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Differentiation of Border Cells during Oogenesis in *Drosophila melanogaster*

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노랑초파리 난자 형성과정 동안의 경계세포의 분화

계명찬 · 조경상* · 이정주**

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ABSTRACT : An enhancer detector line (EDL) having P[lArB] insertion in X chromosome with expression of reporter gene (*lacZ*) in the polar cells and border cell of egg chamber was established and used to monitor the differentiation and migration of border cells during the oogenesis of Drosophila. Differentiation of border cell from the anterior polar follicle cells was evident in stage-9 egg chamber of EDL149 which was chracterized by migration of columnar follicle cells toward posterior of egg chamber surrounding the oocyte. Migration of border cells was observed in the stage-9 and -10 egg chambers. β-galactosidase activities were rapidly increased during the first 4 days after eclosion, and it coincided with the timing of border cell differentiation in the ovary during adult life. Homozygote of EDL149 showed some retardation of border cell migration, resulting absence of migration of some border cells in the anterior part of egg chamber or delayed migration of some border cells in the stage-10 egg chamber. These results suggest that the P[lArB] of EDL149 is inserted at the locus of the structural gene required for the border cell migration. In addition to the expression in egg chambers, *lacZ* expression was also detected in the meiotic germ cells of testis and antenna, suggesting the possible requirement of the trapped gene function in these organ. This EDL and enhancer trapped gene might be useful for the study of developmentally regulated cell migration.

Key words: P-element, Border cell, Oogenesis, *Drosophila*.

요 약 : 강화인자 검출법을 이용 X염색체에 P[lArB]이 형질전환되어 극세포 및 경계세포에서 표시유전자 *lacZ*를 발현하는 노랑초파리 (EDL149)를 이용하여 난자형성과정 동안의 경계세포의 분화 및 이동을 조사하였다. 경계세포는 9기 난포의 선단에 위치한 난포세포로부터 분화하여 9기와 10기에 이동하는 것을 확인하였다. 난소내 β-galactosidase의 활성은 우화 후 처음 4일간 급격히 증가하는 것을 확인하였으며 이 시기는 난포 내에서 경계세포가 분화하는 시기와 일치하였다. EDL149의 P[lArB] 삽입의 동형접합체의 난포 내에서 일부 경계세포의 불완전한 이동 또는 지연이 관찰되었다. 감수분열을 진행 중인 정소내 세포 및 더듬이에서 확인된 lacZ 유전자의 발현양상은 P[lArB]의 삽입부위가 난소특이 유전자부위가 아니지만 경계세포 이동의 조절에 역할을 하는 유전자임을 암시한다. 이 형질전환초파리 및 삽입위치 부근의 유전자는 발생중 진행되는 세포이동의 연구에 좋은 모델로 생각된다.

INTRODUCTION

Two basic cell types can be distinguished during oogenesis of *Drosophila*: the germ line, consisting of fifteen nurse cell and an oocyte, and the somatically derived follicle cells which surround the oocyte and nurse cells. Early in oogenesis, the follicular epithelial cells divide as the follicle enlarges. Before the secretion of the vitelline membrane around the developing oocyte, there is a general migration of follicle cells to produce a dense columnar epithelium over the oocyte itself and a thin sqamous epithelium over the nurse cells (King, 1970). During this period, a group of 6-10 follicle cells (the 'border' cells) leave the anterior end of the epithelium and migrate between the nurse cells, to the anterior pole of oocyte within 3-5 hr (Montell, 1994). Later, these border cells will make the micropylar apparatus

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at the anterior end of the egg (Zarani & Margaritis, 1985). Finally, the follicle cells and nurse cells degenerate, leaving the mature egg alone (reviewed in King, 1970).

During the oogenesis, inductive interactions between the germline cells and the somatic follicle cells determine the polarities of the anterior-posterior (AP) and dorsal-ventral axes of *Drosophila* (Spradling, 1993). The function of the polar cells is still unclear but it has been proposed that they might be involved in the overall control of the polarity of the developing egg chamber (Brower et al., 1981; Gonz lez-Reyes et al., 1995). During the fertilization, sperm invasion occurs through micropyle, and border cells synthesize and shape both of the vitelline and chorion components of this structure (Zarani & Margaritis, 1985). Posterior follicle cells including polar cells are known to be involved in the formation of aeropyle of the oocyte (Zarani & Margaritis, 1985). Another important feature of border cells is thier secretion of Torso-like protein, which is an essential patterning signal (Savant-Bhonsale & Montell, 1993).

