Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders that include Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy, scrapie, and chronic wasting disease in animals. Intraneuronal vacuolization, severe neuronal cell death, astroglisis, and microglia activation are the main hallmarks of TSEs (1). At the molecular level, TSEs are characterized by progressive cerebral accumulation of a misfolded protease-resistant isoform (PrPres) of the host-encoded cellular prion protein (PrPc). No amino acid sequence or posttranslational differences have been detected between the normal PrPc and its pathological PrPres isoform (2). The molecular changes resulting in the formation of amyloid aggregates of PrPres have been extensively studied in vitro as well as in vivo. The PrPres fibril growth is thought to occur by binding of PrPc molecules followed by their conversion into a conformation physicochemically undistinguishable to PrPres (3). Conformational changes involved in the conversion step result in a lowering in α-helical content and an important increase of the β-sheet structures (4, 5).

For decades, TSEs have been widely regarded as infectious diseases lacking inflammatory component (6). However, the accumulation of PrPres is correlated with the appearance of reactive microglia and astrocytes concomitantly with neuronal cell loss (7–9). Because they are frequently present in the vicinity of PrPres aggregates, activated microglial cells could play an important role in brain damages (10, 11). Growing evidence support the hypothesis that the recruitment of microglia may be one of the early steps of TSEs pathogenesis. Activated microglia release various factors such as cytokines and free radicals that regulate neuronal and glial survival (12).

Nitrict oxide release by microglia is thought to be responsible, at least in part, to neuronal cell death likely via the induction of apoptotic pathways (13). Alternatively, microglial cells might assume other roles including a carrier for the TSE infectious agent participating to the spread of the disease throughout the brain (14, 15). The involvement of microglial cells is well described in TSEs as well as in a variety of other neurodegenerative disorders (for review, see Ref. 16).

Recruitment and correct positioning of microglia in the vicinity of cerebral lesions are under control of chemokines acting through the activation of specific G-protein-coupled receptors (17). Expression levels of a broad spectrum V of chemokines and chemokine receptors undergo variations in the course of TSE infection development (18, 19). We have previously demonstrated that PrPres triggers the recruitment of microglial
cells by interacting with neurons and up-regulating the mRNA expression level of the chemokine RANTES (20). Our data strongly suggested the existence of functional interactions between PrPres and the neuronal cell surface that might activate transduction signal pathways leading to the up-regulation of RANTES expression. Prion synthetic fragment 106–126 activated the mitogen-activated protein kinase (MAP kinase)-dependent inflammatory signal transduction cascades in monocytic cell line (21). Moreover, the MAP kinase pathway is activated in vulnerable neurons in patients with Alzheimer’s disease raising the possibility of an important role in the pathogenesis of neurodegenerative diseases (22). The major aim of the present study was to characterize the PrPres-induced signaling pathways in neurons involved in the microglial cell recruitment. We identified a MAP kinase-dependent signaling cascade in primary cultured neurons that is activated within minutes after exposure to PrPres and is directly responsible for RANTES expression. PrPres provokes the activation of extracellular signal-regulated kinase 1/2 (Erk1/2), members of the MAP kinases family (23), independently of the presence of both PrPc and laminin receptors, the putative PrPres receptors on neurons (19, 22). Specific inhibition of kinases and transcription factors within the PrPres-mediated activation pathway prevents microglia migration and could offer novel strategies for TSEs treatment.

EXPERIMENTAL PROCEDURES

Materials—Opti-MEM, serum-free medium Neurobasal, B27 supplement, G418, and penicillin-streptomycin mixture were purchased from Invitrogen. RPMI 1640 medium, L-glutamine, and fetal calf serum were purchased from BioWhittaker (Walkersville, MD) and phosphate-buffered saline (PBS) from Roche Applied Science. Antibodies against the phosphorylated or total forms of Erk1/2 were from purchased Santa Cruz Laboratory (Santa Cruz, CA); antibodies against the phosphorylated form of Elk-1 were from Cell Signalling. Horseradish peroxidase-conjugated goat anti-rabbit and anti-mouse antibodies were from Jackson Immunoresearch (West Grove, PA). Bovine serum albumin, streptomycin, and penicillin were from Roche Applied Science. Bovine serum albumin, streptomycin, and penicillin were from Roche Applied Science.

