Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie

David Westaway,1 Vincent Zuliani,1 Carol Mirenda Cooper,1 Maria Da Costa,1 Sara Neuman,1 Allen L. Jenny,3 Linda Detwiler,4 and Stanley B. Prusiner1,2

Departments of 1Neurology, 2Biochemistry and Biophysics, University of California, San Francisco, California 94143 USA; 3U.S. Department of Agriculture Animal and Plant Health Inspection Services (USDA-APHIS), Veterinary Services, National Veterinary Service Laboratories, Ames, Iowa 50010 USA; 4US-APHIS, Veterinary Services, Trenton, New Jersey 08619 USA

Natural scrapie has been viewed both as a recessive trait and as a contagious disease modulated by a host locus. To address this conundrum, we determined the structure of the sheep prion protein (PrP) gene, which contains three exons and extends over 20 kb of DNA. In the United States 86.4% of scrapie cases occur in Suffolk sheep, and within this breed 49% of healthy animals carry one or more PrP alleles encoding Arg (R)-171. Four scrapie-affected sheep were homozygous for wild-type PrP open reading frames encoding the alternative Gln (Q)-171 allele. Analysis of additional cases revealed that all were Q/Q-171 homozygotes (n=31), yielding a probability of 0.000004 that PrP genotype is unrelated to susceptibility. These data imply that homozygosity for Q-171 codons is necessary but not sufficient for the development of natural scrapie, echo reports of recessive manifestation, and parallel over-representation of PRNP codon 129 homozygotes in Creutzfeldt-Jakob disease of humans. Whereas progress has been substantial regarding experimental scrapie in rodents, the occurrence and spread of disease in flocks of sheep has remained enigmatic. Appreciation of the relationship between codon 171 genotype and susceptibility may help define the molecular basis of natural scrapie.

[Key Words: Prion replication; PrPSc; PrP promoter]

Received December 16, 1993; revised version accepted March 7, 1994.

The origins of natural scrapie have been the subject of heated debate. Although experimental transmission was demonstrated by Cuillé and Chelle [Cuillé and Chelle 1939], Parry produced evidence that the disease was an autosomal recessive trait, the spread of which could be controlled by selective breeding [Parry 1960, 1962]. Studies by Dickinson and co-workers favoring horizontal and vertical (maternal) contagious spread, plus identification of the scrapie incubation time gene \(S_C\) in mice, turned the tide against Parry and led to a prevailing viewpoint of scrapie as a viral-like illness, the outcome of which is modified by host-susceptibility genes [Dickinson et al. 1965, 1968, 1974]. Subsequently, biochemical analyses of the experimental disease revealed that infectious scrapie prions are distinct from viruses in that the major, and possibly only, constituent of prions corresponds to an abnormal isoform of a host protein designated PrP\(^{Sc}\) [Prusiner 1991]. Prion replication is now thought to be synonymous with post-translational conversion of the benign cellular prion protein PrP\(^{C}\) into PrP\(^{Sc}\). The synthesis of PrP\(^{C}\) occurs in the endoplasmic reticulum and it is subsequently transported through the Golgi to the cell surface where it is bound by a glycoinositol phospholipid anchor [Stahl et al. 1987; Caughey et al. 1989, Borchelt et al. 1990]. Subsequently, PrP\(^{C}\) is converted into PrP\(^{Sc}\) by a post-translational process [Borchelt et al. 1990, 1992; Caughey and Raymond 1991] that appears to involve the unfolding of PrP\(^{C}\) and its refolding into PrP\(^{Sc}\). Attempts to identify a post-translational chemical modification that features in the synthesis of PrP\(^{Sc}\) have been unrewarding [Stahl et al. 1993]. Spectroscopic studies have shown that PrP\(^{C}\) is rich in \(\alpha\)-helices and virtually devoid of \(\beta\)-sheet while PrP\(^{Sc}\) has a high \(\beta\)-sheet content [Pan et al. 1993].

Although PrP\(^{Sc}\) has now been demonstrated in the brains of most, if not all, sheep with natural scrapie [Rubenstein et al. 1987; Farquhar et al. 1989, Serban et al. 1990; Katz et al. 1992; Race et al. 1992], our understanding of the parameters controlling disease dissemination and susceptibility is modest. Attempts to define the physical mechanism of spread have yielded conflicting results [Pattison et al. 1972, 1974; Hadlow et al. 1982, Hourrigan et al. 1979]. Similarly, whereas PrP alleles encoding Val-136 may be a risk factor in some breeds [Laplanche et al. 1993b] and in experimental scrapie [Goldmann et al. 1991a], they are not a prerequi-
site; such alleles are absent from three autochthonous (indigenous) French breeds endemically affected with natural scrapie (Laplanche et al. 1993a).

By drawing upon a large sample comprising all histopathologically confirmed scrapie cases reported in the United States, we demonstrate predominance of the Suffolk breed. Whereas codon 136 Val alleles were found to be absent from these animals, a homozygous genotype at codon 171 was strongly correlated with disease development. These findings evoke themes from the work of both Parry and Dickinson and offer a striking parallel to sporadic Creutzfeldt-Jakob disease (CJD), a natural prion disease of humans (Palmer et al. 1991).

Results

Epidemiological aspects of natural scrapie in the United States

When data are compiled retrospectively to 1947, Suffolk sheep are found to account for 86.4 ± 1.0% of scrapie-affected animals (n = 1106). Similar results are obtained if this information is treated on a year-by-year basis, with figures for the last 2 years compiled being 81% and 72%, respectively. Hampshire sheep, genealogically related to Suffolk sheep (Hall and Clutton-Brock 1989) account for an additional 6% of cases. Although a large literature exists on the genetics of scrapie in Cheviot sheep, this breed comprises only 2% of cases in the United States. Within Suffolk sheep, disease onset was most common in 3- to 4-year-olds, in close accord with the incidence in the United Kingdom (Parry 1962). As there are no accurate figures available for the total number of Suffolk sheep in the United States, it is unclear whether this breed is especially susceptible to natural scrapie. However, there is a long-standing association between scrapie and Suffolk sheep, with disease documented in English Suffolks since the turn of the century and in Suffolks exported from the United Kingdom to Australia, Canada, and New Zealand (Parry 1983). The first three outbreaks in the United States were also traced to imported Suffolk sheep (Hourrigan et al. 1979). The propensity for natural scrapie in the United States to remain within the confines of the Suffolk breed is perhaps surprising given the accepted view of an infectious etiology, but may reflect unique features of husbandry, physiology, or genetics.

