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Abstract. Quantitative trait loci (QTL) associated with fat deposition have been identified on bovine Chromosome 27 (BTA27) in two different cattle populations. To generate more informative markers for verification and refinement of these QTL-containing intervals, we initiated construction of a BTA27 comparative map. Fourteen genes were selected for mapping based on previously identified regions of conservation between the cattle and human genomes. Markers were developed from the bovine orthologs of genes found on human Chromosomes 1 (HSA1), 4, 8, and 14. Twelve genes were mapped on the bovine linkage map by using markers associated with single nucleotide polymorphisms or microsatellites. Seven of these genes were also anchored to the physical map by assignment of fluorescence in situ hybridization probes. The remaining two genes not associated with an identifiable polymorphism were assigned only to the physical map. In all, seven genes were mapped to BTA27. Map information generated from the other seven genes not syntenic with BTA27 refined the breakpoint locations of conserved segments between species and revealed three areas of disagreement with the previous comparative map. Consequently, portions of HSA1 and 14 are not conserved on BTA27, and a previously undefined conserved segment corresponding to HSA8p22 was identified near the pericentromeric region of BTA8. These results show that BTA27 contains two conserved segments corresponding to HSA8p, which are separated by a segment corresponding to HSA4q. Comparative map alignment strongly suggests the conserved segment orthologous to HSA8p21-q11 contains QTL for fat deposition in cattle.

Introduction

Fat deposition is an economically important trait in cattle. For US beef producers, fat deposition ultimately determines carcass yield grade and quality, thus affecting profit margin. In dairy cattle, a cow's proficiency to store adequate fat and then utilize these depots appropriately during lactation is genetically correlated with productive life and incidence of metabolic disease (Rogers et al. 1999). Furthermore, selection for more extreme dairy form (a conformation trait based upon body conditioning and moderately correlated with mild production) results in increased incidence of metabolic disease. Marker-assisted selection (MAS) for loci affecting fat deposition and dairy form that do not adversely affect meat or milk production could greatly improve the current selection of seed stock in both beef and dairy cattle.

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The density of microsatellite (ms) markers on the current bovine genetic maps has permitted numerous laboratories to analyze experimental cattle populations for a wide variety of quantitative trait loci (QTL). Before MAS can be employed, the QTL must be validated and refined with respect to map position. One way to improve the validity of detected QTL is to compare results between studies from different populations. An example of comparable results occurs on bovine Chromosome (Chr) 27 (BTA27), where potential QTLs associated with fat deposition were detected in two different populations. A genome-wide analysis of the USDA/Meat Animal Research Center (MARC) F_1 bull resource population (Casas et al. 1998) revealed a QTL for marbling with nominally significant effects close to the telomere of BTA27 (Casas et al. 2000). In this study, individuals inheriting a Belgian Blue allele had more intramuscular fat than those inheriting a MARC III composite allele. Comparatively, strong evidence for an association between genotypes for the BTA27 market *BM203* and dairy form ($P = 0.000021$) was detected in a US Holstein grandsire family by using the granddaughter design and analysis of variance (Ashwell et al. 1998). Interval analysis (Haley and Knott 1992) of this family with genotypes generated from additional BTA27 markers revealed a significant QTL effect for dairy form localized to the telomeric region, while no effect on milk production traits was detected (Van Tassell et al. 1998).

The current map of the BTA27 is insufficient to support efficient use of the gene-rich human map to either select positional candidate genes or develop additional informative markers based on Type I loci for refining genetic intervals containing QTL. Previous comparative mapping with Zoo-FISH revealed regions of conserved synteny between BTA27 and human Chrs 4 (HSA4) and 8 (Hayes 1995; Solinas-Toldo et al. 1995). Somatic cell mapping of six bovine genes confirmed the Zoo-FISH results and suggested additional genomic conservation between BTA27 and HSA1 (Ryan and Womack 1995) and 14 (Womack unpublished). A conserved segment of BTA27 corresponding to HSA4q35.1 was confirmed by the assignment of melatonin receptor 1a (*MNTR1A*) to the genetic map (Messer et al. 1997). Physical assignment by fluorescence in situ hybridization (FISH) of a beta-defensin gene cluster suggested the pericentromeric region of BTA27 corresponded to HSA8p23-p22 (Gallagher et al. 1995). On the basis of this preliminary information, the conserved segment of the human genome orthologous to the telomeric end of BTA27 could not be accurately identified. Therefore, we selected and positioned 14 genes on the bovine maps to improve the comparative alignment with the human gene maps and more precisely identify the conserved segment between these species that is likely to contain dairy form and marbling QTL.

Table 1. Primer, genomic clone, and preliminary map information for target genes.

