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Molecular Cloning and Sequence Analysis of the Porcine Insulin-like Growth Factor Binding Protein-5 Complementary Deoxyribonucleic Acid[1]

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We report here for the first time the isolation of a cDNA clone containing the open reading frame sequence for porcine insulin-like growth factor binding protein-5 (pIGFBP-5) and the complete deduced amino acid sequence for this porcine IGFBP. The cDNA sequence shares 94%, 90% and 91% identity to its human, mouse and rat counterparts, respectively. The deduced amino acid sequence consists of 252 amino acids and a putative 19 amino acid signal and shares 97%, 96% and 96% identity to the human, mouse and rat peptides, respectively. The mature peptide contains the 18 conserved cysteines found in all of the IGFBPs. Northern blot analysis of total RNA isolated from porcine heart, muscle and spleen using a 315 base pair cDNA insert derived from the pIGFBP-5 open reading frame sequence revealed a single mRNA transcript of 6.0 kilobases.

Insulin-like growth factors (IGF) are potent regulators of cellular growth and differentiation (1–4) and the bioactivity of the IGFs is known to be regulated by six specific IGF binding proteins (IGFBP-1-6) (5). The IGFBPs have been reported to have a number of essential roles in the regulation and coordination of IGF action including transport and partitioning of the IGFs in the circulation, regulating the clearance of the IGFs, increasing IGF half-life, regulating localization of IGFs within tissues and around cells, and modulating the interaction of IGFs with their receptors (5). Certain IGFBPs are secreted and localized around specific tissues and specific cells within tissues. A number of IGFBPs are expressed in muscle tissue and our laboratory is interested in the role of the IGFs and IGFBPs in muscle growth and development. IGFBP-5 is of particular interest with respect to muscle development since it is expressed in muscle tissue (6) and has been shown to enhance the in vitro activity of the IGFs in vitro. In addition, IGFBP-5 mRNA expression is altered during myogenic differentiation of some muscle cell lines (7–9) and it has been suggested that IGFBP-5 may play a specialized role during myoblast proliferation and differentiation (7). Consequently, IGFBP-5 may crucially important in the growth and development of muscle tissue.

Until now, a porcine-specific cDNA for IGFBP-5 has not been available for use in investigating the regulation and role of IGFBP-5 in porcine muscle development. In order to facilitate our research on the role of the IGFs and IGFBPs in porcine skeletal muscle development, we have cloned and sequenced a porcine IGFBP-5 cDNA from a pig skeletal muscle cDNA library. Here we report for the first time the complete cDNA sequence of the open reading frame for porcine IGFBP-5 as well as the deduced amino acid sequence for the porcine homolog of IGFBP-5. Northern blot analysis of porcine tissues with a cDNA probe derived from the entire sequence shows a single transcript of approximately 6.0 kb in size.

MATERIALS AND METHODS

cDNA Cloning and Sequencing

A neonatal porcine skeletal muscle Lamda ZapII cDNA Library was prepared by Stratagene Cloning Systems (La Jolla, CA) and plated onto 20, 130 cm^2 plates (1×10^6 pfu total). Duplicate lifts were screened using a human IGFBP-5 (hBP-5)

