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Tetraspanins shape the synapse

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ABSTRACT

Tetraspanins are a family of proteins largely expressed in mammals. These proteins share very similar structures and are involved in several biological processes spanning from the immune system to cancer growth regulation. Moreover, tetraspanins are scaffold proteins that are able to interact with each other and with a subset of proteins involved in the regulation of the central nervous system, including synapse formation, function and plasticity.

In this review, we will focus on the analysis of the literature on tetraspanins, highlighting their involvement in synapse formation and function through direct or indirect modulation of synaptic proteins.

1. Introduction

Tetraspanins, also called transmembrane 4 superfamily (TM4SF), represent a large family of proteins highly conserved among species. Phylogenetic studies discovered the presence of tetraspanins in humans, *Drosophila melanogaster, Caenorhabditis elegans,* plants, fungi and amoebas (Huang et al., 2005). Some of them have ubiquitous expression (e.g., CD9, CD63, and CD82) while others, such as CD53 and CD37, are specifically expressed in the immune system with cell type specificity (de Winde et al., 2015; Maecker et al., 1997). Tetraspanins are able to associate dynamically with numerous partner proteins in tetraspanin-enriched microdomains (TEMs), which are organized in the so called “tetraspanin web” (Box 1) (Boucheix and Rubinstein, 2001).

TM4SF proteins consist of 200–300 amino acids and contain four transmembrane domains (TM), two loops exposed in the lumen or extracellularly, one larger and one smaller, also referred to as EC2 and EC1, and two short intracellular N- and C-terminal domains (Boucheix and Rubinstein, 2001; Charrin et al., 2009). EC2 contains a conserved Cys-Cys-Gly sequence plus 2–6 cysteines that are bound through intramolecular disulfide bridges for correct protein folding. EC2 is divided in one conserved and one more variable region (Fig. 1). The conserved part of the EC2 domain, present in almost all tetraspanins (Kitadokoro et al., 2001), contains 3 alpha-helices (A, B and E) and seems to be important for tetraspanin protein dimerization (Kitadokoro et al., 2001), while the variable region of EC2, located between B and E helices, encloses the majority of protein-protein interaction sites of tetraspanins and seems to be determinant for the choice of interaction partners (DeSalle et al., 2010; Stipp et al., 2003).

Moreover, almost all tetraspanins, except for CD81 and NET2, undergo glycosylation at the level of the EC2 domain. The level of glycosylation seems to be important for tetraspanin-integrin interaction (Berditchevski, 2001). For instance, CD82 glycosylation regulates cell motility, and mutations in its N-glycosylation sites enhance the binding with integrin α5 (Ono et al., 1999, 2000).

On the other hand, the function of EC1 is less characterized. Monoclonal antibodies raised against the extracellular portion of tetraspanins usually recognize the EC2 domain but not EC1, suggesting that it is likely inaccessible. Moreover, this small extracellular loop seems not to be involved in tetraspanin binding properties (Stipp et al., 2003). EC1 was reported to be glycosylated in some tetraspanins, such as CD9 (Boucheix et al., 1991). Furthermore, the EC1 of the tetraspanin CD81 is necessary for the correct surface expression of the protein (Masciopinto et al., 2001).

Glycosylation is not the only post-translational modification of tetraspanins. Indeed, tetraspanins can also undergo palmitoylation through the attachment of palmitic acid to intracellular juxtamembrane cysteine residues (Resh, 2006). This post-translational modification of tetraspanins was reported to regulate protein-protein interactions and tetraspanin web assembly (see Box 1) (Hemler, 2003).

The inner portions of tetraspanins are also involved in interaction with other proteins. For example, the C-terminal tail of CD63 was described to interact with the PDZ domain of Syntenin-1 (Latysheva et al., 2006), whereas that of Tspan7 binds to PICK1 (Bassani et al., 2012). The N-terminus of CD53 was found to interact with protein kinase Cβ (Zuidscherwoude et al., 2017).

The expression pattern and the interplay between tetraspanins and

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Box 1
Tetraspanin-enriched microdomains

Tetraspanins are able to cluster together through direct interactions, creating specialized membrane regions named tetraspanin-enriched microdomains (TEMs).

TEMs are described to act as molecular facilitators (Hemler, 2008) by positioning transmembrane proteins and promoting their clusterization to increase the likelihood of interactions.

The most characterized interactors of tetraspanins in TEMs are integrins, Ig family proteins, proteoglycans, growth factor receptors and metalloproteases (Boucheix and Rubinstein, 2001). These interactions occur mainly through the variable region of the EC2 domain (Bassani and Cingolani, 2012).