Structure of the sheep PrP gene

To define the structure of the wild-type sheep PrP gene, we prepared molecular clones from the brain DNA and RNA of a phenotypically normal 7-year-old Suffolk ewe. Two observations argue against the possibility that this animal represented a preclinical case of scrapie: (1) Only 3% of scrapie cases have an age of onset of 7 years or greater (Parry 1962); and (2) we were unable to detect PrPSc in brain samples from this animal under conditions where this macromolecule was easily detected in brains from scrapie cases (not presented). Also, oligonucleotide typing and sequence analysis defines this animal as a PrP codon 171 heterozygote (see below). Comparative mapping and sequence analysis of cosmids and cDNA clones from this animal reveals that the Suffolk sheep PrP gene contains three exons and extends over 20 kb of genomic DNA (Figs. 1 and 2).

Promoter/exon 1 The sheep PrP gene shares four motifs ~250 nucleotides upstream from the transcriptional start sites in common with the Syrian hamster, mouse, and human PrP genes (Fig. 2A; Westaway et al. 1994a). These motifs may correspond to binding sites for transcription factors. The sheep promoter diverges between these motifs and the mRNA start sites: this area of the gene includes an AP-2-like consensus sequence while Sp1 and AP-1 sites present in the rodent and human genes are absent.

Exons 2 and 3 Exon 2, encoding 98 nucleotides of the mRNA 5'-untranslated region, has no equivalent in the Syrian hamster and human PrP genes (Basler et al. 1986; Puckett et al. 1991), but a cognate is present in the mouse PrP gene (Westaway et al. 1994a). PrP-coding sequences in exon 3 are uninterrupted by introns, as first

Figure 1. Structure of the Suffolk sheep PrP gene. Exons are numbered. Within exon 3 the PrP ORF is shown by an open box and the mRNA 3'-untranslated region by hatching. Polymorphisms discussed in this paper are indicated with arrows. (S) SmaI. Bracketed areas A, B, and C correspond to sequences presented in Fig. 2.
we have not mapped the 3' terminus of exon 3, our reported by Goldmann and co-workers (1990). Although we have not mapped the 3' terminus of exon 3, our

Figure 2. Sequences of the sheep PrP gene deduced from a cosmId clone. Intron and 5'-flanking sequences are presented in lowercase letters. Intron/exon boundaries were assigned by comparison of genomic sequences and the sequence of four 5' CDNA clones, and are in accord with consensus splice donor and acceptor sites. The longest of these 5' CDNA clones was checked in a side-by-side comparison with primer-extended cDNAs. As expected, the strongest primer extension product migrated at the same position as the terminus of cDNA clone, that is, immediately adjacent to the beginning of the synthetic oligo[dC] tail. In turn, the authenticity of primer-extended sheep PrP cDNA was assessed using RNA from the brain of a Tg1855 transgenic mouse harboring the Shecos1 insert [Westaway et al. 1994b], as well as RNA from a non-Tg littermate (data not shown). [A] Promoter and exon 1. [A] The approximate positions of major transcriptional start sites (deduced using a heterologous sequencing ladder), [V] 5' termini of cDNA clones 3, 11, 12, and 4. cDNA clones 3 and 12 have a G residue at their 5' terminus that does not correspond to the genomic exon 1 sequence, and clone 11 has an extra AG dinucleotide at this position. Two upstream termination codons in the same phase as the PrP ORF are underlined. Sequences both 5' and 3' of exon 1 are G+C rich, and the frequency of GpC dinucleotides in this region approximates to that of GpC (1:1.2), a sequence composition associated with eukaryotic promoters (Bird 1986). In sequence alignments, exon 1 and adjacent sequences are most homologous to the human PrP gene (data not shown). This similarity is paralleled by a paucity of transcriptional start sites (2 and 1 in sheep and humans [Puckett et al. 1991], respectively, compared with the multiple initiation sites observed in rodent PrP genes [Basler et al. 1986, Westaway et al. 1987]). Four blocks of nucleotides conserved in all four PrP promoters sequenced to date are located —250 bp upstream of the transcriptional start sites [motifs 1–4 [Westaway et al. 1994a]]. The area 5' of the start sites includes a motif resembling an AP-2-binding site (AP-2-like).

Northern blot analyses [Westaway et al. 1994b] are in accord with previous results that the sheep PrP mRNA

CCAGGGAG and CCAGGGG, are also underlined. (B) Exon 2, three upstream termination codons in the same phase as the PrP ORF are underlined. This 98-nucleotide exon has no equivalent in the human and hamster PrP genes, but a related sequence is present in the mouse PrP gene [Westaway et al. 1994a]. (C) Exon 3: the PrP ORF is uninterrupted and is located at the 5' end of this exon. This ORF sequence, deduced from an asymptomatic 7-year-old ewe does not differ from a Gln-171 allele described previously (Goldmann et al. 1990), nor from eight alleles present in four scrapie-affected sheep (Table 1).
3'-untranslated region is significantly longer than that of the human and rodent genes (Goldmann et al. 1990).

**PrP gene polymorphisms in scrapie-affected sheep**

The *Sip* gene controls incubation time for experimental scrapie in Cheviot sheep (Dickinson et al. 1968). *Sip* alleles may also modulate manifestation of the natural disease (Foster and Dickinson 1988). As *Sip*, in turn, is thought to be linked to PrP (Hunter et al. 1989), we scrutinized two types of *Sip*-associated restriction fragment length polymorphisms (RFLPs; see Fig. 1) in four arbitrarily selected scrapie-affected Suffolk sheep drawn from flocks in Illinois, Michigan, and New Hampshire (Table 1). At least three of four of these cases were found to be related to other diseased animals, underscoring the propensity for natural scrapie to present in a familial manner. Detailed genealogical records were not available for the fourth animal, 27.

**Flanking region polymorphisms** In Cheviot sheep a 3.4-kb sheep PrP HindIII fragment is associated with the short incubation period allele of *Sip*, SA (Dickinson and Fraser 1979; Hunter et al. 1989). This fragment, corresponding to the presence of a polymorphic site in intron 2, was present in only two of four animals and was therefore excluded as a diagnostic marker (Table 1). All four scrapie-affected animals exhibited a 6.8-kb *EcoRI* *Sip* SA-associated fragment, though one also contained a 5.2-kb allele. However, the 6.8-kb RFLP is not unique to scrapie-affected sheep; 7/12 alleles examined by in healthy Suffolk sheep corresponded to this fragment. Similar results were obtained for animals in the United Kingdom where frequencies in unaffected animals of two Suffolk flocks were 32% and 39% (Hunter et al. 1991).

