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NG2 cells: properties, progeny and origin

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Abstract

The NG2 proteoglycan is a type 1-transmembrane protein expressed by a range of cell types within and outside the mammalian nervous system. NG2-expressing (NG2) cells are found in grey and white matter tracts of the developing and adult CNS and have previously been assumed to represent oligodendrocyte precursor cells: new work using transgenic mice has shown that NG2 cells generate oligodendrocytes, protoplasmic astrocytes and in some instances neurons *in vivo*. NG2 cells express GABAA receptors and the AMPA subtype of glutamate receptors. They make intimate contact to neurons prior to myelinating axons and also form electron-dense synaptic specialisations with axons in the cerebellum, cortex and hippocampus and with non-myelinated axons in the corpus callosum. These synaptic NG2 cells respond to neuronal release of glutamate and GABA. This neuron-glia interaction may thus regulate the differentiation and proliferation of NG2 cells. The C-terminal PDZ-binding motif of the NG2 protein binds several PDZ proteins including Mupp1, Syntenin and the *Glutamate Receptor Interacting Protein* (GRIP). Since GRIP can bind subunits of the AMPA receptors expressed by NG2 cells, the interaction between GRIP and NG2 may orientate the glial AMPA receptors towards sites of neuronal glutamate release. The origin, heterogeneity and function of NG2 cells as modulators of the neuronal network are important incompletely resolved questions.

Keywords

NG2; Oligodendrocyte progenitor; Neuron-glia synapse; AMPA-receptor; GABA; Cell lineage

1. Introduction

Cells expressing the proteoglycan NG2 make up 5–10% of all glia in the developing and adult CNS. They are evenly distributed in white and grey matter and some cells proliferate even in the adult, implying a continual turnover of this cell population. NG2 protein expression is down-regulated upon maturation of the cells. Expression of NG2 has in the past been used as a marker for oligodendrocyte precursor cells (OPC; see Nishiyama et al., 2009) and recent evidence discussed below has demonstrated that indeed NG2 cells give rise to

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oligodendrocytes, but also to subpopulations of astrocytes during normal development. The discovery at the turn of the last century of the unusual synaptic association between NG2 cells and neurones in multiple regions of the developing and adult CNS (Bergles et al., 2000; Jabs et al., 2005) has spurred widespread interest in this cell population and suggested that in addition to acting as a plastic progenitor pool for more differentiated cells, NG2 glia may form a unique glial network in continual parlance with neurones. In addition to the expression on immature myelinating glia in the CNS, NG2 is expressed in the PNS by immature Schwann cells.

In this review we will focus on the properties, progeny and origin of NG2 cells but first summarise current knowledge regarding the structure and function of the NG2 protein, the defining feature of this class of cells.

2. Structure of NG2 and its partner molecules

The NG2 protein was originally defined by antibodies directed against surface proteins on a rat cell line with glial and neuronal properties (Stallcup, 1981). Independently NG2 homologues were discovered in human and mouse (Harper et al., 1984; Niehaus et al., 1999; Pluschke et al., 1996; Schneider et al., 2001; Stegmuller et al., 2002). The mammalian protein of 330 kDa (also termed CSPG-4) is encoded by a single gene with multiple exons coding for 2327 amino acids. To date no alternatively spliced variants have been described. The amino terminus exhibits two *Laminin G/Neurexin/Sex Hormone Binding Globulin* (LNS) domains (Fig. 1), thus placing NG2 firmly in the large family of the neurexins; cell adhesion molecules dictating synaptic specificity in neurones and exhibiting a large degree of alternative splice forms (Missler and Sudhof, 1998). The large extracellular domain of NG2 includes sites near the single transmembrane domain which are readily cleaved by a variety of proteases *in vitro* and *in vivo* (Nishiyama et al., 2009). This can result in the deposition of the ectodomain in the extracellular matrix, thus making the identification of NG2-expressing cells by antibodies, especially in lesions rich in proteases, particularly difficult. The biological relevance of this cleavage is unclear: one possibility is that it could be a mechanism to release NG2 from a putative receptor via regulated proteolysis. The intracellular domain is rather short (76 Aa) and includes a C-terminal type I Postsynaptic density 95/Discs Large/Zonula-occludens-1 (PDZ) domain recognition motif, as well as several threonines whose phosphorylation state regulates cell behaviour such as spreading and migration (Fang et al., 1999; Lin et al., 1996; Majumdar et al., 2003; Tillet et al., 2002). In addition, a type II PDZ domain binding motif, a *Src Homology type 2* (SH2) domain binding motif and a WW-domain binding motif all underline interactions of NG2 with intracellular signalling and structural proteins. The NG2 protein is a part-time proteoglycan; chondroitin sulfate gylcosaminoglycan (GAG) chains are linked to the extracellular domain (Nishiyama et al., 2009). The amount of GAG chains carried by the core protein varies with cell type and developmental stage (Schneider et al., 2001).