TEMs have also been shown to present a particularly lipidic composition enriched in cholesterol; however, it was demonstrated that TEMs and lipid rafts, which are known to be present at synapses, are distinct membrane regions (Charrin et al., 2003; Hemler, 2003). Consistently, tetraspanins are able to undergo palmitoylation of the juxtamembrane cysteine residues. This modification, which binds the 16-carbon saturated fatty acid palmitate, promotes the binding of tetraspanins to membranes. Moreover, it increases the interaction between tetraspanins and the other proteins enclosed in the TEMs, as shown in cell lines overexpressing palmitoylation-deficient tetraspanins or depleted in cholesterol with MβCD (Charrin et al., 2003).

The precise nature of TEMs has recently been studied by means of super-resolution microscopy (STED) (Zuidscherwoude et al., 2015). This work, which focuses on the immune system-related tetraspanins CD53, CD81 and CD82, demonstrated that, in contrast to what was thought before, individual tetraspanins form small domains, containing between 4 and 10 molecules of the same tetraspanin. These data are in agreement with the tendency of tetraspanins to be involved in homotypic interactions. Such small domains can then interact with each other, creating the so-called “tetraspanin web” (Boucheix and Rubinstein, 2001).

The domains, studied in immune system cells, had an average size of 120 nm, similar to results obtained previously by electron microscopy (Nydegger et al., 2006) and by STORM (Termini et al., 2014).

Even though the role of TEMs in synapses has never been studied, it is tempting to speculate that the membrane-compartmentalization ability of these domains could participate in the organization of the pre- and/or post-synapse, which are sites at which the correct and dynamic organization of membrane proteins is crucial.

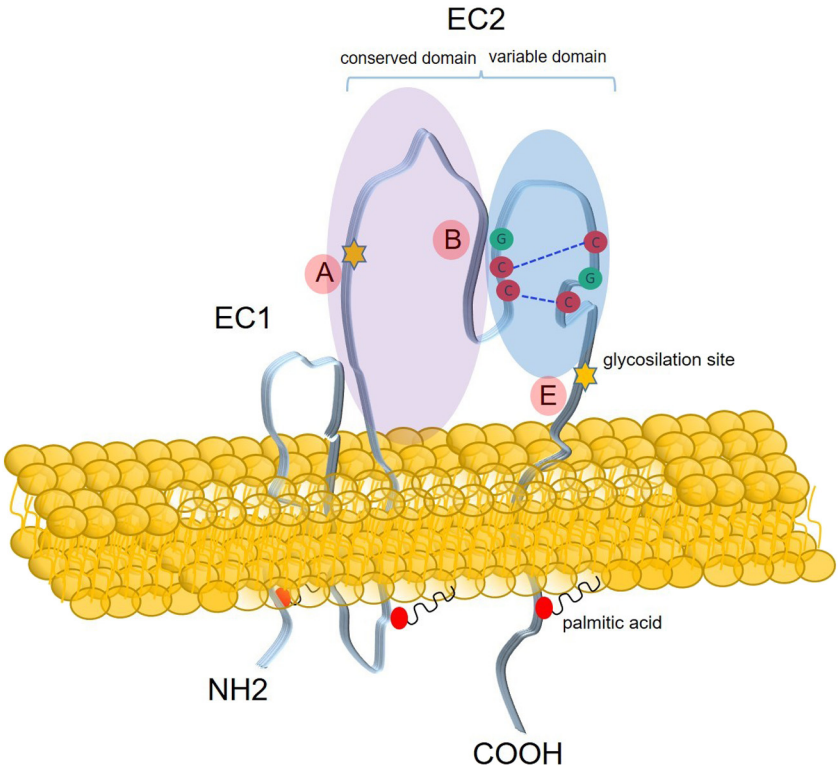

Fig. 1. Tetraspanin structure.
Shared features of tetraspanins are depicted here. Tetraspanins possess 4 conserved transmembrane domains, 2 short intracellular tails and 2 extracellular loops (EC1 and EC2). Disulfide bonds (blue dotted lines), important for correct protein folding, and variable/conserved domains are shown in EC2. Palmitoylation, occurring on cysteines (red dot), and glycosylation sites (yellow stars) are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

other cytosolic or surface-exposed proteins suggest a key role for this superfamily in several biological functions. Indeed, tetraspanins are involved in immune system functions (Jones et al., 2011; van Spriel, 2011), in tumor progression regulation, acting as an inducer or a suppressor of the metastatic process (Hemler, 2014; Romanska and Berditchevski, 2011; Zoller, 2009), in HIV-1 replication (Li et al., 2014), in diabetes (McLaughlin et al., 2016) and in hepatitis (Farquhar et al., 2011).

In this review, we will focus on recent evidence for the involvement of tetraspanins specifically in synaptic functions.

2. Tetraspanins at the synapse

In the last few years, increased attention has been focused on the role of tetraspanins in synapse functions. These proteins are widely expressed in the central nervous system. Indeed, recent studies have