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SHORT COMMUNICATION

Mapping of porcine ESTs obtained from the anterior pituitary

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Abstract: We report the physical mapping of porcine expressed sequence tags (ESTs) from anterior pituitary clones isolated by differential display PCR in a study using lines selected for reproduction. These ESTs were mapped using a somatic cell hybrid panel (SCHP) and a radiation hybrid panel (IMpRH) as follows (SCHP position, nearest marker on the RH map): *SPARCL1* (8q23–q27, *SSP1*); *ATF4* (5p11–p15, *AC02*); *MEF2C* [2(1/2q21)–(1/2q22), *SW2134*]; *FTH1* (2p14–p17, *SWR783*); *FRAP1* (6q22–q23, *SW1355*); *PBP* (14, *SW2508*); *LOC92004* [13q23-(1/2q41), *CP*]; and *PGRMC1* [Xq22, *SW1943*]. All RH assignments were at LOD score >6.0 except for *PGRMC1* at LOD score 5.4. ESTs *TCP1* [12p11-(2/3p13)], *SF3B1* (15q23–q26) and *Clock* (8q11–q12) were assigned using only the SCHP. The map position of *SPARCL1* coincides with a quantitative trait loci (QTL) for age at puberty found in the University of Nebraska selection lines. Physical mapping of ESTs reported in the present study contributes to characterization of the transcriptome of anterior pituitary of pigs, adds new information to the public database of the porcine genome expression map, and further develops the porcine–human comparative map.

Keywords: anterior pituitary, ESTs, mapping, swine.

Genetic maps generated by the use of somatic cell hybrid panels (Yerle *et al.* 1996) provide information on the physical location of genes and, along with painting studies (Goureau *et al.* 1996), provide information on synteny conservation across species. Although genetic and physical maps are important for positional cloning and positional candidate gene approaches, they are not comprehensive and significant gaps exist. Higher resolution maps can be obtained by means of radiation hybrid panels (Yerle *et al.* 1998), and inclusion of temporally and spatially characterized expressed genes onto the radiation hybrid (RH) map help to merge genetic and physiological approaches to gene discovery. The present study reports somatic cell hybrid panel (SCHP) and RH panel mapping of EST markers from clones isolated in an anterior pituitary differential display project (Bertani 2001) designed to identify genes differentially expressed between a line selected for enhanced reproduction and a randomly selected control line (Johnson *et al.* 1999).

The 11 ESTs described herein represent previously unmapped genes from a total of 168 ESTs that were characterized (excised from gels, purified and sequenced) in the differential display study. Primers for amplifying the ESTs were designed using Primer3 (Rozen & Skaletsky 2000). Most of the primers were designed in the 3' UTR. For *TCP1* and *FTH1*, only one primer was designed in the 3' UTR and the *Clock* and *SPARCL1* primers were designed within coding sequence. Primers were then optimized for PCR (Table 1). The SCHP used in this study included 27 hybrids (Yerle *et al.* 1996). Data were submitted to the somatic cell hybrid panel common database for analysis (http://0-www.toulouse.inra.fr.library.unl.edu/lgc/pig/pcr/pcr.htm). Clones of the IMpRH ($n = 118$; Yerle *et al.* 1998) were genotyped for each EST in duplicate. Data were submitted to the IMpRH server (Milan *et al.* 2000) for analysis against the RH map built by Hawken *et al.* (1999). Mapping results for SCHP and RH panels are presented in Table 2. Of the mapping assignments for the 11 genes in the present study, all are in agreement with painting studies (Goureau *et al.* 1996) and confirm previously known pig–human conservation, except for *TCP1*. In the case of *TCP1*, results from RH mapping place this gene in the extremity of a linkage group (Hawken *et al.* 1999) on chromosome 1 with a LOD score of 4.4, or alternatively, linked to *SW943* on chromosome 12 with a LOD score of 3.04. While assignment of *TCP1* to SSC12 is in agreement

Table 1 Information on mapped ESTs isolated from pig anterior pituitary and their PCR amplification conditions.

