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THE JAK-STAT PATHWAY IS REQUIRED FOR MULTIPLE EARLY EVENTS IN DROSOPHILA OOGENESIS

Jennifer Renee Matlock
University of Kentucky, jrmcgr0@uky.edu

ABSTRACT OF THESIS

THE JAK-STAT PATHWAY IS REQUIRED FOR MULTIPLE EARLY EVENTS IN *DROSOPHILA* OOGENESIS

The Janus kinase (JAK) pathway is an integral part of signaling through a variety of ligands and receptors in mammals. The extensive reutilization and pleiotropy of this pathway in vertebrate development is conserved in other animals as well. In *Drosophila melanogaster*, JAK signaling is involved 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. A primary defect of chambers with reduced JAK activity is fusion of successive chambers. These chambers exhibit an expansion of the polar cell population and concomitant loss of interfollicular stalk cells. Mosaic analysis of both JAK pathway transducers, *hopscotch* and *stat92E*, reveals that JAK signaling is specifically required in the somatic follicle cells. Another role of JAK signaling is in oocyte localization. In chambers mosaic for loss of *hop* activity, oocyte mislocalization results. Proper localization occurs only when the posterior follicle cells are wild type for *hop*.

KEYWORDS: Drosophila, JAK, Oogenesis, Follicle cells, Oocyte localization

Jennifer Renee Matlock
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By

Jennifer Renee Matlock

Dr. Douglas Harrison
Co-Director of Thesis

Dr. John Rawls
Co-Director of Thesis

Dr. Peter Mirabito
Director of Graduate Studies

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THESIS

Jennifer Renee Matlock

The Graduate School

University of Kentucky

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THESIS

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By

Jennifer Renee Matlock

Lexington, Kentucky

Co-Directors: Dr. Douglas A. Harrison, Professor of Biology
and Dr. John Rawls, Professor of Biology

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Chapter One

Introduction

Drosophila Oogenesis

The *Drosophila* ovary consists of approximately 15 chains of progressively developing eggs called ovarioles that are more mature toward the posterior. The ovariole is broken down into two distinct parts, the germarium and the vitellarium. The germarium, located at the anterior end of each ovariole, houses the germline and somatic stem cells, support cells, cap cells, and the earliest developing cysts. Formation of a cyst begins with the asymmetric division of a germline stem cell giving rise to a daughter stem cell and a cell that will become a cystoblast. This cystoblast undergoes four incomplete divisions, resulting in a 16-cell germline cyst that remains interconnected by structures called ring canals. This is the last division the germline will undergo. One of these germ cells will become the oocyte and the remaining 15 germ cells will become nurse cells that will eventually provide essential materials to the mature egg. As the cyst progresses posteriorly through the germarium it will become enveloped in a monolayer of immature somatic follicle cells (Spradling 1993). The somatic cells are made up of several subpopulations, each of which will have its own unique role in the development of the egg. Communication between the germ cells and the somatic cells is essential for proper patterning of the egg and ultimately the embryo. As encapsulation is completed, a subset of cells is set aside which will become the polar cells and stalk cells. Prior to exit from the germarium, one germ cell takes position at the posterior of the chamber and assumes the oocyte fate. This process requires the cell-cell adhesion molecules E-cadherin and Armadillo (Godt and Tepass 1998; Gonzalez-Reyes and St Johnston 1998). As the cyst pinches off from the germarium, two distinct types of cells arise. Two cells at each end of the chamber, from the special pool of cells, become polar cells and six to eight cells, also from this pool, become stalk cells. The stalk cells are flattened, disc shaped cells that separate developing chambers from one another. Once the chamber has pinched off from the germarium, molecular markers can distinguish the stalk cells and polar cells from one another and from other somatic cells. (Margolis and Spradling 1995; Tworoger, Larkin et al. 1999). After exit from the germarium the stalk cells and polar cells cease proliferation while the remaining follicle cells, the epithelial follicle cells, will continue to divide up until stage six (Lopez-Schier and St Johnston 2001). The epithelial cells later differentiate into various subpopulations, each with specific functions in the patterning of the egg and ultimately the embryo (Spradling 1993; Gonzalez-Reyes and St Johnston 1998).

The JAK-STAT pathway

The Janus Kinase (JAK) pathway is an integral part of signaling through a variety of cytokines and growth factors in the development of many vertebrate tissues. JAK signaling has been implicated in several types of cancers such as breast cancer and leukemia as well as some immune deficiency diseases. This highly reutilized intracellular signaling cascade is essential during developmental events such as hematopoiesis, immune system development, mammary development and lactation, and overall growth (Zeidler, Bach et al. 2000). Interestingly, the Janus Kinase pathway has also been implicated in mammalian oogenesis (Russell and Richards 1999; reviewed by Imada and Leonard 2000). In vertebrates four JAKs and seven STATs have

been identified so far. The pathway is conserved across many organisms, including *Dictyostelium*, humans, and fruit flies. The *Drosophila* JAK pathway includes one known ligand, Unpaired, one JAK, Hopscotch, one STAT, Stat92E, and one known receptor, Domeless. In *Drosophila melanogaster*, JAK signaling is involved in embryonic pattern formation, sex determination, larval blood cell development, spermatogenesis, and formation of other adult structures. The pathway is initiated by binding of the ligand, Upd, to the receptor, Dome, which causes dimerization, transphosphorylation and activation of the JAKs as well as phosphorylation of the receptor. The JAKs recruit and phosphorylate STATs which dimerize and translocate into the nucleus where they activate transcription of target genes (reviewed by Dearolf 1999; Zeidler, Bach et al. 2000). Maternal loss of *hop* or *stat92e*, or zygotic loss of *upd* results in a unique embryonic patterning defect (Binari and Perrimon 1994; Hou, Melnick et al. 1996; Yan, Small et al. 1996; Harrison, McCoon et al. 1998). Subsequent analysis has implicated the JAK pathway in larval hematopoiesis, wing vein development, thoracic development, sex determination, and planar polarity in the eye (Harrison, Binari et al. 1995; Luo, Hanratty et al. 1995; Yan, Luo et al. 1996; Luo, Asha et al. 1999; Zeidler, Perrimon et al. 1999; Jinks, Polydorides et al. 2000; Sefton, Timmer et al. 2000). The broad utilization of JAK signaling in many facets of development directed us to oogenesis to investigate its potential role there.

In this thesis, some roles of JAK signaling in *Drosophila* oogenesis are described. The JAK pathway ligand, *upd*, is normally expressed in a restricted fashion, exclusively at the poles of the follicular epithelium. Reduction or complete removal of JAK signaling components 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. Also seen and described is oocyte mislocalization within a single chamber.

Chapter Two

Materials and Methods

Fly stocks

Unless otherwise stated, the flies in the experiments described were reared at 25°C. Descriptions of *hop* and *stat92E* alleles used in these experiments can be found in FlyBase. Enhancer marker lines were characterized by various sources: 93F (Ruohola, Bremer et al. 1991), PZ80 (Karpen and Spradling 1992), and A101 (Clark, Giniger 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, Harrison 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 three hour heat shock of adult females at 37°C. Animals were then examined for morphological or molecular alterations in the ovaries two to eight 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 due to expression of FLP recombinase in the somatic cells of the germarium (Duffy, Harrison et al. 1998). Adult females were dissected for ovary analysis between three and seven days after eclosion.

Immunological and histochemical staining

X-gal staining for β-galactosidase activity was performed as previously described (Harrison and Perrimon 1993). Briefly, 1-3 day old males and females were placed in vials containing yeast paste for two days. Ovaries were dissected in PBS, then fixed for 1-2 minutes in 2.5% glutaraldehyde (Sigma) in PBS. Ovaries were washed in PBT (1x PBS, 0.1% Triton-X 100), rinsed in X-gal staining solution (Klambt, Jacobs 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, McCoon et al. 1998) except that proteinase K digestion was performed for one 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 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, rat α-cadherin E (DCAD2, gift of T. Uemura) at 1:50, mouse α-Armadillo (N2 7A1 Armadillo, Eric Wieschaus) at 1:100, and rabbit α-phospho-Histone H3 (PH3, Upstate Biotechnology, Inc.) at 1:500. Secondary antibodies were Texas Red-α-mouse, FITC-α-rabbit, Cy-5-α-mouse, Cy-2-α-rabbit, Rhodamine Red-X-α- mouse 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) and E-cadherin (Godt and Tepass 1998) 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.

Chapter Three

JAK signaling determines the ratio of stalk cells and polar cells

As the germline cyst progresses through region two of the germarium, it becomes encapsulated by a layer of somatic follicle cells. It is also at this time that a subpopulation of follicle cells is set aside that will later become the polar cells and stalk cells. These cells are necessary for proper formation and separation of consecutive cysts. Failure of these cells to take on their fates results in compound chambers. This pool of cells potentially receives signals from several signal transduction pathways. The best characterized is the Notch pathway (Lopez-Schier and St Johnston 2001). The loss of Notch activity causes chamber fusions that appear to be due to a failure to produce stalk cells. But these mutants also fail to make polar cells (Grammont and Irvine 2001; Lopez-Schier and St Johnston 2001). JAK pathway mutants are slightly different. In JAK mutants, fused chambers display an expansion in the polar cell population at the cost of stalk cells.

unpaired is expressed specifically in polar cells

Signaling between the soma and the germline and between various somatic follicle cells is essential for proper patterning of the developing egg. To investigate the potential for JAK-STAT involvement in oogenesis, in situ hybridization to whole ovaries were performed by Gabriella Rennebeck and Rongwen Xi to determine the expression of genes in the pathway. Interestingly, *unpaired*, a ligand for the pathway, is expressed in a very specific pattern in the ovary (Figure 3.1). After chambers pinch off from the germarium, *upd* expression is restricted to the pairs of polar cells at each end of the cyst (Figure 3.1A). In the germarium, *upd* is expressed in a cluster of somatic cells at the posterior of region 3 (Figure 3.1B and C). These cells could potentially be the pool of cells that gives rise to the polar cells and stalk cells. The specific expression pattern of *upd* in ovaries indicates a role for the JAK pathway in oogenesis.

