bimC motor proteins play an essential role in organizing microtubules within the spindle during mitosis (*6*). These data support the Mayer findings and corroborate the accuracy of their screening strategy, which successfully identified an antimitotic compound targeting a nontubulin spindle protein. Although the clinical benefits of Monastrol require further evaluation, this compound will certainly be a useful molecular tool for studying the involvement of the bimC class of kinesin-related proteins in spindle assembly.

Mayer and co-workers have added several new, and potentially very important, molecular tools to the antimitotic toolbox. Clearly, the targets of the four remaining antimitotic compounds identified in this screen need to be characterized. Regardless of whether the four targets turn out to be proteins that are already known or new protein components of the spindle, their identification will shed more light on the steps of spindle assembly. Also, the group of compounds that blocked cells in mitosis without altering microtubule organization in any discernible way should not be ignored. Finally, the success of this screening strategy should stimulate similar screening approaches aimed at identifying small molecules that perturb other stages of the cell cycle. It is easy to envision how a suitable phosphorylation-specific antibody against the Rb tumor suppressor protein or one of the many proteins involved in DNA replication could be used in such a screen to identify new compounds that perturb other aspects of the cell cycle.

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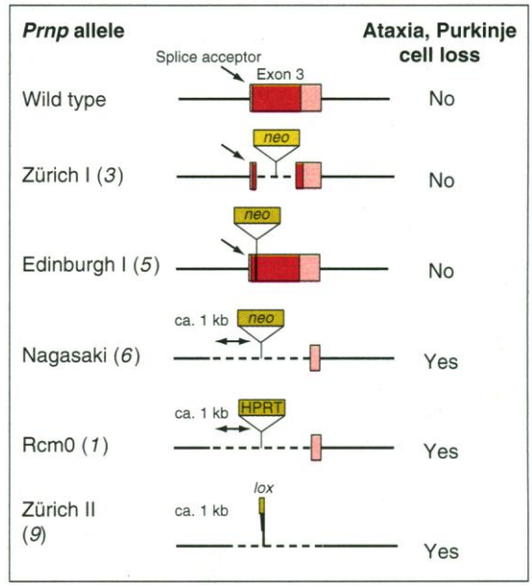
PERSPECTIVES: NEUROBIOLOGY

PrP's Double Causes Trouble

Charles Weissmann and Adriano Aguzzi

Disrupting ("knocking out") a mouse gene by homologous recombination is a widely used strategy for elucidating the function of that particular gene. However, the phenotype (or lack of it) displayed by the knockout mouse is not always conclusive. Although knockout experiments are conceptually simple—disrupt a gene and see what happens—the interpretation of their results is beset with pitfalls. For example, insertion of an interrupting sequence into an exon could result in the modified exon being spliced out after transcription; the truncated protein that is expressed could still be active or could be capable of interfering with the biochemical pathway in which the wild-type protein participates (dominant negative). If the promoter of a gene rather than the gene itself is deleted, transcription from an unrecognized upstream promoter could compensate for this loss. Deletion of the entire gene could result in ablation of an unrecognized open reading frame (the sequence that encodes the protein; ORF) or of important regulatory sequences, entailing loss of a function that is unrelated to the targeted ORF. Restoration of a gene's function in a knockout mouse by introduction of a transgene expressing the targeted protein is good evidence for linkage of the function to the gene in question. However, even then, the conclusion that a phenotype is due to the disruption of a particular gene can be erroneous, as demonstrated by a fascinating paper from Moore *et al.* published in a recent issue of the *Journal of Molecular Biology* (*1*). These investigators identify a second *Prnp*-like gene located 16 kb downstream of the murine *Prnp* gene, which encodes the prion protein (PrP). Prion proteins have been implicated in the propagation of the neurodegenerative prion diseases. Moore and colleagues call the new gene *Prnd* and the protein it encodes Dpl (Doppel, German for double). They go on to show that Dpl is overexpressed in certain strains of PrP knockout mice, possibly causing a neurological disease that some researchers had previously attributed to ablation of PrP.

