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Comparative mapping of *IGHG1, IGHM, FES,* and *FOS* in domestic cattle

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Abstract. The immunoglobulin genes have not been genetically characterized as thoroughly in cattle as in other mammals, particularly humans and mice. Comparative gene mapping in mammals suggests that the bovine immunoglobulin heavy chain genes, *IGHG4* and *IGHM* might be syntenic with the *FOS* oncogene. Interestingly, however, when these genes were assigned to bovine syntenic groups utilizing a panel of bovine : hamster hybrid somatic cells, *IGH* genes were shown to be syntenic with the *FES* oncogene rather than *FOS*. In this study *IGH* and *FES* were assigned to *Bos taurus* chromosome 21 while *FOS* was assigned to chromosome 10. In addition, bovine-specific immunoglobulin-like sequences were observed in the hybrid somatic cells, and one, IGHML1, was mapped to bovine syntenic group U16. The probes used for somatic-cell mapping were also used to screen a small number of cattle of several different breeds for restriction fragment length polymorphisms. *IGHG4* and *IGHM* were shown to be highly polymorphic, while *FOS* and *FES* were not.

Introduction

Antibodies, which are produced by mature B-cells after stimulation by an antigen, are an integral part of the mammalian immune system. The basic mammalian antibody structural unit consists of four chains: two heavy chains, and two light chains. Each chain is additionally divided into two regions; an N-terminal variable (*V*) region, which is responsible for antibody binding, and a constant (*C*) region that determines antibody effector function. Antibodies are coded by immunoglobulin genes, which, through DNA recombination provide the antibody diversity necessary to efficiently combat the complexity of environmental antigens.

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The immunoglobulin (Ig) heavy chain genes code for five different classes of antibodies in mammals, IgM, IgD, IgG, IgA, and IgE, which are coded by the genes *IGHM, IGHD, IGHG, IGHA,* and *IGHE,* respectively. While the immunoglobulins of mouse, human, and other species have been well characterized as to structure, organization, and expression, those of cattle have not been subject to such intense molecular scrutiny.

The immunoglobulin heavy chain genes have been mapped in mice, humans, rats, and rabbits, and are found on chromosomes 12, 14q32, 6, and 16, respectively (Honjo 1983; Liu et al. 1980; Pear et al. 1986; Medrano and Dutrillaux 1986). The basic organization of the heavy chain regions in these species is similar, consisting of variable (V_H) regions upstream to diversity (*D*) regions, joining (J_H) regions, and constant (C_H) regions. A mature heavy chain molecule results from the joining of these regions (Alt et al. 1984; Takahashi et al. 1981).

Despite the similarities of the expression and organization of the immunoglobulin heavy chain genes in mammals, there are some striking differences. Murine heavy chain genes consist of eight *C* regions, *Igm-C, Igd-C, Igg-C3, Igg-C1, Igg-C2b, Igg-C2a, Igl-C,* and *Iga-C,* (Nishida et al. 1981; Shimuzu et al. 1982), four J_H regions (Shimuzu et al. 1982), 10–20 *D* regions (Kurosawa and Tonegawa 1982; Wood and Tonegawa 1983), and nine V_H gene families containing 1000–2000 genes (Givol et al. 1981). In humans, there are eleven *C* region genes, *IGHM-C, IGHD-C, IGHG-C3, IGHG-C1, IGHE-psC1, IGHA-C1, IGHG-psC, IGHG-C2, IGHG-C4, IGHE-C,* and *IGHA-C2,* two of which are pseudogenes (Flanagan and Rabbitts 1982; Migone et al. 1984), nine J_H genes, three of which are pseudogenes (Elliott et al. 1982; Ravetch, et al. 1981), five *D* regions, and at least six V_H families, containing approximately 100 genes (Matthyssens and Rabbits 1982; Rabbitts et al. 1980; Siebenlist et al. 1981; Kodaira et al. 1986).

