

A comparative linkage and physical map of bovine chromosome 24 with human chromosome 18

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Abstract. Polymorphic microsatellites have been developed in the vicinity of nine genes on bovine chromosome (BTA) 24, all orthologous to genes on human chromosome (HSA) 18. The microsatellites have been isolated from bacterial and yeast artificial chromosome clones containing the genes. A linkage map was developed including these polymorphic markers and four anonymous, published microsatellites. Yeast artificial chromosomes containing six of these genes have also been mapped using fluorescent in situ hybridization (FISH), thereby tying the linkage map together with the physical map of BTA24. Comparing gene location on HSA18 and BTA24 identifies four regions of conserved gene order, each containing at least two genes. These genes identify six regions of conserved order between human and mouse, two more than in the human-bovine comparison. The breakpoints between regions of conserved order for human-bovine are also breakpoints in the human-mouse comparison. The centromere identifies a fifth conserved region if the BTA24 centromere is orthologous with the HSA18 centromere.

Introduction

Gene mapping in cattle has progressed well with two medium resolution linkage maps published recently (Barendse et al. 1997; Kappes et al. 1997). All linkage groups have been assigned to individual chromosomes, and their orientations have been established relative to the centromere (Barendse et al. 1997). However, the development of linkage maps has relied heavily on anonymous microsatellites, as exemplified by the presence of only 160 and 55 gene polymorphisms on the recent linkage maps (Barendse et al. 1997; Kappes et al. 1997).

The bovine linkage map is now sufficiently dense and well covered to permit a large scale search for QTL (quantitative trait loci) underlying phenotypic variation among animals. Recently published examples include searches in cattle for QTL influencing milk yield (Georges et al. 1995) and fertility traits (Blattman et al. 1996). The resolution of the results obtained is generally rather coarse, in part because markers are not very polymorphic in the resource populations of interest for trait mapping, and in part because building the large resource populations needed for fine scale mapping is prohibitively expensive. It is therefore difficult to directly identify the gene underlying an identified QTL. An alternative would be to investigate genes that potentially could influence the trait. An efficient approach would be to investigate only such candidate genes already known to map in the region where a QTL

has been mapped, i.e., the positional candidate approach (Ballabio, 1993). However, a sufficiently dense bovine gene map is not likely ever to be obtained for this approach to be directly applicable. Therefore, drawing on the information collected in the human and mouse genome projects via use of comparative gene maps is a prerequisite for making the positional candidate gene approach a viable alternative.

Comparative gene mapping is the effort to obtain map positions for the same gene in several species. The framework for the syntenic relationship between human and bovine genomes has already been established, most efficiently by fluorescent in situ hybridization (FISH) using a collection of clones from a whole human chromosome as probe (ZOO-FISH) (Solinas-Toldo et al. 1995; Hayes 1995; Chowdhary et al. 1996). However, several examples demonstrate that the linear order of genes along a chromosome is often not conserved even though the overall gene complement is (e.g., Johansson et al. 1995). It is therefore necessary to determine the linear order of genes along chromosomes in cattle as well as in the human and mouse in order to use information obtained in the human and mouse genome projects.

ZOO-FISH results for cattle indicate that the only bovine chromosome painted by human chromosome (HSA) 18 is BTA24 (Solinas-Toldo et al. 1995; Hayes 1995; Chowdhary et al. 1996). A small additional part of BTA24 corresponds to HSA4 (Solinas-Toldo et al. 1995; Chowdhary et al. 1996). No gene present on HSA4 has yet been mapped to BTA24, while our recent synteny mapping of seven genes from HSA18 to BTA24 (Larsen et al. 1996) as well as two genes previously mapped to BTA24 (Womack et al. 1991) have corroborated the similarity between HSA18 and BTA24. To establish gene order within this region of conserved synteny we report the development of polymorphic, bovine markers in the vicinity of nine genes found on HSA18, and all mapping to BTA24. Six of the genes have also been mapped using FISH. In order to obtain polymorphic markers, we have devised a new strategy for isolating microsatellites from bovine YACs. The results demonstrate a major difference in linear order of genes between the two species. Additionally, the break-points between conserved segments are conserved when the human-bovine comparative map is extended to include mouse; however, more break-points were observed between human-mouse using the same markers.

Materials and methods

Identifying bovine sequence tagged sites (STS's). Seven loci (Thymidylate Synthase (TYMS), Adenylate Cyclase Activating Peptide, Pituitary 1 (ADCYAP1), Melanocortin-2 Receptor (MC2R), Neural Cadherin (CDH2), Gastrin Releasing Peptide (GRP), Transthyretin (TTR), and Plasminogen Activator Inhibitor 2 (PAI2) were amplified as described (Larsen

Accession numbers of sequences from this study: AF010230–AF010232, AF018087–AF018092, AF019947–AF019949, AF026036, and U78859
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Table 1. PCR conditions for three genes from HSA18.

