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Prion expression is activated by Adenovirus 5 infection and affects the adenoviral cycle in human cells

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ABSTRACT

The prion protein is a cell surface glycoprotein whose physiological role remains elusive, while its implication in transmissible spongiform encephalopathies (TSEs) has been demonstrated. Multiple interactions between the prion protein and viruses have been described: viruses can act as co-factors in TSEs and life cycles of different viruses have been found to be controlled by prion modulation.

We present data showing that human Adenovirus 5 induces prion expression. Inactivated Adenovirus did not alter prion transcription, while variants encoding for early products did, suggesting that the prion is stimulated by an early adenoviral function. Down-regulation of the prion through RNA interference showed that the prion controls adenovirus replication and expression.

These data suggest that the prion protein could play a role in the defense strategy mounted by the host during viral infection, in a cell autonomous manner. These results have implications for the study of the prion protein and of associated TSEs.

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Introduction

The human prion gene (*PRNP*) contains two exons, of which a single one codes for the protein (PrP^C). This is a glycophosphatidylinositol anchored cell surface glycoprotein normally associated with neurons, but expressed in many tissues, including, in humans, spleen, kidney, stomach, blood and muscle (Linden et al., 2008 and references therein). Control of *PRNP* expression has been attributed to sequences in the 5′ and 3′ untranslated regions. No TATA box was identified linked to the gene, which, together with the presence of CpG islands and of Sp1 binding sites, suggests that *PRNP* is a housekeeping gene (Mahal et al., 2001). Nevertheless, several studies have indicated that *PRNP* expression can be modulated both by chromatin structure and by transcription factors (Cabral et al., 2002; Linden et al., 2008; Zawlik et al., 2006).

Some information on the functions performed by PrP^C has been derived from knock-out mice (*Prn-p*$^{-/-}$). Six lines of *Prn-p*$^{-/-}$ mice were developed based on different genetic constructs: they are all resistant to the TSE associated with the PrP^C conformer PrP^{Sc}, but display normal development and behavior. Neurodegeneration in three of these mouse lines (with a deletion extending to mouse *Prn-p* exon 3) has been related to alterations in the expression of the PrP^C like protein Doppel, rather than to the absence of PrP^C itself (Bueler et al., 1993; Bueler et al., 1992; Kuwahara et al., 1999; Manson et al., 1994; Moore and Melton, 1997; Sakaguchi et al., 1996; Weissmann and Aguzzi, 1999). Mice overexpressing wild type PrP^C display different phenotypes, according to the genetic construct chosen for transgenesis. Neurodegeneration, including ataxia and paralysis, has been observed in mice engineered with a large part of the *Prn-p* gene (reviewed in Martins et al., 2002 and Linden et al., 2008).

Studies at the cellular level have suggested that PrP^C is implicated in basic cellular processes, such as proliferation, differentiation and apoptosis. Immortalized hippocampal neurons from *Prn-p*$^{-/-}$ mice are more susceptible to apoptotic and oxidative stress (Kuwahara et al., 1999). In contrast, other studies have suggested that PrP^C can exert proapototic functions (Paitel et al., 2002). Conditions for either cytoprotective or proapoptotic effects of PrP^C have been related to the experimental system chosen to assay PrP^C bioactivity (Linden et al., 2008).

It has been suggested that PrP^C may be involved in modulating the intracellular levels of Ca^{++} via interaction with Ca^{++} channels (Whatley et al., 1995), in activating T-cell lymphocytes (Cashman et al., 1990), in acting as a major transporter of Cu^{++} ions, since PrP^C binds copper via its unique octapeptide sequence repeats (Brown et al., 1997; Rachidi et al., 2003), and in mediating signal transduction (Mouillet-Richard et al., 2000; Spielhaupter and Schatzl, 2001).

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In sum, abundant information has been accumulated over the last few years on PrP^C. Nevertheless, while PrP^C involvement and that of its pathological conformer PrP^{Sc} into TSEs has been conclusively assessed (Cashman and Caughey, 2004; Legname et al., 2004; Prusiner et al., 1998; Roucou and LeBlanc, 2005; Weissmann and Aguzzi, 2005), the precise physiological role of this protein remains elusive.

Different sets of experimental data published over the past few years have attracted our attention with regard to the mutual interaction between PrP^C (or PrP^{Sc}) and viral infection.

Experiments in *Prn-p*$^{-/-}$ mice derived brain cells have shown that Coxsackievirus B3 replication is more effective in *Prn-p*$^{-/-}$ cells, possibly as a consequence of a retarded type I interferon response attributed to the absence of PrP^C (Nakamura et al., 2003). Vesicular stomatitis virus injection in mice has been shown to induce a strong increase in PrP^C in the follicular dendritic cell network (Lotscher et al., 2003), which has been linked to endogenous retrovirus activation and control (Lotscher et al., 2007). Recent experiments in *Prn-p*$^{-/-}$ mice have shown that PrP^C may play certain roles in induction of inflammation and inhibition of apoptosis in relation to infection with the encelophalomyocarditis virus B variant (Nasu-Nishimura et al., 2008). These results suggest an antiviral role for PrP^C. A specific situation has been described for Herpes simplex virus, type I: in vivo, lack of PrP^C expression has been linked to the establishment of Herpes simplex virus type 1 latency, whereas viral replication has been related to its over-expression (Thrackay and Budjoso, 2002b).

Viruses have been proposed as co-factors involved in the spread of the prion related TSE, scrapie (Leblanc et al., 2006). Retroviral infections enhance the release of scrapie infectivity in the supernatant of co-infected cells. In the same line, adenoviral infection has been implicated in scrapie: a study has in fact indicated that mouse adenoviral infection accelerates the onset of scrapie disease in vivo (Ehresmann and Hogan, 1985).

From the micro-array analysis of wild type Adenovirus 5 (Ad5) infected human cells we observed the up-regulation of *PRNP* expression (Piersanti et al., 2004). Given the specific knowledge available on adenoviral products and on their effects on the host, and since the full characterization of the biological processes related to PrP^C, as well as their contribution to the onset of viral infection are still under investigation, we decided to make a thorough examination of Ad5 PrP^C reciprocal interaction in human cells.

The genome of Ad5 consists of a linear 36 kb double stranded DNA molecule. Both strands are transcribed and code for a range of viral proteins that are expressed in temporal succession: the early-activated genes express the proteins involved in viral replication (E2A and E2B) and host/virus interaction (E1, E3 and E4), while the late-activated genes code for structural viral proteins (L1–L5). Adenoviral E1A (12s and 13s), E1B (19K and 55K) and E4 (orfs 1, 2, 3, 4, 6, 3/4 and 6/7) products play a dominant role in the in vitro remodeling of the host biology. E1A proteins inactivate the pRb checkpoint, allowing the E2F transcription factor to activate genes involved in nucleotide metabolism and DNA replication which are required in the S phase. E1A also interacts with transcriptional modulators, including histone acetyltransferases, histone deacetylases, and other chromatin remodeling factors (Ben-Israel and Kleinberger, 2002). These interactions affect the transcription of several cellular and viral genes, some of which are involved in cell cycle control (Gallimore and Turnell, 2001). Cell cycle deregulation by E1A results in stabilization and accumulation of p53. To prevent cell cycle arrest and the apoptosis triggered by p53, the adenoviral E1B and E4 gene products employ various mechanisms to inactivate the tumor suppressor. The E1B 55K protein binds p53 and blocks its transcriptional activity (Martin and Berk, 1998), and, in combination with the E4 orf6 product, promotes p53 degradation (Querido et al., 2001a; Querido et al., 2001b). The adenoviral Bcl-2 homologue E1B 19K inhibits TNF-alpha-mediated apoptosis, interacting with Bax (Sundararajan and White, 2001). On the top of E4 orf6, the E4 region encodes a set of proteins interacting with diverse cell products (Ben-Israel and Kleinberger, 2002; Bridge and Ketner, 1989), including the E4 orf3 which relocalizes the Mre11–Rad50–Nbs1 (MRN) complex, a process required for viral replication (Evans and Hearing, 2005), and the E4 orf4 which associates with protein phosphatase 2A, regulating apoptosis (Shtrichman et al., 1999).

