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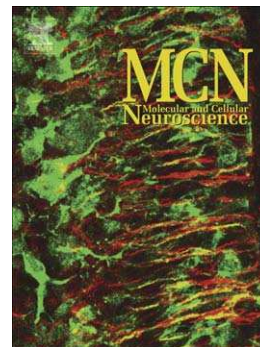
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THE ROLE OF GPI-ANCHORED AXONAL GLYCOPROTEINS IN NEURAL DEVELOPMENT AND NEUROLOGICAL DISORDERS

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- 2. Families of morphoregulatory molecules.**
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Abstract:

This review article focuses on the Contactin (CNTN) subset of the Immunoglobulin supergene family (IgC2/FNIII molecules), whose components share structural properties (the association of Immunoglobulin type C2 with Fibronectin type III domains), as well as a general role in cell contact formation and axonal growth control. IgC2/FNIII molecules include 6 highly related components (CNTN 1-6), associated with the cell membrane via a Glycosyl Phosphatidyl Inositol (GPI)-containing lipid tail. Contactin 1 and Contactin 2 share ~50 (49.38)% identity at the aminoacid level. They are components of the cell surface, from which they may be released in soluble forms. They bind heterophilically to multiple partners in *cis* and in *trans*, including members of the related L1CAM family and of the Neurexin family Contactin-associated proteins (CNTNAPs or Casprs). Such interactions are important for organising the neuronal membrane, as well as for modulating the growth and pathfinding of axon tracts. In addition, they also mediate the functional maturation of axons by promoting their interactions with myelinating cells at the nodal, paranodal and juxtapanodal regions. Such interactions also mediate differential ionic channels (both Na⁺ and K⁺) distribution, which is of critical relevance in the generation of the peak-shaped action potential. Indeed, thanks to their interactions with Ankyrin G, Na⁺ channels map within the nodal regions, where they drive axonal depolarization. However, no ionic channels are found in the flanking Contactin1-containing paranodal regions, where CNTN1 interactions with Caspr1 and with the Ig superfamily component Neurofascin 155 in *cis* and in *trans*, respectively, build a molecular barrier between the node and the juxtapanode. In this region K⁺ channels are clustered, depending upon molecular interactions with Contactin 2 and with Caspr2.

In addition to these functions, the Contactins appear to have also a role in degenerative and inflammatory disorders: indeed Contactin 2 is involved in neurodegenerative disorders with a special reference to the Alzheimer disease, given its ability to work as a ligand of the Alzheimer Precursor Protein (APP), which results in increased Alzheimer Intracellular Domain (AICD) release in a γ -secretase-dependent manner. On the other hand Contactin-1 drives Notch signalling activation via the Hes pathway, which could be consistent with its ability to modulate neuroinflammation events, and with the possibility that Contactin 1-dependent interactions may participate to the pathogenesis of the Multiple Sclerosis and of other inflammatory disorders.

Adhesion molecules and neural developmental events: the definition of morphoregulatory molecules.

The development of the nervous tissue requires the coordination of several processes, including precursor proliferation and migration, neurite growth and fasciculation, axonal pathfinding control and synaptogenesis (see for instance Gerrow and El-Husseini, 2006; Mitsuhashi and Takahashi, 2009; Borodinsky et al., 2015; Missaire and Hindges, 2015). Most of them implicate interactions of neuronal surface glycoproteins with components of the adjacent cell surfaces or of the extracellular matrix (Schmidt and Rathjen, 2010; Giger et al., 2010; Barros et al., 2011; Vitriol and Zheng, 2012; Hirano and Takeichi, 2012; Frei and Stoeckli, 2014; Petrovic and Shumker, 2015). The molecular players in such events fulfil the operational definition of adhesion molecules since a relevant role for them is to promote and/or stabilize cell interactions. While being of structural relevance, such interactions may also have a more functional meaning, since cell adhesion is also a source of signals known to modulate events involved in nervous tissue development (Ebel et al., 2014; Hildebrandt and Dityatev, 2015), which in turn arise from the physicochemical interactions among such molecules. On this basis, such molecules fulfil the operational definition of morphoregulatory molecules proposed in earlier studies (Edelman, 1992; Edelman and Jones, 1997).

In this review, we consider the properties of a family of adhesion molecules whose expression is tightly regulated during development, consistent with their roles during both early neurogenesis and later stages of neural differentiation, and whose functions extend beyond simple adhesion, encompassing the generation and modulation of morphoregulatory signalling that controls the molecular composition and organisation of the cell surface according to context. We also consider emerging evidence that changes in the expression of these molecules underlie the genesis of specific neurological disorders.

Families of morphoregulatory molecules.

Morphoregulatory molecules may belong to distinct families of adhesion receptors, the main grouping being the Cadherins (Basu et al., 2015; Friedman et al., 2015; Gartner et al., 2015), the Integrins (Gardiner, 2011) and the Immunoglobulin superfamily (IgCAM, Maness and Schachner, 2007). Based on their interactions, these molecules may be differentially involved in distinct aspects of neural developmental control (Yogev and Shen, 2014). Broadly speaking, adhesion mediated by Cadherins is strong, overcoming that of other adhesion molecules (Thiery et al., 2012). Classically, Cadherins are thought to mediate cell-cell interactions, while integrins mediate those between cell and matrix, though this simple classification is confounded by complex interplay among the molecules (Weber et al., 2011; Mui et al., 2016). In any case, the critical developmental role for such molecules depends upon their ability to mediate homo-or heterophilic interactions. For example, in the earliest developmental stages, strong homophilic calcium-dependent cell interactions involving the function of Cadherin family components contribute to the control of events of key ontogenetic relevance such as germ layer separation (Stepniak et al., 2009; Hirano and Takeichi, 2012; McKeown et al., 2013; Barriga and Mayor, 2015; Hayashi and Takeichi, 2015; Duband et al., 2015). However, it is also worth mentioning that members of the same family may mediate the control of later developmental events as neurite growth (Gartner et al., 2012; 2015; Hayashi et al., 2014; Stoeckli, 2014). The functional complexity of these events and their articulated role in neural development is further supported by the evidence that components of distinct gene families may contribute to such events, which may involve either adhesive as the Immunoglobulin (Ig) supergene (Maness and Schachner, 2007) or repellent as the Semaphorin (Pasterkamp et al., 2012; Jongbloets and Pasterkamp, 2014; Battistini and Tamagnone, 2016) families components. As far as axonal growth is concerned, such a molecular and functional complexity suggests that regulating the expression of the genes encoding such molecules represents an aspect of critical functional relevance (Yogev and Shen, 2014).

Axonally expressed adhesive glycoproteins of the immunoglobulin supergene family (Igsf).

This review article focuses on those members of the Immunoglobulin supergene family (IgSF), which are composed of Ig C2-type (IgC2) domains at their N-terminus and Fibronectin type III (FNIII) domains grouped in tandem in the membrane-proximal region. Because of this overall organization, those adhesive glycoproteins may be collectively denominated IgC2/FNIII molecules, and represent a typical model for adhesive/morphoregulatory molecules expressed at the axonal level. The IgC2 and FNIII domains have similar structural cores (Cota et al., 2001) and likely share a common evolutionary origin. This family undergoes complex interactions with members of the same and different families (Özkan et al., 2013) and the genomic complexity of family members reflects organismal complexity (Vogel and Chothia, 2006), suggesting roles in elaborating developmental complexity.

The contactin family of cell adhesion molecules (CNTNs).

Like many cell surface molecules, members of the IgC2/FNIII family can be found anchored to the membrane via a typical transmembrane domain, or via a Glycosyl Phosphatidyl Inositol (GPI)-containing lipid tail. In this review we focus on the latter, specifically the Contactins.

Contactins were named to reflect their preferential location at sites of cell to cell contact (Ranscht, 1988). This subfamily comprises six different members (CNTN1-CNTN6; Shimoda and Watanabe, 2009), which share the same overall organization, including 6 N-terminal IgC2 domains, associated with 4 C-terminal FNIII repeats, followed by a hydrophobic C-terminal aminoacid sequence, characteristic of most GPI-linked proteins, the processing of which results in their lipid anchorage (Low, 1989). Where it has been studied, the Contactins are secreted from cells as well as being found at the cell surface (Ruegg et al., 1989; Furley et al., 1990; Gennarini et al., 1991), apparently the result of specific secretion processing rather than cleavage from the cell surface (Ruegg et al., 1989).

Contactins share in the order of 45-65% protein sequence identity (Yoshihara et al., 1995;

Ogawa et al., 1996). The preservation of a clear overall topology of domain organisation suggests that this family arose as the result of a gene duplication event, which occurred after divergence of the ancestral gene from its nearest relatives, the transmembrane-bearing L1 family components, DsCAMs and SideKicks (Yamagata and Sanes, 2012), reflected in the presence of just one member of each of these families in *Drosophila* (Faivre-Sarrailh et al., 2004; Yamagata, et al., 2002; Schmucker et al., 2000; Bieber et al., 1989).