The molecular structures of the heavy chain genes of other domestic species are not well characterized, with

most of our knowledge limited to studies regarding immunoglobulin isotypes. Horses express IgM, IgA, IgE, and five IgG subisotypes, IgGa, IgGb, IgGc, IgG(B), and IgG(T). Pigs have four IgG subisotypes, IgG1, IgG2, IgG3, and IgG4, in addition to IgE, IgM, IgA, and IgD. Sheep possess IgG1, IgG2, and IgG3, IgM, IgA, and a M_r 210000 IgE, while cats and dogs have IgG1, IgG2, IgM, IgA, and IgG1, IgG2a, IgG2b, IgG2c, IgA, IgM, and IgE, respectively (Tizzard 1987; Trebichavsky et al. 1983; Porter 1979; Barlough et al. 1981; Halliwell et al. 1975; Heddle and Rowley 1975). Rabbits differ markedly from the other species studied thus far in that they possess at least 12 IgA isotypes (Schneiderman et al. 1989). Some rabbits also express IgN, a M_r 118000 molecule that consists of two light chains, and two small heavy chains (Tizzard 1987). Fish, turtles, ducks, and amphibians have also been shown to express IgN (Tizzard 1987).

Immunoglobulins have been characterized in several non-mammalian species as well. Chickens have been shown to have homologs to mammalian immunoglobulins, possessing IgG1, IgG2, IgG3, IgA, IgM, and IgD. In contrast to the DNA rearrangements in mammals, however, chickens derive their antibody diversity from gene conversion events that combine pseudogene sequences with those of a single *V(D)J* unit (Reynaud et al. 1985; Carlson et al. 1990; McCormack and Thompson 1990). *Xenopus laevis* possesses at least 80 unique V_H genes, seven J_H genes, and multiple D segments, in addition to *IGM* and *IGY* constant regions, and shows similarity with mammals in its generation of antibody diversity (Hsu et al. 1989). Horned sharks, *Heterodontus francisci*, have been shown to contain 100–200 V_H sequences, organized as repeated V_H-D-J_H-C_H units (Shamblott and Litman 1989). Until recently, all of the data available regarding bovine immunoglobulins was generated by cross-reacting antisera for bovine antibodies with antibodies from other species. The structure of the molecules was determined by obtaining their amino acid composition, and as late as 1987, only five classes of immunoglobulin molecules had been characterized: IgM, IgA, IgG1, IgG2, and IgE. (Butler 1983, 1986; Butler et al. 1987). IgM, IgG1, IgG2a, and IgG2b were recently assayed in cattle using monoclonal antibodies (mAbs; Estes et al. 1990), and several other recent studies have broadened our knowledge regarding the structure of the immunoglobulin genes in cattle.

By using human heavy chain switch regions as probes Knight and Becker (1987) have shown that the bovine heavy chain constant region is made up of at least seven genes; *IGHM, IGHD, IGHG1, IGHG2, IGHG3, IGHA,* and *IGHE.* IgG3 antibodies were not detected in circulating B-cells, so *IGHG3* is probably a pseudogene. The presence of an *IGHG4* gene was also postulated, but unproven in this study.

Recently, Symons and co-workers (1989) cloned and sequenced *IGHG1* and *IGHG2* in cattle. The genes were revealed to consist of four exons, as in all other mammalian species studied thus far (Calame et al. 1980). There were significant sequence differences between the C_{H2}-hinge exons of these genes. This was an interesting observation, as the antibody IgG1 is selectively transported in large amounts from the serum to the colostrum in cattle, goats, and pigs, due to receptors on the mammary alveolar cells that show specificity for the IgG1 *C* region. Thus, the hinge region sequence differences could be responsible for the differential secretion of IgG1 into the bovine colostrum.

There are many reasons to need to know more about the organization of bovine immunoglobulin genes, not the least of which is their potential involvement in differential responses to pathogens. Towards this goal, the immunoglobulin heavy chain genes *IGHG4* and *IGHM* were assigned to bovine syntenic groups utilizing bovine: hamster hybrid somatic cell clone panels previously characterized for gene markers of all 29 bovine autosomal syntenic groups (Womack and Moll 1986; Womack 1990). In addition, bovine sperm DNA from three different breeds of cattle (Brahma, Hereford, and Holstein) was analyzed to determine the extent of variation present in the germ line configurations of the immunoglobulin genes.

Extensive conservation between syntenic groups of cattle, humans, and mice has been demonstrated (Womack 1982; Womack and Moll 1986; O'Brien et al. 1988). In order to determine the extent of similarity between the bovine syntenic groups in this study and their human chromosomal homologs, the FBJ murine osteosarcoma virus (FOS) and the Snyder-Theilen strain of the feline sarcoma virus (FES), which have been mapped to human chromosomes 14q24.3 and 15q25-qter respectively, (Cox et al. 1989; Harper et al. 1983) were also mapped and analyzed for restriction fragment length polymorphisms (RFLPs).