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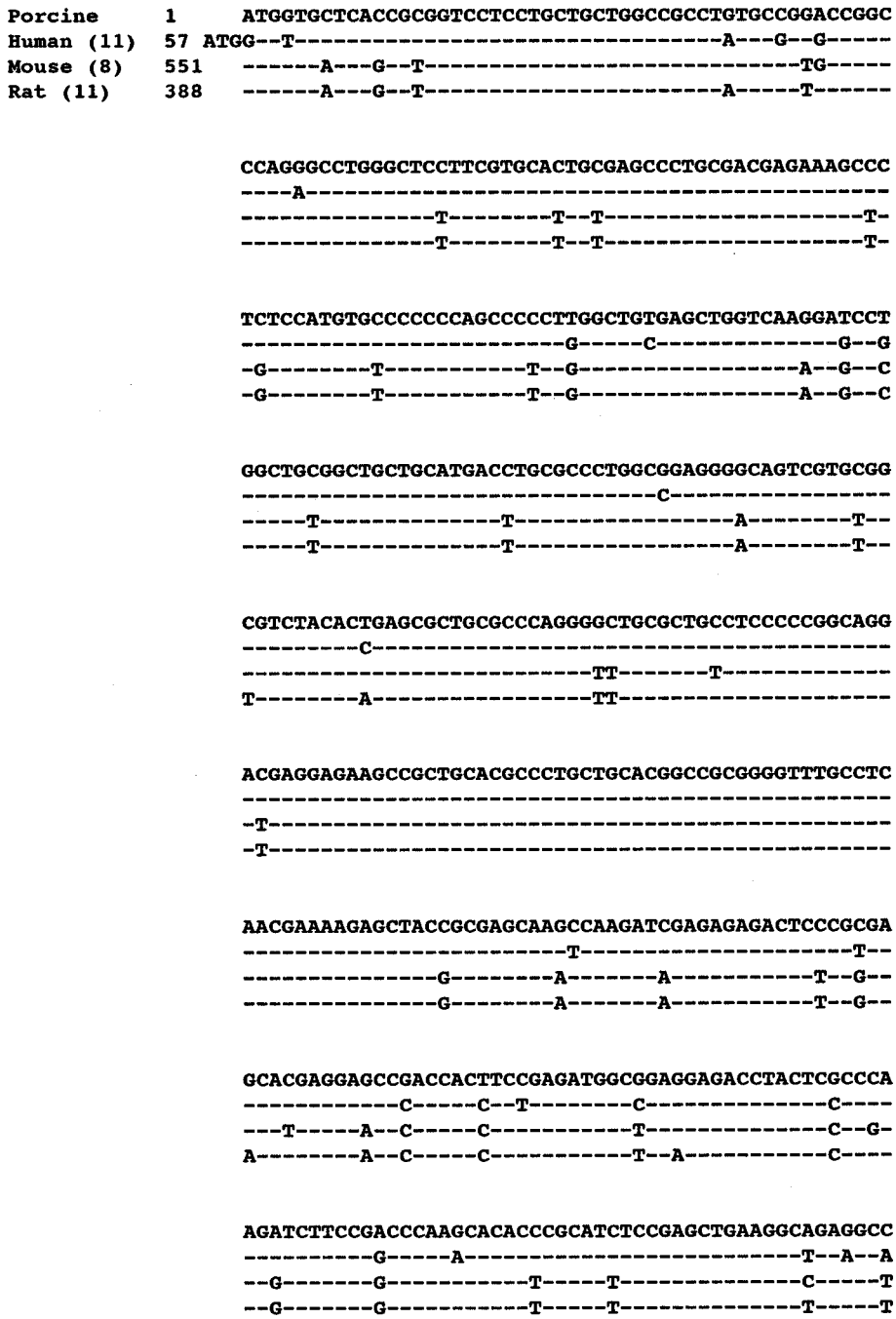

FIG. 1. cDNA sequence of porcine IGFBP-5 open reading frame compared with those of human, mouse and rat. Dashes indicate homology with the porcine cDNA sequence.

cDNA kindly provided by Dr. S. Shimasaki (Whittier Institute for Diabetes and Endocrinology, La Jolla, CA). Thirty positive plaques were selected, re-plated and re-screened using the hBP-5 cDNA probe. Six positive plaques were chosen and the Bluescript SK phagemids containing cDNA inserts were recovered. Plasmids were purified and the inserts sequenced in both directions using the dideoxy Sanger termination DNA sequencing method with α-^{35}S labeled dATP. The Porcine-Specific IGFBP-5 open reading frame cDNA sequence and deduced amino acid sequence were analyzed and