Border cell migration is a good model to study the regulation of cell movement. Several factors have been known to govern the border cells migration (reviewed in Montell, 1994). Among the genes expressed in the border cells, *slow border cell migration* (*slbo*) encoding the *Drosophila* homologue of the CCAAT/enhancer-binding protein (C/EBP), a transcription factor, was reported to be involved in border cell migration (Montell et al., 1992). *breathless* (*btl*) is known to be a target for C/EBP in the regulation of border cell mgration (Murphy et al., 1995). Several cytoskeletal components and proteins stabilizing them are known to be expressed in both the germline and/or somatic cells and supposed to play important roles in various aspects of oogenesis of *Drosophila* (for review Mahajan-Miklos & Cooley, 1994). Some of genes such as *chickadee* encoding profilin (Cooley et al., 1992) are expressed in border cell and somatic follicle cells around the egg chamber. Singed protein which is similar to sea urchin fascin (Bryan et al., 1993) was detected to be colocalized with subcortical actin in the nurse cells and border cells (Cant et al., 1994). Recently Drosophila homologue of catalytic subunit of protein kinase A encoded by *DCO* was localized in the border cells and suspected to function in their migration (Lane & Kalderon, 1995). On the other hand, membrane receptor tyrosine kinase (RTK) signaling has been implicated in various regulated cell migrations. RTK functions as a developmental switch, converting cells from an immotile to a motile state or it acts as a chemotatic receptors, navigating cells toward increasing concentrations of ligand (Kundras et al., 1994). Although various cell surface molecules and protein kinases are supposed to be involved in the border cell migration, detailed feature of border cell migration such as spatiotemporally regulated gene expression in these cells and involvement of the other unidentified factors governing the border cell migration are not well elucidated because the useful marker for border cell is limited.

In an effort to isolate the EDL with *lacZ* expression in the specific set of follicle cells, an enhancer detector line marking the polar follicle cell-originated cells was obtained (Gye et al., 1995). Easy visualization of border cells within the egg chamber after X-Gal staining of this line provides an useful tool to study the border cell differentiation during the oogenesis of *Drosophila* and the mechanisms of developmentally regulated cell migration. Here, we report the detailed morphological feature of border cells during their migration and their fate during the oogenesis in *Drosophila*. Phenotype related to border cell migration in this EDL was also characterized.

MATERIALS AND METHODS

1. Flies

Flies were reared in uncrowded half-pint glass bottles on standard medium (cornmeal, sugar, agar and yeast) containing propionic acid as mold inhibitor and maintained 70-80% humidity at 24±1℃ under a photoperiodic regime (12L:12D). EDL149 carrying the P[lArB] (Bellen et al., 1989; Wilson et al., 1989) on a X chromosome were establisehed previousely (Gye et al., 1995). Establishment of homozygote was done by genetic cross according to Bellen et al (1989).

2. X-Gal staining

Brain, testis, and ovaries were dissected in the Ringer solution. The dissected organs were fixed for 5 min in fixation

solution (1% formamide in Ringer) and washed for 5-10 min with Ringer solution. Embryos and larva at various developmental stages were collected. Embryos were dechorionated with lax solution and rinsed with Ringer. Mixture of haptane and fixative (1:1) was added and vortexed for 10 min. Larva were dissected inside out and fixed for 10 min. After washing, X-Gal staining solution (0.2% X-Gal, 50 mM sodium phosphate, monobasic, 50 mM sodium phosphate, dibasic, 150 mM NaCl, 1 mM $MgCl_2$, 9.3 mM $K_4[Fe(CN)_6]$, 9.3 mM $K_3[Fe(CN)_6]$, 0.3% Triton X-100) was applied to sample on slides and incubated for overnight at 37℃ in the 100% humidity chamber. After staining, samples were rinsed with Ringer and mounted with 50% glycerol in Ringer. Photographs were made under stereo microscope or phasecontrast microscope (Olymphus BX-40).

