A Final Report to the

National Pork Producers Council

Title: Isolation of the Genes that Create Fat Cells in Pigs

Research Category: Breeding and Genetics

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Research Contract: **98-186**

Amount Funded: **$5,000**

Research Objective

Isolate the protein coding sequences for porcine adipose determination and differentiation factor 1 gene and the CAAT/Enhancer Binding Protein gene.

Research Expenditures

The entire funded amount of $5,000 was spent on research supplies, DNA sequencing services and undergraduate labor.

Results

The protein coding sequences were isolated from cDNA using reverse transcriptase-polymerase chain reaction. Based on the mammalian sequences for adipose determination and differentiation factor 1 gene (ADD1) and CAAT/Enhancer Binding Protein Alpha gene (C/EBPα) in the Genbank database, three pairs of PCR primers were designed to produce three overlapping PCR products (amino terminal, middle and carboxyl terminal) for each protein coding sequence. After initial attempts to amplify the PCR products using eLongase™ , only the middle and carboxy terminus fragments of C/EBPα produced an appropriate product. Due to the very high GC nucleotide content of both ADD1 and C/EBPα we used a commercial product, Advantage GC polymerase from Clontech. This product was able to amplify all three products from C/EBPα gene and the middle and carboxy terminal fragments of ADD1. The products from independent PCR reactions were cloned into Bluescript II SK+ and sequenced using ABS Prism automated fluorescence sequencing system operated by the Purdue University Cancer Center Core Facility.

The sequences from the independent PCR products were assembled into a consensus sequence and aligned with the corresponding human gene. Sequences amplified by the middle pair of ADD1 primers did not match the human gene to a significant degree and was therefore

some kind of nonspecific product. The carboxyl terminal PCR product of 828 bp had 84% identity with human ADD1 indicating that it was a specific ADD1 PCR product. Additional attempts to amplify the ADD1 sequence using various PCR techniques including touchdown PCR and alternate 5' sense strand and 3' anti-sense strand primers did not produce any ADD1 PCR products.

The sequences from independent PCR products produced for C/EBPα were also assembled into consensus sequences. The cloned products for the amino terminal PCR product would only produce about 50-60 bases of readable sequence out of the 400 bp product. This same result was obtained using two different automated sequencing protocols and one manual sequencing protocol that are designed to read sequences with high GC nucleotide content. This same region of the human sequence has over 80% GC nucleotide content. The middle fragment of 298 bp had two regions of very high identity (94%-95%) to human C/EBPα that was separated by a 60 bp region with very little identity between the pig and human sequences. This was found in independent PCR sequences and represents an island of evolutionary divergence in an otherwise highly conserved sequence. The sequence of the carboxyl terminal PCR product was 96% identical to human.

With the funding from the NPPC, we have isolated pig DNA sequences for two genes that induce the formation of mature fat cells. Additionally, a "hands on" learning experience on the application of molecular genetics to animal agriculture was provided for an undergraduate student. The sequences for the carboxyl terminal PCR products will be submitted to the national database for future use by those involve in pig adipose tissue research and will certainly aid in the identification of expressed sequence tagged sites generated for these two genes. The work

with ADD1 will not be pursued further as we have recently learned that the USDA group in Texas is working in this area. Although some further sequencing is required, we have sufficient sequence information to generate a full length sequence for C/EBPα for functional studies of this genes role in inducing adipogenesis. At this time, we do not have funding for the future objective.