Materials and methods

Extraction of genomic DNA. DNA was extracted from bovine : hamster hybrid somatic cell panels (Womack and Moll 1986) that segregate bovine chromosomes and bovine skin and blood using the procedure of Maniatis and co-workers (1982) as modified in our laboratory (Adkison et al. 1988). Sperm DNA was extracted in the following manner: 1 straw of sperm DNA (approximately 600 µl/straw) was extracted at 37 °C overnight in 1.15 ml STE, 165 µl Proteinase K (10 mg/ml), 165 µl 20% sodium dodecyl sulfate (SDS), and 75 µl 0.88 *M* dithiothreitol (DTT), extracted twice with phenol : chloroform : isoamyl alcohol (25 : 24 : 1), twice with chloroform : isoamyl alcohol (24 : 1), and precipitated with 1/10 vol. 2 *M* NaCl and 2 1/2 vol. of cold 95% EtOH. 100–200 µg of high molecular mass genomic DNA was generally extracted in this manner. In order to determine the extent of polymorphism present in the germline configuration of the bovine *IGHG4* and

IGHM genes, bovine sperm DNA was digested with *Eco* RI, *Hin* dIII, *Bam* HI, and *Bgl* II, blotted on nylon filters, and probed with *IGHM*, and *IGHG4*. *FOS* and *FES* were also analyzed in this manner.

Southern Blotting. The DNA was digested to completion with restriction enzymes that allowed cattle and hamster restriction fragments to be distinguished, and blotted on Zetabind nylon filters (CUNO, Meriden, CT) using the method of Southern (1975). The filters were then prewashed in 0.1 × standard sodium citrate (SSC), 0.5% SDS for 55 min at 65 °C, and prehybridized in 5 × SSC, 10 × Denhardt's solution, 0.05 *M* $NaPO_4$ (pH 6.8), 500 µg/ml denatured salmon sperm DNA, 5% dextran sulfate, 0.5% SDS, and 40–50% formamide for 2 h at 42 °C.

Hybridizing Probes. The immunoglobulin probes were labeled to a specific activity of at least 10^8 counts/µg with [α^{32}]P-dCTP by random oligonucleotide primed DNA synthesis (Feinberg and Vogelstein 1983a, b) and hybridized to Southern blots overnight at 42 °C in 5 × SSC, 1 × Denhardt's, 0.02 *M* $NaPO_4$, pH 6.7, 40% formamide (50% for the bovine probes), denatured salmon sperm DNA (0.5 mg/ml), and 10% dextran sulfate in distilled water. Non-specifically bound probe was removed by washing for 15 min at room temperature with 2 × SSC (1 × SSC for bovine probes), 1% SDS, then for 30 min at 65 °C with 2 × SSC (or 1 × SSC), 1% SDS. The filters were then loaded on Kodak XAR 5 diagnostic X-ray film at −70 °C overnight, or until the film was sufficiently exposed (up to one week). The filters were reused by stripping the hybridized probe for 20 min in 0.01 × SSC, 0.5% SDS at 90 °C.

Preparation of Probes. A 250 base pair (bp) bovine genomic *IGHM* probe was kindly provided by B. Hague (University of Massachusetts at Amherst). The *IGHG4*, human genomic probe, and the *v-fos* and *v-fes* probes were obtained from ATCC (Rockville, MD). Restriction maps were performed on all of the probes used in this study to verify that the correct gene was indeed mapped. Plasmid DNA was isolated by the alkaline lysis/polyethyleneglycol (PEG) precipitation procedure (Birnboim 1983; Lis 1980).

Results

The *IGHM* probe was applied to *Hin* dIII digested DNA from 37 hybrid somatic cell clones under conditions in which the bovine probe did not hybridize to hamster DNA. The *IGHM* probe hybridized to at least two asyntenic families of bovine fragments in the hybrid somatic cells (Fig. 1A). These asyntenic fragments were temporarily designated genes A and B as indicated in the Figure. Gene A consisted of at least three hybridizing fragments, ranging from 9.4–18.9 kilobases (kb). The exact number and size of these fragments was resolved during the RFLP analysis presented below. The fragments could alternatively represent two or more closely linked genes, although Knight and Becker (1987) have indicated that only one *IGHM* gene is present in cattle. Gene B consisted of a single *Hin* dIII fragment of 6.2 kb. Gene B showed 100% concordance with U16, while gene A showed 94% concordance with U4. U16 is an unassigned bovine syntenic group (Womack 1990) and U4 has been assigned to *Bos taurus* (BTA) chromosome 21 (Georges et al. 1991). In order to determine which of the fragments represented *IGHM* and which represented related sequences, a human *IGHG4* probe was obtained. It was reasoned that since *IGHG* and *IGHM* are closely linked in all mammalian species studied thus far, they should also be so in cattle.

