Radiation hybrid mapping of genes and newly identified microsatellites in candidate regions for bovine arthrogryposis-palatoschisis and progressive ataxia based on comparative data from man, mouse and rat

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ORIGINAL ARTICLE

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Summary

A comparative pathology and mapping strategy was used to initiate a study on two bovine genetic diseases: arthrogryposis–palatoschisis and progressive ataxia, which affect mainly Charolais cattle. Bibliographic studies provided information on the pathology of these diseases, which helped to define similar diseases in other species. Animals affected by bovine arthrogryposis–palatoschisis display similar symptoms to those of muscular dysgenesis, mouse mutants and animals with progressive ataxia to those of Long Evans Shaker rat mutants. Candidate regions are respectively human chromosome 1q32 (BTA16) containing the gene CACNA1S and human chromosome 18q23 (BTA24) containing the gene myelin basic protein (MBP). Primer pairs were designed for 15 loci around each candidate gene, in a region of about 20 megabases and were used to screen a bovine Bacterial Artificial Chromosome (BAC) library. Eighteen microsatellites were found in the identified BAC clones, 11 on BTA24 and seven on BTA16. The genes and microsatellites were mapped by radiation hybrid (RH) analysis and a RH map was obtained for each region with 18 new localizations on BTA16 and 23 on BTA24. Comparative human–bovine analysis of the MBP region shows a good conservation of gene order while that of the CACNA1S region shows several breakpoints.

Introduction

Over the past decades, animal breeders, veterinarians and scientists have been interested in improving the description of the phenotypes of their favourite animals: breeders in order to increase production levels (milk, meat), veterinarians to better understand the biological processes causing pathologies, and scientists to decipher the genomic information underlying the observed phenotypic variations (of both production traits and pathologies). With increased population surveys in cattle, a growing number of genetic disorders has been identified and described (Huston *et al.* 2000). Currently, 357 bovine genetic disorders are described (http://www.angis.org.au/Databases/BIRX/omia/) and for 27 of these, the causative mutation has been identified at the DNA level. According to Huston (1993), most of the disorders are autosomal recessive (60%) or dominant (20%). While genetic abnormalities are described in almost every breed of cattle, the occurrence varies among breeds. For example, because of artificial insemination (AI) and to the particular structure of the dairy bovine population (heavy use of a few very popular AI sires in dairy populations like the Holstein breed resulting in increased inbreeding), dairy breeds present a large palette of different disorders with some at a

relative high frequency. This results in economical losses but also has a negative impact on selection image: this is best illustrated by the recent outspread of the complex vertebral malformation syndrome in Holstein cattle (Agerholm *et al.* 2001).

A closer physiological study of the 357 disorders shows that 117 could provide potential animal models for human disorders, justifying therefore the need to better describe and understand the ontogenesis of these disorders. However, as detailed physiological studies for these disorders are not always available in cattle, it is necessary to use the often well described phenotypes in man and the growing information coming from intensive studies in other mammals, namely mouse and rat: in these two species, systematic gene analyses are in progress using a variety of modern investigation techniques such as systematic knock-out, homologous recombination and genome sequencing (Nolan *et al.* 2000; The Mouse Genome Sequencing Consortium 2002; Rat Genome Sequencing Project Consortium 2004). Furthermore, as genomes of different mammalian species exhibit a high degree of similarity, it is appropriate to make proper use of comparative mapping and comparative physiology.

Here, we describe a combined comparative mapping and functional candidate gene approach in order to initiate the genetic characterization of two bovine genetic disorders affecting the Charolais cattle: arthrogryposis–palatoschisis syndrome and progressive ataxia. First, bibliographic studies provided information concerning the pathology of these disorders, most probably autosomal and recessive, (Palmer *et al.* 1972; Jackson 1978; Huston *et al.* 2000), which helped to identify similar diseases in other species. As a second step, comparative linkage and radiation hybrid (RH) maps together with a BAC-based physical map of the bovine genome were used to identify target chromosomal regions from which polymorphic markers were detected and to determine the extent of similar gene content and/or gene order between specific segments of the human and the bovine genomes. Both disorders could serve as potential animal models to better understand and dissect human disorders where similar phenotypes exist as being part of a more complex syndrome. In the near future, with the availability of the sequence of the bovine genome, comparative mapping combined with identification of candidate genes will be more effective and will permit faster identification of relevant markers and putative positional and functional candidate genes for genetic disorders.

