

# Genome mapping in ruminants and map locations for genes influencing reproduction

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Genetic maps provide a critical link between genes and phenotypes and are essential tools in the search for the genetic basis of variation in reproductive traits. Genes coding for hormones, growth factors, receptors, binding proteins, transcription factors and enzymes that influence the development and function of the reproductive axis have been assigned to genetic maps of ruminants and locations can be found in the respective genome databases. In addition, comparative information on gene structure and map location will help define the functions of essential genes. Gene locations from other species can be used because of extensive comparative links among mammalian gene maps. Large-scale projects to sequence genes and the ability to map these genes in parallel in radiation hybrid panels of different species will greatly improve the maps and our ability to translate between them. Cloning the genes responsible for genetic differences in fertility and fecundity in ruminants is likely to provide valuable clues to understanding ovarian function and germ cell development.

Successful reproduction is the outcome from complex interactions of genes and environment to transfer the genetic 'blueprint' to the next generation. One goal of reproductive biology is to understand the key events that regulate the development and function of the reproductive axis. Information about genes and their protein products, variation in genes that lead to significant physiological differences (or phenotypes), and the interaction of genes and the environment is required to achieve this understanding. Important resources for this task are a complete description of all the genes in the mammalian genome, genetic maps, and physiological models that define critical steps causing variation in reproductive traits.

Mammalian genome projects are making progress towards complete gene transcript maps. The number of genes in mammals is unknown and estimates vary from 70 000 to 150 000 genes. The recent human gene map includes locations for 30 181 genes (Deloukas *et al.*, 1998). Complete transcript maps and the complete DNA sequence are expected for the human genome in the near future. Efforts in livestock species are more modest, but gene sequences, gene order and chromosome organization are highly conserved among mammalian species (Andersson *et al.*, 1996). Consequently, rapid progress in the human and mouse genome projects can be translated readily to ruminants through comparative genomics.

The human DNA sequence will be available in the near future, but it will be much longer before functions can be ascribed to all the genes. Many genes contribute to successful reproduction and additional complexity comes from the interactions of genes with one another and with environmental variables. One approach to defining the set of genes for immediate study is to examine genetic variation causing differences in reproduction in animal models. Genetic variation resulting in phenotypic differences among breeds and selection

lines in livestock will help identify key steps in some pathways. Genetic maps are a critical link between the genes and the phenotypes. They provide essential tools in our search for the genetic basis of variation in reproductive traits.

Reproductive success is critical for efficient livestock production, and research in ruminants has contributed significantly to our knowledge of genetic and environmental factors influencing reproduction. This review describes the methods used in the building of gene maps, summarizes data on the locations of genes and phenotypes affecting reproduction in ruminants, provides links to the databases in which relevant data can be found, and describes how information from other mammalian species can be related through comparative genomics.

## Genetic maps

Genetic maps are important in many areas of biology and medicine. They provide tools with which important disease genes can be located and cloned, and new diagnoses and treatments developed. Maps are essential for dissecting out genetic and environmental components of complex traits. Physical maps provide the backbone for large-scale projects to sequence mammalian genomes. Maps for different species can be aligned and knowledge about genetic variation translated between distantly related species.

Genetic maps describe the assignment of genes or loci to each chromosome, and provide information on gene order and on the distances separating genes. Genes can be mapped by a variety of methods and at different levels of resolution down to the ultimate resolution of the DNA sequence (Silver, 1995; Broad *et al.*, 1997; Graves, 1998). Linkage maps and physical maps are two types of map with different applications and scales of measurement.

### Box 1. Genomes, genes and genetic markers

The genome is the DNA complement of germ cells. These cells are haploid and have one copy of each gene. Mammalian somatic cells are diploid and contain two full genomes or two copies of genes (except for the genes on the sex chromosomes). In mammals, the genome is essentially the same size with the total information content of the haploid genome being approximately  $3 \times 10^9$  base pairs (bp) or 3000 megabase pairs.

In classical genetics, genes were defined by alternative physical characteristics or *phenotypes*, which could be observed and were transmitted to different offspring in genetic crosses. Molecular biology has greatly expanded the range of methods to define genes and distinguish individual DNA segments. Any DNA segment (gene or other sequence) that can be specifically identified using some genetic method is defined as a *locus*.

Any difference in DNA sequence for a given locus produces *alleles* that can be followed in genetic crosses. An example of a DNA probe for a particular gene that identifies two different alleles is shown (Fig. 1a). The inheritance of these two alleles could be followed in the family used in the example, although the grandparental origin could not be determined in all offspring. The polymorphisms that generate these alleles can influence gene function where the DNA sequence differences change the protein, alter gene expression, or affect RNA stability. However, most polymorphisms lie outside the coding or control regions of genes and do not have detectable effects on gene function.

The development of comprehensive linkage maps has been made possible by rapid methods for detecting and assaying variation in DNA sequence. Rapid methods are necessary to screen large numbers of genetic markers in many families.

#### Restriction fragment length polymorphism

The first widely used markers for the construction of linkage maps were restriction fragment length polymorphisms (RFLPs; Botstein *et al.*, 1980). Restriction enzymes cut DNA at specific recognition sequences. Changes in DNA sequence caused by point mutations of individual bases at these sites can create or destroy restriction-cutting sequences which, in turn, leads to changes in the size of DNA fragments generated by restriction digests of DNA from different individuals. Early methods to detect RFLPs used Southern blotting (Southern, 1975). In the example given (Fig. 1a), one polymorphic restriction site, outside of the coding region for the gene, produces bands of different size. A constant band and two variable bands are detected using the gene sequence to a probe on a Southern blot of DNA samples for each individual in the pedigree. The inheritance of the variable bands can be followed through the pedigree.

An alternative source of variation is the presence of insertions or deletions of DNA fragments that can also be detected by Southern blots (Fig. 1b). RFLPs generally have few alleles and are not

informative in many pedigrees. Southern blots are labour intensive and require large amounts of DNA sample for each test. The development of the polymerase chain reaction provides alternative methods to detect RFLPs. PCR primers flanking the region can be used to amplify a fragment containing the restriction site. A restriction digest of the product reveals the variable band sizes.

#### Microsatellites

The marked improvement in linkage maps came with the development of highly polymorphic microsatellite markers (Weber and May, 1989). These markers are based on variation in the length of short repeated sequences (di-, tri- and tetra-nucleotide repeats). An example of DNA sequence for two possible repeats is shown (Fig. 1d). The lengths of the repeat sequences can vary greatly among individual sequences generating multiple alleles. PCR primers are designed to the unique sequence flanking the repeat to amplify across the repeat sequence. Size differences caused by changes in the repeat length are resolved on polyacrylamide gels. Microsatellite markers have several advantages and have become the most common markers used in map construction. Large numbers of markers can be cloned, are highly variable (have many alleles), require only small amounts of DNA for analysis, and methods can be automated readily. One disadvantage of microsatellites is that they are mostly anonymous DNA fragments and work only in closely related species.

