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Paracrine Signaling through the JAK/STAT Pathway Activates Invasive Behavior of Ovarian Epithelial Cells in *Drosophila*

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Summary

The JAK/STAT signaling pathway, renowned for its effects on cell proliferation and survival, is constitutively active in various human cancers, including ovarian. We have found that JAK and STAT are required to convert the border cells in the *Drosophila* ovary from stationary, epithelial cells to migratory, invasive cells. The ligand for this pathway, Unpaired (UPD), is expressed by two central cells within the migratory cell cluster. Mutations in *upd* or *jak* cause defects in migration and a reduction in the number of cells recruited to the cluster. Ectopic expression of either UPD or JAK is sufficient to induce extra epithelial cells to migrate. Thus, a localized signal activates the JAK/STAT pathway in neighboring epithelial cells, causing them to become invasive.

Introduction

The JAK/STAT (*Ja*nus *K*inase/*S*ignal *T*ransducer and *A*ctivator of *T*ranscription) signaling pathway plays critical roles in mammalian immune system function and mammary development (Akira, 1999; Catlett-Falcone et al., 1999a; Imada and Leonard, 2000). In addition, constitutive activation of members of the STAT family, most commonly STAT3, is found in many human cancers (Campbell et al., 2001; Catlett-Falcone et al., 1999b; Gao et al., 2001; Huang et al., 2000). Activated STAT3 is sufficient to cause transformation of fibroblasts and tumor formation in nude mice (Bromberg et al., 1999).

The canonical JAK/STAT signaling pathway is initiated upon binding of interferon or one of a number of cytokines to a transmembrane receptor. These receptors do not possess intrinsic catalytic activity, but rather associate constitutively with nonreceptor tyrosine kinases of the JAK family. Ligand binding induces dimerization of the receptor/kinase complexes, thus activating the kinases to phosphorylate each other as well as the receptor. This latter phosphorylation creates a pair of docking sites for the SH2 domain of STAT proteins, which are thus recruited to the active receptor complex. The STAT proteins are then phosphorylated by the JAKs, dimerize, and translocate to the nucleus, where STAT activates transcription of downstream target genes (reviewed in Darnell, 1997; Horvath, 2000).

The effects of several members of the JAK/STAT family on cell proliferation and survival are well established (Bromberg, 2001). In certain circumstances, STAT1 promotes growth arrest and/or apoptosis (Bromberg et al., 1996), whereas STAT3 and STAT5 protect against apoptosis and promote proliferation (Shen et al., 2001). Expression of a constitutively activated form of STAT3 in 293T cells results in increased expression of cyclin D1, BCL-XL, and c-*myc*, which promote cell cycle progression, cell survival, and proliferation, respectively. Inhibition of STAT3 in myeloma cells results in apoptosis (Catlett-Falcone et al., 1999b), and dominant-negative STAT3 prevents v-src mediated cellular transformation (Bromberg et al., 1998). Taken together with the observation that STAT3 is frequently constitutively activated in cancer cells, it is likely that STAT3 plays a role in tumorigenesis and/or cancer progression.

Most tumors derive from cells of epithelial origin; in order to become metastatic, and thereby a serious threat to human health, these cells must detach from the epithelium of origin and invade surrounding tissues, ultimately reaching the bloodstream. This step in cancer progression, the conversion of stationary, epithelial cells to invasive, migratory cells, is poorly understood compared to the mechanisms regulating proliferation and survival of tumor cells.

In order to investigate the mechanisms controlling the development of invasive behavior by cells of epithelial origin in vivo, we are employing a systematic genetic approach to study the border cells of the *Drosophila* ovary (reviewed in Montell, 1999, 2001). The *Drosophila* ovary is composed of egg chambers, each of which contains 16 central germline cells surrounded by an epithelium of between 650 and 900 follicle cells (Spradling, 1993) (Figure 1A). One of the germline cells develops into the oocyte, whereas the remaining 15 nurse cells function to supply the oocyte with cytoplasm. During the early stages of egg chamber development, a specialized pair of follicle cells, known as polar cells, differentiates at each end of the chamber, while the rest of the follicle cells form a nearly uniform cuboidal epithelium (Ruohola et al., 1991) (Figure 1A). As oogenesis proceeds, the vast majority of follicle cells change to a columnar shape and stack up in the posterior of the chamber in contact with the oocyte. Most of the remaining follicle cells stretch to cover the nurse cells, while at the anterior pole, four to eight follicle cells surround the anterior polar cells, delaminate from the rest of the epithelium, and invade the nurse cell cluster. This group of cells, referred to as the border cells, migrates about 150 microns until they reach the anterior border of the oocyte (Figure 1).

Previous studies have suggested that border cell migration is initiated by a signal from the polar cells that "recruits" adjacent cells into the cluster, stimulates their detachment from neighboring epithelial cells, and endows them with the ability to migrate (Han et al., 2000; Liu and Montell, 1999; Niewiadomska et al., 1999). The polar cells develop earlier in oogenesis than border cells, and polar cells are located in the center of the cluster throughout migration. Polar cells themselves are not able to migrate following ablation of the outer border cells (Han et al., 2000). However, extra polar cells, which can form in response to excessive hedgehog signaling,

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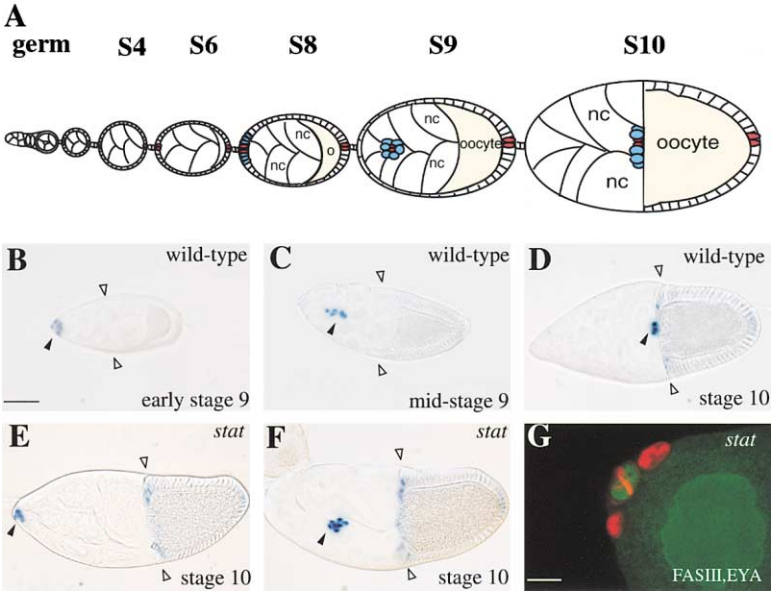


Figure 1. Ovarian Development and Border Cell Migration in Wild-Type and *stat* Mosaic Egg Chambers

(A) Schematic diagram of an ovariole showing the germarium (germ) and egg chambers of stages 2 through 10 (s2–s10). The nurse cells (nc) and oocyte (o) are indicated. Polar cells are red. Border cells are indicated in blue.

