

## SMAD SEQUENCES IN PPAR-GAMMA2 PROMOTER

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### SUMMARY

*PPAR-gamma2 is a critical gene in the development of fat cells where it acts as transcription factor that regulates the expression of fat specific genes such as FABP4. How PPAR-gamma2 is regulated in fat tissue development or in other tissues in which it is found is not known. To understand how this gene is regulated we sequenced the promoter region of PPAR-gamma2 in the pig and identified response elements that could bind transcription factors. These elements were compared to elements found in mouse and human sequences to determine which response elements are conserved between species.*

### INTRODUCTION

Obesity as a health issue continues to grow in the United States claiming a greater percentage of our adults and children every year. In the area of animal production we are concerned the development of products with greater health benefit where partitioning for lean meat with low fat content is important. We have approached the problem of obesity and lean meat production by investigating fat cell development. Fat cell development is driven by sequences in DNA called promoters that regulate gene expression. Transcription factors are proteins that bind specifically to the DNA sequences in the promoter areas of a gene and regulate its expression.

PPAR gamma 2 is a transcription factor which is critical in fat cell development and is induced when fat cell development is initiated ( Tontonoz, et al., 1994). We are interested in how PPAR gamma 2 is activated. To do this we have looked for transcription factors that bind to specific sequences in the PPAR gamma 2 gene promoter so that we may determine how this gene is regulated in the development of fat tissues. Putative enhancer or regulatory sequences can be identified with several programs that match unknown sequences to known consensus sequences for response elements. These matches based on consensus sequences often give many elements that are not functional. Thus, additional criteria are necessary to identify sequences that have a high probability of functional significance. We searched for promoter sequences that were conserved between species, and showed coupling with other conserved response elements. In addition, we tried to relate our findings to known responses of adipocytes and preadipocytes to signaling molecules

We found conserved sequences in the promoter region between all three species. Further analysis using Microsoft Excel identified three closely grouped conserved response elements representing two SMAD sites and one AP1 site. Conserved response elements would indicate highly important functions.

### MATERIALS AND METHODS

Human and mouse sequences for PPAR-gamma2 were obtained from GenBank. Promoter response elements were identified based on sequence matching to known consensus promoter elements using the MatInspector Pro program. Sequences from pig, human and mouse were aligned using the Jellyfish alignment program to find sequences conserved in all three species. Putative response elements were aligned using Microsoft Excel.

## RESULTS

The alignment between the proximal promoter (400 bp) of the mouse, human and pig PPAR gamma 2 sequence is shown in figure 1.

