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Polysialylation of the Neural Cell Adhesion Molecule: Setting the Stage for Plasticity Across Scales of Biological Organization

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1. Introduction

In vertebrates, the neuronal cell adhesion molecule (NCAM/CD56) has 3 isoforms resulting from alternative splicing that differ by their size (120, 140 and 180 kDa) and their anchoring at the membrane. Whereas NCAM120 is glycophosphatidyl inositol anchored, NCAM140 and NCAM180 are transmembrane molecules. NCAM180 has an additional intracellular 267 amino acids insert, that differentiate it from NCAM140, but its role remains to be fully elucidated. There are differences regarding the specificity and the level of expression of these isoforms in different cells of the nervous system. Whereas NCAM120 and NCAM140 are preferentially expressed in glial cells NCAM180 seems to be prevalent on neurons. Although differences in expression and function of the NCAM isoforms exist, one common denominator is that they can be post-translationally modified by the addition of long, linear chains of α2,8-linked *N*-acetylneuraminic acid (Neu5Ac) residues. In vertebrates, NCAM is the major acceptor of this unique carbohydrate. This modification occurs on the fifth immunoglobulin domain of NCAM located on the extracellular part of the membrane and common to all 3 isoforms. These polysialylated isoforms have emerged as particularly attractive candidates for promoting plasticity in the central nervous system (CNS). The large negatively charged polysialic acid (PSA) chain of NCAM is postulated to be a spacer that reduces adhesion forces between cells allowing dynamic changes in membrane contacts. However, recent studies indicate that a crucial function of PSA resides in controlling interactions mediated by NCAM. Accumulating evidence also suggests that PSA-NCAM-mediated interactions lead to activation of intracellular signals fundamental to biological functions. An important role of PSA-NCAM appears to be during development, when its expression level is high and where it contributes to the regulation of cell shape, growth or migration. However, PSA-NCAM does persist in adult brain structures such as the hippocampus that display a high degree of plasticity where

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it is involved in activity-induced synaptic plasticity. Recent advances in the field of PSA-NCAM research have not only consolidated the importance of this molecule in plasticity processes but also suggested a role for PSA-NCAM in the regulation of higher cognitive functions and psychiatric disorders, metastasis and tissue repair. The presence of PSA-NCAM also outside the nervous system in specific cell types or in pathological conditions, such as cancer, suggests that it is an important feature of NCAM to be investigated.

An underlying theme in this review will be the potential role that polysialylation, a post-translational modification of NCAM, can play in translating plasticity across scales of biological organization. Indeed, the formation of any complex tissue like the CNS involves adaptive changes such as fate determination, differentiation, proliferation, happening via cell dynamic autonomous processes or dynamic interactions with adjacent cells and tissues. In this chapter, we will update interpretations of some earlier findings and highlight how PSA exerts effects on plastic events at the molecular, cellular and tissue levels.

2. Polysialylation controls plasticity at the molecular scale by regulating the molecular behavior of its own carrier, NCAM

Insight into the function of NCAM at the molecular level came from observations based upon NCAM gene knock out, PSA removal/modulation by enzymatic digestion of PSA by endoneuraminidase (EndoN), knock out of the polysialyltransferase coding genes responsible for addition of PSA to NCAM, or the use of mimotope peptides of PSA. Altogether, they converge to indicate that glycosylation of NCAM may be more critical to account for the biological functions than the presence or absence of the core protein itself. The length of the PSA chains isolated from brain NCAM varies widely with development and has been estimated to exceed 50 sialic acid residues (Galuska et al. 2006). Two enzymes, the polysialyltransferases ST8SiaII and ST8SiaIV, are involved in the biosynthesis of PSA chains in mammals. These enzymes share <60% similarity at the amino acid sequence level and are, during postnatal development, differentially expressed in a tissue- and cell type-specific manner with overlapping expression patterns. In vitro studies showed that each enzyme is independently capable of synthesizing PSA on NCAM although ST8SiaII was found to synthesize shorter PSA polymers than ST8SiaIV. Additional studies involving fine structure analysis suggest a comparable quality of polysialylation by ST8SiaII and ST8SiaIV and a distinct synergistic action of the two enzymes in the synthesis of long PSA chains at N-glycosylation site 5 in vivo (Galuska et al., 2008). By analyzing defects in mice with selected combinations of mutant NCAM and polysialyltransferase alleles, Hildebrandt et al. (2009) revealed that the extent of the fiber tract deficiencies was not linked to the total amount of PSA or NCAM, but correlated strictly with the level of NCAM erroneously devoid of PSA during brain development. Hence, PSA is the key regulator of central NCAM functions.

At the molecular level, modelling of light scattering data, with the assumption that the proteins are spherical, led to the conclusion that PSA increased the hydrodynamic radius of NCAM. Moreover, intercellular space was increased by 10 to 15 nm (Yang et al, 1992; Rutishauser, 1996). In this vein, it was accepted that polysialylation was a mechanism controlling the range and magnitude of intermembrane repulsion and thereby cell-cell interactions. Nevertheless, early studies gave no indication on the forces of interactions mediated by NCAM, whether polysialylated or not. The dependence on ionic strength for

adhesion of PSA-NCAM expressing cells however suggested that this was due to change in hydrodynamic volume and repulsive forces (Pincus, 1991; Yang et al., 1994). The link between repulsive forces and adhesion suggested that this concept could be generalized to other adhesion molecules (Fujimoto et al., 2001).

