Function of the ETS Transcription Factor Yan in Border Cell Migration

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Markus Schober[1,4,*], Ilaria Rebay[3] and Norbert Perrimon[1,2,*]

[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
[2]Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA
[3]University of Chicago, 5801 South Ellis Avenue, Chicago, IL 60637, USA
[4]The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA
*Authors for correspondence (e-mail: schobem@rockefeller.edu and perrimon@receptor.med.harvard.edu)

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Summary

Invasive cell migration in both normal development and metastatic cancer is regulated by various signaling pathways, transcription factors and cell-adhesion molecules. The coordination between these activities in the context of cell migration is poorly understood. During *Drosophila* oogenesis, a small group of cells called border cells exit the follicular epithelium to perform a stereotypic, invasive migration. We find that the ETS transcription factor Yan is required for border cell migration and that Yan expression is spatiotemporally regulated as border cells migrate from the anterior pole of the egg chamber towards the nurse cell-oocyte boundary. Yan expression is dependent on inputs from the JAK/STAT, Notch and Receptor Tyrosine Kinase pathways in border cells. Mechanistically, Yan functions to modulate the turnover of DE-Cadherin-dependent adhesive complexes to facilitate border cell migration. Our results suggest that Yan acts as a pivotal link between signal transduction, cell adhesion and invasive cell migration in *Drosophila* border cells.

Key words: Cell migration, Oogenesis, *Drosophila*, ETS, Notch, JAK/STAT, RTK, Border cells

Introduction

Morphogenetic movements such as cell migration are crucial for the development of multicellular organisms. Cells that are born at distinct locations in the developing animal often undergo precise, spatiotemporally regulated migration to distant sites where they eventually build specialized tissues and organs. How input from multiple signaling pathways is coordinated to ensure proper cell movement remains one of the main challenges in the field of cell migration and developmental biology.

The migration of border cells (BCs) in the *Drosophila* egg chamber provides a unique system with which to genetically dissect the mechanisms regulating invasive cell migration in vivo (Montell, 2003; Rorth, 2002). During oogenesis, a group of approximately eight cells, called BCs, is specified at the anterior pole of the ovarian follicular epithelium (Montell et al., 1992). At stage 9 of oogenesis, BCs change their shape, exit the epithelium and become migratory (Fig. 1A). BCs comprise two inner cells, called polar cells, surrounded by six to eight outer BCs (Niewiadomska et al., 1999). The highly dynamic and polarized cytoskeleton of outer BCs enables them to migrate (Fulga and Rorth, 2002) and transport the non-motile polar cells with them. BCs migrate in between nurse cells (NCs) until they reach the NC-oocyte boundary at stage 10 of oogenesis.

A variety of molecules have been identified to regulate BC migration. The *Drosophila* C/EBP transcription factor homolog Slow border cells (Slbo) is a key regulator of BC migration (Montell et al., 1992). Slbo is specifically expressed in BCs at the time when BCs become migratory. Slbo promotes the expression of *shotgun* (*shg*), the gene encoding the *Drosophila* homolog of the cell-cell adhesion molecule E-Cadherin (DE-Cad), which has been shown to be crucial in both BCs and NCs for BC motility (Niewiadomska et al., 1999). Specifically, loss of *shg* leads to delayed BC migration, and it has been speculated, although not tested, that an increase in DE-Cad expression might also result in BC migration defects. These findings have led to a model proposing that DE-Cad-containing adhesive complexes, together with myosins, provide the traction between BC and NC surfaces that allows BCs to migrate (Fulga and Rorth, 2002; Geisbrecht and Montell, 2002).

Genetic screens have further identified four signaling pathways that control the stereotypic migration of BCs. During early oogenesis, the cytokine-like molecule Unpaired (Upd) is secreted from the pair of anterior polar cells to activate the JAK/STAT pathway in the surrounding cells. JAK/STAT activation induces the expression of *slbo*, which specifies BCs (Silver and Montell, 2001). Like JAK/STAT, Notch signaling is also required for the expression of *slbo* in anterior follicle cells (Gonzalez-Reyes and St Johnston, 1998), indicating that Notch signaling might be crucial for BC specification and migration. Once specified, BCs exit the follicular epithelium and become migratory, a process that relies on nuclear hormone signaling (Bai et al., 2000). Ecdysone hormone co-receptor *taiman* (*tai*) mutants have defects in BC migration even though Slbo is expressed and DE-Cad-containing adhesive complexes are formed at the BC-NC interface, leading to the proposal that the migration defects of *tai* mutant BCs are due to problems in the turnover of adhesive complexes (Bai et al., 2000).

Two RTK pathways, the Platelet-Derived Growth Factor/Vascular Endothelial Growth Factor Receptor (PVR) and the Epithelial Growth Factor Receptor (EGFR) pathways, control the migration of BCs. Pvf1, one of three secreted PVR ligands (Duchek et al., 2001), is expressed in an increasing gradient along the anteroposterior (AP) axis of the egg chamber. This gradual expression, together with the delayed or misrouted migration that results when dominant-negative forms of both EGFR and PVR are co-expressed in BCs, or when the ligand Pvf1 is ectopically expressed in ovarian egg chambers, have led to a model whereby PVR and EGFR exert redundant functions in guiding BC migration (Duchek and Rorth, 2001; Duchek et al., 2001; McDonald et al., 2003). Although the MAPK pathway is activated in migrating BCs (Duchek and Rorth, 2001), it is not known whether its target genes are crucial for BC migration.

The ETS (E-26) transcription factor gene *yan*, which encodes the *Drosophila* homolog of the TEL oncogene (Laudet et al., 1999), is one of the main target genes of the Notch and MAPK signal transduction pathways during photoreceptor specification in the *Drosophila* developing eye (O'Neill et al., 1994; Rohrbaugh et al., 2002). While Notch-mediated Suppressor of Hairless [Su(H)] activity promotes *yan* expression (Rohrbaugh et al., 2002), MAPK pathway stimulation above a critical threshold triggers Yan phosphorylation (Rebay, 2002; Rebay and Rubin, 1995). This post-translational modification of Yan stimulates its nuclear export and subsequent degradation, enabling photoreceptor cells to differentiate. Thus, Yan, which is antagonistically regulated by Notch and RTK signaling, probably provides a negative-feedback loop that allows the tight regulation of RTK-mediated cell differentiation.

Although many molecules involved in BC migration have been accounted for, it is not yet clear how input from multiple signaling pathways is integrated to ensure the fidelity and precise orchestration of cell movements. Here, we describe a novel function for Yan in BC migration. We find that Yan is dynamically expressed and regulated in migrating BCs. Specifically, we find that upregulation of Yan expression in the follicular epithelium depends on the Notch and JAK/STAT signaling pathways, and precedes BC motility and exit from the follicular epithelium, whereas downregulation of Yan in response to PVR and EGFR is important for continued invasive migratory behavior. We further demonstrate that BC migration can be delayed by increasing DE-Cad levels and that Yan may influence this process by regulating the turnover of DE-Cad-containing adhesive complexes at the plasma membrane.

Materials and methods

Fly strains

$yan^{P(lacZ)}$, yan^{1}, tai^{61G1}, $pnt^{P(lacZ)}$, N^{ts} and *UAS-Ser* were obtained from the Bloomington stock center. $stat92E^{85C9}$, $stat92E^{397}$ and *UAS-DE-Cad* were obtained from Denise Montell. *UAS-DE-Cad*$^{5.9}$ was obtained from Benedicte Sanson. *UAS-Rab5*^{S43N} was obtained from Marcos Gonzalez-Gaitan. yan^{443} and yan^{884} were identified in a genetic screen for Ras modifiers in the eye (Karim et al., 1996). yan^{443} is a non-sense mutation in Q111*, deleting most of the protein including all of the MAPK phosphorylation sites and the entire ETS DNA-binding domain. yan^{443} is presumably a null allele. yan^{884} has an identical phenotype to yan^{443} in eye clones, embryos and border cell clones. Negatively marked follicle cell clones were generated by crossing *ubGFP FRT40A/CyO; T155-Gal4 UAS-Flp/TM6c* virgin females to *yan FRT40A /CyO* males. Positively marked clones were generated by crossing *y w UAS-GFP hs-Flp; tub-Gal4; FRT82B tub-Gal80* virgin females to $stat^{85C9}$ *FRT82B /TM3, Sb* males. Mosaic mutant females were fed for 5 days at 25°C before dissection. Overexpression experiments were performed using *slbo-Gal4*; *UAS-*yan^{WT}, *UAS-*yan^{ACT}, *UAS-λpvr*, *UAS-λtop*, *UAS-*raf^{F179}, *UAS-λBtl*, *UAS-DE-Cad*, *UAS-DE-Cad*$^{5.9}$ and *UAS-*Rab^{S43N}. Epistasis experiments between *slbo* and *yan* were performed using $slbo^{1310}$; $slbo^{1310}$, *slbo-Gal4/CyO*; $slbo^{1310}$, *UAS-*yan^{WT}*/CyO* and $slbo^{1310}$, *UAS-*yan^{ACT}*/CyO*. All experiments were performed at 25°C. N^{ts} flies were grown and used for control experiments at the permissive temperature of 18°C. N^{ts} female flies were shifted to the restrictive temperature of 29°C for 8, 12 and 16 hours prior to dissection in the *Notch* mutant analysis.

