INTRODUCTION

The conversion of stationary, epithelial cells to migratory, invasive cells is an important feature of embryonic development and tumor metastasis. The border cells of the *Drosophila* ovary have emerged as a useful model system for a forward genetic approach to this problem. The border cells are a group of four to eight follicle cells that form at the anterior pole of the somatic follicular epithelium, which surrounds the germ-line-derived nurse cells and oocyte (Fig. 1A). These cells, which are recruited by two non-migratory polar cells to form a cluster (Han et al., 2000; Liu and Montell, 1999; Silver and Montell, 2001), subsequently delaminate from the epithelium and migrate through the nurse cell cluster towards the anterior border of the oocyte. Border cells complete their migration when they reach the oocyte border and align with the oocyte nucleus on the dorsal side of the egg chamber (Fig. 1A) (Montell, 1999b; Montell, 2001). It is clear that border cell migration is tightly regulated both temporally and spatially.

The border cells migrate over a long distance (~150 μm) to reach the oocyte border, raising the question of how the border cells are able to find their correct target. A putative guidance factor has been reported, platelet-derived growth factor/vascular-endothelial growth factor (PDGF/VEGF) related factor 1 (PVF1; also known as VEGF1 and Vegf17E) (Cho et al., 2002; Duchek et al., 2001; Heino et al., 2001). PVF1 protein accumulates at the highest levels in the oocyte and, to a lesser extent, in nurse cells, whereas the PVF1 receptor (PVR) is expressed in all follicle cells (Duchek et al., 2001). Loss-of-function mutations in *Pvf1* result in only mild migration defects, whereas expression of dominant-negative forms of PVR and the GRK receptor [the epidermal growth factor receptor (EGFR)] together affect border cell migration more dramatically than either receptor alone (Duchek et al., 2001). PVF1, a PDGF/VEGF homolog, is sufficient to guide border cells and interacts genetically with Taiman

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However, it is unknown whether other ligands, such as the two other PVF homologs PVF2 (also known as Vegf27Cb) and PVF3 (also known as Vegf27Ca), participate in guiding border cell migration.

Regulation of border cell migration by a second signaling pathway might contribute to the timing of migration. The *Drosophila* steroid hormone ecdysone provides a signal for the border cells to begin migrating at stage 9 (Bai et al., 2000) and controls the movements of other follicle cells at this stage (J.A.M. and D.J.M., unpublished). The role of this pathway in border cell migration was suggested by identification of loss-of-function mutations in the ecdysone receptor co-activator *taiman* (*taim*; Bai et al., 2000). The ecdysone pathway, via the ecdysone receptor, *tai*, and as-yet-unidentified transcriptional target genes, regulates the distribution of *Drosophila* E-cadherin (DE-cadherin) in border cells (Bai et al., 2000). DE-cadherin protein is expressed at the cortex of all follicle cells and nurse cells, and is upregulated in the border cells (Bai et al., 2000; Niewiadomska et al., 1999). During migration, DE-cadherin appears to localize in a punctate pattern at the interface between border cells and nurse cells, whereas a higher level and more uniform distribution are observed between cells within the cluster (Bai et al., 2000; Niewiadomska et al., 1999).

DE-cadherin is important because loss of DE-cadherin in either the border cells themselves or the nurse cells disrupts migration (Niewiadomska et al., 1999; Oda et al., 1997). Loss of *tai* in the border cells results in an abnormal accumulation of DE-cadherin at the interface between border cells and nurse cells (Bai et al., 2000). Thus, disruption of either the normal expression or distribution of DE-cadherin is associated with defective migration. An important unresolved question is how DE-cadherin is dynamically regulated at the interface of border cells and nurse cells.

To identify genes that play a role in border cell migration, we undertook a screen for mutations that exhibit dominant genetic interactions with a mutation in *tai*. 199 deficiencies were screened, 16 of which showed some degree of inhibition of border cell migration when heterozygous with *tai*. In one case, we identified a P-element insertion that showed dominant interactions with *tai* and also exhibited border cell migration defects when homozygous mutant. This P-element causes a null mutation of *Pvf1* (Duchek et al., 2001). In order to test whether PVF1 or other growth factors are capable of guiding the border cells, we ectopically expressed PVF1, PVF2 or GRK in random cells to see whether the border cells could be attracted to a new source of growth factor. PVF1 was capable of attracting the border cells to a new target but GRK and PVF2