In the present work we report on the effect of wild type Ad5 on *PRNP* expression and vice versa. We present data showing that Ad5 early gene expression is responsible for *PRNP* induction. We show that a UV-inactivated adenoviral genome and an E1/E3-deleted adenovirus do not alter *PRNP* transcription, while adenoviral variants selectively deprived of the E1B (19K or 55K), or of the E4 products, exert a *PRNP* inducing activity. We also present data to the effect that PrP^C down-regulation through RNA interference significantly enhances Ad5 replication and expression.

These results suggest that *PRNP* is stimulated by Ad5 infection and that this effect is a specific response by the cell to an early adenoviral function, which could be related to the E1A products; the attenuation of this response through PrP^C down-regulation allows the adenoviral life cycle to proceed more vigorously.

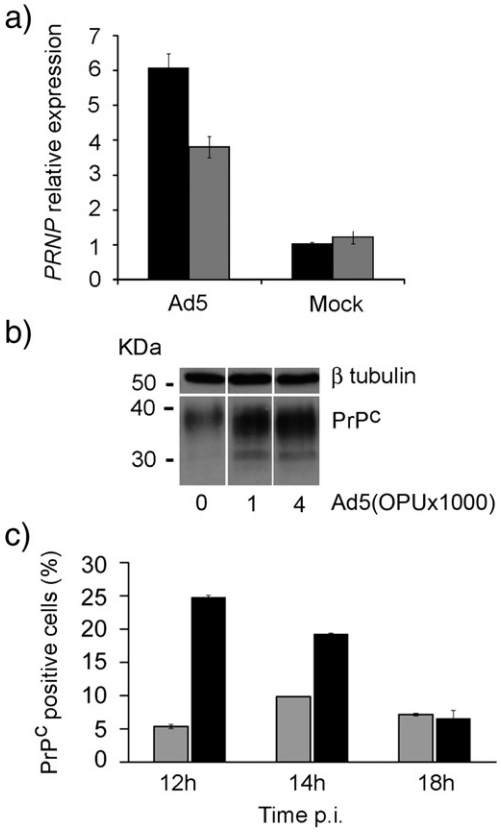

Fig. 1. Ad5 induces *PRNP* mRNA and protein expression. a) Cells were infected with Ad5 at a moi of 1000, mRNA was collected at 18 (black bars) and 48 h (grey bars) p.i., PrP^C mRNA was monitored by quantitative PCR. Results are normalized to GAPDH values and expressed as relative fold change, with an arbitrary value of 1 assigned to the mock sample collected at the 18 h time point. Ad5 versus mock, both at 18 and 48 h p.i.: $p<0.01$. b) Cells were infected with Ad5 at a moi of 1000 or of 4000, protein extracts were collected at 18 h p.i. and analyzed by western blotting using an anti-PrP^C antibody and an anti-β tubulin antibody as internal control. Normalized signals from infected samples correspond to a 3 and 3.6fold change for the moi 1000 and 4000, respectively, as compared to mock. c) FACS analysis of PrP^C was performed on cells infected with Ad5 at a moi of 1000 (black bars) or mock treated (grey bars). Significant activation of PrP^C was observed at 12 and 14 h p.i. Quantitative PCR and FACS data are expressed as mean values of two experiments with triplicate samples; SD is shown. Altogether the data indicate that Ad5, starting from 12 h p.i., up-regulates total and membrane PrP^C expression.

Results

PRNP induction by Ad5

Several published data attracted our interest concerning the mutual interaction between PrP^C (or PrP^{Sc}) and viral infection (Leblanc et al., 2006; Nakamura et al., 2003; Nasu-Nishimura et al., 2008; Thrackay and Budjoso, 2002b). In particular, from gene chip studies we observed that Ad5 infection up-regulates *PRNP* 7fold in human cells at 12 h p.i. (Piersanti et al., 2004). To analyze this interaction more thoroughly, we infected human cells with Ad5 and performed quantitative PCR, western blotting and FACS analysis on PrP^C at various times p.i. We observed that Ad5 indeed significantly increased *PRNP* expression: this was detected both at 18 and at 48 h p.i. (respectively: ~6fold and ~4fold change Ad5 versus mock treated cells; Fig. 1a). *PRNP* induction was detected also at the protein level: Ad5 induced the accumulation of total-cytosolic and membrane PrP^C (Fig. 1b). FACS analysis of infected cells showed that Ad5 also induces an increase in membrane PrP^C, at the 12 and 14 h time points (Fig. 1c). At 18 h p.i. no further increase in membrane PrP^C was observed in Ad5 treated cells as compared to mock specimens. Altogether, these data confirm that Ad5 up-regulates membrane PrP^C expression and induces PrP^C cytosolic accumulation, presumably as a consequence of the observed transcriptional induction.

PRNP activation by Ad5 could be related to a general stress response by the cell to microbial invasion, i.e. a response to viral DNA or to input viral proteins, or to a specific reaction of the cell to adenoviral gene expression. In order to distinguish between these two scenarios, we infected human cells with Ad5, with the genetically attenuated Ad5 H14 (dlE1E3), or with physically inactivated (UV-treated) adenoviral particles. Inactive viruses were unable to induce *PRNP* activation (Fig. 2) even when used at high dosage (moi 10000, Fig. 2a). These results indicate that active viral infection is needed for *PRNP* activation, which matches the second scenario indicated above; conversely, the sole presence of viral DNA or of adenoviral proteins was not sufficient for *PRNP* activation.

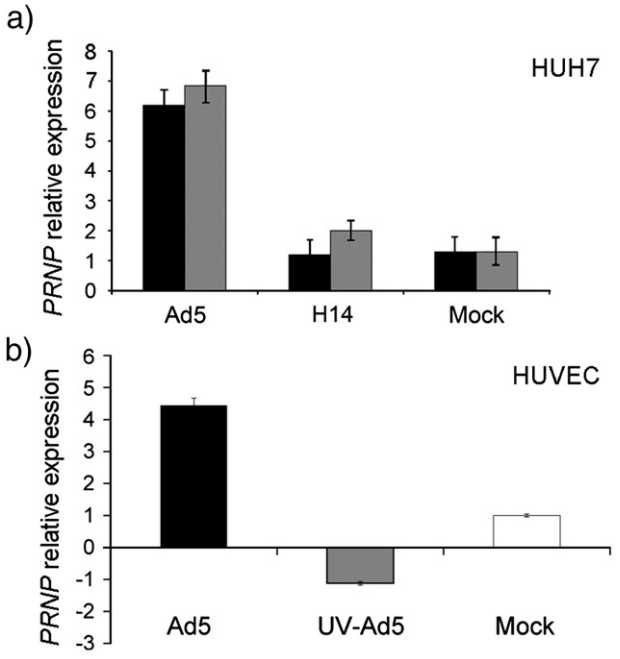

Fig. 2. Active adenoviral infection is needed for *PRNP* induction. a) HuH7 cells were infected with Ad5, or dlE1E3 Ad5 (H14) at a moi of 1000 (black bars) or 10,000 (grey bars) or mock treated. b) HUVEC (human umbilical vein endothelial) cells were infected with Ad5 (black bar) and UV-inactivated Ad5 at a moi of 1000 (grey bar), or mock treated (white bar). In both cell systems mRNA was collected at 18 h p.i. and *PRNP* mRNA was monitored by quantitative PCR. Results are normalized to GAPDH values and expressed as relative fold change, assigning to mock samples an arbitrary value of 1. Data are expressed as mean values of two experiments with duplicate samples; SD is shown. Ad5 versus mock in a) and in b): $p<0.01$. Only wild type Ad5 induces *PRNP* transcription both in HuH7 and in primary cells (HUVEC), while neither the genetically attenuated adenovirus (H14) nor the UV-inactivated virus induces its modulation, even when used at high dosage (moi 10,000).

