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The mouse and human Liprin-α family of scaffolding proteins: Genomic organization, expression profiling and regulation by alternative splicing

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ABSTRACT

In the nervous system the Liprin-α protein family plays an important role in the regulation of dendrite development, the targeting of photoreceptor axons, and the formation and structure of synapses. To gain a better understanding of Liprin-α regulation we have comparatively analyzed the genomic organization of the human and mouse *Liprin-α* genes, characterized the alternative exon use in human and mouse, and studied their expression in adult rodent tissues and brain regions. Our results show that Liprins-α1–4 share multiple properties in their genomic structure, exhibit an identical modular organization, and are highly conserved within certain structural domains, indicating strong evolutionary cohesion. We demonstrate that all *Liprin-α* genes are subject to alternative splicing, which is regulated in a developmental manner. Interestingly, regulation via alternative splicing is not conserved between isoforms and across species and represents a post-transcriptional mechanism to independently diversify the properties of the individual isoforms.

Introduction

Synapses are specialized sites of contact and communication between a neuron and its target. Both sites of signal transduction at the synapse, the presynaptic active zone (AZ), where synaptic vesicles dock and fuse, and the postsynaptic density (PSD) that contains the neurotransmitter reception apparatus, are highly organized structures consisting of proteinaceous networks [1–3]. In spite of the progress that has been made in recent years to identify the essential components of these networks the molecular mechanisms underlying synapse formation and function are still not understood in detail.

The evolutionary conserved Liprin-α family of scaffolding proteins plays a crucial role in synapse assembly and function [3–5]. Liprins-α were originally identified by their interaction with the LAR-RPTPs (leukocyte common antigen-related family of receptor protein tyrosine phosphatases) [6,7]. Whereas in the invertebrates *C. elegans* and *Drosophila* only a single (*Liprin-α*) gene was described, *syd-2* (synapse-defective-2) [8] and *Dliprin* [9], respectively, four Liprin-α isoforms were found in vertebrates, Liprins-α1, α2, α3, and α4 [10]. Liprins-α exhibit a striking degree of conservation, with 40% amino acid identity between the human Liprin-α1 and worm syd-2. Liprin-α proteins are composed of an N-terminal region predicted to form coiled-coil structures and three C-terminal SAM (sterile-α-motif) domains that constitute the LH (liprin homology) region [6,7,10]. The SAM-domains have been reported to interact with the intracellular domain of LAR-RPTPs [7], the MAGUK (membrane-associated guanylate kinase) protein CASK (calcium/calmodulin-dependent serine protein kinase) [11,12], CaMKII (Ca^{2+}/calmodulin-dependent protein kinase II) [13], Liprin-β and ATP [14]. The N-terminal coiled-coil region mediates homo- and hetero-dimerization as well as interactions with various synaptic proteins, including the presynaptic active zone proteins RIM (RIMS1, Rab3-interacting molecule) [15] and ELKS (ERC1(ELKS/RAB6-interacting/CAST)) [16], the GTPase activating protein for the family of ADP-ribosylation factor GTPases GIT1 (G protein-coupled receptor kinase interactor) [17], and the kinesin motor protein KIF1A (kinesin family member 1A) [18]. At their C-terminus vertebrate Liprins-α contain a PDZ-binding motif that interacts with GRIP1 (glutamate receptor interacting protein) [19]. Through these interactions Liprins-α are directly linked to essential components of the presynaptic active zone and the postsynaptic density as well as the machinery required for vesicular transport.

In loss-of-function mutants of the *C. elegans* Liprins-α homolog SYD-2 synaptic vesicles are diffusely localized, presynaptic active zones are abnormal in size and appearance, and synaptic transmission

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Table 1
Chromosomal locations and sizes of *Liprin-α* genes

Gene (symbol)	Human location	Size (kb)	Protein names	Gene ID	Mouse location	Size (kb)	Protein names	Gene ID
Liprin-α 1 (PPFIA1) (PTPRF interacting protein alpha 1)	11q13.3	114.041	Liprin-α1 LIP.1 LIP1 MGC26800	8500	7F5	76.975	Liprin-α1 LIP.1 LIP1 CO30014K08Rik	233977
Liprin-α 2 (PPFIA2) (PTPRF interacting protein alpha 2)	12q21.31	500.523	Liprin-α2 MGC132572 FLJ41378	8499	10D1	463.108	Liprin-α2 E130120L08Rik	327814
Liprin-α3 (PPFIA3) (PTPRF interacting protein alpha 3)	19q13.33	31.620	Liprin-α3 KIAA0654 LPNA3	8541	7B4	27.805	Liprin-α3 2410127E16Rik	76787
Liprin-α4 (PPFIA4) (PTPRF interacting protein alpha 4)	1q32.1	40.138	Liprin-α4 KIAA0897	8497	1E4	36.146	Liprin-α4 1110008G13Rik	68507

Official name: protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha.

