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# The *Drosophila* cytokine receptor Domeless controls border cell migration and epithelial polarization during oogenesis

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## SUMMARY

**In mammals, the JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription) signaling pathway is activated in response to cytokines and growth factors to control blood cell development, proliferation and cell determination. In *Drosophila*, a conserved JAK/STAT signaling pathway controls segmentation in embryos, as well as blood cell development and other processes in larvae and adults. During embryogenesis, transduction of the Unpaired [Upd; also known as Outstretched (Os)] ligand through the JAK/STAT pathway requires Domeless, a putative membrane protein with distant homology to vertebrate type I cytokine receptors. We have isolated *domeless* (*dome*) in a screen to identify genes essential in epithelial morphogenesis during oogenesis. The level of *dome* activity is critical for proper border cell migration and is controlled in part through a negative feedback loop. In addition to its essential role in border cells, we show that *dome* is required in the germarium for the polarization of follicle cells during encapsulation of germline cells. In this process, *dome* controls the expression of the apical determinant Crumbs. In contrast to the ligand Upd, whose expression is limited to a pair of polar cells at both ends of the egg chamber, *dome* is expressed in all germline and follicle cells. However, the Dome protein is specifically localized at apicolateral membranes and undergoes ligand-dependent internalization in the follicle cells. *dome* mutations interact genetically with JAK/STAT pathway genes in border cell migration and abolish the nuclear translocation of Stat92E in vivo. We also show that *dome* functions downstream of *upd* and that both the extracellular and intracellular domains of Dome are required for JAK/STAT signaling. Altogether, our data indicate that Dome is an essential receptor molecule for Upd and JAK/STAT signaling during oogenesis.**

Key words: JAK/STAT pathway, Cytokine, Crumbs, Border cells, Follicle cells, Egg chamber, *Drosophila melanogaster*

## INTRODUCTION

The temporal and regional control of epithelial morphogenesis underlies the assembly of many organs in metazoans. In *Drosophila*, the egg chamber provides a useful model to study genes and signaling networks controlling numerous stages of epithelia formation, from stem cell development to the final organ shape (Dobens and Raftery, 2000). The egg chamber is a composite unit that develops in a succession of discrete developmental stages (Spradling, 1993). Each egg chamber is made of a central germline cyst, from which the oocyte is selected, surrounded by a monolayer epithelium, that ultimately makes the external envelope of the egg. Several signaling pathways are essential to control egg chamber development, including Notch, EGFR, Hedgehog, TGFβ, steroid hormones and JNK cascades (Dequier et al., 2001; Dobens et al., 2001; Dobens and Raftery, 2000; Nilson and Schupbach, 1999; Riechmann and Ephrussi, 2001; Suzanne et al., 2001; van Eeden and St Johnston, 1999). Among the diverse cellular processes taking place during oogenesis, the delamination and migration of border cells (BCs), a group of anterior follicle cells, represent a powerful model to study cell invasion in a normal tissue (Montell, 1999; Montell, 2001). During stage 9 of oogenesis, BCs detach from the outer epithelium to invade the germline cyst compartment (Fig. 1A). The BC cluster contains two centrally located polar cells surrounded by approximately six outer border cells and undergoes a nearly 6-hour long posteriorward migration to reach the anterior part of the growing oocyte. Together with centripetal cells, they assemble the micropyle, a specialized structure required for sperm entry. Recent work has shown that the EGFR and its ligand Gurken, together with a novel PDGF/VEGF transduction pathway are involved in the directional control of BC migration to the anterior-dorsal corner of the oocyte (Duchek and Rorth, 2001; Duchek et al., 2001). In addition, a novel co-activator of the ecdysone receptor, Taiman, has been shown to be essential for BC migration, thus providing an interesting parallel with the known role of steroids in mammary gland metastasis (Bai et al., 2000). In order to identify new genes and signaling

pathways that are involved in egg chamber development and BC migration, we performed a tissue-targeted mosaic screen using a collection of X-linked lethal mutations (unpublished results; see Materials and Methods). Here, we report the identification of *domeless*, which encodes a putative receptor of the JAK/STAT pathway.

The JAK/STAT signaling pathway is involved in many developmental and physiological processes in mammals, particularly in blood cell differentiation or proliferation and in mammary gland development (Darnell et al., 1994; Ihle, 1995; Leonard and O'Shea, 1998; O'Shea et al., 2002). Moreover, constitutive activation of STAT3 and STAT5, for example, is responsible for numerous human epithelial cancers and leukemia (Bowman et al., 2000; Levy and Gilliland, 2000). Signaling through the JAK/STAT cascade involves membrane receptors the intracellular domain of which holds a tyrosine kinase of the JAK family. Ligand binding promotes receptor dimerization and its subsequent transphosphorylation by the associated JAK molecules. Phosphorylation of the receptor on specific tyrosine residues creates docking sites for the STAT transcription factors, which in turn are phosphorylated and activated by receptor-bound JAK. The final step in the transduction cascade is the release of activated STAT, which then translocates as a dimer into the nucleus to regulate target gene transcription.

In *Drosophila*, homologs of the mammalian JAK/STAT pathway components have been isolated. *hopscotch* (*hop*) encodes a *Drosophila* JAK homolog (Binari and Perrimon, 1994) and *marelle* (*mrl*) codes for a STAT factor (also known as *Stat92E*) (Hou et al., 1996; Yan et al., 1996). The only known ligand for the *Drosophila* JAK/STAT pathway is encoded by the *unpaired* (*upd/os*) gene (Harrison et al., 1998). Only recently, Domeless (Dome; also known as Mom) (Chen et al., 2002) has been identified as the first putative receptor for the JAK/STAT pathway in *Drosophila* embryos (Brown et al., 2001). The reduced number of JAK/STAT pathway genes in *Drosophila* contrasts with the multiplicity of JAK/STAT homologs found in mammals, making *Drosophila* a suitable system to study JAK/STAT signaling (Luo and Dearolf, 2001; Mathey-Prevot and Perrimon, 1998; Zeidler et al., 2000). The *Drosophila* JAK/STAT pathway was originally identified for its role in embryonic segmentation. Further work has shown that this pathway also participates in blood cell determination and proliferation, eye and wing development (Luo and Dearolf, 2001; Zeidler et al., 2000; Zeidler et al., 1999), sex determination (Jinks et al., 2000; Sefton et al., 2000; Zeidler and Perrimon, 2000) and stem cell differentiation (Kiger et al., 2001; Tulina and Matunis, 2001). More recently it has been shown to play a role in oogenesis for stalk and BC differentiation (Baksa et al., 2002; Beccari et al., 2002; McGregor et al., 2002; Silver and Montell, 2001). Thus, as in mammals, the *Drosophila* JAK/STAT pathway has a pleiotropic role and a common function in cell differentiation and blood cell development.

Here we show that *dome* is involved in BC determination and migration. In addition, *dome* is required for follicle cell polarization through the control of the apical determinant Crumbs. The Dome protein is associated with apicolateral membranes in follicle cells, and becomes internalized upon exposure to Upd, its putative ligand. Our loss- and gain-of-function analyses show that *dome* is autoregulated in follicle cells, and that both the intracellular and extracellular domains are required for Upd transduction and BC migration. Our data show that Dome is an essential receptor molecule for Upd and JAK/STAT signaling during oogenesis.