Immunohistochemistry and immunofluorescence

Ovarioles were dissected from 5-day-old, well-fed females in Schneider S2 medium. For β-galactosidase staining, ovaries were dissected, fixed for 10 minutes in 0.1% glutaraldehyde in PBS-0.1% Triton X-100 and stained as described previously (Bai et al., 2000). For immunofluorescence analysis, ovaries were fixed with 4% formaldehyde in PBS-0.1%Triton X-100 for 30 minutes. Ovaries were incubated in primary antibody overnight, washed twice for 20 minutes in PBS-0.1% Triton X-100, followed by a 2-hour incubation in secondary antibody containing Alexa-568-Phalloidin (1:100, Molecular Probes) to stain the actin cytoskeleton. After washing the tissue twice for 30 minutes with PBS-0.1% Triton X-100, it was mounted in Slow Fade (Molecular Probes). The following primary antibodies were used: mouse anti-FAS III at 1:10 (2B5, Developmental Hybridoma Bank); mouse anti-Yan at 1:200 (I. Rebay); rat anti-Slbo at 1:3000 (P. Rorth); rat anti-DE-Cad at 1:100 (H. Oda); and rabbit anti-β-Gal at 1:2000 (Cappel). As secondary antibodies, Alexa-488 and Alexa-598 were used at 1:500 (Molecular Probes). Images were obtained using a Leica-SP2 confocal microscope.

FM1-43 uptake experiment

Double-stranded RNA was generated against the following regions using the Ambion MegaScript kit: Yan (NM_078731) nucleotides 394-1359 and GFP nucleotides 3-549 of the open reading frame. SL2 cells were seeded in serum-free Schneider's medium (Gibco) into a 96-well plate, with 8×10^5 cells and 1 μg dsRNA per well). Serum was added after 1 hour of serum starvation and cells were grown for 4 days at 25°C. SL2 cells (8×10^5) were transfected with 0.1 μg *MT(metallothionin)-*Yan^{ACT} using effectene transfection reagents. Expression was induced with 1 mM $CuSO_4$ 12 hours before the endocytosis assay. Cells were replated on eight-well coverslip chambers coated with concavidin A and washed with HL3 medium (Kuromi and Kidokoro, 1999). HL3 medium was exchanged with HL3 medium containing 10 μM FM1-43 (Molecular Probes). FM1-43 incorporation was observed in real time. Eight confocal sections per minute were acquired on a Leica-SP2 inverted confocal microscope over a time period of 30 minutes. A maximum projection was generated for each frame and the relative fluorescence intensity was plotted against time to assess the kinetics of FM1-43 incorporation. Addition of $CuSO_4$ in mock-transfected cells did not alter the endocytic rate when compared with untreated control cells.

Results

Yan expression is dynamic in migrating border cells and is required for their migration

In our search for genes that regulate *Drosophila* follicle cell morphogenesis (M.S., unpublished), we identified a *lacZ* P-element insertion in the *yan* gene, $yan^{P(lacZ)}$, that is expressed

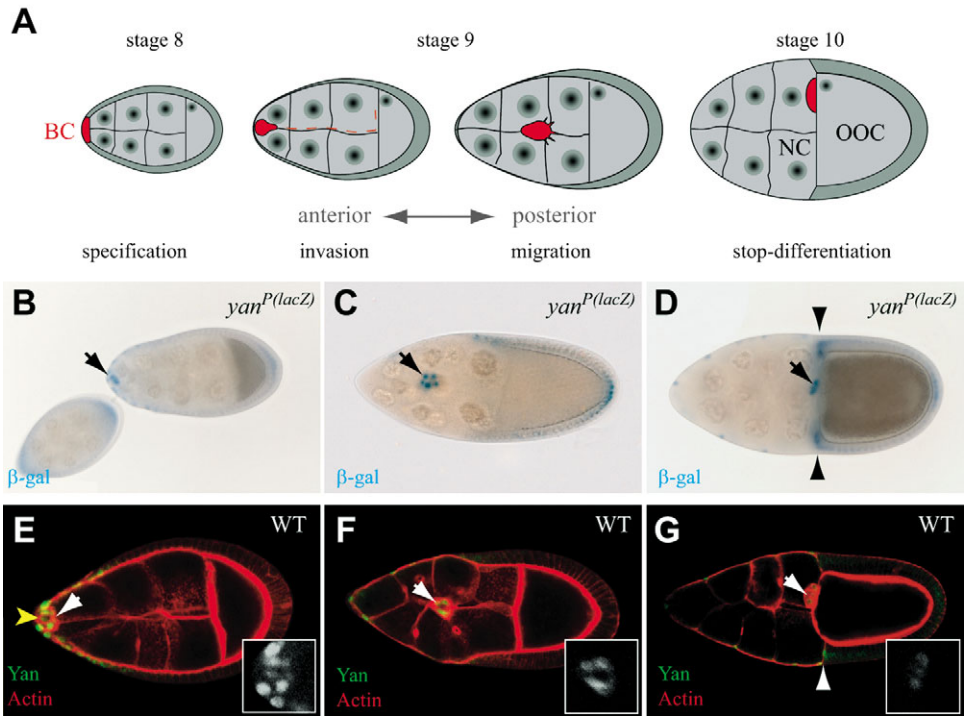


Fig. 1. Yan is dynamically expressed in migrating border cells. (A) BCs are specified at stage 8 of oogenesis at the anterior pole of the ovarian follicle cell epithelium. They exit from the epithelium at stage 9 to migrate in between the NCs to reach the NC-oocyte (OOC) boundary at stage 10 of oogenesis. (B-D) Expression of $yan^{P(lacZ)}$ in egg chambers. Arrows indicate BCs and arrowheads centripetal cells. (B) $yan^{P(lacZ)}$ is expressed in anterior and posterior terminal cells at early stages of oogenesis, and in BCs that exit the epithelium. (C) At stage 9, $yan^{P(lacZ)}$ is strongly expressed in outer BCs, while it is weakly detectable in the main body follicle cells. (D) At stage 10, $yan^{P(lacZ)}$ expression broadens and shows the highest levels in BCs and centripetal cells. (E-G) Staining of egg chambers with anti-Yan antibodies (green) and Alexa-568-phalloidin (red). Arrows mark BCs. Magnified boxes show the progressive decrease in Yan protein (gray) levels during migration. (E) Yan is strongly expressed in BCs, whereas it is hardly detectable in polar cells (yellow arrowhead). (F). Yan expression becomes weaker after BCs exit from the epithelium and progressively decreases thereafter. (G) Yan is hardly detectable in BCs that have reached the NC-oocyte boundary at stage 10 of oogenesis.

in both anterior and posterior terminal cells during early oogenesis. At stage 8 of oogenesis, prior to the exit of BCs from the follicular epithelium, $yan^{P(lacZ)}$ is strongly upregulated in BCs (Fig. 1B). $yan^{P(lacZ)}$ expression appears to be elevated in outer, migratory BCs when compared with the pair of enclosed non-migratory polar cells (Fig. 1C, see Fig. S1 in the supplementary material). At stage 10, when BCs reach the NC-oocyte boundary, $yan^{P(lacZ)}$ is strongly expressed in both BCs and centripetal cells (Fig. 1D).

Interestingly, Yan protein expression levels are dynamic during the course of BC migration. At early stages, Yan is expressed in anterior terminal cells and becomes strongly upregulated in outer BCs before they delaminate from the follicular epithelium (Fig. 1E). Subsequently, Yan expression becomes weaker in BCs once they exit the follicular epithelium (Fig. 1F), and appears to be severely decreased when they reach the NC-oocyte boundary (Fig. 1G).

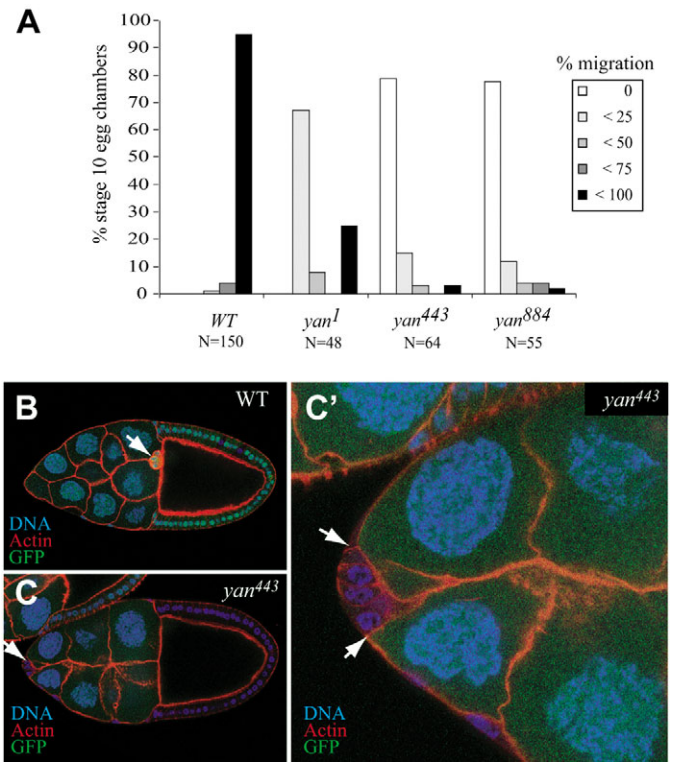


Fig. 2. Yan is required for the invasive migration of border cells. (A) Comparison of BC migration in stage 10 egg chambers between wild type (WT) and three different *yan* mutant alleles. Ninety-five percent of wild-type BCs reach the NC-oocyte boundary. Seventy-nine percent of completely mutant yan^{443} or yan^{884} mutant BC clusters are detectable at the anteriormost pole of the follicular epithelium and do not show any signs of migration, while approximately 15% of yan^{443} or yan^{884} mutant BCs migrate less than 25% of the way towards the oocyte. Only 2% of yan^{443} or yan^{884} mutant BCs reach the NC-oocyte boundary on time. BCs of the weaker yan^{1} allele exit from the epithelium but are delayed in their migration to the oocyte. Sixty-seven percent of mutant BCs migrate less then 25% of the way towards the oocyte, while 25% reach the NC-oocyte boundary. (B,C) Stage 10 egg chambers stained with Alexa-568-phalloidin (red) and DAPI (blue). Wild-type cells are marked by expression of GFP and *yan* mutant cells by lack of GFP expression. (B) Wild-type BC clusters reach the NC-oocyte boundary at stage 10 of oogenesis. (C) yan^{443} mutant BC clusters remain part of the anterior follicle cell epithelium. (C′) High magnification image of a yan^{443} mutant BC cluster. Arrows indicate the boundary between BCs and squamous follicle cells.

