Genetics of Disease Resistance

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Abstract

A role for genes of the host in resistance or susceptibility to specific diseases is generally accepted by those who work with cattle but difficult to document scientifically. The necessary scientific experiments are expensive and plagued with difficulties in design. Several such experiments are in place, however, each with the potential to not only document the existence of such genes but to either identify the genes or to locate them on chromosomes relative to mapped markers. The "candidate gene" approach is to find genetic polymorphism in and around genes with a known or suspected involvement in host-pathogen interactions and to look for associations of allelic forms of the gene with clinical response. The alternative is a "genomic" approach in which crosses made to segregate the response to pathogens and a battery of markers, spanning as much of the genome as possible, are followed to look for genetic linkage of the trait with a specific marker. The latter approach will be facilitated by a complete marker map of the bovine genome. The complete marker map is rapidly becoming a reality. The rapid development of microsatellite and other hypervariable markers and the availability of a common set of reference families for linkage analysis has almost achieved the goal of a 20 cM linkage map. Immediate needs for development are (1) markers to anchor physical maps to linkage maps, (2) resource families segregating economic trait loci, and (3) chromosome-specific libraries to develop densely saturated linkage maps over genomic regions shown to contain economic trait loci. These needs are being addressed by organized national and international programs, particularly in the USDA's NAGRP program and the latest European genome effort called BovMap.

Introduction

Differences between breeds and individuals within breeds in response to pathogens has long been recognized by cattle breeders, feedlot managers and bovine practitioners. The scientific documentation of these differences has not come easy, requiring experiments that are both expensive and fraught with problems in design. New technologies in molecular genetics have given recent momentum to the effort, however, and several experimental approaches are now being applied to the genetics of different cattle diseases.

The "candidate gene" approach is an obvious one. Understanding the cellular mechanisms involved in a host response predicts the involvement of specific gene products. Variation in those genes is then sought and population sampling may be sufficient to find associations between a variant form of the gene and response to the pathogen. Success with this approach has been achieved with leucocyte adhesion deficiency (19) and response to bovine herpesvirus I (18).

The "genomic" approach relies on the co-segregation of a clinical phenotype with a molecular marker to which it is linked. The probability of success in such a search is dependent on the number of markers tested and success can only be assured if the genome is saturated with such markers at regular intervals. A fail-safe collection of markers requires a map of the bovine genome. The remainder of this paper is devoted to the goals and the status of ongoing genome mapping activities in cattle.

Goals of Bovine Genome Mapping

The goal of the current international initiative to map the bovine genome is to produce a map which will describe linkage relationships of economic trait loci (ETL) with highly polymorphic DNA markers. Such has not always been the case. The initial goal of bovine gene mapping, at least in our laboratory, was to study mammalian chromosome evolution. Homologous genes mapped in humans and mice by the early 1980s demonstrated extensive conservation of synteny. Mapping many of these same genes in a prototypic genome would answer questions regarding the evolutionary significance of the chromosomal conservation being revealed by comparative mapping of mouse and man. A panel of bovine-rodent hybrid cells was generated and analyzed for segregation of the bovine homologues of genes mapped in the other mammals. Our initial study[27] and series of subsequent studies[4, 20, 21, 22, 23] have demonstrated that the conservation of synteny between cattle and humans is even more extensive than that between mice and humans.

The economic significance of domestic cattle was not lost in our selection of *Bos taurus* (and *Bos indicus*) as a target for genomic mapping. It was largely a matter of faith, however, that prompted our early expectations that a better understanding of organization of the bovine genome would eventually translate to genetic improvement of meat and milk production. A linkage map

sufficient to map quantitative trait loci or even to present a reasonable probability of mapping single gene traits was beyond comprehension because of the paucity of known genetic polymorphisms in the species. 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 centimorgan (cM) genomic spacing is generally regarded as sufficient for the use of multiple linked markers for interval mapping of quantitative trait loci (QTL)[13] and is the target of most organized initiatives for animal genome mapping. A 20 cM linkage map became a realistic goal with the availability of cloned DNA probes and the discovery of restriction fragment length polymorphisms (RFLP)[2, 3] and even more promising with the subsequent discovery of hypervariable loci such as minisatellites[12] and microsatellites.[8, 11, 25] The availability of such markers and a common set of reference families (Barendse *et al.*, personal communcation) make a linkage map of polymorphic loci a realistic short-term goal. A 20 cmM map is likely to be produced in the next 12 months. The longer term goal of mapping ETL and marker-assisted selective breeding for improvement of these traits is also realistic, as will be described in this presentation.

