EMBO reports

scientific report

Drosophila RalA is essential for the maintenance of Jak/Stat signalling in ovarian follicles

Christian Ghiglione, Olivier Devergne†, Delphine Cerezo & Stéphane Noselli+
Institute of Developmental Biology and Cancer, University of Nice Sophia-Antipolis, UMR6543-CNRS, Nice, France

Small GTPases of the Ras-like (Ral) family are crucial for signalling functions in both normal and cancer cells; however, their role in a developing organism is poorly understood. Here, we identify the *Drosophila* Ral homologue RalA as a new key regulator of polar-cell differentiation during oogenesis. Polar cells have a crucial role in patterning the egg chamber and in recruiting border cells, which undergo collective and guided migration. We show that *RalA* function is essential for the maintenance of anterior and posterior polar-cell fate and survival. *RalA* is required cell autonomously to control the expression of polar-cell-specific markers, including the Jak/Stat ligand Unpaired. The loss of RalA also causes a cell non-autonomous phenotype owing to reduced Jak/Stat signalling in neighbouring follicle cells. As a result, border-cell assembly and migration as well as the polarization of the oocyte are defective. Thus, *RalA* is required in organizing centres to control proper patterning and migration *in vivo*.

Keywords: RalA; polar cell; border cell; Jak/Stat
EMBO reports (2008) **9**, 676–682. doi:10.1038/embor.2008.79

INTRODUCTION

The migration of border cells (BCs) is a powerful model system to study collective migration, which occurs during normal development and also in some epithelial cancers. BCs migrate as a cohort of cells with mixed epithelial and mesenchymal attributes. Each cluster is made up of two cell types organized radially with two central polar cells (PCs) encircled by 6–8 outer border cells (oBCs; Fig 1A,B; Rorth, 2002; Montell, 2003). PCs have been shown to be crucial for organizing function in egg chambers (Grammont & Irvine, 2002; Xi *et al*, 2003), whereas oBCs are involved in the migratory activity of BCs. Because the organization of BC clusters is functionally important and dynamic (Bianco *et al*, 2007; Prasad & Montell, 2007), it is crucial to identify the genes that control PC and oBC differentiation and to understand how these groups of cells are assembled during development, as well as to explain how the coordination of assembly and migration is carried out.

Institute of Developmental Biology and Cancer, University of Nice Sophia-Antipolis, UMR6543-CNRS, Parc Valrose, 06108 Nice cedex 2, France
†Present address: Yale School of Medicine, Boyer Center for Molecular Medicine (Room 337), New Haven, Connecticut 06519, USA
+Corresponding author. Tel: +33 4 92 07 64 33; Fax: +33 4 92 07 64 03;
E-mail: noselli@unice.fr

Received 3 September 2007; revised 9 April 2008; accepted 9 April 2008; published online 13 June 2008

RESULTS AND DISCUSSION

To isolate new genes involved in BC organization and migration, we conducted a genetic mosaic screen in *Drosophila* egg chambers using a set of 311 independent P-element-mediated lethal mutations, targeting individual genes on the underscreened X chromosome (Bourbon *et al*, 2002; Ghiglione *et al*, 2002; see Methods). One mutation, PG69, induced a complete lack of BC migration (Fig 1C). The determination of PG69 chromosomal position showed that it is inserted in the 5′ untranslated region of *RalA/CG2849*, the unique *Drosophila* homologue of human *RAL* genes (Fig 1D). Another insertion, PG89, in the first intron of *RalA* (Fig 1D) was analysed and showed phenotypes similar to those of PG69 (data not shown). As PG69 and PG89 are Gal4-expressing enhancer-trap lines (Bourbon *et al*, 2002), they were used to drive expression of diverse UAS lines in a *RalA* mutant background. Expression of UAS–*RalA* (a wild-type form of RalA) or UAS–$RalA^{G20V}$ (a constitutively activated form; Sawamoto *et al*, 1999) rescued lethality and migration (Fig 2E; 29 out of 30 rescued egg chambers showed normal migration). However, expression of UAS–$RalA^{S25N}$ (a dominant-negative form; Sawamoto *et al*, 1999) did not, indicating that PG69 and PG89 are mutations targeting the *RalA* gene. Furthermore, a crossreacting polyclonal antibody directed against the human RALB protein allowed detection of RalA in wild type, which was strongly reduced in mutant ovaries taken from a viable $RalA^{h1}/RalA^{PG89}$ combination (Fig 1E).

Overexpression of UAS–$RalA^{S25N}$, but not UAS–*RalA*, in BCs induced by using *USG*–Gal4 (a combination of two Gal4 lines, *Upd*–Gal4 and *slbo*–Gal4) led to a high proportion of BC clusters that did not migrate at all or migrated only slightly (>70%, $n=298$; Fig 1F,G), thereby confirming our genetic clonal analysis. Consistently, RalA is expressed in follicle and border cells, as shown by *in situ* hybridization and specific enhancer-trap lines (Fig 2A–D″). The RalA protein is localized to the plasma membrane in ovaries and we showed that a carboxy-terminal sequence containing a putative CAAX box (198-CTLL-STOP), important for prenylation of H-Ras and other small GTPases, is essential for RalA subcellular localization and function. Indeed, the deletion or hiding of this motif was sufficient to abolish