Cell Culture and Adenovirus Infection of Neurons—Cortical neurons from embryonic day 13–14 of C57-black wild-type or PrP gene knock-out (PrP0/0; Zurich I line; (27)) were prepared as described previously (21). Moreover, the MAP kinase pathway is activated in vulnerable neurons in patients with Alzheimer’s disease raising the possibility of an important role in the pathogenesis of neurodegenerative diseases (22). The major aim of the present study was to characterize the PrPres-induced signaling pathways in neurons involved in the microglial cell recruitment. We identified a MAP kinase-dependent signaling cascade in primary cultured neurons that is activated within minutes after exposure to PrPres and is directly responsible for RANTES expression. PrPres provokes the activation of extracellular signal-regulated kinase 1/2 (Erk1/2), members of the MAP kinases family (23), independently of the presence of both PrPc and laminin receptors, the putative PrPres receptors on neurons (19, 22). Specific inhibition of kinases and transcription factors within the PrPres-mediated activation pathway prevents microglia migration and could offer novel strategies for TSEs treatment.

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Cell Culture and Adenovirus Infection of Neurons—Cortical neurons from embryonic day 13–14 of C57-black wild-type or PrP gene knock-out (PrP0/0; Zurich I line; (27)) were prepared as described previously (21). Disassociated cells were plated at a density of 1 × 10^6 cells in 12-well and 5 × 10^5 in 24-well tissue plastic dishes, precoated with polylysine (10 μg/ml). Cells were grown for 24 h in Neurobasal supplemented with B27 and 10% inactivated fetal calf serum, then the medium was replaced with fresh Neurobasal/B27 mixture. GFP-expressing adenovirus infections of neurons were performed by adding to the Neurobasal/B27 mixture, 5 or 15 μl of adenoviruses preparation at 3 multiplicities of infection/m.o.i./w/vl/1 × 10^5 neuronal cells. In these conditions, no neurotoxicity was observed (data not shown). Cultures were pure of neurons up to 98% and used after 4–5 days of differentiation.

The N11 microcellar line cell was grown in RPMI 1640 containing 10% inactivated fetal calf serum, penicillin-streptomycin, 0.5 × 10^-4 M-mercaptoethanol and 2 × M L-glutamine. Neuroblastoma cells chronically infected with the murine Chandler strain (N2asc-) were grown in Opti-MEM supplemented with 10% inactivated fetal calf serum, penicillin-streptomycin, and 1 μg/ml G418. As described previously (28), PrPres-cured neuroblastoma cells (N2asc-) were obtained by treatment with Congo Red (1 μg/ml) for several passages of the cells. All cell cultures were grown at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. N2asc homogenate preparations (used as source of PrPres) were obtained in detergent-free conditions, mainly consisting of one purification step using protease K digestion as previously described (20). Typically, the preparation was used in PrPres up to >75%, and its concentration was about 20 μg/μl, as estimated by silver staining and Western blot analyses on SDS-PAGE. N2asc homogenate preparations (hgtsc) performed in the same conditions are PrPres-free and were used as a negative control in all the experiments.

Microglial Cell Migration Assays—Four-day-old cortical neurons (5 × 10^5 cells/well) plated in 24 wells of the Boyden’s chamber were incubated with 5 μl of PBS, hgtsc-, or hgtsc- (i.e., ~100 μg of PrPres) for 24 h at 37 °C. Mouse microglial cells N11 were added on the top chamber of the Boyden’s chamber (5 × 10^5 in 200 μl) and allowed to migrate through polyester filters (pore size, 8 μm; Falcon) for 6 h at 37 °C. Cells remaining in the upper surface of the filter were delicately removed with a cotton swab, whereas migrating cells were fixed in 3% paraformaldehyde and stained for 10 min at room temperature with 0.5% crystal violet in PBS plus 10% methanol. Filters were mounted on glass slides, and stained cells were counted (5 random fields/Filter) under an inverted microscope. Statistical analysis between each experimental condition was made from the number of filters (n = 6) by using the Student’s t test. Differences were considered significant for p < 0.01.

Western Blot Analyses—Neurons (1 × 10^6 cells/well) were pre-incubated in presence or in absence of PD98059 (25 μM) for 2 h then treated with 10 μl of PBS (0), hgtsc-, or hgtsc- at 37 °C for the indicated times. Then, cells were washed in PBS and then lysed in 180 μl of ice-cold lysis buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 5 mM EDTA, 0.5% Na-deoxycholate, 0.5% Nonidet P-40) in the presence of 1 μg NaOx, 10 μM NaF, and complete mixture of protease inhibitors (Roche Applied Science). Identical amounts of total cell-solubilized proteins (~40 μg) were analyzed on SDS-PAGE, electrotransferred onto nitrocellulose membrane, and subjected to immunoblotting using antibodies directed against early growth response factor-1 (Egr-1) protein or the active phosphorylated forms of Erk1/2 or Elk-1. Blots were revealed using an enhanced chemoluminescence system (Pierce) and exposed on films (X-Omat AR, Kodak). To ensure equal loading of proteins in each lane, the same blots were rerolled with the total anti-Erk1/2 antibodies.