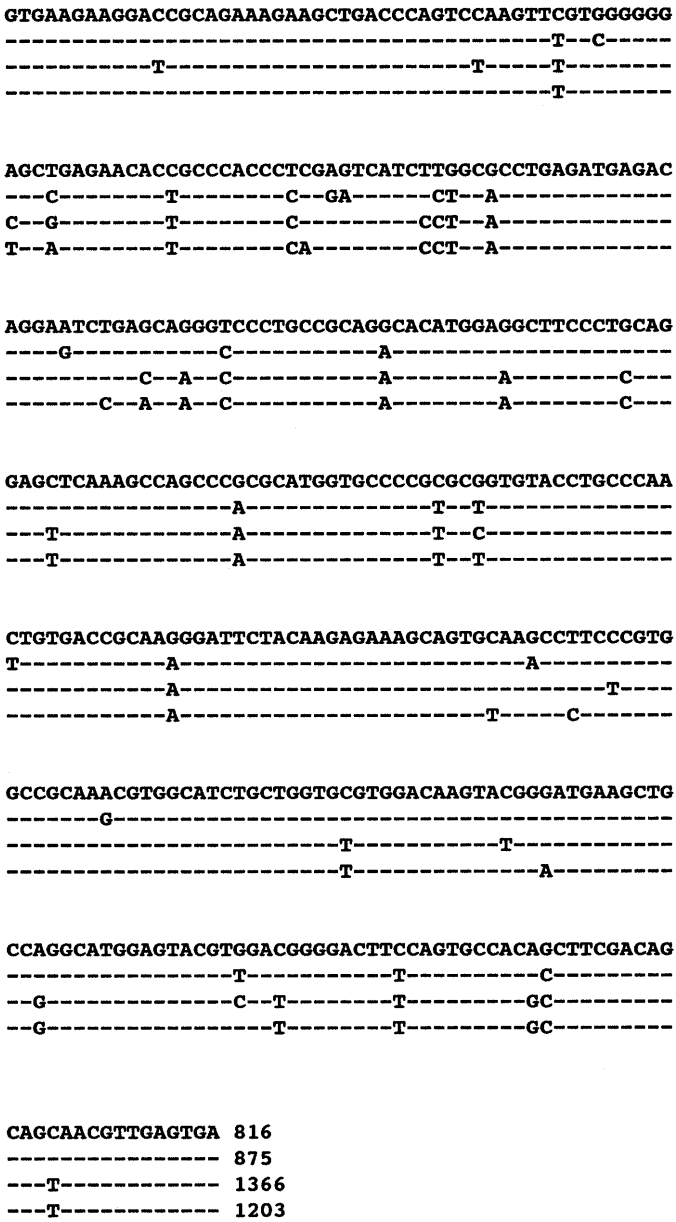

FIG. 1.—Continued.

compared with those of human, mouse and rat using the GCG Program Manual for the Wisconsin Package, Version 8, September 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711.

Northern Blot Analysis

Total RNA was isolated using the single step guanidinium thiocyanate procedure (10). Total RNA (75 μg) was denatured (24 mM HEPES, 6 mM Na-acetate, 1.2 mM EDTA, 50% deionized formamide, and 2.2 M formaldehyde) and fractionated on 1.2% agarose gel containing 2.2 M formaldehyde in 20 mM phosphate buffer, pH 7.0 RNA was transferred to a nylon membrane and hybridized with the porcine IGFBP-5 317 base ^{32}P-labeled c-DNA probe. Hybridization (1×10^6 cpm/ml) was conducted for 18 hrs at 42°C and blots were washed once in 1 × SSC, 0.1% SDS at 55°C for 15 min and twice in 0.1× SSC, 0.1% SDS at 60°C for 15 min each time and the hybridization signal was detected using autoradiography.

