Abstract
The organisation of the domestic bovid genomes is very similar, thus ovine and caprine genome mapping may benefit from the most advanced bovine map. At present, there have been identified over 2000 markers in the sheep and over 700 in the goat. Recently, several spectacular examples of the genome map application for the identification of loci controlling important traits have been reported, for instance: muscular hypertrophy and high fecundity in the sheep, and polled intersex syndrome in the goat. Searching for genes controlling other traits (resistance to gastro-intestinal parasites, wool quality, milk productivity, etc.) is performed with the use of the genome scanning approach, which is based on advanced genome maps. Also other molecular tools, such as species-specific sheep and goat genome libraries cloned in bacterial artificial chromosomes (BACs) and cDNA libraries, bring an opportunity for a detailed study of the sheep and goat study. In the near future another important tool – the cattle genome sequence – will also be available.

Key Words: sheep, goat, genome map, molecular tools

Zusammenfassung
Titel der Arbeit: Genomkartierung für Schaf und Ziege

Schlüsselwörter: Schaf, Ziege, Genomkartierung, molekulare Methoden
derived from one species, and chromosomes or chromosome fragments of the compared species. Using this technique the sheep was compared with the human, cattle and pig genomes. The use of human chromosome specific probes on sheep chromosome preparations facilitated the identification of 48 homeologous (versus human) segments (IANNUZZI et al., 1999). Also sheep chromosome specific probes were used to compare the ovine genome with the bovine and porcine ones (FRONICKE and WIENBERG, 2001). This study revealed that sheep probes, representing one-armed chromosomes, painted entire cattle chromosomes, whilst the probes for the ovine bi-armed chromosomes painted six pairs of one-armed cattle chromosomes. This result was concordant with earlier data originating from banding studies. However, an exception was noticed. The probe for OAR9 painted BTA14 and in addition a segment, localised close to the centromere, of the BTA9. The remaining part of BTA9 was painted by a probe for OAR8. This observation indicates that during evolution a reciprocal translocation between OAR8 and OAR9 took place. There are no such differences while comparing almost identical cattle and goat karyotypes – both species have the same number of 29 one-armed autosomes with similar banding patterns. Minor differences concern the morphology of the X chromosome, which is submetacentric in cattle and acrocentric in the sheep and goat. Detailed cytogenetic studies, performed with the use of the bovine painting probe for a short arm of the X chromosome (Xp), demonstrated the presence of an interstitial homologous segment in the sheep and goat long arm (Xq). It was hypothesised that it was due to chromosome inversion (PONCE DE LEON et al., 1996).

Extensive studies on karyotype homology revealed, already a long time ago, that the similarity of chromosome banding patterns between cattle and sheep reflects the similarity of gene contents (HEDIGER et al., 1991). In the last decade of the 20th century the localisation of polymorphic microsatellite markers became the main approach towards establishing advanced marker genome maps. These type II markers have appeared to be very useful for linkage mapping. One could expect that the cattle-derived markers will be simultaneously used for sheep and goat genomes mapping. An extensive study on the use of the bovine-derived PCR primers for the amplification of DNA fragments harbouring microsatellites, was performed by DE GORTARI et al. (1997). The authors found that 58% of the bovine primers were useful for the amplification of homeologous loci in sheep. Moreover, only 67% of the amplified fragments were polymorphic. These observations showed that extensive mapping of the ovine genome would also require a species-specific source of markers.

An important tool facilitating the identification of nucleotide sequences of interest (microsatellites, genes, etc.) are genomic libraries. The most commonly used are libraries constructed with the use of vectors called Bacterial Artificial Chromosomes (BACs). Such libraries were established for the sheep (VAIMANN et al., 1999), as well as the goat (SCHIBLER et al., 1998a). These libraries are also a source of probes used in cytogenetic mapping by FISH.

The latest linkage genome map of the sheep genome was published by MADDOX et al. (2001). This map includes 1062 loci and among them there are 935 anonymous loci (mainly microsatellite markers – 890), 121 genes and 6 ESTs (expressed sequence tags). A majority of microsatellites were bovine-derived (63%) and sheep-derived (32%). Only 5% of them were of goat origin. The total length of the map exceeded 3600 cM (sex-average) and the mean distance between neighbour loci was 3.4 cM. It
should be pointed out that the length of the ovine genome was significantly larger than the bovine one. There is also another surprising observation which concerns differences between the so-called male (estimated on crossing over events in spermatogenesis) and female genome lengths. In a majority of mammalian species, including cattle, the female map is larger than the male one. In case of the sheep the situation is reversed and the estimated length of the female map was 3374 cM, while the male one – 3932 cM.

Knowledge about the goat linkage genome map is less advanced. The latest caprine map was presented by SCHIBLER et al. (1998b). The authors mapped 307 loci, which covered 2737 cM. Since the coverage of the genome was 88%, thus an extrapolated total genome length was 3100 cM. An average distance between neighbour loci was 10 cM.