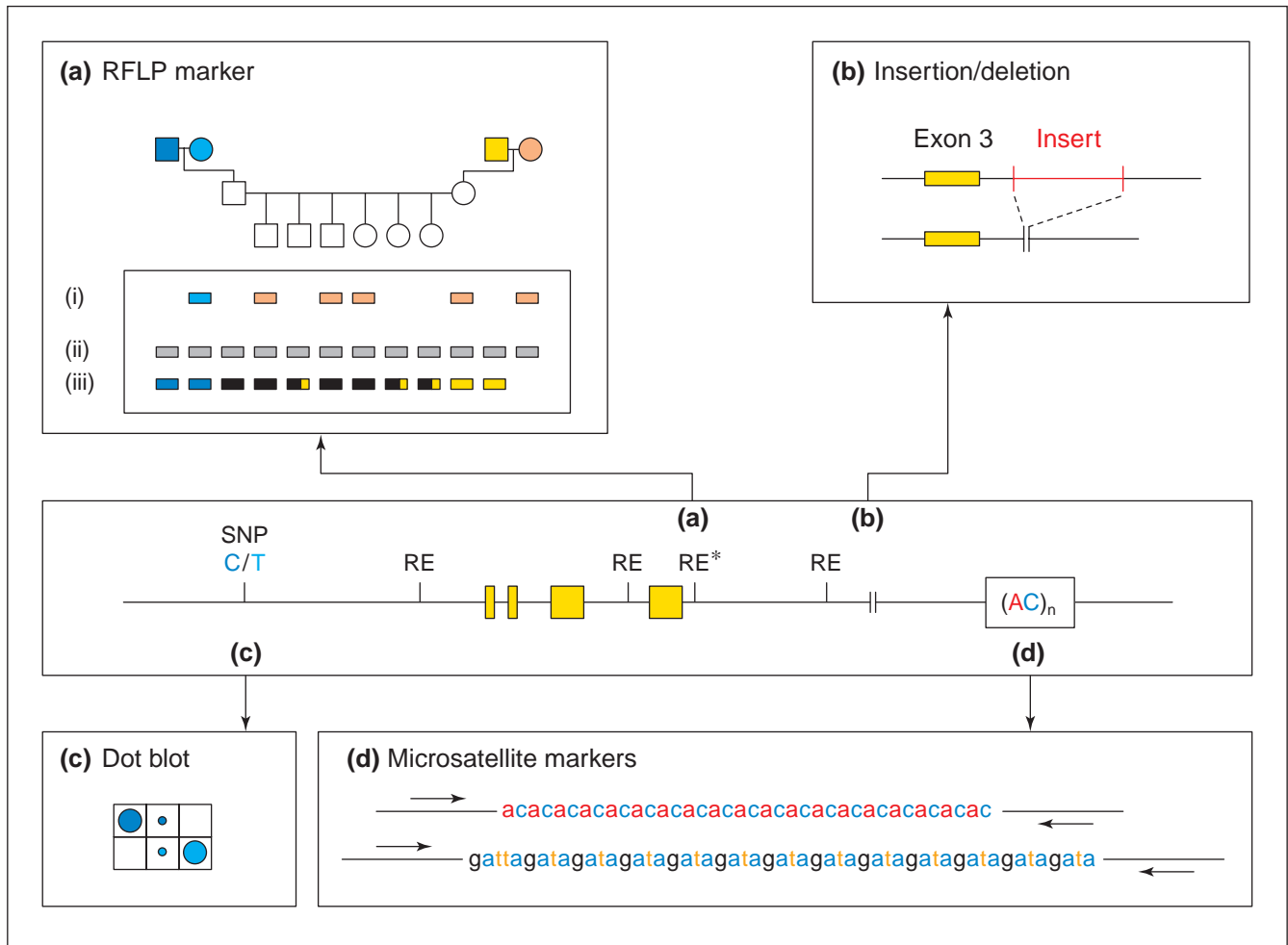
#### Allele-specific oligonucleotides

Single base pair variation can be detected by hybridization with allele-specific oligonucleotides (Landegren *et al.*, 1999). Oligonucleotide probing depends on the synthesis of two DNA probes of 20–30 base pairs. One probe is complementary to the normal gene sequence and the other is complementary to the mutated sequence. Each oligonucleotide is radiolabelled and hybridized to samples of DNA fixed to a membrane (a dot blot) from normal individuals, and those heterozygous and homozygous for the mutation (Fig 1d). Hybridizing conditions must be carefully controlled. The DNA from individuals carrying two copies of the normal sequence will hybridize with only the normal probe. Conversely, DNA from individuals homozygous for the mutation will hybridize only to the oligo complementary to the mutation. Heterozygous individuals will show hybridization to both probes. Allele-specific oligonucleotide techniques can be applied with either the DNA samples or the oligonucleotides arrayed on a surface. The two approaches are suited for screening either large numbers of individuals for a few variants or one or two individuals for sequence differences at many sites. For a review of other methods for detecting single nucleotide polymorphisms, see Landegren *et al.* (1999).

#### Linkage maps

Linkage maps can only be constructed using polymorphic loci with two or more heritable forms or 'alleles' (Box 1). Linkage maps are also known as recombination maps and define the order and distance of loci along a chromosome on the basis of inheritance in families. During meiosis, one random copy of each chromosome pair is passed to the gamete. Consequently,

grandparental copies of genes located on different chromosomes are inherited independently. Genes on the same chromosome are not inherited independently, but are passed on together or 'linked'. Only genes next to each other are tightly linked. A physical exchange of chromosome segments (crossing-over) takes place when homologous chromosomes pair during meiosis. Crossing-over or recombination results in exchange of grandparental alleles for genes further apart on that chromosome.



**Fig. 1.** Schematic representation of methods commonly used to detect variation in DNA sequences. The centre panel depicts a gene with four exons shown in yellow. The DNA sequence has several polymorphisms. Four sites have DNA sequences that are recognized and cut by a specific restriction enzyme (RE). One of these sites (a) has a single base pair that varies among individuals (RE\*). The variation results in a polymorphism and the restriction enzyme will only cut on some chromosomes. This variation generates a restriction fragment length polymorphism (RFLP). Other types of variation depicted are an insertion/deletion polymorphism (b) and microsatellite repeat (d) shown downstream of the gene. The single base pair change shown upstream of the gene (c) is a single nucleotide polymorphism (SNP). However, the sequence at this point is not a restriction enzyme site and some other method must be used to detect the SNP. (Methods to detect these polymorphisms are described in Box 1.)

**DNA markers.** The copy or allele inherited from each parent needs to be identified in order to follow the inheritance or segregation of a locus or chromosome segment (Box 1). DNA-based methods (Botstein *et al.*, 1980; Weber and May, 1989) provide genetic markers with sufficient variability in populations to track the inheritance of all chromosome segments. Details of DNA marker types used in the construction of linkage maps and methods of detection are given (Box 1; Fig. 1). Microsatellite markers (Weber and May, 1989) remain the markers of choice for the construction of linkage maps because they are highly polymorphic and require small amounts of DNA for each test.