(B–F) Nomarski optics images of egg chambers. Border cells (filled arrowheads) are stained for β-gal activity (blue) in egg chambers from the enhancer trap, PZ1310. Open arrowheads indicate the anterior border of the columnar follicle cell group, which is even with the anterior border of the oocyte at stage 10. (B–D) Wild-type egg chambers in early stage 9, mid-stage 9, and stage 10, respectively. (B) Prior to border cell migration. (C) During migration. (D) After migration. (E–F) Stage 10 egg chambers containing $stat^{397}$ mosaic clones.

(G) A stage 10 $stat92E^{06346}$ mosaic egg chamber, in which homozygous mutant cells are labeled by the absence of GFP and polar cells are labeled by the presence of FasIII (red, membrane staining) and the absence of EYA (red, nuclear staining).

Scale bar is 50 μm in (A)–(F) and 10 μm in (G).

recruit ectopic border cells, which often migrate (Liu and Montell, 1999). The nature of the signal from the polar cells to the surrounding epithelial cells has been unclear.

Here we report the identification of four mutant alleles of *Drosophila Stat92E* from a genetic screen for mutations that cause defects in border cell migration. Mutations disrupting JAK also lead to migration defects, as do mutations affecting the only known ligand for the JAK/STAT pathway in flies, UPD. UPD is expressed specifically in the polar cells, and ectopic expression of UPD, or of JAK, is sufficient to cause additional follicle cells to express border cell markers and invade the nurse cell cluster. These results suggest that, in addition to regulating cell proliferation and survival, signaling through the JAK/STAT pathway can convert stationary epithelial cells to migratory, invasive cells.

Results

Mutations in *Stat92E* Cause Border Cell Migration Defects in Mosaic Clones

In order to identify genes required for border cell migration, we performed a screen of the right arm of the third chromosome for mutations that cause defective border cell migration in mosaic clones (see Experimental Procedures). Mosaic clones are patches of homozygous mutant cells within an otherwise heterozygous organism. Screening for phenotypes using mosaic clones allows the identification of mutations that would otherwise lead to lethality and/or pleiotropic defects. In this mutant screen, clones were induced in random subsets of follicle cells.

At stage 9 of oogenesis, six to ten anterior follicle cells differentiate as border cells (Figures 1A and 1B). The border cell cluster detaches from the epithelium, and migrates between the nurse cells toward the posterior of the egg chamber during stage 9 (Figure 1C). Border cells complete their journey at stage 10, when they reach the nurse cell-oocyte boundary (Figure 1D).

6,184 lines containing newly induced mutations were screened, and roughly 100 mutant lines exhibited some migration defects. Four mutant lines, 397, 56D3, 80A7, and 85C9, which exhibited particularly dramatic migration defects (Figures 1E and 1F), were tested for complementation with respect to lethality and found to comprise a single complementation group. Border cells frequently failed to penetrate into the nurse cell cluster at all, and could be found at the anterior tip (Figure 1E) or along the sides of the egg chamber. Alternatively, the cells migrated partially, but failed to reach the oocyte by stage 10 (Figure 1F).

To determine whether the migration defects were autonomous to the migratory cells, mutant clones were marked. A transgene expressing GFP in all cells of the egg chamber was included on the wild-type chromosome arm, so that homozygous mutant cells could be recognized by the absence of GFP. Border cell clusters in which the polar cells were wild-type, but the outer border cells were mutant, exhibited migration failure (Figure 1G). Large mutant clones in the remaining follicle epithelium did not produce border cell migration defects, nor did germline mutant clones (not shown). Thus, the affected gene was required autonomously in the outer, migratory border cells for normal migration.

Using meiotic recombination and deficiency mapping (see Experimental Procedures), two of the alleles, 397 and 85C9, were mapped to the 92D3-92F13 region (Figure 2A). Complementation testing with 12 P elements within this region identified one, known as $Stat92E^{06346}$, which failed to complement all four alleles (Figure 2A). This P element also caused a border cell migration phenotype in mosaic clones (Figure 1G).

$Stat92E^{06346}$ is a loss-of-function allele of *Stat92E* (Hou

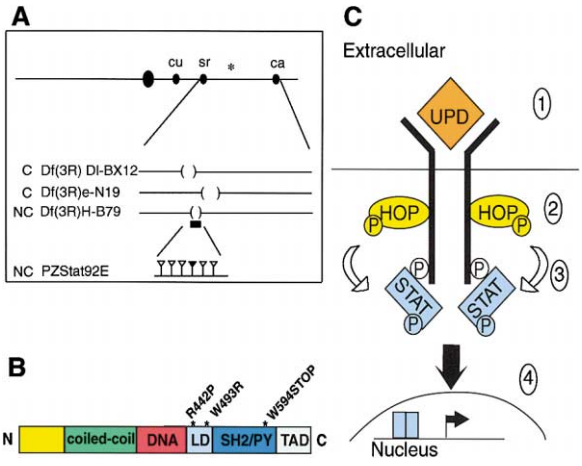


Figure 2. Genetic Mapping of *stat* Mutants

(A) A cartoon of chromosome 3 and the mapping results. The brackets indicate regions deleted in each deficiency. C; complementing, NC; noncomplementing. The mutation causing the border cell migration phenotype (*) mapped between stripe (sr) and claret (ca).

(B) A schematic of STAT protein domains. The amino terminus (N) contains a coiled-coil domain, a DNA binding domain (DNA), and a linker domain (LD). The carboxy terminus (C) contains a src homology 2 (SH2) domain, tyrosine 711 (PY), and the transcriptional activation domain (TAD). The indicated mutations (*) were found within the coding sequences for $stat^{85C9}$, $stat^{56D3}$, $stat^{397}$, respectively.

(C) Steps in the JAK-STAT pathway. (1) UPD binds its receptor. (2) The JAK tyrosine kinase (HOP) phosphorylates the receptor and itself. (3) STAT is recruited to the receptor. (4) Phosphorylation of STAT causes it to enter the nucleus.

et al., 1996), the *Drosophila* homolog of mammalian STATs. *Drosophila* STAT is activated by phosphorylation of tyrosine 711, which is located near the carboxy terminus (Figure 2B). Phosphorylated STAT dimerizes through interaction of the SH2 domain of one molecule with the phosphotyrosine residue of another molecule, and this dimerization is necessary for STATs to enter into the nucleus and activate transcription (Figure 2C). We sequenced the coding region of the *Drosophila Stat92E* locus from each of the four ethylmethane sulfonate (EMS) induced *stat* alleles and identified mutations in three of the four alleles (Figure 2B). $Stat^{397}$ contains a G to A mutation at residue 594, changing a highly conserved tryptophan within the SH2 domain to a stop codon. This mutation would be expected to truncate the protein short of the Y711, and thus produce either an unstable protein or a nonfunctional protein that cannot dimerize or enter the nucleus. $Stat^{85C9}$ has a G to C mutation at residue 442, resulting in a nonconservative arginine to proline substitution. $Stat^{56D3}$ contains a T to A mutation resulting in an arginine in place of tryptophan at residue 493. Both of these missense mutations affect a linker domain thought to be required for transcriptional activation, since mutations in this region of mammalian STAT1 inhibit STAT transactivation (reviewed in Bromberg, 2001).

