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JAK signaling is somatically required for follicle cell differentiation in *Drosophila*

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SUMMARY

Janus kinase (JAK) pathway activity is an integral part of signaling through a variety of ligands and receptors in mammals. The extensive re-utilization and pleiotropy of this pathway in vertebrate development is conserved in other animals as well. In *Drosophila melanogaster*, JAK signaling has been implicated in embryonic pattern formation, sex determination, larval blood cell development, wing venation, planar polarity in the eye, and formation of other adult structures. Here we describe several roles for JAK signaling in *Drosophila* oogenesis. The gene for a JAK pathway ligand, *unpaired*, is expressed specifically in the polar follicle cells, two pairs of somatic cells at the anterior and posterior poles of the developing egg chamber. Consistent with *unpaired* expression, reduced JAK pathway activity results in the fusion of developing egg chambers. A primary defect of these chambers is the expansion of the polar cell population and concomitant loss of interfollicular stalk cells. These phenotypes are enhanced by reduction of *unpaired* activity, suggesting that Unpaired is a necessary ligand for the JAK pathway in oogenesis. Mosaic analysis of both JAK pathway transducers, *hopscotch* and *Stat92E*, reveals that JAK signaling is specifically required in the somatic follicle cells. Moreover, JAK activity is also necessary for the initial commitment of epithelial follicle cells. Many of these roles are in common with, but distinct from, the known functions of Notch signaling in oogenesis. Consistent with these data is a model in which Notch signaling determines a pool of cells to be competent to adopt stalk or polar fate, while JAK signaling assigns specific identity within that competent pool.

Key words: *Drosophila*, JAK, Oogenesis, Follicle cells, Notch

INTRODUCTION

The *Drosophila* ovary consists of approximately 15 ovarioles, chains of developing eggs that are progressively more mature toward the posterior of the structure. At the anterior tip of each ovariole is the germarium, a structure that contains the germline and somatic stem cells, other support cells, and the youngest developing cysts. Each cyst comprises 16 interconnected germ cells covered with a somatic monolayer epithelium (reviewed by Spradling, 1993). The germ cells include one oocyte fed by fifteen nurse cells that provide essential components for the mature egg. The somatic cells consist of multiple subpopulations, each with its own functions in the developing egg. While the germline cyst is dividing and developing within the germarium, a monolayer of somatic cells surrounds the cyst as it moves posteriorly through the germarium (Spradling, 1993). As the cyst becomes enveloped by the somatic cells, the egg chamber pinches off from the germarium, entering the vitellarium. At that time, approximately 5-8 somatic cells differentiate into stalk. These flattened, disc-shaped cells are stacked together to form the spacer between successive cysts. Stalk cells connect the anterior end of a more mature egg chamber to the posterior end of the next younger chamber. Also at that time, molecular markers can distinguish the stalk cells from the polar cells, which arise from the same precursors (Margolis and Spradling, 1995; Tworoger et al., 1999). The polar cells are arranged as two pairs of follicle cells, one pair at either end of each chamber near the stalk cells. While the stalk cells and polar cells cease proliferation at the end of the germarium, the remaining follicle cells, which we will refer to here as epithelial follicle cells (Lopez-Schier and St Johnston, 2001), divide approximately five times to expand the pool of follicle cells. Those epithelial cells later differentiate into various subpopulations with specific functions in the vitellarium (Gonzalez-Reyes and St Johnston, 1998; Spradling, 1993). Those subpopulations are pre-patterned with mirror image symmetry along the anterior-posterior axis of the egg. Imposed on that pre-pattern, signaling from the oocyte by the TGFα molecule Gurken stimulates the induction of posterior polarity on the somatic cells at that end. The result is an egg with coordinated polarities of the somatic and germline cells. This coordination is essential for the proper localization of maternal determinants that pattern the resulting embryo.

One signaling pathway recently implicated in gametogenesis in mammals is the Janus kinase (JAK) pathway (Herrada and Wolgemuth, 1997; Matsuoka et al., 1999; Russell and Richards, 1999). This is an important and re-utilized signaling

cascade that has been well characterized in many other tissues. JAK pathway activity is essential for the response of many tissues to a broad array of cytokines and growth factors. The JAK cascade provides a means for rapid cellular response to these signals utilizing only a small number of components for signal transduction (reviewed by Imada and Leonard, 2000). The intracellular cascade consists of the JAKs, which associate with receptor subunits specific for the ligand inducing the signal, and the STATs, latent transcription factors that are phosphorylated by activated JAKs. The phosphorylated STATs translocate to the nucleus and bind DNA to regulate transcription of target genes. In vertebrates, the pathway is used for multiple developmental events, including hematopoiesis, immune system development, mammary development and lactation and regulation of overall growth. The JAK pathway is evolutionarily conserved in metazoans, with both JAKs and STATs found in the fruit fly *Drosophila melanogaster* (reviewed by Dearolf, 1999; Zeidler et al., 2000).

The *Drosophila* JAK pathway components previously described include one JAK, *hopscotch* (*hop*), one STAT, *Stat92E*, and one ligand, *unpaired* (*upd*). Mutations in these genes of the *Drosophila* JAK pathway were originally characterized with regard to their requirement in embryogenesis. Maternal loss of *hop* or *Stat92e*, or zygotic loss of *upd* results in a striking and unique embryonic patterning defect (Binari and Perrimon, 1994; Harrison et al., 1998; Hou et al., 1996; Perrimon and Mahowald, 1986; Yan et al., 1996b). Subsequent analysis has implicated the JAK pathway in male fertility, larval hematopoiesis, wing vein development, thoracic development, sex determination, and planar polarity in the eye (Harrison et al., 1995; Jinks et al., 2000; Luo et al., 1999; Luo et al., 1995; Perrimon and Mahowald, 1986; Sefton et al., 2000; Yan et al., 1996a; Zeidler et al., 1999). The broad utilization of this signaling cascade in many facets of development prompted the investigation of potential roles of JAK signaling in oogenesis.

We have investigated the roles of JAK signaling in *Drosophila* oogenesis. The JAK pathway ligand, *upd*, is normally expressed in a restricted fashion, exclusively at the poles of the follicular epithelium. Reduction or removal of JAK signaling components only from the somatic cells of the ovary results in multiple developmental defects. The most penetrant phenotype is the fusion of multiple germline cysts into a single egg chamber. Coincident with the fusions is the production of excess polar cells at the expense of stalk cells. Furthermore, mutations of JAK components can cause mis-specification of epithelial follicle cells. These data indicate that the JAK pathway is utilized by somatic follicle cells to respond to signals in multiple oogenic events.

MATERIALS AND METHODS

Fly stocks

Unless otherwise stated, flies were reared at 25°C. Descriptions of *hop*, *upd* and *Stat92E* alleles used in these experiments can be found in FlyBase. Enhancer marker lines were characterized by various sources: 93F (Ruohola et al., 1991), PZ80 (Karpen and Spradling, 1992) and A101 (Clark et al., 1994).

Generation of mosaic animals

Mosaic animals carrying mutations in JAK pathway genes were generated using either synchronous induction of recombination (Chou and Perrimon, 1992) or the directed mosaic method (Duffy et al., 1998). The genotype of animals in which clones were induced by heat shock were *y w v hop^{msv} FRT101/ Ub-nGFP FRT101; hs-FLP99, MKRS/ +* or *y w hop^{c111} FRT101/ y w histone-GFP FRT101; hs-FLP38/ +*. Clones were induced by a 3-hour heat shock of adult females at 37°C. Animals were then examined for morphological or molecular alterations in the ovaries 2-8 days post-heat shock (phs).

The genotypes of directed mosaic animals were as follows:

y w v hop^{msv} FRT101/ Ub-nGFP FRT101; e22C-GAL4 UAS-FLP/ +

y w hop^{c111} FRT-L46B/ y w Ub-nGFP FRT-L46B; e22C-GAL4 UAS-FLP/ +

e22C-GAL4 UAS-FLP/ +; FRT82B Stat92E^{06346}/ FRT82B π-Myc

e22C-GAL4 UAS-FLP/ +; FRT82B Stat92E^{j6C8}/ FRT82B π-Myc

These animals continuously produce new clones owing to expression of FLP recombinase in the somatic cells of the germarium (Duffy et al., 1998). Adult females were dissected for ovary analysis between 3 and 7 days after eclosion.

Immunological and histochemical staining

X-gal staining for β-galactosidase activity was performed as previously described (Harrison and Perrimon, 1993). Briefly, 1- to 3-day old males and females were placed in vials containing yeast paste for 2 days. Ovaries were dissected in PBS, then fixed for 1-2 minutes in 2.5% glutaraldehyde (Sigma) in PBS. Ovaries were washed in PBT (1× PBS, 0.1% Tween 20), rinsed in X-gal staining solution (Klambt et al., 1991), then stained in X-gal staining solution with X-gal (0.5 mg/ml) at room temperature until color developed. Staining solution was washed out with PBT and ovaries were mounted in 70% glycerol.

