TARGETING A SPECIFIC GENOMIC INTERVAL TO IDENTIFY RAPD MARKERS LINKED TO THE HIGH GROWTH (*hg*) LOCUS IN MICE

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SUMMARY

The high growth (*hg*) locus is a major locus that increases weight gain and mature body size in mice. A previous mapping study positioned *hg* to an interval (*hg* candidate interval) on mouse chromosome 10. To saturate this interval with more markers, we utilized the random amplified polymorphic DNA (RAPD) assay to analyze two DNA pools created from F_2 mice. The pools differed for the alleles in the *hg* candidate interval, while being genetically similar outside this interval. Screening of 888 RAPD primers identified eight markers that map to the *hg* candidate interval. These RAPD markers will be used for fine genetic mapping of *hg*, and will facilitate future map-based cloning of this locus. The approach used in this study was successful in targeting markers to a specific genomic interval in mice, and should be also applicable in domestic animal species.

INTRODUCTION

Genetic markers are of primary importance in gene mapping studies, gene cloning, DNA diagnostics, and for the introgression of specific traits of interest in plant and animal breeding. Recently, a new type of DNA marker, random amplified polymorphic DNA (RAPD) marker (Williams *et al.* 1990), has been introduced. The RAPD marker is based on the Polymerase Chain Reaction (PCR) and the use of primers (~10-mers) of arbitrary sequences to analyze DNA samples. The polymorphisms between assayed DNAs result from sequence differences at a priming site or from other chromosomal changes (*e.g.* insertions, deletions etc.) in the amplified regions. In plants, the RAPD-PCR technique has been efficiently used for identification of markers in specific genomic regions (Giovannoni *et al.* 1991, Michelmore *et al.* 1991, Paran *et al.* 1991). In this approach, two pools of DNAs are constructed from selected individuals of a segregating population. The two pools differ in allelic composition within the genomic region of interest, while they are genetically similar outside this region. Differences in the electrophoretic patterns of RAPD-PCR products between the pools identify polymorphisms potentially linked to the targeted region.

The *hg* locus increases weight gain and mature body size by 30-50% in homozygous individuals (Bradford and Famula, 1984). Previous genetic crosses (Medrano *et al.*, 1992) have detected linkage with loci on mouse chromosome 10. Our recent work (unpublished results) using interval mapping analysis (Lander and Botstein, 1989) with several microsatellite markers (Dietrich *et al.*, 1992) has determined the *hg* maximum likelihood position between microsatellites *D10Mit9* and *D10Mit12* (*hg* candidate interval). The long-term research goals of our laboratory are to clone the high growth (*hg*) locus in mice, functionally characterize it, and study the effects of the *hg* genomic region in domestic animals. For efficient and successful cloning of *hg* based on its map position, a fine genetic map (*i.e.* subcentiMorgan resolution) is required. Crucial for the construction of such a high resolution *hg* map will be the

availability of genetic markers around *hg*. We present here the identification of eight markers mapping to the *hg* candidate interval utilizing RAPD-PCR and pools of DNAs from F_2 mice. Our study demonstrates the value of this approach to identify markers needed for fine genetic mapping in animals.

MATERIALS AND METHODS

Construction of DNA pools: Two pools of DNA were constructed from F_2 mice of a cross between line C57BL/6J-*hg* and an inbred line of wild mice, *Mus musculus castaneus*. The so-called *hghg* pool contained DNAs of eight F_2 mice that were homozygous for *hg* alleles in the *D10Mit9* to *D10Mit12* interval, but were recombinant in the flanking intervals. In contrast, the eight F_2 mice in the ++ pool were homozygous for wild type alleles in the *D10Mit9* to *D10Mit12* interval but were also recombinant in the flanking intervals. Thus, the two pools of DNAs differed for alleles in the *hg* candidate region but were genetically identical (heterozygous) outside this interval. We estimate the size of the genetic window defined by these two pools for screening to be approximately 8 cM.

