HHS Public Access

Author manuscript

Science. Author manuscript; available in PMC 2017 July 06.

Published in final edited form as:

Science. 2016 January 29; 351(6272): 514–516. doi:10.1126/science.aad1795.

Two genes substitute for the mouse Y chromosome for spermatogenesis and reproduction

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Abstract

The mammalian Y chromosome is considered a symbol of maleness, as it encodes a gene driving male sex determination, *Sry*, as well as a battery of other genes important for male reproduction. We previously demonstrated in the mouse that successful assisted reproduction can be achieved when the Y gene contribution is limited to only two genes, *Sry* and spermatogonial proliferation factor *Eif2s3y*. Here, we replaced *Sry* by transgenic activation of its downstream target *Sox9*, and *Eif2s3y*, by transgenic overexpression of its X chromosome–encoded homolog *Eif2s3x*. The resulting males with no Y chromosome genes produced haploid male gametes and sired offspring after assisted reproduction. Our findings support the existence of functional redundancy between the Y chromosome genes and their homologs encoded on other chromosomes.

Many sexual characteristics are influenced by sex chromosome constitution, with mammalian females typically carrying XX and males XY. We recently reported that in the mouse, only two Y-chromosome genes—testis-determinant *Sry* and spermatogonial proliferation factor *Eif2s3y*—are needed for successful assisted reproduction (1). Here, we asked if these two genes could be replaced by transgenic activation of their homologs encoded on other chromosomes.

For *Sry* replacement, we chose *Sox9* (*Sry*-related high-mobility–group box gene 9), a direct target of SRY (2). Prior work showed that transgenic overexpression of *Sox9* driven by the *Wt1* promoter results in female-to-male sex reversal in XX mice (3). We placed the *Wt1-Sox9* transgene in the context of a single X chromosome carrying the *Eif2s3y* transgene (fig.

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SUPPLEMENTARY MATERIALS

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Supplementary Text
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Movie S1

S1A) (4) and found that it generated males ($X^{E}O$*Sox9*). In these males, the Y-chromosome gene contribution is limited to *Eif2s3y* (table S1).

XO*Sry* males, which carry an autosomally encoded *Sry* transgene, develop testes containing spermatogonia that are unable to proliferate, which results in seminiferous tubules appearing empty when compared with those from males with an intact Y chromosome (XY) (Fig. 1, A and B). This defect can be overcome by transgenic *Eif2s3y* addition to the X chromosome ($X^{E}O$*Sry*) (table S1 and fig. S4A) (1, 5). To replace *Eif2s3y*, we transgenically overexpressed its X chromosome–encoded homolog, *Eif2s3x* (fig. S2). We then placed the *Eif2s3x* transgene in the context of XO*Sry* (fig. S1B and supplementary text). The resulting XO*Sry,Eif2s3x* males (carrying autosomally encoded *Sry* and *Eif2s3x* transgenes) had the Y-chromosome contribution limited to *Sry* (table S1).

$X^{E}O$*Sox9* and XO*Sry,Eif2s3x* males had small testes (fig. S3), but spermatogenesis was initiated and progressed through meiosis and arrested at the round spermatid stage (fig. S4, B and C). Spermatogonia/Sertoli ratios in $X^{E}O$*Sox9* and XO*Sry,Eif2s3x* and spermatid/Sertoli ratio in $X^{E}O$*Sox9* were comparable to $X^{E}O$*Sry* but lower than those in XY (Fig. 1, D and E). Round spermatids in XO*Sry,Eif2s3x* were dramatically depleted; $X^{E}O$*Sry* and XY had 10 and 88 times as many, respectively (Fig. 1E and table S2). The spermatids from both $X^{E}O$*Sox9* and XO*Sry,Eif2s3x* males were functional in assisted fertilization, and live offspring were obtained after embryo transfer (Table 1).

We next tested whether spermatogenesis can take place in males with a complete absence of Y-chromosome genes. We used the same transgenes that were successful in single–Y gene substitutions (*Wt1-Sox9* and *Eif2s3x* Tg1) to generate mice transgenic for *Sox9* and *Eif2s3x* in the XO context (XO*Sox9,Eif2s3x*) (fig. S1C and table S1) The majority (35 out of 48) of XO*Sox9,Eif2s3x* males had testicular defects and essentially no germ cells (fig. S5 and supplementary text). In the remaining males, spermatogonial proliferation arrest was overcome (Fig. 1C and fig. S5), and spermatogenesis progression was comparable to that of XO*Sry,Eif2s3x* (Fig. 1, D and E, and table S2). Using assisted reproduction [round spermatid injection (ROSI)], we injected oocytes with spermatids from 13 males and obtained zygotes with two well-developed pronuclei and normal two-cell embryos (fig. S6, A to C, and movie S1). Embryos from 11 males were used for transfer. Ten resulted in pregnancy, and nine yielded offspring (Table 1). Among the males that yielded progeny, there were F_1, F_2, and F_3 generation XO*Sox9,Eif2s3x* ROSI males (fig. S6D). ROSI offspring from males with one or no Y-chromosome genes were all normal and healthy (figs. S7 to S9 and supplementary text).