Gene	Primary PCR primers	Annealing conditions ^a	Size of PCR product (bp)	Secondary PCR primers ^b	Annealing conditions ^a	Size of PCR product (bp)
MADH4	TGGTGTTCATTGCTTACTTT CTCTCTCAATGGCTTCTGTCC	50 C, 1.5 mM	169 bp	N.A. ^c N.A. ^c		
LAMA1	gtttCCTGTTTCTGCCATTCTC ^d gtTTGTGACCCAGTGATATTCTCTC ^d	58 C, 1.5 mM	437 bp	TGGAAATGAACAGCGGTAAG GCAATGCACACATCAGCAC	58 C, 1.5 mM	244 bp
MBP	CCGGGGAGGGAGGACAACACC GAAGCGCCGATGGAGTCAAG	62 C, 1.8 mM	231 bp	AATGGCGGCCAGAAAG GGAGTCAAGGATGCCAGTGTC	66 C, 1.5 mM	118 bp

^a Annealing temperature and concentration of MgCl₂.

^b Bovine specific primers designed at sites interior to the primary PCR primers.

^c No secondary primers were designed for this gene.

^d Several nucleotides (PIGtail) were added to the 5' end of this primer to facilitate non-templated adenylation during PCR (Brownstein et al. 1996).

et al. 1996). Bovine STS (Table 1) were developed for three more genes from HSA18 for which sequences were available in GenBank/EMBL (accession numbers in parentheses). A bovine fragment from the Laminin Alpha 1 (LAMA1) gene was cloned using primers from a human cDNA sequence (X58531) aligned with a mouse cDNA sequence (J04064). The expected size of this fragment was 263 bp based solely on the cDNA sequence (the intron/exon structure of the LAMA1 gene was unknown). It was not surprising that the cloned PCR product was larger than predicted (437 bp) and contained an intron. A second, bovine-specific primer pair was designed with one primer in the intron. Primers for the Myelin Basic Protein gene (MBP) were designed using a human genomic sequence (M63599) of the 5' flanking region, aligned with a mouse cDNA sequence (X67319) for Golli-MBP, a transcript overlapping the MBP gene. Bovine-specific primers were developed from the cloned product. Primers for the gene Deleted in Pancreatic Carcinoma-4 (MADH4, previously known as DPC4) were designed within exon 8 of a human cDNA sequence (U44378) without any second species sequence. This human-specific primer pair worked so well that no bovine-specific primer pair was designed. PCR conditions for amplifying these three genes were 0.125 mM dNTP, 0.3 μM of each primer (Table 1), 0.35 U *Taq* DNA polymerase (Promega), 1x Mg-free buffer (Promega) and MgCl₂ (concentration according to Table 1) in a 10 μl reaction volume. Amplification profile was 2 min at 95°C; 30 cycles of 30 s at 94°C, 30 s at annealing temperature, and 40 s at 72°C; 5 min at 72°C in a MJ PTC100 thermal cycler.

Screening large insert libraries. Large insert libraries were screened by PCR to identify positive colonies. The first library screened was a bovine BAC library containing 23,040 clones (Cai et al. 1995). There was an expected success ratio of 0.7 for finding a clone containing any given gene from this library (Cai et al. 1995). We also screened a bovine YAC library containing 21,500 clones (Libert et al. 1993). The much larger average insert size led to a virtually 100 percent chance of finding a gene within this library, however the expected level of chimerism is approximately one third (Libert et al. 1993).

Isolating microsatellites. BAC DNA was purified with alkaline miniprep and digested overnight with restriction enzyme *AluI* or *HaeIII* according to the manufacturer's recommendations. The fragments were then ligated into Ready-to-go™ pUC18/*SmaI*/BAP (Pharmacia). Ligated DNA was then transformed into competent cells and the resulting colonies screened for microsatellites by probe hybridization as described below. Positive clones were sequenced and primers designed flanking microsatellites.

A major problem when preparing ligations from YAC DNA is that only about 5% of the total DNA is bovine with the balance coming from the yeast host cell. Preparative pulsed field gel electrophoresis was not an option for us. Initial experiments aimed at enriching for bovine DNA employed PCR with one or more primers specific for bovine short interspersed nuclear element (SINE) sequences. However, the PCR produced either too few fragments from YAC-containing yeast or too many fragments from yeast control DNA. We therefore devised the method, described in detail below, which relies on cloning of restriction fragments into a plasmid vector followed by PCR with one SINE primer and one vector primer. This seems to produce a better specificity and a better coverage of the bovine DNA compared with PCR using only SINE primers.

Total DNA from the YAC clone was purified with a miniprep procedure (Kaiser et al. 1994). 0.25–0.75 μg DNA was digested overnight with

restriction enzyme *EcoRI* or *HindIII* in a total volume of 20 μl. The whole volume was ligated into Ready-to-Go pUC18/*re*/BAP (where *re* means either *EcoRI* or *HindIII*) as recommended by the supplier (Pharmacia). 0.5 μl of the ligation was used as template using one of two primers from pUC18 (BSF (TTT TCC CAG TCA CGA CGT TG) or BSR (TGT GGA ATT GTG AGC GGA TAA)) and one of three primers from bovine SINE sequences (retrol (GAA TAC TGG AGT GGG TTG CCA), retro2 (AGG CTA CAG TCC ATG GGA TT) or AMSI (CAA GAA TCT TCT CCA ACA CCA CAG TTC A) (Kaukinen and Varvio, 1992; Lenstra et al. 1993)). The PCR was set up as above, but using 2 units *Taq* DNA polymerase per 25 μl reaction. The temperature profile for this PCR was 2 min at 95°C; 35 cycles of 30 sec at 94°C, 2 min at 60°C, and 2 min at 72°C; 10 min at 72°C. The PCR products were analyzed by the hybridization of a biotin labeled (CA)₂₀ oligonucleotide using the Phototope kit (New England Biolabs), and CA-repeat positive fragments were gel purified and ligated into the T/A cloning vector pCR2.1 (Invitrogen). This method for enriching for fragments containing CA-repeats is in principle slower than a method employing solution hybridization and reamplification (Brown et al. 1995). However, the method described here worked more reliably in our hands.

