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Asymmetric localisation of cytokine mRNA is essential for JAK/STAT activation during cell invasiveness

Véronique Van De Bor, Geordie Zimniak, Delphine Cérézo, Sébastien Schaub and Stéphane Noselli*

SUMMARY

The transition from immotile epithelial cells to migrating cells occurs in all organisms during normal embryonic development, as well as during tumour metastasis. During *Drosophila* oogenesis, border cells (BCs) are recruited and delaminate from the follicular epithelium. This process is triggered by the polar cells (PCs), which secrete the cytokine Unpaired (Upd) and activate the JAK/STAT pathway in neighbouring cells, turning them into invasive BCs. Interestingly, either a decrease or an increase in BC number alters migration, indicating that mechanisms controlling the level of JAK/STAT signalling are crucial in this process. Here, we show that PCs have a highly stable and polarised network of microtubules along which *upd* transcripts are asymmetrically transported in a Dynein-dependent manner. We demonstrate that in the absence of *upd* mRNA localisation the ligand is no longer efficiently secreted, leading to a loss of signalling strength as well as recruitment and migration defects. These findings reveal a novel post-transcriptional regulatory mechanism of JAK/STAT signalling in the control of epithelial cell invasiveness.

KEY WORDS: Cell migration, JAK/STAT, *Drosophila*

INTRODUCTION

In many cell types, the switch from stationary to migratory behaviour is associated with changes in gene expression (Thiery et al., 2009; Yang and Weinberg, 2008). During this transition, migrating cells have to detach from the epithelium, develop novel interactions with the extracellular environment, become competent to extend protrusions and decipher new guidance cues (Yilmaz and Christofori, 2009). Studying migration under physiological conditions is crucial to understand the molecular mechanisms that control how subsets of cells become motile during development or tumour metastasis.

Border cell (BC) migration in *Drosophila* ovaries provides a powerful model with which to study regulated invasive cell migration in vivo. The egg chamber is the functional unit of the fly ovary and is composed of an oocyte, 15 nurse cells and surrounding follicle cells assembled into an epithelial monolayer. BCs are organised into a cluster of about eight epithelial cells [two polar cells (PCs) and six outer border cells (oBCs)], which delaminate and migrate in between the nurse cells to reach the oocyte and contribute to the formation of the micropyle, a structure required for fertilisation (Fig. 1A). Delamination and migration are initiated at stage 9 and occur over a period of 6 hours. This process has been well characterised both in fixed and living egg chambers and has proven to be robust and stereotyped (Bianco et al., 2007; Jang et al., 2007; Prasad et al., 2007; Prasad and Montell, 2007; Rorth, 2007).

The well-conserved JAK/STAT (Janus kinase/Signal transduction and activator of transcription) signalling pathway is known to promote cell proliferation, survival and cell fate differentiation in different organisms. In *Drosophila*, JAK/STAT signalling is essential for embryonic segmentation, eye and wing development, immune response, stem cell development and BC specification and migration (Hombria and Brown, 2002; Hou et al., 2002). oBCs are recruited by a pair of specialised cells, the PCs, which secrete the cytokine Unpaired (Upd; Outstretched – FlyBase), the main ligand of the pathway. Upd binding to its receptor Domeless (Dome) (Chen et al., 2002; Harrison et al., 1998; Ghiglione et al., 2002) in neighbouring cells activates the JAK/STAT cascade. Activated Hopscotch (Hop), the JAK orthologue, phosphorylates the transcription factor Stat92E, which then enters the nucleus and induces the expression of target genes including *slow border cells* (*slbo*), which encodes a transcription factor related to mammalian C/EBPs, the expression of which is essential to promote BC invasiveness (Montell et al., 1992). Indirect evidence suggests that Upd activates adjacent cells in a graded manner. Indeed, receptor endocytosis shows a gradient of internalisation, with its maximal level close to the PCs (Devergne et al., 2007; Ghiglione et al., 2002; Xi et al., 2003).

It is essential that the correct number of oBCs be specified because an excess or a deficit of recruited cells prevents migration and reduces fertility (Silver et al., 2005). Hence, loss of function of any component of the JAK/STAT pathway impairs oBC recruitment, whereas overactivation of the pathway is sufficient to induce ectopic follicle cells to become invasive (Silver and Montell, 2001). These findings suggest that JAK/STAT activity needs to be tightly controlled and that a specific level of signalling must be reached close to the ligand source, allowing the selection of an appropriate number of oBCs. One control mechanism involves the DNA-binding protein Apontic (Apt), which spatially regulates JAK/STAT signalling in the egg chamber follicular epithelium and prevents the recruitment of cells distant from the ligand source (Starz-Gaiano et al., 2008). In addition, it was shown that JAK/STAT activity has to be maintained during the entire process of migration to prevent splitting of the cluster (Silver et al., 2005). However, no mechanism involved in the positive regulation of JAK/STAT activation has been demonstrated.

Many signalling pathways, including JAK/STAT, have been shown to be partially polarised (Hombria and Sotillos, 2008), and polarisation or subcellular localisation of pathway components

Institute of Developmental Biology and Cancer UMR6543/CNRS, University of Nice Sophia-Antipolis, Parc Valrose, 06108 Nice cedex 2, France.

*Author for correspondence (noselli@unice.fr)

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emerge as key mechanisms to control signalling specificity and/or efficiency. In the ectoderm, JAK/STAT signalling is modulated by the cell polarity protein Par3 (Bazooka – FlyBase) (Sotillos et al., 2008). Furthermore, in egg chambers, both Dome and Dome-containing endocytic vesicles localise apicolaterally, suggesting that the ligand has to be presented to the apical membrane for correct pathway activation (Devergne et al., 2007; Ghiglione et al., 2002). Finally, *upd* mRNA is apically enriched in the PC, suggesting a polarised synthesis and release of the protein (Ghiglione et al., 2002). mRNA localisation is a well-characterised and conserved mechanism that plays an important role in targeting proteins to their site of function in different organisms (Kardon and Vale, 2009; Martin and Ephrussi, 2009; Tekotte and Davis, 2002; Wade, 2009). Recent studies revealed that the substantial majority of *Drosophila* mRNAs have specific subcellular localisations (Lecuyer et al., 2007), indicating that mRNA targeting is more widely used than first anticipated. Nonetheless, it is not yet clear whether mRNA localisation can be involved in signalling regulation.

In this study, we identify a novel positive-regulatory mechanism that controls JAK/STAT signalling through intracellular trafficking and apical localisation of the *upd* cytokine mRNA. We show that PCs possess a dense and polarised microtubule network, the integrity of which is required for BC specification and migration. *upd* mRNA appears to be apically concentrated in the PC in a Dynein- and Bicaudal D (BicD)/Egalitarian (Egl)-dependent manner. We show that in the absence of *upd* transcript localisation, secretion of the Upd protein is affected and JAK/STAT signalling is dramatically reduced, leading to recruitment and migration defects. We identified the *upd* localisation element in the open reading frame (ORF) region, suggesting an important selection constraint on this motif. We propose that *upd* transcript localisation is essential to optimise Upd apical secretion as well as JAK/STAT activation in epithelial cells that require the highest levels of ligand. This work reveals a novel post-transcriptional regulatory mechanism controlling cell invasiveness.

MATERIALS AND METHODS

Fly strains

D. melanogaster stocks were raised on standard corn meal agar medium at 25°C. w^{1118} or nlsGFP strains were used to inject fluorescently labelled mRNA. The following strains were used: UAS-SpastinGFP (Jankovics and Brunner, 2006), Slbo-gal4, Upd-gal4 (gift from D. Montell), 10×STAT92E-GFP (Bach et al., 2007), Nod-lacZ (Clark et al., 1997), UAS-Upd (M. Zeidler and N. Perrimon, Sheffield, UK and Boston, MA) and *Sas-4* null mutants (gift from J. Raff, Oxford, UK). UAS-BicD RNAi, UAS-Egl RNAi, UAS-Glued RNAi and UAS-Lis-1 RNAi strains are from the Vienna Drosophila RNAi Center. Dynein mutant clones were generated by crossing the *Dhc4-19, FRT80/TM6b* females with *e22cgal4, UAS-FLP/CyO; FRT80, UbiGFP* males. *e22cgal4, UAS-FLP/+; Dhc4-19, FRT80/UbiGFP, FRT80* females were kept for 48 hours at 30°C before dissection (Li et al., 2008).

Immunohistochemistry and RNA in situ hybridisation

Ovaries were dissected in 1×PBS and fixed in 3.7% formaldehyde. The samples were blocked in 1×PBS containing 0.3% Triton X-100 and 2% BSA prior to incubation with antibodies and then mounted in Vectashield (Vector Laboratories). For extracellular staining, ovaries were dissected on ice and incubated with the primary antibody in Schneider medium containing 10% foetal calf serum (FCS) for 1 hour 30 minutes at 4°C. Ovaries were washed three times in 1×PBS at 4°C and fixed for 20 minutes in 3.7% formaldehyde. The following primary antibodies were used: mouse anti-α-tubulin-FITC (1/250; Sigma); mouse anti-α-tubulin clone DM1A (1/500; Sigma); rabbit anti-Slbo (1/2000); mouse anti-Fas3 7G10 [1/100; Developmental Studies Hybridoma Bank (DSHB)]; rabbit anti-Upd [1/1000 for conventional staining, 1/300 for extracellular staining; gift from D. Harrison (Ghiglione et al., 2008)]; sheep anti-digoxigenin-POD (1/1000; Roche); and rabbit anti-PLP (1/500; gift from J. Raff). Cy3-, Alexa Fluor 546-, Alexa Fluor 488- and Cy5-conjugated secondary antibodies (Molecular Probes, Jackson ImmunoResearch) were used. Hoechst was used to visualise DNA. In situ hybridisation signal was revealed using the Fluorescent Tyramide Detection Kit (TSA, NEN LifeSciences). Digoxigenin-labelled antisense probes were prepared using full-length *upd* cDNA (gift from D. Harrison, Lexington, KY).

