Gene Mapping Report

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Assignment[1] of the Homeobox A10 gene (HOXA10) to porcine chromosome SSC18q23→q24 by FISH and confirmation by hybrid panel analyses

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[1] To our knowledge this is the first time this gene has been mapped in swine.

Rationale and significance

Homeotic or homeobox genes represent selector genes involved in the genetic control of development and organization of the body plan. They are characterized by a common highly conserved sequence stretch of approximately 60 amino acids, the homeodomain (McGinnis et al., 1984). In vertebrates, four homeobox gene clusters (HOXA, HOXB, HOXC, and HOXD) have been described (Scott, 1992). In each cluster, the Hox genes are organized along the chromosome corresponding to their function along the anteroposterior axis of the individual (McGinnis and Krumlauf, 1992). Using heterologous HOXA11 probes Lahbib-Mansais et al. (1996) mapped the HOXA cluster to porcine chromosome SSC18q21→q24. Here we report the localization of the porcine HOXA10 gene to chromosome SSC18q23→q24 by FISH and confirm the position by the use of hybrid panels.

Materials and methods

Isolation of the porcine HOXA10 gene from a PAC library

PCR amplification to generate a probe for screening of a porcine PAC library (Al-Bayati et al., 1999) was done with primers A (forward: 5′-CAG CCA ACT GGC TCA CGG CA-3′) and B (reverse: 5′-AGT TGG CTG TGA GCT CCC GG-3′) designed from the murine *HoxA10* mRNA (EMBL accession no: L08757). PCR amplification was performed using 50 ng of porcine DNA in a total volume of 25 µl. DNA was preheated at 95 °C for 5 min, and the PCR profile used was: 30 cycles of 95 °C for 60 s, 58 °C for 60 s, and 72 °C for 60 s. The final cycle had an extension time of 10 min. The resulting fragment of 239 bp was bidirectional sequenced (with tagged primers) and a similarity of 91 % between the probe and the mouse mRNA confirmed the sequence identity. A PAC clone of approximately 120 kb was identified by PCR screening of the PAC-library with the above mentioned primers. *Xba*I subclones were generated. Southern blots were done and the probe hybridized to a 15.5-kb fragment, which was sequenced and found to contain the complete sequence of the porcine HOXA10 gene.

Fluorescence in situ hybridization (FISH)

FISH was performed as described previously by Toldo et al. (1993) and Solinas-Toldo et al. (1995) using swine metaphase spreads (prepared from peripheral lymphocytes) obtained from a normal, healthy boar. Prior to FISH, the QFQ-banded spreads were photographed using a cooled CCD camera. Hybridization signals were detected and amplified by incubation with Streptavidin-Cy3 (Rockland, Gilbertsville). The chromosomes were then DAPI-counterstained (Sigma, Deisenhofen). The relative positions of the signals on the chromosomes were measured considering the distance to the centromere and the length of the entire chromosome enabling the calculation of the fractional length (Flcen).

Hybrid panel analyses

A porcine-rodent somatic cell hybrid panel (Yerle et al., 1996) and a porcine whole-genome radiation hybrid panel (Yerle et al., 1998) were screened for porcine HOXA10 by PCR. Primers (forward: 5′-TGC AGG CAG GCT GAC CTT GT-3′; reverse: 5′-AGA TGA GGC GAA CGC GGA G-3′) originated from intron 1 of porcine HOXA10. PCR amplifications of a 116-bp fragment were performed in a total volume of 25 µl with 25 ng of panel DNA as template. Cycling conditions were 95 °C for 45 s, 54 °C for 30 s, and 72 °C for 45 s for 33 cycles. PCR involved a preheating step at 95 °C for 5 min plus a final extension at 72 °C for 10 min. PCR results were evaluated using the interpreting web pages http://imprh.toulouse.inra.fr (radiation hybrid panel) and http://www.toulouse.inra.fr/lgc/pig/hybrid.htm (somatic cell hybrid) at INRA.

