coated these silent *ura4*$^+$ genes and association was abolished in *swi6*Δ cells. This dissociation of Rad21 cannot be attributed to transcriptional interference because Rad21-3xHA also coats the telomere adjacent sequence, which is transcribed in *swi6*$^+$ and *swi6*Δ cells (Fig. 4C). Therefore, these data indicate that silent Swi6 chromatin alone forms domains with a high affinity for Rad21-cohesin and that the high concentration of cohesin over centromeric regions occurs outside of the context of a functional kinetochore.

Clr4 [equivalent to mammalian and fly Su(var)39] methylates histone H3 on lysine-9 (*21*, *22*), Swi6 association with centromeres requires this activity, and the chromo-domain of Swi6 binds specifically to histone H3 NH_2 termini only when methylated on lysine-9 (*23*). The assembly of this silent Swi6 chromatin is required to attract cohesin to centromeres, because centromere association of Rad21 and another cohesin component, Psc3 (*5*), were dependent on Swi6 (*19*). Many mutants known to alter this heterochromatin coating the outer repeats of fission yeast centromeres lose chromosomes and have a high incidence of lagging chromosomes on late anaphase spindles (*12*, *14*, *16*). Figure 1D shows that the major segregation defect observed when Rad21-cohesin function is perturbed is also anaphase-lagging chromosomes. Lagging chromosomes are mostly single separated chromatids (*16*). A similar defect is observed in trichostatin A–treated cells, where mouse Heterochromatin protein 1 (HP1) or *S. pombe* Swi6 are also mislocalized (*15*, *24*). In rat kangaroo PtK1 cells, most lagging chromosomes are single chromatids resulting from the attachment of an individual kinetochore on the laggard to microtubules from both poles (*25*) (merotelic attachment). In metazoans, cohesin persists only between the heterochromatic domains of sister centromeres at metaphase (*6*, *7*). Thus, as in fission yeast, lagging chromosomes may result from defective centromeric cohesion due to HP1 dispersal. Intriguingly, lagging chromosomes have not been reported in any mutant defective in centromere or cohesin function in budding yeast. Indeed, the major mitotic defect observed in *S. cerevisiae* cells lacking cohesin is nondisjunction (*26*). *S. cerevisiae* kinetochores have been shown to bind only one microtubule (*27*), and centromeric heterochromatin has not been described. In contrast, *S. pombe* centromeres are packaged in heterochromatin and bind two to four microtubules (*28*) and, thus, have the capacity for merotelic attachment.

We propose that one function of silent Swi6 chromatin at fission yeast centromeres is to attract a high concentration of cohesin so that sister kinetochores face away from each other. The architecture formed by this silent chromatin in cooperation with cohesin might, therefore, aid the arrangement of multiple microtubule attachment sites at each sister kinetochore so that all sites at one kinetochore capture microtubules from the same pole. Assuming that this role for centromeric heterochromatin is conserved, we expect that deficiencies in heterochromatin formation will contribute to aberrant mitotic and meiotic chromosome segregation in humans, driving tumor formation and the production of aneuploid offspring.

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Stem Cell Self-Renewal Specified by JAK-STAT Activation in Response to a Support Cell Cue

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Stem cells generate many differentiated, short-lived cell types, such as blood, skin, and sperm, throughout adult life. Stem cells maintain a long-term capacity to divide, producing daughter cells that either self-renew or initiate differentiation. Although the surrounding microenvironment or "niche" influences stem cell fate decisions, few signals that emanate from the niche to specify stem cell self-renewal have been identified. Here we demonstrate that the apical hub cells in the *Drosophila* testis act as a cellular niche that supports stem cell self-renewal. Hub cells express the ligand Unpaired (Upd), which activates the Janus kinase–signal transducer and activator of transcription (JAK-STAT) pathway in adjacent germ cells to specify self-renewal and continual maintenance of the germ line stem cell population.