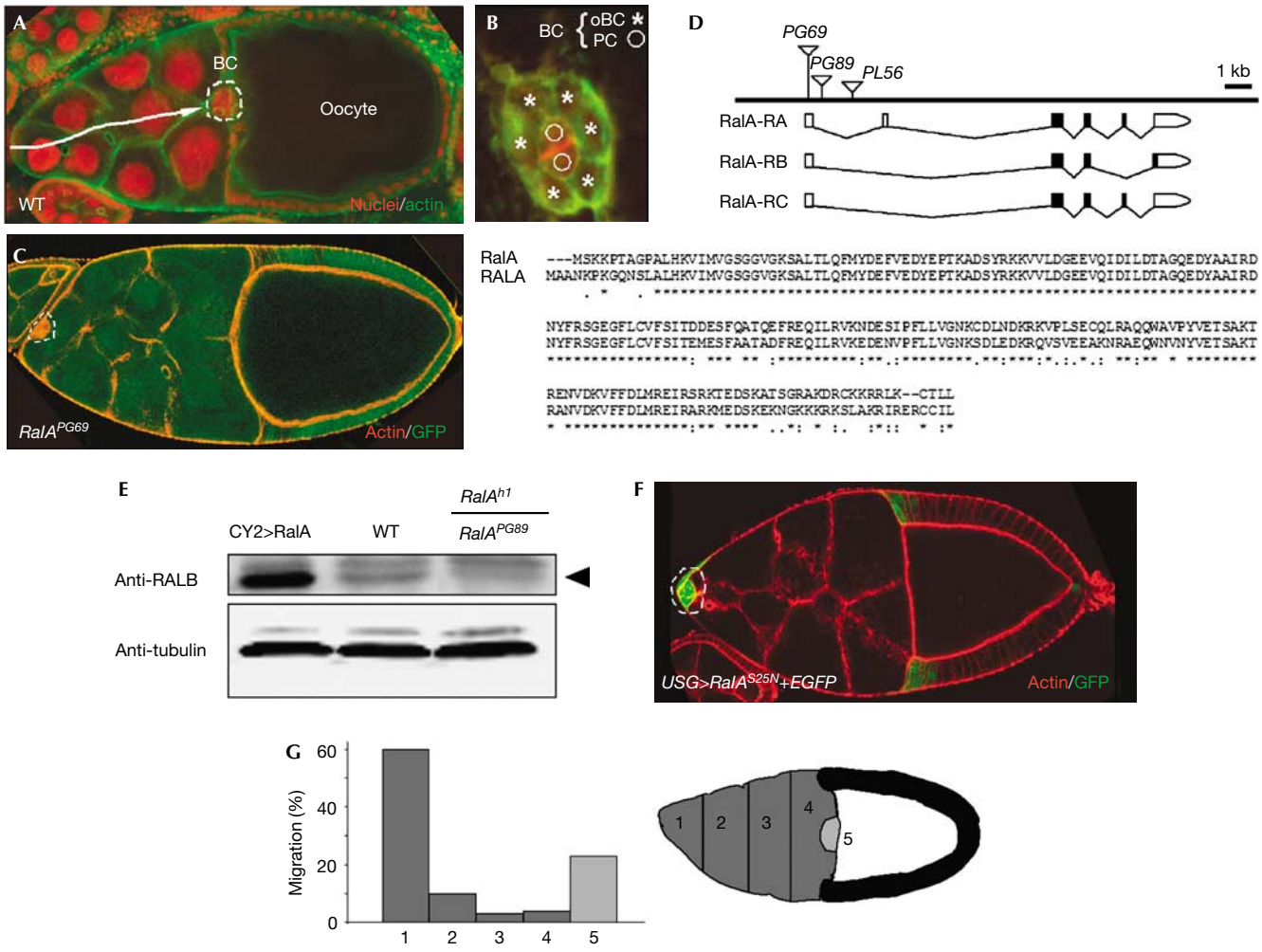


Fig 1 | RalA controls border-cell migration in egg chambers. (**A**) Wild-type (WT) stage-10 egg chamber stained with phalloidin (green) and propidium iodide (red). Border cells (BCs; enclosed by dotted lines) have reached the anterior region of the oocyte, after delaminating from the anterior epithelium and migrating through the nurse-cell compartment (arrow). (**B**) BC cluster organization showing two centrally located polar cells (PCs; circles) surrounded by six outer BCs (oBCs; asterisks). (**C**) Mosaic stage-10 egg chamber containing homozygous *RalA*^{PG69} cells (GFP–negative). Note the absence of migration of BCs (enclosed by dotted lines). Actin is shown in red. (**D**) Schematic view of the genomic organization of the *RalA* locus and ClustalW alignment of RalA and human RALA (accession no. EAL23994) protein sequences. (**E**) Western blot of ovary extracts. RalA (black arrowhead) is detected using a polyclonal antibody directed against the human RALB protein. Tubulin antibodies were used to assess protein levels in each lane. (**F**) Expression of UAS–*RalA*^{S25N} in BCs (enclosed by dotted lines) blocks their migration. (**G**) Stage-10 migration defects following expression of *RalA*^{S25N} in BCs. The nurse-cell compartment (dark grey) has been divided into five regions (1–5) to determine the extent of migration. GFP, green fluorescent protein.

both the rescue activity and plasma membrane localization of RalA (Fig 2E,H–J).

RalA has been shown to be downstream from *Drosophila* Rap1 for bristle development (Mirey *et al*, 2003). We thus tested whether Rap1 might also be crucial in egg chambers. Interestingly, expression of a dominant-negative form of Rap1 (Rap1^{N17}) blocked migration of BCs, similar to RalA^{S25N} (compare Fig 1 and supplementary Fig 2 online), suggesting that Rap1 and RalA are part of a common pathway for BC migration.

