chromosome II. Because PCR (40 cycles) is sensitive enough to detect single copies if the DNA of 20 chromosomes is used as the template (per PCR reaction), no other region of the field bean genome harbors vicilin genes. Our in situ hybridization data specify this result and indicate that all members of this low copy gene family map at a single locus in the short arm, near the centromere, at a distance of ~15% of the arm length

From the Institut für Pflanzengenetik und Kulturpflanzenforschung, D-06466 Gatersleben, Germany (Fuchs and Schubert), and Abteilung Organisation Komplexer Genome, Deutsches Krebsforschungszentrum, Heidelberg, Germany (Joos and Lichter) Address correspondence to Dr Schubert at the address above
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Minute Fragment Observed in a Bovine Pedigree With Robertsonian Translocation

Y-I. Miyake, K. Kawakura, R-K. Murakami, and Y. Kaneda

A minute fragment was found in the metaphase plates obtained from one Holstein bull carrying Robertsonian translocation The pedigree investigation indicated that three out of 34 offspring had the same minute fragment as their sire Fluorescence in situ hybridization (FISH) studies with bovine satellite DNA revealed that the minute fragment was hybridized with this DNA and was part of the bovine centromere We propose that the minute fragment is the product of centric fusion and is transmitted to offspring without being lost in the subsequent cell divisions

Robertsonian translocation (centric fusion) has been postulated to occur by three different mechanisms (Eldridge 1985; Long 1985). It is presumed that in any case of centric fusion, by any of the three different mechanisms, the small fragment is lost (Eldridge and Balakrishnan 1977; John and Freeman 1975) in the subsequent cell divisions. Presumably if the animal remained viable, no serious loss of genetic material occurred. So, in many types of bovine Robertsonian translocation (Eldridge 1985; Gustavsson 1979; Popescu 1990), minute fragments were not observed in the metaphase plates.

Here, we report a minute fragment together with Robertsonian translocation in the metaphase plates of a bull and his offspring. This minute chromosome was further investigated with a bovine satellite DNA probe using the fluorescence in situ hybridization (FISH) technique.

Materials and Methods

We used 40 Holstein cattle in the study reported here, as shown in Figure 1. Bull (II 6), who carried the Robertsonian translocation and a minute fragment, was the proband in this study (Miyake et al. 1991). The five half-sib brothers (II 1–5) of bull (II 6) by different cows and 34 progeny were examined cytogenetically.

Chromosome Analysis

We collected and cultured heparinized blood at 38°C in Eagle's MEM supplemented with 10% FCS and PHA-M. In addition, a piece of skin, kidney, and spleen of the bull (II 6) was asceptically collected and cultured. To harvest the cells, the cultures were treated with colcemid (final concentration of 5 µl/ml). Hypotonic treatment, fixation of cells, and preparation of slides were performed according to routine procedures (Eldridge 1985). More than 50 metaphase plates per case were examined under a light microscope.

Preparation of the Probe

The bovine satellite DNA probe was made by the polymerase chain reaction (PCR). We designed a set of primers (5-TGGA-AGCAAAGAACCCCGCT, 5-AAGCCCTCGAG-AGACCTTCT), according to the sequence of the bovine 1.715 satellite DNA by Plucienniczak et al. (1982). For the synthesis of biotin-labeled probe, the following mix-