The ligated DNA was transformed into One-shot™ competent cells (Invitrogen). Resulting colonies were gridded onto LB-plates (150 mm diameter) and colonies lifted onto nylon filters (MSI Magnagraph). DNA was released and bound to the filters as described (Maniatis et al. 1982). A (CA)₂₀ oligonucleotide was labeled with Fluorescein as recommended in the ECL 3' oligolabeling and detection system (Amersham) and used as hybridization probe at 45°C. Blots were washed in 1% SDS, 25 mM NaCl, 5 mM NaPhosphate (pH 7.2) for 5 min at room temperature and 2 × 15 min at 45°C and detection was performed as recommended in the ECL 3' oligolabeling and detection system (Amersham). Colonies showing a hybridization signal were further analyzed for insert size and for presence and position of the CA-repeat by PCR with primer-pairs BSF/BSR, BSF/(CA)₂₀ and BSR/(CA)₂₀. The hybridization conditions employed were of relatively low stringency, and consequently, most of the CA-repeats identified were short. However, some of the CA-repeats were part of a larger structure with several repeats. Primers were designed for all repeats longer than (CA)₅ (Table 2). In addition, primers were designed for an (AT)₈ repeat present in the 3' untranslated region of the ADCYAP1 gene, and for a T₁₅ repeat from a clone also carrying a CA-repeat. For each primer-pair one primer was labeled with a fluorescent dye (FAM, HEX, or TET) for automated genotyping. A few nucleotides (PIGtail) were added to the 5'-end of the other primer to facilitate non-templated adenylation during PCR (Brownstein et al. 1996).

Sequence was determined using the Prism Dye-deoxyterminator kit with *Taq* polymerase FS (Perkin-Elmer/ABI) and standard vector primers M13-reverse, M13-forward and T₇-promoter primer or a pCR2.1-specific primer, pCR2.1-reverse (CTA TGA CCA TGA TTA CGC C). Templates were plasmid DNA from alkaline minipreps with PEG precipitation (according to the Prism protocol). The reactions were run on an ABI model 373 automated sequencer. Sequences were analyzed using the GCG program suite (GCG, 1996). DNA sequences generated in this study have been submitted to GenBank (accession numbers AF010230–AF010232, AF018087–AF018092, AF019947–AF019949, AF026036, and U78859).

FISH. Two μg of total YAC/yeast DNA from a YAC miniprep was labeled with biotin by nick-translation (BioNick, Life Technologies). This labeled DNA was hybridized to R-banded chromosomes and detected essentially as described (Hayes et al. 1992) except that it was prehybridized

Table 2. Microsatellites developed in this study.

Locus name	Associated gene	D-numbers	Primer sequences	Observed size of PCR product	Number of alleles ^a	H ₀ ^a	Repeat structure
ADCYAP1	ADCYAP1 ^b	—	GCTCAGGCGAATCTTCAATC ^c gtTTACGAAGCACTAGAGAATGCAC ^f	154–162 bp	4	0.32	(AT) ₈
UW64	CDH2	D24S48	CAAACCTCAGTCTCCATATTGC ^c gttCCCCCTGGTGGCTAAGATG ^f	65–68 bp	4	0.50	(T) ₁₅
UW65	MADH4	D24S49	GGACACAATTCAGCACTAACAC ^a gttTGGAGCACCATAAGGAAAC ^f	177–187 bp	4	0.53	(CA) ₁₄
UW67	TTR	D24S50	ACAGGGCTAAGATCCACAC ^a gttTGGGGGAAGTAGAGGAGGT ^f	229–241 bp	9	0.53	(T) ₁₁ (N) ₅₀ (GT) ₁₀ (AT) ₈ (T) ₁₂
UW69	LAMA1	D24S51	GCAAACCTCACTAATCTTCTTCA ^d gttTCTCAGCATTCGCTGCTT ^f	117–138 bp	6	0.62	(CA) ₉
UW72 ^e	MBP	D24S52	ACGTTGTTTTATAGTTAATGACGGG ^d gttCTGTATTGCACCTCCTCCAC ^f	160–185 bp	7	0.86	(CA) ₂₁
UW74	TYMS	D24S53	TGGGATTGCACAGTCAGAC ^d gttTCACCCCACTAATCTTCCACATAC ^f	195–197 bp	2	0.20	(CA) ₁₁
UW75	TYMS	D24S54	CAGACATGACTGAGCATGAGTG ^d gttCTGCACTACCCTGAGTGATACC	91–97 bp	4	0.60	(CA) ₉
UW76 ^e	PAI2	D24S55	TACAGTAGCCCAAGGGACC ^d gttGTGAGTCAGTGGCCTTATTGAG ^f	181–189 bp	2	0.18	CA-compound
UW77	GRP	D24S56	GTGACATGATCTGGTTTGTGATT ^c gTTTCACTTACTGAGCCATCTCC ^f	126–132 bp	4	0.18	(CA) ₁₀
UW78	ADCYAP1	D24S57	ACCTCTAAGTCCATCCATGTTG ^d gttGAAATGCTTCATGTTTCACCTG ^f	247–249 bp	2	0.42	(AC) ₄ (CA) ₆

^a among the parental animals in the IBRP families.