Materials and methods

Comparative pathology

For both diseases, arthrogryposis–palatoschisis syndrome and progressive ataxia, bibliographic studies provided information concerning the pathology, which helped to identify similar diseases in other species (Palmer *et al.* 1972; Jackson 1978; Huston *et al.* 2000). Animals affected by bovine arthrogryposis–palatoschisis display similar symptoms to those of muscular dysgenesis (mdg) mouse mutants: in both species, newborn animals are smaller than normal ones, with a fixation of posture at multiple joints and a cleft palate in around 70% of the cases. They suffer from generalized muscular weakness, originating from the disorganization of the muscle's structure, particularly in the Z-band. Additionally, there is an abnormal differentiation of neuromuscular junctions in mouse, and this may also be the case in cattle (Pai 1965; Banker 1977; Drouet *et al.* 1993; Luo *et al.* 1996). Animals with progressive ataxia display similar symptoms to those of Long Evans Shaker (LES) rat mutants. Clinical signs are weakness of the hind limbs, which slowly progress to ataxia, and sometimes to total paralysis. These two ataxia are disorders of the central white matter, and the main abnormality is dysmyelination (Delaney *et al.* 1995; Kwiecien *et al.* 1998, 2000). Based on this information and on the demonstration that *mdg* in mice is caused by a genetic disruption of the coding sequence for the CACNA1S mRNA (calcium channel, voltage-dependent, L-type, alpha-1S subunit; Chaudari 1992) and *LES* in rat is because of the insertion of an endogenous retrotransposon into a non-coding region (intron 3) of the Myelin Basic Protein (MBP) gene (O'Connor *et al.* 1999), a functional candidate gene was identified for each bovine disorder. By comparative mapping between mouse and man for *mdg* and between rat and man for *LES*, candidate regions were identified and used as a basis to find the homologous bovine segment. Candidate regions were thus defined, respectively, human chromosome 1q32 (containing the gene CACNA1S and corresponding to bovine chromosome 16, BTA16) and human chromosome 18q23 (containing the gene MBP and corresponding to bovine chromosome 24, BTA24) as defined by chromosome painting (Hayes 1995).

Primers

Fifteen loci were selected around each candidate gene (CACNA1S and MBP), in a segment of about

20 megabases. Primer pairs were designed from available bovine EST sequences using the 'Interspecific Comparative Clustering and Annotation foR Est' user interface (ICCARE: http://genopole.toulouse.inra.fr/bioinfo/Iccare/). Primer sequences and sizes of the amplified target segments are given in Table 1.

Bovine BAC identification

The PCR-based screening for each locus was performed on the four-genome equivalent INRA bovine BAC library containing 105 984 clones (Eggen *et al.* 2001). PCR conditions are described in Gautier *et al.* (2002).

Microsatellite isolation

Microsatellites were isolated from BAC clones containing each selected locus, after digestion with *Sau*3AI (Promega, Madison, WI, USA), subcloning and hybridization with fluorescent $(TG/TC)_{12}$ (Roche Diagnostics, Mannheim, Germany). After partial sequencing of the positive clones, primers for PCR amplification were designed to specifically amplify these microsatellite regions. The polymorphism of 10 BTA24 microsatellites was tested on 44 animals from different breeds on 5% acrylamide denaturating gels. Microsatellites characteristics are given in Table 2.

Bovine whole-genome RH panel genotyping and map construction

The loci (genes and microsatellites) were mapped by PCR on the 94-cell lines RH panel described by Williams *et al.* (2002) and the CARTHAGENE software (Schiex & Gaspin (1997) was used to perform two-point and multipoint analyses of the RH data and to provide comprehensive maps of the two regions.

Results

BAC library screening and microsatellite identification

Of the 30 selected genes used to screen the BAC library, at least one BAC clone was identified for 25 genes (see Table 1). After sub-cloning of the BAC clones, 45 microsatellites were identified by sequencing, five of them being duplicates: the ratios of the structures of the repeat motifs were similar to those described by Vaiman *et al.* (1994) with 80% of $(TG/CA)_n$, 9% of $(TC/GA)_n$ and 9% of $(GTC)_n$ or $(TGC)_n$. Because of the proximity of the *Sau*3AI restriction site to the microsatellite motif for some sequences, primers were designed for 23 microsatellites and 18 were found useful, as reported in Table 2.