To address whether Yan is required for BC migration, we generated mitotic clones of BCs that lack Yan activity. Migration is significantly delayed in hypomorphic *yan*^{1} clones (Fig. 2A). Strikingly, clones of *yan*^{443}, a protein null allele, exhibit stronger migration defects, where the majority of mutant BCs fail completely to migrate and remain part of the anterior follicular epithelium (Fig. 2A,C). High-magnification confocal microscopy of *yan*^{443} mutant egg chambers labeled with Alexa-568-phalloidin indicated that mutant BCs are connected laterally to squamous follicle cells, suggesting that they remain attached to the follicular epithelium (Fig. 2C′).

Yan modulates DE-Cad-containing adhesive complexes

To address whether the *yan* mutant phenotype is caused by a misspecification of BCs, we examined Slbo expression in *yan*^{443} mutant BCs. Removal of Yan function had no effect on Slbo expression (Fig. 3A,A′). In addition, expression of the polar cell-specific marker Fasciclin 3 (Fas3) was unaffected, indicating that the fate of migratory outer BCs was not transformed into non-migratory polar cells (Fig. 3B,B′). Together, these data suggested that Yan does not function to specify BCs or to discriminate between inner versus outer BC identity.

To test whether Yan regulates cell motility, we analyzed DE-Cad expression in *yan* mutant BCs. DE-Cad is a key regulator of BC migration (Niewiadomska et al., 1999) and the dynamic regulation of E-cadherin is crucial for epithelial-to-mesenchymal transitions and morphogenetic cell movements in many systems (Thiery, 2002). In wild-type BC clusters, DE-Cad localized primarily to the surface between polar cells and outer BCs, as well as to the interface between outer BCs (Fig. 3C). It is further detectable at a significantly lower level at the margins between BCs and NCs. In *yan*^{443} mutant BC clusters, DE-Cad was strongly expressed in both polar cells and mutant outer BCs (Fig. 3C′). Specifically, DE-Cad was strongly enriched at the boundary between BCs and squamous follicle cells (arrow, Fig. 3C′, see also Fig. S2 in the supplementary material). Interestingly, even partially *yan*^{443} mutant BC clusters can show severe migration defects, and *yan* mutant BCs stay connected to squamous follicle cells, showing elevated DE-Cad accumulation at their plasma membrane interface (arrows, Fig. 3D,D′).

To test whether Yan might suppress DE-Cad expression at the initiation of BC migration, we overexpressed Yan specifically in outer BCs using *slbo-Gal4*. Although overexpression of wild-type Yan had no effects on BC migration, overexpression of a dominant-active form of Yan (*UAS-yan*^{ACT}) strongly delayed migration (Fig. 4B,D). Interestingly, outer BCs expressing activated Yan showed decreased cortical DE-Cad staining (Fig. 4B,B′, Fig. S3 in the supplementary material). To genetically test whether the BC migration defects associated with *slbo-Gal4/UAS-yan*^{ACT} are due to reduced cortical DE-Cad expression, we co-expressed *UAS-DE-Cad* and observed a significant, but incomplete, rescue of the BC migration defects (Fig. 4D). BC migration defects observed in *yan* mutant egg chambers could therefore be due to elevated and/or mislocalized DE-Cad. Indeed, overexpression of *UAS-DE-Cad*^{5,9} in BCs severely delayed their migration and in many cases blocked their detachment from the anterior follicular epithelium (Fig. 4C,D). Confocal immunofluorescence analysis further indicated that, upon overexpression, DE-Cad was localized in a polarized fashion and was primarily concentrated at the trailing edge where BCs and squamous cells interface (arrowheads, Fig. 4C′). Together, these data suggest that DE-Cad expression is regulated, either directly or indirectly, by Yan in BCs, and that precise amounts and/or localization of DE-Cad are important for BC migration.

The transcriptional suppression of E-Cadherin is a crucial step in mediating epithelial-mesenchymal transitions (Thiery, 2002). We therefore tested whether Yan might function to suppress *shg* transcription to allow BCs to exit the follicular layer and become migratory. However, *shg* transcription was not affected in *yan* mutant BCs, nor could we find significant

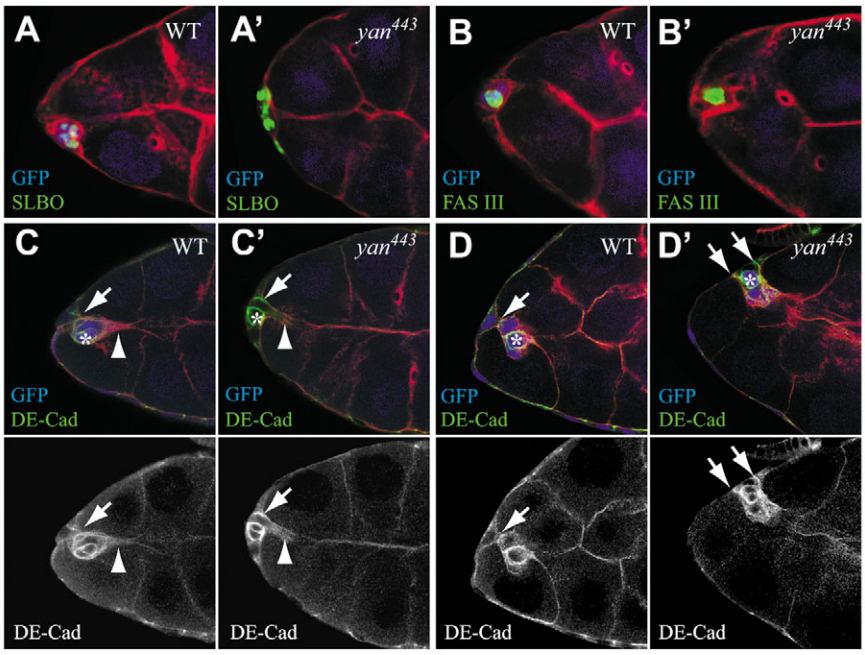
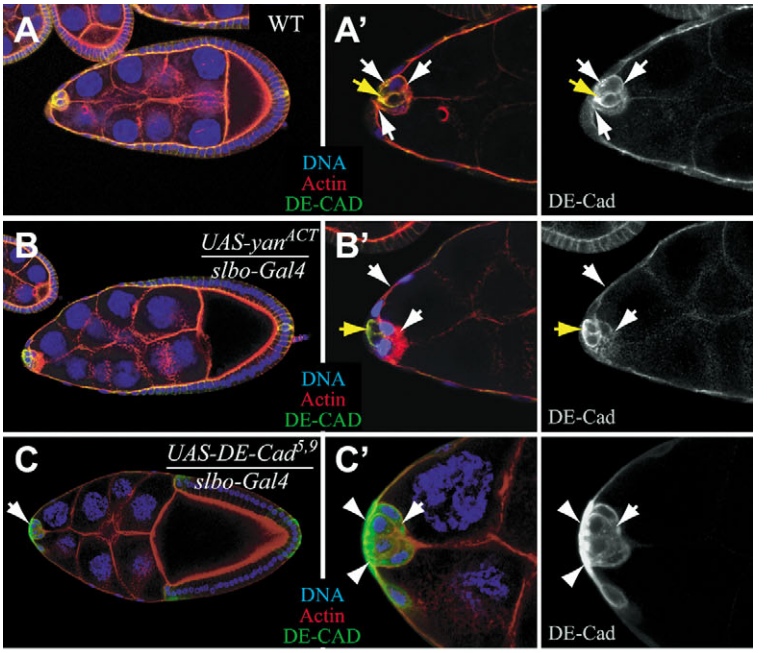


Fig. 3. Accumulation of DE-Cad in *yan* mutant border cells. The expression of Fas3 and DE-Cad was compared between *yan*/+ (WT) and *yan* mutant clones (see Materials and methods for details). (A-D′) Actin is visualized by Alexa-568-phalloidin (red). (A-D) Expression of GFP (blue) marks non-mutant cells in stage 9 mosaic mutant egg chambers. (A′-D′) *yan*^{443} mutant follicle cell clones are marked by the absence of GFP (blue). (A,A′) Slbo is expressed both in *yan* mutant and non-mutant BCs. (B,B′) The polar cell marker Fas3 is restricted to polar cells, even when outer BCs lack Yan expression. (C,C′) DE-Cad accumulates in *yan*^{443} mutant BCs at the boundaries between outer BCs and polar cells (asterisk), as well as at the interface between BCs and squamous follicle cells (arrow). (D,D′) Mutant BCs accumulate high levels of DE-Cad on their surface (arrows) and connect non-mutant BCs and the follicular epithelium. Asterisks mark polar cells.