Gene locus[a] Gene	Primer sequences (5′ → 3′) Forward (Top) Reverse (Bottom)	Position[b]	GenBank accession[c]	T_a[d] (°C)	Pred. size	Actual size	BAC/YAC Clone ID[e]	Previous assignments[f,g]
ADRA1C adrenergic alpha-1C-receptor	ATCGGCGTGAGCTATCCTC GTTCTTGGCGCTGGTCA	472–490 855–839	J05426 (bovine cDNA)	64	384	384	BAC 172 L1	ND[h] HSA8p21
CLU clusterin	GACGCCCTGAATGACACC GATCCGCTTCTGCACACC	298–315 449–432	J05391 (bovine cDNA)	62	152	152	ND	ND HSA8p21
CTSB cathepsin B	CGGCAACTCCTGGAACAC CTAGTACTGATGAGTGCACGGC	888–905 1008–987	L06075 (bovine cDNA)	64	121	718	ND	BTA8 HSA8p22
DEFB1 enteric beta-defensin 1	GGAAGACAGGAAGGCCTCTGG CCTCACGTTTTCAGAACCAC	1816–1836 2219–2200	AF016539 (bovine DNA)	60	404	404	ND	BTA27q1.3-q1.4 HSA8p23.2-p23.1
F11 coagulation factor XI	GCCCATATGCCTACCTTCC CATCTTGCTGGTTATTCTGTGC	137–155 871–850	U85057 (Bovine DNA)	64	735	736	ND	BTA17 HSA4q35
FGFR1 fibroblast growth factor receptor 1	ACAGGATGGTCCCTTGTATGTC CTGGTAGGCGCAGGACAC	66–87 228–211	AJ004952 (bovine cDNA)	58	163	163	cosmid	ND HSA8p11.2-p11.1
FNTA farnesyltransferase, CAAX box, alpha	TGTGACAACAAGGAGGACATTC TGCTGTGTTTGCTTTGAAGG	864–885 990–971	M74083 (bovine cDNA)	60	127	385	BAC 17 C5	ND HSA8p22-q11
MNTR1A melatonin receptor 1a	(Messer et al. 1997)	285–304 1108–1089	U14109 (sheep DNA)	60	824	824	BAC 16 B18	BTA27 HSA4q35.1
NPY2R neuropeptide Y receptor Y2	TTATCGACAGCACCAAGCTG CACTGTCAAGGTGATGGTGG	270–289 580–561	U50144 (bovine cDNA)	60	311	311	BAC 2 B23	ND HSA4q31
PENK proenkephalin	AACCTGCTCAAGGAGCTGC TCCATCCACCACTCTGGAC	563–581 771–753	V00109 (bovine cDNA)	66	209	209	ND	ND HSA8q11.23-q12
PLAT plasminogen activator, tissue	AGACTTTGTCTGCCAGTGCC CTCTCTGCCGTGCTCCAC	324–343 443–426	X85800 (bovine cDNA)	64	110	387	YAC 182 B12	BTA27 HSA8p12
RNS1 ribonuclease, RNase A family, 1 (pancreatic)	ACTGCCTTCTCTCTCTCAGACATC AGGACCAGGGACTTCAGAGC	718–741 1538–1519	X07283 (bovine DNA)	58	821	821	ND	BTA27 HSA14
SKI v-ski avian sarcoma viral oncogene homolog	ACATCTACTGCTCGCGCTG CCGTCTTGGTGATGAGCC	512–530 613–596	U14173 (murine cDNA)	56	102	102	BAC 216 G6	BTA27 HSA1q22-q24
STAR steroidogenic acute regulatory protein	TACGCTGTACCAAGCGCC AGCCAGGTGAGTTTGGTCC	5877–5894 6770–6752	Y17260 (bovine DNA)	56	893	893	BAC 16 L13	ND HSA8p11.2

[a] Locus name equivalent to human gene symbol. Bovine enteric beta defensin has no defined human ortholog by BLASTn comparison. However, BLASTx suggests closest human ortholog at amino acid level is DEFB1 (44% similarity, 19/43 amino acids match).
[b] Position of oligonucleotide primers is based upon the corresponding nucleotide sequence obtained from GenBank.
[c] DNA template source of accession number shown in parentheses. GenBank accession number for bovine *SKI* AF238294.
[d] T_a, annealing temperature used for PCR amplification.
[e] Clone address in RPCI-42 BAC or USDA/MARC YAC Libraries.
[f] Previous assignment of locus on bovine:human comparative map (http://bos.cvm.tamu.edu/htmls/BHM.html).
[g] Physical map location of human ortholog (http://gdbwww.gdb.org/).
[h] ND, not determined.