To determine which cells among the BC cluster might require *RalA* function, we analysed mosaic clusters containing zero, one or two mutant PCs with wild-type or mutant oBCs. We found that the BC cluster formed and migrated normally when all oBCs were mutant for *RalA* (Fig 3A–B′), indicating that *RalA* function is not required in oBCs. Consistently, mosaic analysis showed an essential function of *RalA* in PCs, with a varied phenotype depending on the number of *RalA* mutant PCs present in the BC cluster. When only one of the two PCs was mutant, the BC formed but did not migrate (Figs 1C,3C–D′). In this case, the number of oBCs was reduced with an average of 3.5 ± 0.7 oBCs ($n = 26$) recruited instead of 6.1 ± 0.9 in wild type ($n = 24$; see the table in Fig 3). These data indicate that one PC cannot compensate for the loss of the other to recruit a full set of oBCs. As one PC has been shown to be sufficient for BC migration, these results indicate that

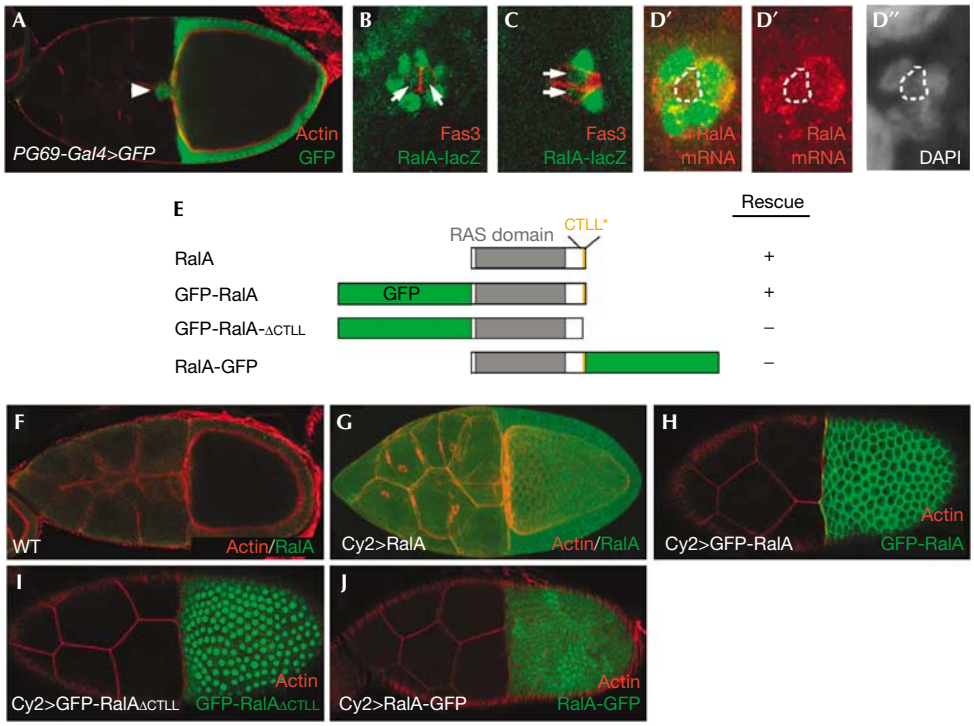


Fig 2 | Expression of RalA and sequence determinants for RalA subcellular localization. (**A**) Expression of RalA in border cells (BCs) and follicle cells was detected using a *RalA*–Gal4 enhancer trap ($RalA^{PG69}$) crossed to UAS–GFP. Expression of RalA–LacZ in BCs (**B**) during or (**C**) when migration has completed using the $RalA^{PL56}$ enhancer-trap line. Expression of β-galactosidase is detected in outer BCs (oBCs) as well as in polar cells (PCs). Fas3 staining is used to mark PCs (red). (**D**–**D**″) Fluorescent *in situ* hybridization of a stage-9 egg chamber using an antisense *RalA* probe, showing expression in PCs and oBCs. Red indicates *RalA* mRNA and green EYA (marks oBCs but not PCs, enclosed by dotted lines). (**E**) Schematic representation of RalA wild-type and GFP fusion proteins, showing the RAS domain and the CTLL motif at the C terminus. Each RalA construct was used in rescue experiments to assess their activity (see text for details). (**F**) The human RALB antibody (Fig 1E) does not detect endogenous levels of RalA expression but recognizes overexpressed wild-type RalA (using *CY2–Gal4*), which localizes to the plasma membrane (**G**). GFP–RalA expressed using *CY2*–Gal4 also localizes to the plasma membrane (**H**). (**I**) GFP–$RalA^{\Delta CTLL}$, in which the last four C-terminal amino acids are deleted, is mislocalized and accumulated predominantly in the nucleus. (**J**) RalA–GFP fusion protein is also mislocalized in the nucleus. GFP, green fluorescent protein.