If Unpaired's roles in oogenesis involves activation of the JAK pathway, then expression of the other JAK signaling components would be expected as well. In situ hybridization to *stat92E* RNA reveals that the *Drosophila* STAT is expressed in both the germarium and the vitellarium (Figure 3.1D). Expression in the germarium includes all follicle cells in region 2a and 2b, then is restricted to terminal follicle cells in region three. In the vitellarium, *stat92E* is expressed weakly at the termini of the egg chamber, but unlike *upd*, in a broader domain than only the two polar cells. Also, 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 and warranted further investigation.

hop mutant ovaries contain egg chamber fusions

Homozygosity for complete loss-of-function alleles of any of the known JAK pathway components, *upd*, *hop*, and *stat92e* results in lethality prior to adulthood. 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. The morphological defects ranged from essentially wild-type for hop^{msv}/hop^{M4} to severely compounded chambers for hop^{msv}/hop^{M38} (Figure 3.2). Compound chambers can be due to two main events: overproliferation of the

germline or failure of consecutive chambers to separate from one another. In moderate *hop* mutant combinations, compound chambers contain twice the number of germ cells (Figure 3.2B). In some cases it is possible to detect a follicle cell layer that bisects the compound chamber (Figure 3.2B, also Figures 3.3E, 3.4B, and 3.4C). This indicates that the defect is due to fusion of consecutive chambers. However, to rule out the possibility of excessive germline proliferation, mutant chambers were stained with an antibody to the Orb protein which accumulates at highest concentrations in the oocyte. Orb staining shows that there are multiple distinct cysts within each compound chamber (Figure 3.2E). If there had been overproliferation of the germline, presumably there would be only one oocyte per compound chamber, however, this is not the case with *hop* mutant chambers. To verify these data, Kelch staining of the ring canals was performed. An extra round of germline division 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 a fusion event. In a normal chamber the oocyte is connected to the nurse cells by four ring canals, if an extra division had taken place, there would be five. Observation of the ring canals in *hop* mutants ovaries failed to detect more than four ring canals per oocyte in any chamber (n=69). Other supporting data from this experiment show that in chambers in which all ring canals could be definitively counted, there were a multiple of 15 ring canals per chamber (Figure 3.2F). From these data we conclude that compound egg chambers seen in *hop* mutants are due to fusions of consecutive cysts rather than overproliferation of the germline as described in mutants for genes such as *encore* (Hawkins, Thorpe et al. 1996).

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

Fusions of egg chambers in other mutants have been linked to alterations in the differentiation of follicle cells (Ruohola, Bremer et al. 1991; Forbes, Lin et al. 1996; Keller Larkin, Deng et al. 1999; Zhang and Kalderon 2000; Lopez-Schier and St Johnston 2001). To investigate the production of follicle cell subpopulations, antibody and enhancer trap markers were used to identify specific cell fates in *hop* mutant ovaries. Polar cell fate was assayed using antibodies to Fasciclin III (Fas III). In the wild-type ovaries, Fas III is expressed in all immature follicle cells until stage three, after which it becomes restricted to the two polar cells at the anterior and posterior of each chamber. Fas III staining is expanded in ovaries from *hop* mutant females (Figure 3.3).

Due to the fact that high levels of Fas III mark both undifferentiated follicle cells and polar cells, a second cell fate marker was used to verify the cells as being polar cells rather than immature follicle cells. PZ80 is an enhancer trap that is expressed only in terminally differentiated polar cells (Karpen and Spradling 1992). Expansion of PZ80 positive cells was observed in *hop* mutant ovaries, reinforcing the results seen with Fas III staining. Given the distinct nuclear localization of the β-galactosidase marker in the enhancer trap, it was possible to detect the most subtle expansion of the polar cell populations in very weak *hop* mutants (Figure 3.3B). Depending on the severity of the *hop* mutant combination, egg chambers can contain anywhere from the normal two PZ80 positive cells to more than a dozen (Table 3.1). Nearly half of the polar cell clusters from the hop^{msv}/hop^{M4} eggs contain more than two cells with no effects on chamber morphology (Table 3.1). In hop^{msv}/hop^{M75}, a slightly more severe mutant combination, the non-fused chambers have polar cell clusters that contain an average of 4.6 cells at each end.

Since stalk cells and polar cells arise from the same precursor population, a possible cause for extra polar cells is the mis-specification of stalk cells. To investigate this possibility, another enhancer trap marker, 93F was used. 93F is an enhancer trap with specific expression of lacZ primarily in the stalk cells and also in the terminal filament cells at the anterior tip of the ovariole (Figure 3.3D) (Ruohola, Bremer et al. 1991). *hop* mutants were generated in a 93F background to examine the effects of reduced *hop* activity on the stalk cell population. As one might expect, *hop* mutant ovaries show a reduced number of stalk cells (Figure 3.3E). The degree of stalk cell loss is directly related to the severity of the *hop* mutant combination. Chambers with fusions have few or no stalk cells present. In the case of hop^{msv}/hop^{M75} ovaries where there is a moderate frequency of fusions, no 93F positive stalk cells are detected between fused chambers, but there are between distinctly separated chambers. In the most severe combination observed, hop^{msv}/hop^{M38}, there are very few 93F positive cells (Figure 3.3F). These data, together with the data from the polar cell marking experiments, show that loss of JAK pathway activity causes fused chambers due to a loss of interfollicular stalk cells and an increase in the polar cell population.

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

As described earlier, *upd* is expressed in the polar cells of the ovary, suggesting a role in oogenesis. To ensure that Upd is in fact the signal that stimulates the JAK pathway in oogenesis, ovaries from females mutant for *hop* were compared with ovaries from females mutant for *hop* and heterozygous mutant for *upd*. Reducing *upd* activity by approximately one half dramatically enhances the fusion rate of egg chambers seen in various heteroallelic mutant combinations of *hop*. This is particularly striking for the hop^{msv}/hop^{M4} combination in which fusions are rarely seen (compare Figure 3.3G and 3.3H). However, in females that are $hop^{msv}\ upd^{YM55}/hop^{M4}$ or $hop^{msv}\ upd^{YC43}/hop^{M4}$ the rate of ovarioles with fused chambers rises to 75% or more (Table 3.2). In addition to the increased rate of fusion, the reduction of *upd* activity enhances the adoption of polar cell fates (Fig. 3.3H), just as seen for strong allelic combinations of *hop*. We conclude from this enhancement that *upd* is the ligand that functions to positively stimulate JAK signaling in the follicle cells.

Loss of *hop* does not promote excess proliferation

Similar to JAK pathway mutants, ectopic Hedgehog (Hh) signaling also results in the expansion of the polar cell population (Forbes, Lin et al. 1996; Tworoger, Larkin et al. 1999; Zhang and Kalderon 2000). Polar cells and stalk cells cease division prior to pinch off from the germarium, but persistence of Hh signaling causes excess proliferation beyond exit from the germarium. This prolonged proliferation causes an expansion of the polar cell/stalk cell precursor population, which ultimately leads to an overabundance of these cells later in oogenesis (Zhang and Kalderon 2000). To determine whether this could be the reason for the increased number of polar cells in JAK mutants, ovaries were stained with antibodies against phospho-histone H3 (PH3) which marks actively dividing cells. While polar cells and stalk cells cease division prior to exit from the germarium, epithelial follicle cells divide up until around stage six. Therefore, there is no PH3 staining past stage six in wild type ovaries. When JAK mutants were stained with PH3, the same was observed, indicating no prolonged proliferation beyond normal. In addition, there is no PH3 staining observed in the polar cells or stalk cells

(Figure 3.4). This suggests that the phenotypes observed in *hop* mutants are not the result of excess proliferation of follicle cells in general.

Despite there being no indication of extended proliferation of the follicle cell population in general, *hop* could have effects on just the stalk cells and polar cells. To examine this possibility the number of polar cells, as indicated by the PZ80 marker, at each stage were counted in *hop* mutants with distinct and separate chambers. If the proliferative program were changed, we would expect to see the number of polar cells to increase over time; there would be more polar cells in older chambers than in younger chambers. The number of polar cells in chambers from stage 4 to stage 9 is approximately constant in *hop* mutant ovaries (Table 3.1). In wild-type ovarioles, the average number of polar cells at each pole is 2.24 at stage 4, and drops slightly in stage 8 to 9 chambers to approximately 2.06. In hop^{msv}/hop^{M4} mutant ovaries, most polar cell clusters have more than two cells, with an average of 2.46 at stage four and 2.25 at stage 8 to 9. The expansion of polar cells is more pronounced in the hop^{msv}/hop^{M75} mutant ovaries, with an average of 4.56 cells per cluster at stage 4. Consistent with wild-type, there is no expansion of polar cell cluster size at later stages in mutants, in fact the number tends to decrease over time. 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 cell death near the poles of the chambers (not shown). It can be concluded that the extra polar cells in *hop* mutants are not due to proliferation after exit from the germarium.

