Cellular prion protein is required for neuritogenesis: fine-tuning of multiple signaling pathways involved in focal adhesions and actin cytoskeleton dynamics

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Abstract: Neuritogenesis is a dynamic phenomenon associated with neuronal differentiation that allows a rather spherical neuronal stem cell to develop dendrites and axon, a prerequisite for the integration and transmission of signals. The acquisition of neuronal polarity occurs in three steps: (1) neurite sprouting, which consists of the formation of buds emerging from the postmitotic neuronal soma; (2) neurite outgrowth, which represents the conversion of buds into neurites, their elongation and evolution into axon or dendrites; and (3) the stability and plasticity of neuronal polarity. In neuronal stem cells, remodeling and activation of focal adhesions (FAs) associated with deep modifications of the actin cytoskeleton is a prerequisite for neurite sprouting and subsequent neurite outgrowth. A multiple set of growth factors and interactors located in the extracellular matrix and the plasma membrane orchestrate neuritogenesis by acting on intracellular signaling effectors, notably small G proteins such as RhoA, Rac, and Cdc42, which are involved in actin turnover and the dynamics of FAs. The cellular prion protein (PrP^C), a glycosylphosphatidylinositol (GPI)-anchored membrane protein mainly known for its role in a group of fatal neurodegenerative diseases, has emerged as a central player in neuritogenesis. Here, we review the contribution of PrP^C to neuronal polarization and detail the current knowledge on the signaling pathways fine-tuned by PrP^C to promote neurite sprouting, outgrowth, and maintenance. We emphasize that PrP^C-dependent neurite sprouting is a process in which PrP^C governs the dynamics of FAs and the actin cytoskeleton via $\beta 1$ integrin signaling. The presence of PrP^C is necessary to render neuronal stem cells competent to respond to neuronal inducers and to develop neurites. In differentiating neurons, PrP^C exerts a facilitator role towards neurite elongation. This function relies on the interaction of PrP^C with a set of diverse partners such as elements of the extracellular matrix, plasma membrane receptors, adhesion molecules, and soluble factors that control actin cytoskeleton turnover through Rho-GTPase signaling. Once neurons have reached their terminal stage of differentiation and acquired their polarized morphology, PrP^C also takes part in the maintenance of neurites. By acting on tissue nonspecific alkaline phosphatase, or matrix metalloproteinase type 9, PrP^C stabilizes interactions between neurites and the extracellular matrix.

Keywords: prion, neuronal differentiation, neurite sprouting, neurite outgrowth, signaling, multiprotein complexes

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Introduction

Neuritogenesis is a complex morphological phenomena accompanying neuronal differentiation. Neuritogenesis relies on the initial breakage of the rather spherical symmetry of neuroblasts and the formation of buds emerging from the postmitotic

neuronal soma.¹ Buds then evolve into neurites, which later convert into an axon or dendrites.² At the distal tip of neurites, the growth cone integrates extracellular signals and guides the neurite to its target. The acquisition of neuronal polarity depends on deep modifications of the neuroblast cytoskeleton characterized by the remodeling and activation of focal adhesions (FAs) and localized destabilization of the actin network in the neuronal sphere.^{1,3,4} Actin instability in unpolarized neurons allows neurite sprouting, ie, the protrusion of microtubules, and subsequent neurite outgrowth.⁵ Once the neurite is formed, actin microfilaments recover their stability and exert a sheathed action on neurites, a dynamic process necessary for the maintenance and integrity of neurites.⁶

A combination of extrinsic and intrinsic cues pilots the architectural and functional changes in FAs and the actin network along neuritogenesis. This process includes neurotrophic factors (nerve growth factor, brain derived neurotrophic factor, neurotrophin, ciliary neurotrophic factor, glial derived neurotrophic factor) and their receptors,^{7–12} protein components of the extracellular matrix (ECM) (laminin, vitronectin, fibronectin),^{13–15} plasma membrane integrins and neural cell adhesion molecules (NCAM),^{5,16} and intracellular molecular protagonists such as small G proteins (RhoA, Rac, Cdc42) and their downstream targets.^{17,18}

The cellular prion protein (PrP^C), whose conversion into pathogenic prions (PrP^{Sc}) is at the root of transmissible spongiform encephalopathies, a group of neurodegenerative diseases affecting both animals (scrapie in sheep, bovine spongiform encephalopathy in cattle) and humans (Creutzfeldt–Jakob disease and its variant, Gerstmann–Sträussler–Scheinker),¹⁹ has been shown to take part in neuronal differentiation and notably to influence neuritogenesis.

