Mammalian Genome 9, 853–855 (1998).

Mammalian Genome
Incorporating *Mouse Genome*

Comparative mapping of the prion gene (PRNP) locus in cattle, sheep and human with PCR-generated probes

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Received: 13 April 1998 / Accepted: 29 May 1998

Prions are the unconventional causative agent of sub-acute transmissible spongiform encephalopathies in human and various mammals; for example, Scrapie of sheep, Bovine Spongiform Encephalopathy (BSE) of cattle, Cruetzfeldt-Jakob disease (CJD) and Fatal Familial Insomnia (FFI) of human (Prusiner 1994). These diseases are characterized by the accumulation in the brain tissue of a prion protein, PrP^{Sc} that is a partially proteinase-resistant isoform of the cellular protein, PrP^{c}. Prions are the product of a single gene that is highly conserved in mammals. Hamster prion gene is composed of two exons; the entire coding region of the gene is contained within the second exon (Goldfarb et al. 1991). On the contrary, the genes of human, cattle, sheep, rat, and mouse have three exons, with the entire protein-coding region contained within exon 3 (Inoue et al. 1997). Cytogenetic analysis of the prion genes has been reported in human, where the gene has been localized on HSA 20p12-pter by a combination of somatic cell and in situ hybridization (Sparkes et al. 1986). In cattle, the gene has been mapped to syntenic group U11 (BTA 13) with a panel of bovine–rodent hybrid somatic cells (Ryan and Womack 1993; Hawkins et al. 1995) and has been recently incorporated into a radiation hybrid framework map of BTA 13 (Shläpfer et al. 1997). Here we report the direct localization of the PrP gene in cattle, sheep, and human by means of fluorescence in situ hybridization (FISH) with PCR-generated probes.

Three pairs of oligonucleotides were designed, spanning the protein coding region to the 3′ UTR region, on the basis of available sequences in cattle, sheep, and human (Yoshimoto et al. 1992, GenBank accession number D10612, nucleotides 153–1723; Goldmann et al. 1990, accession number M31313, nucleotides 61–1651; Puckett et al. 1991, accession number X83416, nucleotides 1–2131). The primer sequences were 5′ GAAGTCATCATGGTGAAAAGC 3′/5′ TCACATCTCTAAACAATGTCAAA 3′ (cattle), 5′ GAAGTCATCATGGTGAAAAGC 3′/5′ AAGCATGAACTCTTCAGCACT 3′ (sheep), 5′ TTTTGCAGAGCAGTCATTAT 3′/5′ AATTTCAGTCAGATATTAAACATT 3′ (human), and produced PCR products of 1570, 1590, and 2130 bp, respectively. Amplifications were done on 50 ng of genomic DNA, in the presence of 10 pmoles of each primer, 0.2 mM dNTPs, 1.5 mM of $MgCl_2$. Thermal profiles were: 94°C × 5 min, 35 cycles of 94°C × 30 s, 54°C × 60 s, 72°C × 90 s, and 72°C × 10 min. The amplified fragments were run on agarose gels, purified, and sequenced to confirm their identity.

In addition, oligonucleotide primers were synthesized that amplify a 738-bp product corresponding to the ORF region of the human PrP gene (X83416, nucleotides 1–738), which was used in the comparative FISH mapping. The primer sequences were 5′ TTTTGCAGAGCAGTCATTAT 3′ and 5′ TCATCCCACTATCAGGAAGA 3′. PCR conditions were as above except for the thermal profile: 94°C × 2 min, 35 cycles of 94°C × 30 s, 59°C × 45 s, 72°C × 1 min, and 72°C × 10 min.

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All the probes were either nick-translated or directly labeled by PCR in the presence of biotin 16-dUTP and digoxigenin 11-dUTP (Boehringer; Richard et al. 1994) with the above described primers.

Chromosome preparations were arranged from fibroblast cultures by standard procedures, except for the hypotonic treatment, 0.02 M KCl, 37°C, 13 min (bovine and ovine). After a few days at −20°C, the slides were stained with 0.005% Quinacrine Mustard for 10 s (QFQ-banding), and well-spread metaphases, with distinctive banding, were imaged on a Leitz Aristoplan microscope connected to a CCD-camera (Photometrics) controlled by a Macintosh Quadra 950 computer. The probes generated by PCR were hybridized in situ to metaphase chromosomes at a final concentration of 0.5 ng/μl (5 ng/slide) in the presence of 3 μg of Cot-1 DNA, whereas nick-translated probes were used as described in Mezzelani and coworkers (1995). For two-color FISH, the probes were detected via avidin-conjugated FITC (Vector) and anti-digoxigenin-rhodamine (Boehringer). DAPI was used to counterstain the chromosomes. Digitized images were taken separately for each fluorochrome and merged with the software IPIab Spectrum MultiProbe (Signal Analytics) and Gene Join (Office of Cooperative Research, Yale University).