Ubiquitous Upd stimulates stalk cell production

Given that loss of JAK pathway activity results in the loss of stalk cells with an increase in the number of polar cells, one might expect the reciprocal to occur with an increase in JAK activity. An expectation would be that there would be an increase in stalk cell production and a loss of polar cells. Work to investigate this possibility was performed by Rongwen Xi (McGregor, Xi et al. 2002). *upd* was ubiquitously expressed in adult females to examine the effects on follicle cell fates. The increased levels of *upd* produced throughout the ovary resulted in phenotypes that are reciprocal to the loss-of-function phenotype for *hop*. Specifically, polar cells are often missing from one pole of the developing egg chambers and cells positive for stalk cell markers are expanded (Figure 3.5B). In extreme cases, rope-like stalks are produced that have two or more layers of cells rather than the normal single string of cells (Figure 3.5C-F). These cells stain strongly for 93F and α-spectrin, markers of mature stalk cells, but they also stain strongly for Fas III (Figure 3.5D), a marker of immature follicle cells. These cells also lack the characteristic flattened, disc-shaped morphology of mature stalk cells This indicates that the extra stalk cells produced by misexpression of *upd* are incompletely differentiated. In addition to production of abnormal stalks, chronic *upd* expression also resulted in some chamber fusions and oocyte mislocalizations (Figure 3.5F), similar to loss-of-function mutations.

Unlike loss-of-function mutants, the number of extra stalk cells seen in these chambers is much greater than the size of the normal stalk cell/polar cell precursor pool. This indicates a defect beyond mis-specification of cells from this pool. Ectopic Hedgehog activity results in excess cells that express both polar cell and stalk cell markers. This has been explained to be due to a proliferative defect in the precursor pool which causes these cells to divide beyond their

normal point (Forbes, Lin et al. 1996; Zhang and Kalderon 2000). However, this is not the case in hs-upd ovarioles. The mitotic cell marker PH3 was not detected in chambers beyond stage six, nor in the abnormal stalks (not shown). Thus, as with reduced JAK pathway activity, increased JAK activity does not alter the proliferation of the polar cell/stalk cell precursor cells. One possible explanation for the nature of the abnormal stalks is that cells outside the precursor pool, from the epithelial cell population, get recruited to become part of the stalk, but cannot completely take on the stalk cell fate, and therefore retain the immature follicle cell marker.

Cyst encapsulation requires only somatic JAK activity

The Notch signaling pathway has been shown to be necessary both in the germline and in the soma in oogenesis for proper egg development, in particular, for proper cyst encapsulation and separation (Grammont and Irvine 2001; Lopez-Schier and St Johnston 2001; Roth 2001). To determine whether JAK function in oogenesis is 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, Harrison et al. 1998). The e22C-GAL4 used to stimulate FLP recombinase expression is abundant in follicular stem cells and early follicle cells (Duffy, Harrison et al. 1998). Homozygous mutant *hop* mosaic patches of tissue were identified by the loss of a GFP marker that expresses in all follicle cells (Davis, Girdham et al. 1995). Mosaics of *stat92E* were generated in a similar manner, but wild-type tissues were marked by the presence of the π-Myc marker (Xu and Rubin 1993). The results observed were similar with all alleles tested hop^{msv}, hop^{c111}, $stat92e^{06346}$, and $stat92E^{j6C8}$. As with the *hop* heteroallelic mutants, the most common defect seen in mosaic ovarioles was the fusion of adjacent egg chambers (Figure 3.6). In most fused chambers, mutant clones include what should be the termini of the combined chambers. Also of interest, clones are never seen in the stalk cells separating adjacent chambers (Figure 3.6). Given that the UAS-FLP system used to create these mosaics is specific for somatic cells, we can conclude that that *hop* function is necessary in these cells. Using a comparable system, hsFLP, which is active in the soma and in the germline, hop^{c111} mosaics were generated. Chambers that had *hop* mutant cells restricted to the germline (n=90) showed no mutant phenotype (not shown). This further supports the conclusion that JAK signaling is required only in the soma for proper egg development, despite *hop* and *stat92e* expression in the germline.

Conclusions

The loss of JAK pathway function in somatic cells of the *Drosophila* ovary results in the fusion of adjacent chambers and/or the mislocalization of the oocyte within a cyst. Based on cell specific markers, mutations in *hop* or *stat92E* causes an increase in the number of polar cells and a decrease in the number of stalk cells. This alteration in cell fates results in fusion of adjacent egg chambers. A series of *hop* mutant combinations shows a wide range of phenotypes, from nearly wild type with occasional fusions of adjacent chambers to complete fusion of all cysts with no morphological distinction between germarium and vitellarium. Effects on fate range from the emergence of one extra polar cell in the weakest mutant combination to consistent appearance of a dozen or more extra polar cells in more severe mutants. 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, Harrison 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 soma.

Table 3.1

	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.

Table 3.2

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.

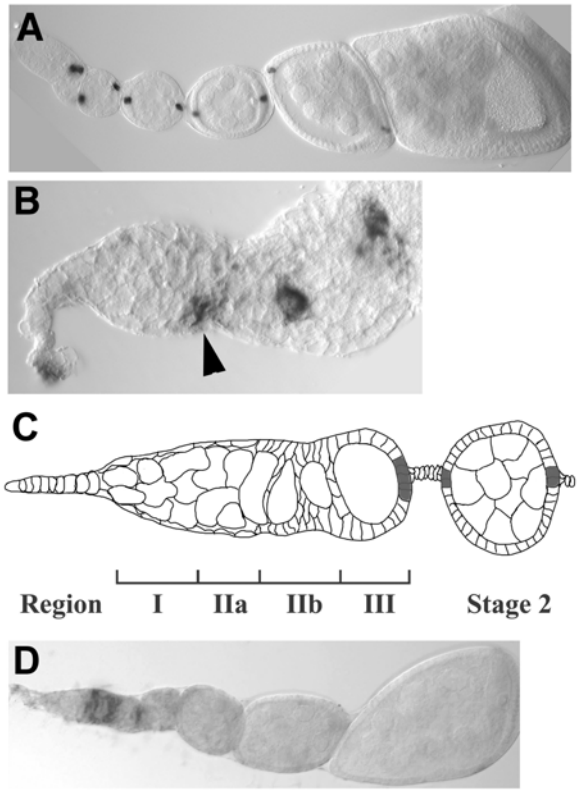

Figure 3.1. The JAK ligand, Upd, is expressed in the follicular epithelium.
(A) Expression of *upd* in the vitellarium is restricted to the two polar cells at the anterior and posterior of each egg chamber. Within region 3 of the germarium (B), *upd* is expressed in the most posterior follicle cells (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 up to stage 4, then weakly in later stages.

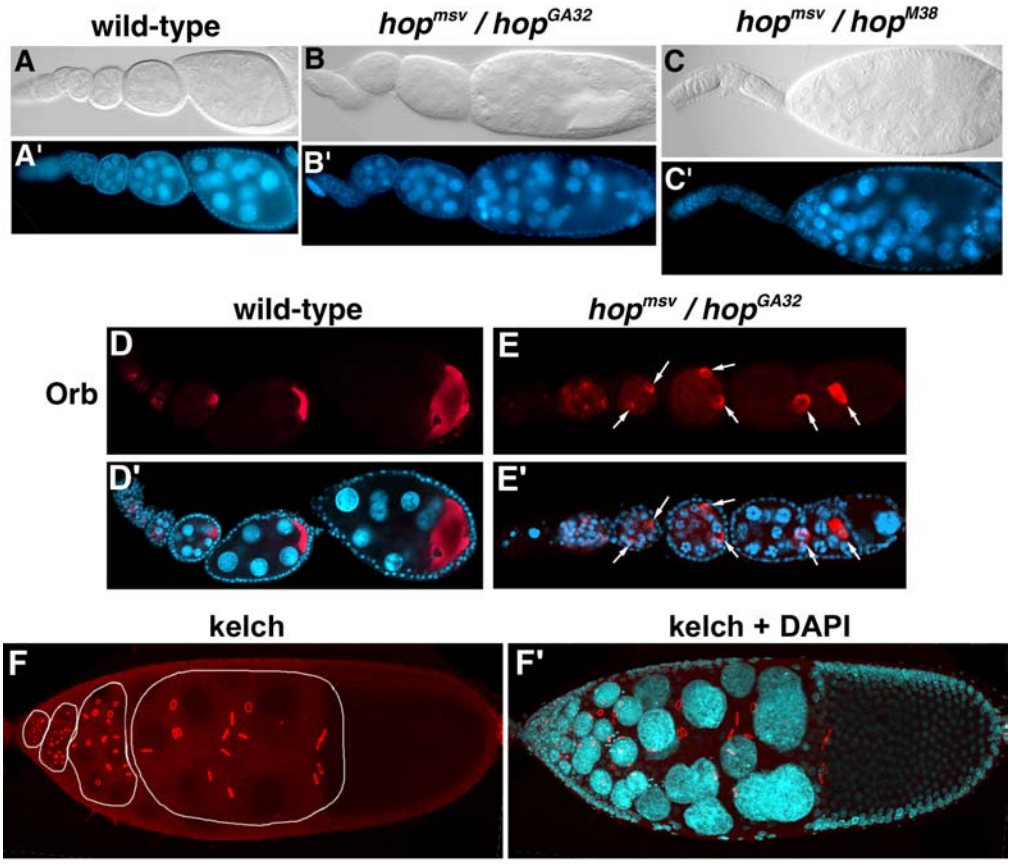

Figure 3.2. *hop* mutant ovaries contain fused egg chambers.
Heteroallelic combinations of *hop* alleles show a range of severity of phenotypes. (A-C) In comparison with wild type (A), loss of *hop* function results in chambers with additional germ cells, with penetrance and severity determined by allelic 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 is 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.