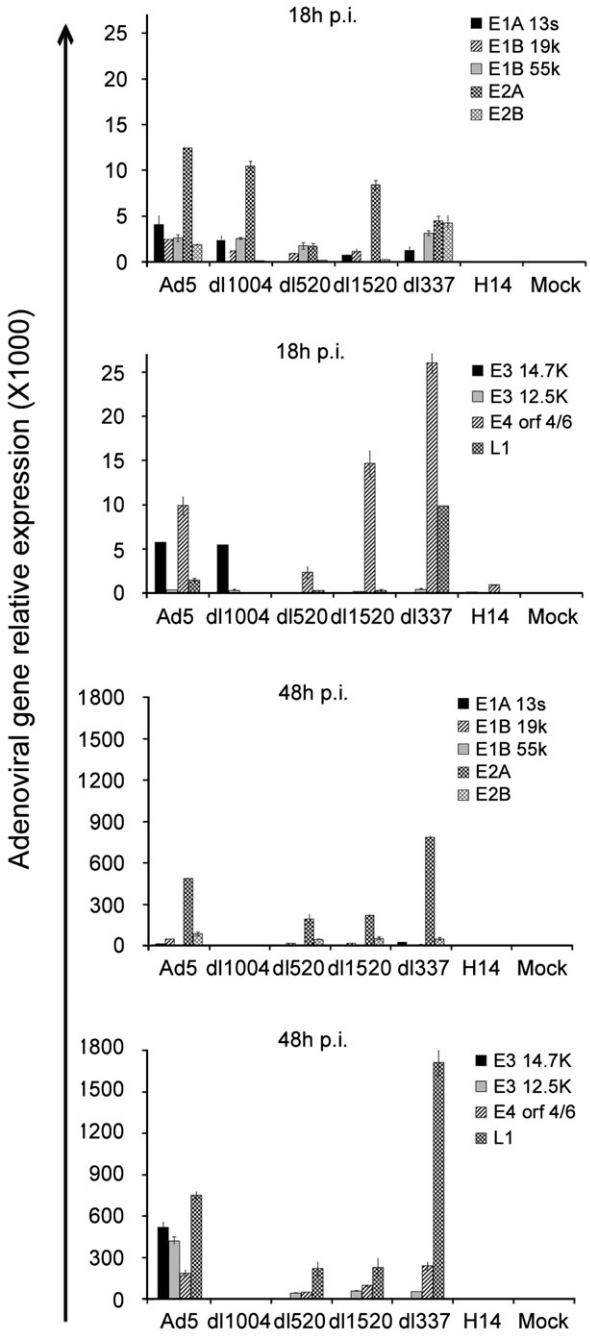

Fig. 3. Characterization of viral gene expression in HuH7 infected with differently deleted adenoviral mutants. Cells were infected with Ad5, or with the adenoviral mutants dl1004 (dlE4), dl520 (dlE1A 13s), dl1520 (dlE1B 55K), dl337 (dlE1B 19K) at a moi of 1000. mRNA was collected at 18 and 48 h p.i. and adenoviral early (E1A 13s, E1B 19K, E1B 55K, E2A and E2B, E3 14.7K, E3 12.5K and E4 orf4/6) and late (L1) gene expression was monitored by quantitative PCR. Results are normalized to GAPDH values and expressed as relative fold change, having assigned an arbitrary value of 1 to mock treated cells. Data are mean values of two experiments with duplicate samples; SD is shown. Expression data are concordant with the genotype of the single adenoviral mutants. In particular: i) in all cases the mutated viral genes are not expressed, ii) the dl1004 infection cycle does not reach the late phase, iii) the dl337 has an accentuated infectious cycle, while the dl520 and dl1520 have mildly attenuated cycles.

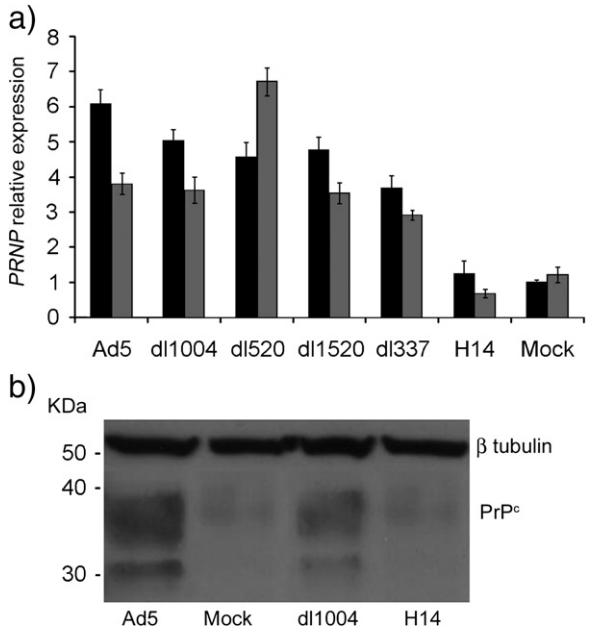

Fig. 4. Early viral gene expression is required for *PRNP* activation. Cells were infected with Ad5 or with adenoviral mutants at a moi of 1000. a) mRNA was collected at 18 (black bars) and 48 h (grey bars) p.i. and *PRNP* mRNA was monitored by quantitative PCR. Results are normalized to GAPDH values and expressed as relative fold change, with an arbitrary value of 1 assigned to the mock sample collected at the 18 h time point. Data are mean values of two experiments with duplicate samples; SD is shown. For all viruses but H14, as compared to mock, $p<0.05$. For Ad5 versus dl520 at 48 h and for Ad5 versus dl337 at 18 h, $p<0.05$. b) Protein extracts were collected at 18 h p.i. and analyzed by western blotting using an anti-PrP^C antibody and an anti-β tubulin antibody as internal control. The band intensity (normalized to β tubulin) of Ad5, H14 and dl1004 were, respectively, 6fold, 1fold and 3fold the mock signals. In sum, viruses competent for early gene transcription were able to activate *PRNP*.

PRNP induction was observed in human primary cultures (HUVEC) as well as in HuH7 (Fig. 2): this result implies that *PRNP* induction is neither a specific event of the hepatic cell line HuH7, nor, more in general, a response related to a transformed cell status (characteristic of HuH7 but not of HUVEC).

Induction of PRNP by differently deleted adenoviral mutants

Having assessed that active adenoviral infection is required for *PRNP* induction, we investigated which viral open reading frames (orfs) were crucial to this process. A battery of adenoviral mutants was thus selected and extensively characterized by quantitative PCR for early and late gene expression at 18 and 48 h p.i. in HuH7 cells (Fig. 3). The adenoviral mutants tested include the following: the dl520 (dl13s; Haley et al., 1984), the dl337 (dlE1B 19K; Pilder et al., 1984), the dl1520 (dlE1B 55K; Barker and Berk, 1987) and the dl1004 (dlE4; Bridge and Ketner, 1990). Expression data on specific adenoviral orfs were concordant with the genotype of the single adenoviral mutants. Indeed, none of the mutated viral genes were expressed. In addition, dl1004 infected cells did not express L1 at either 18 or 48 h, or any other orf at 48 h p.i.. This is concordant with the blocking of the infectious process. This halting of the viral cycle has been ascribed mainly to the formation of viral DNA concatemers, which, in turn, is caused by the absence of the DNA repair machinery counter-activation function orchestrated by the adenoviral E4 products (Bridge and Ketner, 1989; Carson et al., 2003). As already described the dl520 and dl1520 have moderately attenuated cycles because of the E1A 13s and E1B 55K deletions, respectively (Cherubini et al., 2006; Haley et al., 1984). The dl520 can express the E1A 12 s coding region, which partially compensates the E1A 13s functions (Haley et al., 1984).