Additional JAK/STAT Pathway Components Are Required for Border Cell Migration

Components of the JAK-STAT pathway have been delineated previously in *Drosophila* (Hou et al., 1996; Yan et al., 1996b), where they have been shown to participate in embryonic segmentation (Yan et al., 1996b), hematopoiesis (Luo et al., 1997), wing veination (Yan et al., 1996a), cell polarity (Zeidler et al., 1999), and sex determination (Jinks et al., 2000; Sefton et al., 2000). UPD is the one extracellular ligand known to activate the JAK-STAT pathway in a variety of tissues (Harrison et al., 1998). Likewise, to date, only one JAK gene, called *hop*, and one *stat* gene, make up the pathway in flies (Hou, 1997). In order to gain insight into the possible roles of *upd*, *stat*, and *hop* in border cell migration, we first examined their expression patterns.

Within the ovary, *stat* is widely expressed. Studies in other tissues have revealed that β-galactosidase (β-gal) expression from the enhancer trap line $Stat92E^{06346}$ is an accurate indicator of a subset of *stat* expression (Zeidler et al., 1999). In the ovary, $Stat92E^{06346}$ shows expression in cap cells at the extreme anterior end of the germarium, as well as in follicle cells that envelop germ cell clusters (Figure 3A). As egg chambers bud from the germarium, β-gal expression becomes restricted to a few cells at the anterior and posterior poles of the egg chamber (Figure 3A). At stage 8 and early in stage 9, expression is observed in both pairs of polar cells and in a larger group of follicle cells adjacent to the anterior polar cells (Figure 3B). Border cell and polar cell expression persists as the border cells migrate (Figures 3C and 3D). In addition, at stage 9 and later stages, β-gal from $Stat92E^{06346}$ is expressed in most if not all follicle cells (Figure 3C). In situ hybridization using a *stat* probe shows a similar pattern of expression, but also indicates strong expression of STAT in the germline beginning at stage 9 (McGregor et al., 2002). HOP, the *Drosophila* JAK, appears to be ubiquitously expressed in the ovary, as assessed by antibody staining (data not shown).

UPD expression was examined by in situ hybridization. *UPD* RNA is expressed specifically in the anterior and posterior polar cells of the egg chamber (Figures 3E–3H). The specific expression of *UPD* in polar cells, and requirement for *stat* in neighboring follicle cells, suggested that *UPD* might signal from the polar cells to the surrounding follicle cells, recruiting them to become border cells and endowing them with the ability to migrate.

We examined the requirement for *upd* in the ovary, taking advantage of a hypomorphic allele of *upd*, upd^{sisC5}, which is homozygous viable but exhibits reduced female fertility (Sefton et al., 2000). In upd^{sisC5} egg chambers, border cell migration was dramatically inhibited (Figures 4A–4C). To quantify this effect, we calculated a migration index, which is a measure of the extent of migration for all stage 10 egg chambers examined expressed as a percentage of wild-type (see Experimental Procedures). Normal border cell migration produces a migration index of 100, whereas complete failure of migration in all egg chambers would lead to a migration index of 0. The migration index of upd^{sisC5} was 22 (Figure 4O).

To assess the null *upd* phenotype, and to test the hypothesis that *upd* function is required only in the polar cells, egg chambers containing mosaic clones of a lethal *upd* allele, upd^{YM55}, were analyzed. In each case that the polar cells were homozygous mutant for upd^{YM55}, marked by absence of GFP expression, dramatic migration defects were observed (Figures 4D and 4G), and the migra-

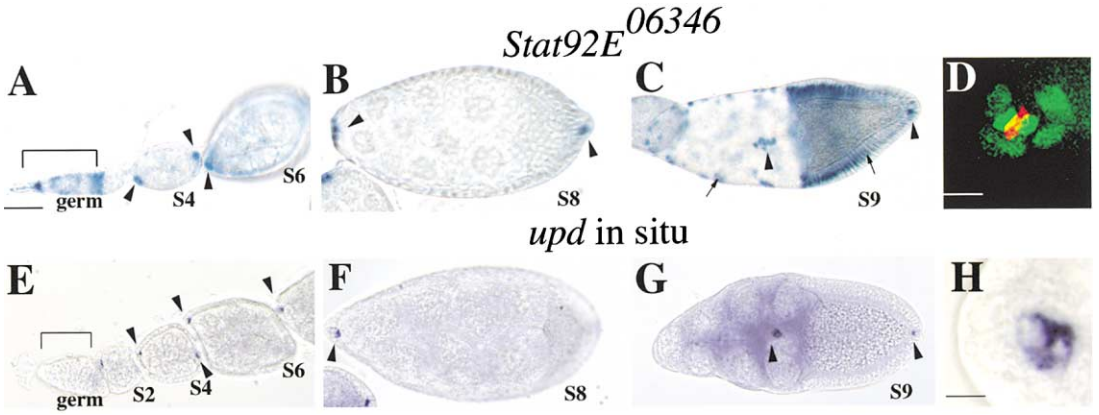


Figure 3. Ovarian Expression of *stat* and *UPD*

(A–D) Beta-gal expression pattern in the *stat* enhancer trap line *Stat*^{06346}. (A and B) Beta-gal activity staining (blue) is observed early in the germarium (germ, bracket) and at the anterior and posterior poles of stage 4–8 egg chambers (arrowheads). (C) A late stage 9 egg chamber. Border cells (arrowhead) as well as the oocyte- and nurse cell-associated follicle cells (arrows) are indicated. (D) Double labeling with anti-β-gal (green) and FasIII (red) antibodies in a migrating border cell cluster.

(E–H) In situ hybrization to *UPD* mRNA. (E and F) *UPD* expression in anterior and posterior polar cells is indicated (arrowheads). The posterior polar cells are out of the plane of focus in (F). (G) Polar cell expression during migration. (H) A higher magnification view of an early stage 9 egg chamber, showing *UPD* expression in anterior polar cells.

Scale bar in (A) is 50 μm. Scale bar in (D) and (H) is 10 μm.

tion index was 0 (Figure 4O). In contrast, egg chambers in which the germline was homozygous for *upd*^{YM55} showed no migration defect (Figures 4E and 4H). Likewise, clones in the outer border cells and in other follicle cells did not affect migration (Figures 4F and 4I).

