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Gal4 in the *Drosophila* female germline

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Abstract

The modular Gal4 system has proven to be an extremely useful tool for conditional gene expression in *Drosophila*. One limitation has been the inability of the system to work in the female germline. A modified Gal4 system that works throughout oogenesis is presented here. To achieve germline expression, it was critical to change the basal promoter and 3′-UTR in the Gal4-responsive expression vector (generating UASp). Basal promoters and heterologous 3′-UTRs are often considered neutral, but as shown here, can endow qualitative tissue-specificity to a chimeric transcript. The modified Gal4 system was used to investigate the role of the *Drosophila* FGF homologue *branchless*, ligand for the FGF receptor *breathless*, in border cell migration. FGF signaling guides tracheal cell migration in the embryo. However, misexpression of *branchless* in the ovary had no effect on border cell migration. Thus border cells and tracheal cells appear to be guided differently.

Keywords: UAS; Ovary; Border cells

1. Introduction

The Gal4/UAS system is widely used to drive tissue-specific expression of cloned genes in *Drosophila* (Brand and Perrimon, 1993). The system makes use of two types of transgenes, a Gal4 ‘driver’ and a Gal4-responsive UAS expression vector. The driver directs tissue specific expression of the yeast Gal4 protein, a sequence-specific transactivator. The UAS vector contains multiple binding sites (upstream activating sequences) for Gal4 and is designed to drive expression of inserted cDNA sequences when Gal4 is present. Combining appropriate Gal4 driver and UAS transgene allows tissue-specific conditional expression of cloned genes. In addition, Gal4 dependent transactivation can be used to target endogenous genes for activation and thus carry out systematic gain-of-function screens (Rørth, 1996; Rørth et al., 1998).

Driving ectopic expression in a conditional manner is an important advantage of the Gal4 system, as many genes cause severe developmental defects when misexpressed. Conditional misexpression is particularly important for an essential tissue such as the germline. Unfortunately, the original Gal4/UAS system does not work in the germline during oogenesis (Brand and Perrimon, 1993). Overcoming this problem would clearly improve the experimental strategies available for studying oogenesis and early embryogenesis. Here a modified UAS vector which works in the germline during oogenesis is described.

2. Results and discussion

2.1. Making the Gal4 system work in the germline

One possible explanation for the Gal4 system being inactive in the germline during oogenesis is that, in this tissue, the UAS construct cannot drive productive transcription in response to Gal4. To test this idea, a modified UAS vector, which should be optimal for expression in the germline, was constructed. The original pUAST vector (Brand and Perrimon, 1993) contains in addition to five Gal4 binding sites, a basal promoter from the hsp70 gene, cloning sites and the SV40 3′ region (Fig. 1). As basal promoter in germline competent vector, the P transposase minimal promoter and first intron was chosen, which has been shown to drive efficient, enhancer-dependent expression in the germline during oogenesis (Grossniklaus et al., 1989; Spradling, 1993). In addition, to avoid using a 3′-UTR which might

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destabilize expressed transcripts, the 3′-UTR from K10 was used (Serrano et al., 1994). The resulting vector, UASp, is shown in Fig. 1. A UASp-lacZ reporter was constructed and transformed into flies by germline transformation. As expected, UASp-lacZ showed no expression in the absence of Gal4 and reported the same expression pattern and level as conventional UAS-lacZ when tested with somatic drivers such as *slbo*-Gal4 and *ptc*-Gal4 (data not shown).

In order to express Gal4 protein in the female germline, the open reading frame of Gal4 was cloned into the pCOG expression vector (Robinson and Cooley, 1997), which utilizes the *otu* promoter to direct germline expression (Rodesch et al., 1995). When combined with UASp-lacZ, pCOG-Gal4 drives b-galactosidase expression in the germline (Fig. 2A). Gal4 expressed under control of the *armadillo* promoter (arm-Gal4) also induced germline expression from UASp-lacZ (Fig. 2B). Thus the Gal4 protein does function in germline cells of the ovary, but an appropriate UAS vector such as UASp is required to achieve Gal4-induced gene expression.

