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Prion formation correlates with activation of translation-regulating protein 4E-BP and neuronal transcription factor Elk1

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ABSTRACT

Cellular mechanisms play a role in conversion of the normal prion protein PrP^{C} to the disease-associated protein PrP^{Sc}. The cells provide not only PrP^{C}, but also still largely undefined factors required for efficient prion replication. Previously, we have observed that interference with ERK and p38-JNK MAP kinase pathways has opposing effects on the formation of prions indicating that the process is regulated by a balance in intracellualar signaling pathways. In order to obtain a "flow-chart" of such pathways, we here studied the activation of MEK/ERK and mTORC1 downstream targets in relation to PrP^{Sc} accumulation in GT1-1 cells infected with the RML or 22L prion strains. We show that inhibition of mTORC1 with rapamycin causes a reduction of PrP^{Sc} accumulation at similar low levels as seen when the interaction between the translation initiation factors eIF4E and eIF4G downstream mTORC1 is inhibited using 4EGI-1. No effect is seen following the inhibition of molecules (S6K1 and Mnk1) that links MEK/ERK signaling to mTORC1-mediated control of translation. Instead, stimulation (high [KCl] or [serum]) or inhibition (MEK-inhibitor) of prion formation is associated with increased or decreased phosphorylation of the neuronal transcription factor Elk1, respectively. This study shows that prion formation can be modulated by translational initiating factors, and suggests that MEK/ERK signaling plays a role in the conversion of PrP^{C} to PrP^{Sc} via an Elk1-mediated transcriptional control. Altogether, our studies indicate that prion protein conversion is under the control of intracellular signals, which hypothetically, under certain conditions may elicit irreversible responses leading to progressive neurodegenerative diseases.

Introduction

Prion diseases are fatal neurodegenerative diseases associated with accumulation of a misfolded prion protein designated PrP^{Sc} in the brain. PrP^{Sc} is derived from a normal cellular protein, PrP^{C}, through a post-translational conversion process and linked to the transmissibility of the disease (Prusiner, 1982). PrP^{C} is expressed at the cell surface where it is attached in rafts through a glycolipid anchor. The conversion of the normal PrP^{C} to the misfolded PrP^{Sc} during disease, may occur either at the cell surface, in early endosomal organelles (Borchelt et al., 1992; Taraboulos et al., 1995) or in endosomal recycling compartments (Marijanovic et al., 2009). The rate of degradation of PrP^{Sc} is slower than that of its formation (Borchelt et al., 1992), which could lead to accumulation of prions in the infected brain. The formation of prions can be described in biophysical terms as a process that converts PrP^{C} to PrP^{Sc}. However, like other pathogens, prions maintain a complex, two-way relationship with the host cell. It is clear that prions form more efficiently in their natural host cells than they do in the test tube. The host cell provides both the molecular species PrP^{C} and auxiliary factors required for the conversion process. For instance, the lipid composition of rafts (Bate et al., 2004, 2008; Hagiwara et al., 2007; Taraboulos et al., 1995), heparan sulfates (Ben-Zaken et al., 2003; Díaz-Nido et al., 2002; Horonchik et al., 2005; Löfgren et al., 2008; Schonberger et al., 2003), and expression of laminin receptors (Gauczynski et al., 2001a, 2001b; Hundt et al., 2001; Rieger et al., 1997) and lipoprotein receptor-related protein 1 (Parkyn et al., 2008; Taylor and Hooper, 2007) can affect the level of PrP^{Sc} accumulation in a cell. The protein misfolding cyclic amplification (PMCA) technique employs cell lysates as a vital component to accelerate the conversion (Abid et al., 2010), and fatty acids, heparin, synthetic poly-RNA, proteins and heparin can partially replace cell

Abbreviations: PrP^{C}, normal cellular prion protein; PrP^{Sc}, disease-associated prion protein; GT1-1 cells, immortalized hypothalamic gonadotropin-releasing hormone neurons; BDNF, brain-derived neurotrophic factor; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; mTORC1, mammalian target of rapamycin complex 1; TSC, tuberous sclerosis complex; S6rp, ribosomal protein S6; S6K1, p70 ribosomal S6 Kinase 1; Mnk1/2, MAP kinase-interacting kinases; eIF4E, eukaryotic initiation factor 4E; 4E-BP, eukaryotic initiation factor 4E binding protein; PF-4708671, S6K1-inhibitor 2-((4-(5-ethylpyrimidin-4-yl)piperazin-1-yl)methyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole; CGP 57380, Mnk1-inhibitor N3-(4-fluorophenyl)-1h-pyrazolo[3,4-d]pyrimidine-3,4-diamine; leupeptin, leupeptin hydrochloride; U0126, MEK-inhibitor (1,4-diamino-2,3-dicyano-1,4-bis(o-aminophenylmercapto) butadiene ethanolate); DMSO, dimethyl sulfoxide; BrdU, bromodeoxiuridine.