Cells infected with Ad5 or with all adenoviral mutants but the fully attenuated H14 (dlE1E3) did activate *PRNP*, as assessed by quantitative PCR analysis of *PRNP* mRNA (Fig. 4a), both at 18 and 48 h p.i. Interestingly, the timing of early gene expression was concordant with that of *PRNP* induction. This is in particular suggested by the data observed in cells infected with the dl520 mutant. This virus, as described above, has a slower cycle, and unlike other mutants, has a moderately higher impact on *PRNP* at 48 h p.i. than at 18 h p.i.

Western blotting on extracts from cells infected with Ad5, dl1004 or H14, further confirmed that early adenoviral expression activates *PRNP* (Fig. 4b). Accumulation of PrP^C was actually observed in cells infected with the adenoviral mutant dl1004. Conversely, but in accordance with mRNA data (Fig. 2), the fully attenuated mutant H14 did not induce PrP^C accumulation.

Taken together, these data suggest that early adenoviral gene expression is needed for *PRNP* activation, while late gene expression and viral production are not indispensable. Furthermore, among early adenoviral products, the E4 (orfs 2, 3, 3/4, 6 and 6/7), the E1B 19K, the E1B 55K and the E1A 13s are not strictly required for *PRNP* activation. The *PRNP* activating candidate orfs thus appear to be restricted to the E1A 12s, E2, and E4 orf1.

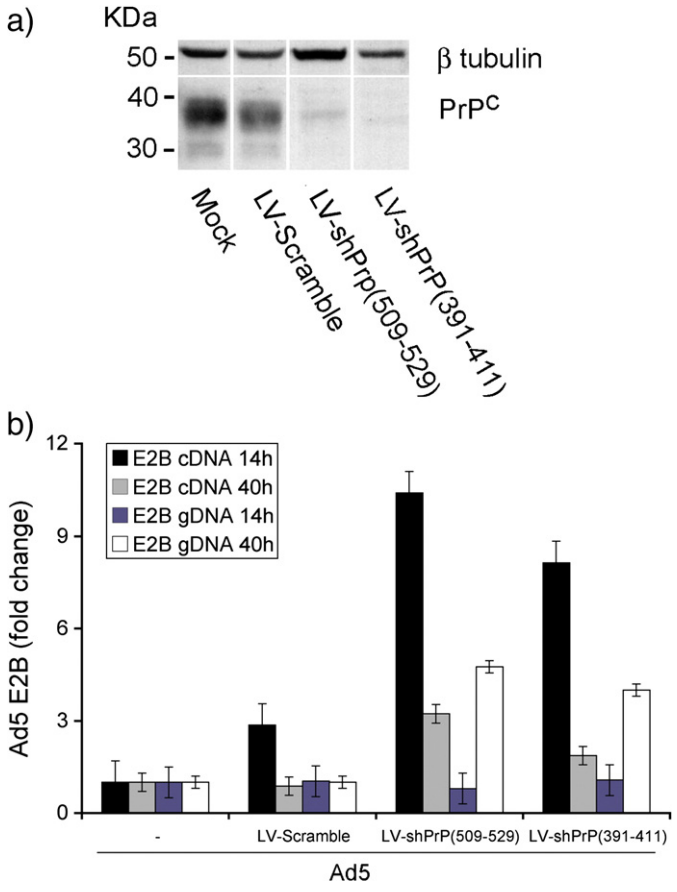

Fig. 5. *PRNP* down-regulation aids adenoviral expression and replication. Cells were infected with control lentivirus (LV-Scramble) or PrP^C interfering lentivirus [LV-shPrP (509–529) and LV-shPrP(391–411)], subjected to puromycin selection and (a) analyzed by western blotting for PrP^C expression; (b) super-infected with Ad5 and monitored for adenoviral E2B mRNA (E2B cDNA) and genomic DNA (E2B gDNA) by quantitative PCR at 14 h and 40 h p.i. Quantitative PCR data are normalized to GAPDH values and expressed as relative fold change, with an arbitrary value of 1 assigned to the Ad5-only treated sample at the relative time point. Results are expressed as mean values of two experiments with duplicate samples; SD is shown. Efficient and specific down-regulation of PrP^C obtained with the lentiviral vector directed towards PrP^C favors adenoviral early gene expression and improves adenoviral DNA replication. $p<0.01$ in LV-shPrP versus LV-Scramble infected relative samples in all cases but in the E2B gDNA sample collected at 14 h p.i.

PrP^C expression controls adenoviral replication

In order to answer the question of whether the effect of Ad5 on *PRNP* affected the viral cycle, a battery of recombinant lentiviruses encoding short hairpin precursors of siRNA sequences directed towards *PRNP* under control of H1 promoter and containing the puromycin resistance coding sequence were produced and tested for their ability to down-regulate PrP^C (data not shown). The best performing lentivirus were those encoding shRNAs directed towards the region 509–529 or 391–411 of *PRNP* [LV-shPrP(509–529) and LV-shPrP(391–411)]. As shown in Fig. 5a, cells infected with LV-shPrPs showed efficient and specific down-regulation of PrP^C as compared to those infected with lentivirus coding for a scramble hairpin sequence (LV-Scramble) or mock treated. Puromycin selected populations of LV-shPrP or LV-Scramble infected cells, or untreated HuH7 were infected with Ad5. Quantitative analysis of viral (E2B) mRNA and of genomic adenoviral DNA content was performed 14 and 40 h after adenoviral infection. PrP^C reduction positively affected adenoviral replication and adenoviral expression. Timing of activation by PrP^C interference was shifted for adenoviral DNA as compared to mRNA: mRNA accumulation was particularly evident at 14 h p.i., while viral DNA accumulation was observed at 40 h p.i. (Fig. 5b). These results, taken together, suggest an intracellular antiviral role for PrP^C, possibly related to other cell defense pathways.

Discussion

The interaction between viruses and PrP^C has been investigated in recent years in the context of two main frames: the exploitation of viral infection as a way of investigating PrP^C physiological functions, and the search for a direct or indirect role of eukaryotic viruses in PrP^C related pathologies. Research in the latter area has led to the commonly accepted concept that viral co-infection is not a necessary condition for the development of prion diseases (Prusiner, 1998). Nevertheless, different microbes have been shown to contribute to the onset of TSEs (Ehresmann and Hogan, 1985; Leblanc et al., 2006; Lotscher et al., 2003). The research field aimed at analyzing viral components and viral infection to add insights to our knowledge of PrP^C physiological functions is an open area of investigation, since the full characterization of the biological role of PrP^C is still elusive. Data indicate that the stress/defense response and the apoptotic process are the main functions associated with PrP^C linking this protein to the modulation of viral infections. The question is more difficult to interpret at the organism level where the immune response creates a further stage of complexity in the analysis of phenomena related to the reciprocal interaction between PrP^Cs and viruses, nevertheless a link between PrP^C induction, immune response and viral replication has been assessed (Lotscher et al., 2003, 2007; Nakamura et al., 2003; Nasu-Nishimura et al., 2008; Thackray and Bujdoso, 2002a).

