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From stem cells to prion signalling

Odile Kellermann[a]*, Claire Lafay-Chebassier[a,b], Myriam Ermonval[a], Sylvain Lehmann[c], Sophie Mouillet-Richard[a]

[a] *Laboratoire de différenciation cellulaire et prions, CNRS UPR 1983–Institut Pasteur, BP 8, 94801 Villejuif, France*

[b] *CR Claude Bernard, hôpital Lariboisière, 75010 Paris, France*

[c] *Biologie des EST, CNRS UPR 1142, 34396 Montpellier cedex 5, France*

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Abstract – A strategy based upon the introduction of an adenovirus-SV40 plasmid into multipotential cells was designed to immortalize clones displaying properties of lineage stem cells. The murine 1C11 cell line behaves as a neuroepithelial progenitor. Upon appropriate induction, almost 100 % of 1C11 precursor cells develop neurite extensions and convert into either serotonergic or noradrenergic neurons. The two mutually exclusive neuronal programs are autoregulated by serotonergic or adrenergic receptors. PrP^c is constitutively expressed by 1C11 cells. Antibody-mediated cross-linking of PrP^c promotes the dephosphorylation of the tyrosine kinase Fyn associated to a Fyn kinase activation. The coupling of PrP^c to Fyn is dependent on caveolin-1. It is restricted to the fully differentiated serotonergic or noradrenergic cells and occurs mainly at neurites. Thus, PrP^c may represent a signal transduction protein which may fine-tune neuronal functions. Since the 1C11 stem cell supports prion replication, it may provide a tool to investigate whether PrP^{Sc} accumulation interferes with PrP^c signalling activity. To cite this article: O. Kellermann et al., C. R. Biologies 325 (2002) 9–15. © 2002 Académie des Sciences/Éditions scientifiques et médicales Elsevier SAS

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Résumé – Une cellule souche neuronale pour étudier le rôle fonctionnel de la protéine prion. L'introduction d'un plasmide immortalisant dans des cellules multipotentielles, a permis d'obtenir des cellules souches des différents lignages. La lignée murine 1C11 a les propriétés d'un précurseur neuroectodermique bipotentiel qui, après induction, acquiert l'ensemble des fonctions des neurones sérotoninergiques ou catécholaminergiques. Les programmes de différenciation sont mutuellement exclusifs et régulés par des autorécepteurs bioaminergiques. Le clone 1C11 exprime naturellement la protéine prion (PrP^c). La ligation de la PrP^c avec des anticorps anti-PrP provoque une activation de la tyrosine kinase Fyn. Ce couplage, qui dépend d'une interaction PrP^c/cavéoline, n'est détecté que dans les cellules ayant acquis un phénotype sérotoninergique ou noradrénergique complet. Il implique principalement les molécules de PrP^c localisées sur les neurites. La PrP^c pourrait donc jouer un rôle dans le réseau des voies de transduction qui contrôlent l'homéostasie des neurones. La lignée 1C11 est infectable par les prions, elle constitue un nouveau modèle pour comprendre comment l'accumulation de PrP^{Sc} interfère avec le rôle fonctionnel de la PrP^c. Pour citer cet article : O. Kellermann et al., C. R. Biologies 325 (2002) 9–15. © 2002 Académie des Sciences/Éditions scientifiques et médicales Elsevier SAS

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**Correspondence and reprints.*
E-mail address: okellerm@vjf.cnrs.fr (O. Kellermann).

1. Introduction

The infectious agent associated to transmissible spongiform encephalopathies (TSE) is mainly, if not entirely, composed of a protein termed PrP^{Sc} which stands for scrapie isoform of the cellular prion protein or PrP^{C} [1]. The term 'prions' used to designate the infectious agent may be defined as pathogenic isoforms of the host-encoded PrP^{C} protein. Prion propagation strictly depends on the presence of PrP^{C} since *prnp* knock-out mice are resistant to prion infection [2]. It is now accepted that prions could act as a template for the conversion of newly synthesized PrP^{C} into PrP^{Sc} in neuronal cells [3]. However, the mechanisms of conversion accounting for prion replication and accumulation are still unclear, and the molecular and cellular events leading to TSE-associated neurodegeneration remain to be elucidated.