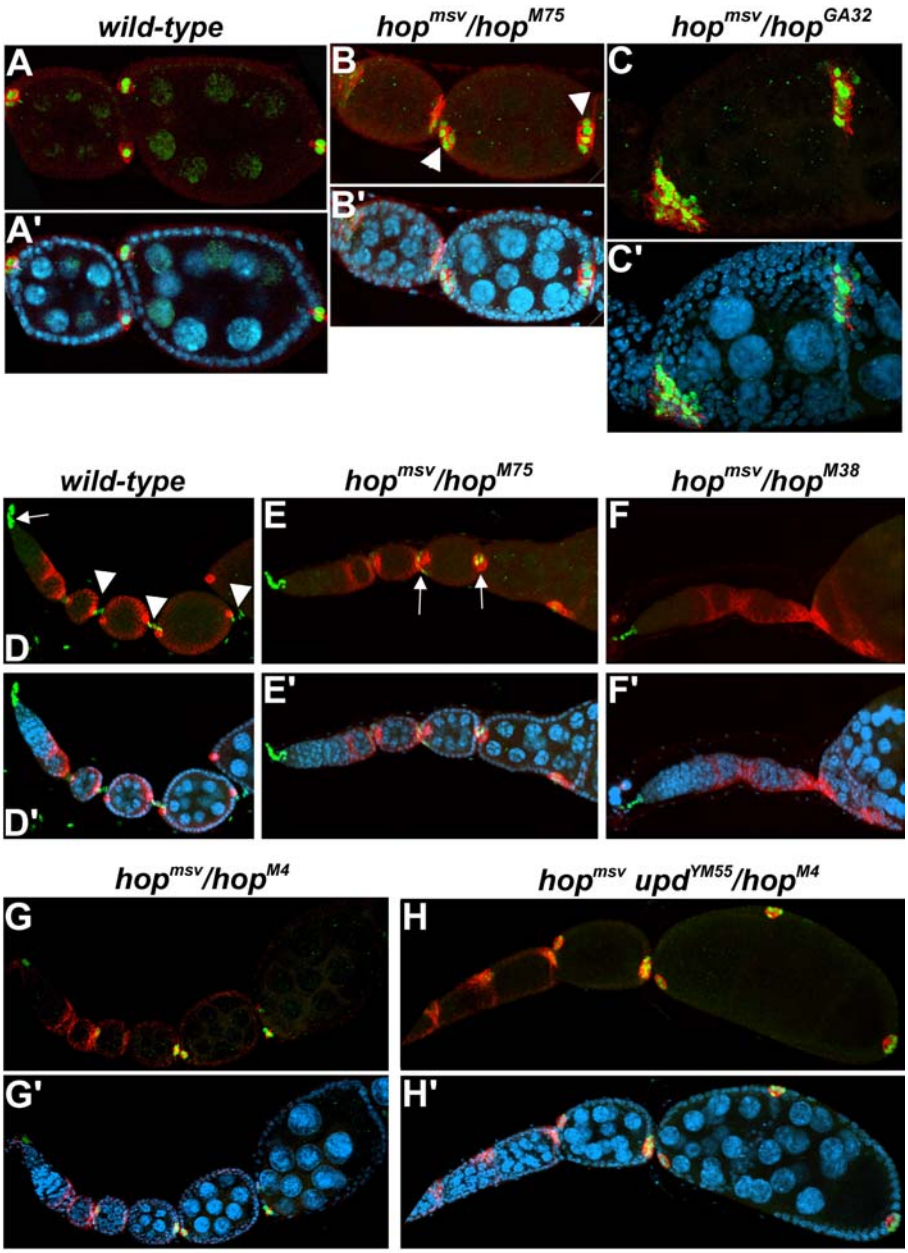

Figure 3.3. *hop* mutants produce polar cells at the expense of stalk cells.
The identity of polar cell fates was assayed using the molecular markers Fas III (in red) and PZ80 (in green) with nuclear staining by DAPI (in blue). In wild-type ovaries (A), Fas III protein is found at high levels in all follicle cells of the germarium, but is markedly reduced in all but the polar cells of egg chambers of the vitellarium. PZ80 in the two polar cells at each end of the egg chamber. In the intermediate (B) and severe (C) mutant combinations, there are extra polar cells, as indicated by the appearance of both Fas III and PZ80 (arrowheads). 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 intermediate (E) and severe (F) mutants, there are consistently fewer β-galactosidase positive interfollicular cells (arrows).

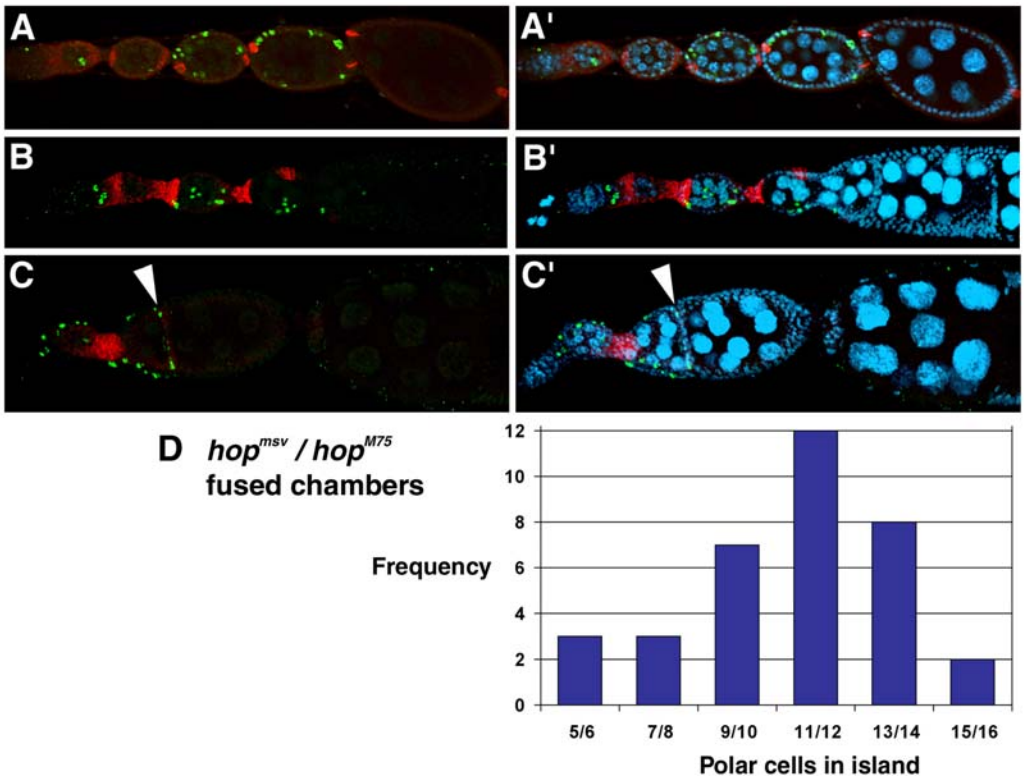

Figure 3.4. Proliferation of follicle cells is not extended in *hop* mutants.
In wild type(A) ovarioles, follicle cells cease proliferation after stage 6, as indicated by the lack of any PH3 staining (in green, with Fas III in red, and DAPI in blue). As in wild type, hop^{msv}/hop^{GA32} (B) and hop^{msv}/hop^{M38} (C) ovarioles no PH3 staining can be seen after stage 6. This restriction is visible even within the 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 interface of the fused cysts. The number of polar cells in those islands is represented in the graph.

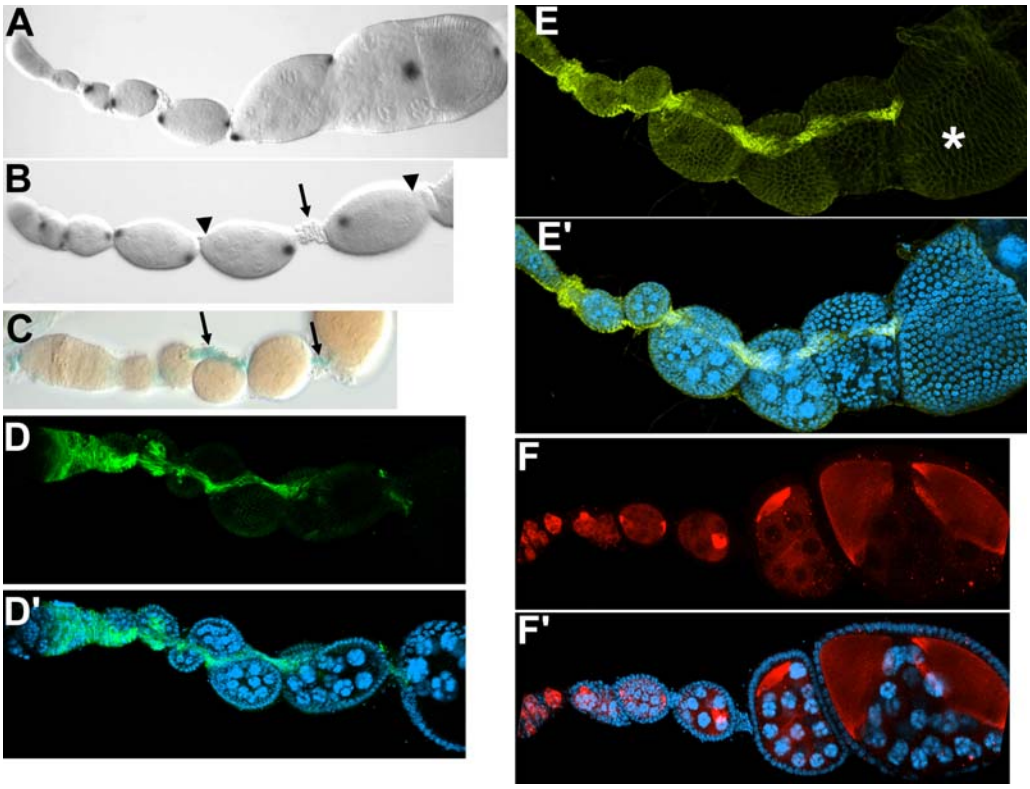

Figure 3.5. Upd misexpression stimulates stalk production.
In wild type ovaries (A) enhancer trap marker A101 marks polar follicle cells in the vitellarium. When *upd* is misexpressed (B-F), polar and stalk cells are mis-specified. Chronic expression from *hs-upd* results from shifting adults to 30°C (B-F). (B) This treatment causes the frequent loss of polar cells (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).

