Assignment¹ of *SOX1* to platypus chromosome 20q by fluorescence in situ hybridization

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¹ To our knowledge this is the first time this gene has been mapped in platypus.

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Rationale and significance

The platypus belongs to the subclass of monotreme mammals that is most distantly related to humans, so data on gene arrangement and sequence will be of unique value for comparative studies (Grützner and Graves, 2004). Sequencing of the platypus genome (www.genome.gov/12512287) is almost complete. The task is now to identify comparative anchor points on the platypus genome, which is almost entirely unmapped. We isolated and mapped a platypus BAC containing *SOX1* (SRY-like HMG box containing gene 1). *SOX1* has roles in neural cell fate determination (Pevny et al., 1998) and lens development (Nishiguchi et al., 1998), and orthologues are highly conserved throughout vertebrates (Koopman et al., 2004). The assignment of *SOX1* in this study is the first gene from human chromosome 13 to be mapped in platypus and the first sequence mapped to platypus chromosome 20. *SOX1* will therefore provide a valuable anchor locus for aligning the platypus and human genomes.

Materials and methods

A platypus (*Ornithorhynchus anatinus*) genomic BAC library (Clemson University Genomics Institute) was screened with a *SOX3* probe amplified by PCR from male platypus genomic DNA using the primers 5'CACAACCTCGGAGATCAGCAA3' and 5'GTTGGTCCAGCCGTTGAC3'. Cycling parameters were: 94°C for 2 min, 35 cycles of 94°C for 30 s; 58°C for 30 s; 72°C for 1 min, then a final cycle of 72°C for 10 min. A positive BAC clone was obtained (117-J7) and verified by PCR using the previously described primers. Cycling parameters were: 94°C for 2 min, 35 cycles of 94°C for 30 s; 54°C for 30 s; 72°C for 1 min, then a final cycle of 72°C for 10 min. The PCR product was sequenced and confirmed to contain *SOX1*. The positive BAC clone was labeled with digoxigenin-11-dUTP by nick translation, pre-annealed with 1 µg platypus Cot-1 DNA and hybridized to male and female platypus metaphase chromosomes. Hybridization was detected with Cy3 conjugated to anti-digoxigenin antibodies; chromosomes were counterstained with DAPI.

Probe name: 117-J7

Probe type: platypus genomic DNA BAC clone

Insert size: ~240 kb

Vector: pCUGI-Bac1

Proof of authenticity: partial sequencing

Gene reference: DQ157771

Results

Mapping data:

Most precise location: 20q, identifiable by size (the second smallest autosome) and morphology (submetacentric) (Grützner et al., 2005)

Number of cells examined: male (10), female (10)

Number of cells with specific signal: Male cells 1 (0), 2 (0), 3 (4), 4 (6) chromatids per cell

Female cells 1 (0), 2 (2), 3 (5), 4 (3) chromatids per cell

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Fig. 1. (a) A female platypus metaphase spread showing hybridization of the *SOX1*-containing BAC to chromosome 20 (in female platypus the third smallest pair). Insert: Chromosome 20 pair from a male metaphase spread. **(b)** Inverted DAPI image of **a**.

References

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