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Gephyrin: does splicing affect its function?

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Abstract

Gephyrin is a protein involved in both synaptic anchoring of inhibitory ligand-gated ion channels and molybdenum cofactor synthesis. Substantial progress has been made in understanding its gene and protein structures. Furthermore, numerous binding partners of gephyrin have been identified. The mechanisms by which these interactions occur are unclear at present. Alternative splicing has been proposed to contribute to gephyrin's functional diversity within single cells as well as in different cell types and tissues.

Introduction

Gephyrin is a ubiquitously expressed protein with an apparent molecular mass of approx. 93 kDa. In neurons, it is involved in clustering of inhibitory ligand-gated ion channels, GlyRs (glycine receptors) and certain $GABA_A$ (γ-aminobutyric acid A) receptor subtypes [1,2]. Additionally, gephyrin is required for molybdoenzyme activities such as sulphite oxidase and xanthine dehydrogenase [2]. The generation of knockout mice suggested that gephyrin is dispensable for embryonic development, but absolutely essential for postnatal survival [2]. Numerous gephyrin variants have been found and described previously. Alternative splicing of gephyrin is at least partially tissue-specific. One exon, C5, seems to be much more abundant in muscle compared with other tissues [3], whereas a different cassette, C3, has been observed to be very prominently expressed in liver [4]. The same cassette is largely repressed in neurons by Nova proteins, neuronal regulators of pre-mRNA splicing [5]. This tissue-specific expression of at least some cassettes is a clear indication that gephyrin's functions may be modified by alternative splicing. Hence, a better understanding of gephyrin's gene and protein structures is likely to provide clues as to how gephyrin manages to fulfil complex and distinct functions in a whole organism.

Gephyrin gene structure

The structure of the gephyrin gene has been studied most extensively in mouse and human [3,6]. In mouse, the gephyrin gene encompasses 457 kb on chromosome 12 D2 containing 30 exons. Ten of these exons, called cassettes, can be spliced out, giving rise to numerous splice variants. These cassettes have been named C1–C7 and C4′–C6′ (see Figure 1A). In human, the gephyrin gene is located on chromosome 14q23.3, with a size of 668 kb and including 27 exons [6]. All constitutively expressed exons are common to mouse and human. However, for the cassettes some differences have been observed. The cassettes C1, C6, C7 and C5′ seem not to be transcribed in the human gene. Additionally, the 'C4 cluster' encompassing C4′, C4 and C5 in mouse contains one more exon in the human gene. C4A, corresponding to C4′ in mouse, is followed by a cassette (C4B) specific to human, and two further cassettes, C4C and C4D (C4 and C5 in mouse respectively).

Gephyrin protein structure

The gephyrin gene encodes a protein consisting of three domains, an N-terminal domain, a C-terminal domain and a central linker region (see Figure 1B). Both the N- and C-terminal domains have been crystallized [7,8]. The N-terminal domain has a high structural similarity to the *Escherichia coli* MogA protein and forms trimers, whereas the C-terminal domain shows similarity to the *E. coli* MoeA protein and is dimeric. Full-length gephyrin is thought to be a trimer, which, triggered by an as yet unknown signal, can undergo a conformational change that allows dimerization of the C-terminal domains. Both the N-terminal trimerization and the C-terminal dimerization have been suggested to be involved in the submembranous gephyrin scaffold formation [8].

Gephyrin-binding proteins

Among several proteins reported to bind gephyrin, it is the interaction of gephyrin with the GlyR that provides the basis for synaptic GlyR clustering [8]. Collybistin, a guanylate exchange factor for cdc42, interacts with the C-terminal domain of gephyrin. This interaction has been shown to be important for postsynaptic localization of gephyrin and inhibitory ligand-gated ion channels [9]. Gephyrin also interacts with tubulin and is believed to anchor the GlyR in the postsynaptic membrane through this interaction [10]. Based on amino acid sequence analysis, the tubulin-binding site has been proposed to be located in the linker region [3]. Mena/VASP (vasodilator-stimulated phosphoprotein) and profilin are further proteins shown to bind gephyrin [11]. Hence, the interactions with VASP and profilin might serve as a link between the synaptic gephyrin scaffold and the microfilament system. Additionally, binding of Dlc (dynein light chain) 1 and 2, components of motor protein complexes, has been proposed to be involved in retrograde gephyrin

Key words: collybistin, gephyrin, glycine receptor, oligomerization, splicing, vasodilator-stimulated phosphoprotein (VASP).

