William & Mary
CHARTERED 1693

W&M ScholarWorks

Arts & Sciences Articles

Arts and Sciences

2014

The Jak-STAT Target Chinmo Prevents Sex Transformation of Adult Stem Cells in the Drosophila Testis Niche

Qing Ma

Erika L. Matunis

Matthew Wawersik
College of William and Mary

Follow this and additional works at: https://scholarworks.wm.edu/aspubs

Recommended Citation

Ma, Qing; Matunis, Erika L.; and Wawersik, Matthew, The Jak-STAT Target Chinmo Prevents Sex Transformation of Adult Stem Cells in the Drosophila Testis Niche (2014).
https://scholarworks.wm.edu/aspubs/458

This Article is brought to you for free and open access by the Arts and Sciences at W&M ScholarWorks. It has been accepted for inclusion in Arts & Sciences Articles by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.

CellPress

Developmental Cell
Article

The Jak-STAT Target Chinmo Prevents Sex Transformation of Adult Stem Cells in the *Drosophila* Testis Niche

Qing Ma,[1] Matthew Wawersik,[2] and Erika L. Matunis[1,*]
[1]Department of Cell Biology, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA
[2]Department of Biology, College of William and Mary, Williamsburg, VA 23185, USA
*Correspondence: matunis@jhmi.edu
http://dx.doi.org/10.1016/j.devcel.2014.10.004

SUMMARY

Local signals maintain adult stem cells in many tissues. Whether the sexual identity of adult stem cells must also be maintained was not known. In the adult *Drosophila* testis niche, local Jak-STAT signaling promotes somatic cyst stem cell (CySC) renewal through several effectors, including the putative transcription factor Chronologically inappropriate morphogenesis (Chinmo). Here, we find that Chinmo also prevents feminization of CySCs. Chinmo promotes expression of the canonical male sex determination factor DoublesexM (Dsx^M) within CySCs and their progeny, and ectopic expression of DsxM in the CySC lineage partially rescues the *chinmo* sex transformation phenotype, placing Chinmo upstream of Dsx^M. The Dsx homolog DMRT1 prevents the male-to-female conversion of differentiated somatic cells in the adult mammalian testis, but its regulation is not well understood. Our work indicates that sex maintenance occurs in adult somatic stem cells and that this highly conserved process is governed by effectors of niche signals.

INTRODUCTION

Male versus female fate is controlled by a variety of mechanisms across taxa (Kopp, 2012). In mammals, this choice was recently found to be labile even in adults; loss of sex-specific transcriptional regulators in the adult mouse gonad causes differentiated somatic cells to transdifferentiate into somatic cells of the opposite sex (Matson et al., 2011; Uhlenhaut et al., 2009). This indicates that sexual identity must continuously be maintained in specific differentiated cell types long after sex determination has occurred. Whether sexual identity is plastic in undifferentiated adult stem cells remains unknown. Because adult stem cells have the capacity to rebuild entire adult organ systems, altering the sexual identity of a stem cell could conceivably cause widespread changes to the tissue.

In *Drosophila*, a well-studied sex determination cascade culminates in the sex-specific splicing of mRNA encoding the conserved transcription factor Doublesex (Dsx); this binary choice cell autonomously dictates somatic sexual identity (Whitworth et al., 2012). While upstream regulation of sex determination differs between flies and mammals, downstream control converges on *Dsx/mab-3-related transcription factor* (*Dmrt*) genes (Matson and Zarkower, 2012). The Janus kinase-Signal Transducer and Activator of Transcription (Jak-STAT) pathway is also required for the initial choice of somatic sexual identity in *Drosophila* embryos and promotes male germline sexual behavior in embryonic testes (Jinks et al., 2000; Wawersik et al., 2005). However, it is not known whether Jak-STAT signaling is required for sex maintenance in *Drosophila*, and the link between the Jak-STAT pathway and the canonical sex determination pathway is unknown.

The *Drosophila* ovary and testis provide excellent models for studying adult stem cell behavior in vivo (Fuller and Spradling, 2007; Matunis et al., 2012). In the testis, Jak-STAT signaling maintains two types of stem cells: sperm-producing germline stem cells (GSCs) and supporting somatic stem cells called cyst stem cells (CySCs). Both of these cell types attach to a single niche created by quiescent somatic hub cells at the testis apex and divide asymmetrically to produce differentiating progeny (spermatogonia and cyst cells, respectively) that are displaced from the niche (Matunis et al., 2012). Several factors, including the Jak-STAT targets Zinc-finger homeodomain-1 (Zfh-1) and Chronologically inappropriate morphogenesis (Chinmo), are required for CySC self-renewal (Amoyel et al., 2013; Flaherty et al., 2010; Issigonis and Matunis, 2012; Leatherman and DiNardo, 2008; Michel et al., 2012). Here, we reveal an unexpected function of Chinmo: it acts through the canonical sex determinant DoublesexM (Dsx^M) to maintain the male identity of adult CySCs.

RESULTS

Reduction of Chinmo Triggers the Appearance of Cells Resembling Ovarian Follicle Cells in the Adult Niche and throughout the Testis

While screening for *Drosophila* testis phenotypes, we identified a spontaneous mutation causing a striking transformation of the adult testis. Adult mutant males are fertile, indicating testes develop normally. Consistent with this observation, testes from young males (0–1 days old) are indistinguishable from wild-type testes in overall morphology (Figures 1C, 1D, 1I, and 1J). With age, however, a progressive change in the testis morphology occurs. Initially, subtle changes are detected at the testis apex, where aggregates of epithelial somatic cells

CrossMark

(defined as eight or more closely apposed cells expressing high levels of adhesion proteins) appear adjacent to the hub, whereas the remainder of the tissue is unaffected (Figures 1E, 1K, 1P, and 1Q). With time, somatic cell aggregates acquire additional cells and extend away from the testis apex, whereas older differentiating germ cells and cyst cells are displaced toward the basal end of the testis (Figures 1F, 1G, 1L, and 1M). In 7- to 9-day-old males, an obvious transformation is apparent throughout the testis: somatic cell aggregates adjacent to the hub remain but now a monolayer of columnar epithelial cells lines the testis periphery, whereas germ cells are restricted to the lumen of the tissue (Figures 1G, 1M, and 1R). The progression of this phenotype from the testis apex to the basal end suggests a stem cell origin. This testis phenotype had not been described before. However, the arrangement of the somatic cells in the mutant testes bears a striking resemblance to that of somatic follicle cells within the wild-type *Drosophila* ovary: both are arranged in a columnar monolayer that surrounds developing germ cells (Mahowald and Kambysellis, 1980) (Figures 1B, 1H, 1N, and 1S). Therefore, we refer to these somatic cells in the mutant testes as "follicle-like cells." We also find that germ cells in 7- to 9-day-old mutant testes are arrested as early male germ cells (spermatogonia), based on their morphology, branching fusomes (de Cuevas et al., 1997; Hime et al., 1996; Lin et al., 1994) (open arrowheads, Figure 1R), expression of the male-specific early germ cell marker M5-4 (Tran et al., 2000) (Figures S1A–S1C available online), and the undifferentiated germ cell marker Bam (data not shown) (McKearin and Spradling, 1990). By 2–3 weeks, degeneration of differentiation-arrested germ cells is observed (Figure S1D). Because germ cell arrest occurs when the germline and somatic sex are mismatched (Whitworth et al., 2012), this phenotype is consistent with a defect in somatic sexual identity maintenance in the adult testis.

Sex maintenance has not been characterized previously in the adult *Drosophila* testis or ovary but was recently found to occur in the adult mammalian testis and ovary (Matson et al., 2011; Uhlenhaut et al., 2009). The underlying mechanisms are of interest, because defects in this process may cause testicular cancer or disorders of sexual development (Hanson and Ambaye, 2011). Therefore, we sought to identify this spontaneous mutant. Our genetic complementation (Figure 2A; Table S1) and rescue experiments (Figures 2B and 2C; Table S2) indicate that the mutation is a partial loss-of-function allele of the essential BTB-Zinc-finger cell-fate determinant *chinmo* (Flaherty et al., 2010; Zhu et al., 2006), which we denote as $chinmo^{Sex\ Transformation}$ or $chinmo^{ST}$. We did not find any changes in the coding region (data not shown), suggesting that the mutation is in a regulatory region of the gene. Consistent with this hypothesis, Chinmo is only depleted in the CySC lineage in $chinmo^{ST}$ mutant testes (Figures 2D–2H; Figures S1E and S1F). Together, these data support the hypothesis that Chinmo promotes male sexual identity in the adult testis soma.

Reduction of Chinmo in Adult Testes Causes Somatic Stem Cells and Their Progeny to Acquire Female Somatic Identity

Because testes with reduced Chinmo acquire somatic cells that morphologically resemble ovarian follicle cells, we directly tested whether these follicle-like cells underwent a male-to-female sex transformation. Specifically, female-specific gene expression and cell behavior was examined in $chinmo^{ST}$ testes. Castor, Cut, and a *slbo-GFP* gene reporter are normally expressed in adult ovaries (Chang et al., 2013; Jackson and Blochlinger, 1997) (described below), but not in testes (Figures 3A, 3D, and 3G). Castor, the earliest marker in the ovarian follicle stem cell lineage, marks follicle stem cells and their early progeny (Figure 3C), whereas more differentiated follicle cells express high levels of Cut and then *slbo-GFP* (Figures 3F and 3I). In $chinmo^{ST}$ testes, these three markers are sequentially expressed as the phenotype progresses (Figures 3B, 3E, and 3H). In testes from young (1- to 3-day-old) $chinmo^{ST}$ males, follicle-like cells have not formed yet (described above). However, the earliest ovarian marker, Castor, is detected in most testes (61.7%; n = 47). Importantly, Castor is always restricted to a subset of somatic cells within the testis at this time point: CySCs and their earliest daughters (Figure 3B). To ask whether Castor expression originates in CySCs, their earliest daughters, or both, we next stained testes from even younger males (newly eclosed virgins). At this time point, fewer testes contained Castor-positive cells (38.8% of testes; n = 49), and the number of Castor-positive cells ranged from 3 to around 30 (data not shown). Castor was detected in at least one CySC in all testes, where it was expressed, further supporting the hypothesis that CySCs, but not their differentiating progeny (cyst cells), which have exited the stem cell niche, are the cells within the testis that undergo sex transformation. These data further suggest that follicle-like cells arise from sex-transformed CySCs. Consistent with this hypothesis, the ovarian somatic cell marker Cut becomes strongly expressed in follicle-like cells in 7- to 9-day-old $chinmo^{ST}$ testes (Figure 3E). In contrast, the earlier marker, Castor, becomes depleted from follicle-like cells (described below; Figure 4), a pattern consistent with its absence in older ovarian follicle cells (Chang et al., 2013). Finally, the *slbo-GFP* reporter, which normally becomes highly expressed in follicle cells at stage 10 of oogenesis (Cai et al., 2014) (Figure 3I), is also expressed in a subset of follicle-like cells in older $chinmo^{ST}$ testes (Figure 3H). Together, these data suggest that follicle-like cells can progress through the normal follicle cell differentiation program to a surprising extent, in an otherwise male gonad. Consistent with this hypothesis, *Yolk protein1* (*Yp1*) transcripts, which become highly expressed in follicle cells at stage 10 but are not transcribed in the testis (Brennan et al., 1982; Logan et al., 1989), are present in testes with reduced Chinmo (Figure 3J).