^b This marker is in the 3' untranslated region of the ADCYAP1 gene.

^c Fluorescent dye TET added to the 5' end of this primer during synthesis.

^d Fluorescent dye FAM added to the 5' end of this primer during synthesis.

^e Fluorescent dye HEX added to the 5' end of this primer during synthesis.

^f Several nucleotides (PIGtail) were added to the 5' end of this primer to facilitate non-templated adenylation during PCR (Brownstein et al. 1996).

^g Null-alleles observed at this locus.

with 100 µg sonicated salmon sperm DNA and 200 µg sonicated bovine total genomic DNA at 37°C for 30 min.

Genotyping. The international bovine reference family panel comprises 347 individuals in full-sibling pedigrees sired by 15 bulls (Barendse et al. 1997). Microsatellite genotypes were obtained using PCR amplification with up to three primer-pairs simultaneously using the following PCR conditions: 0.125 mM dNTP, 0.8 µM of each primer (Table 2), 0.35 U AmpliTaq Gold polymerase (Perkin-Elmer), 1× Mg-free buffer (Perkin-Elmer) and 1.5 mM MgCl₂ in a 10 µl reaction volume. The temperature profile was 15 min at 95°C; 5 cycles of 40 s at 95°C, 40 s at 56°C, 50 s at 72°C; 30 cycles of 40 s at 95°C, 40 s at 57°C, 50 s at 72°C; 20 min at 72°C. PCR products were diluted and mixed with other PCR products before electrophoresis on a Perkin-Elmer/ABI 310 Genetic Analyzer. Peak sizes were determined using the ABI-Prism Genescan Analysis Software v. 2.0.2. The ABI Genotyper Software v. 1.1 was used for semiautomatic allele identification and data files for linkage analysis were prepared using Microsoft Excel.

Inspection of the genotypes for UW76 revealed a pattern in two families that indicated a null allele (an allele that was not amplified using the primers employed here) in each case originating in the sire. Genotypes in these families were recoded with the sire homozygous for a “dummy” allele to allow the inheritance of the dam alleles to be analyzed. Likewise, both alleles for UW72 in one dam were null alleles, and this family was also recoded. The resulting inheritance was in agreement with Mendelian expectations, and these recoded genotypes were therefore used in the linkage analyses. Genotypes of all other locus/family combinations adhered to the expectations for co-dominant inheritance.

Linkage analysis. Linkage was analyzed using the program CRI-MAP v. 2.4 (Green et al. 1990) running on a HP9000 mainframe. In order to provide ties with the published linkage map (Barendse et al., 1997), genotypes for four anonymous microsatellites were included in these analyses (CSSM23, CSSM31, TGLA351, and AGLA269; Barendse et al., 1997). Initially, pairwise linkage was assessed using the option TWOPOINT with a threshold for significant linkage of 3 LOD-units. Four loci (ADCYAP1, UW74, UW75, UW78) known to reside within the same YAC were grouped into a haplotype system with no recombination between loci. A multipoint map was constructed using the option BUILD and double recombinants that indicate possible data-errors were localized using the option CHROMPIC. Data were rechecked and construction of the multi-point map repeated. The FLIPS option was used to compare the relative probability of alternative orders.

Table 3. Seven YACs mapped by FISH to BTA24.

Gene	YAC	Specific location ¹	Other locations ^{1,2}
ADCYAP1, TYMS	ABS011021	24q23	
ADCYAP1, TYMS	ABS088020	24q23	2q43
TTR	ABS018003	24q21–22	6q19–21
CDH2	ABS125029	24q21–23	
CDH2	ABS026035	24q21–23	
GRP	ABS038091	24q27	2q35–41
MBP	ABS067117	24q12	8q18 and 2q23–24

¹ All locations according to the Texas nomenclature (Popescu et al. 1996).

² Secondary FISH signals from presumably chimeric YACs.

Results

The bovine BAC library was screened with PCR for seven loci (TYMS, ADCYAP1, GRP, MC2R, CDH2, TTR, and PAI2). One BAC was identified for each of ADCYAP1, GRP, and PAI2, and two BACs for MC2R. This is close to the 70% success ratio expected from this library (Cai et al. 1995). The bovine YAC library was screened by PCR for the above mentioned loci as well as for LAMA1, MADH4, and MBP. One YAC was identified for MBP, two YACs for LAMA1 and GRP, and more than two YACs for each of the other genes investigated. Among the 12 YACs containing either ADCYAP1 or TYMS, 8 YACs contained both loci. This indicates that the two genes are close together in the bovine. Indeed, the size of the smallest YAC measured that contains both genes as well as the three microsatellites isolated from this region is about 550 kb (Larsen & Eggen, personal communication). A fine scale map of this region was made using information on the STS content of each YAC. The locus order was determined to be ADCYAP1/UW78-TYMS-UW74-UW75; the order of ADCYAP1 and UW78 could not be distinguished by this means. The YAC ends have not been mapped relative to each other.

