Summary

For higher organisms the central nervous system is the key organ to ensure rapid adaption to environmental cues by controlling the expression of neuronal genes. The ability to react to external stimuli has to be fast, as it is of great importance for learning and memory formation due to the fact that the patterns of neural connections are continuously in the process of change. Gene expression can be controlled at different levels including transcription, mRNA processing, mRNA export from the nucleus, translation and mRNA degradation. mRNA-transport into defined cell compartments occurs both in neurons and non-neuronal cells. For neurons, which each are connected with neighboring cells via thousands of synapses, the directed and fast mRNA-transport into dendrites and local translation at synapses is of outstanding importance. Dendritic mRNA transport is mediated by messenger ribonucleoprotein particles (mRNPs) that are dragged along cytoskeletal structures by motor proteins. It is specified by sequences (*cis*-acting elements) within mRNAs that interact with certain proteins (*trans*-acting factors) to collectively constitute mRNPs. A *trans*-acting factor described previously is the multifunctional poly(A)-binding protein (PABP), which among others specifically binds to the *cis*-acting element within vasopressin mRNA, thus presumably mediating its transport into dendrites. PABP is a protein highly conserved throughout evolution and exerts numerous functions in eukaryotic cells. N-terminally it consists of four non-identical RNA binding RRM-type domains (RNA-recognition motif), while C-terminally it comprises a proline-rich region and a structured C-terminal domain called PABC or MLLE.

In the experimental work presented here, a novel PABP-interacting partner has been identified using a yeast two-hybrid screen. It represents a shorter isoform of the human Makorin RING-zinc finger of protein 1 (Makorin1, MKRN1). In addition to the artificial yeast two-hybrid system, the interaction could be confirmed in a mammalian cell culture system, both by co-immunoprecipitation and a method called PDZ-pulldown.

Makorin1 is a modular protein: N-terminally it consists of three zinc fingers and a Makorin-type zinc finger, C-terminally it harbors a RING finger domain and a another zinc finger motif. The shorter protein variant identified here (Makorin1 short) is truncated at the C-terminus and lacks the C-terminal zinc finger as well as the last six amino acids of the RING finger domain. In order to characterize the sites of interaction between the proteins, experiments using Makorin1- as well as PABP deletion mutants were carried out. A significant result was revealed: an area within Makorin1 spanning amino acids 111-234 is necessary for the

PABP/Makorin1-short interaction. This sequence includes the zinc finger domain 3 and the amino acids linking zinc finger domains 2 and 3. The RING finger domain and the Makorin1-type zinc finger do not bind to PABP. As far as PABP is concerned at least two of the four RRMs are involved in the interaction between both proteins.

So far, hardly anything is known about the function of Makorin1. Because of the presence of zinc fingers it is supposed that Makorin1 is an RNA-binding protein. The long Makorin1 variant acts as E3 ubiquitin ligase for some proteins and is capable of autoubiquitination, whereas the short protein variant does not appear to catalyze its own ubiquitination because of its incomplete RING finger domain.

When investigating mRNA expression of both Makorin1 variants in different rat tissues (heart, brain, spleen, lung, liver, muscle, kidney, testis) it could be clearly demonstrated that the mRNA expression is strongest in the brain and the testis. In addition, corresponding rat brain Makorin1 protein expression was demonstrated by Western blot experiments as well as immunocytological and immunohistochemical analyses, in which the Makorin1-short isoform predominated.

The intracellular distribution of endogenous Makorin1 in mammalian HeLa cells included the nucleus and cytoplasm. In rat neurons it could be detected in the nucleus, somata and dendrites. The short Makorin1 isoform was enriched both in isolated hippocampal synaptosomes and the postsynaptic density (PSD). These structures play a central role in synaptic plasticity as well as in learning and memory processes.

Furthermore, it could be shown that Makorin1-short, is not involved in ongoing translation. Rather, the results point to a possible role in regulating protein synthesis at the translation-initiation level. It is still to be resolved, whether Makorin1-short takes part in the translational regulation of vasopressin mRNA in dendrites. However, it could be shown that it binds to vasopressin mRNA *in vitro* by performing affinity chromatography studies with immobilized RNA. Whether this binding occurs directly or is mediated by PABP, remains to be shown by further experiments.

Moreover, the results of this work suggest another possible role of Makorin1-short, namely in mRNA decay by the NMD (nonsense-mediated mRNA decay) mechanism, because the UPF1 protein, a key factor of the NMD process, could be identified as a potential interaction partner of Makorin1-short. Further experiments are needed in order to elucidate this function in more detail.