## MATERIALS AND METHODS

### *Drosophila* genetics

A description of genetic markers and chromosome balancers can be found in Lindsley and Zimm (Lindsley and Zimm, 1992) and FlyBase (http://flybase.bio.indiana.edu). *dome* has been identified in an ongoing P element screen using a targeted mosaic approach in ovaries (unpublished). Homozygous follicle cell clones for *dome* or *hop* were induced using the UAS-FLP method (Duffy et al., 1998). *dome* FRT19A/FM7 or $hop^{C111}$ FRT101/FM7 females are crossed to UB-GFP, FRT19A/Y; *e22c*-GAL4, UAS-*flp*/CyO or UB-GFP, FRT101/Y; e22c-GAL4, UAS-*flp*/CyO males, and mosaic egg chambers are analysed in *dome*, FRT19A/UB-GFP, FRT19A; *e22C*-GAL4, UAS-*flp*/+ or $hop^{C111}$, FRT19A/UB-GFP, FRT19A; *e22C*-GAL4, UAS-*flp*/+ females. The expression of the FLP recombinase under the control of the *e22c*-GAL4 line induced mutant follicle cell clones, which were identified by the absence of GFP expression (Duffy et al., 1998). The following *Drosophila* stocks have been used: $dome^{PL100}$ FRT19A/FM7i; $dome^{12030}$ FRT19A/FM7i; $dome^{PG14}$ FRT19A/FM7i. Other stocks used in this study are: $hop^{C111}$ FRT101/FM7; UAS-*hop*; UAS-*Stat92E*; UAS-*upd* (a gift from M. Zeidler and N. Perrimon). S. Brown and J. Castelli-Gair kindly provided us with UAS-*dome* and UAS-*domeΔCYT* (Brown et al., 2001). The *CY2*-GAL4 and *slbo*-GAL4 lines used in this study have been described elsewhere (Queenan et al., 1997; Rorth, 1998).

### Molecular biology and transgenic lines

Several independent P-element insertions (PL58, PL100, PG14, 12030, PG5, PG35) (Bourbon et al., 2002) have been identified in *domeless* and mapped in the 5′UTR region of the gene between position –20 and +405 of the putative transcription start site. A full-length cDNA (LD46805) was obtained from the Berkeley Drosophila Genome Project (BDGP) and fully sequenced using an automated ABI DNA sequencer (Accession Number, AY147847). The *dome* cDNA is 4805 bp long and contains 478 bp of 5′UTR, 3846 bp of coding region and 480 bp of 3′UTR. It encodes a protein of 1282 amino acids, whose sequence is identical to the one described previously (Brown et al., 2001; Chen et al., 2002). The pUAS-*dome* construct was made by cloning a full-length *dome* cDNA (LD46805) cut with *Eco*RI and *Xho*I and ligated into the *Eco*RI and *Xho*I sites of the transformation vector pUAST. pUAS-*domeΔEXT* was constructed by cloning a *Eco*RI-*Xho*I fragment containing the 5′UTR, and the sequence encoding amino acids 1-27 (signal peptide) fused to amino acids 873-1281 (transmembrane and intracellular region) into the transformation vector pUAST cut with *Eco*RI and *Xho*I.

pUAS-*domeGFP* was constructed by single step ligation of the following DNA fragments: (i) *Eco*RI-*Xho*I fragment containing the 5′UTR and coding region (1-1281), (ii) a *Xho*I-*Xba*I fragment encoding EGFP and (iii) the transformation vector pUAST cut with *Eco*RI and *Xba*I. This results in the insertion of GFP at the C terminus of a wild-type Dome protein. For each construct, several independent transgenic lines have been generated and tested.

### Protein purification and antibody production

A glutathione S-transferase (GST) fusion protein containing Dome amino acids 918-1113 was produced in *E. coli* and used to immunize female New Zealand rabbits, according to standard protocols. The sera were collected and tested by western blot analysis to ensure specificity (data not shown). To purify the sera, a His-tagged-Dome (aa 918-1113) protein was produced in *E. coli*. 1 mg of this protein was separated by SDS-PAGE, and blotted onto a nitrocellulose filter. A

strip containing the purified protein was used to affinity purify the Dome antibodies. Following extensive washing, the bound antibodies were eluted with elution buffer (200 mM glycine and 1 mM EDTA, pH 2.8). The eluate was neutralized immediately with one-tenth volume of 1 M Tris (pH 8.0). This antibody was used on western blots and for immunohistochemistry.

### Immunohistochemistry and X-gal staining

Staining of egg chambers with X-gal or antibodies were performed as described previously (Lasko and Ashburner, 1990; Suzanne et al., 2001; Tanentzapf et al., 2000). The following primary antibodies have been used: rabbit anti-Dome (1:200), mouse anti-Fas3 (1:40; 7G10, Developmental Studies Hybridoma Bank-DSHB), mouse anti-β-galactosidase (1:1000, Promega), mouse anti-Crumbs (1:50; Cq4, DSHB); rat anti-DE-cadherin (1:50, a gift from Hiroki Oda), rabbit anti-Stat92E (1:500, a gift from Steven Hou). Secondary antibodies used in this study were anti-rabbit FITC (fluorescein)-tagged (1:400), anti-mouse CY3 (1:400; Molecular Probes). Confocal images were taken with a Leica TCS-SP1 confocal microscope. Other images were taken using a Nikon Coolpix 990 digital camera and processed with Photoshop 6.0 (Adobe).

## RESULTS

### *domeless* controls egg chamber formation and migration of border cells during oogenesis

In a screen for mutations on the X chromosome affecting follicle cell development (Bourbon et al., 2002) (unpublished results), we identified several independent P-element insertions leading to defective migration of border cells (Fig. 1C-E). All P elements are inserted in the 5′UTR of the CG14226 gene, at cytological position 18D13-E1 (see Materials and Methods). While this study was being conducted, this gene was independently identified for its role in JAK/STAT signaling during embryogenesis and named *domeless* [*dome;* a.k.a. *mom* (Brown et al., 2001; Chen et al., 2002)]. *dome* encodes a putative transmembrane protein with distant homology to vertebrate cytokine receptors, and is proposed to function as a receptor molecule in the *Drosophila* JAK/STAT pathway during embryogenesis (Brown et al., 2001; Chen et al., 2002) (data not shown).