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lysates in PMCA (Abid et al., 2010). However, such molecules have a relatively low efficiency and factors with large effects on prion conversion or propagation have still to be identified. Furthermore, the cellular susceptibility to a prion infection seems to be dependent on the integrity of the living cell, since lysates from resistant and susceptible cells support prion amplification equally well by PMCA (Herva and Weissman, 2012).

We have previously observed that stimulation or inhibition of MAP kinase signaling pathways can interfere with the accumulation of the RML strain of prions in immortalized hypothalamic gonadotropin-releasing hormone neurons (GT1-1 cells). Treatment with brain-derived neurotrophic factor (BDNF) (Nordström et al., 2005) or depolarization with high [KCl] (Nordström et al., 2009), which both activate the MEK (mitogen-activated protein kinase kinase)/ERK (extracellular signal-regulated kinase) MAP (mitogen-activated protein) kinase pathway, increase the accumulation of PrP^{Sc} in the cells. Inhibition of the MEK/ERK cascade clears prion-infected cells from PrP^{Sc} (Nordström et al., 2005), while the blockade of two other MAP kinase pathways, the stress-activated protein kinase cascades p38 and JNK, has the opposite effect (Nordström et al., 2009). The changes in prion accumulation reflect changes in the formation rather than in the degradation of PrP^{Sc} as observed by combining the treatments with known inhibitors of prion formation and degradation, and, since PrP^{C} levels are not altered by the treatments (Nordström et al., 2009, 2005).

The MEK/ERK pathway may exert both transcriptional and translational controls through the phosphorylation of a number of regulatory molecules (Yoon and Seger, 2006). The pathway may regulate protein synthesis via the mTORC1 (mammalian target of rapamycin complex 1) signaling pathway. Thus, ERK may in certain systems promote mTORC1 activity via the inhibition of the TSC (tuberous sclerosis complex) and activation of the small GTP binding protein Rheb (Ma et al., 2005). In addition, the MEK/ERK pathway may converge with mTORC1 through the S6rp (ribosomal protein S6), which is regulated by mTORC1 via the S6K1 (p70 ribosomal S6 Kinase 1) (Roux et al., 2007), and through the Mnk1/2 (MAP kinase-interacting kinases), which phosphorylates eIF4E (eukaryotic initiation factor 4E) downstream of mTORC1/4E-BP (eukaryotic initiation factor 4E binding protein; Fig. 1).

In order to obtain a "flow-chart" of intracellular signaling pathways that regulate prion formation, we here studied the activation of MEK/ERK potential translational and transcriptional downstream targets in relation to PrP^{Sc} accumulation in GT1-1 cells infected with either the RML or 22L strain of prions. We show that mTORC1-signaling via the 4E-BP and activation of the eIF4E participates in the formation of PrP^{Sc} in a MEK/ERK-independent way. The study also indicates a potential role of the MEK/ERK-activated neuronal transcription factor Elk1 in the cell-mediated regulation of PrP^{Sc} formation.

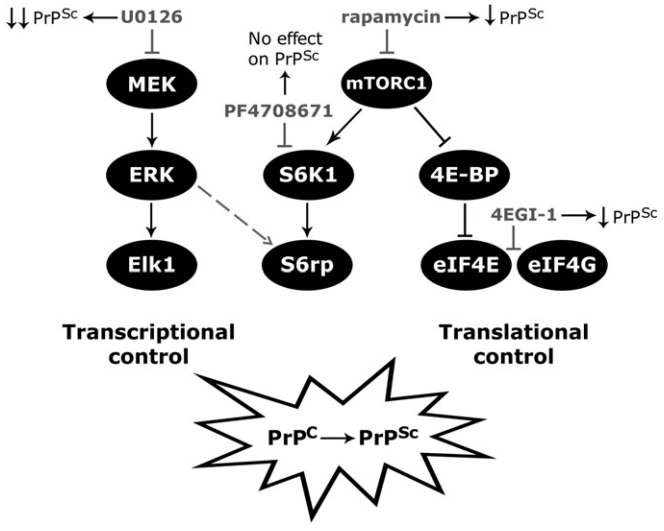

Fig. 1. Overview of the MEK/ERK and the mTORC1 signaling pathways involved in prion formation.