Figure 1B shows hybrid somatic cell clones digested with *Msp* I, and screened with *IGHG4* for bovine sequences. Despite entensive hybridization to hamster DNA, several (at least three) sets of asyntenic bovine

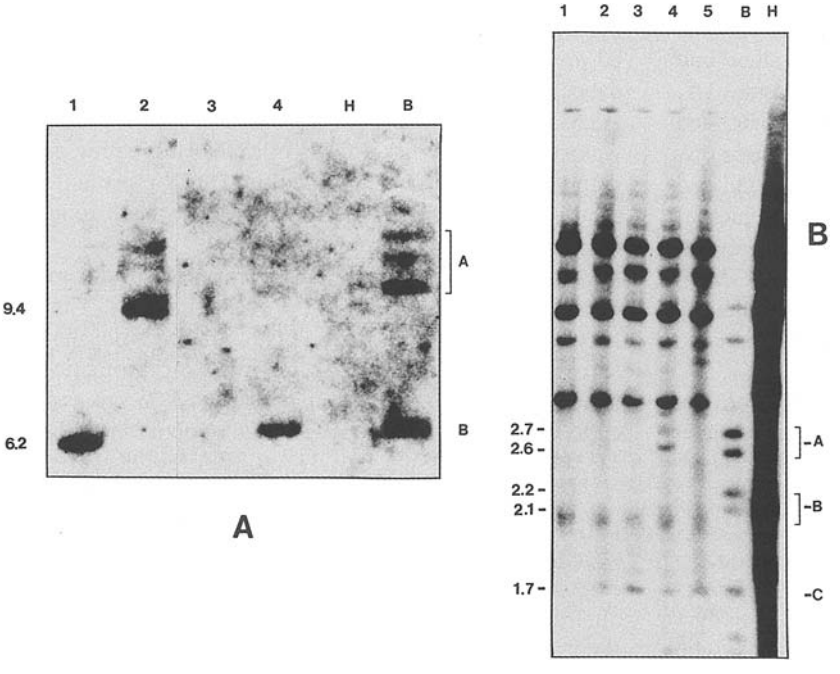

Fig. 1A, B. Bovine : hamster hybrid somatic cell DNA digested with *Hin* dIII and probed with *IGHM* **A** and digested with *Msp* I and probed with *IGHG4* **B**. Numbered lanes contained DNA from different hybrid cells. H and B are hamster and bovine controls respectively. Fragment sizes are indicated in kb and groups of fragments marked by A, B, and C segregate as units (genes) described in the text. Panels 1A and 1B show DNA from a different subset of hybrid cells.

Table 1. Concordancy of *IGHG4, IGHM, FES, FOS, IGHML1,* and *IGHG4L2* with bovine syntenic groups.

Syntenic groups	% Concordant					
	IGHG4	*IGHM*	*FES*	*FOS*	*IGHML1*	*IGHG4L2*
U 1	71	71	69	63	53	72
U 2	49	49	49	37	47	56
U 3	60	60	54	37	67	66
U 4	**94**	**94**	**89**	54	50	44
U 5	61	61	55	**85**	38	47
U 6	74	74	77	49	58	31
U 7	80	80	77	43	53	38
U 8	66	66	66	71	47	34
U 9	71	71	69	46	44	41
U10	69	69	69	46	64	72
U11	58	58	68	65	66	71
U12	66	66	63	69	31	41
U13	74	74	66	57	49	29
U14	71	71	74	51	50	53
U15	78	78	75	50	54	30
U16	50	50	57	43	**100**	52
U17	74	74	77	60	53	50
U18	80	80	83	54	58	50
U19	66	66	69	46	72	66
U20	66	66	69	29	56	44
U21	78	78	76	51	55	29
U22	78	78	76	51	55	29
U23	34	34	36	67	48	45
U24	74	74	74	51	54	70
U25	52	52	52	79	38	48
U26	79	79	74	54	54	32
U27	79	79	76	50	57	29
U28	56	56	54	66	63	55
U29	N/A	N/A	N/A	N/A	N/A	N/A
X	29	29	31	49	67	59