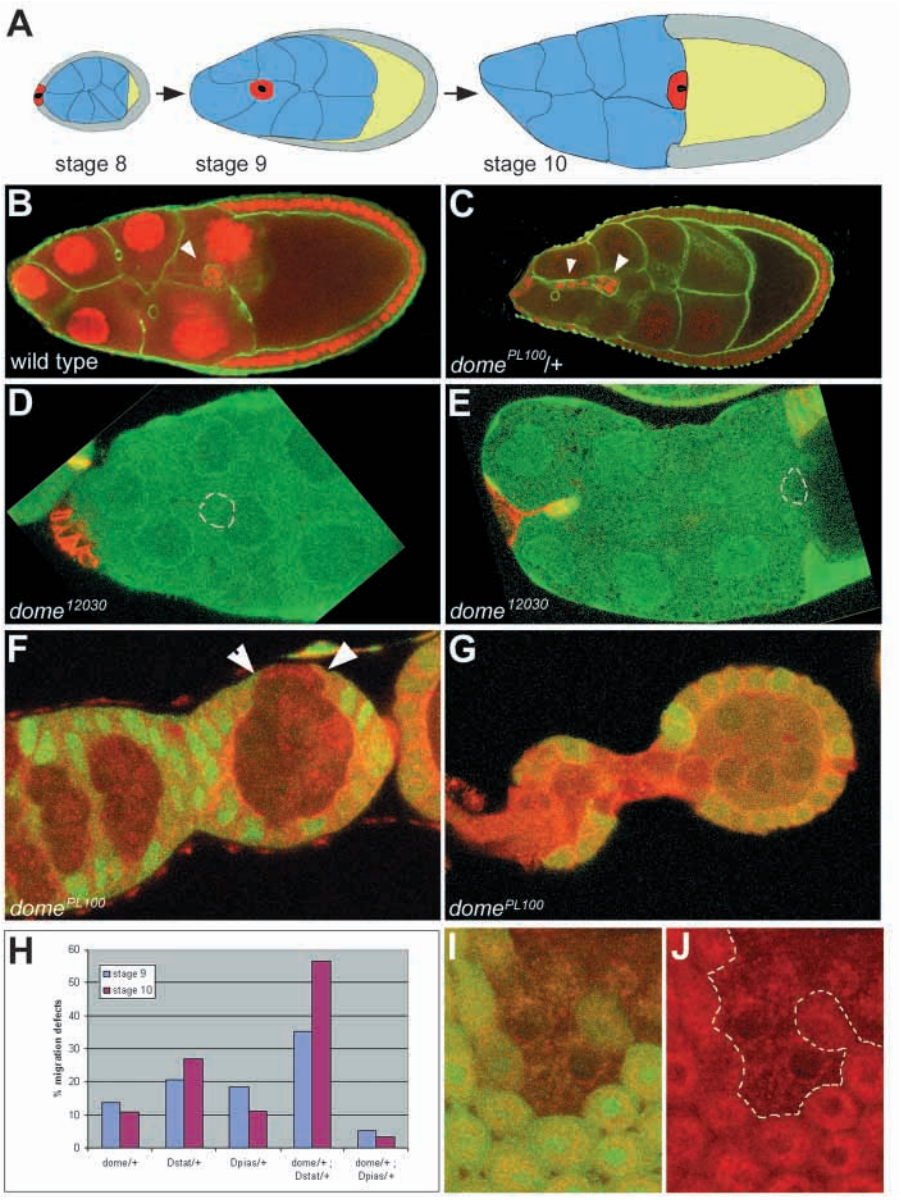


**Fig. 1.** Domeless controls border cell migration and interacts with the JAK/STAT pathway.(A) Schematic representation of BCs (in red) during stages 8 to 10. The BC cluster, containing the polar cells (in black), starts to delaminate from the follicle cell layer (in grey) during stage 9 and invades the nurse cell compartment (in blue). At stage 10, BCs reach the anterior of the developing oocyte (in yellow). (B) Wild-type stage 10 egg chamber stained with phalloidin-FITC (green; staining the actin cytoskeleton) and propidium iodide (red; staining the nuclei). Note the round BC cluster at the anterior of the oocyte (arrowhead). (C) Stage 9 egg chamber from an heterozygous $dome^{PL100}$/+ female. The BC cluster is not correctly assembled with some cells being left behind (arrowheads). (D,E) Mosaic egg chambers containing *dome* mutant clones (recognized by the absence of the GFP clonal marker and upregulation of the Fas3 marker (in red; see also Fig. 7). Loss of *dome* in BC precursors leads to defective, or absence of, BC migration. For comparison, the normal position of the cluster as it would be in a wild-type chamber is represented by a dashed circle. (F,G) Early stage 2-3 *dome* egg chambers showing incomplete encapsulation (arrowheads, F) or fusion (G). Anti-Dome staining is in red. (H) *dome* interacts genetically with *Stat92E* and *dpias*. Histogram representing the percentage of egg chambers of various genotypes with BC migration phenotypes similar to those shown in C or with defective BCs at stages 9 and 10. (I,J) *dome* homozygous mutant follicle cells (GFP negative) show a dramatic reduction in nuclear Stat92E (in red). Anterior is to the left.

In *dome* mosaic egg chambers, some BC clusters are not formed, migrate aberrantly and/or contain less cells than normal (Fig. 1D,E). In these cases, *dome* mutant cells are always found in the anterior region of the egg chamber, indicating that they cannot be determined to become migratory BCs. In mosaic BC clusters, wild-type cells usually migrate normally or ahead of *dome* mutant cells (Fig. 1E; data not shown), suggesting differences in the migratory ability and adhesiveness of mutant and wild-type cells.

Interestingly, in heterozygous females the BC cluster shows a slow border cell migration phenotype, aberrant shape and/or incomplete number of cells in the cluster due to the detachment of some BCs (Fig. 1C; data not shown). This suggests a semi-dominant, dose-sensitive effect of *dome* in this process and a possible role of *dome* during migration itself.

In addition to an aberrant migration of the BCs, we also found defects in the encapsulation of early egg chambers (Fig. 1F). In some cases, this can lead to the fusion of adjacent egg chambers (Fig. 1G), suggesting that *dome* may also be involved in the assembly of egg chambers during early oogenesis (see below).

### *domeless* interacts with members of the JAK/STAT pathway in BC migration

*dome* is a maternal effect gene controlling tracheal development and segmentation in embryos where it participates in JAK/STAT signaling (Brown et al., 2001; Chen et al., 2002). Interestingly, members of the JAK/STAT pathway, including *upd*, *hop* and *Stat92E*, have recently been shown to be involved in oogenesis for proper stalk and BC differentiation (Baksa et al., 2002; Beccari et al., 2002; McGregor et al., 2002; Silver and Montell, 2001). However, it is not known whether Dome is a receptor for JAK/STAT signaling during oogenesis and whether it is activated by the Upd ligand. In order to address these questions, we tested for genetic interactions between *dome* and other members of the JAK/STAT pathway using BC migration as an assay. Since *dome*, *upd* and *hop* genes are all located on the X chromosome, we have only been able to test interactions with *Stat92E* (Hou et al., 1996; Yan et al., 1996) and the recently identified *dpias* (a.k.a. *Su(var)2-10*) gene (Betz et al., 2001), which are positive and negative regulators of the JAK/STAT pathway, respectively. Removing one copy of *Stat92E* aggravates *dome/+* BC migration phenotypes, while removing one copy of *dpias* suppresses them (Fig. 1H). This supports a model in which *dome*, *Stat92E* and *dpias* would participate in the same pathway. In order to test the role of *dome* in JAK/STAT signaling more directly, we analysed Stat92E expression in follicle cells. Upon activation, Stat proteins translocate into the nucleus to regulate target gene transcription. In *dome* mutant follicle cells, nuclear Stat92E is dramatically reduced (Fig. 1I,J), as it is in cells mutant for $hop^{C111}$ (data not shown). Since the active, nuclear form of Stat92E is strongly affected, these data indicate that *dome* is required cell autonomously to activate JAK/STAT signaling during oogenesis.

### Dome is expressed in all follicle cells and localizes to apicolateral membranes

In egg chambers, *upd* expression is restricted to two pairs of polar cells, which are located at the most anterior and posterior parts of the follicle cell layer (Fig. 2A,B) (Beccari et al., 2002; McGregor et al., 2002; Silver and Montell, 2001). Interestingly, we found that the *upd* mRNA is concentrated at the apical side of the polar cells, which is suggestive of a polarized synthesis and secretion of this ligand during oogenesis (Fig. 2A,B). Upd is proposed to signal to neighboring cells, committing them to the BC fate (Beccari et al., 2002; Silver and Montell, 2001). It is thus important to determine the expression pattern of *dome* relative to its putative ligand. In situ hybridization suggests that *dome* is expressed at low level in all germline and follicle cells (Fig. 2C). Indeed, *dome* transcripts can only be detected over background level after overexpression using the UAS-GAL4 system (Fig. 2D) (Brand and Perrimon, 1993). To determine the subcellular localization of the Dome protein in follicle cells, we raised antibodies directed against an intracellular fragment of Dome (aa 918-1113; see Materials and Methods). Consistent with in situ data, immunostaining of egg chambers reveals that Dome is expressed in all germline and follicle cells. Dome is a membrane protein whose localization is restricted to apicolateral regions (Fig. 3). The membrane staining is specific, since it is absent in cells that are mutant for *dome* (Fig. 3A,B).