Time Polynucleotide Chain Reaction—mRNAs were prepared using the method of Chomczynski and Sacchi (29). Briefly, after treatment with DNase I, 0.5 μg of mRNA were reverse-transcribed with M-MLV reverse transcriptase kit (Invitrogen). Real-time PCR was performed in an ABI PRISM 5700 Sequence Detector System (Applied Biosystem) using the SYBR Green detection protocol as outlined by the manufacturer. RANTES gene-specific primers were designed using the Primer Express software (Applied Biosystem). To investigate the role of PrPres in the RANTES gene expression in the PrPres-induced chemotactism mechanism, the RANTES gene was normalized for RNA concentrations with four different housekeeping genes (glyceraldehyde-3-phosphate dehydrogenase, β-actin, hypoxanthine-guanine phosphoribosyltransferase, and ubiquitin).

Confocal Microscopy Experiments—Primary cultures of mouse neurons were grown on glass coverslips in the appropriated medium for 24 h at 37 °C and then infected with adGFP and adGFP-Nab2 as described above. Three days post-infection, coverslips were washed twice with PBS and then fixed with 3% paraformaldehyde in PBS for 10 min at room temperature. Coverslips were mounted on glass slides with Mowiol for confocal microscopy examination. Cells were analyzed using a laser scanning confocal inverted microscope (Leica, Nussloch, Germany) equipped with an argon-krypton laser. The samples were scanned under 488 excitation wavelength. The images were acquired as single transcellular optical sections and averaged over at least 4 scans/frame.

RESULTS

Neurons Trigger Microglia Migration in Response to PrPres Exposure by Activation of a MAP Kinase/Erk1/2 Signaling Pathway Independently of the PrPc Expression—We previously demonstrated that neurons respond to PrPres exposure by up-regulating the expression of the chemokine RANTES leading to the recruitment of microglial cells (20). This study strongly suggested the existence of specific interactions between PrPres and putative receptors on the cell surface and the subsequent induction of transmigration signal pathways. Based on specific binding observed in vitro between PrPres and PrPc (3) or PrPres and laminin receptor (30) both proteins might act as cellular PrPres receptors. To investigate the role of PrPc expression in the PrPres-induced chemotactism mechanism, in vitro microglial cell migration assays were performed in the presence of wild-type or PrP-depleted cortical neurons. Cultured neurons from wild-type or PrP00 mice grown in the Boyden bottom chamber were incubated in the presence of PBS, hgtsc-, or hgtsc- for 24 h, and then the microglial cells plated in the top insert of the chamber were allowed to migrate
for 6 h at 37 °C (Fig. 1A). In the presence of hgtsc−, the level of migrating cell was increased by 3.3-fold when compared with PBS and hgtsc− conditions. The rate of microglia migration was similar when assays were performed with wild-type and PrP0/0 neurons suggesting that the PrPres-induced chemotactism was independent of the presence of PrPc on the neuronal cell surface. To test whether the PrPres-induced microglia migration was mediated though the binding to the laminin receptor polyclonal antibodies (10-40 μg/ml) were added to the incubation medium. B and C, the experiments were performed as described above except that the primary cultures of neurons from wild-type mice were pre-incubated in the absence or in the presence of 25 μM PD98059 for 2 h at 37 °C prior to stimulation with 5 μl of hgtsc− or hgtsc+. B, histograms represent the means of five independent experiments ± S.D. performed in duplicate, and results were expressed as the percentage of the microglia migration rate obtained with 5 μl of hgtsc−. C, RANTES mRNA expression was determined by real time PCR analysis as described under “Experimental Procedures.” mRNA values are expressed as arbitrary units and represent mean ± S.D. of triplicates and are representative of two independent experiments.