Materials and methods

Polymerase chain reaction (PCR) amplification and SNP detection. Primer pairs for amplification of bovine genomic DNA (Table 1) were designed from sequences stored in the GenBank database (Benson et al. 1993). Amplification was performed by PCR as described by Smith et al. (1997). Thermocycling conditions were modified for use on the PTC-200 DNA engine (MJ Research Inc, Watertown, Mass.) by reducing step times to 15 s. PCR products were electrophoresed on 3% agarose gels and visualized with ethidium bromide. Gel-isolated PCR products were purified by using a Qiaquick column (Qiagen, Valencia, CA) and were directly sequenced. Sequencing reactions (5 μl total volume) were performed as recommended with 2 μl of ABI Big Dye (PE Corporation, Foster City, Calif.), 1–20 ng of DNA template, and 3.2 pM primer. Reaction products precipitated with 20 μl 70% isopropanol were resuspended in blue dextran:formamide dye (1:5) for analysis on an ABI-377 (PE Corporation, Foster City, Calif.). Sequencing files were analyzed with Chromas 2.1 (http://www.technelysium.com.au/chromas.html). Sequences searched against the GenBank database via BLAST search (Altschul et al. 1990) verified that each PCR product was specific to the target gene and human orthology. For single nucleotide polymorphism (SNP) discovery, PCR products generated from four F_1 bulls and eight cows in the MARC reference population (Bishop et al. 1994) were sequenced and compared. SNPs associated with recognition sites for restriction enzymes (Table 2) were detected and mapped as described by Sonstegard and Kappes (1999a).

Somatic cell mapping. Amplified products generated from bovine and rodent control DNA were compared. If the bovine product was unique or distinguishable, primers were used to PCR amplify DNA from 57 bovine x rodent cells lines of the Texas A&M (College Station, TX) bovine somatic cell hybrid panel, and amplification products were compared for concordance to previously mapped loci (Womack and Moll 1986).

Large-insert clone isolation. Yeast artificial chromosome (YAC) clones were identified and isolated from the USDA bovine YAC library by PCR (Smith et al. 1996). Cosmid clones were identified and isolated by iterative PCR (Heaton et al. 1997). For screening of the Roswell Park Cancer Institute (RPCI)-42 bovine bacterial artificial chromosome (BAC) library (Warren unpublished), radiolabeled probes were synthesized from gel-isolated PCR amplicons with the MegaPrime DNA Labelling System (Amersham Pharmacia Biotech, Piscataway, NJ). BAC clone positions in the library plates were detected by hybridization of the probes to the high-density replica filters. Hybridization results correlated with estimated genome coverage of the library (10x) by yielding an average of one clone/probe/filter hybridized. Single colonies grown on Luria broth plates containing 20 μg/ml chloramphenicol (CAP) were inoculated into 5 ml of SuperBroth (DiGene, Beltsville, Md.) with 20 μg/ml CAP and grown for 22 h at 37°C. Correspondence between BAC clones and gene probes was determined by hybridization to BAC DNA purified by ProPrep BAC purification kit (LigoChem Inc., Fairfield, N.J.) before being confirmed by direct sequencing (Kelley et al. 1999).

Table 2. Single nucleotide polymorphism (SNP) identification and mapping.

Marker[a] (Gene locus)	Polymorphic sequence (5′ → 3′)	SNP position	GenBank accession	PCR-RFLP allele sizes	Restriction enzyme	Chr	Meioses	Linkage analysis[b] LOD	Rec. Frac.	Marker[c]
CTSB-1	GCAGG(A/G)GGCGG	212	AF230197	Detection by sequence analysis		8	68	7.22	0.00	Z27077
CTSB-2 (*CTSB*)	TGAGC(A/G)TGCAC	563		185/153–32	*Nla*III		55			
DEFB1-1 (*DEFB1)*	AGTAA(A/G)TTCTC	1978	AF016539	403/243–161	*Tsp*509I	27	74	21.98	0.00	BMS2104
F11-1	TAATA(T/A)CATTA	197	U85057	Detection by sequence analysis		27	91	24.11	0.05	INRA016
F11-2 (*F11*)	TACAG(C/T)GGAAG	118		735/616–119	*Tsp*RI		193			
FNTA-1	CTAGG(G/T)ACTGA	250	AF230196	385/250–135	*Rsa*I	27	60	19.87	0.00	BB701
FNTA-2 (*FNTA*)	CAGTG(C/T)GCATG	91		385/294–91	*Hha*I		70			
PENK-1 (*PENK*)	GGAGC(C/T)GGGGA	589	V00109	125/115–10	*Alu*I	14	91	23.23	0.01	RM011
PLAT-1 (*PLAT*)	GTGGC(A/G)CTGAG	249	AF230195	387/249–138	*Hha*I	27	68	17.60	0.01	MSBQ
RNS-1 (*RNS1*)	GCCTC(T/C)CTGGC	1428	X07283	557/474–83	*Bst*NI	10	89	23.52	0.01	MB077

[a] All markers listed are SNPs. The associated gene symbol is listed below the marker name in parentheses.
[b] Data presented only for the largest LOD scores from two-point linkage analysis with Cri-Map. Genotypic data generated from SNP markers associated with the same gene were treated as haplotypes for linkage analysis. Estimated marker position on the bovine linkage map can be accessed at (http://www.marc.usda.gov/genome/cattle/cattle.html).
[c] Marker identified to have the largest LOD score with marker in locus column.