The quantification of *Eif2s3x/y* transcripts in males transgenic for *Eif2s3y* or *Eif2s3x* revealed a correlation between spermatogenesis progression and *Eif2s3x/y* expression level (Fig. 2, figs. S10 to S12, and supplementary text). All transgenic males had their respective transgene transcript levels elevated when compared with XY (Fig. 2, A and B). Compared with *Eif2s3x* transgenic males, *Eif2s3y* transgenic males showed higher *Eif2s3x/y* transcript levels (Fig. 2C) and increased incidence of round spermatids (Fig. 1E). When spermatogenesis and *Eif2s3x* expression were examined in XO*Sry,Eif2s3x* males with varying numbers of *Eif2s3x* transgene copies, one (Tg2 and Tg6) and four (Tg1), no

differences in spermatogonia/Sertoli cell ratio were observed, but round spermatids were found only in Tg1 males, in which *Eif2s3x* transcript levels are 2.4 to 2.9 times those in Tg2 and Tg6 males (Fig. 2, D to F, and fig. S4, D to G).

We have shown that a male mouse without any Y-chromosome genes but with transgenically activated *Sox9* and *Eif2s3x* can generate haploid gametes and father offspring with the help of assisted fertilization. *Sox9* is not unique in being able to take over the *Sry* function in sex determination. Manipulation of expression of other genes can lead to sex-fate change [reviewed in (6–8)]. A surrogate sex-determination mechanism can also be activated without human input, as shown by two rodent species that lost the Y chromosome and *Sry* (9, 10).

Eif2s3y and *Eif2s3x* represent a typical, formerly autosomal, single-copy, X-Y homologous gene pair and were hypothesized to have interchangeable function (1, 5). Our data support this hypothesis: A single additional copy of *Eif2s3x* can functionally replace *Eif2s3y* in spermatogenesis initiation. For progression through meiosis, however, at least four *Eif2s3x* transgene copies are necessary, and the number of global *Eif2s3x/y* transcripts must reach a certain threshold. Our data also suggest that *Eif2s3x/y* may play roles in gonad formation. We observed severe abnormalities of mature testes, indicative of impaired gonadal development, in XO*Sox9,Eif2s3x* but not $X^{E}O$*Sox9* males. Because the global *Eif2s3x/y* expression is lower in the former, this suggests that a critical level of *Eif2s3x/y* may be required for efficient testis differentiation.

Our data support a model where *Eif2s3y* and *Eif2s3x* are functionally interchangeable in spermatogenesis, but each homolog has evolved a distinct expression level. *Eif2s3y* transcript amounts are ~5 to 7 times those in premeiotic and meiotic cells (11), which explains why the addition of one *Eif2s3x* transgene copy could not replace the function of endogenous *Eif2s3y* in driving spermatogenesis through meiosis. Our finding that a single *Eif2s3x* transgene copy was sufficient to substitute for *Eif2s3y* in overcoming spermatogonial proliferation arrest suggests that the strong *Eif2s3y* expression in spermatogonia is required for the subsequent meiotic stages but not for mitotic proliferation. Our observations contradict the accepted dogma of X-Y gene pairs evolving by decay on the Y chromosome and compensation on the X chromosome (12), because here, it is a beneficial overexpression of the Y gene *Eif2s3y* and not its X homolog that appears to have evolved to meet the needs of spermatogenesis. This might be the result of a selective advantage during oogenesis for reduced *Eif2s3x* levels or a selection for male germ cell beneficial effects on the Y chromosome.

It is generally believed that widely expressed genes on the human Y chromosome with X homologs that escape X inactivation are dosage-sensitive (13). Dosage sensitivity explains why genes are conserved on the Y chromosome: A certain combined X-Y dose is critical at certain stages in certain tissues, and the X-gene dose cannot simply be increased globally to compensate for the loss of the Y gene because this would have detrimental effects in females. To lose the Y gene safely, the X gene, or the genome, or the developmental systems must adapt. Possible strategies might include incremental increases in X-gene dosage, relaxing constraints on dose-sensing, or retrogenes (14). The mouse *Eif2s3x/y* gene pair is not sensitive to overexpression; substantial elevation of *Eif2s3y* (5) or *Eif2s3x* in the XY

context (this study) has no obvious somatic effects, nor does it affect spermatogenesis and fertility. These findings suggest that dosage sensitivity may appear mainly in association with underexpression and/or may vary between different X-Y gene pairs.