Johnson et al. (2005) later showed, by introducing physical forces measurements, that PSA decreased cell-cell interactions by playing a repulsive role on trans-homophilic NCAM-NCAM interactions, as well as on trans-interactions between other membrane-bound proteins, at the contact between cell membranes. Surface force apparatus can be used to quantify the distance dependence of the force between extended surfaces such as membranes (Shi et al., 2010). This technique revealed that PSA was able to overcome NCAM-NCAM homophilic and NCAM-cadherin heterophilic attraction. Physical forces measured to demonstrate such role of PSA were dependent on the amount of PSA and ionic strength. The magnitude of the repulsive effect of PSA is significant since it overwhelms a 3-fold excess of cadherin and at 3 binding sites (Johnson et al., 2005). Furthermore, the properties of the polymer of PSA are independent of other proteins on the membrane. We also demonstrated using two different molecular imaging techniques, Fluorescence Correlation Spectroscopy (FCS) and spot Fluorescence Recovery after Photobleaching (FRAP), that when two live cells were in contact, PSA increased the lateral mobility of NCAM, suggesting more fluidity at cell-cell contacts (Conchonaud et al., 2007). Since the polymer of PSA occupies a tridemensional volume, it was likely that the steric influence of PSA would be at play not only in trans-interactions but also in cis-interactions, involving NCAM or other membrane proteins (Figure 1A). We therefore asked whether PSA affected the molecular mobility of NCAM locally within cell membrane microdomains in isolated cells. Indeed, this could also be a reflection of increased molecular repulsion between adjacent proteins in the same membrane, as suggested by Johnson et al. (2005). By using FRAP and FCS to study NCAM dynamics, we brought the evidence that PSA increased the mobility of NCAM itself at the cell membrane without effect on its trafficking or confinement in small domains. The later depends on the actin cytoskeleton as when the cytoskeleton was disrupted, NCAM like PSA-NCAM were no longer confined in microdomains and acquired a Brownian type mobility with a lateral diffusion 60% of that with intact cytoskeleton. Moreover, without intact cytoskeleton, PSA no longer facilitated NCAM mobility (Figure 1B). Polysialylation effect on NCAM lateral diffusion thus requires the integrity of the link of NCAM with the actin cytoskeleton and/or the confinement of NCAM by the cytoskeleton. Furthermore, PSA effect on NCAM lateral mobility was maintained even when NCAM diffused less rapidly due to cell-cell contacts. Importantly, PSA increased NCAM lateral diffusion when cells were activated by an extracellular factor such as GDNF (Conchonaud et al., 2007). Altogether, these data showed that polysialylation conveys to NCAM an intrinsic capacity to increase its lateral diffusion within the membrane even when the molecules are engaged in interactions or signaling. This leads to postulate that not only molecular interactions, but also the duration of NCAM interactions, are reduced by PSA.

Thus, these data place the addition of a carbohydrate by post-translational modification to a cell surface receptor as an efficient way to control its lateral diffusion in a cell autonomous fashion, and most likely cis-interactions with other proteins in the membrane. Figure 1 recapitulates the modulation of cis and trans-homophilic and heterophilic interactions at cell membranes by PSA via repulsive forces and enhanced mobility. Ultimately, as the

resolution of fluorescent imaging improves, it will be exciting to see the imaging of macromolecular assemblies. One of the compelling prospects of this integration is that it should provide molecular models for new mechanistic insights into the signaling processes and the resulting cell behavior.
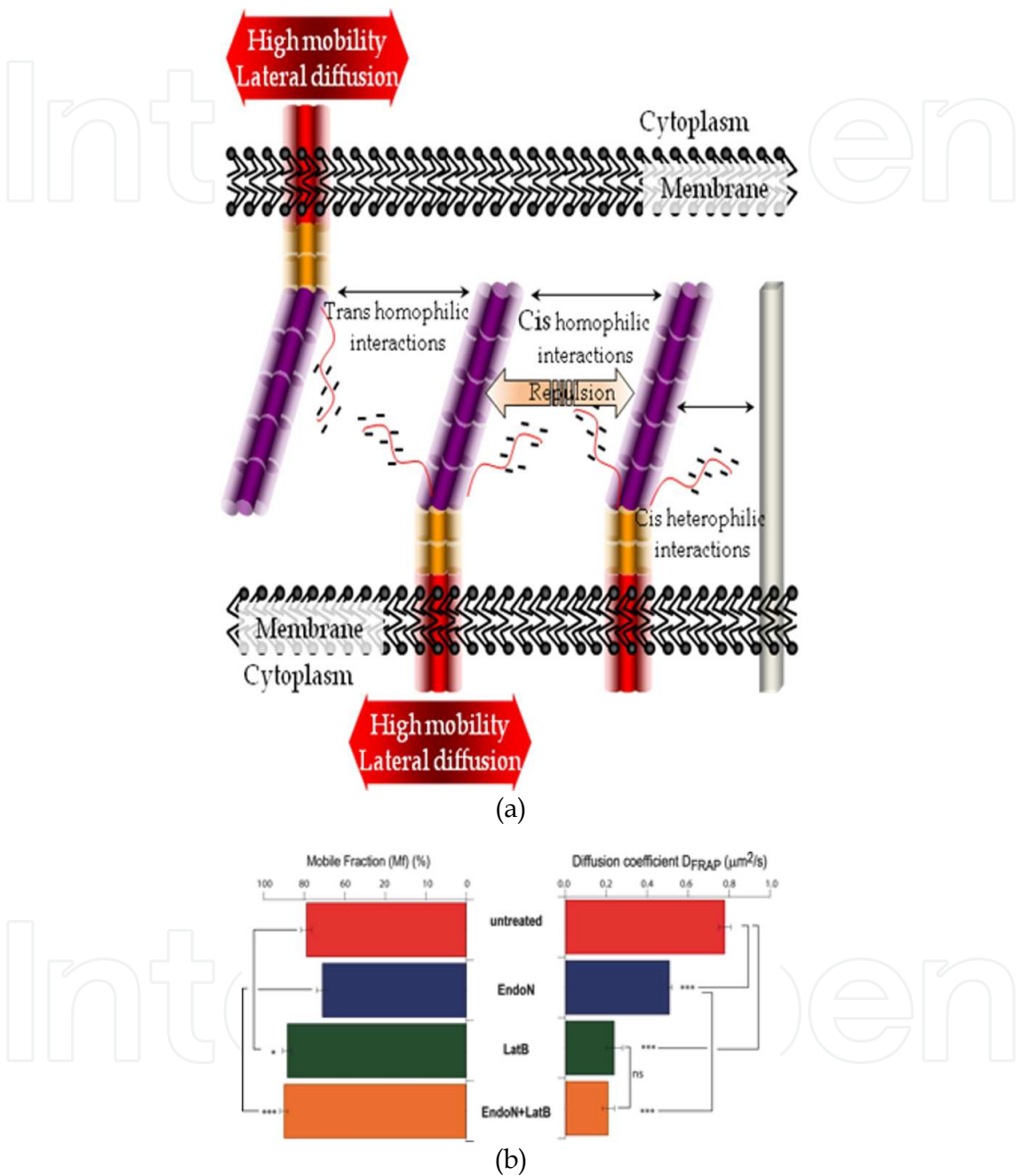

(a)

(b)

Fig. 1. (a) Modulation of NCAM lateral diffusion at the membrane, and homophilic and heterophilic trans and cis molecular interactions by PSA. (b) Tracking of GFP-tagged PSA-NCAM140 molecules by FCS without or after treatment of the expressing cell by EndoN allows to calculate the mobile fraction and by FRAP its diffusion. PSA-NCAM or NCAM molecules were as mobile (80%) but PSA increased NCAM diffusion. Disrupting actin organization by Latrunculin B (Lat B) increases the mobile fraction independently of PSA, but suppresses the enhancing effect of PSA on NCAM lateral diffusion.