Recent developments include improvements in automation to increase speed and throughput for detection of single base pair changes in DNA sequence. Single nucleotide polymorphisms

(SNPs) with alternate bases at a single nucleotide position (Fig. 1c) provide the most common source of variation in the genome. A base change that alters a restriction site, and generates variable restriction fragments (RFLP), is a special case of a single nucleotide polymorphism. Restriction enzymes only sample a small proportion of the SNPs in the genome. A number of methods for detecting single nucleotide polymorphisms have been developed, including allele-specific oligonucleotides (Fig. 1), automated methods (such as the TaqMan assay), and DNA sequencing (for a review of these methods, see Landergren *et al.*, 1999).

Individual SNPs are less informative than other marker systems, but they are more abundant and have a great potential for automation (Wang *et al.*, 1998; Cargill *et al.*, 1999; Griffin *et al.*, 1999; Li *et al.*, 1999). Two major technologies are being

developed for SNP mapping. One method is hybridization to miniaturized arrays of densely packed oligonucleotides 'DNA chips'. This method is related to allele specific oligonucleotide methods (Box 1). Specific oligonucleotide sequences that detect the variable base pairs are synthesized covalently bound at defined locations to glass slides using photolithographic techniques (Fodor *et al.*, 1991; Southern, 1996). The high-density arrays provide a powerful and rapid method for SNP detection (Wang *et al.*, 1998). Large-scale mapping has been demonstrated with construction of a map of over 2000 SNPs and development of a DNA chip capable of genotyping 500 SNPs in a single assay (Wang *et al.*, 1998).

Mass spectrometry provides an alternative for rapid, automated, large-scale genotyping of SNPs (Griffin *et al.*, 1999; Li *et al.*, 1999). This method uses single base extension of a primer immediately adjacent to the site of the SNP. The polymorphism at the SNP site will determine the base added in the extension reaction. Products of the primer extension assay are detected in a mass spectrometer using specifically developed mass tags and a method to cleave the mass tags after the primer extension assay.

Large scale SNP typing will be essential for a number of gene mapping applications. Most of the variation within small chromosomal regions will be single base changes. Methods to find and detect this variation will be important for mutation detection and resolving genetic effects on complex characteristics. Single nucleotide polymorphism detection may replace microsatellites as the marker of choice in the future. However, major efforts would be required to identify large numbers of SNP markers for livestock.

*Linkage map construction.* Genetic markers tag individual chromosome segments and a high density of markers spread over each chromosome can be assembled into a linkage map. Co-segregating markers are placed into linkage groups, and the proportion of recombinants (crossover events) detected between linked markers is used as a measure of the distance between them. Genetic distance is usually measured in centi-Morgans (cM), where 1 cM is equivalent to 1% recombination between markers. Linkage maps are constructed by analysing large numbers of polymorphic markers in DNA samples from large families. Gene mapping algorithms analyse the co-segregation of markers in the families and assemble the markers into linkage groups. The partial linkage map for sheep chromosome 6 (Lord *et al.*, 1996; Lumsden *et al.*, 1999a) is illustrated (Fig. 2). This map is typical of maps for ruminant chromosomes, with a framework map made up mainly of microsatellite markers. The locations for a small number of genes are well defined, but the order for many genes with respect to the framework markers is not known. The genes are only mapped to a region of the framework map.

### Physical maps

Physical maps provide the location and order of genes on chromosomes by the physical assignment of genes to chromosomal segments (for a review of methods, see Broad *et al.*, 1997). Genes can be assigned directly through *in situ* hybridization of DNA fragments to chromosome spreads. A cloned

probe with a tag or label is specifically bound to a complementary DNA sequence on chromosomes bound to a glass slide.

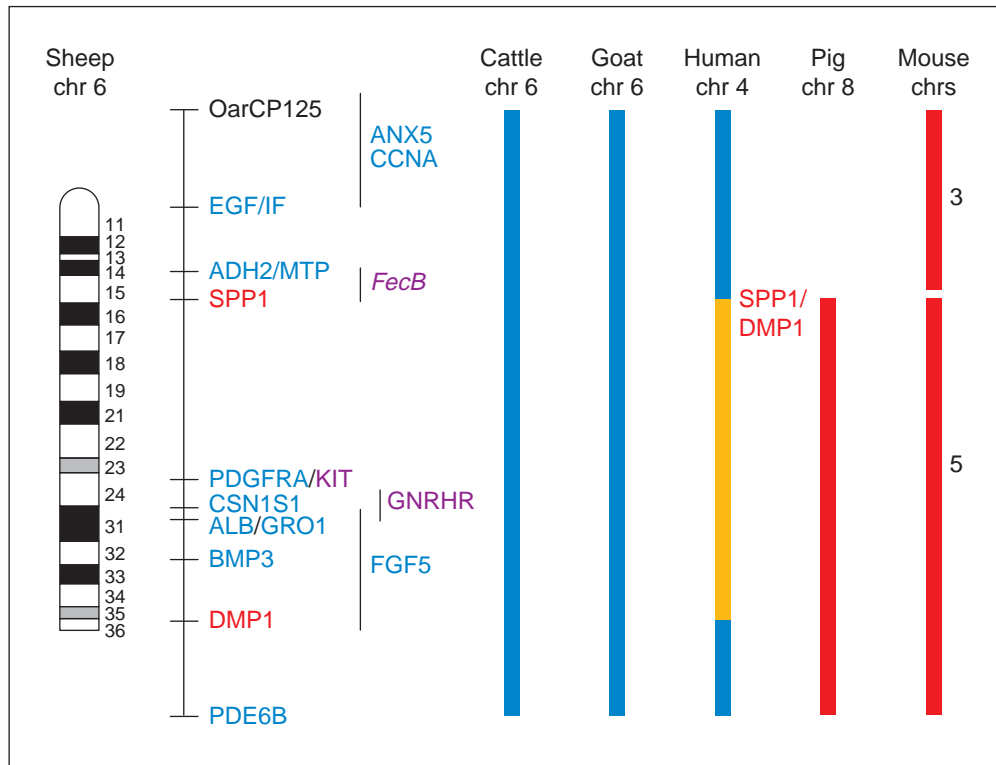
*Fluorescence in situ hybridization.* The use of fluorescence tags on the fragments or probes (fluorescence *in situ* hybridization; FISH) has greatly improved the speed and resolution of this method for mapping genes (Trask, 1991). Large cloned fragments provide the most suitable probes, but steps must be taken to mask repeated DNA sequences in the probe. The fluorescent tag is bound to the probe and detected with a sensitive ultraviolet microscope. Large cloned DNA fragments (cosmids) containing inhibin genes were used in FISH experiments to assign the  $\beta$ A-inhibin gene to cattle chromosome 4 (Neibergs *et al.*, 1993) and the  $\beta$ B-inhibin gene to sheep and cattle chromosome 2 (Goldammer *et al.*, 1995).