Several partner molecules have been identified for the NG2 protein. These include ß1 integrins in melanoma and astrocytoma and the receptor for PDGFα: these associate in cis with NG2. PDGFAA, FGF2, Collagen V and VI, MT3MMP, Plasminogen, tPA and galectin 3 have all been described as binding directly to the large NG2 ectodomain (summarised in Nishiyama et al., 2009). Interestingly, no cell adhesion molecule ligands for the LNS domains have been described to date. In the light of the interaction of NG2 cells with axons during myelination and at synapses in development and in the adult, it is likely that neuronal receptors exist. Several binding partners for the C-terminal type I PDZ domain-binding motif have been identified. These are *Multi-PDZ Domain protein 1* (MUPP1), *Glutamate Receptor Interacting Protein* (GRIP) and Syntenin-1(Barritt et al., 2000; Chatterjee et al., 2008; Stegmuller et al., 2003). In particular, the latter two partners may be relevant for the function of NG2 cells at synapses and

in wrapping axons at early stages of myelination. GRIP binds to the GluR2/3 subunits of the AMPA receptor, glutamate receptors expressed by NG2 cells which are activated by neuronally released glutamate at the neuron-glial synapse and also influence oligodendrocyte differentiation (see articles in this issue by Steinhauser et al., (Gallo et al., 1996) and also Fig. 2). Syntenin provides connections to the cell cytoskeleton, which may be relevant for migration of NG2 cells to axons prior to myelination as well as process movement of synaptic NG2 glia. Furthermore, NG2 has been shown to recruit the small GTPase cdc42 and p130cas (crk-associated substrate) in melanoma cells (Eisenmann et al., 1999), intracellular molecules regulating diverse processes in migration and cell polarisation

3. NG2 in evolution

NG2-like proteins are found in non-vertebrates including C.Elegans and D. Melanogaster. Interestingly, in both these species the C-terminal PDZ binding domain is conserved, furthermore in Drosophila NG2 two LNS domains are recognisable. Two recent papers have reported that Drosophila NG2 (called Kon-tiki or Perdido) associates with Drosophila GRIP demonstrating the evolutionary conservation of this interaction (Estrada et al., 2007; Schnorrer et al., 2007). Both groups reported a role of NG2 expressed by immature muscle cells in attaching to tendons. In vertebrates NG2 expression outside the nervous system includes muscle progenitor cells and pericytes (Nishiyama et al., 2009). It remains to be seen whether Drosophila glia express NG2, similar to their mammalian counterparts.

4. Functions of NG2 cells

The study of NG2 cells in situ as well as their progeny has been facilitated by the generation of new mouse lines. The DsRed protein chromophore or the Cre recombinase with the NG2 promoter has been inserted using Bacterial Artificial Chromosome technology to generate transgenic mouse lines (Nishiyama et al., 2009). Alternatively the EYFP protein has been inserted after the start AUG of the first exon of the endogeneous NG2 gene to yield a “knock-in” mouse line (Karram et al., 2008). Use of these mice to study the generation of oligodendrocytes and astrocytes from NG2 progenitors is discussed below.

Synaptic contacts formed by NG2 cells have been described with axons of neurones in the hippocampus and cerebellum (Bergles et al., 2000; Lin et al., 2005), as well as with unmyelinated axons in the corpus callosum (Kukley et al., 2007; Ziskin et al., 2007). In addition to these synapses which appear largely on the processes of NG2 cells, the cell bodies of NG2 cells are very closely apposed to neurons in many brain regions including the hippocampus, cerebellum and cortex (Fig. 3). Intimate contact between NG2 cells and neurons has been observed earlier in an electron microscopic analysis of cortex in rats, where NG2 glia were defined as beta glial cells and were considered a fourth glial cell type (Peters, 2004). In contrast to published literature where double-labelling of brain sections was carried with polyclonal antibodies to NG2 and neuronal markers (Belachew et al., 2003; Dayer et al., 2005) we have never observed expression of neuronal markers by EYFP cells in the EYFP-NG2 knockin mouse or by DsRed+ cells in the NG2-DsRed transgenic mice (Fig. 4)