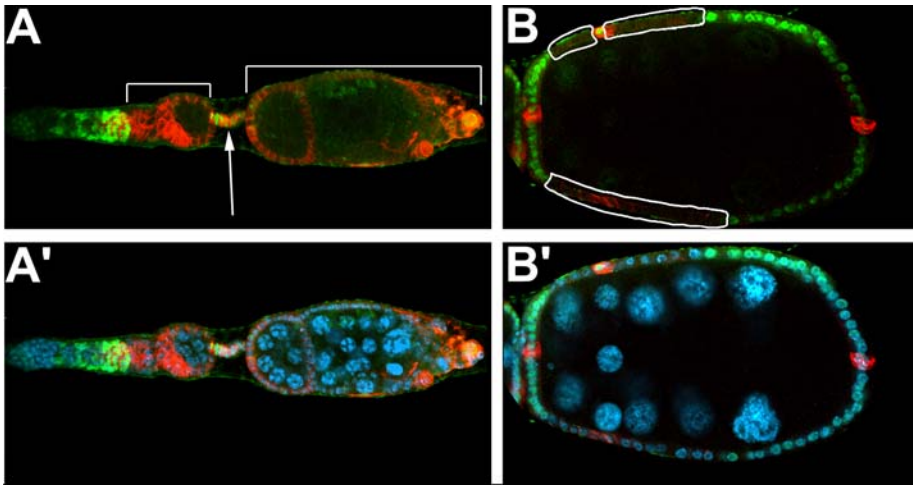

Figure 3.6. JAK pathway activity is required in the soma.
Somatic mosaics of *Stat92E*^{j6C8} show the same chamber fusion phenotype as the *hop* heteroallelic ovaries. Clones are marked by the loss of π-Myc (green, mutant cells outlined) and Fas III staining shown in red. (A) In an ovariole with nearly 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.

Chapter Four

JAK signaling in the posterior is required for proper oocyte localization

At the anterior tip of the germarium, a germline stem cell divides to give rise to a daughter stem cell and a cystoblast. The cystoblast then undergoes four incomplete divisions, remaining interconnected by cytoplasmic bridges called ring canals. As these cells divide, they share a common germline specific mitotic spindle structure called a fusome that results in a characteristic pattern of intercellular connections (Lin, Yue et al. 1994; Lin and Spradling 1995). Given the nature of their divisions, there will be two cells, the pro-oocytes, that contain four ring canals. Since these two pro-oocytes are the result of the first division, it is thought that an asymmetry of the mitotic spindle structure causes one of these cells to become the oocyte and begin to accumulate oocyte specific markers such as BicD, Orb, Cup, and Egl while the remaining 15 cells become nurse cells (Lantz, Chang et al. 1994; Ran, Bopp et al. 1994; Keyes and Spradling 1997; Mach and Lehmann 1997). It has been shown that several factors, including an intact microtubule-cytoskeleton, several *spindle* genes, proper centrosome migration, and *vasa* are required for proper fate determination of the oocyte (Gonzalez-Reyes, Elliott et al. 1997; Styhler, Nakamura et al. 1998; Bolivar, Huynh et al. 2001; Wodarz 2002). Disruption of any of the factors above can lead to misspecification or mislocalization of the oocyte, resulting in the formation of two oocytes (both pro-oocytes developing as oocytes), no oocyte, or a mispositioned oocyte.

Cell adhesion is known to play a necessary role in posterior positioning of the oocyte. Cadherin is expressed in all of the germ cells and the surrounding follicle cells in the germarium. The oocyte reaches the posterior between regions 2b and 3 in the germarium through events coordinated with the germline and soma. *D*E-cadherin is expressed in the germ cells and in the surrounding follicle cells with the highest concentrations between the oocyte and the posterior follicle cells. In region 2b of the germarium, E-cadherin is expressed at high levels in the posterior follicle cells, the oocyte preferentially binds to these cells (Godt and Tepass 1998; Gonzalez-Reyes and St Johnston 1998). Determination of the oocyte triggers an increase in the cadherin adhesiveness of this cell, giving the oocyte an advantage over the nurse cells for adhesion to the cadherin expressing cells at the posterior position. Concurrently, the follicle cells at the posterior up regulate E-cadherin, Armadillo, and α-catenin to further increase adhesiveness between the cell surfaces (Gonzalez-Reyes and St Johnston 1998). Of interest in this study thus far has been the role of the adhesion molecules cadherin and armadillo. Cadherin is a large transmembrane glycoprotein that mediates adhesion between cells of the same type. Armadillo (β-catenin) and α-catenin are also required parts of these adhesion structures. Germline mutants for any of these genes result in mispositioned oocytes (Peifer, Orsulic et al. 1993). It has been shown that cadherin mediates the localization of the oocyte in the germarium, and for this reason we decided to investigate its expression in JAK mutants. In ovaries mosaic for *hop*, specifically those with *hop* mutant posterior follicle cells, mislocalized oocytes occur. We have found, however, that when cells are mutant for *hop*, there is no effect on Cadherin or Armadillo accumulation, even in chambers where the oocyte is mislocalized.

<u>Somatic JAK activity is required for posterior oocyte localization</u>

While the most prevalent defects seen in *hop* and *stat92e* mosaic animals is fusion of consecutive egg chambers (Figure 3.1), there are additional, less frequent defects detected in these mosaic animals. Most notably, the oocyte fails to localize at the posterior of some egg chambers (Figure 4.1). In some cases, the oocyte comes to lie at the anterior end of the egg chamber rather than the posterior (Figure 4.1). Rarely, the oocyte lies in the center of the egg chamber to create a bicephalic egg (Figure 4.1). To determine what cells are responsible for the correct localization of the oocyte, marked clones were examined. Specifically, clones of hop^{c111} were generated and analyzed for the location of the oocyte. Clones were scored for oocyte position only for chambers with large clones (approximately 25% or more of the total) in the follicle cells (Figure 4.2). Three classes of chambers consistently emerged from these mosaics. The largest class by far (I and II, totaling 91%) contained the oocyte located at the normal posterior position. Within this class, a significant number (28% of total) were mutant for most or all of the follicle cells at the anterior end of the chamber, but wild type for cells at the posterior end. All of these had normal oocyte position. Only 9% of the mosaic chambers had the oocyte located at an abnormal position. Of these chambers, 5% had the oocyte at the anterior. In each case, all of the posterior cells were *hop* mutant, while most or all of the cells at the anterior were wild type. The mislocalized oocytes in the other mosaic chambers, 4%, showed a lateral position. In all, most follicle cells at both the anterior and posterior were mutant. We conclude that the oocyte has a preference to lie adjacent to one of the poles of the encapsulated follicle, though posterior is obviously preferred. If JAK function is lost from the posterior of the overlying epithelium, the oocyte will typically align adjacent to the anterior pole. However, if both poles are comprised substantially of *hop* mutant cells, the oocyte comes to lie at a lateral position. In all cases in which the oocyte moved to a lateral position, it was located adjacent to wild type cells. In one chamber, there were only approximately 20 wild type follicle cells, yet the oocyte was positioned directly adjacent to those cells (Figure 4.2). It should also be noted that all of the chambers with hop^{c111} germline clones had the oocyte correctly positioned at the posterior. We conclude that JAK activity is only required in the soma for proper oocyte localization.

Cell adhesion molecules Cadherin and Armadillo are not disrupted in *hop* mutant ovaries

Several other mutations that cause mislocalization of the oocyte have also been noted. Some of these are somatically required, while others act in the germline. Maternal mutations in the *spindle* genes, as well as somatic loss of E-cadherin or misexpression of the *argos* gene result in mispositioned oocytes (Gonzalez-Reyes, Elliott et al. 1997; Godt and Tepass 1998; Zhao and Bownes 1999). In particular, mutants in the *Drosophila* E-cadherin gene, *shotgun*, have been extensively analyzed for effects on the follicle. As with *hop*, loss of E-cadherin in the posterior follicle cells results in mispositioning of the oocyte. In region 2b of the germarium, E-cadherin protein is noticeably more abundant at the anterior and posterior poles of the cyst. In follicle cells mosaic for *shg* mutations, the oocyte fails to localize at the posterior when the overlying follicle cells are mutant for *shg* (Godt and Tepass 1998). To determine whether mislocalization of the oocyte in *hop* mutants might be due to an influence on E-cadherin, ovaries mosaic for *hop* were stained with E-cadherin antibody. As can be seen in figure 4.3, follicle cells mutant for *hop* have normal levels of E-cadherin at the normal apical locations. Next we looked at Armadillo expression in *hop* mutants. Armadillo is the fly homologue to β-catenin which is a required part of the adhesion complex formed by Cadherin. Consistent with Cadherin function, germline

mutants of Arm result in mispositioned oocytes (Oda, Uemura et al. 1997). To investigate whether Arm localization is altered in JAK mutants, *hop* mutant mosaic ovaries were stained with Arm antibody. Similar to the Cad staining, Arm accumulation also appears normal, as can be seen in Figure 4.4 where ovaries with *hop* mutant clones are stained with the Arm antibody. In figure 4.4, an egg chamber with the posterior cells all mutant for *hop*, portrays the oocyte mispositioned to the anterior of the chamber, shows normal distribution of Arm.