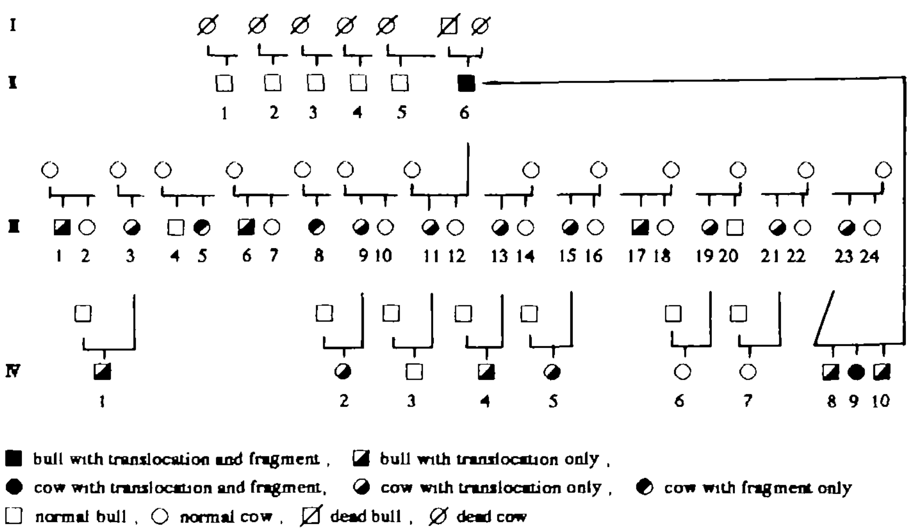

Figure 1. The family for Robertsonian translocation and minute fragment

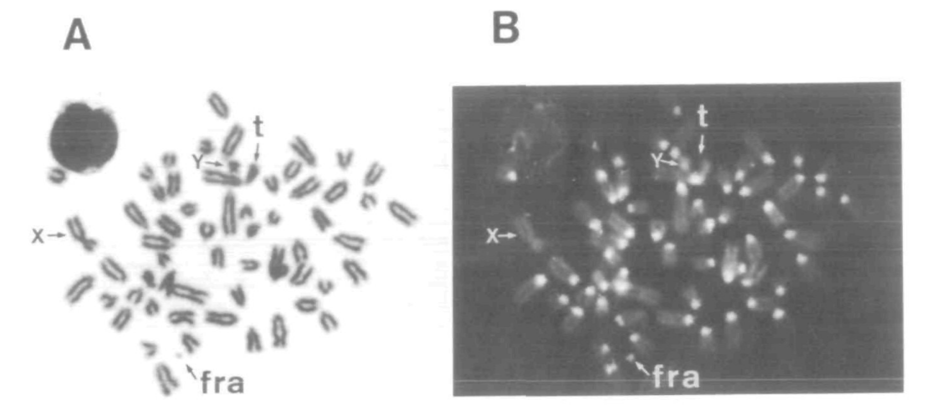

Figure 2. Giemsa-stained metaphase plate (**A**) of Bull (II 6), carrying both translocated chromosome (arrow with "t") and a minute fragment (arrow with "fra"), (**B**) the same metaphase plate after FISH. X = X chromosome, Y = Y chromosome

ture was prepared: 10 ng of bovine genomic DNA; 100 μM each of dATP, dCTP, and dGTP; 40 μM of dTTP; 60 μM of biotin-16-dUTP; 0.5 μM of each primer; and 5U *Taq* polymerase in a total volume of 100 μl. The mixture was followed by 30 cycles consisting of 1.2 min denaturation at 94°C, 1.5 min annealing at 55°C, and 1 min extension at 72°C in an automated thermal cycler. PCR-amplified products were precipitated with ethanol and then used as probe.

In situ Hybridization

For FISH, we prepared metaphase chromosomes from bull (II 6), carrying both the translocation and a minute fragment, and from his daughter (III 5), carrying only a minute fragment. FISH was carried out basically according to Pinkel et al. (1986) with a slight modification. In brief, 10 μl of a hybridization cocktail consisting of 50% formamide/2× SSC, 10% dextran sulfate, 1 mg/ml of sonicated salmon sperm DNA, and biotynylated DNA was applied to each slide, covered with a 24 × 22 mm coverslip, and sealed with rubber cement. Denaturation of probe and chromosomes was performed simultaneously on the hot plate at 74°C for 8 min. The slides were incubated overnight at 39°C in a moist chamber. After washing in 50% formamide/2× SSC three times at 42°C for 5 min and in 2× SSC three times at room temperature for 5 min, the hybridized probe was detected by fluorescein isothiocyanate (FITC)-labeled avidin (Vector Laboratory, U.S.A.: A-2011). The slides were counterstained with propidium iodide (1 μg/ml) and observed under the fluorescence microscope (Nikon) using a B2 filter.