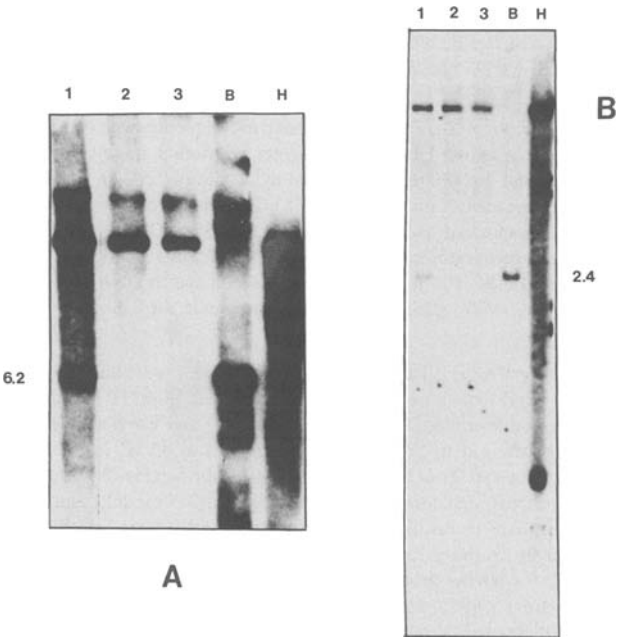

Fig. 2A, B. Bovine : hamster hybrid somatic cell DNA digested with *Bgl* II, Southern blotted, and probed with *FES* **A** and with *FOS* **B**. Clone 1 is positive for both *FES* and *FOS*. Bovine and hamster DNA was run as a control, and the fragment sizes are indicated in kb.

fragments were identified in the hybrid panel. The fragments designated gene A segregated together and showed 100% concordance with gene A from Figure 1A, and thus these two genes are thought to be the *IGHG* and *IGHM* genes, respectively. Cattle have at least three different *IGHG* genes that hybridize to *IGHG4* (Knight and Becker 1987), and it is impossible to determine which of these sequences was mapped in this study. Gene B revealed by the *IGHM* (Fig. 1A) probe was designated *IGHML* (immunoglobulin M-like 1). Genes B and C revealed by the *IGHG4* probe (Fig. 1B) were likewise designated *IGHG4L1* and *IGHG4L2*.

When 37 hybrid somatic cell clones were assayed, the bovine immunoglobulin heavy chain genes were assigned to U4 (BTA21), with which they showed 94% concordance (Table 1). BTA21 consists of homologous markers from the distal arms of HSA14 and 15 (Fig. 5). *IGHML1* was assigned to U16, with which it showed 100% concordance (Table 1). U16 consists solely of HSA9 markers thus far (Womack 1990). *IGHG4L2* was likewise analyzed on the *Msp* I-digested panel, but did not segregate with any known syntenic group markers (Table 1). *IGHG4L1* was not found in any of the hybrid somatic cell clones analyzed in this study.

Figure 2A shows hybrid somatic cell clones digested with *Bgl* II, in which the 6.2 kb bovine band can clearly be distinguished from the 19 kb hamster band. When 37 hybrid somatic cell clones were analyzed, *FES* was assigned to U4 (BTA21), with which it showed 89% concordance (Tables 1 and 2). *FES* had been mapped to HSA15q25-q26 and MMU7 (Kozak et al. 1983), and thus was a more distal marker for HSA15 than those previously mapped to U5 (Fig. 5). In addition, *FES* represented a mouse chromosome that had not previously been assigned to a bovine syntenic group.

Figure 2B shows the bovine : hamster hybrid somatic cells digested with *Bgl* II, and probed with *v-fos*. The 2.4 kb bovine band can easily be distinguished from the 8.4 and 0.7 kb hamster bands. Thirty-five hybrid somatic cell clones were analyzed in this manner, and *FOS* was assigned to U5, with which it showed 85% concordance (Table 1). U5 has been assigned to BTA10 (Georges et al. 1991).

All of the immunoglobulin sequences showed polymorphic bands in several digests, while the protooncogenes showed no variation. The *IGHM* probe revealed extensive polymorphism. Figure 3A illustrates a two allele polymorphism in the 8 kb region revealed by *Eco* R1.