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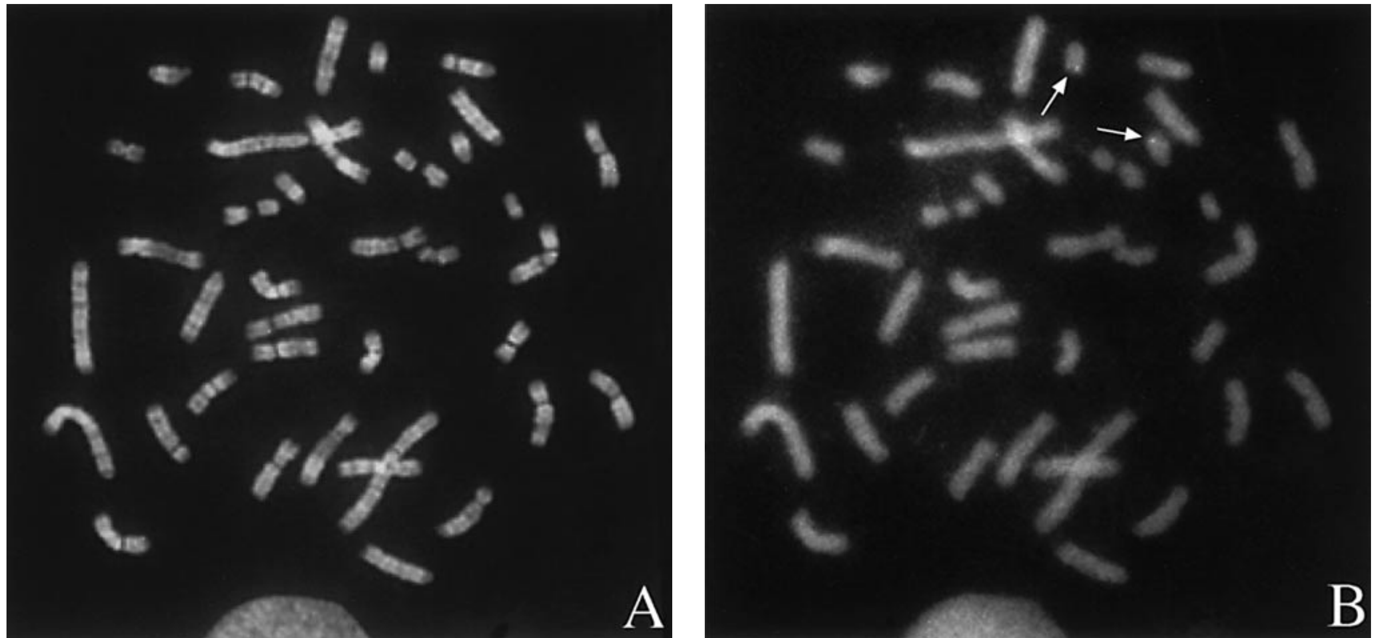

Fig. 1. Chromosome assignment of HOXA10 by FISH. (**A**) Q-banding of the metaphase spread. (**B**) Detection of signals on porcine chromosome SSC18.

Results

Fluorescence in situ hybridization
Most precise location: SSC18q23→q24
Flcen: 0.78 ± 0.049 (Fig. 1)
Chromosomes measured: 8

Somatic cell hybrid panel

Somatic hybrid panel analysis gave the following vector: 00001 10110 00000 00011 01000 00. Statistical evaluations revealed a significant correlation of 1.0 between the HOXA10 gene and SSC18 (error risk lower than 0.1 % and maximum correlation of 0.90). Within SSC18, chromosome region q13→q21 indicated the highest probability of 0.80 with a correlation of 0.9035.

Radiation hybrid panel

Radiation hybrid panel analysis resulted in the following vector: 11011 11001 10010 11000 11000 01110 01001 01000 00101 01000 10010 00111 00100 01010 00000 01010 00000 00010 01000 00000 00100 01101 10011 100. The most significantly linked marker (two-point-analysis) is S0120 on SSC18 (56 cR and LOD score of 7.65). Multi-point-analysis leads to linkage group SW1682–S0062–S0120–HOXA10–S0177. SW1682 is assigned to SSC18q23, whereas S0062 and S0177 are assigned to SSC18q24 confirming the localization of HOXA10 to SSC18q23→q24.

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