Even with the germline competent UASp, Gal4 only induced low levels of β-galactosidase expression and limited to certain stages of oogenesis. This was unexpected as the *otu* (Rodesch et al., 1995) and *armadillo* promoters are active at both earlier and later stages as well. To increase the potency of transactivation, a Gal4:VP16 fusion was cloned into the pCOG vector in place of Gal4. pCOG-Gal4:VP16 directed expression of UASp-lacZ at higher levels than pCOG-Gal4, and also at later stages of oogenesis (Fig. 2C). Finally, a driver with Gal4:VP16 transactivator under control of the nanos promoter and 3′-UTR (Van Doren et al., 1998) was tested with UASp-lacZ. Nanos-Gal4:VP16 gave high level expression in the germline from the tip of the germarium (germline stem cells) to the mature egg (Fig. 2D–2F). A comparison of Otu-Gal4: VP16 and Nanos-Gal4:VP16 indicates that Gal4:VP16 inducible expression functions throughout oogenesis. It was also clear that different Gal4 and Gal4:VP16 drivers give distinct temporal expression patterns. pUAST-lacZ was inactive in the ovary with all germline drivers, including nanos-Gal4:VP16 (Fig. 2G), showing that expression in the germline was absolutely dependent on the modifications in UASp.

2.2. Role of the basal promoter in the UAS construct

The rationale for changing the promoter in the UAS vector was as follows. In *Drosophila*, many genes have separate maternal (germline) and zygotic promoters, instead of different enhancers controlling the same promoter. It has been shown that core promoter sequences can influence the ability of enhancers to engage specific promoters in the Drosophila genome (Merli et al., 1996; Ohtsuki et al., 1998), which could explain the need for different promoters in the germline. Alternatively, basal promoters per se may be restrictive and not act in all tissues. There is increasing evidence indicating that the so-called basal transcription machinery includes components which differ in different cell types and during the cell cycle. In *Drosophila*, selective usage of the two Adh promoters is at least in part due to differences in efficiency of the basal promoter at different stages (Hansen and Tjian, 1995). A tissue-specific TATA binding protein has also been described (Hansen et al., 1997). The original pUAST construct includes the basal promoter of hsp70. However heat shock induced expression

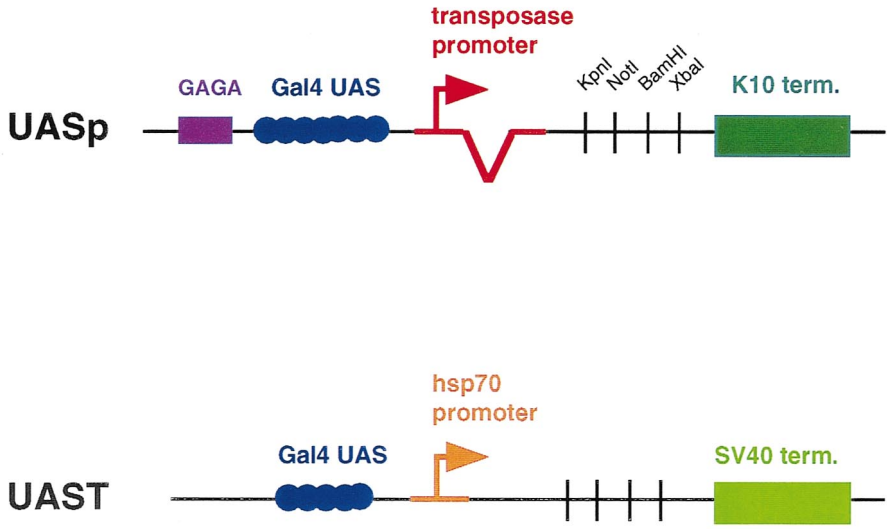

Fig. 1. The UASp vector. For comparison, pUAST, redrawn from Brand and Perrimon (1993), is shown below. In UASp, 14 Gal4 UAS sites and adjacent GAGA sites from the EP vector (Rørth, 1996) were placed upstream of the promoter. This change alone did not make a UAS-lacZ construct work in the germline. UASp contains P transposase promoter and first intron, but a construct without the intron was also active in the germline. Downstream of the cloning sites are 3′-UTR sequences and terminator from the K10 gene. The 3'UTR used here stabilizes transcripts in the germline but does not affect localization (Serrano et al., 1994).

using the full hsp70 promoter is inefficient in the germline. One possible reason is that the hsp70 basal promoter is poorly active in this tissue.