3. Quantitative analysis of β-galactosidase

β-galactosidase enzyme activity was measured using the chlorophenicolred-β-D-galactopyranoside (CPRG) as substrate (Simon & Lis, 1987). Ovaries were dissected from heterozygotes under ether anesthesia (see above). After homogenization with plastic pestle (50 strokes) fitted to microcentrifuge tube in 100μl of assay buffer (50 mM potassium phosphate, pH 7.5, 1 mM magnesium chloride, 0.

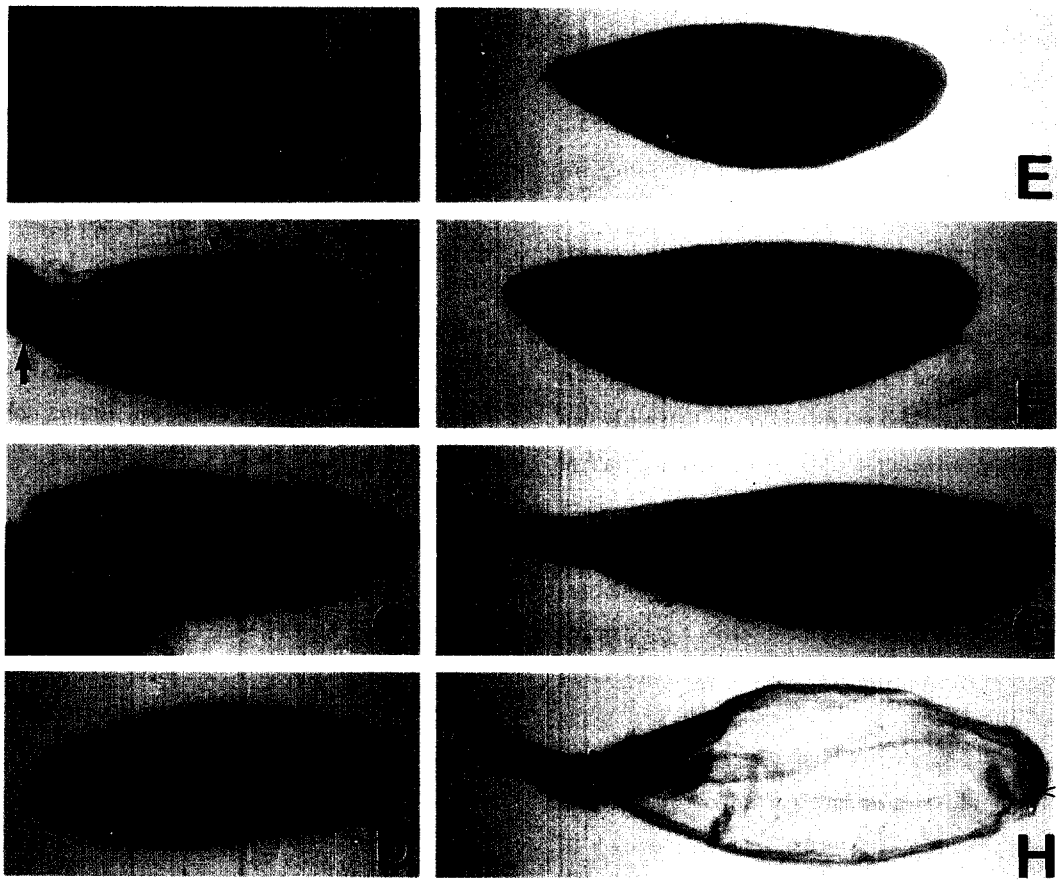

Fig. 1. *lacZ* expression in the egg chambers during oogenesis in the EDL149.
(A) Germarium to stage-6 egg chambers showing *lacZ* expression in the polar follicle cells. Anterior polar cells (filled arrowheads). Posterior polar cells (open arrowhead). Germarium is free from staining (arrow). (B) Border cells (arrow) just before the start of their migration are seen in the anterior pole of stage-9 egg chamber. Follicle cells surrounding the egg chamber are shown with arrowhead. (C) Migrating border cells (arrowhead) between the surounding nurse cells in the stage-9 egg chamber. (D) Stage-10 follicle. Border cells completed thier migration (arrow). (E) Stage-11 follicle. Regression of nurse cells (asterisks) is shown. (F) Stage-12 follicle showing regression of nurse cells and follicle cells. (G) Stage-13 follicle. Most of nurse cells are degenerated and chorionic appendages are developed (arrowhead). Border cells still showing strong expression of *lacZ*. (H) Chorion isolated from stage-13 follicle. β-galactosidase activities are seen both at the anterior (micropyle, filled arrowhead) and posterior ends (aeropyle, open arrowhead) of egg chamber.