Colchicine treatment

Microtubule alteration was induced by Colchicine treatment (65 μg/ml; Sigma). Colchicine was mixed with dry yeast and added to the corn meal agar medium at room temperature. Flies were fed for 48 hours on this medium before dissection.

Synthesis of fluorescently labelled RNA and injection

RNA was transcribed in vitro using T3, T7 or SP6 RNA polymerase and UTP-Alexa 546 (Molecular Probes) (Wilkie and Davis, 2001). Plasmids containing *gfp* cDNA were used as a negative control. Plasmids containing full-length or truncated fragments of *upd* cDNA were used. Constructs were generated by PCR amplification and cloned into the pGEM-T Easy vector (Promega). The plasmids were sequenced to confirm the final products. Embryo injections were performed as described (Wilkie and Davis, 2001) at 250-500 ng/μl. P1H4 antibody was pre-injected 10 minutes before RNA injection.

Image analysis and statistical methods

Imaging was performed on a Zeiss LSM 510 confocal microscope or on a wide-field DeltaVision microscope (Applied Precision, DV 3.0 system, IX70 Olympus microscope and CH350 camera) and deconvolved using SoftWorx software. Particle tracking was performed manually using ImageJ and SoftWorx Explorer. The speed of particles was calculated in micrometers per second according to the distance travelled in each time interval. ImageJ was used to quantify the GFP level relative to Hoechst staining. Experiments were performed at least twice in parallel, with the control processed under the same conditions and on the same day. Image acquisitions were performed using the same parameters. Quantifications were made with ImageJ. Statistical analysis of the number of oBCs was performed in Excel using a paired *t*-test to compare wild-type and mutant conditions. Statistical significance of migration defects was evaluated using a χ^2 test of the mutant conditions versus control (5% critical value, one degree of freedom).

RESULTS

Microtubules are strongly polarised in the polar cells and are involved in border cell recruitment and migration

In order to characterise microtubule (MT) organisation in BC clusters, we examined their overall distribution and density as well as their polarity using anti-α-tubulin antibodies and a Nod-lacZ construct, respectively. The Nod-lacZ flies carry a MT motor fusion leading to β-gal accumulation at the minus end of MTs (Clark et al., 1997). At stage 8 (i.e. before BC cluster delamination), the α-tubulin staining was apically enriched and revealed a dense network of MTs in the PCs and oBCs, confirming previous observations (Dahlgaard et al., 2007; Doerflinger et al., 2003) (Fig. 1B). Double β-gal and α-tubulin stainings revealed a colocalisation between apical α-tubulin and the minus ends of MTs that was particularly visible in the PCs (Fig. 1B). At stage 9, when migration starts, PCs rotate 90° from their initial anteroposterior orientation and are found roughly orthogonal to the direction of migration (Pinheiro and Montell, 2004). Interestingly, their MT network maintained the same strongly polarised architecture (Fig.

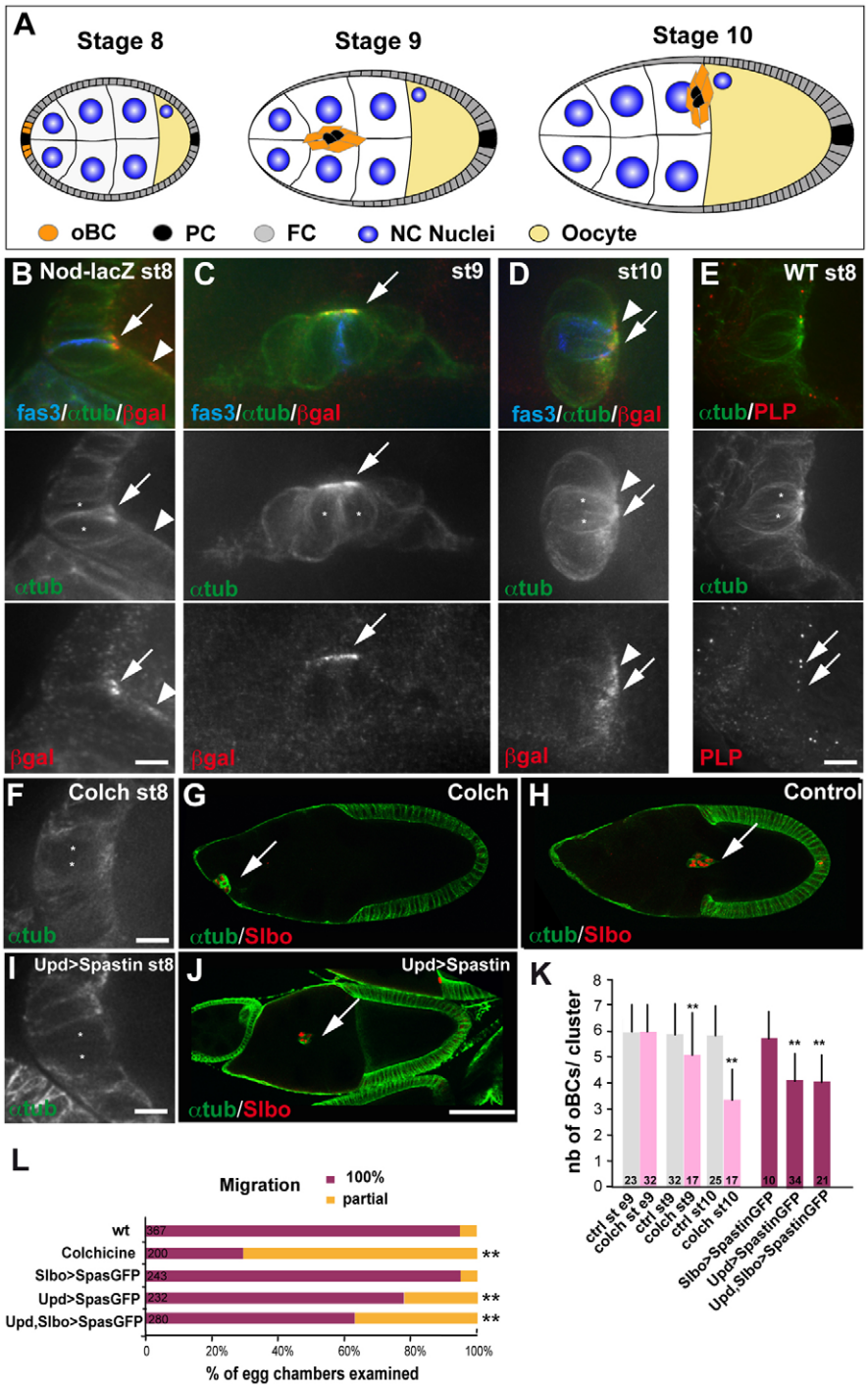

Fig. 1. Microtubule organisation in the BC cluster. (**A**) *Drosophila* egg chambers at different stages of development. oBCs are recruited from the epithelium by the two PCs (stage 8), then migrate through the nurse cells (stage 9) and reach the oocyte (stage 10). oBC, outer border cell; PC, polar cell; FC, follicle cell; NC, nurse cell. (**B-D**) High magnification of Nod-lacZ egg chambers stained for Fas3 (blue) to identify the PCs, and for α-tubulin (green) and β-Gal (red) to visualise microtubules (MTs) and their minus ends, respectively. In this and all subsequent figures posterior is to the right. Arrows and arrowheads indicate the strong accumulation of β-Gal staining at the apical side of PCs and oBCs, respectively. (B) Stage 8, before delamination from the epithelium. (C) Stage 9, migration has started. (D) Stage 10, the cluster has reached the oocyte. Asterisks indicate the PCs. (**E**) Wild-type (WT) stage 8 egg chamber stained for α-tubulin and PLP to visualise centrioles. (**F**) Stage 8 Colchicine-treated egg chamber stained for α-tubulin, which shows alteration of the MT network. (**G**,**H**) Colchicine-treated and control egg chambers stained for α-tubulin (green) and Slbo (red). Colchicine treatment induces a partial migration phenotype (compare G with H, arrow). (**I**) Stage 8 Upd-gal4xUAS-Spastin egg chambers show a dramatic reduction in MT staining in the PCs (asterisks). (**J**) Stage 10 Upd-gal4xUAS-Spastin egg chamber stained for α-tubulin and Slbo shows partial migration (arrow). (**K**) The number of oBCs per cluster in the indicated genotypes. Error bars indicate s.d.; the number of clusters analysed (*n*) is indicated. **, $P<0.01$ versus controls; paired *t*-test. (**L**) The percentage of BCs showing migration defects in the indicated genotypes. **, $P<0.005$; χ^2 test of the mutant conditions versus control (5% critical value, one degree of freedom). Scale bars: 10 μm in B-F,I; 50 μm in G,H,J.

1C). By contrast, the oBC MT cytoskeleton showed no clear orientation as revealed by the dispersed β-gal staining (Fig. 1C). When the cluster reached the oocyte at stage 10, both the PCs and oBCs showed an accumulation of MT minus ends toward the posterior (Fig. 1D). Hence, in comparison to the oBCs, which seem to have a very dynamic cytoskeleton, the PCs exhibit a very dense and stable MT network during the entire process of migration. In order to analyse centrosome positioning, we stained egg chambers using anti-PLP (Pericentrin-like protein; Cp309 – FlyBase) antibody (Martinez-Campos et al., 2004). Pericentrin was found at the apical side, confirming that MTs emerge from the apical surface of follicle cells (Fig. 1E). However, it appears that centrosomes are not essential for BC migration because acentriolar *Sas-4* mutant females (Stevens et al., 2007) showed normal MT organisation and BC migration (data not shown).

