

## 5 Summary

To identify proteins that are differentially expressed in pain model systems, a proteome assay was established based on the separation of proteins by classical two-dimensional gel electrophoresis (IEF/SDS-PAGE) or by 16-BAC/SDS-PAGE, which is suitable for the separation of membrane proteins, in combination with MALDI mass spectrometry. The limits of the methods with respect to the particular question were described. Synaptosomal membranes of rat dorsal spinal cord tissue (from control and from pain-model animals) were analysed with a particular focus on membrane-bound proteins. When comparing the protein patterns after 2DE, differences on the amount of particular proteins were detectable but were not reproducible. Moreover, integral membrane proteins of the plasma membrane were strongly under-represented on 2D-IEF/SDS gels. Based on the whole data set, a provisional protein map of dorsal-horn synaptosomal membranes is provided, which comprises 30 proteins within a pH range from 3-10.

The coding sequences of *Trpv1* and *Trpv2* were inserted into the eukaryotic expression vector pcDNA3.1(+). As a neuronal expression system, the F11 fusion cell line of rat dorsal root ganglion cells and a mouse neuroblastoma cell line N18TG2 was established. F11 cells express both TRPV2 of rat and TRPV2 of mouse endogenously. The existence of the two channels in the same cell was shown by single-cell RT-PCR (O. Bender).

After heterologous expression of TRPV1 and TRPV2 in F11 and HEK293 cells, it was demonstrated that both channels are N-glycosylated, and show high-mannose-type glycosylation as well as complex glycosylation. The glycosylation sites for TRPV1 and TRPV2 were identified as amino acid Asn 604 and as amino acids Asn 571 and Asn 572, respectively. They are located extracellularly between membrane-spanning sequences TM5 and TM6 and confirm the predicted topology of the channels with both the N- and C-terminus positioned in the cytoplasm. The glycosylation has no influence on the subcellular localization of the channels. In transfected F11 cells, TRPV1 and TRPV2 are located at the plasma membrane of the soma and of neurite-like extensions. In HEK293 cells, TRPV1 is primarily located at intracellular membranes. The localization of TRPV1 at the plasma membrane is

disturbed when the cytoplasmic N-terminus is missing or when it is coupled to GFP. The determination of the plasma membrane targeting sequences is still pending. Based on its analysis by two-dimensional gel electrophoresis applying BN-PAGE as first dimension and SDS-PAGE as second dimension, TRPV1 complex forms a homotetramer. This complex shows a higher apparent molecular weight than predicted, which indicates the existence of additionally associated proteins. A search for putative interacting proteins for TRPV1 and TRPV2, which used fusion proteins of the cytoplasmic C-termini and MBP, resulted in the identification of actin for TRPV1, and of actin,  $\beta$ -tubulin and P450-(cytochrome)-oxidoreductase for TRPV2. Meanwhile,  $\beta$ -tubulin has been identified and verified as a direct TRPV1-interacting protein. The calcium-dependent interaction of the two proteins was analysed in detail by C. Goswami. Additional putative TRPV1-interacting proteins, identified by analysing pull-down assays, will be characterized soon.