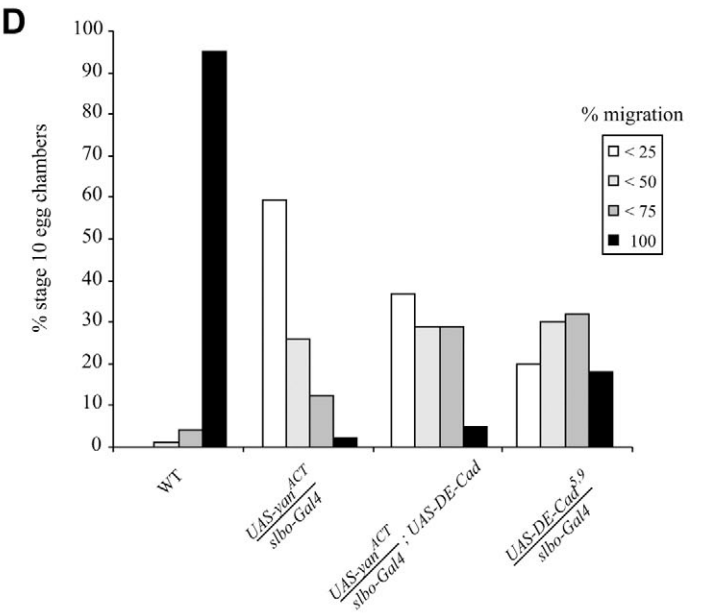


Fig. 4. Yan regulates DE-Cad expression levels. (A-C) Egg chambers are stained for actin (with Alexa-568-phalloidin, red), anti-DE-Cad (green) and DAPI (blue). (A′-C′) Higher magnification views of A-C; grayscale images of DE-Cad allow the visualization of DE-Cad expression levels. Yellow arrows point towards polar cells, white arrowheads point at outer BCs, white arrows point at the interface between BCs and squamous follicle cells. (A,A′) In wild type, high levels of DE-Cad are found at the boundary between polar cells and outer BCs, as well as between outer BCs. DE-Cad is also strongly detectable at the edge of outer BCs. (B,B′) DE-Cad surface expression is reduced in outer BCs where UAS-yan^{ACT} is ectopically expressed using *slbo-Gal4*. (C,C′) Expression of *UAS-DE-Cad*^{5,9} in BCs by *slbo-Gal4*; arrow in C indicates arrested BCs. (D) Functional interaction between DE-Cad and Yan to regulate BC migration. In wild type, 95% of BCs reach the NC-oocyte boundary. Ectopic expression of *UAS-*yan^{ACT} induces strong BC migration defects (60% of BCs migrate less than 25%, 26% migrate 25-50%, 12% migrate 50-75% and 2% complete their migration). Weak expression of *UAS-DE-Cad* partially suppresses *UAS-*yan^{ACT} migration defects (37% migrate less than 25%, 29% migrate 25-50%, 29% migrate 50-75% and 5% reach the nurse cell-oocyte boundary). Forced expression of *UAS-DE-Cad*^{5,9} delays BC migration (20% migrate less than 25%, 30% migrate 25-50%, 32% migrate 50-75% and 18% complete their migration by stage 10 of oogenesis). n>100.

alterations in the activity of a *shg-luciferase* reporter construct in S2R+ cells, either when *yan* expression was knocked down by RNA interference using a *yan*-specific double-stranded RNA (yan^{RNAi}) or when Yan^{ACT} was overexpressed (data not shown), indicating that Yan does not affect *shg* transcription.

Changes in E-cadherin expression at the cell surface can alter the adhesive strength between cells (Yap et al., 1997). Endocytosis has recently surfaced as a crucial step in modulating E-Cadherin turnover at the cell surface, which might be crucial in remodeling adhesive complexes in morphogenetic movements and cell migration where E-Cadherin expression is not transcriptionally suppressed (Paterson et al., 2003). We thus tested whether Yan might modulate endocytosis to potentially affect DE-Cad turnover. Using *Drosophila* SL2 cells, we performed an endocytosis assay in which we followed the uptake of the membrane dye FM1-43 over time. Interestingly, expression of Yan^{ACT} strongly enhanced endocytic FM1-43 incorporation (Fig. 5B,D), whereas it was reproducibly decreased after yan^{RNAi} treatment (Fig. 5A,D). These data suggest that Yan might influence endocytosis and that way impinge on DE-Cad expression and BC migration. To further test whether alterations in the endocytic machinery affect DE-Cad turnover in vivo, we expressed a dominant-negative form of the small GTPase Rab5 (UAS-$Rab5^{(S43N)}$) (Entchev and Gonzalez-Gaitan, 2002) in BCs to block endocytosis. In support of our hypothesis, the migration of BCs expressing *UAS-Rab5*$^{(S43N)}$ was severely delayed and elevated DE-Cad levels could be clearly detected at the cell cortex (Fig. 5F-H).

yan functionally interacts with *slbo*

Slbo is specifically expressed in BCs and is a crucial regulator of *shg* expression and BC migration. Similar to Slbo, Yan is upregulated in BCs just before they become migratory (Fig. 1). To test whether the expression and upregulation of Yan requires Slbo function, we analyzed Yan expression in *slbo*^{1310} mutant egg chambers. Interestingly, Yan was strongly expressed in BCs of stage 9 egg chambers (Fig. 6A), indicating that Yan expression does not require Slbo activity. We further noticed that Yan remained strongly expressed in *slbo*^{1310} BCs that failed to migrate (Fig. 6B), suggesting that migration towards the NC-oocyte boundary is required for Yan downregulation in BCs.

Our data indicate that the two transcription factors Yan and Slbo do not regulate the expression of one other (Fig. 3A′, Fig. 6A), suggesting that they might not operate in a direct, linear pathway. However, Yan and Slbo could instead function in independent pathways that converge to regulate BC migration.

We therefore tested whether *yan* and *slbo* functionally interact in BCs. While overexpression of Yan^{WT} had no or little effect on BC migration, expression of constitutively active Yan^{ACT} severely delayed their migration (Fig. 6C). Furthermore, overexpression of Yan^{WT} in heterozygous *slbo*^{1310} mutants weakly enhanced BC migration defects, whereas ectopic expression of Yan^{ACT} in heterozygous *slbo*^{1310} mutant BCs caused BC migration defects that were even stronger than the defects observed in homozygous *slbo*^{1310} mutant egg chambers. Strikingly, BC migration was completely blocked in homozygous *slbo*^{1310} mutants overexpressing Yan^{WT} (Fig. 6C). Altogether, these data indicate that Yan and Slbo functionally interact to control BC migration without influencing the expression of each other.

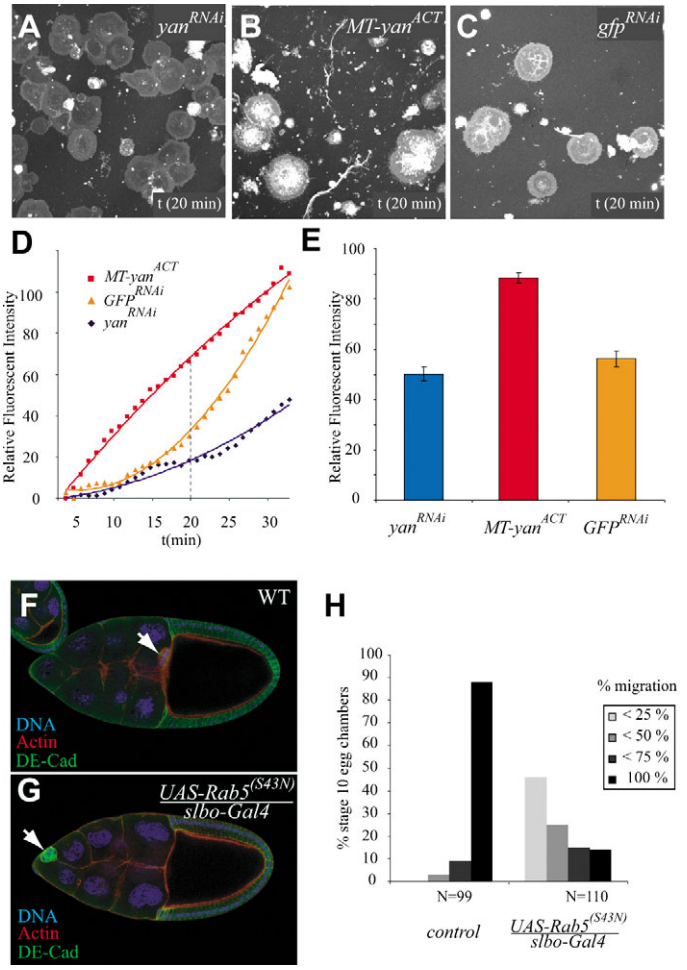


Fig. 5. Yan modulates endocytosis. (A-C) Live FM1-43 uptake in *Drosophila* SL2 cells. FM1-43 membrane incorporation in 20 minutes after *yan*^{RNAi} (A), overexpression of *yan*^{ACT} (B) and *gfp*^{RNAi} control (C). (D) Kinetics of FM1-43 uptake is accelerated in cells overexpressing *yan*^{ACT} (red) and decreased in *yan*^{RNAi} cells (blue), when compared with a *gfp*^{RNAi} control (orange). (E) Twenty-minute FM1-43 uptake after *yan*^{RNAi}, *gfp*^{RNAi} and *MT-Yan*^{ACT} in triplicate. (F,G) Stage 10 egg chamber, stained with anti-DE-Cad (green), Alexa-568-phalloidin (red) and DAPI (blue). *slbo-Gal4*, *UAS-Rab5*$^{(S43N)}$-expressing BCs (G) show impaired ability to migrate and elevated levels of cortical DE-Cad expression when compared with control egg chambers (F). (H) Overexpression of dominant-negative Rab5 in BCs delays their migration (46% migrate less than 25%, 25% migrate between 25-50%, 15% migrate 50-75% and 14% complete their migration by stage 10 of oogenesis).