RH mapping and comparative maps

Specific amplification of the bovine DNA in RH was obtained for 77% of the genes and 90% of the microsatellites. For the remaining loci, three kinds of results were observed: a PCR product of the same size for both the hamster and the bovine DNA (C3IP1, HSPC060, MYOG and FLJ20307), a PCR product without a clear pattern (INRA297 and INRA319) or no PCR product (FHR, RNPEP and LAMB3). Data were analysed with the CARTHAGENE software and comprehensive maps were built for both regions integrating markers previously reported by Williams *et al.* (2002), the bovine genes and the newly developed microsatellites (see Figure 1 for BTA16 and Figure 2 for BTA24).

Of the 15 genes mapping to the flanking regions of the candidate gene CACNA1S in man (HSA1-BTA16), 11 were successfully mapped with the bovine RH panel and could be integrated in a comprehensive RH map for BTA16 based on markers already mapped by Williams *et al.* (2002). The comprehensive comparative map between BTA16 and HSA1 (1q25–1q32 region) exhibits three conserved syntenic segments (S1, S2, S3) (Figure 1): S1 and S3 are next to each other in cattle and localized in the centromeric region of BTA16, while S2, localized in human between the S1 and S3, maps to the telomeric region of BTA16. It should be noted that the S2 segment containing the candidate gene CACNA1S, maps to the distal part of BTA16, therefore, despite the seven newly identified microsatellites, we found no markers flanking the candidate gene. Therefore, it is necessary to identify more markers in the telomeric region of BTA16. Furthermore, markers are unequally spread over the three bovine segments, with 85% in the first centromeric segments (S1 + S3).

For BTA24, 23 loci (12 genes and 11 microsatellites) were mapped to the bovine region corresponding to HSA18 containing the candidate segment around the MBP gene. The bovine gene order obtained is in perfect agreement with the human map for the studied region, except for very close genes, for which discrimination is impossible because of the limit of resolution of the RH panel. Our RH map is perfectly integrated to the previous published one (Williams *et al.* 2002) and has a mean density of one marker every 543 kb (as measured on the

