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In the QTL region surrounding porcine MHC, gene order is conserved with human genome

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Abstract. On the porcine genome, the region surrounding the Major Histocompatibility Complex, also called Swine Leukocyte Antigens (SLA), is of particular interest not only owing to its role in the control of immune response, but also because of its influence on many traits such as growth, fatness, and meat quality. To help in the identification of responsible genes, detailed comparative maps of the MHC region in mammalian species and powerful mapping tools allowing accurate ordering of genes and markers in this region are needed. In this report, we describe the use of the recently developed IMpRH radiation hybrid panel, to construct a higher density radiation hybrid map of swine Sscr 7p-q12, containing 23 additional loci. Our results show that the gene order is conserved between the two MHC-containing regions, even if an inversion is observed above the QTL region in the region containing *DEK*, *SCA1*, and *EDN1* genes. The framework map produced shows that the IMpRH panel permits the ordering of genes and markers in the three MHC classes and would thus allow accurate localization of ESTs and candidate genes.

Introduction

Swine Chromosome 7 (Sscr 7) contains the MHC also called Swine Leukocyte Antigens (SLA). As in other mammalian species, SLA is divided into three classes (for review see Chardon et al. 1999) and is highly polymorphic. The class I and class III regions were assigned to 7p1.1, while the class II region was assigned to 7q1.1. Thus, unlike human complex, SLA is split by the centromere. The SLA spans about 2.4 megabases of DNA excluding the centromere, which is half the size of human MHC. Following the development of BAC libraries, the construction of a BAC contig of SLA region and development of a physical map of this region are under way. In some herds, SLA haplotypes have been found associated with a higher or a lower level of performance for traits including birth and weaning weight, growth, backfat thickness, ovulation rate, and litter size (Rothschild et al. 1986). More recently, these results were confirmed by identification of QTLs on Chr 7 (Rothschild 1998 for a review). In Large White–Meishan crosses realized at INRA, QTLs for growth and for backfat thickness were detected around SLA, in a region situated between microsatellites *Sw1354* and *S0102* (Milan et al. 1998; Bidanel et al. in preparation). From the chromosomal location of these markers, genes responsible for the observed effects are expected in the Sscr 7p1.2-q1.2 region.

Correspondences between human and pig chromosomal segments have already been studied. In particular, bidirectional ZOO-FISH painting established that Sscr 7pter-q12 shares conserved synteny with Hsap 6p (Goureau et al. 1996). Recently INRA-University of Minnesota porcine Radiation Hybrid panel (IMpRH), a swine 7000 rad whole genome Radiation Hybrid (RH) panel, was developed (Yerle et al. 1998). For Chr 7, 41 microsatellite markers from the genetic map were used to establish a first-generation map (Hawken et al. 1999), containing seven linkage groups and five unlinked markers. In this report, we present a higher density RH map of the region surrounding SLA, produced by mapping 23 additional markers (13 genes, 1 gene-associated microsatellite, 8 microsatellites, and one anonymous EST).

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Materials and methods

Markers. Information for microsatellites *Sw1344, S0013, Sw1409, Swr74, Sw2019, Sw1856, Sw1681,* and *Swr946* was available at http://sol.marc.usda.gov/. We also used primers already defined for the following genes: *MOG, SCA1, BF, GST, BAT1,* and *EST Det9* (cf. Table 1). We develop new primers for *DEK, EDN1, PRL, BT, MS-SLA11, CYP21, SLA-DRα, RXR*β, and *CLPS* (cf. Table 1). PCR products were purified with Wizard PCR Preps DNA purification system (Promega) and were cloned with pGEM-T vector system I kit (Promega). Clones were sequenced on ABI 377 (PE Biosystems) by using ABI Prism dRhodamine Terminator cycle sequencing kit. Pig specific primers were then defined. Primer sequences and PCR conditions are described in Table 1. PCR reactions were performed in standard conditions as described in Robic et al. (1999). Amplifications were carried out on a GeneAmp System 9700 (PE Biosystems, Countaboeuf, France) or on an Omnigene (Hybaid, Paris, France) thermocycler. Touch Down PCR was performed for BAT1: denaturation at 94°C for 5 min, followed by three cycles at each annealing temperature of 62°, 60°, and 58°C and 30 cycles with an annealing temperature of 56°C.