<table>
<thead>
<tr>
<th>Deficiency (Bloomington stock no.)</th>
<th>Cytology</th>
<th>% border cell migration defect</th>
<th>% border cell migration defect</th>
<th>Interacting region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(1)v-N48 (3560)</td>
<td>9F;10C3-5</td>
<td>19 (333)</td>
<td>13 (192)</td>
<td>9F-10A9</td>
</tr>
<tr>
<td>Df(1)KA7 (957)</td>
<td>1A9;10F6-7</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Df(1)N19 (970)</td>
<td>1A1;1KA2</td>
<td>8 (184)</td>
<td>10 (77)</td>
<td>17A1-1A82</td>
</tr>
<tr>
<td>Df(2L)Dd-2 (3571)</td>
<td>27A:28A</td>
<td>15 (209)</td>
<td>10 (88)</td>
<td>27A-C1</td>
</tr>
<tr>
<td>Df(2L)BSC7 (6374)</td>
<td>26D10-E1:27C1</td>
<td>10 (98)</td>
<td>15 (162)</td>
<td></td>
</tr>
<tr>
<td>Df(2L)J-H (1357)</td>
<td>27C2-9:28B3-4</td>
<td>0 (0)</td>
<td>1 (123)</td>
<td></td>
</tr>
<tr>
<td>Df(2L)J39 (1469)</td>
<td>31C-D:32D1-5</td>
<td>47 (131)</td>
<td>Lethal</td>
<td></td>
</tr>
<tr>
<td>Df(2L)J2 (3366)</td>
<td>31B1:32A1-2</td>
<td>34 (85)</td>
<td>18 (62)</td>
<td></td>
</tr>
<tr>
<td>Df(2R)M60E (2471)</td>
<td>60E2-3:60E11-12</td>
<td>9 (200)</td>
<td>Semi-lethal</td>
<td>60E2-12</td>
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<tr>
<td>Df(3L)G24 (3686)</td>
<td>63F6-7:64C13-15</td>
<td>22 (271)</td>
<td>37 (82)</td>
<td>63F6-64B12</td>
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<td>Df(3L)10H</td>
<td>64B10-12:4C5-7</td>
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<tr>
<td>Df(3L)AC1 (997)</td>
<td>67A2:67D7-13</td>
<td>26 (231)</td>
<td>14 (66)</td>
<td>67B1-D13</td>
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<td>Df(3L)29A6 (2479)</td>
<td>66F5:67B1</td>
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<tr>
<td>Df(3L)fz-GF3b (3124)β</td>
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<td>10 (281)</td>
<td>6 (200)</td>
<td>70C1-D5</td>
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<td>Df(3R)3-4 (4787)</td>
<td>82F3-4:82F10-11</td>
<td>12 (180)</td>
<td>4 (265)</td>
<td>82F3-11</td>
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<tr>
<td>Df(3R)by10 (1931)</td>
<td>85D8-12:85E7-1F</td>
<td>14 (206)</td>
<td>15 (125)</td>
<td>85D11-F1</td>
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<tr>
<td>Df(3R)by62 (1893)</td>
<td>85D11-14:85F6</td>
<td>20 (153)</td>
<td>7 (97)</td>
<td></td>
</tr>
<tr>
<td>Tp(3);Yi506-85C (1534)</td>
<td>87D1-2:8E5-6</td>
<td>16 (146)</td>
<td>Semi-lethal</td>
<td>87D1-8E6</td>
</tr>
<tr>
<td>Df(3R)H-B79 (4962)</td>
<td>92B3:92F13</td>
<td>20 (185)</td>
<td>12 (169)</td>
<td>92B3-F13</td>
</tr>
<tr>
<td>Df(3R)cub87-4 (4432)</td>
<td>95D7:11:95F15</td>
<td>12 (272)</td>
<td>12 (146)</td>
<td>95E8-F7</td>
</tr>
<tr>
<td>Df(3R)cub87-4 (2362)</td>
<td>95F8-1:95F15</td>
<td>13 (304)</td>
<td>22 (189)</td>
<td></td>
</tr>
<tr>
<td>Df(3R)cub87-5 (2363)</td>
<td>95F7:96A17-18</td>
<td>0 (0)</td>
<td>4 (124)</td>
<td></td>
</tr>
<tr>
<td>Df(3R)cub87-5 (430)</td>
<td>98E3:99A6-8</td>
<td>11 (278)</td>
<td>10 (114)</td>
<td>98E3-99A8</td>
</tr>
</tbody>
</table>