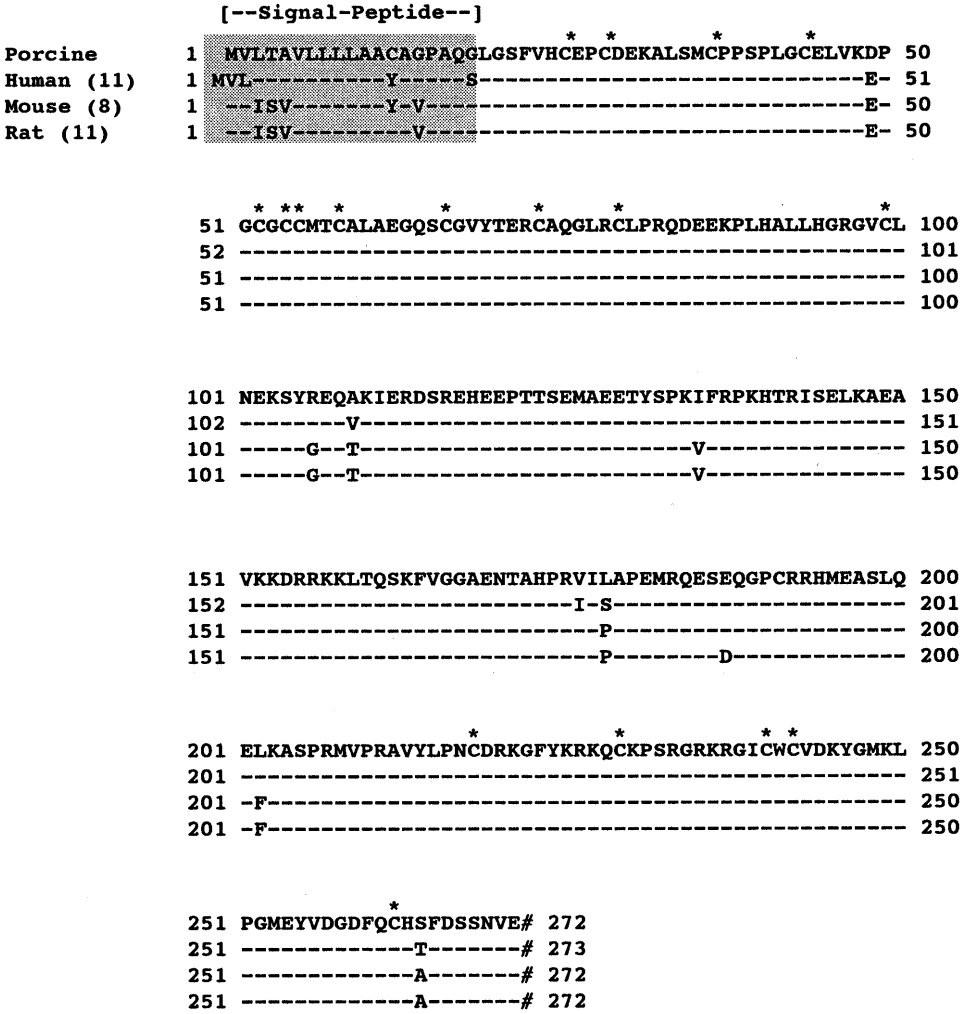

FIG. 2. Deduced amino acid sequence of porcine IGFBP-5 compared with those of human, mouse and rat. Dashes indicate homology with the porcine deduced amino acid sequence. Termination codon is indicated by (#). (*)Asterisks mark the 18 conserved cysteine residues in the mature peptide.

RESULTS AND DISCUSSION

We report here for the first time the isolation of a cDNA clone containing the open reading frame sequence for porcine GFBP-5 and the complete deduced amino acid sequence for this porcine IGFBP. A porcine skeletal muscle cDNA library was screened using a human IGFBP-5 cDNA probe and positive clones were isolated. Of the positive clones isolated two were purified to homogeneity. The clone with the largest insert was sequenced and contained the open reading frame for porcine IGFBP-5. Figure 1 shows the cDNA sequence of the porcine IGFBP-5 open reading frame and provides a comparison with the reported cDNA sequences for human, mouse and rat IGFBP-5. The porcine IGFBP-5 cDNA clone contained an open reading frame of 816 bases, coding for a signal peptide of 19 amino acids and a mature protein of 252 amino acids. The IGFBP-5 cDNAs for human (11), rat (11) and mouse (8) have been reported and the nucleotide sequence of the porcine IGFBP-5 cDNA is 94%, 91% and 90% homologous with the published DNA sequence for human, rat and mouse IGFBP-5 respectively (Table 1). Figure 2 shows the

TABLE 1
Comparison of Porcine IGFBP-5 cDNA and Deduced Amino Acid Sequence Characteristics