One of the current challenges in the field of TSE is the identification of the physiological role of the cellular prion protein. PrP-deficient mice develop normally, are viable [4] and have but yielded minor clues as to PrP^{C} function. PrP^{C} is a ubiquitous glycoprotein anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) moiety. Cell surface PrP^{C} may be present either in its native form or as an N-terminally truncated isoform, both isoforms carrying two, one or no Asn-linked sugar chain [5]. Moreover, the proteolytic cleavage involved in the generation of truncated PrP^{C} produces an amino-terminal peptide, which is secreted in the extracellular space. The attachment of PrP^{C} to the outer leaflet of the plasma membrane is consistent with a role in cell surface signalling, cell adhesion [6], or ligand binding [7]. Candidate PrP^{C} cellular partners have been identified such as the laminin receptor precursor [6], members of the dystroglycan complex [8], the Hsp chaperone [9], an enigmatic protein identified through complementary hydropathy [10] as well as PrP^{C} itself [11]. Much interest has been raised by the copper binding-ability of PrP^{C}, although the accurate functional link between copper and the prion protein remains to be established. Up to 4 Cu^{2+} ions may bind to the histidine-containing octarepeats present in the N-terminal domain of PrP^{C}, with a 5- to 10-μM affinity [12]. Recently, an involvement of PrP^{C} in the regulation of the prepsynaptic copper concentration and of synaptic transmission has been proposed [13]. Besides, copper ions appear to stimulate PrP^{C} endocytosis [14], and, under oxidative conditions, to favour the cleavage of the protein in the octapeptide region [15]. These overall data raise the question as to the involvement of PrP^{C} in the neuronal response to oxidative stress. Indeed, $PrP^{-/-}$ mouse-derived cerebellar cells show an increased susceptibility to oxidative stress as compared to their wild type counterparts [16]. Brown et al. [16] have also reported a reduced level of Cu–Zn superoxyde dismutase (SOD) activity in knock-out mice. Actually, the PrP^{C}-induced regulation of Cu–Zn SOD may be an indirect effect resulting from copper uptake from the synaptic cleft into the cytosol [13].

PrP^{C} is abundantly expressed in neurons, which constitute the major targets of TSE-associated cellular damage. The identification of PrP interacting molecules in neuronal cells therefore constitutes a useful approach to unravel PrP^{C} biological function, as well as the phenotypic elements involved in neuronal susceptibility to TSE agents.

2. A model of neuronal differentiation derived from multipotential cells

In order to isolate committed precursors of the neuroectodermal, mesodermal or endodermal lineage, we have developed an immortalisation strategy based upon the introduction of an adenovirus-SV40 recombinant plasmid (PK4) into multipotential cells [17, 18]. The selected clones behave as somatic stem cells [19–21]. They display a stable and undifferentiated phenotype and express genes that are involved in early embryonic development. Upon induction, they have the capacity to undergo differentiation along distinct pathways of a same lineage. Depending on the inducers, they follow mutually exclusive programs up to the end stages of differentiation.

The 1C11 clone, which derives from the F9 teratocarcinoma cell line [17, 20], is a bipotential progenitor (*figures 1A and 1B*) with the properties of a neuronal stem cell [22]. 1C11 precursor cells display an epithelial phenotype, express nestin, a neuroepithelial cell marker, as well as genes involved in early neurogenesis such as Notch 1 to 4 and their ligands Delta and Jagged, Krox 20, Mash-1, msx-1, … 1C11 cells do not differentiate in the absence of induction, even when grown in 10 % fetal calf serum, and lack neuron-associated functions.

Within 4 days of treatment with dibutyryl cyclic AMP (dbcAMP) and cyclohexane carboxylic acid (CCA), 1C11 cells convert into $1C11^{*/5\text{-HT}}$ cells expressing a complete serotonergic phenotype. As soon as day 2 after the addition of the inducers, $1C11^{*/5\text{-HT}}$ cells have acquired the ability to synthesize, store and catabolize serotonin (5-HT), and express 2 serotonergic receptors of the $5\text{-HT}_{1B/D}$ and 5-HT_{2B} subtypes. At day 4, a third receptor (5-HT_{2A}) as well as the membranous transporter allowing extracellular serotonin uptake,