Linkage maps assemble markers into linkage groups. At least one marker from each group must be mapped by physical assignment to determine the chromosome corresponding to each linkage group. FISH analysis, using DNA sequences that contain framework markers from the linkage map, has been used to anchor the linkage and physical maps of bovine chromosomes (Hawkins *et al.*, 1995; Toldo *et al.*, 1993; Ferretti *et al.*, 1997).

*RH maps.* An alternative method of physical mapping is to assign genes to chromosome fragments contained in somatic cell hybrids created from fusing lymphocytes or fibroblasts from a target species with permanent cell lines. Somatic cell hybrids from cattle and sheep containing chromosomes or large chromosome fragments provided early assignments of genes to ruminant chromosomes (Womack and Moll, 1986; Saidi-Mehtar *et al.*, 1981).

Recently, somatic cell genetics has been greatly extended with radiation hybrid (RH) mapping (Cox *et al.*, 1990; Walter *et al.*, 1994). Hybrids are constructed from donor cells that have been lethally irradiated to cause fragmentation of the chromosomes. The dying cells are fused with recipient cells (such as hamster cells) and grown in a medium to select the hybrids. Hybrid cells will retain an unknown number of fragments as separate minichromosomes or recombined in the host chromosomes. The small fragments of donor chromosomes in RH cell lines provide high resolution maps. Most PCR-based markers can be successfully mapped on RH panels using rapid high throughput techniques (James *et al.*, 1994; Schuler *et al.*, 1996; Stewart *et al.*, 1997). Whole genome RH panels are valuable tools to map large numbers of genes and markers, and to generate dense maps of genes and marker maps supporting large scale sequencing projects (Hudson *et al.*, 1995; Deloukas *et al.*, 1998; Yang and Womack, 1998).

RH panels have been constructed for many livestock and other species (Womack *et al.*, 1997; Hawken *et al.*, 1999). Only limited success has been achieved in ordering genes on linkage maps of cattle and sheep (Fig. 2). RH maps have much better resolution (Fig. 3). Moreover, RH maps can integrate linkage and physical maps by mapping framework microsatellite markers on the same map together with many genes (Fig. 3). Consequently, dense RH maps determine the likely order of genes within segments of the linkage map, and provide valuable resources for gene discovery projects by creating links to the high-density human gene maps (Fig. 3).



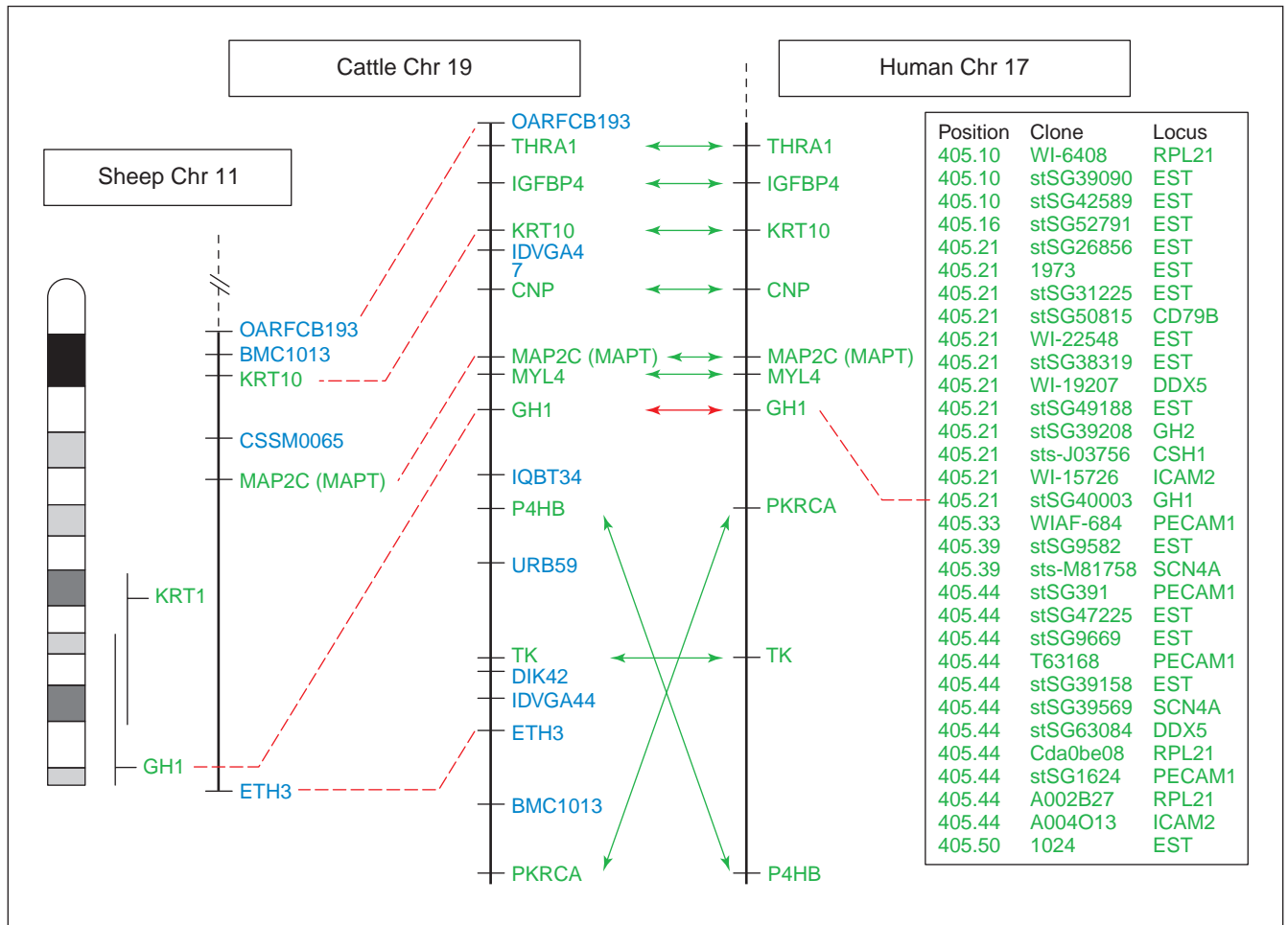
**Fig. 2.** A map of genes located on sheep 6 (redrawn from Lord *et al.*, 1996 and Lumsden *et al.*, 1999a) with a schematic representation of comparative gene orders on the equivalent cattle, goat, human, pig and mouse chromosomes (chr). The blue bars represent a similar gene order, the yellow bar represents an inversion of gene order and the red bars represent more complex rearrangements. Genes are shown in blue and those influencing reproduction are shown in purple. The genes *SPP1* and *DMP1* (shown in red) identify one breakpoint in the inversion in gene order between the sheep and human gene maps (see text). Symbols follow the human genome nomenclature and names for genes located on the map are: alcohol dehydrogenase beta polypeptide (*ADH2*), albumin (*ALB*), annexin V (endonexin II; *ANX5*), bone morphogenetic protein 3 (*BMP3*), cyclin A (*CCNA*), casein, alpha S1 (*CSN1S1*), dentin-specific acidic phosphoprotein (*DMP1*), epidermal growth factor (*EGF*), fibroblast growth factor 5 (*FGF5*), Fecundity Booroola (*FecB*), gonadotrophin-releasing hormone receptor (*GNRHR*), *GRO1* oncogene (*GRO1*), complement factor 1 (*IF*), Hardy-Zuckerman 4 feline sarcoma viral (*v-kit*) oncogene (*KIT*), microsomal triglyceride transfer protein (*MTP*), phosphodiesterase, cGMP (*rod receptor*), beta polypeptide (*PDE6B*), platelet derived growth factor receptor, alpha (*PDGFRA*), and secreted phosphoprotein 1 (*SPP1*). OarCP125 is a sheep microsatellite marker near the centromeric.