Seven YACs containing a total of six genes have been regionally mapped on BTA24 using FISH (Table 3). One major motive for using FISH was to avoid isolating microsatellites from chimeric YACs, but for genes where only one or two YACs were identified, this was not possible. Additionally, the FISH assignments provide ties between the linkage and physical maps of

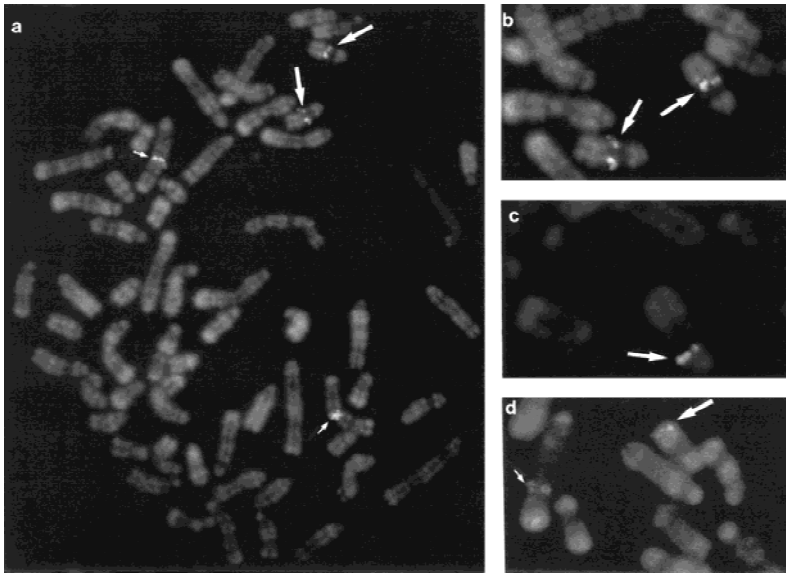


Fig. 1. R-banded whole (a) and partial (b, c, d) metaphase spreads after in situ hybridization with YACs containing (a, b) TTR, (c) MBP, and (d) GRP. Large arrows indicate specific signals on BTA24 and small arrows indicate signals due to chimerism. Bovine chromosomes are numbered according to the Texas nomenclature (Popescu et al. 1996).

BTA24. The YACs mapped include No. ABS067117 containing MBP that maps in band q12, very close to the centromere, and No. ABS038091 containing GRP that maps near the telomere (Fig. 1). This is the first FISH assignment of a polymorphic marker near the BTA24 telomere.

A polymorphic CA-repeat was cloned from the BAC for each of the genes ADCYAP1, GRP, and PAI2, and developed into microsatellite markers (Table 2). Two polymorphic CA-repeats were cloned from YACs containing TYMS, and one from YACs containing LAMA1, CDH2, and TTR. Two chimeric YACs yielded microsatellites mapping to two different chromosomes: (1) from a YAC containing MADH4 (No. ABS097155), one microsatellite mapped to the expected location on BTA24 and one mapped to BTA17, and (2) from the YAC carrying MBP (No. ABS067117), one microsatellite mapped to BTA24 and one mapped to BTA8. The BTA8 and BTA17 microsatellites have been published (Larsen et al. 1997a, 1998). One additional marker was developed from a TA-repeat present in the 3' un-translated region of the ADCYAP1 gene. This TA-repeat was present in the original STS for ADCYAP1 reported previously (Larsen et al. 1996).

Two-point linkage analysis showed that each locus studied here was linked to at least one of the other loci with a LOD score >3 . A multipoint linkage map was constructed including these loci as well as four anonymous microsatellite loci (CSSM23, CSSM31, TGLA351 and AGLA269; Barendse et al. 1997). The linkage map produced here covers 76.7 cM (Fig. 2), from UW72 to UW77. The loci shown in boldface in Fig. 2 constitute a framework map with a statistical support of 3 LOD units against inversions, and linkage distances, obtained using the option FIXED, are given for these markers only. The approximate locations are shown for the remaining markers (not bold). These markers could not be localized with a LOD score higher than 3 (TGLA351 and UW65) or their inclusion resulted in a large increase in map size (TGLA351 and AGLA269).

The gene order as determined by a combination of linkage and FISH is shown in Fig. 2. Fig. 2 also shows the relationship of this order with the gene order on HSA18. The centromeric end of the BTA24 linkage map, marked by PAI2 and MBP, shows correspondence with HSA18 from q22.1 to near the telomere (HSA18 map positions are from Silverman et al. 1996) but the region is inverted on BTA24. These two genes are separated to two mouse chromosomes, PAI2 on mouse chromosome (MMU) 1{61} and MBP on MMU18 {55} (numbers in bracket { } after MMU# denote map position in cM counted from the centromeric end of

the mouse chromosome according to the Mouse Genome Database, 1997). Next in the linkage map is an evolutionarily conserved segment including CDH2 and TTR that corresponds to HSA18q12 and MMU18{6-7}. These genes map to BTA 24q21-23 with FISH but the most probable order is reversed relative to the HSA18 order. Following is a region mapping to BTA24q23 including TYMS, ADCYAP1, and LAMA1. This region seems conserved between bovine and human, in which it maps close to HSA18pter, but these loci are separated onto MMU5{18} (TYMS) and MMU17{38} (LAMA1) indicating at least one translocation specific for that lineage. The last region is defined by the interval MADH4-GRP on BTA24qter, corresponding to HSAq21.1-q21.3 and MMU18{47.5}-18{40}. The results given here imply that several inversions and/or translocations have taken place in one or all lineages since the latest common ancestor. The region containing loci orthologous with HSA4 would be expected to fit between PAI2 and TTR in the map developed here. It is possible that investigating more loci would identify further rearrangements.

