Review

Gephyrin: where do we stand, where do we go?

Jean-Marc Fritschy[1], Robert J. Harvey[2] and Günter Schwarz[3]

[1] Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
[2] Department of Pharmacology, The School of Pharmacy, 29–39 Brunswick Square, London WC1N 1AX, UK
[3] Institute of Biochemistry, University of Cologne, Otto-Fischer-Strasse 12–14, 50674 Cologne, Germany

Gephyrin is a multifunctional protein responsible for molybdenum cofactor synthesis and the clustering of glycine and $GABA_A$ receptors at inhibitory synapses. Based on the structure of its two conserved domains, G and E, gephyrin is thought to form a hexagonal lattice serving as a scaffold for accessory proteins at postsynaptic sites. However, important aspects of gephyrin gene expression, protein structure and regulation, as well as the role of gephyrin in synapse formation and plasticity, remain poorly understood. Here we review the current state of knowledge about gephyrin, highlighting new research avenues based on a different structural model and a revised nomenclature for gephyrin splice variants. Unraveling the biology of gephyrin will further our understanding of glycinergic and GABAergic synapses in health and disease.

Introduction

Functional chemical synaptic transmission requires a precise matching of the presynaptic active zone and release machinery with the postsynaptic density. Bidirectional trans-synaptic communication is necessary for synapse formation and maintenance. At the molecular level, scaffolding proteins ensure the proper subcellular location of functionally interconnected proteins and serve as anchors to maintain the long-term stability of synapses, despite continuous turnover of individual components. Proteomic analysis of purified synaptosomal preparations has identified several hundred proteins at glutamatergic synapses [1,2], among which PDZ domain-containing proteins play a major role in building molecular scaffolds. By contrast, much less is known about the molecular constitution of type II GABAergic and glycinergic synapses, which are not readily purified biochemically. A major component of these synapses is gephyrin [3–5] (Figure 1), initially discovered as a constituent of affinity-purified glycine receptor (GlyR) preparations. This multidomain protein lacks PDZ domains but forms aggregates by auto-oligomerization, potentially providing a scaffold for postsynaptic proteins and an anchor to the cytoskeleton [6]. Gephyrin is extensively conserved across the entire living kingdom and mediates molybdenum cofactor (Moco) biosynthesis, in addition to its role as a postsynaptic protein [7]. Despite the successful elucidation of crystal structures of two constituent domains of gephyrin [8–10] and identification of many interacting proteins, little progress has been made regarding the biology of gephyrin. It is also unresolved whether its two major functions are interrelated. Given the fundamental role of GABAergic and glycinergic transmission for normal brain function and its relevance for a large spectrum of neurological, neurodevelopmental and neuropsychiatric diseases, it is a high priority to further our knowledge about the assembly, maintenance and regulation of inhibitory synapses in the CNS. A recent meeting devoted to the molecular and cell biology of gephyrin was aimed at stimulating research in this field. The present review summarizes the current state of knowledge and presents a consensus opinion as to how unmet challenges should be addressed to deepen our understanding of GABAergic and glycinergic transmission in health and disease.

Gephyrin structure and aggregation

Gephyrin consists of three major domains, two of which were named the G and E domains because of sequence similarities with the bacterial Moco-synthesizing enzymes MogA and MoeA. The 20 kDa N-terminal G domain and the 43 kDa C-terminal E domain in gephyrin are linked by a central domain (C domain; also referred to as the linker region) of 18–21 kDa. Multiple isoforms of gephyrin are generated by alternative splicing of several exons (see Box 1). The isolated G domain forms stable trimers that are also found in purified, nonaggregated holo-gephyrin. G domain trimerization depends on identified residues [11] and is disrupted by a specific splice cassette (C5′) that is present also in nonneuronal tissues [12]. By contrast, the isolated E domain forms dimers in solution and presents the high-affinity binding site for the M3-M4 intracellular loop of the GlyR β subunit (β loop). The E domain crystal structure shows binding of the GlyR β loop to gephyrin at the dimerization interface, with residues F330, Y673 and P713 being crucial for the formation of a high-affinity binding site [9,13,14]. The C domain is absent from gephyrin-homologous proteins from prokaryotes and plants. The finding that holo-gephyrin is sensitive to proteolytic degradation within the C domain suggested a flexible solvent-exposed structure, for which reason it was also called the 'linker region.' However, the C domain contains binding sites for several gephyrin-interacting proteins, such as Pin1, dynein light chain 1 and 2, and collybistin (see Figure I in Box 1). Furthermore, recent data suggest the presence of a well-structured folded C domain (G.S., unpublished), which might be of relevance for protein–protein interactions .

Corresponding author: Fritschy, J.-M. (fritschy@pharma.uzh.ch).