Screening of DNA pools with RAPD-PCR: The *hghg* and ++ pools were screened with 888 RAPD primers: 288 Operon (OP) primers, sets A through K, O, S, W, AD (Operon Tech., Alameda, CA), and University of British Columbia (UBC) RAPD primers (#1 to #600). The RAPD primers were stored as 1.2 μM stocks in 96-well Serocluster plates (Costar, Cambridge, MA). The PCR components using the Taq DNA polymerase (Promega, Madison, WI) were 25 ng DNA, 0.4 μM RAPD primer, 1X Taq DNA polymerase reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH=9.0, 0.1% Triton X-100, Promega), 100 μM each of dNTPs, 2 mM $MgCl_2$, and 0.6 units of Taq DNA polymerase in a final volume of 15 μl. The PCR amplification components using the *AmpliTaq* DNA polymerase, Stoffel fragment (Perkin Elmer Cetus, Norwalk, CT) differed in the use of 1X Stoffel buffer (10 mM KCl, 10 mM Tris-HCl, pH=8.3, Perkin Elmer Cetus), 4 mM $MgCl_2$ and 1.5 units of the Stoffel fragment. The amplification profile (D. Lavelle & R.W. Michelmore, personal communication) was one cycle at 94 °C (2 min), 3 cycles at 94 °C (1 min), 35 °C (1 min) 72 °C (2 min) followed by 32 cycles at 94 °C (10 sec), 35 °C (30 sec) 72 °C (1 min); in the last cycle the extension time at 72 °C was increased to 5 min. PCR reactions were run in 96-well V-bottom plates (MJ Research, Watertown, MA) on PTC-100-96V Thermal Cycler (MJ Research). PCR products were analyzed in gels of 1.5% agarose (IBI, New Haven, CT) 0.5% Synergel (Diversified Biotech, Boston, MA). Multichannel pipetting was used in all steps from PCR preparation to gel loading.

RESULTS

Eight RAPD primers out of 888 RAPDs screened yielded polymorphisms linked to the ~8 cM interval harboring the *hg* locus: OPJ12, OPG12, UBC13, UBC29, UBC119, UBC135, UBC327, and UBC350 (Figure 1). These markers were identified by analyzing two pools of DNAs constructed from F_2 mice: one homozygous for *hg* alleles in the *hg* candidate interval, the other homozygous for wild type alleles in this interval. Seven primers produced dominant genetic markers (presence or absence of a band), while one primer, UBC-119 (Figure 1, lane 6), produced a codominant marker (length polymorphism). Genetic linkage between the eight polymorphic RAPDs and the *hg* candidate interval was verified by analyzing DNAs of individuals constituting the DNA pools and other F_2 mice of known genotype in the *hg* candidate interval. Expected results were obtained with all eight markers (*e.g.*

a dominant marker amplified in all individuals from one pool, but was absent in all individuals from the other pool), which confirmed their assignment to the *hg* candidate interval.