To test whether MTs are required for oBC specification and/or migration, we used Colchicine to prevent MT polymerisation. Wild-type females were fed for 2 days on standard medium supplemented with Colchicine and their ovaries analysed. We observed that MTs were strongly affected and that BC clusters arrested before they reached the oocyte in 70% of the chambers (compare Fig. 1F,G with 1H). At early stage 9, the number of oBCs expressing Slbo, a marker of oBCs in delaminating clusters, was identical to that of the control (6), but dropped to 3.3 at stage 10

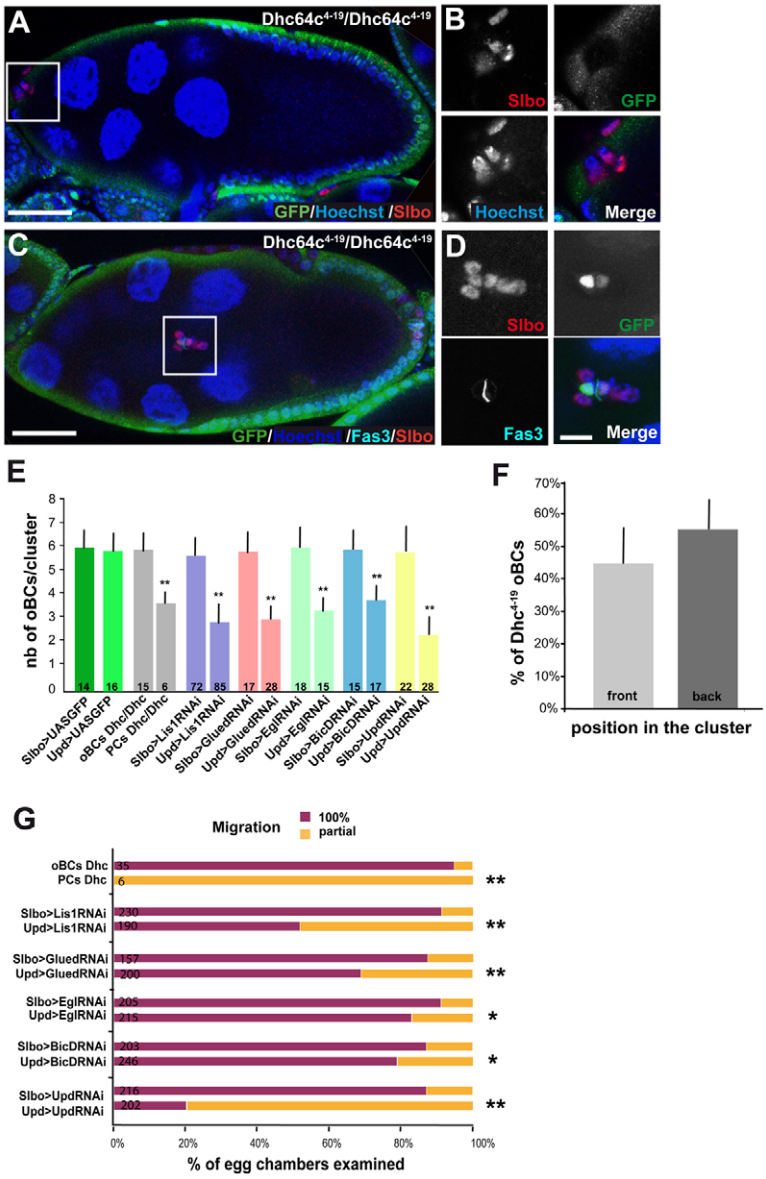

Fig. 2. Dynein motor function is required in polar cells for the proper specification and migration of border cells. (**A-D**) *Drosophila* late stage 9 egg chambers showing a *Dhc64C*$^{4-19}$ mutant clone stained with GFP (green) to identify the mutant cells, with Hoechst (blue) to visualise the DNA, for Slbo (red) to mark the BCs and for Fas3 (cyan) to label the PCs. *Dhc64C* homozygous mutant cells do not express the GFP marker. (A) Homozygous mutant clone comprising the entire BC cluster. The mutant cluster is found attached to the anterior tip. (B) High-magnification views of the boxed region in A. (C) Mosaic clone in which all the oBCs are mutant and the PCs are wild-type. Cluster migration is normal. (D) High magnification of the boxed region in C. (**E**) The mean number of oBCs per cluster for the indicated genotypes + s.d. The number of clusters analysed (*n*) is indicated. **, $P<0.01$ versus controls; paired *t*-test. (**F**) The position of *Dhc64C*$^{4-19}$ mutant oBCs in clones. Error bars indicate s.d. (**G**) The migration phenotype in the indicated genotypes. *, $P<0.05$; **, $P<0.005$; χ^2 test of the mutant conditions versus control (5% critical value, one degree of freedom). The number of egg chambers analysed (*n*) is indicated. Scale bars: 50 μm in A,C; 10 μm in B,D.

(Fig. 1K). Thus, after Colchicine treatment, the Slbo marker is not efficiently maintained (see below). As Colchicine feeding alters MTs in the whole egg chamber, we expressed Spastin, a MT-severing protein, to disrupt MTs specifically in the BCs. Spastin expression has been shown to cause very efficient MT disassembly in larvae and embryos (Jankovics and Brunner, 2006; Sherwood et al., 2004). Expression of Spastin in the oBCs (using a Slbo-gal4 driver) did not cause any phenotype (Fig. 1K,L). By contrast, MT disruption in the PCs (using a Upd-gal4 driver) specifically induced a decrease in oBC recruitment (4.5) as well as migration defects, with ~30% of the clusters not reaching their final destination at stage 10 as compared with 5% in the control (Fig. 1J-L). These results demonstrate that a polarised MT network is required in the PCs for normal BC specification and migration.

Dynein and the BicD-Egl complex are involved in border cell recruitment and migration

The dynein motor complex is responsible for cargo transport towards the minus ends of MTs in all eukaryotic cells. To investigate whether intracellular transport along MTs is involved in BC specification and migration, we analysed the effects of cytoplasmic Dynein motor loss-of-function in egg chambers. Dynein heavy chain 64C (Dhc64C) is a component of the dynein complex, the disruption of which prevents all forms of cargo transport and is embryonic lethal (Vale, 2003). To bypass lethality, we performed clonal analysis using two *Dhc64C* lethal alleles, *Dhc*$^{4-19}$ and *Dhc*^{902} (Li et al., 2008), which showed similar phenotypes. Interestingly, clusters of *Dhc64C* mutant BCs did not migrate and were found at the anterior tip of the egg chambers (Fig. 2A,B). The number of cells per cluster was determined by observing the Slbo and Fas3 markers, which are expressed in the oBCs and the PCs, respectively. *Dhc64C* mutant clusters comprised only 3.5 oBCs on average as compared with six in the wild type (Fig. 2E), indicating that Dynein is involved in BC specification and migration. To determine whether Dynein function is required in the PCs and/or the oBCs, we analysed mosaic BC clusters consisting of wild-type and mutant cells. Interestingly, clusters in which only oBCs were mutant for *Dhc64C* showed no migration defects (Fig. 2C,D,G) and had the normal number of oBCs. Furthermore, *Dhc64C* mutant oBCs were randomly distributed at

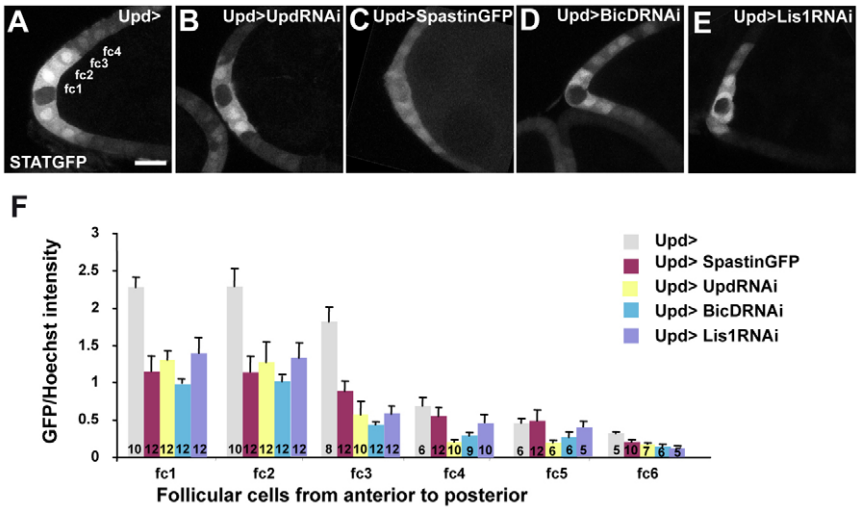

Fig. 3. JAK/STAT signalling is altered in the absence of Dynein transport. (**A-E**) *Drosophila* stage 8 egg chambers expressing GFP under the control of ten STAT binding sites. (A) Upd-gal4; 10×STAT92E-GFP/+. (B) Upd-gal4; 10×STAT92E-GFP/UAS-Upd RNAi. (C) Upd-gal4; 10×STAT92E-GFP/UAS-SpastinGFP. (D) Upd-gal4; 10×STAT92E-GFP/UAS-BicD RNAi. (E) Upd-gal4; 10×STAT92E-GFP/UAS-Lis-1 RNAi. fc, follicular cell. (**F**) GFP nuclear staining intensity relative to Hoechst staining intensity in the different follicular cells, fc1 being the most anterior cell as indicated in A. Each experiment was repeated at least twice. The number of follicular cells analysed (*n*) is indicated. Error bars indicate s.e.m.

the front or at the rear, indicating that oBC migration and shuffling behaviour does not require Dynein (Fig. 2F) (Bianco et al., 2007; Prasad et al., 2007). We conclude that Dynein function is required in the PCs, but not in the oBCs, for normal specification and migration of the cluster.

