THE BOVINE GENOME STUDY AND ITS GOALS

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The consensus conclusion of several recent major international livestock genetics conferences is that genomic localization of economic trait loci and subsequent marker-assisted selection for these traits will rapidly facilitate genetic improvement of livestock. Genetic improvement of cattle, specifically for efficiency of food utilization, reproductive performance, resistance to disease, and higher quality of beef and dairy products ranks high among the world's agricultural research needs. The necessary tool for large scale improvement of such a diverse array of phenotypes by marker-assisted selection is a 20 centiMorgan map of the bovine genome, consisting of highly polymorphic loci that can be easily scored by teams of investigators studying the segregation of desirable traits. This same map can subsequently be developed to a high level of resolution in regions around genes of particular interest and used to isolate and clone genes involved in the expression of economically important traits.

Experiments in our laboratory (1, 2, 3) and others (4, 5) demonstrate that the methods that have revolutionized human genetics in the last decade can do the same for cattle genetics. Bovine cells readily hybridize with mouse L cells or hamster E36 cells and segregate cattle chromosomes (1). Genetic analysis of these segregating hybrid cells has produced the current syntenic map of the cow which consists of more than 270 genes including markers of every bovine chromosome. Fifteen of the autosomal syntenic groups have been assigned to chromosomes. Our laboratory has participated in these assignments (6, 7). We have selected genes for physical mapping based on their potential role in key physiological processes and their value in defining the limits of chromosomal conservation with the human and mouse maps. We have used Southern blotting of more than 200 bovine and heterologous probes and more recently, the polymerase chain reaction (PCR) with published primer sequences to generate these maps.

Comparative mapping.

The human and mouse gene maps contain over 3000 and 1200 loci, respectively, representing investments of several hundred scientist-years and millions of dollars. This investment need not be totally repeated with cattle. It has become apparent that sizable portions of most chromosomes have been conserved in mammalian evolution. The homology of the cow and human genomic maps is remarkable (1, 2). Thus, the chromosomal location of genes in cattle is predictable - if the same genes have been mapped in humans to one of the highly conserved regions. Similarly, there is sufficient conservation of the cow and mouse genomes to predict, with only slightly less confidence, the location of cattle genes whose homologues are mapped in mice.

Linkage mapping.

A high density linkage map of marker loci will be the ultimate tool in the use of marker assisted selection for the improvement of disease resistance and productivity in cattle. The number of markers required for a linkage map of the bovine genome useful for the detection of economically important traits is dependent on the desired informativeness and distribution of markers. Twenty cM genomic spacing is generally regarded as sufficient for the use of multiple linked markers for interval mapping of quantitative trait loci (8) and is the target of most organized initiatives for animal genome mapping. A marker spacing of 20 cM requires a minimum of 125 evenly spaced, totally informative markers. Over 230 randomly spaced markers are required, however, for 90% coverage of the 25 Morgan bovine genome at 20 cM intervals (9). The effective use of physical mapping should reduce the required number to below 230 but perfect spacing is an unrealistic expectation. Currently, there are only a few highly polymorphic markers available for bovine linkage analysis and common public reference families have not been available; consequently, only a few isolated linkage studies have been conducted (4, 5, 10, 11). The development of

additional polymorphic markers and the application of these markers to common meiotic products is a necessity for the development of the bovine linkage map.

A new type of polymorphic marker, commonly referred to as a microsatellite, has recently become available that exploits the variation in simple tandemly repeated DNA (12, 13, 14). The variation between individuals is visualized by PCR and gel electrophoresis. Initial studies indicate that microsatellites are found throughout genomes of all mammals studied, including cattle (15, 16, 17), and demonstrate high levels of polymorphism (12, 13, 14). Many genotypes can be simultaneously analyzed and since the technique is based on the polymerase chain reaction, preparation of samples is simplified (18). Microsatellites also serve as the sequence tagged sites (STS) described by Olson et al. (19) and generally regarded as the ideal marker for a truly public linkage map of agriculturally important species (20). A map of such markers antiquates the need for storage and shipment of probes, requiring only a public list of primer sequences and optimum PCR conditions. Consequently, microsatellites appear to be the markers of choice for development of the bovine linkage map. With this in mind, we have begun to lay the foundation for a microsatellite linkage map with anchor points to the valuable physical map of the species and comparative maps of other mammals.

Genomic mapping.

Mature genomic maps are composites of data derived in different laboratories with different genetic/molecular tools and biological resources. These disparate data can only be tied into a common map by anchor loci, i.e., unique sequences common to two or more sets of mapping data. Two types of anchor loci are generally recognized as essential to the genomic map of a species (21). Type I anchor loci are evolutionarily conserved loci, usually coding genes, whose homology between species can be clearly established. These loci provide the predictive power of comparative gene maps and help resolve break points in evolutionary chromosomal conservation. Type II anchor loci are species-specific markers that are highly polymorphic and consequently likely to be scored in multiple linkage studies of that species. They anchor linkage maps generated from analysis of different meiotic events and thus facilitate the merger of these data into a consensus map. These loci should have a high polymorphic information content (PIC $\geq .5$) (22) and should utilize probes or PCR primers from the public domain. The best of these loci are often minisatellites or microsatellites which do not demonstrate sufficient evolutionary conservation to double as Type I anchor loci.