1% PMSF) on the ice water, homogenate of 10 ovaries at different stages of oogenesis was sonicated for 30 sec and vortexed for 30 sec. After centrifugation at 12,000g for 5 min at 4℃, 20μl of the supernatant was mixed with 1 mM solution of CPRG in assay buffer. The mixture was incubated at 37℃ and OD_{574} was read at 1 and 2 hr after addition of the enzyme source. OD changes from the EDL 149 were subtracted with that of control (cantonized $ry^{506\text{-}iso}$) and average β-galactosidase activities were calculated from 4 trials.

RESULTS

1. *lacZ* expression in the egg chamber during the oogenesis in EDL149

EDL149 showed staining in the polar follicle cells at both ends of egg chamber throughout up to stage-6 but only the posterior end was stained in germarium (Fig. 1A). In the early stage of egg chamber (stage 2-6), Stain-positive polar follicle cells were observed in the both ends of egg chamber. A group of border cells that emerged from anterior polar follicle cells were observed in the stage-9 follicle (Fig 1B, arrow) which was characterized by migration of columnar follicle cells contacting with nurse cells in the anterior half of egg chamber toward oocyte (Fig. 1B, arrowheads). They left the anterior tip of the egg chamber and migrated the intercellular space between nurse cells and reached to the border between nurse cells and oocyte in stage-10 egg chamber (Figs. 1C and D). Border cells completed their migration and located at the border between the oocyte and nurse cells in stage-10 egg chambers which were characterized by completion of migration of follicle cell toward centripetal region in the oocyte-nurse cell border (Fig. 1D, arrow). In stage-11 and -12 follicles, border cells between degenerating nurse cells and growing oocyte were stained (Fig. 1E and F). At stage-12, X-Gal staining occurred in the border cells around developing micropyle between degenerating nurse cells and growing oocyte (Fig. 1G). X-Gal staining was detected in region corresponding to micropyle of the isolated chorion of stage-14 follicle (Fig. 1H). Posterior polar follicle cells showed consistent expression of *lacZ* throughout the oogenesis (Fig. 1).

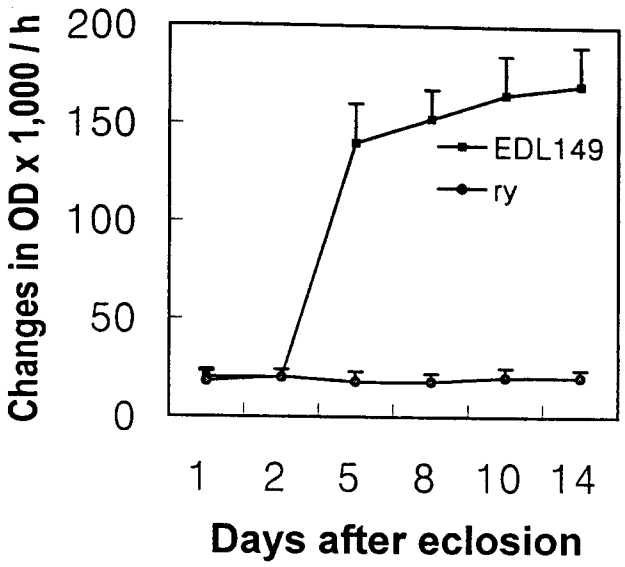

Fig. 2. β-galactosidase activity in the ovary during oogenesis.
β-galactosidase activities were increased exponentially, reached at the peak 8 days after eclosion, and then slightly decreased. Error bar=SD.