![Figure 1](image1)

**Fig. 1.** Effect of PrPc expression and MAP kinase inhibitor PD98059 on microglial cell migration induced by PrPres exposure on neurons. A, four-day-old cortical neurons from wild-type or PrP0/0 mice were incubated with 5 μl of PBS (white bars), hgtsc− (gray bars), or hgtsc+ (black bars) for 24 h at 37 °C. Migration experiments were initiated by plating N11 microglial cells on an 8-μm pore size insert for 6 h. When indicated (+αLRP) specific anti-laminin receptor polyclonal antibodies (10-40 μg/ml) were added to the incubation medium. B and C, the experiments were performed as described above except that the primary cultures of neurons from wild-type mice were pre-incubated in the absence or in the presence of 25 μM PD98059 for 2 h at 37 °C prior to stimulation with 5 μl of hgtsc− or hgtsc+. B, histograms represent the means of five independent experiments ± S.D. performed in duplicate, and results were expressed as the percentage of the microglia migration rate obtained with 5 μl of hgtsc−. C, RANTES mRNA expression was determined by real time PCR analysis as described under “Experimental Procedures.” mRNA values are expressed as arbitrary units and represent mean ± S.D. of triplicates and are representative of two independent experiments.

![Figure 2](image2)

**Fig. 2.** Effect of PrPres exposure on MAP kinases ERK1/2 phosphorylation in primary cultures of neurons. A, four-day-old cortical neurons were stimulated with 10 μl of hgtsc− or hgtsc+ for 0 (unstimulated)–60 min at 37 °C. The phosphorylation of MAP kinases (p-Erk1/2) was determined by immunoblotting using an antibody directed against the phosphorylated active form of Erk1/2. The same blots were subsequently reprobed with anti-total Erk proteins to ensure equal loading of samples. B, data were standardized from three different experiments using the labeling obtained on the same blot with the anti-total Erk antibody and expressed as means ± S.D. C, cultures of neurons were pre-incubated with 25 μM PD98059 for 2 h before being exposed to 10 μl of hgtsc− for the indicated times. Immunoblots shown in A and C are representative of typical experiments.

migration assays were performed by pre-incubating wild-type neurons in the presence of the MEK-specific inhibitor, PD98059 (31) prior to the induction of microglia migration by hgtsc−. As shown in Fig. 1B, the addition of 25 μM PD98059 in the incubation medium provoked a ~46% decrease in the rate of microglia migration. Because we identified RANTES as the main neuronal chemokine involved in the PrPres-induced chemotactism (20), we assessed the possible involvement of the MAP kinase cascade in the up-regulation of RANTES mRNAs. PD98059-treated or non-treated neurons were stimulated with 5 μl of hgtsc− or hgtsc+; then RANTES mRNAs were quantified by using a real time PCR assay (Fig. 1C). RANTES mRNA expression was highly up-regulated following hgtsc− stimulation in comparison to the PBS or hgtsc− treatments. A 66% decrease in the RANTES mRNA levels was measured from hgtsc−-stimulated neurons pretreated with PD98059 compared with the non-treated cells suggesting that the RANTES mRNA expression is under the control of MAP kinase activation. To confirm that PrPres exposure on neurons activates the MAP kinase signaling pathway, neurons were incubated in the absence (t = 0) or in the presence of hgtsc− or hgtsc+ for various times. Then, Western blot analyses were performed on cell lysates to detect phosphorylated i.e. activated forms of mitogen-activated kinases Erk1/2 (Fig. 2). Hgtsc− rapidly and transiently stimulated the phosphorylation of MAP kinases Erk1/2 in neurons with a maximal effect after 2.5 min that was main-
lead to a complete inhibition of the MAP kinase pathway as phosphorylation (activation) of Elk-1 and Egr-1 expression i.e. (Figs. 2A or with hgtsc in the presence of 25 μM PD98059 (Fig. 2C). Surprisingly, PD98059 that seems to lead to a complete inhibition of the MAP kinase pathway as shown by Western blot analyses (Fig. 2C), gives only a partial inhibition of RANTES mRNA expression and microglia migration (Fig. 1, B and C). This discrepancy could be explained by the fact that primary cultures contain astrocytes (2–4%) and heterogeneous neurons in terms of differentiation stages and brain areas origin. We cannot rule out the fact that astrocytes and a minor part of neurons respond to PrPPres exposure in a MAP kinase pathway-independent manner (20). In summary, our data strongly suggest that PrPPres stimulates the MAP signaling pathway leading to the up-regulation of chemokine levels and the subsequent microglia recruitment in a way that does not require the presence of PrPc or the laminin receptor.