*Physical maps of overlapping clones.* High-resolution physical maps are produced from overlapping clones of large DNA fragments arrayed end to end to produce contiguous (contig) segments of cloned DNA. Contig maps that span whole chromosomes or large regions of the genome are developed from clone libraries with large DNA inserts. Yeast artificial chromosome (YAC) libraries were constructed for cattle (Libert *et al.*, 1993; Smith *et al.*, 1996; Takeda *et al.*, 1998; Hills *et al.*, 1999) and sheep (Broom and Hill, 1994). YACs can contain large DNA inserts of more than 1 mb and a small number of YACs can span a large genomic region. However, they are difficult to work with. DNA inserts in YAC clones are often deleted or rearranged through recombination in the yeast host. Bacterial artificial chromosomes (BACs) are an alternative, and BAC libraries have also been constructed for ruminants (Schibler *et al.*, 1998a; Vaiman *et al.*, 1999a; Zhu *et al.*, 1999). BACs hold smaller DNA inserts (100–300 kb), but are more stable and easier to work with.

YAC and BAC maps are being developed in sheep and cattle for several positional cloning projects, including the search for the goat polled-intersex syndrome (*PIS*) locus (Vaiman *et al.*, 1999b) and the Booroola fecundity (*FecB*) and Inverdale (*FecXI*) loci in sheep (Lumsden *et al.*, 1999b). Contig maps for human chromosomes are available (Hudson *et al.*, 1995) and provide a useful resource to determine overlapping human clones in target regions for positional cloning in livestock (Montgomery and Kinghorn 1997; Vaiman *et al.*, 1999b).

### Current ruminant gene maps

Genetic maps for ruminants are now in the second and third generation (Barendse *et al.*, 1997; Kappes *et al.*, 1997; de Gortari *et al.*, 1998; Schibler *et al.*, 1998b). In some species, there are several different maps and many individual gene assignments. This information is summarized and available through a number



**Fig. 3.** An example of use of comparative maps to identify genes close to the growth hormone locus in sheep. Parallel mapping of genes on cattle and human radiation hybrid maps connects linkage maps for ruminants to the high-density gene maps for the human. The growth hormone locus has been mapped to sheep chromosome (chr) 11, but is not located on the linkage map (<http://zaphod1.agresearch.cri.nz:8002/>). Common genes, including the growth hormone locus, map to cattle chromosome 19 and human chromosome 17 (Yang and Womack, 1998), but show chromosomal rearrangements between the species. The growth hormone locus maps close to the end of one conserved segment. Thirty genes from the dense radiation hybrid map for human chromosome 17 (<http://www.ncbi.nlm.nih.gov/genemap/map.cgi?CHR=17>) immediately adjacent to the growth hormone locus are shown. These would be expected to map close to the growth hormone locus in cattle, depending on the exact position of the evolutionary breakpoint.

of sites on the World Wide Web (Table 1). These sites comprise results from individual research programmes, such as the extensive dataset available at the US Meat Animal Research Center and summaries of mapping data in the individual species databases (Table 1). The Bioinformatics Group at the Roslin Institute developed software for the storage and graphical representation of genome maps (ARKdb). This is now used for all the livestock species. The editorial copy of the database for each species is updated at individual sites (Table 1) and distributed to a number of other sites to provide easy access to the data. Links to the range of different sites are included on many web pages. ARKdb currently holds both linkage and physical maps, together with bibliographic information. The databases are expanding to hold additional data, as it becomes available. A gene map has been developed for deer using an interspecies cross (Tate *et al.*, 1995) and has been made available through the Roslin web site.

Markers on the deer map have been assembled into linkage groups, but not yet assigned to the deer chromosomes.

### Assignment of genes influencing reproduction

The number of genes located on the different maps for ruminant species ranges from 164 in deer to 579 in cattle. The genes mapped include those for hormones, growth factors, receptors, binding proteins, transcription factors, and enzymes influencing the development and function of the reproductive axis (Table 2). Locations on the human map are known for most of these genes and assignments on the human and pig gene maps have been included for comparison (Table 2). Few genes are assigned to chromosomes in all species.

Genes coding for hormones were among the earliest assigned to the maps of ruminants. The prolactin gene was

**Table 1.** Mapping and comparative mapping sites for ruminants on the World Wide Web

Site	Species	Genome data	URL
Animal Genome Database in Japan	Cattle Sheep	Locus data Genetic maps Comparative data	<a href="http://ws4.niai.affrc.go.jp/">http://ws4.niai.affrc.go.jp/</a>
Cattle Genome Database	Cattle	Locus data Genetic maps Phenotypic traits	<a href="http://spinal.tag.csiro.au/cgd.html">http://spinal.tag.csiro.au/cgd.html</a>
Deer Gene Mapping Database, Roslin, Edinburgh	Deer	Locus data Genetic maps	<a href="http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/browsers/browser.sh?species=deer">http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/browsers/browser.sh?species=deer</a>
Livestock maps, INRA, France	Cattle Goat	Locus data Genetic maps Comparative data	<a href="http://locus.jouy.inra.fr/cgi-bin/bovmap/livestock.pl">http://locus.jouy.inra.fr/cgi-bin/bovmap/livestock.pl</a>
Meat Animal Research Center (MARC) USDA	Cattle Sheep	Locus data Genetic maps	<a href="http://sol.marc.usda.gov/">http://sol.marc.usda.gov/</a>
SheepBase, AgResearch, New Zealand	Sheep	Locus data Genetic maps	<a href="http://zaphod1.agresearch.cri.nz:8002/">http://zaphod1.agresearch.cri.nz:8002/</a>
US Bovine ARKdb	Cattle	Locus data Genetic maps	<a href="http://bos.cvm.tamu.edu/bovarkdb.html">http://bos.cvm.tamu.edu/bovarkdb.html</a>
Online Mendelian Inheritance in Animals (OMIA), Sydney	Cattle Goats Sheep	Phenotype data Genes	<a href="http://www.angis.su.oz.au/Databases/BIRX/omia/">http://www.angis.su.oz.au/Databases/BIRX/omia/</a>
MedVet Homepage	Cattle Sheep	Comparative maps	<a href="http://www.bioch.ox.ac.UK/~jhe/">http://www.bioch.ox.ac.UK/~jhe/</a>
National Center for Biotechnology Information	Human Other Species	Genetic maps DNA sequences Phenotype data	<a href="http://www.ncbi.nlm.nih.gov/">http://www.ncbi.nlm.nih.gov/</a>
Mouse Genome Informatics, Jackson Laboratory	Mice	Locus data Phenotype data Genetic maps Comparative data	<a href="http://www.informatics.jax.org/">http://www.informatics.jax.org/</a>
Online Mendelian Inheritance in Man (OMIM)	Human	Locus data Phenotype data	<a href="http://www3.ncbi.nlm.nih.gov/Omim/">http://www3.ncbi.nlm.nih.gov/Omim/</a>