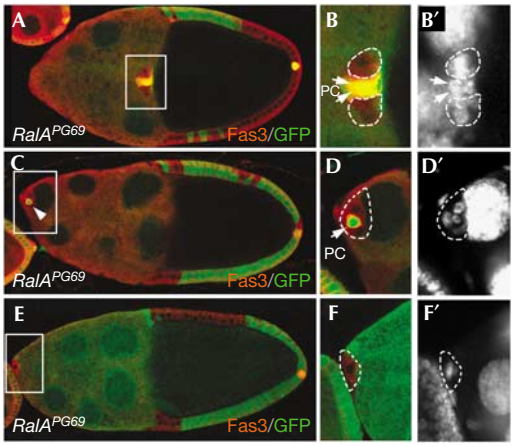
the presence of a RalA mutant PC inhibits migration. In egg chambers in which both PCs were mutant for RalA, almost no oBCs were recruited, with an average of 0.6 ± 0.6 oBC per cluster ($n = 18$; Fig 3E–F′). This result was confirmed using targeted expression of RalA RNA-mediated interference specifically in PCs using the *Upd*–Gal4 driver (data not shown). The lack or reduction of oBCs was confirmed using terminal follicle cell markers, including *slbo–lacZ* and *E(Spl)–lacZ* enhancer-trap expression (Fig 4G–H′; supplementary Fig 1 online). Note that the expression of these oBC markers remained unaffected when only oBCs were mutant for *RalA* (data not shown), indicating a cell non-autonomous function of *RalA* in PCs to control recruitment of oBCs and migration of the cluster.

To analyse further the role of *RalA* in PC function, we stained egg chambers using a set of PC-specific markers. These markers are expressed both at the anterior and posterior poles, so clones made at either side of the egg chamber have been analysed indifferently.

When anterior or posterior PCs were mutant for *RalA*, the expression of Fas3, PZ80 and A101 were abolished, indicating that *RalA* is essential for proper PC differentiation (Fig 4A–F). The effect of *RalA* mutations on Fas3 protein was stage specific: before stage 6–7, Fas3 staining was normal, whereas later on, staining was strongly reduced or absent, suggesting that RalA controls the maintenance of PC differentiation.

It has been shown that PCs originate from a larger group of pre-PCs in which cell number is reduced from 3–5 to 2 through apoptosis at early stages (Besse & Pret, 2003). To assess whether de-differentiated PCs could initiate apoptosis, we stained egg chambers with antibodies raised against activated caspase 3 (Casp3). Interestingly, some of the *RalA* mutant PCs (20%, $n = 30$), but not other follicle cells, induced strong activated Casp3 expression (Fig 4I–J′), which is indicative of cells undergoing cell death. As not all the mutant PCs showed activated Casp3 expression, this suggests that either this expression is transient or mutant PCs might show distinct behaviours. It is interesting to note that *RalA* was recently shown to protect cells from apoptosis in the sensory lineage (Balakireva *et al*, 2006), suggesting that this gene might have an anti-apoptotic role in differentiated cells during *Drosophila* development. Altogether, our results identify *RalA* as a new regulator to maintain PC fate and survival.

RalA shows a cell non-autonomous phenotype originating from the PC. PCs are essential anteriorly for recruiting a ring of around



PCs	oBCs	Migration
2	6.1	+
1	3.5	−
0	0.6	−

Fig 3 | *Drosophila* RalA is essential in polar cells for border-cell recruitment and migration. (**A**) Mosaic border-cell (BC) cluster in which the two polar cells (PCs; arrows in **B**,**B**′) are wild type, whereas outer BCs (oBCs; enclosed by dotted lines) are mutant for *RalA*^{PG69}. Mutant cells are negative for GFP (green). PCs are marked by Fas3 (red). Stage-10 egg chamber is shown. (**B**,**B**′) Enlargement of the boxed region shown in (**A**). (**C**) BC cluster in which only one PC is wild type (arrow; GFP and Fas3 positive) does not migrate. Early stage-10 egg chamber is shown. (**D**,**D**′) Enlargement of the boxed region shown in (**C**). (**E**) The two PCs are mutant in this egg chamber and BCs did not form. (**F**,**F**′) Enlargement of the boxed region shown in (**E**). Stage-10A egg chambers are shown in (**A**,**C**) and a stage-10B egg chamber is shown in (**E**). Green indicates GFP clonal marker and grey 4′,6-diamidino-2-phenylindole (DAPI) staining. The dotted lines indicate clonal limits. The table shows a summary of *RalA*-induced BC phenotypes shown in (**A**–**F**′). Left column, number of wild-type PCs; middle column, number of recruited oBCs; right column, migration phenotype (+, wild type; −, no migration). GFP, green fluorescent protein.

six oBCs that make a mature BC cluster, which depends on the secretion of the Unpaired (Upd) ligand from the PC and subsequent Jak/Stat activation in the oBC (Silver & Montell, 2001; Beccari *et al*, 2002; Ghiglione *et al*, 2002; McGregor *et al*, 2002). Secretion of Upd and binding to Domeless (Dome), the *Drosophila* Jak/Stat receptor, induces ligand-dependent internalization of Dome in cells surrounding PCs, both in BCs and posterior follicle cells (Fig 5A,A′; Ghiglione *et al*, 2002; Devergne *et al*, 2007). When PCs were mutant for *RalA*, Dome-containing endocytic vesicles were no longer observed, both anteriorly and posteriorly (Fig 5B,B′), suggesting that Jak/Stat signalling was not activated. In wild-type egg chambers, Stat is localized in the nucleus as a gradient, with higher levels of nuclear Stat close to the PC (Fig 5C,C′), thus reflecting Jak/Stat pathway activation. The nuclear localization of Stat was normal when PCs were wild type with adjacent follicle cells mutant for *RalA* (Fig 5D,D′), indicating that RalA does not have a role in the function of oBCs and posterior follicle cells in controlling Jak/Stat signalling. By contrast, in egg chambers with mutant PCs, Stat nuclear localization was completely abolished (Fig 5E). To discriminate between a role of *RalA* in Upd expression or activity, we stained egg chambers using a Upd antibody, which shows a gradient of this ligand in egg chambers (Fig 5F). Using this assay, we showed that *RalA* mutations in PCs strongly affect the expression of the Upd protein (Fig 5G).

