Loss of functional prion protein: a role in prion disorders?

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To understand the normal function of the prion protein (PrP) and its role in prion disorders, several groups have generated mice lacking PrP. Some of these mice develop symptoms associated with prion diseases, but other experimental evidence suggests that the loss of functional PrP is not the instigating factor in these disorders.

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Human prion diseases are rare neurodegenerative disorders with genetic, sporadic or infectious etiologies (reviewed in [1]). The initial clinical presentation of affected individuals can vary from ataxia to dementia, with brain pathology dominated by spongiform degeneration and gliosis. A fraction of cases also show 'amyloid' plaques that are birefringent when stained with Congo Red, and immunoreactive with antiserum raised against the host-encoded prion protein (PrP). Creutzfeldt–Jakob disease (CJD) is the most common form of sporadic prion disorders, but familial forms of CJD have also been described. Gerstmann–Straussler–Scheinker disease (GSS) and fatal familial insomnia are other examples of familial prion disorders, and kuru is an example of a transmitted prion disease. Familial CJD and GSS are the only inherited human diseases in which nervous tissues from affected individuals can transmit disease to nonhuman primates and rodents. Prion disorders also are endemic to domestic sheep (scrapie), and more recently have become prevalent in cattle in the United Kingdom (bovine spongiform encephalopathy (BSE)).

The ease of transmissibility of prions led to the establishment of the first rodent models of human neurodegenerative disease, and these models have greatly facilitated investigations of the underlying molecular mechanisms of disease. In both humans and other animals a critical event in the pathogenesis of prion diseases is the conversion of the normal cellular prion protein (PrP^C), to an infectious form, termed scrapie prion protein (PrP^{Sc}) [2,3]. Although the mechanisms by which PrP^C is converted to PrP^{Sc} are not fully defined, several lines of evidence indicate that PrP^{Sc} is generated from PrP^C via alterations of protein conformation [4–6]. Despite the advances in our understanding of the cell biological and biochemical events involved in the conversion of PrP^C to PrP^{Sc}, the function of PrP^C and the role of PrP^C in scrapie propagation has, until recently, been enigmatic and controversial. Several groups have approached these issues by generating mice in which the mouse prion protein gene was modified using gene-targeting strategies; here, we provide some perspectives on the lessons learned from these approaches.

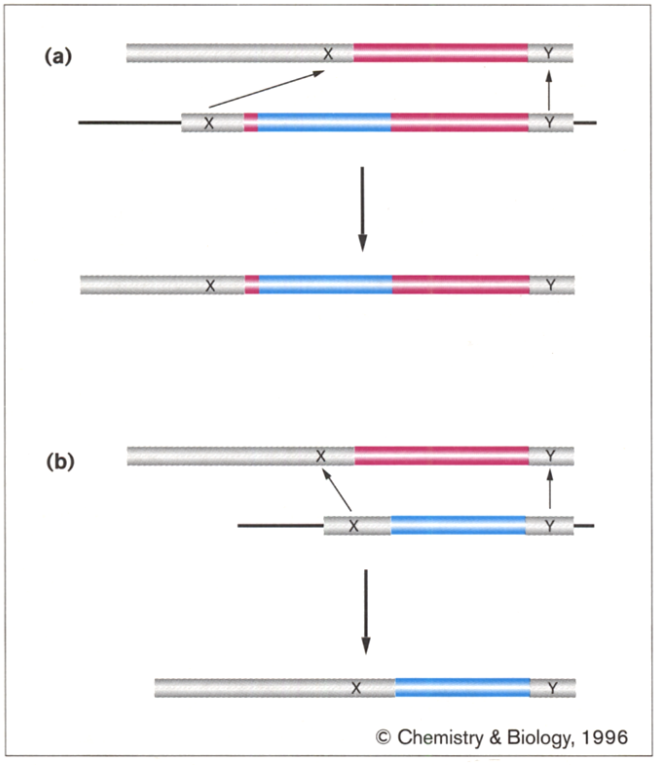
PrP^C is required for infection with PrP^{Sc}

Elegant studies by Büeler *et al.* [7] convincingly demonstrated that mice devoid of PrP^C ($PrP^{0/0}$ or PrP knockout mice) do not succumb to prion infection when inoculated with mouse PrP^{Sc}, and do not support the clandestine replication of prions. These data firmly established that the host susceptibility to scrapie infection and prion propagation require the expression of PrP^C. In these studies, $PrP^{0/0}$ mice developed normally up to the age of two years and did not exhibit behavioral abnormalities or neurological impairments [7]. These data supported the idea that PrP^C is not critical either for normal development or for central nervous system function. Collinge *et al.* [8] reported, however, that $PrP^{0/0}$ mice exhibit neurophysiological defects in synaptic inhibition and long term potentiation, and that these defects in synaptic function could be rescued following cross-breeding to transgenic mice expressing human PrP [9]. Although these studies strongly suggested that the observed abnormalities in $PrP^{0/0}$ mice were the result of the loss of normal PrP^C, a recent report demonstrated that the same $PrP^{0/0}$ animals do not exhibit defective synaptic physiology [10]. The discrepancies in phenotypes may be explained by the fact that the original knockout animals were generated in hybrids of C57BL/6J and 129/Sv mouse strains. Reassortment of alleles during subsequent matings would then lead to the generation of sublines of mice that respond differently to diminished PrP^C expression.