In situ hybridizations to ovaries were performed as previously described for embryos (Harrison et al., 1998) except that proteinase K digestion was performed for 1 hour. Strand-specific probes for *upd*, *hop* and *Stat92E* were generated by linearizing pBS-GR51, phop5.1 and pNB40-Stat, respectively, then making digoxigenin-labelled DNA with Taq polymerase by using appropriate primers from the polylinkers of the cloning vectors and subjecting them to 30 cycles of synthesis. This generated separate single-stranded sense and antisense probes.

With the exceptions noted below, antibody staining of ovaries was performed using standard procedures (Patel, 1994). Primary antibodies and dilutions used were: rabbit α-β-galactosidase (5′-3′) at 1:1000, rabbit α-Myc (sc789, Santa Cruz Biotech.) at 1:60, rabbit α-GFP (Torrey Pines Lab) at 1:500, mouse α-Fasciclin III (7G10, Developmental Studies Hybridoma Bank-DSHB) at 1:30, mouse α-α Spectrin (3A9, Developmental Studies Hybridoma Bank) at 1:20, mouse α-Orb (4H8, DSHB) at 1:30, mouse α-Kelch (gift from L. Cooley) at 1:1, and rabbit α-phospho-histone H3 (PH3; Upstate Biotechnology, Inc.) at 1:500. Secondary antibodies were Texas Red-α-mouse, FITC-α-rabbit, and Texas Red-α-rat each used at 1:200 (Jackson Immunolabs). For anti-β-galactosidase stainings, ovaries were fixed for 15 minutes in 50% methanol in PBS. Staining protocols for Kelch (Xue and Cooley, 1993) have been described by others.

Epifluorescence and Nomarski (DIC) images were captured using a Spot Camera (Diagnostic Instruments) on a Nikon E800 microscope. Captured images were processed and annotated in Adobe Photoshop. Confocal micrographs were collected on a Leica TCS-SP laser scanning confocal microscope using Leica TCS software. Images were exported to TIF format and processed as above.

RESULTS

unpaired is expressed specifically in polar cells

Cell signaling between somatic follicle cells and germline cells and signaling between various follicle cells is essential for the proper establishment of pattern in the developing *Drosophila* egg. To investigate the potential for activity of the JAK

pathway in these processes, in situ hybridization to whole ovaries was used to determine the expression patterns of genes in the pathway. Strikingly, *unpaired*, which encodes an extracellular ligand that stimulates JAK pathway signaling, is expressed very specifically within the ovary (Fig. 1). After chambers pinch off from the germarium, *upd* is restricted to the two pairs of polar cells found at the anterior and posterior tips of the egg (Fig. 1A). In the germarium, *upd* is expressed in a cluster of somatic cells at the posterior of region 3 (Fig. 1B,C). Presumably these are the cells that give rise to the stalk and polar cells. Expression in the polar and border cells persists until egg maturation. No staining was detected using a sense control probe (not shown). The expression of *upd* in a specific pattern in the ovaries suggests a role for the JAK pathway in the development of the egg chamber.

If Upd has a role in oogenesis that involves activation of the JAK pathway, then expression of *hop*, the *Drosophila* JAK, and *Stat92E* in the ovaries would also be expected. In situ hybridization to *Stat92E* RNA reveals that the *Drosophila* STAT is expressed in both the germarium and the vitellarium (Fig. 1D). Expression in the germarium occurs in all follicle cells in region 2a and 2b, it then begins to be restricted to terminal follicle cells in region 3. In the vitellarium, *Stat92E* is expressed weakly at the termini of the egg chamber, but in a broader domain than only the two polar cells. After stage 9, *Stat92E* is strongly expressed in the nurse cells, consistent with the maternal role of *Stat92E* in the segmentation of the early embryo (not shown). Moreover, weak ubiquitous expression of *hop* is detectable in the follicular epithelium (not shown). These data are consistent with a potential role for JAK signaling in oogenesis.

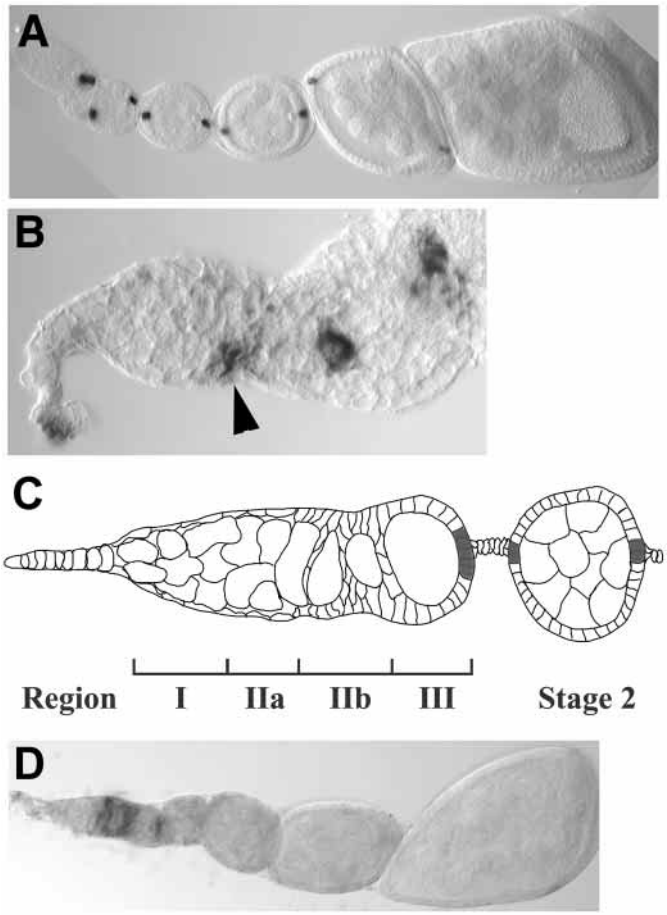

Fig. 1. The JAK ligand, Upd, is expressed in the follicular epithelium. (A) Expression of *upd* in the vitellarium is restricted to the two polar cells at the anterior and posterior end of each egg chamber. Within region 3 of the germarium (B), *upd* is expressed in the most posterior follicle cells (arrowhead). (C) A schematic representation of *upd* expression (shaded) illustrates the expression in the polar/stalk cell precursors at the posterior of the germarium and the polar cells in the vitellarium. (D) *Stat92E* is expressed strongly in the follicle cells of the germarium and terminal cells of chambers of the vitellarium up to stage 4, then weakly in later stages.

hop mutant ovaries contain egg chamber fusions

Homozygosity for complete loss-of-function alleles of any of the known JAK pathway genes, *upd*, *hop* and *Stat92e*, results in lethality prior to adulthood. Therefore, to examine potential roles for the pathway in oogenesis, heteroallelic combinations of reduced-function *hop* mutations were generated to recover adult females for ovarian analysis (Perrimon and Mahowald, 1986). The morphological defects ranged from essentially wild type for hop^{msv}/hop^{M4} to severely compounded chambers for hop^{msv}/hop^{M38} (Fig. 2). The compound egg chambers consist of greater than the normal 16 germ cells encapsulated within a single cyst. In the moderately affected mutant combinations, compound chambers typically consist of twice the normal number of germ cells (Fig. 2B). In some instances, it is possible to detect a follicle cell layer that bisects the compound chamber (Fig. 2E, also see Fig. 3E, and Fig. 4B,C), suggesting that the defect is due to the fusion of consecutive chambers in the vitellarium. The alternative explanation for twice the normal number of germline cells within a cyst is the overproliferation of germ cells. Such a phenotype has been described for mutants of genes such as *encore* (Hawkins et al., 1996). To distinguish between these alternative possibilities, mutant egg chambers were stained with an antibody to Orb, a protein that accumulates in the germline, and at the highest levels in the oocyte. Visualization of Orb protein shows that there are multiple distinct cysts of sixteen germline cells found within each compound chamber (Fig. 2E). In addition, there is one oocyte for each 16-cell germline cluster, suggesting that the cysts are developing independent of one another within a single follicular epithelium. Furthermore, an extra round of germline proliferation would result in an additional ring canal for the oocyte and a total of 31 ring canals per chamber, rather than the 30 expected from fusion of two individual cysts. Staining of ring canals in fused cysts of compound chambers failed to detect more than four ring canals per oocyte in any chamber (n=69). Moreover, chambers in which all ring canals could be definitively counted contained a multiple of 15 ring canals per chamber (Fig. 2F). We conclude that the compound chambers observed in *hop* mutants are the result of fusions or improper encapsulation of germline cysts.

