

## Identification and characterization of the *C. elegans* proteome

Michael Hengartner

Center for Model Organism Proteomes and Institute of Molecular Biology, University of Zurich,  
Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. michael.hengartner@molbio.uzh.ch

The nematode *Caenorhabditis elegans* (*C. elegans*) is a popular model system in biomedical research. To generate a comprehensive inventory of its proteome, we used shotgun proteomic approaches to search for proteins expressed during the various stages of *C. elegans* development. To date, we identified almost 11'000 different proteins, corresponding to 54% of the annotated *C. elegans* gene models. I will describe the generation of this proteome dataset as well as its many uses, including improved genome annotation, comparative proteomics between species, and targeted quantitative proteomics.

# System-wide Analysis of Phosphatase Knock-down in *Drosophila* by Quantitative Proteomics

Maximiliane Hilger<sup>1</sup>, Tiziana Bonaldi<sup>2</sup>, Florian Gnad<sup>1</sup>, Chanchal Kumar<sup>1</sup>, Jürgen Cox<sup>1</sup>, Matthias Mann<sup>1</sup>

<sup>1</sup>Department of Proteomics and Signal Transduction, Max-Planck Institute for Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

<sup>2</sup>Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16, 20139 Milan, Italy

## Introduction

Protein phosphorylation is a dynamic post-translational modification and the result of a coordinated action of kinases and phosphatases. The elucidation of kinase and especially phosphatase substrates is crucial to fully understand their functions. In this study we show proof of principle that by combination of RNAi with SILAC-based quantitative proteomics in *Drosophila* we can identify novel potential substrates of a protein tyrosine phosphatase in a site-specific manner as well as detect global proteome changes induced by its knock-down.

## Methods

Semi-confluent *Drosophila* SL2 cells (Schneider cells) were transferred into SILAC medium without FCS and bathed for one hour in presence of dsRNA targeting PTP61F ("heavy" SILAC media) or mock dsRNA ("light" SILAC media) respectively. After dilution in FCS containing heavy or light SILAC media the cells were grown for 8 days, mixed 1:1 and lysed. Phosphoproteome (1) and proteome-specific digestion and separation steps followed. The resulting peptide fractions were analyzed by nano-LC-MS/MS on an LTQ-Orbitrap.

## Results

We chose the *Drosophila* model system for functional studies because it has less genomic complexity and less functional redundancy and because RNA interference (RNAi) is very efficient in this system. To characterize the homolog of the prototypic human tyrosine phosphatase PTP1B (called PTP61F in *Drosophila*) both the phosphoproteome and the proteome were measured in depth by high accuracy MS. We identified 6751 unambiguously identified phosphorylation sites on 1997 proteins. Twenty tyrosine phosphorylation sites on 16 proteins show a significant upregulation upon PTP61F RNAi. Proteins with upregulated tyrosine phosphorylation sites are enriched for certain molecular functions: GTPase regulator activity, protein tyrosine activity, structural component of cytoskeleton and ATP binding. Interestingly, two in vivo upregulated tyrosine phosphorylation sites on the kinase Abl were detected, both in the protein kinase domain and the C-terminus. PTP61F may have a general role in regulating actin cytoskeleton organization, perhaps acting together with Abl as already shown by for the PTP61F substrate Abi (2).

The most upregulated tyrosine phosphorylation site was detected on the transcription factor STAT92E, the sole *Drosophila* STAT, which is a substrate of PTP61F (3). This demonstrates the power and robustness of our global phosphorylation analysis method.

## Innovative aspects

- Quantitation of global phosphorylation and proteome changes upon RNAi of a single phosphatase
- In-vivo, site-specific analysis of potential phosphatase substrates

## References

- (1) J.V. Olsen et al., Global, In Vivo, and Site-Specific Phosphorylation Dynamics in Signaling Networks; *Cell* 127, 635-648 (2006).
- (2) C-H. Huang et al., The Involvement of Abl and PTP61F in the Regulation of Abi Protein Localization and Stability and Lamella Formation in *Drosophila* S2 Cells. *J. Biol. Chem.* 282, 32442-32452 (2007).
- (3) P. Mueller et al., Identification of JAK/STAT signalling components by genome-wide RNA interference; *Nature* 436, 871-875 (2005).

## STAT92E RQDPVTGpYVK

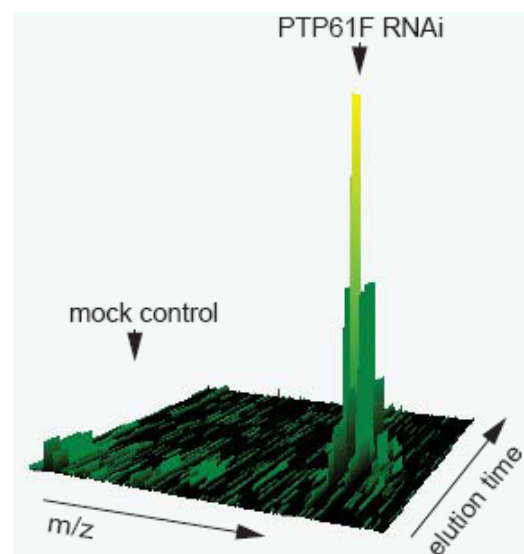


Figure 1. Shown is the mass spectrum of a STAT92E phosphopeptide which is the most upregulated upon PTP61F RNAi.