Altogether, our analyses of the *Eif2s3x/y* gene pair support their importance for spermatogenesis. It will now be imperative to explore the mechanisms whereby *Eif2s3x/y* factors exert their functions during testicular development and spermatogenesis. Our work also paves the way and prompts future evaluations of other ancestral X-Y gene pairs to clarify the dosage requirements for spermatogenesis and beyond. Finally, our demonstration that offspring can be obtained from males with no Y-chromosome genes shows that for assisted reproduction in the mouse, the Y chromosome is no longer necessary. However, there is extensive evidence from both phenotype characterization (15–17) and genomic analyses (13, 14, 18) unequivocally supporting the importance of Y-chromosome genes for normal, unassisted fertilization. So, although our data demonstrate that it is possible to bypass the requirement for the Y chromosome in male assisted reproduction, the Y clearly remains the genetic determinant of full natural masculinity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The work was supported by NIH HD072380 and Hawaii Community Foundation 14ADVC-64546 grants to M.A.W. and INSERM core funding to M.J.M.

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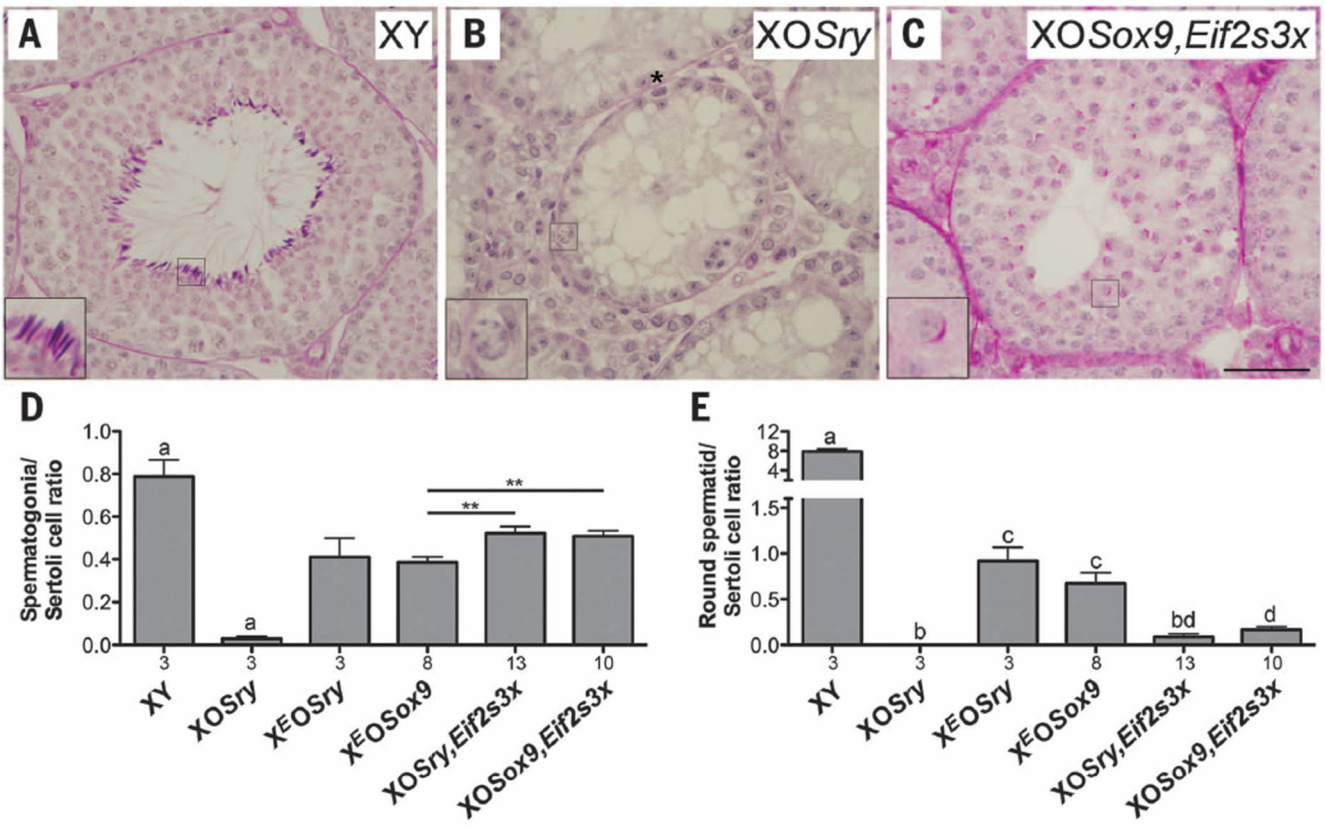