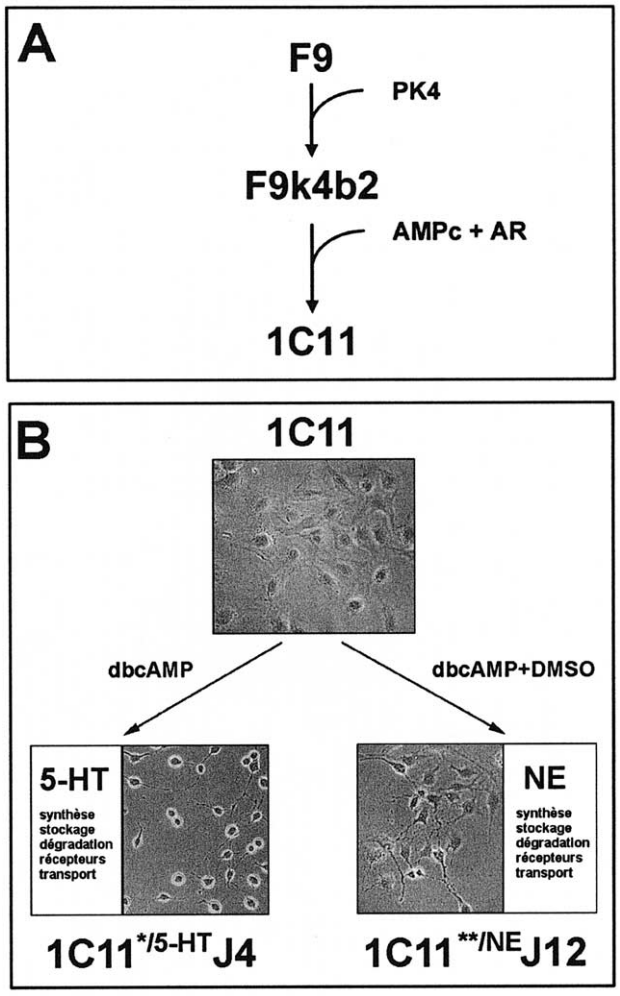

Figure 1. Neuronal differentiation of neuroepithelial 1C11 cells. **A.** The 1C11 clone has been isolated upon differentiation of F9 embryonal carcinoma cells in vitro. **B.** Depending on the inducers, 1C11 precursor cells acquire the overall functions of serotonergic ($1C11^{*/5\text{-HT}}$) or noradrenergic ($1C11^{**/NE}$) neurones.

become functional. Moreover, the receptors selectively induced in the time course of differentiation promote an autoregulation by 5-HT of $1C11^{*/5\text{-HT}}$ cells overall serotonergic functions [22].

Dimethylsulfoxide (DMSO, 2 %) in combination with dbcAMP and CCA triggers within 12 days the differentiation of 1C11 cells into $1C11^{**/NE}$ cells, expressing a complete noradrenergic phenotype. $1C11^{**/NE}$ cells synthesize, store and catabolize catecholamines from day 4 of differentiation, and acquire a functional noradrenergic transporter at day 12. An α_{1D}-adrenoceptor is selectively induced at day 8 of the programme and controls the onset of the noradrenergic transporter [22].

The two programmes are mutually exclusive. The differentiation events are synchronous within the cell population and concern nearly 100% of the cells (*figure 1B*). The time sequence of implementation of the neurotransmitter-associated functions is similar to that observed in vivo during neurogenesis. Along either pathway, $1C11^{*/5\text{-HT}}$ and $1C11^{**/NE}$ cells develop bipolar extensions and express neuronal markers such as neurofilaments, N-CAM, γγ-enolase, synaptophysin and Met-enkephalin.

This differentiation model therefore constitutes a useful tool to investigate the transcriptional and post-transcriptional regulations underlying the onset of neuronal functions and to characterize the involvement of the transduction pathways coupled with the receptors in the expression and/or the maintenance of a bioaminergic phenotype.

3. The 1C11 cell line endogenously expresses the prion protein

The regulation of expression of the *prnp* gene has been monitored by real-time quantitative PCR analysis (Taqman) [23]. The level of PrP^C transcripts present in 1C11 precursor cells is significant as compared to the very low level of expression (< 7 %) measured in multipotential cells, i.e. embryonic stem (ES) cells or the F9 teratocarcinoma cell line, from which the 1C11 clone is derived. These observations are in agreement with those obtained in the time course of embryonic development [24], and demonstrate that the commitment of multipotential cells to the neuronal lineage is accompanied by a strong increase in the level of PrP^C expression.

While no significant variation in the level of PrP trancripts may be monitored during the differentiation of 1C11 cells along the noradrenergic pathway, the entry of 1C11 cells into the serotonergic program is associated with a decrease in the level of PrP mRNA (67 %). This regulation observed at the transcriptional level is paralleled by a modulation of the level of the protein, as assessed by immunoprecipitation experiments [23]. All PrP^C isoforms are present in the 1C11 cell line (*figure 2*).

In vivo, the expression of PrP^C differs according to brain regions [25] and the vacuolation pattern observed in TSE-infected brain depends on the agent strain [26]. This suggests a differential expression of PrP^C mRNA or isoforms according to the neuronal cell type [27]. However, the accurate distribution of PrP transcripts and protein in relation to the phenotypic specificity of neuronal cells (serotonergic, catecholaminergic, GABAergic, cholinergic, etc.) remains to be characterized. Similarly, besides several studies highlighting a NGF-mediated modulation [23, 28–30], little is known

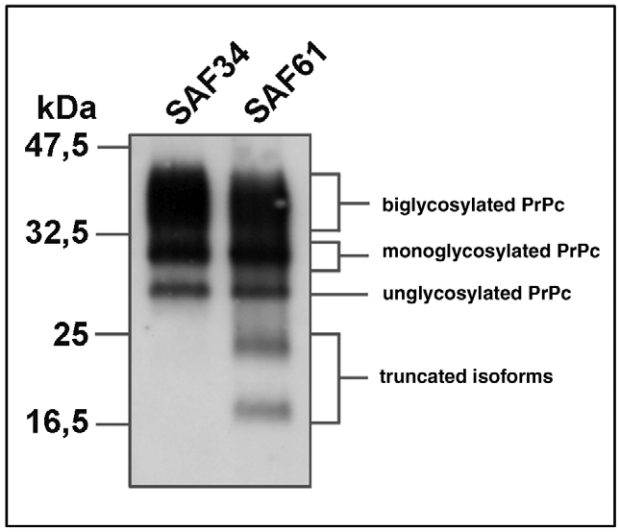

Figure 2. Detection of PrP^C isoforms in $1C11^{*/5\text{-HT}}$ serotonergic cells. PrP^C immunoprecipitation with monoclonal antibodies directed against the N-terminal (SAF34) or C-terminal (SAF61) domain of PrP^C (J.Grassi, CEA Saclay) reveals the presence of PrP isoforms : native, N-terminally truncated, unglycosylated or mono- or bi-glycosylated.

concerning the regulation of PrP^C expression by neuronal factors.