FISH analyses. DNA isolated from selected genomic clones was biotinylated with BioNick Labeling System (GibcoBRL, Gaithersburg, Md.) and purified through a G-50 fine sephadex column (5Prime3′, Boulder, Colo.) R-banded metaphase chromosomes were prepared as reported by DiBerardino and Iannuzzi (1982). FISH analysis of these probes was performed essentially as described by Lichter et al. (1990). Approximately 200 ng of probe was co-precipitated in ethanol with 5 μg sonicated salmon sperm DNA and 10 μg bovine $C_{ot}1$ DNA (Applied Genetics Lab, Melbourne, Fla.). FITC and PI signals were detected with the 488- and 568-nm line, respectively, of an argon-krypton laser by using a Zeiss LSM 410 confocal microscope. To aid chromosomal positioning, Co-FISH was performed with a probe generated from a cosmid containing BMS2104 (Heaton unpublished) for all the BTA27 specific probes.

Microsatellite markers. Preparation of BAC subclone libraries was performed essentially as reported by Sonstegard et al. (1998) using *Sau*3AI-digested BAC DNA. Plasmid inserts containing ms (GT > 11) were identified (Stone et al. 1995) and sequenced on an ABI-377 according to the manufacturer's protocol (PE Biosystems, Foster City, Calif.). Primer pairs, PCR conditions, and parental heterozygosity were determined for each ms locus as described by Stone et al. (1995). Allele sizes were determined with ABI-Genotyper software after ABI-GeneScan analysis of PCR amplicons electrophoresed on an ABI-3700 (PE Corporation, Foster City, Calif.). Linkage analysis of genotypic data was performed as described by Kappes et al. (1997) to the existing linkage groups in the MARC database (Keele et al. 1994; http://www.marc.usda.gov/cattle) with Cri-Map version 2.4 (Green et al. 1990).

Results

Target gene amplification. Selection of the 14 genes for map development of BTA27 was based on comparative alignment of the existing bovine and human maps available from public databases on the World Wide Web. Seven of the genes had been previously mapped (Table 1). We elected to further analyze these genes, since some exhibited potential inconsistencies in positioning when bovine somatic cell mapping and Zoo-FISH results were compared (http://bos.cvm.tamu.edu/htmls/BHM.html). Sequence analysis of the bovine genomic amplification products revealed proper targeting of all selected genes (Table 1). The *SKI* primers designed from murine sequence amplified a bovine product 97% and 100% similar to a 102-bp segment of murine and human *SKI* exon 1, respectively. Only the amplification products for *CTSB*, *FNTA*, and *PLAT* differed from the predicted size, as the primer pairs for these genes each amplified across a single intron not defined by bovine cDNA accessions.

Mapping of SNP markers. Gene amplification products generated from 12 MARC reference parents were compared by sequence analysis to detect SNPs that could be exploited for linkage mapping. SNPs were found in the coding sequence of *PENK* and the noncoding sequences of *CTSB*, *DEFB1*, *F11*, *FNTA*, *PLAT*, and *RNS1* genes. The SNP used to previously map *MNTR1A* to BTA27 was not detected in the MARC reference parents (Messer et al. 1997). In all, genotypes were generated from 10 SNP markers that corresponded to seven genes (Table 2). Linkage analysis of these genotypes positioned *DEFB1*, *F11*, *FNTA*, and *PLAT* in the BTA27 linkage group. The results for *DEFB1* and *PLAT* agreed with previous physical results (Gallagher et al. 1995; Threadgill and Womack 1991; respectively). The linkage map positions of *FNTA* and *PLAT* were estimated to be less than 10 cM from the putative fat deposition QTL found at the telomeric end of BTA27. In contrast, the linkage map position for *F11* did not correspond to a previous physical assignment of this gene to BTA17 (Zhang et al. 1992). A similar inconsistency was revealed when *RNS1* mapped to BTA10, which disagreed with a previous physical assignment to BTA27 obtained by Womack (unpublished). Positioning of *CTSB* correlated with previous somatic cell mapping to BTA8 (Guerin et al. 1994). A third gene not syntenic with BTA27 was *PENK*, which mapped to BTA14. The most current positions on the linkage map for these gene-associated markers can be accessed at http://www.marc.usda.gov/cattle.

Physical mapping by somatic cell hybrid panel. Alternative mapping strategies were used to position the seven genes for which no SNPs were detected. To avoid developing ms markers for loci not syntenic to BTA27, we attempted to assign the remaining genes by somatic cell mapping. Primers for *ADRA1C*, *CLU*, *FGFR1*, *NPY2R*, and *SKI* were tested on bovine and rodent control DNA.