The BTA24 centromere may be the same as the HSA18 centromere, as an inversion specific for the human lineage involving the pericentric region and the present p-arm has been demonstrated (Clemente et al. 1990, McConkey, 1997). This would change an acrocentric chromosome found in most primates into the metacentric chromosome HSA18, however, another rearrangement would be necessary to place MBP near the centromere instead of the distal telomere.

Discussion

The recently published linkage maps of the bovine genome differ in their orientation of the BTA24 linkage map relative to the centromere (Barendse et al. 1997, Kappes et al. 1997). This is probably because both markers physically assigned in one of the maps are located in the middle of BTA24 (Barendse et al. 1997). Our FISH assignments of YACs carrying the GRP and MBP genes and associated microsatellites near the telomere and centromere, respectively, support the orientation in Kappes et al. (1997). It was also possible to estimate that the linkage group covers 90-95% of the physical length of the chromosome, mainly excluding the centromere from coverage. The coverage might be even more complete when expressed in recombination units, as recombination often is suppressed near the centromere (e.g., Johansson Moller et al., 1996).

The genes on HSA18 investigated here showed completely

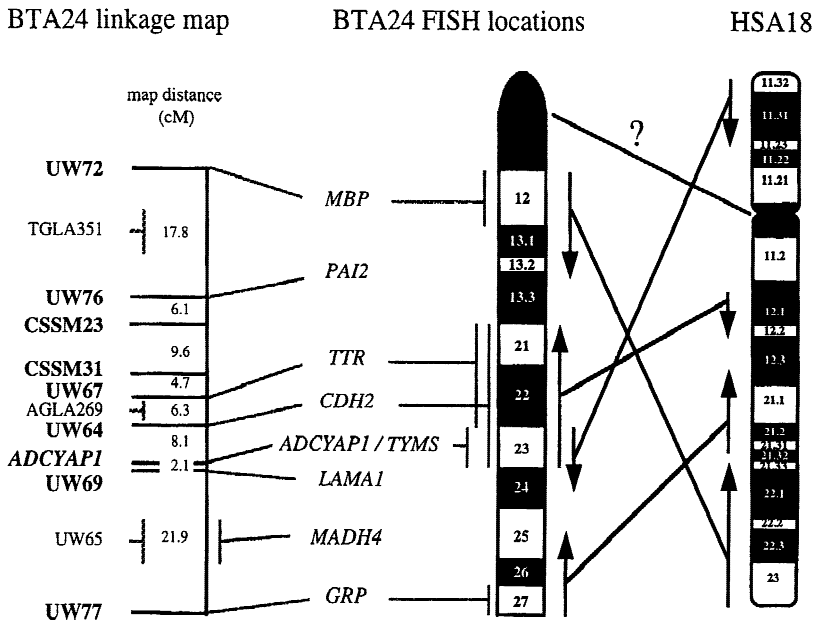


Fig. 2. Combined map showing the linkage between polymorphic markers (left), markers mapped to bovine chromosome 24 by FISH (middle), and the comparative relationship with human chromosome 18 (HSA18; right). In the linkage map, distances (in cM) are given between markers in boldface only. These constitute a framework map with LOD-score >3 against inversions. The remaining markers cannot fulfill this criterion or inclusion leads to map inflation, but an approximate location is given. Included under the name of ADCYAP1 are UW74, UW75 and UW78. In the comparative part, the relative length and orientation of each region with conserved order as defined by two or more genes is shown by arrows connected by a line. The homology between the centromeres has not been determined.

conserved synteny with BTA24, but Figure 2 demonstrates that this conserved synteny does not translate into conserved order. Instead, the genes define four regions of conserved order, and the centromere may constitute a fifth region. Similarly, the human-mouse comparative map shows extensive rearrangement in the mouse relative to the human for regions corresponding to HSA18 (Carver and Stubbs, 1997). There is greater similarity in order of the genes investigated here between human and cattle than between either of these and mouse, in contrast to the conclusion based on genes mapped to all the bovine chromosomes (Barendse et al. 1997).

Most genes reported on recent BTA24 maps support the regions of conserved order defined here. The exception is one gene (mitochondrial NADH-ubiquinone reductase, 24 kDa subunit; NDUFV2) between MC2R of the HSA18 p-arm and the centromere, mapping to BTA5 (Agaba et al. 1997). This result must be viewed as provisional until supporting evidence emerges, but it indicates the existence of a small sixth region, that has undergone an independent translocation (we have previously mapped the MC2R gene to BTA24 using synteny (Larsen et al. 1996)). Additionally, a small region of BTA24 was painted with a HSA4 specific probe (Solinas-Toldo et al. 1995; Chowdhary et al. 1996). This chromosome therefore seems unusually rearranged. A study of the chromosome pair HSA13/BTA12 indicated only a single inversion within a region of conserved synteny covering the whole chromosome (Sun et al. 1997).