Fig. 1. Testis histology analysis

Tubules of periodic acid–Schiff–hematoxylin–stained sections of testis from XY (**A**), XO*Sry* (**B**), and XO*Sox9,Eif2s3x* (**C**) males. XY have normal spermatogenesis with expected germ cell types present, including step 16 spermatids [inset (A)]. XO*Sry* have spermatogonial proliferation arrest, resulting in tubules lacking germ cells except for occasional normal [inset (B)] and abnormal (*) spermatogonia. XO*Sox9,Eif2s3x* have meiotic and postmeiotic arrests that occasionally allow formation of round spermatids [insets (C)], arresting at step 7. Scale bar, 50 μm; insets, ×3 magnification. (**D** and **E**) Quantitative analysis of spermatogenesis progression. Bars are averages ± SEM, with *n* under the x axis. Statistical significance (*t* test): (D) bars marked with[a] are different from all others ($P < 0.05$), ** $P <$ 0.01; (E) bars with different letters are significantly different ($P < 0.05$).

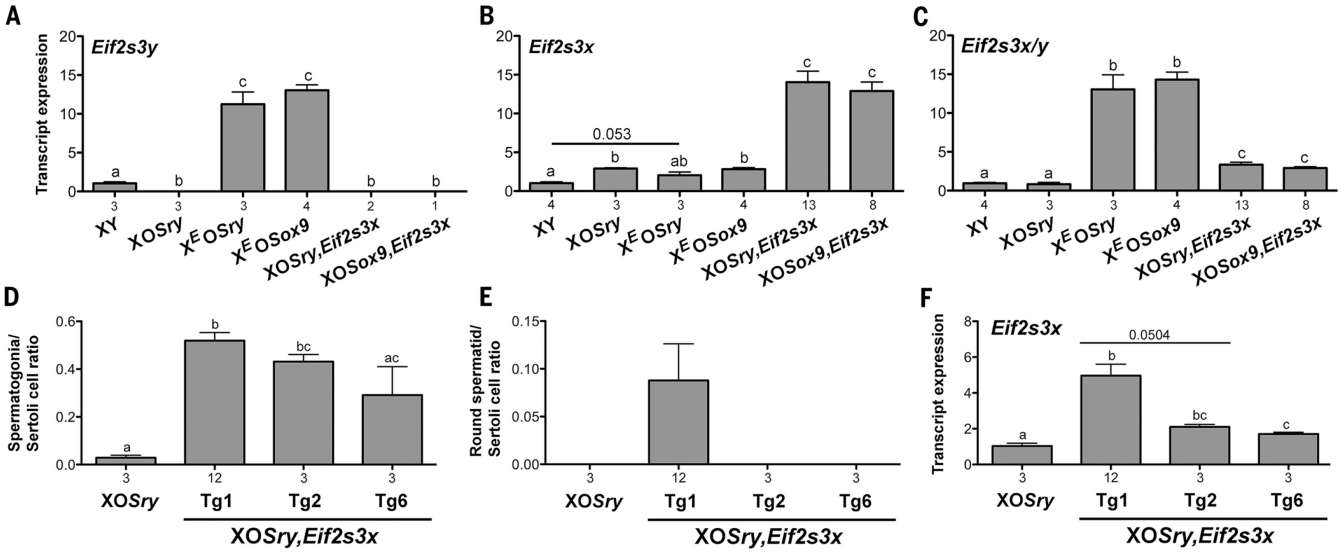

Fig. 2. Relation between *Eif2s3x/y* expression and spermatogenesis progression

(**A** to **C**) Transcript levels of endogenous and transgenic spermatogonial proliferation factors quantified by real-time polymerase chain reaction with *Actb* as a loading control and XY serving as reference control. (**D** to **F**) Analysis of spermatogenesis progression (D and E) and *Eif2s3x* expression (F) in XO*Sry,Eif2s3x* males with 4 (Tg1) and 1 (Tg2 and Tg6) *Eif2s3x* transgene copies. Means ± SEM, with *n* under the x axis; bars with different letters are statistically different (*t* test, $P < 0.05$).

Table 1

The results of ROSI with spermatids from males with a single or no Y-chromosome genes

Column heads 2 to 4: Males with round spermatids identifiable in live testicular cell suspension out of males examined. Males yielding progeny out of the number of males that yielded embryos used for embryo transfer and induced pregnancy. Live offspring as a percentage of embryos transferred.

Male genotype	Y gene contribution	Males with round spermatids (%) (no.)	Males yielding progeny	Live offspring (%) (no.)
$X^{E}O$*Sox9*	*Eif2s3y*	80 (8/10)	7/8	15.7 (18/115)
XO*Sry,Eif2s3x*	*Sry*	55 (17/31)	16/17	22.5 (57/253)
XO*Sox9,Eif2s3x*	None	27 (13/48)[a]	9/10	20.7 (46/222)
XY control	Intact Y	100 (6/6)	6/6	25.0 (22/88)

Statistical significance (Fisher's exact test, $P < 0.05$):

[a]Different from all others.