*Number of egg chambers examined.
†Overlapping deficiencies are indented; deficiencies from the deficiency kit are not indented.
‡Fewer than 100 egg chambers examined.
§Semi-lethal in two alleles of *Drosophila*; not indented.
¶Df(3R)H-B79 removes *Star29E*, which is haploinsufficient for border cell migration (Silver and Montell, 2001); Df(3R)H-B79/TM2 females exhibit ~10% border cell migration defect (n=101).
when they reach the anterior border of the oocyte. (B,C) Representative examples of border cell (arrows) migration defects observed in stage 10 egg chambers heterozygous for tali61G1 (tai) and either Df(1)N19 (B) or Df(3)GN24 (C). (D) Crossing scheme used for the tai genetic interaction screen. The arrowheads in A–C indicate the extent of rearrangement of the outer follicle cells (ofc). Anterior is towards the left in all panels. Df, deficiency.

were not. Our results indicate that PVF1 acts as a guidance cue but additional factors likely contribute to the robust ability of these cells to find their target.

**MATERIALS AND METHODS**

_Drosophila_ strains and genetics

For wild-type analysis, we used the w1118 strain. The following mutant alleles were used: 27C1 (Liu and Montell, 1999); tali61G1, an EMS-generated null allele (Bai et al., 2000); tali61G0, a P-element inserted upstream of the tai locus (Bai et al., 2000; Spradling et al., 1999); Pvf1EP1624, a null allele of Pvf1 (CG7103) (Duchek et al., 2001); chie113G1 (Spradling et al., 1999); slbro and slboL5 (Montell et al., 1992); ecd1 (Garen et al., 1977); pvr25947 (Cho et al., 2002); and pvr2193 (Cho et al., 2002). All deficiencies and lethal P-element alleles used in the interaction screen were obtained from the Bloomington _Drosophila_ stock center except for Df(3L)10H1 (a gift from T. Hays). Phenotypic analyses were performed at 25°C and border cell migration was assayed at stage 10, when the outer follicle cells have completed their rearrangement (King, 1970; Spradling, 1993).

We used the FLP/FRT system (Xu and Rubin, 1993) to generate mosaic mutant follicle cell clones of tali61G1, FRT10A and Pvr2193, FRT10A. Mosaic mutant clones marked for loss of GFP were induced as described (Bai et al., 2000; Silver and Montell, 1999) using females of the genotype hs-FLP; ubiquitin-nuclear-GFP, FRT10A. Positively marked clones were induced using the MARCM system (Lee and Luo, 2000) as described (Silver and Montell, 2001).

**Screen for dominant genetic interactions with tai**

The tali61G1 allele was recombined with a viable enhancer trap expressed in the border cells, PZ8685 (D.J.M., unpublished). The tali61G1, PZ8685/Cyo stock was crossed to the deficiency kit lines from the Bloomington stock center (http://fly.bio.indiana.edu/). We dissected ovaries from three to five female progeny lacking the balancer chromosomes and performed β-galactosidase activity staining. Egg chambers double heterozygous for tai and the deficiency were examined for delays in border cell migration compared with tali61G1, PZ8685/Cyo. A deficiency was considered phenotypic if ≥28% of the egg chambers scored showed delay of border cell migration. The average of at least two experiments is reported in Table 1. Two deficiencies were identified that exhibited partial haploinsufficiency with respect to border cell migration (Table 1).

**Ectopic expression**

To express genes ectopically, we used the GAL4/UAS system (Brand and Perrimon, 1993). _slbo-GAL4_ is expressed specifically in border cells (Rørth et al., 1998) and _c306-GAL4_ is expressed in a subset of anterior follicle cells, including the border cells (Manseau et al., 1997). To generate UAS-Pvf1 flies, we subcloned a full-length _Pvf1_ cDNA into pUAST (Brand and Perrimon, 1993) and injected this construct into _w1118_ flies along with the pnr25.7 (wings-clipped) source of transposase as described (Spradling, 1986). Two independent insertions were obtained, a viable insertion on chromosome 2 and a lethal insertion on chromosome 3. Additional UAS lines used were UAS-grkΔTC (UAS-s-grk; secreted GRK) (Queenan et al., 1999) and UAS-pvf2 (pvf2624) (Cho et al., 2002).