	961	971	981	991	1001	1011	1021	1031
<b>Mus mus PPAR</b>	-----	-----	-----	-----	-----	-----	-----	-----
<b>HS ppar g2 p</b>	-----	-----	-----	-----	-----	-----	-----	-----
<b>ppar seq 2 i</b>	CTTAGAACTTCC	ATATGCTGCAGGTACAGCCCTGAAAAGACACAAAA	AGAAAGCAA	GTGGATGTTGAACAGCCTC	---	TT		
Consensus		atat				aaaaagcaagtggatattgaacagctctctgctc		
	1041	1051	1061	1071	1081	1091	1101	1111
<b>Mus mus PPAR</b>	TGGTAATTC	CAACTACTGTACAGTT	CACGCCCTCACA	GACAGTGAAT	GTGGGTC	ACTGGCGAGAC	CAATGTAGCAAC	
<b>HS ppar g2 p</b>	TGATAATTC	TAATAACAGTACAGTT	CACGCCCTCACA	AAGACACTGAACAT	GTGGGTC	CACGGCGAGAC	AGTGTGCCAAT	
<b>ppar seq 2 i</b>	TGAGAGTTC	GAACACTGC	ACTGCTTGTGCCCTCACA	AAGACACTGAACAT	GTGGGTC	ACTGGCGAGAC	AGTGTGCCAAT	
Consensus	tgataattctaaatactgtacagttcacgcccctcacaagacactgaacatgtgggtcactggcgagacagtggtgcaat							
	1121	1131	1141	1151	1161	1171	1181	1191
<b>Mus mus PPAR</b>	GTTTTCCTT	GTAAATGTACCAAGTCT	TGCCAAAGCAGCAGAC	CAGCAATTATGACAC	CCATTTTGT	CACA	ACTGGCTCTCAG	
<b>HS ppar g2 p</b>	ATTTTCCTT	GTAAATGTACCAAGTCT	TGCCAAAGCAGTGAACA	---	TTATGACACA	ACTTTTGT	CACAGCTGGCTCCTAA	
<b>ppar seq 2 i</b>	GTATTCCTT	GTAAATGTACCAAGTCT	TGCCAAAGCAGTGAACA	AATATTATGACACA	ACGTTTTTGT	CGCAGCTGGCCCTAA		
Consensus	gttttcctt	gttaatgtaccaagtccttgccaaagcagtggaaca	attatgacacac	actttt	gttcacagctggtgctcctaa			
	1201	1211	1221	1231	1241	1251	1261	1271
<b>Mus mus PPAR</b>	TCAGGACAGT	GCCAGCCAA	ATTTCAGGCCTG	ATTTCTGTGTT	TATCCCACTCT	CTCCCAA	ATAATTTG	AAAAGTGGTGC
<b>HS ppar g2 p</b>	T-AGGACAGT	GCCAGCCAA	ATTTCAGGCCTG	ATTTCTGTGTT	TATCCCACTCT	CTCCCAA	ATAATTTG	AAAAGTGGTGC
<b>ppar seq 2 i</b>	C-AGGAGT	GTCAGCCAG	TTTCAGCCCTG	TCCTGTGGAGTT	GATTCCCACTCT	CTCC	--AGTATTG	GAAAAGTGGTGC
Consensus	t aggcagctgcccagccaaatttcaggcctgatttctgtgtttatcccaactctcccaaatatttggaaaactgatgctc							
	1281	1291	1301	1311	1321	1331	1341	1351
<b>Mus mus PPAR</b>	TTGACTTAT	TAGCATATTCATAAGCT	CGATGACCATAAGC	TTTTTCTTTA	ACCAACCAAT	CTTTTGC	AAGCATAGAC	
<b>HS ppar g2 p</b>	TTGACTCAT	GGTGTATTCCAAATTC	TGTTACTTCAAGT	TTTTTCTTTTAA	CGGAATTGAT	CTTTTGT	AGATAGAGAC	
<b>ppar seq 2 i</b>	CTGACTCAT	TGTCCTTCCAGT	TCTACAACAGGAAT	TTTTTCTTTA	TGGAATTGGC	TTTTTGC	AAGAAATAGAC	
Consensus	ttgactcattggtgtattcacaagttctat	acc	aagtcctttctttaa	cggattgatcttttgc	aaga	atagac		
	1361	1371	1381	1391	1401	1411	1421	1431
<b>Mus mus PPAR</b>	AAAA	CACAGTGTGAATTACAGCAA	ATCTCTGTTTATGCTGTTATG	-----				
<b>HS ppar g2 p</b>	AAAA	TACAGTGTGAATTACAGCAA	ACCCTTATCCATGCTGTTATG	-----				
<b>ppar seq 2 i</b>	AAAA	TACAGTGTGAATTACAGCAA	AGCCCTTATCCATGCTGTTATG	GGTGAAACTCTGGGAGATTCTCTTATTGACCCA				
Consensus	aaaatatcagtggtgaattacagcaaaccttattccatgctggttatg							
	1441	1451	1461	1471	1481	1491	1501	1511

Figure 1. Sequence Alignment for mouse PPAR (Mus mus), human PPAR (HS ppar g2 p) and pig PPAR (ppar seq 2 i). Sequences that are shaded are conserved across all species. The ATG start codon begins at base pair 1405.

Several regions of homology can be observed in the alignment shown in figure 1. Further analysis of these homologous sequences identified SMAD core elements (GTCT) at 1142-1146 and (AGAC) at 1105-1109. Matrix similarity determined by the MatInspector program was at 94 to 96%. These sequences are conserved between the three species. Other sequences that

have been reported in the PPAR gamma 2 promoter did not show conservation between the species. In addition an AP1 site was found to be conserved at 1095-1099. These sites are all within 50 bp of each other and could work together to provide gene regulation.

## **DISCUSSION**

The PPAR gamma 2 gene is important in fat development. The transcription factors responsible for directing the expression of this gene can be identified by examining the sequences in the promoter region of this gene. We have found two tandem SMAD response elements which are conserved in mouse, pig and human. In addition we have found a conserved AP1 transcription factor binding site in very close proximity to the conserved SMAD sites which further indicates that the conserved SMAD sites actively participate in the regulation of PPAR-gamma 2

If the PPAR-gamma2 responds to SMAD transcription factors then it is likely that it is being regulated by TGF-beta. TGF beta is known to activate SMAD transcription factors and in enhance cell proliferation and prevent fat cell differentiation in pig stromal-vascular cells (Frederick et al., 2006; Norwitz et al., 2002). This suggest that the SMAD response elements in the PPAR-gamma2 promoter may act to inhibit the expression of PPAR-gamma 2. If this is the case then these sites may be manipulated to enhance the inhibition of fat cell development.

## **LITERATURE CITED**

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