In addition to distinct morphological and molecular characteristics, somatic cells in the testis and ovary differ in potency and proliferation status. CySCs are unipotent, giving rise to cyst cells that exit the cell cycle (Gönczy and DiNardo, 1996). In contrast, follicle stem cells are multipotent, yielding both stalk cells and mitotically active follicle cells (Margolis and Spradling, 1995). By examining markers of cell proliferation in $chinmo^{ST}$ testes, we find that follicle-like cells express mitotic markers (Figures 3K–3O). Furthermore, somatic cells with the molecular and morphological characteristics of stalk cells become apparent in older $chinmo^{ST}$ testes (Figure 4), suggesting that reduction of Chinmo causes CySCs to transdifferentiate into cells resembling multipotent female follicle stem cells. Together, these data support the hypothesis that

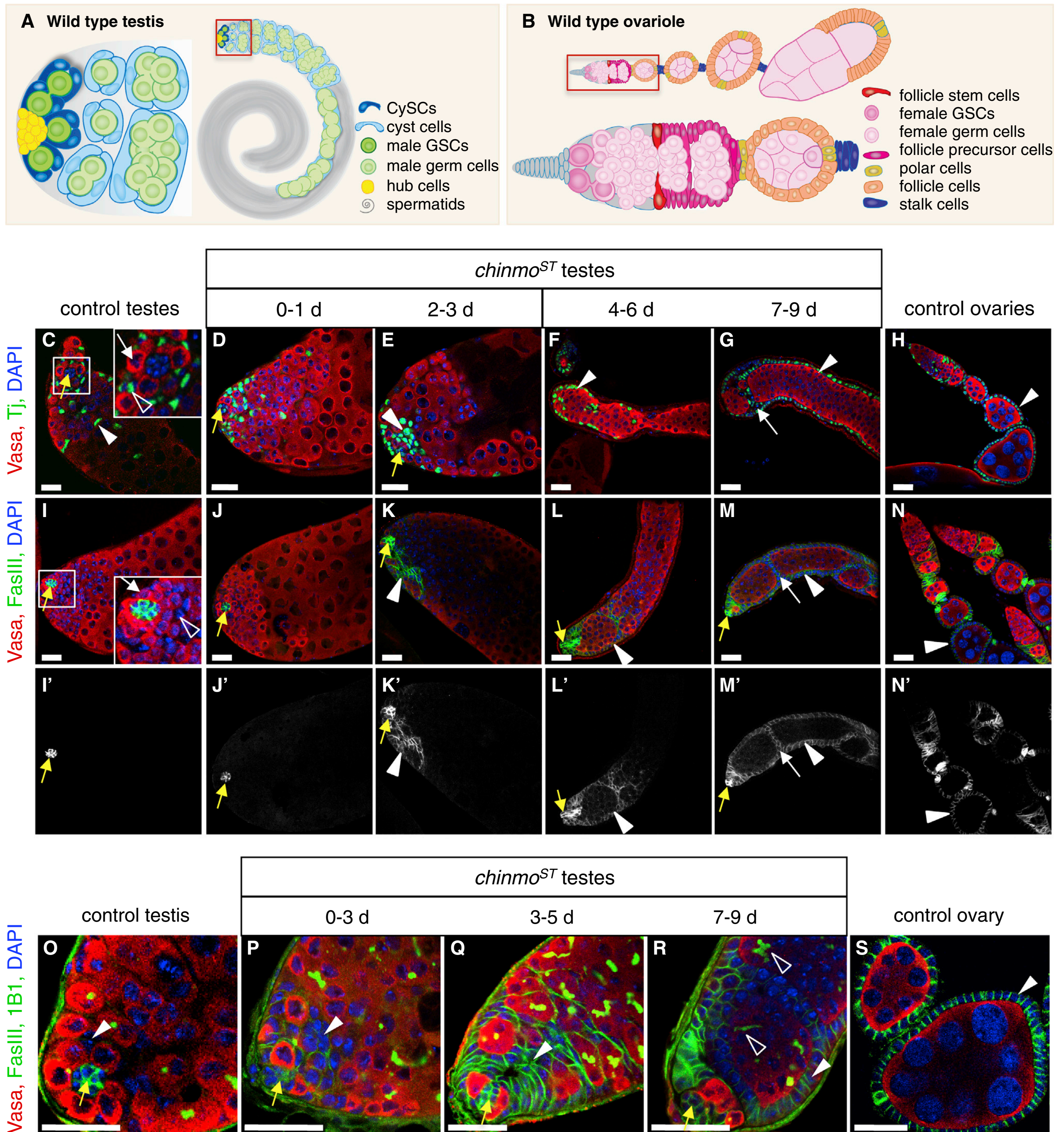


Figure 1. Reduction of Chinmo Causes Somatic Cells in Adult Testes to Be Gradually Replaced by Cells Resembling Ovarian Follicle Cells

(A) Illustration of a wild-type *Drosophila* testis (right) with the apex magnified (left). Germline stem cells (GSCs; dark green) and somatic cyst stem cells (CySCs; dark blue) adhere to the hub (yellow). GSCs produce differentiating male germ cells (spermatogonia and spermatocytes, green) that are displaced from the hub and form elongated spermatids (gray) and mature sperm (not shown). Approximately two CySCs flank each GSC; CySCs produce squamous, quiescent cyst cells (light blue), which encase differentiating germ cells.

(B) Illustration of a wild-type *Drosophila* ovariole (top) comprised of a germarium (magnified, bottom), followed by a series of developing egg chambers. In the germarium, anterior niche cells (cap cells; gray) support GSCs (dark pink), which produce differentiating female germ cells (light pink). Two somatic follicle stem cells (red), located near the middle of the germarium, produce follicle precursor cells (magenta), which differentiate into follicle cells (orange), stalk cells (purple), and polar cells (yellow). Each egg chamber contains 16 germ cells surrounded by a monolayer of columnar epithelial follicle cells. Polar cells are located at each end; egg chambers are linked by chains of stalk cells.

(legend continued on next page)

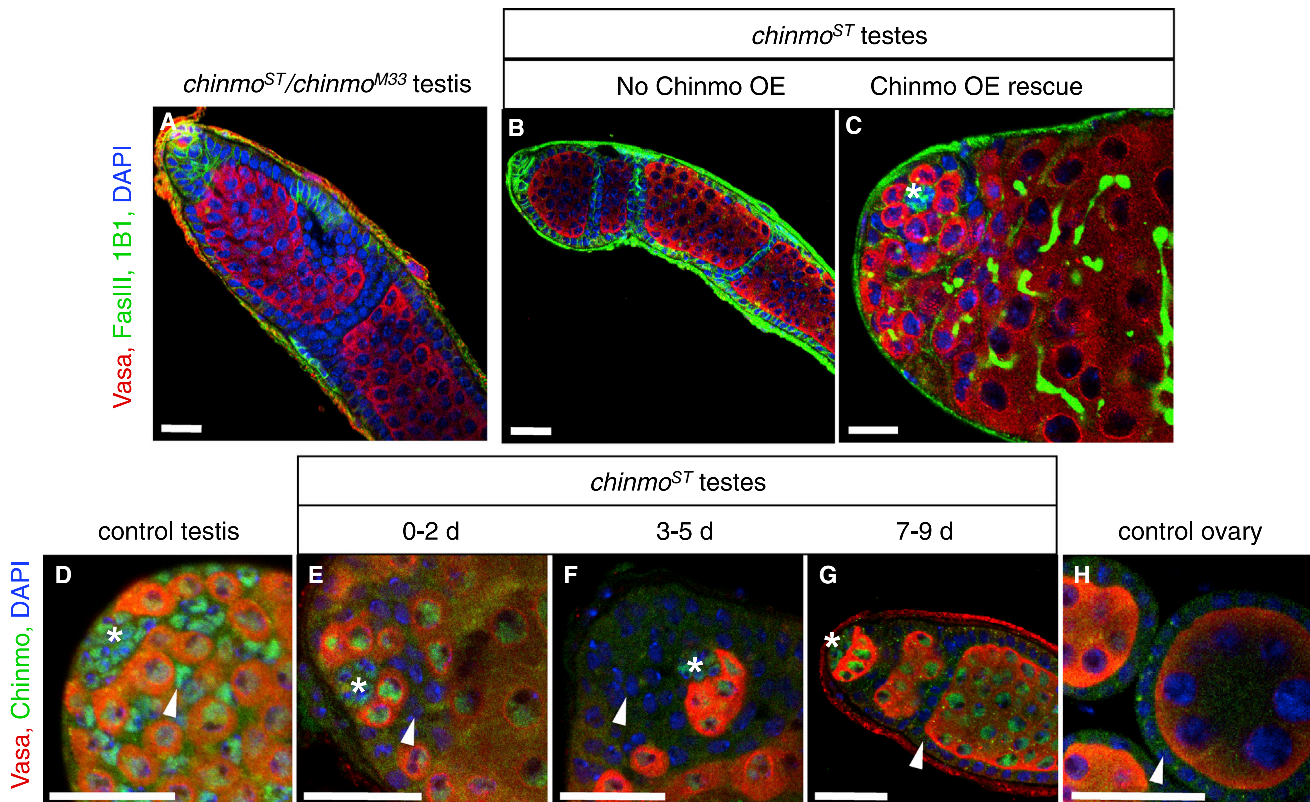


Figure 2. Genetic Complementation and Rescue Experiments Indicate that *chinmo*ST Is a Partial Loss-of-Function Allele of *chinmo*

(A) Immunofluorescence detection of FasIII (green at cell periphery) to visualize follicle-like cells in testes and Vasa (red) to visualize germ cells. Testes from *chinmo*ST/*chinmo*^{M33} males resemble *chinmo*ST homozygous testes (compare to Figure 1R).