**Regulation of Elk-1 Phosphorylation and Egr-1 Expression in PrPPres-exposed Neurons**—Transcription factor Elk-1 is the main target of activated p-Erk1/2 (32). Because the expression of early gene transcription factor Egr-1 is dependent on Elk-1 activation (33), we investigated whether PrPPres induces the phosphorylation (i.e. activation) of Elk-1 and Egr-1 expression in neurons in a MAP kinase-dependent manner. PD98059-treated or untreated neurons were stimulated with 10 μl of hgtsc for different times; total proteins from neuronal cell lysates were then submitted to Western blot analysis for p-Erk and Egr-1 detection (Fig. 3). Whereas p-Erk was barely detectable in the absence of PrPPres exposure (t = 0), its level increased significantly as a function of hgtsc incubation time (Fig. 3A). Interestingly, in the same experimental conditions, Egr-1 expression was up-regulated and reached its maximal value around 60–90 min (Fig. 3B). As expected, in the presence of PD98059 in the incubation medium, neither p-Erk nor Egr-1 immunoreactivity was observed. These results demonstrated that soon after PrPPres exposure on the neurons, Elk is activated by phosphorylation leading to the expression of Egr-1, its early gene transcription factor target, in a MAP kinase-dependent pathway.

Because Egr-1-mediated transcription is negatively controlled by a specific corepressor, Nab2 (34), we analyzed the effect of the neuronal overexpression of Nab2 on the PrPPres-induced microglia migration. First, we estimated the percent of fluorescent neurons 2 days post-infection with adGFP expressing GFP-Nab2 at 15 and 45 m.o.i. (Fig. 4A). We observed an increase in the number of fluorescent neurons with higher concentrations of adGFP-Nab2 used for the infection (15 m.o.i., 70 ± 5%; 45 m.o.i., 90 ± 4%). In the course of these adGFP infection experiments, neither significant neuronal cell death nor cellular morphological changes were observed (Fig. 4A). Then we evaluated the effect of neuronal overexpression of Nab2 on the microglial cell migration induced by PrPPres (Fig. 4B). Two days post-infection with adGFP expressing GFP-Nab2 or GFP-Nab-2, neurons were challenged with 5 μl of hgtsc for 24 h before being assayed in the in vitro cell migration assay. Infection with adGFP-Nab2 resulted in a decrease in the rate of N11 microglial migration in an adGFP concentration-dependent manner. A low but significant decrease in the rate of microglial cell migration was measured in adGFP-infected neurons, likely because of the GFP overexpression. The basal cell migration rate determined by incubating non-infected neurons stimulated with hgtsc was up-regulated and reached its maximal value around 60–90 min (Fig. 3). As expected, in the presence of PD98059 in the incubation medium, neither p-Erk nor Egr-1 immunoreactivity was observed. These results demonstrated that soon after PrPPres exposure on the neurons, Elk is activated by phosphorylation leading to the expression of Egr-1, its early gene transcription factor target, in a MAP kinase-dependent pathway.
hgtsc-stimulated neurons overexpressing GFP-Nab2 as compared with neurons overexpressing GFP alone. Thus, neuronal overexpression of Nab-2, a corepressor of Egr-1, leads to a decrease of RANTES mRNA level that is concomitant with impaired microglial migration. No significant difference was observed in hgtsc-stimulated neurons in both types of adenovirus infections. In summary, RANTES mRNA production might be under the control of the transient expression of transcription factor Egr-1 induced in PrPres-stimulated neurons in a MAP kinase activation-dependent way.

**DISCUSSION**

Although there is compelling evidence that inflammatory processes play a significant role in the pathology of neurodegenerative diseases such as Alzheimer’s disease and TSEs (35, 36), little is known concerning the nature of molecular signals responsible for microglia recruitment in the vicinity of amyloid deposits. Recently, we have shown in vitro and in vivo that neurons respond to PrPres exposure by up-regulating the expression of chemokines mainly RANTES. Chemokines released by neurons provoke a rapid recruitment of microglial cells in the vicinity of PrPres aggregates. In contact with PrPres, re-active microglia synthesize diffusible neurotoxic factors that could participate in neuronal cell death. In our attempt to characterize molecular events leading to the increase of chemokine expression, we showed that PrPres activation of MAP kinase/Erk1/2 pathways mediates RANTES expression and microglia recruitment by inducing Elk-1 phosphorylation and Egr-1 expression. Although, in vitro specific binding has been demonstrated between PrPres aggregates and PrPc, the PrPres-induced microglia migration was independent of the presence of PrPc at the neuronal cell surface. As well, the laminin receptor has been shown to bind and internalize PrPc (30) and is thought to be also involved in PrPres binding. Specific antibodies developed against the laminin receptor inhibit the accumulation of the pathological isoform, PrPres in N2a neuroblastoma cells (37). In our model, neither PrPc nor the laminin receptor are involved in the neuronal cell response induced by PrPres. Further investigations will be required to define the exact nature of neuronal receptors implicated in the PrPres-mediated response. However, direct insertion of PrPres into the cell membranes, particularly in cholesterol-rich lipid rafts, might result in disturbances, and subsequent specific cell response cannot be totally ruled out. The importance of intact organization in these microdomains is well illustrated by the elevated Erk1/2 activity following cholesterol depletion in lipid rafts (38).