Stalk cell/polar cell differentiation is altered in *hop* mutant ovaries

Fusions of egg chambers in other mutants have been linked to alterations in differentiation of follicle cells (Forbes et al., 1996; Keller Larkin et al., 1999; Lopez-Schier and St Johnston, 2001; Ruohola et al., 1991; Zhang and Kalderon, 2000). To

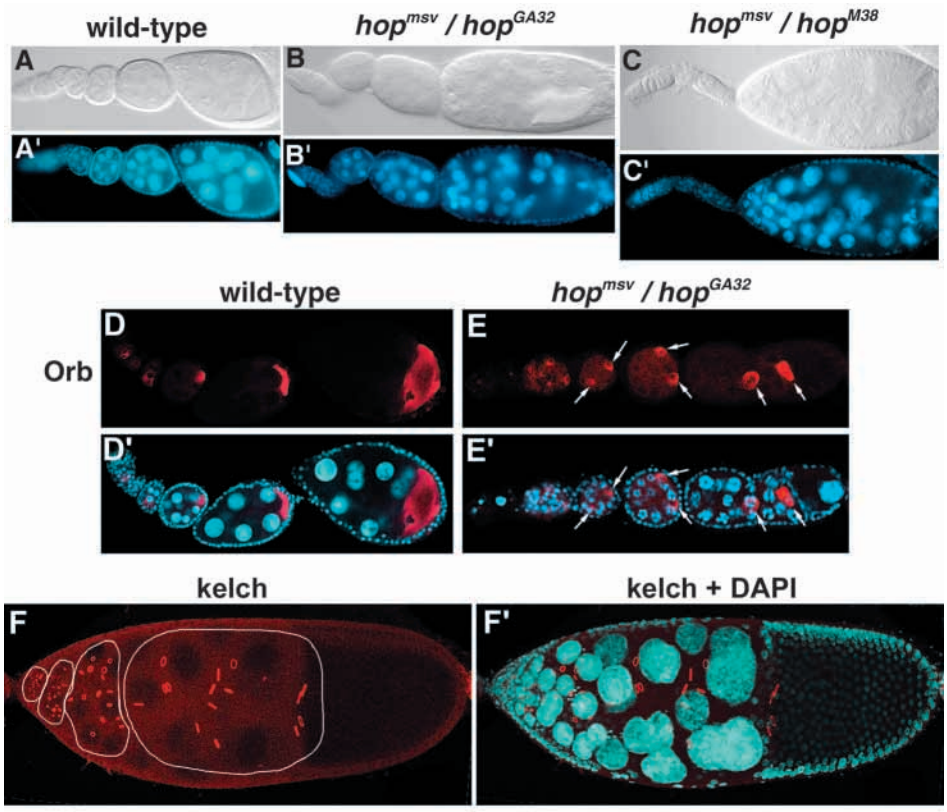

Fig. 2. *hop* mutant ovaries contain fused egg chambers. Heteroallelic combinations of *hop* alleles show a range of severity of ovarian defects. (A-C) In comparison with wild type (A), loss of *hop* function results in chambers with additional germ cells, with penetrance and severity determined by allelic combination. Moderate allele combinations, such as hop^{msv}/hop^{GA32} (B), show frequent compound chambers. Severe allele combinations, such as hop^{msv}/hop^{M38} (C), result in extensive fusion of chambers, with no distinct, separated cysts. Orb antibody staining shows that the additional germ cells are the results of multiple germline cysts encapsulated within a single follicular epithelium. In wild-type ovarioles (D), Orb protein is dispersed throughout the germ cells of cysts within the germarium, but is concentrated in the oocyte of chambers in the vitellarium (Orb in red, DAPI in blue). In hop^{msv}/hop^{GA32} ovarioles (E), fused chambers contain multiple germline cysts (arrows), each with its own oocyte. Moreover, a chamber containing 4 fused cysts (F) has 60 ring canals (Kelch staining in red). The multiples of 15 ring canals (F′, red) are consistent with chamber fusions rather than extra rounds of germline proliferation.

investigate the production of follicle cell subpopulations, antibody and enhancer trap markers were used to identify specific cell fates in mutant ovaries. Polar cell fate was assayed using antibodies to Fasciclin III (Fas III). In the wild-type germarium, Fas III is highly abundant in all immature follicle cells. In the vitellarium, Fas III protein is dramatically reduced in all epithelial cells, but remains abundant in the pairs of polar cells at the anterior and posterior of each chamber. Egg chambers from *hop* mutant females show an expansion of the Fas III-staining cells in the vitellarium (Fig. 3). Depending on the severity of the heteroallelic *hop* mutant combination, egg chambers can have anywhere from the normal number of two Fas III-staining cells in a polar cell cluster to more than a dozen (also see Table 1).

Because high levels of Fas III mark both polar cells and undifferentiated follicle cells, a second marker of cell fate was used. Expression of an enhancer trap marker that is only expressed in mature polar cells, PZ80 (Karpen and Spradling, 1992), was examined in mutant and wild-type ovaries. The loss of *hop* activity in the ovaries results in the expansion of PZ80-positive cells (Fig. 3), similar to the results seen with Fas III. Because of the distinct nuclear localization of the β-galactosidase marker in the PZ80 enhancer trap it was also possible to detect the subtle expansion of the polar cell populations in weak *hop* mutant combinations (Fig. 3B). Nearly half of the polar cell clusters from hop^{msv}/hop^{M4} eggs have more than two cells, despite no morphological consequence in the egg (see Table 1). In non-fused chambers of a slightly stronger mutant combination, hop^{msv}/hop^{M75}, nearly all polar cell clusters contain more than two cells, with an average cluster size of 4.5 cells in stage 4 cysts.

Because stalk and polar cells arise from the same precursor population, a possible cause of extra polar cells is the mis-specification of stalk cells. To address this hypothesis, *hop* mutant ovaries were generated in the background of the 93F enhancer trap, an insertion line that results in specific expression of *lacZ* primarily in the stalk cells of the vitellarium (Ruohola et al., 1991). The terminal filament cells at the anterior tip of the ovariole and all the follicle cells of stage 10 or later chambers also express *lacZ* in the 93F line (Fig. 3D). In ovaries from flies with reduced JAK pathway signaling, there is a consistent reduction of stalk cells, as identified by expression of the 93F marker. The degree of stalk cell loss is correlated with the severity of the *hop* alleles examined. In hop^{msv}/hop^{M75} ovaries, there is a moderate frequency of chamber fusions. In particular, chambers that show fusions have few or no obvious stalk cells, while surrounding chambers that are distinctly separated typically have several 93F-positive cells. In a more severe mutant combination in which nearly all chambers are fused, hop^{msv}/hop^{M38}, there are few 93F marked cells. Occasional β-gal-positive cells are seen amongst the follicular layer that encapsulates the fused cysts, but these do not have the characteristic flattened, disc-shaped morphology of normal stalk cells.

upd genetically interacts with *hop* to control follicle cell differentiation

While the experiments described here clearly demonstrate requirement of the intracellular JAK pathway in cyst encapsulation, the signal that stimulates the pathway is not identified. However, *upd*, the gene encoding an embryonic ligand for the pathway, is expressed specifically in the polar follicle cells, raising the possibility that Upd may also be a ligand for the JAK pathway in oogenesis. To test this

Table 1. Expansion of the polar cell population in *hop* mutants occurs early in oogenesis

	Stage 4		Stage 5/6		Stage 8/9	
	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
	(*n*=79)		(*n*=92)		(*n*=87)	
Wild type						
Chambers with 2 pfc	83%	80%	88%	92%	97%	98%
Chambers with 3 pfc	16%	20%	12%	8%	3%	2%
Chambers with 4 pfc	1%	0%	0%	0%	0%	0%
Defective chambers	17%	20%	12%	8%	3%	2%
Average no. of pfc	2.23	2.24	2.15	2.1	2.06	2.05
	(*n*=69)		(*n*=73)		(*n*=75)	
hop^{M4}/hop^{msv}						
Chambers with 2 pfc	58%	54%	60%	44%	87%	64%
Chambers with 3 pfc	41%	45%	37%	56%	13%	35%
Chambers with 4 pfc	1%	1%	3%	0%	0%	1%
Defective chambers	42%	46%	40%	56%	13%	36%
Average no. of pfc	2.43	2.48	2.42	2.56	2.13	2.37
	(*n*=36)		(*n*=46)		(*n*=42)	
hop^{M75}/hop^{msv}						
Chambers with 2 pfc	0%	8%	6%	20%	7%	33%
Chambers with 3 pfc	14%	28%	33%	41%	50%	38%
Chambers with 4 or more pfc	86%	64%	61%	39%	43%	29%
Defective chambers	100%	92%	94%	80%	93%	67%
Average no. of pfc	5.08	4.03	4.3	3.52	3.62	3.05

In females of *hop* heteroallelic combinations, the number of polar cells (pfc), as indicated by PZ80 staining, both at the anterior and posterior ends of the chamber was determined. Only distinct chambers (not fused) were scored and results are expressed as the percentage of total chambers of that genotype. The average number of polar cells for each pole at each stage is listed at the bottom of each genotype panel.

hypothesis, ovaries from females mutant for *hop* were compared with ovaries from females mutant for *hop* and heterozygous for *upd*. The reduction of *upd* activity by approximately half dramatically enhances the fusion of egg chambers seen in various heteroallelic combinations of *hop*. This is particularly striking for the hop^{msv}/hop^{M4} combination in which fusions are rarely seen (compare Fig. 3G and 3H). However, in females that are hop^{msv} upd^{YM55}/hop^{M4} or hop^{msv} upd^{Y43}/hop^{M4} the proportion of ovarioles with fused chambers rises to 75% or more (Table 2). Moreover, the reduction of *upd* activity enhances the adoption of polar cell fates (Fig. 3H), just as seen for strong allelic combinations of *hop*. We conclude from this enhancement that normal *upd* function positively influences JAK signaling in the follicle cells. The simplest explanation of this outcome is that the Upd ligand stimulates JAK signaling in the ovary, just as proposed for the embryo.