(B and C) The follicle-like cell phenotype in *chinmo*ST testes (B) can be rescued by overexpressing *chinmo* in the CySC lineage (*c587-Gal4; chinmo*ST*; UAS-chinmo/tub-Gal80*^{ts}) (C). The position of the hub is marked with an asterisk.

(D–H) Immunofluorescence detection of Chinmo (green) in testes and ovaries. In control testes (D), Chinmo is found in the nuclei of hub cells (asterisk), CySC lineage cells (arrowhead), and germ cells (Vasa, red) (Flaherty et al., 2010). In both young and old *chinmo*ST testes (E–G), Chinmo is present in hub cells and germ cells but is absent from CySC lineage cells (arrowhead). Chinmo is absent from follicle cells (arrowhead) in control ovaries (H). DAPI marks nuclei (blue). Hubs marked by asterisk. Scale bars, 20 μm.

See also Figure S1 and Tables S1 and S2.

chinmo actively maintains a male sexual identity in adult CySCs.

Chinmo Autonomously Maintains Male Sexual Identity in Adult Somatic Stem Cells

The above data indicate that CySCs specifically require Chinmo for maintenance of male sexual identity. However, this requirement could be direct or indirect. To distinguish between these possibilities, we used cell-type-specific RNA interference (RNAi) knockdown of *chinmo*. Consistent with previous mosaic analysis (Flaherty et al., 2010), knockdown of *chinmo* in the germline during development does not yield a testis phenotype (Figure 5F), ruling out a germline requirement for *chinmo*. Next, we allowed testes to develop normally and then conditionally induced *chinmo* RNAi in subsets of adult somatic cells. Knockdown of *chinmo* in adult hub cells does not yield testes with the *chinmo*ST phenotype (Figure 5G). In contrast, the *chinmo*ST phenotype is fully recapitulated by

(C–S) Immunofluorescence detection in adult testes and ovaries of Tj (C–H, green) to visualize somatic cell nuclei, or FasIII (I–S, green) to highlight the hub in all testes (yellow arrows), and somatic cell membranes in ovaries and *chinmo* mutant testes (arrowheads). (I')–(N') show the FasIII signal alone. Vasa (red) marks germ cells, and DAPI (blue) marks nuclei in all panels. In control testes (C, I, and O), somatic CySC lineage cells (arrowheads) are squamous and interspersed among germ cells. Insets (C and I) show GSCs (white arrows) and CySCs (open arrowheads) surrounding the hub. In *chinmo*ST testes (D–G, J–M, and P–R), a distinct phenotype develops over time. Testes from young mutant males (D, E, J, K, P, and Q) resemble those from controls, except that most (~77%; n = 61), contain aggregates of eight or more somatic cells (arrowheads); these always appear near the hub (yellow arrows). As flies age (F, G, L, M, and R), aggregates expand beyond the testis apex and become columnar and peripheral (arrowheads) in 82% of testes (n = 545), forming FasIII-positive "follicle-like cells" that resemble somatic follicle cells (arrowheads) in control ovaries (H, N, and S). Follicle-like cells occasionally invaginate (G and M, white arrows) to envelop groups of germ cells. 1B1 (O–R, green) marks fusomes; branching fusomes in older germ cells in *chinmo*ST testes indicate spermatogonial arrest (R, open arrowheads). Scale bars, 20 μm.

See also Figure S1.

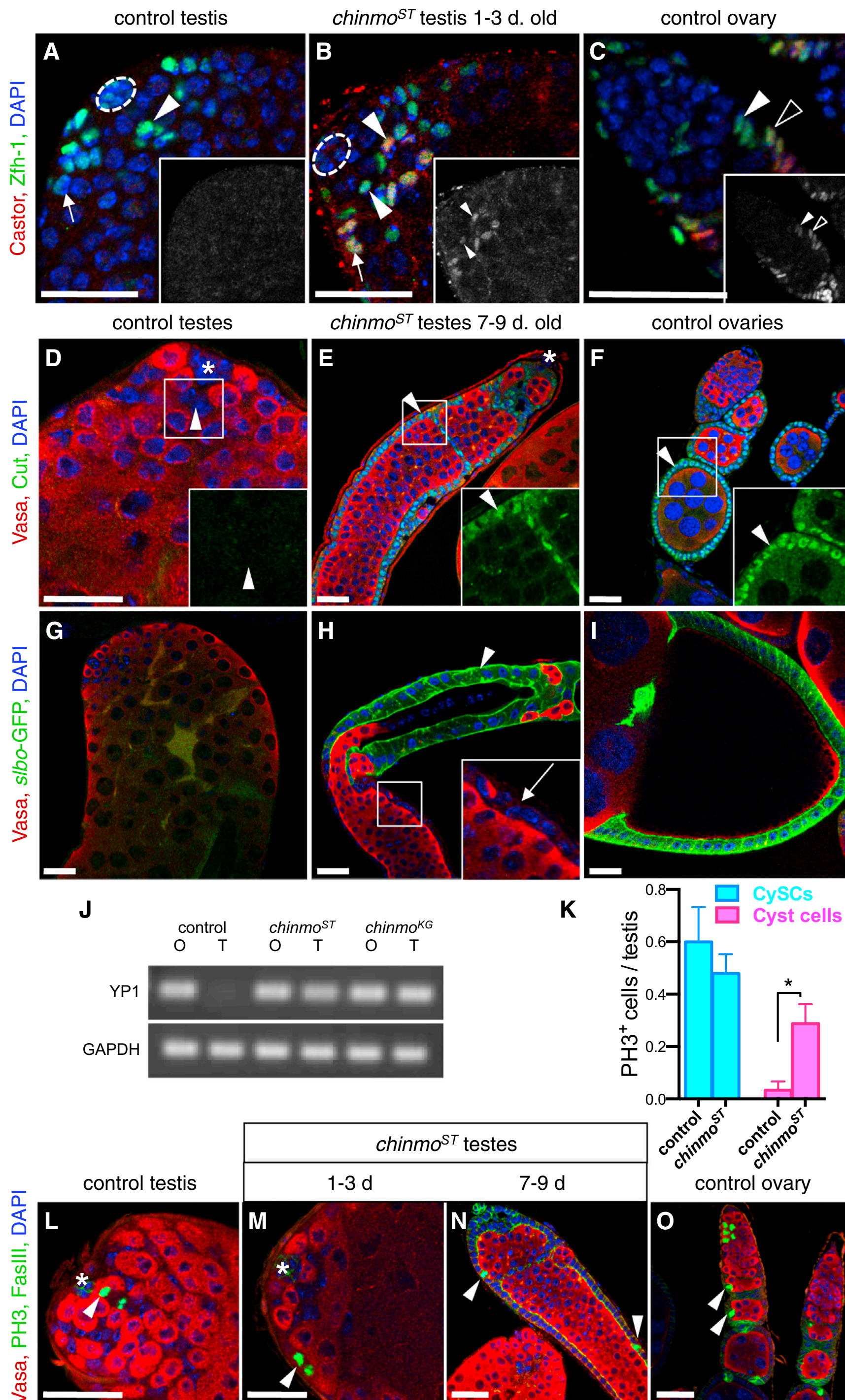


Figure 3. Reduction of Chinmo in Adult Testes Causes Somatic Stem Cells and Their Progeny to Acquire Female Somatic Markers

(A–C) Immunofluorescence detection of Zfh-1 (green) and Castor (red) in testes and ovaries. Insets, red channel only shown in grayscale. In control testes (A), Zfh-1 marks CySCs (arrowhead) and early cyst cells (arrow), but Castor is not detected. In testes from young (1- to 3-day-old) *chinmo*ST males (B), some CySCs (arrowheads) and cyst cells (arrow) express both Zfh-1 and Castor, as do ovarian follicle stem cells (solid arrowhead) and their early progeny (follicle cell progenitors, open arrowhead) in control ovaries (C).

(D–F) Immunofluorescence detection of Cut (green) and Vasa (red). Insets, green channel only, magnified. In control testes (D), Cut is absent from all cells, including CySCs (arrowhead), but it is present in follicle-like cells in *chinmo*ST testes (E, arrowhead) and follicle cells in control ovaries (F, arrowhead).

(G–I) Immunofluorescence detection of *slbo*-GFP (green). *slbo*-GFP is not expressed in control testes (G) but marks later somatic follicle cells (around stage 10) in control ovaries (I). In *chinmo*ST testes (H), *slbo-GFP* marks a subset of follicle-like cells (arrowhead); adjacent follicle-like cells (inset, arrow) are not marked.

(J) *Yolk protein 1* (*YP1*) expression is detected by RT-PCR in ovaries (O) and testes (T) from 7- to 9-day-old *chinmo*ST and *chinmo*^{KG} adults, but not in control testes. GAPDH is used as a control.

(K) Bar graph showing the number of CySCs and cyst cells containing the mitotic marker phosphohistone H3 (PH3) in testes from 1- to 3-day-old control or *chinmo*ST flies. Somatic cells within two cell diameters of the hub were scored as CySCs; those more than two cell diameters away were scored as cyst cells. Significantly more mitotic cyst cells are found in *chinmo*ST testes than in control testes (*p = 0.0168). There is no significant difference in the number of mitotic CySCs between *chinmo*ST and control testes. Data represented as mean ± SEM.

