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Visions & Reflections (Minireview)

Teneurins, a transmembrane protein family involved in cell communication during neuronal development

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Abstract. Teneurins are a unique family of transmembrane proteins conserved from *Caenorhabditis elegans* and *Drosophila melanogaster* to vertebrates, in which four paralogs exist. In vertebrates, teneurin expression is most prominent in the developing brain. Based on their distinct, complementary expression patterns, we suggest a possible function in the establishment of proper connectivity in the brain. Functional studies show that teneurins can stimulate neurite outgrowth, but they might also play a role in axon guidance as well as in target recognition and synaptogenesis, possibly mediated by homophilic interactions. Though teneurins are transmembrane proteins, there is evidence that the intracellular domain has a nuclear function, since it can interact with nuclear proteins and influence transcription. Therefore, we speculate that teneurins might be processed by proteolytic cleavage (possibly regulated intramembrane proteolysis), which is triggered by homophilic interactions or, alternatively, by the binding of a still unknown ligand.

Keywords. Neural circuit, regulated intramembrane proteolysis, brain, nervous system, odz, ten-m.

Teneurins – an emerging family of transmembrane proteins

The goals of this article are to introduce the teneurin family of transmembrane proteins and to discuss possible functions based on gene expression patterns and cell culture studies. Additionally, we will summarize evidence indicating that teneurins could be a novel substrate for regulated intramembrane proteolysis (RIP).

Originally discovered in *Drosophila (ten-m* and *ten-a)*, the teneurin protein family is conserved from *Caenorhabditis elegans (ten-1)* to vertebrates, in which four paralogs exist (teneurin-1 to -4 or odz-1 to -4). Their distinct domain architecture is highly conserved between invertebrate and vertebrate teneurins, particularly in the extracellular part [1, for reviews see refs. 2, 3]). The large C-terminal extracellular domain is composed of eight tenascin-type, epidermal growth factor (EGF)-like repeats, a region of conserved cysteines and 26 YD-repeats, which are not present in any other eukaryotic protein. The N-terminal intracellular domain of vertebrate teneurins contains two EF-hand-like calcium-binding motifs and two polyproline domains involved in protein-protein interactions, followed by a single-span transmembrane domain (Fig. 1a).

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A role in establishing and maintaining neuronal connections?

Teneurin expression in vertebrates is most prominent in the brain and has been studied mainly in the chicken and mouse [4–12]. In addition, one study reports the distribution of the teneurin-3 and -4 homologs in zebrafish [13]. All of these studies agree that each of the four teneurins is expressed most strongly in a subset of neurons or in subregions of the developing brain, and that these patterns are strikingly distinctive and largely non-overlapping. In the chicken embryo, teneurin-1 is expressed in brain regions and nuclei belonging to the tectofugal pathway, whereas teneurin-2 is present in the thalamofugal pathway, coinciding temporally with periods of target recognition and synaptogenesis (Fig. 1b) [8]. A recent study examined teneurin expression in the developing mouse brain and revealed defined patterns in the cortex and in the corresponding thalamic nuclei to which they connect. Moreover, the four murine teneurin paralogs exhibit unique, complementary expression patterns that are temporally regulated during development [5].
Taken together, these results suggest that teneurins could be involved in the following aspects of neuronal connectivity.

Neurite outgrowth. Cell culture studies provide evidence that teneurins promote neurite outgrowth. Teneurin-2 overexpressed in neuronal cells enhances neurite elongation and causes enlarged growth cones, and it localizes to actin-containing filopodia [9]. Accordingly, chick dorsal root ganglia explants plated on recombinant teneurin-1 YD-repeats dramatically increase the number of outgrowing neurites [6]. In addition, recent data suggest that the most C-terminal part of teneurin-1, which is called TCAP-1, is able to enhance growth and fasciculation of neurites in cell culture and in brain slices [14].

Axon guidance. Teneurins could either act as receptors for guidance molecules, or may provide cues for other axons, or a combination of both. Cytoskeletal rearrangements are crucial for axon pathfinding, and the interaction of the teneurin-1 intracellular domain with the adaptor protein CAP/Ponsin represents a possible link between these transmembrane proteins and the actin cytoskeleton [15]. Conversely, a cleaved teneurin extracellular domain (see below), which seems to be deposited in the extracellular matrix, might act as a guidance cue for adjacent axons [9]. Axons can also be guided through fasciculation, and the knockdown of the teneurin homolog *ten-1* in *C. elegans* leads to defasciculation and aberrant pathfinding [16].

Target recognition and synaptogenesis. Once axons reach their appropriate target, neuronal connections need to be established and stabilized. One way that teneurins could be involved is suggested by the fact that members of the teneurin family can form homo- or heterodimers [17], allowing migrating neurons to identify targets that are also expressing teneurins. Homophilic interaction is further supported by the detection of teneurin-binding activity in tissue sections using a labeled extracellular domain, which largely coincides with teneurin expression [12].