Early Yan expression is regulated by both the JAK/STAT and Notch pathways

Our observation, that both gain- and loss-of-Yan function influence DE-Cad expression and BC migration, prompted us to re-examine *yan* expression during the course of BC migration. As described above, *yan* is expressed in anterior terminal cells, becomes upregulated in BCs before they become migratory and then the Yan protein levels decay as BCs approach the NC-oocyte boundary (Fig. 1E-G). This complex spatiotemporal expression pattern suggests that different signal transduction pathways are likely to cooperate to control Yan expression. Previous studies have shown that *yan* is a transcriptional target of the Notch/[Su(H)] pathway in

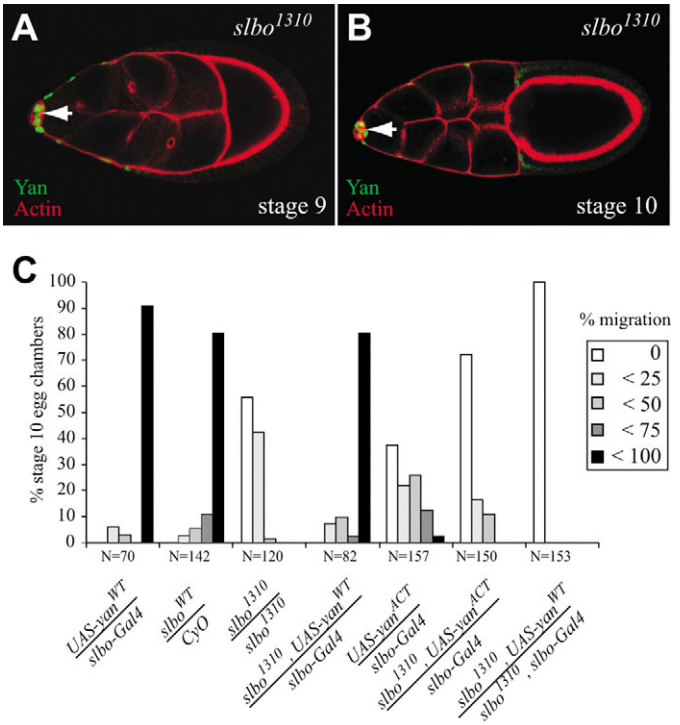


Fig. 6. Activated Yan enhances *slbo* mutant border cell migration defects. (A,B) *slbo*^{1310} mutant egg chamber stained with Alexa-568-phalloidin (red) and anti-Yan (green). Arrows indicate BCs. (A) Yan is normally expressed in *slbo*^{1310} mutant egg chambers at stage 9. (B) Yan is strongly expressed in border cells of *slbo*^{1310} mutant egg chambers that fail to migrate towards the oocyte at stage 10. (C) Statistical representation of BC migration defects. Overexpression of wild-type Yan in BCs has only minor effects on BC migration. By contrast, expression of Yan^{ACT} specifically in BCs delays their migration; 38% completely failed to migrate, 22% migrated less than 25% and 26% of BC clusters migrate less than 50% towards the NC-oocyte boundary. Interestingly, this phenotype can be enhanced by removing one copy of *slbo*^{1310} resulting in 72% of BCs that completely failed to migrate, 17% that migrated less than 25% and 11% that migrated less than 50% towards the oocyte. Expression of Yan and Yan^{ACT} enhances the BC migration defects of *slbo* mutant egg chambers. These BC migration defects are significantly stronger than the defects observed in homozygous *slbo*^{1310} mutant egg chambers, where 56% of mutant BCs show no migration and 42% migrate less than 25% of the way towards the oocyte.

photoreceptor cell development (Rohrbaugh et al., 2002). Furthermore, during oogenesis Notch signaling functions at multiple times to control axis specification, proliferation and the determination of various follicle cell fates (Gonzalez-Reyes and St Johnston, 1998; Grammont and Irvine, 2001; Grammont and Irvine, 2002; Keller Larkin et al., 1999; Larkin et al., 1996; Lopez-Schier and St Johnston, 2001; Ruohola et al., 1991) (Fig. 7A). To test whether the Notch pathway is active at the time of BC migration, we analyzed the expression of a β-galactosidase reporter construct that contains both Su(H)- and Grainy Head-binding sites (Furriols and Bray, 2001). Interestingly, expression of this Notch activity reporter largely resembled the *yan*$^{P(lacZ)}$ expression pattern (Fig. 1B-D). Su(H)-lacZ was strongly expressed in the anterior and posterior terminal cells at early stages of oogenesis (Fig. 7B). At stage 9 and 10 it was strongly active in BCs (Fig. 7B,C). Furthermore, we detected an asymmetric distribution of the Notch ligand Delta. Delta was predominantly expressed in anterior follicle cells that express Yan in stage 8 egg chambers (data not shown).

Because Notch signaling appeared to be active at the time of BC migration, we analyzed the function of Notch on BC development using a temperature-sensitive (ts) allele of Notch (N^{ts}) that allows egg chamber development. To prevent defects in oogenesis prior to stage 8, we shifted N^{ts} females for 8, 12 and 16 hours to the restrictive temperature of 29°C. Whereas BC migration was normal in N^{ts} females raised at the permissive temperature (Fig. 7D), we observed severe BC migration defects in a significant number of egg chambers in N^{ts} females raised at the restrictive temperature (Fig. 7E). Staining of N^{ts} egg chambers with anti-Slbo and anti-Yan antibodies revealed that, in these egg chambers, Slbo

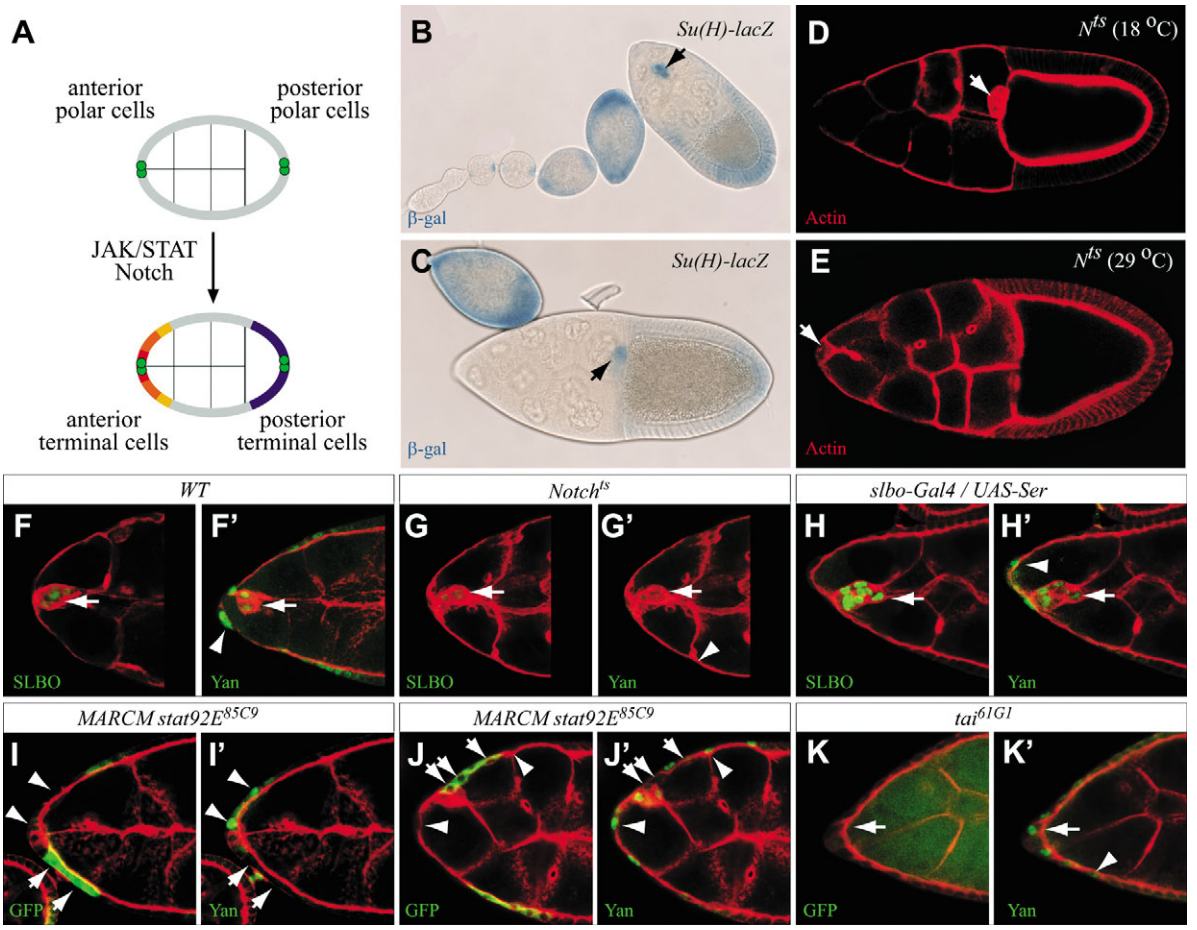


Fig. 7. Yan expression depends on JAK/STAT and Notch pathways. (A) JAK/STAT and Notch signaling around the anterior and posterior polar cells (green) specify terminal cell populations during oogenesis. (B,C) β-galactosidase (β-gal) staining of a Su(H)-lacZ Notch activity reporter. Arrows indicate BCs. (B) β-Gal is dynamically expressed during early oogenesis, strongly in BCs of stage 9 egg chambers. (C) β-Gal is strongly expressed in BCs of stage 10 egg chambers. (D-K′) Egg chambers are stained with Alexa-568-phalloidin (red) to visualize actin. Arrows indicate BCs and arrowheads mark squamous cells. (D) BCs migrate normally in N^{ts} egg chambers at the permissive temperature. (E) BCs have migration defects in N^{ts} egg chambers at the restrictive temperature. (F-H) Slbo expression is shown in green; (F′-K′) Yan is shown in green. (F,F′) Wild-type control BC cluster expressing Slbo and Yan. (G,G′) N^{ts} BCs have reduced Slbo and Yan expression. (H,H′) Slbo and Yan are normally expressed in BCs where *Serrate* (*Ser*) is ectopically expressed. (I,J) *stat92E* mutant follicle cells are positively marked by GFP expression (green, arrows), whereas wild-type cells do not express GFP (arrowhead). (I′,J′) *stat92E* mutant BCs do not express Yan (arrows), whereas Yan expression (green) is normal in wild-type cells (arrowhead). (K,K′) tai^{61G1} mutant BC clones are negatively marked by lack of GFP expression. (K) *tai* mutant BCs do not express GFP (green). (K′) The expression of Yan in *tai* mutant BCs and squamous follicle cells was similar to that observed in wild-type control egg chambers (F′).

expression was strongly reduced (Fig. 7G), whereas Yan was not detectable (Fig. 7G′). Ectopic activation of Notch following expression of one of its ligands Serrate (Ser), or ectopic expression of the constitutively active intracellular domain of Notch (N^{intra}) in BCs using *slbo-Gal4* resulted in robust expression of Slbo and Yan (Fig. 7H,H′ and data not shown) and normal BC motility. Thus, Notch signaling controls the expression of Yan and Slbo in anterior terminal cells and BCs, respectively.