Dynein associates with adaptor proteins, such as the large multi-protein dynactin complex, which facilitates Dynein processivity and interaction with its cargoes, and Lissencephaly-1 (Lis-1), which modulates Dynein association with the MTs (Kardon and Vale, 2009). We tested the role of the dynactin component Glued (also known as p150) and Lis-1 using double-stranded RNA-mediated knockdown specifically in the PCs or oBCs. Interestingly, when Glued or Lis-1 was depleted in the PCs (Upd-gal4 driver), but not when depleted in the oBCs (Slbo-gal4 driver), the number of oBCs was reduced by half and migration defects were observed as for the *Dhc64C* mutant clones or after Upd RNAi expression (Fig. 2E,G). BicD and Egl proteins are required for the Dynein-dependent localisation of many mRNAs in *Drosophila*. Egl is an mRNA-binding protein that associates with the BicD Dynein co-factor, thus bridging mRNAs to motors (Claussen and Suter, 2005; Dienstbier et al., 2009; Nashchekin and St Johnston, 2009). To test their role in BC migration, BicD and Egl were specifically depleted in the PCs or oBCs by RNAi. Absence of BicD or Egl in the PCs induced a reduction in oBC recruitment as well as migration defects (Fig. 2E,G). By contrast, depletion of BicD or Egl in the oBCs alone showed no defect as compared with the wild type (Fig. 2E,G). Altogether, these data indicate that the dynein complex as well as the adaptor proteins BicD and Egl are specifically required in the PCs for cluster differentiation and migration.

JAK/STAT signalling is altered in the absence of Dynein cargo transport

Slbo is a known target of the JAK/STAT pathway. Therefore, reduction in the number of Slbo-expressing cells in egg chambers through alteration of the MT network or Dynein function (Fig. 1G,J,K) suggests that JAK/STAT signalling might be affected. To further investigate the role of MT or Dynein in oBC specification through JAK/STAT signalling we used the 10×STAT92EGFP reporter line, which accurately reflects JAK/STAT activity (Bach et al., 2007; Starz-Gaiano et al., 2008). At stage 8, GFP accumulates in the nuclei of follicular cells surrounding the PCs in a graded manner. We quantified the level of nuclear GFP relative to Hoechst staining in anterior follicular cells (fc) as a function of their position, fc1 being the closest to the PC. In the absence of Upd ligand there was a strong reduction in GFP intensity from fc1 to fc6 compared with the control, which correlated with the reduction in Slbo expression in this context (Fig. 2E, Fig. 3A,B,F). Interestingly, when MT integrity or Dynein motor function was altered in the PCs using SpastinGFP or by BicD, Egl or Lis-1 RNAi, we observed a substantial decrease in GFP intensity from fc1 to fc3 (Fig. 3A,C-F). These data show that, in the absence of Dynein transport along MTs, JAK/STAT signalling is dramatically decreased leading to a reduction in oBC recruitment. To confirm the genetic interaction between the JAK/STAT pathway and the transport machinery, we tested whether the Egl and Glued RNAi phenotypes were enhanced in a *upd* heterozygous mutant condition. Using three different *upd* alleles, we found that the number of oBCs per cluster was significantly lower in all three conditions than in the control (see Fig. S1 in the supplementary material).

We next looked directly at the distribution of the Upd protein by immunostaining and found that in the wild type, Upd is present in the PCs as well as in two to three adjacent cells in a punctate pattern, most likely representing exocytic and endocytic Upd vesicles, respectively (Fig. 4A-C,I). Strikingly, the staining revealed an apical enrichment of Upd protein in PCs throughout the entire process of migration, suggesting that the protein is preferentially secreted from their apical region (Fig. 4I,J).

This result is consistent with previous indirect evidence suggesting that Upd is secreted from the apical surface of the PCs to act on adjacent cells in a graded manner (Devergne et al., 2007). In order to confirm this hypothesis, a specific immunostaining protocol (Strigini and Cohen, 2000) was performed that allows the exclusive visualisation of secreted Upd protein in the extracellular space. An accumulation of extracellular Upd was detected in the apical region of the PCs (Fig. 4D,I,J; data not shown) from delamination to the end of migration, confirming that Upd is constantly apically secreted to activate the JAK/STAT pathway (Silver et al., 2005). Antibody specificity was validated using Upd RNAi knockdown, a condition in which no antibody signal was detected (Fig. 4H-J). After either Colchicine or Spastin treatment, or in Glued, BicD and Egl loss-of-function conditions, apical secretion of the Upd protein was completely lost and the punctate signal in adjacent cells reduced, suggesting that less Upd was available in the extracellular space for signalling and for endocytosis by receiving cells (Fig. 4E-J; data not shown).

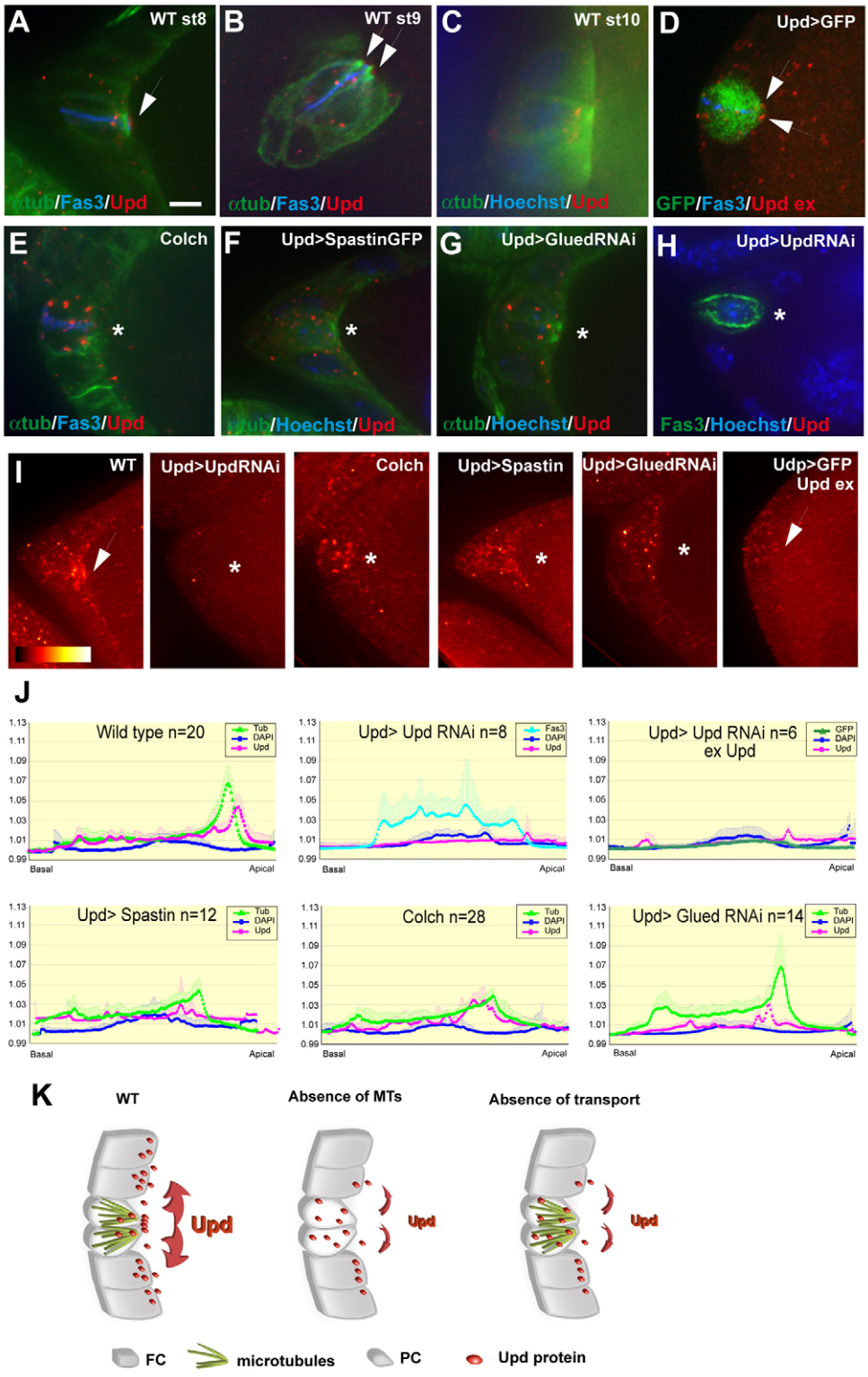

Fig. 4. Absence of Dynein transport affects Upd protein secretion. (**A-H**) Wild-type *Drosophila* egg chambers at stage (st) 8, 9 and 10 stained for Upd (red) and for α-tubulin (green), Fas3 or with Hoechst (blue) as labelled. Arrowheads indicate secreted Upd protein. (D) Stage 9 Upd-gal4; UAS-GFP (Upd>GFP) egg chamber showing extracellular Upd staining. (E) Colchicine (Colch)-treated egg chamber at early stage 9. No apical Upd secretion was observed (asterisk). (F) Stage 8 Upd-gal4; UAS-SpastinGFP (Upd>SpastinGFP) egg chamber showing no Upd apical secretion (G) Stage 8 Upd-gal4; UAS-Glued RNAi (Upd>Glued RNAi) egg chamber showing no Upd apical secretion. (H) Stage 8, Upd-gal4; UAS-Upd RNAi (Upd>Upd RNAi) egg chamber showing that Upd staining is dramatically decreased. Scale bar: 10 μm. (**I**) Upd staining (red) in a modified colour scale (thermal1). Arrowheads indicate secreted Upd; asterisks indicate a lack of Upd secretion. The bar indicates the thermal1 LUT (Look Up Table). (**J**) Quantification of Upd staining variation in the PCs along the apicobasal axis in the indicated genotypes. Wild-type PCs show an apical accumulation of Upd protein towards the apical side, which is not found or is reduced in the other conditions. Error bars indicate s.d. (**K**) Schematic representations of the different phenotypes observed.