A third declared purpose of a bovine map is to use a map of markers and ETL to isolate and clone the ETL by positional cloning (reverse genetics). A 20-cM map will not serve this purpose and even a one cM map in regions of interest may be minimal for positional cloning. The goal is a valid one, however, and the rewards will be worth the effort required to construct one cM maps in chromosomal regions around ETL. Some of the requirements of this goal will also be discussed.

Status of the Map

The bovine synteny map has grown to approximately 350 genes placed on all syntenic groups. These loci identify 59 independent segments of chromosomal evolution relative to the human map, some spanning almost the entire length of individual human chromosomes.[4, 20, 21, 23] Our panel of hybrid cells and those now being developed and characterized in other laboratories will continue to be a valuable resource for the initial assignment of new genes to bovine syntenic groups or chromosomes and for the further identication of the boundaries of conservation of chromosomal segments relative to other mammals.

Genes, and consequently syntenic groups, have been assigned to 18 bovine chromosomes, including the X and Y. Thus, 13 syntenic groups remain unassigned. This number is diminishing rapidly, primarily due to the development of in situ hybridization technology in several laboratories. Especially notable in this regard is the laboratory of R. Fries in Zürich and D. Gallagher in our laboratory, who have targeted the assignment of the remaining 14 syntenic groups, most of which will likely be done in the coming year. A major advance in this technology is fluorescent in situ hybridization (FISH) with biotinylated probes. Post hybridization Q-banding by fluorescence using Hoechst 33258 permits a very precise localization of genes to bovine chromosomes.[17]

A linkage map with marker spacing of 20 cM will require a minimum of 125 evenly spaced, totally informative markers. As many as 250 randomly spaced markers may be required, however, for 90% coverage of the 25 bovine genome at 20 cM intervals.[1] The effective use of synteny and chromosomal mapping should reduce the required number to below 200 but perfect spacing is an unrealistic expectation. Until recently, only a few highly polymorphic markers have been available for bovine linkage analysis and common public reference families have not been available; consequently, only a few isolated linkage studies have been conducted.[5, 6, 7, 9] Fortunately, polymorphic markers are now being identified at a rapid pace, and the application of these markers to common meiotic products is now possible.

A relatively new type of polymorphic marker, commonly referred to as a microsatellites, is now available to exploit the ubiquitous variation in simple tandemly repeated DNA in mammalian chromosomes.[11, 24, 25] Allelic variation can be visualized by the polymerase chain reaction (PCR) and gel electrophoresis. Microsatellites are found throughout genomes of all mammals studied to date, including cattle,[8, 10, 14] and generally demonstrate high levels of polymorphism.[11, 24, 25] Many genotypes can be simultaneously analyzed and since the technique is based on the PCR, preparation of samples is simplified.[26] Microsatellites also serve as bovine sequence tagged sites (STS)[16] and are generally regarded as the ideal class of marker for a truly public linkage map of agriculturally important species.[2] A map of such markers dispells the need for storage and shipment of probes, requiring only a public list of primer sequences and optimal PCR conditions. Consequently, microsatellites appear to be the markers of choice for development of the bovine linkage map.

Needs

Highly developed genomic maps are composites of data derived in different laboratories by different methods 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 have been recognized as essential to the genomic map of a species.[15] Type I anchor loci are evolutionarily conserved loci, the homology of which can