The observation that UASp drives expression in the germline supports the idea that basal promoters can have inherent tissue specificity. To test this more directly, I compared basal promoters from genes which use distinct maternal and a zygotic promoters for their ability to substitute for the P transposase in the UASp vector. As the transactivator used (Gal4 or Gal4:VP16) remains the same in each case, this experiment directly tests whether the basal promoter itself has any inherent tissue specificity or restriction. The following promoters were tested: *chickadee* germline (distal) and somatic (proximal) promoters (Cooley et al., 1992) as well as *hunchback* maternal and zygotic promoters (Treier et al., 1989). Basal promoters were arbitrarily defined as encompassing from –45 to +30 basepairs relative to the transcription start site, which includes the TATA boxes. All four promoters were competent to drive lacZ expression in response to Gal4 in both germline and soma (data not shown). Thus the basal promoter sequence per se does not determine which promoter of the *chickadee* and *hunchback* genes is activated in the germline. Instead it is likely that each promoter interact selectively with endogenous enhancers.

In summary, Gal4 transactivation works in the germline of the ovary when acting on a permissive UAS construct. What defines a permissive UAS vector is not completely understood but appears to depend on both the promoter and the 3′-UTR sequences.

2.3. The role of branchless in border cell migration

Several useful models for studying directed cell migration during development have emerged in *Drosophila*, such as the migration of tracheal cells in the embryo (Manning and Krasnow, 1993) and the migration of border cells in the ovary (Montell et al., 1992). Migration of tracheal cells require the FGF receptor homologue encoded by *breathless* (*btl*), expressed in the migrating cells (Klambt et al., 1992). *branchless* (*bnl*) encodes an FGF ligand for *btl* and is also required for tracheal migration(Sutherland et al., 1996). Bnl acts as an instructive guidance cue, a direct chemoattractant, for at least some of the migrating cells. Localized ectopic expression of *bnl* causes cell migrations and tubular outgrowths towards the source of expression. General ectopic expression of *bnl* disrupts the tracheal pattern completely.

During stage 9 of oogenesis a group of 6–8 somatic follicle cells, called border cells, migrate from the anterior of the egg chamber, between the germline nurse cells, to the posteriorly located oocyte (Fig. 3C). The transcription factor C/EBP, encoded by the *slow-border-cells* (*slbo*) locus, is expressed in border cells and required for their migration (Montell et al., 1992). In addition, two enhancer-traps in the *btl* locus drive expression in border cells, and the level of Btl expression affect migration in *slbo* mutant females (Murphy et al., 1995). Heat shock induced overexpression of Btl dramatically suppresses the *slbo* phenotype. This has lead to the suggestion that *btl* is downstream of *slbo* and important for border cell migration (Murphy et al., 1995). If so, the *btl* ligand, *bnl* might be expected to direct border cell migration similar to the situation in tracheal cells. Since border cells invariantly move towards the oocyte, Bnl would need to be expressed posteriorly in the egg chamber, for example in the oocyte, to serve as an instructive attractant.

The modified Gal4 system allowed me to address whether Bnl is an instructive attractant for border cells. Specifically, nanos-Gal4:VP16 drives high expression in nurse cells before and during stage 9; expression is uniform or higher at the anterior end of the egg chamber (Fig. 2E). If Bnl were