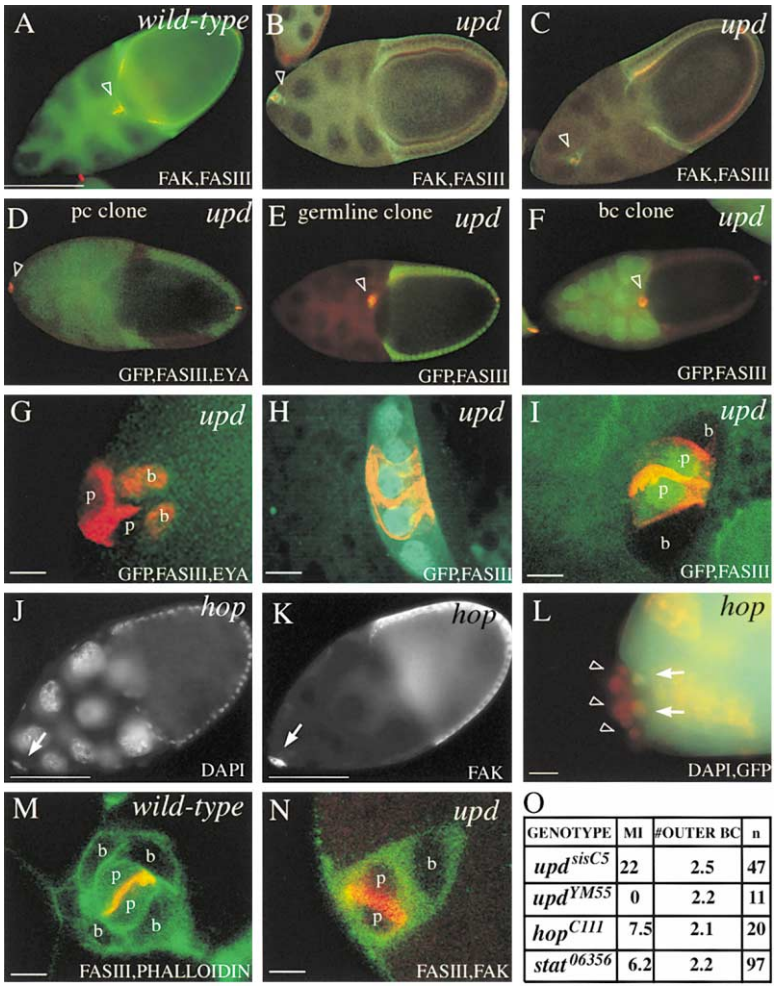
Loss of *hop* in border cell clusters also caused border cell migration defects (Figures 4J and 4K). Like *stat*, *hop* function was required autonomously in the outer migratory border cells (Figure 4L). No defect in migration was observed when the germline was mutant for *hop* (not shown). Nor were border cell defects observed when large clones were generated in the rest of the follicle cell epithelium (not shown). When the entire border cell cluster was mutant for *hop*, the migration index was 7.5 (Figure 4O), which was similar to the migration index of 6.2 for *stat*.

In addition to the migration defects, *upd*, *hop*, and *stat* mutants also contained fewer outer border cells compared to controls. Wild-type border cell clusters consist of an average of six outer, migratory cells, plus the two central, nonmigratory polar cells (Figure 4M). Border cell clusters in egg chambers from *upd*^{sisC5} mutant females contained an average of 2.5 outer border cells (Figures 4N and 4O). For the *upd*^{YM55} allele, the average number was 2.2. In egg chambers in which all border cells were mutant for *hop* or *stat*, the average number of outer cells was 2.2 and 2.1, respectively (Figure 4O). These results show that *upd*, *hop*, and *stat* are required for recruitment of the normal number of cells to the cluster as well as for migration.

Overexpression of UPD or HOP Is Sufficient to Cause Additional Follicle Cells to Migrate

We addressed the question of whether signaling through this pathway might be sufficient to cause epithelial cells to become invasive, by ectopically expressing UPD, HOP, or the constitutively active form of HOP, HOP^{Tum1}, using the GAL4/UAS expression system (Brand and Perrimon, 1993). In this method, the yeast transcriptional activator GAL4 is expressed under the control of a cell type-specific enhancer, in this case *slbo*-GAL4 and *c306*-GAL4. In stage 9 egg chambers, *slbo*-GAL4 induces expression of genes that are under the control of the yeast *u*pstream *a*ctivating *s*equence (UAS) in approximately 20 anterior follicle cells, a subset of which normally become the border cells (Rørth et al., 1998) (Figure 5B). This is nearly identical to the β-gal expression from an enhancer trap insertion into the *slow border cells (slbo)* locus (Figure 5A), even though SLBO protein expression is normally restricted to the border cells at stage 9 (Montell et al., 1992). *C306*-GAL4 drives expression in a larger number of anterior, as well as posterior, follicle cells, compared to *slbo*-GAL4 (Figure 5C). *C306*-GAL4 also begins expressing earlier in oogenesis than *slbo*-GAL4 (not shown).

Egg chambers from c306-GAL4; UAS-*hop* females exhibited a dramatic increase in the number of border cells compared to wild-type (Figures 5D and 5G). Up to 90 *slbo* expressing cells were produced, about 60 of which invaded the nurse cell cluster and 20 of which had completed migration by early stage 10. Similar, though less dramatic, phenotypes were observed when the constitutively activated kinase was expressed with either *slbo*-GAL4 or *c306*-GAL4 (Figures 5E and 5F). Likewise, *slbo*-GAL4;UAS-*upd* and *c306*-GAL4;UAS-*upd* females contained numerous extra *slbo*-expressing cells compared to wild-type, in the absence of extra polar cells (Figures 5H and 5I, compared to 5A). This is in marked contrast to the effect of excessive Hedgehog pathway signaling, which causes ectopic border cells to form as a secondary consequence of ectopic polar cell specification (Liu and Montell, 1999). Overexpression of *upd* did not appear to cause excess cell proliferation, as no difference in phospho-histone H3 antibody labeling, which marks mitotic cells, was detected compared to



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GENOTYPE	MI	#OUTER BC	n
upd^{sisC5}	22	2.5	47
upd^{YM55}	0	2.2	11
hop^{C111}	7.5	2.1	20
$stat^{06356}$	6.2	2.2	97

Figure 4. Defects in Border Cell Migration and Recruitment in *upd* and *hop* Mutant Egg Chambers

(A–C) Egg chambers are stained with anti-FAK antibody (green) and anti-FasIII antibody (red), which is expressed at highest levels at the interface between the two polar cells. (A) A wild-type stage 10 egg chamber. Border cell migration is complete (arrowhead). (B and C) Representative stage 10 egg chambers from females homozygous for the hypomorphic allele of *upd*, upd^{sisC5}.

(D–I) Stage 10 egg chambers mosaic for a null allele of upd^{YM55}. Homozygous *upd* mutant cells lack GFP. (D and G) Polar cells (p) are labeled by the presence of FasIII (red, membrane staining) and the absence of EYA (red, nuclear staining). (E and H) The germline is mutant for upd^{YM55}, whereas follicle cells are wild-type. (F and I) The outer, migratory border cells (b) are mutant for *upd*, whereas polar cells are wild-type. (G)–(I) are high magnification views of the egg chambers shown in (D)–(F).