In addition to Notch signaling, the JAK/STAT pathway has recently been shown to control the specification of various follicle cell fates (Keller Larkin et al., 1999; Xi et al., 2003) and BC migration (Beccari et al., 2002; Silver and Montell, 2001) (Fig. 7A). To test whether JAK/STAT signaling regulates Yan expression in BCs, we generated positively marked *stat92E* mutant clones. *stat* mutant follicle cells showed strongly reduced Yan expression (arrows, Fig. 7I,I′) when compared with the corresponding wild-type follicle cells (arrowheads, Fig. 7I,I′) in stage 8 egg chambers. *stat* mutant BCs, as well as squamous follicle cells, did not express Yan (arrows, Fig. 7J,J′), whereas it was normally expressed in wild-type anterior terminal cells (arrowhead, Fig. 7J,J′). Similar results were obtained in clones that were mutant for *hopscotch*, the JAK kinase that activates Stat92E (data not shown). Interestingly, BCs of hypomorphic $stat^{F}/stat^{P1608}$ mutant egg chambers showed severe BC migration defects and a significant reduction in Yan expression (data not shown), suggesting that Yan is a crucial target gene of the JAK/STAT pathway in BCs and anterior terminal cells.

Finally, we found that the ecdysone nuclear hormone pathway, which coordinates BC migration with egg chamber development (Bai et al., 2000), did not influence Yan expression. Staining of tai^{61G1} mutant BC clusters with anti-Yan antibodies revealed that Yan was normally expressed in the absence of *tai* gene activity (Fig. 7K,K′). Altogether, we conclude that the JAK/STAT and Notch signaling pathways control the expression of Slbo and Yan in the developing egg chamber to promote BC migration.

Yan is downregulated in response to increasing PVR and EGFR signaling

Yan expression decreases in wild-type BCs as they migrate from the anterior pole of stage 9 egg chamber towards the NC-oocyte boundary (Fig. 1), but remains strongly expressed in *slbo* mutant BCs, which fail to migrate and remain at the anterior tip of stage 10 egg chambers (Fig. 6). Furthermore, overexpression of yan^{WT} had little or no effects on BC

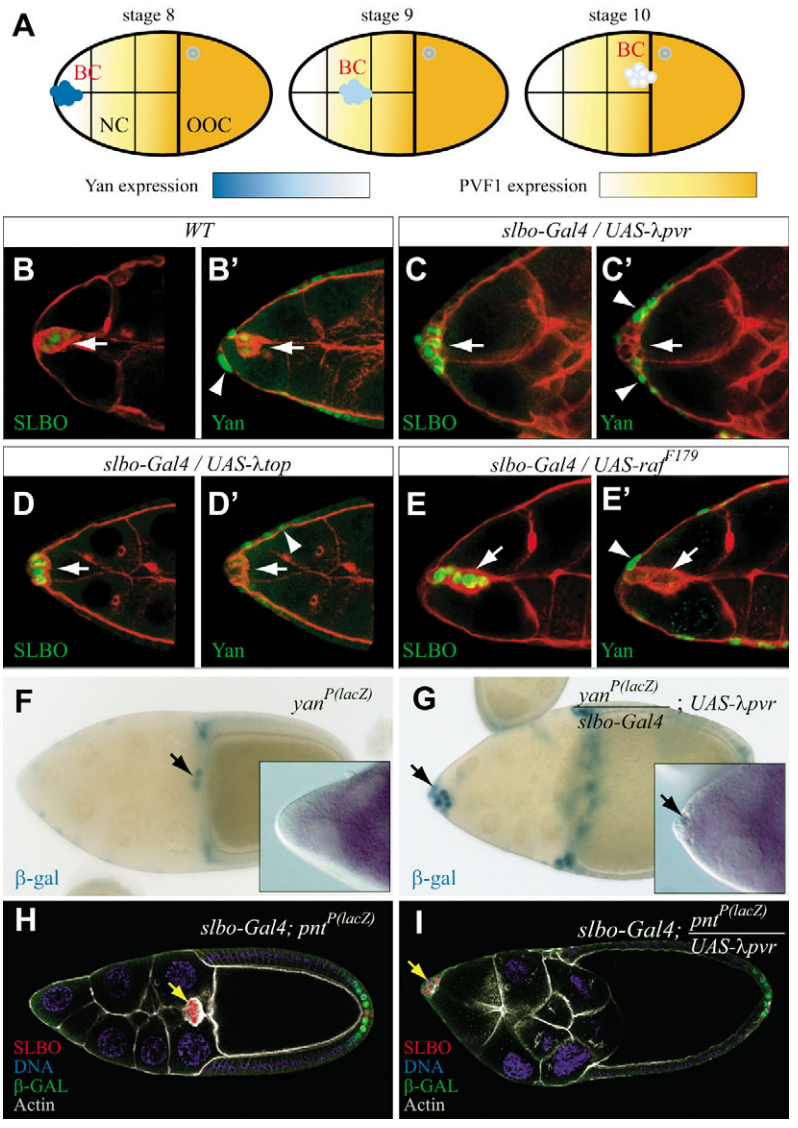


Fig. 8. RTKs signaling downregulates Yan. (A) Yan (blue) is strongly expressed in BCs that delaminate from the follicular epithelium, but expression gradually decreases as BCs migrate along the increasing gradient of Pvf1 (orange). The inverse expression levels of Yan and Pvf1 suggest that PVR activity counteracts Yan expression in migrating BCs. (B-E′) Egg chambers are stained with Alexa-568-phalloidin (red) to visualize actin. Arrows indicate BCs and arrowheads the squamous follicle cells. (B-E) Anti-Slbo (green) and actin (red) staining; (B′-E′) anti-Yan (green) and actin (red) staining. (B,B′) Slbo and Yan are strongly expressed in wild-type BCs that delaminate from the follicular epithelium. (C) Slbo is normally expressed in BCs that ectopically express activated PVR. (C′) By contrast, Yan is undetectable in BCs but is expressed normally in squamous follicle cells where *slbo-Gal4* is inactive. Similarly, expression of either activated EGFR (D,D′) or activated Raf (E,E′) in BCs downregulates Yan but not Slbo. All experiments and stainings were done in parallel, and images were taken with the same settings, together with experiments in Fig. 7F-H. The expression levels of Yan in squamous follicle cells serve as an internal control. (F,G) β-Gal staining indicates *yan* expression in $yan^{P(lacZ)}$ (F) and $yan^{P(lacZ)}$/*slbo-Gal4*; *UAS-λPVR* (G) stage 10 egg chambers. Arrows indicate BCs; insets show *yan* mRNA expression by in situ hybridization. (H,I) Stage 10 egg chamber stained with anti-Slbo antibody (red), anti-β-Gal antibody indicating *pnt-lacZ* expression (green), DAPI (blue) and Alexa-568-phalloidin (gray). (H) Pnt-lacZ is expressed in posterior cells and is absent in wild-type BCs. (I) Expression of activated PVR in BCs arrests their migration. These BCs express Slbo but do not express *pnt-lacZ*.

migration, whereas expression of activated *yan* (*yan*^{ACT}), a form of Yan that cannot be post-translationally regulated by phosphorylation via the MAPK pathway (Rebay and Rubin, 1995), showed a severe delay in BC migration. These data, together with the recent finding that Pvf1 is expressed in an increasing gradient along the AP axis towards the NC-oocyte boundary, and that the PVR and EGFR signaling pathways are active in BCs and are crucial for their migration (Duchek and Rorth, 2001; Duchek et al., 2001; McDonald et al., 2003), suggested that the gradient of PVR and EGFR activity might control the spatiotemporal expression of Yan during the course of BC migration (Fig. 8A).

To test whether the PVR and EGFR pathways can trigger Yan downregulation in BCs, we expressed activated forms of PVR, EGFR and the RTK signal transducer RAF in BCs, and assayed Slbo and Yan expression. Whereas Slbo was expressed at normal levels in BCs that expressed activated forms of PVR, EGFR or RAF (Fig. 8B-E), Yan protein levels were strongly reduced (Fig. 8B′-E′). As an internal control, normal Yan expression levels were observed in squamous follicle cells where *slbo-Gal4* is not expressed. Furthermore, expression of activated FGFR hardly affected BC migration and Yan expression levels were comparable to wild type (data not shown).

To distinguish whether PVR and EGFR regulate Yan expression at the transcriptional or post-transcriptional level, we expressed activated PVR using *slbo-Gal4* in a *yan*$^{P(lacZ)}$ background. BCs that do not express activated PVR (Fig. 8F) are clearly distinguishable from activated PVR-expressing BCs (Fig. 8G) by their migration defects in stage 10 egg chambers. Yan has previously been described to suppress its own transcription (Rohrbaugh et al., 2002), and loss of Yan protein might thus result in activation of the *yan*$^{P(lacZ)}$ reporter due to an autoregulatory feedback loop. Interestingly, BCs that express activated PVR strongly express β-Gal, suggesting that PVR activation controls Yan expression post-transcriptionally, which is further supported by RNA in situ hybridization data using a *yan*-specific probe (see insets, Fig. 8F,G).

Our data support a model where JAK/STAT and Notch signaling specify anterior terminal cells including BCs, resulting in a strong expression of Yan in BCs; increasing RTK activity can decrease Yan expression as BCs approach their destination (Fig. 9).