Radiation hybrid mapping. Reactions were performed in independent duplicate on IMpRH panel. PCR products were analyzed on 2% agarose gels in 1× TBE buffer after staining with ethidium bromide. A third amplification was carried out on clones for which discordant results were obtained. When products of similar size were amplified from hamster and pig genomic DNAs, pig fragments were identified following Single Strand Conformational Analysis (SSCA) carried out according to Lahbib-Mansais et al. (1999). Vectors of amplification results were submitted to IMpRH database accessible at http://imprh.toulouse.inra.fr. (Milan et al. 2000).

RHMAP 3.0 statistical package was used to build radiation hybrid maps (Lange et al. 1995). Analyses were performed under the equal retention probability model. Using RH2PT program, two-point distances were calculated between all markers. Linkage groups were built by using a LOD score threshold of 4. Multipoint analyses were performed with RHMAXLIK. For each linkage group, a framework map was built, with a likelihood difference threshold of 1000:1 to incorporate a new marker in the framework of previously ordered markers. Finally, the resulting map was checked again, by removing one marker at a time from the framework, and analyzing the likelihood of all its possible locations on the map of other markers.

Results

Mapping of new markers on IMpRH panel. Our first goal was to densify the map around SLA complex to determine in the QTL

Table 1. Primer sequences and PCR conditions used to map new genes on Chromosome 7. TD means Touch Down PCR (from 56° to 62°C), as described in Materials and methods.

Marker or gene	No. Genbank	Gene name	Forward/Reverse primers or reference	Annealing Tp	$MgCl_2$ (mM)	Size of pig fragment (bp)
DEK	AJ277648	Dek oncogene	TTC CTG CTG ACC TTT TAA TCT CAA TGT ACA AAA TAC AAC TAT	50	3	142
EDN1	X07383	Endothelin 1	CGC TTG GCA GGG GCT GAG GCA GGT GGC ATC CGT GTC	64	3	234
PRL	X14068	Prolactin	AAT GAC CCG CTT GTA TCA C CCG CTA TCT TCT CCA TGC C	59	2	130
BT	Z97409	Butyrophilin	ACT GCC TGA GAA ACC TGA GAG CTC CAC AGC CCA GAA CCC ATT	60	2	193
MS-SLA11	AJ131112	microsatellite associated to SLA11	CCT GTG TTT CTA TGG CTG TGC CAG GGA AGG AAC CCA CAT C	61	1.5	234
CYP21	M83939	Cytochrome P450 steroid 21-hydroxylase	ACG CAG AGA CTC GGG CCT ATC TAC A ATG GTA TCT GGG GTC TGC CGG CAA A	57	3	214
*SLA-DR*α	M93028	Class II SLA-DRα	CGA CAA GTT CTC CCC GCC AGT TGT CCA AAC CCC AGT GCT CGA	65	2	150
*RXR*β	AF1463018	Retinoic X receptor β	CAG GAT AAT GCT GAT TTC AAGG TGA GTT GCT GAG GCT GCT AC	61	1.5	347
CLPS	AF012819	Colipase	GAG TTG ATG AAG AGA GGG AG CTT GCC CCT GTC TGA CTG	60	3	249
GST	Z69586	Glutathion S transferase	Tosser-Klopp et al. 1996	52	1.5	178
SCA1	X79204	Ataxin	Lahbib-Mansais et al. 1999	58	2	123
BAT1	Z34846	Nuclear RNA helicase	Peelman et al. 1995	TD 62–56	2	96
MOG	Z97404	Myelin Oligodendrocyte glycoprotein	Velten et al. 1999	55	1.5	297
BF	M59240	Complement Factor B	Peelman et al. 1991	71	2	390
Det9	X91764	Porcine EST det9	Tosser-Klopp, in preparation	58	1.5	90

region whether order is conserved or not between human and pig genomes. To fill gaps between groups of linked markers, we were able to localize, on IMpRH, eight additional microsatellites mapped on the genetic map between *Sw1354* and *Swr946*. We also mapped on IMpRH panel EST *Det9* previously mapped in this region (Tosser-Klopp, in preparation) on INRA Somatic Cell Hybrid panel http:www.touloose.imra.fr/lgc/pig/hybrid.htm

To increase the number of genes mapped in this region, we succeeded in amplifying eight genes from the homologous region on human map: *EDN1, SCA1, DEK, PRL, BF, BT, CLPS,* and *GST*. For *DEK*, human primers amplified several bands on pig DNA. After cloning, amplified fragments were sequenced. A homology of 87.5% on 139 upon 142 bp was identified with human DEK gene. Partial porcine *DEK* sequence was deposited in EMBL under accession number AJ277648. Previous to their analysis on IMpRH panel, *BF, DEK,* and *CLPS* were first assigned onto INRA somatic hybrid cell panel. They were respectively assigned to Sscr 7p11-p12, Sscr 7p13 or p11, and Sscr 7q12-q23 or SSC 7q26. *EDN1, SCA1, DEK, PRL, BT, BF, CLPS,* and *GST* have been successfully mapped on IMpRH panel.