(L–O) Immunofluorescence detection of PH3 (green in nuclei). FasIII (green) marks cell membranes in the hub (asterisk, L–M), follicle-like cells (N), and follicle cells (O). In control testes (L), PH3 is detected in CySCs (arrowhead); cyst cells away from the hub are nonmitotic and do not express PH3. Cyst cells in young *chinmo*ST testes (M) and follicle-like cells in older *chinmo*ST testes (N) express PH3 (arrowheads), as do follicle cells (arrowheads) in control ovaries (O). Hubs marked by dashed line (A and B) or asterisk (D, E, L, and M). DAPI marks nuclei (blue, all panels). Scale bars, 20 μm.

knockdown of *chinmo* in all adult CySCs and early cyst cells (Figures 5A–5E; Figures S2A–S2D; Tables S3A and S3B). To determine whether *chinmo* is required primarily in CySCs or in their cyst cell progeny, we compared the phenotypes that developed when various CySC lineage drivers were used to induce *chinmo* RNAi. Drivers expressed in CySCs and their intermediate cyst cell daughters are sufficient to recapitulate the *chinmo*ST phenotype, whereas drivers expressed solely in later cyst cells are not (Figure 5H; Figures S2E–S2I; Table S3A). Together, these data indicate that *chinmo* is required in CySCs, but not in their differentiated progeny, for the maintenance of adult somatic sexual identity.

Follicle-like Cells Arise from the Adult Somatic Stem Cell Lineage, Not from Hub Cells

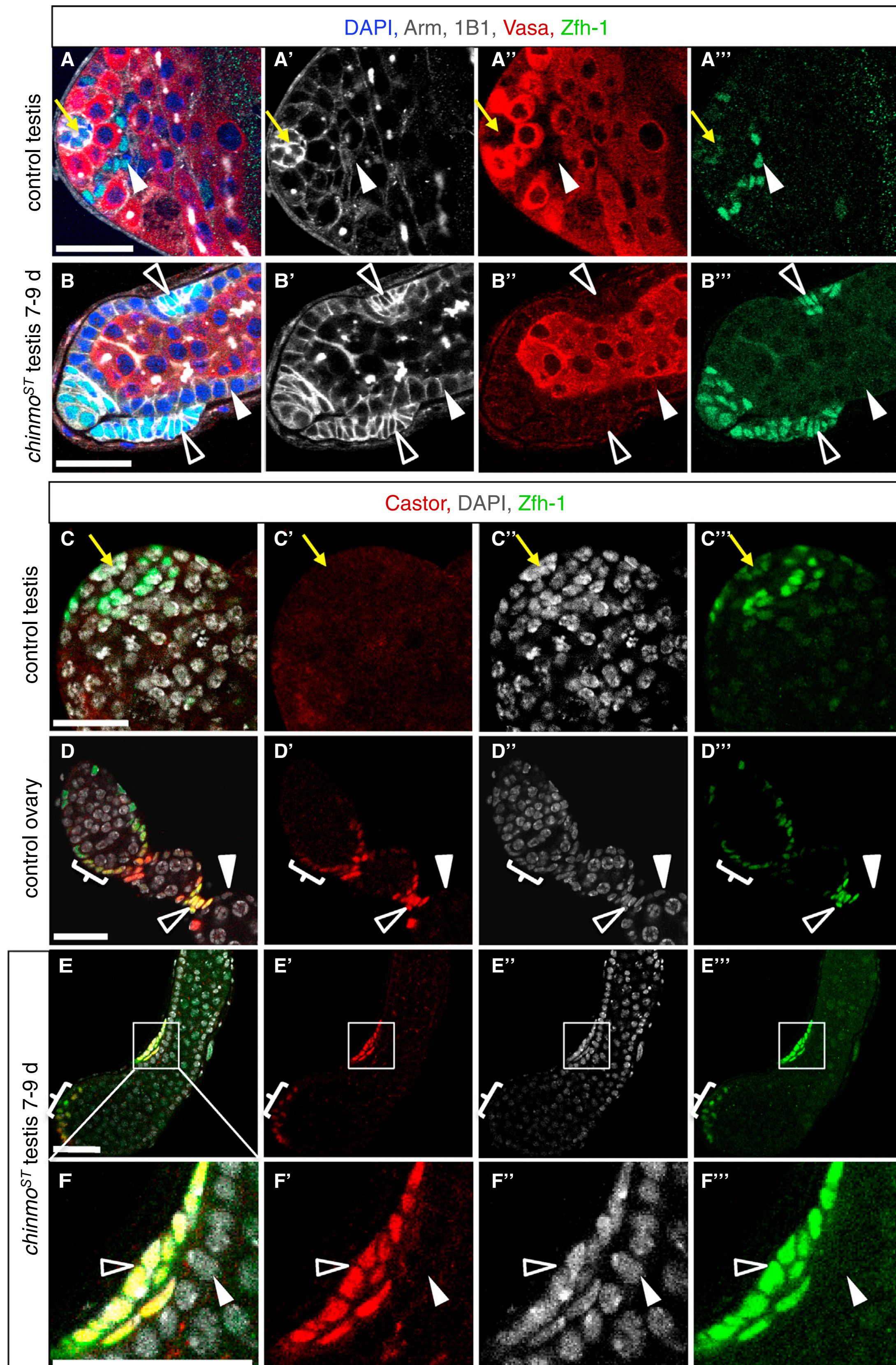
The fact that CySCs autonomously require *chinmo* for maintenance of adult sexual identity suggests that follicle-like cells arise directly from CySC lineage cells. However, it is also possible that *chinmo* is required in CySCs to prevent hub cells, the only other somatic cells in the testis niche, from giving rise to follicle-like cells. To distinguish between these possibilities, we used genetic lineage tracing to permanently mark hub cells or CySCs and early cyst cells in $chinmo^{ST}$ males during development, and then we assayed for the presence of permanently marked cells in adult testes. Permanently marked hub cells do not produce marked follicle-like cells in $chinmo^{ST}$ testes (Figures 6A and 6B; Table S4), ruling out hub cells as a source of follicle-like cells. In contrast, testes with permanently marked CySCs and early cyst cells acquire marked follicle-like cells over time (Figures 6C–6E). Because follicle-like cells do not express the CySC and early cyst cell driver used in this experiment (Figures S2J–S2L), the marked follicle-like cells must be descendants of CySCs and early cyst cells. We conclude that CySCs and early cyst cells, but not hub cells, give rise to follicle-like cells when *chinmo* is reduced. Consistent with this finding, hub cells within $chinmo^{ST}$ testes remained quiescent when assayed for proliferation using extended in vivo bromodeoxyuridine (BrdU) labeling (Figures 6F–6H). Because all CySC drivers are also expressed in early cyst cells, we cannot exclusively mark CySCs via lineage tracing. However, because CySCs, but not cyst cells, require *chinmo*, and CySCs are the earliest cells to express ovarian somatic markers, our data strongly support the hypothesis that somatic stem cells, rather than differentiated cells, undergo sex transformation when levels of Chinmo are reduced.

Chinmo Maintains Male Sexual Identity through the Canonical Sex Determination Pathway

Sex determination in both mammals and *Drosophila* converges on the transcription factor Dsx/Dmrt1. Our finding that *chinmo* promotes adult somatic sex maintenance prompted us to ask whether Chinmo works in concert with the canonical sex determination pathway, in which a splicing cascade mediated by Sex-lethal (Sxl) and Transformer (Tra) promotes the formation of either a female or male isoform of Dsx (Dsx^F or Dsx^M, respectively) (Whitworth et al., 2012). Although female forms of *Sxl*, *tra*, and *dsx* mRNA were not detected in $chinmo^{ST}$ testes (Figure S3A), changes in Dsx^M protein expression were observed. Dsx^M is absent from the ovary (Figure S3B) but present in the somatic cells of the testis, including hub cells, CySCs, and cyst cells (Hempel and Oliver, 2007) (Figure 7A). Testes from young $chinmo^{ST}$ males closely resemble wild-type testes, but a few CySCs and their immediate progeny lack Dsx^M, whereas older cyst cells retain this male determinant. As somatic cell aggregates and follicle-like cells arise, they all lack Dsx^M, and the remaining older Dsx^M-positive somatic cells become displaced from the testis apex (Figures 7B–7D). Similar results were obtained in testes with *chinmo* RNAi knockdown in the CySC lineage (data not shown). Although Dsx^M protein is decreased in the CySC lineage in $chinmo^{ST}$ testes, a decrease in dsx^M mRNA is not detectable at the whole-testis level (Figure S3A), suggesting that a decrease in dsx^M mRNA in CySCs and cyst cells might be masked by the presence of dsx^M mRNA from other cells in the testis, such as sheath, basal epithelial, and seminal vesicle cells. Therefore, we used *dsx-Gal4* to detect changes in *dsx* transcription levels, specifically in CySC lineage cells (Robinett et al., 2010). Two independent *dsx-Gal4* lines are active in CySC lineage cells in control testes (Figures 7E and 7G), but not in follicle cells in normal ovaries (Figures S3C and S3D). In $chinmo^{ST}$ testes, however, somatic aggregates and follicle-like cells no longer express these transgenic reporters (Figures 7F and 7H). These results suggest that Chinmo either directly or indirectly regulates dsx^M transcription levels. Because Dsx^M directly represses *Yp1* expression (Burtis et al., 1991; Coschigano and Wensink, 1993), the appearance of ectopic *Yp1* transcripts in $chinmo^{ST}$ testes likely reflects loss of Dsx^M (Figure 3J). Taken together, these results suggest that Chinmo is required for maintenance of dsx^M expression in the CySC lineage and that loss of Dsx^M contributes to the male-to-female somatic sex transformation in $chinmo^{ST}$ testes. In support of this hypothesis, expression of Dsx^M in the CySC lineage in *chinmo-RNAi* testes partially rescues the phenotype (Table 1). We have also found, using two independent *dsx-RNAi* lines, that knockdown of *dsx* in the CySC lineage partially phenocopies the *chinmo* mutant phenotype. In most *dsx-RNAi* testes, germ cells overproliferate and arrest at early spermatogonial stages (Figures 7J, S3I, and S3J), as do germ cells in $chinmo^{ST}$ testes (Figure 1R), consistent with a mismatch of germline and somatic sex. Some testes also contain small aggregates of somatic cells (Figure S3J, arrows) that resemble the follicle-like cells in *chinmo* mutant testes, but they never develop a full layer of follicle-like cells. Together, these results suggest that Chinmo has targets in addition to Dsx^M that maintain other aspects of CySC fate and prevent them from transforming into follicle stem cell-like cells. We conclude that male sexual identity is actively maintained in the CySC lineage of the adult testis and that this requires the concerted action of Chinmo and Dsx^M.