PrP^C expression starts early, during murine embryogenesis (E8.5).²⁰ In adult tissues, PrP^C is present in virtually all cell types and is most abundantly expressed in neurons. It is located at the outer leaflet of the plasma membrane, to which it is anchored via a glycosylphosphatidylinositol (GPI) moiety.²¹ Because PrP^C is subject to diverse post-translational modifications, including heterogeneous glycosylations on two Asn residues and proteolytic cleavages, a variety of PrP^C isoforms exists at the cell surface. As for numerous GPI-anchored proteins, PrP^C distributes in detergent-resistant microdomains,^{22–25} ie, lipid-rafts or caveolae, of the plasma membrane, known to act as signal transduction platforms.^{26,27} The recruitment of PrP^C within raft multimolecular complexes and interaction with several partners²¹ fits in with the notion

that PrP^C is associated with signaling events and behaves as a receptor or co-receptor. Indeed, in 2000, our work allowed us to assign a signaling function to PrP^C in bioaminergic neurons by showing that PrP^C controls p59Fyn tyrosine kinase activity through interaction with the caveolin-1 membrane protein.²⁸ During the last decade, signaling targets downstream from PrP^C have been identified in neuronal as well as nonneuronal cells. These include PI3 (phosphoinositide-3) kinase, protein kinase C (PKC), NADPH oxidase, extracellular regulated kinases 1/2 mitogen activated protein kinases, cAMP Responsive Element Binding (CREB) transcription factor, TNF α converting enzyme, Ca²⁺ and protein kinase A (PKA).^{29–34} Beyond its own signaling activity, PrP^C is also assumed to exert the role of a scaffolding protein regulating the assembly of various interactors and signaling modules in rafts.²¹ According to the cellular context and local environment (lipid-raft), PrP^C interacts with various partners and thereby exerts a wide array of functions, dealing with cell adhesion, stress protection, stem cell proliferation and differentiation, and homeostasis of neurons and nonneuronal cells.^{21,35–38} Here, we review the current knowledge on the contribution of PrP^C to neuritogenesis focusing on how PrP^C takes part in the three steps of neuritogenesis: (1) neurite sprouting; (2) neurite outgrowth; and (3) neurite maintenance.

PrP^C contribution to neurite sprouting: regulatory function of focal adhesions and actin dynamics via integrins

Neurite sprouting depends on an optimal concentration of PrP^C expression in neuronal stem cells

The involvement of PrP^C in the initial phase of neuritogenesis is supported by the observation that siRNA-mediated silencing of PrP^C in the 1C11 cell line or PC12 cells (PrP^{null}-cells) impairs the sprouting of neurites accompanying neuronal differentiation.¹⁵ The 1C11 cell line behaves as a neuroepithelial progenitor, which lacks neuron-associated functions and acquires, upon differentiation, the overall functions of serotonergic (1C11^{5-HT}) or noradrenergic (1C11^{NE}) neurons in 4 days or 12 days, respectively.³⁹ Along the two bioaminergic differentiation programs, almost 100% of 1C11 neuronal stem cells develop bipolar extensions. Neurite formation starts 3 to 6 hours after exposure to neuronal inducers. At the end of the serotonergic program (1C11^{5-HT}, day 4), neurites reach ~5-fold the length of the small ovoid cell body. With fully differentiated

noradrenergic cells (1C11^{NE}, day 12), neurites are shorter, thicker, and widely branched on a pyramidal cell body. Of note, 1C11 progenitors as well as their neuronal progenies endogenously express PrP^C at similar levels.⁴⁰ The impairment of neurite sprouting in PrP^{null}-1C11 neuronal stem cells strictly depends on the absence of PrP^C since the reintroduction of PrP^C in a PrP^{null} context restores neuritogenesis and neuronal differentiation. However, we should note that restoration of neurite sprouting can be observed only when PrP^{null} cells re-express PrP^C to a level similar to that of 1C11 parental cells. Precursor cells that overexpress or underexpress PrP^C fail to develop neurites¹⁵ and to differentiate in response to neuronal inducers. This suggests that the competence of 1C11 neuronal stem cells to respond to neuronal inducers and to engage in a differentiation program depend on a concentration window of PrP^C expression. In line with this idea, Watanabe et al recently reported that neurite sprouting and outgrowth in N2a cells is influenced by PrP^C expression level.⁴¹