Since the role of CNTNs 3-6 are considered elsewhere in this issue, here we focus specifically on CNTNs 1 and 2, the first discovered and consequently most widely studied. However, we begin with a brief overview reflecting on the general properties of the subfamily. The Contactins, notably CNTN1 and 2, were identified initially as axonal glycoproteins expressed during neural development that had the property of stimulating or inhibiting axonal growth according to context (Furley et al., 1990; Gennarini et al., 1991; Durbec et al., 1992; Buttiglione et al., 1996; 1998). The other striking feature of such molecules is in their differential developmental profile. Indeed CNTN2 was initially named TAG-1 (Transient Axonal Glycoprotein), being expressed relatively early in neuronal maturation (Dodd et al., 1988), when it appears to be engaged in neuronal polarisation (Namba et al., 2014), while CNTN1 was found associated with more mature stages (Gennarini et al., 1989; Perrin et al., 2001; Stoeckli, 2010). Subsequently, it has become clear that while some members are expressed in most neurons at some stage (for example, late stage expression of CNTN1 is common to most if not all neurons), others are restricted to subsets of neurons (CNTN5, for instance, is preferentially expressed in central auditory pathways and its loss leads to auditory deficits; Li et al., 2003). The differential expression of these related proteins gives exquisite control of the construction of neuronal circuits, as is beautifully shown in the case of the developing retina, where the family, together with its close relatives Sidekicks and Dscams, is found to play a critical role in the definition of laminar specificity, dictating both the location of cell bodies and the development of axons tracts in specific sublaminae (Yamagata et al., 2002; 2008; Yamagata and Sanes, 2012).

Mechanistically, laminar specificity in the retina is thought to be mediated in part by homophilic interactions among the Contactins (and among Sidekicks and Dscams), which are expressed by largely non-overlapping subsets of distinct retinal subpopulations and project to the same sublaminae. Thus, differential expression of the genes encoding these homophilically binding surface proteins allows the sorting of neurites expressing the same proteins, effectively constituting an IgSF code for layer-specific neurite targeting (Yamagata and Sanes, 2012). Whether this sorting is the result of selective fasciculation or selective synapse formation, or some combination of these is unclear, as Contactins are known to be involved in both processes (Sittaramane et al., 2009; Walsh & Doherty, 1997; Takeda et al., 2003; Murai et al., 2002).

However, although CNTN2 and CNTN4 bind homophilically (Furley et al., 1990; Rader et al., 1993), CNTN1, 3 and 5 do not (Yamagata & Sanes, 2012). Nonetheless, neurites expressing the latter still project together with other neurites expressing the same Contactin, implying that these may promote laminar specificity via heterophilic interactions. Indeed, many heterophilic partners of Contactins have been identified, varying according to family member, but in some cases these partners fall into families themselves. Notable among these are the related L1 family, comprising 4 members (L1/NgCAM, NrCAM, Neurofascin and CHL1; Maness & Schachner, 2007), Amyloid Precursor Proteins (APP, APLP1, APLP2; Ma et al., 2008; Osterfield et al., 2008) and the Contactin-associated proteins (CNTNAP/Caspr1-5; Gollan et al., 2002; 2003; Horresh et al., 2008; 2010; Arroyo et al., 2001; Traut et al., 2006). These interactions can occur in trans or in cis (in some cases both) and provide the means by which the GPI-anchored proteins can interact with the cytoskeleton. The significance of these will be discussed in detail in relation to CNTN1 and 2 below.

Overall, the above data indicate an important role for the coordinated expression of Contactins and their associated molecules in the organisation of neuronal perikaria, in the modulation of axonal growth and in synapse formation, dependent upon homo- and heterophilic interactions. It is clear, however, that the Contactins continue to play a role at later stages, not only

in neurons but also in myelinating cells and other glia. Most significantly, the Contactins, in molecular complex with L1 family members and Casprs, play a critical role in the organisation of interactions between neurons and myelinating cells at the level of the nodal, paranodal and juxtapanodal regions, critically governing the positioning of both Na⁺ and K⁺ channels to the node and juxtapanode respectively (Falk et al., 2002; Labasque and Faivre-Sarrailh, 2010; Buttermore et al., 2013; Salzer, 2015), a separation which is vital to the mechanisms leading to action potential generation and conduction.

Much of the focus of the above has been on the role of Contactins in post-mitotic events, but increasing evidence indicates that they have roles in the modulation of proliferating precursor behaviour. This appears to take the form either of Contactins being markers on mature cells that are detected by precursors as feedback signals to trigger or inhibit differentiation (Bizzoca et al., 2003; Xenaki et al., 2011), or that the Contactins are themselves present in the proliferating precursors as part of the apparatus modulating precursor behaviour (Ma et al., 2008; Bizzoca et al., 2012; Okamoto et al., 2013).

Physicochemical and molecular features of CNTN-dependent interactions

The breadth of the known functions of Contactins is reflected in the complexity of the molecular interactions in which they are involved, which in turn may be mapped to the different domains from which these molecules are built. In molecular terms such complexity may be addressed by studying the interactions of Contactin family components with their ligands, in particular with the Receptor Protein Tyrosine Phosphatases (RPTP), mostly with their V subgroup components RPTP ζ and RPTP γ (Johnson and Van Vactor, 2003; Mohebiny et al., 2013; Nikolalenko et al., 2016) and with their submolecular domains. The extracellular regions of RPTP are built of a Carbonic Anhydrase (CA) domain, associated with Fibronectin type III domains. On the other hand its intracellular region includes two phosphatase domains. This domain complexity is associated with specific molecular interactions and differential expression profiles. RPTP ζ is expressed on glia (astrocytes and oligodendrocytes) while RPTP γ in neurons. They have also

different binding partners: RPTP ζ has been found as a relevant binding partner for Contactin1 while RPTP γ has been found to bind CNTN 3, 4, 5 and 6 (Bouyain and Watkins, 2010). As for the Contactin interacting domains, in the case of CNTN1 its IgC2 domains 2, 3 and 4, adopt a horseshoe-like conformation and are involved in binding to RPTP ζ (Lamprianou et al., 2011). However, for other interactions, simple division of the molecule into binding cassettes may be involved, as is evident in the recently described binding of CNTN1 to Caspr2 (see below).

Since much of the work on the early roles of contactins, and on their roles in dictating the distribution of ion channels in myelinated axons, relates to Contactins 1 and Contactin 2, this review article focuses on these proteins, while the functional roles of Contactins 3 to 6 have recently been explored (Mohebiany et al., 2014; Zuko, 2011), and will be part of a different review article from Burbach and coworkers in this same Special Issue.

The Contactin 1-2 glycoproteins.

Contactin 1 is the name given to an axonal adhesive glycoprotein originally denominated Contactin or F11 in chick (Ranscht, 1988; Brummendorf et al., 1989) and F3 in rodents (Gennarini et al., 1989 a,b), while Contactin 2 was formerly denominated Transient Axonal Glycoprotein (TAG-1) in rodents (Yamamoto et al., 1986; Dodd et al., 1988; Furley et al., 1990), Axonin1 in chick (Ruegg et al., 1989; Zuellig et al., 1992) and TAX-1 in humans (Tsiotra et al., 1993; Hasler et al., 1993).

Contactins 1 and 2 are closely related to each other in structural terms, sharing a nearly 50% identity at the aminoacid level (Figure 1) and a similar overall organization (Figure 2A). Classically, both molecules have been shown to stimulate the outgrowth of neurites when presented as substrates (Rathjen et al., 1987; Furley et al., 1990; Stoeckli et al., 1991; Gennarini et al., 1991) or as soluble molecules (Durbec et al., 1992) and antibodies to them cause defasciculation (e.g. Chang et al., 1987). As noted above, CNTN2 can bind homophilically to cause cell and bead aggregation (Felsenfeld et al., 1994; Rader et al., 1993; Yamagata & Sanes, 2012), which is not true of CNTN1 which instead binds heterophilically (see Figure 2). However,

it is unclear whether homophilic binding occurs in *cis* or *trans* (Mortl et al., 2007) and several studies indicate that CNTN2 neurite outgrowth stimulation critically involves heterophilic interactions with other CAMs, including $\beta 1$ integrins, L1/NgCAM and NrCAM (Kuhn et al., 1991; Felsenfeld et al., 1994; Stoeckli et al., 1996; Lustig et al., 1999). Thus, both CNTN1 and CNTN2 stimulate axon growth using heterophilic interactions.

Importantly, the heterophilic interactions of CNTN1 and CNTN2 are both similar and distinct (Figure 2B), including shared partners such as L1 and NrCAM, but also interactions that are specific to each, such as Notch with CNTN1 (Hu et al., 2003) and CNTN2 with APP (Ma et al., 2008). In some cases, although apparently not shared, interactions are with sister molecules; for example CNTN1 binds to Caspr1 while CNTN2 binds to Caspr2 (Poliak et al., 2003; Lu et al., 2016), although recent data has drawn into question the exclusivity of these interactions, suggesting that CNTN1 also binds Caspr2 (Rubio-Marrero et al., 2016). The interaction of Caspr1 with CNTN1 was found to be essential for the association of this complex with RPTP β and for transport of CNTN1 to the cell surface (Bonnon et al., 2003), and therefore for myelination. The newly reported binding of CNTN1 to Caspr2 (Rubio-Marrero et al., 2016), greatly increases the complexity of these interactions, both in myelination and in other Contactin-dependent functions.

The functional consequences of this variety of interactions is by no means fully understood, but it is clear that the combination of interactions involved and the relative disposition of the components can change the functional outcome significantly. For example, while a *cis* interaction between CNTN1 and NrCAM stimulates the extension of dorsal root ganglion (DRG) sensory axons on an RPTP β substrate (Sakurai et al., 1997; 2012), cerebellar granule neuron axon extension is inhibited by a Tenascin-R substrate for which CNTN1 is the neuronal receptor (Pesheva et al., 1993), indicating that the role played by Contactins varies according to cellular and molecular context. This is very much reflected in phenotypes arising from both loss of function and gain of function experiments *in vivo* which will be considered below.