Conclusions

Cell adhesion molecules such as Cadherin and Armadillo are required for posterior positioning of the oocyte. In chambers that contain *hop* mutant posterior follicle cells, oocytes are mislocalized. It is easy to imagine the adhesion molecules in *hop* mutant cells would be disrupted, decreasing the amount of adhesion between the posterior follicle cells and the oocyte, causing the oocyte to become mislocalized. Investigations into this possibility suggest otherwise, however. In chambers that contain a significant number of *hop* mutant cells, adhesion molecules, specifically Cadherin and Armadillo, retain their wild-type expression pattern. This does not, however, rule out the possibility of JAK altering the function of these proteins. Cad and Arm may maintain wild-type expression, but they may lose their ability to interact with other vital molecules. Additional experiments will be necessary to address this possibility.

We are unsure of the exact cause of oocyte mislocalization in *hop* mutants, but there are several possible scenarios. Since it has been determined that JAK is required somatically for proper localization, it is likely that the effects seen are secondary, since most known cues for oocyte localization are required in the germline. Therefore there must be something linking JAK signaling to the germline events of oocyte localization. One action that specifies the oocyte is distribution of the fusome, a structure that arises from a remnant of the mitotic spindle. It is unlikely, however, that JAK affects the specification of the oocyte, as that would be an action that takes place within the germline, and loss of JAK in the germline has no effect on phenotype. Other possibilities include disruption of localization cues, or mis-specification of posterior cells early in the germarium. Further investigation is currently being performed to determine the cause of the mislocalized oocytes in JAK mutants.

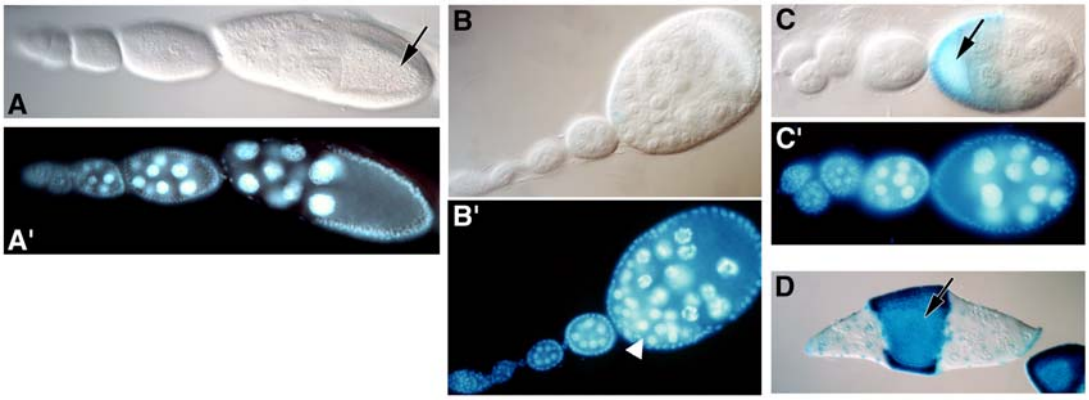

Figure 4.1. Mosaic ovaries of JAK pathway mutants show multiple phenotypes. Corresponding DIC (A-D) and DAPI (A'-C') images are shown in the comparison of wild-type (A) and *hop*^{msv} mosaic ovarioles (B-D). Clones of *hop* mutant somatic cells are marked by the loss of B-galactosidase activity, which stains blue in upper panels. Location of oocyte is indicated by the black arrows. Fusions of adjacent chambers are the most common defect seen in mosaics (white arrowhead in B). Less frequently, mislocalizations of the oocyte to the anterior end (C) or even the center (D) of the egg chamber are seen.

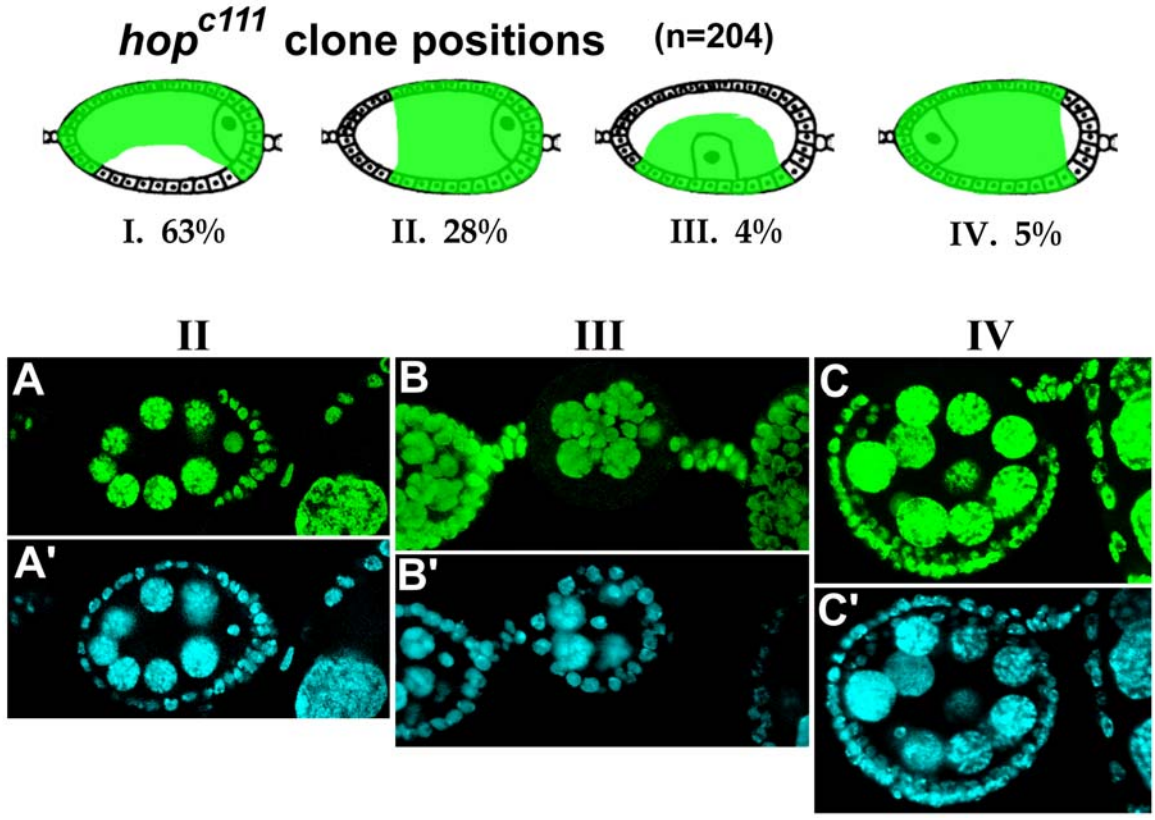

Figure 4.2. Hop in follicle cells directs localization of the oocyte.
In 204 egg chambers that contained at least one-quarter *hop*^{c111} mutant cells in the epithelium, the localization of the oocyte was examined relative to the position of the clones. The results are depicted schematically, with the position of wild-type cells indicated by green shading (analogous to the GFP-marked clones in A-C), and the oocyte location outlined in the germline. In A-C, histone-GFP is shown in green, and DAPI in blue. Mutation of cells laterally (I) or at the anterior (II) did not affect oocyte localization, even where all of the anterior and most of the lateral cells were mutant (A). But mutation of most of the posterior cells (III and IV) caused the oocyte to be improperly localized. In chambers with mutant cells at both the anterior and the posterior (III), the oocyte was located laterally. In B, only approximately 20 cells within the epithelium are wild-type (green), yet the oocyte (arrowhead) is found directly underneath those cells. In chambers mutant at the posterior, but with wild-type cells at the anterior (IV), the oocyte was located at the anterior (C).

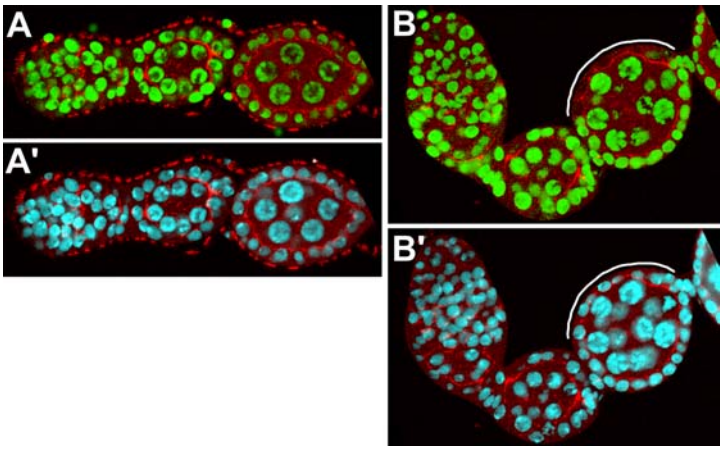

Figure 4.3. JAK does not influence E-cadherin accumulation in early follicle cells
In region 2b of the germarium, E-cadherin is expressed asymmetrically at high levels in the posterior and anterior follicle cells. Others have demonstrated that E-cadherin is necessary for proper localization of the oocyte. We looked at E-cadherin expression in *hop* mutant clones and have yet to find clones at the right stage in the germarium. However, E-cadherin is still expressed in its normal pattern at later stages of oogenesis. In wild-type ovarioles (A), E-cadherin (shown in red) is located on the apical side of all follicle cells, but is transiently enriched at the anterior and posterior ends of the cyst in region 2b of the germarium. Cells that are mutant for hop^{c111} (bracket in B, loss of GFP) retain apical accumulation of E-cadherin, though the mutant cells are more flattened than wild-type. DAPI is blue, GFP is green.