The results of the hybridization of the PrP genes on bovine, ovine, and human chromosomes are shown in Fig. 1. The bovine and ovine PrP genes were mapped on the chromosomes of the two species either as single probes (Fig. 1a–d) or cohybridized in a dual-color FISH experiment (Fig. 1g–j). Fifty metaphase spreads were examined in both cases, with 70% showing a specific signal on at least one of the homologous chromosomes. The intensity of the hybridization signal was weak in the case of nick-translated probes; a much stronger signal was obtained when the same probes were directly labeled by PCR (Richard et al. 1994). The chromosomal assignment for the *PrP* gene was 13q17 in cattle and 13q17/q18 in sheep (Fig. 1a–d). The ovine and bovine hybridization spots on the same metaphases overlapped, as anticipated from the high conservation of the loci in the two species (Fig. 1g–j). FISH with the human probe extending to the 3′ UTR region showed that the PRNP gene is located on HSA 20p12/p13 (Fig. 1e, f). The data are in agreement with the results of Sparkes et al. (1986), who mapped the gene to 20p12-pter. Of 55 metaphases examined, 60% exhibited a specific signal on one or both of the homologous chromosomes. The regional localization of the probes was established by determining the FLcen values on cattle and sheep chromosomes (0.55 ± 0.07 and 0.46 ± 0.06, respectively) and FLpter on human chromosomes (0.14 ± 0.04), as described by Lichter and colleagues (1990). In an additional experiment, the protein coding region of the human PrP gene, that is, the 738-bp fragment described above, was used as a hybridization probe on sheep and cattle chromosomes. Thirty bovine and ovine metaphases were examined, with 30% showing specific spots on at least one of the two Chromosomes (Chrs) 13 (Fig. 1k–n). The low efficiency of this comparative FISH was probably owing to the very small size