In contrast to observations that the NG2 protein appeared to be a repulsive molecule for cerebellar neurons in vitro (Tan et al., 2005; Tan et al., 2006), axons are clearly not repulsed by NG2 cells but in fact appear to be actively contacting them (Butt et al., 2005; Nishiyama et al., 2005; Yang et al., 2006). We have observed that axons of rat hippocampal neurones readily grow over the plasma membrane of HEK cells expressing a truncated version of NG2 lacking a large part of the extracellular domain but containing the LNS domains (Chatterjee et al., 2008); (Griemsmann and Trotter, unpublished observations). In contrast, the chondroitin sulfate side chains of NG2 are likely to be inhibitory to axonal growth (Galtrey and Fawcett, 2007). Neurones exclusively form synapses in vivo with NG2 cells but not with neighbouring

astrocytes, oligodendrocytes or microglia: NG2 glial cells are thus unique in promoting presynaptic specialisation in neurones. Could the NG2 protein itself be a synapse-promoting molecule? Furthermore, it is likely that NG2 cells release synapse-modulatory substances such as *Brain Derived Neurotrophic Factor* (BDNF), as suggested by a recent publication (Tanaka et al., 2009)

Electrophysiological and imaging evidence has demonstrated that excitation of NG2 glia by neuronal release of GABA acting on the GABAA receptors of NG2 cells, or via glutamate acting on the AMPA receptors, invokes a calcium signal (Gallo et al., 2008; Hamilton et al., 2009; Paukert and Bergles, 2006). Recent work has linked the GABA-induced signals in OPC to activation of sodium channels prior to changes in calcium and a stimulation of migration (Tong et al., 2009). It will be important to define events subsequent to this rise in calcium, for example changes in gene expression in NG2 cells.

5. Heterogeneity of the NG2 population

Since NG2 cells can give rise to different cell types (see below), an obvious question is whether NG2 cells are a heterogeneous population. Are there regional or developmental differences in the functions and differentiation potential of NG2 cells? All NG2 cells appear to express the receptor for PDGF AA. Analysis of transcription factor expression by NG2 cells in different regions of the brain have shown that almost all NG2 cells express Olig 2 and Sox 10 (Karram et al., 2008; Kitada and Rowitch, 2006; Ligon et al., 2006a; Ligon et al., 2006b). The NG2 population is heterogeneous when examined for the expression of glutamine synthestase, at least in the hippocampus (Karram et al., 2008). Study of a transgenic mouse in which EGFP was fused to the 3' UTR of the PLP gene, demonstrated two populations of NG2 cells in the developing subventricular zone: one population expressed EGFP and another lacked EGFP expression. The authors suggested that one population generated oligodendrocytes while the other population was more immature (Mallon et al., 2002). Several groups have shown heterogeneity in the NG2 cell population based on electrophysiological measurements. NG2 cells in the white and grey matter areas of the developing mouse were shown to differ in morphology and electrophysiological properties: furthermore, a few NG2+ cells in the grey matter elicited depolarization-induced spikes similar to immature action potentials (Chittajallu et al., 2004). In contrast, two other groups (Ge et al., 2009; Karadottir et al., 2008) reported subpopulations of NG2 cells in white matter which were able to generate action potentials; a finding which has generated controversy in the field as it would force a reclassification of these NG2 cells as bona-fide neurons. Within the hippocampus, a grey matter area, the EYFP+ cells are heterogeneous at a given developmental stage based on their electrophysiological properties (Karram et al., 2008). Unfortunately, neither the immunohistochemical nor the electrophysiological studies permit a distinction of functional diversity within the lineage from lineage heterogeneity. This question can better be addressed by studies using the transgenic mouse lines described below.

6. Progeny of NG2 cells

The fate of NG2 cells has been a subject of intense debate and has been studied using a variety of approaches. Here we will review primarily the recent literature on in vivo fate mapping of endogenous NG2 cell using the Cre-loxP technology. This method utilizes transgenic mouse lines that express the site-specific recombinase Cre driven by various promoters that are active in NG2 cells. When these mouse lines are crossed to Cre reporter mouse lines, the expression of the reporter gene is activated permanently in cells that express Cre, thereby allowing identification of their progeny by persistent reporter expression.

In NG2creBAC transgenic mice, constitutively active Cre is expressed from a large BAC (bacterial artificial chromosome) transgene in the context of 200 kb of sequence including all

of the regulatory sequences of the 34 kb NG2 (CSPG4) gene. Using these mice, Zhu et al. (Zhu et al., 2008a; Zhu et al., 2008b) demonstrated that NG2 cells generate oligodendrocytes throughout the gray and white matter of the brain and spinal cord. Quantification of oligodendrocytes that expressed the reporter in these mice revealed that the percentage of oligodendrocytes that expressed the reporter gene was similar to the Cre recombination efficiency, indicating that all of the oligodendrocytes are derived from cells that express NG2 at some time in their life. In addition, a subpopulation of protoplasmic astrocytes in the gray matter of ventral forebrain and spinal cord appear to be derived from NG2 cells. Surprisingly, none of the GFAP+ astrocytes in the white matter were generated from NG2 cells under normal conditions, suggesting heterogeneity of the source of astrocytes.

