Minireview

New clues to the puzzle of mammalian sex determination

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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

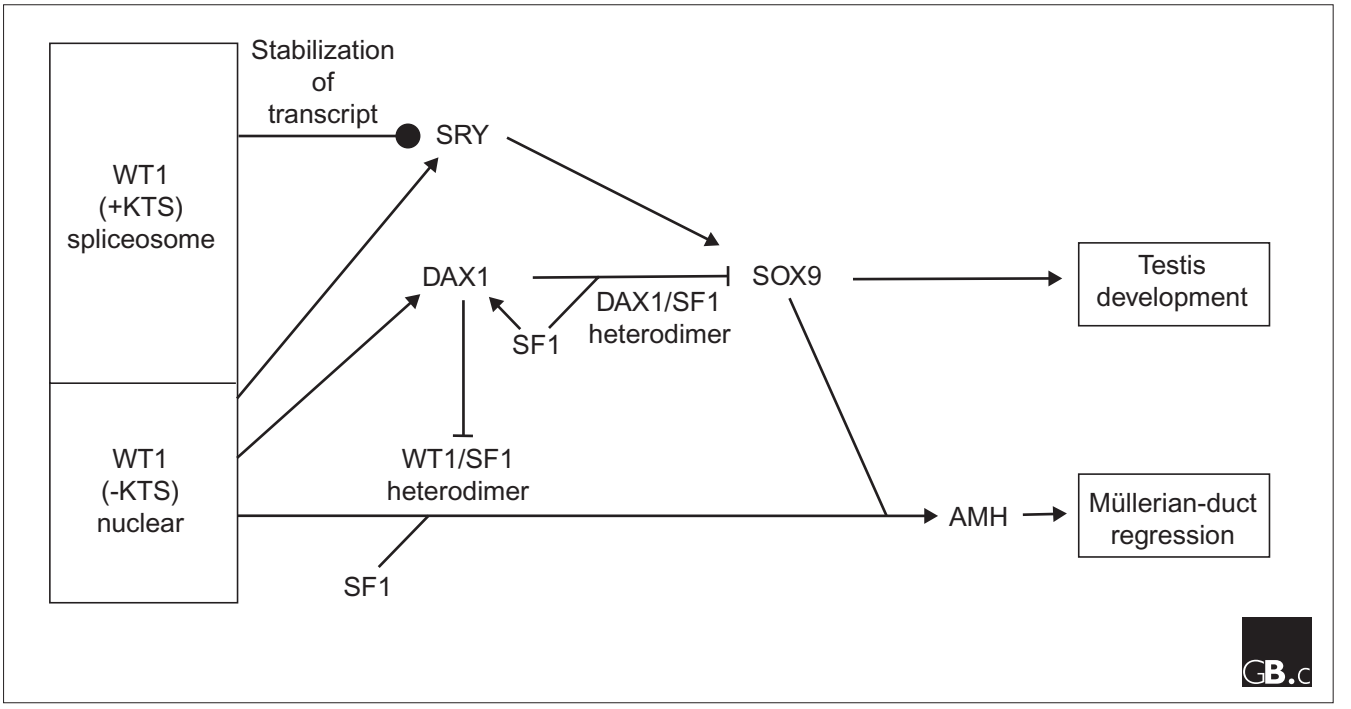

Figure 1
Proposed interactions among transcription factors during mammalian male sex determination and gonadal development (for further details, see text). Arrowed lines represent activation; blunt-ended lines represent repression. The unequal size of the boxes around the WT1 isoforms represents the observation that the total amount of WT1 remains constant and that WT1^{+KTS} is normally the predominant WT1 isoform.

gonads [19]. In Frasier syndrome, mutations that prevent the production of WT1^{+KTS} act dominantly to cause male-to-female sex reversal, male pseudohermaphroditism and gonadal dysgenesis. In Denys-Drash Syndrome (DDS), mutations are found in the zinc-finger region of the protein, and act dominantly to cause urogenital abnormalities and small, degenerated ('streak') gonads.

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^{+KTS} and WT1^{-KTS}, Schedl and co-workers made isoform-specific deletions in mice by gene targeting [3]. The authors refer to mice lacking WT1^{+KTS} as Frasier mice, and to those lacking WT1^{-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^{KTS} isoform simultaneously causes overexpression of the other.

Nevertheless, some important observations resulted from this study. In homozygous Frasier mice (no WT1^{+KTS}, increased WT1^{-KTS}), complete sex reversal of XY animals to female was observed, but XX ovaries were normal. This confirms a critical role for WT1^{+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^{-KTS} activates, rather than suppresses, *Sry* expression *in vitro* [8,9] and that WT1^{+KTS} binds to RNA (as discussed above), a likely explanation is that WT1^{+KTS} is required to stabilize the *Sry* transcript (Figure 1). Given that WT1^{-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^{-KTS}, increased WT1^{+KTS}) died within 24 hours of birth. Both XX and XY KTS mice had small, poorly differentiated gonads and abnormal genital

Table 1

Phenotypes of WT1-isoform-specific knockout mice

	Sex	Gonadal phenotype	*Sry* (11.5 dpc)	*Sox9* (12.5 dpc)	*Dax1* (11.5 dpc)	*Amh* (12.5 dpc)
Wild-type mice						
	XY	Testis	+	+++	+	++++
	XX	Ovary	–	–	+	–
Frasier mice (no $WT1^{+KTS}$)						
	XY	Ovary	25%	–	Similar or slight increase	ND
	XX	Ovary	–	–	relative to wild type*	ND
KTS mice (no $WT1^{-KTS}$)						
	XY	Small, undifferen-	ND	– (+ in rare cells)	Lower than	– (+ in rare cells)
	XX	tiated gonads	–	–	wild type	–

Based on data from Hammes *et al.* [3]. ND, not determined; dpc, days *post coitum*. * Seven arbitrary units in XY Frasier compared to five in XY wild type, with unknown standard errors, as measured by quantitative PCR.

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 $WT1^{-KTS}$ 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 $WT1^{-KTS}$ (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.

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