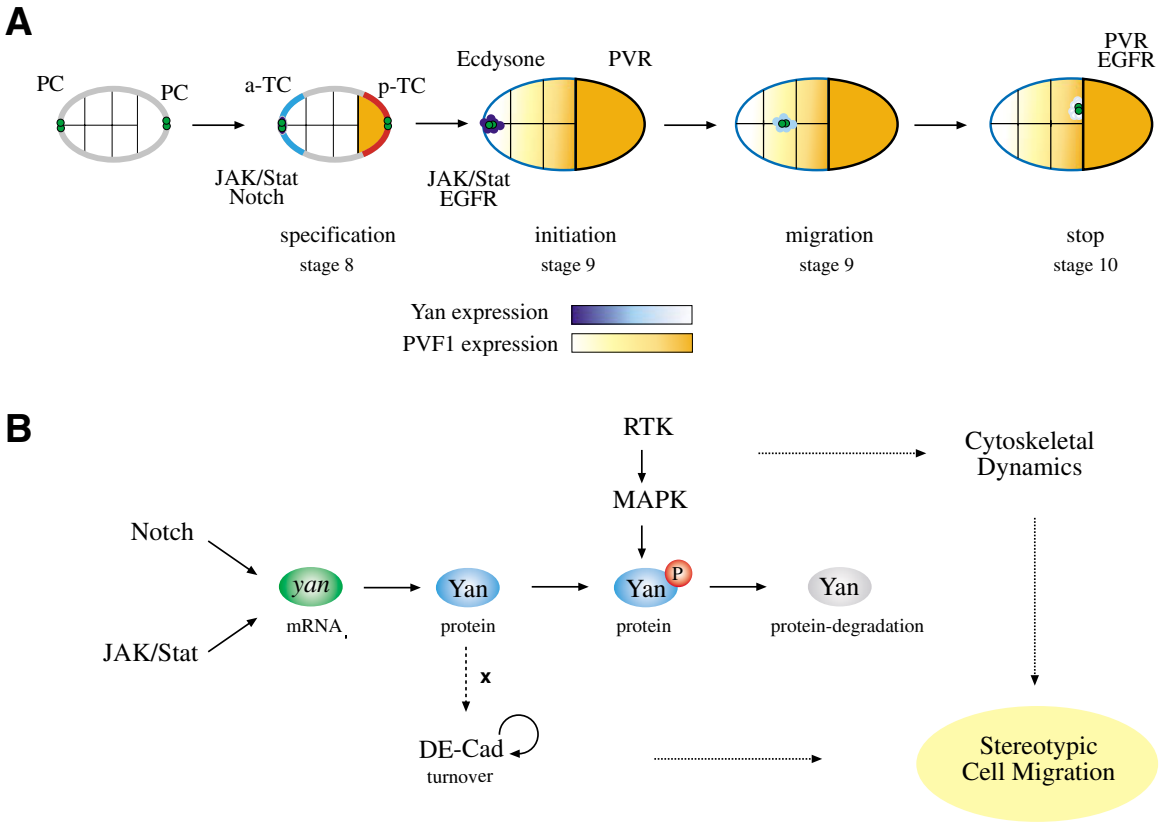


Fig. 9. Integrative model of Yan regulation and function during border cell migration. (A) The JAK/STAT and Notch pathways specify the group of anterior terminal cells (a-TC) around the pair of polar cells (PC, green). Cells that are directly adjacent to the anterior polar cells are specified as BCs expressing Slbo. With the exception of the polar cells, Yan (blue) is expressed in all a-TCs, and becomes upregulated immediately prior to their transit from a static, epithelial state to a migratory state (dark blue). Posterior terminal cells (red) are specified by Gurken (EGF) signaling. Pvf1 (orange), secreted from the oocyte, guides BCs towards the oocyte. As BCs face increasing Pvf1 levels from anterior to posterior, Yan expression levels decrease (light blue circles). (B) Regulatory relationships between signal transduction pathways that control BC migration. JAK/STAT and Notch signaling pathways regulate the expression of *slbo* and *yan*, whereas PVR and EGFR induction lead to Yan phosphorylation and its inactivation. The transient upregulation of Yan at the initiation of BC migration facilitates DE-Cad turnover at the plasma membrane to enable BCs to make and break adhesive contacts, and to promote detachment from the epithelium and cell movement. Coordinated, dynamic changes in cell adhesion and cytoskeletal organization enable BCs to migrate in a stereotypic fashion.

The Notch and RTK signaling pathways function to control AP axis specification at early stages of oogenesis, resulting in expression of the ETS transcription factor *pointed* (*pnt*) at the posterior pole. In photoreceptor cells, RTK activation induces the downregulation of Yan, which subsequently allows *pnt* expression and a switch in cell fate. Thus, we tested whether Yan expression at the initiation of BC migration might suppress *pnt* expression, and Yan downregulation at the NC-oocyte boundary might lead to *pnt* expression, and therefore, potentially, induce BC differentiation. RNA in situ hybridization data and analysis of $pnt^{P(lacZ)}$ expression in ovaries revealed that *pnt* is not expressed in BCs at any stage of oogenesis, and ectopic expression of *slbo-Gal4::UAS-pntP2* does not alter BC motility (data not shown). Furthermore, ectopic activation of PVR in BCs downregulated Yan expression (Fig. 8C′) and delayed BC migration without induction of $pnt^{P(lacZ)}$ in BCs (Fig. 8I). We thus conclude that although the Notch and RTK signaling pathways modulate Yan expression levels in both photoreceptor cells and BCs, the mechanisms used are not identical, and the transcriptional responses and downstream mechanisms depend, at least in part, on the developmental context.

Discussion

Directed cell migration is a complex process whereby extracellular cues stimulate distinct signal transduction pathways to modulate cytoskeletal dynamics, as well as cell-cell and cell-substratum adhesion. Despite considerable advances in recent years, the relationships between the activities of signaling pathways, transcription factors and cell adhesion molecules in the context of cell migration and metastatic cancer remain poorly understood. Here, we describe a function for the ETS transcription factor Yan in BC migration. Yan expression levels are spatiotemporally regulated during the course of BC migration by the Notch, JAK/STAT and RTK signaling pathways (Fig. 9). Either an increase or a decrease in Yan activity delays BC migration and is associated with an alteration in DE-Cad-dependent adhesive complexes, which themselves are crucial for BC motility. The finding that Yan functions as a key regulator of BC migration is an important step towards understanding the molecular mechanisms by which extrinsic cues regulate cell adhesion and cytoskeletal dynamics to control invasive cell migration in vivo.

Yan is dynamically expressed in migrating border cells

Our study reveals that during oogenesis *yan* mutant BCs are defective in their invasive migratory behavior. In addition, we found that Yan is upregulated as BCs exit the epithelium to become migratory, and that subsequently Yan protein levels decay as BCs approach the NC-oocyte boundary (Fig. 1). Because Yan has previously been shown to function as a transcriptional repressor and an inhibitor of neuronal differentiation, we tested whether it regulates BC identity. Although we cannot completely exclude this possibility, BC markers are properly expressed in the absence of Yan. Thus, we propose that Yan promotes BC motility, an hypothesis which is supported by the observations that: (1) Yan is upregulated prior to the BCs exiting the follicular epithelium to become migratory; (2) Yan protein levels decrease progressively as BCs approach their final destination; and (3) *yan* mutant BCs exhibit a delay in migration. Interestingly, ectopic expression of constitutively activated Yan in BCs also delays their migration, suggesting that the spatiotemporal activity of Yan protein needs to be precisely controlled during the migratory process.

Yan coordinates signal inputs from different pathways

The dynamic expression of Yan is crucial for BC migration, as indicated by the migratory defects associated with both gain- and loss-of-function alleles of *yan*. Analysis of mutations in the JAK/STAT (Silver and Montell, 2001; Xi et al., 2003) and Notch (Gonzalez-Reyes and St Johnston, 1998) signaling pathways revealed that they are required for the expression of at least two transcription factors that are crucial for BC migration and which themselves influence DE-Cad activity. Slbo is specifically expressed in BCs and enhances *shg* transcription. Yan, by contrast, is expressed in anterior terminal cells, but becomes upregulated in BCs at the time they exit from the epithelium to become migratory. Yan might enhance DE-Cad turnover to facilitate the transition from an immobile epithelial state to a migratory one. Enhanced BC migration defects of hypomorphic *slbo* mutant egg chambers overexpressing Yan further underscore their interaction to regulate DE-Cad expression and BC migration.

Interestingly, we find that Yan expression levels gradually decrease as BCs move along an increasing PVR/EGFR activity gradient (Duchek and Rorth, 2001; Duchek et al., 2001; McDonald et al., 2003). Yan has been shown to be phosphorylated by the EGFR-MAPK pathway, which triggers its nuclear export and protein degradation (Rebay and Rubin, 1995). Consistent with these previous studies, expression of dominant-active PVR and EGFR in BCs blocks BC migration and abrogates Yan protein expression, whereas *yan* transcript or enhancer trap expression is still detectable. Expression of activated Ras and Raf similarly induced Yan downregulation, consistent with an involvement of the canonical Ras/MAPK pathway in mediating PVR/EGFR signaling. We note, however, that although BC migration was significantly delayed upon ectopic expression of activated Ras, activated Raf hardly affected their ability to migrate. The basis of this difference, which might be due to complex feedback loops between the implicated signaling pathways, is unclear at the present time and will need to be investigated further.