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3. Polysialylation results in plasticity at the cellular scale

There is still a big gap to uncover before getting a full understanding on how the effect of polysialylation of NCAM at the molecular level translates into cellular plasticity. For the purpose of this chapter, only studies describing changes in cellular behavior that occur in a cell autonomous manner or in which the cell carrying PSA is considered as the system of reference will be reviewed. PSA effect during development or on axon regeneration, or in diseased conditions will be addressed in part 4 in an integrated tissue system undergoing changes as a whole. As defined, plasticity at the cellular scale can encompass cell fate decisions, cell division, cell death, cell shape and cell migration.

PSA-NCAM is most conspicuously involved in the change of shape or the migration of different cell types (Decker et al., 2000; Hu et al., 1996). PSA-NCAM is required for the migration of group of cells as chains in the rostral migratory stream (RMS) over millimeters (Durbec & Rougon, 2001). In vivo, ectopic expression of PSA in Schwann cells increases their migration (Bachelin et al., 2010; Papastefanaki et al., 2007). PSA-NCAM can also be considered a cell-mobility enhancing/permissive factor. Specifically, several experiments revealed that PSA–NCAM is required for directional migration in response to concentration gradients of chemoattractants such as PDGF, BDNF, CNTF or GDNF. For example, removal of PSA from purified oligodendrocyte progenitor cell (OPC) significantly reduced lamellipodia formation in response to low concentrations of PDGF, raising the possibility that under these conditions OPC are less responsive to PDGF (Zhang et al., 2004). These observations raised the intriguing possibility that PSA–NCAM could modify the ability of cells to sense accurately growth factor gradients and thereby play a role in the guidance process, as also initially suggested for retinal ganglion axons in vivo (Monnier et al., 2001). We examined the requirement of polysialylation using transfilter migration assays of TE671 cells. We observed that removal of PSA decreased by half the ratio between migrating and non-migrating cells (Conchonaud et al., 2007). Furthermore, like for OPC removal of PSA dramatically reduced the enhancing effect of GDNF on migration. Thus, polysialylation of NCAM plays a role in promoting cell migration but also in efficiently potentiating the chemotactic effect of GDNF in TE671 cells. PSA potentiation of GDNF or PDGF effect illustrates that it may affect NCAM relationship either directly with other molecular partners such as growth factors or their receptors or by priming cells at the level of their cytoskeleton to respond more efficiently. In this cellular system, we found an inter-dependence between migration, cytoskeletal changes, and NCAM lateral diffusion in membranes (Part 2). Polysialylation of NCAM increased actin stress fibers formation, showing that PSA had consequences on the actin cytoskeleton involved in plastic cellular events. Moreover, showing that NCAM lateral diffusion and a directional cell migration both require the cytoskeleton, makes the latter appear as a physical link to NCAM that is required for plasticity at the molecular and cellular level.

Cell differentiation is also characterized by a differential expression of PSA-NCAM (Figure 2). Although being preferentially committed to a restricted either glial or neuronal fate, several studies revealed that cultured PSA-NCAM+ neural progenitors isolated from brain do preserve a relative degree of multipotentiality. With this regard, it is noteworthy that growth factors and neurotransmitters, which belong to the micro-environment of neural cells in vivo, regulate morphogenetic events preceding synaptogenesis such as cell differentiation and death. PSA-NCAM seems to play an active role in the differentiation

program of neural cells. For instance, PSA limits the differentiation of progenitors from the subventricular zone (SVZ) (Petridis et al., 2004; Rockle et al., 2008). In this specific system, the effects of PSA removal on cell differentiation and migration seem to be uncoupled as differentiated SVZ neuroblasts due to PSA removal is associated with dispersion into surrounding CNS tissues (Battista & Rutishauser, 2010). It is likely that other factors are also involved in controlling these processes, much like it is the case for neuroblasts reaching the olfactory bulb where signals like reelin influence migration in the OB layers (Courtes et al., 2011; Hack et al., 2002). PSA down regulation in OPC seems to be critical for proper maturation of OPC into myelinating mature oligodendrocytes (Charles et al., 2000, 2002; Koutsoudaki et al., 2010). Polysialylation of NCAM appears to be regulated at the post-translational level since overexpression of polysialyltransferases by ectopic expression is not sufficient to maintain PSA expression and only delays myelination (Coman et al., 2005). These experiments however did not elucidate whether this occurs in a cell-autonomous manner or following interactions with axons to be remyelinated.
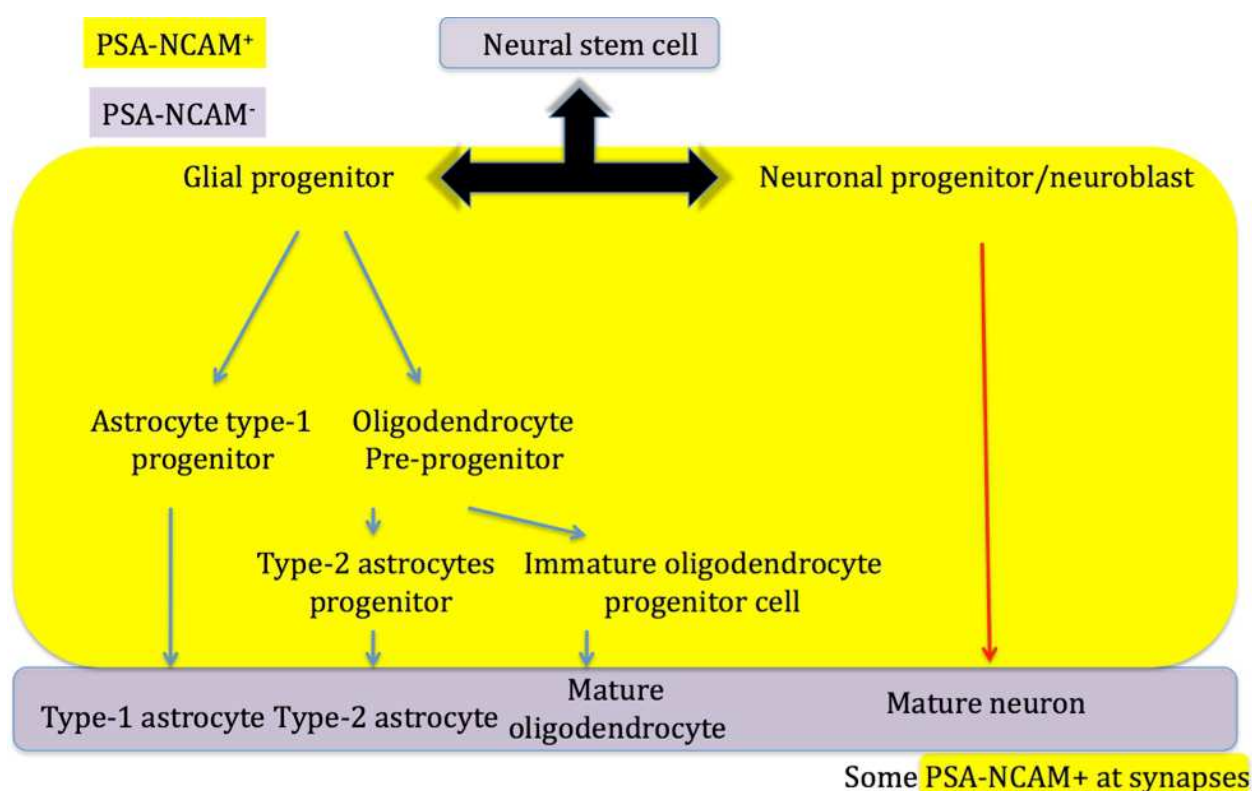