Such a drastic impact of PrP^C depletion on neuritogenesis has never been described before and contrasts with the lack of major phenotypic abnormalities in PrP^C knockout mice. The absence of an obvious developmental phenotype, regarding neurite initiation and outgrowth in PrP knockout mice,³⁵ supports the hypothesis that another host-encoded protein is able to compensate for the lack of PrP^C very early during embryogenesis.^{42,43} That PrP^C plays a key role during embryogenesis is supported by the work of Malaga-Trillo et al, who describe a strong PrP loss-of-function in zebrafish embryos, characterized by the loss of embryonic cell adhesion and arrested gastrulation.⁴⁴ Injection of either zebrafish or mouse PrP mRNAs can partially rescue this knockdown phenotype, further indicating conserved PrP functions in vertebrate embryogenesis.⁴⁴

Negative regulatory function of PrP^C towards β 1 integrins allows neurite sprouting

The impairment of neurite sprouting in PrP^C-depleted 1C11 ectodermal stem cells and PC12 cells revealed that PrP^C exerts a negative regulatory function towards β 1 integrins: (1) PrP^C deficiency is associated with a clustering of β 1 integrins and a raise in β 1 integrin signaling activity; (2) antibody-mediated neutralization of β 1 integrins partly rescues neuritogenesis in PrP^{null}-1C11 cells;¹⁵ and (3) manganese-induced β 1 integrin overactivation in PrP^C expressing 1C11 neuronal stem cells impairs neurite sprouting (our unpublished data).

How the sole presence of PrP^C is sufficient to limit β 1 integrin aggregation and modulate β 1 integrin activation remains enigmatic. The activation of integrins relies on deep structural modifications, ie, the acquisition of an extended conformation pointing towards the extracellular space and changes in the orientation of integrin transmembrane domains allowing the recruitment of vinculin and talin adaptors.⁴⁵ Because PrP^C interacts with β 1 integrins,⁴⁶ one possibility is that PrP^C would restrict the conformational changes associated with β 1 integrin activation. Alternatively, the regulatory function of PrP^C towards β 1 integrins may operate within and/or just beneath the plasma membrane. As mentioned, PrP^C may act in rafts as a scaffolding protein interacting with partners whose identity depends on the cell context.²¹ Through stoichiometric interactions, PrP^C can modulate the signaling activity of its partners in lipid-rafts, underlying the involvement of PrP^C in the cell adaptative response (ie, migration, adhesion, cell division, stress protection and so on) to diverse stimuli.²¹ This specific regulatory function of PrP^C possibly relies on its interaction with the membrane protein caveolin-1.^{28,47,48} The recruitment to rafts and the increase in signaling activity of β 1 integrins (ie, activation of Src kinases, focal adhesion kinase [FAK], and paxillin) were also shown to depend on caveolin-1.^{49,50} Therefore, by interacting with caveolin-1, we may propose that PrP^C controls the level of caveolin-1 bioavailable for β 1 integrin recruitment and activation.⁴⁷ Disruption of the PrP^C/caveolin-1 contact upon PrP^C silencing would authorize the redistribution and interaction of caveolin-1 with β 1 integrins. This would favor the clustering of β 1 integrins and their engagement in highly functional signaling complexes⁴⁹ that block neurite sprouting.

By controlling β 1 integrin clustering and activity, PrP^C optimizes the dynamics of focal adhesions and actin cytoskeleton

The initiation of neurite formation relies on the recruitment and assembly of FAs with high turnover rates (Figure 1). In PrP^C-deficient precursor cells, the increased activity of FAK and Src kinases,^{15,51} two direct targets of β 1 integrin signaling,⁵² associated with the overphosphorylation of the FA component paxillin on Tyr31 and Tyr118,¹⁵ enhance the stability of FAs and slow down their turnover.⁵³ Of note, neurite sprouting and outgrowth depend on the constant spatiotemporal regulation of FA stability in the growth cone:⁵⁴ (1) excessive instability of FAs at the front of the growth cone hinders progression of the distal region; and (2) high stability of FAs at the end of the growth cone impairs the

Figure 1 PrP^C depletion in neuroectodermal stem cells impairs neurite sprouting and differentiation. In PrP^C expressing neuroectodermal stem cells, PrP^C exerts a negative regulatory function towards the clustering and activation of β1 integrins, which optimizes the dynamics of FAs and the actin network. This regulatory role of PrP^C supports cell competence for neurite sprouting and neuronal differentiation. In the absence of PrP^C, clustering and activation of β1 integrins reduce turnover of FAs and overactivate the RhoA-ROCK-LIMK-Cof signaling pathway. Phosphorylated cofilin loses its severing activity toward F-actin microfilaments, which causes tension in the actin cytoskeleton. Reduction of FAs and F-actin dynamics blocks neurite sprouting. β1 integrin overactivity is further fueled by an excess of fibronectin in the surrounding milieu of PrP^C-deficient neuroectodermal stem cells, originating from the upregulation of fibronectin gene transcription.