The subcellular localization of Dome protein was further analysed using a Dome-GFP construct which retains wild-type activity (see below; Fig. 5D,G,H). Expression of Dome-GFP in follicle cells targets the fusion protein to apicolateral membranes, in a pattern similar to wild-type Dome (compare Fig. 3B and 3C). In addition, the fusion protein accumulates in intracellular vesicles (Fig. 3C). This specific vesicular pattern may reflect an enhanced trafficking of Dome in the secretory and/or endocytic pathway(s) due to overexpression. Consistently, the overexpression of the wild-type Dome protein, or a truncated form (DomeΔEXT, a deletion of the

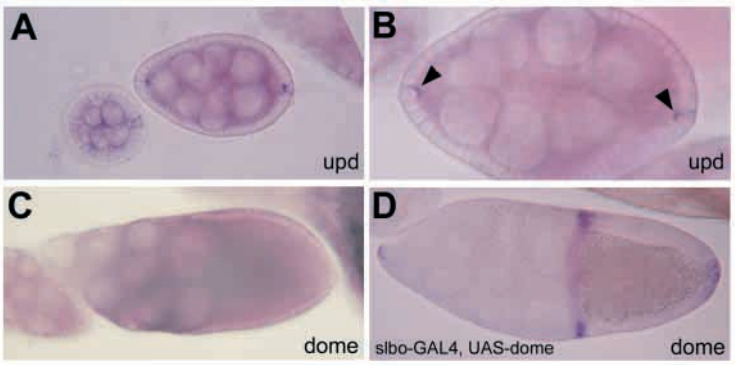


**Fig. 2.** Expression of *upd* and *dome* in egg chambers. In situ hybridization using *upd* (A,B) or *dome* (C,D) antisense riboprobes. The *upd* mRNA is detected in the 2 pairs of polar cells and is preferentially accumulated in the apical region (B, arrowheads). The *dome* transcripts are widely and weakly expressed in follicle and germline cells (C).
(D) Overexpression of *dome* using the *slbo*-GAL4 line and a UAS-*dome* construct.

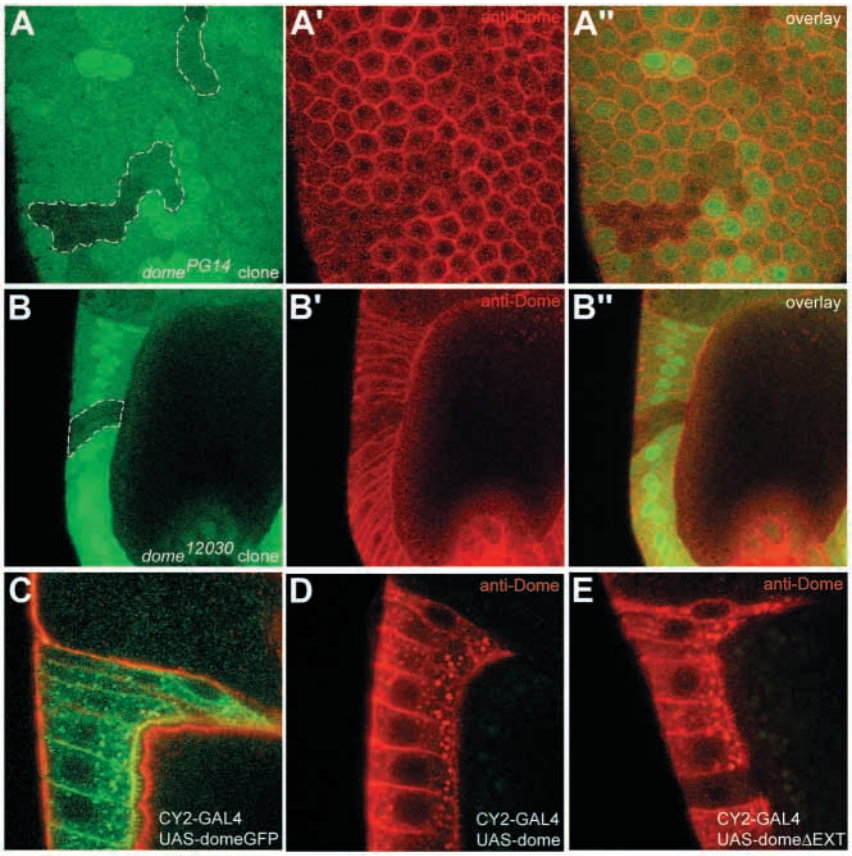


**Fig. 3.** Dome subcellular localization in follicle cells. (A,B) Immunostaining of *dome* mosaic egg chambers using an anti-Dome antibody (in red). Mutant clones are identified by the loss of the GFP clonal marker (dashed lines; see Materials and Methods). The Dome protein is targeted to the apicolateral membranes in all follicle (A,B) and germline cells (not shown). Membrane staining is absent in cells that are mutant for 2 different *dome* alleles ($dome^{PG14}$, $dome^{12030}$). A dome-GFP fusion protein is also targeted to the membrane (C; red is phalloidin-TRITC). Overexpression of Dome-GFP (C), wild-type Dome (D) or a truncated Dome (domeΔEXT; E), promotes the formation of Dome-containing intracellular vesicles apically.

extracellular domain; see Fig. 5D) also gives rise to similar Dome-containing vesicles (Fig. 3D,E).

## Upd-dependent internalization of the Dome receptor

The presence of Dome in intracellular vesicles after overexpression prompted us to check whether Dome could undergo detectable trafficking in normal conditions. Indeed, detailed analysis of Dome protein localisation in follicle cells reveals a specific accumulation of endogenous Dome in intracellular vesicles, starting from stages 2-3 of oogenesis. In early stages (Fig. 4A,B), Dome vesicles are present in all follicle cells, but later they become restricted to regions where the Upd ligand is most abundant, i.e., close to the polar cells (Fig. 4C,F-I). These vesicles contain Dome protein, since they are absent in *dome* mutant follicle cell clones (Fig. 4B,H,I). Dome-containing vesicles are present in BCs before and during migration (Fig. 4C-E), thus following the migration pattern of *upd*-expressing polar cells (Fig. 1A). In the posterior region where polar cells also express *upd* (Fig. 2A,B), the same pattern of vesicles is visible, though these cells do not migrate (Fig. 4F,H).

Interestingly, the abundance and distribution of Dome vesicles depend on the distance from the polar cells, which are the source of the Upd ligand (Fig. 4F,G). To test whether vesicle formation is Upd dependent, we generated flies in which *upd* was ectopically expressed. Cells newly expressing *upd* accumulate a high level of Dome vesicles (Fig. 4J,K). In addition, overexpression of *upd* in the BCs leads to an elevated number of vesicles (Fig. 4L), as compared to wild-type cells (Fig. 4C). Thus, Upd can promote the internalization of the Dome receptor. Vesicles may likely reflect active endocytosis of the Dome receptor after binding to its ligand, a known mechanism by which receptor-ligand complexes are inactivated or recycled. Consistently, the intracellular domain of Dome contains several tyrosine-based and di-leucine motifs, which have been shown to work as internalization sorting codes in several vertebrate receptors (Trowbridge et al., 1993). It is noteworthy that both endogenous and ectopic Dome vesicles are found preferentially at the apical side of the cells, a region where the *upd* mRNA, and probably the Upd protein, are most abundant. In this respect, the pattern of endogenous Dome vesicles may well reflect the level and pattern of Dome and JAK/STAT pathway activation in follicle cells.

## Both the intracellular and extracellular domains of Dome are essential for its function

To assess the contribution of specific domains in Dome activity, we performed a structure-function analysis by expressing truncated Dome proteins in BCs using *slbo*-GAL4 as a driver. In this study we used UAS-*dome*, UAS-*domeGFP*, UAS-*domeΔCYT* (deletion of the cytoplasmic domain) (Brown et al., 2001), and UAS-*domeΔEXT* (deletion of the extracellular domain; see Fig. 5D). The fate and migratory phenotype of BCs were assessed using the *slbo-lacZ* marker (Fig. 5A) (Montell et al., 1992). Overexpression of *Stat92E* or *hop* leads to migration defects, while expression of the *upd* gene causes the formation of extra BCs (Fig. 5B,C,M) (Beccari et al., 2002; Silver and Montell, 2001). Excess of Dome or DomeGFP in BCs blocks their migration (Fig. 5E-H), which never takes place, as indicated by the presence of *slbo-lacZ*-positive cells at the tip of old egg chambers, and female sterility (data not shown). This phenotype indicates a dose-sensitive effect of *dome* on BC migration, reminiscent of the dose sensitivity of the *slbo* gene (Rorth et al., 2000), another gene that is crucial for BC migration.