Contactin 1 developmental role. Contactin 1 null mutant mice (Cntn1ko; Berglund et al., 1999) exhibited a cerebellar phenotype in which cerebellar volume was reduced by ~25% and the parallel fibres of granule cell neurons were misoriented, indicating an effect on axon guidance, but not growth. There was no evidence of gross parallel fibre defasciculation, but axons within bundles were slightly less compact, and mutant granule neurons in culture grew completely defasciculated. Dendritic elaboration from both granule cells and from Golgi neurons were also severely affected, although that of Purkinje neurons was not. However, the cerebellar size was reduced and the parallel fibres were misoriented. In addition, an axonal defasciculation occurred in mutant mice and Purkinje neuron axons exhibited aberrant morphologies. Therefore the cerebellar circuitry was heavily perturbed in Contactin 1 null mice and functionally this morphological phenotype resulted in severe ataxia and postnatal lethality (Berglund et al., 1999).

It seems unlikely that Cntn1 KO lethality is due simply to cerebellar dysfunction and indeed further analysis revealed myelination defects in the peripheral (Boyle et al., 2001) nervous system, specifically changes in the organisation of nodal and paranodal regions of the node of Ranvier. Normally, CNTN1 is located in the PNS in the paranode, the septate-like junction that separates the node, where the Na⁺ channels required to propagate the action potential are located, from the juxtaparanode where the Shaker-type voltage-gated K⁺ channels (Kv1.1/1.2) required for re-polarisation reside (Figure 3). Loss of CNTN1 widens the gap between the axolemma and the myelin loop at the paranode and disrupts the junction resulting in the mislocalisation of the Kv1.1/1.2 channels into the paranode and to some extent also in the node, together with a concomitant reduction in conduction velocity. Within the paranode, CNTN1 loss leads also to loss of its cis-binding partner Caspr1 on the axolemma and of their trans, L1-like binding partner, Neurofascin 155 (NF-155) from the myelin loops. This is mirrored in Caspr1 null mice, which also have defects in conduction velocity, mislocalised Kv1.1/1.2 channels and, importantly, an absence of CNTN1 on the axolemma (Bhat et al., 2001), consistent with CNTN1 and Caspr1 being mutually dependent for trafficking to the cell surface (Peles et al., 1997). Caspr1 mutants also

display ataxia and have similar swellings in their Purkinje cell axons, which appears to be due to the aberrant accumulation of mitochondria and smooth endoplasmic reticulum at the nascent paranodes, but exhibit no defects in parallel fibre orientation (Pillai et al., 2007; 2009). Together this indicates that although CNTN1 and Caspr1 co-operate in the organisation of the paranode, CNTN1 functions without Caspr1 to establish parallel fibre orientation and, moreover, that the latter defect is unlikely to contribute to the lethality seen in these mutants.

Contactin 2 developmental role. Contactin 2 expression was first characterised by antibody staining in developing rodents, where it is seen transiently on subsets of neurons in the developing spinal cord and brain, associated with the early stages of neuronal differentiation and axonogenesis (Yamamoto et al., 1986; Dodd et al., 1988). Most famously it is expressed by commissural spinal relay neurons as they extend to the floor plate, but it is turned off after these axons turn orthogonally to ascend to the brain after crossing the midline, after which they instead express L1/NgCAM (Dodd et al., 1988; Karagogeos et al., 1991) and CNTN1 (Perrin et al., 2001). However, CNTN2 is also seen transiently on a number of other developing axon types, including spinal motor and sensory axons, retinal ganglion cell axons, axons in the olfactory nerve, the cortex, midbrain and cranial nerves (Yamamoto et al., 1986; Dodd et al., 1988). It is not on all axons, for instance it is never seen in sympathetic neurons, on Purkinje cells or in ipsilaterally projecting spinal relay neurons, and in some tracts, the corpus callosum for example, only subsets of axons are labelled (Dodd et al., 1988; Yamamoto, 1986; Stottmann and Rivas, 1998). In some regions, notably the cerebellum, it is also found on the cell bodies of migrating granule neuron precursors (Pickford et al., 1989; Furley et al., 1990; Yamamoto et al., 1990), including on cells which label with cell cycle markers (Xenaki et al., 2011), indicating that it can be found on proliferating precursors.

Knockout of *Cntn2* (*Cntn2ko*) in mice has not revealed evidence of a critical role in some of the key systems in which it has been implicated from the above-described expression profile. Two independent *Cntn2ko* mutants are each homozygous viable and display no gross phenotypes

in the brain or spinal cord (Fukamauchi et al., 2001; Poliak et al., 2003), although a predisposition to seizures (Fukamauchi et al., 2001) and failure to gain weight in response to high fat diet (Buchner et al. 2012) have been reported. Extensive axon tracing analysis of commissural axon trajectories revealed no evidence (Yeomans, Kiernan and Furley, unpublished) of the dramatic pathfinding defects found using a variety of acute function blocking methodologies in chick (Stoeckli & Landmesser, 1995), where CNTN2 has been implicated in interactions with midline-expressed NrCAM in the switching of C axon sensitivities to floor plate-derived repellants (Stoeckli et al., 1997; Pekarik et al., 2003), one of which has been identified to be Semaphorin3B (Nawabi et al., 2010). Similarly, antisense RNA and antibody blocking techniques have suggested a role for CNTN2 in parallel fibre alignment or extension in cerebellar granule neurons (Baeriswyl & Stoeckli, 2008; Wang et al., 2011), yet *Cntn2ko* animals display only subtle effects on granule neuron progenitor migration (Xenaki et al., 2011). While some of these differences may be due to the species used for investigation (chick vs rodent), more likely is that compensatory mechanisms operate in germline nulls that are not able to function in acute knockdown experiments, as has recently been demonstrated in zebrafish (Rossi et al., 2015).

Nonetheless, *Cntn2ko* animals do exhibit subtle phenotypes that have given important insight into CNTN2 function. Consistent with earlier work in chick that used function blocking antibodies to disrupt CNTN2 function in ovo (Shiga et al., 1997; Perrin et al., 2001), Law et al. (2008) demonstrated that nociceptive sensory fibres from the DRG enter the dorsal horn of the spinal cord prematurely in *Cntn2ko* mice. Although Perrin et al. (2001) had interpreted this to represent premature defasciculation of nociceptive fibres from the dorsal root entry zone, in vitro analysis of DRG from *Cntn2ko* animals revealed these fibres to be insensitive to repellants in the spinal cord that normally repel wild type sensory axons, including Semaphorin3A to which *Cntn2ko* growth cones showed reduced sensitivity (Law et al., 2008), as had been previously shown for growth cones lacking L1 (Castellani et al., 2000). Functionally, *Cntn2ko* animals also exhibit behavioural deficits in sensori-motor gating and motor co-ordination (Savvaki et al., 2008).

Significance of Contactin 2 interactions. Subsequent studies revealed that like L1 (Castellani et al., 2002, 2004), CNTN2 forms a complex with neuropilin1 (NRP1) on the cell surface (Dang et al., 2012); together with PlexinA4, NRP1 is a core component of the Sema3A receptor. However, unlike L1, CNTN2 was not required for NRP1 endocytosis upon Sema3A binding, but instead facilitated the intracellular trafficking of NRP1 and PlexinA4 into lipid raft-enriched vesicles away from co-endocytosed L1, which instead is trafficked into Rab11⁺ recycling endosomes (Dang et al., 2012; Dang & Furley, unpublished). In the absence of CNTN2, Sema3A-induced CRMP2 phosphorylation, a key signalling readout of PlexinA4 activation (Uchida et al., 2005) does not occur.

There remains some uncertainty about which semaphorins are critical to the guidance of spinal sensory axons in vivo: whereas similar defects are seen in Sema3A mutants in the central branches of nociceptive axons in NRP1 knockouts animals (Gu et al., 2003), loss of NRP1 or of Sema3A also affects the peripheral branches, whereas loss of CNTN2 does not (Law et al., 2008; Liu & Halloran, 2005), suggesting that the in vivo effects of CNTN2 loss may reflect sensitivities to other Semas, for example Sema5B (Liu et al., 2014).

The fact that CNTN2 loss in sensory axons affects just one branch of their bipolar projection suggests the intriguing possibility that it may play a role in the establishment or maintenance of axonal polarity. This is coherent with observations that antibodies to CNTN2 block the emergence of parallel fibres from granule neuron progenitors and inhibit radial migration, leading to an accumulation of immature cells in the external granular layer (EGL; Wang et al., 2011), consistent with the delayed maturation of granule neurons precursors (GNPs) seen in *Cntn2ko* mice (Xenaki et al., 2011). Similar observations have been made in the pioneering of the medial longitudinal fascicle of zebrafish (Wolman et al., 2008). Moreover, recent data reveals that CNTN2 shRNA knockdown during cortical neurogenesis affects both interkinetic nuclear migration of neural precursors (Okamoto et al., 2013) and the polarisation of newly born neurons (Namba et al., 2014), depending on the timing of the knockdown. In early

cortico genesis, CNTN2 is found restricted to the basal process of radial neural progenitors and shRNA knockdown results in the accumulation of undifferentiated progenitors near the apical (ventricular) surface due to a failure of knockdown cells to extend a basalward process after apparently symmetric division at the ventricle (Okamoto et al., 2013). At later times, when such divisions become asymmetric and give rise to neuronal progenitors, the nascent neurons fail to polarise to generate an axon (Namba et al., 2014). In both situations, it appears that CNTN2 is required to establish a polarised, basally-projecting process.

Given that CNTN2 is continually endocytosed and can affect the trafficking of heterologous surface molecules, including L1s, NRPs and Plexins (Dang et al., 2012) in addition to Casprs, this suggests that homophilic contact with CNTN2⁺ early-born neurons, stabilises CNTN2 at the surface and begins the process of recruiting the components required to establish polarity by trapping of molecules being co-trafficked with CNTN2. Given that CNTN2/L1-mediated contacts might also negate or reverse responses to Semaphorin3A (Castellani et al., 2000; Dang et al., 2012), it is interesting to note that Sema3A also has a role in establishing neuronal polarity (Shelly et al., 2011).