Table 1 Characteristics of the designed primer pairs

Locus name[1]	BTA[2]	HSA[3]	HSA Mb[4]	Primer sequence 5′–3′	Size (bp)	BAC clones adresses
PTGS2	16	1q25.2–q25.3	218.91	GGTAGAAGCTGGAGCACCATT	199	0172F02
				GGTGAGGTGCGTATCTTGAAC		0531F07
RGS2	16	1q31	225.59	CAAAACTCTGATTGCCCAAAA	152	0013G06
				TGATCTGGGGCTTTTTACACA		0591G03
UCH37	16	1q32	225.76	AAGCAGAATGCAAAGAAAGCA	182	0486E10
				CCATGCATTAAAGACAAGACAGA		
B3GALT2	16	1q31	225.91	TTGGAAAGAAACTGTCCCTCA	192	0277B07
				CACTTTTCGGGTTCGTTGATA		0930H10
FHR	16	1q32	229.73	ACCTTGTCATTTTCCCGACAT	165	–
				CCATCTTGTGTGCAATGAATG		
ZNF281	16	1q32.1	233.86	GCGTTTGGTTCTCAGTTCAAG	202	0126H02
				CACTCCTGGGACCTTACCTGT		0375C04
CACNA1S	16	1q32	234.39	CTTCCTGGAAAGGACCAACTC	160	0114F06
				GTTGTTGGTGTTGGCACGAG		0340A11
PKP1	16	1q32	234.76	GTACCAGCTGGGTGGCATCT	205	0183H11
				GGTCAGCTGCTTCTGGATCT		0942G10
RNPEP	16	1q32	235.55	ACCTTACCTCCCGGACCTCT	174	–
				GGGGACTTCTGCAGGATCTTA		
C3IP1	16	1	236.87	GCTTGATGAGAGGCATTATGG	191	0245E06
				GAACATGGCGCAGAAGTAATC		0427D04
MYOG	16	1q31–q41	237.01	GGGCGTGTAAGGTGTGTAAGA	194	0605D06
				CAGGCGCTCTATGTACTGGAT		0765E05
BTG2	16	1q32	237.23	TGGACCCCATCATCAGTAAAG	205	0685B06
				ATCATTTGGTTCTTGCAGGTG		0808F03
ELK4	16	1q32	239.92	GCTATCACCCTGTGGCAGTT	176	0644C07
				GCTCGGCTGAGTTTGTCATAA		0968D06
DYRK3	16	1q32	240.96	GTGCCTCTGACTCCAGAACAG	193	0967H01
				CTCGAGGCACGTGAATATAGG		0976C09
LAMB3	16	1q32	244.27	CTCAGCAGGTCTCGGACAG	170	–
				GTTGATGGTGGGCGTCAG		
FLJ20281	24	18q22	70.25	ACTTTGGCAACAGTTCACCAC	100	0927C07
				CTTAGTGACGGGACACTGACA		
HSPC060	24	18q22	78.62	ATCTCCTTCCCGTAGCAGCTC	132	–
				GACCTTCCGGAAATCTTTCAA		
HSPC154	24	18q23	83.32	AGACTGAGCCCAGGTGGAG	153	0194G06
				TGTGCACAGACACTTGCTTG		0543H04
CYB5	24	18q23	83.44	CTGGTGGGGAGGAAGTCTTA	129	1088G11
				CCAATGATGAACGTTTTGGAC		
FLJ10830	24	18q23	83.73	CTGGAGGGCATGGAGGAGT	141	0619C08
				GTAGGTGATGCAGGGCTTGT		0772C09
						0778B03
FLJ20307	24	18q23	84.19	GTCAAACTGCGCTGAAAACAT	200	–
				CGTAGGACTTGGACACAATGC		
ZNF236	24	18q22–q23	86.36	GCAAGGGGTTTGTTTAATTCC	200	0569C03
				ACATCAATGTCTTGGGTTTGC		
MBP	24	18q23	86.51	ACAGAAGAGACCCTCCCAGAG	113	0791A05
				AGTCGAGGATGCCAGTGTCT		0894H05
NFATC1	24	18q23	89.30	GTGCAACAAGCGCAAGTACG	144	0514B05
				GAGCTGGTGTACTGTGTGGTG		0525C02
						0547B02
CTDP1	24	18q23	89.58	TATGCCAAGTATGACCGCTTC	120	0812E02
				CAGCCCGCTGAAGATGATG		0872H11
FLJ22378	24	18q23	89.82	CTTCAAGACGGCCTACTTCCT	133	0470G09
				GGCTGGCTTCAGAGGGTAG		0763C06
DIM1	24	18q23	89.88	ACCGGCAACAACAACAAGAT	127	0460F08
				TGGAGTAGTCCTTCGGAGACA		0470G09
						0696H10

Table 1 Continued

Locus name[1]	BTA[2]	HSA[3]	HSA Mb[4]	Primer sequence 5′–3′	Size (bp)	BAC clones adresses
KIAA0863	24	18q23	90.07	AACTGCGTGTTTTCCTCTCAA GCTACCAGCACATGCTTCTTC	202	0553F07 0712E08 0104F06 0371F07
FLJ21172	24	18q23	90.08	CACAAGTCATCCCAGTTGGAG GTTCAAGTCGCACAACTCCTG	159	0068F05 0556E12
PARD6G	24	18q23	90.13	ACGTTGTTCCTCTGGTTGG GTCAATGACGAAGTCCTGGAG	134	0316H10

[1]The exact denomination of each locus can be found at: http://www.ncbi.nlm.nih.gov.
[2]Bovine localization.
[3]Human cytogenetic localization.
[4]Position in megabases on the corresponding human chromosome (NCBI build 30).

Table 2 Characteristics of the microsatellites identified for the two candidate chromosome segments