(J–L) A stage 10 mosaic egg chamber containing a border cell cluster mutant for hop^{c111}. (J) DAPI staining, (K) FAK staining highlights the border cells (arrow). (L) A high magnification view of a stage 10 hop^{c111} mosaic egg chamber. Homozygous mutant *hop* cells are marked by the absence of GFP (green). Nuclei are labeled with DAPI (red). Arrowheads indicate homozygous mutant cells. Arrows indicate two wild-type cells.

(M) A high magnification image of a wild-type border cell cluster stained with phalloidin (green) and FasIII (red). Two polar cells (p) and four outer migratory cells (b) are indicated.

(N) A high magnification image of the mutant border cell cluster in (B). Polar cells (p) and one additional cell (b) are indicated. (O) A table summarizing the migration indices (MI) and the number of outer border cells observed in a given number (n) of stage 10 egg chambers of the indicated genotypes. In wild-type egg chambers, the average number of outer border cells is 6 and the migration index is 100.

The scale bars for (A)–(F), (J), and (K) are 50 μm and for panels (G)–(I) and (L)–(N) are 10 μm.

wild-type (Supplementary Figure S1 at http://www.cell.com/cgi/content/full/107/7/831/DC1).

Some of the extra border cells migrated as single cells, whereas others formed multiple small clusters, and still others formed one large cluster. The ability of the cells to migrate varied according to which protein was being expressed as well as with the timing and level of expression. High levels of ectopic UPD resulted in egg chambers in which both normal and extra border cells frequently failed to migrate (Figures 5H and 5I), whereas high levels of wild-type HOP produced the most migratory cells. Thus, ectopic activation of the JAK/STAT pathway was sufficient to cause extra epithelial follicle cells to invade the nurse cell cluster.

Mechanism of Regulation of Cell Migration by STAT

In order to gain further insight into the mechanism by which STAT regulates border cell migration, we examined the expression of a number of proteins that are highly expressed in border cells, some of which are also required for migration. The first gene identified to play a critical role in border cell migration was *slow border cells* (*slbo*) (Montell et al., 1992), which encodes *Drosophila* C/EBP, a basic region/leucine zipper transcription factor (Rørth and Montell, 1992). SLBO protein expression was undetectable in *stat* mutant border cells, which were identified using a positive clone marking system known as MARCM (Lee and Luo, 1999), suggesting that STAT regulates SLBO expression (Figures 6A–6C). We confirmed this result by examining several additional proteins, the expression of which is reduced in *slbo* mutant border cells. In wild-type stage 8 and 9 egg chambers, FAK expression is upregulated in migratory border cells (Bai et al., 2000; Fox et al., 1999) (Figure 6D). Border cells that lack *stat* exhibited reduced levels of FAK (Figures 6E and 6F). In wild-type stage 9 egg chambers, *D*E-cadherin is enriched throughout the border cell cluster and is expressed to the highest level in the polar cells (Niewiadomska et al., 1999) (Figure 6G). *Stat* mutant border cells exhibited reduced *D*E-cadherin expression compared to wild-type border cells of the same cluster (Figures 6H and 6I). The polar cells, though mutant, did not show reduced *D*E-cadherin staining,

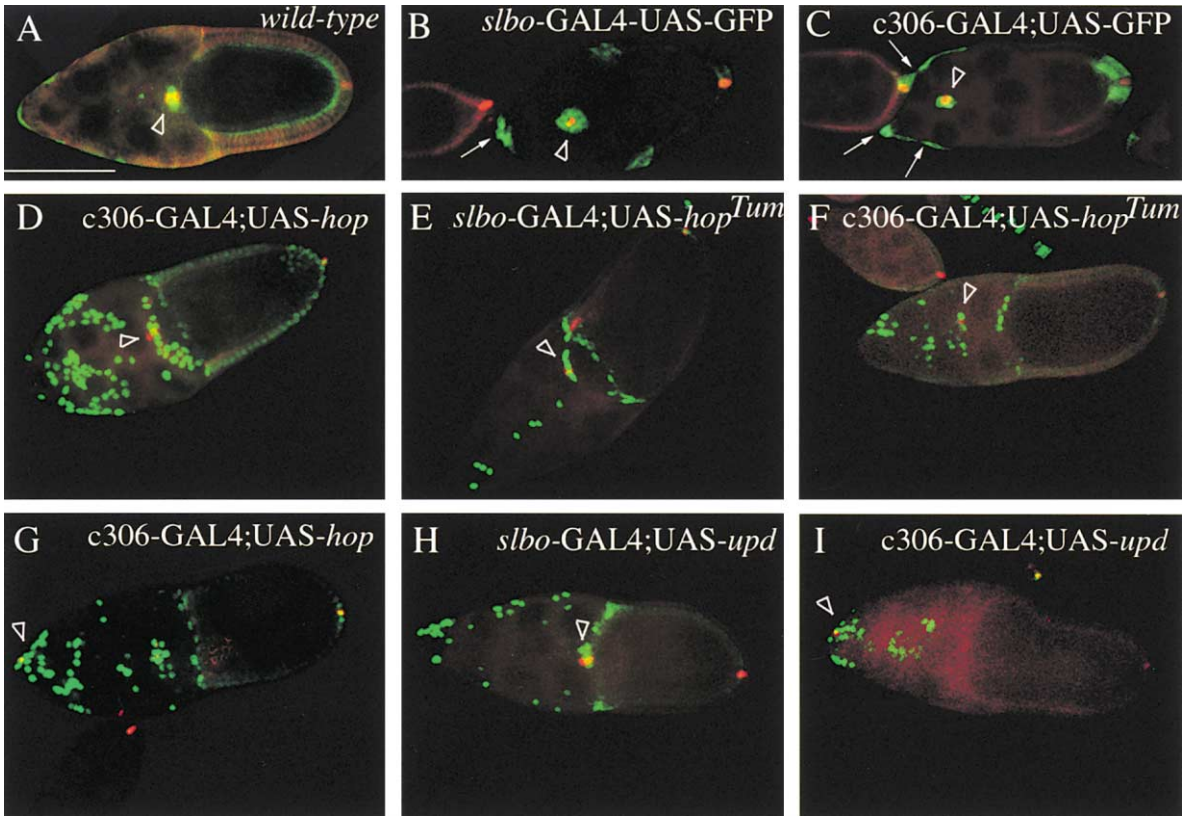

Figure 5. Extra Border Cells Form and Migrate upon Ectopic Expression of HOP, UPD, and HOP^{TUM}

Confocal micrographs of egg chambers stained with an antibody against β-gal, showing expression from the enhancer trap PZ1310 (green in [A] and [D]–[I]) or GFP (green in [B] and [C]). Polar cells are labeled with FasIII (red). The border cell clusters containing the anterior polar cells are indicated by arrowheads.