To make clones of cells ectopically expressing a gene under the control of GAL4/UAS, we used the ‘FLP-out’ GAL4 system (Ay-GAL4) (Ito et al., 1997). To induce clones, adult females were heat-shocked at 37°C for 1 hour and incubated for 2 days at 25°C before having their ovaries dissected. Clones were detected by expression of UAS-lacZ using an anti-β-galactosidase antibody. For UAS-Pvf1 and UAS-s-grk, we confirmed by antibody staining that the respective proteins were expressed.

**Production of antisera, β-galactosidase activity and immunofluorescence**

The last 311 amino acids of PVR were cloned into the pGST-Parallel 1 expression vector (Sheffield et al., 1999). The PVR C-terminal fragment was expressed as a fusion protein in _Escherichia coli_ [strain BL21(DE3)] and affinity purified. Purified protein was used to immunize rabbits and rats (Covance). Animals were boosted a total of five times, resulting in optimal signal in egg chambers at 1:1000 to 1:2000 dilution.

Ovarian dissection and fixation, β-galactosidase activity staining and antibody staining were performed essentially as described (Bai et al., 2000; Montell, 1999a). The following primary antibodies were used: mouse anti-Armadillo monoclonal (N27A1; 1:75) (Developmental Studies Hybridoma Bank); rabbit anti-β-galactosidase monoclonal (1:3000; Cappel); rat anti-DE-cadherin monoclonal (DCAD2; 1:10) (Uemura et al., 1996); rabbit anti-GFP serum (1:4000; Molecular Probes); mouse anti-PVF1 serum (1:200) (Duchek et al., 2001); rat anti-PVR serum (1:1500); mouse anti-Singed monoclonal (7C; 1:25) (Cant et al., 1994); and rabbit anti-TAI serum (1:1000) (Bai et al., 2000). Secondary antibodies conjugated to alexa fluor 488 and alexa fluor 568 (Molecular Probes) were used at a dilution of 1:400. To visualize actin, rhodamine-phalloidin (Molecular Probes), at a dilution of 1:400, was added during secondary antibody incubation. Images were
We found that a P-element insertion allele, \( \text{ta}^{-}\), which is a P-element insertion allele (Table 1). Most of the deficiencies interacted with both \( \text{ta}^{-} \) alleles with a few exhibiting different strengths of interactions (Table 1). In several instances, we were able to define smaller interacting regions by testing overlapping deficiencies (Table 1).

In order to identify individual genes that were responsible for the dominant interactions, several lethal P-element alleles that map to ten of the regions were tested but none exhibited an interaction with \( \text{ta}^{-} \) (data not shown). In a separate screen, we identified a homozygous viable P-element insertion that caused mild border cell migration defects (E.M.P. and D.J.M., unpublished; Fig. 3C). This P-element allele, EP1624, maps to 17E4-6, a region deleted by the interacting deficiency Df(1)N19 (Table 1). EP1624 has been shown to be a null allele of the \( Pvf1 \) locus (Duchek et al., 2001). We tested whether EP1624 interacted with \( \text{ta}^{-} \) and found that about 6% of the double heterozygous egg chambers had border cell migration defects, compared with 1% of \( \text{ta}^{61G1}/\text{CyO} \) egg chambers (Fig. 3A-C; \( P<0.006 \), Student's \( t \)-test). This effect was similar in strength to the interaction of Df(1)N19 with \( \text{ta}^{-} \) (Table 1).

