Minireview

New clues to the puzzle of mammalian sex determination

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Published: 29 August 2001

Genome Biology 2001, 2(9):reviews1025.1—1025.4

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2001/2/9/reviews/1025

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Abstract

WT1 and SOX9 are transcription factors with critical roles in mammalian sex determination and gonadal development. Recent studies in vivo clarify the roles of two alternative splice isoforms of WT1, and demonstrate that SOX9 can induce male sex determination.

In mammals, testis determination is normally initiated in males by expression of the Y-chromosomal HMG-box transcription factor gene SRY in the bipotential gonad common to males and females. A handful of other genes, including those encoding the SRY-related transcription factor SOX9, the zinc-finger transcription factor WT1, the orphan nuclear receptors DAX1 and SF1, and the TGFβ-like signaling molecule AMH, are also known to play roles in sex determination and subsequent gonadal development (for reviews see [1,2]). Although the number of genes known to be involved in sex determination and gonadal development is not large, protein-protein interactions and regulatory relationships among their protein products are complex (see below and Figure 1) and are the subject of intense study. Two recent reports from the laboratory of Andreas Schedl [3,4] shed some light on the roles played by WT1 and SOX9. Hammes et al. [3] addressed the individual molecular functions of two splice-generated isoforms of WT1 (designated WT1-KTS and WT1-KTS) by creating isoform-specific deletions in mice. In a separate but related study [4], the Wt1 promoter was used to drive the overexpression of Sox9 in XX genital ridges, in order to test the hypothesis that Sox9 is sufficient to induce the full spectrum of male sexual development in mammals.

Activation, repression, and interaction - the evidence so far

Previous studies have shed some light on the complex interrelationships among the sex determination and sexual differentiation genes in mammals (Figure 1). During male development, SRY activity leads to the expression of SOX9, which in turn activates transcription of Amh and genes required for testis development (reviewed in [5]). WT1 and SF1 form heterodimers and, together with SOX9, synergistically activate the expression of Amh [6,7]. WT1-KTS isoforms appear to play a role in the initial activation of SRY expression [8,9]. In female development, DAX1 antagonizes the activation of both Amh [6,10] and Sox9 (see [11]), probably through a direct association with SF1 [12,13]. Dax1 itself may be activated by WT1-KTS and SF1 [14,15].

The many roles of WT1

At least 24 different isoforms of WT1 have been described. The best-studied variants are known as WT1-KTS and WT1-KTS; they differ by the presence or absence, respectively, of a lysine-threonine-serine motif between the third and fourth zinc fingers. WT1-KTS associates with the spliceosome and binds to RNA, suggesting a role in RNA processing, whereas WT1-KTS exhibits diffuse nuclear staining and appears to act as a classical transcription factor [6,9,15-17]. Clinical and experimental data indicate that WT1 plays important roles in kidney and gonadal development. Wt1-null knockout mice die in utero with a complete absence of kidneys and gonads [18]. Gonadal development is initiated but is then arrested at a very early stage, indicating a role for WT1 in early establishment of the genital ridges, in addition to roles in regulating sex-specific gene expression. WT1 mutations are associated with Wilms’ tumor and several human conditions that involve developmental abnormalities of the kidneys and
KTS isoform-specific deletions of WT1 in mice

In order to clarify the etiology of Frasier and Denys-Drash syndromes, and to understand the distinct functions of WT1<sup>+</sup>KTS and WT1<sup>−</sup>KTS, Schedl and co-workers made isoform-specific deletions in mice by gene targeting [3]. The authors refer to mice lacking WT1<sup>+</sup>KTS as Frasier mice, and to those lacking WT1<sup>−</sup>KTS as KTS mice.

Although heterozygous Frasier mice showed similar kidney pathology to human Frasier syndrome patients, two features of the mice generated in this study compromised their usefulness as models of human disease and tools for molecular analysis of the sex-determining pathway. First, neither mutant mouse showed gonadal abnormalities in the heterozygous state, unlike human Frasier and DDS patients. This observation is in accord with growing evidence that there are species differences with respect to critical gene threshold levels during sex determination [5,13]. Secondly, overall levels of Wt1 expression were not altered in either Frasier or KTS mice. This situation complicates analysis of the mice, because deletion of one WT1<sup>+</sup>KTS isoform simultaneously causes overexpression of the other.