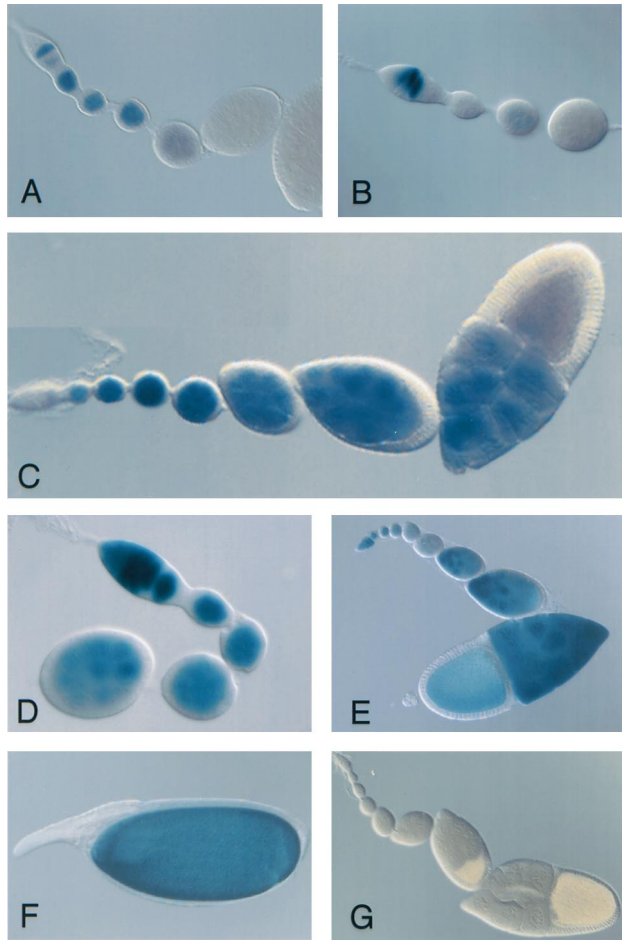

Fig. 2. Gal4 inducible expression in the germline of the ovary. Xgal staining of ovaries from females containing the following Gal4 transgenes (A) pCOG-Gal4, (B) armGal4, (C) pCOG-Gal4:VP16, (D) to (G) nos-Gal4:VP16. In (A–F) the reporter gene was UASp-lacZ, in (G) pUAST-lacZ (Brand and Perrimon, 1993). Ovaries in (A), (B) and (G) were stained with Xgal for 3 h, (C) for 30 min and (D–F) for 15 min, all at 37°C. With UASp-lacZ, nosGal4:VP16 drives expression from earliest stages in the germarium (E) to late stages resulting in dumping of β-galactosidase into the maturing oocyte (F), but pUAST-lacZ shows no expression (G).