\( Pvf1 \) is normally expressed only in the germ line but misexpression of \( Pvf1 \) in the border cells disrupts border cell migration in a similar way to that seen with \( Pvf1 \) loss of function (Fig. 3D) (Duchek et al., 2001). Because the loss-of-function and misexpression phenotypes of \( Pvf1 \) are similar, we
examined whether loss of \textit{tai} could enhance the PVF1 misexpression phenotype. As previously reported (Duchek et al., 2001), PVF1 misexpression in the border cells caused a minor delay in border cell migration (23% of egg chambers; Fig. 3D), with most clusters migrating varying distances along the length of the egg chamber (Fig. 3D), whereas \textit{tai} heterozygotes did not exhibit migration delays (Fig. 3C,D). By contrast, misexpression of PVF1 in a \textit{tai} heterozygous mutant background resulted in 90% of the egg chambers exhibiting border cell migration defects (Fig. 3D). Most of the delayed border cell clusters (81%) migrated ≤50% the normal distance (Fig. 3D). We further lowered the dosage of \textit{tai} using a viable combination of \textit{tai} alleles (\textit{taik}\textsuperscript{61G1} \textit{taik}\textsuperscript{5809}), which on its own exhibited a minor delay in border cell migration (26% of the egg chambers; Fig. 3D). Lowering \textit{tai} gene dosage in this genetic background dramatically enhanced the PVF1 misexpression migration defect, such that all egg chambers were defective (Fig. 3D). Most border cell clusters (91%) migrated less than 25% of the normal distance, resembling the \textit{slbo} mutant phenotype (Fig. 3D). If this were simply an additive phenotype, we would expect approximately 50% of the border cells to fail to migrate and just a few clusters (13%) to migrate less than 25% of the normal distance. Identical results were obtained using a different GAL4 driver, \textit{c306}-GAL4 (data not shown). The interaction between misexpressed PVF1 and loss of \textit{tai} appeared to be specific because lowering the gene dosage of \textit{chickadee} (\textit{chic}), another gene required for border cell migration (Verheyen and Cooley, 1994), did not enhance the PVF1 misexpression phenotype (Fig. 3D). These data together demonstrate that \textit{tai} and \textit{Pvf1} genetically interact to control border cell migration.

\textbf{\textit{tai} and \textit{Pvf1} affect DE-cadherin distribution in the border cells}

To investigate the basis for the genetic interaction between \textit{Pvf1} and \textit{tai}, we first tested whether ecdysone signaling affected expression of either PVF1 or its receptor PVR. TAI is expressed in all follicle cells (Fig. 4A) (Bai et al., 2000), as is PVR (Fig. 5A,B) (Duchek et al., 2001). Because TAI encodes a transcriptional regulator, we examined the expression of PVR protein in follicle cells that were mutant for \textit{tai}. No change in the levels of PVR expression was observed either in the columnar follicle cells surrounding the oocyte (Fig. 4C,D) or in the border cells (Fig. 4F). PVF1 was also expressed normally in \textit{ecdysoneless} mutant egg chambers (data not shown), which are defective in the synthesis of ecdysone. We then examined the expression of TAI protein in \textit{Pvf1} mutant egg chambers (Fig. 4A,B). \textit{Pvf1} mutant egg chambers displayed normal levels of TAI protein in border cells and other follicle cells (Fig. 4C,D).
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4B). We conclude that ec dysone signaling does not affect PVF or PVR protein expression in follicle cells, nor does the Pvf1 pathway regulate the expression of TAI in follicle cells. Because tait has been shown to regulate the distribution of DE-cadherin in the border cells, we tested whether PVF1 might also regulate the distribution of DE-cadherin in border cells. In wild-type border cells undergoing migration, DE-cadherin is detected at high levels in the central, non-migratory polar cells and between border cells, whereas it is detected at lower levels at the interface between border cells and nurse cells (Fig. 4G) (Bai et al., 2000; Niewiadomska et al., 1999). In tait mutant border cells, the distribution of DE-cadherin is altered and variable, and the protein can accumulate abnormally at the interface between nurse cells and border cells (Fig. 4H) (Bai et al., 2000). In Pvf1 mutant egg chambers, DE-cadherin distribution in the border cell cluster was disrupted to various degrees (Fig. 4J). In some Pvf1 mutant egg chambers, we detected DE-cadherin mainly to one side of the cluster at junctions between nurse cells and border cells (Fig. 4J). We also observed Pvf1 mutant egg chambers in which DE-cadherin was detected at high levels overall in the border cells and in a few junctions between border cells (Fig. 4J). Misexpression of PVF1 (sibo-GAL4/UAS-Pvf1) in the border cells altered DE-cadherin distribution in a manner similar to that of Pvf1 mutant egg chambers (Fig. 4K,L). The distribution of Arm, the Drosophila homolog of β-catenin, is indistinguishable from that of DE-cadherin in wild-type border cells (Bai et al., 2000; Oda et al., 1997). We found that Arm

Table 2. Quantitation of phenotypic classes observed following misexpression of PVF1, PVF2 and GRK in anterior follicle cells