Accession number	Gene symbol	Gene name	Primers 5′ → 3′	PCR conditions[1]
BE344576	*SPARCL1*	SPARC-like 1 (*mast9, hevin*)	F – TCCTTGATTACCCTGCTCAAA R – AGCGAGCAGGAAGAAGACAG	62 / 2.0 / 111
BE344562	*ATF4*	Activating transcription factor 4 (tax-responsive enhancer element B67)	F – TTGCAAACTCTTCCCCTTTC R – GGGCTCATACAGATGCCACT	60 / 1.5 / 197
BE344561	*MEF2C*	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)	F – GGTTCTTTAATGGGATTTCTTGA R – TTGCTTCATCATCCGTTTTT	58 / 1.5 / 190
BE344556	*FTH1*	Ferritin, heavy polypeptide 1	F – GTATGGCATGGCCGAGTATC R – GTGACGGTAACCCGAAACAT	60 / 1.5 / 139
BE344575	*FRAP1*	FK506 binding protein 12-rapamycin associated protein 1	F – CCGACACAGAGAAGGAAGGT R – TCGATGTCATTTATTGGCACA	60 / 2.0 / 143
BE344574	*PBP*	Prostatic binding protein	F – GCAAACAGCAGAATTAAAGCAATG R – CAGAAATCTGAAAGTCTGTGCCTGT	60 / 1.0 / 101
BE344552	*PGRMC1*	Progesterone receptor membrane component 1	F – CATTGTATTTTTCTTGTGAACCGTGT R – CCGGGTAAAACCATTTTATTAACTGAC	65 / 1.5 / 97
BE344563	*LOC51714*	Selenoprotein T	F – CGGGACTGACGTTATGAAGG R – CTGAATGGATTTGGGGAAGA	60 / 1.5 / 199
BE344560	*TCP1*	T-complex 1	F – TCAATGGTAAACCCCGAGAC R – TCCAGCTTGTTGCACTTTTAATG	60 / 2.0 / 278
BE241029	*SF3B1*	Splicing factor 3b, subunit 1	F – GCGTAGAACTGGTCATAGAAGAA R – TCTTGGTCACTACTGGCGTTT	60 / 2.0 / 106
BE344559	*Clock*	Clock homologue (mouse)	F – GAGCAATTCAAATGGCAACA R – CACCAAAAGAGACCACTGAGC	60 / 1.5 / 197

[1]Optimal annealing temperature in °C/$MgCl_2$ concentration in mM/length of amplified fragment in base pairs.

Table 2 Somatic cell hybrid panel and radiation hybrid panel mapping results, and comparative mapping data, for ESTs isolated from pig anterior pituitary.

Gene symbol	Accession number[1]	Percent homology	SCHP assignment	R^2	RHP assignment	Linked marker	LOD score	Human chromosome[2]
SPARCL1	NM_004684[h]	87	8q23–q27	0.85	8	SSP1	22.87	4q21.3[a]
ATF4	NM_001675[h]	86	5p11–p15	0.86	5	*AC02*	7.78	22q13.1[a]
MEF2C	NM_002397[h]	93	2(1/2q21)–(1/2q22)	0.85	2	*SW2134*	13.26	5q14[a]
FTH1	D15071[p]	95	2p14–p17	0.87	2	*SWR783*	18.42	11q13[a]
FRAP1	U88966[h]	89	6q22–q23	1.00	6	*SW1355*	18.39	1p36.2[a]
PBP	BC031102[h]	85	14	0.87	14	*SW2508*	13.42	12q24.22[a]
rid *PGRMC1*	X99714[p]	100	Xq22	0.84	X	*SW1943*	5.35	Xq22–q24[a]
LOC51714	NM_016275[h]	94	13q23-(1/2q41)	1.00	13	*CP*	8.51	3[b]
TCP1	BC000665[h]	92	12p11-(2/3p13)	0.73	1	*SW1824*	4.41	6q25–q27[a]
TCP1[3]					12	*SW943*	3.04	
SF3B1	NM_012433[h]	94	15q23–q26	0.81	–	–	–	2q33.1[a]
Clock	AF000998[m]	82	8q11–q12	0.69	–	–	–	4q12[a]

[1]Accession number of DNA sequences of human[h], pig[p], and mice[m] that have homology with the anterior pituitary ESTs. These data were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) using the nr nucleotide sequence database (September, 2002).
[2]Human chromosome location retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) at unigene[a] or locuslink[b] (September, 2002).
[3]Alternative RHP assignments were obtained for this gene.

with the SCHP results, an assignment to SSC1 is supported by known pig–human conservation. Markers *SF3B1* and *Clock* were not analysed using the IMpRH.

This report has placed 11 new EST markers on nine pig chromosomes. These data contribute to characterization of the porcine anterior pituitary transcriptome during a defined peri-

od of follicular development. In the present study, *SPARCL1* was assigned within a QTL region for age at puberty previously identified in these selection lines (Cassady *et al.* 2001). While integration of functional analysis and mapping information identifies *SPARCL1* as a positional candidate gene for age at puberty in pigs, the function of this gene is not yet well understood.

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