Physical mapping of four porcine 20S proteasome core complex genes (PSMA1, PSMA2, PSMA3 and PSMA6)

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Rationale and significance

Proteasomes are protein complexes in eukaryotic cells that degrade proteins into peptides, some of which are involved in presentation by major histocompatibility complex class I molecules. The 20S proteasome has a columnar shape with four rings which act as a catalytic core in protein degradation. The 20S proteasome consists of 28 protein subunits that are encoded by 17 protein subunit genes. In this study, we successfully isolated four genes, PSMA1, PSMA2, PSMA3, and PSMA6. Furthermore, the four genes are assigned to porcine chromosomes.

Materials and methods

Isolation and sequencing of four porcine gene fragments

Human cDNA sequences of the four genes were obtained from NCBI (GenBank accession numbers are NM_002786, NM_002787, NM_002788, and NM_002791) and were compared with all sequences respectively in the EST-other databases by using the BLAST (www.ncbi.nlm.nih.gov/blast/) algorithm. Pig ESTs with at least more than 80% similarity with the corresponding human mRNA were chosen and aligned together using SaqMan. The primers were designed from the pig ESTs by using primer design software (Primer 1.0) (Table 1).

The PCR was conducted in a 20- μ l reaction volume consisting of 50 ng of porcine genomic DNA, 1 $\times$ PCR buffer, 0.3 μ M of each primer, 75 μ M of each dNTP, 1.5 mM MgCl₂ and 2 U Taq DNA polymerase (Promega, Madison WI, USA). The PCR parameters were 5 min at 94 °C followed by 20 s or 30 s at 94 °C, 20 s or 30 s at the annealing temperature (Table 1) and 20 s or 30 s at 72 °C for 35 cycles and an extension of 8 min at 72 °C. The PCR products were purified with Wizard prep PCR purification system (Promega, Madison, WI, USA), cloned to Promega pGEM-T easy vector and sequenced by a commercial service. Comparisons of pig exon sequences of the four gene's PCR products with the corresponding human mRNA sequences in the GenBank were done using the BLAST2 software provided by the NCBI server (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>) to confirm that the expected pig sequences were isolated.

Radiation hybrid (RH) mapping

The same primers were used in mapping (Table 1) and the mapping results are shown in Table 2. The 25-ng RH template was used in 10 μ l PCR reactions (94 °C for 30 s, 58–60 °C for 30 s, then 72 °C for 20 s for 31 cycles in a Mastercycler gradient with a final extension of 72 °C for 8 min). The PCR products were size-separated on a 1.5% agarose gel and then stained with 0.5 μ g/ml ethidium bromides. The PCR typing of each gene was done twice.

Results and discussion

The PCR results for the four genes were analyzed using the IMpRH mapping tool available through the IMpRH Web Server (<http://imprh.toulouse.inra.fr/>). In this study, we reported

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Table 1. Primer pairs used for porcine PSMA1, PSMA2, PSMA3 and PSMA6

Gene symbols	GenBank Acc. No. (porcine)	Sequence similarity ^a	Primer sequences (5'-3')	Primer binding region	Size (bp)	Tm value (°C)
PSMA1	AY680661	94%	PF-CTCTGCCTGTGTCTCGTCTT PR-GTACGAGCTGATTGAGAACG	exon 6 exon 8	1487	59
PSMA2	AY680662	93%	PF-GGAACCTGAAGATGCCATTC PR-GGATTTTCAGTCACTTCGTT	exon 7 exon 8	266	58
PSMA3	AY680663	92%	PF-CAAAAAGATATAAGGGAAGAG PR-TAGATGCTGAAGTAGGTGTT	exon 10 3'UTR	489	58
PSMA6	AY680664	96%	PF-GGTGGCCTTACATCAGTAGC PR-GCTGTCATTCTGTTCATCAC	exon 2 exon 3	634	60

^a The pig sequences were compared to the corresponding human sequences.

Table 2. RH mapping of porcine PSMA1, PSMA2, PSMA3 and PSMA6

Gene symbol	GenBank Acc. No. (human)	Human cytogenetic location ^a	Porcine RH mapping result				
			Retention (%)	Lod score	Closed marker	Chr	Dist (cR)
PSMA1	NM_002786	11p15.1	26	5.66	SW1026	2p11	68
PSMA2	NM_002787	7p13	34	12.77	SWR414	18q24	33
PSMA3	NM_002788	14q23	24	10.78	S0357	1q21→q27	38
PSMA6	NM_002791	14q13	18	10.4	NFKB	7q	39

^a The locations of genes of the human map were collected from <http://www.ncbi.nlm.nih.gov/Locuslink/>

locations of the four genes by choosing the closest markers with LOD scores higher than 5.0. Porcine gene PSMA1 was assigned to SSC2p11 and the linked marker was SW1026. PSMA2 was assigned to SSC18q24 with the marker SWR414. Both PSMA3 and PSMA6 were located on HSA14, but the porcine genes PSMA3 and PSMA6 were assigned to SSC1q21→q27 and SSC7q separately, with markers SWR414 and NFKB. The results were consistent with the known homology between HSA14 and SSC1 and SSC7 respectively (Table 2).

References

- Coux O, Tanaka K, Goldberg AL: Structure and functions of the 20S and 26S proteasomes. *A Rev Biochem* 65:801–847 (1996).
- Schweisguth F: Dominant-negative mutation in the b2 and b6 proteasome subunit genes affects alternative cell fate decisions in the *Drosophila* sense organ lineage. *Proc Natl Acad Sci USA* 96:11382–11386 (1999).
- Ottosen S, Herrera FJ, Triezenberg SJ: Transcription. Proteasome parts at gene promoters. *Science* 296:479–481 (2002).
- McCusker D, Jones T, Sheer D, Trowsdale J: Genetic relationships of the genes encoding the human proteasome beta subunits and the proteasome PA28 complex. *Genomics* 45:362–367 (1997).
- Milan D, Hawken R, Cabau C, Leroux S, Genet C, Lahbib Y, Tosser G, Robic A, Hatey F, Alexander L, Beattie C, Schook L, Yerle M, Gellin J: IMpRH server: an RH mapping server available on the Web. *Bioinformatics* 16:558–559 (2000).
- Yu M, Wang Y, te Pas MFW, Yerle M, Liu B, Fan B, Xiong T, Li K: Investigation of the porcine PA28 activator γ -subunit (*PSME3*) gene: isolation, polymorphism and its chromosomal localization. *J Anim Bred Genet* 121:142–148 (2004).
- Wang Y, Yu M, Yerle M, Liu B, Zhao S, Xiong T, Fan B, Li K: Mapping of genes encoding four ATPase genes and three non-ATPase components of the pig 26S proteasome. *Anim Genet* 34:393–395 (2003).
- Wu X, Yu M, Liu B, Yerle M, Zhao S, Wang Y, Fan B, Li K: Mapping of three porcine 20S proteasome genes using the IMpRH panel. *Cytogenet Genome Res* 106:142E (2004).