PrP^C may be monitored by immunofluorescence at the surface of 1C11 cells, whatever their state of differentiation [23, 31]. Anti-PrP antibodies stain both the cellular bodies and the neuritic varicosities of the serotonergic and noradrenergic cells. The subcellular localization of PrP^C in neurones is still imperfectly characterized. In vivo, immunocytochemistry experiments have revealed a strong concentration of the protein in the neuropile [32], where it may be conveyed through fast axonal transport [33, 34]. A synaptic localization of PrP^C in the hamster hippocampus has also been evidenced through electron microscopy [35]. The distribution of PrP^C at the surface of the soma and the processes of $1C11^{*/5\text{-HT}}$ and $1C11^{**/NE}$ cells prompted us to investigate into a putative role of PrP^C as a cell-surface receptor.

4. PrP^C is coupled with a signal transduction pathway

Our experimental approach to identify a signalling pathway coupled with PrP^C lies on antibody-mediated ligation, used as a means to mimic an endogenous signal. PrP^C cross-linking does not lead to any p21ras or PLA2 activation, or to any IP3 or NO production within a 30-min time frame. By contrast, PrP^C ligation induces the dephosphorylation of Fyn, a member of the SRC tyrosine kinase family. This effect is reproducibly observed with different anti-PrP antibodies. Fyn dephosphorylation correlates with the induction of a Fyn kinase activity. The level of Fyn expression, as measured by Western blot, does not significantly vary during differentiation. However, PrP-mediated Fyn activation is restricted to $1C11^{*/5\text{-HT}}$ or $1C11^{**/NE}$ differentiated cells, and is not observed in 1C11 precursor cells [31].

PrP^C is a GPI molecule, anchored to the outer leaflet of the plasma membrane, while Fyn is an intracellular kinase. We therefore assumed the involvement of intermediate membrane factor(s) in the coupling of PrP^C to Fyn. Co-immunoprecipitation experiments allowed us to identify caveolin as a PrP^C cellular partner. The PrP^C/caveolin interaction is specific of $1C11^{*/5\text{-HT}}$ and $1C11^{**/NE}$ cells, although caveolin is also present in 1C11 undifferentiated cells. The PrP^C-induced activation of Fyn may be abolished following immunosequestration of caveolin, demonstrating that caveolin indeed is one of the protagonist involved in the signalling pathway coupled with PrP^C in these neuronal cells [31]. A colocalization of caveolin together with PrP^C may be monitored through immunofluorescence experiments, with a prominent labelling of the neurites varicosities of $1C11^{*/5\text{-HT}}$ and $1C11^{**/NE}$ cells (*figure 3*).

5. The coupling of the prion protein to Fyn is dependent upon neuronal differentiation

The kinetics of PrP^C-mediated Fyn activation in the time-course of 1C11 differentiation shows that the ability of 1C11 cells to respond to PrP^C cross-linking is strictly associated with the acquisition of a complete serotonergic or noradrenergic phenotype [31]. However, the three identified partners of the signalling cascade are present in the 1C11 cell line from the stem cell state. The PrP/caveolin interaction does appear to be a critical event since it is observed as soon as two days after the induction of the cells towards either differentiation program.

The onset of a PrP^C/caveolin/Fyn complex, therefore, appears to depend on the implementation of the overall neurotransmitter-related functions. Furthermore, the phenotypic conversion of the cells is most likely accompanied by a maturation of the membrane properties, which may also contribute to the spatial organization of the signalling partners. The onset of neuronal polarity and of fast axonal transport may be critical as well. Indeed, cell fractionation experiments demon