BTA	Microsatellite	Structure	In locus	Isolated from BAC clone	Primer sequence 5′–3′	Size	Polymorphism: number of alleles
16	INRA297	$(CA)_{10}$	BTG2	0685B06	CAGTTTCTGCTGCTCTCAACA ACACAACCTCACCTCACCTTT	149	–
16	INRA298	$(CA)_{21}$	RGS2	0591G03	GGAGGAGGCTCTCGGTAAAC GTTGGACACGACTGAGCAACT	189	–
16	INRA299	$(TG)_{12}TACA(TG)_5TATA(TG)_6$	DYRK3	0486E10	AGCTCATGTTCCTGCTCTCTG TTCCAATCATGAGAACACAGC	205	–
16	INRA301	$(CA)_{18}$	ELK4	0644C07	AAGTCAGCTGTGGAAAAACCA TTTCTCCAATATCCAGCCAGA	202	–
16	INRA306	$(CA)_{11}$	ELK4	0644C07	GAGTGACAGGAAAATGGGAGT GATTTTGAGCTCATCCAGTTTTT	205	–
24	INRA309	$(TG)_{16}$	CYB5	1088G11	GGGAGCTCTCTGGAGTCTCTG CAACCCATTCCAGTGTTCTTG	196	5
24	INRA310	$(GT)_{10}$	MBP	0791A05	GTAGCCTGAGCCCTCTCACTT GAAATGCCATGACCAGAGAAG	216	1
24	INRA311	$(TG)_{14}$	FLJ10830	0619C08	CAGTCTGAGAACACATATTAC TTCCAGATTACAGGAGGCTGA	189	5
24	INRA312	$(TG)_{15}TC(TG)_4$	KiAA0863	0553F07	CAGGCGGGTTCTTTACCACTA TCCACAAGAATGCTTTTCACC	192	7
16	INRA313	$(TC)_8$	MYOG	0605D06	AGATACCTTGCAACCCGTCTC AGCAGGACGGTAAGGGCTAT	195	–
24	INRA316	$(CA)_{11}$	FLJ20281	0927C07	AGAATCCCATGGACAGAGGAG TGGGAACCATTCCTGTAAAAG	198	2
24	INRA317	$(CA)_{14}$	FLJ10830	0772C09	TTACAGGAGGCTGAGGAGACA CAGTCTGAGAACACATATCACA	182	6
24	INRA318	$(TGC)_3TAC(TGC)_4$	DIM1 FLJ22378	0470G02 0460G09 0783G06	GCTGTGAGGCAACACATTTCT ATGGGGTCACACAAGAGTCAG	210	1
24	INRA319	$(GTC)_5AA(GTC)$	ZNF236	0569C03	TGAGGCATCTCACTGATGACA ACAGAGTCGGACACGACTGAA	146	6
24	INRA320	$(CA)_{15}$	KIAA0863 FLJ21172	0371F07 0556E12	TTTGTGTGTTGCCAGGTACAG GAGCTCTTTGGGAGGGTACTG	199	7
24	INRA321	$(GT)_{20}$	KIAA0863	0371F07	CGTACCTTGAGGCTGAGACTG AGCCTGGTGAGTGAGCTACAG	191	7
24	INRA323	$(AC)_{21}$	MBP	0894H05	TCTGTATTGCACCTCCTCCAC GGCTGCTCTGCTGTAGTTTCA	198	–