To produce denser radiation compared maps in MHC region, seven additional genes located in HLA or microsatellite developed from porcine BACs containing these genes were mapped on IMpRH.

Multipoint mapping. Retention frequencies of markers mapped in this study range from 19.1% to 49.1% with an average retention frequency of 29.5%. A slightly higher retention frequency was observed for markers located close to the centromere (data not shown). Six non-significantly linked linkage groups were built with RHMAP 3.0 (Lange et al. 1995). A framework map 1000:1 was established for each independent linkage group. A global framework map merging framework of independent linkage groups was assembled by using 30 markers out of 41 (Fig. 1). A comprehensive map with the 41 loci was finally built, adding each remaining marker at the most likely location. Globally our results show a conservation of gene order from *PRL* to *GST* between human and pig. An inversion was, however, identified for region containing *DEK, SCA1,* and *EDN1* genes. More markers between *DEK* and *PRL* need to be mapped in order to confirm or weaken this inversion.

Map resolution. In this study, the total length of Sscr 7 is about 2650 cR_{7000} (data not shown). Based on an estimation of physical size of 141 Mb, we observed a ratio of 53 kb/cR. Focusing on the region in which we mapped additional markers, the framework map covers 1335 cR for a genetic distance of 81.5 cM; a ratio of 16 cR/cM was then observed in this region. The cloning of SLA region in BAC contig permits an estimate of the physical size between genes. On our RH map, the upper limit of MHC class I was represented by *MOG*, superior and inferior borders of MHC class III were defined by *BAT1* and *CYP21/BF* genes, class II was represented by *SLA-DR*α and *RXR*β. Globally in this region, excluding the centromere, whose size remains undetermined, we observed a ratio of 14.6 kb/cR (2150 kb for 147 cR; data not shown).

Discussion

Localization of 23 new loci on IMpRH presented in this paper allowed us to improve the 7000-rad IMpRH map of Sscr 7p-q12. More specifically in the QTL region, we increased the number of known genes mapped on IMpRH from 1 to 13 genes. A framework map (1000:1) containing 30 markers or genes was proposed. The comprehensive map of this region contains 41 markers; 3/4 of the markers were thus included in the framework map.

Only two pairs of markers (*SSC11F02*/*BT* and *CYP21*/*BF*) were totally linked on IMpRH panel. Physical distance between *CYP21* and *BF* is estimated at 100 kb (Chardon et al. 1999); total linkage of these two genes is thus not surprising, as a breakage is expected to occur in one hybrid of IMpRH panel each 145 kb (Hawken et al. 1999). In four cases, markers totally linked on the USDA genetic map were mapped at a distance ranging from 8 to 35 cR_{7000}. This result is consistent with the ratio of 18 cR/cM observed on the whole genome by Hawken et al. (1999).

PRL was previously genetically mapped on Chr 7 approximately in the middle of an interval of 26 cM. A very precise linkage mapping of *PRL* can be deduced from our results (Fig. 1). In pig, *GST* was roughly mapped on INRA somatic cell panel in region 7q1.2-1.4. By heterologous painting, this region was shown to be in correspondence with fragments of Hsap 6p but also to Hsap 15q24-26 (Goureau et al. 2000). Mapping of *GST* allows identifying microsatellites located in this region. Isolation of BACs containing these microsatellites will provide material very useful

