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Quantitative proteomics

Previous and current research

Mass spectrometry is an important tool in proteomics, where it is essential for the identification of proteins. Coupling of a mass spectrometer to chromatographic separation of peptide dramatically expands the capacity in large scale experiments, leading to the identification of hundreds of proteins in a single experiment. The technique has matured to the stage that we can now start thinking of capturing entire proteomes. At the same time, alternative strategies are sought that complement the standard LC-MS approach, and that are targeted at the isolation of specific classes of proteins/peptides by pre-fractionation or selective enrichment. While such developments indicate that the 'proteomic toolbox' is still expanding, they also reflect the ongoing integration of proteomics into the larger domains of molecular biology and biochemistry. A major leap has been the introduction of quantitative techniques in mass spectrometry using stable isotope-labeling, allowing to monitor the dynamics of protein expression levels over time. This has opened the doors to explore a plethora of biological phenomena.

Our interest has been both in the development of proteomic techniques and their application to dynamic biological systems, primarily in developmental biology. The tools that we developed and explored includes isoelectric focusing (IEF) of peptides that can be used as a peptide separation strategy, but that also exploits the pI of peptides as an additional criterion aiding in the identification process. Enrichment strategies are often required to capture proteins that are of low abundance, but of high biological importance. The prime example are phosphopeptides that can be selectively enriched for by metal affinity chromatography such as titanium dioxide. We use this technique to identify kinase-specific phosphorylation sites in Mediator, a the multi-subunit protein complex involved in transcription regulation. In addition, we have a longstanding interest in the use and development of quantitative approaches using stable isotopes, where we have introduced the labeling of multicellular organisms *in vivo*, including *C. elegans* and fruit flies. More recently, we have also explored this extensively in embryonic stem cells.

Our biological interest is in developmental biology, but extends to other areas as well. Currently we are studying the dynamics of protein expression during fruit fly development by comparing distinct embryonic stages. In conjunction with gene expression data this provides insight in profiles and regulation of individual or functionally related proteins, and helps explaining regulatory mechanisms in early embryonic development. Another major effort is in application of quantitative proteomic techniques to stem cell pluripotency and differentiation. We have identified a number of proteins that are associated with pluripotency in human and mouse embryonic stem cells. Currently we are studying the dynamics of phosphorylation upon inducing differentiation of human stem cells. This should provide an initial blueprint of the cascades activated during differentiation, and a starting point for further exploration in other cell lines and organisms.

Future projects and goals

Our work on stem cells awaits extension to other cell lines, as well as refinement of temporal expression profiles of previously identified proteins. In addition, this will be extended to enrichment protocols targeting nuclear proteins that most likely includes important players in stem cell maintenance and differentiation. This will be approached by both 'discovery' and targeted approaches, the latter invoking prior knowledge of identified proteins in MRM (multi-reaction monitoring)-type of experiments. Our general interest remains in the application of quantitative proteomic approaches to developmental processes in other systems as well. In addition, we anticipate to start a line of research focusing on protein-DNA interactions aiming to identify proteins binding conserved and/or regulatory domains in DNA.

Selected publications

1. W. Dormeyer, D. van Hoof, S.R. Braam, A.J.R. Heck, C.L. Mummery, J. Krijgsveld. Plasma membrane proteomics of human embryonic stem cells and human embryonal carcinoma cells (2008) [J. Proteome Res](#) (in press)
2. W. Dormeyer, S. Mohammed, B. van Breukelen, J. Krijgsveld, A.J.R. Heck. Targeted analysis of protein termini (2007) [J. Proteome Res. 6\(12\):4634-45](#)
3. J. Krijgsveld, A.D. Whetton, B. Lee, I. Lemischka, S. Oh, M. Pera, C. Mummery, and A.J.R. Heck. Proteome biology of stem cells, a new joint HUPO and ISSCR initiative (2008) [Mol. Cell. Proteomics 7\(1\): 204-5](#)
4. D. Van Hoof, M.W.H. Pinkse, D. Ward-Van Oostwaard, C.L. Mummery, A.J.R. Heck, J. Krijgsveld. An experimental correction for arginine-to-proline conversion-artifacts in SILAC-based quantitative proteomics (2007) [Nature Methods 4\(9\):677-678.](#)
5. D. Van Hoof, C.L. Mummery, A.J.R. Heck and J. Krijgsveld. Embryonic stem cell proteomics (2006) [Expert Reviews of Proteomics 3\(4\): 427-437](#)
6. D. Van Hoof, R. Passier, D. Ward-Van Oostwaard, M.W.H. Pinkse, A.J.R. Heck, C.L. Mummery and J. Krijgsveld. A quest for human and mouse embryonic stem cell-specific proteins (2006) [Mol. Cell. Proteomics 5\(7\): 1261-1273](#)
7. J. Krijgsveld, S. Gauci, W. Dormeyer and A.J.R. Heck. Iso-electric focusing of peptides as a tool for improved protein identification and quantitation (2006) [J. Proteome Res. 5\(7\): 1721-1730](#)
8. P.A. Everley, J. Krijgsveld, B.R. Zetter and S.P. Gygi. Quantitative cancer proteomics: Stable isotope labeling with amino acids in cell culture (SILAC) as a tool for prostate cancer research (2004). [Mol. Cell. Proteomics. 3 \(7\): 729-735](#)
9. A.J.R. Heck and J. Krijgsveld. Mass spectrometry-based quantitative proteomics (2004). [Expert Reviews of Proteomics. 1\(3\): 317-326](#)
10. J. Krijgsveld, R.F. Ketting, T. Mahmoudi, J. Johansen, M. Artal-Sanz, C.P. Verrijzer, R.H.A. Plasterk and A.J.R. Heck. Metabolic labeling of *C. elegans* and *D. melanogaster* for quantitative proteomics (2003). [Nature Biotechnology 21 \(8\): 927-931.](#)