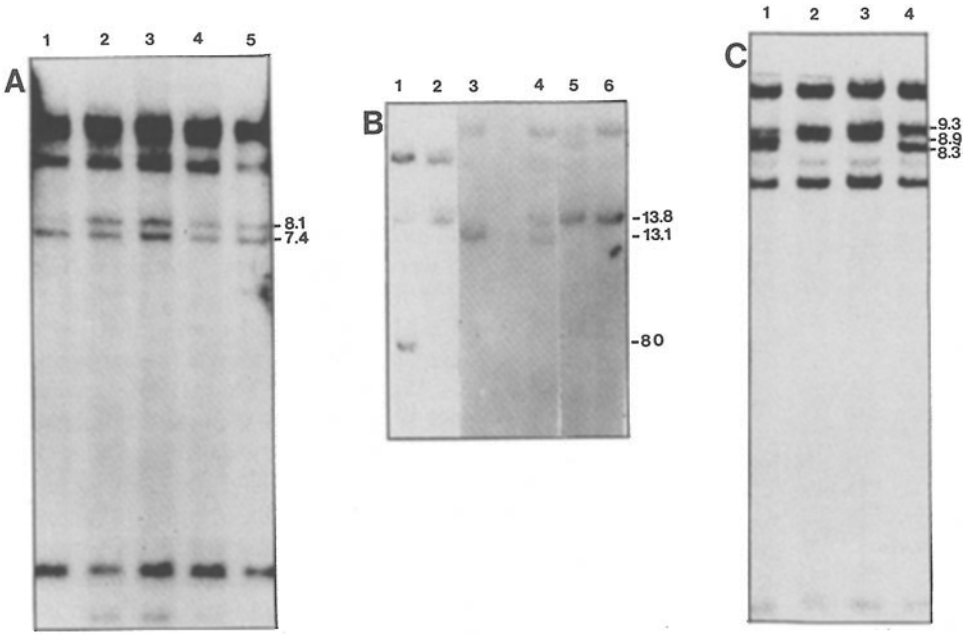

Fig. 3A, B, C. Bovine sperm genomic DNA digested with *Eco* RI **A**, *Hin* dIII **B**, and *Bgl* II **C** and probed with *IGHM*. Fragment sizes are indicated in kb to the *right* of each figure. *Bos indicus* samples are in lanes 4 and 5 of panel A; lanes 3, 4, and 6 of panel B; and lane 4 of panel C. All others are *Bos taurus*.

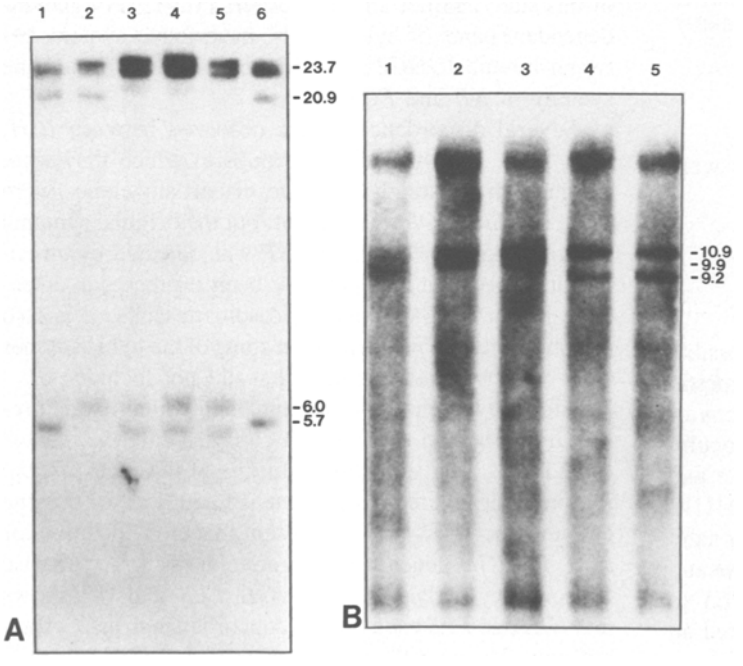

Fig. 4A, B. Bovine sperm genomic DNA digested with *Eco* RI **A** and *Bgl* II **B** and probed with *IGHG4*. Fragment sizes are indicated in kb to the *right* of each figure.

When the sperm DNA was digested with *Hin* dIII, six to eight restriction fragments were seen, ranging from 8.0–22.4 kb in length (Fig. 3B). The 22.4 kb fragment was only seen in the *Bos taurus* breeds, while the 8.0 kb fragment was seen only in *Bos indicus*.

Bgl II generated restriction fragments of 15.2, 9.3, 8.9, 8.3, 7.3, and 1.0 kb, respectively (Fig. 3C). The 8.9 kb fragment was only seen in Herefords and Holsteins, and, in addition to the 8.3 kb fragment, was the only polymorphic restriction fragment seen in these breeds. No polymorphisms were observed in the Brahmas with this enzyme-probe combination.

