Guidance of Cell Migration by EGF Receptor Signaling During *Drosophila* Oogenesis

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Directed cell migration is important for many aspects of normal animal development, but little is known about how cell migrations are guided or the mechanisms by which guidance cues are translated into directed cell movement. Here we present evidence that signaling mediated by the epidermal growth factor receptor (EGFR) guides dorsal migration of border cells during *Drosophila* oogenesis. The transforming growth factor–α (TGF-α)–like ligand Gurken appears to serve as the guidance cue. To mediate this guidance function, EGFR signals via a pathway that is independent of Raf-MAP kinase and receptor-specific.

Border cells are a cluster of 6 to 10 specialized somatic follicle cells that perform a stereotypic migration during *Drosophila* oogenesis (*1*). At the beginning of stage 9, border cells delaminate from the anterior follicular epithelium and initiate their migration between the germline derived nurse cells, toward the oocyte (Fig. 1, A and B). About 6 hours later, at stage 10, the border cells reach the oocyte and then migrate dorsally toward the germinal vesicle (GV) (Fig. 1C). The migration of border cells is essential for female fertility (*2*); however, it is not known what guides this migration. Spatial information may be provided by the surrounding tissue in the form of cell-associated or secreted guidance cues, for example, as attractive gradients (Fig. 1, D to F). The posterior and dorsal migration phases might be guided by separate cues (Fig. 1, D and E), or by a single cue and a fixed migration path (Fig. 1F).

To identify guidance cues, we reasoned as follows: The gradient of spatial information would be perturbed if a key attractant or repellant were uniformly overexpressed (Fig. 1G). This would be expected to cause the cells to migrate inefficiently as there would be no difference between signaling in the front and the back of the cell. To identify genes capable of perturbing border-cell migration when expressed uniformly, we used a modular misexpression screen (*3*) with the P element EPg (*4*). Expression was induced in the germline (*nanosGAL4:VP16*) and in the border cells themselves (*slboGAL4*) (*5*). Of 8500 independent insertion lines, three showed defects in border-cell migration but no detectable morphological abnormalities in the egg chamber. In one of these, EPg35521, the single EPg element is inserted in such a way that it drives expression of the gene encoding the neuregulin-like EGFR ligand Vein (*6*). Border-cell migration was affected both when Vein was expressed in the germline tissue and when it was expressed in the border cells themselves, as might be expected of a secreted molecule (Fig. 1H).

To determine whether the effect on migration was specific to Vein or common to EGFR ligands, secreted forms of the TGF-α–like ligands Gurken (*7*) and Spitz (*8*) were expressed in border cells. Both affected border-cell migration, with the potent ligand secreted Spitz having the strongest effect (Fig. 1H). Border-cell expression of an activated, ligand-independent, form of EGFR [λ−top (*9*)] also severely affected migration (Fig. 1H). Thus, constitutive stimulation of EGFR signaling in border cells effectively inhibits their migration.

To determine whether EGFR signaling was required for normal border-cell migration, we expressed a dominant negative form of the receptor (DN-DER) (*10*) or the transmembrane EGFR inhibitor Kekkon-1 (*11*) in border cells. Both specific EGFR inhibitors severely inhibited dorsal migration of border cells (Fig. 2, A to D), with only minor effects on the initial posterior migration. Most eggs from these females did not hatch (Fig. 2D) and appeared unfertilized. This phenotype mimics loss of border-cell function (*2*), suggesting that the dorsal aspect of migration may be essential. The requirement for EGFR in border cells was confirmed by looking at clones of *Egfr* mutant cells (*12*). When all