β-galactosidase activity in the ovarian homogenate of EDL 149 was rapidly increased during the first 4 days (Fig. 2), and it coincided with the timing of border cell diffrentiation in the ovary during adult life.

2. Migration of border cell was retarded in the homozygote of EDL149

Progression of border cell migration was somewhat different in stage-8 to 10 egg chambers. Therefore the migration of BC was graded into 4 groups according to relative position of border cells in the egg chambers (Figs. 3A-3C). Overall progression of border cell migration in the egg chambers of EDL149 carrying the homozygous insertion of P[lArB] was retarded compared to that of heterozygote. The incidence of delay in the onset of migration was evident in the stage-9 egg chamber of EDL149 with homozygous insertion of P[lArB] (Fig. 3B). Several types of abnormal migration of border cells were also observed in the stage-10 egg chambers such as no sign of migration and delayed or slow migration of border cells (Fig. 3C). In a few cases, border cells migrated over the nurse cell-oocyte border. Other abnormalities of oogenesis were not observed

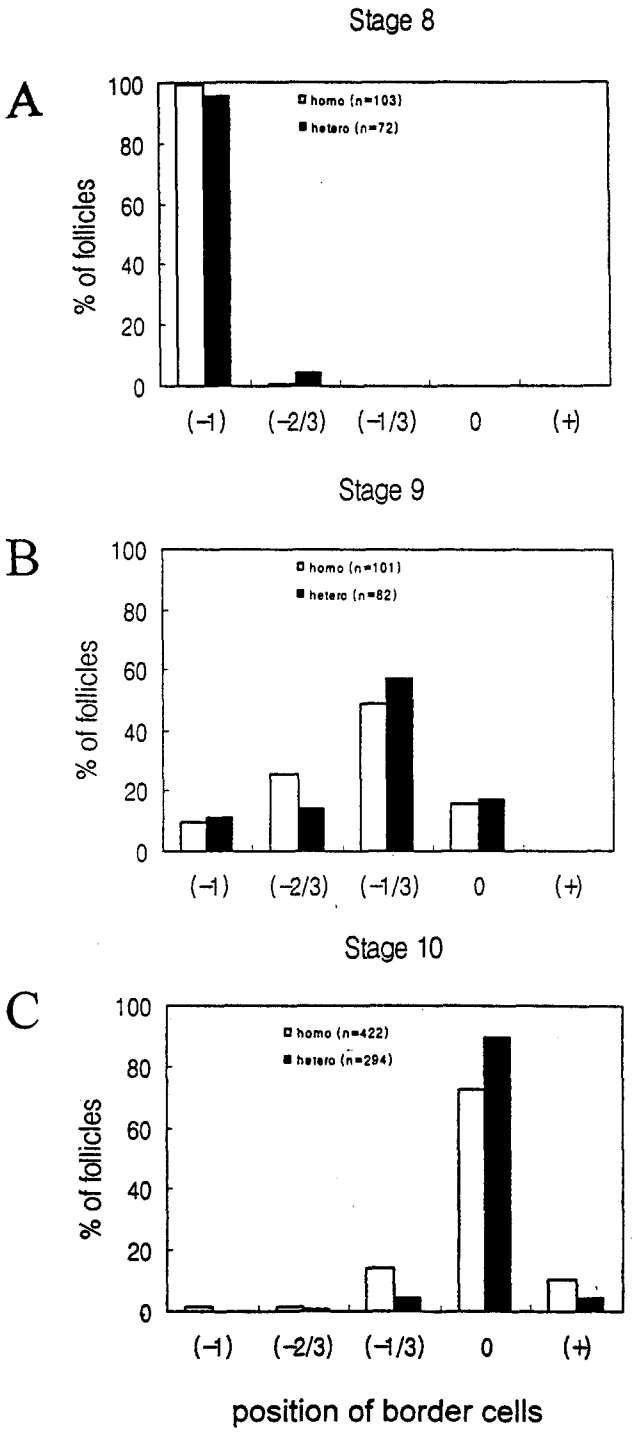

Fig. 3. Migration of border cell in EDL149.
Migration of border cells in the stage-8 (A) -9 (B), and −10 (C) egg chambers of EDL149 was monitored in heterozygote (+/P[lArB]) and homozygote (P[lArB]/P[lArB]) after X-Gal staining. Degree of border cell migration was graded according to their relative position of border cells in the anterior half of egg chamber; −1, no migration; −2/3, appearence of border cells and one third of migration; −1/3, two third of migration; 0, completion of migration; +, migration over the oocyte-nurse cell border.

in the EDL149 homozygote (Fig. 3C).