Abbreviations: PrP^C, cellular prion protein; Cav-1, caveolin-1; Cof, cofilin; FA, focal adhesion; F-actin, fibrillar actin; G-actin, globular actin; ROCK, RhoA-associated coiled-coil containing kinases, or Rho kinases.

recycling of FA components necessary for neurite formation and outgrowth. Thus, the presence of PrP^C appears essential for the fine-tuned regulation of FA stability and dynamics in neural stem cells,^{15,51} a prerequisite for the onset of neuronal polarity accompanying neuronal differentiation.

Beyond the impact on neuritogenesis, alteration of FA dynamics caused by the absence of PrP^C would also affect proliferation and migration of neuronal stem cells, since these two events were shown to depend on the fine-tuning of FA stability.^{55,56} Accordingly, it was shown *in vivo* that neuronal progenitors of PrP knockout mice display reduced proliferation capacities.³⁵

PrP^C depletion in 1C11 neuroectodermal stem cells also affects the structure and dynamics of the actin cytoskeleton. Upon PrP^C silencing, actin fibers have lost their parallel orientation, and their stability is greatly increased, compared

with PrP^C expressing 1C11 precursor cells.¹⁵ Thus, PrP^C exerts a regulatory function on the organization and turnover of the actin meshwork in neuronal stem cells¹⁵ (Figure 1). PrP^C indirectly promotes the severing of actin microfilaments through negative control of RhoA-GTPase signaling and notably the RhoA-Rho-associated kinase (ROCK)-LIMK1 and 2-cofilin signaling pathway,¹⁵ known to play a central part in neuritogenesis.^{57–61} By downregulating the signaling activity of the RhoA-ROCK-LIMK1 and 2 cascade, PrP^C maintains cofilin in a poorly phosphorylated state,¹⁵ which in turn triggers the active conversion of F-actin into G-actin^{62,63} and thereby renders neuronal stem cells competent for neuritogenesis. The interplay between PrP^C and β1 integrins may favor relaxed actin stress fibers, which can rapidly disintegrate when neurite buds start forming in differentiating neurons. Indeed, actin turnover and FA dynamics are

functionally related. $\beta 1$ integrin-induced FAK overactivation in PrP^{null} -cells may trigger the recruitment and activation of Rho guanine nucleotide exchange factors, a class of proteins that switch on small G-RhoA proteins.^{64,65} Excessive mobilization of these factors would lead to overactivation of the RhoA-ROCK-LIMK1 and 2-cofilin pathway, the formation of tensed actin cytoskeleton with reduced turnover, and impairment of neurite sprouting. Alternatively, overstimulation of the RhoA-ROCK-LIMK1 and 2-cofilin cascade could also account for the increased stability of FAs, since RhoA-induced cell contractility was shown to increase the phosphorylation level of FA protagonists, leading to enhanced adhesion maturation.^{66,67}

Neurite sprouting also depends on PrP^{C} -dependent control of fibronectin expression

The nature and concentration of ECM components as well as the three-dimensional organization of ECM are known to influence the differentiation properties of neuronal progenitors.⁶⁸ The ECM environment that permits neuronal differentiation was shown to differ according to the neuron type.^{69,70} Depletion of PrP^{C} in 1C11 neuronal stem cells is associated with changes in the ECM, with an enrichment of secreted fibronectin surrounding PrP^{null} -cells. The increase in secreted fibronectin level originates from enhanced fibronectin gene transcription.¹⁵ Since fibronectin is a main $\beta 1$ integrin ligand, PrP^{C} -mediated control of fibronectin expression in neuronal stem cells is critical for neurite sprouting and neuronal differentiation (Figure 1). Artificially enhancing the density of fibronectin in the environment of PrP^{C} expressing 1C11 precursor cells (by coating increasing levels of fibronectin on culture dishes) hence impairs the initiation of neurites in a concentration-dependent manner. A fibronectin-induced defect of neurite formation originates from reduced turnover of FAs and overactivation of the RhoA-ROCK-LIMK1 and 2-cofilin signaling pathway, and thereby mimics PrP^{C} deficiency.¹⁵