be clearly established between species. These loci, usually coding genes, provide the predictive power of comparative gene maps and help to resolve break points in evolutionary chromosomal conservation between mammals. Type II anchor loci are species-specific markers that are highly polymorphic and consequently useful for the development of linkage maps of that species. They anchor linkage maps generated from analysis of different meiotic events, possibly from different laboratories, and thus facilitate the merger of these data into a consensus map. Ideally, loci should have a high polymorphic information content (**PIC** $\geq .5$)[3] and should utilize probes or PCR primers from the public domain. The best of these loci are likely to be minisatellites or microsatellites or other classes of marker 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 not necessarily coupled. Moreover, chromosome maps, as generated by somatic cell genetics or in situ hybridization, are often not anchored to the linkage map of the same species simply because Type I anchor loci historically predominate in the development of chromosomal maps. This situation is unfortunate in that it limits the extrapolation to cattle of the genetic data available (and anticipated) for humans and mice and, consequently, stifles the efficient development of a 20 cM bovine linkage map of Type II loci, the ultimate tool for mapping ETL. Efforts should be made to incorporate a sufficient number of Type II loci into the physical and comparative maps to utilize the physical map of cattle chromosomes as a gauge for spacing and terminalization of the linkage map and to utilize human and mouse comparative data better in the development of the bovine map.

A 20 cM linkage map will soon be a reality. The mapping of ETL will require large families segregating the traits of interest. These will likely be independent (and expensive) studies. It is imperative that investigators and funding agencies anticipate the reality of the linkage map and begin construction of the "resource families" segregating various ETL. Several laboratories are well into such breeding programs. Several years ago M. Soller and J. Beckmann suggested a program in the Gambia to map the genes for trypanotolerance in N'Dama cattle. Under the direction of A. Teale this program is well underway in Nairobi, where F2 offspring will be scored for response to Tsetse challenge. Investigators world-wide will receive DNA for marker typing. The magnitude of QTL mapping begs for collaboration of laboratories, as exemplified by this international effort.

Positional cloning, using a linked marker to clone an unidentified gene by chromosome walking and jumping, is no trivial task. The amount of DNA in an average cM demands a 1-cM map around ETL of interest to present any reasonable hope of successful cloning from a linked marker. One cM maps can be generated around ETL after they are placed on the 20 cM map if chromosome and subchromosome libraries are available to saturate desired regions with polymorphic markers. It is not too early for investigators to begin development of techniques to produce such libraries.

Several national and international programs are now underway to promote the development of the bovine map. An Australian program within the C.S.I.R.O. is headed by Jay Hetzel. The European Economic Community has approved and funded Bovmap, an organized network of more than 30 laboratories dedicated to producing a foundation map of the bovine genome. In the USA, a National Animal Genome Research Program has been organized to map several agriculturally important species, including cattle. Other strong programs involving individual laboratories are brought together with these within the International Society for Animal Genetics (ISAG), the scientific society which appears to have taken a leadership role in the organizational scheme of animal genome programs. Active committees within ISAG address critical issues of nomenclature and recently the development of an international panel of reference families for linkage analysis. The use of these families by the international community will make quick work of the linkage map providing a battery of mapped markers for application to resource families segregating ETL.

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Abstract

A long term epidemiological study of bovine viral diarrhoea infections in a large herd of dairy cattle

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Epidemiological aspects of bovine viral diarrhoea virus (BVDV) infections were studied longitudinally in a large dairy herd for three years. At the start of the study, practically all the cows more than four years old had BVDV antibody titres, whereas the younger stock were almost all seronegative. The spread of the virus was monitored in a part of the population that contained only transiently viraemic cattle and in another part that contained persistently viraemic calves. Among the lactating cows the virus circulated for two-and-a-half years, although they had no direct contact with persistently viraemic cattle during this period. The highest transmission rate occurred when a large number of susceptible heifers was added to the population of cows that contained transiently viraemic cattle. The circulation of BVDV among the lactating cows ceased while 27 seronegative cows were still present. Both findings are in accordance with predictions from simple epidemic models. The susceptibility of the cows that remained seronegative was confirmed experimentally. In contrast with the limited circulation of BVDV caused by transiently viraemic cattle, virtually all susceptible cattle that came into contact with a persistently viraemic calf became seropositive within three months. Transplacental BVDV infections were not detected in the calves born to cows that had antibodies against the virus due to an infection that had occurred at least four years earlier. Transplacental transmission of BVDV did not occur in most of the pregnant cows that were infected before approximately the 60th day of gestation, but when cows became infected later in gestation the virus virtually always invaded the fetus. Clear conclusions on transplacental infection were not always possible in fetuses infected in late gestation. The precolostral sera of six of 42 prenatally infected calves contained both virus and antibodies; the antibody titres were low. After retesting four to five months later, the two calves remaining on the farm were still viraemic, but they had become seronegative.