# Genome-Wide Characterization of Proteins Involved in Cell Cycle Regulation

Mikael Bjorklund, Mikko Turunen, Martin Bonke, Maria Sokolova, Minna Taipale and Jussi Taipale  
Genome-Scale Biology Program, Institute of Biomedicine and High Throughput Center, University of Helsinki,  
and Department of Molecular Medicine, National Public Health Institute (KTL), Biomedicum, P.O.Box 63  
(Haartmaninkatu 8), FI-00014 University of Helsinki, Finland

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## Introduction

The generation of a high-quality list of cell-cycle regulators is essential for the systems biology analysis of the metazoan cell cycle. Although parts lists such as this can be obtained for practically any biological process through genomics methods such as gene-expression profiling or RNAi, functional analysis of the proteins encoded by the identified genes is a major challenge. Here we present our attempt to understand cell cycle regulatory network on a proteomic level based on genes identified in a genome-wide RNAi screen.

## Methods

We identified *Drosophila* cell cycle regulators using a genome-wide RNAi screening in *Drosophila* Schneider S2 cells using flow cytometry. The open reading frames (ORFs) of the identified genes and of those genes which are known to be involved in the identified cell cycle regulatory protein complexes and pathways were cloned into a recombination-based expression system. The ORF collection was fully sequence verified using a second-generation sequencer. The proteins were expressed in S2 cells and systematically analyzed using various proteomic methods.

## Results

We identified genes that control cell size, cytokinesis, cell death/apoptosis, and G1 and G2/M phases of the cell cycle. In addition to classical regulatory mechanisms such as Myc/Max, Cyclin/Cdk and E2F, the cell cycle progression in S2 cells is controlled by vesicular and nuclear transport proteins, COP9 signalosome activity, and four extracellular signal-regulated pathways, Wnt, p38 $\beta$ MAPK, FRAP/TOR, and JAK/STAT.

The genes we identified include novel components of the identified pathways, ubiquitin ligase and kinase substrates, and targets of transcription factors such as E2F and Myc/Max that are relevant for cell cycle progression.

To this end, we have cloned approximately 650 *Drosophila* genes which were identified by RNAi or which are known to be involved in the identified cell cycle regulatory protein complexes and pathways. We have studied the expression of these proteins using DNA microarrays, and analyzed the stability, post-translational modifications of these proteins using high-throughput Western blotting. We have also analyzed the subcellular localization of these proteins in cultured *Drosophila* cells.

## Innovative aspects

- Comprehensive identification and cloning of a collection of genes required for cell cycle progression in *Drosophila* cells
- Expression and initial characterization of cell cycle proteome and its post-translational modifications using the ORF collection
- Functional platform for the detailed systematic analysis of the cell cycle proteome network in higher organisms

## References

- (1) M. Bjorklund et al, Identification of pathways regulating cell size and cell-cycle progression by RNAi; Nature 2006 439, 1009-1013

# Quantitative Proteome Atlas of Adult Honey bee Castes

Q.W.T. Chan

Y. Fang, M.Y. Chan, M.M. Logan, H. Higo, L.J. Foster  
Department of Biochemistry and Molecular Biology, University of British Columbia  
301-2185 East Mall, V6T 1Z4, Vancouver, Canada

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## Introduction

The motivation to study *Apis mellifera* (honey bee) is two-fold: firstly, they are key players in global ecology and agriculture; and secondly, they serve as a model organism for social behavior, innate immunity, and neurobiological processes. Research in bee biology has been hindered by the lack of molecular biological knowledge of this insect compared to the fruit fly and mosquito. To this end, we are currently constructing a proteome atlas of adult bees by relative quantitation of proteins from the worker, drone (males) and queen honey bees.

## Methods

We obtained 34 different honey bee organs from workers, drones, and queens to make worker-to-drone (W/D) and worker-to-queen (W/Q) comparisons. Protein extracts were digested by trypsin. Peptides were labeled with formaldehyde: worker samples with heavy ( $CD_2O$ ), drone and queen with light ( $CH_2O$ ) [1]. Differentially-labeled samples were mixed, concentrated samples were fractionated by ion exchange chromatography, then separated by a reverse phase column coupled to an LTQ-Orbitrap. Data was searched against the bee protein database [2] using Mascot, with a score cutoff corresponding to  $p < 0.05$ . MsQuant was used to quantify peak volumes to derive relative peptide ratios from the W/D and W/Q comparisons. Protein ratios were calculated if  $>1$  unique peptide was quantified among triplicate samples.

## Results

To date, we have quantified  $>1500$  proteins from W/D and W/Q comparisons across 10 bee organs. Overall, our results support previous research. For example, analysis of the legs showed that workers have heightened levels of metabolic enzymes compared to the queens, in line with the workers' extensive motor activity as they clean, nurse, and forage – tasks which queens do not partake in. From the antenna, data corroborates the caste-regulated expression of odorant binding proteins (pheromone transport) as seen in [3]. The high levels of one isoform of alpha-glucosidase present in the queen midgut is consistent with their constant feeding by attendant workers, who express 8-fold less of this enzyme. Numerous unreported findings are also observed, such as the higher abundance of phenoloxidase (pigmentation

and immunity) in workers' legs with respect to the drones', the queens' overexpression of an axon guidance protein in the brain over the workers', and an excess of translation machinery enzymes in the salivary glands of drones compared to workers. To maximize wide usage of this dataset, results will be made accessible on the internet upon the project's completion and publication.

## Innovative aspects

- Construction of a whole bee proteome atlas
- Insight into caste-dependent protein expression regulation
- Provides protein evidence for *in silico* gene annotations from the honey bee genome

## References

1. Hsu, J.L., et al., *Stable-isotope dimethyl labeling for quantitative proteomics*. Anal Chem, 2003. 75(24): p. 6843-52.
2. Elsik, C.G., et al., *Creating a honey bee consensus gene set*. Genome Biol, 2007. 8(1): p. R13.
3. Foret, S. and R. Maleszka, *Function and evolution of a gene family encoding odorant binding-like proteins in a social insect, the honey bee (Apis mellifera)*. Genome Res, 2006. 16(11): p. 1404-13.