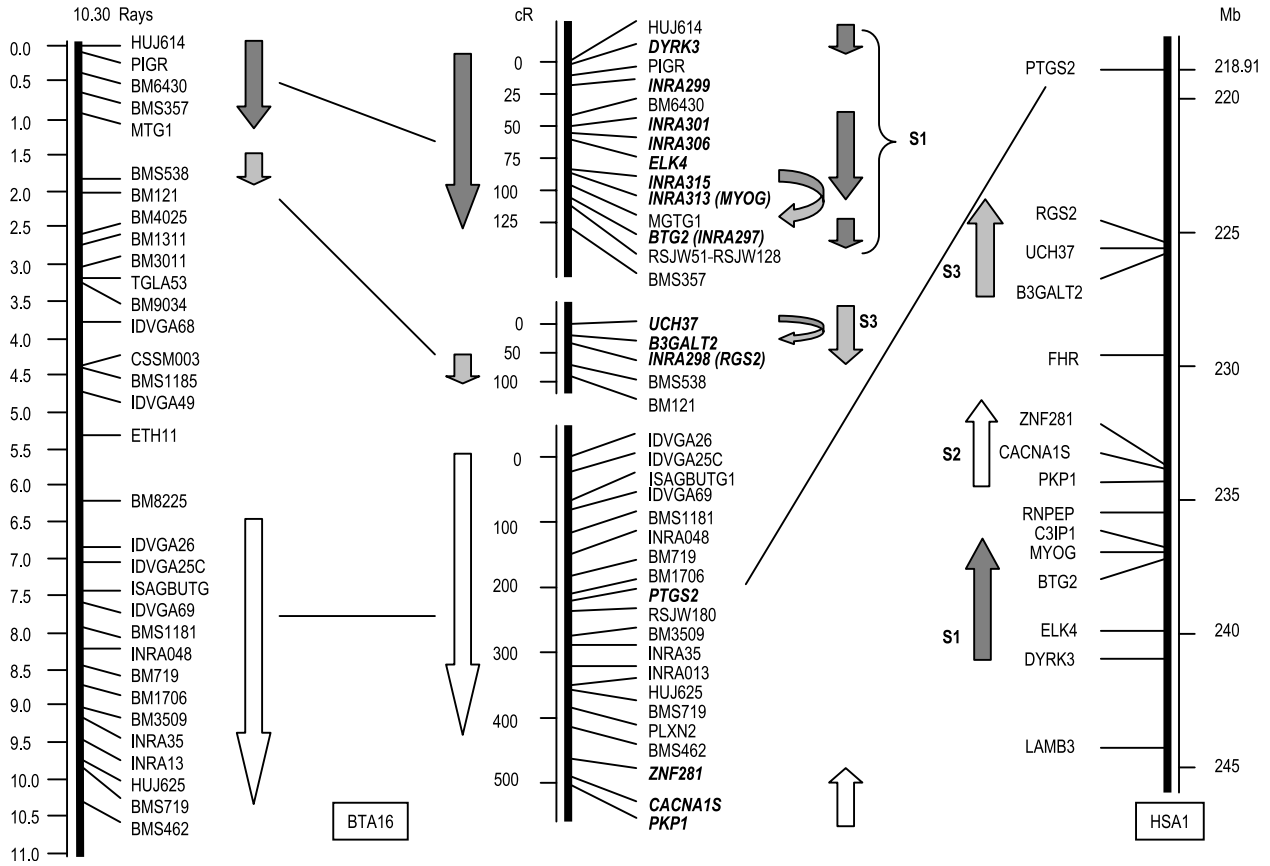

Figure 1 Comparative mapping between human chromosome 1 (HSA1) and bovine chromosome 16 (BTA16). Distances are expressed in centi-Rays for BTA16 and megabasepairs for HSA1 (NCBI build 30). Localization uncertainties are shown by curved arrows. Loci in bold and italic were mapped during this work; others are from Williams *et al.* (2002). Mapping information for the loci in brackets were inferred from BAC information content.

human genome). The informativity of 10 of the newly identified microsatellites (BTA24) was assessed by testing their polymorphism: seven are highly polymorphic displaying five to seven alleles and will therefore be useful for further linkage studies.

Discussion

We described a combined comparative mapping and functional candidate gene approach in order to initiate the genetic characterization of two bovine genetic disorders, arthrogryposis–palatoschisis syndrome and progressive ataxia, affecting the Charolais cattle. Using the available comparative mapping information, we were able to map, respectively, 11 + 12 new genes on BTA16 and BTA24 and to identify and map 7 + 11 new microsatellite markers in the candidate comparative segments on BTA16 and 24. Therefore, useful information has been added to the bovine map, confirming and refining, for those chromosomes, a recent comparative study published by Hayes *et al.* (2003).

As our candidate regions are based on comparative physiological studies, the next step in the identification process of the underlying genes for these bovine disorders is to test the candidate region with the obtained polymorphic microsatellites in affected pedigree either by linkage analysis, homozygozity mapping or linkage disequilibrium studies. This is certainly a limiting step, because even if the disorder is relatively frequent in the population, collecting samples from affected animals is not easy. Indeed, breeders do not systematically report cases, and breeder organizations are not always motivated to exchange information on genetic disorders, especially if some famous genitors are involved in the disease. This is typically the case for the two disorders reported in this paper. In the future, systematic collection of such data together with biological samples will be enhanced by national surveillance programmes and international collaborations.