The observation that PrPres induces phosphorylation of Erk1/2 in primary cultures of neurons supports a potential physiopathological role of the interaction PrPres/putative receptor in modulating neuronal functions. Erk1/2 activation has been associated with a variety of physiological functions like neuronal growth, differentiation, survival, and long term memory (39, 40). However, in a cell culture model of a seizure, the inhibition of Erk1/2 activation could protect hippocampal neurons against the damage resulting from focal cerebral ischemia (41, 42). Abnormal sustained activation of Erk has been reported in neurons from Alzheimer’s disease-affected brains (43). Basal Erk1/2 phosphorylation was significantly increased in neuroblastoma cells chronically infected with scrapie (44). Our study is the first demonstration of MAP kinase cascade activation induced by PrPres exposure on neurons, suggesting that a perturbation in the balance of intracellular signaling might be responsible for the set up of TSE pathogenesis.

Activation of the MAP/Erk1/2 signaling cascade results in a rapid up-regulation of the transcription factor Egr-1 (32). Egr-1 belongs to a wide family of early genes rapidly induced in response to various cell stimuli. The Egr family plays a key role in coordinating subsequent waves of gene expression that underlie long term changes in several cell biological processes like proliferation and differentiation. Furthermore, a number of data support a major role of Egr-1 in the acute response to various kinds of stress such as ischemic injury (45). Nuclear corepressor Nab2 selectively inhibits the activity of Egr-1 by binding on a regulatory domain. Nab2 is mostly expressed in the brain and induced by the same stimuli that up-regulate Egr-1 (34). In N2a neuroblastoma cells, Nab2 expression lags behind that of Egr-1, raising the possibility that Nab2 may act as a negative feedback signal on Egr-1 activity (24). Using a recombinant adenovirus to overexpress Nab2, and therefore inhibit endogenous Egr-1 activity of neurons, we showed that Egr-1 is required in the cascade of events leading to the PrPres-induced microglia recruitment. It appears at least in neuronal cell cultures that mainly Egr-1 can be considered a mediator of PrPres-induced gene transcription; nevertheless, the participation of other transcription factors non-regulated by Nab2 is possible. Based on the presence of the Egr-1 binding site on the RANTES gene sequence, it is likely that Egr-1 controls RANTES expression by direct interaction on regulatory sequence gene. However, indirect mechanisms of regulation cannot be totally ruled out. Currently, this point is the subject of a thorough investigation in the laboratory.

Based on our findings, we propose a pathway for PrPres-induced chemotaxis (Fig. 5). The first step would be the interaction of PrPres with a yet unidentified neuronal cell surface receptor. This interaction would trigger a MAP kinase activation pathway leading to the phosphorylation (i.e. activation) of the transcription factor Elk, the main target of p-Erk. Phospho-Elk up-regulates the expression of other transcription factors, among them Egr-1, inducing a subsequent elevation of chemokine expression. It is noteworthy that recombinant PrPc was unable to stimulate the MAP kinases pathway as well as
the directional microglia migration (data not shown (20)). This observation suggests that the conformational changes occurring during the conversion PrPc–PrPres could lead to a gain of function. Additionally, PrPres but not PrPc, induces an increase of the intracellular calcium concentration and the subsequent caspase-12 activation in N2a neuroblastoma cells (46).

In summary, the frequent association of reactive microglial cells with PrPres aggregates suggests that active chronic inflammatory response related to amyloid deposits occurs in TSE-affected brains. We hypothesize that abnormal up-regulation of MAP kinases signaling pathway upon PrPres stimulation could be an intraneuronal mechanism disturbing the Erk1/2 balance and could play a significant role to microglia recruitment and neuronal injury. Understanding the PrPres-induced intracellular signaling would provide important mechanistic insights into the earliest molecular events of prion infection. The MAP kinase pathway offers multiple activation steps as many promising ways of investigation for gene therapies of diseases with neuro-inflammatory component.

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