The Cattle Gene Map
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INTRODUCTION

Cave paintings in Europe and North Africa depict wild cattle, the aurochs (Bos primigenius), among the prized prey of Stone Age hunters. These majestic animals stood nearly 2 m at the withers and had horns up to 1 m long. Bulls are usually shown as dark brown or black with cows and calves reddish brown, although white and speckled animals also appear in the paintings (Felius 1985). The aurochs spread out of western Asia after the Ice Age (250,000 yr ago) and are believed to have survived in Europe until early in the 17th century. Sometime before their extinction, probably 7000 to 10,000 yr ago, aurochs were domesticated. At least 2 domestications are likely represented in breeds of cattle at the time of this writing. Bos primigenius primigenius, a European auroch subtype evidenced by the fossil record, is thought to be the ancestor to today's humped breeds of cattle given the species designation Bos taurus. An Asian auroch subtype, Bos primigenius namadicus, probably gave rise to today's humped zebu breeds classified as Bos indicus.

Although domestic breeds of cattle are divided into 2 species, they are totally interfertile; in fact, several synthetic breeds have arisen in this century from intercrosses of taurus and indicus breeds. Chromosome numbers are identical: 29 autosomes, all of which are acrocentric, and a submetacentric X. Morphology of the Y chromosomes is the only recognized structural difference in B. taurus and B. indicus karyotypes. Thus, the “cattle” gene map developed to date and discussed in this article is for 2 species that many people, including myself, believe should be reclassified as 1. Cattle are members of the subfamily Bovinae, the family Bovidae, the suborder Ruminantia, and the order Artiodactyla.

A standard karyotype for cattle was resolved by Popescu and others (1996), with gene nomenclature following the guidelines for human gene nomenclature as recommended by the International Society for Animal Genetics (ISAG). Loci without human equivalents are named according to the recommendations of the Committee on Genetic Nomenclature of Sheep and Goats (COGNOSAG 1995).

REASONS FOR MAPPING THIS SPECIES

The rapid development of genome maps in cattle, as in other livestock species, has been driven by several motivating forces. The extensive use of comparative gene mapping as a major tool for the study of chromosomal evolution in animals affords ample reason to map the bovine genome. The internationally funded and highly organized human genome initiative has already provided the standard mammalian genomic map with which all others will ultimately be compared. The genome map of the laboratory mouse continues to develop rapidly and will undoubtedly be the first measure of comparison for evaluating mammalian chromosomal conservation and genomic evolution. However, other mammals, including cattle, play extremely important roles in helping to understand the pathways of chromosomal rearrangement that have accompanied mammalian evolution. At the time of this writing, it has become obvious through comparative mapping that some mammals such as cattle and cats have genomes more highly conserved relative to the human genome than the most often compared mouse genome. These more highly conserved genomes probably most accurately reflect the chromosomal arrangement of the ancestral mammal. At the least, they demonstrate that the total picture of mammalian chromosome evolution cannot be fully represented by differences in the genomes of humans and mice. Expanded comparative mapping including the genomes of cattle will continue to make a valuable contribution to understanding mammalian chromosomal evolution in a universal context.