We observed that Ad5 infection of human liver carcinoma cell line or of human umbilical vein endothelial cells induces a specific increase in *PRNP* mRNA, and that this occurs in the early phase of infection concomitantly with the transcription of early viral genes. The induction of PrP^C by Ad5 was observed at protein level too: this may be a direct consequence of transcriptional activation, or else a stabilization effect. Interestingly, a different result was observed for total and membrane PrP^C. We observed that, while at 18 h total PrP^C did accumulate on viral infection, and that this occurred to the same extent as *PRNP* mRNA, at the membrane level, the adenoviral effect on PrP^C blew over at 18 h p.i. This suggests that membrane expression of PrP^C is not linearly correlated with PrP^C mRNA or cytosolic expression of the protein. These results are in agreement with data suggesting that trafficking of PrP^C to and from the cell surface is a complex process subject to regulation through mechanisms that are as yet unclear (Magalhaes et al., 2002a, 2002b).

Importantly, *PRNP* mRNA up-regulation was not found to be dependent on cell type or cell status. Both primary and transformed human cells responded to adenoviral infection in terms of PrP^C activation, as well as cells of different tissue origin.

Our data indicate that Ad5 induced *PRNP* transcription, followed by PrP^C accumulation, is a process specifically determined by viral gene expression: neither virus attachment, nor its internalization determined PrP^C alterations, since neither a UV-inactivated virus nor an E1-attenuated Ad5 were able to activate *PRNP* transcription. The analysis of viral mutants allowed us to restrict the candidate viral functions implicated in *PRNP* activation to the early genes E1A 12s, E2, and E4 orf1. Through the analysis of the dl1004 mutant, which expresses early orfs but most of the E4 region, and which neither completes the viral cycle nor expresses late adenoviral orfs, we further observed that viral production and late products are not indispensable in the process of PrP^C induction. These results are in line with the dominant role of adenoviral early genes in the alteration of host cell biology preceding viral production.

Among the orfs that are candidates for PrP^C activation, the E1A 12s could be suggested as key actor in the process. The E1A 12s accomplishes cell cycle deregulation by acting on transcriptional regulation through the interaction with a number of host cell proteins that function as transcription co-activators, including CBP/300, nucleosomal remodeling factors (SWI/SNF) or comprise the general transcription machinery (TBP) (Ben-Israel and Kleinberger, 2002). These effects could be directly implicated in the modulation of *PRNP*, or, alternatively, act indirectly on PrP^C through the activation of other cellular functions. Interestingly, it has been recently demonstrated that an immune stimulus, including viral infection, can transactivate endogenous retroviruses in mice and cells, which, in turn induces PrP^C (Lotscher et al., 2007). Adenoviral infection, and possibly the E1A 12s protein, could induce endogenous retroviruses too, and consequently activate PrP^C. Further experiments are under way to elucidate the role of E1A 12s in *PRNP* activation.

An effect of PrP^C on viral cycles, and more in general a microbe/prion interaction, has been previously described. Several agents are implicated, including retroviruses (Leblanc et al., 2006; Lotscher et al., 2007), Herpes simplex virus (Thrackay and Budjoso, 2002b), Coxsackievirus B3 (Nakamura et al., 2003) the encelophalomyocarditis virus B variant (Nasu-Nishimura et al., 2008), and the gram-negative pathogen *Brucella abortus* (Watarai et al., 2003). Various roles are hypothesized for PrP^C in the different infectious processes. Taken together the data in this field indicate that viral infection can be influenced by PrP^C modulation linked to the alteration in the cell or organism of several processes, including apoptosis, cell cycle activation, interferon response, and activation of the immune system.

In order to assess whether PrP^C actually plays a role of resistance to adenoviral infection, we performed RNA interference experiments with lentiviral vectors encoding hairpin sequences directed towards *PRNP*. RNA interference worked efficiently to down-regulate the protein, and, indeed, this system has been proposed as a strategy to cure or prevent scrapie (Daude et al., 2003; Pfeifer et al., 2006), since the reduction of the substrate protein for PrP^{Sc}, i.e. PrP^C, is a general approach conceived to combat the disease.

In cells down-regulated for PrP^C we detected an increase in adenoviral mRNA and DNA, which is concordant with other studies suggesting that replication and release of infectious particles is rendered more efficient by the absence of PrP^C and is inhibited by its over-expression (Lotscher et al., 2007; Nakamura et al., 2003), indicating an antiviral role of PrP^C.

Finally, this study is conceptually in accordance with data by Ehresmann and Hogan (1985) describing how, in mice, the injection of replication competent mouse Ad accelerates the onset of scrapie. The contribution of Ad to scrapie observed by these authors could be related to the Ad induced PrP^C (cytosolic) production described herein: PrP^C (over-) expression is in fact considered the key factor

implicated in the development of TSEs (Bueler et al., 1993; Pfeifer et al., 2006; Prusiner et al., 1998; Vilotte et al., 2001). In view of the high incidence of adenoviral infection in the human population (Nwanegbo et al., 2004), the fact that Ad infection has an effect on the PrP^C and on the onset of TSEs suggests a possible interest in conducting further investigations in this field.

In conclusion, this study shows that adenoviral early genes activate the transcription of *PRNP* as well as PrP^C accumulation in the cytoplasm and on the cell membrane, and PrP^C expression appears to control adenoviral replication. Our data add further insights to our knowledge of PrP^C functions as well as to the study of virus–prion interactions.

Materials and methods

Cells

HuH7 (Nakabayashi et al., 1982), 293 (ATCC # CRL-1573), 293-T (ATCC # CRL-11268) and HeLa cells (ATCC # CCL-2) were cultured in complete medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Invitrogen). WI-62 cells (provided by G. Ketner, John Hopkins University, Baltimore, MD) were cultured in MEM (Invitrogen) supplemented with 10% FBS (Invitrogen). Human umbilical vein endothelial cells obtained from healthy patients (HUVEC, provided by A. Orecchia, IDI, Rome, Italy) were cultured in EGM-2 (Clonetics, San Diego, CA) supplemented with 10% FBS (Invitrogen). All cells were maintained at 37 °C and 5% CO_2.

Vectors and viruses

Stocks of wild type Ad5 (ATCC #VR-5) were prepared in HeLa cells as previously described (Curiel and Douglas, 2002). H14 (dlE1E3 adenovirus, provided by Merck & Co. M.P. Neeper, Westpoint, PA) (Sandig et al., 2000), dl520 (dl13s, provided by M. Crescenzi Istituto Superiore di Sanità, Rome, Italy) (Haley et al., 1984), dl337 (dlE1B 19K, provided by T. Shenk Princeton University, Princeton, NJ) (Pilder et al., 1984) and dl1520 (dlE1B 55K, provided by A. Berk University of Southern California, Los Angeles CA) (Barker and Berk, 1987) were prepared in 293 cells. dl1004 (dlE4) was provided by G. Ketner (John Hopkins University, Baltimore, MD) and prepared from WI-62 cells (Bridge and Ketner, 1989). Briefly, all viruses were amplified in the respective lines, cell pellets were collected 48 h post infection (p.i.) and, after 3–5 freezing/thawing cycles, supernatants were purified on CsCl gradients and desalted on PD-10 Sephadex G-25 columns (Amersham Pharmacia, Uppsala Sweden). Stocks were titrated by optical particle units (OPU) (Trapnell, 1993). The ratio between OPU and plaque forming units (PFUs), in our hands, and according to Mittereder et al. (1996) ranges from 9:1 to 245:1, depending on viral preparation and on viral titration conditions. In all experiments the multiplicity of infection (moi) is intended as OPU/cell. UV-inactivation of Ad5 was performed as described (Gerba et al., 2002). Adenoviral infections were performed by incubating cells for 1 h at 37 °C with viruses diluted in DMEM supplemented with 2% FBS.