Does the reduction of Upd lead to non-autonomous defects posteriorly? It was shown previously that the Jak/Stat pathway is essential for specifying posterior follicle cells, which then signal back to the oocyte for anterior–posterior polarization (Xi *et al*, 2003; Fig 4G–H′). When polarity is normal, the Staufen protein forms a posterior crescent in the oocyte (Fig 5H). In egg chambers containing posterior *RalA* mutant PCs, the localization of Staufen was not normal and it was found centrally in strongly affected oocytes (Fig 5I), similar to mutants that fail to reorganize the microtubules (van Eeden & St Johnston, 1999).

Among follicle cells, PCs have been shown to be important in patterning the egg chamber and in establishing BCs (Xi *et al*, 2003). In this study, we identified *RalA* as a new key regulator of PC fate. *RalA* is essential both cell autonomously for maintaining PC differentiation and cell non-autonomously for patterning terminal follicle cells through Jak/Stat signalling. However, *RalA* mutations do not reproduce the full range of Jak/Stat mutations (Silver & Montell, 2001; Beccari *et al*, 2002; Ghiglione *et al*, 2002; McGregor *et al*, 2002; Xi *et al*, 2003), consistent with the fact that some Upd is still produced by *RalA* mutant PCs (Fig 5G). For example, the follicle cell markers MA33 and *dpp–lacZ*, the expression of which in stretched cells is controlled by Jak/Stat signalling (Xi *et al*, 2003), are expressed normally when PCs are mutant for *RalA* (data not shown). Altogether, our data indicate that the function of *RalA* is essential for maintaining the PC fate and for ensuring high levels of Upd expression, which are required for patterning the most terminal follicle cells, including BCs and posterior follicle cells. The *RalA* phenotype suggests the existence of a maintenance signal taking place around stage 6–7—that is, following egg chamber proliferation phase—which would be necessary to complete egg chamber patterning by providing sustained Jak/Stat activation.

Previous studies have shown that Ral proteins interact with Sec5 (Moskalenko *et al*, 2002; Sugihara *et al*, 2002) and Exo84 (Moskalenko *et al*, 2003; Formstecher *et al*, 2005) to regulate the exocyst function during proliferation and tumorigenesis (Camonis & White, 2005; Vitale *et al*, 2005; Rosse *et al*, 2006). Our *in vivo* study suggests a role for *RalA* in cell differentiation and patterning, independent of secretion. Not only Upd but also several