DISCUSSION

The male-to-female sex transformation phenotype that we observe in *Drosophila* testes with reduced Chinmo demonstrates that adult somatic stem cells actively maintain their sexual identity in a cell-autonomous manner (Figure 7K). In vertebrates, sexual phenotypes were thought to be determined exclusively by systemic hormones, but recent studies have found exceptions to this rule. In birds, for example, somatic sexual identity may be largely cell autonomous, as illustrated by lateral gynandromorph chickens; these male:female chimeras have one side that appears male and the other female, even though both sides are exposed to the same blood system and hormone environment (Zhao et al., 2010). Another example is the adult mouse gonad, where loss of sex-specific transcriptional regulators can cause differentiated somatic cells to transdifferentiate into cells of the opposite sex (Matson et al., 2011; Uhlenhaut et al., 2009). Other recent work has found that adult stem cells can respond differently to the different levels of hormones found in male and female mice: hematopoietic stem cells, which were thought to function similarly in both sexes, respond to the higher levels of estrogen in females, resulting in differences in self-renewal ability (Nakada et al., 2014). We now show, using the *Drosophila* testis as a model, that adult somatic



(legend on next page)

Cell Press

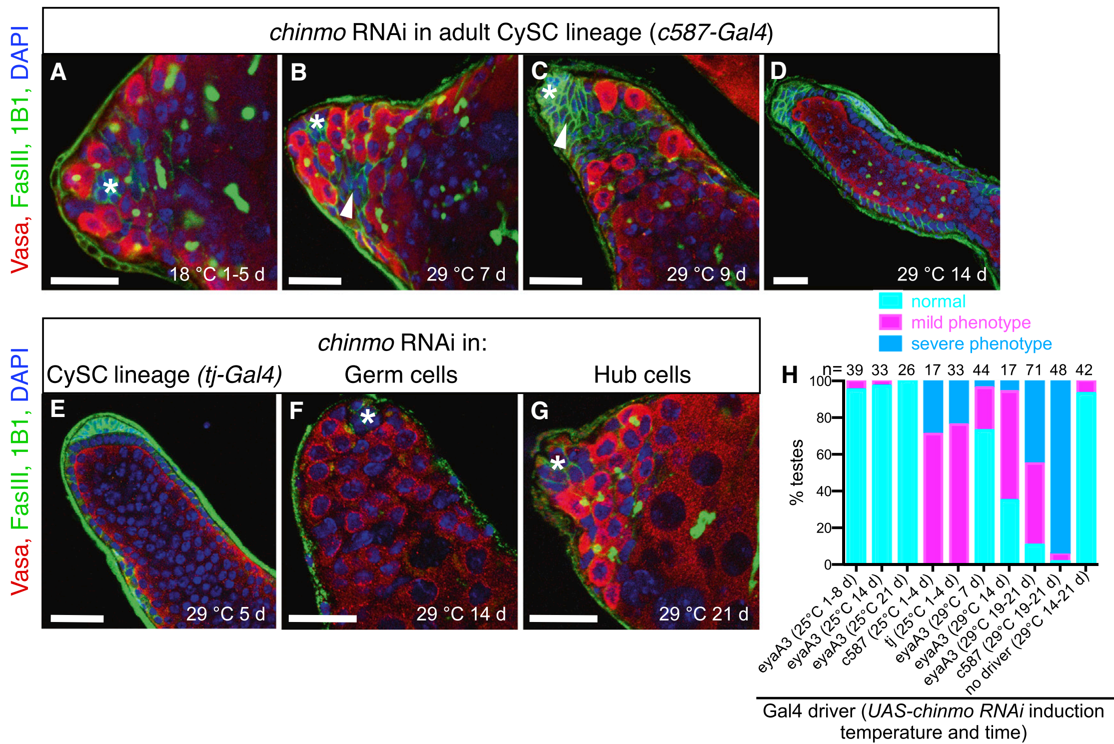


Figure 5. Chinmo Is Required Autonomously in Adult CySC Lineage Cells to Prevent Their Transformation into Female Soma

(A–G) Immunofluorescence detection of FasIII (green at cell periphery) and the germ cell marker Vasa (red) to visualize the morphology of adult testes after expression of *chinmo*-RNAi in different cell types. Before RNAi induction in the CySC lineage (A), testes appear normal. After RNAi induction in the CySC lineage, testes resemble *chinmo*ST testes: somatic cells initially form aggregates (97% of testes; n = 36/37) (B and C, arrowheads) and then follicle-like cells (76% of testes; n = 68/90) (D). RNAi induction in the CySC lineage with a different Gal4 driver also phenocopies *chinmo*ST testes (E). RNAi induction in germ cells (F) or in hub cells (G) does not phenocopy *chinmo*ST. Hubs marked by asterisk. Scale bars, 20 μm.

(H) Composite bar graph showing the percentage of testes with normal, mild, or severe phenotypes after expression of *chinmo*-RNAi with different somatic drivers. Testes remain morphologically wild-type after *chinmo*-RNAi expression only in cyst cells (*eyaA3-Gal4* at 25°C), but after expression in both CySCs and cyst cells (*eyaA3-Gal4* at 29°C, *c587-Gal4*, or *tj-Gal4*), testes display a range of *chinmo*ST phenotypes, as defined in the Experimental Procedures.

See also Figure S2 and Table S3.

stem cells can also autonomously maintain their sexual identity. Chinmo regulates sexual identity by promoting expression of the male sex determination factor Doublesex^{M} (Dsx^{M}) in CySCs and their progeny. Because *dsx*-family members play central roles in the maintenance of somatic sexual identity in diverse organisms, including mice, fish, and amphibians (Masuyama et al., 2012; Matson et al., 2011; Shibata et al., 2002), but their upstream regulators are not well understood, it will be interesting to determine whether Chinmo is required for sex maintenance at other times in development and in other tissues in *Drosophila*, and whether Chinmo homologs function more broadly in sex maintenance in other organisms.

Our data suggest that Chinmo is the central regulator of CySC sex maintenance and implicate Dsx^{M} is an important target of Chinmo in this process. However, because expression of Dsx^{M} only partially rescues the sex maintenance phenotype in *chinmo* mutant testes, and *dsx* knockdown only partially phenocopies the *chinmo* mutant phenotype, Dsx^{M} is unlikely to be the only target of Chinmo. Together, our data support a model in which Chinmo promotes expression of Dsx^{M}, which in turn

Figure 4. Ovarian Stalk Cell Markers Are Expressed in a Subset of Somatic Cells in *chinmo*ST Testes

(A and B) Immunofluorescence detection of Arm (white) and Zfh-1 (green) reveals stalk-like cells in *chinmo*ST testes. In control testes (A), hub cells (yellow arrow) express high levels of Arm and low levels of Zfh-1, and CySCs and their immediate daughters (arrowhead) express low levels of Arm and high levels of Zfh-1. In all other cyst cells, Arm is low and Zfh-1 is absent. In *chinmo*ST testes (B), Arm is low and Zfh-1 is absent in follicle-like cells lining the periphery (solid arrowhead). Other somatic cells (open arrowheads) form aggregates that resemble ovarian stalk cells morphologically and express high levels of Arm and Zfh-1, which are characteristic of stalk cells (data not shown). These aggregates are typically located just beneath the testis sheath, sandwiched between follicle-like cells and the basement membrane.

(C–F) Immunofluorescence detection of Castor (red) and Zfh-1 (green) reveals stalk-like cells in *chinmo*ST testes. Castor is absent from control testes (C; also see Figure 2A). Hubs marked by yellow arrow. In control ovaries (D), Castor is expressed in follicle stem cells and early follicle cell progenitors (bracket; also see Figure 2C). After egg chamber formation, Castor is restricted to polar cells and Zfh-1^{+} stalk cells (open arrowhead) and is no longer expressed in main-body follicle cells (solid arrowhead). In *chinmo*ST testes (E and F), Castor is expressed in Zfh-1^{+} cell aggregates at the testis apex that resemble follicle cell progenitors (bracket) and in stalk-like cells (open arrowheads), but not in Zfh-1^{-} follicle-like cells (solid arrowhead). (F) is an enlargement of the boxed area in (E). Scale bars, 20 μm.