In egg chambers expressing DomeΔEXT or DomeΔCYT, outer BCs are absent and only polar cells are formed and express the *slbo-lacZ* marker. This phenotype, which is similar to a complete loss of *dome* in BCs (Fig. 1), is consistent with these truncated Dome proteins behaving as dominant negative forms. We confirmed this conclusion by looking at the

embryonic cuticle phenotypes induced in early embryos using a maternal GAL4 line (Brown et al., 2001; data not shown).

To date, Dome is the only known receptor for the JAK/STAT pathway in *Drosophila*. In order to test the absolute requirement of *dome* in *upd* signaling during oogenesis, we used an epistasis test in egg chambers. When the *upd* gene is overexpressed, extra BCs are recruited and the level of *slbo-lacZ* expression is strongly enhanced, indicating that *slbo* is positively regulated in conditions of high JAK/STAT activity (compare Fig. 5A and 5M) (Silver and Montell, 2001). The co-expression of *upd* and a *dome* dominant negative construct (*UAS-DomeΔCYT*) completely suppresses the gain-of-function phenotypes associated with *upd* overexpression, including recruitment of extra BCs and enhanced *slbo-lacZ* expression (compare Fig. 5M and 5N). The resulting phenotype is similar to the simple expression of DomeΔCYT (Fig. 5I). These results demonstrate that *dome* is downstream of *upd* to activate JAK/STAT signaling in follicle cells, and that *dome* is essential for *upd* function.

## Dome expression is autoregulated in follicle cells

Either reduction, as in *dome* heterozygotes (Fig. 1C), or elevation, as in overexpression experiments (Fig. 5E-H), of *dome* expression leads to BC migration defects, suggesting that *dome* function is tightly regulated in normal egg chambers. To further test this hypothesis, we examined Dome expression in cells that are mutant for *JAK/hop*. In *hop* mutant follicle cell clones, the level of Dome protein is higher than in the neighboring wild-type cells (Fig. 6A), suggesting that *hop* normally downregulates Dome expression. Since this effect could be due to either transcriptional or post-transcriptional regulation, we tested whether *dome* gene expression itself is affected. For this purpose, and because endogenous *dome* transcripts are barely detectable (Fig. 2C), we used a *dome*-Gal4 line as a reporter of *dome* expression. *dome-Gal4* is a pGAL4 enhancer trap element that is inserted in the promoter region of the *dome* gene (20 bp upstream of the transcription start site; Fig. 6E). *dome*-GAL4 is expressed weakly in anterior and posterior follicle cells, but much more highly in polar cells and BCs (Fig. 6B). Expression of UAS-*dome* leads to a strong reduction of *dome*-GAL4 driven GFP expression in BCs (Fig. 6C), suggesting that elevated *dome* activity can downregulate its own expression. In contrast, expression of the dominant negative form DomeΔEXT did not significantly affect the level of *dome*-GAL4 activity (Fig. 6D). These data suggest that in normal egg chambers, *dome* expression is negatively controlled by the JAK/STAT pathway. In agreement with this proposal, the analysis of the *dome* promoter region (12 kb upstream to the transcription start site) revealed the presence of 2 short

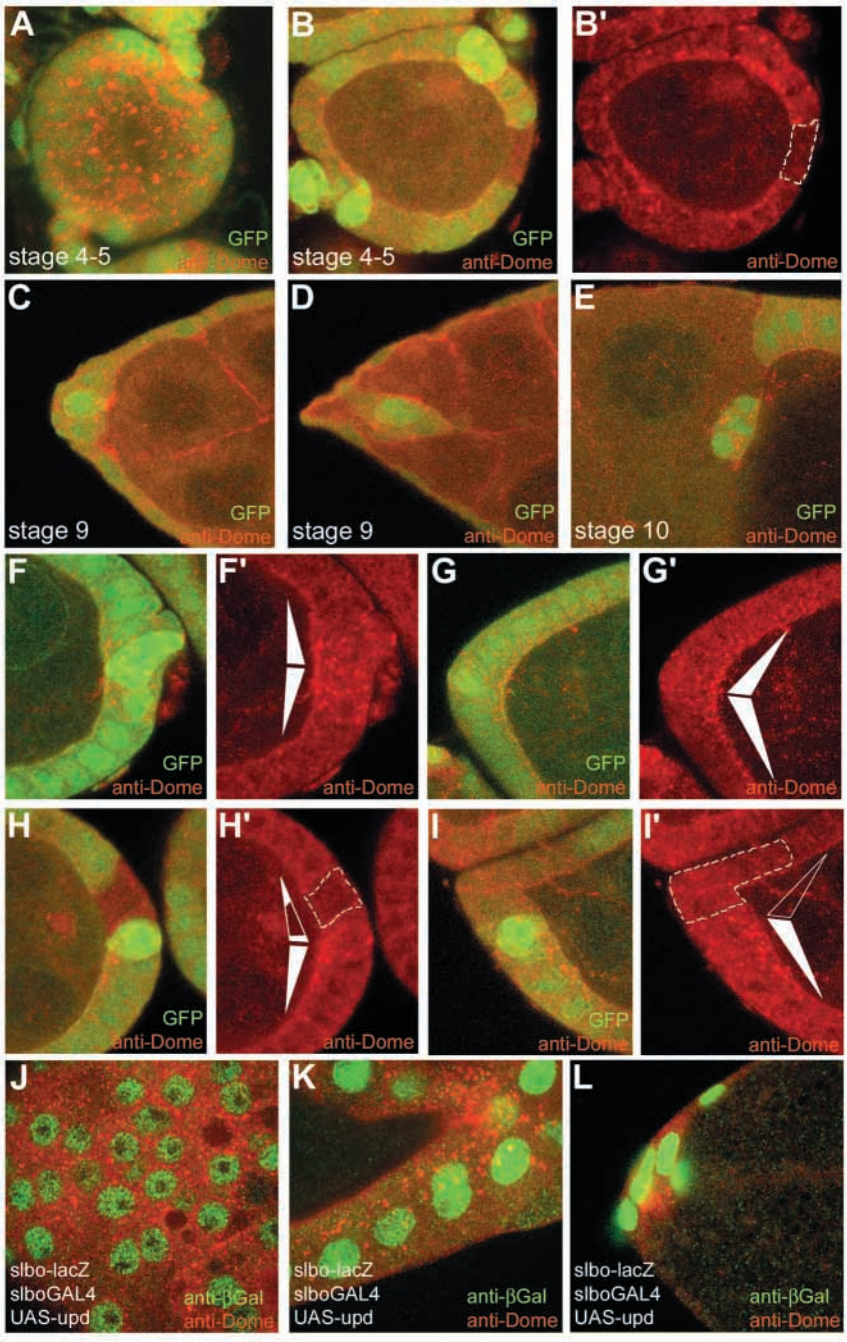


**Fig. 4.** Upd-dependent internalization of Dome. From stages 4 to 10, endogenous Dome (in red) is detected in intracellular vesicles. (A,B,B′) In early stages, vesicles are present in all follicle cells (A is a stack of several optical sections; B,B′ represent an optical section through a stage 4-5 egg chamber), but later, they become restricted to the poles of the egg chambers (C,F-I), where the Upd ligand is most concentrated. (C-E) BCs contain a high level of Dome vesicles before, during, and after migration. (G,I,F,H) Dome is expressed in a gradient (represented by the white wedges) of apically located vesicles at the anterior (G,I) and posterior poles (F,H). (B,H,I) Vesicles contain the Dome protein, since they are absent in *dome* mutant follicle cells (broken outlines) at any stage. (J-L) Ectopic expression of the Upd ligand in main body follicle cells (J,K) or BCs (L) causes the accumulation of extra Dome-containing vesicles.

sequences that perfectly match the consensus Stat92E binding site, TTCNNNGAA (Fig. 6E) (Yan et al., 1996). These putative Stat92E sites are similar to those present in the *eve* promoter, which have been shown to respond to JAK/STAT signaling (Yan et al., 1996).