The potential for marker-assisted selection (MAS) of desirable and marketable traits drives gene mapping in livestock species. Genetic markers of advantageous alleles for economic trait loci (ETL), including quantitative trait loci (QTL), have the potential to enhance the rate and efficiency of genetic gain through selective breeding, a concept advanced long before the technical tools became available for its implementation (Smith and Simpson 1986; Soller and Beckmann 1982; Weller and others 1990). The best markers for use in selective breeding programs are obviously the gene variants actually responsible for phenotypic differences in the important traits. Although such markers are still rare, a few have been identified by thorough searches for variation in genes believed to be involved in the physiological pathways leading to the phenotype of interest. This so-called “candidate gene” approach to marker identification requires a sound fundamental knowledge of physiology un-
derlying the trait followed by extensive and usually expensive screening for variation in candidate genes related to these processes. Ideally, sequence variation related to the trait can ultimately be incorporated directly into the marker assay. Unfortunately, physiological bases for many economic traits remain unresolved, and candidate genes are not obvious. Alternatively, even when the physiology is understood, the complexity of the trait may present a long and cumbersome list of candidate genes. However, ETL, and even QTL, can be mapped by linkage analysis (Andersson and others 1994; Georges and others 1993a,b, 1995; Lander and Botstein 1989; Paterson and others 1989). Markers mapped in proximity to ETL can be used to assist in selection for the ETL if the recombination frequency is sufficiently small and the chromosomal phase of marker and ETL alleles is known. Efficiency of MAS can be increased by identifying markers on either side of the ETL since recombination in the region spanned by 2 markers can be detected. One major early goal of cattle gene mapping was therefore to produce maps of highly polymorphic markers spaced at intervals of approximately 20 cM (1 cM = 1% recombination) or less across every chromosome. These markers could then be available for mapping studies in families segregating QTL, hopefully resulting in linkage associations of the QTL with 1 or more markers. Under appropriate breeding protocols, linked markers can then be used for MAS.

Another goal of gene mapping is to identify and clone genes responsible for ETL. It is obvious from the discussion above that MAS is much more efficient when it utilizes variation in the genes actually responsible for the ETL. More importantly, a complete understanding of the potential interaction of the trait with other physiological processes is possible only when the genes involved are known. The term “reverse genetics” has been replaced in common usage by “positional cloning” or “map based cloning” to describe the process whereby the application of map information is used to clone a gene responsible for a specific trait in the absence of information about the biochemical or molecular basis of the trait. Although the task of positionally cloning genes in any species is formidable, cloning genes for ETL in livestock is almost prohibitive. Animal maps will most certainly never be as dense as those of the human. Large insert libraries for livestock species are only beginning to be developed and used. Naturally occurring chromosomal deletions of important genes, important tools in many of the human and mouse successes, have not been identified and propagated in livestock. The task is further complicated by the quantitative nature of most traits of economic interest in farm animals and the paucity of worldwide research support for animal agriculture relative to the human genome initiative. Alternative strategies to conventional positional cloning must be planned and developed. One proposed approach is “comparative candidate positional cloning,” which takes advantage of knowledge of the evolutionary history of chromosomes and rapid advances in the human and mouse maps.

CURRENT MAP STATUS

Physical Mapping

More than 400 type I loci have been mapped in cattle (Fries and others 1993; O’Brien and others 1993; Womack and Kata 1995) primarily through somatic cell genetics (Arruga and others 1992; Womack and Moll 1986). These synteny maps have provided a foundation for genome maps in cattle, indicating the boundaries of chromosomal conservation relative to the map-rich genomes of mice and humans. However, the comparative map is incomplete, addressing neither conservation nor rearrangement of gene order.

In situ hybridization, especially with fluorescence, has been used effectively to address the order of type I loci, to assign syntenic groups to specific chromosomes, and to anchor the rapidly growing linkage map to chromosomes (Fries and others 1993; Gallagher and others 1993; Iannuzzi and others 1993; Solinas-Toldo and others 1993). At the time of this writing, more than 100 in situ localizations of unique sequences have been identified on cattle chromosomes. All bovine syntenic groups are now anchored to specific chromosomes by fluorescence in situ hybridization (FISH1), and the linkage map is physically anchored at approximately 100 sites on all the bovine chromosomes.

Linkage Mapping

First generation bovine linkage maps (Barendse and others 1994; Bishop and others, 1994) have been expanded in 3 recently published maps containing markers totaling 1250 (Kappes and others 1997), 746 (Barendse and others 1997), and 269 (Ma and others 1996). These combined maps contain almost 1400 unique markers with an average spacing of 2 to 2.5 cM. Although the majority of markers represented are microsatellites, almost 200 are within or near coding sequences. Sex-averaged total genome size is 2990 cM (Kappes and others 1997) and 3532 cM (Barendse and others 1997) on the 2 larger maps. Unlike the human and mouse maps, very little sex difference in recombination was observed in these 2 bovine maps. The male-specific map of Ma and others (1996) covers 1975 cM, which will undoubtedly grow as markers are added.