3. *lacZ* expression in the other organs of EDL149

lacZ expression in the reproductive organs was examined in both sexes. Interestingly, strong signal of *lacZ* expression was found in testis except the proximal part which was occupied by spermatogonia (Figs. 4A and 4B). Ejaculatory bulb also showed positive X-Gal staining, but it was also in the cantonized $ry^{506\text{-}iso}$ male, indicative of non specific staining. *lacZ* expression in the female reproductive organs was found in ovary (Figs. 4C and 4D). All of the genital tracts except oviducts also showed postive staining but also in the cantonized $ry^{506\text{-}iso}$ female. *lacZ* expression was also found in antenna (Fig. 4E).

DISCUSSION

Border cell differentiaion and migration during the all stages of oogenesis was able to be monitored by X-Gal staining of the EDL149 heterozygote. The shape of border cells just leaving the anterior pole of the stage-8 to stage-9 egg chambers was round. However the proximal end of border cells migrating between the surrounding nurse cells were elongated toward the oocyte in stage-9 egg chamber. It suggests that certain signal(s) which direct movement of border cells toward oocyte possibly exist in nurse cell surface, and the putative interaction(s) between molecules present in the surface or extracellular matrix of these two different cells might regulate precise movement of border cells.

Migration of border cell was directed to navigate intercellular space between the nurse cells along the main axis of egg chamber during the stage-9 to stage-10. Why the border cells navigate this way? It is the shortest way between anterior pole to oocyte-nurse cell border. The period of transition from stage-9 to stage-10 is relatively short and border cell migration completes within 3 to 5 hours from initiation (Montell et al., 1994). So border cells are needed to run the shortest way to accomplish migration within limited time. Cell to cell contact between the migrating border cell and nurse cells is possibly important for correct migration and it probably directs border cell migration along this axis. The nurse cell junctions may simply provide an unobstructed