Yan regulates the accumulation of DE-Cad containing adhesive complexes

Is the function of Yan to facilitate the transition of BCs from an epithelial to a migratory state, or to promote their motility? Although E-Cadherin is often downregulated as cells transit from an epithelial to a mesenchymal-like migratory state (Thiery, 2002), this may not be the case in BCs, as DE-Cad is strongly expressed in BCs and *shg* mutant BCs fail to migrate (Niewiadomska et al., 1999). However, BCs mutant for *yan* or *tai* accumulate ectopic DE-Cad-containing adhesive complexes (Bai et al., 2000). Consistent with these observations, ectopic stimulation of PVR in BCs, which enhances *tai* mutant BC migration defects, also results in elevated, cortical DE-Cad staining (McDonald et al., 2003). Even though the observed

BC migration defects in these mutants might not be due to altered surface levels of DE-Cad only, we found that overexpression of DE-Cad alone can cause migration impaired BCs. E-cadherin not only mediates homophilic cell-cell adhesion but also functions together with its binding partners as a key regulator of the cortical actin cytoskeleton. It is therefore interesting to note that follicle cells overexpressing DE-Cad show severely enhanced filamentous actin staining (data not shown).

Our experiments revealed that DE-Cad was elevated in *yan* mutant BCs and suppressed upon expression of *UAS-yan*^{ACT}, suggesting that Yan controls, at least in part, DE-Cad expression in BCs. These observations find further support in the partial rescue of *slbo-Gal4::UAS-yan*^{ACT}-induced BC migration defects upon co-expression of *UAS-DE-Cad*. How does Yan affect DE-Cad expression in BCs? Although the function of Yan as a transcriptional repressor in various tissues (Rebay, 2002) suggests that it may act as a transcriptional regulator of *shg*, we could not detect a change in *shg* transcription in *yan* mutant follicle cells. However, increased FM1-43 incorporation in *Drosophila* SL2 cells overexpressing Yan^{ACT}, and a decrease in incorporation after *yan*^{RNAi}, suggests a change in endocytic activity. E-Cadherin has previously been found in endocytic compartments and endocytosis has been speculated to modulate E-Cadherin activity regulation during morphogenetic movements (Lanzetti et al., 2004; Paterson et al., 2003). Interestingly, blocking endocytosis by the expression of dominant-negative Rab5 lead to severe BC migration defects and increased DE-Cad staining. Consistent with our observations, expression of *shg* under a heterologous promoter has recently been shown to rescue *shg* mutant BC migration defects, suggesting that the dynamic expression of DE-Cad in BCs might depend on both transcriptional and post-transcriptional mechanisms (Pacquelet et al., 2003). Based on these results, we favor a model whereby Yan might, at least in part, function to regulate DE-Cad turnover, possibly through the transcriptional regulation of as-yet-unidentified components of the endocytic machinery.

ETS factors during epithelial-mesenchymal transition and metastatic cancer

ETS transcription factors are not only regulators of morphogenetic processes but have also been identified as oncogenes. Indeed, several ETS factors are upregulated in invasive cancers and are currently used as molecular markers to grade their invasiveness (Dittmer and Nordheim, 1998; Oikawa and Yamada, 2003; Sharrocks, 2001). The molecular function of ETS factors in tumorigenesis is not clear, as they can act as both oncogenes and tumor suppressors. Our observations that *yan* is associated with similar gain- and loss-of-function phenotypes support both a positive and negative function on invasive migration, dependent on activity levels and possibly on available cofactors. Furthermore, the complexity of invasive tumors makes it difficult to assess what function ETS factors have, as they are upregulated not only in the cancerous tissue but also, for example, in forming blood vessels during tumor angiogenesis. Finally, our finding that Yan levels are regulated by JAK/STAT, Notch and RTK signaling pathways, which have been implicated in metastatic cancer, is another strong connection between Yan-like ETS factors and tumorigenesis.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/132/15/3493/DC1

References

Bai, J., Uehara, Y. and Montell, D. J. (2000). Regulation of invasive cell behavior by taiman, a Drosophila protein related to AIB1, a steroid receptor coactivator amplified in breast cancer. *Cell* **103**, 1047-1058.

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.

Dittmer, J. and Nordheim, A. (1998). Ets transcription factors and human disease. *Biochim. Biophys. Acta* **1377**, F1-F11.

Duchek, P. and Rorth, P. (2001). Guidance of cell migration by EGF receptor signaling during Drosophila oogenesis. *Science* **291**, 131-133.

Duchek, P., Somogyi, K., Jekely, G., Beccari, S. and Rorth, P. (2001). Guidance of cell migration by the Drosophila PDGF/VEGF receptor. *Cell* **107**, 17-26.

Entchev, E. V. and Gonzalez-Gaitan, M. A. (2002). Morphogen gradient formation and vesicular trafficking. *Traffic* **3**, 98-109.

Fulga, T. A. and Rorth, P. (2002). Invasive cell migration is initiated by guided growth of long cellular extensions. *Nat. Cell Biol.* **4**, 715-719.

Furriols, M. and Bray, S. (2001). A model Notch response element detects Suppressor of Hairless-dependent molecular switch. *Curr. Biol.* **11**, 60-64.

Geisbrecht, E. R. and Montell, D. J. (2002). Myosin VI is required for E-cadherin-mediated border cell migration. *Nat. Cell Biol.* **4**, 616-620.

Gonzalez-Reyes, A. and St Johnston, D. (1998). Patterning of the follicle cell epithelium along the anterior-posterior axis during Drosophila oogenesis. *Development* **125**, 2837-2846.

Grammont, M. and Irvine, K. D. (2001). fringe and Notch specify polar cell fate during Drosophila oogenesis. *Development* **128**, 2243-2253.

Grammont, M. and Irvine, K. D. (2002). Organizer activity of the polar cells during Drosophila oogenesis. *Development* **129**, 5131-5140.

Karim, F. D., Chang, H. C., Therrien, M., Wassarman, D. A., Laverty, T. and Rubin, G. M. (1996). A screen for genes that function downstream of Ras1 during Drosophila eye Development. *Genetics* **143**, 315-329.

Keller Larkin, M., Deng, W. M., Holder, K., Tworoger, M., Clegg, N. and Ruohola-Baker, H. (1999). Role of Notch pathway in terminal follicle cell differentiation during Drosophila oogenesis. *Dev. Genes Evol.* **209**, 301-311.

Kuromi, H. and Kidokoro, Y. (1999). The optically determined size of exo/endo cycling vesicle pool correlates with the quantal content at the neuromuscular junction of Drosophila larvae. *J. Neurosci.* **19**, 1557-1565.

Lanzetti, L., Palamidessi, A., Areces, L., Scita, G. and Di Fiore, P. P. (2004). Rab5 is a signalling GTPase involved in actin remodelling by receptor tyrosine kinases. *Nature* **429**, 309-314.

Larkin, M. K., Holder, K., Yost, C., Giniger, E. and Ruohola-Baker, H. (1996). Expression of constitutively active Notch arrests follicle cells at a precursor stage during Drosophila oogenesis and disrupts the anterior-posterior axis of the oocyte. *Development* **122**, 3639-3650.

Laudet, V., Hanni, C., Stehelin, D. and Duterque-Coquillaud, M. (1999). Molecular phylogeny of the ETS gene family. *Oncogene* **18**, 1351-1359.

Lopez-Schier, H. and St Johnston, D. (2001). Delta signaling from the germ line controls the proliferation and differentiation of the somatic follicle cells during Drosophila oogenesis. *Genes Dev.* **15**, 1393-1405.

McDonald, J. A., Pinheiro, E. M. and Montell, D. J. (2003). PVF1, a PDGF/VEGF homolog, is sufficient to guide border cells and interacts genetically with Taiman. *Development* **130**, 3469-3478.

Montell, D. J. (2003). Border-cell migration: the race is on. *Nat. Rev. Mol. Cell Biol.* **4**, 13-24.

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.

Niewiadomska, P., Godt, D. and Tepass, U. (1999). DE-Cadherin is required for intercellular motility during Drosophila oogenesis. *J. Cell Biol.* **144**, 533-547.

Oikawa, T. and Yamada, T. (2003). Molecular biology of the Ets family of transcription factors. *Gene* **303**, 11-34.

O'Neill, E. M., Rebay, I., Tjian, R. and Rubin, G. M. (1994). The activities of two Ets-related transcription factors required for Drosophila eye development are modulated by the Ras/MAPK pathway. *Cell* **78**, 137-147.

Pacquelet, A., Lin, L. and Rorth, P. (2003). Binding site for p120/{delta}-catenin is not required for Drosophila E-cadherin function in vivo. *J. Cell Biol.* **160**, 313-319.

Paterson, A. D., Parton, R. G., Ferguson, C., Stow, J. L. and Yap, A. S. (2003). Characterization of E-cadherin endocytosis in isolated MCF-7 and chinese hamster ovary cells: the initial fate of unbound E-cadherin. *J. Biol. Chem.* **278**, 21050-21057.

Rebay, I. (2002). Keeping the receptor tyrosine kinase signaling pathway in check: lessons from Drosophila. *Dev. Biol.* **251**, 1-17.

Rebay, I. and Rubin, G. M. (1995). Yan functions as a general inhibitor of differentiation and is negatively regulated by activation of the Ras1/MAPK pathway. *Cell* **81**, 857-866.

Rohrbaugh, M., Ramos, E., Nguyen, D., Price, M., Wen, Y. and Lai, Z. C. (2002). Notch activation of yan expression is antagonized by RTK/pointed signaling in the Drosophila eye. *Curr. Biol.* **12**, 576-581.

Rorth, P. (2002). Initiating and guiding migration: lessons from border cells. *Trends Cell Biol.* **12**, 325-331.

Ruohola, H., Bremer, K. A., Baker, D., Swedlow, J. R., Jan, L. Y. and Jan, Y. N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in Drosophila. *Cell* **66**, 433-449.

Sharrocks, A. D. (2001). The ETS-domain transcription factor family. *Nat. Rev. Mol. Cell Biol.* **2**, 827-837.

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.

Thiery, J. P. (2002). Epithelial-mesenchymal transitions in tumour progression. *Nat. Rev. Cancer* **2**, 442-454.

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.

Yap, A. S., Brieher, W. M. and Gumbiner, B. M. (1997). Molecular and functional analysis of cadherin-based adherens junctions. *Annu. Rev. Cell Dev. Biol.* **13**, 119-146.