Fig. 2. Schematic view of the progression of CNS cell specification, showing relationships between intermediary phenotypes. Cells expressing PSA-NCAM were specifically shaded on a yellow background and PSA-NCAM- cells shaded in purple. The level of PSA-NCAM expression decreases with the progression towards more differentiated phenotypes.

In vivo, PSA downregulation seems to be associated with cell fate and or functional changes. A most convincing example is given by the experiments showing that the developmental and activity-dependent decline of PSA expression regulates the timing of the maturation of GABAergic inhibition and the onset of ocular dominance plasticity (Di Cristo et al., 2007). Concentrations of PSA significantly decline shortly after eye opening in the adolescent mouse visual cortex; this decline is hindered by visual deprivation. The developmental and

activity-dependent regulation of PSA expression is inversely correlated with the maturation of GABAergic innervation. Premature removal of PSA in visual cortex results in precocious maturation of perisomatic innervation by basket interneurons, enhanced inhibitory synaptic transmission, and earlier onset of ocular dominance plasticity. Intrigingly, PSA is also expressed in a subpopulation of adult cortical interneurons characterized by reduced structural features and connectivity (Gomez-Climent et al., 2011). Birth-dating analyses reveal that these interneurons are generated during embryonic development. They show a reduced density of perisomatic and peridendritic puncta expressing synaptic markers and receive less perisomatic synapses, when compared with interneurons lacking PSA-NCAM. Moreover, they have reduced dendritic arborization and spine density. Altogether these data indicate that PSA-NCAM expression is important for the connectivity of interneurons in the adult and that its regulation at the cellular level may play a role in the structural plasticity of inhibitory networks.

The environment also plays a role on the fate of PSA expressing cells. PSA+ neuroblasts become mostly PSA- glial cells after transplantation in a non-neurogenic environment (Seidenfaden et al., 2006). Some of the effects of PSA on cell fate could however be attributable to NCAM since the NCAM knock mouse displayed glial cells accumulating in the SVZ due to altered cell fate and/or disorganized RMS (Chazal et al., 2010), while the fate of neuroblasts remained mostly neuronal after PSA removal with endoN, despite some dispersion in the surrounding adult tissue (Battista & Rutishauser, 2010). PSA-NCAM has also been shown to be a prosurvival factor for immature neurons (Gascon et al., 2007). The mechanism underlying the effect of PSA on cell fate could thus involve the potentiating effect of PSA with regard to growth factors evidenced in other cell types or systems (Conchonaud et al., 2007; Muller et al., 2000; Vutskits et al., 2001; Zhang et al., 2004), the differentiation of neuroblasts induced by NCAM (Amoureux et al., 2000), or an upregulation of the p75 dependence receptor (Gascon et al., 2007).

Polysialylation of NCAM has also been shown to regulate neuritogenesis (Seidenfaden, 2006), and axonal growth (Doherty et al., 1990; Zhang et al., 1992) synaptic plasticity and activity-dependent cell remodeling, which has been extensively reviewed (Bonfanti & Theodosis, 2009; Muller et al., 2010; Kochlamazashvili et al., 2010). The effects of PSA-NCAM on axonal fasciculation and defasciculation in different systems have been reported and seem to depend on a combination of both intrinsic and context/environment dependent effects (reviewed by Durbec & Cremer, 2001).

At the cellular level, plastic events involve changes in physical/mechanical and chemical/molecular properties. Indeed, cell shape-dependent functions result from complex mechanical interactions between the cytoskeleton architecture and external conditions, be they cell-cell or cell-extracellular matrix adhesion contact-mediated, and their corresponding (mutually interactive) signaling machinery. It is noteworthy that polysialylation of NCAM influences signaling pathways such as ERK phosphorylation (Conchonaud et al., 2007) or FAK (Duveau & Fritschy, 2010), indicating that it is likely that these two types of signals cross-talk. It is important for future studies to elucidate how cross talk between these signals is coordinated to control neural cells structure and function. Ultimately, understanding how the highly interactive mechanical signaling can give rise to phenotypic changes is critical for targeting the underlying pathways that contribute to cell plasticity.