Finally, CNTN2 has a role in myelination, in many respects mirroring that of CNTN1. CNTN2 also associates with a Caspr, Caspr2, and is localised to a subregion of the nodal structure, though in this case the juxtaparanode, where it is found complexed with Kv1.1/1.2 channels (Figure 3). Loss of CNTN2 does not lead to a breakdown of the paranode, but instead localisation of the K⁺ channels to the juxtaparanode is disrupted (Poliak et al., 2003), which is mirrored in the Caspr2 knockout (Poliak et al., 2003; Traka et al., 2003). In these studies, loss of Kv1.1/1.2 channel localisation was found to have no apparent effects on nerve conduction.

As for CNTN1 and Caspr1 at the paranode, CNTN2 and Caspr2 localisation at the juxtaparanode is also co-dependent (Poliak et al., 2003; Traka et al., 2003). A significant difference, however, is that in this instance an L1-like ligand for CNTN2 is not involved. Instead,

CNTN2 expression on the enveloping glia is sufficient to localise Caspr2 and the Kv1.1/1.2 channels (Savvaki et al., 2010), although CNTN2 is also expressed on the enveloped axon.

Together, these experiments show that Contactins (and the related L1-like CAMs) have important roles to play in the trafficking and positioning of interacting membrane components into specific domains of the cell surface and also in the modulation of responses to heterologous signals.

Contactins expression in non-neuronal cells and the generation of action potentials.

The studies reviewed above point to important roles for Contactins 1 and 2 in the generation of neuronal action potentials in the peripheral nervous system, though, as noted, CNTN2 expression is only required in the myelinating glia, not the axolemma (Savvaki et al., 2010). In fact, glial expression of Contactins was first described for CNTN1 (Koch et al., 1997) where it was found in oligodendrocyte-lineage cells, suggesting there may be differences between the PNS and CNS. Indeed, while bearing some features in common in the central nervous system, Contactin-1 was found to be expressed not only at the paranode, but also in the juxtaparanode and in the node itself (Peles and Salzer, 2000; Boyle et al., 2001; Savvaki et al., 2010). This is especially relevant for ion channel distribution: in the central nodes, CNTN1 was found associated with Neurofascin 186 (Nfasc186) and NrCAM, in a complex that includes the Na⁺ channel β_1 subunit, an interaction that is required for functional activity and expression of the latter (Kaplan et al., 2001; Rios et al., 2000, 2003; McEwen and Isom, 2004 a, b). However, this interaction also required submembrane interactions with Ankyrin G (AnkG; Berghs et al., 2000) and β -IV spectrin (Bennett and Lambert, 1999; Ogawa et al., 2006; Chang et al., 2014; Boiko et al., 2001; Komada and Soriano, 2002; Salzer, 2003; McEwen et al., 2004), which in turn are stabilized by Tenascin R (Weber et al., 1999, Figure 3). By contrast in the peripheral nervous tissue, where CNTN1 is not found in the node, Gliomedin was proposed to play a role in ionic channels clustering, in particular through interactions with NrCAM (Feinberg et al., 2010, Figure 3). As we have seen above, in the periphery instead CNTN1 plays a role in the integrity of the paranode,

which separates the nodal Na⁺ channels from the flanking juxtaparanodal region where the delayed rectifier potassium channels Kv1.1 and 1.2 are located so that a barrier between these channels is generated (Rhodes et al., 1997; Wang et al., 1993; Poliak et al., 2003; Traka et al., 2003; Chatzopoulou et al., 2008; Hivert et al., 2016)(see Figure 3).

These interactions are then of critical relevance in the generation of functional myelin, and therefore of action potentials (Zhou et al., 1998; Tait et al., 2000; Buttermore et al., 2013; Gordon et al., 2014), indicating a critical role for the regulated expression of Contactin family components in these processes (Boyle et al., 2001; Kazarinova-Noyes et al., 2001; 2002; Freeman et al., 2016; Buttermore et al., 2013; Salzer, 2015). In addition, a comparable organization is shared by the axonal initial segment (AIS), in which action potentials are generated based on the overall input reaching this region from the perikarya. This region extends about 40 μm from the axon hillock (Hedstrom and Rasband, 2006) and it includes both Na⁺ channels, which span the whole region, and K⁺ channels, which are rather clustered in the distal juxtaparanodal region. As in the node, the location of Na⁺ Channels (Na_v1.2 and Na_v1.6) in the AIS involves AnkG (Hedstrom et al., 2007; Zhou et al., 1998; Gasser et al., 2012; Buttermore et al., 2013), and also Neurofascin 186 (Zonta et al., 2011; Sherman et al., 2005). To date, there is no evidence to indicate that CNTN1 is present in the AIS, although CNTN2 is found together with Caspr2 in the juxtaparanode of the AIS (Ogawa et al., 2008). However, unlike in the node of Ranvier, in the AIS loss of CNTN2 (and therefore of Caspr2) does not appear to affect the distribution of the Kv1 channels with which they normally cluster (Duflocq et al., 2011). Together, these data indicate that the axon initial segment bears several features in common with the node of Ranvier, including the involvement of one of the Contactins (CNTN2), although in this instance their function is not critical, perhaps reflecting some redundancy with other Contactins known to be expressed in glia (Cui et al., 2004; Hu et al., 2006).

Potential significance of regulated expression of Contactins 1 and 2.

As noted above, although Contactin 1 and Contactin 2 have distinct molecular interactions, they also share a number of partners and properties. This suggests that their differential expression may be a critical feature of their distinct function in development. Contactins 1 and 2 are encoded by different genes, *Cntn1* and *Cntn2*, mapping to human chromosomes 12q12 (Berglund and Ranscht, 1994) and 1q32 (Tsiotra et al., 1993; Kozlov et al., 1995), respectively (15E3 and 1E4 in mouse). As we have seen, *CNTN1* is associated with later stages of neuronal differentiation and is more generally expressed than *CNTN2*. This is especially evident as GNPs of the cerebellar cortex mature: *CNTN2* expression begins earliest, on premigratory precursors in the external granular layer in developing cerebellar cortex, a subset of which, located at the boundary between the outer and inner EGL (iEGL), are proliferating (Xenaki et al., 2011, see also Figures 4A-D). By contrast, *CNTN1* expression overlaps only with the innermost *CNTN2*-expressing cells (Figure 4Ab) in the iEGL, which do not express cell cycle markers.

As an alternative approach to understanding the role of these proteins in this differentiation process, gain of function experiments were conducted using elements of the *Cntn2* regulatory apparatus to drive *Cntn1* gene expression (Bizzoca et al., 2003). The *Cntn2* gene, comprising 23 exons, spans more than 40 Kbp of human chromosome 1 (1q32.1), where it lies ‘head to head’ with the gene encoding Neurofascin, its L1-like relative (Hadas et al., 2013). The basal promoter region of the human gene maps upstream of the first non-coding exon which, together with the 11kb first intron, is sufficient to largely recapitulate the profile of the endogenous mouse *Cntn2* gene in neural cell lines and transgenic animals (Kozlov et al., 1995; Bizzoca et al., 2003).

In the cerebellum, ectopic expression of *CNTN1* using this promoter element, in so-called ‘TAG/F3’ transgenic mice, had a transient, but dramatic effect on cerebellar growth (Figure 5), delaying its expansion by 25% due to an inhibition of GNP proliferation in the first postnatal week (Bizzoca et al., 2003, 2009). Subsequent analysis of wild type GNP proliferation in culture demonstrated that a soluble form of *CNTN1* was able to suppress proliferation induced by the

mitogen Sonic Hedgehog (SHH; Xenaki et al., 2011), which normally is produced by Purkinje cells in vivo to support granule neuron expansion in the early postnatal period (Wallace, 1999; Wechsler-Reya & Scott, 1999; Dahmane & Ruiz I Altaba, 1999). Suppression of SHH-induced GNP proliferation by soluble CNTN1 was shown to be due to its binding to NrCAM (Xenaki et al., 2011). The suppressive effect of premature CNTN1 expression in the cerebellum was interpreted to reflect that CNTN1, which is normally expressed on maturing post-mitotic granule neurons, may provide a feedback signal to proliferating GNPs to co-ordinate cell cycle exit and orderly differentiation (Bizzoca et al., 2003).

However, this phenotype is not obviously consistent with the reduced cerebellar size of *Cntn1*ko mutants (Berglund et al., 1999), although the reason for the latter reduction has not been explored in detail. The knockout is a complete null, affecting expression in all cell types, not just neurons, and so the phenotype may also be due to earlier effects on glia or progenitor cells, as indeed may be the case for the TAG/F3 phenotype. Indeed, as we have seen, CNTN1 is expressed in neurons and myelinating glia and, in specific circumstances, also on cells of the astroglial lineage as shown in the case of glioblastoma (Eckerich et al., 2006) and we shall see below that its ectopic expression in other neurogenic areas affects the behaviour of neural progenitors. It is also likely that CNTN1 has different effects according to context and the binding partners (*cis* and *trans*) that are present. Moreover, it is clear that CNTN1 can both elicit signals in a cell to which it binds and generate signals in the cell in which it is expressed (Revest et al., 1999), making it possible that ectopic expression in novel molecular environments cannot directly be equated to loss of function.