Figure 4 shows the restriction fragments generated when the same sperm DNA was probed with *IGHG4*. *Eco* RI generated polymorphism of fragments in the 20–23 kb range and also in the 6 kb range, apparently the result of independent RFLPs (Fig. 4A). Finally, *Bgl* II generated multiple *IGHG4* restriction fragments (Fig. 4B). The

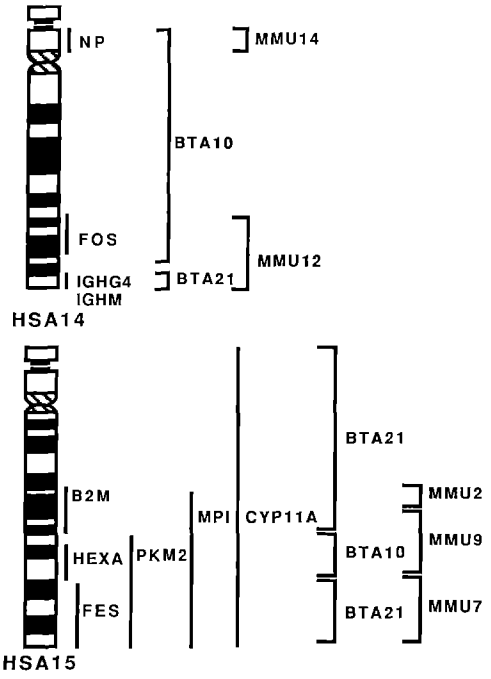

Fig. 5. Comparison of gene markers from BTA10 and BTA21 with their known chromosomal locations on HSA14, HSA15, MMU2, MMU7, MMU9, MMU12, and MMU14.

polymorphic fragments in the 9–10 kb range were presented in all three of the breeds studied.

Discussion

Womack and Moll (1986) showed that it is often possible to predict the chromosomal locations of genes in domestic cattle by utilizing the known chromosomal locations of the genes in mouse and human. The immunoglobulin heavy chain genes and *FOS* are syntenic in mouse and human, and have been mapped to HSA14 and MMU12 (D'Eustachio 1984), respectively. *FES*, on the other hand is asyntenic to the *FOS* and *IGH* genes in both mouse and human. The syntenic assignment of *IGH* and *FES* to BTA21, and of *FOS* to BTA10, therefore uncovered an unexpected area of genomic dissimilarity between these species. The relationships of BTA21 and BTA10 to human and mouse chromosomes are shown in Figure 5.

BTA21 contains markers from two human and three mouse chromosomes. *IGH* has been mapped to HSA14q32.33 and MMU12. *FES* has been mapped to HSA15q22-q26 and MMU7 (Kozak et al. 1983). Womack and Moll (1986) also assigned mannose phosphate isomerase (*MPI*) to BTA21. MPI has been mapped to HSA15q22-qter (Chun et al. 1977) and MMU9 (Nichols et al. 1973). The cytochrome P450 cholesterol side chain cleavage enzyme (CYP11A), which was mapped to BTA21 by Bolch and Skow (personal communication), has also been mapped to HSA15 (Chung et al. 1986) and remains unmapped in mice. Finally, the β2-microglobulin gene (*B2M*) was recently mapped to BTA21 (Nan and Womack, unpublished data), and maps to HSA15q21-q22.2 (Cohen et al. 1990) and MMU2 (Goding 1981; Michaelson, 1981).

In contrast to its chromosomal assignments in humans and mice, *FOS* was not found to be syntenic to *IGH* in cattle. Rather, it maps to BTA10, which consists of HSA14, HSA15, MMU9, MMU14, and MMU12 gene markers. In previous studies, the muscle form of pyruvate kinase (PKM2; Womack and Moll 1986), a keratin homolog (KRT8L1; Womack 1990), hexoseaminidase A (HEXA; Womack 1990), and nucleoside phosphorylase (*NP*; Womack and Moll 1986) have also been localized to BTA10. PKM2 maps to HSA15q22-qter (Chun et al. 1977) and MMU9 (Johnson et al. 1981), HEXA maps to HSA15q23-q24 (Nakai et al. 1987) and is unmapped in the mouse, and *NP* maps to HSA14q11.2 (Cohen et al. 1990) and MMU14 (Womack et al. 1977). KRT8L1 has not been mapped in mouse or human. Since the beginning of this study, Miller and co-workers (1991), using an independent panel of hybrid cells, have found synteny between bovine *IGHG2* and *MPI* and also confirmed the synteny of *NP* and *FOS*.