Table 2. Reduction of *upd* enhances *hop* mutant phenotypes

Genotype	Frequency of fusions
v hop^{msv}/ *w* hop^{M4}	5% (*n*=102)
v hop^{msv} upd^{YC43}/ *w* hop^{M4}	75% (*n*=380)
v hop^{msv} upd^{YM55}/ *w* hop^{M4}	92% (*n*=179)

Ovarioles of each genotype were examined to determine the number that contained at least one egg fusion. The frequency of affected ovarioles is indicated for each.

Loss of *hop* does not promote excess proliferation

Similar to the phenotypes described here for JAK pathway mutations, ectopic Hedgehog (Hh) signaling also results in the expansion of the polar cell population (Forbes et al., 1996; Tworoger et al., 1999; Zhang and Kalderon, 2000). Persistence of Hh signaling results in excessive proliferation of follicle cells beyond region 3 of the germarium, at which point the polar and stalk cell precursors normally cease to divide (Tworoger et al., 1999; Zhang and Kalderon, 2000). The extended proliferation delays differentiation and causes the expansion of the stalk cell/polar cell precursor population. Consequently, when the stalk and polar cells finally differentiate, too many cells adopt those fates (Zhang and Kalderon, 2000). To test whether this may be true for mutations in the JAK pathway, mutant ovaries were stained with antibodies against phospho-histone H3 (PH3), a marker for mitotic cells. In ovaries from heteroallelic combinations of *hop*, there were no PH3-positive cells detected beyond stage 6 (Fig. 4). This suggests that the defects seen in *hop* mutants are not the result of excess proliferation of follicle cells in general.

While there is no detectable extension of the proliferative program of the general follicle cell population, this does not exclude the possibility of specific effects of *hop* mutations on just the stalk cells and polar cells. Therefore, the ability of the polar cells in *hop* mutants to continue proliferation after release from the germarium was examined. If there were a loss of proliferative arrest in those cells, we would expect to see more polar cells in later stage egg chambers than at earlier stages. Contrary to the hypothesis, the number of polar cells seen in stage 4 to stage 9 egg chambers, as marked by PZ80 staining, remains approximately constant in *hop* mutant ovaries (Table 1). In wild type, the average number of polar cells at each pole is 2.24 at stage 4, and drops slightly by stage 8-9. In both weak (hop^{msv}/hop^{M4}) and moderate (hop^{msv}/hop^{M75}) mutant

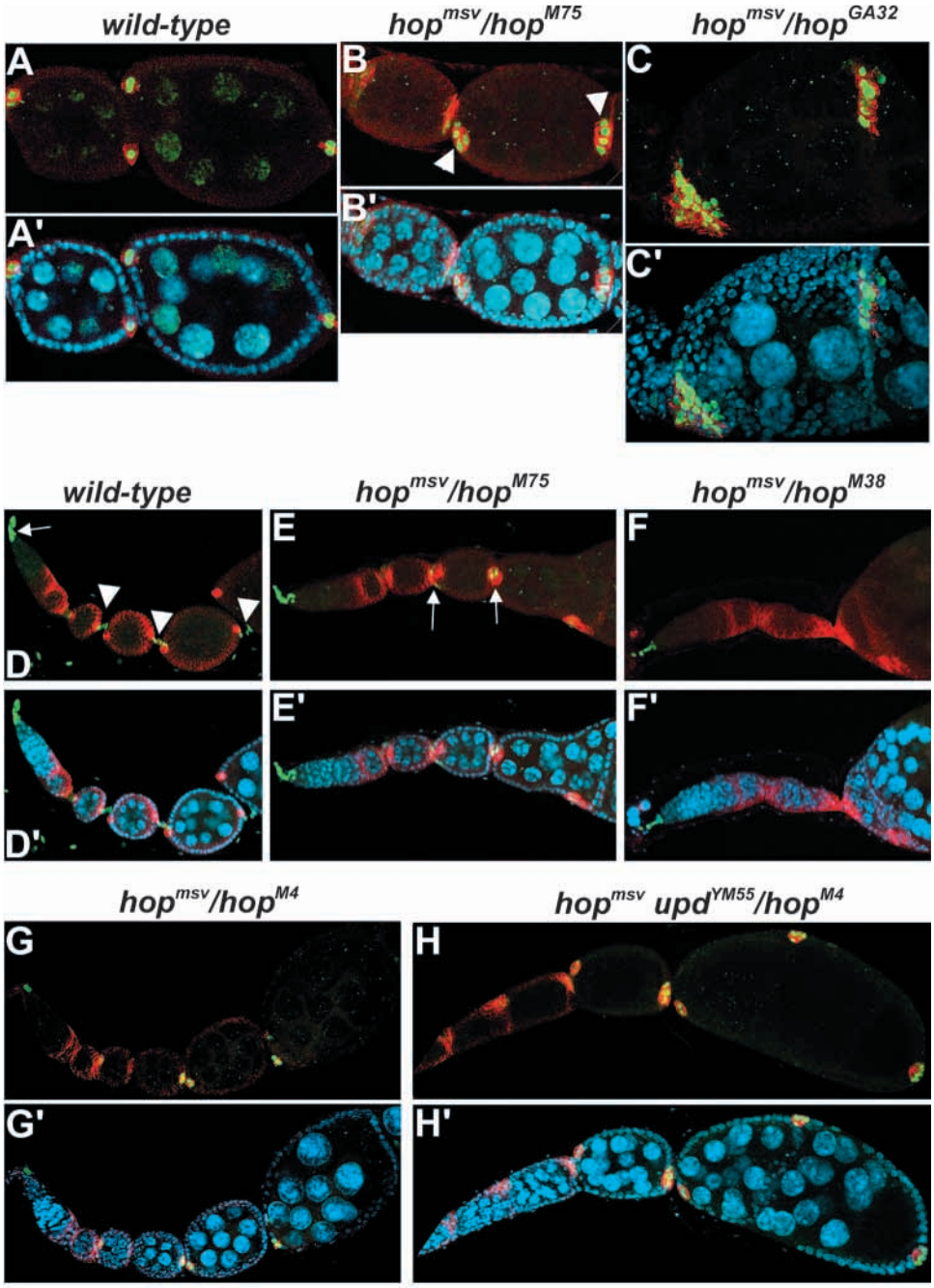

Fig. 3. *hop* mutants produce polar cells at the expense of stalk cells. The identity of polar cell fates was assayed using the molecular markers Fas III (in red) and PZ80 (in green) with nuclear staining by DAPI (in blue). In wild-type ovaries (A), Fas III protein is found at high levels in the membranes of all follicle cells of the germarium, but is markedly reduced in all but the polar cells of egg chambers of the vitellarium. β-galactosidase produced in the PZ80 enhancer trap is not detectable until approximately stage 4, after the egg chamber has exited the germarium. At that time, β-galactosidase is visible specifically in the two polar cells at each end of the egg chamber. (B) In the intermediate mutant combination hop^{msv}/hop^{M75}, there are extra polar cells, as indicated by the appearance of both Fas III and PZ80 (arrowheads). The number of polar cells is even greater in more severe mutant combinations, such as hop^{msv}/hop^{GA32} (C). The expression of the *lacZ* enhancer trap line, 93F, was used to mark the stalk cells in wild-type (D), and *hop* mutant (E and F) ovarioles. In wild type (D) 93F strongly marks the terminal filament (arrow) and the interfollicular stalk cells (arrowheads). In hop^{msv}/hop^{M75} (E), there are consistently fewer β-galactosidase positive interfollicular cells (arrows). In strong mutant combinations, such as hop^{msv}/hop^{M38} (F), stalk cells are rare or absent in extensively fused ovarioles. Additional loss of one copy of the *upd* gene enhances the phenotype of *hop* mutants. The hop^{msv}/hop^{M4} heteroallelic combination shows nearly normal ovarioles (G), with only occasional extra polar cells, as indicated by Fas III (red) and PZ80 (green) and marked by arrows (see Table 1) and rare chamber fusions. However, these phenotypes are dramatically enhanced in $hop^{msv}\ upd^{YM55}/hop^{M4}$ females (H).

combinations, many polar cell clusters start with more than two cells, 2.46 and 4.56 on average, respectively. However, just as with wild type, there is no expansion of cluster size at later stages. This suggests that extra polar cells observed in *hop* mutant egg chambers are not due to continued proliferation of the polar cell population in the vitellarium. An alternative explanation would be that the polar cells do continue to proliferate, but then die. TUNEL staining of mutant ovarioles showed no more cell death in mutant ovaries than in wild type, and that there were no clusters of dead cells near the poles of the chambers (not shown). We conclude that it is unlikely that the polar cells in *hop* mutant ovaries continue to proliferate after exit of the cyst from the germarium.

