Brief Gene Mapping Report

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Mapping of three porcine 20S proteasome genes using the IMpRH panel

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Rationale and significance

The 26S proteasome is a multicatalytic enzyme complex that plays a central role in all regulatory pathways such as cell cycle regulation, differentiation, and apoptosis. The 26S proteasome has three components, 20S proteasome, PA28 (Proteasome Activator 28) and PA700 (Proteasome Activator 700) subunits. The combination of PA28 and 20S proteasomes greatly increases the proteasome-mediated proteolysis activity, which is important in the processing and presentation of some antigens. The three genes related to PA28 and seven genes related to PA700 have been mapped by our group (Wang et al., 2003, 2004; Yu et al., 2004). Here we report our mapping results on the PSMA5, PSMB3 and PSMB6 genes, which were thought to be part of the 20S proteasome, the core of the 26S proteasome.

Materials and methods

Isolation and sequencing of the three porcine gene fragments

Human cDNA sequences for these three genes were gained from NCBI (GenBank accession numbers are NM_002790, NM_002795 and NM_002798) and were compared with all sequences respectively in the EST-other databases by using the BLAST (www.ncbi.nlm.nih.gov/blast/) algorithm. One porcine EST was selected for each gene from the BLAST analysis and primer pairs were designed by using primer design software (Primer 1.0) (Table 1). PCR amplification was successfully carried out for the three markers using porcine genomic DNA as template. The PCR products were cloned and sequenced to verify their identity.

Radiation hybrid (RH) mapping

The INRA-University of Minnesota porcine radiation hybrid (IMpRH) panel consisting of 118 hybrid clones was used to map these genes, using the same primer pairs (Table 1). Twenty five nanograms of RH DNA was used as template in 10 µl polymerase chain reaction (PCR) reactions (94 °C for 40 s, 58–62 °C for 30 s, then 72 °C for 30 s, 35 cycles in a Mastercycler gradient and a final extension of 72 °C for 5 min) containing 1.5 mM $MgCl_2$, 1× PCR buffer (Promega, Madison,WI, USA), 2.0 Units *Taq* DNA Polymerase (Promega, Madison,WI, USA), 150 µM of each dNTPs and 0.3 µM of each primer (see Table 1). The PCR products were size-separated on a 2.0% agarose gel stained with 0.5 µg/ml ethidium bromide. The PCR typing was done twice for each gene.

Results and discussion

The statistical analyses of the PCR results were performed with the IMpRH mapping tool accessible at http://imprh.toulouse.inra.fr/ (Milan et al., 2000). Two-point RH analyses were used for the identification of linkage groups using LOD score threshold of 5.0. The mapping results are presented in Table 2. Our results indicated that PSMA5 is located on SSC4 and both PSMB3 and PSMB6 are located on SSC12.

The cytogenetic positions of these genes can be deduced from the cytogenetic position of closely linked markers already localised on the cytogenetic map. For instance, PSMA5 is tightly linked to SW818, which is very close to SW445, already map-

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Table 1. Primer pairs used for porcine PSMA5, PSMB3 and PSMB6

Gene	Primer sequence	Binding region	Size (bp)	PCR (T_m)
PSMA5	PL:5'-ATAGGTTGTGCCATGAGTGG-3'	Exon 3	1084	60
	PR:5'-GATCTGCATCCTCTTCTCCA-3'	Exon 4		
PSMB3	PL:5'-GGTCCTAATACACTGCAATC-3'	Exon 1	455	58
	PR:5'-ATCTTCTGGAAGTCCGTGGT-3'	Exon 2		
PSMB6	PL:5'-GCTGACTCCTATTCACGACC-3'	Exon 3	584	62
	PR:5'-TGTCTCACCATCATACCTCC-3'	Exon 5		

Table 2. RH mapping of porcine PSMA5, PSMB3 and PSMB6

Gene	GenBank accession number	Linked marker	LOD score	Distance (cR)	Retention (%)	Deduced cytogenetic position	Sequence similarity with human sequences (accession number)	Human Localization
PSMA5	AY462282	SW818	8.51	0.47	24	4q15-q16	95% (NM-002790)	1p13
PSMB3	AY462280	SW943	17.29	0.18	23	12p13-p11	98% (NM-002795)	17q12
PSMB6	AY462281	S0160	8.54	0.37	12	12q13	92% (NM-002798)	17p13

ped to 4q15→q16. PSMB3 is linked to SW943 which is in the close vicinity of SW874 mapped to 12p13→p11 and the closely linked marker of PSMB6 is S0160 already mapped to 12q13. Consequently, our results suggested that PSMA5 is located on 4q15→q16, PSMB3 on 12p13→p11 and PSMB6 on 12q13. These results are totally in accordance with comparative mapping data available as SSC4q15→q16 has been shown to be in correspondence with HSA1p13 and SSC12 with HSA17 (Goureau et al., 1996).

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