**Coding region polymorphisms** To date, Northern blot analysis and primer extension reactions (using an exon 3 primer) have failed to reveal unusual PrP transcripts in cerebellar RNA from the four scrapie-affected animals (not shown). Thus, alternative pathways of PrP mRNA maturation are unlikely to feature in the pathogenesis of natural scrapie. We therefore turned our attention to the PrP-coding region. The entire open reading frame (ORF) sequence for these animals was established by direct sequencing of polymerase chain reaction (PCR)-amplified brain DNA [Table 1]. No unusual missense polymorphisms were detected in the ORFs of the four scrapie-affected Suffolks when compared with the cosmids isolated from the phenotypically normal ewe (Fig. 2C) or to a previously published sequence (Goldmann et al. 1990). All four scrapie-affected animals were homozygous for Gln-171, although some PrP alleles are known to encode Arg at this position (Goldmann et al. 1990). The only silent replacements in the ORF were found in animal 15, which was heterozygous for changes at codons 231 (AGG → CGG) and 237 (CTC → CTG). This animal was also the only carrier of a 5.2-kb *EcoRI* fragment encompassing the ORF (Table 1). Negative controls included in our PCR experiments rule out the possibility that wild-type PrP-coding sequences were "carried over" into DNA from the scrapie-affected animals. Similarly, demonstration of heterozygosity for two silent substitutions in animal 15, as well as facile detection of codon 171 heterozygotes by oligonucleotide typing (Table 2) or direct sequencing (not presented), argues against the possibility that variant alleles are amplified inefficiently.

**PrP codon 171 genotype is related to scrapie susceptibility**

**Putative scrapie-free sheep** As PrP missense mutations are often correlated with susceptibility to prion disease, we established the frequency of the Gln (Q)-171 allele within a sample of 69 phenotypically normal sheep (Table 2). There are considerable difficulties in establishing animals as "scrapie-free." We note that flocks 1 and 2 were recommended to us by a breeder attuned to the problem of scrapie and are presumed, but not proven, to be scrapie-free. We also analyzed a putative scrapie-free flock from a research facility [flock 3]. In addition to being assembled from bloodlines thought to be free of scrapie, flock 3 was under continuous observation for a period of 5 years, and necropsies performed to date have excluded that intercurrent deaths were due to scrapie (J. Call, pers. comm.). To reduce further the risk of includ-

| Animala (no.) | Age at onset (year) | PrPsc | EcoRia | HindIIIa | Exon 3 coding sequence | Codon 171 |
|---------------|---------------------|-------|--------|----------|------------------------|-----------|
| 15            | 4.0                 | +     | 6.8 + 5.2 | 4.8 + 3.4 | wild typec            | Q/Q       |
| 27            | 4.0                 | + +   | 6.8     | 4.8 + 3.4 | wild type              | Q/Q       |
| 139           | 3.8                 | +     | 6.8     | 4.8      | wild type              | Q/Q       |
| 340           | 3.7                 | +     | 6.8     | 4.8      | wild type              | Q/Q       |

aNeuropathology for each animal is detailed in Materials and methods.
bExon 3 polymorphisms in kb.
cThis animal was heterozygous for silent replacements in codons 231 and 237 [see text].

962 GENES & DEVELOPMENT
years, mean±s.n. at death but the sample included one from 12 states. With regard to the ages of our scrapie-affected Suffolk sheep, most were <4 years (3.6±0.15 animals was drawn from a total of 23 flocks originating in Louisiana, Minnesota, Ohio, Oregon, Texas, Virginia, and Wisconsin. Thus, our sample of 31 scrapie-affected animals defined all as Q/Q homozygotes. These diseased animals were selected at random from recent scrapie outbreaks in California, Iowa, Louisiana, Minnesota, Ohio, Oregon, Texas, Virginia, and Wisconsin. Thus, our sample of 31 scrapie-affected animals was drawn from a total of 23 flocks originating from 12 states. With regard to the ages of our scrapie-affected Suffolk sheep, most were <4 years (3.6±0.15 years, mean±s.e.) at death but the sample included one 7-year-old and one adult of unspecified age.

The allelic composition of these scrapie-affected sheep was significantly different from that of the putative scrapie-free animals [Fisher’s “exact” test (Mehta and Patel 1983)]. In one instance, we were able to obtain blood samples from the phenotypically normal flockmates of scrapie-affected animals. R-171 alleles were present in many animals of this flock (flock 4, Table 2, f=0.23±0.08), and the distribution of genotypes was not significantly different from that found in the other healthy animals. Our findings therefore define a relationship between PrP Q/Q-171 genotype and disease susceptibility. Because the R/R-171 genotype is probably rare, the absence of such homozygotes from our sample of affected animals is not statistically significant. It will be necessary to analyze more animals to ascertain whether scrapie ever occurs in this genotype, and if heterozygosity for R-171 alleles prevents or merely delays clinical manifestation of scrapie.

Discussion

Mutations controlling initiation and propagation of prion diseases

The ability of natural scrapie to present both as an infectious disease and also as a monogenic trait perplexed and divided early investigators. With the benefit of hindsight this dualism can be seen to reflect the host-encoded origin of PrPSc, the major and potentially sole component of prions. Analysis of other prion diseases has confirmed the importance of host genotype and has defined two types of mutations that influence pathogenesis. The first type are dominant, linked to loci specifying disease, and correspond to rare nonconservative missense substitutions in the PrP ORF, for example, the PRNP

| Table 2. PrP codon 171 polymorphisms in Suffolk sheep |
|-----------------------------------------------|
| Animals | Codon 171 genotype [%] | Q/Q | Q/R | R/R | total |
| Putative scrapie-free* | | | | | |
| flock 1 | | 6 | 1 | 0 | 7 |
| flock 2 | | 6 | 10 | 1 | 17 |
| flock 3 | | 20 | 10 | 3 | 33 |
| Total | | 32 | 21 | 4 | 57 |
| All healthy animalsb | | 35 | 30 | 4 | 69 |
| Scrapie flock-matesc | | 35 | 30 | 4 | 69 |
| flock 4 | | 7 | 6 | 46 | 13 |
| Total | | 46 | 37 | 50 | 133 |
| (23 flocks of origin) | | 31 | 10 | 0 | 41 |

*aAll animals were 4 years or older at time of sampling.
*bAlso includes animal M132 and a Suffolk of unspecified age, both from northern California, and younger animals from flocks 1–3.
*cThe 13 phenotypically normal animals in flock 4 were flock-mates of scrapie-affected animals 139 and 340. Of these, all but one animal were 4 years or older.
*dIncludes four animals genotyped by direct sequencing of amplified genomic DNA. Val-136 alleles were absent from all 31 animals.

Scrapie-affected sheep Analysis of codon 171 in 27 additional scrapie-affected animals defined all as Q/Q homozygotes. These diseased animals were selected at random from recent scrapie outbreaks in California, Iowa, Louisiana, Minnesota, Ohio, Oregon, Texas, Virginia, and Wisconsin. Thus, our sample of 31 scrapie-affected animals was drawn from a total of 23 flocks originating from 12 states. With regard to the ages of our scrapie-affected Suffolk sheep, most were <4 years (3.6±0.15 years, mean±s.e.) at death but the sample included one 7-year-old and one adult of unspecified age.