Consistent with the data described above, the number of cells that can adopt polar cell fate in the hop^{msv}/hop^{M75} egg chambers is similar to the expected size of the precursor pool in wild-type chambers. Mosaic analysis suggests that the precursor pool for the stalk cell-polar cell cluster consists of the anterior polar cells of the more mature chamber, the stalk cells bridging the two chambers, and the posterior polar cells of the less mature chamber (Tworoger et al., 1999). In wild-type chambers, this would correspond to two cells for each polar cell cluster and 5-8 stalk cells, for a total of approximately 9-12 precursor cells in each pool. In *hop* mutant egg chambers resulting from fusion of two consecutive chambers, there is a distinct island of PZ80-staining cells near the point of fusion of the chambers. Consequently, if *hop* mutation causes the presumptive stalk cells to adopt a polar cell fate, then the size of these PZ80-

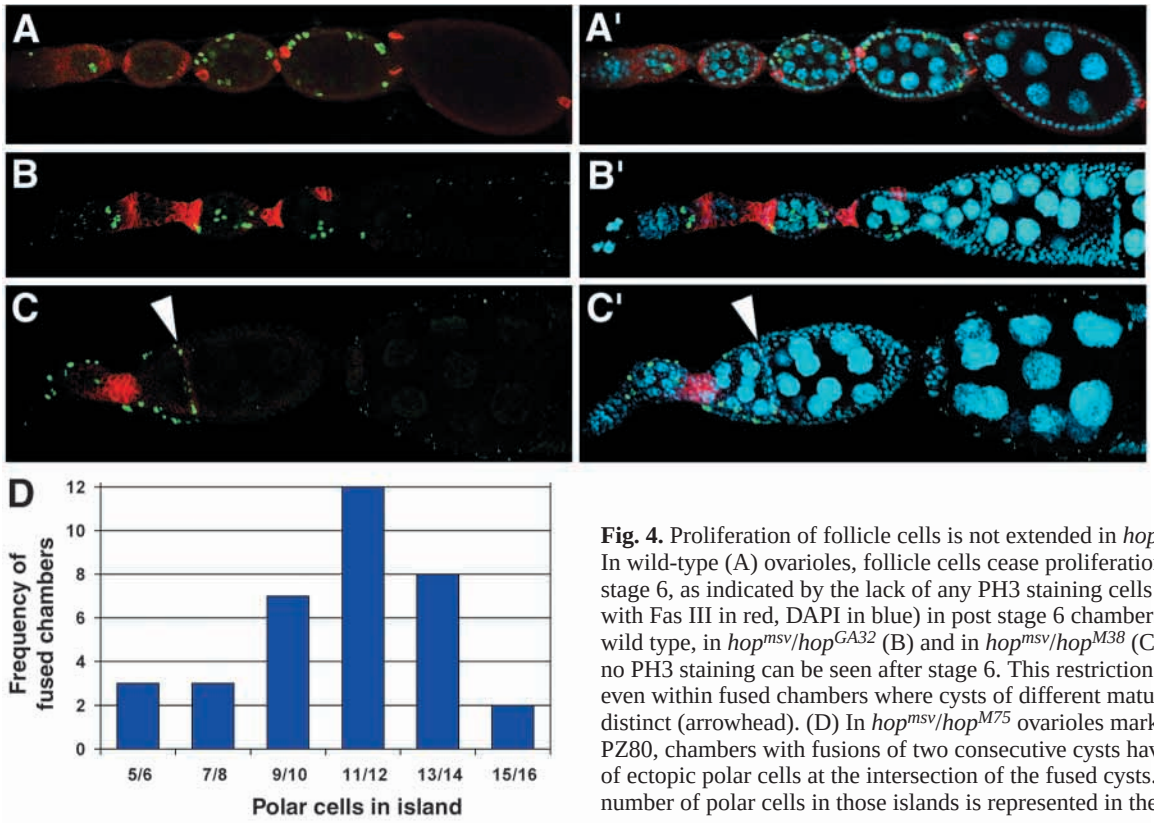

Fig. 4. Proliferation of follicle cells is not extended in *hop* mutants. In wild-type (A) ovarioles, follicle cells cease proliferation after stage 6, as indicated by the lack of any PH3 staining cells (in green, with Fas III in red, DAPI in blue) in post stage 6 chambers. As in wild type, in hop^{msv}/hop^{GA32} (B) and in hop^{msv}/hop^{M38} (C) ovarioles, no PH3 staining can be seen after stage 6. This restriction is visible even within fused chambers where cysts of different maturity are distinct (arrowhead). (D) In hop^{msv}/hop^{M75} ovarioles marked with PZ80, chambers with fusions of two consecutive cysts have islands of ectopic polar cells at the intersection of the fused cysts. The number of polar cells in those islands is represented in the graph.

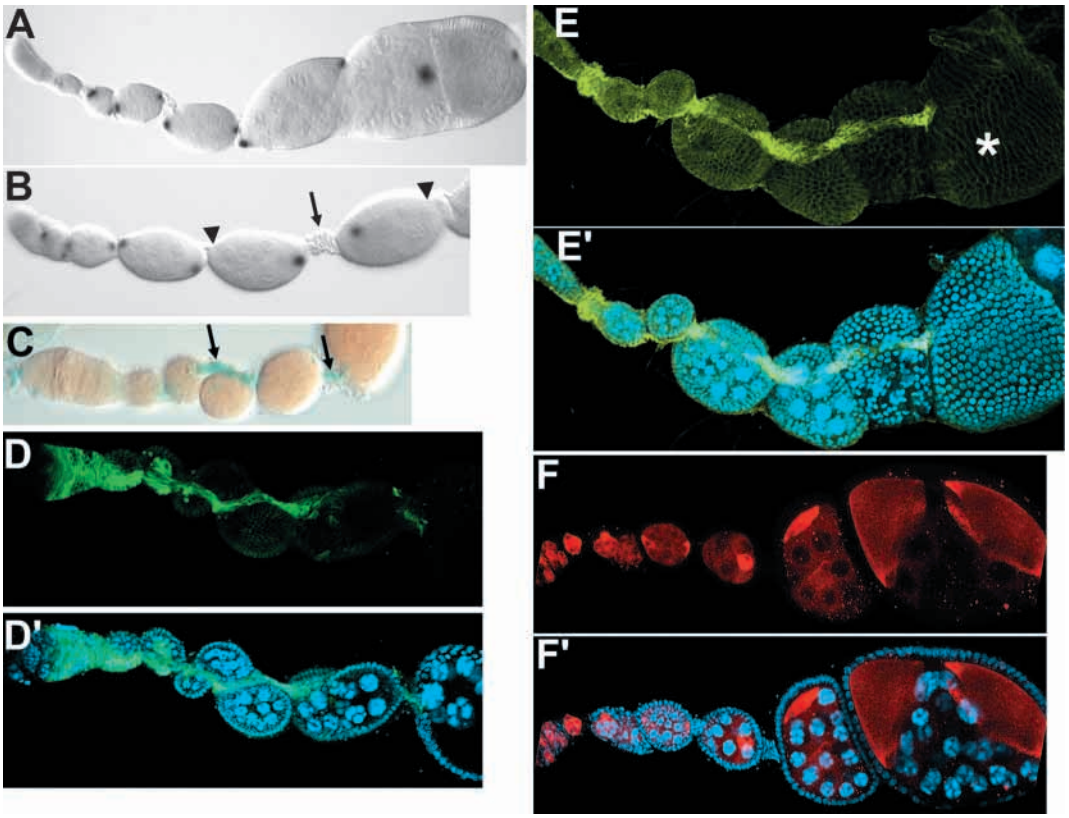

Fig. 5. Upd misexpression stimulates stalk cell production. In wild-type ovaries (A) enhancer trap A101 marks polar follicle cells in the vitellarium. When *upd* is misexpressed (B-F), polar and stalk cells are mis-specified. Chronic expression of *hs-upd* results from shifting adults to 30°C (B-F). (B) This treatment causes the frequent loss of polar cell clusters (arrowheads) and development of expanded and morphologically abnormal stalks (arrow). (C-F) The abnormal stalks are not monolayer and often traverse the outside of the chambers to form a continuous 'rope'. (D, D′) The cells in these ropes strongly express Fas III (green) which does not mark normal stalks. However, markers for mature stalk cells, 93F (blue stain in C) and α-spectrin (yellow, E, E′), are also abundant in ropes. (F, F′) Fusions of egg chambers and mislocalization of the oocyte (asterisk), similar to loss-of-function phenotypes, can occur in chronic *upd* misexpression, as revealed by Orb accumulation (red).