Stem cell self-renewal must be regulated to avoid either stem cell loss or hyperproliferation. In some systems stem cell numbers are limited by asymmetric cell division, where one daughter cell retains stem cell identity, while the other initiates differentiation. However, stem cells can also divide symmetrically to expand stem cell numbers after wound-

ing or transplantation (*1*, *2*). Stem cells frequently lose the capacity for self-renewal when removed from their niche (*3*), suggesting that cues from the local microenvironment influence stem cell fate (*4*–*6*). In most systems, however, the difficulty in identifying stem cells in situ has precluded investigation of mechanisms by which microenvironments regulate stem cell self-renewal.

The *Drosophila* male germ line provides an excellent system for analyzing the relation between stem cells and their microenvironment. Asymmetric division of male germ line stem cells begins by late embryogenesis and maintains spermatogenesis throughout adulthood. Germ line stem cells lie at the apical tip of the testis, surrounding a cluster of somatic cells called the hub (Fig. 1A) (*7*). Upon stem cell division, the daughter cell maintaining contact with the hub retains stem cell identity, whereas the cell displaced away from the hub initiates differentiation as a gonialblast (*7*, *8*).

Here we demonstrate that the JAK-STAT signal transduction pathway is required for stem cell self-renewal in the *Drosophila* male germ line. JAKs mediate signaling downstream of many mammalian cytokine and growth factor receptors, often by phosphorylation and activation of STAT proteins. *Drosophila melanogaster* has one known JAK, Hopscotch (Hop), and one known STAT, Stat92E, which act together in essential developmental processes ranging from embryonic segmentation to larval hematopoiesis. The viable, male sterile hop^{25} allele contains a point mutation in a conserved region implicated in receptor binding leading to activation (*9*, *10*).

Adult hop^{25} males lacked renewing germ line, suggesting a role for JAK activity in the maintenance of germ line stem cells. Wild-type testes are long, coiled tubes containing small germ cells undergoing stem cell renewal and mitotic amplification at the apical tip, followed by cysts of interconnected spermatocytes and elongating spermatid bundles (Fig. 1B). hop^{25} testes were shorter and contained several bundles of elongated spermatids but completely lacked early germ cell stages (Fig. 1C). Constitutive expression of a *hop* cDNA restored normal germ line renewal and testis length in hop^{25} males, demonstrating that these defects were due to the hop^{25} mutation (Fig. 1D).

Analysis of embryonic and larval testes indicated that JAK function may be required for stem cell maintenance as early as the onset of spermatogenesis. Normal numbers (~10) of primordial germ cells populated each embryonic gonad in hop^{25} mutants (*11*). However, loss of stem cells in hop^{25} mutant testes was observed by the first larval instar (L1), becoming more apparent by second larval instar (L2). In wild-type testes, germ line stem cells and gonialblasts appear as single cells at the testis apical tip, whereas later spermatogonia are clustered in cysts (Fig. 1E). Single germ cells were not detected in

