Construction of a bovine gene map

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ABSTRACT

Gene maps of livestock will play an important role in the development of new animal breeding technologies based on molecular techniques.

The current status of the bovine gene map is reviewed and various methods of mapping genes are discussed. The task of constructing the bovine gene map will be aided by the conservation of chromosomal segments amongst mammalian species. In particular, the detailed maps of man and the mouse can be used to rapidly establish a provisional, but extensive bovine gene map.

Keywords Gene mapping; reverse genetics; linkage; cattle

INTRODUCTION

Until recently, gene mapping in domestic animals has received little attention. However with the recent advent of recombinant DNA techniques and subsequent explosion of activity in the human gene mapping field, the feasibility of rapidly mapping genes and the power of the information has become apparent. Consequently there is now considerable interest and activity in the development of gene maps for livestock.

WHAT IS A GENE MAP?

A gene map comprises information on the location of genes and genetic markers with respect to each other. (In this context, a genetic marker is defined by a non-coding DNA sequence). The bovine genome is made up of 29 autosomes and two sex chromosomes. Thus determination of gene order and the genetic/physical distance between genes and genetic markers is the objective of gene mapping. In a broader sense however, gene mapping includes the isolation of individual genes once closely linked genetic markers are found.

There are more than 100 000 genes within the mammalian genome. Clearly it will be some time before a gene map includes even a moderate proportion of all genes. However as will be explained later, the ease of mapping new genes increases rapidly as the density of the map rises. The immediate task confronting bovine gene mapping is to construct a primary map comprising several hundred genes or genetic markers.

USES OF GENE MAPS

Once genes are mapped, diagnostic tests can be developed and/or genes can be modified using genetic engineering technologies. However the principal use of a gene map is for the identification and subsequent mapping of new genes. Genes already on the map are used to follow segregation of specific chromosomal regions within families in which the gene of interest is also segregating. This method of identifying genes is known as reverse genetics (Ruddle, 1984) since it leads to the cloning of genes followed by determination of the primary gene product, i.e. the reverse of the classical method. Reverse genetics will play an important role in understanding the genetic basis of traits which animal breeders have until now been attempting to manipulate at the phenotypic i.e. observable, level. It will be especially appropriate because, for the vast proportion of genes which control traits such as growth, reproduction, disease/parasite resistance, carcass quality and fibre quality, the primary gene products are unknown. Reverse genetics, discussed in detail by Hetzel and Fries (1988), relies on a large number of chromosomal markers, defined by genes or

genetic markers. Thus the construction of at least primary gene maps is of high priority since it will lead to the identification of a large number of genes potentially useful for animal breeders.

CURRENT STATUS OF GENE MAPS

TABLE 1 Status of the genetic maps of man, cattle, sheep, pigs and the horse in 1984 and 1987.

Species	Haploid chromosome	Numer of mapped genes 1984	1987
Man	23	824	3500
Cattle	30	25	55
Sheep	27	14	26
Pig	19	30	43
Horse	32	11	21

Sources: O'Brien (1987); Human Gene Mapping Workshop 9, 1988.

The current status of genetic maps of the major domestic livestock species is poor (Table 1). In 1987, while over 3500 genes and markers had been mapped in humans, the genetic maps of livestock consisted of less than 60 genes. Furthermore, whilst in man, the majority of genes had been mapped to a specific chromosomal region, livestock maps largely comprised unassigned syntenic groups.

In a very recent review, Fries *et al.* (1989) have summarised the current status of the bovine map (Table 2). Gene loci have been assigned to six chromosomes and there are 26 autosomal synteny groups established. Thus a minimum of three bovine chromosomes are unmarked by at least one gene. This situation will improve in the near future.

GENE MAPPING METHODS

There are basically three methods for producing genetic maps. Firstly, linkage mapping estimates the genetic distance between loci in terms of meiotic recombination units. Secondly, physical mapping defines the chromosomal localisation of genes/markers with respect to standard cytogenetic karyotypes. Although the resolution from linkage mapping is greater, physical mapping

TABLE 2 Synteny groups and chromosomal assignments in cattle.

Synteny group[1]	Gene symbol	Position[2]
U1	ENO1	U1
	PGD	U1
U2	ME1	U2
	PGM3	U2
	SOD2	U2
U3	GAPD	U3 (19)
	IFNG	U3 (19)
	KRTA	19q14-q23 (U3)
	LDHB	U3 (19)
	PEPB	U3 (19)
	TPI1	U3 (19)
U4	MPI	U4
U5	NP	U5
	PK3	U5
U6	PGM1	U6
U7	LDHA	U7
U8	MDH2	U8
U9	DIA4	U9
	GPI	U9
U10	CRYA1	U10 (13)
	IFNAR	U10 (13)
	PAIS	U10 (13)
	SOD1	U10 (13)
	SST	U10 (13)
U11	ADA	U11
	ITPA	U11
U12	ACY1	U12
U13	KRTB	5q12-q23 (U13)
	PEPC	U13 (5)
U14	GSR	U14
U15	PEPS	U15
	PGM2	U15
U16	AK1	U16
U17	CRYG	U17
	FN1	U17
	IDH1	U17
U18	ACO1	U18
	IFF	U18
	IFL	U18
U19	GUK	U19
U20	CAT	U20
U21	BOLAA	23q21-qter (U21);
	BOLAD	23q21-qter (U21);
	C4	U21 (23)
	CYP21	U21 (23)
	GLO1	U21 (23)
	M	U21 (23)
	PRL	U21 (23)
	TCP1	U21 (23)
U22	GDH	U22
U23	A	U23 (15)
	FSHB	15q22-qter (U23)
	HBB	15q21-qter (U23)
	PTH	15q21-qter (U23);
U24	GHC	U24
U25	TK1	U25
	UMPH2	U25
U26	RHO	U26
X	G6PD	X
	GLA	X
	HPRT	X
	PGK1	X
Y	DYZB	Y