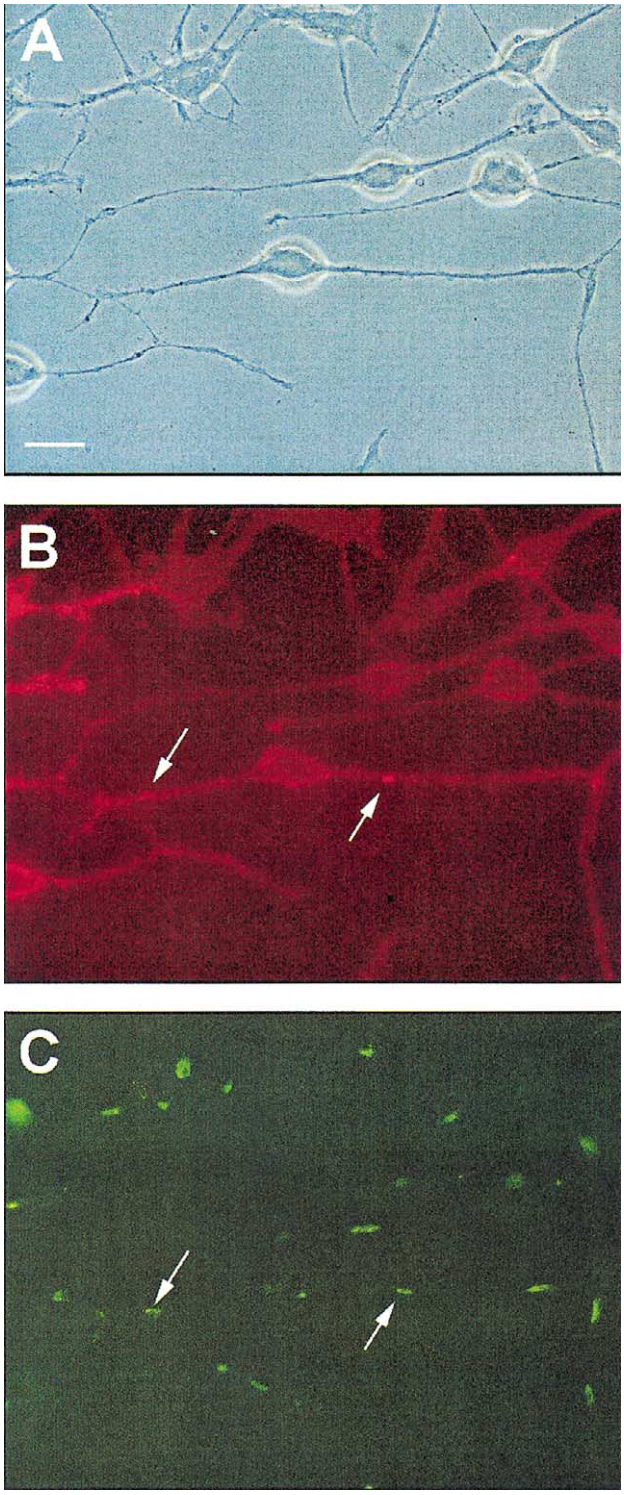

Figure 3. Membrane expression of PrP^C and caveolin on $1C11^{*/5\text{-HT}}$ serotonergic cells. Double-labeling experiments suggest a colocalization (arrows) of PrP^C (B) and caveolin (C) on neurites. (A) Corresponding phase contrast of $1C11^{*/5\text{-HT}}$ cells, 4 days after the addition of the inducers. Scale bar = 25 μM.

strate that PrP^C molecules located on neurites mainly account for Fyn activation [31].

The identification of a signalling pathway coupled with PrP^C defines the prion protein as a cell surface receptor (*figure 4*). It provides a foundation for uncovering the extracellular signal activating PrP in vivo and for dissecting the intracellular targets downstream of Fyn, which are chemically modified in response to PrP activation.

6. Discussion

Due to the paucity of cell lines expressing ex vivo a neuronal phenotype, the cellular biology of PrP^C has mainly been investigated using transfected CHO cells, N2a or HaB neuroblastoma cells or PC12 pheochromocytoma cells [5, for review].

The teratocarcinoma-derived 1C11 inducible cell line constitutes a novel in vitro model to gain insight into the biological function of PrP^C in relation to the onset and the control of bioaminergic neuronal functions. The restriction of the PrP^C-mediated activation of the Fyn kinase to fully differentiated serotonergic or noradrenergic cells suggests that the implementation of a neuronal phenotype and of neurotransmitter-associated functions is not dependent upon this signalling pathway. By contrast, this PrP^C-dependent signal could participate to neuronal homeostasis. This idea is in line with knock-out experiments which argue against a critical role of PrP^C in neurogenesis and neuronal differentiation. Besides, an interesting observation is that mice expressing an N-terminally truncated version of PrP^C (PrP Δ32-121) suffer from neurodegeneration soon after birth [36]. According to Shmerling, the lesions monitored in these mice would result from a signalling deficiency. Our data obtained with the 1C11 cell line supports the notion that PrP^C, or at least some isoforms of the protein, plays a role in the signalling network involved in the maintenance and/or the fine-tuning of neuronal functions.

The PrP^C-mediated Fyn activation provides a foundation for identifying the effectors downstream of this kinase, which may control neuronal functions. Fyn is majorly expressed in the brain and appears to be involved in multiple signalling pathways. Some targets of Fyn have been characterized and include: (i) the focal adhesion kinase FAK, a key component, together with paxillin, of focal adhesions, which form a structural link between the extracellular matrix and the cytosqueleton and constitute important sites of signal transduction [37]; (ii) α-synuclein, a pre-synaptic protein associated with vesicular transport and involved in neurotransmitter release at the synapse [38]; (iii) protein kinase C θ (θ PKC) which has been reported as a Fyn substrate in T cells [39].