Since the promoter element used in the TAG/F3 mice recapitulates a substantial part of normal CNTN2 expression, ectopic expression of CNTN1 is not limited to the cerebellum (Bizzoca et al., 2003; Bizzoca et al., 2012; Puzzo et al., 2013). In contrast to results from the cerebellum, in the early developing cerebral cortex, ectopic CNTN1 expression from the *Cntn2* regulatory region – known to be expressed in cortical neural progenitors (see above) - was found

to promote neural precursor proliferation in the subventricular zone and reduce cell cycle exit (Bizzoca et al., 2012). Upon closer inspection, however, it was also clear that this was accompanied by a suppression of neurogenesis, in a manner reminiscent of the effect of activating Notch in this region (Gaiano et al., 2000; reviewed in Imayoshi et al., 2013; Tiberi et al., 2012; Pierfelice et al., 2011; Cau and Blader, 2009), which is discussed in detail below. Importantly, these studies highlighted that CNTN1 itself, often assumed to be a post-mitotic marker, in fact is normally expressed in proliferating cells of the cortical ventricular zone, though the precise identity and state of these cells has not been determined. Thus, these transgenic experiments demonstrate that the precise regulation of Contactin expression at all stages of neurogenesis is critical.

Similar effects were seen in the developing hippocampus, where CNTN1 expression is normally observed in the neurogenic region and on both granule and pyramidal neurons and on their axonal extensions (Virgintino et al., 1999). Ectopic expression of CNTN1 in development causes a similar suppression of neurogenesis to that seen in the cortex (Puzzo et al., 2013). However, in the adult, where neurogenesis is known to continue (Kemperman et al., 2015) the opposite was seen, where neurogenesis was sharply increased as a consequence of the presence of the TAG/F3 transgene, resulting in increased hippocampal size and, in older mice, evidence of enhanced learning and memory, including elevated long term potentiation in CA1, increased pCREB levels (Lonze and Ginthy, 2013) and enhanced performance in spatial and object recognition tests (Puzzo et al., 2013).

Seemingly at odds with the suppression of neurogenesis in early development, these results suggest either that the mode of CNTN1 action changes with time just as the potential of the neural stem/progenitor cells is known to vary, for example, in the layering of the developing cortex. Alternatively, because the TAG/F3 transgene is expressed throughout development, it is also possible that early expression, through its inhibition of early neurogenesis, has 'set aside' larger numbers of long-lived neural stem cells that survive into the adult.

In any event, together these observations strongly support the view that the mechanisms which drive regulated expression of the contactins is a critical component of their precise function *in vivo*. In the next section we briefly review what is known about the transcriptional regulation of the genes encoding the Contactins.

Mechanisms which drive differential Contactin genes activation.

The regulatory regions of the mouse *Cntn1* and *Cntn2* genes have both been characterized. These regions display a different level of complexity: compared to the *Cntn2* gene (described above) the mouse *Cntn1* promoter was found to span a very large genomic region, overcoming 100 Kbp in size and including four alternative non-coding exons, three of which associated with distinct alternative 5' neuro-specific promoters, provided with a differential activation profile and undergoing a complex splicing mechanism (De Benedictis et al., 2001; Cangiano et al., 1997; Figure 6 A-C). The activity of the most 5' A1 exon-associated promoter was found to undergo only minor developmental changes, suggesting that it contributed to the basal *Cntn1* gene expression; on the other hand, the exon C1- and exon 0-associated promoters were significantly and transiently upregulated at the end of the first postnatal week (Figure 6D). The demonstration of multiple, alternative and developmentally-regulated promoter elements suggests that regulated developmental activations of the *Cntn1* gene implies both transcriptional and posttranscriptional mechanisms. In turn, such an organization was found to be of critical relevance in the definition of the Contactin 1 gene expression profile for which it carried all necessary molecular features. This was demonstrated by combining these elements into a single *Cntn1* promoter/EGFP reporter-construct, which essentially recapitulated endogenous *Cntn1* gene expression (Figure 7, see De Benedictis et al., 2006). These data demonstrate that the *Cntn1* gene does indeed breakdown into components that are differentially regulated during development, but it remains to be seen whether different elements control expression in different cell types (e.g. neurons vs glia, astrocytes vs oligodendrocytes), or whether this is achieved by differential expression of common transcription factors.

A surprising result of this regulatory element characterisation was that mice carrying the condensed EGFP reporter construct exhibited a behavioural circling defect typical of basal ganglion dysfunction, which correlated with a downregulation of the endogenous CNTN1 protein in cells in which the transgene was expressed at high level, which led to a significant downregulation of the protein particularly in the basal ganglia of adult animals and a concomitant increase in the generation of dopaminergic neurons (Massaro et al., 2012). Exactly how this downregulation occurred is not understood, and whether this phenotype is directly due to CNTN1 downregulation awaits the generation of a conditional *Cntn1* mutant that can be directed to the basal ganglia, but such a phenotype at face value remains consistent with the general hypothesis that CNTN1 exerts an inhibitory effect on developmental neurogenesis.

Signalling mechanisms involved in axonal adhesive glycoproteins developmental function.

As noted above, (Bizzoca et al., 2012) the inhibition of neurogenesis observed as a consequence of Contactin 1 overexpression was strongly suggestive of, and demonstrated to correlate with Notch pathway activation, known for its ability to counteract neurogenesis (Imayoshi et al., 2013; Tiberi et al., 2012; Pierfelice et al., 2011; Cau and Blader, 2009). Indeed, the Contactin 1 effects on the neuronal lineage in the cerebral cortex typically reflected activation of the canonical Notch pathway through the Hes transcription factor (Bizzoca et al., 2012). In fact, a relevant indication of Contactin 1 ability to activate the Notch pathway was already available, thanks to the studies from the Zhicheng Xiao (Hu et al., 2003, 2006) laboratory, who demonstrated the Contactin 1 ability to promote oligodendrocyte differentiation, depending upon direct interactions of the molecule with the Notch receptor. This was in contrast, however, with the evidence that activation of Notch by its canonical ligands Delta or Serrate rather results in inhibitory effects on oligodendrocyte differentiation and therefore on myelination, still implying the Hes-dependent pathway activation (Wang et al., 1998; Jessen and Mirski, 2008; Woodhoo et al., 2009), thus sharing the same effects and mechanism activated on the neuronal lineage.

However, at the same time the evidence was achieved that Contactin-1 interactions with oligodendrocyte Notch receptors resulted in the activation of the alternate Deltex1-dependent pathway which, in turn, promotes oligodendrocyte commitment and differentiation (Hu et al., 2003; 2006), thus resulting in positive effects on myelination. This indicated that Notch pathway activation was a general consequence of Contactin 1 expression, and that this resulted in specific and opposite effects on the neuronal and, respectively, on the oligodendrocyte lineages, depending upon the activation of different signalling pathways.

Therefore, both Hes-1 and Deltex-1 dependent pathways were found to be activated by CNTN1 interactions, the former being responsible for early Contactin 1 inhibitory effects on the neuronal lineage (Bizzoca et al., 2012) and the latter for its effects in promoting oligodendrocyte differentiation (Hu et al., 2003). This strongly indicated that Contactin 1 interactions with the Notch receptors represents a pleiotropic effect, which, in fact, differentially contributes to the modulation of neural developmental events.

How these differential responses are elicited in these different scenarios is not understood. The work of Hu et al. (2003) is interpreted to reflect interactions of CNTN1 on the axolemma with Notch on oligodendrocyte precursors, i.e. in a *trans* interaction. However, oligodendrocytes themselves express CNTN1 (Haenisch et al., 2005; Çolakoğlu et al., 2014) making it possible that *cis* interactions are involved, which may affect how Notch is trafficked; the trafficking pathways taken by Notch during signalling affects both the level and type of signal generated (Yap & Winckler, 2015). This is pertinent to the TAG/F3 experiments in the cortex since ectopic expression of CNTN1 is induced in cells that may already express both CNTN1 and CNTN2 (it is not clear whether this is in the same or different cells), which suggests that it may be the levels, or the subcellular localisations of these proteins that is critical to the signalling outcome. Of particular interest, therefore, is the suggestion that CNTN1 and CNTN2 may compete for common receptors (Xenaki et al., 2011) and that the intracellular trafficking pathways subsequently taken by those proteins may be altered dependent on their association with Contactins (Dang et al.,

2012). It will be of interest in future to determine whether Notch trafficking is affected by its association with CNTN1.

Overall significance of Contactin 1 regulated expression.

The overall idea therefore is that the *Cntn1* gene represents a typical example of a developmentally regulated component of the Immunoglobulin superfamily, whose expression regulates neurogenesis in different regions of the nervous tissue and that, in doing so, distinct signalling pathways are in turn activated. The most likely interpretation of the Contactin developmental role is therefore that the function of this molecule mostly results from its ability to promote signalling rather than driving adhesion *per se*. In turn, this supports the idea that the molecule fulfils the definition of morphoregulatory besides of adhesive molecule (Edelman GM, 1992; Edelman and Jones, 1997).

Potential significance of Contactins expression in neurological disorders.

The use of transgenic mice models indicated that changes in the expression of Contactin family components may affect neural developmental events. In turn, such changes may reproduce the phenotype of specific neurological disorders, either inflammatory or degenerative in nature, consistent with the existence of a behavioural phenotype, indicative of changes in the cerebellar function in TAG/F3 mice (Coluccia et al., 2004). These disorders may correlate with signalling pathways activation, which, in the case of Contactin 1, may concern either Notch or pCREB factors-associated pathways (Hu et al., 2003; 2006; Bizzoca et al., 2012; Puzzo et al., 2013), known to affect neural precursor proliferation/differentiation events and to be involved in either neurodegenerative or neuroinflammatory (Dragunow, 2004; Shen, 2014; Pozueta et al., 2013; Wei et al., 2011) disorders.