Of note, the increase in fibronectin level may change the structure of ECM through the conversion of soluble fibronectin into fibrillar fibronectin.¹⁸ The formation of fibrillar fibronectin would be catalyzed directly by $\beta 1$ integrins through their conformational changes induced by the binding of fibronectin to $\beta 1$ integrins (outside-in signaling).⁷¹ Alternatively, the increase in actin contractility triggered by the elevation of fibronectin level would also provoke the activation of $\beta 1$ integrins (inside-out signaling) and subsequent conversion of soluble fibronectin

into fibrillar fibronectin.⁷² In turn, changes in fibronectin organization in the environment of PrP^{C} depleted cells in combination with tensed actin would sustain $\beta 1$ integrin clustering and favor the conversion of dynamic FAs into static fibrillar adhesion contacts,⁷³ thus contributing to neurite sprouting failure.

How PrP^{C} exerts a transcriptional control on fibronectin expression level remains unclear. The cAMP-responsive element binding (CREB) transcription factor is assumed to pilot fibronectin gene expression.^{74,75} CREB is a downstream target in the PrP^{C} signaling pathway that promotes the expression of *Egr-1* and *c-fos* immediate early genes in 1C11 precursor cells, underlying a potential role of PrP^{C} in the survival, proliferation, and differentiation of neuronal stem cells.³¹ The coupling of PrP^{C} to the CREB transcription factor may finely adjust the level of fibronectin in the microenvironment of neural stem cells, thus rendering cells competent for neuritogenesis. Of note, CREB is also controlled by $\beta 1$ integrin signaling.⁷⁶ We may hypothesize overactivation of CREB activity downstream from $\beta 1$ integrins in PrP^{null} -cells, leading to excessive fibronectin expression. In agreement, pharmacological inhibition of CREB lowers the fibronectin mRNA level in PrP^{null} -1C11 cells (our unpublished data). Oversecreted fibronectin would in turn self-sustain $\beta 1$ integrin activation and induce vicious circle conditions that impair neurite onset.

As a whole, these data argue for a role of PrP^{C} in neurite sprouting. Endogenous expression of PrP^{C} is necessary for regulating the level of ECM fibronectin, the clustering and activation of $\beta 1$ integrins, and the turnover of FAs and actin microfilaments via RhoA-GTPase signaling. PrP^{C} -dependent optimal dynamics of FAs and actin cytoskeleton render neuronal stem cells competent to respond to neuronal inducers and to develop neurites (Figure 1).

PrP^{C} and neurite outgrowth

Apart from its contribution to the initial phase of neuritogenesis, PrP^{C} also takes part in the outgrowth of neurites. This role of PrP^{C} was initially supported by a set of experiments indicating that: (1) during mouse embryogenesis, PrP^{C} is mainly detected in elongating axons;⁷⁷ (2) neuronal differentiation and neurite outgrowth are displayed more slowly in primary cultures of neuronal progenitors derived from $\text{PrP}^{-/-}$ mice than from wild type mice;^{35,78,79} and (3) neurites of cultured hippocampal neurons from $\text{PrP}^{-/-}$ mice are shorter than those of PrP^{C} expressing cells.⁸⁰ In these paradigms, PrP^{C} depletion alters neuritogenesis but does not cancel the acquisition of neuronal polarity. As mentioned before, this likely underlines

the occurrence of compensatory mechanisms for the lack of PrP^C in mice that permit neurite sprouting but do not allow the full execution of the neuritogenesis process. Based on these observations the concept emerges that PrP^C facilitates neurite outgrowth along neuronal differentiation.⁷⁹

A set of PrP^C partners sustains its facilitator role in neurite outgrowth

The facilitator role of PrP^C towards neurite outgrowth depends on its interactions with a set of distinct partners, reflecting the dynamic scaffolding function of PrP^C according to its immediate membrane environment.²¹ By interacting with ECM components, soluble ligands, and/or neighboring cell surface proteins, PrP^C functions as a dynamic platform for the assembly of various signaling modules orchestrating neurite elongation.