The allelic composition of these scrapie-affected sheep was significantly different from that of the putative scrapie-free animals [Fisher’s “exact” test (Mehta and Patel 1983)]. In one instance, we were able to obtain blood samples from the phenotypically normal flockmates of scrapie-affected animals. R-171 alleles were present in many animals of this flock (flock 4, Table 2, f=0.23±0.08), and the distribution of genotypes was not significantly different from that found in the other healthy animals. Our findings therefore define a relationship between PrP Q/Q-171 genotype and disease susceptibility. Because the R/R-171 genotype is probably rare, the absence of such homozygotes from our sample of affected animals is not statistically significant. It will be necessary to analyze more animals to ascertain whether scrapie ever occurs in this genotype, and if heterozygosity for R-171 alleles prevents or merely delays clinical manifestation of scrapie.

Discussion

Mutations controlling initiation and propagation of prion diseases

The ability of natural scrapie to present both as an infectious disease and also as a monogenic trait perplexed and divided early investigators. With the benefit of hindsight this dualism can be seen to reflect the host-encoded origin of PrPSc, the major and potentially sole component of prions. Analysis of other prion diseases has confirmed the importance of host genotype and has defined two types of mutations that influence pathogenesis. The first type are dominant, linked to loci specifying disease, and correspond to rare nonconservative missense substitutions in the PrP ORF, for example, the PRNP

| Figure 3. Oligonucleotide typing of a Q/R polymorphism at PrP codon 171. Panels represent gridded filter arrays of duplicate amplified DNA samples probed with Q-171- or R-171-specific oligonucleotides. Six scrapie-affected animals are represented in positions 1A to 2C. The remaining samples correspond to animals from flock 3. Those samples hybridizing to both probes represent Q/R heterozygotes and can be seen in positions 3C, 4B and 4C, and 5B. R/R homozygotes are represented in positions 6C and 7A. |
Pro → Leu-102 substitution of Gerstmann–Sträussler syndrome [GSS] [Hsiao et al. 1989, 1990]. These mutations may initiate disease by inducing de novo prion biosynthesis: In mechanistic terms the mutant PrP molecules mimic or give rise to PrPSc–like molecules, which then template replicas of themselves using PrPC molecules as precursors. The second type of mutation corresponds to common missense polymorphisms within the PrP ORF. Normally phenotypically silent, in the event of prion infection these mutations modulate the tempo of disease: Alleles can be codominant or behave as recessives [Bruce and Fraser 1991]. These common polymorphisms probably affect the rate of prion replication, and examples include the scrapie incubation time genes Pni/sinc and Sip, associated with codon 108/189 and codon 136 PrP in mice and Cheviot sheep, respectively [Dickinson et al. 1968; Westaway et al. 1987; Goldmann et al. 1991a].

**Homzygosity for Q-171 PrP alleles predisposes to natural scrapie**

In comparisons with sequences from wild-type alleles [Fig. 2; Goldmann et al. 1990], the PrP-coding regions of three scrapie-affected animals with affected bloodstream relatives failed to reveal any unique missense mutations. We therefore conclude that familial natural scrapie is distinct mechanistically from GSS and familial Creutzfeldt–Jacob disease [CJD] (Brown et al. 1991; Hsiao et al. 1989, 1990). Unexpectedly, of 31 scrapie-affected animals, all were scored as Q/Q-171 homozygotes, a highly significant result given the presence of the R-171 allele in ~44% of putative scrapie-free animals [Table 2].

Could the homogeneity of PrP allelic type in scrapie-affected Suffolk sheep reflect mechanistic shortcomings? This seems unlikely. The PCR technique used here does not give information about gene copy number, but heterozygosity for flanking region polymorphisms [Table 1; Hunter et al. 1991] formally establishes that many scrapie-affected animals carry two copies of the PrP gene and that the perceived genotypic homogeneity of these animals is not attributable to allelic loss. “Carryover” of amplified Q/Q samples into DNAs from affected animals is excluded by analyses of negative controls lacking genomic DNA, as well as by facile detection of interspersed Q/R samples from healthy sheep. It is plausible that some R-171 alleles are not diagnosed because of base substitutions outside of codon 171, within the regions of homology to either the R-171 allele-specific oligonucleotide or the PCR primers. However, such hypothetical polymorphisms would introduce a systematic error affecting diagnosis of both scrapie-affected and control animals. Also, we did not detect substitutions adjacent to the CAG/CGG polymorphism at codon 171 in direct sequencing of amplified DNA from the four animals described in Table 1, nor in sequences from a scrapie-affected crossbred Suffolk [not presented]. We conclude that the Q/Q-171 genotype seen in all 31 scrapie-affected sheep does not reflect a diagnostic artifact.

It may be that most Suffolk flocks have a very low frequency of R-171 alleles and that the Q/Q genotype of scrapie-affected animals is therefore unexceptional. This scenario can be addressed by genotyping flock mates of affected animals. In practice, retrospective sampling of flocks is complicated by the U.S. Department of Agriculture policy of flock eradication. In one instance we were able to obtain samples from 13 flock mates of scrapie-affected animals 139 and 340 (flock 4, Table 2). Six of these animals contained R-171 alleles, and the distribution of genotypes in Flock 4 [Q/Q : Q/R : R/R] was not obviously different from that of the sum of Flocks 1–3 [P = 0.79 for the null hypothesis that genotypic distribution is the same, using Fisher’s exact test). Overall, our data indicate that the R-171 allele is routinely present in Suffolk flocks, with an allelic frequency of 0.27 ± 0.038 [± S.D., n = 138 alleles from 69 healthy animals]. We conclude, in turn, that over representation of the Q/Q-171 genotype in scrapie-affected animals is not a sampling artifact.

As Sip alleles may modulate natural scrapie in Cheviot/Suffolk crosses [Foster and Dickinson 1988], we also appraised three Sip sa/PrP markers. Two RFLPs flanking the PrP gene were imperfectly correlated with disease [Table 1; Fig. 1], and by DNA sequencing we were unable to detect Val-136 coding region alleles [n = 8, Table 1, Goldmann et al. 1991a]. The analysis of Val-136 alleles was extended by challenging amplified PrP-coding sequences with the restriction endonuclease BspHI. To date we have been unable to detect Val-136 alleles in any of our scrapie-affected animals [0/62 alleles tested]. Because we now know that only one of three PrP-coding region alleles present in Cheviot sheep has a counterpart in the Suffolk breed, the imperfect correlation between Sip-associated markers and scrapie susceptibility in Suffolk sheep is perhaps unsurprising [Goldmann et al. 1990, 1991a]. On the other hand, comparison of the 31 scrapie-affected animals with animals from flocks 1–3 yields a probability of 0.00004 for the null hypothesis that PrP codon 171 genotype is unrelated to disease incidence. Thus, our data closely conform to the hypothesis that Suffolk sheep harbor a recessive incubation time gene that is closely associated with the PrP Q-171 polymorphism.