Nevertheless, some important observations resulted from this study. In homozygous Frasier mice (no WT1<sup>+</sup>KTS, increased WT1<sup>−</sup>KTS), complete sex reversal of XY animals to female was observed, but XX ovaries were normal. This confirms a critical role for WT1<sup>+</sup>KTS in testis determination but not in initial formation of the genital ridges, a stage that is identical in both males and females. Levels of Sry transcripts in the Frasier XY mice were only 25% of normal, and consequently expression of Sox9 was similar to that observed in female wild-type or mutant mice (Table 1). In view of evidence that WT1<sup>−</sup>KTS activates, rather than suppresses, Sry expression in vitro [8,9] and that WT1<sup>−</sup>KTS binds to RNA (as discussed above), a likely explanation is that WT1<sup>−</sup>KTS is required to stabilize the Sry transcript (Figure 1). Given that WT1<sup>+</sup>KTS levels are increased in these mice, one would also expect increased Dax1 expression; although expression was measured by quantitative PCR, it is not clear whether or not levels were increased in XY gonads relative to wild-type (Table 1).

Homozygous KTS mice (no WT1<sup>−</sup>KTS, increased WT1<sup>+</sup>KTS) died within 24 hours of birth. Both XX and XY KTS mice had small, poorly differentiated gonads and abnormal genital
ducts at birth, a phenotype not unlike that of DDS patients. The reduction in gonadal size was less pronounced at 12.5 days post coitum (dpc), and was probably due to apoptosis, which the authors showed to be more prevalent than in the wild-type at 11.5 dpc. This observation suggests that WT1KTS is required for differentiation and survival of gonadal cells in both sexes. Sry expression levels were not determined, but expression levels of Dax1, Sox9 and Amh were low, perhaps directly resulting from a lack of activation of Sry, Dax1 and Amh by WT1KTS (Figure 1, Table 1). The authors suggest that these low expression levels indicate that the sex-determination pathway is compromised but not completely blocked in KTS mice. Intriguingly, neither Frasier nor KTS mice showed the complete blockage of genital-ridge development shown by Wt1-null mice. This observation suggests that some form of WT1, irrespective of the presence or absence of KTS, is essential for early genital-ridge development.

**Sox9 induces testis development in XX mice**

Sox9 is expressed specifically in the developing male gonad in a number of species; mutations in SOX9 are associated with male-to-female sex reversal in humans, and derepression of Sox9 in ‘Odsex’ XX mice and duplication of the SOX9 gene in a human can induce female-to-male sex reversal (reviewed in [20]). These and other lines of evidence have indicated that SOX9 is necessary and sufficient to induce male sex determination in mammals.

A new study, also from Schedl’s group, shows that ectopic expression of Sox9, driven by the regulatory sequences of Wt1 in a yeast artificial chromosome (YAC), is sufficient to induce female-to-male sex reversal in XX mice [4]. The phenotype of these mice is not detectably different from that of sex-reversed XX mice produced by overexpression of Sry using its own regulatory sequences [21]. It remains formally possible that SOX9 achieves this effect by acting as a molecular mimic of SRY, by virtue of the similar HMG domains of the two proteins; it will be of interest to determine whether other SOX proteins, when similarly mis-expressed, can elicit the same phenotype. This seems unlikely, however, given the differences in biochemical properties, interacting factors and target genes of the various SOX proteins, all of which share a similar HMG domain.

In combination with previous observations, it seems more likely that this study underlines the essential role Sox9 plays in mammalian sex determination, and implies that all downstream effects of Sry expression must feed through Sox9. In this scenario, Sry is merely a Y-chromosomal switch whose role is to activate Sox9, which is then responsible for initiating differentiation of bipotential supporting cells of the gonad into Sertoli cells, marking the beginning of male sexual differentiation in mammals. Further investigation is required, however, to determine just how similar the Wt1-Sox9 transgenic mice are to Sry transgenic mice, or indeed to normal XY mice, at the molecular and cellular levels.

The molecular genetics of sex determination in mammals is often described as a puzzle with many missing pieces. Although no new pieces have been identified in these studies, they do provide new information regarding the range of function of two known proteins, WT1 and SOX9.

**Acknowledgements**

Our work is supported by the National Health and Medical Research Council of Australia and by the Australian Research Council. P.K. is an Australian Senior Research Fellow of the Australian Research Council.

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