

Fast synaptic transmission in the CNS depends on ligand-gated ion channels that are densely clustered in the postsynaptic membrane. Gephyrin is the major scaffolding protein at inhibitory synapses where it anchors glycine-receptors (GlyRs) and a certain proportion of gamma-aminobutyric-acid-receptors (GABA(A)Rs) to the cytoskeleton. Both receptor types are hetero-pentamers that share significant topological homologies. However, considerable diversity is generated by the fact, that GABA(A)R subtypes can be assembled from a large variety of different subunits.

GlyRs bind via the cytoplasmatic loop of their beta subunits to the gephyrin E-domain. Further *in vivo* characterization of the gephyrin GlyR interaction was performed in this study, based on a co-crystal structure of the E-domain dimer harbouring a GlyRbeta-loop derived peptide. The gephyrin GlyR interface is mainly stabilized by a hydrophobic core structure formed by Phe 398 and Ile 400 of the beta-Loop and Phe 330 of the E-domain. In addition, a hydrogen bond links Tyr 673 with Ile 400. P713 is a critical part of a loop in the left wall of the GlyR binding pocket. All essential residues identified are highly conserved among vertebrates.

Much less is known about the interaction of gephyrin with GABA(A)Rs - although colocalization in various brain regions and studies of gephyrin/GABA(A)R knock out mice implicated gephyrin function in the synaptic clustering of selected types of GABA(A)Rs. Here, direct binding of the large intracellular loops of the GABA(A)R subunits alpha2, beta2/3 and delta to the gephyrin E-domain was observed in cosedimentation experiments. The GABA(A)R binding site on GephE is not identical with the GlyR binding pocket. Notably, the binding characteristics also vary between different GABA(A)R subunits and gephyrin. Binding of gephyrin to N- and C-terminal parts of the beta2 subunit is based on ionic interactions. The binding motif overlaps with the AP2 complex binding site, which might suggest an additional regulatory function of gephyrin in receptor internalization. In contrast to the strong interdependence between gephyrin and GlyRs at glycinergic synapses, a role for gephyrin in stabilizing existing GABA(A)R clusters is proposed.

The gephyrin gene is intensely spliced resulting in the tissue specific expression of various isoforms. However, the function of these variants is poorly understood. In this study, the influence of splice cassette insertions was analyzed regarding the second, metabolic function of gephyrin - the catalysis of the last steps in the synthesis of the essential molybdenum containing cofactor (Moco). Insertion of the G2 cassette in the gephyrin G-domain abolished biosynthetic function of gephyrin in reconstitution experiments. *In vitro* data confirmed defects in adenylation of the cofactor intermediate MPT whereas substrate binding was not affected. Taken into account that G2-containing variants are expressed in liver, a hotspot of cofactor synthesis, a regulatory function of G2 can be assumed. In line with a high gephyrin expression level, Moco was also observed in brain. In this organ, cofactor formation is restricted to gephyrin expressed in glia cells - probably mainly catalyzed by an isoform harbouring the C3 cassette in the gephyrin C-domain. Variants present in neurons, like Geph-C4c, are biochemically able to catalyze cofactor formation but do not contribute to neuronal Moco. Apparently, different isoforms are responsible for the diverse functions of gephyrin in the organism. Splice cassette specific antibodies generated here recognize C3 and C4a and will provide further insights in understanding the specific functions of different gephyrin isoforms in brain and peripheral tissue.