Altogether, these results show that in the absence of Dynein transport Upd apical secretion from PCs is much less efficient, leading to a reduction in JAK/STAT signalling and oBC specification.

Interestingly, overexpression of Spastin protein using Upd-gal4 led to an outstretched wing phenotype similar to the *upd* mutant phenotype (see Fig. S1B,C in the supplementary material). The outstretched phenotype was rescued by co-expression of *upd* (see Fig. S1E in the supplementary material), whereas misexpression of *upd* alone had no effect on wing posture (see Fig. S1A in the supplementary material). Similarly, expression of Glued RNAi (Upd>Glued RNAi) produced outstretched wings, which were rescued by co-expressing *upd* (see Fig. S1D,F in the supplementary material). Hence, an excess of ligand is able to compensate for the lack of transport resulting from a loss of Glued function or from Spastin treatment. These data indicate that intracellular trafficking

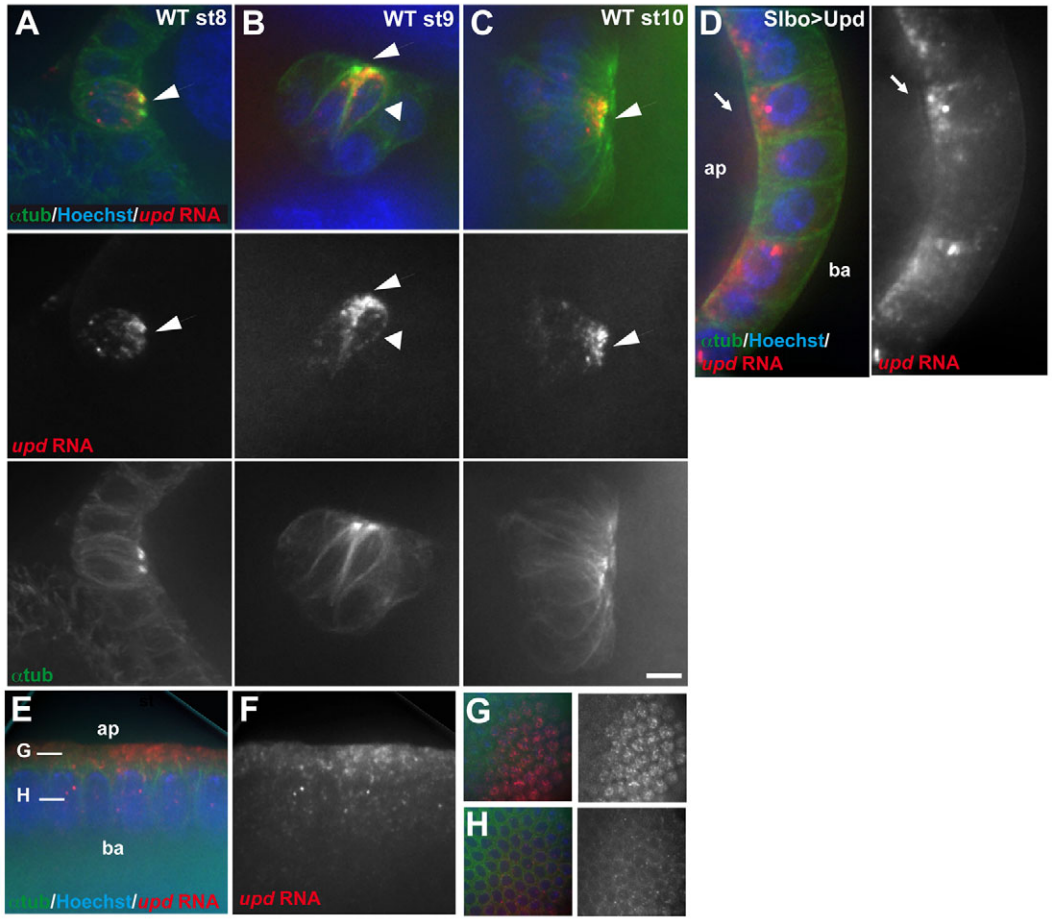

Fig. 5. *upd* transcript localises to the minus ends of microtubules in oocytes and embryos. (**A-C**) High magnification of wild-type *Drosophila* egg chambers stained for α-tubulin (green), *upd* mRNA (red) or with Hoechst (blue) at stage 8 (A), 9 (B) and 10 (C). Arrowheads indicate the apical localisation of *upd* transcripts at all stages. The arrowhead in B indicates *upd* mRNA and MT colocalisation. (**D**) Slbo-gal4;UAS-Upd stage 9 egg chamber stained as above. Arrow indicates apical accumulation of the *upd* transcript. ap, apical; ba, basal. (**E-H**) Wild-type syncitial embryos stained as above. (E,F) *upd* mRNA is enriched apically. (G,H) Apical views of the embryo at the levels indicated in E. Scale bar: 10 μm in A-F; 50 μm in G,H.

involving Dynein transport positively regulates JAK/STAT signalling and ligand delivery efficiency in different developmental contexts.

upd mRNA localises apically in a MT- and Dynein-dependent manner

We next investigated the basis for abnormal Upd secretion in the absence of Dynein cargo transport. BicD and Egl are required for Dynein-dependent localisation of many mRNAs in *Drosophila* (Dienstbier et al., 2009; Nashchekin and St Johnston, 2009), facilitating protein targeting to their site of function (Lecuyer et al., 2007; Martin and Ephrussi, 2009). This prompted us to investigate *upd* mRNA subcellular distribution by performing fluorescent in situ hybridisation coupled with MT staining. We found that *upd* mRNA is apically enriched and colocalises with the MTs at all stages of BC migration (Fig. 5A-C). Hence, apical localisation of *upd* transcript provides a means to concentrate the Upd protein towards the apical side of the PCs, thus promoting efficient activation of the JAK/STAT pathway in adjacent cells. To determine whether MTs and the Dynein motor are required for *upd* mRNA localisation, their functions were altered through pharmacological or genetic means. After Colchicine treatment, *upd* mRNA was no longer apically enriched (compare Fig. 6A with Fig. 5B). Moreover, although Spastin expression in the oBC had no effects on *upd* mRNA localisation (Fig. 6B), its expression in the PCs led to a homogenous distribution of the mRNA in these cells (Fig. 6C). Finally, in loss-of-function conditions for Lis-1, Glued or the BicD-Egl complex, *upd* mRNA was distributed evenly throughout the cell (Fig. 6D-F), consistent with the loss of apical Upd protein secretion (Fig. 4). These results indicate that the apical enrichment of *upd* mRNA is MT and Dynein dependent.

Dynein transports *upd* mRNA in different tissues through a localisation element present in the ORF

To test whether *upd* mRNA localisation is a unique property of the PCs, we ectopically expressed *upd* in follicular cells. Interestingly, *upd* transcripts localised apically in follicle cells, which also have a polarised MT network and a functional dynein complex (Horne-Badovinac and Bilder, 2008; Li et al., 2008) (Fig. 5D). Furthermore, early during embryonic development *upd* mRNA is expressed broadly throughout the trunk of the embryo and in a dorsal crescent in the head (Harrison et al., 1998) to activate the JAK/STAT pathway and regulate the expression of pair-rule genes. At the same stage, Dynein is known to transport *wingless* (*wg*) and pair-rule mRNAs to the apical cytoplasm of blastoderm embryos (Bullock and Ish-Horowicz, 2001; Simmonds et al., 2001; Wilkie