<table>
<thead>
<tr>
<th>Genotype* (n)†</th>
<th>Anterior follicle cell clones</th>
<th>Class I (%)</th>
<th>Class II (%)</th>
<th>Class III (%)</th>
<th>Class IV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAS-Pvf1 (1x)† (148)</td>
<td>87</td>
<td>5</td>
<td>8</td>
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</tr>
<tr>
<td>Pvf1+/+;UAS-Pvf1 (124)</td>
<td>80</td>
<td>2</td>
<td>18</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pvr+/+;UAS-Pvf1 (128)</td>
<td>90</td>
<td>0</td>
<td>13</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>UAS-Pvf2 (130)</td>
<td>57</td>
<td>11</td>
<td>19</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>UAS-Pvf1 + UAS-Pvf2 (94)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>UAS-Pvf1 (2x)† (95)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Genotypes are hsflp+/++; AyGAL4/x, where “x” is the indicated UAS-transgene.
†Total number of egg chambers examined for each genotype.
‡1x and 2x represent total number of copies of the transgene.
See Fig. 6 legend and text for description of classes.
In order to better understand the role of PVF1 receptor, PVR (Cho et al., 2002; Duchek et al., 2001; Heino et al., 2001). A dominant-negative version of PVR causes an incompletely penetrant border cell migration defect (Duchek et al., 2001). It is not clear whether the mild phenotype actually represents the effect of a null mutation in the receptor or reflects a partial loss-of-function effect of the dominant negative mutation. Moreover, the Drosophila genome encodes two additional ligands related to PVF1, called PVF2 and PVF3 (or VEGF27Cb and VEGF27Ca), which are expressed in the ovary (Duchek et al., 2001) and could possibly contribute to border cell migration. However, there is only a single receptor, therefore we tested to what extent a Pvr mutation disrupted border cell migration (Fig. 5). An allele of Pvr (Pvr<sup>2195</sup>) was recently reported (Cho et al., 2002). We generated homozygous mutant clones in follicle cells using this allele. We found that PVR was undetectable in mutant cells (Fig. 5C,D) and so Pvr<sup>2195</sup> is a strong loss-of-function, and possibly null, allele. Border cell clusters in which all cells were homozygous for the mutation displayed delays in border cell migration, but the phenotype was incompletely penetrant (Fig. 5E,G) and many border cell clusters completed their migration to the oocyte (Fig. 5F). This phenotype is indistinguishable from the null phenotype for Pvf1 (Fig. 5G).

**Ectopic expression of PVF1 is sufficient to misguide the border cells**

PVF1 is expressed in the oocyte and its receptor is expressed in all follicle cells (Fig. 5A,B), and so it has been proposed to guide the border cells (Duchek et al., 2001). However, this has not been directly tested. We used the 'FLP-out' GAL4 system (Ito et al., 1997) to express PVF1 in random groups of follicle cells in order to test whether PVF1 was sufficient to guide the border cells to a new source of ligand (Fig. 6; Table 2). We scored border cell migration in egg chambers that expressed PVF1 in the anterior follicle cells that surround the nurse cells (squamous follicle cells) but not in border cells (Fig. 6). Antibody staining confirmed that PVF1 was actually expressed in the follicle cell clones (Fig. 6A-C). The levels of ectopic PVF1 expressed in clones of anterior follicle cells appeared to exceed the concentration of endogenous PVF1 in the nurse cells.

We observed four classes of phenotypes when PVF1 was ectopically expressed in clones of anterior follicle cells. In the class I phenotype, border cell clusters migrated normally to the oocyte (Fig. 6D); most of the egg chambers misexpressing PVF1 fell into this class (Table 2). The class II phenotype was characterized by border cells that did not migrate away from the anterior pole (Fig. 6E). In all examples of this class, we observed that the border cells were adjacent to a group of follicle cells expressing ectopic PVF1 (Fig. 6E); 5% of egg chambers misexpressing PVF1 fell into class II (Table 2). In class III egg chambers, border cells were located on the side of the egg chamber, not far from the anterior pole and adjacent to cells expressing ectopic PVF1 (Fig. 6F); we observed 8% class III phenotypes (Table 2). In the class IV phenotype, the border cells were found on the side of the egg chamber at least two nurse cells or more away from the anterior pole, adjacent to a clone of cells expressing ectopic PVF1 (Fig. 6G). Therefore it was possible for border cells to be guided to a source of PVF1, but this class was relatively rare (Table 2).

