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Proteomics: from protein complexes, via cells to whole organisms

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The sequencing of the human genome and many other genomes heralds a new age in human biology, offering unprecedented opportunities to improve human health and to stimulate scientific, industrial and economic activity. Following this historical landmark in science, the emphasis is now rapidly moving to the biological interpretation of the genome-sequence information. This biological interpretation, which encompasses the immense tasks of identifying structure, function and interactions of the gene-products, i.e. the proteins, and their role in biological processes, will heavily rely on the fast-emerging field of proteomics. Proteomics is the application of evolving technologies to analyze proteins on a large scale to measure protein-expression profiles and protein modifications and networks related to development, health/disease and other biological processes. Basically, proteomics is for proteins what genomics is for genes. In this paper we will highlight several of our current applications in the use of proteomics to unravel life-processes at the molecular protein level. These include the characterization of protein complexes involved in chromatin remodeling, studies on differentiation of B cells, and the identification of immunogenic proteins secreted by a parasite. Additionally, we will describe a new method for improved quantification of protein expression.

Chromatin remodeling complex

Chromatin remodeling is crucial for many developmental processes, and is carried out by multi-protein complexes, which are conserved across many species. The exact composition of these protein complexes remains elusive. The aim of the current study was to identify the components of the *Drosophila brahma* (BRM) chromatin-remodeling complex.

After extensive purification from Drosophila-embryo nuclear extracts, co-precipitating proteins were analyzed by proteomics technologies. Several proteins known to be part of the BRM complex were identified. Furthermore, a number of proteins previously unknown to this complex, or without any previous known function, were identified. These novel proteins had putative DNA-binding properties. Possibly, these proteins could assist in the DNA-binding property of the BRM complex. In conclusion, our proteomics strategy has allowed us to reveal in depth the constituents of the BRM complex, enhancing our understanding of the role of this complex in chromatin remodeling.

B-cell differentiation

We have followed, using proteomics, B-cell differentiation at the molecular protein level. When dormant B-lymphocytes are challenged by antigen, they differentiate into plasma cells. B-lymphocytes do not secrete IgM, whereas plasma cells secrete large amounts of pentameric IgM. In the course of a few days, IgM secretion increases massively, reaching a peak at four days after activation. Also, cell morphology changes dramatically: dormant B-lymphocytes hardly contain any cytosol or organellar structures surrounding the nucleus, whereas plasma cells have evolved into cells that virtually only consist of secretory organelles, notably the endoplasmic reticulum. Protein expression in differentiating B-lymphocytes was followed for five consecutive days. We could cluster proteins, according to expression patterns and correlate these to functional groups. By similarity of expression kinetics, we can classify unknown proteins into one of these clusters. New proteins involved in protein folding could be identified (1).

Identification of immunogenic proteins

In recent years, a shift may be observed in approaches used in proteomics, away from 2D gels as protein-separation tool towards multi-dimensional chromatography. Although, multi-dimensional chromatography has several advantages, 2D gels will not easily disappear. In this contribution we present data that prove that 2D gels have great advantages in analyzing mixtures of 'alike' proteins. The data focus on the identification and characterization of host-exposed proteins by the nematodal parasite *Haemonchus contortus*, which is a nematode infecting small ruminants (such as sheep), giving significant economical losses due to its blood-feeding behavior in the gastrointestinal tract. The nematode releases a variety of molecules into the host environment, which are referred to as excretory/secretory products (ESP). ESP contain proteins that apparently are crucial for the infective process, since immunization with this fraction induces up to 90% of protection in

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sheep. Only a few proteins have been identified as being part of the ESP, and most of them have never really been proved as being secreted. To cast light on the mechanism of infection and to provide information for future vaccine development, the aim of the present study was to characterize the proteins in the ESp, and to determine which proteins are immunogenic (2).

Protein expression quantification

One of the major aims of proteomics is to provide quantitative data on differential protein-expression levels, for instance in healthy and diseased states. Conventially, the proteomics approach uses protein separation by 2D-gel electrophoresis, followed by staining of the proteins. The image analysis of the 2D gels provides quantitative data on protein expression levels. More recently, mass spectrometry based methods have been introduced that can provide quantitative data on differential protein expression, mostly using stable isotope labeling. A very promising method is the so-called isotope-coded affinity-tags method. In our work we have however chosen to use metabolic labeling to introduce stable isotopes in the proteins. We have found that metabolic labeling with stable isotopes provides efficient means to quantify differential protein expression by mass spectrometry. It has the advantage over chemical-labeling methods that no derivatization is needed and that all proteins are labeled universally. Stable isotope labeling has so far been limited to lower organisms because of their ability to grow in defined media. In the present study we aimed to label the multicellular organisms *Drosophila melanogaster* (fruitfly) and *C. elegans* with ^{15}N. The method is applied to the analysis of a *C. elegans* mutant unable to generate a germline. Furthermore we show that this can be approached by 2D-gel electrophoresis and MALDI-TOF MS as well as LC-MS/MS based methods.

Literature

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Expression profiling changes treatment in breast cancer

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Microarray gene expression profiling combined with advanced bio-informatics is beginning to show its power in delineating disease entities that are otherwise indistinguishable. This refinement in tumor classification allows a more accurate prediction of outcome of disease for patients that present with the same stage of disease based on conventional clinical and histopathological criteria. Gene activities determining the biological behaviour of the tumor may indeed be more likely to reflect the aggressiveness of the tumor than general parameters like tumor size, age of the patient, or even tumor grade. Therefore, the immediate clinical consequences are that treatment schemes can be tailored based on the gene-activity patterns of the primary tumor.

We used gene expression profiling with DNA microarrays harboring 25.000 genes on 78 primary breast cancers of young lymph-node negative patients to establish a signature, predictive for a short interval to distant metastases. This 'poor prognosis' signature consists of genes involved in cell cycle, invasion and angiogenesis. The prognosis signature is superior to currently available clinical and histo-pathological prognostic factors in predicting outcome of disease (OR=18 (95%CI 3.3-94), $p<0.001$, multivariate analysis). At present we have validated our findings of this poor-prognosis profile on a large independent series of LN0 as well as LN+ (lymph-node positive) young breast-cancer patients (n=187). Preliminary analysis confirms that the profile is a strong factor in predicting outcome of disease for LN0 patients (OR=17). Furthermore, the profile is as powerful for LN+ patients (OR=12).

Nowadays, consensus guidelines in the management of breast cancer select up to 90% of lymph-node negative young breast-cancer patients for adjuvant systemic therapy (e.g., St Gallen). As 70-80% of these patients would have remained disease-free without this adjuvant treatment, these patients are 'overtreated'. Our 'poor prognosis' signature provides a novel strategy to accurately select patients who would benefit from adjuvant systemic therapy and can greatly reduce the number of patients that receive unnecessary treatment.

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