mapped in somatic cell hybrids in 1988, establishing a segment of the bovine genome conserved with human chromosome 6 (Hallerman *et al.*, 1988). The beta subunit of follicle-stimulating hormone (*FSHB*) gene was assigned to bovine chromosome 15 by *in situ* hybridization the following year (Fries, 1989).

Gene locations provide information on the evolution of genes that may be useful for understanding relationships between structure and function. In some cases, hormones or receptors are located together on chromosomes and probably arose through gene duplications. For example, the LH and FSH receptors map close together in several species and probably originated from local gene duplication (Montgomery *et al.*, 1995a). This event was probably preceded by a duplication event that gave rise to the thyroid-stimulating hormone receptor and the ancestral gonadotrophin receptor.

A further example of gene duplication is the growth hormone (*GH*) locus in sheep and goats. In most mammals, a single gene codes for pituitary GH. Exceptions occur in humans, sheep and goats. In humans, a cluster of five genes on chromosome 17 codes for GH-like proteins, including placental

lactogens (Chen *et al.*, 1989). The *GH* locus is duplicated in sheep and goats (Valinsky *et al.*, 1990; Ofir and Gootwine, 1997; Wallis *et al.*, 1998) and the duplication exists as a polymorphism. Some individuals carry the single copy of the *GH* locus, while some carry the duplication. The reason why both alleles are retained in the population is not clear, but may relate to the expression of the two genes in different tissues or variation in physiological responses from the two alleles (Fleming *et al.*, 1997; Gootwine *et al.*, 1997; Ofir and Gootwine 1997).

### Comparative genomics

Map locations of genes are also useful for comparing the maps of different species. Since genes are conserved, they provide points of reference between the species. The number of genes on ruminant maps is limited, but map density is now sufficient to use maps from other species as a valuable source of information (Fig. 3). Comparisons of mammalian gene maps show large regions of conserved synteny where the same genes map to equivalent regions in different species (Chowdhary *et al.*,

**Table 2.** Chromosomal assignments for genes influencing reproduction in ruminants together with assignments for these genes in pigs and humans<sup>a</sup>

Gene symbol	Gene Name	Cattle	Deer <sup>b</sup>	Goat	Sheep	Pig	Human
ACR	Acrosin	5				5	22
ACVR2	Activin A receptor, type II	2					
AMH	Anti-Mullerian hormone	7				2	19
AR	Androgen receptor			X		X	X
CGA	Glycoprotein hormones, alpha polypeptide		LG28	9		1	6
CYP19	Cytochrome P450, subfamily XIX (aromatization of androgens)	10		10	7		15
CYP21	Cytochrome P450, subfamily XXI (steroid 21-hydroxylase)	23				7	6
EGF	Epidermal growth factor	6			6	8	4
ESR1	Oestrogen receptor 1	9			8	1	6
FGF2	Fibroblast growth factor 2	17			17	8	4
FSA	Follistatin		LG25		16	16	
FSHB	Follicle stimulating hormone, beta polypeptide	15	LG01	15	15	2	11
FSHR	Follicle stimulating hormone receptor		LG11		3	3	2
GDF-9	Growth differentiation factor 9				5		5
GH1	Growth hormone		LG05	19		12	17
GHR	Growth hormone receptor		LG25	20		16	5
GNRHR	Gonadotrophin-releasing hormone receptor			6	6	8	4
IGF1	Insulin-like growth factor I (somatomedin C)	5		5	3	5	12
IGF1R	Insulin-like growth factor I receptor	21	LG13		18	1	15
IGF2	Insulin-like growth factor II (somatomedin A)	29	LG02		21	2	11
IGF2R	Insulin-like growth factor II, receptor	9					6
IGFBP3	Insulin-like growth factor binding protein 3	4		4		18	7
INHA	Inhibin, alpha	2			2	15	2
INHBA	Inhibin, beta A (activin A, activin AB alpha polypeptide)	4	LG18		4	18	7
INHBB	Inhibin, beta B (activin AB beta polypeptide)	2	LG23		2	12	2
KIT	v-Kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	6	LG06		6	8	4
LEP	Leptin	4				18	7
LEPR	Leptin receptor	3					1
LHB	Luteinizing hormone, beta polypeptide	18	LG04	18	14	6	19

1996; Burkin *et al.*, 1997; Graves, 1998; Schibler *et al.*, 1998b; Womack, 1998). The maps for ruminants are highly conserved and, at current map densities, marker order is generally the same for most chromosomes. A small number of rearrangements have been documented among ruminant species (Crawford *et al.*, 1995; Schibler *et al.*, 1998b).

Comparison of ruminant maps with those of more distantly related species also show conservation of large segments. An analysis of approximately 250 genes, mapped in goats and humans, identified 103 evolutionary breakpoints between the maps, with an average size of 27 cM for the conserved segments (Schibler *et al.*, 1998b). More rearrangements were identified between ruminant and rodent chromosomes, with an average size of 8 cM for conserved segments (Schibler *et al.*, 1998b). The map densities and links between mammalian maps have developed to the point where the location of a gene in one species gives a strong indication of the location in other well-mapped species.

Large genome segments are conserved, but the order of genes within these segments may be rearranged. Numerous

examples of internal rearrangements have been documented. The genes located on cattle, goat and sheep chromosome 6 all map to human chromosome 4 (Fig. 2). However, gene order is not conserved. There is a large inversion in gene order between sheep chromosome 6 and human chromosome 4 over a portion of the chromosome (Lord *et al.*, 1996). The breakpoint for one end of the inversion has been mapped to a small region of 150 kb pairs between the genes for secreted phosphoprotein 1 (*SPP1*) and dentin-specific acidic phosphoprotein (*DMPI*) on human chromosome 4 (Lumsden *et al.*, 1999a). Genes from chromosome 6 in sheep also map to pig chromosome 8, but show further rearrangements in gene order (Johansson *et al.*, 1992; Ellegren *et al.*, 1993).