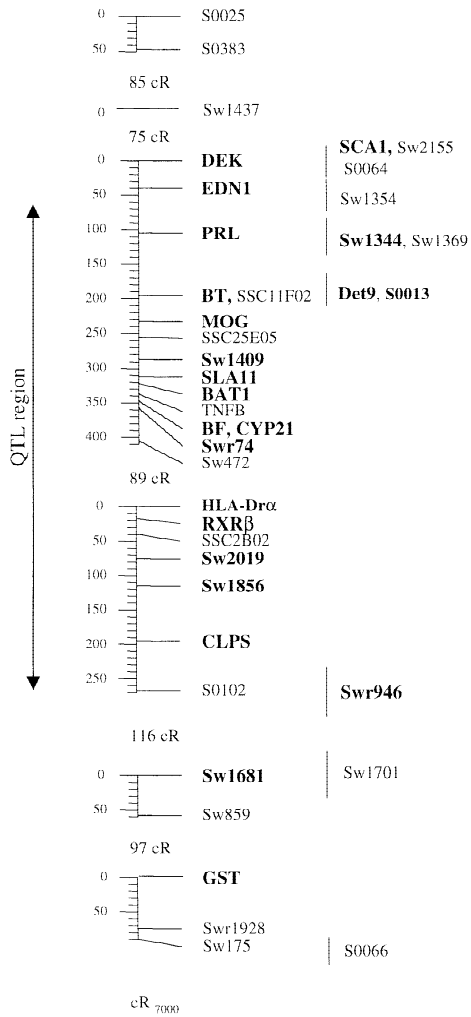

Fig. 1. Framework radiation hybrid map of swine Chr 7. The 23 markers mapped in this study were in bold type characters. Distances between linkage groups were determined from multi-point analyses. Markers not included in the framework map were indicated on the right of the map.

to refine comparative mapping knowledge in this complex region. This will be particularly interesting as we could not totally exclude that genes responsible for the observed QTL effects can be located in that region.

In the first-generation map, *SSC2B02* was only assigned to Sscr 7, but was not significantly linked to other markers. Following this study, *SSC2B02* belongs to a linkage group of eight markers containing genes of SLA Class-II. Another linkage group containing class-I and class-III genes consists now of 23 genes and markers instead of 4 in the first-generation map. However, these two linkage groups separated by the centromere are only weakly linked. In the SLA region, the order of genes determined on IMpRH panel is in agreement with physical map data (Velten et al. 1999). The highest retention frequency was observed for marker *Sw472*, which is likely to be the marker closest to the centromere on the q arm. Such a higher frequency in centromeric region has been similarly observed for human and bovine chromosomes.

On average, on the 2.15 Mb covered by genes of SLA complex, a ratio of 14.6 kb/cR was observed. This resolution is very high compared with the 53 kb/cR observed on the whole Sscr 7. It is due both to a high frequency of chromosomal breakage following cell irradiation and to a high retention frequency of chromosomal fragments in the hybrids. The mapping resolution within centromeric region could not be accurately estimated, as the size of centromere remains unknown. Janzen estimated the size of SSC 9 centromere at 2.2 Mb in Large White and Piétrain pigs (Janzen et al. 1999), which is similar to indications given in human. Considering this value as a possible size of Chr 7 centromere, the kb/cR ratio could be estimated at 16 kb/cR, which is quite similar to the value observed on the surrounding SLA region, but different from the average value of 55 kb/cR for the whole Chr 7.

In human, the sizes of SLA11-MOG and MOG-BT intervals were reported to be respectively 600 kb and 3.5 Mb. In pig, the distance between *SLA11* and *MOG* was estimated at 450 kb. On pig RH map, the distance between *SLA11* and *MOG* was of 80 cR, whereas the distance between *MOG* and *BT* was only 36 cR. Considering a similar ratio of 15 kb/cR in this region, we can estimate that physical distance between *MOG* and *BT* could be around 540 kb, which is significantly lower than the 3.5 Mb distance observed in human. Such an observation is important for the comparative study of the MHC complex region in pigs and human.

This study will be useful for the identification of genes involved in QTL. It reveals that gene order seems conserved in the QTL region between human and pig. Even if chromosomal rearrangements are complex between human and mouse genomes, we can also take into account data obtained on the mouse. Genes from *EDN1* to *BT* are located on Mmus 13, whereas *MHC* and *CLPS* are found on Mmus 17 (MGD, April 2000). In mouse, QTLs affecting reproduction and growth have been mapped close to mouse MHC on Mmus 17, whereas no QTL have been mapped to Mmus 13 region. If homologous genes are involved, it is thus more likely to expect genes responsible for QTL effect in pigs in a region below *BT* including *SLA.* We have shown that IMpRH panel permits very accurate mapping of genes located in this region. This panel will thus be a tool of choice for mapping new genes or ESTs that may be considered as candidate genes, possibly involved in the regulation of growth, fatness, and meat quality in the pig.

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