 and after 2h the dorsal skin was sectioned and the proteins were evaluated by 2-DE. HF3 was also injected in the thigh muscle of mice and after 4h plasma proteins were analyzed by 2-DE.

Results

As expected, some of the results observed corroborate previous data generated using *in vitro* assays, however, new extracellular, intracellular and plasma proteins have been found to be cleaved *in vivo* by the directly and/or indirectly effect of HF3. On the other hand, some plasma proteinase inhibitors showed higher abundance compared to control. Some proteins affected *in vivo* by HF3 were confirmed to be directly cleaved *in vitro* by HF3. Moreover, gelatin-zymography of skin proteins injected with HF3 showed *in vivo* activation of pro-metalloproteinases by HF3. In summary, these findings further extend our understanding of the effect of SVMPs at the level of the tissue/organism and underscore new mechanisms by which the SVMPs participate in the development of the hemorrhage typically observed with viperid envenoming.

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