Prion proteins play a central role in transmission of the prion diseases (transmissible spongiform encephalopathies), a group that includes sheep scrapie, mad cow disease, and Creutzfeldt-Jakob disease in humans. A conformational variant of the normal cellular protein PrP^C is either an essential component of the transmissible agent (the prion), or the prion itself (*2*). A crucial role for PrP was revealed by demonstrating that mice lacking PrP (*3*) were resistant to prion disease, failing to develop the illness when mouse scrapie prions were inoculated into their brains (*4*). These so-called Zürich I $Prnp^{0/0}$ mice, as well as a later PrP knockout line designated Edinburgh $Prnp^{-/-}$ (*5*), were clinically healthy, although they showed discrete neurophysiological changes and demyelination of peripheral nerves as they aged. However, mice of a third PrP knockout strain, Nagasaki $Prnp^{-/-}$ (*6*), developed ataxia and loss of cerebellar Purkinje cells at 6 to 12 months of age. Because the disease was prevented by introduction of a cosmid encoding PrP, this phenotype was attributed to PrP ablation (*7*). A comparison of the knockout strategies (see figure, this page) reveals that in the Zürich I mice, two-thirds of the ORF of *Prnp* was replaced by a cassette containing the neomycin resistance gene. In the Edinburgh mice a similar cassette was inserted into the ORF. However, in the Nagasaki mice not only the ORF, but also 0.9 kb of intron 2, the 5′ noncoding region, and 0.45 kb of the



Knock, knock. Which phenotype is there? Various strategies used to disrupt the locus of the *Prnp* gene (which encodes PrP) in the creation of different strains of *Prnp* knockout mice. The dotted line indicates the segment of *Prnp* DNA that has been deleted, and the green box indicates a sequence inserted into the gene. Dark red, ORF; pink, noncoding region; *neo*, neomycin phosphotransferase; HPRT, hypoxanthine phosphoribosyltransferase; *lox*, a 34-bp recombination site from phage P1.

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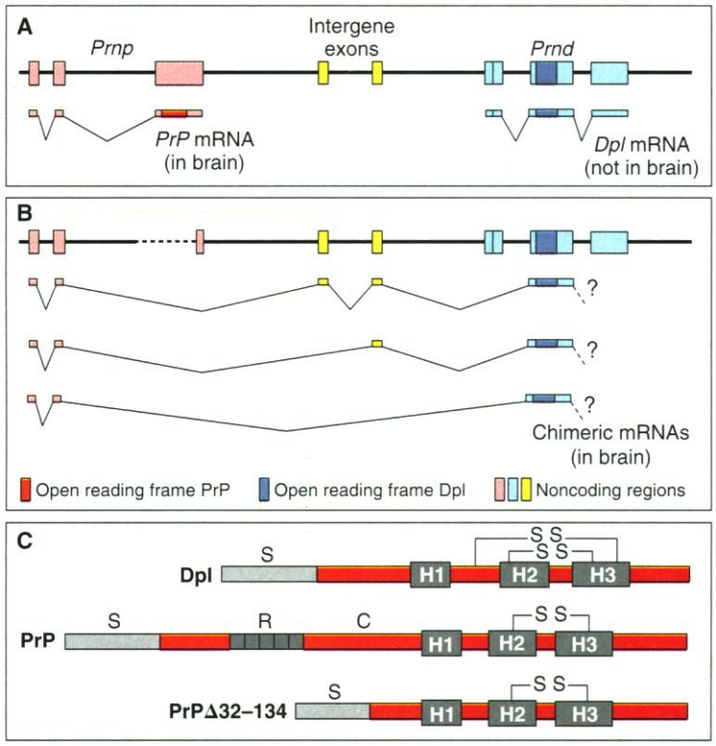
3′ noncoding region of *Prnp* were deleted. Not in any of these strains could PrP or a fragment thereof be detected. It therefore seemed likely that the removal of the flanking sequences, and not ablation of the PrP ORF, was responsible for the phenotype (*8*). Two additional PrP knockout lines, Zürich II (*9*) and Rcm0 (*1*), in which the PrP ORF and its flanking regions were replaced by a *lox* sequence and an HPRT cassette, respectively, confirmed the observations on the Nagasaki line (see figure, previous page).