Materials and methods

Cell cultures, prion strains and infection procedure

The GT1-1 cell line is derived from immortalized mouse hypothalamic gonadotropin-releasing hormone neurons and was a generous gift from Prof. Pamela Mellon (Department of Reproductive Medicine, University of California, San Diego, CA, USA). The cells were cultivated and infected with the mouse-adapted scrapie strains RML (a kind gift from Prof. Stanley B. Prusiner, Institute for Neurodegenerative Diseases, University of California, San Francisco, CA, USA) or 22L (provided by TSE Resource Centre, Institute for Animal Health, Newbury, UK) as previously described (Nordström et al., 2009). The presence of proteinase K (PK; Boehringer Mannheim, Mannheim, Germany) resistant PrP^{Sc} was confirmed by Western blotting (see below) after 6 passages and these infected cells will be referred to as ScGT1-1/RML or ScGT1-1/22L cells.

Western blot analyses

The cells were lysed on ice and Western immunoblotting was performed as previously described (Nordström et al., 2009). Protein concentration was determined using spectrophotometry and samples were normalized to contain the same protein concentration. In some experiments one part of the lysates was normalized to protein concentration and the other part was instead normalized to the number of cells seeded, and no significant difference in PrP levels was observed between these on Western blots. PrP was labeled with monoclonal anti-prion protein antibody IPC1 (Sigma-Aldrich Chemie, Steinhem, Germany). Phospho-ERK1/2 (#9101), total-ERK1/2 (#9102), phospho-S6rp $Ser^{235/6}$ (#2211), phospho-S6rp $Ser^{240/4}$ (#2215), total-S6rp (#2317), phospho-Mnk1 $Thr^{197/202}$ (#2111), phospho-4E-BP Ser^{65} (#9451) and phospho-Elk1 Ser^{383} (#9181) were detected using antibodies from Cell Signaling Technology (Cell Signaling Technology, Inc., Danvers, MA, USA) and phospho-Histone H3 Ser^{10} by antibodies from Millipore (Bedford, USA). Secondary anti-rabbit- or mouse-HRP antibodies were obtained from Dako (Denmark A/S, Glostrup, Denmark). The levels of different phospho-proteins were analyzed 60 min, 24 h and 5 d after exposure to the various compounds (below).

Optical densities of the bands were determined using the software ImageLab (Bio-Rad Laboratories AB, Hercules, CA, USA). All samples were normalized to the mean densities of the controls.

Treatment of cells with various compounds

The cells were treated with the following compounds: rapamycin (inhibitor of mTORC1; 20 nM; Merck KGaA, Calbiochem, Darmstadt, Germany), 4EGI-1 (eIF4E/eIF4G interaction inhibitor; 25 μM; Merck KGaA, Calbiochem; (Moerke et al., 2007)), PF-4708671 (S6K1-inhibitor; 2-(4-(5-ethylpyrimidin-4-yl)piperazin-1-yl)methyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole; 0,1–10 μM; Sigma-Aldrich (Pearce et al., 2010), CGP 57380 (Mnk1-inhibitor; N3-(4-fluorophenyl)-1h-pyrazolo[3,4-d] pyrimidine-3,4-diamine;1 μM; Sigma-Aldrich), leupeptin (leupeptin hydrochloride; 15 μM; Sigma-Aldrich), U0126 (MEK-inhibitor; (1,4-diamino-2,3-dicyano-1,4-bis(o-aminophenylmercapto) butadiene ethanolate), 2–10 μM; Promega Corporation, Madison, WI, USA), and KCl (35 mM; Sigma-Aldrich).

All the inhibitors were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) prior to cell treatments, except leupeptin that was dissolved in H_2O. Our previous studies on the MEK/ERK pathway experiments were carried out in DMEM supplemented with 1% serum, but for studies on the mTORC1 pathway DMEM with 10% serum