PHYSICAL MAPPING OF THE PRION GENES IN CATTLE, SHEEP AND MAN BY FLUORESCENCE IN SITU HYBRIDISATION

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SUMMARY

Polymerase chain reaction (PCR) primers were designed to amplify the region of the prion gene in cattle, sheep and man. Using the amplified fragments as hybridisation probes we established the following chromosomal localizations: BTA 13q17, OAR 13q17-18 and HSA 20p12-13. The FISH data confirm the strong conservation of the prion genes in the three species and are in agreement with a comparative conservation of the same locus in man, sheep and cattle.

Keywords: Prions, FISH, cattle, sheep, human

INTRODUCTION

Prions are the unconventional causative agent of sub acute transmissible spongiform encephalopathies in man and animals, e.g. Scrapie of sheep, Bovine Spongiform Encephalopathy (BSE) of cattle, Creutzfeldt-Jakob disease (CJD) and Fatal Familial Insomnia (FFI) of man. These are rare neurodegenerative disorders characterised by the accumulation in the brain tissue of a prion protein, PrP^{Sc}, that is a proteinase-resistant isoform of a cellular protein, PrP^{c}. Prions are the product of a single gene, which is highly conserved in mammals (>90% at the amino acid level). Prion genes are generally composed of two exons, a GC-rich 5' region and a fairly long 3' untranslated region. The entire coding region of the gene is contained within the second exon. Mutations in the coding region have been linked to familiar cases of human encephalopathies (Goldfarb *et al.*, 1991). Cytogenetic analysis of the prion genes has mostly focused on man, where the gene has been localized on chromosome 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 using a panel of bovine-rodent hybrid somatic cells (Ryan *et al.*, 1993). Although U11 has been subsequentely assigned to bovine chromosome 13 (Hawkins *et al.*, 1995), no other data of direct localization of the PrP gene in cattle have been reported.

In this paper, we established the physical localization of the bovine and ovine PrP genes and confirmed the assignment of the gene in man. With a comparative FISH approach we demonstrated the conservation of the prion gene locus in man, sheep and cattle.

MATERIALS AND METHODS

Chromosome preparation. Metaphase spreads were prepared from cultured fibroblasts with 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 and the metaphases were imaged on a Leitz Aristoplan microscope connected to a CCD-camera (Photometrics) controlled by a Macintosh Quadra 950 computer.

DNA probes. *PCR amplification of the PrP genes.* In order to generate specific probes for the FISH localization of PrP genes in man, sheep and cattle, three couple of PCR primers were designed on the basis of available cDNA sequences in cattle, sheep and man (Goldmann *et al.*, 1990; Puckett *et al.*, 1991; Yoshimoto *et al.*, 1992).

Bovine (GAAGTCATCATGGTGAAAAGC; TCACATCTCTAAACAATGTCAAA), ovine (GAAGTCATCATGGTGAAAAGC; AAGCATGAACTCTTCAGCACT) and human (TTTTGCAGAGCAGTCATTAT; AATTTCAGTCAGATATTAAACATT) oligonucleotides were used to amplify a 1570, a 1590 and a 2130 bp fragment, respectively. Amplifications were done on 50 ng of genomic DNA, in the presence of 10 pmoles of each primer, 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$. PCR conditions were: 94°C x 5', 35 cycles of 94°C x 30", 54°C x 60", 72°C x 90", and 72°C x 10'. The amplified fragments were run on agarose gels, purified, sequenced and used as probes for FISH.

PCR amplification of the second exon of the PrP. Oligonucleotide primers complementary to the entire second exon of the PrP genes were synthesized and used to amplify the complete PrP coding region in cattle, sheep and man. The primers were identical for cattle and sheep (GAAGTCATCATGGTGAAAAGC, CTATCCTACTATGAGAAAAATGA, 783 and 779 bp fragments respectively); the human primers (TTTTGCAGAGCAGTCATTAT; TCATCCCACTATCAGGAAGA) generated a 756 bp product. PCR conditions were as above except for the amplification profile: 94°C x 2', 35 cycles of 94°C x 30", 58°C x 45", 72°C x 1', and 72°C x 10'.