A first ligand identified is laminin,⁸¹ a protein of the ECM and a major constituent of the neuronal basal lamina. The PrP^C-laminin interaction was shown to sustain neuritogenesis: competition experiments using PrP^C antibodies or laser inactivation of cell surface PrP^C interfere with the laminin-dependent neurite outgrowth of PC12 cells.⁸² For instance, binding of laminin- γ 1 chain to PrP^C allows the recruitment and association of PrP^C with group I metabotropic glutamate receptors (mGluR1-5), and thus the formation of a multicomponent complex that transduces intracellular signals for neurite outgrowth in primary hippocampal neurons.^{83,84} In dorsal root neurons, PrP^C interaction with vitronectin, another component of the ECM, is also shown to favor axonal growth through a β 3 integrin dependent mechanism.¹⁴

Several membrane proteins were also reported to influence PrP^C-facilitated neuritogenesis. This includes PrP^C itself, because exposure of primary cultures of post-natal cerebellar neurons to dimers of recombinant PrP^C, mimicking transacting PrP^C originating from neighboring cells or exosomes,⁸⁵ sustains neurite outgrowth.³³ Kanaani et al further showed that incubation of cultured hippocampal neurons with recombinant PrP^C induces rapid polarization and the development of synapses.⁸⁰ Recently, such transacting PrP^C-PrP^C interaction was shown to positively influence neurite outgrowth of hippocampal neurons by enhancing the formation of a co-cluster between PrP^C and the raft-associated reggie protein (also called flotillin). The association between PrP^C and reggie protein pilots the trafficking and cargo-mediated delivery of N-cadherins to the growth cone as well as elongating axons.⁸⁶ Another membrane protein candidate is the neural cell adhesion molecule (NCAM). PrP^C interacts with NCAM and stabilizes NCAM in lipid rafts.

Disruption of the PrP^C-NCAM interaction impairs neurite outgrowth of cultured hippocampal neurons stimulated by exogenous PrP^C.⁸⁷

Stress-inducible protein-1 (STI-1), a co-chaperone that associates with Hsp70 and Hsp90,⁸⁸ is a soluble trophic factor released in the extracellular milieu by astrocytes.⁸⁹ The interaction of soluble STI-1 with PrP^C has been shown to influence neurite outgrowth of hippocampal neurons.^{90,91} The binding of STI-1 to PrP^C recruits and activates the α 7 nicotinic acetylcholine receptor (α 7nAChR) that transduces neurite outgrowth signals.⁹¹ Santos et al further documents that simultaneous binding of the STI-1 and laminin- γ 1 chain to PrP^C promotes a synergistic activation of α 7nAChR and mGluR1-5, which potentiates axonal growth of dorsal root ganglia neurons.⁹²

Although PrP^C is widely assumed to facilitate neurite outgrowth, one study illustrates that PrP^C exerts the opposite role depending on the neuron type and PrP^C partner involved.⁹³ Indeed, PrP^C is shown to negatively modulate in vivo and in vitro neurite outgrowth of neurons from the central nervous system through the interaction with plasma membrane contactin-associated protein (CaspR).⁹³ CaspR is an adhesion molecule required for the formation of axoglial paranodal junctions surrounding Ranvier nodes in myelinated axons.⁹⁴⁻⁹⁶ PrP^C-CaspR interaction inhibits the shedding of CaspR by the Reelin protease,⁹⁷ and thereby triggers the accumulation of CaspR at the cell surface of neurons, which exacerbates the inhibitory role of CaspR on neurite outgrowth.⁹³

By interacting with distinct membrane partners and ligands, PrP^C influences neurite elongation. How PrP^C-dependent downstream signaling controls the remodeling of cell cytoskeleton that accompanies neurite outgrowth, remains unresolved however. Because actin and the dynamics of FAs also play a central role in neurite elongation, an attractive hypothesis is that the signaling modules scaffolded by PrP^C at the plasma membrane of elongating neurites participate in neuritogenesis.

Are Rho-GTPases the executive machinery through which PrP^C takes part in neurite outgrowth?

PrP^C role in neurite outgrowth may involve its signaling function since the inhibition of PrP^C-controlled signaling intermediates, ie, Src kinases, including the p59Fyn tyrosine kinase,²⁸ PKC or PI3 kinase,³⁴ partially or fully abolishes neurite outgrowth.^{33,80} Nevertheless, activation of p59Fyn in neurite outgrowth also originates from the interaction of PrP^C

with NCAM.⁸⁷ Whether Fyn activation mediated by PrP^C–PrP^C or PrP^C–NCAM interaction facilitates neurite outgrowth by acting on actin microfilaments dynamics remains unknown. The Rho regulatory proteins, p190RhoGAP (GTPase-activating protein), are major regulators of Rho-GTPase-mediated actin reorganization in neuronal growth cone. By transducing signals downstream from Src kinases

and NCAM, p190RhoGAP contributes to axon outgrowth.¹⁷ Besides, Rho-GTPases are signaling intermediates coupled to other PrP^C binding partners, including laminin/laminin receptor, reggie/flotillin, and mGluR.^{17,98,99} We may propose that the functional interaction between PrP^C and its partners modulates Rho-GTPase signaling and thereby optimizes actin remodeling necessary for neurite outgrowth (Figure 2).