PrP knockout mice can develop symptoms of prion diseases

Recently, two groups independently reported on the generation of mouse strains devoid of PrP^C [11,12] (see Fig. 1), which exhibited striking behavioral and neuropathological alterations. Sakaguchi *et al.* [12] reported that mice with a targeted deletion of the entire open reading frame of the *PrP* gene lose Purkinje cells and exhibit ataxia and deficits in coordination at ~70 weeks of age. In contrast, mice with insertionally disrupted PrP genes showed altered circadian rhythms and disturbances in sleep patterns [13]. Notably, these latter mice were generated in F1 hybrids of 129/Ola and F2 (C57Bl/6J x CBA) [11].

Figure 1



Schematic representation of two different methods for generating knockout mice by homologous recombination between cellular DNA and recombinant DNA. **(a)** Insertional disruption (used by Manson *et al.* [11]), **(b)** targeted deletion (used by Sakaguchi *et al.* [12]). Genomic sequences are shown at the top, with sequences contained in plasmid DNA shown below. Arrows indicate potential sites of recombination. Gray, genomic DNA; magenta, target gene; blue, foreign DNA; black line, plasmid vector DNA. X and Y are used to indicate regions of identical genomic DNA. The cell lines generated by these techniques are heterozygous for the PrP mutation. Mice are produced by injection of the engineered cells into a host blastocyst and are interbred to produce offspring that are homozygous for the mutation.

Interpreting the phenotypes of the knockout mice

Can we relate the phenotypes of the PrP knockout mice to the clinical manifestations of human prion disorders? In view of the varied phenotypes associated with one or more of the human prion disorders including diminished synaptic function, ataxia, and altered sleep patterns, it is tempting to speculate that the loss of functional PrP^{C} (as a result of conversion to PrP^{Sc}) causes the clinical symptoms associated with prion disorders. To address this issue, Brandner *et al.* [14] grafted normal murine brain tissue into the brains of $PrP^{0/0}$ animals, and subsequently infected the graft with PrP^{Sc}. Surprisingly, neurodegenerative pathology was restricted to the graft, and surrounding tissues were spared despite the presence of PrP^{Sc} outside of the boundary of the graft. These data suggested that neurodegeneration is the result of deleterious events intrinsic to cells within the graft (which may, or may not, include the acute depletion of PrP^{C}) and that PrP^{Sc} is not itself neurotoxic to neurons lacking PrP^{C}. Although the graft studies offered the provocative suggestion that neurodegeneration in prion disorders might be the result of depletion of cellular pools of PrP^{C}, several lines of experimental evidence argue against this notion. First, $PrP^{0/0}$ mice do not exhibit pathology typical of prion disorders (gliosis, spongiform degeneration). Second, although a hamster prion inoculum administered into transgenic mice expressing hamster PrP^{C} provokes spongiform degeneration in the host, the only PrP^{C} converted to PrP^{Sc} is of hamster origin, suggesting that the cellular pool of mouse PrP^{C} is unaltered [15,16]. Third, forced overexpression of PrP^{C} in transgenic animals results not in a delay, but rather in the acceleration, of disease onset following inoculation with prions [15,16]. Finally, the presence of high levels of overexpressed PrP^{C} in transgenic mice leads to spontaneous prion disease [17]. Taken together, little biochemical and genetic evidence has accrued that lends support to the notion that depletion of PrP^{C} contributes to pathogenesis of prion disease.

The disparate phenotypes between the PrP knockout mice generated by three different groups underscores the complexities involved in modeling human diseases in transgenic or knockout mice. The knockout mice were generated from hybrid mouse strains with genetically heterogeneous backgrounds, and subsequently interbred to generate mice homozygous for the deleted allele; thus, the genotype of each inbred line will be significantly different. Heterogeneity in genetic background between lines of knockout mice may translate into differing phenotypes due to changes in the levels of expression of molecules that compensate functionally for the missing protein, or molecules that transduce or modify functional information. In support of this view, there are profoundly different phenotypes among various inbred strains of mice with a particular disruption of the gene encoding the transcription factor Hoxb-4 [18]. In addition, each of the PrP knockout lines were generated using different targeting strategies, and hence subtle differences in expression of the remaining PrP^{C} coding regions in targeted genes or genes located near (or within) the PrP gene may be responsible for the phenotypic heterogeneity. It is relevant to note that mice with an insertionally inactivated exon of the cystic fibrosis transmembrane regulator gene [19] exhibited a phenotype remarkably different to that of mice in which the identical exon was replaced with foreign sequences [20]. To address the influences of genetic heterogeneity and genomic manipulations, it will be necessary to prove that the observed phenotype(s) can be rescued by the expression of PrP cDNA transgenes.

Conclusions and future directions

Although the phenotypes of $PrP^{0/0}$ mice may mimic a subset of the clinical features of human prion disorders, the weight of evidence suggests that loss of functional PrP^{C} is

not the instigating factor in disease. Moreover, the normal function of PrP^C remains a mystery and attempts to clarify this issue using knockout strategies have been confounded by the complexities of mouse genetics and gene-targeting strategies. With these caveats in mind, we believe that it is essential to generate highly inbred (congenic) mouse strains with disrupted *PrP* genes to fully appreciate the consequences of loss of normal PrP^C. These genetic approaches will greatly facilitate the identification of host gene products that modify the phenotypes of $PrP^{0/0}$ mice, efforts that will no doubt provide insights into the normal biology of PrP^C and its role in the pathophysiology of the enigmatic spongiform encephalomyopathies.

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