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Reversal of Misfolding: Prion Disease Behavioral and Physiological Impairments Recover following Postnatal Neuronal Deletion of the PrP Gene

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The prionoses are fatal neurodegenerative diseases caused by a pathogenic protein, PrP scrapie, that derives from misfolding of a normal form, PrP^c. These diseases progress through stages. A new study by Mallucci et al. in this issue of *Neuron* shows that prion disease may be reversed in mice by selective removal of the gene in neurons after early physiological, cognitive, and pathological features have developed.

In this issue of *Neuron*, Mallucci et al. present a compelling set of observations that illustrate reversal of synaptic and behavioral dysfunction in a mouse prion disease model (Mallucci et al., 2007). In many neurodegenerative diseases, particularly those involving pathogenic protein misfolding such as transmissible spongioform encephalopathies (TSEs) and Alzheimer's and Parkinson's diseases, the window of pathophysiological reversibility has been difficult to define. These distinct disease entities share some pathogenic and biochemical features, including conversion of a normal cellular protein to one with an altered conformation and apparent toxic action. The exact nature of the most toxic protein forms, the cell biologic loci of their toxic action, and the extent to which injured neuronal systems can compensate are all critical issues in the search for disease-modifying therapies for these neurodegenerative diseases. In each disorder, vulnerable synapses appear to be affected early in each disease course through mechanism(s) that are largely unknown. It can be demonstrated in some animal models of these diseases, and presumably human patients, that synaptic dysfunction is attended by cognitive and behavioral abnormalities (Chiti et al., 2006). However, discordance between histopathological features and behavioral deficits has been described (Santacruz et al., 2005), underscoring the importance of measures of clinical relevance of animal models, including physiology and behavior. With disease progression the specific pathogenic proteins lead directly and perhaps indirectly to neuronal death, characterizing a stage of disease likely refractory to functional recovery. Unfortunately, this is the time during which patients currently present clinically. Thus, these potentially discrete epochs of pathogenesis—synaptic dysfunction, neuronal injury, and death—frame the issue of disease reversibility and its relationship to meaningful therapeutic interventions.

The TSEs are pathophysiologically characterized by conversion of a pathogenic protein molecule, termed PrP scrapie (PrP^{Sc}), derived from its normal cellular form, PrP^c, through an ill-defined misfolding process (reviewed in Prusiner, 2001). Importantly, PrP^{Sc} appears capable of catalyzing conversion of PrP^c, thus rendering the progression of disease dependent upon expression of the substrate gene product. Removal of the capacity to express PrP^c from neurons, the principal contributor of CNS prion protein, enables testing of whether PrP^{Sc} damage is reversible and at what stage of disease.

In the current study, the investigators use the cre/*loxP* system to address this issue of reversibility in a mouse model of prion disease (Figure 1A). Mallucci et al. (2007) constructed bigenic mice, termed NFH-Cre/tg37, which harbor floxed *Prnp* alleles encoding the prion (PrP) protein and a neurofilament H (NF-H) promoter driven *cre* recombinase gene. The key to their experimental system is that the NF-H promoter becomes active postnatally at 9–10 weeks, resulting in recombination and permanent loss of PrP expression within neurons thereafter. This temporal feature allowed the investigators to establish prion infection in mice prior to cre-mediated recombination and then to monitor disease evolution. When inoculated intracerebrally with mouse-adapted scrapie prions early in life, at 1 week postnatal, the animals developed progressive behavioral deficits revealed by impaired hippocampal-dependent learning and spontaneous burrowing and nesting. These behavioral changes were accompanied by electrophysiological deficits recorded from contemporaneous hippocampal slices and characteristic histopathological features of spongiosis, gliosis, and PrP scrapie containing amyloid deposits. Coincident with NF-H cre-promoted removal of the *Prnp* gene and loss of PrP^c