Table 3. Microsatellite markers developed from BAC clones containing gene loci.

Locus[a]	Primer sequences (5′ → 3′) Forward (Top) Reverse (Bottom)	T_a (°C)[b]	No. alleles	Allele (bp) Min.–Max.	Obs. Het.[c]	Chr	Meioses	Linkage analysis[d] LOD	Rec. Frac.	Marker[e]
BB700 (*FNTA*)	TTTATGAGTCCCTCTTTCTGCC GCTGTATCCTAGGCGAGCAC	62	17	146–280	0.82	27	310	79.10	0.01	BB701
BB701 (*FNTA*)	GCATTTTCAGATGGCTTACTCC TGTTTGTTTGTTATCTGCTGCC	60	12	144–230[e]	0.82	27	340	73.49	0.01	MSBQ
BB703 (*ADRA1C*)	AACTCCCAGGGGATGGAC GCATGCAAACCTGCAATG	58	12	154–180	0.89	8	309	76.68	0.01	IDVGA-52
BB708 (*FGFR1*)	CACAGCTGCAGACACATGG GTCAGGGAAAGCTGGATTAGC	60	4	171–181	0.43	27	157	33.11	0.00	INRA134
BB709 (*SKI*)	GCTGGGTTTTGTAGACGCTC GGATTCAAGAATTAGCCAGTGC	60	11	220–247	0.56	16	225	38.91	0.06	IDVGA-49
BB716 (*MNTR1A*)	GATCTTTCTACAGACTGCCAC ACATTTGCTGATATGGTTGG	60	5	148–166	0.64	27	286	65.02	0.00	BMS641
BB718 (*NPY2R*)	TCTCTCTCCAAGGGCTTCAC GGAGCATTCTGTTTGAAATGC	60	5	141–151	0.68	17	279	41.49	0.04	BMS1825

[a] All markers listed are ms. Microsatellite sequences can be accessed in dbSTS of GenBank (Accession numbers G63884-9 and G63905). The symbol for the gene found on the same BAC clone as the ms is listed below the marker names in parentheses.
[b] T_a, Annealing temperature used to generate genotypes via PCR amplification.
[c] Marker heterozygosity observed in MARC reference population parents (n = 28).
[d] Data presented only for the largest LOD scores from two-point linkage analysis with Cri-Map. Estimated marker position on the bovine linkage map can be accessed at (http:www.marc.usda.gov/genome/cattle/cattle.html).
[e] Marker identified to have the largest LOD score with marker in locus column.
[f] Some animals found to have a null (unamplified) allele.

Only *CLU* amplified a distinguishable bovine specific product. *CLU* was 98% concordant with the interferon omega locus on BTA8 (data not shown); therefore, no additional markers were developed.

Mapping of ms markers. Large insert clones containing *ADRA1C, FGFR1, NPY2R, MNTR1A, SKI,* and *STAR* were isolated for marker development (Table 1). Genomic clones containing *PLAT* and *FNTA* were also isolated, because the SNP markers for these genes were positioned near the telomere of BTA27. Seven unique and informative gene-associated ms markers were successfully developed (Table 3). Only the YAC containing *PLAT* and BAC containing *STAR* did not yield ms makers. Linkage analysis of marker genotypes positioned BB700 and BB701 (*FNTA*), BB708 (*FGFR1*), and BB716 (*MNTR1A*) in BTA27 linkage group (Table 3). BB703 (*ADRA1C*), BB709 (*SKI*), and BB718 (*NPY2R*) linked to BTA8, BTA16 and BTA17, respectively. Similar to *RNS1,* the positioning of BB709 (*SKI*) on BTA16 was inconsistent with previous somatic cell mapping to BTA27 (Ryan and Womack 1995).

Physical mapping by FISH. In order to integrate the physical and genetic maps, DNA from the large insert clones used as a resource for marker development was also used to generate FISH probes. Before mapping these clones, the physical locations for the ends of the BTA27 linkage group were established by performing FISH analyses on cosmid clones containing BMS2104 and BM203 (Fig. 1A). The FITC signal localizations of BMS2104 to BTA27q12 were consistent with a previous physical assignment (Kappes unpublished). For BM203, the physical assignment was extended from a previous assignment (Masabanda et al. 1996) to include BTA27q24. No chimerism was detected for the prepared probes based on detection of only one signal for each probe hybridized (Fig. 1B-H). The physical locations of these FISH probes corresponded to the genetic locations of the marker loci contained within the probes. In all, unique physical assignments were made for eight genes (Fig. 1B-H). *FNTA, PLAT, FGFR1, MNTR1A,* and *STAR* were located on BTA27, and these assignments integrated six loci positioned on the BTA27 linkage map (Fig. 2).