0166-2236/$ – see front matter doi:10.1016/j.tins.2008.02.006 Available online 9 April 2008

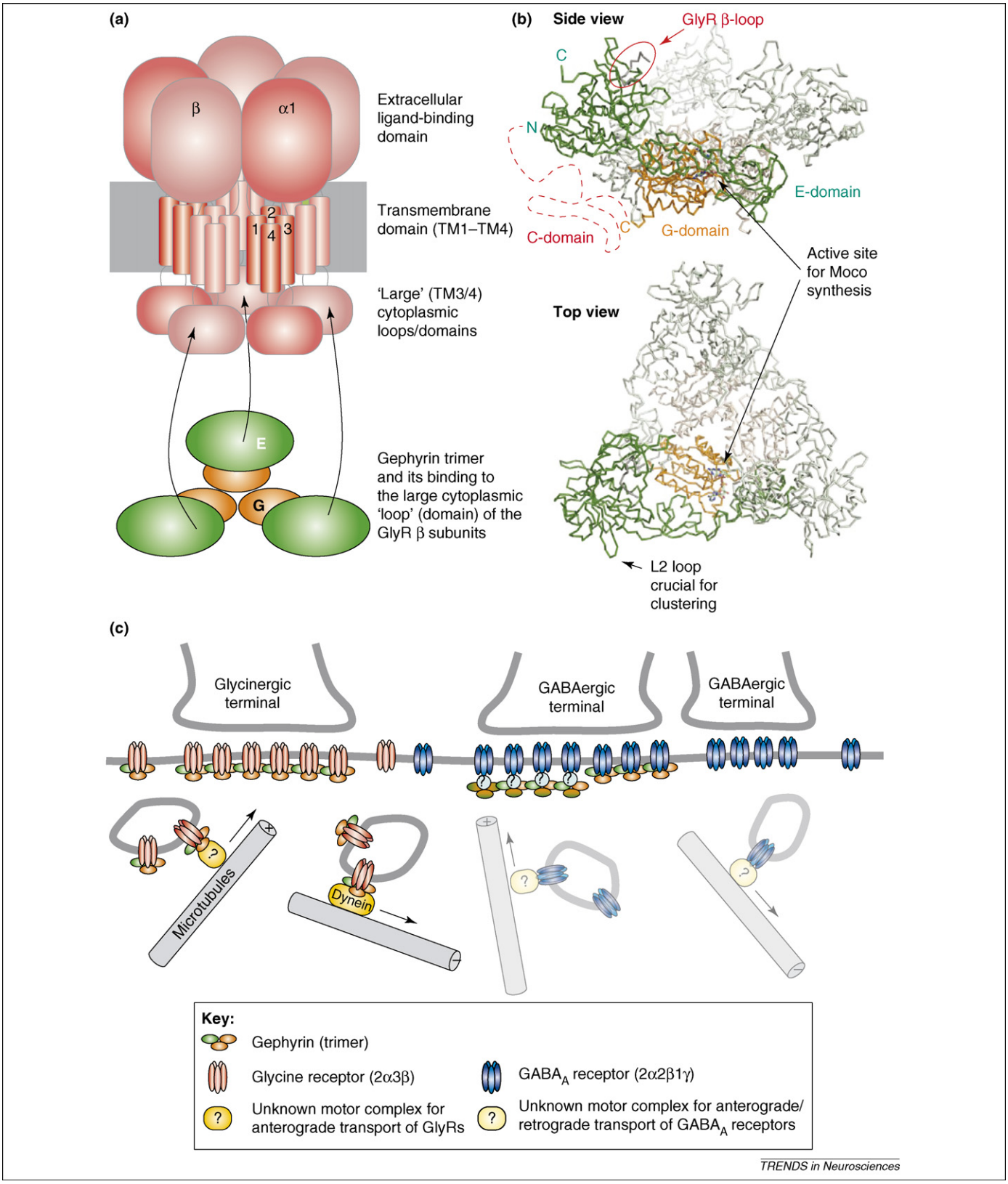

Figure 1. Model of gephyrin structure and postsynaptic targeting and clustering with GlyR and GABA$_A$ receptors. **(a)** Cartoon of the pentameric GlyR with its three functional domains, the extracellular ligand-binding, the transmembrane and the cytoplasmic (loop) domains. Interaction with the gephyrin trimer is depicted. **(b)** Structural model of a gephyrin trimer. The model was generated based on the orientation of the two subunits within the E domain dimer. One of the subunits was replaced by the G domain structure, which was overlaid onto the structurally related subdomain 3 of the other E domain. By doing so, one can arrange each E domain monomer relative to the G domain, thereby projecting a possible conformation for the gephyrin trimer. In this model, the binding sites for GlyR β loops are fully exposed on one side of the protein, both active sites essential for Moco synthesis face each other, and the L2 loop, identified to be important for aggregation and clustering [18], is pointing into the horizontal plane of the trimer. The position of the C domain is indicated by the dotted red line connecting the G and E domains. **(c)** Gephyrin trafficking and clustering in glycinergic and GABAergic synapses. GlyR is shown in complex with gephyrin trimers either at the synapse when they are clustered or in complex with transport proteins along microtubules. Although retrograde transport via Dlc1/2 was described recently [24], the proteins involved in anterograde transport are not known. GABA$_A$ receptor clusters are depicted to interact either directly or via an intermediate protein with gephyrin or are completely independent of gephyrin. The mechanism of GABA$_A$ receptor trafficking is poorly understood and therefore shown in gray shading.