[1] U1-U26: Unassigned synteny groups; 5, 13, 14, 15, 19, 23, X, Y refer to chromosome number

[2] Numbers refer to chromosomal assignments

Source: Fries *et al.* (1989).

is sometimes faster and provides chromosomal landmarks from which genetic distances can be estimated. Physical and genetic distances will only differ significantly where recombination hotspots or suppressors are present. The third method, known as comparative mapping, allows provisional mapping based on evolutionary conservation of genes.

Linkage Mapping

Linkage is determined by measuring the meiotic recombination frequency in informative families. The recombination fraction (r) giving the highest lod (logarithm of the odds) score is taken as the maximum likelihood estimate. If any one of the genes in a syntenic linkage group has been assigned to a chromosome or chromosomal region the whole group can be assigned. Since loose genetic linkage, e.g. r greater than 0.4, can only be measured with large numbers of meioses (more than 250), in practice linkage mapping is most useful over shorter genetic distances.

When linkage between more than two loci is to be estimated, gene order as well as recombination rates need to be estimated. As the number of loci increase, the number of possible gene orders increases rapidly. Various computation strategies and algorithms have been developed for analysing human data (Lalouel *et al.* 1986) and with some adaptation will be useful for similar analyses in animals.

Physical Mapping

Physical assignment to chromosomes can be accomplished using either a somatic cell hybrid panel or *in situ* hybridisation. A hybrid panel consists of a number of stable hybrid cell lines, each containing a small number (e.g. 1-5) chromosomes from the species of interest. The panel is produced by first fusing cells of one species, e.g. bovine, with cells of a rodent species, e.g. mouse or hamster. During subsequent culture, there is preferential and random loss of the non-rodent chromosomes. Eventually the hybrid cells stabilise and clones containing only a few non-rodent chromosomes can be selected to make up a panel. By screening such a panel with a gene probe or marker sequence, rapid chromosomal assignment is possible. Human panels which also contain known chromosome fragments have also been constructed, thereby allowing assignment to a specific chromosomal region. The development of somatic cell hybrid panels for our livestock species is essential for future gene mapping studies because once constructed, rapid chromosomal assignments are possible. However past studies have been hampered by the difficulty in chromosome identification in species such as cattle and sheep where most of the chromosomes are morphologically similar. Improved banding techniques and chromosome specific DNA probes have recently facilitated this task and new hybrid panels are now under development in most species.

Chromosomal assignment to a particular region can be achieved by *in situ* hybridisation. This technique involves the hybridisation of labelled DNA probes to fixed metaphase chromosomes and the subsequent visualisation of the signal as silver grains after autoradiography. Single copy genes can be mapped with a resolution of 5-10 cM. The use of this technique in animals has also been hampered by the requirement for unequivocal identification of the chromosomes. However, given improved banding techniques and the imminent development of satisfactory non-radioactive labels, *in situ* hybridisation will become more widely used. The physical assignment of at least 2 genes/markers per chromosome is required to provide landmarks from which more detailed genetic maps can be developed.

Comparative Mapping

It is clearly established that during the course of mammalian evolution, certain chromosomal segments have been conserved. Thus within these segments, the proximity of genes in different mammalian species is the same, although in some cases, gene order may be altered. For example, the total number of conserved segments for man and mouse is now 68 with an average length of 10 cM in linkage groups on the mouse map (Nadeau,

1989). In a mapping study of 32 bovine genes which had previously been mapped in man and mouse, Womack and Moll (1986) found the conservation of cattle and human chromosomes to be even more extensive than of man and mouse. Therefore, an important strategy in bovine mapping will be to identify conserved chromosomal segments with either man or the mouse, and to consequently predict the location of genes in cattle, based on relative position in the other two species.

Further extrapolation of the bovine map to the ovine (sheep) map appears to be likely because of the extremely high chromsome banding homology. In an elegant study, Hediger (1988) was able to almost perfectly match up sheep and cattle chromosomes using identical G-banding patterns. If this level of homology holds up at the gene level, the sheep map can be predicted from the cattle map and vice versa.

THE FUTURE

Techniques for mapping genes have improved dramatically with the advent of recombinant DNA methods. The next generation of animal breeding technologies will follow from an understanding at the molecular level of the genetic basis of traits important in animal production. Gene maps for use in analysing genetic variation are an important part of the necessary research effort. Accordingly there must be a concerted effort to develop a bovine gene map as well as further refinement of the techniques for using them.

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