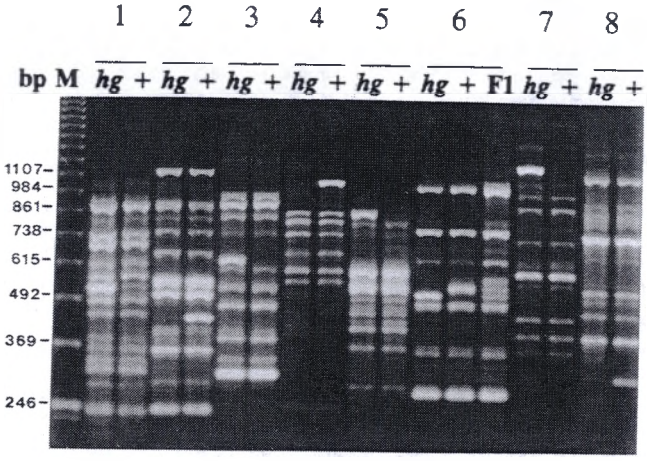

Fig. 1. Agarose gel electrophoresis of RAPD polymorphisms linked to the *hg* candidate interval; *hg* = *hghg* DNA pool, + = ++ DNA pool; lane M = 123-bp ladder, lanes 1-8 correspond to primers OPJ12, OPG12, UBC135, UBC327, UBC350, UBC119, UBC29, UBC13, respectively. Approximate sizes of polymorphic bands in lanes 1-8 are 540bp, 460bp, 645bp, 1080bp, 875bp, *(hg)* 535bp/*(+)* 555bp, 1190bp, and 310bp, respectively. Primers in lanes 1-6 were amplified using the Stoffel fragment of Taq DNA polymerase, and in lanes 7 & 8 with Taq DNA polymerase.

In the RAPD-PCR reactions, we used Stoffel fragment, a derivative of the native form of Taq DNA polymerase (Lawyer *et al.*, 1993). Bassam *et al.* (1992) reported that this enzyme produced clearer RAPD fingerprints and more informative profiles than the native Taq DNA polymerase in PCR reactions using bacterial DNA and short (~8 mers) arbitrary primers. Using the Stoffel fragment, we screened 888 RAPDs and identified seven polymorphic RAPDs (Figure 1, lanes 1-7). Rerunning these seven RAPDs with a native form of Taq resulted in a loss of the original polymorphism for three primers (OPJ12, UBC135, UBC327). One primer (UBC119) produced fainter polymorphic bands than the Stoffel reaction, two primers (OPG12, UBC350) retained the same strong polymorphic bands produced in the Stoffel reaction, while the primer UBC-29 produced a stronger polymorphic band with the native Taq enzyme than with the Stoffel fragment. Furthermore, primer UBC13 (Figure 1, lane 8) generated a polymorphism with the native Taq enzyme only; we identified this marker in our initial screens of ~100 RAPDs when the native Taq was used. These data suggest that the native Taq DNA polymerase and its derivative, the Stoffel fragment, may or may not produce the same polymorphisms with a particular primer. When the two enzymes produce the same polymorphic band, one type of enzyme may generate a stronger and clearer marker than the other enzyme.

DISCUSSION

Our study demonstrates successful application of RAPD-PCR technology and the use of pools of DNAs from selected F_2 individuals for identification of genetic markers in the *hg* candidate interval in mice. Eight RAPD markers were identified in the targeted ~8 cM interval by screening 888 RAPD primers. The screening methodology employed in our study (the use of Stoffel fragment, 96-well plates to store primers and run PCR reactions, and the application of multichannel pipetting for PCR preparation and gel loading) has proven to be technically simple and very efficient for analyzing large numbers of primers. We are currently mapping the eight RAPDs within the *hg* candidate interval. These RAPD markers will increase the density of markers around *hg*, which will facilitate future fine genetic mapping of the *hg* locus.

The comparison of two DNA pools from segregating populations using RAPD-PCR to generate markers in targeted intervals was originally introduced in plants (Giovannoni *et al.* 1991, Michelmore *et al.*, 1991). Our study shows that this approach can also be successful in mice, and hence most likely in other animal species. In domestic animals, this approach may be useful in identifying markers linked to genes of interest (*e.g.* disease resistance genes, major genes controlling economically important quantitative traits), or in searching for markers in gaps from existing genetic maps. The construction of pools to define target intervals may, however, differ. We defined the target interval by markers bordering the locus of interest and constructed pools from selected individuals of an F_2 population originating from a single cross. Such genetic material and preexisting map information on the gene of interest may not always be available in domestic animals. Alternative pooling strategies based on phenotypic data (Plotsky *et al.*, 1993, see also discussion in Michelmore *et al.* 1991) may be more feasible for domestic animals. Nevertheless, regardless of the pooling strategy used, the analysis of DNA pools with the RAPD-PCR screening technique employed in our study can be applied to other animal species to efficiently search for markers linked to genomic regions of interest.

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