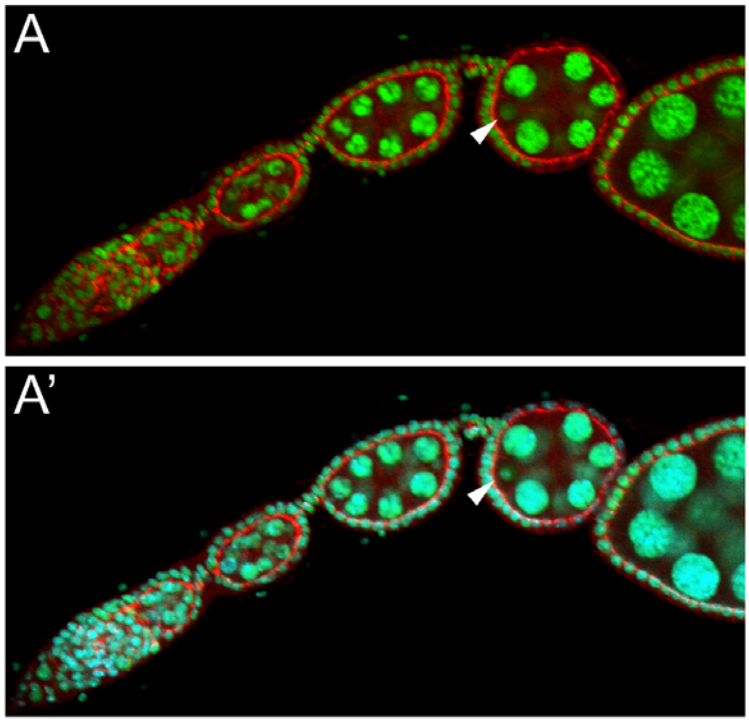

Figure 4.4. Armadillo expression is not altered in JAK mutants.
In region 2b of the germarium, E-cadherin is expressed at high levels in the anterior and posterior follicle cells. The oocyte preferentially associates with the E-cadherin expressing cells at the posterior of the developing cyst. Also necessary in this adhesion complex is the β-catenin homologue, Armadillo. It has been shown that loss of E-cadherin or Armadillo in the posterior follicle cells of the egg chamber in the germarium causes mislocalization of the oocyte. (A-A') Ovaries that contain *hop* mutant clones in the posterior follicle cells do not affect Arm concentrations. Arm, shown here in red, accumulates just as in wild type chambers. *hop* mutant cells (cells lacking GFP expression) in the posterior of the chamber cause the oocyte to localize to the anterior of the chamber (arrowhead). This mislocalization occurs, however, in the presence of normal Armadillo accumulations in the follicle cells.

Chapter Five

Discussion

The JAK-STAT signal transduction pathway is conserved across many organisms from slime molds to flies to humans. In humans, there are several ligands that activate the pathway such as interferons and cytokines. The human JAK pathway consists of four JAKs and seven STATs that can be utilized in various combinations with the numerous ligands that activate the pathway to achieve different developmental effects. In *Drosophila*, however, there is only one known ligand, one JAK, and one STAT. Upd is a ligand that activates the pathway in most developmental events in which the JAK pathway is known to be involved, such as planar polarity in the eye, sex determination, spermatogenesis, and embryonic pattern formation. The pathway is well conserved in all processes including oogenesis. We have shown that there is a genetic interaction between *hop* and *upd* in oogenesis, suggesting that Upd is the ligand that stimulates the pathway in the ovary.

Several novel roles for the JAK pathway in oogenesis have recently been uncovered including border cell migration (Silver and Montell 2001; Beccari, Teixeira et al. 2002), epithelial follicle cell patterning and fate determination, as well as those mentioned here; polar cell/stalk cell fate determination and oocyte localization (Xi, McGregor, and Harrison, in progress). In the past, several signaling pathways have been implicated in these functions such as Hedgehog, Notch, and EGFR (van Eeden and St Johnston 1999; Dobens and Raftery 2000). It is only recently that JAK has been found to be involved and play such a pivotal role in these processes.

JAK activation regulates stalk cell specification

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 2001). While still in the germarium, a pool of stalk cells and polar cell precursors is set aside from other somatic cells, the epithelial follicle cells (Margolis and Spradling 1995; Tworoger, Larkin et al. 1999). Those precursors then differentiate into either stalk cells or polar cells (see Figure 5.1) while the remaining somatic cells take on various fates in the epithelium.

Mosaics of *Stat92e* and *hop* show very similar phenotypes. A range of phenotypes is observed from a few extra polar cells or simple fusion of two adjacent chambers to inability of a chamber to pinch off from the germarium. The JAK pathway, acting in a typical JAK signaling mechanism, clearly influences the final fate of the stalk cell/polar cell precursors, as demonstrated by the phenotypes of loss of function mutants. 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 and 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 cell 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 cell/polar cell precursor pool (see Figure 5.1). 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 model for JAK pathway functions in the follicle cells

Several signaling pathways have been implicated in the patterning of the follicular epithelium (see Figure 5.1). The best characterized are the Notch, EGFR, and Hedgehog pathways (reviewed by Van Buskirk and Schupbach 1999; van Eeden and St Johnston 1999; Dobens and Raftery 2000). The earliest activity is strong expression of *hh* in the terminal filament and cap cells at the anterior of the germarium which stimulates proliferation of the somatic stem cells (Forbes, Lin et al. 1996; Tworoger, Larkin et al. 1999; Zhang and Kalderon 2000; Zhang and Kalderon 2001). Loss of Hh signaling results in a decrease in overall follicle cell number and therefore a failure to properly encapsulate the germline cyst (Forbes, Lin et al. 1996; Zhang and Kalderon 2000). After Hh activity promotes the production of a pool of follicular precursors, the stalk cell/polar cell precursor pool is set aside from the epithelial cell pool. The stalk cells and polar cells do not proliferate after exit from the germarium (Margolis and Spradling 1995; Tworoger, Larkin et al. 1999).

The process by which the stalk cell/polar cell precursors are determined is unknown, but it has been suggested that Notch signaling may be involved in the process (Grammont and Irvine 2001; Lopez-Schier and St Johnston 2001; Roth 2001). Similar to JAK mutants, the loss of Notch activity causes chamber fusions that appear to be the result of a failure to produce stalk cells and have reciprocal gain of function phenotypes. But unlike JAK mutants, Notch pathway mutants also fail to produce polar cells (Grammont and Irvine 2001; Lopez-Schier and St Johnston 2001). In the Notch pathway, Delta, the ligand, is expressed in the germ cells, fringe, a glycosyltransferase which modifies Notch causing it to be more responsive to the Delta signal, is expressed in a subset of somatic cells, and Notch, the receptor, is expressed in the somatic cells (reviewed by Irvine, 1999). Several models have been put forth to describe the formation of stalk cells and polar cells, but all have failed to include JAK signaling (Grammont and Irvine 2001; Lopez-Schier and St Johnston 2001; Roth 2001). Earlier models suggested that the Notch pathway was solely responsible for the specification of the stalk cells and polar cells (Ruohola, Bremer et al. 1991). We have found, however, that this is not the case. One recent model put forth indicates that in the germarium, Delta signals from the germ cells to the fringe expressing polar cell/stalk cell precursor pool to specify polar cells. It is the presence of fringe in these cells that makes these cells, and not the epithelial cell population, sensitive to Delta signaling (Bruckner, Perez et al. 2000; Jordan, Clegg et al. 2000; Zhao, Clyde et al. 2000; Grammont and Irvine 2001). In this model, Delta then signals from the polar cells to specify stalk cell fate adoption (Lopez-Schier and St Johnston 2001; Roth 2001). This model correlates with the data, but fails to account for JAK activity. So how does JAK fit into this model? Recently it has been discovered that, for other steps in follicle cell differentiation, Notch is simply a switch between proliferation and differentiation.

We demonstrate here that JAK signaling induces the adoption of stalk cell fates in a subset of the stalk cell/polar cell precursor population. 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 (Figure 5.1). However, JAK is limited in defining the fates of polar cells and stalk cells to only a group of "competent cells", those in the polar cell/stalk cell precursor pool. These cells are first instructed by the Notch pathway, via the mechanisms described above, that they are capable of becoming polar cells or stalk cells. Then JAK activity assigns the final fates, either by telling cells they are stalk cells, or that they are not

polar cells. The data support this hypothesis. In Notch null clones, neither stalks nor polar cells are formed because there have been no cells assigned to be competent to receive JAK instruction. With excess Notch stimulation, an increase in polar cells and stalk cells results due to there being an increased number of cells competent to receive instruction from JAK.

It was assumed in the past that the expansion of Fas III staining in late stage chambers of Notch mutants was an increase in the number of polar cells. However, the Fas III positive cells were actually immature, undifferentiated follicle cells. Fas III stains immature follicle cells as well as polar cells, and when a second marker for polar cells was used, it was found that these cells did not express a marker of mature polar cells (Lopez-Schier and St Johnston 2001).