(A) A wild-type stage 10 egg chamber.

(B) Expression pattern of *slbo*-GAL4, UAS-GFP in border cells as well as several cells at the anterior of the egg chamber (arrow).

(C) Expression of *c306*-GAL4;UAS-GFP in border cells and a broader domain of anterior follicle cells (arrows).

(D and I) Stage 10 egg chambers from the indicated genotypes.

The scale bar in (A) represents 50 μm.

which is also true of *slbo* mutants (Niewiadomska et al., 1999). Additional downstream targets of *slbo*, including PZ6356 and *jing* (Liu and Montell, 2001), were also reduced in *stat* mosaic clones (data not shown). Thus, even the few mutant cells that were recruited to the cluster failed to express many border cell proteins required for migration. The effect was specific because expression of Taiman, a protein that is required for border cell migration but is independent of the *slbo* pathway (Bai et al., 2000), was not altered (data not shown). Mosaic clusters containing a mixture of wild-type and mutant cells, such as those described above, showed variable migration defects. On average, the extent of migration was proportional to the number of wild-type cells in the cluster.

Egg chambers from females heterozygous for any of the *stat* alleles had a semi-dominant border cell migration phenotype. We took advantage of this slight haploinsufficiency to test for dominant genetic interactions with other genes required for border cell migration (see Supplementary Table S1 at http://www.cell.com/cgi/content/full/107/7/831/DC1). Dominant genetic interactions were observed with *slbo*, *hop*, and *upd* alleles. A mutation in the gene coding for *D*E-cadherin, *shotgun*, also exhibited a dominant interaction with *stat*. These interactions appeared to be specific, since *stat* did not interact with other known border cell migration genes, such as *tai*, *jing*, or PZ6356.

A Candidate Receptor for UPD, Domeless, Also Affects Border Cell Recruitment and Migration

Recently, a candidate transmembrane receptor for UPD has been identified (Brown et al., 2001). Mutation of this gene, which is named *domeless*, causes embryonic phenotypes that are indistinguishable from those of *upd*, *hop*, and *stat* mutants. In addition, the gene encodes a protein with sequence homology to mammalian cytokine receptors that mediate JAK/STAT signaling. A dominant negative form of Domeless has been generated, which mimics the loss-of-function phenotype (Brown et al., 2001). Upon expression of the dominant negative receptor specifically in the outer border cells, using *slbo*-GAL4, dramatic recruitment and migration defects were observed (Figure 7). The average number of outer border cells in these egg chambers was 0.5 and the migration index was 2.6. These results provided further support

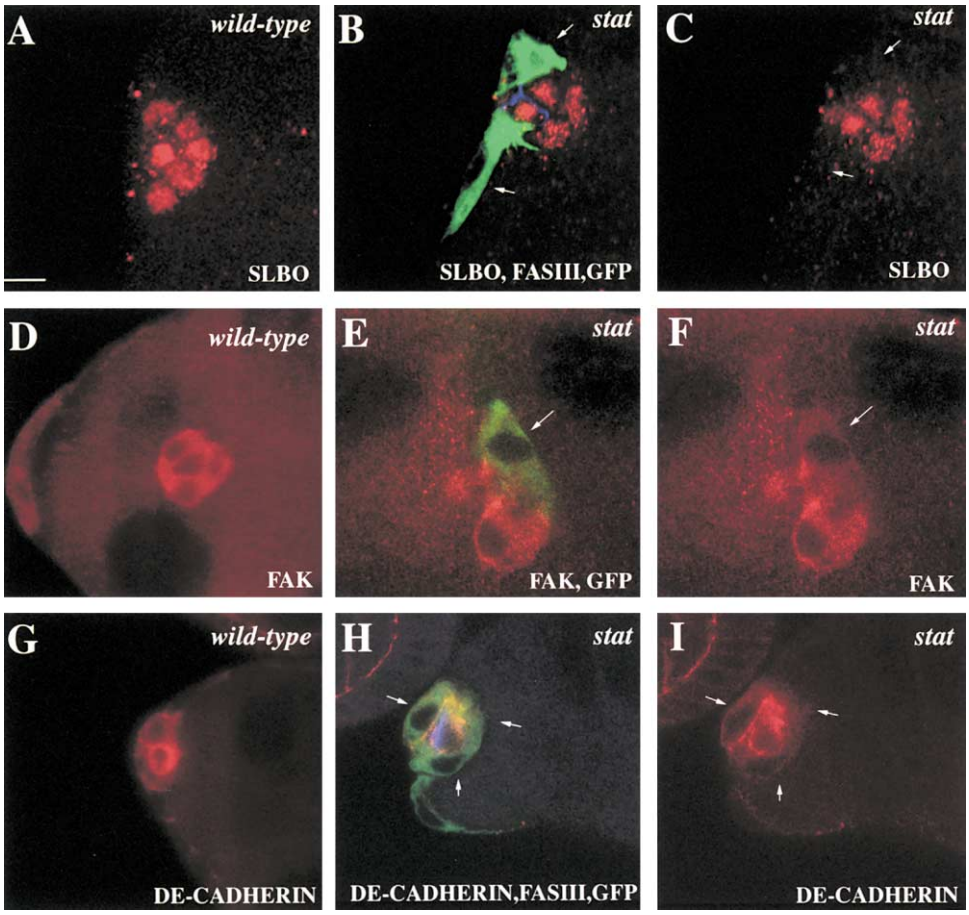

Figure 6. Reduced Expression of SLBO, FAK, and *D*E-cadherin in *stat* Mutant Border Cells

Confocal micrographs of clusters composed of a mixture of wild-type and *stat* mutant border cells. Homozygous *stat* mutant cells were labeled with GFP (green) using the MARCM technique (see Experimental Procedures). White arrows indicate homozygous mutant cells.
(A–C) SLBO expression is shown in red. (A) A wild-type border cell cluster. (B) A mixed cluster. Wild-type cells lack GFP and express SLBO. Mutant cells express GFP and lack SLBO. (C) The same cluster as in (B) showing SLBO staining alone.
(D–F) FAK expression is shown in red. (D) A wild-type border cell cluster. (E) A mosaic border cell cluster in which homozygous *stat* mutant cells express GFP. (F) FAK staining alone in the same cluster.
(G–I) *D*E-cadherin expression is shown in red. (G) Wild-type border cells as they initiate migration. (H) A *stat* mutant border cell cluster. Fas III staining is shown in blue. GFP and *D*E-cadherin colocalize in the mutant polar cells (yellow). (I) *D*E-cadherin expression alone in the same border cell cluster.
Scale bar in (A) is 10 μm.

for the proposal that UPD from the polar cells activates signaling in the surrounding epithelial cells for their recruitment to the cluster and migration.