Comparative Mapping

Approximately 400 loci have been mapped in both cattle and humans. Most of these have also been mapped in mice. Although extensive conservation of synteny has been observed between cattle and humans (Threadgill and Womack 1991; Womack and Kata 1995; Womack and Moll 1986), conservation of linkage (conservation of gene order) may not be as prevalent. Barendse and others (1997) incorporated a sufficient number of type I loci into the linkage map to demonstrate the presence of numerous rearrangements of gene
order within conserved syntenies. An interspecific hybrid backcross (Riggs and others 1997) was used to generate maps of bovine chromosomes 7 and 19 containing sufficient numbers of genes to address this question. Gene order rearrangement within conserved synteny on BTA 7/HSA 5 was demonstrated (Gao and Womack 1997) with a major breakpoint in segment homology identified in the small region between bovine AMH and CSF2. A similar pattern was revealed in bovine chromosome 19 (Yang and Womack 1997). HSA 17 and BTA 19 are completely conserved syntenic groups, yet the linear order of genes has been rearranged. These data support the need for ordered comparative maps to facilitate the extrapolation of candidate genes for bovine traits from the human gene map.

A major contribution to comparative gene mapping is heterologous chromosomal painting or zoological (ZOO)-FISH painting. Solinas-Toldo and others (1995), Hayes (1995), and Chowdhary and others (1996) have "painted" cattle chromosomes with human chromosome-specific libraries to delineate segments of homology. These studies define the boundaries of chromosomal conservation at the "human on cattle" cytogenetic level; and they are very consistent, at the time of this writing, with the results of comparative synteny mapping, which defines "cattle on human" homology. Like synteny mapping, they do not address conservation of gene order in homologous segments.

**APPROACHES USED TO DEVELOP THE MAP**

**Synteny Mapping**

"Synteny" simply denotes "on the same strand" or, in genetic terminology, "on the same chromosome." A synteny map is nothing more than a list of genes known to reside on the same chromosome in a particular species. "Conserved synteny" was used by Nadeau (1989) to describe the location of 2 or more homologous genes on the same chromosome in different species. Synteny should not be substituted for "conserved synteny" in our comparison of maps between species. Synteny mapping is probably associated with comparative mapping because the only maps available for comparison between most animal species have heretofore been synteny maps.

Somatic cell genetics is still the most common method for building synteny maps. Hybrid somatic cells can be constructed so that the chromosomes of practically any progenitor species are preferentially lost. Each hybrid clone will retain a partial genome of that species along with the complete genome of the other that is usually a transformed rodent cell line. Since chromosome loss is more or less random, each clone will retain a different subset of chromosomes from the species being mapped. As in human gene mapping, analysis of pairs of genes in a panel of hybrid cell lines will reveal concordance or discordance of their retention. Concordance of retention is evidence for the location of 2 genes on the same chromosomes. Conversely, discordance of retention is evidence for asynteny (their location on different chromosomes). Gene products or DNA sequences may be mapped by synteny analysis in any species as long as the presence or absence of the gene or gene product of the targeted species can be ascertained against the fully retained rodent genomic background. Enzyme electrophoresis, Southern blotting with unique sequence probes, and polymerase chain reaction amplification with species-discriminating primers have all been effective analytical tools for synteny mapping.

Somatic cell genetics does not typically result in the assignment of markers to specific chromosomal sites or even to chromosomal subregions. Consequently, genes on a synteny map are usually not ordered. Somatic cell methods employing rearranged chromosomes are an exception to this generalization and have been used very effectively to order genes in the human map.

Radiation hybrid mapping (Cox and others 1990; Walter and others 1994) has recently become an important tool for constructing high-resolution maps of human chromosomes. The techniques employed are variations of basic somatic cell genetics in which the donor cells have been irradiated to achieve chromosome fragmentation. Statistical analysis is based on the principles of linkage analysis, that is, greater closeness of 2 loci results in less separation by random chromosomal rearrangement. First used by Goss and Harris (1975), the technique can be used with single chromosome hybrids as the irradiated donor (Cox and others 1990) or with total genome irradiation in a diploid donor cell (Walter and others 1994). Whether used in mapping a single chromosome or a whole genome, the technology is effective for constructing contiguous maps of mammalian chromosomes at a 500-kb level of resolution. This method may prove to be the ideal approach to comparative gene mapping since it provides an ordered map without the requirement of segregating polymorphisms in breeding populations.