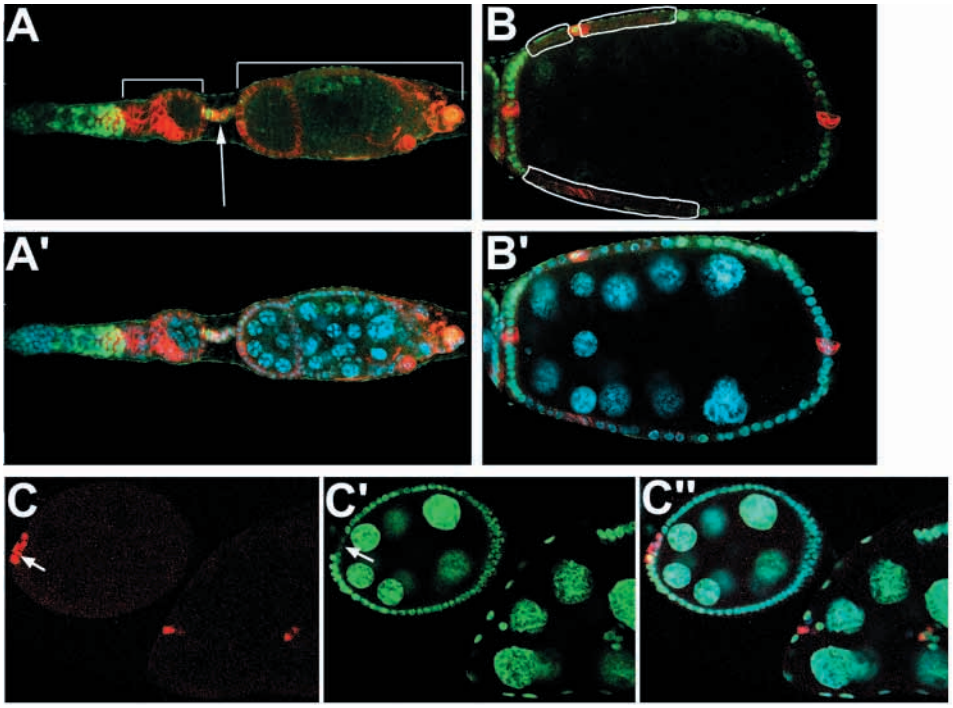

Fig. 6. JAK pathway activity is required in the soma. Somatic mosaics of *Stat92E*^{j6C8} show the same chamber fusion phenotype as the *hop* heteroallelic ovaries. Clones are marked by the loss of π-Myc (green, mutant cells outlined) and Fas III staining is shown in red. (A) In an ovariole with nearly all mutant follicle cells in the vitellarium (brackets), multiple cysts are fused into a single egg (posterior). Approximately 8 stalk cells can be identified (arrow), all of which are wild type for *Stat92E*. (B) In a late stage fused chamber, the mutant clone is in the middle of the fusion, presumably corresponding to the anterior terminus of the older chamber and the posterior terminus of the younger chamber. (C) Consistent with the alteration of fate seen in *hop* mutant heteroallelic combinations, clones of *hop*^{c111} mutant cells (lack of green GFP) express the polar cell marker PZ80 (red).

staining islands should be limited to the size of the original stalk cell-polar cell precursor pool. In the *hop*^{msv}/*hop*^{M75} mutant egg chambers, the number of PZ80-positive polar cells in such islands between two fused chambers were counted and the results appear in Fig. 4D. The average number of polar cells in these clusters was 11.2, with a maximum of 16. These numbers are similar to the expected value of 9-12 precursors in a single pool of precursors. This further supports the idea that mutations in *hop* do not stimulate an expansion of the stalk cell-polar cell precursor cells. Unlike Hh signaling, these data would strongly support a role of JAK signaling in differentiation of the somatic epithelium, rather than regulation of proliferation.

Ubiquitous Upd stimulates stalk cell production

Given that loss of JAK pathway function results in the adoption of polar cell fates at the expense of stalk cells, a logical corollary is that excessive or inappropriate JAK activity may stimulate stalk cell fates at the expense of polar cells. To test this hypothesis, *upd* was ubiquitously expressed in adult females to examine the effects on follicular cell fate. Chronic stimulation of *hs-upd* was achieved by shifting adult females to 30°C for 6 days prior to dissection. The presumably moderate levels of *upd* produced throughout the ovary resulted in phenotypes that appear reciprocal to the loss-of-function phenotype for *hop*. Specifically, polar cells are often missing from one pole of the developing egg chambers (Fig. 5B). Concomitantly, cells marked by stalk cell reporters are expanded. In the extreme, rope-like stalks are produced that have two or more layers of cells rather than the normal monolayer (Fig. 5C-F). These cells lack the flattened, disc-shaped morphology of mature stalk cells. Furthermore, these rope-like stalks are frequently continuous, with stalk cells piled on top of the follicle across the outside of a chamber (Fig. 5C-F). While these cells stain strongly for 93F and α-spectrin, markers of mature stalk cells, they also stain strongly for Fas III (Fig. 5D), a marker of immature follicle cells. Thus the extra stalk cells produced by misexpression of *upd* appear to be incompletely differentiated. Moreover, chronic *upd* expression also resulted in some chamber fusions, similar to loss-of-function mutations. Germ cells were also affected by this treatment, as evidenced by the condensed chromatin morphology characteristic of stage 4 cysts seen in many chambers that were much older (Fig. 5D,F). This is also observed with incomplete penetrance in loss-of-function mutants (see Fig. 2E and Fig. 3E). Thus, while induced JAK pathway activity causes the differentiation of stalk-like cells at the expense of polar cells, JAK activity has additional effects that are not simply reciprocal to loss-of-function mutations. The number of extra stalk cells seen in these chambers is much greater than the size of the normal stalk/polar cell precursor pool. In conjunction with the aberrant nature of these cells, this demonstrates that *hs-upd* is causing defects beyond the simple mis-specification of cells from the stalk cell/polar cell precursor pool. Interestingly, the production of extra cells expressing both polar and stalk cell markers is seen in animals with ectopic Hedgehog (Hh) activity (Forbes et al., 1996; Tworoger et al., 1999). This phenotype has been explained as a proliferative defect in which the polar and stalk cell precursors continue to divide beyond when they are normally specified. This delays differentiation, such that when the stalk and polar cells are eventually specified, there are too many cells to adopt those fates (Zhang and Kalderon, 2000). However, the mitotic marker PH3 was not detected in chambers beyond stage 6, nor was it ever detected in aberrant stalks in the vitellarium of hs-upd ovarioles (*n*=47, data not shown). Thus the expansion of stalk cells in *hs-upd* ovaries is not likely the result of increased proliferation of precursors, but may be a consequence of recruitment of cells from outside that precursor pool. If the additional stalk-like cells are actually from the epithelial cell precursors, then the inability of *hs-upd* to completely transform the cells to a stalk cell (non-epithelial) identity is not surprising.

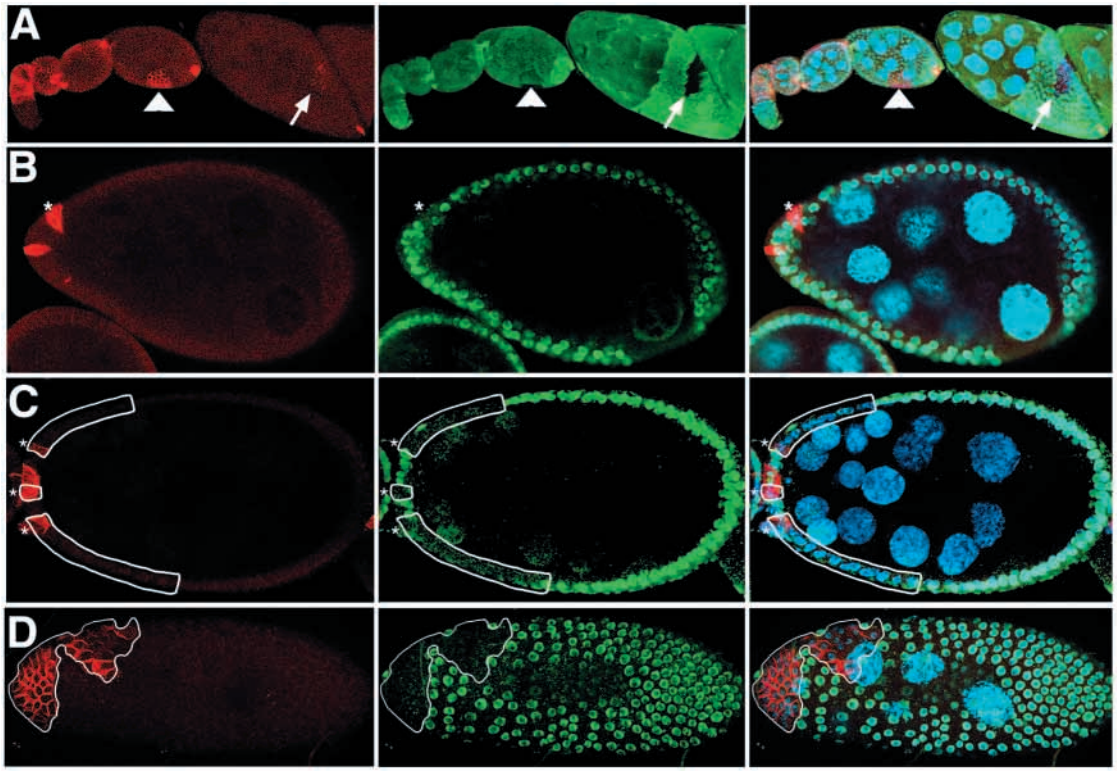

Fig. 7. JAK pathway function affects epithelial follicle cell differentiation. Loss of *hop* (A) or *Stat92E* (B-D) in mosaic animals alters epithelial cell fates. (A) In mutant clones (marked by loss of Ub-nGFP in green) generated 3 days before dissection, only cells of early stage chambers of the vitellarium maintain strong Fas III (red) expression (arrowhead). A mutant clone in a late stage chamber of the same ovariole retains little Fas III (arrow). Interestingly, some mutant cells in stage 7 or later chambers maintain Fas III in part of a clone (B-D). Typically the Fas III-positive cells are at the clonal boundary, adjacent to wild-type cells. Moreover, the Fas III-positive cells are almost always close to the terminus of the egg (asterisks).