Discussion

Polar Cells Recruit Surrounding Cells to Become Migratory via UPD/JAK/STAT

Previous studies indicated that polar cells emit a short-range signal that causes adjacent follicle cells to surround them and acquire the ability to migrate through the nurse cells. The results reported here suggest that UPD is the major signal secreted by the polar cells that both recruits adjacent follicle cells into the cluster and causes them to become migratory. Both of these functions are carried out by activation of JAK and STAT in the neighboring follicle cells. Signaling through this pathway is necessary, both for recruitment of border cells to the cluster and for motility once the cells are recruited. This is based on the observations that in the majority of mutant egg chambers, border cell clusters contain fewer than the normal number of cells, and that even clusters with normal numbers of cells fail to migrate normally.

It is worth noting that while some migration is observed in JAK and STAT border cell mutants, the loss of UPD in the polar cells completely prevents migration. This may reflect greater perdurance of JAK and STAT proteins in the mosaic clones, compared to UPD, if UPD is normally present at lower levels and/or is more labile. Alternatively, these differences may imply that in addition to its activation of JAK and STAT, UPD can activate other signaling pathways.

Activation of the JAK/STAT pathway is not only necessary but is also sufficient to convert epithelial follicle cells to become migratory. Numerous extra border cells

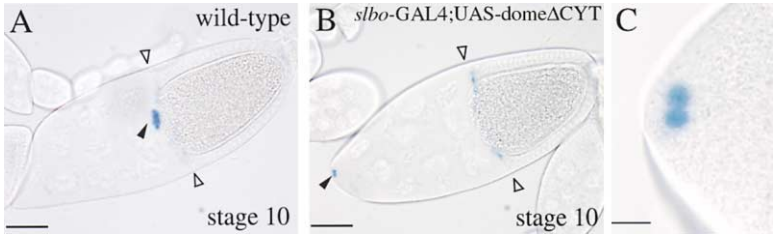

Figure 7. Effect of Dominant-Negative Domeless on Border Cell Recruitment and Migration

Nomarski optics images of egg chambers stained for β-galactosidase activity from the enhancer trap insertion into the *slbo* locus.
(A) A wild-type stage 10 egg chamber.
(B) A stage 10 egg chamber from *slbo*-GAL4; UAS-dome$^{\Delta CYT}$.
(C) A high magnification view of the border cell cluster, showing that only two β-gal positive cells were present. The average number of outer border cells in such egg chambers was 0.5 (wild-type is 6), and the migration index was 2.6 (wild-type is 100).
The scale bar represents 50 μm in (A) and (B), and 10 μm in (C).

were observed following overexpression of *upd*, *hop*, or *hop^{Tum}*, many of which invaded the nurse cell cluster. These extra cells did not result from excess proliferation, as follicle cells cease dividing at stage 6, at least 12 hr prior to border cell differentiation (Spradling, 1993). Furthermore, no difference in phospho-histone H3 antibody labeling was observed in cells overexpressing *upd* or in cells lacking *stat* compared to wild-type. Moreover, it was possible to obtain large clones lacking *upd*, *hop*, or *stat* activity, indicating that homozygous mutant cells retain the ability to divide numerous times. Thus, activation of the JAK/STAT pathway leads to border cell specification and migration, without effects on proliferation. In addition, while it was previously known that extra follicle cells could become migratory as a secondary consequence of ectopic polar cell formation, activation of the JAK/STAT pathway resulted in the appearance of additional migratory cells in the absence of extra polar cells.

Recently, a putative guidance factor for the border cells was reported. A protein with homology to VEGF and PDGF, known as PVF-1, accumulates in the oocyte beginning at stage 7 (Duchek et al., 2001). It is interesting to note that the receptor for this factor is expressed uniformly on the surfaces of all of the follicle cells. Thus, all of the follicle cells are exposed to the ligand, and all of the follicle cells express the receptor. However, only the border cells detach from the epithelium, invade the nurse cell cluster, and migrate. These observations raise the obvious question as to why it is that such a small subset of cells migrates toward the putative chemoattractant. The results reported here indicate that it is activation of the JAK/STAT pathway that defines the invasive population of follicle cells. Thus, exposure of epithelial cells to a chemoattractant does not appear to be sufficient in vivo to cause them to become migratory. Rather, the cells require an additional signal, which results in substantial changes in gene expression, in order to migrate in response to this factor.

Relationship between JAK/STAT and SLBO, *Drosophila* C/EBP

STAT proteins have many biological functions, but the key downstream targets that carry out these functions are largely unknown (Darnell, 1997). The mechanism by which STAT activation leads to border cell migration appears to involve activation of *slbo* expression and its target genes. The evidence for this is that ectopic activation of the JAK/STAT pathway resulted in ectopic *slbo* expression, and loss of STAT led to reduction in the SLBO protein level.

The *slbo* locus encodes *Drosophila* C/EBP, a basic region-leucine zipper transcriptional regulator. It is intriguing to note that mammalian C/EBPβ is expressed in ovarian carcinomas, and its expression increases dramatically with malignancy (Sundfeldt et al., 1999). STAT3 is constitutively active in ovarian carcinomas (Huang et al., 2000), and while it is not known whether STAT3 activates expression of C/EBPβ in these cells, it could be that this relationship has been conserved in evolution.

In addition to SLBO, expression of each of its known target genes was reduced in *stat* mutant cells. The contributions of several of these target genes to cell migration is known. For example, dynamic regulation of *D*E-Cadherin plays a critical role in promoting migration by providing optimal adhesion with the nurse cells (Bai et al., 2000; Niewiadomska et al., 1999). Furthermore, FAK is essential for the migration of numerous mammalian cell types, while *jing* encodes a zinc finger transcription factor that cooperates with SLBO in regulating border cell migration (Liu and Montell, 2001).

We have shown that both loss-of-function and gain-of-function of JAK/STAT pathway activity are detrimental to border cell migration. Interestingly, this is also true for SLBO, since *slbo* mutants show border cell migration defects (Montell et al., 1992) and overexpression of *slbo* also impedes migration (Rørth et al., 2000). This similarity lends further support to the proposal that STAT exerts at least part of its effect on migration by regulating SLBO.

Integration of Local, Global, and Long-Range Guidance Signals Regulates Border Cell Migration

Two transcriptional regulatory pathways have been identified that control the invasive behavior of the border cells in vivo (reviewed in Montell, 2001). In addition to expression of *slbo* and its targets, border cell migration requires a global hormonal signal in the form of ecdysone (Bai et al., 2000). This global hormonal signal appears to function in a *slbo*-independent manner, since the expression of neither *slbo* nor its targets is reduced when ecdysone signaling is compromised, and no genetic interaction has been observed between mutations affecting the ecdysone response and *slbo* or *stat*. Taken together with the putative guidance signal PVF-1 and the data presented here, these results indicate that border cell migration requires the integration of at least three signals. The global hormonal signal coordinates

multiple events that occur at stage 9, including border cell migration, and PVF-1 contributes to the directional cue for the border cells. Finally, the local paracrine signal through JAK/STAT is necessary to define the population of cells capable of responding to the other signals by detaching from the epithelium and invading the nurse cell cluster. Of these three signals, only the signal through the JAK/STAT pathway is spatially restricted to the migratory population.