We know that *upd* is expressed in cells within the polar cell/stalk cell precursor pool and later is expressed exclusively in the polar cells. Unpaired is a secreted molecule (Harrison, McCoon et al. 1998) that signals outward from the polar cells to define the fate of the cells surrounding it. It is possible that Upd, from the polar cells, instructs the stalk cells to become stalks. Notch would work upstream of JAK in this model, acting just as suggested, but as a signal giving those cells the ability to become stalk cells upon proper stimulation. When *upd* is overexpressed, extra cells, probably from the epithelial cell population, are recruited to become stalks, but because they were not in the stalk cell/polar cell precursor pool, they lacked the "competence" signal from Notch, therefore they cannot completely differentiate (Figure 5.2) and retain immature follicle cell markers.

JAK function in oocyte localization

We have shown that loss of JAK activity does not influence E-cadherin or Armadillo accumulation in follicle cells, therefore ruling out disruption of these molecules as being the cause of mislocalized oocytes in JAK mutants. In chambers with all posterior follicle cells mutant for JAK, and the oocyte localized to the anterior of the chamber, Arm distribution is not affected (Figure 4.4). This, however, does not address the issue of Cad and Arm function. These molecules may accumulate properly, but lose their normal function. It is possible, given that JAK is a kinase, that the phosphorylation state of these molecules is altered, changing their interactions with other components of adhesion complexes. Much work remains to be done in this area before a possible link between JAK and these adhesion molecules can be ruled out as a possible explanation for oocyte mislocalization in *hop* mutants.

We have examined molecules that are involved in oocyte localization directly, but the problem could be occurring before localization takes place. Before an oocyte takes position at the posterior it must be chosen from one of the two pro-oocytes to become the oocyte. If this process is delayed or defective at all, the process of localization could also be disrupted.

In the germarium, a cystoblast incompletely divides four times giving rise to 16 interconnected cells, two of which are pro-oocytes. In wild type cysts, one of the pro-oocytes is chosen to differentiate into the oocyte while the other reverts back to the developmental path of a nurse cell. Shown to be involved in these processes are the *spindle* genes. In *spindle* mutants, the mutant phenotypes that arise are the formation of two oocytes or the formation one oocyte that is mislocalized. Chambers with two oocytes are thought to be due to a delay in the choice between the pro-oocytes, resulting in both taking on the oocyte fate, rather than one reverting back to the nurse cell fate. In these chambers, accumulation of Orb and Bic-D proteins occurs in both pro-oocytes rather than one. This phenotype, however, typically disappears by later stages, indicating a slowing of the oocyte determination process, but not preventing it (Gonzalez-Reyes,

Elliott et al. 1997). This delay is also what is said to be the cause of the mislocalization. Delay in oocyte determination can result in the "wrong" pro-oocyte positioning itself at the posterior, preventing the "real" oocyte from taking its proper position. In JAK mutants we do not see chambers with two oocytes, as shown by Orb staining (data not shown), but we do see phenotypes similar to those with mislocalized oocytes.

Other factors indicated in the process of oocyte determination are the organization of the microtubule cytoskeleton, *vasa*, centrosome migration, *encore*, and various other gene products including Bic-D and Egl (Hawkins, Thorpe et al. 1996; Styhler, Nakamura et al. 1998; Bolivar, Huynh et al. 2001). Since JAK is somatically required the possibilities are broad-spanning as to the mechanism by which it acts to influence oocyte localization. It is most likely that JAK signals from the follicle cells to the germline to influence some aspect of either oocyte determination or localization. Oocyte mislocalization in JAK mutants could be a secondary effect of the posterior cells not being properly specified early in the germarium. The posterior cells must somehow be specified in order to accumulate, for example, Cadherin, in order to direct the oocyte to the correct location. If JAK is involved in the specification of the posterior cells in the germarium, this could cause the subsequent mislocalization of the oocyte. Though there are a number of possible reasons for mislocalization of the oocyte in JAK mosaic chambers, we can definitively state that JAK is not essential for Cadherin or Armadillo localization and accumulation, but that it is required in posterior somatic cells to direct oocyte position. Other possibilities must be explored to uncover the exact mechanism of involvement.

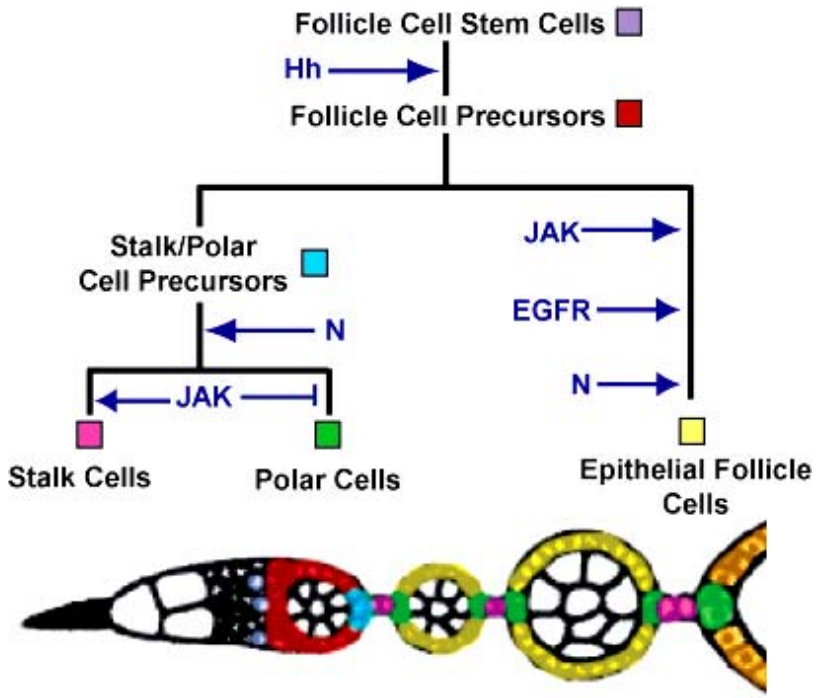

Figure 5.1. A model for JAK signaling in oogenesis.
Anteroposterior patterning of the follicular epithelium is accomplished through a series of cell signaling events. Each event progressively defines somatic fates. Stalk cells and polar cell precursors become distinct from epithelial follicle cells. In the late germarium. JAK signaling specifies stalk cell fates and establishes the correct ratio with polar cells. Though JAK instructs stalk cell fate, Notch signaling is required for differentiation to occur. 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

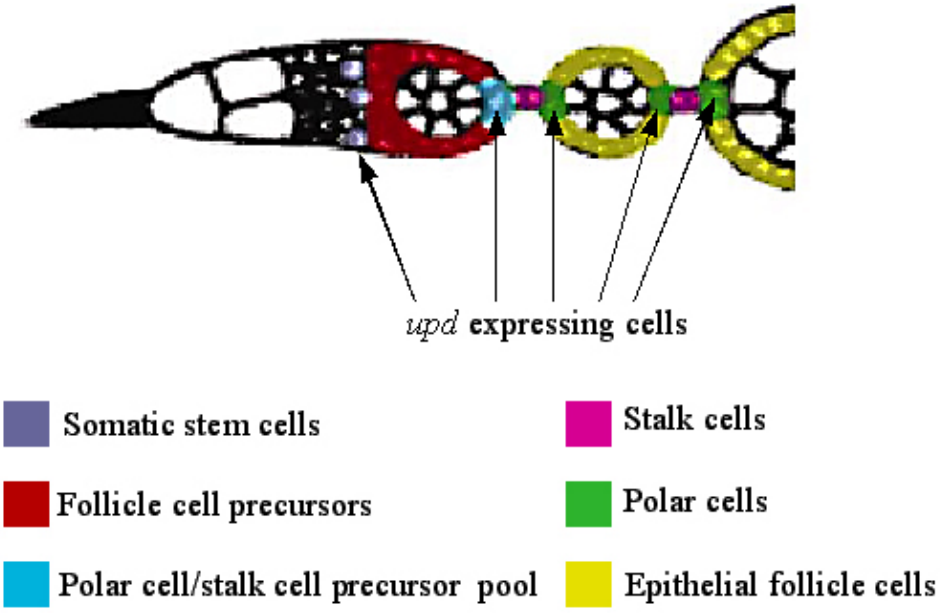

Figure 5.2. Unpaired expressing cells in early oogenesis.
Upd from the polar cells has the potential to signal to adjacent cells, instructing them to become stalk cells, or to prevent them from becoming polar cells.

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Vita

Jennifer Renee Matlock

Born in 1979, Johnson City, New York.

EDUCATION

1998 - 2000	University of Kentucky	Lexington, Kentucky
	BS in Biology with Departmental Honors	
1997-1998	Florida Southern College	Lakeland, Florida

SCHOLASTIC AND PROFESSIONAL HONORS

Graduate School

University of Kentucky

Student Development Award -- January 2001.

Ribble Research Fellowship -- Summer 2001.

Undergraduate

University of Kentucky

Graduated with Honors, August 2000.

Dean's List Fall 1998, Spring 1999, Spring 2000.

Howard Hughes Medical Institute Research Incentive for Undergraduates, Summer 1999.

Florida Southern College

Academic Merit Award, Fall 1997, Spring 1998.

PROFESSIONAL PUBLICATIONS

McGregor*, J.R; Xi, R.; Harrison, D.A. **2002**. "JAK signaling is somatically required for follicular patterning in *Drosophila." Development* 129, 706-717.
*maiden name