Results

The bull (II 6) carrying the Robertsonian translocation had a minute fragment in most of the metaphases made from blood (86.8%), skin (42.3%), kidney (96.0%), and spleen (91.2%). His five half-sib brothers (II 1–5) had no Robertsonian translocation and no minute fragment. In the 34 progeny, 17 cases had the Robertsonian translocation, but none had the minute fragment; 14 cases had neither Robertsonian translocation nor minute fragment; one case had both Robertsonian translocation and minute fragment; and the remaining two cases had only minute fragment (Figure 1). Consequently, 18 progeny (52.9%) had the Robertsonian translocation and 16 (47.1%) did not. Three progeny (8.8%) had the minute fragment, yet 31 (91.2%) did not. The present cytogenetic findings showed an apparent transmission of the minute fragment to some of the progeny of the bull (II 6).

In situ hybridization showed that the hybridized signals were found on the centromeric region of every normal chromosome except the X and Y chromosomes. Figures 2 and 3 show the metaphase spreads before and after hybridization in the bull and the daughter cow. In metaphases derived from bull (II 6) (Figure 2a), the centromeres of all autosomes and the minute fragment had strong signals after hybridization (Figure 2b). In metaphases derived from the daughter who carried only a minute fragment (Figure 3a), centromeres of all autosomes and the minute fragment were clearly visible with strong signals after hybridization (Figure 3b).

Discussion

After the report on a bovine Robertsonian translocation by Gustavsson and Rockborn (1964), many types of translocation were found to occur in many cattle breeds, and the frequency of translocation varied widely among the breeds (Eldridge 1985; Gustavsson 1979; Popescu 1990). However, evidence that the minute fragments appeared together with translocated chromosomes in the same metaphase plates in cattle has not yet been reported. In humans, researchers (Howard and Fielding [1987], among others) reported a case of familial Robertsonian translocation accompanied by an extra marker microchromosome. Using the C- and NOR-banding technique, they demonstrated that the marker chromosome was dicentric and bisatellited, while the translocation had a single centromere.

From the results obtained here, this minute chromosome seems to have a centromere, because the satellite DNA probe consists of a part of the centromere. On the basis of the hypotheses on the formation of Robertsonian translocation in cattle by Eldridge (1985) and Long (1985), we have guessed that this minute fragment was a product of centric fusion in the bull (II 6) de novo or derived from the

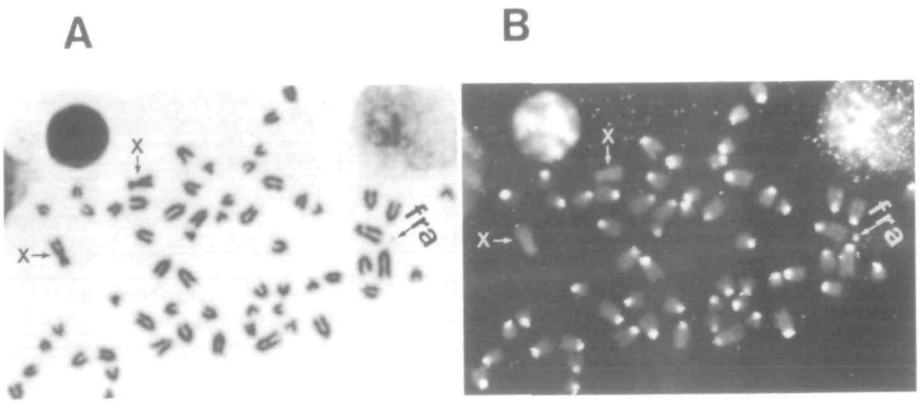

Figure 3. Giemsa-stained metaphase plate (**A**) of a daughter cow, carrying only a minute fragment (arrow with "fra"); (**B**) the same metaphase plate after FISH X = X chromosome, Y = Y chromosome.