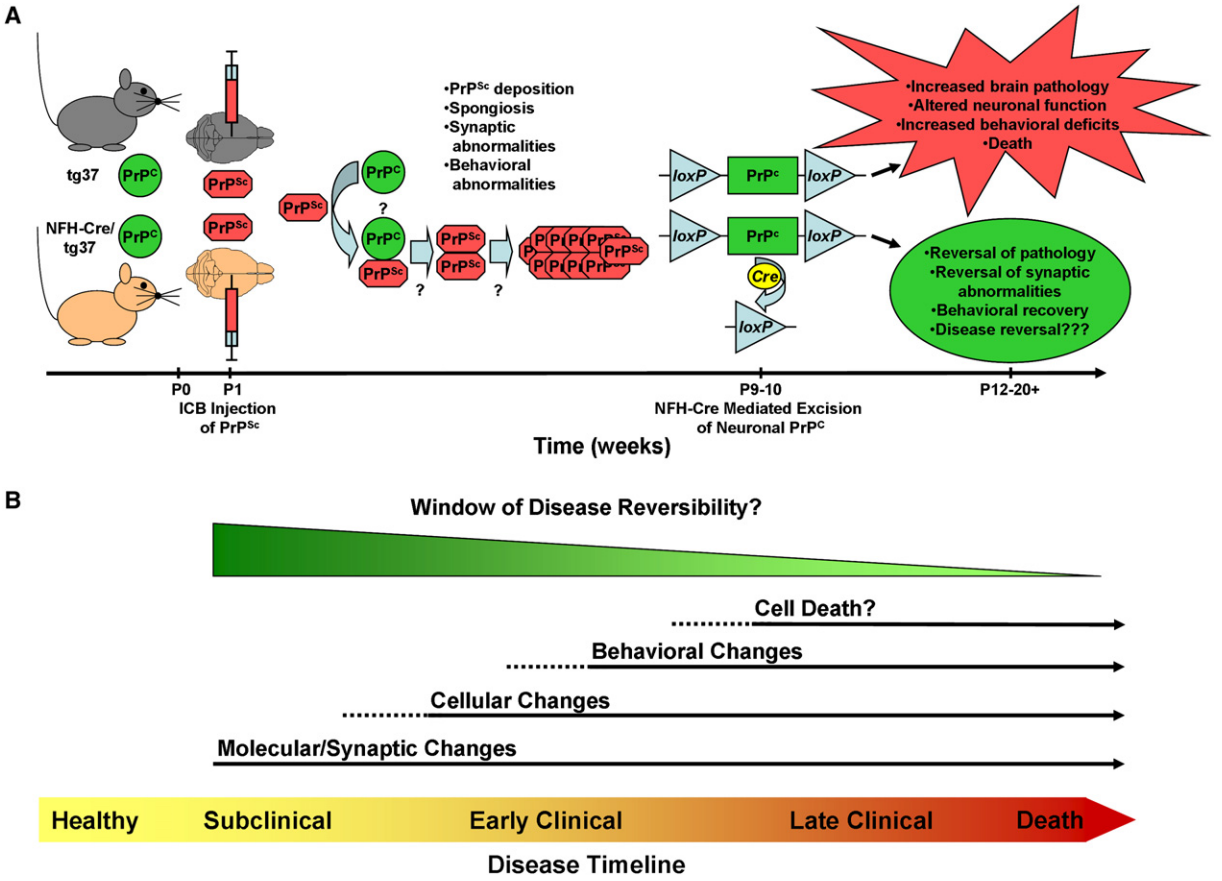

Figure 1. Postnatal Excision of Neuronal PrP^C Reverses Disease Processes in Prion-Infected Mice

(A) Intracerebral (ICB) injection of PrP^{Sc} (Chandler/Rocky Mountain Laboratories mouse-passaged scrapie strain) in 1-week-old tg37 mice results in a stereotyped pattern of disease progression, including deposition of PrP^{Sc} and a variety of histological, synaptic, and behavioral pathologies. In the present study, cre-mediated knockout of neuronally expressed PrP^C (under the control of the murine neurofilament H control elements, NFH-Cre) at 9–10 weeks of age resulted in a halting and reversal of disease processes in these bitransgenic mice.

