Rapid communication: Nucleotide sequences of two isoforms of porcine *micromolar calcium-activated neutral protease 1* cDNA

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Name of Sequences. Porcine *micromolar calcium-activated neutral protease 1* isoforms A and B (***CAPN1A, CAPN1B***) cDNA.

Genus and Species. Sus scrofa (Meishan × White Composite).

Origin of Clones. Primers (mu1725 and mu1840; Smith et al., 2000) developed from the complete cDNA sequence of bovine *CAPN1* (GenBank accession no. AF221129) were used to screen one embryonic and one adult pooled tissue porcine cDNA library (Fahrenkrug et al., unpublished data) by an iterative process as described (Smith et al., 1995). One full-length cDNA clone was obtained from each library. The p*CAPN1A* isoform was obtained from the adult tissue library, and isoform p*CAPN1B* was obtained from the embryonic cDNA library.

Comparison with Related Species. The 2,142-bp open reading frame of p*CAPN1A* cDNA sequence has 94% and 92% identity with the bovine (GenBank accession no. AF221129) and human (GenBank accession no. X04366) sequences, respectively. Conceptual translation predicts a protein of 714 amino acids with 96% and 95% identity to bovine and human proteins, respectively. The p*CAPN1B* cDNA sequence has a 1,941-bp open reading frame that is 100% identical to the first 1,941 bases of the p*CAPN1A* cDNA.

Sequence Data. The truncated reading frame of p*CAPN1B* results from a 496-bp insertion into the sequence of p*CAPN1A*, which introduces a stop codon immediately after the site of insertion. Following the inserted sequence, the sequence of p*CAPN1B* is identical to the remainder of p*CAPN1A* throughout the coding region and the 3′ untranslated region (3′UTR). The position of the insertion is identical to the boundary between exon 17 and exon 18 of the bovine *CAPN1* gene (Smith et al., 2000). Therefore we tested the possibility that the insertion resulted from a retained intron, using primers (mu1896 and mu2239; Smith et al., 2000) that flank intron 17 and 18 of the bovine gene. These primers were used to amplify the corresponding region from a Bacterial Artificial Chromosome clone containing the porcine *CAPN1* gene (data not shown). The sequence of the amplification product confirmed that the origin of the insertion is a retained intron (data not shown) and established the presence of an intron between bases 1,995 and 1,996 of the p*CAPN1A* cDNA sequence.

GenBank Accession Numbers. p*CAPN1A* has been given accession number AF263610; p*CAPN1B* has accession number AF263609.

Comments. The calpain family of genes encode cysteine proteases that have been implicated in processes such as regulation of protein kinases and hormone receptors, cytoskeletal reorganization, cell cycle progression, and apoptosis. The active enzyme includes a small subunit that seems to be shared by all members of the large subunit gene family. The μ-calpain protein is a ubiquitous calpain that requires micromolar levels of calcium for proteolytic activity in vitro. It seems to be the major enzyme involved in postmortem tenderization of beef (Morgan et al., 1993) and has recently been identified as a positional candidate gene for a QTL affecting meat tenderness in cattle (Smith et al., 2000). Human, murine, and bovine *CAPN1* cDNA clones have been described, each predicting proteins with four domains (Aoki et al., 1986; Poirier et al., 1998; Smith et al., 2000). Domain IV contains four motifs with homology to the calcium-binding portions of calmodulin (Imajoh et al., 1988), as well as residues involved with contacting the small subunit. The predicted protein from the p*CAPN1B* cDNA clone lacks two of the four calcium binding motifs of Domain IV and would likely be deficient in binding the small subunit. The possibility that it represents a cloning artifact was tested by RT-PCR using the mu1896-mu2239 primer pair. Total RNA was isolated from spherical and filamentous embryo forms on d 12 of gestation and treated with DNAse to remove residual genomic DNA. Reactions were performed in duplicate in the presence and absence of reverse transcriptase, to rule out the presence of residual genomic DNA. The principal amplification product from Meishan-White Composite porcine embryos, similar to those used in production of the embryonic cDNA library, was the size predicted from p*CAPN1B* isoform, suggesting that the retained intron isoform is expressed in these embryos (data not shown). However, embryos of other breed composition (including the PIC lines C22, 405, and 406, which include Yorkshire, Landrace, Duroc, Large White, Pietrain, and European Large Black ger-

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mplasm) were only found to produce the p*CAPN1A* isoform. In addition, adult tissues from all breeds tested only produced the p*CAPN1A* isoform. We conclude that the aberrant splice form may be a variant allele of the porcine *CAPN1* gene, but it is unlikely that the variant form plays an important function in development because the majority of breeds do not seem to express it.

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