The human teneurin-1 gene is located on Xq25, a region to which several X-linked mental retardation syndromes are mapped. Because of its neuronal expression and probable role in axonal connectivity, teneurin-1 represents a promising candidate gene.
To date, teneurin expression has been studied primarily during embryonic or early postnatal development, and little is known about possible functions of teneurins in the adult brain. In one investigation, rat neurestin (corresponding to teneurin-2) was found in olfactory sensory neurons, which regenerate throughout adulthood. In these cells, neurestin expression normally ceases shortly after birth, but is re-induced after lesioning the olfactory epithelium [7].

Processing of teneurins: a new substrate for RIP?

Teneurins may represent a novel substrate for RIP. Such a signaling mechanism would allow the teneurins to act not only as extracellular adhesion molecules, but also as signaling molecules themselves: reception of a stimulus could lead to cleavage and release of the intracellular domain (Fig. 1c). In fact, it was shown that the intracellular domains of teneurin-1 and -2 localized to the nucleus when transfected into cells [15, 18]. A reporter assay using a transcription activator fused to the teneurin-2 N-terminus demonstrated increased transcriptional activity when transfected into cells constitutively expressing the teneurin-2 extracellular domain, suggesting that release and nuclear localization of the intracellular domain occur after homophilic interaction on the extracellular side. In this case, activation of reporter transcription could either result from a signal initiated by teneurin clustering on one cell, or from an interaction between two teneurin-expressing cells. In addition, overexpressed teneurin-2 intracellular domain colocalized with PML bodies and affected zic-mediated transcription [18]. Finally, interaction of the teneurin-1 intracellular domain with MBD-1, a methyl-CpG-binding protein, could connect teneurin signaling and transcriptional regulation [15].