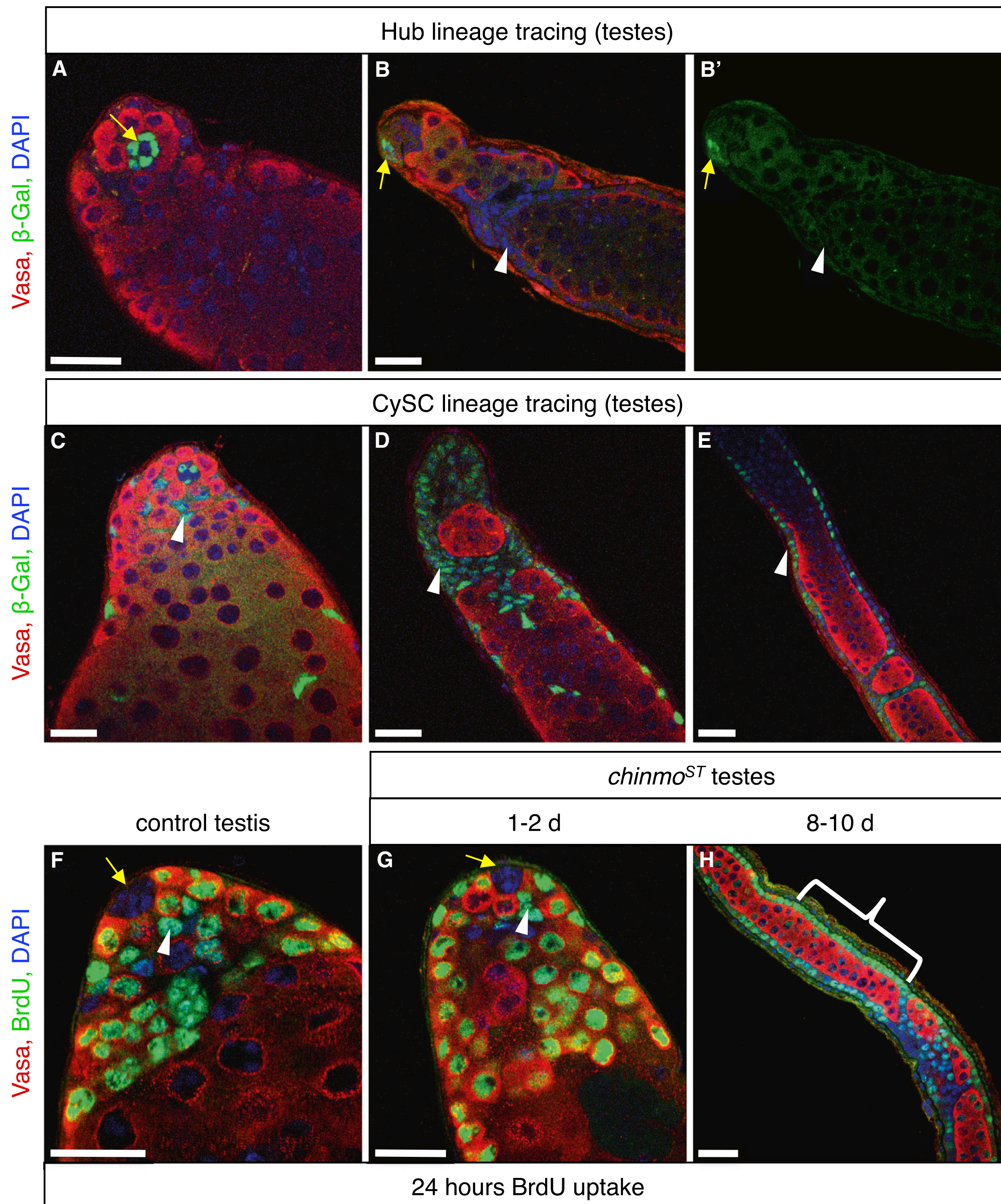


Figure 6. Follicle-like Cells Come from the Cyst Stem Cell Lineage, but Not from Hub Cells

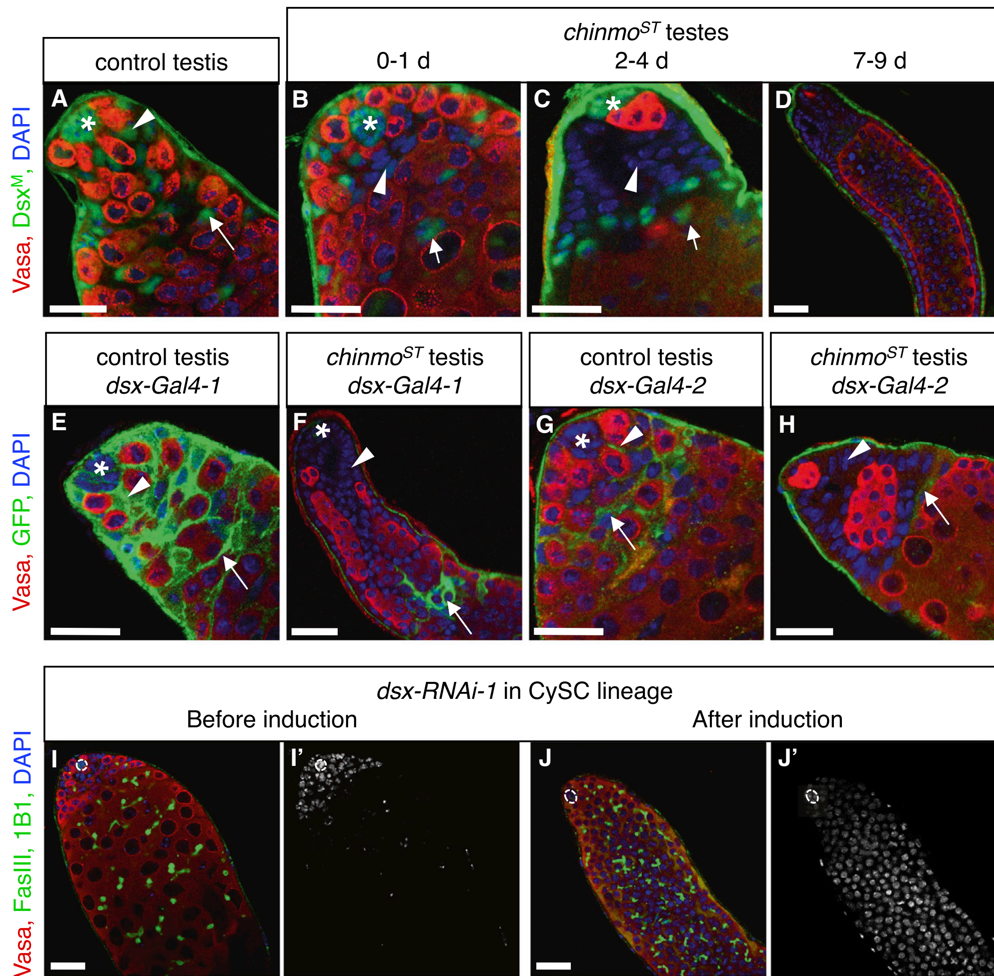
(A–E) Immunofluorescence detection of β-galactosidase (β-gal) (green), which permanently marks either hub cells alone (A-B) or CySC lineage and hub cells (C-E) in *chinmo*ST testes. Somatic cell aggregates and follicle-like cells (B, D, and E, arrowheads) are derived from CySC lineage cells (C, arrowhead), but not from hub cells (A and B, arrows) because they express β-gal in testes with marked CySC lineage cells, but not in testes with only marked hubs.

(F–H) Immunofluorescence detection of the thymidine analog bromodeoxyuridine (BrdU, green). Adult males were fed BrdU for 24 hr prior to dissection to label all cells that traversed S phase during this time. In control (F) and young *chinmo*ST (G) testes, BrdU is not found in any hub cells (arrows), but many germ cells (red) and CySCs (arrowheads) are BrdU^{+}. BrdU is also found in most follicle-like cells in older *chinmo*ST testes (H, bracket). In all panels, DAPI marks nuclei (blue) and Vasa marks germ cells (red). Scale bars, 20 μm.

See also Table S4.

regulates its own set of target genes that are critical for male sexual identity. In addition to promoting Dsx^{M} expression, Chinmo likely affects the expression of Dsx-independent target genes that are important for additional aspects of CySC identity. In this case, resupplying Dsx^{M} does not fully rescue the *chinmo*-RNAi phenotype because these testes still lack Chinmo targets

CellPress



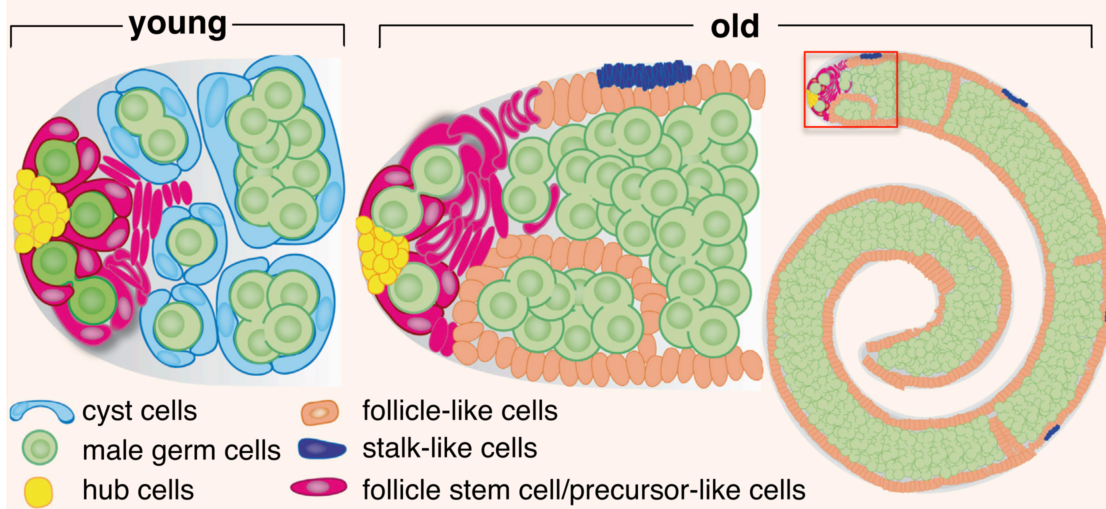


Figure 7. Chinmo Maintains the Male Identity of Adult Somatic Stem Cells through the Canonical Sex Determination Pathway

(A–D) Immunofluorescence detection of Dsx^M (green) in adult testes. In control testes (A), Dsx^M is expressed in the nuclei of hub cells (asterisk), CySCs (arrowhead), and cyst cells (arrow) (n = 59 testes). In young *chinmo*ST testes that are still morphologically normal (B), Dsx^M levels are low in some CySCs and early

(legend continued on next page)

Table 1. Overexpressing DsxM in the CySC Lineage Rescues Chinmo Knockdown Phenotype

Use c587-Gal4 to Express[a]	% Testes with FasIII+ Somatic Aggregates[b]					
	0 Days	4 Days	7 Days	9 Days	11 Days	14 Days
UAS-chinmoRNAi-1	0 (n = 31)	6.3 (n = 64)	73.8 (n = 103)	80.5 (n = 133)	97.1 (n = 35)	100 (n = 59)
UAS-chinmoRNAi-1, *UAS-DsxM*	0 (n = 25)	5.3 (n = 76)	36.6 (n = 153); $p < 0.0001$[c]	55.9 (n = 145); $p < 0.0001$[c]	39.4 (n = 33); $p < 0.0001$[c]	86.3 (n = 124); $p < 0.01$[c]
UAS-DsxM	0 (n = 18)	N/A	0 (n = 24)	5.3 (n = 19)	0 (n = 21)	N/A

[a]UAS-chinmoRNAi-1 = chinmo^{HM04048}.
[b]All flies were raised at 18°C, which suppresses RNAi induction. After eclosion, adult flies were shifted to 29°C for the indicated amount of time to induce RNAi.
[c]Compared to age-matched *UAS-chinmoRNAi-1*.

that are independent of Dsx but are required for CySC self-renewal. Similarly, CySCs lacking Dsx may only partially recapitulate the Chinmo loss-of-function phenotype because, although they lack expression of male determinants downstream of Dsx, they continue to express Dsx-independent Chinmo targets. These Dsx-independent Chinmo targets may permit maintenance of CySCs lacking Dsx and may also prevent the complete conversion of these mutant CySCs into follicle stem or progenitor-like cells. Furthermore, because our data suggest that Chinmo regulates Dsx^{M} at the level of transcription, comparison of targets of Chinmo and Dsx^{M} in the CySC lineage should be informative.

