Assignment¹ of the thymosin beta 4 X/Y chromosome (TMSB4X/Y) gene to tammar wallaby chromosome 5p by fluorescence in situ hybridisation

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¹ To our knowledge this is the first time this gene has been mapped in tammar wallaby or any other marsupial.

Rationale and significance

All mammals have an XX female:XY male sex chromosome system. The X and Y are thought to have evolved from a homologous pair of autosomes by a process of Y degradation. This process left a small, heterochromatic and gene-poor Y, and a relatively unchanged X chromosome (Ohno, 1967).

The origin of the human sex chromosomes has been explored by comparing their gene content with that of sex chromosomes from distantly related mammals. Previous work has defined conserved (XCR, shared with the X of marsupials and monotremes) and added regions of the human X (XAR, homologous to marsupial and monotreme autosomal regions, represented by chromosome 5p in the tammar wallaby) (Watson et al., 1991; Spencer et al., 1991). As a degraded X, the Y also consists of conserved (YCR) and added (YAR) regions (Graves, 1995; Waters et al., 2001).

We cloned and characterized the tammar wallaby homologue of TMSB4X (GenBank accession number AY341342), a gene with a copy on both the X and Y in human, and mapped it by fluorescent in situ hybridisation. No signals were found over the X or Y chromosome, but clear signals were apparent on

chromosome 5p. This location is consistent with the hypothesis that the region of human Xp that includes TMSB4X was recently added (XAR), and that the human Y is largely derived from the same added region.

Materials and Methods

A tammar wallaby lambda genomic DNA library was screened with a labelled mouse *Tmsb4x* cDNA probe (Supplied by Dr. M. Mitchell, INSERM, Marseille, France; GenBank accession number NM021109). Two final positive clones were isolated and sequencing revealed that one, MeugTMSB4X2.1, contained the wallaby TMSB4X gene. The wallaby TMSB4X clone was biotin-14-dCTP labelled by nick translation and hybridised to wallaby metaphase chromosomes. Hybridisation was detected using anti-biotin raised in goat and anti-goat raised in rabbit coupled with FITC. The chromosomes were counter-stained with DAPI.

Probe name: MeugTMSB4X2.1

Probe type: Genomic DNA

Insert size: Approximately 20 kb

Vector: Lambda EMBL3

Proof of authenticity: Sequencing

Results

Mapping data

Location: 5p proximal region (Fig. 1)

Number of cells examined: 28

Number of cells with specific signal: 1(1), 2(5), 3(6), 4(16) chromatids per cell.

Most precise assignment: 5q proximal region

Location of background signals (sites with > 2 signals): none observed

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Fig. 1. FISH mapping of MeugTMSB4X2.1. Hybridisation signals were observed on the short arm of chromosome 5.