Abbreviations used: $GABA_A$, γ-aminobutyric acid A; GlyR, glycine receptor; VASP, vasodilator-stimulated phosphoprotein.

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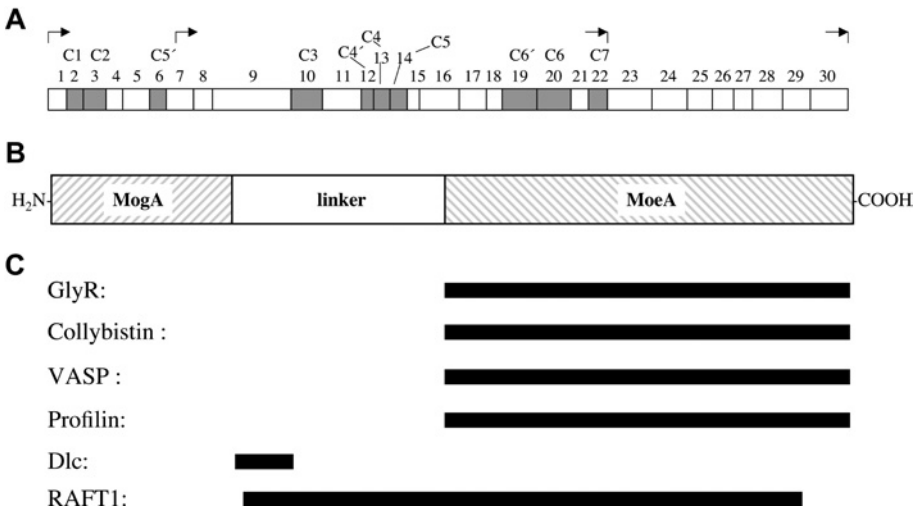
Figure 1 | **Maps of gephyrin exon and domain organization**

(**A**) Exon organization of the murine gephyrin gene. Exons are numbered from 1 to 30. Cassettes subject to alternative splicing are depicted in grey. Arrows represent alternative start and stop codons. (**B**) Gephyrin domain structure. (**C**) Localization of the binding sites of proteins interacting with gephyrin. Regions similar to bacterial and eukaryotic molybdenum cofactor synthesis proteins are striped. Positions of the binding sites are indicated with black bars. Both (**B**) and (**C**) are aligned with respect to (**A**). Dlc, dynein light chain; RAFT1, rapamycin and FK506-binding protein 12 target 1.



transport [12]. On the other hand, the interaction of gephyrin with RAFT1 (rapamycin and FK506-binding protein 12 target 1) is required for rapamycin-sensitive signalling [13]. A tentative map of the known binding sites of the proteins interacting with gephyrin is presented in Figure 1(C). Further binding partners of gephyrin, such as the hypothesized linker protein that mediates gephyrin association with $GABA_A$ receptors, still remain to be discovered.

Effects of splicing on gephyrin function

Gephyrin splicing occurs in all three major domains. Each domain contains at least three variable expression cassettes, making alternative splicing an ideal tool for gephyrin to adjust its abilities to diverse functional demands. However, at present, experimental proof showing the possible importance of this mode of regulation is lacking.

The C7 cassette is expressed in many different organs [3]. It contains a stop codon and, as a consequence, only the first third of the C-terminal domain is translated properly [8]. Therefore, most, if not all, of the known functions of the C-terminal domain such as dimerization, molybdenum cofactor synthesis and clustering of the GlyR would be abolished upon insertion of C7, whereas the N-terminal domain and the linker region are likely to remain unaffected.

The repression of the C3 cassette in neurons by Nova [5] indicates that C3 is important in non-neuronal tissues. Therefore it is likely that C3 might interfere with gephyrin functions in neurons as well.

The function of the C5′ cassette has been studied in the most detail. Although C5′ is located in the N-terminal gephyrin domain and the GlyR binding site lies in the C-terminal domain [8], C5′ has been reported to abolish GlyR binding [14]. Furthermore, evidence has been presented suggesting that C5′ interferes with GlyR anchoring in hippocampal and spinal cord neurons [15].

Alternative splicing has been found to affect the functions of gephyrin in mouse, but not yet in human. One cassette, C3, is a very good candidate to also affect the functions of gephyrin in human. Further studies on the functions of the distinct expression cassettes in gephyrin may unravel their roles in the regulation of molybdenum cofactor synthesis, binding of gephyrin to its interaction partners and gephyrin oligomerization.

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Received 20 September 2005

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