To construct a cytogenetic map two main approaches are applied: FISH and somatic cell hybridisation, including radiation hybrid mapping. The first technique facilitates a precise localisation of a locus. The other identifies loci assigned to the same chromosome (classical somatic cell hybridisation) or chromosome fragment (radiation hybrid map). The localisation of syntenic groups is carried out by FISH of a chosen locus belonging to this group. Recently, several such reports on FISH mapping of microsatellite markers and genes in the sheep and goat genomes were published. TABELT-AOUL et al. (2000) anchored thirty three ovine BAC clones containing microsatellite markers, previously assigned to sheep chromosomes by the linkage and somatic cell hybridisation approaches, to specific sheep and goat chromosomes. DI MEO et al. (2002) used twenty eight bovine cosmids, representing nineteen bovine syntenic groups, to localise them on sheep chromosomes. In another report, 31 bovine BAC clones, harbouring type I markers (genes), were assigned to the sheep and goat chromosomes (DI MEO et al., 2003). Also caprine BAC library was applied to develop a cytogenetic map of the sheep genome. Using the goat BACs, IANNUZZI et al. (2003) mapped sixty loci (five genes and 55 microsatellites), previously localised in the goat genome, onto the sheep chromosomes. It should be emphasised that all the above mentioned loci were localized on homeologous chromosomes and chromosome fragments, which were earlier recognised by the chromosome banding and comparative chromosome painting approaches applied to cattle, the sheep and the goat. These results show that genomic libraries, developed for these species, are very helpful in cytogenetic mapping of all the three species. However, one should remember that the physical localisation of a probe, derived from one species and containing a polymorphic marker (microsatellite), does not mean that in another species this marker is also polymorphic or is present at all. In case of BAC vectors, a cloned insert is quite large (50 – 200 kb), but the microsatellite marker is very small (approx. 50 - 100 bp). Thus, even a lack of the microsatellite in a target chromosome does not inhibit efficient cross-species hybridisation, if the flanking sequences in the probe recognise a homeologous sequence. Since many of the FISH mapped loci are included in syntenic groups, the total number of loci assigned to specific chromosomes is larger: 622 in the goat (http://locus.jouy.inra.fr/) and 512 in the sheep (http://www.thearkdb.org/). Moreover, databases for the sheep and goat genomes contain many more markers – 2030 (sheep) and 731 (goat). It is foreseen that further progress in genome mapping will be achieved when the radiation hybrid mapping will
be employed. Such an approach is currently developed for the sheep (COCKETT, 2003).

Establishing of the marker genome maps is a crucial step towards the identification of genes important from the breeding point of view. Among them quantitative trait loci (QTLs), which significantly influence the variability of quantitative traits, and genes responsible for genetic diseases or resistance to specific pathogens are the most important. Recently several spectacular achievements were reported, concerning the characterisation of the molecular background of three traits: fecundity (Fec) and muscular hypertrophy (CLPG) in sheep, and polled intersex syndrome (PIS) in the goat. Increased ovulation rate in highly prolific Booroola Merino ewes is caused by a nucleotide substitution A>G which changes a single amino acid (glutamine > arginine) in the bone morphogenetic protein receptor –IB gene (MULSANT et al., 2001). Two other mutations occurred in the non-coding sequences, which influence the expression of the genes located in the close vicinity. Muscular hypertrophy is also caused by the substitution (A>G) in a long-range control element (LRCE), which is located close to four imprinted loci (FREKING et al. 2002). Two of them are paternally (DLK1 and PEG11), and two (GTL2 and MEG8) are maternally expressed. The inheritance of the hypertrophic and normal phenotypes is unusual, because only the paternally inherited CLPG allele in a heterozygous genotype is expressed and then muscular hypertrophy is developed. This type of inheritance is called polar overdominance. The molecular background of the observed phenotypes and genotypes seems to be complex. It was proposed that there is a cis effect between the LRCE and imprinted genes, as well as a reciprocal trans effect between polypeptides encoded by the imprinted genes and the genes themselves (GEORGES et al., 2003). Also in case of the PIS locus the causative mutation, responsible for the intersexual development of XX sex-reversed goats, occurred in the non-coding sequence. It was found that the deletion of a 11.700 bp-long sequence affects the expression of two loci (PISRT1 and FOXL2) involved in the differentiation of foetal gonads (PAILHOUX et al., 2001).

An important approach towards the identification of QTLs is the identification and localisation of the Expressed Sequence Tags (ESTs). These sequences (cDNA), obtained by reverse transcription on an isolated mRNA, represent genes, which are expressed in a studied tissue. Among such genes potential QTLs might be present. LE PROVOST et al. (2000) mapped twenty five ESTs derived from the goat mammary gland and found that six of them were localised within the chromosome region, which was indicated in cattle as harbouring QTLs for milk traits. Intensive studies have been undertaken to characterise 2939 sheep ESTs, obtained from skin and wool (ADELSON et al., 2004). Another approach was adopted by DESILVA et al. (2003), who looked for microsatellite markers in 65.000 sheep skin cDNA clones and identified 251 ESTs containing such markers. Twenty seven of them were included in the linkage mapping study and their localisation in the sheep genome map was given. These markers are bi-potential (type I and type II) and thus may play a very important role in further studies on the identification of QTLs influencing wool traits. It can be foreseen that the identification of such markers in ESTs from the mammary gland will stimulate efforts towards the identification of QTLs for milk traits in bovids.

In sheep breeding an important issue is resistance to gastro-intestinal parasites. Searching for genes responsible for the resistance/susceptibility is carried out with the
use of the marker genome map and some chromosomal regions were indicated to harbour QTLs for the resistance (for review see: CHARON, 2004). This review shows that new molecular techniques have been successfully applied to study the ovine and caprine genome organisation and marker maps. Rapid progress of these maps has been based on the well developed bovine map. As it was shown, the similarity between the bovid genomes facilitates the use of probes (cytogenetic mapping by FISH) and primer sequences (physical mapping with the use of the somatic hybridisation technique and linkage mapping in reference families) for the mapping of the sheep and goat genomes. The next step – the cattle genome sequencing has already been started and should be completed in the near future (LEWIN, 2003). This achievement will be another milestone towards understanding cattle, as well as sheep and goat genomes.

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