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Genetics – Short papers

CONSTRUCTION OF AN INTEGRATED GENETIC AND CYTOGENETIC MAP IN THE RABBIT

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ABSTRACT

Rabbit is an important species for meat and fur in French agriculture as well as a valuable animal model for biomedical research. Since genomic data are still rudimentary in this species, the French National Institute for Agronomic Research (INRA) has launched in 2001 a project to map the rabbit genome. The aim is to produce a genetic map with microsatellite markers distributed every 10 to 20 cM along the genome and simultaneously to establish the corresponding cytogenetic map to provide the chromosomal position of all the genetic markers. These data will constitute an integrated genetic and cytogenetic map of the rabbit, which should be available in 2004. As demonstrated in other domestic species, this first generation map should help to identify economic traits and contribute to further applications such as marker-assisted selection.

Key words : genetic map, microsatellite, genome, cytogenetic map.

INTRODUCTION

The rabbit (*Oryctolagus cuniculus*) is a species with a double status: production and laboratory animal. Rabbits are bred for meat, and at a smaller scale, for fur and wool. As a laboratory animal, it is used for the production of antibodies but also as model for certain human pathologies, such as atherosclerosis and hypercholesterolemia (MORTENSEN *et al.,* 1994) or carcinogenesis associated with papillomavirus infection (BREITBURD *et al*., 1997). Its prolificacy and its small size make it a useful alternative to mouse or rat, in certain cases. Rabbit is also a choice animal for transgenesis (FAN *et al*., 1999) and cloning experiments (CHESNÉ *et al*., 2002) and it has a good potential as bioreactor for the production of therapeutic recombinant proteins in large quantities (Fan *et al*., 1999).

Rabbit genomic data are still limited. Until recently, the only mapping data available consisted of a genetic map containing 39 loci (FOX *et al*., 1994) and in 55 genes precisely localized on the chromosomes (ZIJLSTRA *et al*., 2002, HAYES *et al*., 2003). There is a clear need for the development of molecular tools in this species for fundamental research as well as for agronomics, in view of pathology problems occurring in rabbit farms and quality problems of products. The INRA department of animal genetics has invested massively, for the last ten years, in the construction of genetic maps for different domestic species such as the pig, the cow and the chicken, in order to localize traits with economic interest and to develop marker-assisted selection. Recent examples illustrate the efficiency of these approaches, such as the identification of the RN gene involved in pig meat quality (MILAN *et al*., 2000). Rabbit has recently been included in this research effort and an integrated genetic and cytogenetic map is currently under construction. Potential use of a genetic map for meat rabbit breeding was discussed by MULSANT AND ROCHAMBEAU (1996). The original feature of this project is the direct construction of an integrated map, the cytogenetic results providing accurate comparative mapping data with man. Indeed, in bovine and porcine species, cytogenetic and genetic maps were built separately and then integrated.

Our work on rabbit genome mapping has started in 2001 and includes the construction of reference families and the production of microsatellite makers. It has been decided to construct a map with markers every 10 to 20 cM. It is generally considered that the size of the rabbit genome is around 3000 cM therefore our aim is to identify 150 to 300 informative markers in our reference families. The genetic map will be constructed after genotyping the animals and studying the segregation of the markers in these families. The cytogenetic localization of the markers will be done by fluorescent in situ hybridization on chromosomes using rabbit genomic DNA BAC clones. A first generation map should be available by the end of 2004.

MATERIALS AND METHODS

Reference families

Several genes controlling hair structure (rex and angora genes) and color segregate in the families. Three INRA rabbit strains, bred at the Magneraud experimental farm, were used: strain 2066, (RL/RL) wild type for both rex and angora loci, the Orylag® strain, (rL/rL) with a mutation in the rex locus and a new strain, (rl/rl) with mutations at both loci. Among a total of 980 rabbits, 187 were selected to constitute 8 reference families consisting in 20 F0, 16 F1, and 151 F2 individuals.

Gene selection and screening of a rabbit BAC library

Genes were selected for their regular distribution on the human genome, every 10 cM, using public databases (http://www3.ncbi.nlm.nih.gov/Entrez/index.html). Primers were

designed specifically to amplify each gene, either from rabbit cDNAs when available or from other mammalian cDNAs. cDNAs and the corresponding human sequences were aligned to determine the exon-intron structure using the BLAST software through the Iccare tool (http://genopole.toulouse.inra.fr/Iccare/). Primers were designed with the Primer 3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). A library of large rabbit genomic DNA fragments cloned in bacterial artificial chromosomes (BAC) constructed by Rogel-Gaillard and colleagues (2001) was screened for each selected gene, either by PCR, or by hybridization of high density filters.