Other studies have used inducible Cre lines in which Cre-mediated excision is activated by tamoxifen in transgenic lines that express a fusion protein consisting of Cre and various forms of the mutated ligand-binding domain of estrogen receptor engineered to bind tamoxifen with a higher affinity than endogenous estradiol(Metzger and Chambon, 2001), 2001). Rivers et al. (2008) generated Pdgfra-creER^{T2}BAC transgenic mice that express CreER^{T2} under the regulatory sequences of PDGFRA, which is expressed in all NG2 glial cells (Rivers et al., 2008). Induction of Cre in adult Pdgfra-creER^{T2}BAC transgenic mice revealed that the vast majority of the reporter+ cells were either NG2 cells or oligodendrocytes. No astrocytes expressed the reporter when Cre was activated in adult mice. In addition to oligodendrocyte lineage cells, a small number of cells with the morphology of projection neurons were detected in the piriform cortex, and these cells expressed the neuronal antigens NeuN and MAP2 but not markers of interneurons. The observation that the number of reporter+ neurons gradually increased after Cre induction suggests that they had been generated from NG2+/PDGRα+ cells. The identity of these PDGFRα+ neuronal precursors and whether they express NG2 remain unknown.

Using Olig2-creER™ transgenic mice that were generated by inserting CreER™ (Danielian et al., 1993) into the Olig2 gene(Takebayashi et al., 2002), Dimou et al., (2008) observed that when Cre was induced in adult mice, the reporter gene was expressed almost exclusively in either NG2 cells or mature oligodendrocytes and in a few protoplasmic astrocytes in gray matter but not in neurons (Dimou et al., 2008). The number of reporter+ oligodendrocytes in the white matter increased over time to >80% of the total induced cells. By contrast, those in the gray matter reached a plateau at <20% of total reporter+ cells. These findings differ slightly from the results obtained by Cre activation at similar ages in NG2creER™BAC transgenic mice, where the number of reporter+ oligodendrocytes continued to rise in both gray and white matter for 60 days after induction, although the rate of increase was smaller in the gray matter ((Zhu et al., 2008c); manuscript submitted).

Recently, another NG2 cell fate-mapping study was performed using PLP-creER^{T} transgenic mice, which express CreER^{T} under the control of the PLP promoter (Guo et al., 2009). Previous studies using PLP-EGFP transgenic mice had revealed that PLP transcriptional activity is detected in a subpopulation of NG2 cells in adult mice as described above(Mallon et al., 2002). When Cre was activated at postnatal day 7 (P7) in PLP-creER^{T} transgenic mice, 90 – 94% of the reporter+ cells in the forebrain were NG2+, while only 27% of the induced cells were NG2+ in the spinal cord, presumably due to activation of Cre in oligodendrocytes that were more prevalent in the caudal CNS at this age. Eight days after Cre induction, the majority of the reporter+ cells in the white matter of the forebrain were oligodendrocytes, while those in the gray matter consisted of a mixture of oligodendrocytes and NG2 cells. In addition, reporter expression was detected in some protoplasmic astrocytes in the ventral forebrain and scattered neurons throughout the forebrain. However, a prior study using plp-cre transgenic mice had shown that PLP transcription is activated not only in oligodendrocyte lineage cells but also in neuronal progenitor cells prior to glial development (Delaunay et al., 2008).

While all of these studies consistently support the oligodendrocyte fate of NG2 cells in both white and gray matter, the findings related to astrocyte and neuronal fate of NG2 cells vary. The precise reason for the differences observed in the fate of NG2 cells among these studies remains unclear, but it is likely that differences in the specificity of Cre-targeting to NG2 cells and the efficacy of Cre induction contribute to the different results obtained in these studies.

7. Origin of NG2 cells

In the spinal cord, the majority of oligodendrocyte lineage cells arise from discrete ventral domains under the influence of Sonic hedgehog (Shh) (Lu et al., 2000; Orentas et al., 1999; Richardson et al., 2006; Takebayashi et al., 2000; Zhou et al., 2000) et al., 2000). Cells that express Olig1 and Olig2 in the pMN domain and those that express Nkx2.2 in the ventrally adjacent P3 domain comprise the early committed oligodendrocyte lineage cells. NG2 becomes detectable after these cells migrate out of the ventricular zone and expand to occupy the entire spinal cord (Nishiyama et al., 1996a; Pringle and Richardson, 1993). While the majority of oligodendrocytes arise from the ventral sources, some PDGFRα+ oligodendrocyte precursors in the dorsal spinal cord arise from the dorsal structures independently of Shh in the absence of the homeodomain transcription factor Nkx6.1 (Cai et al., 2005; Vallstedt et al., 2005), and some arise from the dorsal domains defined by Dbx1 expression via radial glia (Fogarty et al., 2005).