**Codon 171: possible mechanisms and a parallel to sporadic CJD**

It may be that the PrP codon 171 polymorphism is phenotypically silent, but in linkage disequilibrium with a susceptibility mutation, for example, a mutation affecting levels of PrP mRNA. However several precedents argue for a direct impact of codon 171 on the protein–protein interactions equated with prion replication. Absence of R-171 alleles from affected animals indicates that these alleles impede scrapie pathogenesis and are compatible with observations in Ile-de-France sheep. Whereas heterozygosity or homozygosity at PrP Val-136 appears crucial for natural scrapie to develop in this breed, age at disease onset was nearly doubled in V/A-
lems with Parry’s theory concern the gene frequency for s and reports of lateral transmission (Dickinson et al. 1988; Foster and Dickinson 1991), the shared requirement for PrP homozygosity most likely reflects a common mechanism for replication of scrapie and CJD prions. This is in accord with transgenic analyses of prion replication that implicate interactions between PrP Sc and PrP Sc molecules of the same amino acid sequence (Prusiner et al. 1990; Scott et al. 1993). These studies, as well as the behavior of mice hemizygous for the PrP gene, suggest that sheep prions may replicate less efficiently in Q/R heterozygotes because the concentration of Q-171PrP Sc is reduced by approximately half compared with Q/Q homozygotes (Collinge and Palmer 1992, Büeler et al. 1993; Prusiner et al. 1993). Another possibility is that the R-171 substitution reduces the stability of PrP Sc.

The “recessive-gene hypothesis” revisited

The correlation between homozygosity at codon 171 and the development of natural scrapie brings to mind Parry’s recessive gene hypothesis. The observation that natural scrapie in endemically affected Suffolk flocks manifests in a recessive manner seems sound (Parry 1962; Dickinson et al. 1965; Brown et al. 1987; Harries-Jones et al. 1988; Westaway et al. 1989, 1994b; Prusiner 1991), the shared requirement for PrP homozygosity most likely reflects a common mechanism for replication of scrapie and CJD prions. This is in accord with transgenic analyses of prion replication that implicate interactions between PrP Sc and PrP Sc molecules of the same amino acid sequence (Prusiner et al. 1990; Scott et al. 1993). These studies, as well as the behavior of mice hemizygous for the PrP gene, suggest that sheep prions may replicate less efficiently in Q/R heterozygotes because the concentration of Q-171PrP Sc is reduced by approximately half compared with Q/Q homozygotes (Collinge and Palmer 1992, Büeler et al. 1993; Prusiner et al. 1993). Another possibility is that the R-171 substitution reduces the stability of PrP Sc.

What are the practical consequences of our findings? Diverged sequences within the sheep PrP gene promoter suggest that the tissue tropism of PrP Sc expression may differ from rodent counterparts. Whether such differences in tropism could account for the muted ability of natural scrapie to spread by horizontal and vertical infection is unknown (Dickinson et al. 1965; Westaway et al. 1992).

We can now envisage a genetic approach to the eradication of natural scrapie. We suggest selective breeding for an enhanced frequency of Q/R heterozygotes as an avenue worthy of investigation. Values for Q/Q, Q/R, and R/R genotypes in flocks 1–3 are compatible with the hypothesis that the Q and R alleles are at equilibrium [predicted and observed genotypes Q/Q : Q/R : R/R using the Hardy–Weinberg equation = 31.7 : 21.6 : 3.7 and 32 : 21 : 4, respectively], and by implication that neither allele is associated with a selective advantage in vivo. This is not in conflict with the hypothesis that PrP [R-171] alleles heighten scrapie resistance, as the disease is probably too rare and has an onset too late in life to shape allele frequencies in the general population. Potential caveats to breeding for elevated frequencies of the R-171 allele concern incompletely defined penetrance, emergence of prion isolates tropic for this allele, and the possibility that R/R as well as Q/Q homozygotes are susceptible to scrapie. Another approach to disease eradication is to generate livestock deleted for the PrP gene [Notariani et al. 1991; Büeler et al. 1992, 1993, Prusiner et al. 1993]. Given that cases of natural scrapie may have already given rise to one disastrous epidemic in the form of bovine spongiform encephalopathy [Wilesmith 1992], it may be prudent to pursue both of these strategies.

PrP genotypic effects described here extend the central role for PrP molecules in the pathogenesis of natural scrapie. The impact of PrP allelic composition not only parallels CJD and experimental scrapie but can account for long-standing observations that correlate disease outbreaks with inbreeding and a recessive susceptibility gene (Parry 1983). Our studies indicate that pedigree Suffolk sheep are less heterogeneous regarding PrP genotype than other breeds examined to date (Goldmann et al. 1991a, Laplanche et al. 1993b), which exhibit up to five
Animals for DNA sequencing studies (diagnosed positive for PrP Sc by Western blotting; Table 1) were selected at random from out-breaking scrapie. His sire, a 6-year-old, was apparently normal at time of sacrifice; all four pathological changes were found in the medulla. Ewe 340 from Illinois may also represent a familial instance of scrapie-positive flock. Ewe 27 from Michigan was 4 years old at onset of clinical signs and exhibited the four (Race et al. 1992) pathological changes used for diagnosis of scrapie at the National Veterinary Services Laboratories at Ames, Iowa. These are vacuolated neurons, spongiform change, neuronal necrosis, and astrocytosis. Her sire and dam, both 3 years old at time of culling, were symptomatic for scrapie, although the sire was from a certified scrapie-positive flock.

Ewe 15 from New Hampshire was 4 years old at the time of disease onset. Mild neuropathologic lesions were restricted to the medulla. Ram 139 from Illinois was 3 years and 10 months at time of sacrifice. This animal may reflect a familial manifestation of scrapie. His sire, a 6-year-old, was apparently normal at the time of death but came from a flock that was suspected of having endemic scrapie. The dam of ram 139 died between 3 and 4 years, of an undiagnosed complaint. All four pathological parameters were present in the caudal medulla of ram 139, but only mild spongiform change was visible in sections of the mesencephalon. Gliosis was generalized throughout the brain stem.

Ewe 340 from Illinois may also represent a familial instance of scrapie. The sire died between ages 3 and 4 for unknown reasons, and the parental flock was dispersed in 1991 under suspicion of endemic scrapie. Ewe 340 was 3 years and 8 months old at time of sacrifice; all four pathological changes were found in the thalamus and hypothalamus, and milder pathological changes were found in the midbrain. Animals 139 and 340 originate from flock 4 (Table 1). Twenty-seven additional histopathologically confirmed Suffolk cases were typed for the codon 171 polymorphism (see below). The presence of PrP Sc in the CNS was verified for all but one of these animals, either by dot-blotting (Serban et al. 1990) or immunohistochemistry (Miller et al. 1994). The final sample of 31 scrapie-affected animals was drawn from 23 flocks originating from 12 states.