ancestor of translocation. Namely, the minute fragment is simultaneously formed by the fusion of two different centromeric regions of chromosomes at the time of formation of Robertsonian translocation. And it is not lost in the original cells, in spite of the small amount of centromeric heterochromatin. Therefore, it seems that minute fragments can be transmitted to the progeny through the generations independently of the translocated chromosomes. Buckton et al. (1985) reported 44 cases with additional "marker" chromosomes in humans and showed that the majority of markers involving 15p are the consequence of non-sister chromatid exchanges. The majority of these occur as the result of translocations between homologous chromosome 15. Unfortunately, in our study including other translocations in cattle, there is no evidence of breakage in the centromeric regions of any chromosomes at the time of formation of a balanced translocation. Recently, Cheung et al. (1990) investigated molecular cytogenetic evidence characterizing breakpoint regions in human Robertsonian translocations. They mentioned three different types of breakpoints: between the ribosomal or alphoid sequences, as deduced from the banding and in situ hybridization results, and breaks in two seemingly unrelated region on the two different chromosomes. Further, it was reported by Wolff and Schwartz (1992) that the majority of the breakpoints in human Robertsonian translocations seem to be distal to the centromere and just proximal to the beta-satellite and nuclear-organizing regions.

It is very interesting that only three individuals (8.8%) have a minute fragment in the progeny, compared with about half the individuals that have a translocation. Probably, most of the minute fragments would be eliminated during spermatogenesis of the translocation carrier bull by meiotic error. Several articles (Buckton et al. 1985; Callen et al. 1991; Haaf et al. 1992) discuss the formation and the disappearance of the heterochromatic microchromosomes or fragments in humans over time in somatic cells of over 2 generations. Unfortunately, in our study, we were unable to clarify the existence and/or elimination of the minute fragment during spermatogenesis, because of the death of bull (II 6). We are continuing to study the male progeny of this bull, however, because we have retained some of his frozen semen.

From the Laboratory of Theriogenology, Faculty of Agriculture, Iwate University, Morioka 020, Japan (Miyake and Kaneda), the National Institute of Animal Husbandry, Tsukuba, Japan (Kawakura), and the Morioka Livestock Hygiene Service Center, Takisawa, Japan (Murakami) We are very grateful to the late Professor N. S Fechheimer (Department of Dairy Science, Ohio State University) for his continuous efforts to get the information and samples from the parents of this case in the United States and to Professor M C Yoshida (Chromosome Research Unit, Faculty of Science, Hokkaido University, Japan) for his kind reading of the manuscript This study was supported in part by a Grant-in-Aid for Scientific Research (no. 03660319) from the Ministry of Education, Science and Culture, Japan.

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Mapping the Bovine Homolog of the Human Cystic Fibrosis Gene

D. Wallis and J. E. Womack

Human cDNA probe H1 6 (clone 10-1) encoding cystic fibrosis transmembrane conductance regulator (CFTR) was used to map the bovine homolog of CFTR Using a panel of bovine × rodent hybrid somatic cells, the homolog was mapped to bovine syntenic group U13 which corresponds to chromosome 4 CFTR is 97 7% concordant with syntenic markers T-cell receptor beta (TCRB) and P-glycoprotein 3 (PGY3) The comparative gene maps of CFTR, TCRB, and PGY3 on human chromosome 7, bovine chromosome 4, and mouse chromosomes 5 and 6 indicate conservation of synteny, although internal rearrangements relative to HSA 7 are present in both cattle and mice

Cystic fibrosis (CF) is the most common lethal human genetic disease, affecting 1:2,000 Caucasian and 1:17,000 African-American births in the United States. CF is characterized by abnormal electrolyte transport that primarily affects the exocrine glands and respiratory system. CF is inherited in an autosomal recessive Mendelian fashion. The disease is caused by the production of a defective protein called the cystic fibrosis transmembrane conductance regulator (CFTR), which acts as a chloride conductance channel (Collins 1992). The chief mutation that accounts for almost 70% of all CFTR mutations is a deletion of three base pairs that results in a lost phenylalanine at codon 508 (Kerem et al. 1989). The gene encoding CFTR has been mapped in humans to 7q31.3-32 (Rommens et al. 1989), and the murine homolog has been cloned and assigned to mouse chromosome 6 (Siegel et al. 1992).

In the interest of comparative genome mapping among humans, mice, and cattle, we have mapped the CFTR gene in cattle as described below.

Materials and Methods

Preparation of Genomic DNA

The genomic DNAs of the bovine × rodent hybrid somatic cell lines used in this study were prepared from the fusion of bovine peripheral leukocytes with mutant rodent cell lines as described previously (Womack and Moll 1986). The DNA sam-