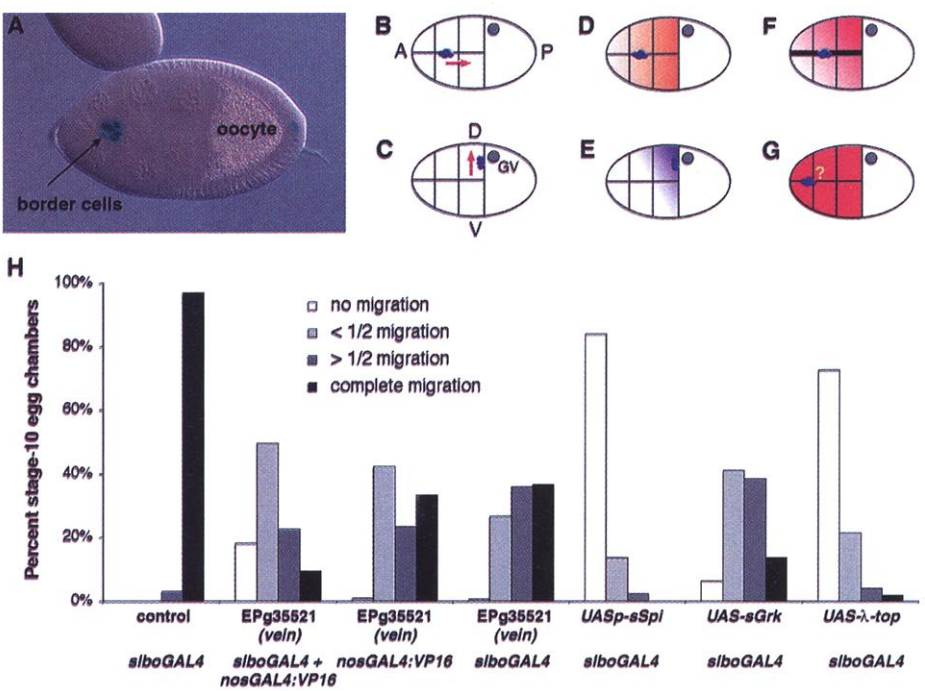

Fig. 1. Identifying EGFR signaling as a putative regulator of border-cell guidance. In this and subsequent images of egg chambers, anterior is to the left and dorsal is up. (**A**) *slbo*^{1}/+ stage-9 egg chamber stained with X-Gal to reveal the *slbo*^{1} *lacZ*-enhancer trap in border cells. Border-cell migration occurs in two phases: posterior (**B**), followed by dorsal (**C**) migration. (**D**) A putative gradient of chemoattractant for posterior border-cell migration. Border cells sense a slightly higher concentration at the leading (front) edge and move in this direction. (**E**) Close to the oocyte, the cells may encounter a second gradient for dorsal migration. (**F**) A single gradient may guide the cells throughout if the path is physically constrained. (**G**) Uniform overexpression of a putative chemoattractant. The nongraded guidance signal makes border cells unable to migrate productively. (**H**) Activating EGFR signaling arrests border-cell migration. The position of border cells along the anterior-posterior axis was scored by X-Gal staining of stage-10 egg chambers from females carrying one copy of the *slbo*^{1} enhancer trap, as well as one copy of the indicated transgenes ($n >$ 100 for each genotype). UAS, upstream activating sequence.

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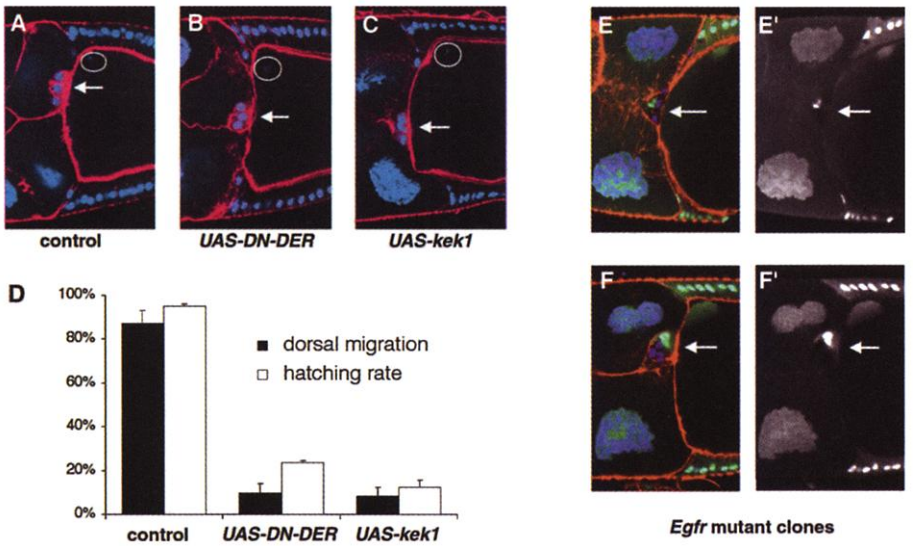