The Moore report (*1*) provides surprising insights into the origin of the different phenotypes of *Prnp* knockout mice. The investigators sequenced downstream of the murine *Prnp* gene and discovered an ORF encoding Dpl, a protein composed of 179 amino acids (see figure, this page). The predicted protein has about 25% identity with the carboxyl-terminal two-thirds of murine PrP. Dpl is predicted to contain three α helices and a disulfide bond between the second and third helix, as is found in PrP (*10*). Strikingly, however, Dpl lacks sequences homologous to the amino-terminal octarepeat region that binds copper ions and, in particular, lacks the highly conserved region between amino acids 106 and 126 that is essential for PrP to sustain prion replication (*11*). Dpl mRNA is expressed at relatively high levels in testis, at lower levels in other peripheral organs, and at very low levels in the brains of wild-type mice. Intriguingly, however—and herein lies the exciting revelation—there is a high level of expression of Dpl-specific mRNA in the brains of Nagasaki and Rcm0 mice, both of which suffer from ataxia, but not in the brains of Zürich I or Edinburgh mice, which are not ataxic. Analysis of brain-derived cDNAs indicates that in wild-type mice Dpl mRNA is weakly expressed, mainly from a promoter upstream of exon 1 of *Prnd* (the gene encoding Dpl), whereas the strong expression in Nagasaki mice is due to a chimeric mRNA that originates at the *Prnp* promoter, runs all the way past the *Prnd* ORF, and is processed by one or more splicing events that link the 3′ end of the second PrP exon directly or indirectly to the Dpl-encoding exon (see figure, this page). This intergenic splicing, which is also detected at very low levels in wild-type mice, is greatly enhanced in the ataxic mice because the splice acceptor site upstream of the PrP-encoding exon is deleted, thus diverting the splice to a downstream acceptor site. The chimeric mRNA is expressed undiminished in Nagasaki mice that had been "cured" by the introduction of a cosmid expressing PrP.



A prion protein's doppelgänger. The *Prnp* and *Prnd* loci and expression of the PrP and Dpl mRNAs and proteins that they encode. **(A)** Coding and noncoding exons of *Prnp*, *Prnd*, and intergenic exons of unknown function. **(B)** Deletion of the exon of *Prnp* containing an open reading frame and its flanking regions results in the formation of several chimeric mRNAs that comprise the first two exons of *Prnp* (*14*), which are spliced directly or indirectly to the exon encoding Dpl. **(C)** Comparison of predicted domains of Dpl with full-length PrP and with PrP in which amino acid residues 32 to 134 have been deleted (*10*).

Why should overexpression of Dpl cause ataxia and concurrent overexpression of PrP restore normal function? Shmerling *et al.* (*12*) found that introduction into Zürich I $Prnp^{0/0}$ mice of a truncated *Prnp* transgene lacking the amino terminus and, hence, devoid of the octarepeats and the conserved 106–126 amino acid region (PrPΔ32–135) resulted in ataxia and degeneration of the cerebellar granule cell layer within weeks after birth. Moreover, introduction of a single intact PrP allele prevented the disease. They proposed that PrP interacts with a ligand to elicit an essential signal and that a PrP-like molecule with lower binding affinity could fulfill the same function in the absence of PrP. According to this hypothesis, in PrP knockout mice the truncated PrP could interact with the ligand, displacing the PrP-like molecule, but without eliciting the survival signal. If PrP has the higher affinity for the ligand, it could displace its truncated counterpart and restore function. Because Dpl resembles truncated PrP, it might cause disease by the same mechanism, namely by competing with PrP for the PrP ligand. However, because the promoter used to express the truncated PrP is active in granule cells but not in Purkinje cells, whereas the wild-type PrP promoter is active in Purkinje cells, the cells targeted in mice expressing truncated PrP may be different from those in Nagasaki mice expressing Dpl. Indeed, targeting the truncated PrP to Purkinje cells causes them to die, leading to ataxia (*13*).

The discovery of PrP's double solves some questions but raises others. Evidence for the expression, proposed structure, and cellular location of the predicted Dpl protein is still lacking. Will transgenes encoding Dpl indeed cause degeneration of cells in which they are overexpressed? Does the time of appearance and intensity of the pathological phenotype depend on the ratio of Dpl to PrP? What is the physiological function of Dpl, and is it important in prion diseases? Are there genetic defects in humans elicited by overexpression of Dpl in the brain? And, now that we know that PrP is not required to maintain Purkinje cell function, what then is its purpose? But perhaps one of the most important lessons from these findings is that disrupting a gene may entail far more than the phenotype that meets the eye.

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