Discussion

Identification of conserved segment containing QTL. The goal of this study was to map 14 genes that would improve the comparative alignment of conserved synteny shared between BTA27 and the human genome. Ten SNP and seven gene-associated ms markers were developed and used to position 12 genes on the linkage map (Tables 2 and 3, respectively). For physical mapping, seven FISH probes encompassing 10 of these markers were used to increase integration between physical and genetic maps (Figs. 1 and 2). The remaining two genes, *STAR* (Fig. 1F) and *CLU* (data not shown), were assigned only to the bovine physical map. Together, these results provided a better comparative alignment between species maps, which allowed for a more accurate identification of the conserved segment of human genome corresponding to the telomeric region of BTA27 (Fig. 2). Our results strongly suggest a physical interval contained within HSA8p21-q11 (http://gdbwww.gdb.org/) and a genetic interval from 54 to 67 cM on HSA8 (Fig. 2) corresponds to the conserved segment of BTA27 containing putative fat deposition QTL.

Comparative map of BTA27. A more accurate assessment of the comparative locations of conserved segments was permitted by increasing the gene marker resolution and density on the BTA27 maps. Six SNP and four ms markers were syntenic with BTA27, and these ten markers (associated with six genes) provided comparative coverage of nearly the entire chromosome (Fig. 2). Beginning at the pericentromeric portion of BTA27, this region corresponds to a conserved segment contained within the genetic interval from 8 to 30 cM on the HSA8 transcript map (Fig. 2). This comparison was supported by lack of detectable recombination between the SNP marker for *DEFB1* and the centromeric marker of the linkage group, BMS2104, that was also assigned by FISH to BTA27q12 (Table 2 and Fig. 1A). This genomic conservation may extend only ~5 cM, because there is a discrepancy in the positioning of *CTSB* on the HSA8 transcript map (Fig. 2). Based on this result, the previous physical assignment by Gallagher et al. (1995) of the defensin gene cluster to BTA27q13-q14 appears to have been too distal with respect to the centromere.

