

MULTI-PROTEIN COMPLEXES STUDIED BY MASS SPECTROMETRY

Jens S. Andersen¹, Juri Rappsilber¹, Hanno Steen¹, Akhilesh Pandey¹, Henrik Molina², Michael Lund², Alexandre Podtelejnikov², Angus I. Lamond³, Matthias Mann^{1,2,*}

¹University of Southern Denmark, Dept. Biochemistry and Molecular Biology, Campusvej 55, DK-5230 Odense M, Denmark; ²MDS-Proteomics, Staermosegaardsvej 6, DK-5230 Odense M, Denmark; ³Department of Biochemistry, Wellcome Trust Building, University of Dundee, Dundee DD1 4HN, U.K.
mann@bmb.sdu.dk

INTRODUCTION. Methods of biological mass spectrometry have improved at a dramatic pace ever since the discovery of electrospray ionization (ES) and Matrix assisted laser desorption ionization (MALDI). These methods now allow the relatively rapid identification of gel-separated proteins as well as the analysis of complex protein mixtures. Continuing efforts aim at increasing the sensitivity of mass spectrometric analysis which currently allows peptide sequencing at the low femtomole level, facilitating the sequencing of silver stained proteins and the detection of medium to low abundant proteins in complex mixtures. One of the goals of our groups has been to apply these methods towards the analysis of protein-protein interactions and the molecular elucidation of multiprotein complexes[1,2].

METHODS. Gel separated proteins were visualized, excised, and digested as previously described[3] and analyzed by nanoelectrospray sequencing as described[4]. For direct complex mixture analysis, peptides were enzymatically degraded and peptides were separated by HPLC and on-line infused into the mass spectrometer. Mass spectrometric analysis was on PE-Sciex QSTAR Pulsar. Protein databases, EST databases and the draft human genome sequence were searched with the MASCOTT program (Matrix Sciences) and the PepSea software suite (MDS-Proteomics).

RESULTS. A large number of multiprotein complexes in mammalian cells have been analyzed by the mass spectrometric methods listed above. These complexes include the spliceosome, the centrosome, mitochondria, and many others. In depth analysis of the nucleolus by a layered approach involving MALDI mass spectrometry and directed sequencing with direct searching of the human draft genome yielded more than 260 proteins. Surprisingly, 30% of these proteins were novel or had no function attached to them (Andersen et al., in press). As in the case of the analysis of the human spliceosome, a number of the novel factors were tagged with the green or yellow fluorescent proteins to determine localization *in vivo*. Based on the results of this experiment, initial fluorescence and electron microscopy and the identity of the found proteins we estimate the purity of the preparation to be very high. Bioinformatic analysis of the found factors revealed no common targeting signal to the nucleolus. Subsequent analysis of the nucleolus by direct mass

spectrometric analysis of the crude mixture served to identify factors which were present or absent in particular cell states such as transcription inhibition.

Mass spectrometry based methods can also be used to study dynamic events such as signaling. Several new members of the EGFR pathway were isolated by antiphosphotyrosine antibodies and some of them are under active study in our laboratory. Novel phosphorylation sites were detected in a number of such factors using a phosphotyrosine specific precursor ion scan[5].

ACKNOWLEDGEMENT. Work in MM's laboratory is supported by a generous grant by the Danish Foundation for Basic Research and A.I.L.'s laboratory is supported by the Wellcome Trust.

REFERENCES

1. Pandey, A. and Mann, M. (2000) *Nature* 405, 837–846.
2. Lamond, A.I. and Mann, M. (1997) *Trends Cell Biol.* 7, 139–142.
3. Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996) *Anal. Chem.* 68, 850–858.
4. Wilm, M., Shevchenko, A., Houthaeve, T., Breit, S., Schweigerer, L., Fotsis, T., and Mann, M. (1996) *Nature* 379, 466–469.
5. Steen, H., Kuster, B., Fernandez, M., Pandey, A., and Mann, M. (2001) *Anal. Chem.* 73, 1440–1448.