**In Situ Hybridization**

Unique DNA sequences, repetitive elements, and whole genomes have all been effectively localized to chromosomal sites by in situ hybridization. This technique employs the attachment of a microscopically detectable marker to a DNA probe followed by hybridization of the probe to denatured DNA of an otherwise intact chromosome. The specificity of hybridization is determined by the uniqueness of the probe. Although radioactive probes dominated the early application of this technology, fluorescent probes are now generally used. In her review of FISH, Trask (1991) notes that it has the following advantages over isotopic labeling: It provides superior spatial resolution, usually requiring visualization of fewer labeled chromosomes; it is faster; and the probe employed is generally more stable. The sensitivities are similar,
Markers for mapping purposes have been categorized as type I or II by O’Brien (1992). Type I markers, which are expressed sequences (genes) usually conserved from 1 mammalian species to another, are favored for use in comparative gene mapping. Unfortunately, they are usually not highly polymorphic and are therefore difficult to incorporate into linkage maps. Type II markers are highly polymorphic anonymous sequences more widely used for linkage mapping. The markers of choice for cattle linkage mapping have been microsatellites, which dominate the linkage maps discussed above.

SCIENTIFIC CONTRIBUTIONS OF THE MAP

A growing number of traits of economic significance are being placed on the bovine genome map. Bovine leukocyte adhesion deficiency (LAD) (Shuster and others 1992; Threadgill and Womack 1991) and uridine monophosphate synthetase deficiency (UMPS) have been mapped to specific sites on chromosome 1 (Ryan and others 1994; Schwenger and others 1993). BoLA was shown to be associated with susceptibility to leukemia virus infection (Lewin and others 1988). Georges and others (1993a) have found linkage of the polled locus to microsatellites on chromosome 1. The Weaver disease maps to markers on chromosome 4 (Georges and others 1993b) and has the added interest of being associated with a quantitative trait for improved milk production. Variation around the prolactin gene on chromosome 23 (Cowan and others 1990) is related to milk production in some holstein sire families, and Georges and others (1995) have used mapped microsatellites to locate an additional 5 QTL for milk production. The number of ETL on the cattle genome map is rapidly expanding, and at least 1 complete success story has emerged. The muscular hypertrophy (double muscling) trait was mapped to microsatellite markers on chromosome 2 (Charlton and others 1995). Comparative candidate positional cloning suggested myostatin as a candidate gene to Grobet and others (1997), who identified an 11-bp deletion responsible for the trait in Belgian blue cattle.

An excellent example of a successful candidate gene search is that of Shuster and others (1992), who identified the genetic defect responsible for LAD in holstein cattle as a missense mutation coding amino acid 128 in CD 18. It then became possible to distinguish the mutant and normal alleles by the polymerase chain reaction, providing the ideal genetic marker of this economic trait locus.

ANTICIPATED FUTURE CONTRIBUTIONS OF THE MAP

The large number of mapped markers that exist in cattle at the time of this writing provides extensive genome coverage for mapping ETL in families segregating the traits. Unfortunately, each ETL usually requires a unique segregating family, generally requiring expensive development and maintenance. Nonetheless, the resource families are integral and necessary in the ultimate application of the gene map to economic improvement. The mapping of ETL to regions of a chromosome between 10 and 20 cM will likely be followed by high-resolution mapping in an effort ultimately to identify and clone the responsible genes. Chromosome-specific libraries are being developed to aid this process. Animal breeders should not and probably will not be satisfied with only an approximation of ETL marker distance (such as 10 cM).

