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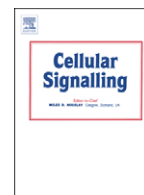

CREB-dependent gene regulation by prion protein: Impact on MMP-9 and β-dystroglycan

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ABSTRACT

Corruption of the normal function of the cellular prion protein (PrP^C) by the scrapie isoform (PrP^{Sc}) emerges as a critical causal event in Transmissible Spongiform Encaphalopathies (TSE) pathogenesis. However, PrP^C physiological role remains unclear. By exploiting the properties of the 1C11 neuroectodermal cell line, able to convert into $1C11^{5-HT}$ serotonergic or $1C11^{NE}$ noradrenergic neuronal cells, we assigned a signaling function to PrP^C. Here, we establish that antibody-mediated PrP^C ligation promotes the recruitment of the cAMP responsive element binding protein (CREB) transcription factor downstream from the MAPK ERK1/2, in 1C11 precursor cells and their $1C11^{5-HT}$ and $1C11^{NE}$ neuronal progenies. Whatever the differentiation state of 1C11 cells, the PrP^C-dependent CREB activation triggers Egr-1 and c-*fos* transcription, two immediate early genes that relay CREB's role in cell survival and proliferation as well as in neuronal plasticity. Furthermore, in 1C11-derived neuronal cells, we draw a link between the PrP^C–CREB coupling and a transcriptional regulation of the metalloproteinase MMP-9 and its inhibitor TIMP-1, which play pivotal roles in neuronal pathophysiology. Finally, the PrP^C-dependent control on MMP-9 impacts on the processing of the transmembrane protein, β-dystroglycan. Taken together, our data define molecular mechanisms that likely mirror PrP^C ubiquitous contribution to cytoprotection and its involvement in neuronal plasticity.

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1. Introduction

The cellular prion protein (PrP^C) is a ubiquitous glycosyl-phosphatidylinositol (GPI)-anchored membrane protein, whose conversion into an abnormal isoform, the scrapie prion protein (PrP^{Sc}), lies at the root of transmissible spongiform encephalopathies (TSE) pathogenesis [1]. While it is well established that PrP^C is absolutely necessary for the development of TSE [2], its physiological function remains enigmatic. PrP-deficient mice are viable and exhibit but minor phenotypic or behavioural alterations at baseline and hence did not permit to assign any specific function to PrP^C [3]. Unravelling PrP^C normal function is all the more challenging since it may present as multiple glycoforms [4] and may serve promiscuous functions depending on the cell type or tissue considered. Increasing evidence actually favors the notion that PrP^C combines a ubiquitous role in cell homeostasis [5,6] as well as some neurospecific function(s). Elucidating the latter may notably provide some clues as to how the PrP^{Sc}-induced deviation of PrP^C function in neurons leads to neuronal cell demise in TSE [7,8].

A substantial body of recent research favors the view that PrP^C acts as a gatekeeper against cellular insults in neuronal cells [9]. Indeed, neuronal cells derived from PrP-deficient mice undergo apoptosis following oxidative stress or serum deprivation more readily than those from wild-type mice [10–12]. From a mechanistic point of view, it is proposed that PrP^C exerts an anti-apoptotic action through interference in the mitochondrial apoptotic pathways, by mimicking the inhibitory effect of Bcl-2 on the pro-apoptotic Bax protein [13,14]. In line with in vitro observations, taking a closer look at PrP^C-deficient mice allowed to reveal an enhanced susceptibility to various brain injuries as compared to wild-type animals [15]. For instance, ischemia induces larger brain infarcts in PrP-null mice than in their wild-type counterparts [16–18]. Interestingly, in wild-type animals, PrP^C accumulates in the injured area following cerebral ischemia, suggesting that PrP^C could participate to posttrauma recovery by counteracting neurodegeneration [19].

