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

Christian Ghiglione*, Olivier Devergne*, Emmanuelle Georgethumb, Fabrice Carbèlès, Caroline Médion, Delphine Cerezo and Stéphane Noselli†

Institute of Signaling, Developmental Biology and Cancer, UMR 6543 – CNRS, Parc Valrose, 06108 NICE cedex 2, France

*These authors contributed equally to this work
†Author for correspondence (e-mail: noselli@unice.fr)

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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 metazoaos. 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 Star92E) (Hou et al., 1996; Yan et al., 1996). The only known ligand for the *Drosophila* JAK/STAT pathway is encoded by the unpaired (upd) 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 (Baksá et al., 2002; Becvari 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*111 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*111, 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: *domeP100* FRT19A/FM7; *dome1208* FRT19A/FM7; *domePG14* FRT19A/FM7i. Other stocks used in this study are: *hop*111 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 EcoRI and XhoI and ligated into the EcoRI and XhoI sites of the transformation vector pUAST cut with EcoRI and XhoI.

pUAS-domeGFP was constructed by single step ligation of the following DNA fragments: (i) EcoRI-XhoI 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 EcoRI and XhoI.

#### 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.
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^{FL100/+} female. The BC cluster is not correctly assembled with some cells being left behind (arrowheads). (D,E) Mosaic egg chambers containing dome^{FL100/+} 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* (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. 3D,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

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**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.
the formation of Dome-containing intracellular vesicles apically. Polar cells also express upd (Fig. 1A). In the posterior region of egg chambers, and female sterility (data not shown). This phenotype indicates a dose-sensitive effect of upd. Slbo-promoted polar cell 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

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-domeCYT (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-DomeCYT*) 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 DomeCYT (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. 5E). *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 DomeEXT 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

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**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,H,I) 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, TTCNNGAA (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 (DomeD_CYT or DomeD_EXT) 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.
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. 11J), 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. 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.
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 Dome\textsubscript{CYT} would titrate the ligand Upd, and Dome\textsubscript{EXT} 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 chambers. Rather, we favor a model by which the JAK/STAT pathway is active in all follicle cells. At this stage, Upd would be more diffusible than later, as suggested by the pattern 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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