fCJD and sCJD patients produced the 21-kDa PrP^{Sc} in mice expressing the same transgene. On second passage, these differences were maintained, demonstrating that chimeric PrP^{Sc} can exist in two different conformations based on the sizes of the protease-resistant fragments, even though the amino acid sequence of PrP^{Sc} is invariant.

This analysis was extended when patients with sporadic fatal insomnia (sFI) were identified. Although they did not carry a *PRNP* gene mutation, the patients demonstrated a clinical and pathologic phenotype that was indistinguishable from that of patients with FFI. Furthermore, 19-kDa PrP^{Sc} was found in their brains, and on passage of prion disease to mice expressing a chimeric human-mouse PrP transgene, 19-kDa PrP^{Sc} was also found. These findings indicate that the disease phenotype is dictated by the conformation of PrP^{Sc} and not the amino acid sequence. PrP^{Sc} acts as a template for the conversion of PrP^{C} into nascent PrP^{Sc}. On the passage of prions into mice expressing a chimeric hamster-mouse PrP transgene, a change in the conformation of PrP^{Sc} was accompanied by the emergence of a new strain of prions.

Many new strains of prions were generated using recombinant (rec) PrP produced in bacteria; recPrP was polymerized into amyloid fibrils and inoculated into transgenic mice expressing high levels of wild-type mouse PrP^{C}; approximately 500 days later, the mice died of prion disease. The incubation times of the "synthetic prions" in mice were dependent on the conditions used for polymerization of the amyloid fibrils. Highly stable amyloids gave rise to stable prions with long incubation times; low-stability amyloids led to prions with short incubation times. Amyloids of intermediate stability gave rise to prions with intermediate stabilities and intermediate incubation times. Such findings are consistent with earlier studies showing that the incubation times of synthetic and naturally occurring prions are directly proportional to the stability of the prion.

Species Barrier Studies on the role of the primary and tertiary structures of PrP in the transmission of prion disease have given new insights into the pathogenesis of these maladies. The amino acid sequence of PrP encodes the species of the prion, and the prion derives its PrP^{Sc} sequence from the last mammal in which it was passaged. While the primary structure of PrP is likely to be the most important or even sole determinant of the tertiary structure of PrP^{C}, PrP^{Sc} seems to function as a template in determining the tertiary structure of nascent PrP^{Sc} molecules as they are formed from PrP^{C}. In turn, prion diversity appears to be enciphered in the conformation of PrP^{Sc}, and thus prion strains seem to represent different conformers of PrP^{Sc}.

In general, transmission of PrP prion disease from one species to another is inefficient, in that not all intracerebrally inoculated animals develop disease, and those that fall ill do so only after long incubation times that can approach the natural life span of the animal. This "species barrier" to transmission is correlated with the degree of similarity between the amino acid sequences of PrP^{C} in the inoculated host and of PrP^{Sc} in the prion inoculum. The importance of sequence similarity between the host and donor PrP argues that PrP^{C} directly interacts with PrP^{Sc} in the prion conversion process.

SPORADIC AND INHERITED PrP PRION DISEASES

Several different scenarios might explain the initiation of sporadic prion disease: (1) A somatic mutation may be the cause and thus follow a path similar to that for germline mutations in inherited disease. In this situation, the mutant PrP^{Sc} must be capable of targeting wild-type PrP^{C}, a process known to be possible for some mutations but less likely for others. (2) The activation energy barrier separating wild-type PrP^{C} from PrP^{Sc} could be crossed on rare occasions when viewed in the context of a population. Most individuals would be spared, while presentations in the elderly with an incidence of ~1 per million would be seen. (3) PrP^{Sc} may be present at low levels in some normal cells, where it performs some important, as yet unknown, function. The level of PrP^{Sc} in such cells is hypothesized to be sufficiently low as to be not detected by routine bioassay. In some altered metabolic states, the cellular mechanisms for clearing PrP^{Sc} might become compromised, and the rate of PrP^{Sc} formation would then begin to exceed the capacity of the cell to clear it. The third possible mechanism is attractive because it suggests PrP^{Sc} is not simply a misfolded protein, as proposed for the first and second mechanisms, but that it is an alternatively folded molecule with a function. Moreover, the multitude of conformational states that PrP^{Sc} can adopt, as described above, raises the possibility that PrP^{Sc} or another prion-like protein might function in a process like short-term memory where information storage occurs in the absence of new protein synthesis.

More than 40 different mutations resulting in nonconservative substitutions in the human *PRNP* gene have been found to segregate with inherited human prion diseases. Missense mutations and expansions in the octapeptide repeat region of the gene are responsible for familial forms of prion disease. Five different mutations of the *PRNP* gene have been linked genetically to heritable prion disease.

Although phenotypes may vary dramatically within families, specific phenotypes tend to be observed with certain mutations. A clinical phenotype indistinguishable from typical sCJD is usually seen with substitutions at codons 180, 183, 200, 208, 210, and 232. Substitutions at codons 102, 105, 117, 198, and 217 are associated with the GSS variant of prion disease. The normal human PrP sequence contains five repeats of an eight-amino-acid sequence. Insertions from two to nine extra octarepeats frequently cause variable phenotypes ranging from a condition indistinguishable from sCJD to a slowly progressive dementing illness of many years in duration to an early-age-of-onset disorder that is similar to AD. A mutation at codon 178 resulting in substitution of asparagine for aspartic acid produces FFI if a methionine is encoded at the polymorphic residue 129 on the same allele. Typical CJD is seen if the D178N mutation occurs with a valine encoded at position 129 of the same allele.

HUMAN *PRNP* GENE POLYMORPHISMS

Polymorphisms influence the susceptibility to sporadic, inherited, and infectious forms of prion disease. The methionine/valine polymorphism at position 129 not only modulates the age of onset of some inherited prion diseases but can also determine the clinical phenotype. The finding that homozygosity at codon 129 predisposes to sCJD supports a model of prion production that favors PrP interactions between homologous proteins.

Substitution of the basic residue lysine at position 218 in mouse PrP produced dominant-negative inhibition of prion replication in transgenic mice. This same lysine at position 219 in human PrP has been found in 12% of the Japanese population, and this group appears to be resistant to prion disease. Dominant-negative inhibition of prion replication was also found with substitution of the basic residue arginine at position 171; sheep with arginine were resistant to scrapie prions but were susceptible to BSE prions that were inoculated intracerebrally.

INFECTIOUS PrP PRION DISEASES

IATROGENIC CJD

Accidental transmission of CJD to humans appears to have occurred with corneal transplantation, contaminated electroencephalogram (EEG) electrode implantation, and surgical procedures. Corneas from donors with unsuspected CJD have been transplanted to apparently healthy recipients who developed CJD after variable incubation periods. The same improperly decontaminated EEG electrodes that caused CJD in two young patients with intractable epilepsy caused CJD in a chimpanzee 18 months after their experimental implantation.

Surgical procedures may have resulted in accidental inoculation of patients with prions, presumably because some instrument or apparatus in the operating theater became contaminated when a CJD patient underwent surgery. Although the epidemiology of these studies is highly suggestive, no proof for such episodes exists.

Dura Mater Grafts More than 160 cases of CJD after implantation of dura mater grafts have been recorded. All of the grafts appear to have been acquired from a single manufacturer whose preparative procedures were inadequate to inactivate human prions. One case of CJD occurred after repair of an eardrum perforation with a pericardium graft.