4. PSA controls plasticity at the tissue scale

4.1 PSA in a dynamic healthy environment: During development and in adult CNS areas of plasticity

High plasticity at the tissue level occurs during development when organogenesis takes place. At this scale, PSA has been known to play a major role in the formation of the nervous tissue and its organization as highly complex structures (Edelman, 1986). PSA critical role is reflected by PSA-NCAM spatiotemporal regulation throughout development and in adulthood. NCAM during development (embryonic and early postnatal) only exists in its polysialylated form, which then disappears in adulthood in most CNS areas (Figure 3).
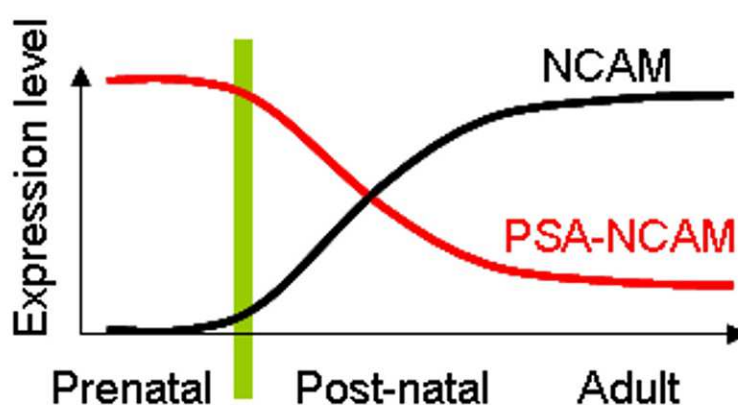

Fig. 3. Regulation of PSA-NCAM throughout development

Conversely to NCAM knock out mice which display a relatively mild phenotype (Chazal et al. 2000), mice lacking the polysialyltransferases ST8SiaII and ST8SiaIV, show a severe phenotype with specific brain wiring defects, progressive hydrocephalus, postnatal growth retardation, precocious death, and severe malformation of major brain axon tracts (corticospinal tract, anterior commissure, corpus callosum and internal capsule hypoplasia). Along with the growth retardation, polysialyltransferase double mutants appear increasingly cachectic and reveal severe deficits in simple tests of motor coordination, strength, and balance (Weinhold et al., 2005). Most of these phenotypes have been attributed to increased adhesion due to absence of PSA on NCAM, and illustrate the prominent aspect of the postranslational modification.

In the adult, following PSA removal by endoN, plasticity is affected in brain regions where PSA is normaly expressed. The first brain region is the SVZ, located at the start of the rostral migratory stream (RMS) pathway followed by PSA-NCAM expressing neuroblasts on their way to the olfactory bulb (OB), the later being constantly exposed to new odors and requiring permanent renewal of neurons for sensing the environment (Gheusi et al., 2000). NCAM deletion results in a 30% decrease in the size of the OB, and 10% in the overall brain size, associated with deficits in learning (Chazal et al., 2000; Cremer et al., 1994; Tomasiewicz et al., 1993).

The second main brain region is the subgranular zone (SGZ), from which newly born neurons emanate and integrate into the granular layer of the hippocampus, involved in memory and long term potentiation, a physiological strengthening of neural connections

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thought to underlie learning and memory. The activity-dependent expression of PSA at the synapse also suggests a role for this molecule in activity-induced synaptic plasticity and memory. Indeed in 2007, Lopez-Fernandez et al. demonstrated that up-regulation of PSA-NCAM in the dorsal hippocampus after contextual fear conditioning was involved in long-term memory formation. This article confirmed the previously published papers in which hippocampal upregulation of the polysialylated form of NCAM was shown to play a key role on spatial memory processes (Venero et al., 2006). The recall of acquired memories is initially dependent on the hippocampus for the process of cortical permanent memory formation, a dependency that decays with time in order to leave room for new memories to be acquired. Production of PSA-positive new neurons in the hippocampus increases the efficiency of the hippocampus, shortens the time where the recall is dependent on the hippocampus, allowing that physiological traces of old memories are promptly removed from the hippocampus to make room for new ones (Kitamura et al., 2009). New neurons enable the hippocampus to work more quickly. Many other studies also point out the functional significance of adult hippocampal neurogenesis in learning and memory (reviewed by Zhao et al., 2008). Therefore in this second brain system, PSA-NCAM is also connected to a brain function that requires plasticity, namely memory. The effect of a PSA-NCAM mimotope, PR21, developed in the laboratory and presented in 4.3. was investigated on spatial memory consolidation following injection of the peptide into the dorsal hippocampus of mice. The mimotope of PSA-NCAM improves memory consolidation requiring hippocampus, suggesting that it enhances the plasticity of this structure (Florian et al., 2006).

The existence of germinative zones associated with high PSA-NCAM labeling has initially been put to light in adult rodents. Apparently, this extends to other species such as the rabbit, the song bird, the primate, and up to the human CNS, although with species-specific organizations of germinative zones and pathways of migration (Bedard et al., 2006; Bernier et al., 2002; Luzzati et al., 2006; Marlatt et al., 2011; Pencea et al., 2001; Sawamoto et al., 2011). The existence of a human RMS, which is organized around a lateral ventricular extension reaching the OB, composed of PSA-NCAM+ neuroblasts, that incorporate 5-bromo-2'-deoxyuridine and become mature neurons has been shown (Curtis et al., 2007; Kam et al., 2009; Sanai et al., 2004). Hipocampal adult human neurogenesis has also been demonstrated to take place (Ni Dhuill et al., 1999). The hypothalamo-neurohypophyseal axis is another system characterized by a dynamic PSA-NCAM expression, and plasticity due to its neuroendocrine physiology related to food intake, sleep-wake cycle and reproduction (Migaud et al., 2010). A strong immunolabeling for PSA-NCAM was initially observed in the rat and monkey hypothalamus (Bonfanti et al., 1992; Perrera et al., 1993; Theodosis et al., 1991) and more recently in the sheep (Franceschini et al., 2010). In this structure estrogen induced synaptic plasticity of gonadotropin releasing hormone (GnRH) neurons is concommittant with PSA-NCAM regulation by activity across the estrous cycle (Parkash & Kaur, 2007). PSA-NCAM+ cells have also been described in the cortex of different species, like the adult rat (Gomez-Climent et al., 2011), rabbit, guinea pig and lizard, particularly in non-spatial learning and memory networks, where the labelled cells are not newly generated (Luzzati et al., 2009).

This indicates that polysialylation of NCAM plays a major role in CNS areas of physiological plasticity encompassing regenerative properties as well as refinement of

neural networks. Long-standing questions include to what extent these regenerative properties recapitulate the events observed in development and to what extent are adult- or species-specific aspects deployed.