Cyst encapsulation requires only somatic JAK activity

To determine whether JAK functions in oogenesis are required in the germline or the soma, females mosaic for *hop* or *Stat92e* were generated. Clones were induced using the UAS-FLP mitotic recombination technique (Duffy et al., 1998). The e22C-GAL4 used to stimulate FLP recombinase expression is abundant in follicular stem cells and early follicle cells (Duffy et al., 1998). Homozygous mutant *hop* mosaic patches of tissue were identified by the loss of a GFP marker driven by the ubiquitin promoter that expresses in all follicle cells (Davis et al., 1995). Mosaics of *Stat92E* were generated similarly, but wild-type tissues were marked by the presence of the π-Myc marker (Xu and Rubin, 1993). The results observed were similar for a weak allele of *hop* (hop^{msv}) a null allele of *hop* (hop^{c111}) and two different strong or null alleles of *Stat92E* ($Stat92e^{06346}$ and $Stat92E^{j6C8}$). The most common defect seen in mosaic ovarioles was a fusion of adjacent egg chambers (Fig. 6). In most chambers with fusions, mutant clones encompassed the presumptive adjacent termini in the region of the fusion.

The generation of mosaics using the UAS-FLP system is specific for somatic cells, as the basal hsp70 promoter used in the pUAST vector does not support expression in the germline (Duffy et al., 1998; Rorth, 1998; Tracey et al., 2000). Consequently, we can conclude that the phenotypes seen in the *hop* and *Stat92e* mutant mosaics are due to loss of gene function specifically in the follicle cells. To complement this analysis, mosaics of hop^{c111} were generated using hsFLP, which is active in both the germline and soma. Chambers that were mutant for *hop* in the germline and not in the soma were not fused (not shown). All chambers (n=90) containing hop^{c111} mutant germline cells with wild-type follicle cells contained 15 nurse cells and one oocyte. We therefore conclude that *hop* function in the germline is unnecessary for proper encapsulation and separation of chambers. Consistent with this conclusion, there have been no reports of defects in oogenesis caused by germline loss of *hop* or *Stat92e* function (Binari and Perrimon, 1994; Hou et al., 1996; Yan et al., 1996b). Thus, despite expression of *hop* and *Stat92E* in the germline, these data indicate that the pathway is required only in the soma for oogenic function.

In small mutant clones of hop^{c111} generated in the background of the PZ80 polar cell marker or the 93F stalk cell marker, alterations in cell fate were consistent with results seen from heteroallelic combinations of *hop* mutations. In mutant cells at the termini, the PZ80 marker was expressed cell autonomously (Fig. 6C). Furthermore, the 93F stalk cell marker was not expressed in any mutant cells. We therefore conclude that JAK signaling in the soma is essential specifically in the presumptive stalk cells to allow determination of that fate. The expansion of PZ80 expression in the mutant cells further suggests that polar cells are the default fate.

Epithelial follicle cell fates are also affected in JAK mutants

The role of JAK signaling in follicle cell differentiation is not

limited to specification of stalk cells from the stalk cell/polar cell precursor pool. In egg chambers that are mosaic for *hop* or *Stat92E* mutations, there is sustained expression of Fas III in epithelial cells of the vitellarium. For both *hop* and *Stat92E* mosaics, the cells that maintain Fas III expression are homozygous mutant (Fig. 7). The level of Fas III protein in the mutant cells is related to the developmental stage of the egg chamber and independent of clone size. In eggs prior to stage 6, mutant clones express high levels of Fas III, comparable to immature follicle cells in the germarium or polar cells in the vitellarium. At approximately stage 7, the levels of Fas III protein begin to drop in the clones until it is essentially undetectable at about stage 10. This temporal limitation is seen regardless of clone size or when the clone is induced. This loss of Fas III at stage 7 coincides with the end of epithelial cell proliferation and the induction of the various epithelial cell fates. The developmental significance of becoming Fas III-positive in clones is not clear. Because expression of Fas III is ambiguous, increased Fas III staining could indicate either that the cells have adopted a polar cell-like fate, or that they have failed to differentiate. However, the fact that the Fas III expression disappears after stage 7 suggests that these cells were not already committed to a specific fate. Thus we favor the hypothesis that the mutant epithelial follicle cells remain immature until the cessation of proliferation. Consistent with the heteroallelic mutant combinations of *hop*, termination of proliferation appears to be unaffected in the mutant clones, as determined by lack of PH3 staining beyond stage 6 (data not shown).

Interestingly, in some mutant clones of either *hop* or *Stat92E*, some cells of later stage (after 7) chambers retain high Fas III levels. The cells with high levels of Fas III are nearly always at the edge of a clone and are frequently the cells closest to the terminus (Fig. 7B-D). At the same time, other mutant cells within a clone reduce Fas III to the levels seen in normal differentiated epithelial follicle cells. This arrangement resembles the normal adoption of polar cell fate seen in the germarium. The staining of such mutant clones in a PZ80 background demonstrates that cells with high levels of Fas III in terminal cells can adopt a polar cell fate (not shown). However, we cannot determine whether the additional polar cells arise from clones that include the stalk/polar cell precursor pool or from the epithelial follicle cell precursor pool. Therefore, it remains possible that the definition of a stalk cell/polar cell precursor pool may not be absolute, and that epithelial cells near the termini might be switched to a polar cell fate in the absence of JAK activity. Additional experiments will be necessary to address the role that JAK signaling, or its loss, plays in epithelial cell differentiation.

Follicular mosaics of strong mutant alleles of *Stat92e* (*Stat92E*^{j6c8} and *Stat92E*^{06346}) and strong or weak alleles of *hop* (*hop*^{c111} and *hop*^{msv}) have very similar phenotypes. Mutants of both genes display a range of phenotypes from simple fusions of two consecutive chambers to inability for any cysts to pinch off from the germarium. Further, all of these mutants show persistence of Fas III staining in early stage chambers of the vitellarium. The fact that mutations in both genes have the same phenotype suggests that these developmental functions in the ovary utilize a typical JAK signaling mechanism, relying on both JAKs and STATs to transduce a signal.

DISCUSSION

The loss of JAK pathway function in the somatic cells of the *Drosophila* ovary results in the fusion of adjacent cysts and/or the mislocalization of the oocyte within a cyst. Based on molecular markers for cell identity, mutations in *hop* or *Stat92E* cause the loss of stalk cells and an increase in the number of polar cell. This shift in cell fates correlates with the fusion of adjacent cysts. An allelic series of *hop* mutant combinations shows a range of phenotypic severity, from occasional fusion of two adjacent chambers to complete fusion of all cysts with no morphological distinction between germarium and vitellarium. The severity of the visible phenotypes is reflective of the severity of the follicle cell fate transformations. Effects on fate range from frequent appearance of one extra polar cell in the weakest mutation to consistent appearance of a dozen or more extra polar cells in more severe alleles. Phenotypes seen in mutant clones of *hop* and *Stat92E* ovaries are similar to those seen in the heteroallelic combinations of *hop* mutations. By using the directed mosaic technique (Duffy et al., 1998), clone production was limited specifically to the somatic cells, thereby demonstrating that the activity of the JAK pathway is required in the follicle cells. Mosaic analysis also demonstrated that the adoption of proper epithelial cell fates requires JAK activity.

JAK activation regulates two cell fate decisions

All follicle cell subpopulations in an egg are derived from approximately three stem cells in the germarium of each ovariole (Margolis and Spradling, 1995; Zhang and Kalderon,

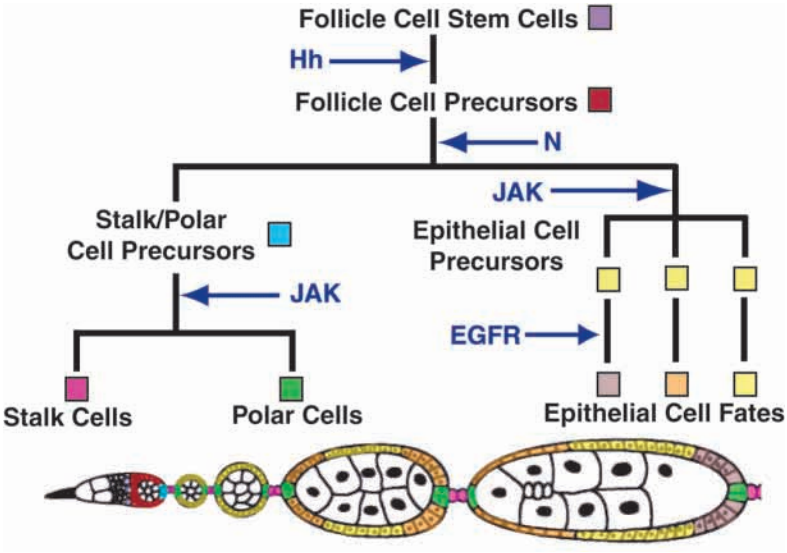

Fig. 8. Model for the functions of JAK signaling in the ovaries. Anteroposterior patterning of the follicular epithelium is accomplished through a series of cell signaling events. Each event progressively defines somatic fates. The differentiation events are represented as a cascade with the signaling pathways involved in each step indicated in blue. A diagram of an ovariole is colored to indicate the somatic cell identities and is keyed to the fates indicated in the cascade. See text for details.