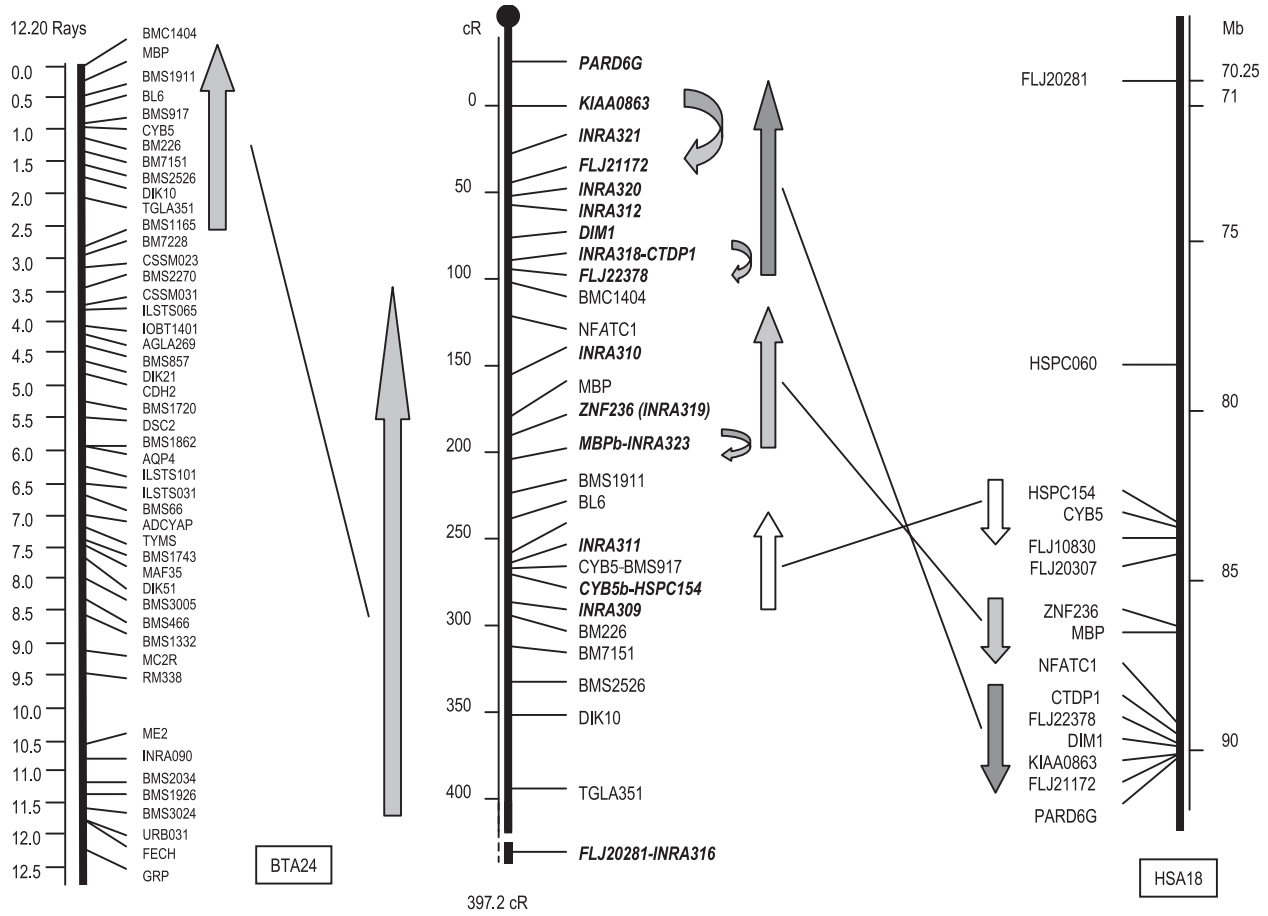

Figure 2 Comparative mapping between human chromosome 18 (HSA18) and bovine chromosome 24 (BTA24). Distances are expressed in centi-Rays for BTA16 and megabasepairs for HSA1 (NCBI build 30). Localization uncertainties are shown by curved arrows. Loci in bold and italic were mapped during this work; others are from Williams *et al.* (2002). Mapping information for the loci in brackets were inferred from BAC information content. MBPb and CYB5b correspond to human genes MBP and CYB5 and have been previously mapped by Williams *et al.* (2002) (MBP et CYB5).

In the near future, with the publicly available sequence of the bovine genome (http://hgsc.bcm.tmc.edu/projects/bovine/) the described approach will evolve into a pure in silico mapping process from the mapping of homologous genes to the identification of microsatellite markers. Furthermore, single nucleotide polymorphisms will be the choice markers as they are very abundant in the cattle genome, easily detected using *in silico* analysis of sequences of interest and can be integrated in high throughput genotyping projects. Comparative sequencing will also play a crucial role as it will help to identify not only the similar coding regions among species but also sequences with significant functional activities as shown by Boffeli *et al.* (2004). This trend best illustrates the fact that we are reaching the end of different mapping activities in livestock, and are now moving towards a sequencing era to further investigate the structural and functional organization of livestock genomes.

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