The origin of NG2 cells in the forebrain is somewhat more complex, but as in the spinal cord, the ventral subpallial regions appear to be the major source of oligodendrocytes. The first PDGFRα+ cells appear in the anterior entopeduncular region (AEP) between the median ganglionic eminence (MGE) and anterior hypothalamus at E13.5 in the rat (Pringle et al., 1992; Tekki-Kessaris et al., 2001) and subsequently expand dorsolaterally. Their appearance is dependent on the homeodomain transcription factor Nkx2.1, which is necessary for the correct expression of Shh (Nery et al., 2001; Tekki-Kessaris et al., 2001).

Prior to E14 in the mouse or E15 in the rat, NG2 expression in the forebrain is confined to the vasculature. The first NG2+ parenchymal cells appear after E15, at least 2 days after the first appearance of PDGFRα+ cells, and all the non-vascular NG2+ cells also express PDGFRα (Nishiyama et al., 1996b). These cells are likely to be the earliest NG2 cells that appear in the forebrain. At this early stage a small number of PDGFRα+ cells without detectable NG2 is seen scattered throughout the parenchyma, along with a larger number of cells that co-express NG2 and PDGFRα. By the end of the embryonic development, there is an almost complete overlap in the expression of NG2 and PDGFRα, with the exception of the SVZ, where there are PDGFRα+NG2− cells (Nishiyama et al., 1996b). This is consistent with the finding that >99% of PDGFRα-immunopanned cells from E19 rat brains were NG2+ (Tekki-Kessaris et al., 2001).

Genetic fate mapping using three different Cre transgenic mouse lines revealed that oligodendrocytes are generated in three waves (Kessaris et al., 2006). The earliest PDGFRα+ cells are generated from Nkx2.1+ cells in the MGE and AEP beginning around E12. Using quail/chick transplantation, Olivier et al. (2001) demonstrated that cells in AEP supplies all the oligodendrocytes in the telencephalon. In the mouse, PDGFRα+ cells derived from Gsh2 + cells in the lateral ganglionic eminence (LGE) appear after E16.5 and eventually replace the earlier Nkx2.1-derived cells. A third wave of PDGFRα+ cells appears mainly postnatally from dorsal Emx1+ cells and generates oligodendrocytes in the pallium including the neocortex and the corpus callosum. The original Nkx2.1-derived cells disappear after birth. It has not been tested whether NG2 is expressed equally in the both the early PDGFRα+ cells derived from Nkx2.1+ cells and the later generated Gsh2-derived PDGFRα+ cells. Both Gsh2-derived and Emx1-derived cells are capable of generating oligodendrocytes, but it has not yet been tested

whether NG2 cells derived from the different sources differ in their ability to interact with neurons or generate astrocytes.

The literature on the development of NG2 cells from ventral progenitor cells that express the DLX family of homeodomain transcription factors varies. Migration studies using DiI labeling and cross transplantation in slice cultures revealed that cells that are generated early from MGE migrate dorsally and become dispersed widely through the neocortex, while those that develop later from LGE and MGE migrate into the proliferative zones of the neocortex to form the future subventricular zone (Anderson et al., 2001. The DLX family of transcription factors that includes Dlx1, Dlx2, Dlx5, and Dlx6 is expressed in the embryonic ganglionic eminences. Short-term fate mapping of Dlx2+ cells using DLX2-tau-lacZ transgenic mice revealed that the location of the cluster of (−galactosidase+ cells representing the progeny of Dlx2+ cells shifts from LGE in late embryonic brain to the central core region of the dorsolateral SVZ in the early postnatal stage {Marshall, 2002 #463). Among the progeny of Dlx2+ cells found at P6–10 were GFAP+ astrocytes and CNPase+ oligodendrocytes in the neocortex and corpus callosum. In another study, cells from the corpus callosum and subcortical white matter from E18 mice were found to coexpress NG2 and Dlx1/2/4/5 (He et al., 2001). By contrast, a recent fate-mapping study using the enhancers URE2 and I12b from DLX1 and 2 genes revealed that DLX1/2+ cells give rise to GABAergic interneurons but very few oligodendrocytes and no astrocytes (Potter et al., 2009). It was also recently shown that Dlx1/2 negatively regulates oligodendrocyte development by repressing Olig2, and that lack of Dlx1/2 increased the number of PDGFRα+ cells in early MGE and LGE (Petryniak et al., 2007). Thus, it appears that Dlx1/2 is upregulated as the progenitor cells become committed to a neuronal fate and is lost in cells that become committed to an oligodendrocyte lineage. The function of Dlx1/2 is in turn repressed by Mash1 (Petryniak et al., 2007) which has been shown to be required for the development of NG2 cells in some regions (Parras et al., 2004).