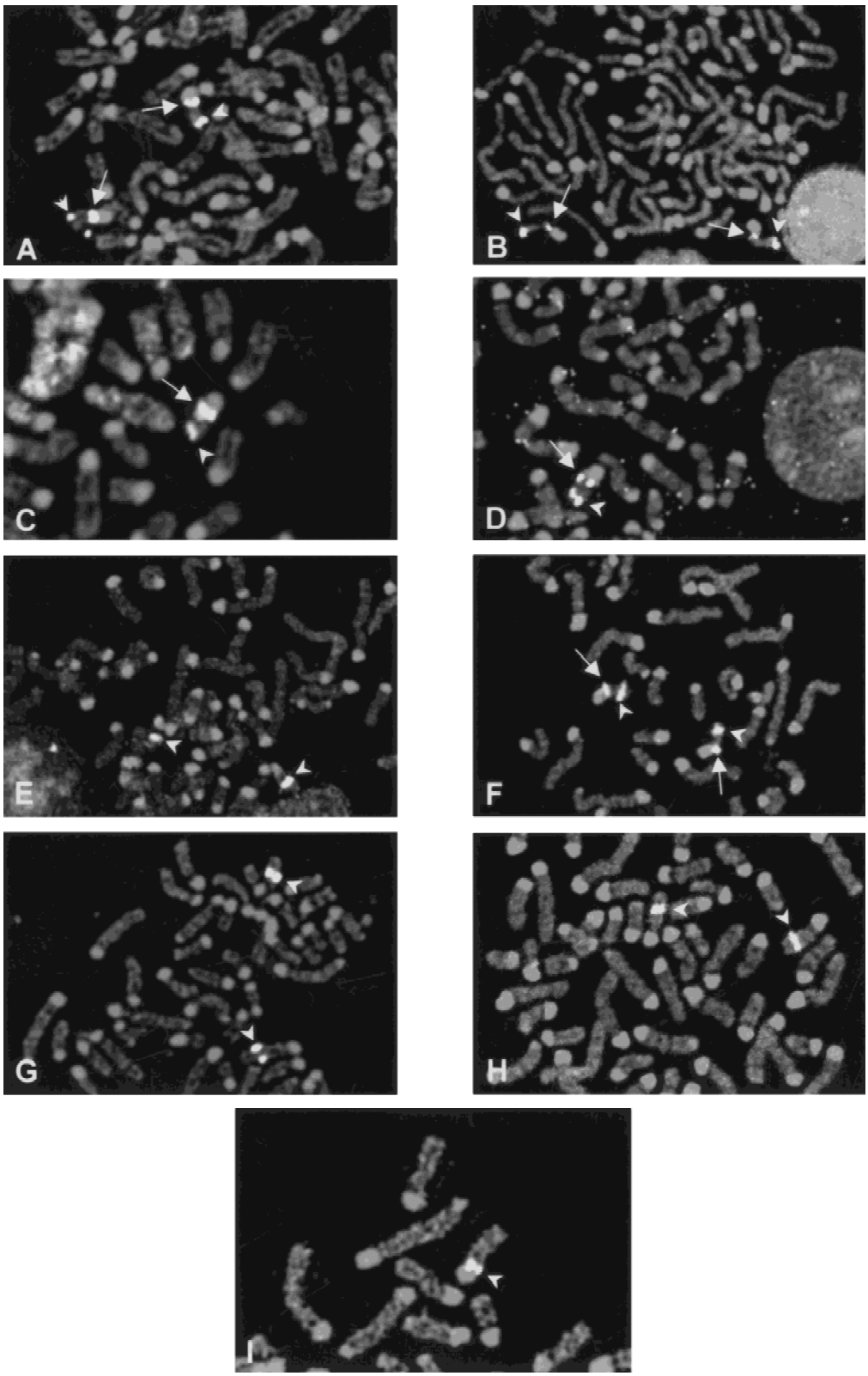

Fig. 1. FISH analyses of eight gene loci. A panel of nine partial bovine R-banded metaphase preparations with FITC hybridization signals derived from biotinylated, bovine genomic DNA probes is shown. White arrowheads point to the location of the detected FITC signal in each panel. The panel displays the following hybridizations: **A**) cosmid probe BMS203 detected at BTA27q23-q24; **B**) BAC 17 C5* (*FNTA*, BB700, BB701) probe detected at BTA27q22-q23; **C**) YAC 182 B12 (*PLAT*) probe detected at BTA27q22-q23; **D**) cosmid containing *FGFR1* and BB708 detected at BTA27q22-q23; **E**) BAC 16 B18* (*MNTR1A*, BB716) probe detected at BTA27q12; **F**) BAC 16 L13* (*STAR*) probe detected at BTA27q22-q23; **G**) BAC 172 L1* (*ADRA1C*, BB703) probe detected at BTA8q21q-22; **H**) BAC 216 G6* (*SKI*, BB709) probe detected at BTA16q21; and **I**) BAC 2 B23* (*NPY2R*, BB718) detected at BTA17q12. For reference, A–D and F also show detection of the co-FISH cosmid probe containing BMS2104 to BTA27q12 (white arrow). Indicates plate and well address of BAC clone in RPCI-42 library.

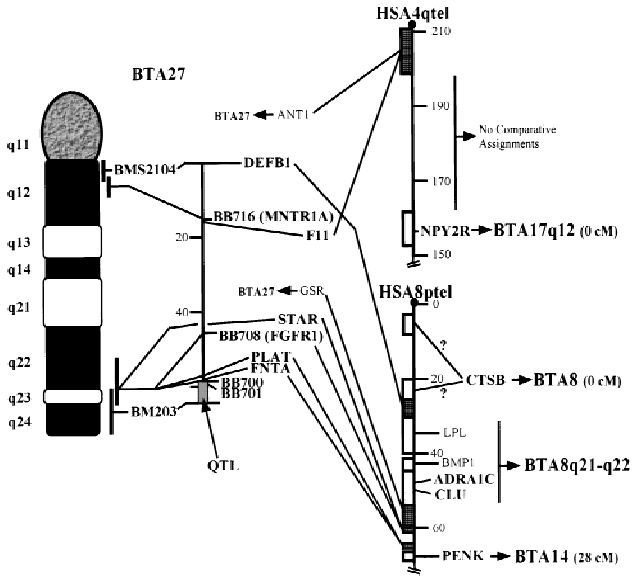

Fig. 2. Comparative alignment of BTA27 comprehensive map (left) and partial transcript maps of HSA4q and HSA8p (right). Thick vertical bars next to ideogram of a G-banded BTA27 indicate positions of physical map assignments. Integration to BTA27 linkage map and alignment with the human maps are indicated by solid and dashed lines between maps and gene symbols. Gene symbols in **bold** represent loci mapped in this study. Estimated positions for bovine and human loci are given in cM. Gray filled box on bovine linkage map represents approximate positions of QTL for marbling and dairy form. Genetic intervals containing human loci are represented by boxes attached to axis of transcript map, and these interval bins were derived from the GB4 series radiation hybrid maps (http://www.ncbi.nlm.nih.gov/genemap99/page.cgi?F = Home.html). Filled boxes represent segments of human genome that appear conserved on BTA27. "?" for the assignments of CTSB to HSA8p represent a positional discrepancy. Citations for the previous assignments of *ANT1*, *BMP1*, *GSR*, and *LPL* are found in the **Discussion**.