Second generation recombinant lentiviruses were produced by co-transfection of 293T cells with the packaging vector pCMV-dR8.74 (15 μg/10 cm dish), the envelope vector pMD2.G (5 μg/10 cm dish), and a transfer vector (20 μg/10 cm dish). The pCMV-dR8.74 and pMD2.G plasmids were provided by D. Trono (Ecole Polytechnique Fédérale de Lausanne, Switzerland). The transfer vector PLKO.1 puro-shScramble (hairpin sequence: 5′ CCGGCAACAAGATGAAGAGCACCAACTCGAGTT-GGTGCTCTTCATCTTGTTGTTTTT3′ Sigma, St. Louis, MO), PLKO.1 puro-shPrP (hairpin sequence: 5′ CCGGCCCATCATACATTTCGGCAGTC-TCGAGACTGCCGAAATGTATGATGGGTTTTT3′, Sigma), and PLKO.1 puro-shPrP (hairpin sequence: 5′ CCGGTCAGTGGAACAAGCCGAGTAC-TCGAGTACTCGGCTTGTTCCACTGATTTTT 3′, Sigma) were used, respectively, for LV-Scramble, LV-shPrP (509–529), and LV-shPrP (391–411) production. 48 and 72 h post transfection 293T supernatants were collected and filtered as described (Piersanti et al., 2006). Lentiviral titers were evaluated by p24 quantification (Alliance HIV-1 p24 antigen ELISA Kit Perkin Elmer, Boston, MA) following the manufacturer's instructions. Infections with recombinant lentiviruses were performed at an moi of 2 pg p24/cell, in complete medium supplemented with 8 μg/ml polybrene (Sigma). After viral addition, cells were centrifuged for 30' at 1800 rpm, incubated 3 h at 37 °C, and then transferred to fresh complete medium. 72 h p.i. cells were subjected to selection in complete medium supplemented with 2 μg/ml puromycin (Sigma) and kept under these conditions for further analyses.

RNA extraction and preparation

Cells were lysed in situ at various times p.i. by addition of TRIzol reagent (LifeTechnologies, Rockville, MD). Total RNA was purified from a TRIzol suspension according to the manufacturer's instructions; after DNase treatment (Sigma), RNA was reverse transcribed into cDNA with oligo d(T) primer and OMNISCRIPT RT KIT (Qiagen, Inc., Valencia, CA).

Quantitative PCR

To quantify viral DNA, cell and viral gene expression, primers (Table 1) were selected using Primer Express software (Applied Biosystems, Foster City, CA). Reactions were performed in a 25 μl volume that included viral DNA [extracted from infected cell pellets treated with 5 ng/μl proteinase K (Roche Diagnostics)] or the cDNA sample, primers, and SYBR Green PCR Master mix (containing nucleotides, AmpliTaq Gold DNA polymerase and optimized buffer components, Qiagen). Quantitative PCR reactions were performed on

Table 1
Sequences used for quantitative PCR reactions

Target	Sequence ref #	Forward	Reverse
Ad5 E1A 13s	AY339865	GAGGACCTGTGGCATGTTTGTC	GTCCTAAAATGGCGCCTGCTA
Ad5 E1B 55K	AY339865	GCTGGCGCAGAAGTATTCCAT	TATGCAAAGGTGGCACTTAGG
Ad5 E1B 19K	AY339865	GGCTCATCCCAGGCAAAGT	GAAGAGCTTTTGAAATCCTGTGG
Ad5 E2A	AY339865	GGATACAGCGCCTGCATAAAAG	CCAATCAGTTTTCCGGCAAGT
Ad5 E2B	AY339865	GCCCTTGATGGAAGGCAATTT	TCGCTTCCAACCCTCATCTTG
Ad5 E3 14.7K	AY339865	TTGCCAACCAAGCGTCAGA	GCTGCATTCACTCACCTTGTCA
Ad5 E3 12.5K	AY339865	CCGGTGAGTTTTGCTACTTTGA	AGCCTGATTCGGGAGTTTACC
Ad5 E4 orf4/6	AY339865	TGATCCT CCAGTATGGTAGCGC	CCGAGAT CGTGTTGGTC GTAGT
Ad5 L1	AY339865	GAGCAAAACCCAAATAGCAAGC	ACTATGTTTAGCAGCGCATCCC
PRNP	NM_183079	AATCAAGCAGCACACGGTCA	TCGGTGAAGTTCTCCCCCTT
GAPDH	NM_002646	TGGGCTACACTGAGCACCAG	GGGTGTCGCTGTTGAAGTCA

Forward and reverse sequences to be used in quantitative PCR were chosen on the indicated target sequences with the Primer Express software.

an Applied Biosystems Prism 5700 sequence detection system. Cycle threshold (Ct) values were exported directly into EXCEL worksheets for analysis and the relative quantification was made using the $2^{-\Delta\Delta Ct}$ method (Tichopad et al., 2003). After cycling, a melting curve was produced by slow denaturation of the PCR end products to validate the specificity of amplification.

Western blotting for total PrP^C quantification

Cell pellets were treated with lysis buffer [Tris–HCl 50 mM pH7.4, 10% NP-40, 0.25% NaDesoxycholate, EDTA1 mM, NaCl150 mM, PMSF1 mM, protease inhibitor cocktail (Roche Diagnostics)]. After freezing and thawing pellets were centrifuged 15' at 13,000 rpm at 4 °C and the supernatants collected. Sample reducing agent (NuPAGE Invitrogen) and LDS sample buffer (NuPAGE Invitrogen) were added to samples and the latter were loaded on to pre-cast 4–12% gradient acrylamide gels (NuPAGE, Invitrogen). The run was performed in MOPS running buffer and antioxidant (NuPAGE Invitrogen). Electroblotting on nitrocellulose filter (Amersham, Psicataway, NJ) was performed in transfer buffer and antioxidant (NuPAGE Invitrogen). After blocking in TBS–Tween 0.1%–Milk 5%, filters were incubated with the anti-PrP^C antibody [PrP (C-20) sc-7693, Santa Cruz Biotechnology Inc.] and with the anti-βeta tubulin antibody (Santa Cruz Biotechnology Inc.). Immunoreactive proteins were detected using horseradish peroxidase conjugated secondary antibodies (Santa Cruz Biotechnology Inc.) and revealed using the enhanced chemiluminescence system (ECL plus, Amersham Biosciences). Protein quantification was performed with the Scion Image software (www.scioncorp.com/): band intensities of the proteins of interest were established, normalized to the relative β tubulin signals, and differences among samples were expressed as the relative fold change of normalized figures.

Flow cytometry analysis for membrane PrP^C detection

Cells were harvested at various times p.i., detached using EDTA solution 0.1 M, washed with PBS and blocked with PBS–5% FBS on ice by shaking. After 1 h, cells were treated with the anti-PrP^C antibody (provided by V. Vetrugno Istituto Superiore di Sanità, Rome, Italy). 90' incubations were performed in PBS–5% FBS by shaking on ice. Cells were then washed four times with PBS and treated with the secondary antibody FITC conjugated (BD Pharmingen™) by 1 h shaking on ice. Cells were washed three times with PBS, resuspended in PBS, and FACS analysis was performed using a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson Biosciences, San Diego, CA).

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using the *t*-test.