2001). While still in the germarium, a common pool of distinct stalk and polar cell precursors is set aside from the epithelial follicle cells (Margolis and Spradling, 1995; Tworoger et al., 1999). Those precursors then differentiate into either stalk or polar cells (see model, Fig. 8). The remaining epithelial cells are pre-patterned with mirror image symmetry along the anteroposterior axis, with three distinct subpopulations at each end. The symmetry is broken at stage 6 when Gurken in the oocyte stimulates EGF receptor in the posterior terminal cells to determine posterior polarity of the egg. The three anterior terminal cell populations then become border cells, stretched (nurse cell-associated) cells, and centripetal cells (Gonzalez-Reyes and St Johnston, 1998; Keller Larkin et al., 1999). The posterior terminal cells are essential for the reorganization of the cytoskeleton in the oocyte. Those cells send an unknown signal to the germline that stimulates the reversal of microtubular polarity in the egg which is necessary for the migration of the oocyte nucleus to the anterior and for the correct localization of polarity determinants in the egg.

Loss of JAK pathway signaling clearly influences the terminal fate of the stalk/polar cell precursors. In heteroallelic mutant combinations of *hop*, the number of polar cells increases while the number of stalk cells decreases. However, the sum of stalk cells plus polar cells remains approximately the same as in wild type, indicating that loss of JAK signaling is not influencing proliferation of the precursor pool, nor is it causing recruitment of epithelial follicle cells to a polar fate. This suggests a model in which the normal function of the JAK pathway is to promote the adoption of stalk cell fate in a subset of the stalk/polar cell precursor pool (see Fig. 8). JAK pathway activation may either instruct the adoption of stalk cell fates or prevent the adoption of polar cell fate. Current data do not distinguish between these alternatives.

A second role for JAK signaling in the follicle cells was highlighted by analysis of mosaics. In chambers of the vitellarium, the immature cell marker Fas III is rapidly downregulated in all but the polar cells. However, the epithelial follicle cells do not begin to express markers of terminal differentiation until stage 7. Indeed, these cells continue to proliferate through stage 6. Nonetheless, the loss of Fas III in the epithelial cells beginning around stage 2 suggests that the identity of these cells has already begun to change. Presumably they become preliminarily committed to an epithelial follicle cell fate. In *hop* or *Stat92E* mutant clones, younger chambers retain high levels of Fas III in all the mutant cells. In more mature egg chambers (stage 7 or later) there is a consistent lack of Fas III expansion in mutant cells. The transient nature of the increase in Fas III expression suggests that the mutant cells remain in an immature State until later stages. In this model, JAK pathway activity would be necessary for the preliminary commitment step in epithelial cell differentiation that occurs after the egg chamber pinches off from the germarium. At approximately stage 7, the normal stage for terminal differentiation, the Fas III-positive JAK mutant cells lose Fas III expression, presumably because they are cued to differentiate by another signal. The consequence of loss of JAK signaling on terminal epithelial cell fates remains to be investigated.

A model for JAK pathway functions in the follicle cells

Several signaling pathways have been implicated in the patterning of the follicular epithelium (see Fig. 8). The best characterized are the Notch, EGFR and Hedgehog pathways (reviewed by Dobens and Raftery, 2000; Van Buskirk and Schupbach, 1999; van Eeden and St Johnston, 1999). In the earliest of these activities, strong expression of *hh* in the terminal filament and cap cells at the anterior tip of the germarium stimulates the proliferation of the somatic stem cells (Forbes et al., 1996; Tworoger et al., 1999; Zhang and Kalderon, 2000; Zhang and Kalderon, 2001). Loss of Hh signaling results in reduced follicle cell number and consequent failure to properly encapsulate the germline cyst (Forbes et al., 1996; Zhang and Kalderon, 2000). Recent work has demonstrated that the normal role of Hh in the ovaries is as a somatic stem cell factor and that it is necessary for the proliferation of somatic stem cells (Zhang and Kalderon, 2001).

After Hh activity promotes the production of a pool of follicular precursors, the stalk/polar cell precursor pool is set aside from the epithelial cell pool. The stalk/polar cell precursor pool is distinct from the epithelial pool because it ceases to proliferate as the cyst reaches the posterior end of the germarium (Margolis and Spradling, 1995; Tworoger et al., 1999). The method by which the stalk/polar cell precursors are determined is not known, but it has been suggested that Notch signaling, enhanced by localized Fringe activity, may be involved in the process (Lopez-Schier and St Johnston, 2001). Similar to JAK mutants, the loss of Notch activity causes chamber fusions that are apparently the result of a failure to produce stalk cells. But unlike JAK mutants, N pathway mutants also fail to produce polar cells (Grammont and Irvine, 2001; Lopez-Schier and St Johnston, 2001). Therefore, N signaling is required for the differentiation of both polar and stalk cell fates.

So what distinguishes stalk and polar cells from each other? We demonstrate here that JAK signaling induces the adoption of stalk cell fates in a subset of the stalk/polar cell precursors. Loss of JAK pathway activity expands polar cells at the expense of stalk cells, while ectopic activation of the pathway causes a reduction of polar cells. Therefore, we propose that it is JAK pathway activity that determines the terminal fate of stalk and polar cells (Fig. 8). However, JAK activity is limited in assigning stalk cell fates to only competent cells, that is, the stalk/polar cell precursor pool. Thus, another activity, perhaps N signaling described above, is necessary to induce competence for stalk and polar fates. Alternatively, N signaling may be primarily responsible for the assignment of polar cell fates (Grammont and Irvine, 2001). One could imagine a mechanism of lateral inhibition, already linked to N signaling in various tissues, in which all the cells of the precursor pool have N activity, but that the signal becomes limited to and maintained only in the polar cells. It may be the activity of the N pathway that then drives stable expression of *upd* and allows the induction of stalk cell fates in neighboring cells.

While polar and stalk cell fates are adopted as chambers exit the germarium, differentiation of the epithelial follicle cell fates is not obvious until later. At approximately stage 7, epithelial follicle cells express markers for each of the terminal identities with a clear anterior-posterior orientation (Gonzalez-Reyes and St Johnston, 1998). But in the absence of Grk/EGFR signaling at the posterior, a symmetrical mirror image pattern of three terminal populations of epithelial fates at each end is revealed (Gonzalez-Reyes and St Johnston, 1998). In wild-type

ovaries, up to approximately stage 6, the oocyte signals to the overlying posterior follicle cells through Gurken, a TGFα molecule that binds the EGF receptor (Egfr) in the follicle cells (Gonzalez-Reyes et al., 1995; Roth et al., 1995). The terminal follicle cells that receive the Grk signal are induced to become posterior follicle cells. The resulting posterior follicle cells then signal to the oocyte to stimulate a cytoskeletal rearrangement. The resulting microtubular polarity drives the migration of the oocyte nucleus from the posterior to the anterior and establishes the AP axis that allows the sequestration of anterior and posterior maternal products to their respective poles. The signal from the soma for polarization of the oocyte microtubules is not yet known.

When the developing cyst exits the germarium, there is a distinct change in the epithelial cell precursors. The level of Fas III, a marker for immature follicle cells, is rapidly reduced in all epithelial cell precursors. However, these cells do not begin to express markers for new cell identities until around stage 7. Therefore, it seems that the epithelial cells become committed to a fate early in the vitellarium, but do not terminally differentiate until later. This is consistent with the fact that the epithelial follicle cells continue to divide until stage 6. Furthermore, Grk/EGFR signaling does not impose posterior identity on epithelial cells until stage 6. So the loss of Fas III in epithelial cell precursors in the early vitellarium marks an intermediate step in specific epithelial identities. Here we demonstrate that JAK signaling is involved in this step, because clones of JAK pathway mutations cause the persistence of Fas III in epithelial cell precursors in the early vitellarium. The normal loss of Fas III expression in epithelial precursors of the early vitellarium may indicate the establishment of a pre-pattern of epithelial identities determined by JAK signaling. It is attractive to speculate such a role because the secreted JAK pathway ligand Upd is expressed symmetrically at the termini of the chamber. It is easy to envision a scheme in which the strength of the Upd signal received by the epithelial cell precursors determines the ultimate epithelial identity. However, these epithelial cells would remain in a proliferative, undifferentiated program until stage 7. The event that allows terminal differentiation is unclear, but could also be a N signal, as suggested above for competence of stalk and polar cells. This is consistent with the report of a pulse of Delta protein, a N ligand, that occurs at stages 5-7 (Deng et al., 2001; Lopez-Schier and St Johnston, 2001). Additional work will determine whether JAK signaling is instructive for specific epithelial fates, but we present a testable model of that role.

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