As for the latter, the most relevant one in which Contactins appear to be involved is Multiple Sclerosis (MS), in which cell- and antibody-mediated immunity against Contactin 2 have been demonstrated (Dorfuss et al., 2009; Boronat et al., 2012). As far as Contactin-1, its interaction with Notch receptors was similarly proposed to be involved in the evolution of such disorder,

given its ability to promote the remyelination events in the damaged nervous tissue (Aparicio et al., 2013). As a general rule, the potential role of Contactin family components in demyelinating disorders was suggested by the demonstration of antibodies to Contactin 1, Contactin 2 and to further components of the nodal complex as Neurofascins, Caspr1 and Caspr2 in chronic inflammatory demyelinating polyneuropathies (Stathopoulos et al., 2015; Miura et al., 2015, Labasque et al., 2014, Querol et al., 2013; Lancaster and Dalmau, 2012), which supports the hypothesis that, in such disorders, these molecules may work as autoantigens. The corresponding autoantibodies were found to belong to either the IgG1 or IgG4 subclasses (Manso et al., 2016) and to affect the integrity of the paranodal region (Kuwabara et al., 2015; Doppler et al., 2015), which could contribute to specific effects observed on the conduction velocity. Besides in demyelinating neuropathies, changes in the expression of the *Cntn1* gene were also demonstrated in some forms of familial and congenital myopathies (Compton et al., 2008).

A relevant aspect concerns the involvement of Contactins in neurodegeneration with a special focus on Alzheimer disease. Indeed, in such disorder, reduced Contactin 2 levels were demonstrated (Mattson and Praag, 2008), suggested to depend upon increased activity of the APP cleaving enzyme BACE1 (Gautam et al., 2014). Indeed, this enzyme was found to recognize Contactin 2 among its potential substrates, which, as a consequence of enzymatic cleavage, resulted in its reduced levels at the cell surface and increased release in soluble form. Indeed, BACE1 activity in Alzheimer neurodegeneration correlated with increased Contactin 2 delivery in soluble form and therefore with decreased tissue levels. In turn, this could suggest that Contactin 2 levels represent a reliable indicator of neurodegeneration (Zoupi et al., 2013). As far as the tissue counterpart of these biological effects, it may be expected that reduced levels of membrane Contactin 2 may result in reduced effects of the molecule on nervous tissue differentiation, thus leading to defects in neurogenesis (Denaxa et al., 2003, 2005; Ma et al., 2008) and in neuronal function (Savvaki et al., 2008). As for the underlying molecular pathway, Contactin 2 has been identified as a functional ligand of APP and this interaction was found to result in increased AICD

release in a γ -secretase dependent manner and, in the ventricular zone, in the activation of the Fe65 pathway in the neural stem cells niche (Ma et al., 2008). Altogether, these data seem to confirm the involvement of Contactin 2-associated signalling in the mechanisms leading to neurodegeneration.

Contactins have also been widely implicated in a variety of neural and other tumours (for review see Katidou et al., 2008). CNTN1 in particular has been said to have oncogene-like properties (Wu et al., 2012) and plays a role in tumour metastasis in a variety of tumours, notably in lung and prostate cancer (Su et al., 2006; Yan et al., 2016). In the former, its expression appears to upregulate AKT, which in turn suppresses E-cadherin expression promoting disaggregation. It has also been found in glioblastoma, where it was found to inhibit cell contacts (Eckerich et al., 2006).

However, besides in specific neural pathologies, which recognize their basis in either neuroinflammatory or neurodegenerative events, a role has been also proposed for Contactin family components in neural regeneration in different systems, and, again, this has been specifically shown in the case of Contactin 2 (Soares et al., 2005; Lin et al., 2012; Devaux et al., 2012; Pang et al., 2012). Altogether, these data thus indicate a complex role for these molecules in modulating the tissue and cellular events underlying neural disorders, either neuroinflammatory, neurodegenerative in nature, but also in neurorepair events.

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Figure legend**Figure 1:**

Alignment of Contactin 1 and Contactin 2 aminoacid sequences by using the CLC sequence viewer 7.6.1, scored by the Jalview 2.9.0b2 software (Waterhouse et al., 2009).

Figure 2:

A: Overall organization of the Contactin 1 and Contactin 2 axonal glycoproteins, showing the Immunoglobulin type C2 domains in the N-terminal, and the Fibronectin type III repeats in the C terminal, premembrane regions as well as the GPI attachment.

B: Schematic summary of the interactions of CNTN1 and CNTN2, illustrating that some of them are held in common while others are distinct. Interacting molecules are grouped into families where relevant, structural similarities are indicated by icon colour and shape. Interactions between some of these and the CNTNs is promiscuous (e.g. with the L1-like family), while others appear to be specific (e.g. with the Casprs). CNTNs 3-6 are also shown with their known interactions, while the whole Caspr family is also shown to illustrate that further interactions with CNTNs seem likely, but have not been documented. In these cases, molecules with no known interactions with CNTN1 or CNTN2 are shown in lighter colours. Abbreviations (with refs not in main text): NCAM, neural cell adhesion molecule (Milev et al., 1996); NRP, neuropilin (Falk et al., 2005); L1, L1CAM; CHL1, Close Homolog of L1 (Ye et al., 2008); Nf, neurofascin; Nr, NrCAM or NgCAM-related CAM (Falk et al., 2005); APP, Amyloid Precursor Protein; APLP, APP-like protein; RPTP, Receptor-like Protein Tyrosine Phosphatase; Phosphacan, splice variant of RPTP β (Milev et al., 1996); Tenascin (Rigato et al., 2002); PTP α , Protein Tyrosine Phosphatase (Zeng et al., 1999); Kv1, Shaker-type, Potassium voltage-gated channel.

Figure 3:

Molecular organization of the Nodes of Ranvier, of the paranode and of the juxtaparanodal regions in both central and peripheral nervous tissue. In central nodes (CNS), contactin-1 (CNTN1) associates *in cis* with Neurofascin186 (NF-186) and with NrCAM as well as with Na⁺ channels β 1 subunit (Nav). In addition, it undergoes submembrane interaction with Ankyrin G and with β IV spectrin, stabilized by Tenascin R. In peripheral neurons (PNS), Contactin-1 is not expressed in the nodal region.

In the paranode, axons and myelinating glia interact via the Contactin1/Caspr1 binary complex with glial Neurofascin 155 (Charles, et al., 2002). This region separates nodal Na⁺ channels from K⁺ channels, restricted to the juxtaparanode by their interactions with the Contactin2/Caspr2 molecular complex. Delayed rectifier potassium channels Kv1.1 and 1.2 are clustered in the same region based on their interaction with such a complex. In the peripheral nervous tissue Gliomedin is involved in NrCAM clustering with ionic channels.

Figure 4:

A. Immunohistochemical localization of the Contactin 2 (TAG1 red, a) and Contactin 1 (F3, green, b) glycoproteins in postnatal day 5 cerebellar cortex. c shows a merged picture, counterstained with DAPI. IGL, Inner Granular Layer; ML, Molecular layer. EGL, External Germinal Layer; Scale bar in c: 40 μ m.

B. Double stain of Contactin 2 (TAG-1) with either Ki-67 (a) or PCNA (b) proliferation markers in postnatal day 5 cerebellar cortex. Arrowheads point to elements displaying different levels of double immunostainings.

C,D: primary cerebellar cultures stained with Ki67 (C, red), Contactin 2 (TAG1) (C, blue) and Tuj1 (C, green) or with Contactin 2 (TAG1, D Red) and BrdU antibodies (D, green). Asterisk indicates Ki67⁺/TAG1⁻, arrowhead Ki67⁺/TAG1⁺ and arrow Ki67⁻/TAG1⁺ granule cells.

Figure 5:

A: Organization of the TAX-1 gene promoter/Contactin 1 cDNA construct. The Contactin 1 regulatory region, including the 5' flanking exons A1, 0, B and C1, and the associated alternative promoter elements was replaced for by the human TAX-1 gene regulatory region, which included the 5' flanking region, the first two exons as well as the intervening intron of the TAX-1 gene, fused to the Contactin 1 cDNA.

B. Phenotype of the postnatal day 6 cerebellum from either wild type (WT) and TAG/F3 transgenic mice, demonstrated by Contactin 1 immunostaining. Note the relevant reduction of the cerebellar size. egl, external granular layer; igl, inner granular layer; PC1, Purkinje cells layer; WM, white matter; CP, Choroid Plexus. Scale bar: 200 μ m (from Bizzoca et al., 2009).

Figure 6:

A. Exon intron organization of the 5' region from the *Cntn1* gene, including the regions surrounding exons A1, 0, and C1, whose 5' ends correspond to the *Cntn1* gene transcription start sites. Exon 1 includes the translation initiation site (ATG). The size of the first 3 introns is also reported.

B. C. The 5' flanking exons of the *Cntn1* gene are shown in B, which undergo the complex splicing events shown in C, resulting in a high level of complexity of the Contactin 1 mRNA.

D. Relative developmental utilization of the Contactin1 5' exons A1, 0, B and C1 as determined by densitometric scan of RT/PCR amplification profiles obtained by using exon-specific primers (from De Benedictis et al., 2001).

Figure 7:

Map of the *Cntn1* promoter/EGFP reporter construct (A) and, in B, its expression in developing cerebellar cortex at postnatal days 0 (P0) and 8 (P8). egl, external granular layer; igl, inner granular layer; PC1, Purkinje cells layer; WM, white matter. Scale bars: P0, P8 = 200 μ m (insets 20 μ m).