Isolation of microsatellite sequences from BAC clones

BAC clones have a standard size of about 110,000 base pairs. Since microsatellite sequences are generally regularly distributed in mammals, about every 20 to 50,000 base pairs, we expected each clone to contain between 1 to 2 microsatellite sequences. Microsatellite sequences corresponding to tandem short motif repeats, i.e. (TG)n and (TC)n motifs are the most easily identified. A microsatellite sequence is defined by the nature of the repeat and by the adjacent genomic sequences in which primers are chosen for PCR amplification. Microsatellite polymorphism depends on the number of repeats at a given locus. This polymorphism is revealed by PCR with primers designed in the flanking genomic sequences. To identify the microsatellite sequences, purified BAC DNA is cut into smaller fragments, which are sub-cloned in a plasmid vector. BAC sub-clones containing microsatellite sequences are identified by hybridization with a probe corresponding to a $(TC)_{12}/(TG)_{12}$ mix and sequenced.

Fluorescence in situ hybridization on chromosomes

BAC DNA is extracted, purified and labeled by nicktranslation with biotin-dATP. After hybridization to rabbit metaphase chromosome spreads, biotinylated probes are revealed by immunodetection with two successive antibodies, the latter coupled with fluorescein. Slides are stained with propidium iodide, mounted in PPD at pH11 to reveal R-banding patterns and observed with an epifluorescence microscope. The position of the probe on rabbit chromosomes gives the cytogenetic localization of the gene contained in the BAC clone and subsequently of the associated microsatellite.

Genotyping animals

Animals are genotyped by fluorescence for each marker. The results, obtained after PCR migration in an automatic sequencer (ABI PRISM 377), are interpreted with the Genotyper software. The genetic map will be constructed with the CriMap software.

RESULTS AND DISCUSSION

Reasoned distribution of anchoring genes on the rabbit genome

The global strategy used to build an integrated genetic and cytogenetic map in rabbit was based on an initial reasoned choice of well-distributed genes on the rabbit genome. BAC clones were then identified for each gene, mapped by FISH to rabbit chromosomes and used to isolate microsatellite makers. This strategy is made possible by the availability of the following tools and data: a rabbit genomic BAC library (ROGEL-GAILLARD *et al.*, 2001), bi-directional correspondences between human and rabbit chromosomes (KORSTANJE *et al.*, 1999), many partial gene and cDNA sequences in rabbit and the complete human genome. Rabbit BAC clones were identified for 310 genes.

Progress in the cytogenetic map

To date, 178 new localizations were obtained, of which 147 are in agreement with the human-rabbit comparative data, 6 concern microsatellites and 25 are gene localizations at unexpected positions. Further studies are necessary to determine whether these unexpected gene localizations reveal chromosomal rearrangements undetectable at the level of resolution obtained with chromosome painting. The distribution of the genes mapped is quite homogeneous with 1 to 17 genes positioned per chromosome depending on the chromosome size. The last few gaps should be completed with a new series of genes in progress. The remaining questions concern the p arm of chromosomes 15 and 20 for which no correspondence with the human chromosomes is available. However, it is expected that, since the chosen genes are regularly distributed on the human genome, some genes will map to these regions.

Progress in the genetic map

To isolate microsatellites, we sub-cloned one BAC per gene. A first series of 188 BAC clones produced 161 microsatellites and a second series of 200 sub-clones are being sequenced. Surprisingly, among these 161 microsatellites, 50% were (TC)n repeats and 50% (TG)n repeats. In mammals, it is more frequent to observe 10% of (TC)n and 90% of (TG)n. In order to diversify the origin of the microsatellites i.e. not only from gene-rich regions, 141 additional microsatellites were identified from a genomic DNA sub-library. Therefore, the total number of microsatellite sequences available amounts to 302.

Preliminary typing analyses on the F0 animals with 32 markers have shown that 18 markers are polymorphic, with a maximum of 4 alleles, which confirms previous reports in rabbit (QUENEY *et al.*, 2001). These first results indicate that around 2/3 of the microsatellites should be informative in our reference families, with a small number of alleles.

At present, it is essential, first to identify informative markers in the reference families by typing the 16 F1 animals and second, to perform the genotyping on all the individuals. We expect to obtain 150 to 200 informative markers and to construct the first global rabbit genetic map by the end of 2004. Availability of this first generation rabbit genome map will make it possible to use tools, developed in other domestic animals, for the identification of genes or QTLs involved in production traits and for marker assisted selection.

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