Mapping the same genes on RH maps for different species is a powerful method for comparison of gene location and order (Yang and Womack, 1998). Twenty-four genes were mapped to either cattle chromosome 19 or human chromosome 17 using parallel RH mapping (Fig. 3). Comparison revealed differences in gene order caused by chromosomal rearrangements in the

Table 2. continued

Gene symbol	Gene Name	Cattle	Deer <sup>b</sup>	Goat	Sheep	Pig	Human
LHCGR	Luteinizing hormone/choriogonadotropin receptor		LG11		3	3	2
MC1R	Melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)	18		18		6	16
MGF	Mast cell growth factor	5			3		12
MTNR1A	Melatonin receptor 1A	27			26	17	4
NPY	Neuropeptide Y			4			7
OSG	Oviduct specific glycoprotein	3					
OXT	Oxytocin, prepro- (neurophysin I)	13					20
PAG1B	Pregnancy-associated glycoprotein 1	29					
PAI1	Plasminogen activator inhibitor, type I	25					7
PL	Placental lactogen	23			20		
PMC	Pro-opiomelanocortin	11		11			2
PRL	Prolactin	23	LG07			7	6
PRLR	Prolactin receptor				16	16	5
PRP@	Prolactin-related protein, cluster	23					
PRP1	Prolactin-related protein 1	23					
PRP10	Prolactin-related protein 10	23					
PRP3	Prolactin-related protein 3	23					
PRP6	Prolactin-related protein 6	23					
SHR	Steroid binding protein	11					
SOX2	SRY (sex determining region Y)-box 2	1		1	1	13	3
SRN	Seminal ribonuclease	10					
SRY	Sex determining region Y	Y				Y	
TGFB1	Transforming growth factor, beta 1	5	LG04	11		6	19
TGFBR1	Transforming growth factor beta, receptor 1	8					
TSHB	Thyroid stimulating hormone, beta polypeptide	3	LG20	3	1	4	1
TSPY	Testis-specific protein, Y-encoded	Y		Y			Y
WT1	Wilms tumour 1	15	LG01	15	15	2	11
ZFY	Zinc finger protein, Y-linked			Y			Y

<sup>a</sup>Mapping data and references for individual loci are held in the relevant databases listed in Table 1.

<sup>b</sup>Genes on the deer map have only been assigned to linkage groups. The relationship between linkage groups and specific chromosomes remains to be determined.

two species. Comparative maps are a valuable source of information for functional studies, but it is essential to map the precise order of genes within chromosomal segments in regions of interest. Locating large numbers of genes on RH maps in many species will significantly improve map resolution for comparing gene orders within and between species.

Comparative map locations are an important consideration in extrapolating functional information from one species to another. Since many genes belong to gene families, positional information in the genome can provide evidence to help decide whether two genes in different species perform the same function (are true orthologs) or are different members of a related gene family. Comparison of maps from several species can detect errors in map locations. Comparative map data is available through several web sites (Table 1). The MedVet site in Oxford and Sydney provides tables and Oxford grids for comparisons with several species, including cattle and sheep. In addition to the mouse mapping data, the Mouse Genome Informatics web site (Table 1) holds extensive

comparative information. The homology database for comparative mapping in Japan uses comparative information to predict likely locations for a given locus. Mapping data are updated steadily and homology data are used in conjunction with the species-specific databases to obtain current information on individual loci.

### Mapping phenotypic traits

One important application for gene maps is the identification of genes and mutations responsible for physiological variation. These traits can show Mendelian segregation associated with a single gene of large effect, or complex inheritance with many genes interacting to produce variation in a quantitative trait. Genes or loci influencing traits that show continuous variation, such as hormone concentrations or birth weight, are known as quantitative trait loci (QTL). Both single gene effects and QTL can be mapped with the current density of genetic markers on ruminant maps.

**Table 3.** Reported locations for monogenic and quantitative trait loci affecting reproduction in ruminants

Trait	Locus	Species	Chromosome <sup>a</sup>				OMIA <sup>b</sup>	Reference
			C	G	P	S		
Ovulation rate	Fecundity Booroola	Sheep	6	6	8	<b>6</b>	000383	Montgomery <i>et al.</i> (1993)
Ovulation rate	Haemoglobin beta	Sheep	15	15	2	<b>15</b>		Glazko <i>et al.</i> (1997)
Ovulation rate/infertility	Fecundity Inverdale	Sheep	X	X	X	<b>X</b>	000386	Davis <i>et al.</i> (1992)
Infertility	Polled intersex syndrome	Goat	1	<b>1</b>	13	1	000483	Viaman <i>et al.</i> (1997a)
Testicular feminization	Androgen receptor	Cattle	X	X	X	X	000991	Peter <i>et al.</i> (1993)
Gonadal hypoplasia	SRY	Cattle	<b>Y</b>	Y	Y	Y	001230	Kawakura <i>et al.</i> (1997)
Ovulation rate	QTL	Cattle	<b>7</b>	7	2	5		Blattman <i>et al.</i> (1996)
Ovulation rate	QTL	Cattle	<b>23</b>	23	7	20		Blattman <i>et al.</i> (1996)

<sup>a</sup>Chromosome locations for the species in which the effect was reported (bold type) and equivalent regions in cattle (C), goats (G), pigs (P) and sheep (S).

<sup>b</sup>MIA entry number for the Online Mendelian Inheritance in Animals (<http://www.angis.su.oz.au/Databases/BIRX/omia/>).

Reproductive traits mapped in ruminants include loci for ovulation rate and infertility. Map locations and information on phenotype traits in animals are available in the database Online Mendelian Inheritance in Animals (OMIA, Table 1) and a subset of phenotypes assigned to chromosomal locations in livestock is listed (Table 3). Phenotypic effects in humans and mice associated with the genes listed (Table 2) can be found in the Online Mendelian Inheritance in Man (OMIM) and the Mouse Genome Informatics database (Table 1).

The goat polled-intersex syndrome (PIS) results in intersexuality associated with the absence of horns or the polled condition (Vaiman *et al.*, 1997a,b). The *PIS* gene maps to goat chromosome 1 (Vaiman *et al.*, 1997a). Genes that increase ovulation rate in sheep map to chromosomes 6 (*FecB*; Montgomery *et al.*, 1993) and X (*FecXI*; Davis *et al.*, 1991). Associations between genetic markers and ovulation rate have been reported in Cambridge sheep (Glazko *et al.*, 1997) and in cattle (Blattman *et al.*, 1996). The reported chromosomal locations for effects on ovulation rate in cattle are different from the locations of genes influencing ovulation rate in sheep (Davis *et al.*, 1991; Montgomery *et al.*, 1993; Blattman *et al.*, 1996).

The increase in ovulation rate in Booroola sheep may result from mutations in a number of genes. Map location provides a good test for whether a particular gene is directly implicated. Genes for gonadotrophin hormones and receptors mapped to different chromosomes and were excluded as candidates for the *FecB* locus (Montgomery *et al.*, 1990, 1992, 1993, 1995a; Penty *et al.*, 1995). Gonadotrophin-releasing hormone receptor (*GNRHR*, Montgomery *et al.*, 1995b) maps to the same chromosome as the *FecB* locus (Tables 2 and 3), but is not closely linked and was excluded as a candidate.