As the preceding paragraph implies, comparative maps and linkage maps are often uncoupled. Moreover, physical chromosome maps, as generated by somatic cell genetics or in situ hybridization, are rarely anchored to the linkage map of the same species simply because Type I anchor loci historically predominate in the development of physical maps. This situation is unfortunate in that it stifles the efficient development of a 20 cM bovine linkage map of Type II loci, the ultimate tool for mapping economic trait loci (ETL), by limiting the extrapolation to cattle of the genetic data available (and anticipated) for humans and mice. Along with the development of a type II locus linkage map, we propose to incorporate a sufficient number of Type II loci into the physical and comparative maps to (1) utilize the physical map of cattle chromosomes as a gauge for spacing and terminalization of the linkage map and (2) to better utilize human and mouse comparative data in the development of the bovine map.

Available Biological Resources.

Hybrid Somatic Cells. We have a panel of 20 bovine-rodent hybrid cell lines (selected from over 150 we have made) that have been characterized for over 270 genetic markers on all bovine syntenic groups. The resulting map, comprised primarily of Type I markers, is the foundation for comparative genomic studies with other mammals and has also proved effective for preliminary assignment of Type II loci, both by probe hybridization (3) and PCR priming (23). STS markers have been mapped in this panel, 24 of them microsatellites. In addition to the live and cryopreserved cells in our laboratory, DNA from this panel has been prepared and stored and is available for distribution to other laboratories.

Reference Families. An international panel of reference families for bovine linkage mapping has been developed, modeled after the very effective C.E.P.H. collaboration in human genetics. Fourteen full-sib families with a total of 212 offspring (Table 1) have been selected from Kenya (A. Teale, I.L.R.A.D.), Australia (J. Hetzel, C.S.I.R.O.) and Texas (J. Womack, T.A.M.U.). Sufficient DNA has

been collected from individuals in these families for analysis of over 5000 markers (more if PCR based) and is currently available as a public scientific resource.

Table 1. Panel of Reference Families for International Use in Developing a Public Linkage Map of the Bovine Genome.

			Offspring
Texas (Brangus)			
♂1	x	♀1	19
		♀2	11
		♀3	10
		♀4	5
♂2	x	♀5	12
Australia (Indicus x Taurus)			
♂1	x	♀1	21
		♀2	13
		♀3	10
♂2	x	♀4	13
		♀5	15
♂3	x	♀6	10
		♀7	16
Kenya (N'Dama x Boran)			
♂1	x	♀1	37
♂2	x	♀2	20
		total	212

Rèsource Families: A 20 cM bovine linkage map of highly polymorphic markers will likely be accomplished by 1994. The next step in utilization of this valuable resource is the mapping of economic trait loci (ETL), many of which will be quantitative trait loci (QTL). Large families segregating these traits must be identified among existing breeding populations or developed under experimental conditions. Several investigators have anticipated the development of a linkage map and initiated the breeding of interesting resource families to coincide with the development of the availability of mapped, highly polymorphic markers. Soller and Beckman (Israel), working with investigators at the I.T.C. in the Gambia, conceptualized the mapping of genes for trypanotolerance in N'Damo cattle. This program has been initiated by Teale (I.L.R.A.D. in Nairobi) and a team of geneticists has been assembled to test marker genotypes on the F_2 animals as they are scored for response to Tsetse challenge. Other resource families have been generated, two at my University, one segregating beef carcass traits and the other segregating resistance to Brucella abortus. These studies will undoubtedly map QTL relative to easily tested DNA markers, making marker-assisted selection a reality. If the linkage map is properly anchored to the physical map, enhanced mapping in the specific chromosomal regions can eventually lead to the identification and cloning of the genes involved.

Summary

An informal international initiative is underway to map the bovine genome. Formal programs have begun in several countries. The overall objective is a linkage map of highly polymorphic DNA markers at intervals no greater than 20 centimorgans (20% probability of recombination in meiosis). This map will be used to identify relationships of markers to economically important traits, and therefore for marker-assisted selection of desirable phenotypes. High density maps of specific chromosomal regions can subsequently be used to identify and clone the genes of interest. The first traits to be mapped will likely be those that are simply inherited, i.e., polled, dwarf and various recessive lethals. The next traits will be those that are multigenic and influenced by environmental factors, i.e., growth, reproduction and disease resistance traits. Markers are rapidly being developed to complete the map and an international panel of reference families isavailable for this purpose. Several resource families segregating specific economically important traits are in various stages of development.

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