### Role of *dome* in follicle cell morphogenesis

In order to better understand how *dome* may control egg chamber development, we examined the expression of different follicle cell markers in *dome* mosaic egg chambers. Previous work has shown that the JAK/STAT pathway controls the accumulation of DE-cadherin and Fas3 in follicle cells (Baksa et al., 2002; McGregor et al., 2002; Silver and Montell, 2001). In *dome* mutant clones, DE-cadherin is strongly reduced (Fig. 7A,B) suggesting that *dome* participates in the formation of a correctly differentiated epithelium throughout oogenesis. Up until stage 3, Fas3 is abundant in all follicle cells (Ruohola et al., 1991), while later, Fas3 level drops sharply in all follicle cells except in the polar cells (data not shown). Therefore, Fas3 serves as a differentiation marker to monitor the maturation of developing egg chambers. The removal of *dome* leads to an abnormally high accumulation of Fas3 in older egg chambers, indicating that these cells did not differentiate normally and remained immature (Fig. 7C-F).

The formation of incompletely encapsulated egg chambers in *dome* mosaic females (Fig. 1F,G) is reminiscent of the loss-of-function phenotype of *crumbs* mutations (Tanentzapf et al., 2000). Indeed, *crumbs* is required for the initial polarization

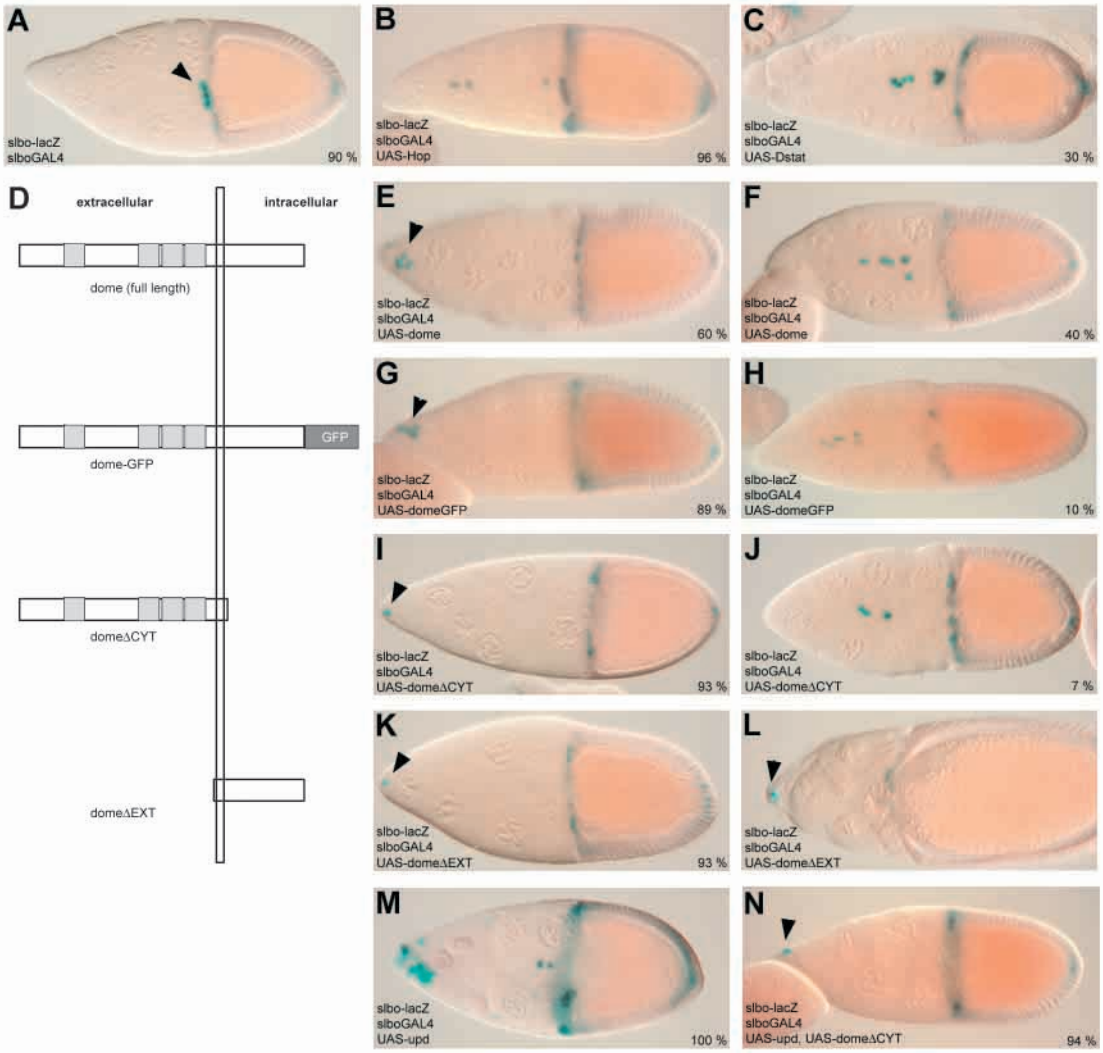


**Fig. 5.** Structure-function analysis of the Dome receptor. The migratory phenotype of BCs after overexpression of the JAK/STAT pathway components is analysed using *slbo-lacZ* as a reporter, which is expressed primarily in the BCs (arrowhead) at stages 9 and 10. (A)At stage 10, *slbo-lacZ* is also expressed in centripetal and posterior polar cells. (B-L) Overexpression, using a *slbo*-GAL4 line (Rorth, 1998), of Hop (B), Stat92E (C) or Upd (M) leads to aberrant BC migration. To test the domain requirements of Dome, several different constructs have been expressed (D) and the resulting phenotypes analysed (E-L, arrowheads indicate BCs). Overexpression of a wild-type Dome or of a Dome-GFP fusion protein leads to an absence of migration of BCs (E-H). Expression of truncated forms (DomeΔCYT or DomeΔEXT) blocks the recruitment of BCs, and only polar cells express *slbo-lacZ* (I-L). Note that even in late stage egg chambers (L; stage 11) polar cells are still present at the anterior pole. Co-expression of Upd and the dominant negative DomeΔCYT proteins in BCs completely suppresses the gain-of-function phenotypes associated with Upd overexpression (compare M and N). The frequency of BC migratory defects is indicated in the lower right corner of each panel. Anterior is to the left.