4.2 PSA in cancer

PSA-NCAM has the status of an oncofetal antigen. Many studies examined tumor versus control expression of NCAM and PSA epitopes in tissue specimens, as well as correlation between tumor expression and clinicopathological features. Generally, results showed a low constitutive expression of PSA-NCAM in control tissue, which reached a statistically significant increase in the tumor tissue. Likewise, the presence and number of metastases at surgery were correlated with PSA-NCAM expression. The data highlight the importance of taking into account PSA epitope when dealing with NCAM cell expression studies in tumor development and progression and the fact that PSA-NCAM could be an intersting biomarker to assess a better knowledge of brain tumors, help prognosis, design and evaluate therapies. We set up a specific and sensitive enzyme linked immunosorbent assay (ELISA) test for PSA-NCAM quantification, which correlated with PSA-NCAM semi quantitative analysis by immunohistochemistry, and thus provides an accurate quantitative measurement of PSA-NCAM content for the biopsies analyzed (Amoureux et al., 2010). Survival of patients with Glioblastoma Multiform (GBM) the most aggressive and frequent brain tumor, albeit without cure, is limited to one year on average, however significant variability in outcome is observed. We studied its expression in GBM and evaluated its prognosis value for overall survival (OS) and disease free survival (DFS). We showed that PSA-NCAM was expressed by approximately two thirds of the GBM at variable levels. On univariate analysis, PSA-NCAM content was an adverse prognosis factor for both OS and DFS. On multivariate analysis, PSA-NCAM expression was an independent negative predictor of OS and DFS. Furthermore, in glioma cell lines, PSA-NCAM level expression was correlated to the one of olig2, a transcription factor required for gliomagenesis (Amoureux et al., 2010). In addition to the prognosis value of PSA-NCAM in GBM, evidence from ours and others' studies have shown that PSA-NCAM is also a reliable marker in many types of cancers of the CNS and other organs, and that a correlation could be established between the level of PSA-NCAM and the severity of the disease and/or the response to treatment and relapse (Daniel et al., 2000, 2001; Figarella-Branger et al., 1990, 1996 ; Gluer et al., 1998; Trouillas et al., 2003). Another focus of intense investigation in the cancer field has been the existence and the role of cancer initiating cells in GBM (reviewed in Stiles & Rowitch, 2008). There is a strong possibility that the glial progenitors that populate the adult CNS are one source of gliomas with plasticity of immature glia (see part 3) underlying the phenotypic heterogeneity one sees in many infiltrating GBM. At present, however, there is no definitive way to distinguish a reactive/recruited progenitor or astrocyte from a glioma cell. There is accumulating evidence that CD133+ cells are not the only elements in GBM with significant tumorigenic potential. Human gliomas also contain an abundance of cells that express markers normally expressed by adult glial progenitors, including PSA-NCAM providing another possible link between glial progenitor like cells and tumorigenesis. In addition, PSA-NCAM could be interesting to study and modulate GBM infiltration as it appears to recapitulate the migration of glial progenitor cells that occurs during brain development (Farin et al., 2006). In addition, treatment of GBM patients as previously

suggested with chemicals that alter PSA expression with the goal to modulate polysialylation in tumors could be envisionned (Mahal et al., 2001).

4.3 PSA-mimicking agents in promoting tissue repair: Example of spinal cord injury

A striking feature of vertebrate regeneration is that it can take place only during development at embryonic and early postnatal stages, when the CNS is still in a phase characterized by high plasticity and remodelling. A specificity of this phase is the overall expression of PSA-NCAM as described above, which then disappears when the adult CNS is stable. From an evolutionary perspective, it is remarkable that the addition of this unique carbohydrate polymer to the membrane bound NCAM only appeared in vertebrates, indicating a role associated to a higher degree of complexity of the nervous system. The evolutionary perspective on PSA-NCAM appearance in vertebrates, placed in the context of repair, also strongly suggests that its disappearance in vertebrate adult nervous system may be one of the key factor that limit regeneration. Indeed, lower organisms have the ability to regenerate after adult CNS lesions and do not require expression of PSA-NCAM during development, whereas vertebrate axons can regenerate while PSA is still expressed but cannot during adulthood when PSA is absent. PSA-NCAM reexpression is common after lesions or induced demyelination of different fiber tracts and has been interpreted as the reactivation of a neurodevelopmental program (Bonfanti et al., 1996; Nait-Ousmenar et al., 1995).

Spinal cord injury (SCI) is a complex pathology, which does not lead to recovery in severe cases. Of specific interest to SCI, PSA-NCAM is expressed in the area lining the central canal of the spinal cord, much like the SVZ lines the brain lateral ventricules and in laminae I, II and X of adult rat spinal cord (Bonfanti et al., 1996). One of the main issues in SCI is the lack of ability of damaged and severed neurons to regenerate. It is undeniable that inhibitors, including chondroitin sulfate proteoglycans, along with non-proteoglycan inhibitors such as myelin-associated glycoprotein and NOGO, present in the disrupted myelin after axonal severing or the glial scar formed after injury constitute a major impediment to axonal re-growth (Silver & Miller, 2004). However, inhibiting one of them will not be sufficient to overcome the system that the vertebrate CNS has put in place to prevent regeneration. In addition, the limitation of such inhibitory strategies is related to the fact that all the inhibitors known to date, lead to the same downstream signaling pathways converging on the activation of Rho small GTPases and Rho-associated, coiled-coil containing protein kinase inhibiting axonal growth. Nevertheless, although not sufficient to overcome the inhibitory signals, spontaneous growth and plastic responses of CNS axons exist after SCI, especially in the first days after injury. Bareyre et al. (2004) and Courtine et al. (2008) unequivocally showed that spontaneous injury-induced structural and functional circuit rearrangements contribute to the spontaneous behavioral recovery after lesions in rodents. Such remodelling of spared system is probably crucial for rehabilitation in humans and offers some hope that a strategy stimulating these self-repair mechanisms may lead to better clinical results (Rosenzweig et al., 2010). It is therefore necessary to stimulate these spontaneous plastic changes. Properties of PSA-NCAM described in Part 3 such as its capacity to enhance cell mobility and promote axonal growth, both necessary to reconstruct functional cell connections after SCI strongly indicate that PSA-NCAM is a valuable, innovative and unique target to overcome the particular set of challenges of SCI. The use of