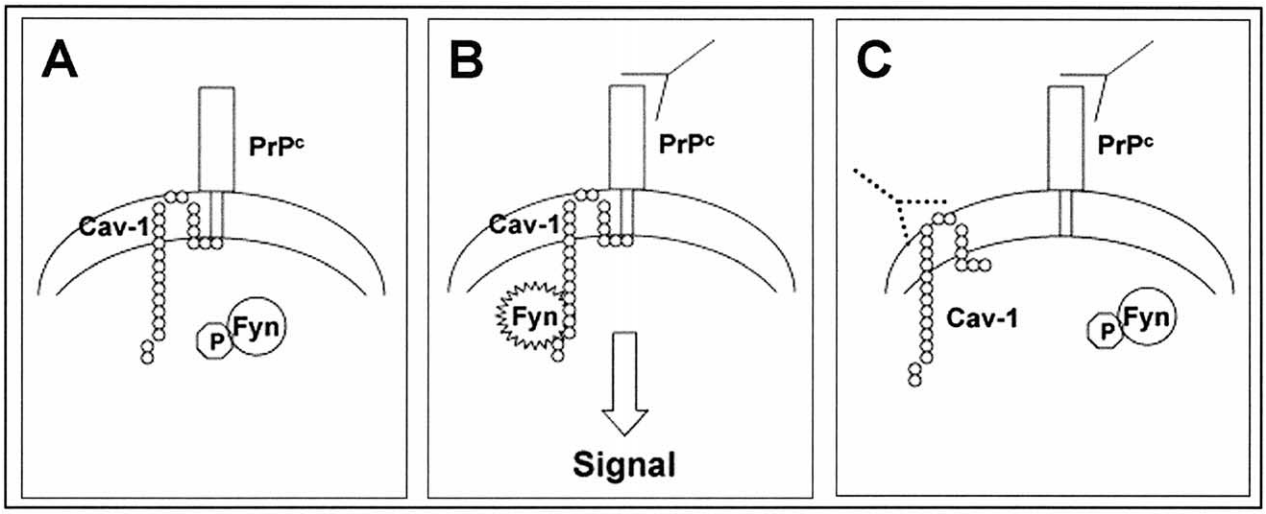

Figure 4. Signal transduction pathway coupled with the prion protein in neuronal cells. **A.** PrP^{C} is anchored to the outer leaflet of the plasma membrane and interacts with the transmembrane protein caveolin. Fyn, an intracellular kinase of the Src family is not in its activated state. **B.** Antibody-mediated ligation of PrP^{C} induces Fyn recruitment, dephosphorylation and activation. The PrP^{C}-caveolin-Fyn signalling complex thus supports the transduction of the signal within the fully dfferentiated neuronal cells. **C.** Caveolin is one of the protagonists involved in the signalling cascade since its immunosequestration abolishes the PrP^{C}-mediated Fyn activation.

Our observation that the activation of Fyn on the neurites of differentiated cells requires a PrP^{C}–caveolin interaction, suggests that the signalling events arise at the level of sphingolipid- and cholesterol-rich membrane microdomains, i.e. 'DIG', also known as major sites of signal transduction. The bioaminergic receptors which autoregulate $1C11^{*/5\text{-HT}}$ and $1C11^{**/NE}$ cells functions are most likely localized on the neurites. The signalling cascade mediated by PrP^{C} may therefore functionally interact with those coupled with the serotonergic or adrenergic receptors and these crosstalks may in fine control neuronal homeostasis.

Identifying the neuronal response to the PrP^{C}-mediated signal and understanding how PrP^{Sc} may interfere with the normal function of PrP^{C} during prion infection hence constitute a great challenge for the next few years. The localization of PrP^{C} in DIG appears necessary for the conversion of PrP^{C} into PrP^{Sc} [40, 41]; PrP^{Sc} formation indeed is abolished in the presence of drugs such as lovastatin which disrupts DIG structure by decreasing the level of cholesterol [42]. By interacting with PrP^{C} within DIG, PrP^{Sc} may hinder its association with cellular partners and thus abrogate the PrP^{C} function. Alternatively, PrP^{Sc} may constitutively recruit the signalling pathways normally coupled with PrP^{C}. PrP^{Sc} accumulation may also indirectly affect the oxidative state of the cell and thereby modify the balance between PrP^{C} different isoforms [43] or alter the membrane environment of the signalling complex [44].

Our recent data indicate that 1C11 cells in the precursor state support prion replication and are able to stably propagate the Chandler, 22L and Fukuoka-1 strains (unpublished data). The 1C11 cell line hence constitutes a new in vitro model to investigate the cellular tropism of the infectious agent. It may also help to unravel the mechanisms related to the toxicity of PrP^{Sc} in neurones. Dissecting the molecular and cellular events underlying TSE-associated pathogeny indeed is a priority in order to design therapeutic agents with the capacity to inhibit PrP^{Sc} formation while preserving PrP^{C} normal function.

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References

[1] Prusiner S.B., Prions, Proc. Natl. Acad. Sci. USA 95 (1998) 13363–13383.

[2] Bueler H., Aguzzi A., Sailer A., Greiner R.A., Autenried P., Aguet M., Weissmann C., Mice devoid of PrP are resistant to scrapie, Cell 73 (1993) 1339–1347.

[3] Prusiner S.B., Scott M.R., DeArmond S.J., Cohen F.E., Prion protein biology, Cell 93 (1998) 337–348.