The neuroprotective role of PrP^C has to be considered in light of its involvement in cell adhesion, neurite outgrowth and signal transduction. By forming a complex with the stress inducible protein 1 (STI1), PrP^C was indeed reported to promote either neuroprotection or neuritogenesis by distinct signaling pathways involving the cAMP-

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dependent protein kinase (PKA) or the mitogen activated protein kinases ERK1/2 [20,21]. Besides, PrP^C interacts with various adhesion proteins including, the extracellular matrix glycoprotein laminin, the 37-kDa laminin receptor precursor (LRP) and its mature form, the 67-kDa laminin receptor (LR) [22–24]. The binding of PrP^C to laminin was shown to promote neuritogenesis and neurite maintenance [23] and was recently proposed to mediate memory consolidation [25]. PrP^C can also associate with the Neural Cell Adhesion Molecule (N-CAM) through both cis and trans interactions, which sustain neurite outgrowth via the activation of the Fyn tyrosine kinase [26,27].

The observations by Santuccione et al. [26] actually fit in with our earlier work substantiating that PrP^C is able to instruct cell signaling events [28]. In that study, we took advantage of the 1C11 neuroectodermal cell line that can differentiate into either serotonergic ($1C11^{5\text{-HT}}$) or noradrenergic ($1C11^{NE}$) neuronal cells upon appropriate induction [29] and showed that antibody-mediated PrP^C crosslinking in differentiated cells triggers the activation of Fyn, through the recruitment of the membrane protein caveolin. We further established that PrP^C ligation promotes the serial activation of NADPH oxidase, a reactive oxygen species (ROS) generating enzyme, and the MEK/ERK1/2 MAP kinase module, not only in 1C11 neuronal derivatives but also in non-neuronal cells, underlying a ubiquitous involvement of PrP^C in cell homeostasis [5]. In the 1C11 precursor and non-neuronal cells, the PrP^C-induced ROS act as second messengers and fully mediate ERK1/2 phosphorylation. In contrast, in $1C11^{5\text{-HT}}$ and $1C11^{NE}$ cells, the PrP^C-caveolin-Fyn platform recruits multiple pathways, converging on ERK1/2, one of which involves NADPH oxidase.

With the aim of shedding further light on PrP^C signaling function, we looked for PrP^C intracellular targets recruited downstream from ERK1/2, focusing on transcription factors. In the present study, we identify the cyclic AMP-responsive element binding protein (CREB) transcription factor as a target of PrP^C signaling, in both the 1C11 precursor and its neuronal progenies. In response to PrP^C ligation, CREB triggers the transcription of two immediate early genes, Egr-1 and c-*fos*. In $1C11^{5\text{-HT}}$ and $1C11^{NE}$ neuronal cells, CREB activation induces a transcriptional regulation of the MMP-9/TIMP-1 metalloproteinase system, which impacts on β-dystroglycan processing.

2. Materials and methods

2.1. Material

All tissue culture reagents were from Invitrogen (Carlsbad, CA, USA). Monoclonal PrP-targeted antibodies (SAF61, SAF32, and Sha31, all IgG) with distinct binding epitopes [30] were obtained from the Service de Pharmacologie et d'Immunologie, Commissariat à l'Energie Atomique (Saclay, France). Polyclonal rabbit IgG antibodies against phospho-Ser133 CREB and total CREB were from Upstate Biotechnology (Lake Placid, NY, USA). Monoclonal rabbit antibodies against Egr-1 and c-*fos* were purchased from Cell Signaling Technology (Danvers, MA, USA). Monoclonal mouse antibody against β-dystroglycan was from Novocastra Laboratories Ltd. (Newcastle, UK). Monoclonal mouse antibody against actin was from Novus Biologicals (Littleton, CO, USA). Dibutyryl cyclic AMP (dbcAMP), cyclohexane carboxylic acid (CCA), forskolin and DPI were purchased from Sigma (St. Louis, MO, USA). Phorbol 12-myristate 13-acetate (PMA), PP2, Wortmannin, Rottlerin, PD98059 and MMP-9 inhibitor were purchased from Calbiochem (San Diego, CA, USA). Naphthol AS-E-phosphate was from Fluka (Diesenhofen, Germany).

2.2. Cell culture, antibody-mediated PrP^C ligation and enzyme inhibition

1C11 cells were grown and induced to differentiate along the serotonergic pathway in the presence of 1 mM dbcAMP and 0.05% CCA or along the noradrenergic pathway by the addition of 1 mM dbcAMP, 0.05% CCA, as in [29]. Ligation of PrP^C at the surface of 1C11 cells was carried out using SAF61, SAF32 antibodies at 10 µg/ml as in [5], or Sha31 antibody at 5 µg/ml. Enzyme inhibition was performed by pretreating cells at 37 °C for 1 h in their culture medium with the appropriate inhibitor prior to exposure to PrP antibody.