The relatively small size of the conserved segment containing *DEFB1* is further supported by the physical assignment of *MNTR1A* to BTA27q12 (Fig. 1E). The FITC signals of the probes for BMS2104 and *MNTR1A* overlapped and were nearly indistinguishable by Co-FISH analysis (data not shown). Aside from the high LOD scores to other BTA27 markers and each other (LOD 15.05, rec. frac. 0.00), placement of BB716 (*MNTR1A*) and *F11* is supported by the somatic cell mapping of adenine nucleotide translocator 1 (*ANT1*) to BTA27 (Li and Womack 1997). These results denote a boundary in conserved synteny between BTA27 and HSA8p and HSA4q, as the human orthologues of *F11*, *ANT1*, and *MNTR1A* are contained on a conserved segment corresponding to HSA4q35 (http://gdbwww.gdb.org/). Additionally, the genetic interval from 199 to 212 cM on the HSA4 transcript map contains *ANT1* and *F11* (Fig. 2).

Continuing distal from the centromere of BTA27, a large gap in the comparative map still exists. Although not proven in this study, the region between BTA27q13-q21 on the physical map and ~20–40 cM on the genetic map may correspond to HSA4q32-q34 and an interval from 160 to 199 cM on the HSA4 transcript map (Fig. 2). This region on the human maps is devoid of comparative gene assignments for cattle. Comprehensive mapping of BB718 (*NPY2R*) to BTA17 (Table 2, Fig 1I) supports this observation. Located just distal to the centromere of BTA17, BB718 designates a breakpoint for a conserved segment corresponding to HSA4q25-q31. Mapping studies that previously defined this comparative genomic conservation revealed that the bovine orthologs for interleukin 2 and 15 and uncoupling protein 1 mapped to BTA17 in reverse gene order with respect to the centromere (Fisher et al. 1997; Sonstegard unpublished; Sonstegard and Kappes 1999b, respectively).

The next distinguishable conserved segment of BTA27 corresponds to a genetic interval from 54 to 67 cM on the transcript map of HSA8p (Fig. 2). The boundary on BTA27 between conserved segments corresponding to HSA4q and HSA8p is defined by the integrated mapping of *FNTA*, *PLAT*, and *FGFR1* and the physical mapping of *STAR* (Fig. 2) just proximal to the telomere. These results are in agreement with the previous somatic cell mapping of *PLAR* and glutathione reductase (*GSR*) to BTA27 (Threadgill and Womack 1991).

Mapping of genes not syntenic with BTA27. Although half of the target genes were not syntenic with BTA27, map information generated from markers associated with these genes improved the resolution of the comparative alignment between species maps. As discussed in the Introduction, previous somatic cell mapping and Zoo-FISH analyses were not in complete agreement. Such a disparity could be overlooked owing to the gross resolution of Zoo-FISH; however, the definitive map placement of BB709 (*SKI*) to BTA16, and RNS-1 (*RNS1*) to BTA10, agreed with the Zoo-FISH results and did not support the existence of conserved synteny between BTA27 and HSA1 and 14. However, the integrated mapping of *SKI* does support extending the genomic conservation shared between HSA1 and BTA16. In addition, the placement of *RNS1* distal to the centromere (~20 cM) on the BTA10 linkage map, together with other supporting comparative map data for this chromosome (http://bos.cvm.tamu.edu/htmls/BHM.html), suggests the genomic segment containing a cluster of ribonuclease genes at HSA14q11 (http://gdbwww.gdb.org/) is conserved in cattle.

Improved breakpoint resolution was also attained by mapping *ADRA1C* (Table 3, Fig. 1G), *CTSB* and *PENK* (Table 2). Previous mapping of lipoprotein lipase (*LPL*; Tank and Pomp 1994) and bone morphogenetic protein 1 (*BMP1*; Martin-Burriel et al. 1997) defined a conserved segment of BTA8 corresponding to 31–45 cM on the HSA8 transcript map (Fig. 2). Somatic cell mapping of *CLU* and integrated mapping of BB703 (*ADRA1C*) extend this conservation to include the genetic interval from 45 to 54 cM on HSA8 (Fig. 2). Placement of *CTSB* on the centromeric end of BTA8 linkage group (Table 2) identified a previously unrecognized region of genomic conservation with a telomeric region of HSA8p (Fig. 2). Finally, the addition of *PENK* to the BT14 linkage group refined the location of a breakpoint on HSA8 for conserved segments found on BTA14 and BTA27.

In summary, a minimum of two segments of HSA8p are conserved on BTA27. These segments are separated by another conserved segment corresponding to HSA4q35 that possibly extends into HSA4q32. Future comparative mapping of bovine orthologs from this region should resolve this possibility. Application of our comparative map for BTA27 will aid future development of informative markers near the QTL locations for dairy form and intramuscular fat, thus enhancing further genetic analysis and interval refinement. The current study has already yielded five genetic markers that are estimated to be within 15 cM of these QTLs.

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