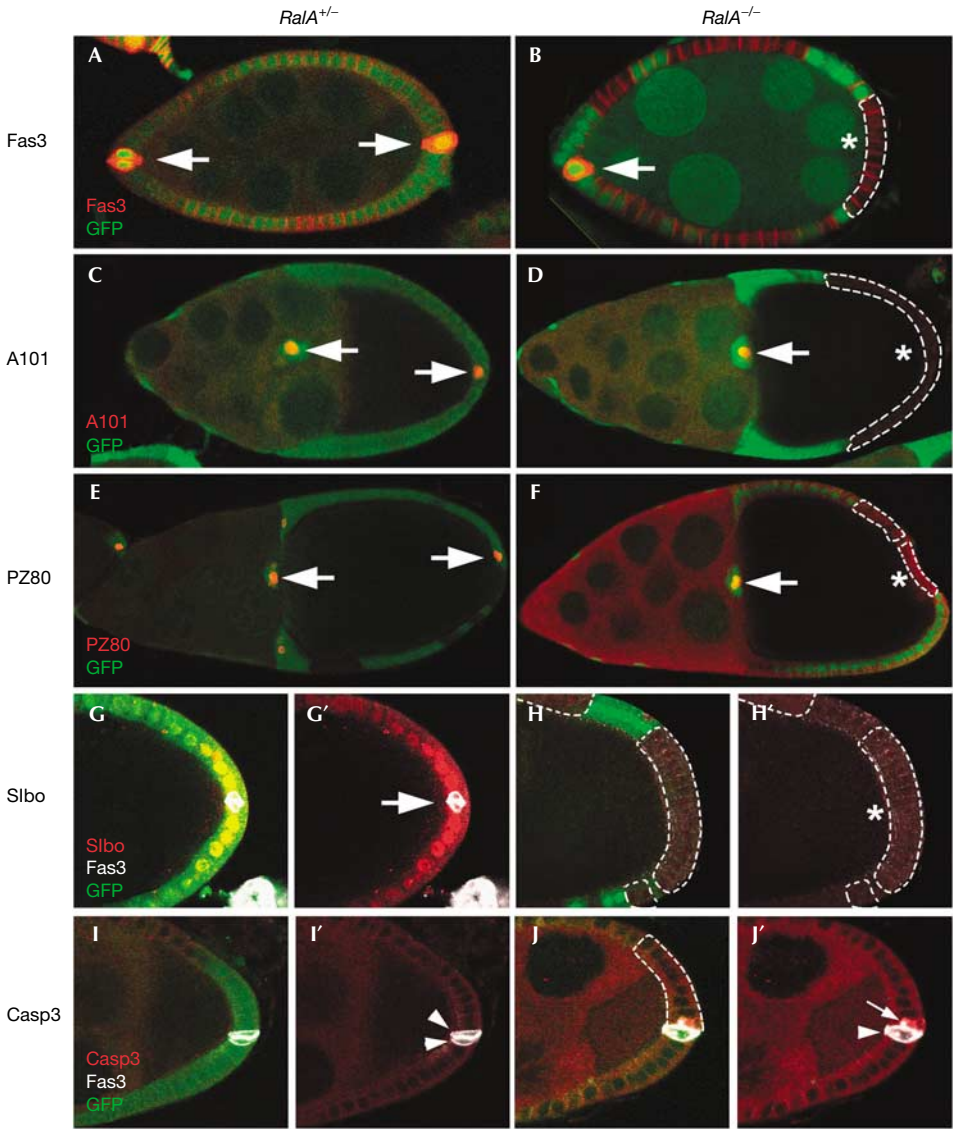


Fig 4 | *Drosophila* RalA controls polar-cell and terminal follicle cell differentiation. Specific markers for polar cells (PCs; Fas3, A101–lacZ, PZ80–lacZ) and terminal follicle cells (Slbo–lacZ) were used to analyse cell fate in *RalA* homozygous follicle cells (**B,D,F,H–H′,J–J′**), as compared with heterozygous cells (**A,C,E,G–G′,I–I′**). The expression of Fas3 (**A,B**), A101–lacZ (**C,D**) and PZ80–lacZ (**E,F**) is completely lost when PCs are mutant for *RalA*. The *RalA* mutations also lead to a complete loss of expression of the terminal follicle cell marker *Slbo–lacZ* (**G–G′,H–H′**). PCs that are mutant for *RalA* show a specific activation of Casp3 (**J–J′**; in red; white arrow) and this is not observed in wild-type PCs at this late stage (**I–I′**; white arrowheads). Green indicates GFP clonal marker and grey Fas3 staining (**G–J′**). The dotted lines indicate clonal limits and the asterisks mutant PCs. GFP, green fluorescent protein.

non-secreted PC markers lose expression following *RalA* loss of function, reminiscent of a more general differentiation phenotype. Contrary to what would be expected of a secretion phenotype, the Upd protein does not accumulate within PCs that are mutant for *RalA* (Fig 5G). The analysis of *sec5* mutations in the follicle cells showed that this gene is required for the positioning of the oocyte and for follicle cell morphology (Murthy & Schwarz, 2004), two phenotypes that we never observed in *RalA* mutant egg chambers. Finally, expression in BCs of RNA-mediated interference against the *sec5*, *sec6*, *sec8* or *sec15* genes did not show any phenotype (data not shown).

Thus, instead of showing a ubiquitous activity, our data indicate a cell-type-specific function for *RalA* in PCs, independent of secretion. Controlling the differentiation of PCs, which have a central organizing role through Jak/Stat ligand production, might represent a way to monitor the number of invasive cells during both normal development and tumour cell invasion. Interestingly, in mouse M1 myeloid leukaemia cells, Stat3 can activate Ral by controlling the expression of its exchange factor (Senga *et al*, 2001). These data suggest a conserved functional link between Ral proteins and Stat activity and provide a basis for the maintenance of Jak/Stat activity in PCs through a positive feedback loop involving RalA and Stat.