Figure 2 PrP^C facilitates neurite outgrowth by fine-tuning the signaling activity of plasma membrane multiprotein complexes. The facilitator role of PrP^C towards neurite outgrowth relies on the capacity of PrP^C to interact with multiple partners (laminin and vitronectin ECM components; PrP^C, NCAM, reggie/flotillin, mGluR1-5, $\alpha 7$ nAChR plasma membrane proteins; soluble ligands such as STI-1) within raft microdomains and to recruit diverse signaling pathways. By orchestrating these signaling networks, PrP^C would favor actin remodeling necessary for neurite outgrowth.

Abbreviations: Cav-1, caveolin-1; ECM, extracellular matrix; FA, focal adhesion; NCAM, neural cell adhesion molecule; mGluR1-5, group I metabotropic glutamate receptors; STI-1, stress inducible protein 1; $\alpha 7$ nAChR, $\alpha 7$ nicotinic acetylcholine receptor; PLC, phospholipase C; PrP^C, cellular prion protein; ?, functional link is not yet determined.

Finally, the increase in calcium concentration triggered by the interaction of PrP^C with laminin- γ 1 or STI-1, and subsequent activation of mGluR1–5 or α 7nAChR, respectively,⁹² also likely contributes to neurite elongation through cytoskeletal rearrangements induced by Rho-GTPases.¹⁰⁰

These combined data indicate that the facilitator role of PrP^C towards neurite outgrowth depends on the neuron type and relies on the capacity of PrP^C to engage multiple partners (laminin and vitronectin ECM components; PrP^C, NCAM, reggie, mGluR, α 7nAChR, and Caspr membrane proteins; soluble ligands such as STI-1) in multiprotein complexes and to recruit promiscuous signaling pathways.²¹ Depending on the partners involved and the stoichiometric equilibria between PrP^C and its partners, PrP^C mainly acts positively on neurite elongation, but may also exert the opposite action.

PrP^C implication in neurite maintenance

Once neurons have reached their terminal stage of differentiation and acquired their polarized morphology, it is suspected that PrP^C positively acts on the maintenance and integrity of the axon and dendrites. Laser-mediated inactivation of cell surface PrP^C promotes neurite retraction of differentiated PC12 cells.⁸² In line with this observation, our unpublished data indicate that transient siRNA-mediated PrP^C silencing in fully differentiated 1C11-derived serotonergic (1C11^{5-HT}) or noradrenergic (1C11^{NE}) neuronal cells triggers neurite retraction. The mechanisms through which PrP^C influences the stability of neuronal polarity have been studied less than those sustaining neurite sprouting and outgrowth, however.

In fully differentiated 1C11^{5-HT} and 1C11^{NE} neuronal cells, two PrP^C interactors, the tissue nonspecific alkaline phosphatase (TNAP) and β -dystroglycan,^{101,102} may both take part in the maintenance of neurites. The restriction of TNAP expression¹⁰¹ and PrP^C-controlled β -dystroglycan cleavage by matrix metalloproteinase type 9 (MMP-9)³¹ to mature 1C11-derived neuronal cells may account for the specific contribution of TNAP and β -dystroglycan to neurite maintenance. These neurospecific interactions underline that PrP^C interactome and coupled effectors differ between neuronal stem cells and fully differentiated neurons. The recruitment of PrP^C in multiprotein complexes, which are specific to the stem cell stage or the neuronal context, may depend on different lipid-raft compositions, the engagement of distinct PrP^C isoforms,¹⁰³ variations of the composition and

structure of the ECM surrounding neuronal stem cells and neurons, or changes in membrane fluidity that determine the dynamics of interaction between partners.