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References

Barker, D.D., Berk, A.J., 1987. Adenovirus proteins from both E1B reading frames are required for transformation of rodent cells by viral infection and DNA transfection. Virology 156 (1), 107–121.
Ben-Israel, H., Kleinberger, T., 2002. Adenovirus and cell cycle control. Front. Biosci. 7, d1369–d1395.
Bridge, E., Ketner, G., 1989. Redundant control of adenovirus late gene expression by early region 4. J. Virol. 63 (2), 631–638.
Bridge, E., Ketner, G., 1990. Interaction of adenoviral E4 and E1b products in late gene expression. Virology 174 (2), 345–353.
Brown, D.R., Qin, K., Herms, J.W., Madlung, A., Manson, J., Strome, R., Fraser, P.E., Kruck, T., von Bohlen, A., Schulz-Schaeffer, W., Giese, A., Westaway, D., Kretzschmar, H., 1997. The cellular prion protein binds copper in vivo. Nature 390 (6661), 684–687.
Bueler, H., Fischer, M., Lang, Y., Bluethmann, H., Lipp, H., DeArmond, S., Prusiner, S., Aguet, M., Weissmann, C., 1992. Normal development and behaviour of mice lacking the neuronal cell surface PrP protein. Nature 356, 577–582.
Bueler, H., Aguzzi, A., Sailer, A., Greiner, R.A., Autenried, P., Aguet, M., Weissmann, C., 1993. Mice devoid of PrP are resistant to scrapie. Cell 73 (7), 1339–1347.
Cabral, A.L., Lee, K.S., Martins, V.R., 2002. Regulation of the cellular prion protein gene expression depends on chromatin conformation. J. Biol. Chem. 277 (7), 5675–5682.
Carson, C.T., Schwartz, R.A., Stracker, T.H., Lilley, C.E., Lee, D.V., Weitzman, M.D., 2003. The Mre11 complex is required for ATM activation and the G2/M checkpoint. EMBO J. 22 (24), 6610–6620.
Cashman, N.R., Caughey, B., 2004. Prion diseases—close to effective therapy? Nat. Rev., Drug Discov. 3 (10), 874–884.
Cashman, N.R., Loertscher, R., Nalbantoglu, J., Shaw, I., Kascsak, R.J., Bolton, D.C., Bendheim, P.E., 1990. Cellular isoform of the scrapie agent protein participates in lymphocyte activation. Cell 61 (1), 185–192.
Cherubini, G., Petouchoff, T., Grossi, M., Piersanti, S., Cundari, E., Saggio, I., 2006. E1B55K-deleted adenovirus (ONYX-015) overrides G1/S and G2/M checkpoints and causes mitotic catastrophe and endoreduplication in p53-proficient normal cells. Cell Cycle 5 (19), 2244–2252.
Curiel, D., Douglas, J., 2002. In: Press, A. (Ed.), Adenoviral Vectors for Gene Therapy. Elsevier Science, San Diego, CA.
Daude, N., Marella, M., Chabry, J., 2003. Specific inhibition of pathological prion protein accumulation by small interfering RNAs. J. Cell. Sci. 116 (Pt 13), 2775–2779.
Ehresmann, D., Hogan, R., 1985. Acceleration of Scrapie disease in mice by an adenovirus. Intervirology 25, 103–110.
Evans, J.D., Hearing, P., 2005. Relocalization of the Mre11–Rad50–Nbs1 complex by the adenovirus E4 ORF3 protein is required for viral replication. J. Virol. 79 (10), 6207–6215.
Gallimore, P.H., Turnell, A.S., 2001. Adenovirus E1A: remodelling the host cell, a life or death experience. Oncogene 20 (54), 7824–7835.
Gerba, C.P., Gramos, D.M., Nwachuku, N., 2002. Comparative inactivation of enteroviruses and adenovirus 2 by UV light. Appl. Environ. Microbiol. 68 (10), 5167–5169.
Haley, K.P., Overhauser, J., Babiss, L.E., Ginsberg, H.S., Jones, N.C., 1984. Transformation properties of type 5 adenovirus mutants that differentially express the E1A gene products. Proc. Natl. Acad. Sci. U. S. A. 81 (18), 5734–5738.
Kuwahara, C., Takeuchi, A., Nishimura, T., Haraguchi, K., Kubosaki, A., Matsumoto, Y., Saeki, K., Matsumoto, Y., Yokoyama, T., Itohara, S., Onodera, T., 1999. Prions prevent cell-line death. Nature 400, 225–226.
Leblanc, P., Alais, S., Porto-Carreiro, I., Lehmann, S., Grassi, J., Raposo, G., Darlix, J.L., 2006. Retrovirus infection strongly enhances scrapie infectivity release in cell culture. EMBO J. 25 (12), 2674–2685.
Legname, G., Baskakov, I.V., Nguyen, H.O., Riesner, D., Cohen, F.E., DeArmond, S.J., Prusiner, S.B., 2004. Synthetic mammalian prions. Science 305 (5684), 673–676.
Linden, R., Martins, V.R., Prado, M.A., Cammarota, M., Izquierdo, I., Brentani, R.R., 2008. Physiology of the prion protein. Physiol. Rev. 88 (2), 673–728.
Lotscher, M., Recher, M., Hunziker, L., Klein, M., 2003. Immunologically induced, complement dependent up-regulation of the prion protein in the mouse spleen: follicular dendritic cells versus capsule and trabeculae. J. Immunol. 170, 6040–6047.
Lotscher, M., Recher, M., Lang, K.S., Navarini, A., Hunziker, L., Santimaria, R., Glatzel, M., Schwarz, P., Boni, J., Zinkernagel, R.M., 2007. Induced prion protein controls immune-activated retroviruses in the mouse spleen. PLoS ONE 2 (11), e1158.
Magalhaes, A.C., Silva, J.A., Lee, K.S., Martins, V.R., Prado, V.F., Ferguson, S.S., Gomez, M.V., Brentani, R.R., Prado, M.A., 2002a. Endocytic intermediates involved with the intracellular trafficking of a fluorescent cellular prion protein. J. Biol. Chem. 277 (36), 33311–33318.
Magalhaes, A.C., Silva, J.A., Lee, K.S., Martins, V.R., Prado, V.F., Ferguson, S.S., Gomez, M.V., Brentani, R.R., Prado, M.A., 2002b. Endocytic intermediates involved with the intracellular trafficking of a fluorescent cellular prion protein. J. Biol. Chem. 277 (36), 33311–33318.
Mahal, S.P., Asante, E.A., Antoniou, M., Collinge, J., 2001. Isolation and functional characterisation of the promoter region of the human prion protein gene. Gene 268 (1–2), 105–114.
Manson, J.C., Clarke, A.R., Hooper, M.L., Aitchison, L., McConnell, I., Hope, J., 1994. 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. Mol. Neurobiol. 8 (2–3), 121–127.
Martin, M.E., Berk, A.J., 1998. Adenovirus E1B 55K represses p53 activation in vitro. J. Virol. 72 (4), 3146–3154.
Martins, V.R., Linden, R., Prado, M.A., Walz, R., Sakamoto, A.C., Izquierdo, I., Brentani, R.R., 2002. Cellular prion protein: on the road for functions. FEBS Lett. 512 (1–3), 25–28.
Mittereder, N., March, K.L., Trapnell, B.C., 1996. Evaluation of the concentration and bioactivity of adenovirus vectors for gene therapy. J. Virol. 70 (11), 7498–7509.
Moore, R.C., Melton, D.W., 1997. Transgenic analysis of prion diseases. Mol. Hum. Reprod. 3 (6), 529–544.
Mouillet-Richard, S., Ermonval, M., Chebassier, C., Laplanche, J., Lehmann, S., Launay, J., Kellermann, O., 2000. Signal transduction through prion protein. Science 289, 1925–1928.
Nakabayashi, H., Taketa, K., Miyano, K., Yamane, T., Sato, J., 1982. Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. Cancer Res. 42 (9), 3858–3863.
Nakamura, Y., Sakudo, A., Saeki, K., Kaneko, T., Matsumoto, Y., Toniolo, A., Itohara, S., Onodera, T., 2003. Transfection of prion protein gene suppresses coxsackievirus B3 replication in prion protein gene-deficient cells. J. Gen. Virol. 84 (Pt 12), 3495–3502.