[4] Raeber A.J., Brandner S., Klein M.A., Benninger Y., Musahl C., Frigg R., Roeckl C., Fischer M.B., Weissmann C., Aguzzi A., Transgenic and knockout mice in research on prion diseases, Brain Pathol. 8 (1998) 715–733.

[5] Lehmann S., Milhavet O., Mange A., Trafficking of the cellular isoform of the prion protein, Biomed. Pharmacother 53 (1999) 39–46.

[6] Rieger R., Edenhofer F., Lasmezas C.I., Weiss S., The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells, Nat. Med. 3 (1997) 1383–1388.

[7] Hornshaw M.P., McDermott J.R., Candy J.M., Lakey J.H., Copper binding to the N-terminal tandem repeat region of mammalian and avian prion protein: structural studies using synthetic peptides, Biochem. Biophys. Res. Commun. 214 (1995) 993–999.

[8] Keshet G.I., Bar-Peled O., Yaffe D., Nudel U., Gabizon R., The cellular prion protein colocalizes with the dystroglycan complex in the brain, J. Neurochem. 75 (2000) 1889–1897.

[9] Edenhofer F., Rieger R., Famulok M., Wendler W., Weiss S., Winnacker E.L., Prion protein PrPc interacts with molecular chaperones of the Hsp60 family, J. Virol. 70 (1996) 4724–4728.

[10] Martins V.R., Graner E., Garcia-Abreu J., de Souza S.J., Mercadante A.F., Veiga S.S., Zanata S.M., Neto V.M., Brentani R.R., Complementary hydropathy identifies a cellular prion protein receptor, Nat. Med. 3 (1997) 1376–1382.

[11] Hundt C., Weiss S., PrP dimerization in the yeast two-hybrid system, International symposium on the characterization and diagnosis of prion diseases in animals and man, 1999.

[12] Viles J.H., Cohen F.E., Prusiner S.B., Goodin D.B., Wright P.E., Dyson H.J., Copper binding to the prion protein: structural implications of four identical cooperative binding sites, Proc. Natl. Acad. Sci. USA 96 (1999) 2042–2047.

[13] Herms J., Tings T., Gall S., Madlung A., Giese A., Siebert H., Schurmann P., Windl O., Brose N., Kretzschmar H., Evidence of presynaptic location and function of the prion protein, J. Neurosci. 19 (1999) 8866–8875.

[14] Pauly P.C., Harris D.A., Copper stimulates endocytosis of the prion protein, J. Biol. Chem. 273 (1998) 33107–33110.

[15] McMahon H.E., Mange A., Nishida N., Creminon C., Casanova D., Lehmann S., Cleavage of the amino-terminus of the prion protein by reactive oxygen species, J. Biol. Chem. 276 (3) (2001) 2286–2291.

[16] Brown D., Schulz-Schaeffer W., Schmidt B., Kretzschmar H., Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity, Exp. Neurol. 146 (1997) 104–112.

[17] Kellermann O., Kelly F., Immortalization of early embryonic cell derivatives after the transfer of the early region of simian virus 40 into F9 teratocarcinoma cells, Differentiation 32 (1986) 74–81.

[18] Kellermann O., Buc-Caron M.H., Gaillard J., Immortalization of precursors of endodermal, neuroectodermal and mesodermal lineages, following the introduction of the simian virus (SV40) early region into F9 cells, Differentiation 35 (1987) 197–205.

[19] Kellermann O., Buc-Caron M.H., Marie P.J., Lamblin D., Jacob F., An immortalized osteogenic cell line derived from mouse teratocarcinoma is able to mineralize in vivo and in vitro, J. Cell Biol. 110 (1990) 123–132.

[20] Buc-Caron M.H., Launay J.M., Lamblin D., Kellermann O., Serotonin uptake, storage, and synthesis in an immortalized committed cell line derived from mouse teratocarcinoma, Proc. Natl. Acad. Sci. USA 87 (1990) 1922–1926.

[21] Poliard A., Nifuji A., Lamblin D., Plee E., Forest C., Kellermann O., Controlled conversion of an immortalized mesodermal progenitor cell towards osteogenic, chondrogenic, or adipogenic pathways, J. Cell Biol. 130 (1995) 1461–1472.

[22] Mouillet-Richard S., Mutel V., Loric S., Tournois C., Launay J.M., Kellermann O., Regulation by neurotransmitter receptors of serotonergic or catecholaminergic neuronal cell differentiation, J. Biol. Chem. 275 (2000) 9186–9192.

[23] Mouillet-Richard S., Laurendeau I., Vidaud M., Kellermann O., Laplanche J.L., Prion protein and neuronal differentiation: quantitative analysis of prnp gene expression in a murine inducible neuroectodermal progenitor, Microbes Infect. 1 (1999) 969–976.

[24] Manson J., West J.D., Thomson V., McBride P., Kaufman M.H., Hope J., The prion protein gene: a role in mouse embryogenesis? Development 115 (1992) 117–122.