Fig. 2. EGFR signaling is required for dorsal migration of border cells. In each panel, DNA (DAPI) is blue, F-actin (phalloidin) is red, and clonal marker [green fluorescent protein (GFP)] is green. The GV is a small DAPI spot in (A) to (C) (circled), and is GFP-positive in (E) and (F). Border cells are marked with a white arrow. Typical stage-10 egg chamber from control (*slboGAL4/+*) (**A**), *slboGAL4/UAS-DN-DER* (**B**), and *slboGAL4/UAS-kek1* (**C**) females. (**D**) Quantification of dorsal migration ($n > 140$ stage-10 egg chambers for each genotype) and egg hatching ($n > 700$ for each genotype) from females as shown in (A) to (C). Stage-10 egg chambers were scored; dorsal indicates border cells were off center and close to the GV. For the hatching test, virgins of the indicated genotype were crossed with wild-type males. Dorsal migration was defective when all border cells [except polar cells, see (*12*)] were mutant for *Egfr* (**E**). Wild-type cells were leading the dorsal migration when some border cells were mutant for *Egfr* (**F**). Mutant cells may be recognized by the absence of the clonal marker (GFP), shown separately in (**E'**) and (**F'**).

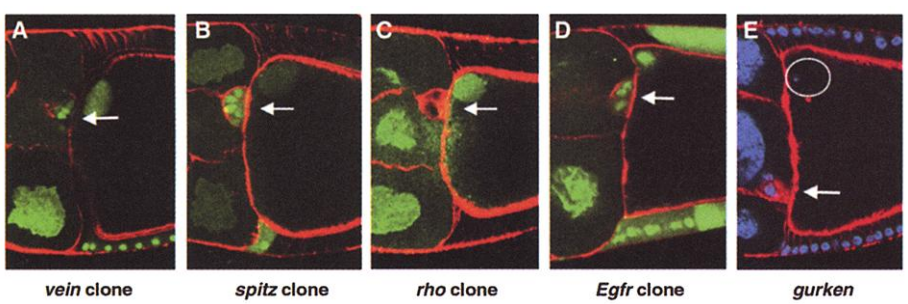

Fig. 3. The Gurken ligand is required for dorsal migration. Border cells migrate dorsally when all dorsal follicle cells are mutant for *vein* (**A**), *spitz* (**B**), or *rhomboid* (**C**) and when patches of dorsal follicle cells are mutant for *Egfr* (**D**), but fail to migrate dorsally in *gurken* (grk^{DC}/grk^{2b6}) egg chambers (**E**). Mitotic clones were induced by heat shock of larvae (A to C) or females (D) with the following genotypes: *hsFLP/+; ; Ubi-GFP, FRT80/vn*^{10567}, *FRT80* (A), *hsFLP/+; Ubi-GFP, FRT40/spi*^{1}, *FRT40* (B), *hsFLP/+; ; Ubi-GFP, FRT80/ru*^{1}, *rho*^{7M43}, *FRT80* (C), and *hsFLP/+*; and *FRT42, Ubi-GFP/FRT42, top*^{CO} (D). In each panel DAPI staining (DNA) is blue, phalloidin (F-actin) red and clonal marker (GFP) green.

outer border cells were mutant for *Egfr*, the cluster remained in the center of the egg chamber at stage 10 (5 out of 5 clusters, Fig. 2E), whereas 90% (129 out of 144) of wild-type clusters were found dorsally. When mixed clusters with both wild-type and mutant cells moved dorsally, the wild-type cells were in the front (14 out of 15 clusters, Fig. 2F). Thus, EGFR signaling is required specifically for dorsal border-cell migration.

When border cells migrate dorsally, activating ligands for EGFR are produced by the oocyte (Gurken) and, in response to Gurken, by dorsal follicle cells [Vein and Spitz (*13*)]. Dorsal migration still occurred when dorsal follicle cells were mutant for *vein* (7 out of 8 egg chambers, Fig. 3A), *spitz* (10 out of 14 egg chambers, Fig. 3B), or *rhomboid*, which is required for Spitz activation (5 out of 6 egg chambers, Fig. 3C). Thus, although ectopic expression of Vein or activated Spitz proteins can affect border-cell guidance, neither is required for the process. Removing EGFR from patches of dorsal follicle cells, which rendered them unable to activate secondary signals, also had no effect (Fig. 3D). In contrast, dorsal migration was perturbed in *gurken* mutants. Ovaries from grk^{DC}/grk^{2b6} mutant females showed a range of defects. In mildly affected egg chambers where the GV had moved anterior and dorsal, border cells completed posterior migration but failed to migrate dorsally (23 out of 25 stage-10 egg chambers, Fig. 3E). In stage-10 oocytes, Gurken protein is detected in a membrane-associated gradient with the highest level at the dorsal anterior over the GV (*14*). These results are most consistent with Gurken serving as the dorsal guidance cue, although contributions from other EGFR ligands cannot be excluded.