Several discordancies were observed between *IGH*, *FES*, *FOS*, and the syntenic groups to which they were assigned. Interestingly, all five discordant clones were those in which *FOS* was present, but the original syntenic group marker, *NP*, was not. *NP* was detected by an enzymatic assay and may not have been expressed at detectable levels in all of the hybrid somatic cells. It is also possible that *FOS* was present in some of the hybrid clones on a chromosomal fragment that did not include *NP*.

FES showed three discordancies in which it was present in the hybrid somatic cell clones, while the marker for BTA21, *MPI* was absent. In one of these clones, the *IGH* markers were also present. In addition, in the one case in which *FES* was not present in a clone positive for *MPI*, the *IGH* genes were negative as well. A pairwise concordancy analysis between *IGH*, *FES*, and *MPI* shows that *IGH* and *FES* have 94.6% concordance to each other, but only 94 and 89% concordance with *MPI* (Table 2).

Table 2. Pairwise concordancy analysis of *IGHM*, *IGHG4*, *FES*, and *MPI*.

	IGHM	*IGHG4*	*FES*	*MPI*
IGHM	–	100	94.6	94
IGHG4		–	94.6	94
FES			–	89
MPI				–

Thus, it is possible that *IGH* and *FES* are present together on chromosomal fragments, that do not contain *MPI*, in at least two of the hybrid somatic cell clones. In the other two clones containing *FES* but not *MPI*, the *IGH* markers were absent, so it is likely that a fragment of BTA21 was present in these hybrids that contained *FES*, but did not contain *IGH* or *MPI*.

It is unclear whether the immunoglobulin-like sequences discovered in this study represent pseudogenes or other sequences closely related to the bovine immunoglobulin heavy chain genes. However, it is likely that as in humans, cattle immunoglobulins have undergone duplications and rearrangements during their evolution resulting in pseudogenes scattered throughout the genome. The evolution of the immunoglobulin gene family has been extensively studied, and appears to be due to gene duplications, translocations, and IVS-mediated domain transfer (Hill et al. 1966; Miyata et al. 1980; Takahashi et al. 1982). In addition, the immunoglobulin genes appear to have arisen from an ancestral gene that gave rise to an entire superfamily of genes that are related to each other by primary nucleotide sequence, as well as protein structure and function (Williams and Barclay 1988). Characterizing the immunoglobulin-like sequences mapped in this study could lead to interesting information regarding the evolution of the immunoglobulin genes in cattle. Of special interest is the fact that an immunoglobulin epsilon heavy chain pseudogene is present on HSA9. It is possible that *IGHML1* is homologous to this pseudogene.

The immunoglobulins were shown to be relatively highly polymorphic genes that could potentially be used in linkage analysis and breeding programs. Since the population size that was used in the polymorphism studies was extremely small, with an average of ten animals per breed and three breeds, the data presented here are preliminary. Further research, including a larger population size with the addition of family studies, must be performed to determine the frequency, mode of inheritance, and breed specificity of the polymorphic immunoglobulin restriction fragments. Since white blood cells are easy to obtain and commonly used in family studies of RFLPs, correlative analysis of germ line and leucocyte patterns of individuals must be performed to distinguish heritable polymorphism from B-cell rearrangement. Our preliminary studies (data not shown) indicate that most of the germ line RFLPs reported here can also be observed in leucocyte DNA.

In contrast to the immunoglobulin genes, the protooncogene markers showed a striking lack of variation when used to probe bovine sperm DNA digested with *Eco* RI, *Hin* dIII, *Bam* HI, and *Bgl* II. In fact, in the eight enzyme : probe combinations studied, neither *FOS* nor *FES* demonstrated any polymorphisms. Previous studies in our laboratory have also failed to show polymorphisms between inbred strains of mice with these probes. The PIC, or polymorphism information content of a gene is a measure of the informativeness of the locus for linkage (Botstein et al. 1980), and may be a valuable screening method for determining which human homologs will be important for bovine linkage mapping. Cohen and coworkers (1990) reported the PIC in humans for *FOS* and *FES* to be 0.12 and 0.09, respectively. The PIC of *IGHG4*, by contrast, is 0.56. Perhaps human PIC values may be important in predicting the relative polymorphism of loci in other mammals. If so, the use of probes that reveal polymorphisms in humans, in addition to proper utilization of comparative gene mapping data, could greatly increase the efficiency of generating a bovine gene map saturated with polymorphic markers.

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