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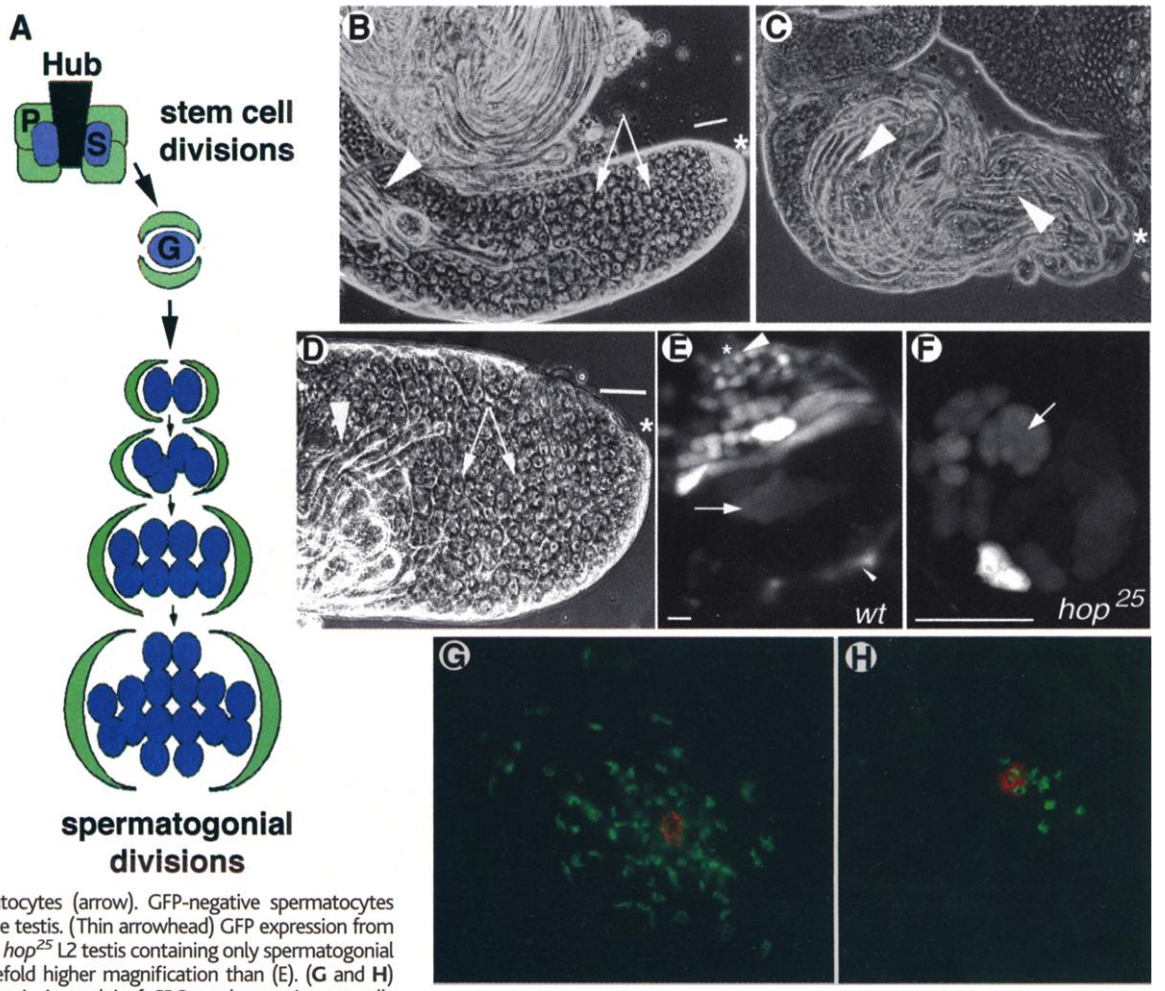

Fig. 1. JAK activity is required for stem cell maintenance in testes. (**A**) Early spermatogenesis. (S) Germ line stem cells and (P) somatic CPCs surround the apical hub. (G) Gonialblasts initiate differentiation with four rounds of mitosis, producing 16 interconnected spermatogonia (blue). CPCs produce somatic cyst cells (green), which encapsulate the gonialblast and do not divide again. (**B**) Wild-type adult testis: (bar) mitotic germ cells at the apical tip (asterisk), (arrows) spermatocytes, (arrowhead) elongated spermatids. (**C**) hop^{25} adult testis containing only elongated spermatids. (**D**) hop^{25};*act5C-hopTr-7* adult testis (*26*). (**E** and **F**) GFP expressed in germ cells by *nanos-GAL4:VP16 (nos-GAL4)*, *UAS-GFP*^{S65T} (*22*, *27*). (E) Wild-type L2 testis. (Thick arrowhead) Single germ cells at apical tip (asterisk), followed by two, four, eight, and sixteen-cell cysts of spermatagonia or spermatocytes (arrow). GFP-negative spermatocytes occupy the basal half of the testis. (Thin arrowhead) GFP expression from *Kruppel-GFP* transgene. (F) hop^{25} L2 testis containing only spermatogonial cysts (arrow). (F) is at fivefold higher magnification than (E). (**G** and **H**) Immunostaining for Tj protein in nuclei of CPCs and somatic cyst cells (green) and Fasciclin III in apical hub (red) (*28*). (G) Wild-type L3 testis. (H) hop^{25} L3 testis. (G) and (H) are at the same magnification.