We next examined which intracellular signaling pathways downstream of EGFR might mediate the effect on border-cell migration. EGFR signaling has been shown to regulate growth and differentiation during *Drosophila* development via activation of the Raf-MAP kinase (MAPK) pathway. Moderate activation of this pathway was observed in migrating border cells at both phases of migration, particularly in the leading cells (Fig. 4A). Mammalian tissue culture studies have indicated, however, that mitogenic and migration-inducing activities of EGFR and other receptor tyrosine kinases (RTKs) may occur via different pathways (*15*, *16*), prompting us to investigate further.

To investigate the role of the Raf-MAPK pathway, we performed clonal analysis with a *raf* null mutant (phl^{11}). When all outer border cells were mutant, migration was normal during stage 9 (11 out of 12 clones, Fig. 4B). Mutant clusters were very rarely recovered at stage 10, but dorsal migration could occur (*17*). Expression of an activated form of Raf [Raf^{GOF} (*11*)] in border cells resulted in robust activation of MAPK but had no effect on border-cell migration (Fig. 4, C and D). Finally, expression of an activated form of the *Drosophila* fibroblast growth factor (FGF) receptor Heartless [λ-htl (*18*)] strongly activated MAPK in border cells but had no effect on migration (Fig. 4, C and D). This contrasts with the effect of EGFR (λ-top). Thus, the effects of EGFR signaling on border-cell migration appear to be specific (not elicited by all RTKs) and independent of Raf-MAPK.

The small guanosine triphosphatase Ras can link RTKs to MAPK pathway or other pathways. Dominant negative Ras (Ras^{N17}) and activated Ras (Ras^{V12}) moderately affected posterior and dorsal border-cell migration (Fig. 4D) (*19*), indicating that Ras has a role in both migrations. Phosphatidylinositol 3–kinase (PI3K) has been implicated directly as regulator of chemotaxis in different systems (*20*, *21*). However, expression of dominant negative or activated forms of the *Drosophila* PI3K catalytic subunit [$p110^{DN}$ and $p110^{CAAX}$ (*22*)] did not affect border-cell migration (Fig. 4D). Phospholipase C–γ (PLC-γ), which can bind directly to RTKs via its SH2 domain, may mediate effects on movement of tissue culture cells (*15*, *16*). In the *Drosophila* genome, there appears to be only one PLC-γ, encoded by the *small wing* (*sl*) locus (*23*). Null mutants in *sl*