Genomic DNA clones

A 7-year-old clinically normal Suffolk ewe [identification number M132] was sacrificed by pentobarbitol injection. The brain was immediately removed, sectioned coronally, and frozen on dry ice. A 1-gram biopsy was thawed and used to prepare genomic DNA by dialysis (Little 1987). This DNA was partially digested with the enzyme MboI, and low-molecular-weight DNA contaminants were removed by centrifugation on a 10-40% sucrose density gradient (Maniatis et al. 1978). DNA was pooled, concentrated by ethanol precipitation, and treated with bacterial alkaline phosphatase (Ish et al. 1981). Dephosphorylation was monitored by a decrease in TCA-precipitable radioactive counts from a 32P-labeled oligonucleotide tracer. After phospho-extraction the target DNA was precipitated and ligated to BamHI digested pWE15 vector (Wahl et al. 1987). The ligation products were packaged in vitro and plated on the McrA-, McrB- host Escherichia coli bacterial strain ED8767 (Raleigh et al. 1988). Screening of 60,000 ampicillin-resistant colonies using a PrP ORF hybridization probe yielded one positive clone, corresponding to the Q allele. Both DNA sequencing and oligonucleotide typing of amplified genomic DNA defined animal M132 as a Q/R-171 heterozygote.

cDNA—PCR cloning

A cortical biopsy from the M132 animal was used to prepare total RNA by the method of Chomczynski and Sacchi (1987). This was converted to cDNA by priming with random hexamer oligonucleotides (PL-Pharmacia), spermine precipitated, and then "tailed" with dG residues. The tailed cDNA was then diluted and amplified using a dC-tailed "anchor" primer as well as an exon 3-specific primer (5'-ACATCTGGCTCCACCA-CGGCC). The resulting heterogeneously sized cDNAs were subjected sequentially to a further two rounds of PCR using nested exon 3 primers, 5'-CACTGCGTGCACAAAGTTGTTTG and 5'-GATCCACTGGCTCTTGTTG. Size-fractionated products of the size anticipated from primer extension reactions [see legend to Fig. 1] were subcloned into pBluescript vector (Stratagene, San Diego, CA) linearized with Smal. The inserts of four plasmid clones were sequenced in their entirety.

Primer extension of PrP mRNA

Primer extension was performed as described previously [Westaway et al. 1987] using a primer 5'-GATCCAACTGGCTAT- GGTGCT and 5'-GATCCACTGGCTCTTGTTG. Size-fractionated products of the size anticipated from primer extension reactions [see legend to Fig. 1] were subcloned into pBluescript vector (Stratagene, San Diego, CA) linearized with Smal. The inserts of four plasmid clones were sequenced in their entirety.

Southern and Northern blotting

Blotting to nitrocellulose or to supported nitrocellulose [Schleicher & Schuell, Keene, NH] was as described previously [Westaway et al. 1991].

DNA sequence analysis

ORF sequences of scrapie-affected animals were determined directly from PCR-amplified genomic DNA. The two oligonucleotide primers for PCR, 5'-AGTGGACGGCATTTGATGC- TGAACC and 5'-TGGTACCACTACAGGGCTGAGTTAGAC, incorporate synthetic SaII and KpmI sites (underlined) and are located in intron 2 and the mRNA 5'-untranslated region, respectively (Boehringer Mannheim, Indianapolis, IN, or SuperScript, Gibco-BRL, Gaithersberg, MD).

Southern and Northern blotting

Blotting to nitrocellulose or to supported nitrocellulose [Schleicher & Schuell, Keene, NH] was as described previously [Westaway et al. 1991].
pol system, as recommended by the manufacturer (Clontech, Palo Alto, CA). Exon 1 and 2 sequences were determined using cosmids subclones corresponding to 2.7-kb HindIII–BamHI and 0.86-kb BamHI–XhoI fragments, respectively.

**Diagnosis of PrP codon 136 and 171 polymorphisms**

Allele-specific oligonucleotides, 5'-GTGGATCGGTATAGT-3' and 5'-GTGGATCAGTATAGT-3', specific for Arg or Gln, respectively, at codon 171 were hybridized to PCR-amplified genomic DNA immobilized on nylon or nitrocellulose membranes, as described previously (Westaway et al. 1987). Final wash temperatures were approximated from the method of Suggs et al. (1981) and then defined empirically at 43°C using molecular clones of Arg- and Gln-171 alleles retrieved from the same chromosomal gene. Cell 46: 417–428.

Bird, A.P. 1986. CpG-rich islands and the function of DNA methylation. Nature 321: 209–213.

Borchelt, D.R., M. Scott, A. Taraboulos, N. Stahl, and S.B. Prusiner. 1990. Scrapie and cellular prion proteins differ in their kinetics of synthesis and topology in cultured cells. J. Cell Biol. 110: 743–752.

Borchelt, D.R., A. Taraboulos, and S.B. Prusiner. 1992. Evidence for synthesis of scrapie prion proteins in the endocytic pathway. J. Biol. Chem. 267: 6188–6199.

Brown, P., F. Cathala, R.F. Raubertas, D.C. Gajdusek, and P. Caistagne. 1987. The epidemiology of Creutzfeldt-Jakob disease: Conclusion of 15-year investigation in France and review of the world literature. Neurology 37: 895–904.

Brown, P., L.G. Goldfarb, and D.C. Gajdusek. 1991. The new biology of spongiform encephalopathy: infectious amyloidoses with a genetic twist. Lancet 337: 1019–1022.

Bruce, M.E. and H. Fraser. 1991. Scrapie strain variation and its implications. Curr. Top. Microbiol. Immunol. 172: 125–138.

Büeler, H., M. Fischer, Y. Lang, H. Bluthmann, H.-P. Lipp, S.J. DeArmond, S.B. Prusiner, M. Aguet, and C. Weissmann. 1992. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 356: 577–582.

Büeler, H., A. Aguzzi, A. Sailer, R.-A. Greiner, P. Autenried, M. Aguet, and C. Weissmann. 1993. Mouse devoid of PrP are resistant to scrapie. Cell 73: 1339–1347.

Casanova, J.L., C. Pannetier, C. Jaulin, and P. Kourilsky. 1990. Optimal conditions for directly sequencing double-stranded PCR products with sequenase. Nucleic Acids Res. 18: 4028.

Caughey, B. and G.J. Raymond. 1991. The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. J. Biol. Chem. 266: 18217–18223.

Caughey, B., R.E. Race, D. Ernst, M.J. Buchmeier, and B. Chesbro. 1989. Prion protein biosynthesis in scrapie-infected and uninfected neuroblastoma cells. J. Virol. 63: 175–181.

Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162: 156–159.