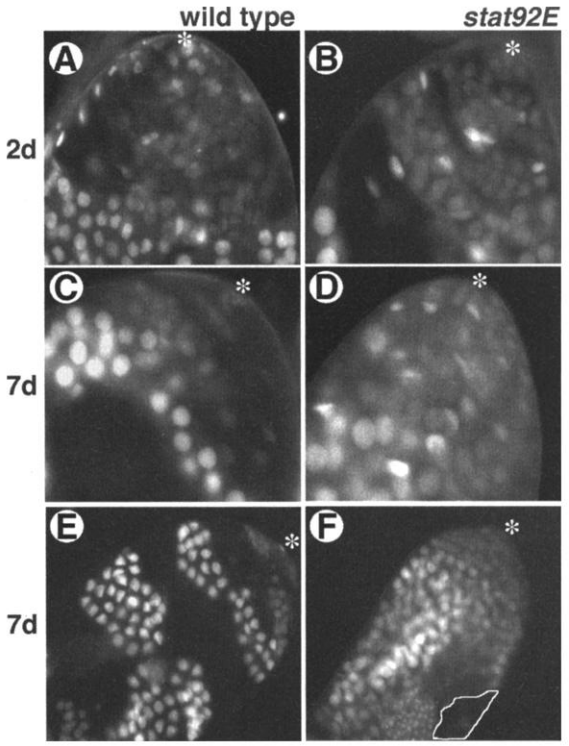

Fig. 2. STAT is required in germ cells for germ line stem cell self-renewal. Germ line clones marked by loss of ubiquitous GFP expression (*29*, *30*). (Asterisk) Apical tip. (**A**, **C**, **E**) Wild-type marked germ cells throughout testis apical tip and lumen. (**B** and **D**) *stat92E*$^{-/-}$ (*stat92E*^{06346}) marked germ cells were observed near the testis tip at 2 (B) but not 7 days (D) after clone induction. (**F**) Differentiating *stat92E*$^{-/-}$ (*stat*^{105}) clones were detected more basally (outline). (E) and (F) Twofold lower magnification for overview of later stages.