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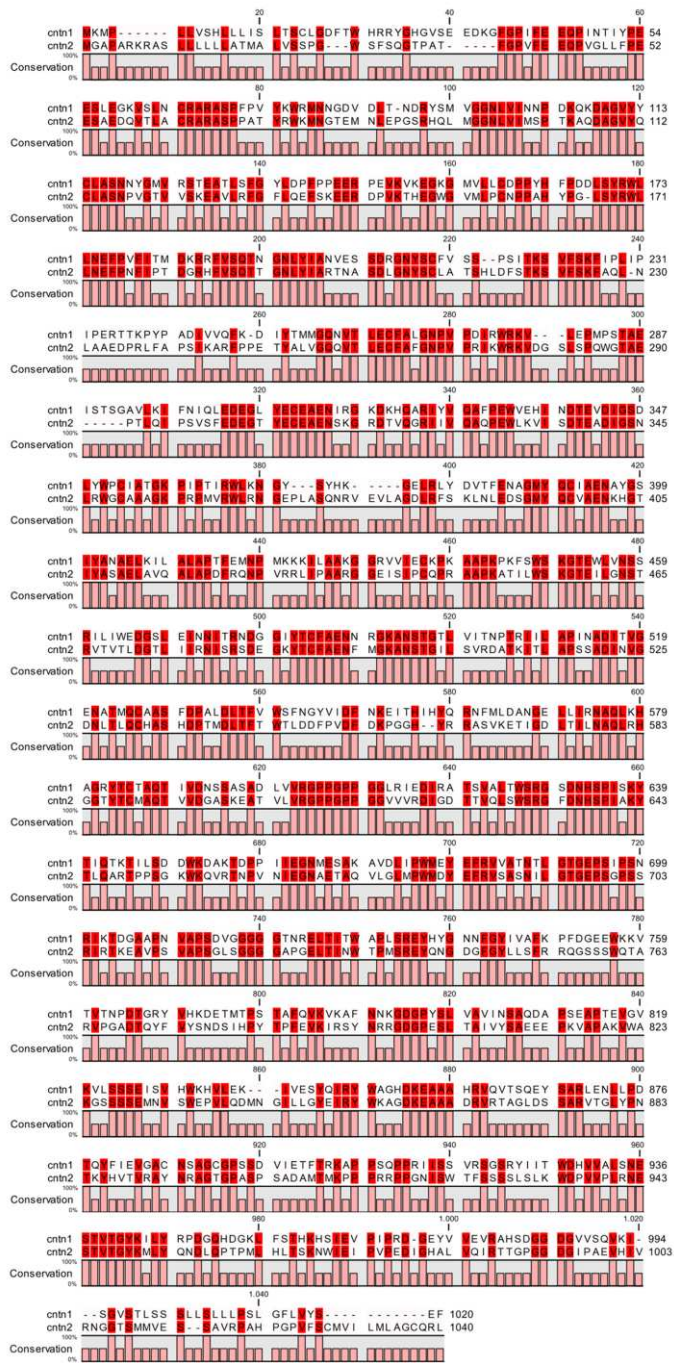