Functional interactions between PrP^C, TNAP, and phosphorylated laminin maintain neurite extensions

Our work establishes that in rafts of fully differentiated 1C11^{5-HT} and 1C11^{NE} neuronal cells, PrP^C interacts with TNAP,¹⁰¹ an enzyme mainly known for its role in bone mineralization. In polarized neuronal cells, TNAP acts as an ecto-phosphatase that catalyzes the dephosphorylation of laminin, which in turn allows PrP^C to interact with laminin.¹⁰¹ The onset of such a functional ternary PrP^C/TNAP/laminin complex sustains the stability of neuronal polarity (Figure 3). Accordingly, pharmacological inhibition of TNAP activity promotes the phosphorylation of laminin, which thus abrogates PrP^C interaction with laminin. Disintegration of the PrP^C/laminin/TNAP complex in rafts of 1C11-derived neuronal cells is accompanied by neurite retraction (our unpublished data). This finding is in line with previous data showing that disruption of the PrP^C–laminin interaction in differentiated PC12 cells triggers neurite retraction.⁸² We may envision that destabilization of the PrP^C/laminin interaction upon laminin phosphorylation in mature neurons corrupts Rho-GTPase signaling¹⁷ and favors the severing of neurite sheathing actin microfilaments, thereby leading to neurite instability and retraction.

Does PrP^C-dependent regulation of β -dystroglycan cleavage by MMP-9 partake in neurite maintenance?

PrP^C-mediated stability of neurites may also originate from PrP^C interaction with β -dystroglycan,¹⁰² a transmembrane protein that forms a bridge from the ECM (notably laminin) to the cell cytoskeleton; the cleavage of this protein triggers a loss of cell adhesion to its substratum.^{104,105} Our work establishes that, in neurites of fully differentiated 1C11^{5-HT} serotonergic neuronal cells, PrP^C signaling protects β -dystroglycan from cleavage by the matrix metalloproteinase MMP-9 by repressing transcription of the MMP-9 encoding gene and inhibiting MMP-9 enzymic activity.³¹ PrP^C interaction and protection of β -dystroglycan may thus enhance the interaction between β -dystroglycan and ECM laminin.¹⁰⁵ This action would contribute to the stability of neurites by maintaining the connection between laminin and the actin meshwork through β -dystroglycan (Figure 3).

Figure 3 PrP^C contributes to the maintenance of neurites in mature neurons. In fully differentiated neuronal cells, PrP^C interacts with TNAP. TNAP catalyzes dephosphorylation of ECM laminin, which then binds to PrP^C. The formation of a neurospecific ternary complex between PrP^C, TNAP, and non-phosphorylated laminin sustains the PrP^C role in neurite stability. PrP^C-mediated control of β-dystroglycan cleavage by MMP-9 may also account for the role of PrP^C in neurite maintenance. By downregulating MMP-9 expression, PrP^C protects β-dystroglycan from cleavage by MMP-9, which would in turn stabilize the connection between ECM laminin and actin cytoskeleton.

Abbreviations: Cav-1, caveolin-1; ECM, extracellular matrix; PrP^C, cellular prion protein; TNAP, tissue nonspecific alkaline phosphatase; MMP-9, matrix metalloproteinase type 9; ?, functional link is not yet determined.

Conclusion

This review illustrates how the cellular prion protein PrP^C takes part in the three steps of neuritogenesis: (1) the initiation of neurite formation or neurite sprouting; (2) the outgrowth of neurites; and (3) the stability and maintenance of neuronal polarity. In future, this particular role of PrP^C related to the

acquisition and the stability of neuronal polarity should be addressed in prion diseases. In neurodegenerative diseases, synapse disconnection as well as retraction and degeneration of axons have been widely recognized as early events in the neurodegenerative process¹⁰⁶ and likely account for behavioral and cognitive impairments observed in patients.^{107,108} In prion

diseases, it is now clearly established that corruption of PrP^C-associated functions by pathogenic prions (PrP^{Sc}) in neurons induces neurodegeneration.¹⁹ Whether PrP^{Sc} triggers a loss-of-function of PrP^C upon its conversion into PrP^{Sc} or promotes a gain-of-function upon PrP^{Sc} interaction with PrP^C, or both, is still debated. In any case, PrP^{Sc} would induce disintegration of multiprotein complexes involving PrP^C and its partners. Because of the critical role of PrP^C in the implementation of neurites and stability of axon and dendrites, it is conceivable that prion infection disturbs the PrP^C-fine-tuned functional relationship between the ECM, and cytoskeleton dynamics, and thereby threatens neuronal connectivity and integrity of neuronal networks. The identification of PrP^C-controlled signaling pathways involved in neuritogenesis that are corrupted by PrP^{Sc} may help to define novel therapeutic strategies towards prion diseases.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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