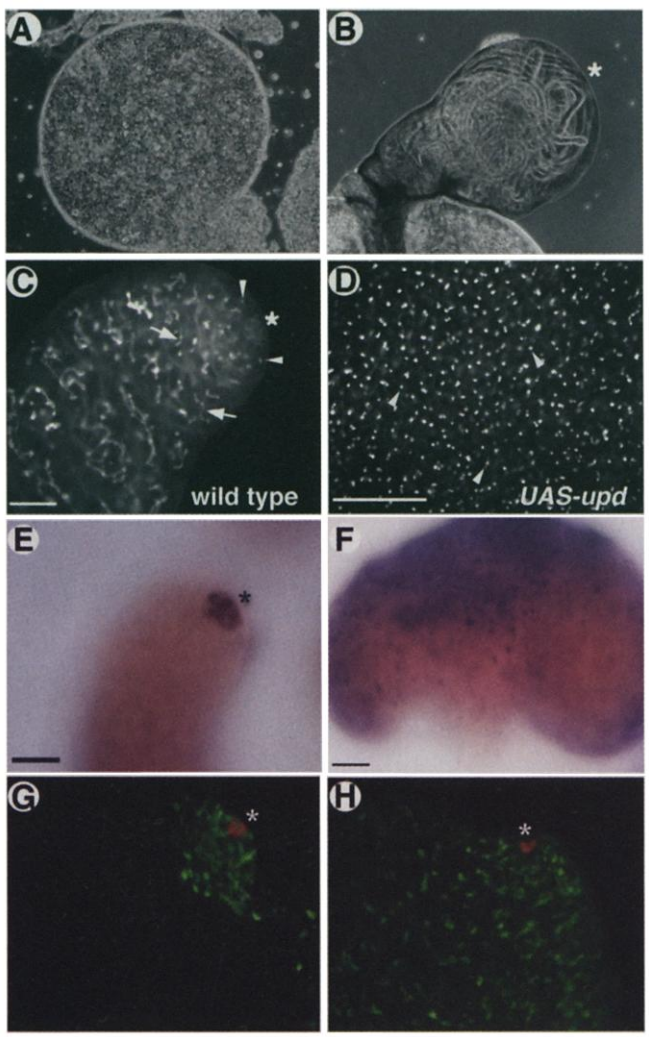

Fig. 3. Unpaired is a potent signal for stem cell proliferation. (**A** and **B**) Phase contrast images of (A) *UAS-upd/+; nos-GAL4* and (B) *hop*^{25}*/Y; UAS-upd/+; nos-GAL4* testes (*31*). (**C** and **D**) Adult testes stained for α-Spectrin. (C) Wild-type apical tip. Spectrosome-containing stem cells and gonialblasts (arrowheads) surround the hub (asterisk). Spermatogonia and spermatocytes contain branched fusomes (arrows). (D) *UAS-upd/+; nos-GAL4* testis. Spectrosome-containing cells (arrowheads). (**E** and **F**) In situ hybridization with *esg* mRNA probe (*32*). (E) Wild type. Apical tip (asterisk). (F) *UAS-upd/+; nos-GAL4* adult testis. (**G** and **H**) Apical tip (asterisk), L3 testes stained with anti-Tj (green) and anti–Fasciclin III (red). (G) Wild type. (H) *UAS-upd/+; nos-GAL4*. (D) is at twofold higher magnification than (C), and (E) is at 1.2-fold higher magnification than (F).

hop^{25} L1 and L2 testes (Fig. 1F). Instead, *hop*^{25} testes contained only a few clusters of differentiating germ cells, which resembled spermatogonia or early spermatocytes. Consistent with early stem cell loss, *hop*^{25} L3 testes lacked markers of germ line stem cell identity, including *escargot* reporter expression and single cells in mitosis (*12*).

JAK activity was also required for maintenance of a second stem cell population in the testis, the somatic cyst progenitor cells (CPCs). Two CPCs flank each germ line stem cell and contact the apical hub (Fig. 1A). CPCs self-renew and give rise to the somatic cyst cells that enclose the gonialblast and ensure spermatogonial differentiation (*7*, *8*, *13*, *14*). Thirty-five percent (9/26) of the *hop*^{25} L3 testes examined had no CPCs, as evidenced by a lack of detectable Traffic jam (Tj), a transcription factor expressed in CPCs and early cyst cells (*15*). In the remaining 65%, the number of early cyst cells was drastically reduced (Fig. 1, G and H). Somatic apical hub cells were present and appeared normal in all *hop*^{25} L3 testes examined, as assessed by Fasciclin III expression (Fig. 1, G and H).

Another component of the JAK signaling pathway, STAT, was required autonomously in germ cells for germ line stem cell maintenance. Marked germ line clones homozygous mutant for either of two *stat92E* alleles were generated at the same frequency as in the wild type, as assessed by the number of testes with at least one marked cyst 2 days after clone induction (Fig. 2, A and B, and Table 1). In control testes, many marked germ line cysts were evident at the apical tip 7 days after clone induction, indicating maintenance of marked stem cells (Fig. 2, C and E). In contrast, *stat92E*$^{-/-}$ marked germ line stem cells did not establish persistent clones. Although clusters of differentiating *stat92E*$^{-/-}$ mutant spermatocytes were detected, no early-stage marked germ cells were evident (Fig. 2, D and F).