is impaired [8]. The *Drosophila* Liprin-α homolog Dliprin-α is required for normal active zone morphology and is involved in the formation of new synaptic boutons at the neuromuscular junction (NMJ) [9]. In Dliprin-α mutants evoked excitatory junctional potentials and quantal content per synaptic event were decreased at the NMJ pointing to a specific impairment of presynaptic function [9]. Furthermore, altered synaptic vesicle movement was observed in these mutants, which implies a role in vesicular trafficking for Liprins-α [20]. Dliprin-α is also required for the targeting of photoreceptor axons in the visual system [21,22]. Mammalian Liprins-α were shown to be localized in axons and dendrites, in agreement with their association with pre- and post-synaptic protein complexes [19]. Liprins-α have been reported to regulate clustering and localization of LAR in cultured cell lines [6,10] and to colocalize with ELKS and RIM at presynaptic active zones in primary cells. In dendrites, the interactions between Liprin-α and GIT1 [17] as well as GRIP [19] are required for AMPA receptor targeting. The LAR-RPTR, GRIP and Liprin-α complex has further been shown to play an important role in synapse morphogenesis and function and to be involved in the dendritic targeting of the cadherin/β-catenin complex [23].

It was recently reported that Liprin1-α is regulated in an activity-dependent manner. Liprin-α1 was degraded either in response to CaMKII phosphorylation or via the E3 ubiquitin ligase anaphase promoting complex (APC) [13]. Expression of non-degradable Liprin-α1 mutants resulted in impaired dendritic targeting of LAR and a reduction of dendritic branching and synapse number. Thus, Liprins-α play three important roles: first, in the assembly, organization, and function of presynaptic active zones; second, in the development and maintenance of dendritic spines and excitatory synapses; and third, in vesicular trafficking. Taken together, studies in both invertebrates and vertebrates point to a critical role of Liprins-α in synapse formation and function.

In view of the important functions exerted by Liprin-α proteins in the central nervous system a detailed characterization of this gene family is necessary. Here we have performed a comprehensive and comparative analysis of human and mouse *Liprin-α* genes with regard to their genomic organization and their regulation by alternative splicing. We show that all four genes encoding Liprin-α family members undergo alternative splicing in humans and mice, but the number and location of alternatively spliced exons vary between the isoforms and the two species. Furthermore, we provide evidence that alternative splicing is regulated during development and results in proteins with diverging properties with regard to potential protein interactions. The relative expression profiles of the individual Liprin-α isoforms show that all four genes are expressed throughout the brain, but that there are variations in the levels of expression.

The aim of this analysis was to provide the framework for further investigations into the diverging and overlapping functional roles of the four mammalian Liprin-α isoforms.

Results

The structure of human and mouse Liprin-α genes

Recent studies have begun to shed light on the neuronal functions of Liprins-α [4,5] and have provided first evidence for potential functional differences between the four isoforms [13]. However, so far the sequence, structures, and alternative splicing of *Liprin-α* genes have not been comparatively and thoroughly analyzed. In this study, we characterized the exon/intron structure of human and mouse *Liprin-α* genes and analyzed the alternative splicing pattern of each *Liprin-α* gene in both human and mouse using bioinformatics and RT-PCR. For each *Liprin-α* a search for mRNA sequences and expressed sequence tags (ESTs) was performed. Furthermore, the identified cDNAs of mouse were cloned and verified by sequencing.

The properties of the human and mouse *Liprin-α* genes are summarized in Table 1, the genomic structure of the human and mouse genes is depicted in Fig. 1, and the sizes of the exons and the sequences of the exon–intron junctions are shown in Suppl. Tables 1–8. The four *Liprin-α* genes are dispersed in the human and mouse genome, but exhibit highly conserved exon/intron structures, suggesting that they are the result of a relatively recent gene duplication. The number of exons differs between the Liprins-α, due to additional exons as well as distinct numbers of alternatively spliced exons. The individual genes are highly conserved between the mouse and human genomes with mostly identical placement of the exons and sizes of the introns (Fig. 1). The human and mouse *Liprin-α2* genes are large with sizes of approximately 500 kb, while the *Liprin-α3* and *Liprin-α4* genes are relatively small, approximately 30 kb and 40 kb, respectively (Table 1, Fig. 1). The human and mouse *Liprin-α1* genes are of intermediate size, 114 kb (human) and 77 kb (mouse). It is notable that in *Liprin-α1* and *Liprin-α2* the exon containing the translation start site is separated from the remaining clustered exons by a large intron, around half the size of the complete genes. In the case of *Liprin-α2* this intron contains an additional exon (exon 3), which is not present in any of the other *Liprin-α* genes.

Liprin-α genes in human and mouse display distinct alternative splicing

Even though the overall gene structure of the *Liprin-α* genes is highly conserved, database analysis and RT-PCR revealed striking variations in

Fig. 1. Comparison of the human and mouse *Liprin-α* gene structures. The organization of the exon/intron structure for the individual human and mouse *Liprin-α1*, *Liprin-α2*, *Liprin-α3*, and *Liprin-α4* genes was determined by bioinformatic analysis of genomic and mRNA data bases and by RT-PCR. Exons are represented by numbered solid boxes. An arrow above an exon indicates the start of translation and a dot marks a stop codon. Alternatively spliced exons are labeled with an asterisk. The rulers above each gene diagram depict the positions in the UCSC genome sequences.