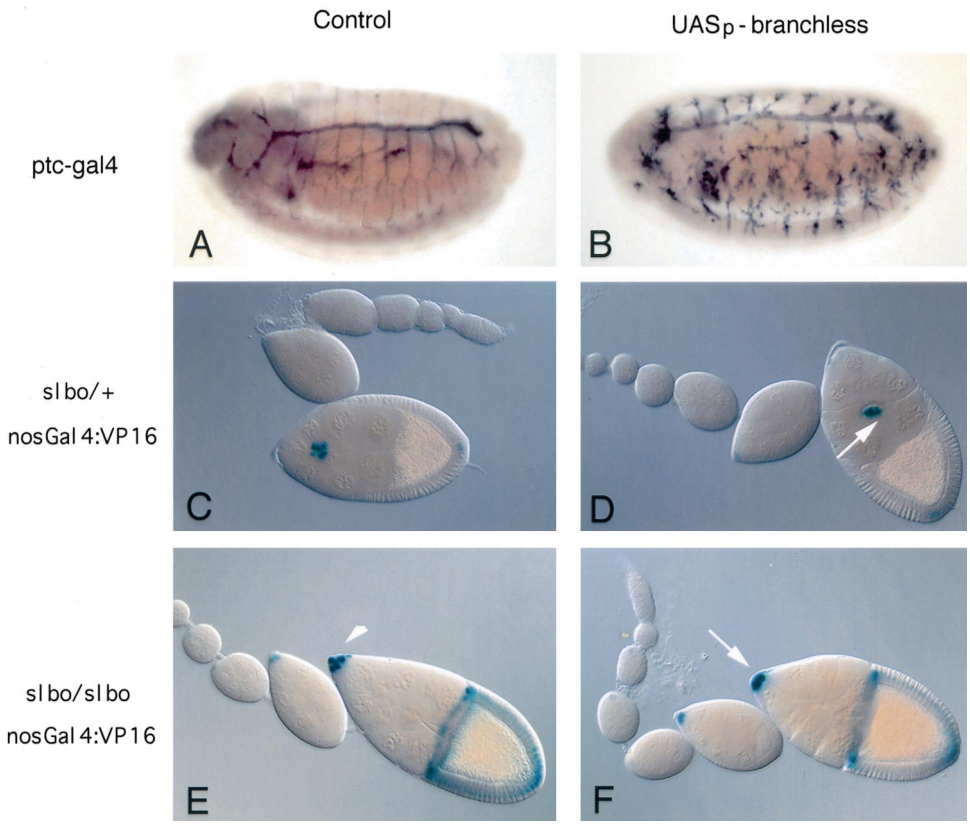

Fig. 3. Effects of ectopic *branchless* expression in the ovary. (A,B) Antibody staining of PtcGal4/+ embryos with an antibody (2A12) recognizing the trachea. Induction of UASp-bnl by the broadly expressed PtcGal4 driver caused severe tracheal branching defects (B), showing that the UASp-bnl transgene is functional. (C–F) Ovarioles from females containing the *slbo*^{1} enhancer trap insertion (Montell et al., 1992); border cells (arrow) are visualized by Xgal staining. (C,D): *slbo*^{1}/+; nosGal4:VP16/+; (D) also one copy of UASp-bnl. Border cell migration, shown here at stage 9, is unaffected by UASp-bnl. Same result was obtained with 6/6 UASp-bnl insertion lines with at least 200 egg chambers scored for each line. (E,F): *slbo*^{1}/*slbo*^{1}; nanosGal4VP16/+. (F) Also one copy of UASp-bnl. Stalled border cell migration in *slbo* mutant females was not suppressed by Bnl expression.

an attractant for border cells, then uniform or predominantly anterior expression of Bnl should perturb their pathfinding. To test this, the coding region of Bnl was cloned by RT-PCR and placed into the UASp vector. Six UASp-bnl insertion lines were generated. They were tested for activity by crossing to a *ptc*-Gal4 driver and visualizing tracheal morphology in the embryo. As expected (Sutherland et al., 1996), the tracheal tree was severely disrupted and had excessive branching (Fig. 3A,B), showing that active Bnl was produced. In contrast, induction of UASp-bnl in the ovary by nanos-Gal4:VP16 had no discernible effect on border cell migration (Fig. 3C and 3D). No precocious, misdirected or stalled migration was observed in any of the 6 UASp-bnl lines. Similarly, forced expression of Bnl in the migrating border cells using slbo-Gal4 (Rørth et al., 1998) had no effect (data not shown). Apparently, ectopic expression of Bnl does not affect border cell migration. A caveat to this interpretation is the possibility that functional Bnl protein is not made either in nurse cells or in border cells. This cannot be directly assessed as no Bnl antibody is available. However, it seems unlikely, as functional Bnl protein is made from UASp-bnl in ectopic locations in the embryo. Furthermore, it has previously been observed that border cells which are mutant for the *btl* receptor continue to migrate normally (Murphy et al., 1995). Thus the most straightforward interpretation is that Bnl does not serve as instructive attractant for border cell migration.

The previously observed effects of *btl* on *slbo* mutant border cells could reflect a simple permissive function for FGF signaling in this migration. If so, overexpression of Bnl in the germline of *slbo* mutant females might also increase Btl signaling and thus suppress the *slbo* mutant phenotype. However, this was not observed (Fig. 3E,F). There are several possible explanations for the apparent discrepancy between this result and the suppression of the same *slbo* allele by overexpression of the receptor (Murphy et al., 1995). It is possible that Bnl protein is not produced, though this seems unlikely for the reasons given above. Alternatively the ligand is already in excess in the *slbo* mutant egg chambers, and therefore only receptor overexpression has a positive effect. Finally, Btl protein may not normally be present in border cells. This would explain why *btl* is not required in these cells and why they cannot respond to ectopic Bnl.

In summary, the results suggest that *bnl/btl* FGF signaling does not guide border cell migration, emphasizing the dif-

ference between border cells and tracheal cells. Whether this is a superficial difference (i.e. another tyrosine kinase and secreted ligand guides border cells) or a more profound mechanistic difference awaits further investigation. With the modifications of the Gal4 system presented here, it is possible to directly test other putative guidance molecules for their effect on border cell migration.

3. Experimental procedures

3.1. Constructing UASp, UASp-lacZ and UASp-bnl

The following was cloned into the pCasper4 transformation vector (see also Fig. 1): (1) Gal4-UAS sites and GAGA sites as a *Not*I-*Xho*I fragment from pEP plasmid (Rørth, 1996); the *Not*I site was made blunt by klenow polymerase. (2) The promoter and first intron of P transposase as an *Xho*I-*Kpn*I fragment (details below). (3) For UASp-lacZ, the lacZ coding region as a *Kpn*I-*Xba*I fragment from Pw15-lacZ (Rørth, 1994), for UASp the polylinker shown in Fig. 1. (4) K10 3′-UTR as a *Hin*dIII-*Pst*I fragment from pGerm8 (Serrano et al., 1994); the *Hin*dIII site was made blunt by klenow polymerase.