The requirement for *hop* and *stat92E* for germ line stem cell maintenance suggested that a signal from surrounding cells might activate the JAK-STAT pathway in germ cells to specify stem cell self-renewal. The secreted factor Upd acts with *hop* and *stat92E* during embryogenesis and activates Hop kinase activity in vitro (*16*). Forced germ line expression of an *upd*$^{+}$ cDNA (*17*) resulted in greatly enlarged testes filled with many small cells (Fig. 3A). The *hop*^{25} mutation suppressed the effects of ectopic *upd*$^{+}$expression, indicating that Upd-dependent hyperproliferation required *hop* activity (Fig. 3B).

Cells that accumulated in response to Upd expression resembled germ line stem cells or gonialblasts. In wild-type testes, only stem cells and gonialblasts have a spherical spectrosome, whereas spermatogonia and spermatocytes have a branched fusome (Fig. 3C). Testes expressing ectopic Upd contained many cells with

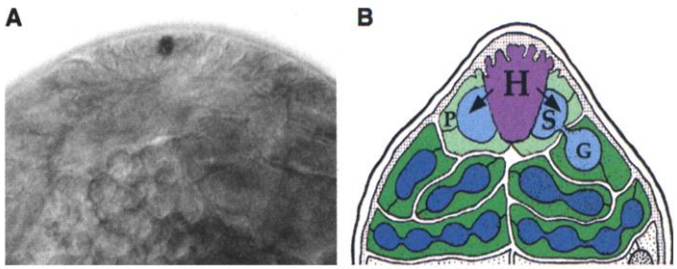

Fig. 4. (**A**) *upd* mRNA is expressed in apical hub cells. Wild-type testis hybridized in situ with antisense *upd* RNA probe. (**B**) The stem cell niche in the *Drosophila* testis: hub cells (purple), germ line stem cells (S), gonialblast (G), and somatic cyst progenitor cells (P). Modified from (*7*) with permission.

Table 1. Effects of *stat92E* mutations on germ line stem cell self-renewal.

Genotype	No. of testes with GFP-negative clones (2 days)*	No. of testes with GFP-negative clones (7 days)	No. of testes with GFP-negative clones at apical tip (7 days)
Wild type	15/22 (68%)	72/81 (89%)	48/81 (59%)
$stat92E^{06346}$†	31/52 (60%)	55/89 (54%)	2/89 (2%)
$stat92E^{105}$‡	ND§	43/80 (62%)	2/80 (2%)

*Number indicates testes with at least one clone 2 or 7 days after clone induction. †The lethal $stat92E^{06346}$ allele is associated with a P-element insertion carrying the *lacZ* gene (*24*). ‡The lethal $stat92E^{105}$ allele has an ethylmethane sulfonate–induced mutation (*25*). §ND, not determined.

a spectrosome, suggesting stem cell or gonialblast identity (Fig. 3D). Cells with branched fusomes were detected occasionally. Immunostaining for phosphorylated Histone H3 revealed many individual cells dividing asynchronously, another characteristic of stem cell or gonialblast identity (*18*). In wild-type testes, mRNA for the transcription factor *escargot* (*esg*) is detected in the apical hub and surrounding germ line stem cells (Fig. 3E) (*13*). Cells positive for *esg* mRNA expression were found throughout the testes upon Upd expression, suggesting that many of the cells retained germ line stem cell characteristics (Fig. 3F). The number of cyst cells expressing the Tj marker also increased markedly upon forced expression of upd^{+} (Fig. 3, G and H), suggesting that CPCs or early cyst cells proliferated in response to Upd. The apical hub remained intact, indicating that uncontrolled stem cell proliferation and self-renewal in response to ectopic Upd occurred independently of contact with the hub (Fig. 3, G and H).

Mutations in the JAK-STAT pathway resulted in stem cell loss, whereas JAK-STAT activation by ectopic expression of Upd caused unrestricted stem cell self-renewal. In situ hybridization revealed that upd^{+} mRNA is expressed in hub cells at the testis apical tip, in close proximity to germ line and somatic stem cells (Fig. 4A). Thus, Upd is expressed at the right time and place to act as a signal to direct stem cell self-renewal.