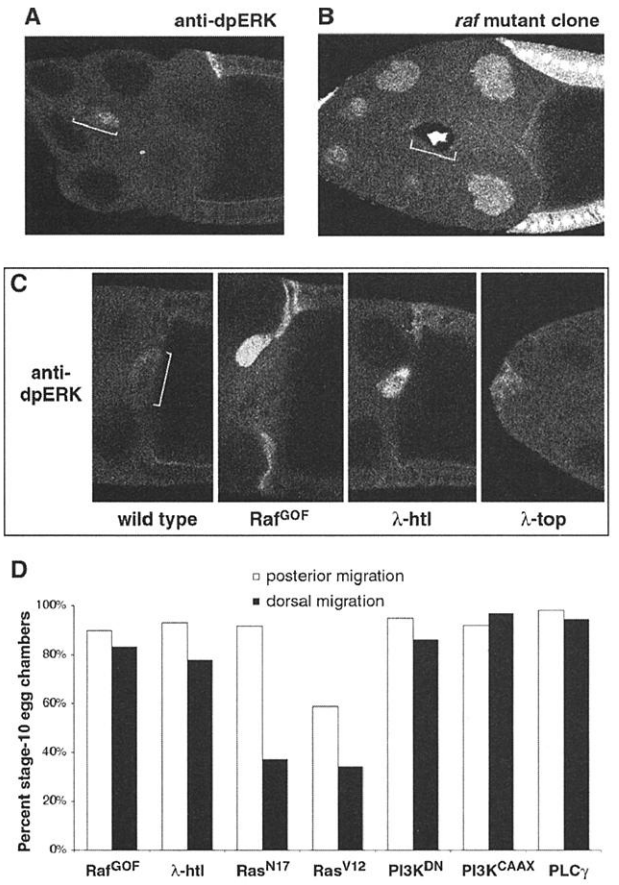

Fig. 4. MAP kinase, PI3K, and PLC-γ do not mediate the effect of EGFR on border-cell migration. Brackets indicate the position of the border-cell cluster. (**A**) Activation of MAP kinase in stage-9 border cells monitored by staining with an antibody specific for the activated state of MAP kinase/ERK [anti-dpERK (*30*)]. (**B**) Normal migration at stage 9 when all outer border cells are mutant for *raf* (phl^{11}). Mitotic clones were induced by heat shock to *Ubi-GFP, FRT18/* phl^{11}*, FRT18; hsFLP/+* females 3.5 days before analysis, and mutant cells are recognized by the absence of the clonal marker GFP. (**C**) Staining with the antibody against dpERK of stage-10 egg chambers from wild-type females or those carrying $slbo^{1}$*,slboGAL4/+* and the indicated UAS transgene. In the λ-top panel, border cells have not migrated. (**D**) Percentage of stage-10 egg chambers with completed posterior migration ($n >$ 100 per genotype), and of these, the percentage that completed dorsal migration ($n >$ 40 per genotype). Genotypes: $slbo^{1}$*,slboGAL4/+* plus the indicated UAS transgene; for PLC-γ, sl^{1}/sl^{2}.

did not affect border-cell migration (Fig. 4D). Thus, neither PI3K nor PLC-γ appear to be key mediators downstream of EGFR in this context.

Border cells are sensitive to EGFR signaling from the onset of migration, which suggests that the posterior migration may be guided by a similar RTK signal. Activated Heartless had no effect on migration. *breathless* mutant border cells migrate normally (*24*), and overexpression of the ligand Branchless has no effect (*25*). In addition, we found that border cells mutant for *dof* (*26*), which is required for signal transduction by both FGF receptors, migrated normally (five clones analyzed). Thus, neither of the two *Drosophila* FGF receptors, Breathless and Heartless, perform this role.

The RTKs of the EGF receptor family are required for growth, survival, differentiation, and migration of various cell types during animal development. EGF signaling also stimulates growth and metastatic potential of human tumors, as well as proliferation and motility of tissue culture cells. Our results demonstrate that EGFR signaling can direct cell migration in vivo. EGFR acts as a guidance receptor for border cells during oogenesis and is specifically required for the second phase of their migration. Another RTK with similar signaling properties may serve this function for the first phase of migration. We have presented evidence that guidance effects of EGFR are mediated by a noncanonical signaling pathway. The challenge is now to determine which pathways and molecules downstream of EGFR translate guidance information into directed cell movement in vivo.