It is interesting that the pericentric inversion specific for the human lineage involving the present p-arm (Clemente et al. 1990; McConkey, 1997) would place ADCYAP1 and TTR not too far apart in the primate ancestor. That is what we observe here for cattle, and a short inter-gene distance has been observed in the pig as well (Larsen et al. 1997b).

LAMA1 was the only Comparative Anchor Tagged Sequence (CATS) locus chosen from HSA18p (Lyons et al. 1997). However, sections of HSA18p correspond to MMU5, 17 and 18. We have included one gene from HSA18p corresponding to MMU5 (TYMS) and one gene corresponding to MMU17 (LAMA1) and previously mapped one gene corresponding to MMU18 to BTA24 by synteny (MC2R). The genes from HSA18q included here are mostly CATS loci, with two exceptions: MADH4 is a proxy for DCC, chosen because the PCR worked well and because MADH4 is slightly more centromeric than DCC (Hahn et al. 1996; Silverman et al. 1996), thereby making the gap towards TTR on HSA18

smaller. PAI2 is a proxy for BCL2, as the initial PCR aimed at cloning a bovine BCL2 fragment did not work in our hands, in spite of testing two different primer pairs. PAI2 was also chosen as an anchored reference locus in the original publication of O'Brien et al. (1993). The two loci map close together on HSA18 (estimated distance is 0.3 ± 0.7 mb; (Genetic Location Database, LDB, 1997) and as well as on MMU1 (1.3 cM apart; Mouse Genome Database, 1997).

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References

- Agaba MK, Kemp SJ, Barendse W, Teale A (1997) Comparative mapping in cattle of genes located on human chromosome 18. *Mammalian Genome* 8, 530-532
- Ballabio A (1993) The rise and fall of positional cloning. *Nature Genetics* 3, 277-279
- Barendse W, Vaiman D, Kemp SJ, Sugimoto Y, Armitage SM, Williams JL, Sun HS, Eggen A, Agaba M, Aleyasin SA, Band M, Bishop MD, Buitkamp J, Byrne K, Collins F, Cooper L, Coppettiers W, Denys B, Drinkwater RD, Easterday K, Elduque C, Ennis S, Erhardt G, Ferretti L, Flavin N, Gao Q, Georges M, Gurung R, Harzelius B, Hawkins G, Hetzel J, Hirano T, Hulme D, Jorgensen C, Kessler M, Kirkpatrick BW, Konfortov B, Kostia S, Kuhn C, Lenstra JA, Levezuel H, Lewin HA, Leyhe B, Lil L, Burriel IM, McGraw RA, Miller JR, Moody JA, Moore SS, Nakane S, Nijman IJ, Olsaker I, Pomp D, Rando A, Ron M, Shalom A, Teale AJ, Thieven U, Urquhart BGD, Vage DI, Van de Weghe A, Varvio S, Velmala R, Vilkki J, Weikard J, Woodside C, Womack JE, Zanotti M, Zaragoza P (1997) A medium-density genetic linkage map of the bovine genome. *Mammalian Genome* 8, 21-28
- Blattman AN, Kirkpatrick BW, Gregory KE (1996) A search for quantitative trait loci for ovulation rate in cattle. *Animal Genetics* 27, 157-162
- Brown J, Hardwick LJ, and Wright AF (1995) A simple method for rapid isolation of microsatellites from yeast artificial chromosomes. *Molecular and Cellular Probes* 9, 53-58
- Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by Taq DNA polymerase, primer modifications that facilitate genotyping. *BioTechniques* 20, 1004-1010
- Cai L, Taylor J, Wing RA, Gallagher DS, Woo SS, Davis SK (1995)