It is intriguing that removing *chinmo* clonally in a few CySCs leads to rapid loss of the mutant CySCs (Flaherty et al., 2010), whereas depleting Chinmo from all CySCs (via RNAi or the *chinmo*ST mutation) allows them to remain but become feminized. We hypothesize that CySC clones lacking *chinmo* (either weak or strong alleles) are outcompeted by wild-type CySCs and that the full sex transformation phenotype only develops when the possibility of competition is removed (i.e., when most or all CySCs lack *chinmo*). Individual CySCs lacking Chinmo may also undergo sex transdifferentiation, but we have not been able to determine whether or not this is the case, because such CySCs are lost quite rapidly. Comparing testes that contain either a few or many CySCs that lack *chinmo* could be informative, as it should reveal the number of stem cells that must be mutant in order to allow the sex transformation of the testis soma to occur. The ability of stem cells to compete for niche access is a poorly understood yet important aspect of adult stem cell biology in general (Stine and Matunis, 2013), and this work provides an avenue for studying the underlying mechanisms.

Because Chinmo is a Jak-STAT effector, we expected that loss of Jak-STAT signaling might give a similar phenotype to loss of Chinmo. However, reduction of Jak-STAT via mutations or RNAi causes only rapid stem cell differentiation and no apparent sex conversion phenotype (Leatherman and DiNardo, 2010; data not shown). Loss of Jak-STAT signaling causes loss of other targets besides Chinmo, for example Zfh-1(Leatherman and DiNardo, 2008). We speculate that loss of these targets causes stem cells to differentiate before they are able to undergo a sex conversion, which our data suggest can arise only from stem cells.

Our finding that stem cells undergo sex transformation may provide unique insight into how transdifferentiation is regulated at a cellular and molecular level more generally. Sex transformation of stem cells in adult *Drosophila* testes provides a highly tractable genetic system to study cellular mechanisms by which conserved *dsx*-related transcription factors and their target genes actively maintain somatic sexual identity. This may provide insight into human testicular cancers, such as granulosa cell tumors, that may be linked to altered somatic sexual identity (Hanson and Ambaye, 2011). Analysis of Chinmo and

cyst cells (arrowhead) but remain high in differentiated cyst cells away from the apex (arrow) (n = 35 testes). In *chinmo*ST testes containing somatic cell aggregates (C), Dsx^{M} levels are low in aggregates (arrowhead) but remain high in differentiated cyst cells (arrow) (n = 37 testes). In *chinmo*ST testes with follicle-like cells (D), Dsx^{M} is absent from all follicle-like cells (n = 42 testes).
(E–H) Immunofluorescence detection of cytoplasmic GFP (green) in adult testes to reflect the transcription of *dsx-Gal4* (two different lines). *dsx-Gal4-1* is expressed in control testes (E) at high levels in CySCs (arrowhead) and cyst cells (arrow) and at lower levels in some hub cells (asterisk) (n = 15 testes). In *chinmo*ST testes (F), *dsx-Gal4-1* expression is absent from somatic aggregates and follicle-like cells (arrowhead) but remains high in differentiated cyst cells away from the apex (arrow) (n = 19 testes). *dsx-Gal4-2* is expressed in control testes (G) in early cyst cells (arrow) and in some CySCs (arrowhead) but is not detectable in hub cells (asterisk) (n = 21 testes). In *chinmo*ST testes (H), *dsx-Gal4-2* expression is absent from somatic aggregates (arrowhead) but present in some differentiated cyst cells away from the apex (arrow) (n = 28 testes).
(I and J) Immunofluorescence detection of FasIII and 1B1 (green) and DAPI (blue) in adult testes before and after induction of *dsx-RNAi* in the CySC lineage. (I') and (J') show the blue channel (DAPI) only in gray scale. Before RNAi induction (I), testes look normal, and cells that stain brightly with DAPI (early germ cells and somatic cells) are restricted to the apex of the testis (n = 32 testes). After RNAi induction (29°C for 10 days) (J), testes fill with germ cells that arrest as early spermatogonia, based on the expansion of the bright DAPI zone and presence of branching fusomes (94% of testes; n = 51). In all panels, nuclei are marked with DAPI (blue); germ cells are marked with Vasa (red); hubs are marked by an asterisk (A–C and E–G) or dashed line (I and J). Scale bars, 20 μm.
(K) Schematic drawings of a young (left) and older (center) *chinmo* mutant testis apex and an older *chinmo* mutant testis (right) with the apex indicated (red box). In young *chinmo* mutant testes, CySCs and their early progeny lose male fate and adopt a follicle stem cell/progenitor-like cell identity (magenta). As flies age, follicle stem cell/progenitor-like cells produce follicle-like cells (orange) and stalk-like cells (purple), which gradually displace normal cyst cells from the niche. The germ cells, which become restricted to the lumen of the tissue, maintain characteristics of their male identity but overproliferate and arrest as spermatogonia because the germline and somatic sex are mismatched.
See also Figure S3.

Dsx/Dmrt1-mediated sex maintenance pathways in somatic stem cells may also yield insight into the maintenance of sexual dimorphism in other organs, such as the mammalian liver, and provide a unique model to study adult stem cell transdifferentiation in vivo.

EXPERIMENTAL PROCEDURES

Fly Stocks and Cultures

Fly stocks were raised at 25°C on standard molasses/yeast medium unless otherwise indicated. The following fly stocks were used: *UAS-FL-chinmo* and *chinmo*[1] (Zhu et al., 2006), *eyaA3-Gal4* (Leatherman and DiNardo, 2008), *M5-4* (Gönczy and DiNardo, 1996), *c587-Gal4* (Kai and Spradling, 2003), *nanos-Gal4-VP16* (Van Doren et al., 1998), *tj-Gal4* (*Drosophila* Genetic Resource Center), *UAS-dsx*^{M} (Lee et al., 2002), *UAS-lifactin-GFP* and *slbo-lifactin-GFP* (from X. Wang and D. Montell), *UAS-dsx RNAi-1* (*P[KK111266] VIE-260B*; from Vienna *Drosophila* RNAi Center [VDRC]), *UAS-dsx RNAi-2* (from T. Shirangi and M. Mckeown), *dsx-Gal4-1* (*w*^{1118}*; P[GMR40A05-Gal4] attP2*; from Bloomington *Drosophila* Stock Center [BDSC]), and *dsx-Gal4-2* (*w*$^{-}$*; dsx-Gal4/TM3,Sb,Ser, twist-gal4, UAS*-2xEGFP; from B. Baker). *y w* and *chinmo*ST*/CyO* flies were used as control flies. Other fly stocks were from the VDRC or BDSC, including the *SOD[x39]/TM3* line from which *chinmo*ST was isolated.

Immunostaining

Testes and ovaries were dissected, fixed, and stained as described previously (Matunis et al., 1997). Tyramide signal amplification (Invitrogen) was used to increase sensitivity of rat anti-Dsx^{M} (from B. Oliver; 1:500 dilution). Antibodies used are described in the Supplemental Experimental Procedures.

Lineage Analysis

For lineage analysis, *chinmo*ST*; actin > stop > lacZ* or *chinmo*^{KG05386}*; actin > stop > lacZ* males were mated to *E132-Gal4; chinmo*ST*; UAS-FLP* or *c587-Gal4; chinmo*ST*; UAS-FLP* females to permanently mark hub cells or the CySC lineage, respectively. Newly eclosed flies were then aged prior to testis dissection and immunostaining. To determine whether the *c587-Gal4* and *E132-Gal4* drivers become re-expressed in follicle-like cells in aged *chinmo*ST testes, *chinmo*ST*; UAS-lifactin-GFP* males were crossed to *E132-Gal4; chinmo*ST*; tub-Gal80[ts]* or *c587-Gal4; chinmo*ST*; tub-Gal80[ts]* females at 18°C. Newly eclosed progeny were aged at 18°C for 1 week to allow development of the follicle-like cell phenotype and were then switched to 29°C to assess Gal4 activity in chinmoST testes.

mRNA Extraction and PCR

mRNA extraction and RT-PCR were performed as previously described (Issigonis and Matunis, 2012). Primer sequences are in the Supplemental Experimental Procedures.

In Vivo BrdU Incorporation

Groups of about 20 age-matched adult males were incubated at 25°C in empty vials for 4 hr and then transferred to vials of fly food overlaid with disks of filter paper soaked with 120 μl of 2.5 mM BrdU (Life Techniologies) and 5 μl of green food coloring (McCormick). After 24 hr, flies that had not eaten the BrdU (and therefore did not have green guts) were discarded, and BrdU was detected in testes from the remaining flies as described (Brawley and Matunis, 2004).

Quantification of Cell Division and Severity of the *chinmo*ST Phenotype

CySC mitoses were quantified by counting the number of PH3^{+} Vasa^{-} cells within two cell diameters of the hub. Cyst cell mitoses were quantified by counting the number of PH3^{+} Vasa^{-} cells more than two cell diameters from the hub. To categorize the severity of the *chinmo*ST phenotype, we used the following criteria. Morphologically wild-type testes were scored as normal. Testes with epithelial aggregates and/or follicle-like cells at the apex near the hub, but not away from the apex, were scored as having a mild phenotype; these testes often contained overproliferating germ cells. Testes with epithelial aggregates and/or follicle-like cells throughout the entire testis were scored as having a severe phenotype; germ cells in these testes were often arrested at an early spermatogonial stage or degenerating. The severity of the *chinmo*ST and *chinmo* RNAi phenotypes both progress with age. Therefore, the presence of a mild versus severe phenotype was used to estimate testis age when not clearly indicated.