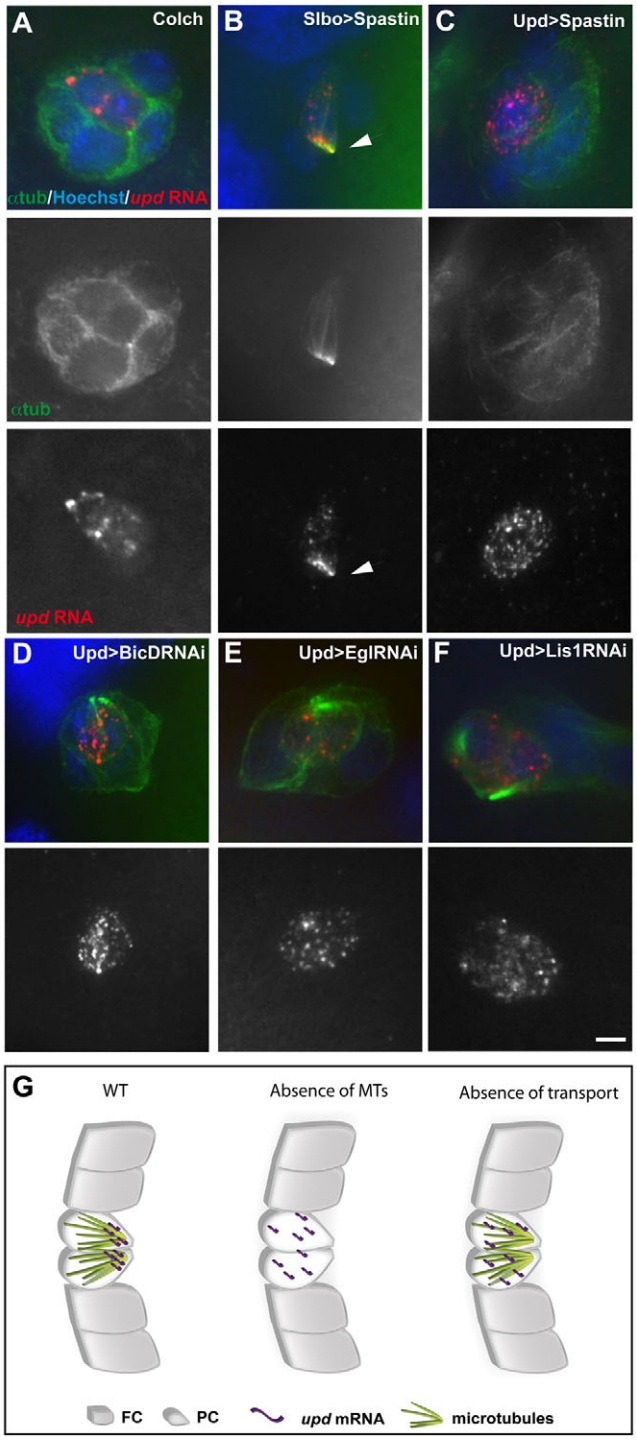

Fig. 6. *upd* mRNA localisation is microtubule and Dynein dependent. (**A-F**) *Drosophila* stage 9 egg chambers stained for α-tubulin (green), *upd* mRNA (red) and with Hoechst (blue). Arrowheads indicate asymmetric localisation of *upd* mRNA. (A) Colchicine-treated egg chambers. After Colchicine treatment, MTs are disorganised and *upd* mRNA distribution is symmetric. (B) Slbo-gal4; UAS-SpastinGFP egg chambers. PCs present a wild-type MT network and *upd* mRNA is apically enriched (arrowhead). (C) Upd-gal4; UAS-SpastinGFP egg chamber in which MTs are absent in the PCs and *upd* mRNA is randomly distributed. (D) Upd-gal4;UAS-BicD RNAi egg chamber. (E) Upd-gal4; UAS-Egl RNAi egg chamber. (F) Upd-gal4; UAS-Lis-1 RNAi egg chambers showing mRNA distributed randomly. Scale bar: 10 μm. (**G**) Schematic representations of the different phenotypes observed.

and Davis, 2001). We found that *upd* mRNA is enriched apically above the nuclei of blastoderm embryos, where MT minus ends are concentrated (Fig. 5E-H), indicating that *upd* mRNA apical localisation is conserved in different tissues.

To confirm that Dynein is required for *upd* mRNA localisation, fluorescently labelled RNAs were injected into syncytial embryos and assayed for their localisation potential. At this stage, the nuclei form a monolayer beneath the apical cortex from which MTs grow and extend into the basal cytoplasm. It has been reported that any transcript carrying a localisation element injected into syncytial embryos is recognised by the transport machinery and moved apically (Bullock and Ish-Horowicz, 2001; Wilkie and Davis, 2001). Injected RNAs assemble into particles, the movement of which can be followed in living embryos. After injection, the full-length *upd* RNA rapidly localised at the apical side of the nucleus, confirming that *upd* RNA is recruited by the localisation machinery and transported along MTs (Fig. 7A,C). *gfp* RNA did not show any apical localisation 20 minutes after injection (Fig. 7E). The movement of *upd*-labelled RNA particles was followed in time-lapse experiments, tracking their speed and direction (Fig. 7D,D′). Interestingly, most particles moved towards the apical side with an average speed of 0.5 μm/second, consistent with Dynein active transport (MacDougall et al., 2003). To further test whether this transport was Dynein dependent, embryos were pre-injected with anti-Dhc64C antibody (P1H4), which completely blocked *upd* RNA apical transport (Fig. 7F). These results indicate that Dynein recruits and transports *upd* mRNA cargoes to the minus ends of MTs.

mRNAs are thought to be sorted in the cytoplasm according to signals present in their sequence (cis-acting elements or ‘Zip’ code) that are recognised by trans-acting factors associated with molecular motors (Martin and Ephrussi, 2009; Van de Bor and Davis, 2004). These signals do not share any obvious consensus sequence or common features, making them difficult to identify. To locate the *upd* mRNA localisation element, different fragments of the *upd* transcript were injected into embryos. No apical localisation of the 3′UTR or 5′UTR fragments was observed (Fig. 7G,H). However, fragments containing the 5′UTR-ORF or the ORF alone did localise apically (Fig. 7I,J), indicating that the *upd* localisation element is located within the ORF region. We further dissected the ORF into five fragments (ORF1 to ORF5) and found that only the 450 bp ORF4 fragment localised apically, indicating that it carries a *upd* localisation signal (Fig. 7K,L). Because ORF4 does not localise as efficiently as the complete ORF (50% of injected embryos showed localisation as compared with 88% for the full-length ORF), we conclude that other element(s) of the mRNA might act in cis with the signal in ORF4 for full recruitment and/or transport by the machinery.

DISCUSSION

The *Drosophila* JAK/STAT pathway is activated by the cytokine Upd, which is a secreted glycosylated protein that is thought to act as a diffusible ligand in several tissues (Arbouzova and Zeidler, 2006; Harrison et al., 1998; Hombria and Sotillos, 2008). In the *Drosophila* adult female gonad, activation of JAK/STAT signalling is essential for BC specification and migration (Beccari et al., 2002; Ghiglione et al., 2002; Silver and Montell, 2001). In this process, JAK/STAT is activated by a high concentration of Upd and is maintained active throughout migration (Silver et al., 2005). However, the mechanisms that ensure proper signalling strength, which is essential for BC determination, remain unclear. Here, we report a new mechanism of JAK/STAT positive regulation that

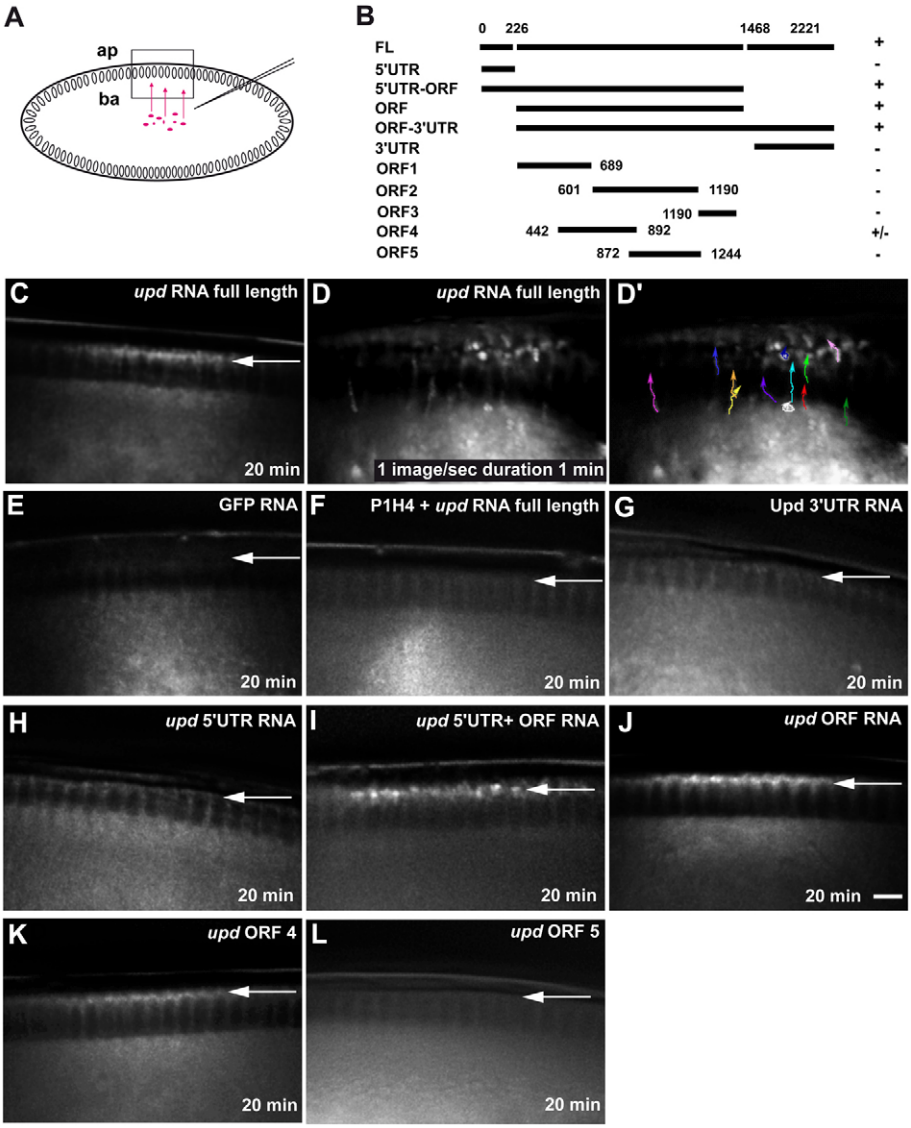

Fig. 7. The *upd* mRNA localisation signal is present in the ORF. (**A**) RNA injection (red) of *Drosophila* embryos, illustrating the region shown in C-L. (boxed). ap, apical; ba, basal. (**B**) The different *upd* RNA fragments injected. FL, full length. Numbering is relative to nucleotides: +, apical localisation; –, no apical localisation; +/–, partial apical localisation. (**C-L**) Syncytial embryos injected with Alexa Fluor 546-UTP-labelled RNAs. (C) Full-length *upd* RNA localised apically 20 minutes after injection (arrow). (D,D′) Tracks of RNA particles (coloured lines in D′) from a movie taken 5 minutes after injection. The movie was 1 minute long at 1 frame/second. (E) *gfp* RNA does not localise 20 minutes after injection. (F) Embryo pre-injected with anti-Dynein antibody P1H4 and 10 minutes later with full-length *upd* RNA; 20 minutes later, the RNA is not transported. (G,H) *upd* 3′UTR (G) and 5′UTR (H) RNA fragments do not localise 20 minutes after injection. (I-K) *upd* 5′UTR+ORF fragment (I), *upd* ORF (J) and *upd* ORF4 (K) show apical accumulation 20 minutes after injection. (L) *upd* ORF5 shows no localisation. Arrows indicate the apical region. Scale bar: 10 μm.