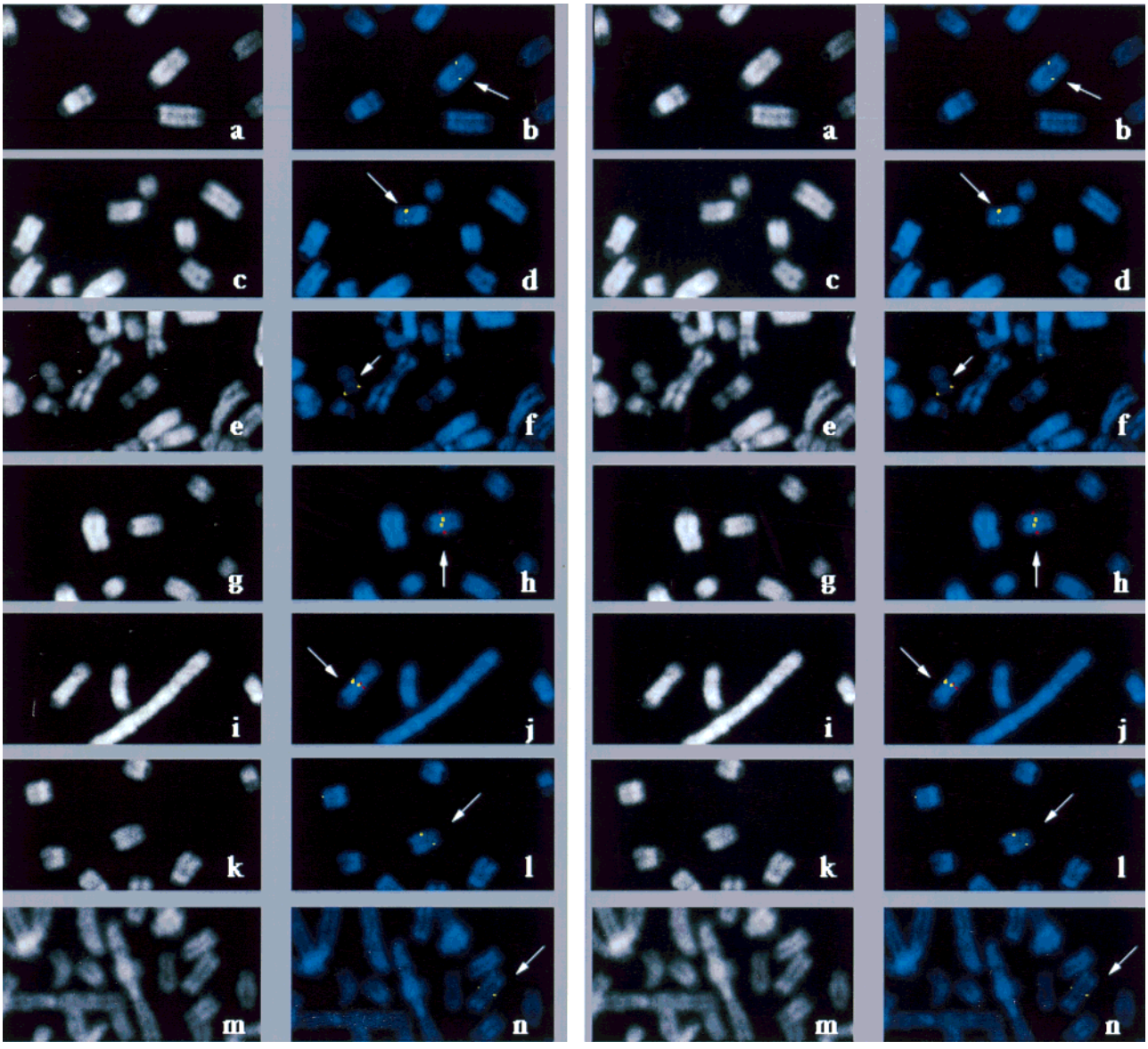

Fig. 1. Partial metaphase spreads showing the results of the hybridization of PrP probes spanning the protein coding region and the 3′ UTR region as described in the text. Banded chromosomes are shown alone to the left and with the hybridization signal to the right. **(a, b)** Bovine probe on cattle chromosomes; **(c, d)** ovine probe on sheep chromosomes; **(e, f)** human probe on chromosomes of human; (**g, h** and **i, j**) cohybridization of the ovine and bovine probes on cattle and sheep chromosomes, respectively. **(k, l** and **m, n**) protein coding region of the human PrP gene on bovine and ovine metaphases, respectively.

of the probe (738 bp) and to a significantly lower level of homology between the human sequence and the bovine and ovine counterparts (66.7% and 65.4% respectively, compared with 94% homology between ovine and bovine DNAs). Our study confirms the adequancy of small-size, PCR-generated probes in physical mapping by FISH and extends the technique to comparative mapping. It is tempting to speculate that, with carefully controlled experiments, even small probes like the expressed sequence tags (EST) that are available in different species might be used to expand the developing animal maps on a comparative basis (Fridolfsson et al. 1997; Comincini et al. 1997).

Acknowledgments. This work was funded by the National Research Council of Italy, Progetto Strategico. ‘‘Encefalopatia Spongiforme Bovine’’.

References

Comincini S, Drisaldi B, Ferretti L (1997) Isolation of coding sequences from bovine cosmids by means of exon trapping. Mamm Genome 8, 486–490

Fridolfsson AK, Hori T, Winters AK, Fredholm M, Yerle M et al. (1997) Expansion of the pig comparative map by expressed sequence tags (EST) mapping. Mamm Genome 8, 907–912

Goldfarb LG, Brown P, McCoumbie WR, Goldgaber D, Swergold GD et al. (1991) Transmissible familial Creutzfeldt-Jakob disease associated with five, seven and eight extra octapeptide coding repeats in the PRNP gene. Proc Natl Acad Sci USA 88, 10926-10930

Goldmann W, Hunter N, Foster JD, Salbaum JM, Beyreuther K et al. (1990) Two alleles of a neural protein gene linked to scrapie in sheep. Proc Natl Acad Sci USA 87, 2476–2480

Hawkins GA, Solinas Toldo S, Bishop MD, Kappes SM, Fries R et al. (1995) Mamm Genome 6, 249–254
Inoue S, Tanaka M, Horiuchi M, Ishiguro N, Shinagawa (1997) Characterization of the bovine prion protein gene: the expression requires interaction between the promoter and intron. J Vet Med Sci 59, 175–183
Lichter P, Tang CJ, Call K, Hermanson G, Evans GA et al. (1990) High resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. Science 247, 64–69
Mezzelani A, Zhang Y, Redaelli L, Castiglioni B, Leone P et al. (1995) Chromosomal localization and molecular characterization of 53 cosmid-derived bovine microsatellites. Mamm Genome 6, 629–635
Prusiner SB (1994) Molecular biology and genetics of prion diseases. Phil Trans R Soc Lond B 343, 447–463
Puckett C, Concannon P, Casey C, Hood L (1991) Genomic structure of the human prion protein gene. Am J Hum Genet 49, 320–329
Richard F, Vogt N, Muleris M, Malfoy B, Dutrillaux B (1994) Increased FISH efficiency using APC probes generated by direct incorporation of labeled nucleotides by PCR. Cytogenet Cell Genet 65, 169–171
Ryan AM, Womack JE (1993) Somatic cell mapping of the bovine prion protein gene and restriction fragment length polymorphism studies in cattle and sheep. Anim Genet 24, 23–26
Shläpfer J, Yang Y, Rexroad C, Womack JE (1997) A radiation hybrid framework map of bovine chromosome 13. Chromosome Res 5, 511–519
Sparkes RS, Simon M, Cohn VH, Fournier RE, Lem J et al. (1986) Assignment of the human and mouse prion protein genes to homologous chromosomes. Proc Natl Acad Sci USA 83, 7358–7362
Yoshimoto J, Iinuma T, Ishiguro N, Horiuchi M, Imamura M et al. (1992) Comparative sequence analysis and expression of bovine PrP gene in mouse L-929 cells. Virus Genes 6, 343–356