2.3. Preparation of cell extracts and Western Blot analyses

1C11 precursor, $1C11^{5\text{-HT}}$ and $1C11^{NE}$ cells were washed in PBS with 1 mM Ca^{2+} and Mg^{2+} and incubated for 30 min at 4 °C in NET lysis buffer [50 mM Tris–HCl (pH 7.4)/150 mM NaCl/5 mM EDTA/1% Triton X-100/1 mM Na_3VO_4 and a mixture of protease inhibitors, Roche, Mannheim, Germany]. Extracts were centrifuged at 14,000 ×*g* for 15 min. Protein concentrations in the supernatant were measured by using the bicinchoninic acid method (Pierce, Rockford, IL, USA). Ten micrograms of proteins was resolved by SDS/10% PAGE and transferred to nitrocellulose membranes (Amersham, Arlington Heights, IL, USA). Membranes were blocked with 0.5% gelatin and 0.5% goat serum or with 3% non-fat dry milk in PBS 0.1% Tween 20 for 1 h at room temperature and then incubated overnight at 4 °C with primary antibody (1 µg/ml). Bound antibody was revealed by enhanced chemiluminescence detection using a secondary antibody coupled to HRP (Amersham).

2.4. Immunofluorescence experiments

Cells grown on labtek chambers (Nunc, Rochester, NY, USA) and exposed to PrP antibodies or Forskolin were washed in PBS with 1 mM Ca^{2+} and Mg^{2+} (buffer A) and fixed with 3.6% formaldehyde in buffer A. Cells were then permeabilized with blocking buffer (buffer A with 2% non-fat dried milk and 0.1% Triton X-100) for 1 h at room temperature. Cells were next incubated with primary antibody (5 µg/ml) diluted in buffer A enriched with 2% BSA and 0.1% Triton X-100 for 1 h at 37 °C. Alexa Fluor 488 immunoglobulins (4 µg/ml) (Molecular Probes, Eugene, OR, USA) were used as secondary antibodies. Immunolabelling was observed with an Axiophot 200 fluorescence microscope (Carl Zeiss, Oberkochen, Germany) equipped with a camera (Nikon).

2.5. Measurement of CREB transcriptional activity

Nuclear proteins were extracted using the Active Motif Nuclear Extract kit (Active Motif Europe, Rixensart, Belgium) according to the manufacturer's instructions, and the total protein concentration of the lysates was determined with the bicinchoninic acid method (Pierce). CREB transcriptional activity was then determined with 2.5 µg of nuclear extract using an enzyme-linked immunosorbent assay (ELISA)-based transcription factor assay kit (TransAM assay) (Active Motif Europe) according to the manufacturer's protocol.

2.6. Isolation of total RNA and reverse transcriptase-polymerase chain reaction analysis

RNA was isolated by using RNase Easy Kit from Qiagen (Hilden, Germany) including a RNase-free DNase I digestion step, as recommended by the manufacturer's instructions. For reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, first-strand cDNA was synthesized from with oligo$(dT)_{17}$ primer, using 400 U of Superscript II™ reverse transcriptase (Invitrogen) according to the manufacturer's protocol. PCR amplifications were then carried out in a 50-µl volume containing 1 µl of the reverse transcription products, using Taq DNA polymerase (Invitrogen). PCR products were analyzed on 2% agarose gels. Primers used for the PCR reactions include: GAPDH forward 5′-TGAAGGTCGGTGTGAACGGAT-3′ and reverse 5′-CATGTAGGCCATGAGGTCCAC-3′; Egr-1 forward 5′-AAGA-CACCCCCCCATGAAC-3′ and reverse 5′-GCGAGAAAAGCGGCGAT-3′; c-Fos forward 5′-ACGGAGAATCCGAAGGGAACGGAATAAATGGC-3′ and reverse 5′-GACAAAGGAAGACGTGTAAGTAGTGCAGC-3′; MMP-9 forward 5′-

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