The *FecXI* locus in sheep increases ovulation rate in heterozygous females, but causes infertility in homozygous females (Davis *et al.*, 1992). The Cambridge sheep was selected from ewes with high litter size (Owen, 1996). Infertility has been reported in this strain (Owen, 1996), with the evidence of streak ovaries, similar to those found in ewes homozygous for the *FecXI* locus (Owen, 1996). Female mice with a deletion of *GDF9* (a member of the *TGF $\beta$*  superfamily and expressed only in the oocyte) develop infertility resulting from a block in follicular development at the primary follicle stage (Carabatsos *et al.*,

1998; Elvin and Matzuk, 1998). This infertility resembles the phenotype in carriers of the *FecXI* gene. *GDF9* maps to sheep chromosome 5 (M. Sadighi and S. M. Galloway, personal communication) and is therefore excluded as a candidate for the *FecXI* locus, which is X-linked. *GDF9* may be a candidate for the infertility seen in the Cambridge sheep. Comparative mapping predicts the location of *GDF9* on human chromosome 5, cattle chromosome 7 and pig chromosome 14. The human cDNA sequence for *GDF9* has not been reported, but a human genomic clone from chromosome 5q contains the complete genomic sequence for *GDF9*. This observation confirms the chromosome location of *GDF9* in humans.

Map locations can be used to compare the location of genes responsible for variation in litter size and fertility in pigs (Rothschild *et al.*, 1996; Lahbib-Mansais *et al.*, 1997; Rathje *et al.*, 1997; Rohrer, 1999; Wilkie *et al.*, 1999) with data for ruminants. Variation around the oestradiol receptor gene (*ESR1*) is associated with increased litter size in pigs (Rothschild *et al.*, 1996). This gene is located on sheep chromosome 8 and excluded as a candidate for loci reported to influence ovulation rate in sheep or cattle (Table 3).

Several studies provide evidence for genes affecting ovulation rate on pig chromosome 8 (Rathje *et al.*, 1997; Rohrer, 1999; Wilkie *et al.*, 1999). This region of the pig genome is syntenic with sheep chromosome 6 and the region containing the *FecB* locus (Fig 1). However, gene order is not conserved between pig chromosome 8 and sheep chromosome 6 (Fig. 1). It will be necessary to clone the genes or complete detailed comparative studies to decide whether the same gene is responsible for effects on ovulation rate in the two species.

Mutations in many of the genes listed (Table 3) are known to affect reproductive function in mice and humans. Mouse knockout studies provide valuable models for studies of ovarian function, particularly during the early stages of follicle development (Elvin and Matzuk, 1998). Mutations in gonadotrophin hormones, receptors and genes involved in steroid biosynthesis result in rare cases of reproductive dysfunction in women (Adashi and Hennebold, 1999; Layman, 1999). One question is whether minor changes in genes for gonadotrophins and their receptors alter gene expression or protein function resulting in variation in ovulation rate or fertility in

livestock. Mutations that result in truncated proteins caused by premature stop codons or exon splice variants are relatively easy to identify. Single base changes in gene control regions that alter gene expression are more difficult to distinguish from non-functional polymorphisms. Glazko *et al.* (1997) reported an association between ovulation rate and alleles at the haemoglobin (*HBB*) locus in Cambridge sheep. *HBB* maps close to the *FSHB* locus on sheep chromosome 15, indicating *FSHB* as a candidate for the observed variation in ovulation rate.

Despite some rearrangements in gene order, comparative maps provide valuable information for gene discovery. The *PIS* locus was mapped to goat chromosome 1 in a region syntenic with human chromosomes 3 and 21 (Vaiman *et al.*, 1997a). The critical region for the location of the *PIS* gene has been reduced to a 1 cM region of goat chromosome 1 (Vaiman *et al.*, 1999b). A human yeast artificial chromosome (*YAC*) contig spans the critical region for a human disease syndrome that includes dysplasia of the eyelids, low nasal bridge and female infertility or premature ovarian failure. The *YAC* contig mapped to goat chromosome 1q43 in the region of the *PIS* locus. The same gene (or genes) may be responsible for the observed infertility in both species (Vaiman *et al.*, 1999b).

### Future directions

The genes that influence reproduction and fertility represent an essential subset of genes since they are necessary for competent development of germ cells and transmission of the genetic blueprint to the next generation. Essential steps in pathways for successful reproduction remain to be determined. Until recently, mammals were thought to have a single *GNRH* gene, located on human chromosome 8. A second copy located on chromosome 20 has now been found in humans (White *et al.*, 1998). This second copy is highly expressed in tissue outside the brain. Inhibins play an important role in the regulation of FSH and there has been debate about the signalling pathway for the inhibins. Strong evidence for specific inhibin receptors has been reported (Hertan *et al.*, 1999).

Genome analysis provides the opportunity to identify key genes more rapidly. Large numbers of partial cDNA clones have been sequenced (expressed sequence tags, ESTs) and are being mapped on radiation hybrid maps for different species. This development will greatly improve the maps and our ability to translate between them. However, genes expressed specifically during different stages of development in tissues such as the ovary, testis and pituitary gland, or in specialized cells such as germ cells, are under-represented in collections of EST sequences and on current maps. Sequencing of 82 genes from the bovine ovary cDNA library generated 11 novel sequences with no significant matches in the sequence databases (Ma *et al.*, 1998). These genes mapped to ten bovine chromosomes. Genes expressed by oocytes play a critical role in synchronizing events in both germ and somatic cells during ovarian follicle development (Elvin and Matzuk, 1998). A method has been devised to use a transgenic approach in mice to separate germ cells at different stages of development for the construction of cDNA libraries (Abe *et al.*, 1998). This resource is being used to sequence novel genes and identify genes that are differentially expressed during development.

The genomic sequence for most human genes will be available in the near future. Defining a function for all the genes will be a major challenge that will require a range of approaches (Clark, 1999). Ruminants have an important contribution to make towards understanding some of the key events that regulate development and function of the reproductive axis. Selection lines and breeds with differences in ovulation rate, fertility and patterns of seasonal breeding provide valuable models for studies of the reproductive axis, although potentially valuable selection lines have been lost or threatened because of funding cuts in a number of countries.

Cloning the genes responsible for the genetic differences in fertility and fecundity is likely to provide valuable clues to the understanding of ovarian function and germ cell development. Genetic maps for ruminants need to be improved and the numbers of genes on the maps increased to make full use of the major genome projects, particularly those in humans and mice. It is possible to map many of the genes responsible for variation in quantitative traits, but identifying the causal mutations will be difficult. Novel genes and gene products will provide new reagents for the development of diagnostic tests, and new targets for contraception and the treatment of infertility.

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