Probes labeling. The probes were directly labelled by PCR in the presence of biotin 16-dUTP and digoxigenin 11-dUTP (Richard *et al.*, 1994) using the above described primers.

FISH. The probes were hybridized *in situ* to metaphase chromosomes at a final concentration of 0,5 ng/µl (5 µg/slide) in the presence of 3 µg of Cot-1 DNA. For single-probe hybridization, biotinylated probes were detected as described elsewhere by Mezzelani *et al.*, (1995). For two-color FISH, the probes were detected via avidin-conjugated FITC and anti-digoxigenin-rhodamine. DAPI was used for counterstaining. Digitized images were taken separately for each fluorochrome and merged using the softwares IPlab Spectrum (Signal Analytics, Vienna, IL) and Gene Join (Office of Cooperative Research, Yale University).

RESULTS AND DISCUSSION

FISH. The results of the hybridization of the PrP genes on cattle, sheep and human chromosomes are shown in Fig.1. The bovine and ovine PrP genes were mapped on the chromosomes of the two species, as single probes and co-hybridized in a dual-color FISH experiment. At least forty metaphase spreads were examined for each probe and for each dual-color experiment, with 70% showing specific signals on one or both of the chromosome homologues. The intensity of the hybridization signals was weak with nick-translated probes; stronger signals were obtained when the probes were directly labelled by PCR (Richard *et al.*, 1994). The chromosomal localization of the PrP gene was 13q17 in cattle and 13q17-18 in sheep. The ovine and bovine hybridization spots on the same metaphases overlapped,

confirming the conservation of the gene in the two species. 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 *et al.*, (1990).