PSA-NCAM mimicking agents appear beneficial to recovery by shifting the balance between inhibitors and stimulators towards stimulators. A cyclic peptide, PSA mimetic peptide, PR-21, was selected from a phage library screening of 100 million dodecapeptides, by its ability to bind an antibody directed against PSA-NCAM (Torregrossa et al., 2004). This peptide was shown to be non immunogenic, and stable and to improve functional recovery after SCI in mice (Marino et al, 2009) as well as regeneration after periheral nerve crush (Mehanna et al., 2009). Earlier studies already had pointed out the importance of the embryonic form of NCAM after nerve injury (Daniloff et al., 1986, 1995). Another PSA mimetic peptide than PR21 was shown to improve SCI recovery in mice (Mehanna et al., 2010). Camand et al. (2004) also showed that PSA-NCAM is expressed by reactive astrocytes in the glial scar and that these molecular changes are correlated with the sprouting of axons, observed for cerebellar neurons (Dusart et al., 1999). Their observations suggest that PSA-NCAM re-expression is accompanied by the acquired permissiveness of axons to regenerate through the glial scar. El Maarouf & Rutishauser (2010) also suggested that PSA expression could be used as a strategy for promoting repair involving rebuilding of neural connections. Data showed that transfection of scar astrocytes with a construct that encodes polysialyltransferases improved the regeneration of corticospinal-tract axons through the glial scar (El Maarouf & Rutishauser, 2010) while overexpression of PSA by grafted Schwann cells, promoted functional recovery after SCI (Papastefanaki et al., 2007; Zhang et al., 2007). After having shown proof of efficacy in a mouse SCI model (Marino et al., 2009), we present herein data showing that the cyclic PSA mimotope PR21 also promotes SCI motor recovery in a clinically relevant rat model of SCI.

PR-21 efficacy was assessed using a chronic delivery paradigm over 14 days in a spinal cord impaction injury rat model. Spinal cord lesions were performed with the NYU impactor. Rats were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). A laminectomy was performed at T9-T10 level exposing the cord without disrupting the dura mater. The spinous processes of T8 and T11 were clamped to stabilize the spine, and the cord was subjected to weight drop impact using a 10 g rod dropped at the height of 12.5 mm. After the injury, the muscles and skin were closed in layers. An entry point for intrathecal cannulation was created immediately after spinal cord contusion injury. An incision of the ligamentum flavum between T12 and L1 was made using a pair of microscissors followed by a L1 laminectomy. The catheter attached to an Alzet minipump was then inserted into the subarachnoid space, and the tip of the catheter was carefully advanced up to 1.25 mm caudal to the injury epicenter. Two doses of PR21 (3 and 12 mg/kg) were tested.

We show, using this closer to human injury model, that PR-21 significantly enhanced motor recovery, measured using the standardised Basso-Bresnaham-Beattie motor BBB test (Basso et al., 1995), in rats when delivered intrathecally (Figure 4). The results show that PR-21 was also able to improve motor coordination.

A BBB score of 12 corresponds to a return to coordinated movements (Basso et al. 1995). This analysis showed that at day 70, 89% of 3mg/kg PR-21-treated animals could walk with occasional coordination versus 50% for vehicle-treated animals (Figure 5A). In addition, if a BBB score threshold of 14 corresponding to permanently coordinated animals (Basso et al. 1995) was applied, a significant higher proportion of 3mg/kg-treated animals could walk with a consistent coordination (Figure 5B) if compared to the vehicle-treated group (57% vs 30%).
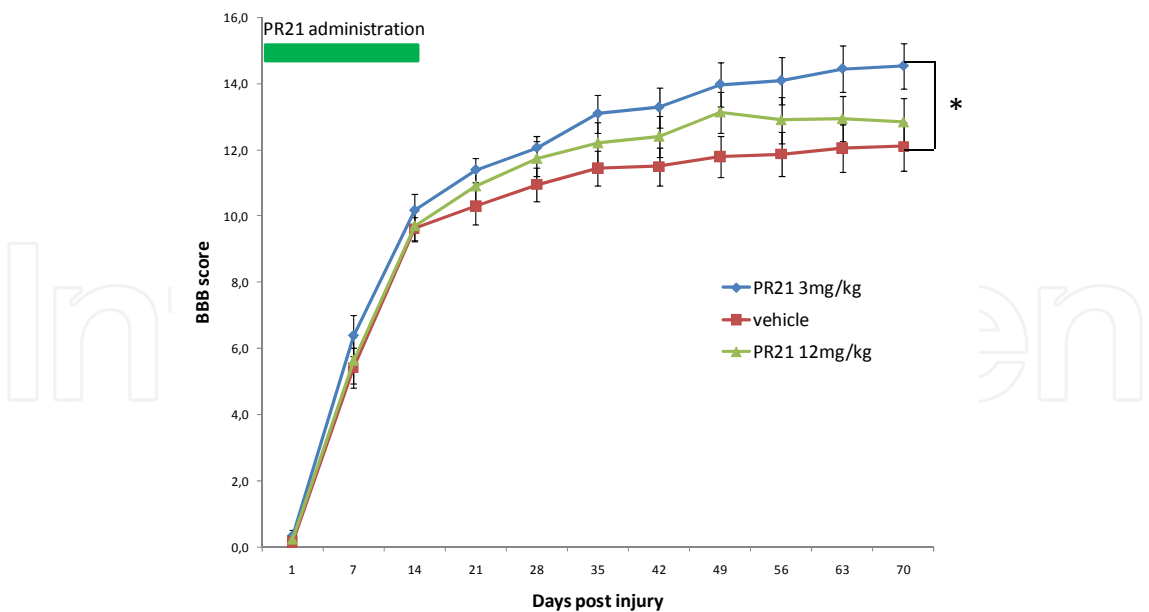
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Fig. 4. Time course recovery of PR-21 treated (3mg/kg and 12mg/kg) and control rats (*p<0.05, one way ANOVA and Dunnet's post-hoc test).
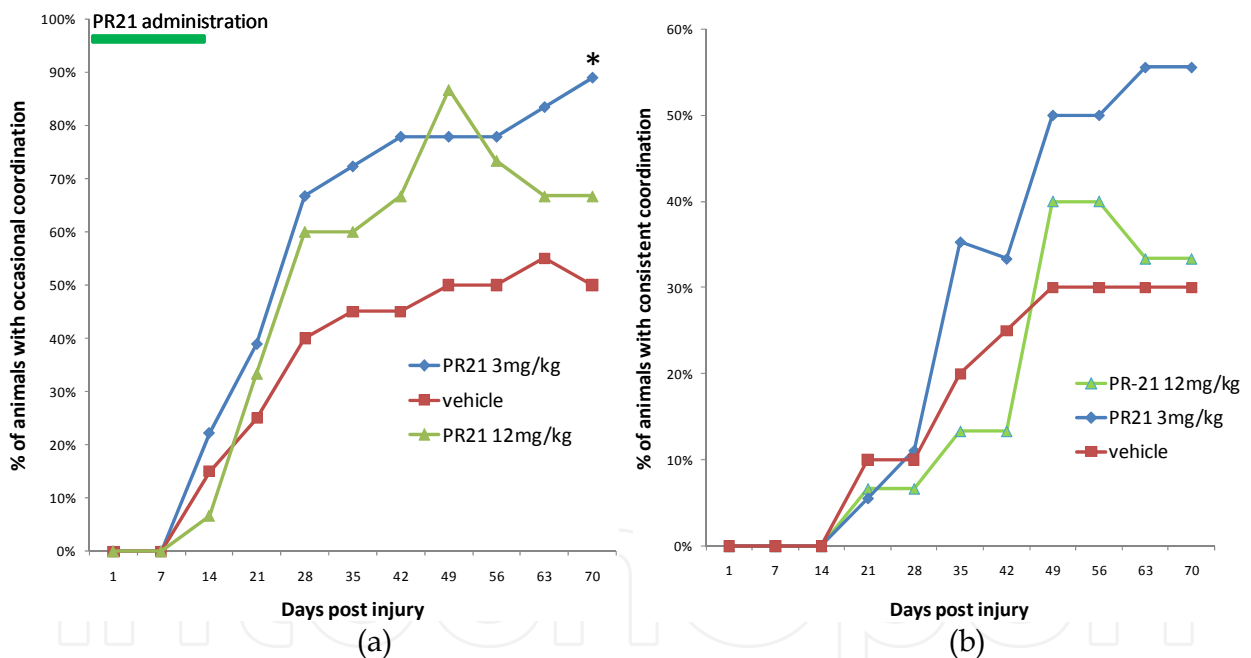