The P transposase promoter was cloned by PCR using the pCasper plasmid as template and the following oligonucleotides: 5′-CCG CTC GAG TCG ATA GCC GAA GCT TACC-3′ and 5′-GGGGT ACC GGT TTT CAA AAA AAA ATT CGTCC-3′ (promoter, position 28–125 according to (O'Hare and Rubin, 1983); 5′-CCACCGGT GAT AGA GCC TGA ACCAG-3′ and 5′-GGGGT ACC AAT GAA CAG GAC CTA ACGCA-3′ (intron, position 380–513 according to (O'Hare and Rubin, 1983). Using these two fragments avoids including the transposase initiator ATG. In UASp the promoter and intron PCR fragments were cut by *Age*I, ligated, then cut by *Xho*I and *Kpn*I; in UASp$^{-intron}$ the promoter PCR fragment was cut by *Xho*I and *Kpn*I. Both fragments were verified by sequencing.

For the UASp variants, the following promoter sequences (oligonucleotides) replaced the P-transposase promoter in UASp-lacZ: chickadee (1.0 kb, maternal) 5′-TCG AGC GAA CAG CGA CTT GTG TTC CAG TAC TAC CGC TTG TCG ACG GTC ACT CTG AAT TTT TAC CGC AGC GTG TGA ACA GTG-3′ and chickadee (1.2 kb) 5′-TCG AGT GGG TTT TCT CGA ATT CAA AAT CGG TTT ATG GTT CTG CTT TGC GCT CAT TCG ACT TTT GAA ATC CGC CTC GAA CGC TTCG-3′ (Cooley et al., 1992) and genomic sequence from BDGP); hunchback (P1) 5′-TCG AGG AGA TTT TCA GCT ATT AGA AGA GCC CGC TGA GCG TGA GTT TGG TCA GTT GTG CTC CGA GTC CCG AAA ACG AAA GTCG-3′ and hunchback (P2) 5′-TCG ATC CGT CTA CCT GAG CGA TAT ATA AAC TAA TGC CTG TTG CAA TTG TTC AGT CAG TCA CGA GTT TGT TAC CAC TGC GAC AAG-3′ (Treier et al., 1989).

The coding region of *bnl* (Sutherland et al., 1996) was cloned by RT-PCR (Stratagene) on embryo mRNA with the following oligos, taking advantage of internal *Eco*RI and *Not*I sites in the cDNA: (1): 5′-GGAGATCT TTG ATG CGA AGA AAC CTG CGC-3′ and 5′-GTGAATTC ACT ATT CTC GTC CTG GGT-3′; (2): 5′-ACC CAG GAC GAG AAT AGT GAA TTCAC-3′ and 5′-CTC GCT TCT CGG CGG CCG CCT TCTC-3′; (3): 5′-GAG AAG GCG GCC GCC GAG AAG CGAG-3′ and 5′-CTCTAGA AAA TAC TAA ATG CTA TAA ATG TAG-3′. Each fragment was verified by sequencing and all three were cloned as *Bgl*II-*Eco*RI-*Not*I-*Xba*I into *Bam*HI-*Xba*I of UASp.

3.2. Gal4 lines, flies and staining

The open reading frame of Gal4 (*Hin*dIII fragment from pGatB (Brand and Perrimon, 1993) and of a Gal4(1-147):VP16 fusion were cloned into pCOG (Robinson and Cooley, 1997) to make pCOG-Gal4 and pCOG-Gal4:VP16. A high variability between different lines carrying the pCOG-Gal4:VP16 transgene was observed, 12/20 lines show no expression; four only at stages 1–6 and 3 as shown in Fig. 2C. In the latter lines expression was usually lowest at stages 6–9 and reappeared at stage 10. pCOG-Gal4:VP16 induced expression was also frequently mosaic, with one or few nurse cells expressing β-galactosidase at stage 10. UAS-lacZ (Brand and Perrimon, 1993) and arm-Gal4 flies were obtained from the Bloomington stock center. All tested stocks (ten) containing UAS-lacZ or UAS[EP]-lacZ (Rørth, 1996) insertions showed no β-galactosidase activity in the germline.

Antibody staining of embryos with MAb 2A12 was done essentially as described in Patel (1994).

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