Do STATs Regulate Cell Motility More Generally?

STAT proteins may regulate epithelial to mesenchymal transitions and cell migration not only in the border cells but also in mammalian cells. Consistent with this proposal, embryos homozygous for deletion of the STAT3 gene fail to gastrulate (Takeda et al., 1997). Gastrulation in the mammal requires that some of the epithelial cells within the epiblast layer become mesenchymal and migrate through the primitive streak to form the mesoderm.

More direct evidence for a role for STAT3 in an epithelial to mesenchymal transition was reported in a study of a tissue-specific knockout of STAT3 (Sano et al., 1999). Loss of STAT3 in epidermal keratinocytes results in defects in re-epithelialization following wounding, where STAT3 appears to be required specifically for the migratory component of the response. JAK2 has also been found to be required for hematopoietic progenitor cell migration in response to stromal cell derived factor 1 (Zhang et al., 2001). Thus, there is evidence that JAK and STAT promote cell migration, though the mechanisms by which they do so have not been elucidated. It may be that the constitutive activation of the JAK/STAT pathway that is observed in a variety of human cancers contributes to invasiveness and mestastasis, in addition to the well established effects on survival and proliferation.

Experimental Procedures

Drosophila Genetics and Identification of *stat* Mutations

The following fly stocks were obtained from Norbert Perrimon: FRT^{82B}, *PStat92E*^{06346} and *FRT*^{18A}, *upd*^{YM55} and *FRT*^{18A}, *hop*^{c111}. *Hop*^{Tum1} flies were obtained from Charles Dearolf. *Upd*^{sisC5} flies were obtained from Thomas Cline.

The mosaic screen was performed essentially as previously described (Liu and Montell, 1999; Bai et al., 2000), except for the use of FRT^{82B}. Expression of the FLP recombinase enzyme was induced with e22c-GAL4, which is expressed in the follicular stem cells (Duffy et al., 1998), and border cells were visualized using the enhancer trap insertion in the *slbo* locus, PZ1310, which is also known as *slbo*^{1} (Montell et al., 1992). Approximately five females were examined for each mutant line.

Two of the alleles, *stat*^{397} and *stat*^{85C9}, were meiotically mapped based on segregation of the border cell migration phenotype with respect to the recessive markers *curved* (86D1-4), *stripe* (90D2-7), and *claret* (99B8-10). 157 recombinant lines for *stat*^{397} and 86 lines for *stat*^{85C9} were scored in mosaic clones for the border cell migration phenotype. *Stat*^{397} was crossed to 34 deficiencies that make up the 3R deficiency kit and are estimated to uncover approximately 70% of the right arm of the third chromosome (Bloomington Stock Center).

Migration indices were calculated by assessing the extent of border cell migration in a large number of stage 10 egg chambers. For each egg chamber analyzed, the extent of migration was measured as a percentage of the normal migration, where 0% indicated no migration at all and 100% indicated migration to the oocyte border. The average % migration is the migration index. Border cell recruitment was defined as cells physically associated with polar cells labeled with DAPI but not necessarily expressing border cell markers.

The MARCM technique was used to positively mark mosaic clones with GFP expression, as described (Lee and Luo, 1999), and the necessary stocks were obtained from Tzumin Lee. Females of the genotype P[hsp70-flp], UAS-mCD8GFP/+; FRT82B, Gal80/FRT82B, *stat*^{397}, with either c306-GAL4 on the X chromosome were heat-shocked for one hour three times a day for two to three consecutive days and were dissected 4–8 days after heat shock. Alternatively, clones were negatively marked by the absence of GFP using females of the genotype hsp70-FLP22; FRT82B, ubGFPnls/ FRT82B, *PStat92E*^{06346}. Border clusters composed entirely of mutant cells were observed most frequently when females were dissected 8–10 days after heat shock. Mosaic clones homozyous for either *upd*, *hop*^{c11}, or *hop*^{va85} mutations were marked by the absence of GFP expression using the following stocks: *FRT*^{18A}, *ubGFPnls*; *hsp70-flp* and *FRT*^{101}, *tubulin lacZ*; *hsp70*-FLP38 stocks, respectively.

Overexpression of the JAK/STAT pathway components was accomplished using the GAL4/UAS system (Brand and Perrimon, 1993). *Slbo*-GAL4 was obtained from Pernille Rørth (Rørth et al., 1998). C306-GAL4 was obtained from Lynn Manseau (Manseau et al., 1997). The following stocks were used for overexpression: [*UAS-upd*] (a gift from Norbert Perrimon), P[UAS-hop] and P[UAS-hop^{Tum}] were provided by Doug Harrison. P[UAS-dome$^{\Delta CYT}$] was obtained from James Castelli-Gair Hombria. This construct encodes a truncated receptor that lacks the cytoplasmic domain and behaves as a dominant negative.

Dominant genetic interactions were examined at 25°C. The *P34* mutant allele of DE-cadherin was obtained from Ulrich Tepass. *ADFcofilin*^{k05633}and *zpr*^{1D16} mutations were obtained from the Bloomington Stock Center.

Molecular Biology

PCR primers were designed based on the *stat92E* genomic DNA sequence in order to amplify the 2283 nucleotide coding region of the *Stat92E* locus, in six separate fragments. DNA from flies heterozygous for each of the four *stat* alleles was amplified and sequenced. The original stock that was mutagenized in the screen was sequenced as a control.

Immunohistochemistry and Immunoflourescence

Ovaries were dissected in Grace's medium (Sigma) containing 10% fetal calf serum. Staining for β-galactosidase activity and with antibodies, DAPI, and phalloidin was performed as described (Bai et al., 2000). The following antibodies were used: rabbit affinity purified anti-FAK at 1:1600 (Palmer et al., 1999), mouse anti-myosin VI at 1:10 (Mermall and Miller, 1995), rat anti-cadherin at 1:75, rabbit anti-βgalactosidase at 1:2000 (Cappel), rabbit affinity purified anti-slbo at 1:200 (Montell et al., 1992), mouse anti-Fascillin III at 1:1 and mouse anti-EYA at 1:100 (Developmental Studies Hybridoma Bank), and rabbit anti-hop at 1:100. The secondary antibodies used at 1:200 were: fluorescein (FITC) conjugated donkey anti-rabbit; CyTM5-conjugated donkey anti-mouse; Rhodamine Red™-X-conjugated donkey anti-rabbit and anti-mouse, and anti-rat (Jackson ImmunoResearch); Alexa Fluor goat anti-rabbit (Molecular Probes); and horse fluorescein (FITC) anti-mouse (Vector Laboratories). In situ hybridization was performed as described (Neuman-Silberberg and Schupbach, 1993). Egg chambers were visualized using either the Noran OZ laser or Ultraview confocal microscopes.

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