Cloning of the complete gene for pig PIT-1 and analysis of PIT-1 protein function

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Summary and Implications

We have cloned and sequenced the complete protein-coding region of the pig PIT-1 gene. The pig PIT-1 gene is highly similar to the PIT-1 gene in other species (human, rodent, cattle). We also found some alternative forms of the pig PIT-1 gene. We have analyzed the protein function of the pig PIT-1 genes and one of its alternative forms; Δ3PIT-1. The functional studies showed that the pig PIT-1 protein can function normally at growth hormone (GH) and prolactin (PRL), whereas the pig Δ3PIT-1 protein cannot. These results imply that the pig PIT-1 gene is important in pig growth through regulation of GH and PRL gene expression, whereas its alternative forms might play different roles on PIT-1 target genes.

Introduction

PIT-1 is a regulatory gene of several important hormone (GH, PRL and thyrotropin-β) genes (1). Dwarf mice and humans have been found that lack PIT-1 gene activity. We have reported that the PIT-1 gene is associated with birth weight and backfat in the pig (2). To understand the biological function of the pig PIT-1 gene and its possible application in swine genetics, we completed cloning the PIT-1 gene in these studies. Several PIT-1 alternative forms also were identified in the pig while we attempted to clone the complete gene of PIT-1. They are the Δ3PIT-1 and the Δ4PIT-1 that are missing different parts of the PIT-1 gene, and PIT-1β that has additional amino acids relative to the PIT-1 gene. The Δ4PIT-1 and PIT-1β have functional differences and have previously been found in other species. Therefore, we investigated the function of the Δ3PIT-1 and the normal pig PIT-1 protein.

Materials and Methods

The cloning of the pig PIT-1 gene and its alternative forms was done by using PCR (polymerase chain reaction) techniques to amplify specific PIT-1 gene sequences from pig pituitary mRNA. Several positive clones for each form of the PIT-1 gene were compared to known genes by using the BLAST molecular biology software. Plasmids containing these genes were engineered to produce recombinant pig PIT-1 protein and Δ3PIT-1 protein in bacteria. These proteins were isolated and used in a DNA-protein gel electrophoresis binding assay to see if the protein could recognize and bind to the regulatory region near the rat GH or PIT-1 genes. (See T.-P. Yu's ISU Ph.D. dissertation for experimental detail.)

Results and Discussion

The complete pig PIT-1 gene (Genbank accession No. AF016251) shows high similarity to the PIT-1 gene of cattle, humans, and rodents (Table 1). Several alternative forms of the pig PIT-1 gene also were found: Δ3PIT-1 (Genbank: AF016382), PIT-1β (Genbank: AF010475), and Δ4PIT-1 (Genbank: AF016348). PIT-1β and Δ4PIT-1, which were previously identified in rodents, have showed different regulatory functions on rodent PIT-1 target genes. The pig PIT-1β and Δ4PIT-1 are highly similar to the rodent PIT-1β and Δ4PIT-1. The Δ3PIT-1 has only been found in pig.

Target gene recognition of pig PIT-1 and Δ3PIT-1 proteins were examined. The coding sequences of each PIT-1 gene form were cloned into plasmids and engineered to express the pig protein in bacteria. Partial purification of the pig PIT-1 and Δ3PIT-1 were used in a DNA-protein binding assay with two targets of the PIT-1 protein identified in rodents. The results showed that pig PIT-1 can recognize GH and PRL regulatory regions, whereas Δ3PIT-1 cannot.

The high similarity in the PIT-1 genes between the pig and other mammals (bovine, human and rat), and the recognition of pig PIT-1 to rGH and rPRL regulatory regions reveal the functional conservation of PIT-1 across species. Therefore, the pig PIT-1 gene should play an essential role in the pig's growth. Our earlier work showed that the pig PIT-1 gene was significantly associated with birth weight and backfat in the ISU Chinese x US pig population (2). Also a region of chromosome 13 associated with the early growth was shown to be very close to PIT-1 (3). More work is required to determine if the PIT-1 or another gene near PIT-1 plays a regulatory role in pig growth and carcass differences between pig breeds, and to understand the possible pig breeding applications of PIT-1.

The PIT-1 alternative form that showed functional differences from normal PIT-1 also could be important in PIT-1 function and potentially have a pig breeding application. However, additional work will be required to demonstrate the level of expression of this alternate PIT-1 form.

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Table 1. The percentage of identity of functional region (exons) between the swine PIT-1 gene and the bovine, human, and rat PIT-1 genes, respectively.asl-14

	exon 1	exon 2	exon 3	exon 4	exon 5	exon 6
bovine	92	94	94	96	95	90
human	90	94	91	94	90	91
rat	86	92	86	88	87	85