Microscopy and Image Analysis

Fixed testes were mounted in Vectashield (Vector Labs), imaged with a Zeiss LSM 5 Pascal or LSM 510 Meta, and analyzed using the Zeiss LSM Image Browser software; panels are single-confocal sections unless stated otherwise. Statistical analysis of pH3 positive cell number was performed with GraphPad Prism 5 software, and averages were compared using a two-tailed Student's t test, assuming unequal variances. Percentages were analyzed for statistical significance using a chi-square test.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2014.10.004.

ACKNOWLEDGMENTS

We thank Helen Salz and Amir Oryan for helpful discussions, Margaret de Cuevas, Leah Greenspan, David Zarkower, and Geraldine Seydoux for comments on the manuscript, Yijie Li for help with dissections, and Nick Sokol, Brian Oliver, Gyunghee Lee, Denise Montell, and Xiaobo Wang for flies or antisera. This work was funded by the NIH (HD040307 and HD052937) (to E.L.M.).

Received: March 14, 2014
Revised: August 29, 2014
Accepted: October 3, 2014
Published: November 13, 2014

REFERENCES

Amoyel, M., Sanny, J., Burel, M., and Bach, E.A. (2013). Hedgehog is required for CySC self-renewal but does not contribute to the GSC niche in the Drosophila testis. Development *140*, 56–65.

Brawley, C., and Matunis, E. (2004). Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. Science *304*, 1331–1334.

Brennan, M.D., Weiner, A.J., Goralski, T.J., and Mahowald, A.P. (1982). The follicle cells are a major site of vitellogenin synthesis in Drosophila melanogaster. Dev. Biol. *89*, 225–236.

Burtis, K.C., Coschigano, K.T., Baker, B.S., and Wensink, P.C. (1991). The doublesex proteins of Drosophila melanogaster bind directly to a sex-specific yolk protein gene enhancer. EMBO J. *10*, 2577–2582.

Cai, D., Chen, S.C., Prasad, M., He, L., Wang, X., Choesmel-Cadamuro, V., Sawyer, J.K., Danuser, G., and Montell, D.J. (2014). Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. Cell *157*, 1146–1159.

Chang, Y.C., Jang, A.C., Lin, C.H., and Montell, D.J. (2013). Castor is required for Hedgehog-dependent cell-fate specification and follicle stem cell maintenance in Drosophila oogenesis. Proc. Natl. Acad. Sci. USA *110*, E1734–E1742.

Coschigano, K.T., and Wensink, P.C. (1993). Sex-specific transcriptional regulation by the male and female doublesex proteins of Drosophila. Genes Dev. *7*, 42–54.

de Cuevas, M., Lilly, M.A., and Spradling, A.C. (1997). Germline cyst formation in Drosophila. Annu. Rev. Genet. *31*, 405–428.

Flaherty, M.S., Salis, P., Evans, C.J., Ekas, L.A., Marouf, A., Zavadil, J., Banerjee, U., and Bach, E.A. (2010). chinmo is a functional effector of the JAK/STAT pathway that regulates eye development, tumor formation, and stem cell self-renewal in Drosophila. Dev. Cell *18*, 556–568.

Fuller, M.T., and Spradling, A.C. (2007). Male and female Drosophila germline stem cells: two versions of immortality. Science *316*, 402–404.

Gönczy, P., and DiNardo, S. (1996). The germ line regulates somatic cyst cell proliferation and fate during Drosophila spermatogenesis. Development *122*, 2437–2447.

Hanson, J.A., and Ambaye, A.B. (2011). Adult testicular granulosa cell tumor: a review of the literature for clinicopathologic predictors of malignancy. Arch. Pathol. Lab. Med. *135*, 143–146.

Hempel, L.U., and Oliver, B. (2007). Sex-specific DoublesexM expression in subsets of Drosophila somatic gonad cells. BMC Dev. Biol. *7*, 113.

Hime, G.R., Brill, J.A., and Fuller, M.T. (1996). Assembly of ring canals in the male germ line from structural components of the contractile ring. J. Cell Sci. *109*, 2779–2788.

Issigonis, M., and Matunis, E. (2012). The Drosophila BCL6 homolog Ken and Barbie promotes somatic stem cell self-renewal in the testis niche. Dev. Biol. *368*, 181–192.

Jackson, S.M., and Blochlinger, K. (1997). cut interacts with Notch and protein kinase A to regulate egg chamber formation and to maintain germline cyst integrity during Drosophila oogenesis. Development *124*, 3663–3672.

Jinks, T.M., Polydorides, A.D., Calhoun, G., and Schedl, P. (2000). The JAK/STAT signaling pathway is required for the initial choice of sexual identity in Drosophila melanogaster. Mol. Cell *5*, 581–587.

Kai, T., and Spradling, A. (2003). An empty *Drosophila* stem cell niche reactivates the proliferation of ectopic cells. Proc. Natl. Acad. Sci. USA *100*, 4633–4638.

Kopp, A. (2012). Dmrt genes in the development and evolution of sexual dimorphism. Trends Genet. *28*, 175–184.

Leatherman, J.L., and DiNardo, S. (2008). Zfh-1 controls somatic stem cell self-renewal in the Drosophila testis and nonautonomously influences germline stem cell self-renewal. Cell Stem Cell *3*, 44–54.

Leatherman, J.L., and DiNardo, S. (2010). Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in Drosophila testes. Nat. Cell Biol. *12*, 806–811.

Lee, G., Hall, J.C., and Park, J.H. (2002). Doublesex gene expression in the central nervous system of *Drosophila melanogaster*. J. Neurogenet. *16*, 229–248.

Lin, H., Yue, L., and Spradling, A.C. (1994). The Drosophila fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. Development *120*, 947–956.

Logan, S.K., Garabedian, M.J., and Wensink, P.C. (1989). DNA regions that regulate the ovarian transcriptional specificity of Drosophila yolk protein genes. Genes Dev. *3*, 1453–1461.

Mahowald, A.P., and Kambysellis, M.P. (1980). Oogenesis. In The Genetics and Biology of *Drosophila*, M. Ashburner and T.R.F. Wright, eds. (New York: Academic Press), pp. 141–224.

Margolis, J., and Spradling, A. (1995). Identification and behavior of epithelial stem cells in the Drosophila ovary. Development *121*, 3797–3807.

Masuyama, H., Yamada, M., Kamei, Y., Fujiwara-Ishikawa, T., Todo, T., Nagahama, Y., and Matsuda, M. (2012). Dmrt1 mutation causes a male-to-female sex reversal after the sex determination by Dmy in the medaka. Chromosome Res. *20*, 163–176.

Matson, C.K., and Zarkower, D. (2012). Sex and the singular DM domain: insights into sexual regulation, evolution and plasticity. Nat. Rev. Genet. *13*, 163–174.

Matson, C.K., Murphy, M.W., Sarver, A.L., Griswold, M.D., Bardwell, V.J., and Zarkower, D. (2011). DMRT1 prevents female reprogramming in the postnatal mammalian testis. Nature *476*, 101–104.

Matunis, E., Tran, J., Gönczy, P., Caldwell, K., and DiNardo, S. (1997). punt and schnurri regulate a somatically derived signal that restricts proliferation of committed progenitors in the germline. Development *124*, 4383–4391.

Matunis, E.L., Stine, R.R., and de Cuevas, M. (2012). Recent advances in Drosophila male germline stem cell biology. Spermatogenesis *2*, 137–144.

McKearin, D.M., and Spradling, A.C. (1990). bag-of-marbles: a Drosophila gene required to initiate both male and female gametogenesis. Genes Dev. *4* (12B), 2242–2251.

Michel, M., Kupinski, A.P., Raabe, I., and Bökel, C. (2012). Hh signalling is essential for somatic stem cell maintenance in the Drosophila testis niche. Development *139*, 2663–2669.

Nakada, D., Oguro, H., Levi, B.P., Ryan, N., Kitano, A., Saitoh, Y., Takeichi, M., Wendt, G.R., and Morrison, S.J. (2014). Oestrogen increases haematopoietic stem-cell self-renewal in females and during pregnancy. Nature *505*, 555–558.

Robinett, C.C., Vaughan, A.G., Knapp, J.M., and Baker, B.S. (2010). Sex and the single cell. II. There is a time and place for sex. PLoS Biol. *8*, e1000365.

Shibata, K., Takase, M., and Nakamura, M. (2002). The Dmrt1 expression in sex-reversed gonads of amphibians. Gen. Comp. Endocrinol. *127*, 232–241.

Stine, R.R., and Matunis, E.L. (2013). Stem cell competition: finding balance in the niche. Trends Cell Biol. *23*, 357–364.

Tran, J., Brenner, T.J., and DiNardo, S. (2000). Somatic control over the germline stem cell lineage during Drosophila spermatogenesis. Nature *407*, 754–757.

Uhlenhaut, N.H., Jakob, S., Anlag, K., Eisenberger, T., Sekido, R., Kress, J., Treier, A.C., Klugmann, C., Klasen, C., Holter, N.I., et al. (2009). Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell *139*, 1130–1142.

Van Doren, M., Williamson, A.L., and Lehmann, R. (1998). Regulation of zygotic gene expression in *Drosophila* primordial germ cells. Curr. Biol. *8*, 243–246.

Wawersik, M., Milutinovich, A., Casper, A.L., Matunis, E., Williams, B., and Van Doren, M. (2005). Somatic control of germline sexual development is mediated by the JAK/STAT pathway. Nature *436*, 563–567.

Whitworth, C., Jimenez, E., and Van Doren, M. (2012). Development of sexual dimorphism in the Drosophila testis. Spermatogenesis *2*, 129–136.

Zhao, D., McBride, D., Nandi, S., McQueen, H.A., McGrew, M.J., Hocking, P.M., Lewis, P.D., Sang, H.M., and Clinton, M. (2010). Somatic sex identity is cell autonomous in the chicken. Nature *464*, 237–242.

Zhu, S., Lin, S., Kao, C.F., Awasaki, T., Chiang, A.S., and Lee, T. (2006). Gradients of the Drosophila Chinmo BTB-zinc finger protein govern neuronal temporal identity. Cell *127*, 409–422.