References and Notes

1. R. C. King, *Ovarian Development in* Drosophila melanogaster (Academic Press, New York, 1970).
2. D. J. Montell, P. Rørth, A. C. Spradling, *Cell* **71**, 51 (1992).
3. P. Rørth, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12418 (1996).
4. J. Mata, S. Curado, A. Ephrussi, P. Rørth, *Cell* **101**, 511 (2000).
5. The EPg P element is a germline-competent, GAL4-inducible expression element; 8500 insertion lines were generated as described (*4*). For the screen, EPg males from each line were crossed to *w;* $slbo^{1}$*,slboGAL4/CyO; nanosGAL4:VP16* virgins, and ovaries from female progeny carrying all transgenes were analyzed. Abnormal migration was identified by visual inspection of egg chambers stained with X-Gal to reveal a border cell–specific *lacZ*-enhancer trap (Fig. 1A) (*2*). *nanosGAL4:VP16* drives expression in the germline tissue and *slboGAL4* in the border cells, as well as in centripetal cells later on. Positive lines were retested with the individual GAL4 drivers. Egg chamber morphology was analyzed by 4′,6′-diamidino-2-phenylindole (DAPI) and phalloidin staining to highlight DNA and F-actin, respectively. Sequencing of a plasmid rescue fragment showed EPg35521 to be inserted into the 5′ untranslated region of *vein*.
6. B. Schnepp, G. Grumbling, T. Donaldson, A. Simcox, *Genes Dev.* **10**, 2302 (1996).
7. A. M. Queenan, G. Barcelo, C. Van Buskirk, T. Schüpbach, *Mech. Dev.* **89**, 35 (1999).
8. R. Schweitzer, M. Shaharabany, R. Seger, B. Z. Shilo, *Genes Dev.* **9**, 1518 (1995).
9. A. M. Queenan, A. Ghabrial, T. Schüpbach, *Development* **124**, 3871 (1997).
10. L. O'Keefe *et al.*, *Development* **124**, 4837 (1997).
11. C. Ghiglione *et al.*, *Cell* **96**, 847 (1999).
12. As EGFR has a role early in oogenesis, we induced clones by mitotic recombination in adult females. This allowed us to recover some clones where all outer border cells (border cells excluding polar cells) were mutant. The central polar cells do not contribute directly to migration (*27*, *28*). Mitotic clones were induced by heat shock to *hsFLP/+; FRT42, Ubi-GFP/FRT42,* top^{CO} females 3.5 days before analysis.
13. C. Van Buskirk, T. Schüpbach, *Trends Cell Biol.* **9**, 1 (1999).
14. F. S. Neuman-Silberberg, T. Schüpbach, *Mech. Dev.* **59**, 105 (1996).
15. P. Chen, K. Gupta, A. Wells, *J. Cell Biol.* **124**, 547 (1994).
16. V. Kundra *et al.*, *Nature* **367**, 474 (1994).
17. In clonal analysis with phl^{11} (*raf* null allele), we recovered 12 stage-9 egg chambers where all border cells or all border cells minus polar cells were mutant; 11 clusters had migrated normally. At stage 10, such clusters were extremely rare. We analyzed more than 2000 stage-10 egg chambers, over half with mutant clones in follicle cells. In total, three mutant clones were recovered at stage 10. In one case, the cluster had normal morphology and had migrated dorsally. In two cases, border cells had stopped immediately before reaching the oocyte and looked abnormal, with little or no detectable cortical F-actin. We tentatively conclude that *raf* mutant cells can migrate dorsally but in most cases die or cause the egg chamber to degenerate at stage 10. As we see degenerating egg chambers, we interpret the inability to recover most mutant clusters at stage 10 to mean that when border cells and/or adjacent follicle cells lack Raf function, most egg chambers degenerate at this point.
18. A. M. Michelson, S. Gisselbrecht, E. Buff, J. B. Skeath, *Development* **125**, 4379 (1998).
19. It has previously been shown that dominant negative Ras (Ras^{N17}) affects posterior border-cell migration (*29*). Activated Ras (Ras^{V12}) was suggested only to affect initiation of border-cell migration. In our experiments, we have driven constitutive expression of Ras^{V12} in border cells, as opposed to a pulse of expression in all follicle cells (*29*). We observed that most border cells initiated migration. However, they failed to move from early stage 10 to late stage 10 ($n >$ 100 in each class), indicating that net movement had stopped. We conclude that Ras^{V12} also negatively affects the actual migration of border cells.
20. C. A. Parent, P. N. Devreotes, *Science* **284**, 765 (1999).
21. S. Wennstrom *et al.*, *Oncogene* **9**, 651 (1994).
22. S. J. Leevers, D. Weinkove, L. K. MacDougall, E. Hafen, M. D. Waterfield, *EMBO J.* **15**, 6584 (1996).
23. J. R. Thackeray, P. C. Gaines, P. Ebert, J. R. Carlson, *Development* **125**, 5033 (1998).
24. A. M. Murphy, T. Lee, C. M. Andrews, B. Z. Shilo, D. J. Montell, *Development* **121**, 2255 (1995).
25. P. Rørth, *Mech. Dev.* **78**, 113 (1998).
26. S. Vincent, R. Wilson, C. Coelho, M. Affolter, M. Leptin, *Mol. Cell* **2**, 515 (1998).
27. D. D. Han, D. Stein, L. M. Stevens, *Development* **127**, 573 (2000).
28. P. Rørth, K. Szabo, G. Texido, *Molecular Cell* **6**, 23 (2000).
29. T. Lee, L. Feig, D. J. Montell, *Development* **122**, 409 (1996).
30. L. Gabay, R. Seger, B.-Z. Shilo, *Science* **277**, 1103 (1997).

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