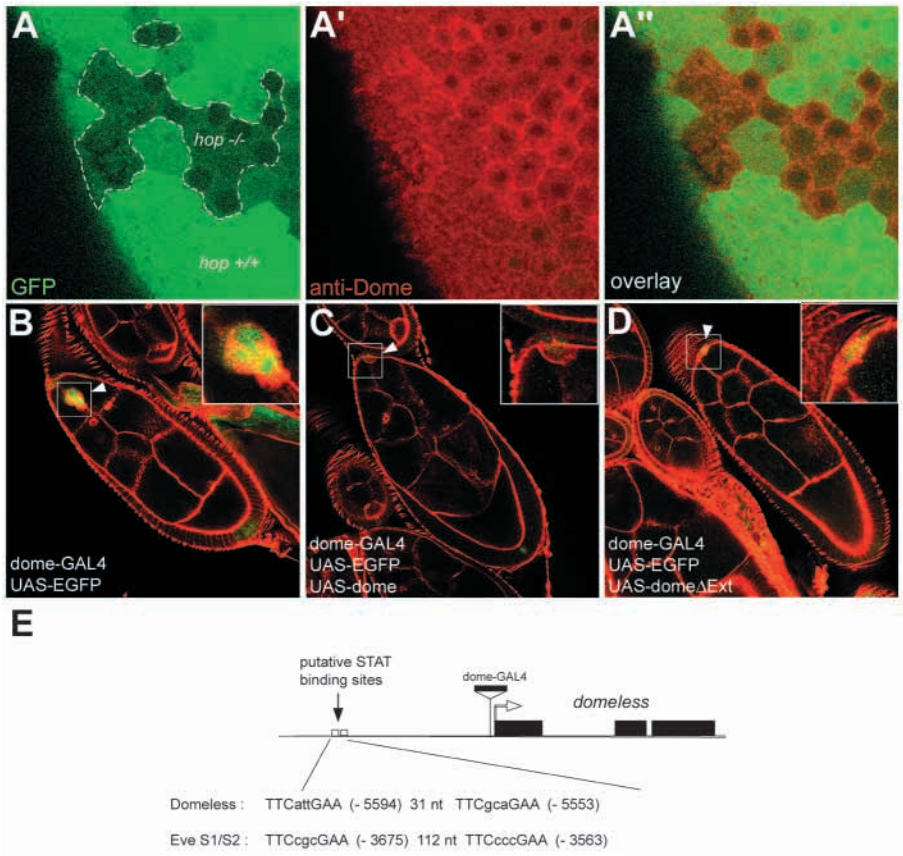


**Fig. 6.** Regulation of *dome* expression by the JAK/STAT pathway. In *hop* mutant follicle cells, Dome protein level is up-regulated (A; Dome is in red). *dome*-GAL4 is a pGAL4 element inserted upstream to the *dome* transcription start site (E) that drives expression in BCs and posterior follicle cells (B). Overexpression of wild-type *dome* (C) strongly reduces the level of GFP driven by *dome*-GAL4 (compare B and C), while expression of *domeΔEXT* has no effect (D). In D, the reduction in GFP expression is due to the absence of BCs induced by the expression of *domeΔEXT* (see Fig. 5I). (E) Schematic view of the *dome* promoter showing the localization and sequence of 2 putative Stat92E binding sites.

of follicle cells, to control their mesenchymal-epithelial transition. In the absence of *crumbs*, precursors of the follicle cells do not encapsulate the germline cells, leading to egg chambers with epithelial discontinuities. In contrast, *crumbs* mutant cells that have been generated after the formation of the follicular epithelium have no apparent abnormalities, indicating that Crumbs is required for initial polarization of the epithelium, but not for its maintenance (Tanentzapf et al., 2000). Interestingly, *dome* mutant cells do not express the apical determinant Crumbs in follicle cells (Fig. 7G-J). We thus conclude that *dome* is necessary for the initial polarization of the follicular epithelium in the germarium through the control, direct or indirect, of *crumbs* expression.

## DISCUSSION

### Dome is a JAK/STAT pathway receptor in egg chambers

The *dome* gene has recently been identified because of its segmentation phenotype which is similar to that of *upd*, *Stat92E* and *hop* mutants (Brown et al., 2001; Chen et al., 2002). As *dome* encodes a putative membrane protein with distant homology to vertebrate type I cytokine receptors, it is proposed to function as a receptor for Upd. This conclusion is supported by experiments made in cell culture showing that nuclear translocation of Stat92E requires Dome in cells treated with Upd-conditioned medium (Chen et al., 2002).

We show here that *dome* interacts genetically with the *Stat92E* and *dpias* genes during BC migration, and that *dome* phenotypes in ovaries are similar to those found in *Stat92E* and *hop* mutants (Baksa et al., 2002; Beccari et al., 2002; McGregor et al., 2002; Silver and Montell, 2001). Furthermore, Stat92E nuclear localization is lost in *dome* mutant follicle cells (Fig. 1I,J), indicating that the mechanisms leading to Stat92E activation and subsequent nuclear translocation require *dome*. Since *dome* is epistatic to *upd* (Fig. 5M,N), our data indicate that *dome* is required downstream of *upd* and upstream of *Stat92E* for JAK/STAT signaling in egg chambers. Altogether, these results provide strong evidence that Dome is a receptor molecule for Upd during oogenesis.

Our study shows that Dome is not uniformly distributed at the membrane but is restricted to apicolateral regions. Other receptor molecules have been shown to preferentially localize to apicolateral membranes, such as the EGF and Notch receptors (Lopez-Schier and St Johnston, 2001; Sapir et al., 1998), suggesting that the apical region is an active signaling interface for several receptors in follicle cells. Indeed, the apical localization of *upd* mRNA, membrane Dome and Dome-containing vesicles support a model in which ligand-receptor interactions take place apically in follicle cells, to activate the JAK/STAT pathway.

Dome is a transmembrane protein with both extracellular and intracellular domains whose functions are unknown. The extracellular part contains a cytokine-binding module (CBM) and 3 fibronectin-type III domains likely participating in ligand binding, while the intracellular domain presumably interacts with Hop, through binding to one or several potentially phosphorylated tyrosines (Brown et al., 2001; Chen et al., 2002; unpublished results). Using truncated forms of Dome we

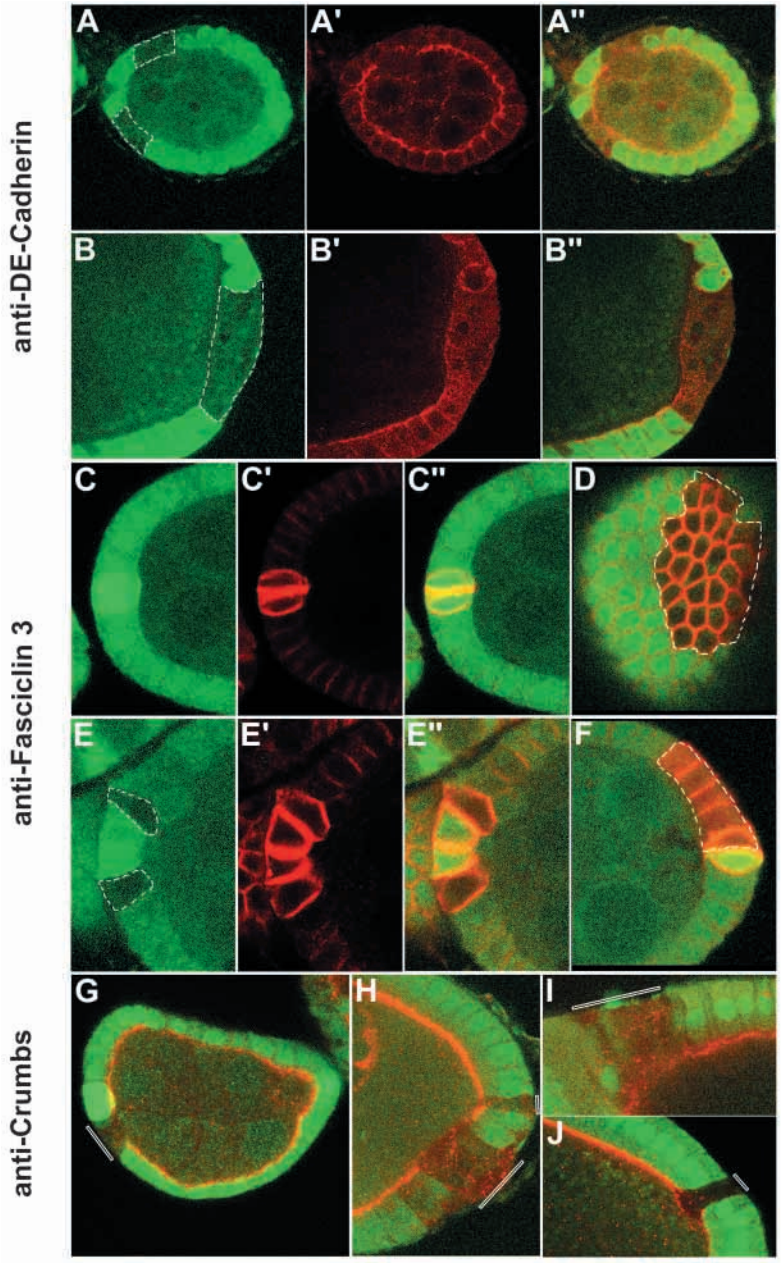


**Fig. 7.** Dome controls the expression of several follicle cell markers. Immunostaining (in red) of *dome* mosaic egg chambers using anti-DE-cadherin (A,B), anti-Fas3 (C-F) and anti-Crumbs (G-J) antibodies. (A,B) Confocal sections of two different egg chambers showing reduction of DE-cadherin. Fas3 is normally expressed in polar cells in wild-type egg chambers (C), and becomes overexpressed in *dome* mutant cells close to the anterior (E) or posterior (F) polar cells. *dome* mutant clones generated away from the poles also accumulate Fas3 (D). Mutant clones have been outlined (dotted lines in A,B,D,E,F and solid bar in G-J). Anterior is to the left.