We propose that the apical hub cells constitute a niche that supports stem cell self-renewal and that Upd, expressed by the hub cells, is a defining molecular component of this stem cell niche. Tissue culture experiments suggested that secreted Upd associates with the extracellular matrix, potentially restricting its diffusion (*16*). Thus, the asymmetric outcome of stem cell divisions may be specified, in part, because only cells that maintain direct contact with the hub receive Upd and activate JAK-STAT signaling (Fig. 4B). The requirement for a signal from the apical hub may also serve to spatially coordinate asymmetric division of both the somatic and germ line stem cell populations.

The stem cell niche may provide a means to regulate stem cell numbers in vivo. Dependence on the niche for stem cell self-renewal, coupled with opposing signals from surrounding cells that promote differentiation of daughter cells displaced outside the niche (*13*, *14*), may provide an important defense against excessive stem cell proliferation. Conversely, if a stem cell niche is unoccupied, both daughters of a neighboring stem cell could self-renew to repopulate the available niche, expanding stem cell numbers. The vast potential of adult stem cells for regenerative medicine and gene therapy can only be realized by discovering the means to control stem cell self-renewal and expand stem cell populations. Identification of the niche and a crucial signaling pathway that supports ongoing spermatogenesis in *Drosophila* offers a model for how stem cell self-renewal may be achieved and regulated.

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26. Testes dissected into phosphate-buffered saline (PBS) were examined live by phase contrast microscopy. hop^{25} mutant phenotypes were complemented by constitutive expression of the *hopT-r7* cDNA expressed under control of the *actin5C* promoter (*19*).
27. Mutant males were distinguished from wild-type males on the basis of lack of somatic green fluorescent protein (GFP) expression from the FM7, $KrGFP^{10}$ balancer chromosome (*20*).
28. Formaldehyde- (4% in PBS) fixed samples were stained with antibody to α-Spectrin (Developmental Studies Hybridoma Bank, IA); anti–Fasciclin III (C. Goodman); anti–Tj-D1 (D. Godt); tetramethyl rhodamine isothiocyanate– or fluorescein isothiocyanate–conjugated anti-mouse or anti-rat immunoglobulin G (Jackson ImmunoResearch Laboratories, West Grove, PA); and 4′,6-diamidino-2-phenylindole by using standard procedures.
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30. Clones were induced by FLP-mediated recombination at FRT sites (*21*) in (i) $y,w,hs\text{-}flp^{122}$; *tub-GAL80*, FRT82B/FRT82B GFP3-13-7 (control); (ii) $y,w,hs\text{-}flp^{122}$; FRT82B, $stat92E^{06346}$/FRT82B GFP3-13-7; and (iii) $y,w,hs\text{-}flp^{122}$; FRT82B, $stat92E^{105}$/FRT82B GFP-3-13-7 flies. Late pupae were heat shocked in vials in a water bath at 37°C for 2 hours on two consecutive days. Testes dissected into PBS 2 and 7 days after the last heat shock were observed by fluorescence microscopy.
31. Males carrying *UAS-upd*$^{+}$/+; *nanos-GAL4:VP16* (*nos-GAL4*), $UAS\text{-}GFP^{S65T}$ (*17*, *22*) were raised at 25°C until they matured into larvae or adults, shifted to 29°C, then held for 5 to 10 days before scoring phenotypes. hop^{25}/FM7, $Kr\text{-}GFP^{10}$; *nos-GAL4*, $UAS\text{-}GFP^{S65T}$ females were crossed to *UAS-upd*$^{+}$ males and larval progeny genotyped by Kr-GFP expression.
32. Sense and antisense mRNA probes made from full-length *esg* cDNA (S. Hayashi) or *upd* cDNA (*16*) were labeled with digoxigenin (Genius System; Roche, Indianapolis, IN) and used according to standard protocols. Testes mounted in 80% glycerol were imaged by Nomarski optics.

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