In the postnatal CNS throughout adulthood, the SVZ appears to play an important role in the generation of NG2 cells. Retroviral marking of perinatal SVZ showed that NG2 cells in the corpus callosum and neocortex are generated from neonatal SVZ (Levison et al., 1999). In adult mice, retroviral marking of GFAP+ SVZ type B cells resulted in generation of NG2 cells in the corpus callosum in normal and demyelinated states (Gonzalez-Perez et al., 2009; Menn et al., 2006). Although a small number of NG2 cells are found in the SVZ, and it has been reported that NG2 cells represent transit-amplifying progenitor cells in the SVZ (Aguirre et al., 2004), recent studies suggest that the majority of these progenitor cells in the SVZ do not express NG2, but NG2 is highly expressed in the parenchyma surrounding the SVZ (Cesetti et al., 2009; Diers-Fenger et al., 2001; Komitova et al., 2009; Platel et al., 2009). Besides the SVZ, local proliferation of NG2 cells also contributes to the maintenance of the NG2 cell population in the mature CNS.

In addition to the ventral sources of tangentially migrating cells in embryonic stages and the neocortical SVZ in postnatal rodents, radial glia have also been implicated as a source for NG2 cells. By using human GFAP-cre fate mapping, the predominant fate of radial glia in the striatum was found to be NG2 cells and oligodendrocytes, while neurons comprised the majority of the progeny of radial glial in the neocortex(Malatesta et al., 2000; Malatesta et al., 2003). In another study, when neonatal dorsal radial glia were marked with adenovirus expressing Cre, Cre reporter was detected in NG2 cells in the neocortex and subcortical white matter, indicating that at least some NG2 cells are derived from dorsal radial glia (Ventura and Goldman, 2007).

These observations from recent studies suggest that the general pattern of NG2 cell development in the forebrain is fundamentally similar to those in the spinal cord in that cells arising from the early ventral source become intermingled with cells that arise later from dorsal

regions. Further studies are needed to determine whether the NG2 cells that originate from different regions are functionally equivalent in various aspects of NG2 cell function described above.

8. Conclusion

Recent insights discussed above address the function and lineage of NG2-expressing cells in the CNS. However, we are still far from a complete understanding of the functional roles they play in the CNS. Multiple questions remain to be answered concerning their heterogeneity, response to CNS damage and most importantly, mechanism of integration into and modulation of the neuronal network. NG2-expressing cells may vary between different CNS regions with regard to expression of proteins and mRNAs. During the next few years, we expect to unravel the mysteries surrounding these exciting NG2-expressing cells.

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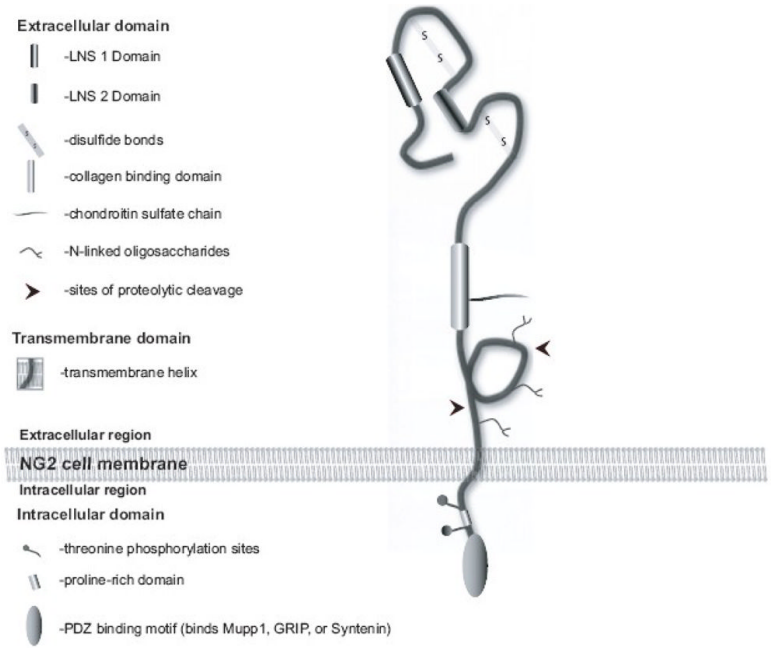

Figure 1. Schematic diagram of the NG2 protein
modified from (Stallcup and Huang, 2008)