Species	Homology with pIGFBP-5 cDNA Open Reading Frame (%)	Homology with pIGFBP-5 Deduced a.a. Sequence (%)	Tissue Source of Library	# Cysteine Residues	# Amino Acids in Mature Peptide
Porcine	100	100	Muscle	18	252
Human	94	97	Placenta	18	252
Mouse	90	96	Myoblast	18	252
Rat	91	96	Ovary	18	252

deduced amino acid sequence of porcine IGFBP-5 compared with that of the human, rat and mouse peptide. The predicted amino acid sequence of the mature porcine IGFBP-5 is highly homologous to the deduced amino acid sequences for human (97%) mouse (96%) and rat (96%) (Table 1). These data are consistent with reports suggesting that IGFBP-5 is one of the most highly conserved of the IGFBPs in mammals (8). The porcine IGFBP-5 amino acid sequence contains the 18 conserved cysteines found in all IGFBPs (5). These cysteine residues in the porcine IGFBP-5 are in the same positions as those found in the human, rat and mouse homologs. These cysteine residues are highly conserved among all IGFBP classes and are thought to be important for specific binding to IGF.

To facilitate studies of IGFBP-5 regulation and function in porcine skeletal muscle, we constructed a porcine-specific IGFBP-5 probe from the original cDNA library insert for use in producing porcine-specific IGFBP-5 cDNA probes. Figure 3 shows the construction of the Porcine IGFBP-5 cDNA probe from the original library phagemid containing the cDNA fragment. A 317 base fragment of the Porcine IGFBP-5 open reading frame was isolated and ligated directionally into the Sac I and Sac II polylinker sights of pBluescript. This plasmid was used to transform competent E. Coli and the 317 base insert used as a porcine-specific IGFBP-5 cDNA to probe Northern Blots containing total RNA isolated from various porcine tissues. Figure 4 shows the results of a northern blot of total RNA isolated from porcine tissues and demonstrates that the porcine-specific IGFBP-5 cDNA probe recognizes a 6.0 kb IGFBP-5 mRNA which is similar to

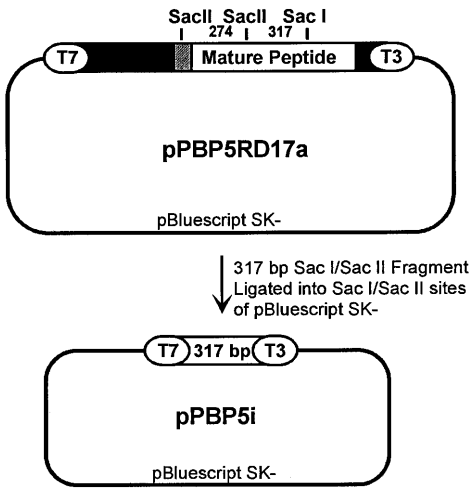

FIG. 3. Construction of the porcine IGFBP-5 cDNA probe from the original library phagemid containing the cDNA insert that included the entire open reading frame and portions of the 5′ and 3′ untranslated regions of porcine IGFBP-5.

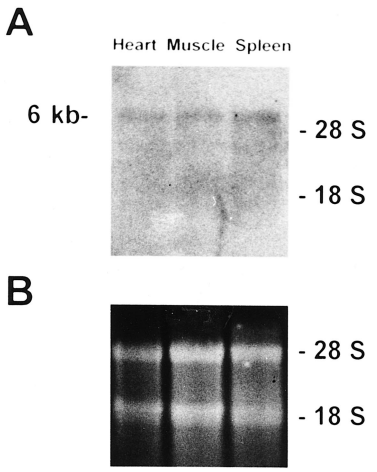

FIG. 4. A: Autoradiograph of northern blot analysis of 75 μg of total RNA isolated from porcine heart (H), Semitendinosus muscle (M), and spleen (S). Total RNA was fractionated on a 2.2 M formaldehyde, 1.2% agarose gel, transferred to a nylon membrane and hybridized with the 317 bp ^{32}P-labeled porcine IGFBP-5 cDNA. **B:** Ethidium bromide staining of the gel demonstrating integrity of ribosomal RNA and relative sample loading.

that reported for other species (11). Development of this porcine-specific IGFBP-5 cDNA probe will allow for further study into the role of IGFBP-5 in porcine muscle growth and development.

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