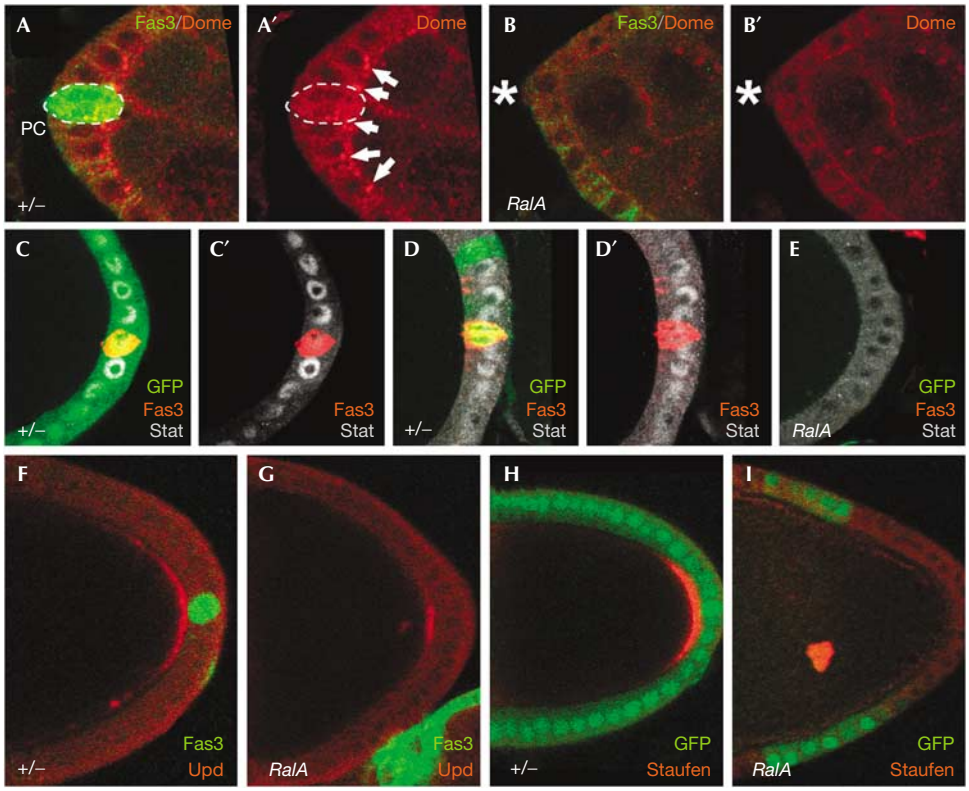


Fig 5 | *RalA* mutant polar cells block Jak/Stat signalling and oocyte anterior–posterior polarization. (**A**,**A**′) Mosaic egg chamber in which the two anterior polar cells (PCs) are wild type (enclosed by dotted lines). Domeless (Dome; in red) is found in intracellular vesicles (arrows). (**B**,**B**′) Mosaic egg chamber in which the two anterior PCs are mutant for *RalA* (asterisk). Note the absence of Dome endocytic vesicles. Mutant cells do not express the clonal marker GFP (green). PCs are marked with Fas3 (green). (**C**,**C**′) Graded nuclear expression of Stat in a heterozygous $RalA^{+/-}$ egg chamber. (**D**,**D**′) Expression of Stat is not affected in a mosaic egg chamber in which the two posterior PCs are wild type (GFP, clonal marker). (**E**) Nuclear Stat is completely lost when the two PCs are mutant for *RalA*. In (**C**–**E**), red denotes Fas3 staining and grey Stat staining. (**F**,**G**) The Unpaired (Upd) ligand (in red) forms a gradient centred at the poles in heterozygous egg chambers (PCs are marked by Fas3 in green). By contrast, Upd expression is strongly reduced when the two PCs are mutant for *RalA* (**G**). (**H**,**I**) When posterior PCs are mutant, the oocyte shows anterior–posterior polarization defects, as evidenced by the mislocalization of the Staufen protein (in red). Green indicates GFP clonal marker. GFP, green fluorescent protein.

METHODS

Genetics. A description of genetic markers and chromosomes can be found at FlyBase (http://flybase.bio.indiana.edu). *RalA* has been identified through P-element mutagenesis using a tissue-targeted mosaic screen in ovaries (Ghiglione *et al*, 2002; this study). $RalA^{h1}$ is a viable, hypomorphic allele generated by imprecise excision of the $RalA^{PG89}$ P-element as per standard protocols. Reverse transcription–PCR (RT–PCR) on both PG69 and PG89 lines showed residual expression, suggesting that these lethal alleles correspond to strong hypomorphic alleles. Green fluorescent protein negatively marked homozygous follicle cell clones for *RalA* were induced using the FLP/FRT (flipase/flipase recognition target) system. The following *Drosophila* stocks have been used: $RalA^{PG69}$ FRT19A/FM7, $RalA^{PG89}$ FRT19A/FM7 and $RalA^{PL56}$ FRT19A/FM7. The other stocks used in this study are *Upd*–Gal4, *slbo*–Gal4, *CY2*–Gal4, *slbo*–lacZ, *E(Spl)*–lacZ, *A101*–lacZ; PZ80–lacZ, UAS–*RalA* wt, UAS–$RalA^{S25N}$ and UAS–$RalA^{G20V}$.

Antibodies, immunostaining and imaging. Immunostaining of egg chambers was performed as described previously (Ghiglione *et al*, 2002). The following primary antibodies were used: rabbit Dome (1:200), mouse Fas3 (1:100; 7G10, Developmental Studies Hybridoma Bank (DSHB), University of Iowa, IA, USA), mouse Fas2 (1:100; 1D4, DSHB), mouse EYA (1:100, 10H6, DSHB), rabbit β-galactosidase (1:1,000, Cappel, Fountain Pkwy, OH, USA), rabbit Stat92E (1:500, a gift from S. Hou), rabbit human RALB (1:500, BD Transduction Laboratories, Franklin Lakes, NJ, USA), rabbit cleaved Casp3 (1:200, Cell Signalling), rabbit Upd (1:1,000, a gift from D. Harrison) and rabbit Staufen (1:5,000, a gift from D. St Johnston). Secondary antibodies were anti-rabbit Alexa 488 (1:400), CY5 (1:100) or Texas Red (1:100), anti-mouse Alexa 488 (1:400) or Texas Red (1:100) from Molecular Probes (Eugene, OR, USA).

Confocal images were taken using a Leica TCS-SP1 or a Zeiss LSM 510 META confocal microscope using ×25 and 0.80 NA and ×40 and 1.3 NA oil immersion objectives. Other images were taken using a Nikon Coolpix 990 digital camera and processed using Photoshop 7.0 (Adobe).

Immunoblot analysis. For preparation of ovarian extracts, ten ovaries were dissected in ice-cold PBS. The PBS was removed and replaced with Laemmli loading buffer. Ovaries were

homogenized using a pestle and then centrifuged briefly to pellet debris. An equivalent of two ovaries per lane was loaded on a 12% SDS–polyacrylamide gel electrophoresis gel under reducing conditions and blotted onto nitrocellulose filter. The rabbit human RALB and the horseradish peroxidase-conjugated secondary antibodies (1:2,000, Vector, Berlingame, CA, USA) were used to detect RalA. The bands were visualized using enhanced chemiluminescence (Pierce, Rockford, IL, USA).