The next major step, identifying and cloning ETL, is formidable. The high-density linkage maps, numerous chro-
mosomal deletions, and large insert contigs that have contributed greatly to positional cloning of human disease loci are simply not available for animal ETL cloning. It is unlikely that this wealth of resources will ever be available for cattle. However, positional cloning of human genes is rapidly shifting toward the positional candidate (Collins 1995) approach, which relies more on the availability of a pool of expressed genes mapped to the same chromosomal regions as the disease gene and less on walking and jumping from a linked marker. In cattle as in humans, the 3-step process for positional candidate cloning of the gene for an important trait will be (1) to localize the trait locus to a chromosomal subregion, (2) to search available databases for reasonable candidate genes, and (3) to test candidate genes for variation correlated with phenotype. Obviously, step 2 is unrealistic in cattle since only 400 of the approximately 70,000 genes have been assigned to chromosomes at the time of this writing. This step was almost as unrealistic in humans until the late 1990s, when several international initiatives targeted the large scale mapping of expressed sequence tags (ESTs).

The success of these efforts suggests that most of the human transcripts will likely be mapped in the next few years (Collins 1995). Thus, the key to bovine ETL cloning may be through comparative genome databases that can translate a 10-cM bovine segment into its human counterpart and can then search for human ESTs in the segment with features that can be applied to the bovine phenotype.

One can hypothesize that with approximately 20 potential candidate genes per centimorgan, 200 genes or ESTs will comprise the total candidate pool. Such a comparative positional candidate cloning strategy provides hope for ETL cloning that is not apparent with the conventional strategies of map-based cloning of human disease genes. This strategy was successfully implemented in the search for the double muscling gene described above. However, systematic use of human EST databases for identification of animal genes responsible for ETL will require comparative maps with greater precision than those currently available. Identification of the boundaries of conserved synteny is not sufficient. We must continue to identify the internal rearrangements that have accompanied mammalian chromosomal evolution and have resulted in rearrangement of gene order within these boundaries of conserved synteny. A promising approach to ordered comparative maps is more extensive use of radiation hybrid mapping.

It is also important to begin to map bovine transcripts and candidate genes for traits localized to specific genomic regions. Again, panels of radiation hybrids will be critical to the mapping of bovine ESTs and for their integration with microsatellite markers from the linkage maps.

USES OF THE MAP AND ACCESSIBILITY

Maps of the cattle genome have grown beyond the scope of a single illustration. These data along with pictorial representations are available, however, on several Web sites (Table 1).

The 3 linkage maps discussed in the foregoing section entitled Current Map Status can be found at the CSIRO, MARC, and IRRF sites, respectively. Summary linkage maps are generated from the NAGRP, Japan, and INRA databases. The NAGRP site also includes “cow on human” and “human on cow” comparative maps with corresponding mouse data.

CONCLUSION

The bovine genome map includes a synteny map, a linkage map, and at least 1 in situ hybridization for each chromosome. More than 1400 markers are placed on at least 3 published linkage maps. These markers are now being used to generate maps of ETL at an accelerating pace. The question of how to identify genes responsible for mapped ETL in cattle (and other livestock species) is formidable. One proposed approach is through comparative candidate positional cloning, in which candidate genes from the human and mouse maps are obtained from the bovine comparative map. It has become apparent that identification of conserved synteny between species is insufficient for comparative candidate positional cloning due to the abundant rearrangements of gene order within conserved syntenic groups. Ongoing experiments to further resolve gene order include an interspecific hybrid backcross and radiation hybrid somatic cell analysis.

### TABLE 1 Web sites for bovine gene mapping databases

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<tr>
<th>Database</th>
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<tr>
<td>US Bovine ArkDB</td>
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<tr>
<td>BovMap Database, INRA, France</td>
<td><a href="http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl">http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl</a></td>
</tr>
</tbody>
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*USDA, US Department of Agriculture.*
REFERENCES


Arruga MV, Monteagudo LV, Tejedor MT. 1992. Assignment of two markers carried by human chromosome 1 to different cattle synteny groups: FH to U1 and PEFCP to H17 (chromosome 8). Cytogeten Cell Genet 59:45-47.