Nasu-Nishimura, Y., Taniuchi, Y., Nishimura, T., Sakudo, A., Nakajima, K., Ano, Y., Sugiura, K., Sakaguchi, S., Itohara, S., Onodera, T., 2008. Cellular prion protein prevents brain damage after encephalomyocarditis virus infection in mice. Arch. Virol. 153 (6), 1007–1012.

Nwanegbo, E., Vardas, E., Gao, W., Whittle, H., Sun, H., Rowe, D., Robbins, P.D., Gambotto, A., 2004. Prevalence of neutralizing antibodies to adenoviral serotypes 5 and 35 in the adult populations of The Gambia, South Africa, and the United States. Clin. Diagn. Lab. Immunol. 11 (2), 351–357.

Paitel, E., Alves da Costa, C., Vilette, D., Grassi, J., Checler, F., 2002. Overexpression of PrPc triggers caspase 3 activation: potentiation by proteasome inhibitors and blockade by anti-PrP antibodies. J. Neurochem. 83 (5), 1208–1214.

Pfeifer, A., Eigenbrod, S., Al-Khadra, S., Hofmann, A., Mitteregger, G., Moser, M., Bertsch, U., Kretzschmar, H., 2006. Lentivector-mediated RNAi efficiently suppresses prion protein and prolongs survival of scrapie-infected mice. J. Clin. Invest. 116 (12), 3204–3210.

Piersanti, S., Martina, Y., Cherubini, G., Avitabile, D., Saggio, I., 2004. Use of DNA microarrays to monitor host response to virus and virus-derived gene therapy vectors. Am. J. Pharmacogenomics 4 (6), 345–356.

Piersanti, S., Sacchetti, B., Funari, A., Di Cesare, S., Bonci, D., Cherubini, G., Peschle, C., Riminucci, M., Bianco, P., Saggio, I., 2006. Lentiviral transduction of human postnatal skeletal (stromal, mesenchymal) stem cells: in vivo transplantation and gene silencing. Calcif. Tissue Int. 78 (6), 372–384.

Pilder, S., Logan, J., Shenk, T., 1984. Deletion of the gene encoding the adenovirus 5 early region 1b 21,000-molecular-weight polypeptide leads to degradation of viral and host cell DNA. J. Virol. 52 (2), 664–671.

Prusiner, S.B., 1998. Prions. Proc. Natl. Acad. Sci. U. S. A. 95 (23), 13363–13383.

Prusiner, S., Scott, M., DeArmond, S., Cohen, F., 1998. Prion protein biology. Cell 93, 337–348.

Querido, E., Blanchette, P., Yan, Q., Kamura, T., Morrison, M., Boivin, D., Kaelin, W.G., Conaway, R.C., Conaway, J.W., Branton, P.E., 2001a. Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. Genes Dev. 15 (23), 3104–3117.

Querido, E., Morrison, M.R., Chu-Pham-Dang, H., Thirlwell, S.W., Boivin, D., Branton, P.E., Morisson, M.R., 2001b. Identification of three functions of the adenovirus e4orf6 protein that mediate p53 degradation by the E4orf6–E1B55K complex. J. Virol. 75 (2), 699–709.

Rachidi, W., Vilette, D., Guiraud, P., Arlotto, M., Riondel, J., Laude, H., Lehmann, S., Favier, A., 2003. Expression of prion protein increases cellular copper binding and antioxidant enzyme activities but not copper delivery. J. Biol. Chem. 278 (11), 9064–9072.

Roucou, X., LeBlanc, A.C., 2005. Cellular prion protein neuroprotective function: implications in prion diseases. J. Mol. Med. 83 (1), 3–11.

Sakaguchi, S., Katamine, S., Nishida, N., Moriuchi, R., Shigematsu, K., Sugimoto, T., Nakatani, A., Kataoka, Y., Houtani, T., Shirabe, S., Okada, H., Hasegawa, S., Miyamoto, T., Noda, T., 1996. Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. Nature 380 (6574), 528–531.

Sandig, V., Youil, R., Bett, A.J., Franlin, L.L., Oshima, M., Maione, D., Wang, F., Metzker, M.L., Savino, R., Caskey, C.T., 2000. Optimization of the helper-dependent adenovirus system for production and potency in vivo. Proc. Natl. Acad. Sci. U. S. A. 97 (3), 1002–1007.

Shtrichman, R., Sharf, R., Barr, H., Dobner, T., Kleinberger, T., 1999. Induction of apoptosis by adenovirus E4orf4 protein is specific to transformed cells and requires an interaction with protein phosphatase 2A. Proc. Natl. Acad. Sci. U. S. A. 96 (18), 10080–10085.

Spielhaupter, C., Schatzl, H.M., 2001. PrPC directly interacts with proteins involved in signaling pathways. J. Biol. Chem. 276 (48), 44604–44612.

Sundararajan, R., White, E., 2001. E1B 19K blocks Bax oligomerization and tumor necrosis factor alpha-mediated apoptosis. J. Virol. 75 (16), 7506–7516.

Thackray, A.M., Bujdoso, R., 2002a. PrP(c) expression influences the establishment of herpes simplex virus type 1 latency. J. Virol. 76 (5), 2498–2509.

Thrackay, A., Budjoso, R., 2002b. PrPc expression influences the establishment of Herpes Simplex Virus type 1 latency. J. Virol. 76, 2498–2509.

Tichopad, A., Dilger, M., Schwarz, G., Pfaffl, M.W., 2003. Standardized determination of real-time PCR efficiency from a single reaction set-up. Nucleic Acids Res. 31 (20), e122.

Trapnell, B.C., 1993. Adenoviral vectors for gene transfer. Adv. Drug Deliv. Rev. 12, 185–199.

Vilotte, J.L., Soulier, S., Essalmani, R., Stinnakre, M.G., Vaiman, D., Lepourry, L., Da Silva, J.C., Besnard, N., Dawson, M., Buschmann, A., Groschup, M., Petit, S., Madelaine, M.F., Rakatobe, S., Le Dur, A., Vilette, D., Laude, H., 2001. Markedly increased susceptibility to natural sheep scrapie of transgenic mice expressing ovine prp. J. Virol. 75 (13), 5977–5984.

Watarai, M., Kim, S., Erdenebaatar, J., Makino, S., Horiuchi, M., Shirahata, T., Sakaguchi, S., Katamine, S., 2003. Cellular prion protein promotes *Brucella* infection into macrophages. J. Exp. Med. 198 (1), 5–17.

Weissmann, C., Aguzzi, A., 1999. Perspectives: neurobiology. PrP's double causes trouble. Science 286 (5441), 914–915.

Weissmann, C., Aguzzi, A., 2005. Approaches to therapy of prion diseases. Annu. Rev. Med. 56, 321–344.

Whatley, S.A., Powell, J.F., Politopoulou, G., Campbell, I.C., Brammer, M.J., Percy, N.S., 1995. Regulation of intracellular free calcium levels by the cellular prion protein. Neuroreport 6 (17), 2333–2337.

Zawlik, I., Witusik, M., Hulas-Bigoszewska, K., Piaskowski, S., Szybka, M., Golanska, E., Liberski, P.P., Rieske, P., 2006. Regulation of PrPC expression: nerve growth factor (NGF) activates the prion gene promoter through the MEK1 pathway in PC12 cells. Neurosci. Lett. 400 (1–2), 58–62.