- Construction and characterization of a bovine artificial chromosome library. *Genomics* 29, 413–425
- Carver EA, Stubbs L (1997) Zooming in on the human-mouse comparative map: genome conservation re-examined on a high-resolution scale. *Genome Research* 7, 1123–1137
- Chowdhary BP, Fröncke L, Gustavsson I, Scherthan H (1996) Comparative analysis of the cattle and human genomes: detection of ZOO-FISH and gene mapping-based chromosomal homologies. *Mammalian Genome* 7, 297–302
- Clemente IC, Ponsa M, Garcia M, Egozcue J (1990) Evolution of the Simiiformes and the phylogeny of human chromosomes. *Human Genetics* 84, 495–506
- GCG (1996) "The Wisconsin Package Version 9.0," Genetics Computer Group (GCG), Madison, Wisconsin
- Genetic Location Database (1997) Available at (http://cedar.genetics.soton.ac.uk/public_html/index.html)
- Georges M, Nielsen D, Mackinnon M, Mishra A, Okimoto R, Pasquino AT, Sargeant LS, Sorensen A, Steele MR, Zhao XY, Womack JR, Hoeschele I (1995) Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics* 139, 907–920
- Green P, Falls K, Crooks S (1990) "Documentation for CRI-MAP, version 2.4.": Washington University School of Medicine, St. Louis
- Hahn SA, Schutte M, Hoque ATMS, Moshaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE (1996) DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 271, 350–353
- Hayes H (1995) Chromosome painting with human chromosome-specific DNA libraries reveals the extent and distribution of conserved segments in bovine chromosomes. *Cytogenetics and Cell Genetics* 71, 168–174
- Hayes H, Petit E, Lemieux N, Dutrillaux B (1992) Chromosome localization of the ovine beta-casein gene by non-isotopic in situ hybridization and R-banding. *Cytogenetics and Cell Genetics* 61, 286–288
- Johansson M, Ellegren H, Andersson L (1995) Comparative mapping reveals extensive linkage conservation—but with gene order rearrangements—between the pig and the human genomes. *Genomics* 25, 682–690
- Johansson Moller M, Chaudhary R, Hellmén E, Höyheim B, Chowdhary B, Andersson L (1996) Pigs with the dominant white coat color phenotype carry a duplication of the KIT gene encoding the mast/stem cell growth factor receptor. *Mammalian Genome* 7, 822–830
- Kaiser C, Michaelis S, Mitchell A (1994) "Methods in Yeast Genetics. A Cold Spring Harbor Laboratory Course Manual", pp. 137–140, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Kappes SM, Keele JW, Stone RT, McGraw RA, Sonstegard TS, Smith TPL, Lopes-Corrales NL, Beattie CW (1997) A second-generation linkage map of the bovine genome. *Genome Research* 7, 235–249
- Kaukinen J, Varvio SL (1992) Artiodactyl retrotransposons: association with microsatellites and use in SINEmorph detection by PCR. *Nucleic Acids Research* 20, 2955–2958
- Larsen NJ, Eggen A, Hayes H, Byla B, Doud L, Bishop MD, Kirkpatrick BW (1997a). UW54 and UW63, two polymorphic bovine microsatellites on chromosome 6 and 8q18, respectively. *Animal Genetics* 28, 377–378
- Larsen NJ, Eggen A, Byla B, Doud L, Bishop MD, Kirkpatrick BW (1998) UW68, a polymorphic bovine microsatellite on chromosome 17. *Animal Genetics* (In press)
- Larsen NJ, Tuggle CK, Yerle M, Rothschild MF (1997b) ADCYAP1 maps to pig chromosome (SCC) 6, not to the expected location on SSC1. In "The 10th North American Colloquium on Gene Mapping and Cytogenetics of Human and Domestic Species, Abstract Book"
- Larsen NJ, Womack JR, Kirkpatrick BW (1996) Seven genes from human chromosome 18 map to chromosome 24 in the bovine. *Cytogenetics and Cell Genetics* 73, 184–186
- Lenstra JA, van Boxtel JAF, Zwaagstra KA, Schwerin M (1993) Short interspersed nuclear element (SINE) sequences of the bovidae. *Animal Genetics* 24, 33–39
- Libert F, Lefort A, Okimoto R, Womack J, Georges M (1993) Construction of a bovine genomic library of large yeast artificial chromosome clones. *Genomics* 18, 270–276
- Lyons LA, Laughlin TF, Copeland NG, Jenkins NA, Womack JE, O'Brien SJ (1997) Comparative anchor tagged sequences (CATS) for mapping of mammalian genomes. *Nature Genetics* 15, 47–56
- Maniatis T, Fritsch EF, Sambrook J (1982) "Molecular Cloning—a laboratory manual." Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- McConkey EH (1997) The origin of human chromosome 18 from a human/ape ancestor. *Cytogenetics and Cell Genetics* 76, 189–191
- Mouse Genome Database (1997) Available at <http://www.informatics.jax.org/mgd.html>
- O'Brien SJ, Womack JE, Lyons LA, Moore KJ, Jenkins NA, Copeland NG (1993) Anchored reference loci for comparative genome mapping in mammals. *Nature Genetics* 3, 103–112
- Popescu CP, Long S, Riggs P, Womack J, Schmutz S, Fries R, Gallagher DS (1996) Standardization of cattle karyotype nomenclature: report of the committee for the standardization of the cattle karyotype. *Cytogenetics and Cell Genetics* 74, 259–261
- Silverman GA, Overhauser J, Gerken S, Aburomia R, O'Connell P, Krauter KS, Detera-Wadleigh DS, Yoshikawa T, Collins AR, Guerts van Kessel A (1996) Report of the fourth international workshop on human chromosome 18 mapping 1996. *Cytogenetics and Cell Genetics* 75, 111–131
- Solinas-Toldo S, Lengauer C, Fries R (1995) Comparative genome map of human and cattle. *Genomics* 27, 489–496
- Sun HS, Cai L, Davis SK, Taylor JF, Doud LK, Bishop MD, Hayes H, Barendse W, Vaiman D, McGraw RA, Hirano T, Sugimoto Y, Kirkpatrick BW (1997) Comparative linkage mapping of human chromosome 13 and bovine chromosome 12. *Genomics* 39, 47–54
- Womack JE, Dietz AB, Gallagher DS, Li L, Zhang N, Neiberghs HL, Moll Y, Ryan AM (1991) Assignment of 47 additional comparative anchor loci to the bovine synteny map. *Cytogenetics and Cell Genetics* 58, 2132