Fig. 5. Percentage of rats able to occasionally walk coordinating their fore- and hindlimbs (a) and with consistent coordination of their fore- and hindlimbs (b). (n=20); *p<0.05: Chi-Square/Fisher exact test.

Altogether, these results demonstrated that PR-21 at a dose of 3mg/kg clearly improves functional recovery of animals with contusion SCI. The dose of 12 mg/kg tended to improve motor performance but the results were not statistically significant. Further analysis by turbidimetry, a method used to assess compound solubility, revealed that PR21 solubilized for the dose of 12 mg/kg did not remain as soluble as for 3 mg/kg, which is a likely explanation for its lower efficiency.

In summary, in two independent animal SCI models (mice dorsal hemisection and rat contusion), and with two types of delivery (one-shot acute delivery in mice and 14 days continuous delivery in rats), a PSA-mimotope improved significantly motor recovery. The improved recovery was observed for global locomotion and hindlimb/forelimb coordination using the Basso Mouse Scale, motor coordination and balance using the Rotarod to test the ability of animals to remain on a rotating rod, and fine locomotor, sensory and proprioceptive performance using the grid walk test. Furthermore, PR-21 shortened the time of return to continence, a major issue in SCI, by 2-fold in the mouse model of SCI. The underlying mechanisms of improved recovery seems to be a decrease of the glial scar, containing regeneration inhibitors at the lesion site, and an increased density of serotonergic axons at and caudal to the lesion, these fibers being major players in the central pattern generators of locomotion (Marino et al., 2009). Other pathways such as the lesioned corticospinal tract seem to have also been positively affected by the treatment since the grid test revealed precise motor placement in which the corticospinal tract plays a significant role in the mouse model (Marino et al., 2009). Accordingly, providing the spinal cord with a mimotope of PSA-NCAM affects plasticity, supporting that PSA-NCAM is an important player in the pathological state of SCI.

5. Conclusion

How tissues regulate their size and morphology remains an incompletely answered question, particularly in the CNS, which shifts from a very plastic structure during embryonic and early postnatal stage to a static organ, isolated by the blood brain barrier in the adult. The presence of PSA-NCAM during developmental tissue morphogenesis or in immature cells throughout life in various species, as well as the use of enzymatic and chemical tools and genetic mice models, point to PSA-NCAM not only as a marker of plasticity, but also a structural and functional factor of plasticity conserved throughout vertebrate evolution. The transitory re-expression of PSA-NCAM after traumatic lesion or demyelination, hormonal or neurotransmitter induction, also suggests a role for PSA-NCAM in glial and neuronal plasticity, even in adulthood. Permanent re-expression such as in cancer illustrate a deregulation of plasticity leading to unwanted cell migration and metastasis. These effects seem mediated by the regulation of the mobility of its carrier, NCAM, within the membrane as well as the repulsive forces between cell membranes and their bound molecules. It is undeniable that these interactions are conditioning and conditioned by other molecular players, such as other ligand-receptor complexes, adhesion molecules, guidance cues, growth factors, locally, or within short or medium range, to coordinate cell and overall tissue homeostasis.

In the context of CNS trauma such as SCI, PSA appears to control the state of damaged neurons submitted to an intrinsic internal potential (repertoire of transcription factors, second messengers, cytoskeleton), exposed to an interactive external environment (extracellular matrix, other cells, growth factors etc.), via an interface identified by the membrane (3D organization of mobile receptors). Adaptive plasticity can be viewed as a physical and mechanical response, at the molecular and cellular level, that can be potentiated by endogenous regulation of PSA or PSA-like agents at the interface between the external and internal parts of the system, making possible plastic changes at the tissue scale.

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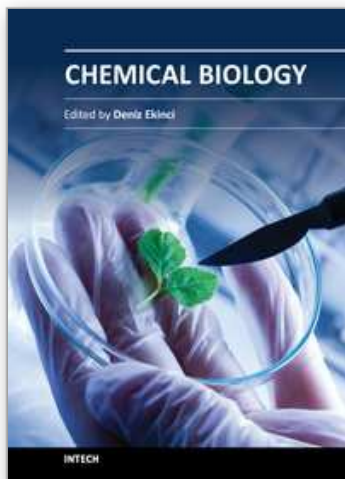
Chemical Biology

Edited by Prof. Deniz Ekinci

Chemical biology utilizes chemical principles to modulate systems to either investigate the underlying biology or create new function. Over recent years, chemical biology has received particular attention of many scientists in the life sciences from botany to medicine. This book contains an overview focusing on the research area of protein purification, enzymology, vitamins, antioxidants, biotransformation, gene delivery, signaling, regulation and organization. Particular emphasis is devoted to both theoretical and experimental aspects. The textbook is written by international scientists with expertise in synthetic chemistry, protein biochemistry, enzymology, molecular biology, drug discovery and genetics many of which are active chemical, biochemical and biomedical research. The textbook is expected to enhance the knowledge of scientists in the complexities of chemical and biological approaches and stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemical biology.

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