relies on polarised accumulation of the ligand mRNA, which in turn allows apical Upd protein secretion from PCs and the strengthening of JAK/STAT activation in oBCs (Fig. 8). The asymmetric accumulation of *upd* mRNAs is driven by an active, Dynein-dependent transport along polarised MTs. We show that this process is essential for BC specification, as conditions that affect the MT network or associated motor complex mimic a loss of JAK/STAT function. Hence, the polarised secretion of Upd protein from PCs provides a peak of ligand that induces efficient activation of the JAK/STAT pathway in neighbouring cells, which is important for oBC determination and migration. These findings reveal a novel post-transcriptional regulatory mechanism controlling JAK/STAT signalling during a process of epithelial cell invasiveness.

mRNA subcellular localisation prior to translation is a conserved mechanism that restricts protein function both spatially and temporally (Martin and Ephrussi, 2009). It plays an important role in the establishment of cell polarity and in the specification of embryonic axes. Here, we show that mRNA localisation also plays a role in the fate determination of migratory cells within an epithelium. Transcripts that are known to be transported encode a wide variety of proteins, including transcription factors, components of the cytoskeleton and signalling molecules. Some well-characterised examples include *Veg1* and *An1* mRNAs in *Xenopus*, the localisation of which determines the vegetal and animal poles of the embryo (Mowry and Melton, 1992), the *Ash1* mRNA which localises to the bud site in yeast (Long et al., 1997), and *Actin* mRNA localisation at the leading edge of migrating fibroblasts and growth cones (Condeelis and Singer, 2005). Transcript localisation can also act as a mechanism to regulate signalling pathways. One example is the Wnt-like protein encoded by the *Drosophila wg* gene, which plays an important role in segment patterning; apical localisation of *wg* transcripts in syncytial embryos restricts the diffusion of the Wg protein and therefore contributes to the definition of sharp boundaries (Simmonds et al., 2001). Our data show that, within the follicular epithelium, mRNA transport and localisation regulate the JAK/STAT signalling cascade to provide a peak of ligand activity that is important for follicle cell patterning and oBC specification. Furthermore, our results show that *upd* mRNA localisation is essential for accumulation of the Upd protein at the apical side of epithelial cells. Hence, as for Wg, Upd is a secreted protein that needs to be restricted to a narrow region, a process that depends on BicD and Egl function (Figs 3 and 6). BicD and Egl form a

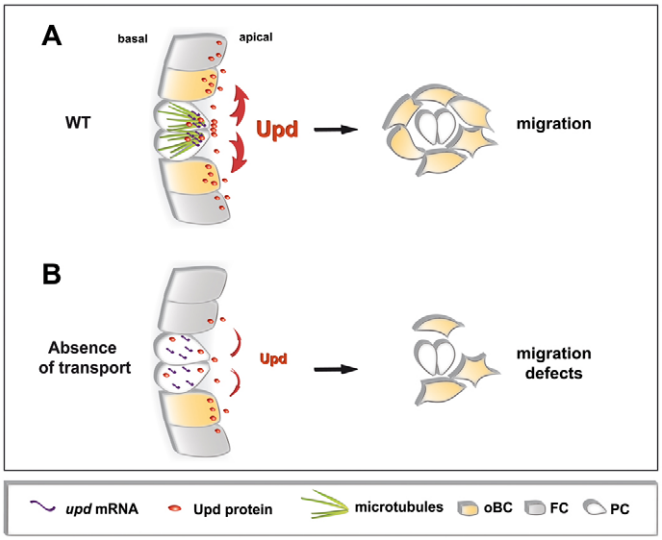

Fig. 8. *upd* mRNA localisation is essential for BC recruitment. (**A**) In the wild type (WT), PCs (white) have a polarised MT network (green) along which *upd* mRNA (purple) localises in a Dynein-dependent manner allowing efficient secretion and accumulation of Upd protein (red) from the apical side of the PCs. This accumulation of Upd establishes the Upd gradient and sets the high-level signal required for the recruitment of oBCs (yellow). (**B**) In the absence of Dynein transport, *upd* mRNA is not localised and Upd protein is not efficiently secreted, adversely affecting oBC specification and migration.

complex that binds the Dynein motor as well as various minus end-directed mRNA signals (Dienstbier et al., 2009). Interestingly, unlike BicD, Egl is specifically required for mRNA transport and for no other cargoes in *Drosophila* (Dienstbier et al., 2009), confirming that *upd* mRNA localisation is a key factor in the control of JAK/STAT pathway activity. It was shown previously that the *Drosophila* JAK/STAT pathway receptor Dome is apically enriched in the follicular epithelium (Ghiglione et al., 2002) and that Dome undergoes ligand-dependent endocytosis apically (Devergne et al., 2007). In addition, a pool of STAT (Stat92E – FlyBase) protein is apically enriched in a Par3-dependent manner in the *Drosophila* embryonic ectoderm (Sotillos et al., 2008). These observations suggest that polarisation and/or subcellular localisation represent a key aspect of JAK/STAT signalling efficiency that serves to avoid misrouting and dilution of the ligand. This is likely to represent a general mechanism utilised in other major signalling pathways, as it has been observed that the Notch and EGF receptors, as well as Patched and Frizzled, also localise apically and/or basolaterally (Assa-Kunik et al., 2007; Levitan and Greenwald, 1998; Strutt, 2003; Whitfield et al., 1999).

Proper patterning of the anterior follicular epithelium by the JAK/STAT pathway requires the establishment of a robust gradient, with an appropriate ligand concentration for follicle cells to become properly determined. The transport of *upd* mRNA and the subsequent accumulation of the Upd protein apically contribute to setting the high concentration of ligand required for oBC determination. Recently, a new negative-feedback mechanism has been identified in oBCs that involves a regulatory circuit between Slbo, STAT and Apt (Starz-Gaiano et al., 2008; Starz-Gaiano et al., 2009). This feedback mechanism inhibits JAK/STAT activity in cells that receive low levels of Upd ligand, i.e. those distant from the source. Therefore, JAK/STAT signalling requires several regulatory mechanisms to select the correct number of migratory BCs. First, in cells sending the signal (PCs), post-transcriptional control of *upd* mRNA and asymmetric protein accumulation are important for setting the correct ligand levels. It is possible that subcellular localisation of the transcript could also control the translation of the protein spatially, which in turn could affect translation efficiency, post-translational modifications such as glycosylation, co-factor association and/or degradation. Second, in receiving cells mechanisms are required to read the Upd gradient and limit the number of migratory cells, which involves spatial regulation of JAK/STAT signalling in anterior follicle cells receiving the Upd ligand through Apt. Additional mechanisms that involve sequestration of the ligand after its secretion might also exist. Indeed, in tissue culture, Upd is mainly found associated with the extracellular matrix (Harrison et al., 1998), which might help to limit Upd diffusion in vivo. Interestingly, it has been reported that a transient cap of extracellular matrix forms at the apical side of PCs when BCs are being recruited (Medioni and Noselli, 2005), raising the possibility of an association of Upd with the extracellular matrix cap for the purposes of building up the gradient.

Future work will need to integrate all these various aspects of signalling regulation in order to fully understand how invasive cells are determined during development and how they can escape control mechanisms in the context of disease and cancer.

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Competing interests statement
The authors declare no competing financial interests.

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References
Arbouzova, N. I. and Zeidler, M. P. (2006). JAK/STAT signalling in Drosophila: insights into conserved regulatory and cellular functions. *Development* **133**, 2605-2616.
Assa-Kunik, E., Torres, I. L., Schejter, E. D., Johnston, D. S. and Shilo, B. Z. (2007). Drosophila follicle cells are patterned by multiple levels of Notch signaling and antagonism between the Notch and JAK/STAT pathways. *Development* **134**, 1161-1169.
Bach, E. A., Ekas, L. A., Ayala-Camargo, A., Flaherty, M. S., Lee, H., Perrimon, N. and Baeg, G. H. (2007). GFP reporters detect the activation of the Drosophila JAK/STAT pathway in vivo. *Gene Expr. Patterns* **7**, 323-331.
Beccari, S., Teixeira, L. and Rorth, P. (2002). The JAK/STAT pathway is required for border cell migration during Drosophila oogenesis. *Mech. Dev.* **111**, 115-123.
Bianco, A., Poukkula, M., Cliffe, A., Mathieu, J., Luque, C. M., Fulga, T. A. and Rorth, P. (2007). Two distinct modes of guidance signalling during collective migration of border cells. *Nature* **448**, 362-365.
Bullock, S. L. and Ish-Horowicz, D. (2001). Conserved signals and machinery for RNA transport in Drosophila oogenesis and embryogenesis. *Nature* **414**, 611-616.
Chen, H. W., Chen, X., Oh, S. W., Marinissen, M. J., Gutkind, J. S. and Hou, S. X. (2002). mom identifies a receptor for the Drosophila JAK/STAT signal transduction pathway and encodes a protein distantly related to the mammalian cytokine receptor family. *Genes Dev.* **16**, 388-398.
Clark, I. E., Jan, L. Y. and Jan, Y. N. (1997). Reciprocal localization of Nod and kinesin fusion proteins indicates microtubule polarity in the Drosophila oocyte, epithelium, neuron and muscle. *Development* **124**, 461-470.