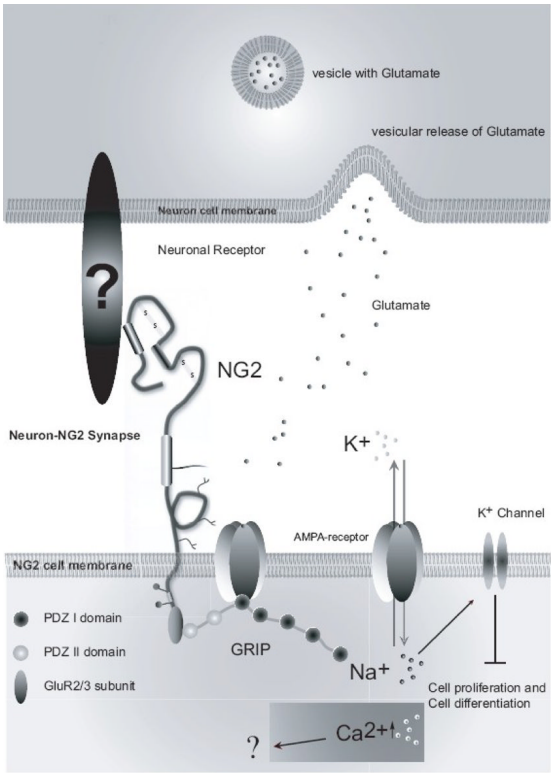

Figure 2. The role of the NG2 protein at the neuron-glial synapse

The NG2 protein could play a role in clustering the glial AMPA receptors towards the site of neuronal glutamate release. Glutamate acting on NG2 cells may thus regulate proliferation and differentiation and also cause a rise in intracellular calcium.