Although it appeared that the levels of ectopic PVF1 were...
higher than the local concentration of endogenous PVF1, it seemed possible that endogenous PVF1 was somehow more effective and therefore that the ectopic protein was not very active because of the presence of endogenous protein. Therefore, we induced clones of cells expressing ectopic PVF1 in a Pvf1EP1624 mutant background, which lacks endogenous PVF1 protein (Duchek et al., 2001). Similar results were obtained whether or not endogenous PVF1 was present (Table 2), making it unlikely that endogenous PVF1 interfered with the ability of ectopic PVF1 to guide the border cells to new targets in the egg chamber. We also considered the possibility that PVF1 expressed in follicle cells might be sequestered by endogenous PVR, which is expressed in all follicle cells, such that little PVF1 ligand could be secreted by these follicle cells. However reducing by half the dose of PVR had little or no effect on the ability of ectopic PVF1 to redirect the migration (Table 2).

Most egg chambers expressing ectopic PVF1 exhibited normal border cell migration, so we tested whether other ligands could misguide the border cells, either alone or in combination with PVF1. PVF2 is a protein homologous to PVF1 that is capable of redirecting hemocyte migration in the Drosophila embryo, even though PVF1 cannot (Cho et al., 2002). We therefore tested whether PVF2 could misguide the border cells when ectopically expressed in follicle cells. PVF2 alone did not cause any border cell migration delays and did not guide the border cells to new targets (Table 2). Specific misexpression of PVF2 in the border cells using slbo-GAL4 did not affect their migration either (data not shown), therefore the border cells appeared to be unresponsive to PVF2 alone. The results of co-expressing PVF1 and PVF2 in follicle cell clones are shown in Table 2. There was a small increase in the proportion of egg chambers that fell into class IV, indicating that PVF2 might be more effective in combination with PVF1 than alone.

The most dramatic effects on border cell migration were observed when two copies of the UAS-Pvf1 transgene were included in the experiment, thus presumably doubling the concentration of ectopic PVF1. The proportion of class IV egg chambers jumped to 13% and that of egg chambers showing normal migration was reduced to 57% (Table 2).

EGFR and PVR might function redundantly to guide the border cells to the oocyte, because expression of dominant-negative EGFR and PVR together disrupt border cell migration more potently than either dominant-negative alone (Duchek et al., 2001). If these factors function redundantly to guide the border cells then either factor should be sufficient on its own to redirect the migration. We tested whether expression of a
secreted form of GRK (s-GRK) (Van Buskirk and Schupbach, 1999) was sufficient to attract the border cells. In all cases, we observed normal border cell migration following expression of s-GRK in follicle cell clones (class I; Table 2). To test whether expression of PVF1 and s-GRK together was a more effective guidance cue than either alone, we coexpressed them in follicle cell clones. We obtained similar results to ectopically expressing PVF1 alone (Table 2), indicating that ectopic s-GRK in anterior follicle cells did not affect border cell migration. Ectopic expression of GRK in border cells, in contrast to PVF2, was able to disrupt border cell migration to a small degree (data not shown) (Duchek and Rørth, 2001).

DISCUSSION

Genetic screen to identify genes that function with tai in border cell migration

We performed a screen to identify genomic regions that exhibit dominant genetic interactions with tai in order to identify new genes that are required for border cell migration and identified 14 different interacting genomic regions. We were able to identify Pvf1 as the locus responsible for one of the deficiency interactions. The interaction of tai with Pvf1 appears to be specific because tai did not interact with either loss-of-function mutations or deficiencies that remove other genes known to regulate border cell migration, such as slbo or shotgun/DE-cadherin (data not shown; Fig. 2). Mutations in slbo or shotgun reduce DE-cadherin levels in the border cells (Niewiadomska et al., 1999), so tai does not interact with every gene that regulates DE-cadherin, possibly because tai regulates the distribution rather than the levels of DE-cadherin in the border cells. Identification of Pvf1 indicates that this screen provides a useful approach for identifying additional loci that affect border cell migration in general and regulated turnover of adhesion in particular.