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org)

ACKNOWLEDGEMENTS

We thank M. Balakireva, D. Harrison, M. Grammont, S. Hou, D. Montell, P. Rorth, K. Sawamoto, T. Sexton, D. St Johnston, the DSHB and the Bloomington Stock Center for providing us with fly stocks and reagents, and M. Balakireva and members of our laboratory for helpful discussions. This work was supported by grants from the Centre National de la Recherche Scientifique, Ministère, the Indo-French Centre for Promotion of Advanced Research, the Association pour la Recherche Contre le Cancer and the European Molecular Biology Organization Young Investigator Program.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Balakireva M *et al* (2006) The Ral/exocyst effector complex counters c-Jun N-terminal kinase-dependent apoptosis in *Drosophila melanogaster*. *Mol Cell Biol* **26:** 8953–8963

Beccari S, Teixeira L, Rorth P (2002) The JAK/STAT pathway is required for border cell migration during *Drosophila* oogenesis. *Mech Dev* **111:** 115–123

Besse F, Pret AM (2003) Apoptosis-mediated cell death within the ovarian polar cell lineage of *Drosophila melanogaster*. *Development* **130:** 1017–1027

Bianco A, Poukkula M, Cliffe A, Mathieu J, Luque CM, Fulga TA, Rørth P (2007) Two distinct modes of guidance signalling during collective migration of border cells. *Nature* **448:** 362–365

Bourbon HM *et al* (2002) A P-insertion screen identifying novel X-linked essential genes in *Drosophila*. *Mech Dev* **110:** 71–83

Camonis JH, White MA (2005) Ral GTPases: corrupting the exocyst in cancer cells. *Trends Cell Biol* **15:** 327–332

Devergne O, Ghiglione C, Noselli S (2007) The endocytic control of JAK/STAT signalling in *Drosophila*. *J Cell Sci* **120:** 3457–3464

Formstecher E *et al* (2005) Protein interaction mapping: a *Drosophila* case study. *Genome Res* **15:** 376–384

Ghiglione C, Devergne O, Georgenthum E, Carballes F, Medioni C, Cerezo D, Noselli S (2002) The *Drosophila* cytokine receptor Domeless controls border cell migration and epithelial polarization during oogenesis. *Development* **129:** 5437–5447

Grammont M, Irvine KD (2002) Organizer activity of the polar cells during *Drosophila* oogenesis. *Development* **129:** 5131–5140

McGregor JR, Xi R, Harrison DA (2002) JAK signaling is somatically required for follicle cell differentiation in *Drosophila*. *Development* **129:** 705–717

Mirey G, Balakireva M, L'Hoste S, Rosse C, Voegeling S, Camonis J (2003) A Ral guanine exchange factor–Ral pathway is conserved in *Drosophila melanogaster* and sheds new light on the connectivity of the Ral, Ras, and Rap pathways. *Mol Cell Biol* **23:** 1112–1124

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

Moskalenko S, Henry DO, Rosse C, Mirey G, Camonis JH, White MA (2002) The exocyst is a Ral effector complex. *Nat Cell Biol* **4:** 66–72

Moskalenko S, Tong C, Rosse C, Mirey G, Formstecher E, Daviet L, Camonis J, White MA (2003) Ral GTPases regulate exocyst assembly through dual subunit interactions. *J Biol Chem* **278:** 51743–51748

Murthy M, Schwarz TL (2004) The exocyst component Sec5 is required for membrane traffic and polarity in the *Drosophila* ovary. *Development* **131:** 377–388

Prasad M, Montell DJ (2007) Cellular and molecular mechanisms of border cell migration analyzed using time-lapse live-cell imaging. *Dev Cell* **12:** 997–1005

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

Rosse C, Hatzoglou A, Parrini MC, White MA, Chavrier P, Camonis J (2006) RalB mobilizes the exocyst to drive cell migration. *Mol Cell Biol* **26:** 727–734

Sawamoto K, Winge P, Koyama S, Hirota Y, Yamada C, Miyao S, Yoshikawa S, Jin MH, Kikuchi A, Okano H (1999) The *Drosophila* Ral GTPase regulates developmental cell shape changes through the Jun NH(2)-terminal kinase pathway. *J Cell Biol* **146:** 361–372

Senga T, Iwamoto T, Kitamura T, Miyake Y, Hamaguchi M (2001) JAK/STAT3-dependent activation of the RalGDS/Ral pathway in M1 mouse myeloid leukemia cells. *J Biol Chem* **276:** 32678–32681

Silver DL, Montell DJ (2001) Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in *Drosophila*. *Cell* **107:** 831–841

Sugihara K, Asano S, Tanaka K, Iwamatsu A, Okawa K, Ohta Y (2002) The exocyst complex binds the small GTPase RalA to mediate filopodia formation. *Nat Cell Biol* **4:** 73–78

van Eeden F, St Johnston D (1999) The polarisation of the anterior–posterior and dorsal–ventral axes during *Drosophila* oogenesis. *Curr Opin Genet Dev* **9:** 396–404

Vitale N, Mawet J, Camonis J, Regazzi R, Bader MF, Chasserot-Golaz S (2005) The small GTPase RalA controls exocytosis of large dense core secretory granules by interacting with ARF6-dependent phospholipase D1. *J Biol Chem* **280:** 29921–29928

Xi R, McGregor JR, Harrison DA (2003) A gradient of JAK pathway activity patterns the anterior–posterior axis of the follicular epithelium. *Dev Cell* **4:** 167–177