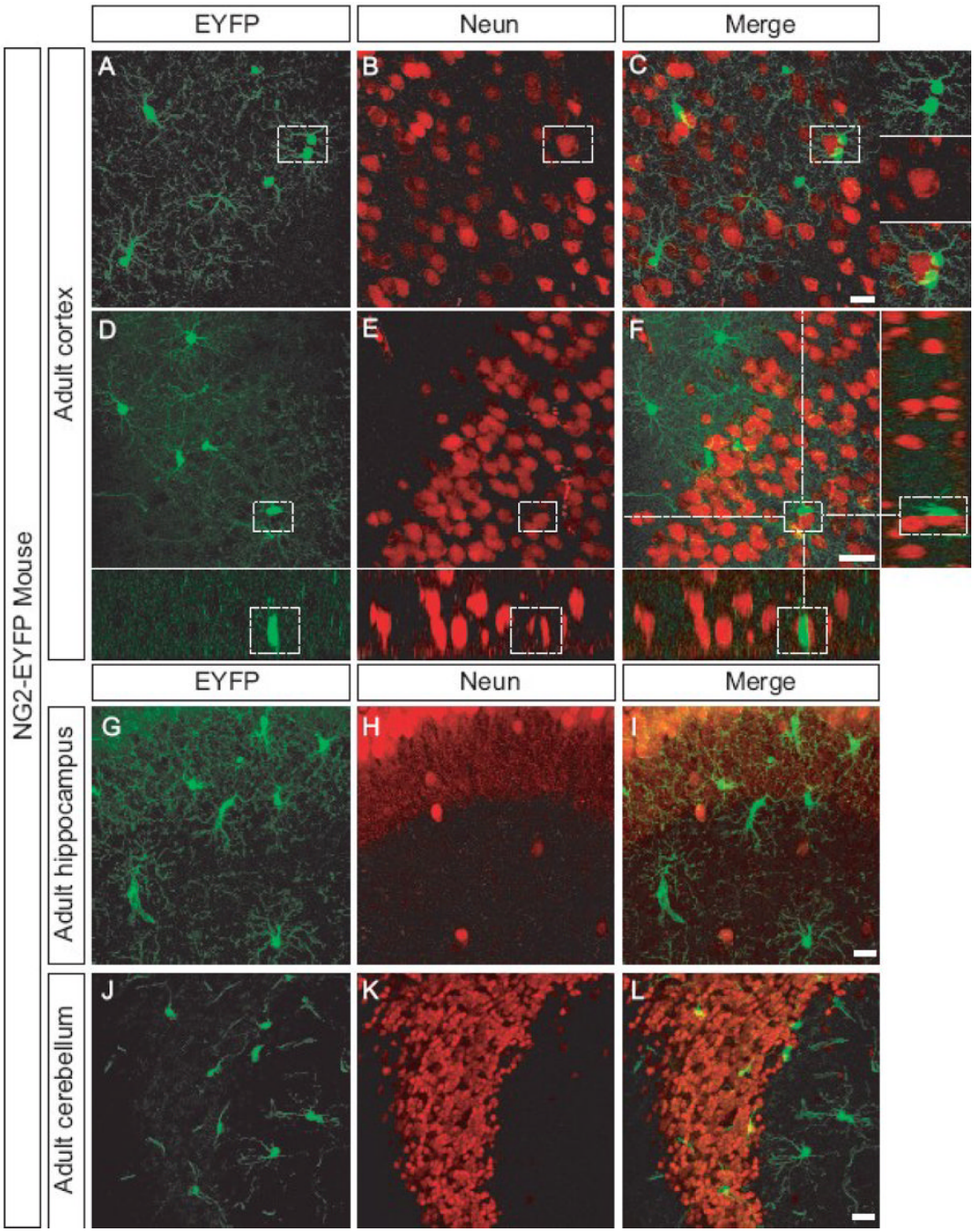

Figure 3. Intimate contact between neurons and NG2 cells in the CNS

Confocal image scan of cortex, hippocampus, and cerebellum of adult mice expressing EYFP (A,D,G, J) stained with an antibody that recognizes Neun (B, E, H, K). Merged images (C, F, I, L) shows no overlap, but close association between EYFP+ cell and Neun + neurons. Inserts at high magnification show EYFP+ cells close to Neun+ neurons.

Scale bars = 20 μm

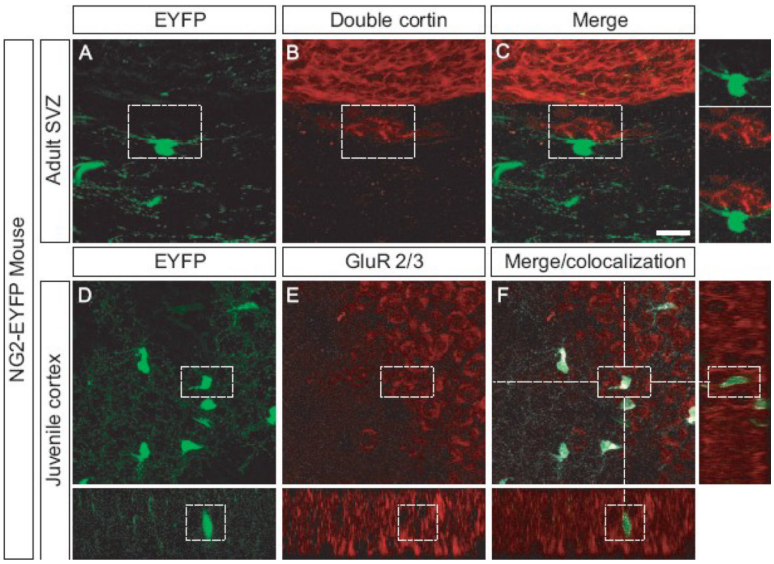

Figure 4. Intimate contact between neurons and NG2 cells in the SVZ and expression of GluR 2/3 by NG2 cells *in vivo*

Confocal image scan of the SVZ of adult mice expressing EYFP (A), stained with an antibody that recognizes Doublecortin (B). Merged images (C) show no overlap but close association between EYFP+ cells and Doublecortin+ neurons. Hogh magnification inserts show EYFP+ cells very close to Doublecortin + neurons. EYFP+ cells (D) in the juvenile cortex stain with an antibody recognising GluR2/3 (E). Merge and co-localization analysis shows expression of the GluR2/3 on the processes and cell body of the EYFP+ cells.

Scale bars = 20 μm

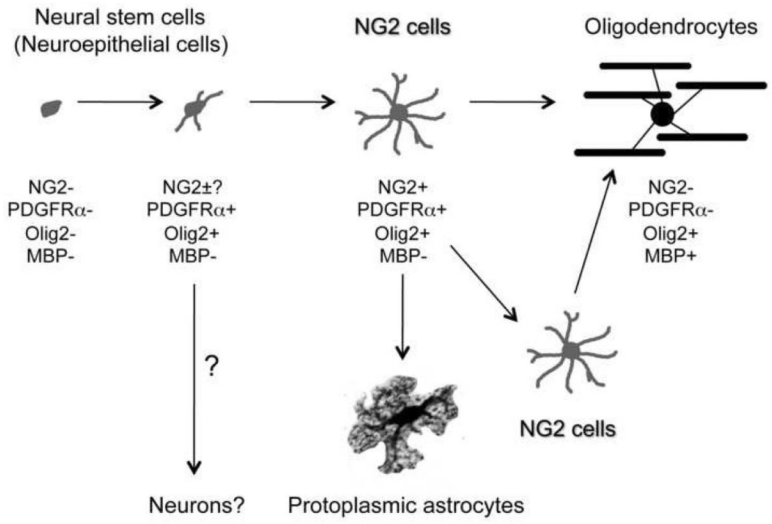

Figure 5. A schematic diagram showing the NG2 cell lineage

NG2 cells originate from NG2−/PDGFRα− cells in the germinal zone and acquire NG2 expression as they migrate to their destination. They have the ability to self-renew and generate oligodendrocytes at all ages. NG2 cells in the immature brain also generate a subpopulation of protoplasmic astrocytes. The neuronal fate of NG2 cells is still debated.