Claussen, M. and Suter, B. (2005). BicD-dependent localization processes: from Drosophila development to human cell biology. *Ann. Anat.* **187**, 539-553.
Condeelis, J. and Singer, R. H. (2005). How and why does beta-actin mRNA target? *Biol. Cell* **97**, 97-110.
Dahlgaard, K., Raposo, A. A., Niccoli, T. and St Johnston, D. (2007). Capu and Spire assemble a cytoplasmic actin mesh that maintains microtubule organization in the Drosophila oocyte. *Dev. Cell* **13**, 539-553.
Devergne, O., Ghiglione, C. and Noselli, S. (2007). The endocytic control of JAK/STAT signalling in Drosophila. *J. Cell Sci.* **120**, 3457-3464.
Dienstbier, M., Boehl, F., Li, X. and Bullock, S. L. (2009). Egalitarian is a selective RNA-binding protein linking mRNA localization signals to the dynein motor. *Genes Dev.* **23**, 1546-1558.
Doerflinger, H., Benton, R., Shulman, J. M. and St Johnston, D. (2003). The role of PAR-1 in regulating the polarised microtubule cytoskeleton in the Drosophila follicular epithelium. *Development* **130**, 3965-3975.
Ghiglione, C., Devergne, O., Georgenthum, E., Carballes, F., Medioni, C., Cerezo, D. and Noselli, S. (2002). The Drosophila cytokine receptor Domeless controls border cell migration and epithelial polarization during oogenesis. *Development* **129**, 5437-5447.
Ghiglione, C., Devergne, O., Cerezo, D. and Noselli, S. (2008). Drosophila RalA is essential for the maintenance of Jak/Stat signalling in ovarian follicles. *EMBO Rep.* **9**, 676-682.
Harrison, D. A., McCoon, P. E., Binari, R., Gilman, M. and Perrimon, N. (1998). Drosophila unpaired encodes a secreted protein that activates the JAK signaling pathway. *Genes Dev.* **12**, 3252-3263.
Hombria, J. C. and Brown, S. (2002). The fertile field of Drosophila Jak/STAT signalling. *Curr. Biol.* **12**, R569-R575.
Hombria, J. C. and Sotillos, S. (2008). Disclosing JAK/STAT links to cell adhesion and cell polarity. *Semin. Cell Dev. Biol.* **19**, 370-378.
Horne-Badovinac, S. and Bilder, D. (2008). Dynein regulates epithelial polarity and the apical localization of stardust A mRNA. *PLoS Genet.* **4**, e8.
Hou, S. X., Zheng, Z., Chen, X. and Perrimon, N. (2002). The Jak/STAT pathway in model organisms: emerging roles in cell movement. *Dev. Cell* **3**, 765-778.
Jang, A. C., Starz-Gaiano, M. and Montell, D. J. (2007). Modeling migration and metastasis in Drosophila. *J. Mammary Gland Biol. Neoplasia* **12**, 103-114.
Jankovics, F. and Brunner, D. (2006). Transiently reorganized microtubules are essential for zippering during dorsal closure in Drosophila melanogaster. *Dev. Cell* **11**, 375-385.
Kardon, J. R. and Vale, R. D. (2009). Regulators of the cytoplasmic dynein motor. *Nat. Rev. Mol. Cell Biol.* **10**, 854-865.
Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T. R., Tomancak, P. and Krause, H. M. (2007). Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell* **131**, 174-187.
Levitan, D. and Greenwald, I. (1998). LIN-12 protein expression and localization during vulval development in C. elegans. *Development* **125**, 3101-3109.
Li, Z., Wang, L., Hays, T. S. and Cai, Y. (2008). Dynein-mediated apical localization of crumbs transcripts is required for Crumbs activity in epithelial polarity. *J. Cell Biol.* **180**, 31-38.
Long, R. M., Singer, R. H., Meng, X., Gonzalez, I., Nasmyth, K. and Jansen, R. P. (1997). Mating type switching in yeast controlled by asymmetric localization of ASH1 mRNA. *Science* **277**, 383-387.
MacDougall, N., Clark, A., MacDougall, E. and Davis, I. (2003). Drosophila gurken (TGFalpha) mRNA localizes as particles that move within the oocyte in two dynein-dependent steps. *Dev. Cell* **4**, 307-319.
Martin, K. C. and Ephrussi, A. (2009). mRNA localization: gene expression in the spatial dimension. *Cell* **136**, 719-730.
Martinez-Campos, M., Basto, R., Baker, J., Kernan, M. and Raff, J. W. (2004). The Drosophila pericentrin-like protein is essential for cilia/flagella function, but appears to be dispensable for mitosis. *J. Cell Biol.* **165**, 673-683.
Medioni, C. and Noselli, S. (2005). Dynamics of the basement membrane in invasive epithelial clusters in Drosophila. *Development* **132**, 3069-3077.
Montell, D. J., Rorth, P. and Spradling, A. C. (1992). slow border cells, a locus required for a developmentally regulated cell migration during oogenesis, encodes Drosophila C/EBP. *Cell* **71**, 51-62.
Mowry, K. L. and Melton, D. A. (1992). Vegetal messenger RNA localization directed by a 340-nt RNA sequence element in Xenopus oocytes. *Science* **255**, 991-994.
Nashchekin, D. and St Johnston, D. (2009). Egalitarian recruitment of localized mRNAs. *Genes Dev.* **23**, 1475-1480.
Pinheiro, E. M. and Montell, D. J. (2004). Requirement for Par-6 and Bazooka in Drosophila border cell migration. *Development* **131**, 5243-5251.
Prasad, M. and Montell, D. J. (2007). Cellular and molecular mechanisms of border cell migration analyzed using time-lapse live-cell imaging. *Dev. Cell* **12**, 997-1005.
Prasad, M., Jang, A. C., Starz-Gaiano, M., Melani, M. and Montell, D. J. (2007). A protocol for culturing Drosophila melanogaster stage 9 egg chambers for live imaging. *Nat. Protoc.* **2**, 2467-2473.
Rorth, P. (2007). Collective guidance of collective cell migration. *Trends Cell Biol.* **17**, 575-579.
Sherwood, N. T., Sun, Q., Xue, M., Zhang, B. and Zinn, K. (2004). Drosophila spastin regulates synaptic microtubule networks and is required for normal motor function. *PLoS Biol.* **2**, e429.
Silver, D. L. and Montell, D. J. (2001). Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in Drosophila. *Cell* **107**, 831-841.
Silver, D. L., Geisbrecht, E. R. and Montell, D. J. (2005). Requirement for JAK/STAT signaling throughout border cell migration in Drosophila. *Development* **132**, 3483-3492.
Simmonds, A. J., dosSantos, G., Livne-Bar, I. and Krause, H. M. (2001). Apical localization of wingless transcripts is required for wingless signaling. *Cell* **105**, 197-207.
Sotillos, S., Diaz-Meco, M. T., Moscat, J. and Castelli-Gair Hombria, J. (2008). Polarized subcellular localization of Jak/STAT components is required for efficient signaling. *Curr. Biol.* **18**, 624-629.
Starz-Gaiano, M., Melani, M., Wang, X., Meinhardt, H. and Montell, D. J. (2008). Feedback inhibition of Jak/STAT signaling by apontic is required to limit an invasive cell population. *Dev. Cell* **14**, 726-738.
Starz-Gaiano, M., Melani, M., Meinhardt, H. and Montell, D. (2009). Interpretation of the UPD/JAK/STAT morphogen gradient in Drosophila follicle cells. *Cell Cycle* **8**, 2917-2925.
Stevens, N. R., Raposo, A. A., Basto, R., St Johnston, D. and Raff, J. W. (2007). From stem cell to embryo without centrioles. *Curr. Biol.* **17**, 1498-1503.
Strigini, M. and Cohen, S. M. (2000). Wingless gradient formation in the Drosophila wing. *Curr. Biol.* **10**, 293-300.
Strutt, D. (2003). Frizzled signalling and cell polarisation in Drosophila and vertebrates. *Development* **130**, 4501-4513.
Tekotte, H. and Davis, I. (2002). Intracellular mRNA localization: motors move messages. *Trends Genet.* **18**, 636-642.
Thiery, J. P., Acloque, H., Huang, R. Y. and Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871-890.
Vale, R. D. (2003). The molecular motor toolbox for intracellular transport. *Cell* **112**, 467-480.
Van de Bor, V. and Davis, I. (2004). mRNA localisation gets more complex. *Curr. Opin. Cell Biol.* **16**, 300-307.
Wade, R. H. (2009). On and around microtubules: an overview. *Mol. Biotechnol.* **43**, 177-191.
Whitfield, C. W., Benard, C., Barnes, T., Hekimi, S. and Kim, S. K. (1999). Basolateral localization of the Caenorhabditis elegans epidermal growth factor receptor in epithelial cells by the PDZ protein LIN-10. *Mol. Biol. Cell* **10**, 2087-2100.
Wilkie, G. S. and Davis, I. (2001). Drosophila wingless and pair-rule transcripts localize apically by dynein-mediated transport of RNA particles. *Cell* **105**, 209-219.
Xi, R., McGregor, J. R. and Harrison, D. A. (2003). A gradient of JAK pathway activity patterns the anterior-posterior axis of the follicular epithelium. *Dev. Cell* **4**, 167-177.
Yang, J. and Weinberg, R. A. (2008). Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev. Cell* **14**, 818-829.
Yilmaz, M. and Christofori, G. (2009). EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev.* **28**, 15-33.