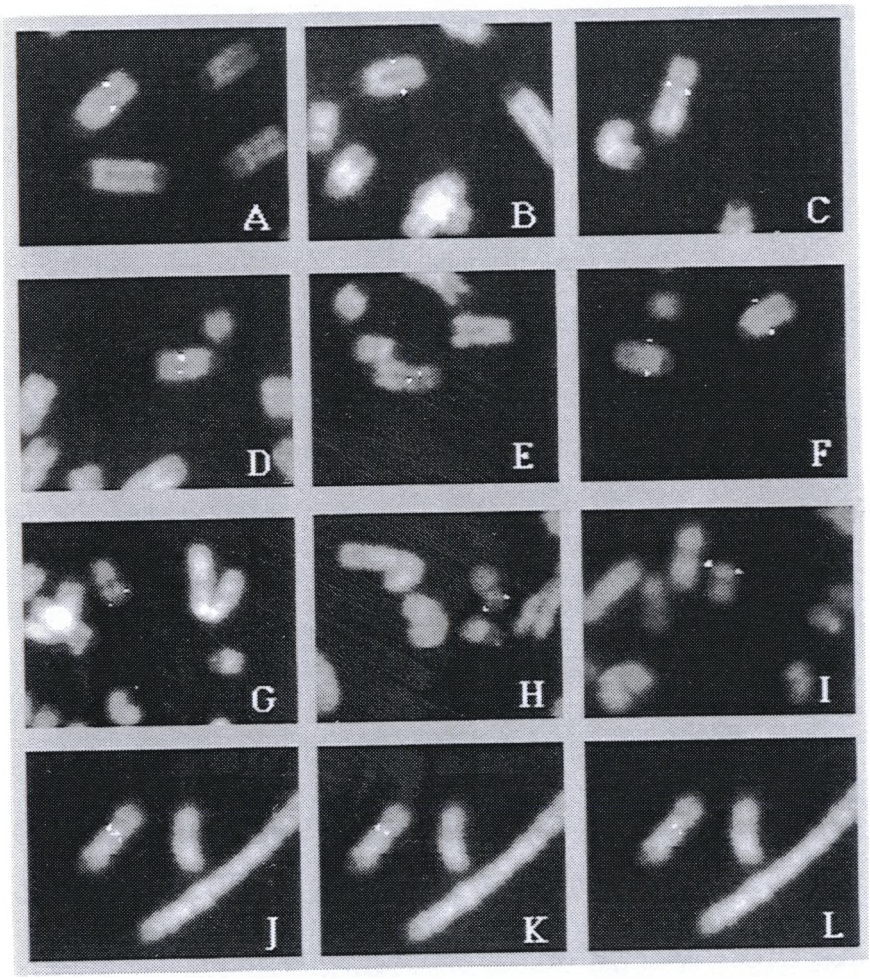

Figure 1. Partial metaphase spreads showing the hybridization of PrP genes. Bovine PrP gene on cattle chromosomes (A, B) and ovine chromosomes (C). Ovine PrP gene on sheep chromosomes (D, E) and bovine chromosomes (F). Human PrP gene on chromosomes of man (G, H, I). Cohybridisation of ovine and bovine PrP gene on sheep chromosomes: both probes (J), ovine PrP gene signal (K) and bovine PrP gene signal (L).

FISH on human PrP probe showed that is located on human chromosome 20p12-13, in agreement with the results of Sparkes *et al.* (1986) who mapped the gene to 20p12-pter. The localization of the PrP gene in cattle, sheep and man is in agreement with the available comparative maps (Solinas Toldo *et al.*, 1995; Hayes, 1995) and suggests that the p-arm of HSA 20 maps on the median q-arm of BTA 13, close to the boundary with the region of homology to HSA 10.

PrP polymorphism. We have analyzed the variability of the octapeptide repeat within the PrP coding region in eight Italian cattle breeds. The data are similar to other reported for UK and US cattle populations, particularly in the high frequency of the genotype 6:6 (AVERAGE FREQUENCIES ARE 6:6, 89%,; 6:5, 10% AND 5:5, 1%). This is a special feature of cattle since in other mammals the alleles with a number of repeats greater than 5 are preferentially associated to the susceptibility to prion diseases (Hunter *et al.*, 1994). Further analysis will focus on the search for new polymorphisms in the 5' and 3' regions of the gene, both on the amplified cDNA as well as in the vicinity of the PrP locus.

REFERENCES

Goldfarb L.G., Brown P., McCoumbie W.R., Goldgaber D., Swergold G.D., Wills P.R., Cervenarkova L., Baron H., Gibbs C.J. Gadjusec D.C. (1991) *Proc.Natl.Acad.Sci.USA* **88**: 10926-10930.

Goldmann W., Hunter N., Foster J.D., Salbaum J.M., Beyreuther K., Hope J. (1990) *Proc.Natl.Acad.Sci.USA* **87**: 2476-2480.

Hawkins G.A., Solinas Toldo S., Bishop M.D., Kappes S.M., Fries R., Beattie C.W. (1995) *Mamm. Genome* **6**: 249-254.

Hayes H. (1995) *Cytogenet.Cell Genet.* **71**: 168-174.

Hunter N., Foster J.D., Benson G., Hope J. (1994) *Veterinary Record* **135**: 400-403.

Yoshimoto J., Iinuma T., Ishiguro N., Horiuchi M., Imamura M., Shinagawa M. (1992) *Virus Genes* **6**: 343-356.

Lichter P., Tang C.J., Call K., Hermanson G., Evans G.A., Houseman D., Ward D.C. (1990) *Science* **247**: 64-69.

Mezzelani A., Zhang Y., Redaelli L., Castiglioni B., Leone P., Williams J.L., Solinas Toldo S., Wigger G., Fries R., Ferretti L. (1995) *Mamm.Genome* **6**: 629-635.

Neibergs H.L., Ryan A.M., Womack J.E., Spooner R.L., Williams J.L. (1994) *Anim.Genet.* **25**: 313-317.

Puckett C., Concannon P., Casey C., Hood L. (1991) *Am.J.Hum.Genet.* **49**: 320-329.

Richard F., Vogt N., Muleris M., Malfoy B., Dutrillaux B. (1994) *Cytogenet.Cell.Genet.* **65**: 169-171.

Ryan A.M., Womack J.E. (1993) *Anim.Genet.* **24**: 23-26.

Solinas Toldo S., Lengauer C., Fries R. (1995) *Genomics* **27**: 489-496.

Sparkes R.S., Simon M., Cohn V.H., Fournier R.E., Lem J., Klisak C., Heinzmann C., Blatt C., Lucero M., Mohandas T. (1986) *Proc.Natl.Acad.Sci.USA* **83**: 7358-7362.