Collinge, J. and M.S. Palmer. 1992. Molecular genetics of inherited, sporadic and iatrogenic prion disease. In Prion diseases of humans and animals (ed. S.B. Prusiner, J. Collinge, J. Powell, and B. Anderton), pp. 93–119. Ellis Horwood, London, UK.

Cuillé, J. and P.L. Chelle. 1939. Experimental transmission of trembling to the goat. C.R. Seances Acad. Sci. 208: 1058–1060.

Dickinson, A.G. and H. Fraser. 1979. An assessment of the genetics of scrapie in sheep and mice. In Slow transmissible diseases of the nervous system, Vol. 1 (ed. S.B. Prusiner and W.J. Hadlow), pp. 367–386. Academic Press, New York.

Dickinson, A.G. and G.W. Outram. 1988. Genetic aspects of unconventional virus infections: The basis of the virino hypothesis. Ciba Found. Symp. 135: 63–83.

Dickinson, A.G., G.B. Young, J.T. Stamp, and C.C. Renwick. 1965. An analysis of natural scrapie in Suffolk sheep. Heredity 20: 485–503.

Dickinson, A.G., V.M.H. Meikle, and H. Fraser. 1968. Identi-
cation of a gene which controls the incubation period of some strains of scrapie agent in mice. *J. Comp. Pathol.* 78: 293–299.

Dickinson, A.G., J.T. Stamp, and C.C. Renwick. 1974. Maternal and lateral transmission of scrapie in sheep. *J. Comp. Pathol.* 84: 19–25.

Farquhar, C.F., R.A. Somerville, and L.A. Ritchie. 1989. Post-mortem immunodiagnosis of scrapie and bovine spongiform encephalopathy. *J. Virol. Methods* 24: 215–222.

Foster, J.D. and A.G. Dickinson 1988. Genetic control of scrapie in Cheviot and Suffolk sheep. *Vet. Rec.* 123: 159.

Goldmann, W., N. Hunter, J.D. Foster, J.M. Salbaum, K. Beyreuther, and J. Hope 1990. Two alleles of a neural protein gene linked to scrapie in sheep. *Proc. Natl. Acad. Sci. USA* 87: 2476-2480.

Goldmann, W., N. Hunter, G. Benson, J.D. Foster, and J. Hope. 1991a. Different scrapie-associated fibril proteins (PrP) are encoded by lines of sheep selected for different alleles of the *Sip* gene. *J. Gen. Virol.* 72: 2411–2417.

Goldmann, W., N. Hunter, T. Martin, M. Dawson, and J. Hope 1991b. Different forms of the bovine PrP gene have five or six copies of a short, C-G-rich element within the protein-coding exon. *J. Gen. Virol.* 72: 201–204.

Hadlow, W.J., R.C. Kennedy, and R.E. Race. 1982. Natural infection of Suffolk sheep with scrapie virus. *J. Comp. Pathol.* 124: 1515–1522.

Hall, S.J.G. and J. Clutton-Brock. 1989. Two hundred years of British farm livestock. British Museum (Natural History), London, UK.

Harries-Jones, R., R. Knight, R.G. Will, S. Cousens, P.G. Smith, and W.B. Matthews. 1988. Creutzfeldt-Jakob disease in England and Wales, 1980–1984: a case-control study of potential risk factors. *J. Neurol. Neurosurg. Psychiatry* 51: 1113–1119.

Hourgirg, J., A. Klinghorns, W.W. Clark, and M. de Camp. 1990. Choice and use of cosmid vectors. In Dev. *Nucleic Acids Res.* 15: 329–342.

Hsiao, K., H.F. Baker, T.J. Crow, M. Poulter, F. Owen, J.D. Tarrant, D. Westaway, J. Ott, and S.B. Prusiner. 1990. Linkage of the gene for the scrapie-associated fibril protein (PrP) to the Sip gene in Cheviot sheep. *J. Vet. Diagn. Invest.* 2: 447–449.

Hunter, N.D. Foster, A.G. Dickinson, and J. Hope. 1989. Linkage of the gene for the scrapie-associated fibril protein (PrP) to the Sip gene in Cheviot sheep. *Vet. Rec.* 124: 364–366.

Hunter, N. and J.D. Foster. 1991. Restriction fragment length polymorphisms of the scrapie-associated fibril protein (PrP) gene and their association with susceptibility to natural scrapie in British sheep. *J. Gen. Virol.* 72: 1287–1292.

Ish, N., D. Horowicz, and J.F. Burke. 1981. Rapid and efficient cosmids cloning. *Nucleic Acids Res.* 9: 2989–2998.

Katz, J.B., J.C. Pedersen, A.L. Jenny, and W.D. Taylor. 1992. Assessment of Western immunoblotting for confirmatory diagnosis of ovine scrapie and bovine spongiform encephalopathy. *J. Vet. Diagn. Invest.* 4: 447–449.

Laplancher, J.L. Chatalet, P. Beaudry, M. Dussaucy, C. Bouneau, and J. Launay. 1993a. French autochthonous scrapie sheep without the 136Val PrP polymorphism. *Mammalian Genome* 4: 463–464.

Laplancher, J.L., J. Chatalet, D. Westaway, S. Thomas, M. Dussaucy, J. Brugere-Picox, and J.M. Launay. 1993b. PrP polymorphisms associated with natural scrapie discovered by de-naturing gradient gel electrophoresis. *Genomics* 15: 30–37.

Little, P.F.R. 1987. Choice and use of cosmid vectors. In *DNA cloning*, Vol. III (ed. D.M. Glover), pp. 19–42. IRL Press, Oxford, UK.

Maniatis, T., R.L. Hardison, R. Lacy, C. Laver, C. O'Cormell, D. Quon, G.K. Sim, and A. Elstratiadis. 1978. Isolation of structural genes from libraries of eukaryotic DNA. *Cell* 15: 687.

Mehta, C.R. and N.R. Patel. 1983. A network algorithm for the exact treatment of Fisher's exact test in RxC contingency tables. *J. Am. Stat. Assoc.* 78: 427–434.

Miller, J.M., A.L. Jenny, W.D. Taylor, R.E. Race, D.R. Ernst, J.B. Kor, and R. Rubenstein. 1994. Detection of prion protein in formalin-fixed brain by hydrolyzed autoclaving, and immunohistochemistry for the diagnosis of scrapie in sheep. *J. Vet. Diagn. Invest.* in press.

Mitchell, P.J., C. Wang, and R. Tijan. 1987. Positive and negative regulation of transcription in vitro: enhancer-binding protein AP-2 is inhibited by SV40 T antigen. *Cell* 50: 847–861.

Notarianni, E., C. Galli, S. Laurie, R.M. Moor, and M.J. Evans. 1991. Derivation of pluriotent, embryonic cell lines from the pig and sheep. *J. Reprod. Fertil. 43: 255–260.

Oesch, B., D. Westaway, and S.B. Prusiner. 1991. Prion protein genes: Evolutionary and functional aspects. *Curr. Top. Microbiol. Immunol.* 172: 109–124.