show that both the extracellular and intracellular domains are essential for BC migration and signal transduction (Fig. 5). The dominant negative phenotypes that are observed are consistent with a model in which DomeΔCYT would titrate the ligand Upd, and DomeΔEXT would titrate Hop, therefore inducing a dramatic reduction in signaling strength. Both constructs may also lead to the formation of non-functional Dome-Dome dimers by capturing the wild-type Dome protein in an inactive complex. Further biochemical work will be necessary to understand the molecular mechanisms underlying Dome signal transduction.

### *dome* is transcriptionally and post-transcriptionally regulated in follicle cells

Previous studies have shown that the migration of BCs is sensitive to gene dosage, making it useful for genetic screens (Beccari et al., 2002; Duchek and Rorth, 2001; Duchek et al., 2001). The reduction or elevation of *slbo*, a gene encoding a C/EBP homolog (Montell et al., 1992), is sufficient to produce BC migration defects. Consistently, recent work has shown that Slbo protein levels are tightly regulated by the ubiquitination pathway (Rorth et al., 2000). Our results show that the BCs are also sensitive to changes in Dome protein levels. Indeed, either a decrease (Fig. 1) or an increase (Fig. 5) of Dome causes BC migration defects. There are several mechanisms by which gene activity can be regulated, including post-translational regulation, as with the Slbo protein (Rorth et al., 2000), or transcriptional regulation. Our data suggest that *dome* expression is regulated in part by a transcriptional negative feedback loop (Fig. 6). Two consensus STAT binding sites (Yan et al., 1996) present in the promoter region of the *dome* gene may prove to be important for this regulation. Interestingly, it has been shown that vertebrate STAT proteins can have both positive and negative regulatory functions (Ramana et al., 2000). Further work will be necessary to determine whether Stat92E is a direct repressor of *dome*.

In addition to a transcriptional control of *dome*, there are also post-translational mechanisms regulating Dome function in follicle cells, through a dynamic pattern of intracellular vesicles. We show that these Dome-containing vesicles are located at a relevant distance from Upd-producing cells, and that Upd can promote the formation of de novo vesicles upon ectopic expression. These results, together with the presence of several tyrosine-based and di-leucine motifs known to sort proteins for internalization (Trowbridge et al., 1993), are consistent with Dome-containing vesicles being the result of endocytosis upon ligand binding. Endocytosis is an important process controlling several signaling pathways during development (Seto et al., 2002), acting on the recycling or desensitization of ligand-receptor complexes. Our work thus provides the first experimental evidence that JAK/STAT signaling in flies may be regulated by endocytosis.

### Roles of *dome* in follicle and border cells

Our analysis of *dome* reveals several important functions in follicle cells. First, we show that during early oogenesis, *dome* is required for the encapsulation of germline cells into a functional egg chamber. *dome* mutant follicle cells made in the germarium are unable to assemble into the nascent follicular epithelium, thus leading to incompletely encapsulated egg chambers at stage 2-3 (Fig. 1F,G). The fusion of some egg

chambers seen in our and other studies (Fig. 1G and data not shown) (Baksa et al., 2002; McGregor et al., 2002), suggests that follicle cells normally separating adjacent egg chambers in the germarium have rapidly degenerated. This conclusion is supported by the fact that mutant cells cannot be observed in early egg chambers (Fig. 1). This is an alternative to the model in which the formation of fused egg chamber would be associated to stalk cell defects (McGregor et al., 2002).

The dramatic, early follicle cell phenotype contrasts with the essentially normal phenotype of *dome* mutant cells observed in later stage egg chambers. In this case, follicle cells are viable and divide normally (Figs 3, 4, 6, 7). A similar, dual phenotype has been reported in *crumbs* mutant chambers. After initial polarization of the follicle cells in the germarium, Crumbs is no longer required and its loss has no visible effects (Tanentzapf et al., 2000). Importantly, we show here that *dome* controls Crumbs expression in follicle cells, thus providing a novel link between the JAK/STAT signaling pathway and epithelial polarity.

In addition to its early function in the germarium, *dome* is required for the normal expression of several follicle cell markers, including DE-cadherin and Fas3. It is important to note that despite a clear defect in the expression of these markers, *dome* mosaic egg chambers are morphologically normal. However, because completely mutant egg chambers cannot be obtained because of the early effect of *dome* in the germarium, one cannot rule out the possibility that large mutant clones would lead to abnormal development of egg chambers.

The pattern of epithelial markers in *dome* mutant cells indicates that the JAK/STAT pathway is active in all follicle cells (Fig. 7), a notion that is reinforced by the wide expression of nuclear Stat92E (Fig. 1). How is Dome activated during egg chamber development and does this activation follow the same profile at all stages? Given the restricted pattern of *upd* expression in the egg chamber and its dramatic effect upon overexpression, it is unlikely that Upd is able to signal long distances in the follicular epithelium of late stage egg chambers. Rather, we favor a model by which the JAK/STAT pathway plays a pre-patterning function, acting early during egg chamber development to activate DE-cadherin and Crumbs expression (see also McGregor et al., 2002). This view is consistent both with the expression pattern of *upd* and the distribution of Dome-containing vesicles described in this study. We have shown that the formation of endogenous vesicles can be promoted by Upd, and that a gradient of such vesicles is present around polar cells. Strikingly, these vesicles, which likely indicate active signaling through Dome, are widespread at early stages and become more restricted later on. We propose that during early development, the Upd signal produced by anterior and posterior polar cells contributes to the differentiation of all follicle cells. At this stage, Upd would be more diffusible than later, as suggested by the pattern of Dome intracellular vesicles (Fig. 4). The study of the mechanisms controlling Dome activation and Upd activity will require additional tools to directly detect Upd, as, for example, Upd-GFP fusion proteins.

In contrast to the situation in main body follicle cells, the role of *dome* in BCs is essential. *dome* and other JAK/STAT pathway components (Beccari et al., 2002; Silver and Montell, 2001) promote the differentiation of a selected group of follicle cells into a cohesive migratory cluster, a process requiring several other inputs (Montell, 2001). Mutations in *dome* induce phenotypes ranging from a complete absence of BCs to non-cohesive BC clusters. This suggests that *dome* may be required during migration itself, in addition to its role in the recruitment of BCs at stages 8-9. Although only conditional mutants could help to address this question, the pattern of Dome vesicles in the BCs before and during migration supports a sustained activation of the JAK/STAT pathway. Such a requirement could also explain the semi-dominant migration phenotype of *dome* heterozygous egg chambers (Fig. 1).

Our study has revealed several new findings about the function of *dome* and the JAK/STAT pathway during oogenesis. Future work will help to understand how Upd and Dome initially interact at the cell surface and transduce the signal to downstream JAK/STAT pathway members.

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