Figure 1

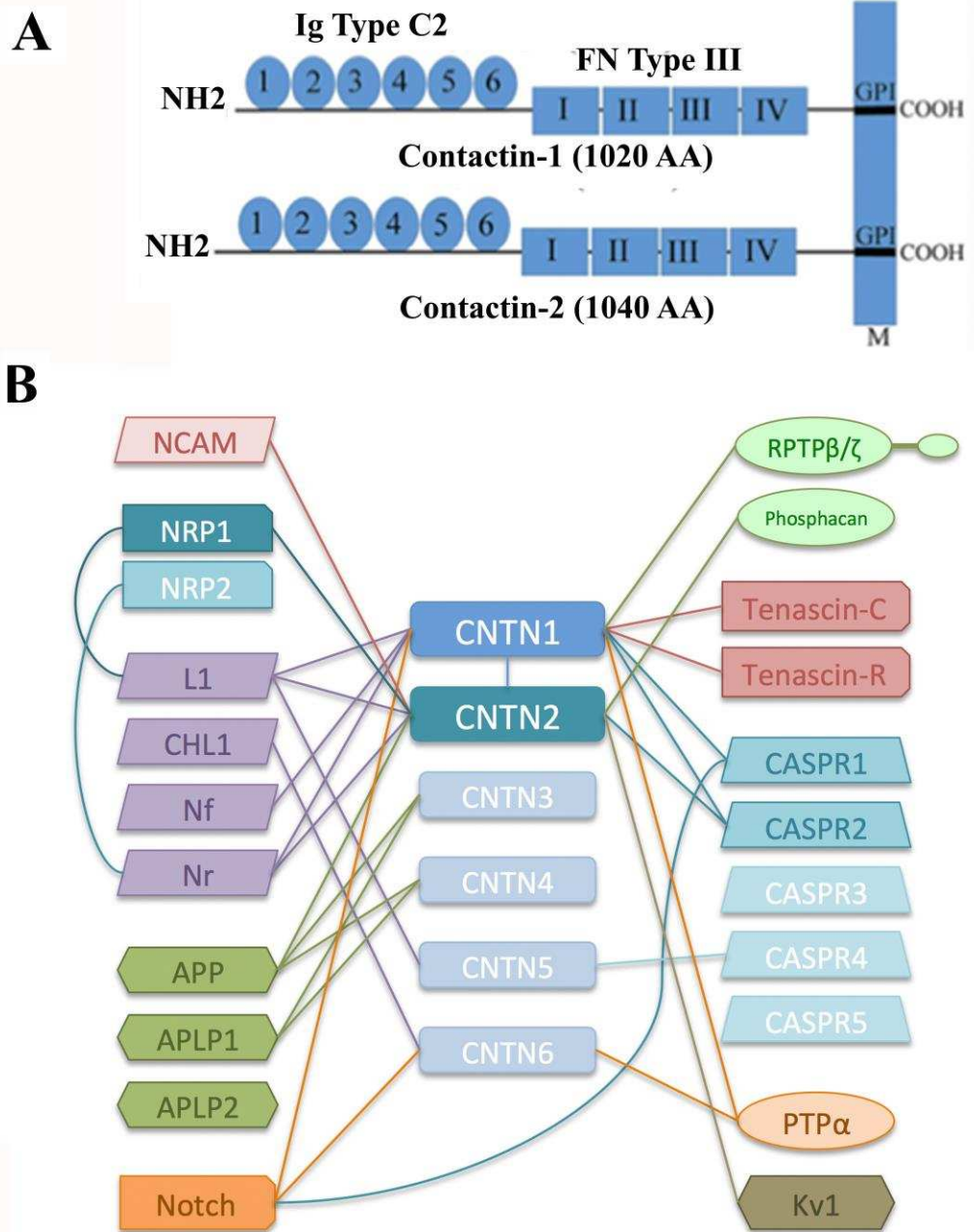


Figure 2

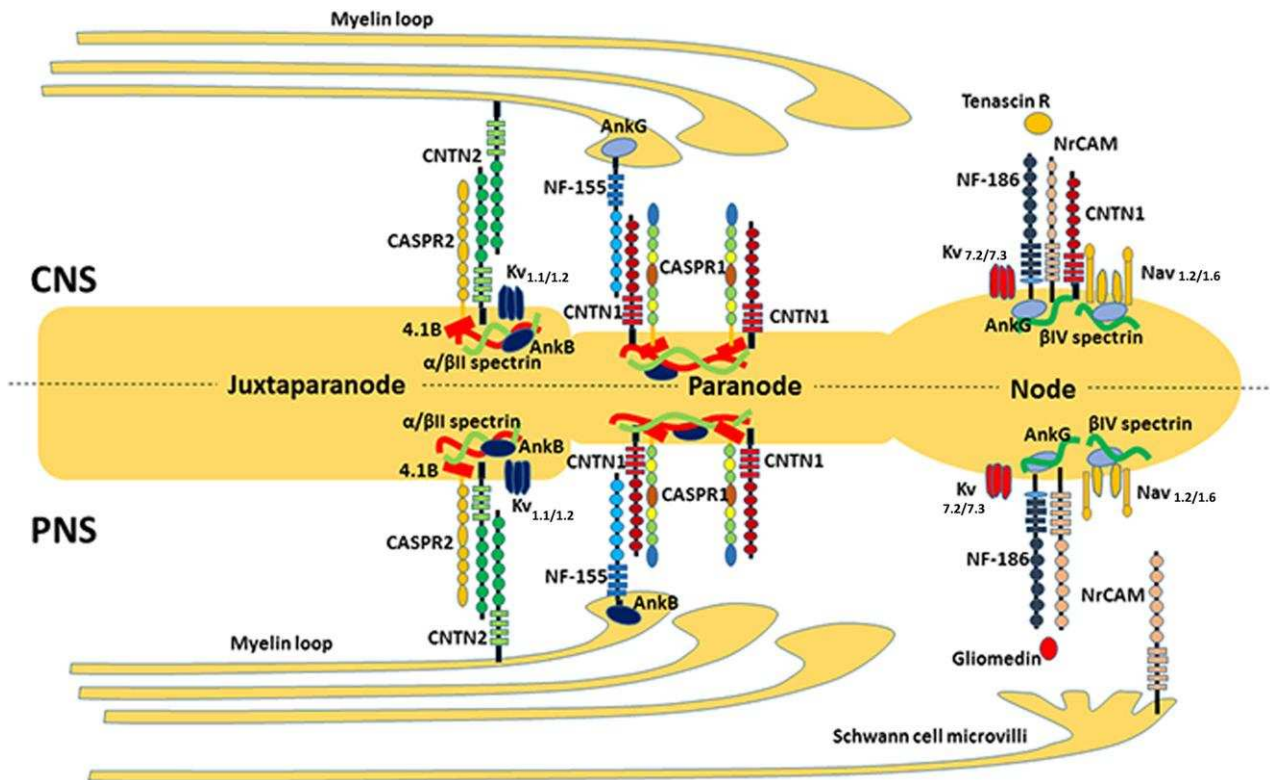


Figure 3

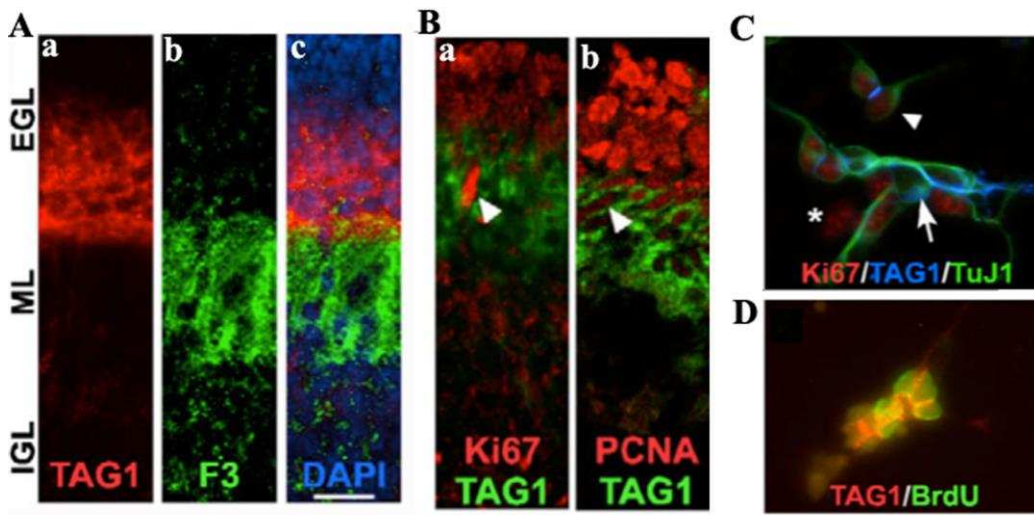


Figure 4

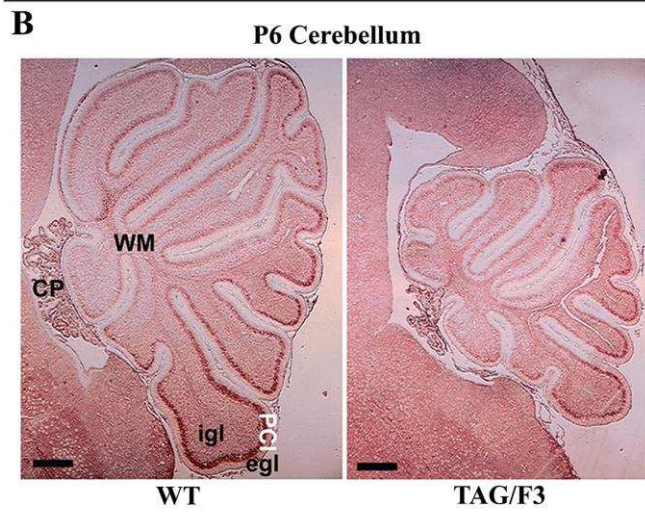
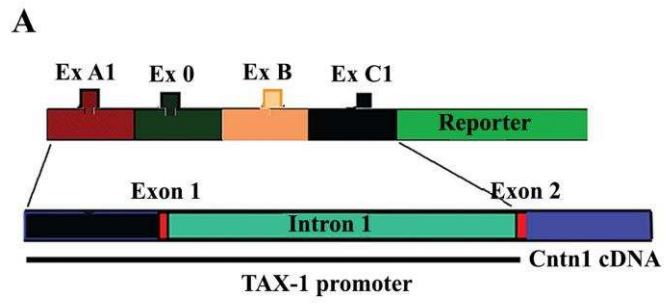


Figure 5

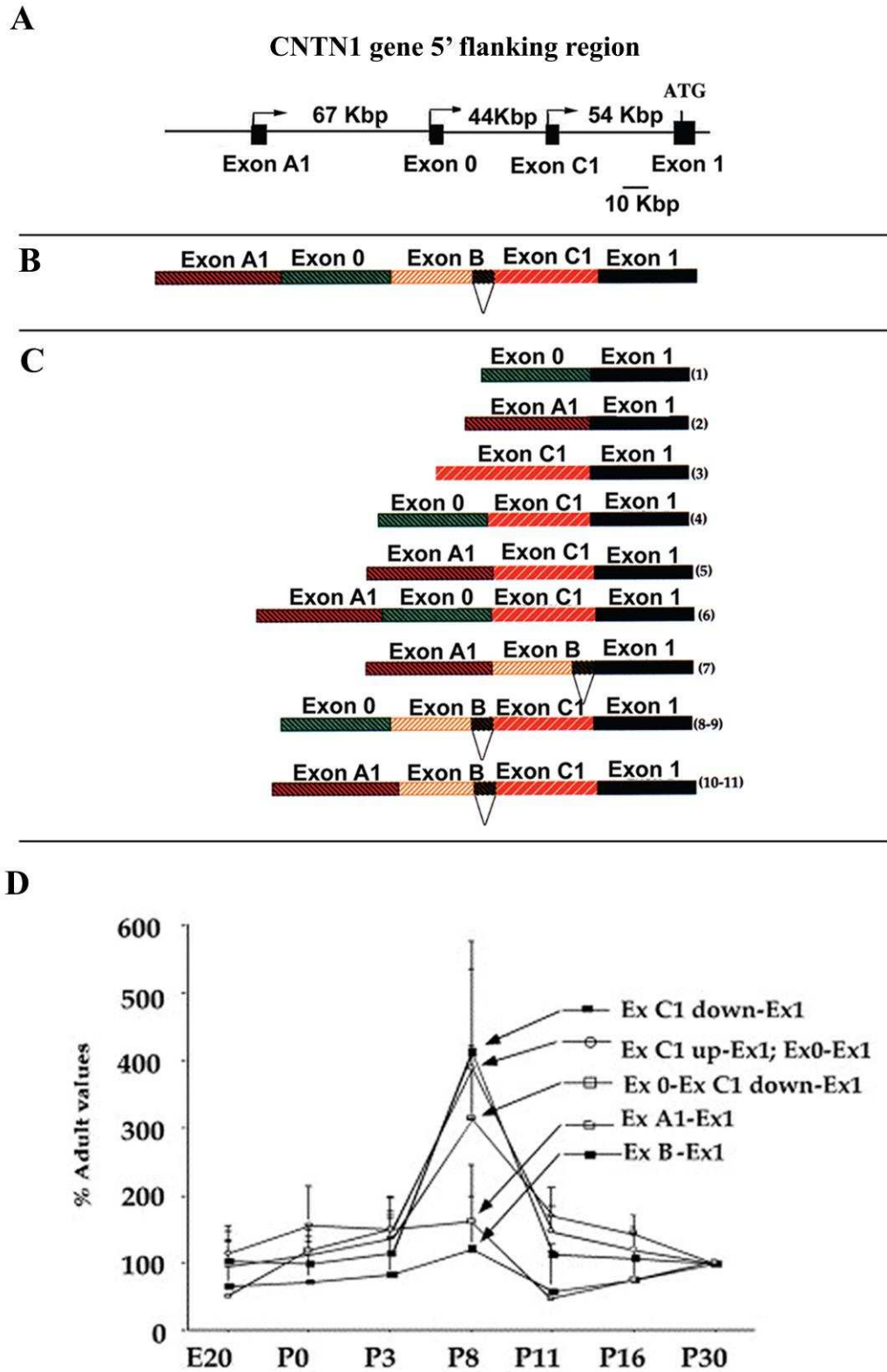


Figure 6

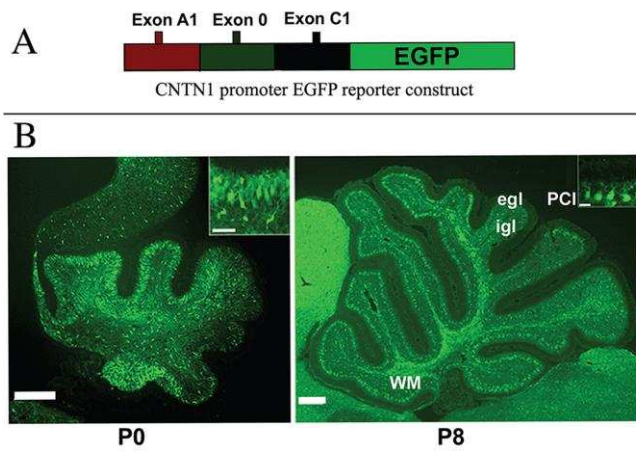


Figure 7

Graphical Abstract

- 1. Adhesion molecules in neural development.**
- 2. Morphoregulatory molecules.**
- 3. Immunoglobulin superfamily adhesive glycoproteins.**
- 4. Contactins (CNTN).**
- 5. Contactins 1-2 and their interactions.**
- 6. Contactins genes differential regulation.**
- 7. Contactins expression and the action potentials generation.**
- 8. Signalling mechanisms in axonal adhesive glycoproteins function.**
- 9. Significance of Contactin 1 regulated expression in development.**
- 10. Significance of Contactins expression in neurological disorders.**

Highlights:

- 1. This manuscript deals with the properties of the GPI anchored adhesive glycoproteins expressed at the axonal level. The main focus is on two components of the Contactin Family (CNTN1 and CNTN2).**
- 2. The developmental significance of the regulated expression of these molecules is mostly described in the attempt to explore their significance in both development of the nervous tissue and in some neurological disorders.**