(B) The successful treatment of the human prionoses and other neurodegenerative diseases will require a better understanding of the stages of disease pathogenesis and "windows" of potential therapeutic intervention. The natural history of a given disease, and the progression of its clinical symptoms and underlying molecular/cellular changes, will necessarily dictate the success of a given treatment. For example, as was the case in this study, interventions at subclinical and early clinical time points in disease, prior to the onset of overt neuronal death, may result in recovery of function and a better prognosis. Alternatively, interventions at later time points where cell death is widespread may prove less efficacious at halting, let alone reversing, disease processes. Thus, future studies will be necessary to answer the questions as to at what point and for how long particular "windows of disease reversibility" are open to treat this and potentially other neurodegenerative diseases.

expression, scrapie-infected mice recovered behaviorally, physiologically, and histopathologically (see Figures 1, 3, and 5 of Mallucci et al., 2007). Control mice that did not carry the cre recombinase gene and that continued to express PrP^C progressively worsened by all measures.

The importance of this work is fourfold. First, it illustrates a clear stage of TSE reversibility that was characterized by synaptic dysfunction and correlated behavioral impairments. Second, it underscores that PrP^{Sc} can be cleared from the scrapie-infected nervous system and its injury compensated by neuronal mechanisms. Third, it heralds an era in therapeutics development for neurodegenerative diseases involving protein misfolding. Finally, the findings of this study make evident the need to better identify patients earlier in their disease course, when reversal and/or treatment of pathophysiological processes may be feasible.

This study, while extending our understanding of the TSEs, raises a number of issues. For example, the temporal dimension of the window to achieve disease reversal is unknown (Figure 1B). Would a greater delay in eliminating PrP^C expression, in this context turning cre recombinase on later within the disease course, still reverse disease or perhaps result in partial recovery? Next, the observations were made in very young mice in which postnatal brain development was still active. As such, the findings may be less clinically relevant since most TSEs present later in life when synaptic plasticity appears more limited and thus the propensity for functional recovery less robust. Lastly,

would hypothetical disease-modifying strategies that could reduce but not eliminate PrP^C expression benefit neurologically impaired patients? Absent such information, it is important that methods including RNAi and PrP antibody based approaches be developed and evaluated (Donofrio et al., 2005; Enari et al., 2001; Golding et al., 2006; Peretz et al., 2001; Pfeifer et al., 2006; White et al., 2003). Testing of candidate therapeutics in animal models could determine the optimal timing for administration (with respect to disease stage), the capacity to attenuate and/or reverse features of disease, the impact of subject age on efficacy, and whether they are effective singly or in combination. It is hoped that a successful demonstration of a preclinical therapy of a prionosis would enable early-stage clinical trials. Moreover, the extension of this general approach of defining a reversible phase and enabling interventions to the more common protein misfolding diseases such as Alzheimer's and Parkinson's should be strongly encouraged.

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Picking Up the Pieces: The Roles of Functional Remnants of Calpain-Mediated Proteolysis

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Calpain-mediated cleavage of neuronal targets has long been associated with excitotoxicity and synaptic plasticity. In this issue of *Neuron*, two papers by Xu et al. and Abe and Takeichi explore the respective roles of mGluR1α cleavage in excitotoxicity and β-catenin cleavage in transcriptional control. Together, these papers show the functional importance of fragments of calpain-mediated cleavage.

Calpain, sometimes referred to as calcium-activated neutral protease, is among the most enigmatic proteases in the body and particularly the nervous system (Wu and Lynch, 2006). This family of proteases is recognized by their requirement for calcium and defined by specific inhibitors, but their substrate specificity defies complete classification. Calpain cleaves at preferred sequences in association with preferred tertiary structures of substrates, but without known absolute rules. Unlike most proteases, substrates of calpain are not usually destroyed but instead become dysregulated. Perhaps because of this unusual specificity, these enzymes have been associated with a diverse set of cellular functions inside and outside the nervous system. In neurons, spectrin has been defined as the prototypic substrate for calpain, inadvertently promoting the idea that the main