[25] Sales N., Rodolfo K., Hassig R., Faucheux B., Di Giamberardino L., Moya K.L., Cellular prion protein localization in rodent and primate brain, Eur. J. Neurosci. 10 (1998) 2464–2471.

[26] Telling G.C., Parchi P., DeArmond S.J., Cortelli P., Montagna P., Gabizon R., Mastrianni J., Lugaresi E., Gambetti P., Prusiner S.B., Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity, Science 274 (1996) 2079–2082.

[27] Kretzschmar H.A., Prusiner S.B., Stowring L.E., DeArmond S.J., Scrapie prion proteins are synthesized in neurons, Am. J. Pathol. 122 (1986) 1–5.

[28] Mobley W.C., Neve R.L., Prusiner S.B., McKinley M.P., Nerve growth factor increases mRNA levels for the prion protein and the beta-amyloid protein precursor in developing hamster brain, Proc. Natl. Acad. Sci. USA 85 (1988) 9811–9815.

[29] Lazarini F., Castelnau P., Chermann J.F., Deslys J.P., Dormont D., Modulation of prion protein gene expression by growth factors in cultured mouse astrocytes and PC-12 cells, Brain Res. Mol. Brain Res. 22 (1994) 268–274.

[30] Wion D., Le Bert M., Brachet P., Messenger RNAs of beta-amyloid precursor protein and prion protein are regulated by nerve growth factor in PC12 cells, Int. J. Dev. Neurosci. 6 (1988) 387–393.

[31] Mouillet-Richard S., Ermonval M., Chebassier C., Laplanche J.L., Lehmann S., Launay J.M., Kellermann O., Signal transduction through prion protein, Science 289 (2000) 1925–1928.

[32] Taraboulos A., Jendroska K., Serban D., Yang S.L., DeArmond S.J., Prusiner S.B., Regional mapping of prion proteins in brain, Proc. Natl. Acad. Sci. USA 89 (1992) 7620–7624.

[33] Borchelt D.R., Koliatsos V.E., Guarnieri M., Pardo C.A., Sisodia S.S., Price D.L., Rapid anterograde axonal transport of the cellular prion glycoprotein in the peripheral and central nervous systems, J. Biol. Chem. 269 (1994) 14711–14714.

[34] Rodolfo K., Hassig R., Moya K.L., Frobert Y., Grassi J., Di Giamberardino L., A novel cellular prion protein isoform present in rapid anterograde axonal transport, Neuroreport 10 (1999) 3639–3644.

[35] Fournier J.G., Escaig-Haye F., Billette de Villemeur T., Robain O., Ultrastructural localization of cellular prion protein (PrPc) in synaptic boutons of normal hamster hippocampus, C. R. Acad. Sci., ser. III 318 (1995) 339–344.

[36] Shmerling D., Hegyi I., Fischer M., Blattler T., Brandner S., Gotz J., Rulicke T., Flechsig E., Cozzio A., von Mering C., Hangartner C., Aguzzi A., Weissmann C., Expression of amino-terminally truncated PrP in the mouse leading to ataxia and specific cerebellar lesions, Cell 93 (1998) 203–214.

[37] Chen L.M., Bailey D., Fernandez-Valle C., Association of beta 1 integrin with focal adhesion kinase and paxillin in differentiating Schwann cells, J. Neurosci. 20 (2000) 3776–3784.

[38] Nakamura T., Yamashita H., Takahashi T., Nakamura S., Activated Fyn phosphorylates alpha-synuclein at tyrosine residue 125, Biochem. Biophys. Res. Commun. 280 (2001) 1085–1092.

[39] Ron D., Napolitano E.W., Voronova A., Vasquez N.J., Roberts D.N., Calio B.L., Caothien R.H., Pettiford S.M., Wellik S., Mandac J.B., Kauvar L.M., Direct interaction in T-cells between thetaPKC and the tyrosine kinase p59fyn, J. Biol. Chem. 274 (1999) 19003–19010.

[40] Kaneko K., Zulianello L., Scott M., Cooper C.M., Wallace A.C., James T.L., Cohen F.E., Prusiner S.B., Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation, Proc. Natl. Acad. Sci. USA 94 (1997) 10069–10074.

[41] Rogers M., Yehiely F., Scott M., Prusiner S.B., Conversion of truncated and elongated prion proteins into the scrapie isoform in cultured cells, Proc. Natl. Acad. Sci. USA 90 (1993) 3182–3186.

[42] Taraboulos A., Scott M., Semenov A., Avrahami D., Laszlo L., Prusiner S.B., Avraham D., Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform, J. Cell Biol. 129 (1995) 121–132.

[43] Capellari S., Zaidi S.I., Urig C.B., Perry G., Smith M.A., Petersen R.B., Prion protein glycosylation is sensitive to redox change, J. Biol. Chem. 274 (1999) 34846–34850.

[44] Wong K., Qiu Y., Hyun W., Nixon R., VanCleff J., Sanchez-Salazar J., Prusiner S.B., DeArmond S.J., Decreased receptor-mediated calcium response in prion-infected cells correlates with decreased membrane fluidity and IP3 release, Neurology 47 (1996) 741–750.