Relationship between TAI and PVF1

The genetic interaction between Pvf1 and tai indicates that the regulation of border cell migration timing and guidance might be linked. What is the nature of the interaction between tai and Pvf1 during border cell migration? Ecdysone signaling did not regulate PVF1 or PVR expression nor did Pvf1 regulate TAI expression, but the ecdysone and Pvf1 pathways both affected the distribution of DE-cadherin and Arm. We favor a model whereby tai and Pvf1 interact because they both regulate adhesion complex localization or turnover. The tai and Pvf1 genes could act independently to regulate cadherin-dynamics. Alternatively, tai and Pvf1 might function in a common pathway. TAI and PVR both function autonomously in the border cells, although they are unlikely to bind directly to each other because TAI localizes to the nucleus and PVR is a receptor tyrosine kinase localized to the membrane. One possibility is that PVR activates (or represses) the function of a protein whose expression is dependent on TAI, and that this protein in turn regulates cadherin dynamics in the border cells. Tyrosine phosphorylation of β-catenin, the Arm homolog, causes destabilization of adhesion complexes in other cell types (Lilien et al., 2002), so perhaps PVR activity destabilizes E-cadherin/Armadillo complexes specifically in the border cells. Identification of additional genes identified in this screen, in particular those that affect adhesion turnover in border cells, should help clarify the biochemical relationship between TAI and PVF1.

PVF1 functions as a concentration dependent guidance cue – evidence for additional guidance cues?

The results reported here demonstrate that ectopic expression of PVF1 is sufficient to redirect border cells even though, in Pvf1 null mutants, border cell clusters migrate normally in the majority of egg chambers. When PVF1 was ectopically expressed in random follicle cells, the border cells were attracted to these sources of PVF1. The border cells were attracted more efficiently to sources of PVF1 signal close to the anterior pole, indicating that they respond better to high concentrations of the ligand. The finding that doubling the dose of ectopically expressed PVF1 dramatically increased the frequency with which the cells responded to the ectopic signal confirmed the idea of a concentration dependent effect.

The concentration of ectopic PVF1 at the anterior end of the egg chamber appeared to exceed the concentration of endogenous PVF1 at that position, even when only a single UAS-Pvf1 transgene was included in the experiment. Consistent with that idea, elimination of endogenous PVF did not significantly alter the response of the border cells to ectopic ligand. The border cells still migrated normally in many cases, apparently ignoring ectopically expressed PVF1. The most likely explanation for this is that there are additional germ-line-derived attractive cues that instruct the cells to migrate correctly in the absence of endogenous PVF1 and in the presence of ectopic PVF1.

PVF2 does not seem to be a good candidate for a redundant guidance cue because loss of function of the PVR receptor produced a phenotype that was indistinguishable from loss of PVF1 alone. Moreover UAS-Pvf2 was not able to redirect the border cells. This finding is surprising because PVF2 is thought to bind and activate the same receptor as PVF1. It is especially surprising because only PVF2 (expressed from the same UAS-PVF2 transgene) and not PVF1 is effective at misguiding hemocytes in the embryo (Cho et al., 2002). Together, these findings suggest a striking, and as-yet inexplicable, specificity of ligand action that will be interesting to study further.

We also found that GRK, the major EGFR ligand in the ovary, was not an effective guidance cue for the border cells, either when expressed alone or in combination with PVF1. The inability of GRK to affect border cells was striking because even class II and III phenotypes were absent, even though these were not uncommon following PVF misexpression. This is consistent with the observation that migration of the border cells to the oocyte is completely normal in grk mutant egg chambers and in mosaic egg chambers in which border cells lack EGF receptor function (Duchek and Rørth, 2001). GRK does, however, have a role in the dorsal migration of the border cells after they reach the oocyte (Duchek and Rørth, 2001). Currently, the evidence supporting a role for GRK in migration of the border cells to the oocyte is the combined effect of dominant-negative PVR and dominant-negative EGFR (Duchek et al., 2001). Taken together with the results supplied here, the evidence in favor of a role for EGFR is somewhat better than the evidence in favor of a role for GRK, possibly suggesting the involvement of other EGFR ligands.
In addition to ligands for PVR and EGF-R, this study might imply the existence of other, as-yet- unidentified cues, that participate in the long-range guidance of the border cells. We propose that PVF1, and possibly additional unknown ligands, guide the border cells to the oocyte. Similarly, in the Drosophila central nervous system, multiple short-range and long-range cues are required to guide motor axons properly to their appropriate muscle targets (Winberg et al., 1998). Perhaps even a simple migration, such as that of the border cells, uses multiple cues, each of which might only have a small contribution. Screens such as the one reported here might help us to identify the full set of border cell migration cues as well as additional genes that function in adhesion complex turnover.

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