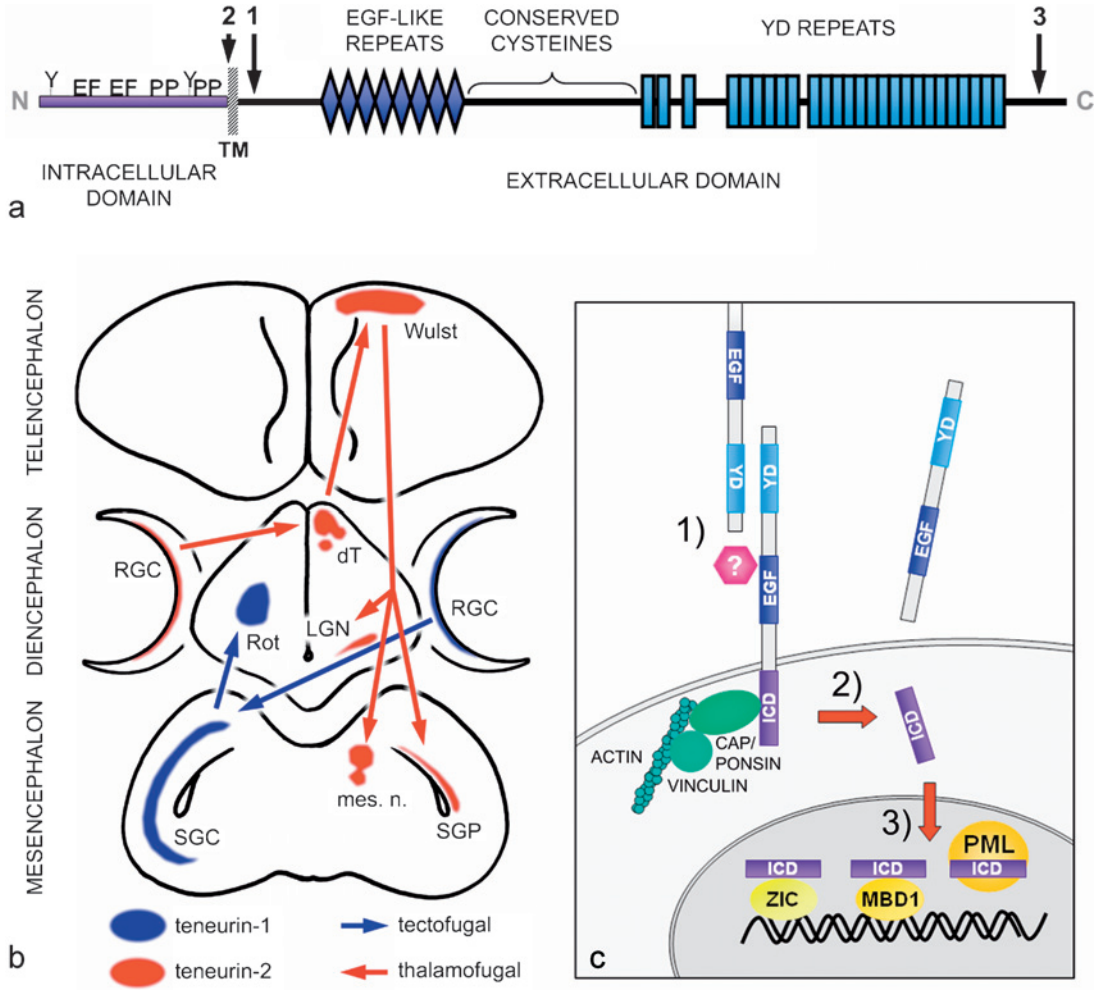

Figure 1. **(a)** Domain organization of teneurins. Teneurins have an N-terminal intracellular domain, a single transmembrane domain (TM) and a large extracellular domain. The intracellular domain contains two EF-hand-like motifs (EF), two polyproline motifs (PP) and several conserved tyrosines (Y) that are predicted phosphorylation sites. The extracellular domain consists of eight tenascin-type EGF-like repeats, a region of conserved cysteines and the YD-repeats. Arrows with numbers indicate postulated cleavage sites: 1) cleavage site for ectodomain shedding (a prerequisite for intramembrane proteolysis), 2) transmembrane cleavage site resulting in the release of the intracellular domain, and 3) an additional cleavage site releasing a teneurin C-terminus associated peptide, which is proposed to have neuromodulatory activity. **(b)** Expression pattern and neuronal connectivity. Teneurin-1 is expressed at key sites along the tectofugal visual pathway, including retinal ganglion cells (RGC), the stratum griseum centrale (SGC) of the optic tectum, and the rotund nucleus (Rot) in the diencephalon. In contrast, teneurin-2 is present in the thalamofugal pathway: RGCs connect to nuclei in the contralateral dorsal thalamus (dT) and further to the Wulst, which in turn projects back to the lateral geniculate nucleus (LGN), various mesencephalic nuclei (mes. n.) and the stratum griseum periventriculare (SGP) of the optic tectum. **(c)** Teneurin signaling hypothesis. 1) A signal, which can be either a homophilic interaction or binding of a yet unknown ligand, triggers the mechanism. 2) After shedding the extracellular domain, the remaining teneurin part becomes a substrate for intramembrane proteolysis, resulting in the release of the intracellular domain. 3) Nuclear translocation of the intracellular domain, where it associates with PML bodies and nuclear proteins such as MBD-1, a methyl-CpG-binding protein, and Zic, a transcription factor family implied in neuronal development. Additionally, CAP/Ponsin can bind to the intracellular domain, which could represent a link to the actin cytoskeleton which is important for neurite outgrowth.

RIP was discovered in studies of the sterol response, where SREBP, a transcription factor residing in the endoplasmatic reticulum (ER) membrane, is cleaved by the intramembrane protease Site-2 and thereby released to translocate to the nucleus [19]. Four families of intramembrane proteases and dozens of substrates have since been identified [reviewed in refs. 20–22]; the best known substrates are Notch and APP, both of which are cleaved by presenilin. Transmembrane proteases exhibit specificity for the orientation of the substrate in the membrane. Presenilin cleaves type I transmembrane proteins, whereas Site-2 protease and signal peptide peptidase (SPP) and SPP-like proteases cleave type II transmembrane proteins and are therefore candidate proteases for teneurin processing. In addition, cleavage by Rhomboid was recently found not to be restricted to type I transmembrane proteins [23]. A

common feature of the RIP mechanism is that a cleavage on the extracellular side has to occur prior to the intramembrane cleavage. Indeed, teneurin-2 can be processed at a furin site between the transmembrane domain and the EGF-like repeats, resulting in shedding of the ectodomain [9]. This furin cleavage site seems to be conserved in teneurin-3 and -4, but probably not in teneurin-1.

Outlook

More work will be needed to prove that RIP and nuclear localization of the intracellular domain take place, and to identify the proteases responsible for the cleavage. It is conceivable that such a cleavage is tightly regulated during development and takes place only in response to a specific stimulus, e.g., by the recognition of a guidance cue or target region by an outgrowing axon. Another possibility to obtain a soluble intracellular domain could be alternative splicing resulting in a teneurin variant that ends before the transmembrane domain.

Their distinctive localization in the developing brain suggests that expression of each teneurin must be intricately regulated by a complex network of transcription factors that govern patterning. Therefore, it will be interesting to identify and investigate the factors that regulate teneurin expression and which genes are, in turn, regulated by teneurins. Teneurins were found to be differentially expressed in the caudal and rostral cortex by microarray analysis together with many other genes, most of which belong to two groups: transcription factors and extracellular signaling molecules [24]. The teneurins might combine both of these functions in a single molecule.

To prove that teneurins are indeed required for proper connectivity and regionalization in the brain and to investigate a potential role in neuronal regeneration, more sophisticated neurobiological studies will be required. Furthermore, analysis of the teneurin-1 gene in X-linked mental retardation patients would reveal if mutations in this gene are responsible for the disease and confirm a role for teneurins in brain development and axonal connectivity.

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