Widespread expression of the testis-determining gene *SRY* in a marsupial

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There is compelling evidence from mutation analysis[1] and transgenesis[2] that the *SRY* gene isolated from human[3] and mouse[4] encodes the testis-determining factor on the mammalian Y chromosome. However, how *SRY* achieves this function is unclear. Although marsupials have been separated from eutherian mammals for ~100 million years, *homologues of* SRY have been localised to the Y chromosome of two unrelated marsupial species, the tammar wallaby and the Darling Downs dunnart[5]. Gonadal development is fundamentally similar in eutherian and marsupial mammals, but the timing of morphological events is different. Fetal *Sry* transcripts are confined to somatic cells of the male mouse genital ridge between 10.5–12.5 days *post coitum*[6], corresponding with the onset of testis differentiation. Analysis of *Sry* gene expression in the genital ridge of normal and germ cell-deficient fetal mice has established that this gene acts in the somatic cell lineage[6], and is presumed to induce the formation of Sertoli cells[7,8]. This assumption can be tested more critically in the tammar, where the equivalent stages of testis differentiation are observed over a 7-day period[9]. We have examined the relationship of *SRY* expression to testis differentiation in the tammar wallaby. We show the the marsupial *SRY* gene cannot be exclusively coupled to Sertoli cell differentiation, as this gene is expressed in the male fetus from several days before genital ridge formation until 40 days after birth. *SRY* transcripts are also present in a variety of extra-gonadal tissues in the developing young and adult male, a pattern of *SRY* expression similar to that observed in humans. These data indicate that, in addition to a role in testis determination, *SRY* may have other functions.

Total RNA was extracted from pooled tammar fetuses and neonatal gonads and analysed for *SRY* expression using reverse transcription and polymerase chain reaction (RT-PCR). In the tammar, the gonadal primordium is first apparent at day 21 of the 26.5 day gestation. By day 25 the cytoplasmic:nuclear ratio in male pre-Sertoli cells is greater than in female cells, but it is not until two days post-partum that seminiferous cords are obvious with putative Leydig cells appearing in the interstitium[9]. We initially tested for the presence of *SRY* transcripts from the day after the genital ridge is established (day 22 of gestation) until two days after the development of seminiferous tubules (day 4 *post partum*). Surprisingly, *SRY* expression was detected throughout this entire developmental period in male fetal tissue and in gonads from male pouch young (Fig. 1*a*). We therefore extended our sampling period from day 19 of gestation (early head-fold; ~20 somites) to 40 days after birth and established that in the tammar *SRY* is expressed from at least 6 days before the appearance of Sertoli cells until long after the immature testis has fully differentiated (Fig. 1*b*). Thus, expression of the tammar *SRY* gene does not result in immediate Sertoli cell differentiation.

Examination of the distribution of tammar *SRY* transcripts showed that *SRY* mRNA is expressed in all male fetal and pouch young tissues assayed, with the exception of liver. Female tissues also were tested to ensure that the *SRY* primers did not amplify sequences of *SRY*-related genes common to both sexes and, as expected, no bands were seen (Figs 1 and 2). *SRY* transcripts were observed not only in fetal gonad with attached mesonephros, but also in heart and brain (Fig. 2*a*), and skin, limb bud, phallus, adrenal, kidney and lung tissues (data not shown). In male pouch young, *SRY* transcripts were found in gonad and brain, at a lower level in heart and mesonephros (Fig. 2*a*), and in phallus, kidney and lung (data not shown). In adult males, *SRY* mRNA was detected in testis, epididymis and brain, at lower levels in kidney and heart, and was undetectable in prostate or liver (Fig. 2*b*). RNase A treatment of RT(+/–) products, as described[10], confirmed that the RT(+) bands in Figs 1 and 2 correspond to transcripts and that the presence of a faint band for some RT(–) samples represents genomic DNA contamination (data not shown). The pattern

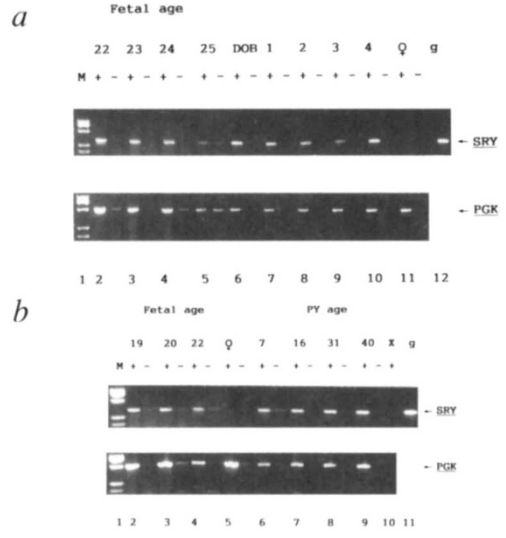

Fig. 1 Time course of tammar *SRY* expression. Total RNA preparations from fetal tissue (posterior to the forelimb bud) or pouch young (PY) gonads were used in a reverse transcription reaction, with (+) and without (–) reverse transcriptase (RT). Separate PCR reactions were performed, using the RT products or tammar genomic DNA, with primer pairs for *SRY* (upper panel) or *PGK* (lower panel). A 405-bp control *PGK* band was present in all RT(+) samples and a 296-bp *SRY* band was found for all male RT(+) and genomic DNA samples. *a*, Lanes 1, molecular size markers (bp) 577, 500, 404, 242 and 190; 2, one male fetus at day 22; 3, three male fetuses at day 23; 4, two male fetuses at day 24; 5, one male fetus at day 25; 6–10, gonads from one male PY at, day of birth (DOB) and days 1–4 after birth; 11, gonads from a single female 1 day after birth; 12, male genomic DNA. *b*, Lanes 1, molecular size markers; 2, two male fetuses at day 19, 3, two male fetuses at day 20, 4, one male fetus at day 22, 5, one female fetus at day 22; 6-9, gonads from a single male PY at 7, 16, 31 and 40 days, respectively, 11, male genomic DNA. A *PGK* band (lower panel) is present for all samples except when template was omitted (lane 10).