Palmer, M.S., A.J. Dryden, J.T. Hughes, and J. Collinge. 1991. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* 352: 340–342.

Pan, K.-M., M. Baldwin, J. Nuyen, M. Gasset, A. Serban, D. Groth, I. Mehlhorn, Z. Huang, R.J. Fletterick, F.E. Cohen, and S.B. Prusiner. 1993. Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. *Proc. Natl. Acad. Sci.* 90: 10962–10966.

Parry, H.B. 1960. Scrapie: A transmissible hereditary disease of sheep. *Nature* 185: 441–443.

———. 1962. Scrapie: A transmissible and hereditary disease of sheep. *Hereditas* 17: 75–105.

———. 1983. Scrapie disease in sheep, (ed. D.R. Oppenheimer). Academic Press, New York.

Pattison, I.H., M.N. Hoare, J.N. Jebbett, and W.A. Watson. 1972. Spread of scrapie to sheep and goats by oral dosing with foetal membranes from scrapie-affected sheep. *Vet. Rec.* 90: 465–468.

———. 1974. Further observations on the production of scrapie in sheep by oral dosing with foetal membranes from scrapie-infected sheep. *Br. Vet. J.* 130: 1x–1xvii.

Prusiner, S.B. 1991. Molecular biology of prion diseases. *Science* 252: 1515–1522.

Prusiner, S.B. and K.K. Hsiao. 1994. Human prion diseases. *Ann. Neurol.* in press.

Prusiner, S.B., M. Scott, D. Foster, K.-M. Pan, D. Groth, C. Mirenda, M. Torchia, S.-L. Yang, D. Serban, G.A. Carlson, P.C. Hoppe, D. Westaway, and S.J. DeArmond. 1990. Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* 63: 673–686.

Prusiner, S.B., D. Groth, A. Serban, R. Koehler, D. Foster, M. Torchia, D. Burton, S.-L. Yang, and S.J. DeArmond. 1993. Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. *Proc. Natl. Acad. Sci.* 90: 10608–10612.

Puckett, C., P. Concannon, C. Casey, and L. Hood. 1991. Genomic structure of the human prion protein gene. *Am. J. Hum. Genet.* 49: 320–329.
Race, R., D. Ernst, A. Jenny, W. Taylor, D. Sutton, and B. Caughey. 1992. Diagnostic implications of detection of proteinase K-resistant protein in spleen, lymph nodes, and brain of sheep. Am. J. Vet. Res. 53:883–889.

Raleigh, E.A., N.E. Murray, H. Revel, R.M. Blumenthal, D. Westaway, A.D. Reith, P.W.J. Rigby, J. Elhai, and D. Hanahan. 1988. McrA and McrB restriction phenotypes of some Escherichia coli strains and implications for gene cloning. Nucleic Acids Res. 16:1563–1575.

Rubenstein, R., P.A. Merz, R.J. Kascsak, R.L. Carp, C.L. Scalici, C.L. Fama, and H.M. Wisniewski. 1987. Detection of scrapie-associated fibrils (SAF) and SAF proteins from scrapie-affected sheep. J. Infect. Dis. 156:36–42.

Scott, M., D. Groth, D. Foster, M. Torchia, S.-L. Yang, S.J. DeArmond, and S.B. Prusiner. 1993. Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. Cell 73:979–988.

Serban, D., A. Taraboulos, S.J. DeArmond, and S.B. Prusiner. 1990. Rapid detection of Creutzfeldt-Jakob disease and scrapie prion proteins. Neurology 40:110–117.

Stahl, N., D.R. Borchelt, K. Hsiao, and S.B. Prusiner. 1987. Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 51:229–240.

Stahl, N., M.A. Baldwin, D.B. Teplow, L. Hood, B.W. Gibson, A.L. Burlingame, and S.B. Prusiner. 1993. Structural analysis of the scrapie prion protein using mass spectrometry and amino acid sequencing. Biochemistry 32:1991–2002.

Suggs, S.V., R.B. Wallace, T. Hirose, E.H. Kawashima, and K. Itakura. 1981. Use of synthetic oligonucleotides as hybridization probes: isolation of cloned cDNA sequences for human β2-microglobulin. Proc. Natl. Acad. Sci. 78:6613–6617.

Wahl, G.M., K.A. Lewis, J.C. Ruiz, B. Rothenberg, J. Zhao, and G.A. Evans. 1987. Cosmid vectors for rapid genomic walking, restriction mapping, and gene transfer. Proc. Natl. Acad. Sci. 84:2160–2164.

Westaway, D., P.A. Goodman, C.A. Mirenda, M.P. McKinley, G.A. Carlson, and S.B. Prusiner. 1987. Distinct prion proteins in short and long scrapie incubation period mice. Cell 51:651–662.

Westaway, D., G.A. Carlson, and S.B. Prusiner. 1989. Unraveling prion diseases through molecular genetics. Trends Neurosci. 12:221–227.

Westaway, D., C.A. Mirenda, D. Foster, Y. Zebarijadian, M. Scott, M. Torchia, S.-L. Yang, H. Serban, S.J. DeArmond, C. Elston, S.B. Prusiner, and G.A. Carlson. 1993. Paradoxical shortening of scrapie incubation times by expression of prion protein transgenes derived from long incubation period mice. Neuron 7:59–68.

Westaway, D., S. Neuman, V. Zuliani, C. Mirenda, D. Foster, L. Detwiler, G. Carlson, and S.B. Prusiner. 1992. Transgenic approaches to experimental and natural prion diseases. In Prion diseases of humans and animals [ed. S.B. Prusiner, J. Collinge, J. Powell, and B. Anderton], pp. 474–482. Ellis Horwood, London, UK.

Westaway, D., C. Cooper, S. Turner, M. Da Costa, G.A. Carlson, and S.B. Prusiner. 1994a. Structure and polymorphism of the mouse prion protein gene. Proc. Natl. Acad. Sci. [in press].

Westaway, D., S.J. DeArmond, J. Cayetano-Canlas, D. Groth, D. Foster, S.-L. Yang, M. Torchia, G.A. Carlson, and S.B. Prusiner. 1994b. Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. Cell 76:117–129.

Wilesmith, J.W. 1992. Bovine spongiform encephalopathy: a brief epidemiography, 1985–1991. In Prion diseases of humans and animals [ed. S.B. Prusiner, J. Collinge, J. Powell, and B. Anderton], pp. 243–255. Ellis Horwood, London, UK.

Williams, T. and R. Tjian. 1991. Analysis of the DNA-binding and activation properties of the human transcription factor AP-2. Genes & Dev. 5:670–682.
Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie.

D Westaway, V Zuliani, C M Cooper, et al.

*Genes Dev.* 1994, 8:
Access the most recent version at doi:10.1101/gad.8.8.959