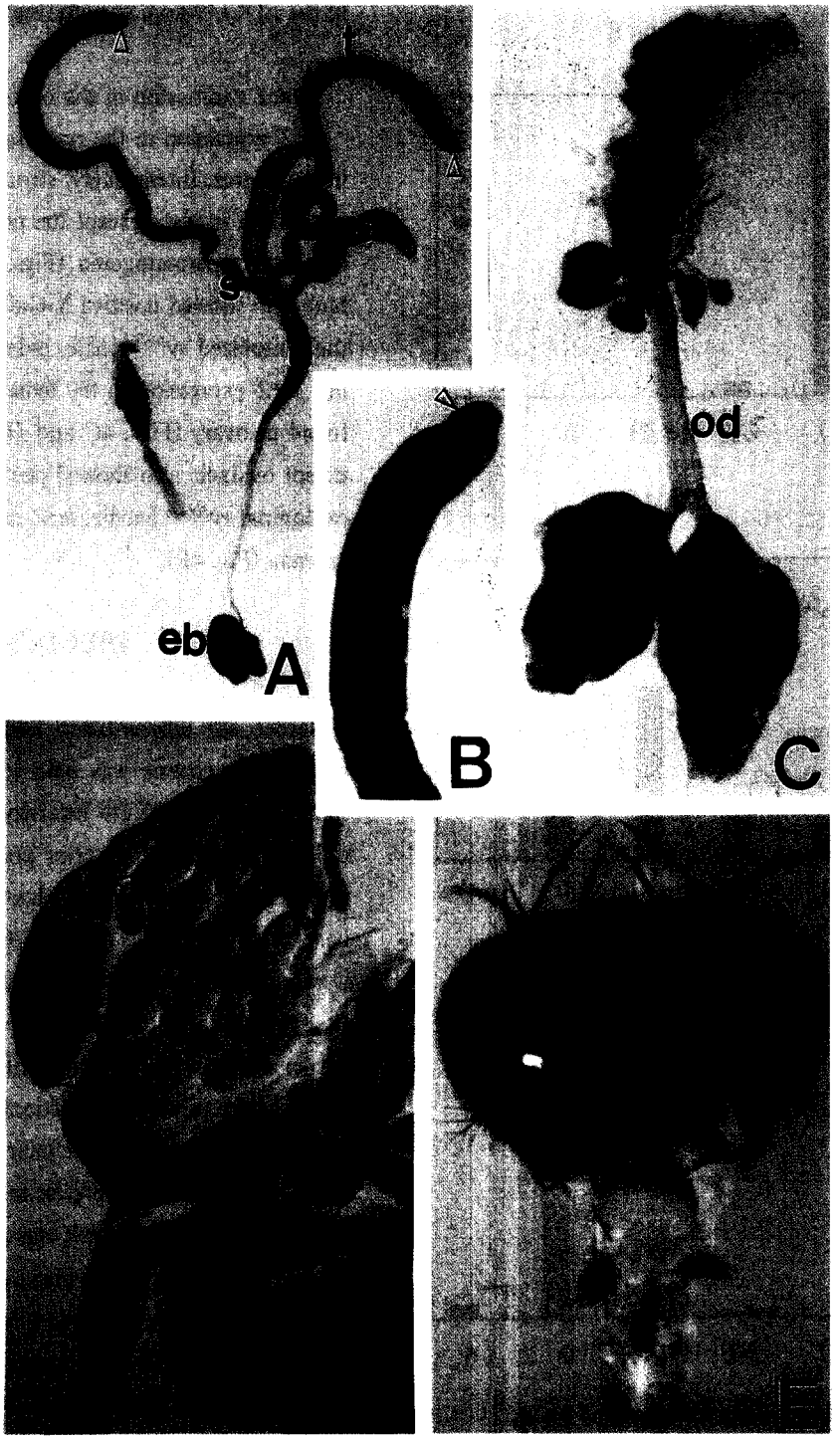

Fig. 4. *lacZ* expression in the adult organs of EDL149.

(A) *lacZ* expression in the male reproductive organs. testis (t) and ejaculatory bulb (eb) are stained but accessory glands (a), seminal vesicle (s), and ejaculatory duct (e) are not. (B) Testicular expression of *lacZ* in EDL149. Proximal part of testis is free from staining (open arrowhead). (C) *lacZ* expression in the female reproductive organs. vagina (v) and uterus (u) are stained but oviduct (od) was not. Spot of positive stain is seen in the ovary (o). (D) Ovarian expression of *lacZ*. Egg chambers except germarium (arrow) are positively stained. *lacZ* expression in the anterior polar follicle cells and border cell (filled arrowheads) are positively stained. Posterior polar follicle cells throughout oogenesis (open arrowheads). (E) *lacZ* expression in the head of EDL149. Antenna is stained (arrowhead).

path for migration but may also be a source of specific molecular cues for the initiation or continuation of migration (Lane & Kalderon, 1995). In this regard, RTK which functions as a chemotatic receptors, may attract navigating cells toward increasing concentrations of ligand. And what is in the case of border cell migration remains to be elucidated.

In addition, intercellular space of nurse cells of stage-8 to -10 egg chamber was filled with eosinophilic material which was also detected in earlier stage of egg chamber (data not presented). Intercellular bridge which acts as a gate of cytoplasm transport from the nurse cell to oocyte is characterized by intensive phalloidin staining and it was sparcely distributed in the nurse cell contact along the migration path (Robinson et al., 1994). Therefore, it can be speculated that migration will be retarded when the migrating border cell is misled to migrates the intercellular space of nurse cells apendicular to anterior-posterior axis of egg chamber. Occasionally, migration of border cells over the oocyte was observed and lateral movement of border cells between the nurse cells and surrounding follicle cells was observed in stage-10 egg chamber. It might be resulted from change of guidence of border cell migration. Although molecular interaction between the migrating border cells and nurse cells are not still understood, spatiotemporal regulation of expression of molecules interacting on the surface of both border cell and nurse cell would influence border cell migration. Taken together, it might be speculated that change of guidence of border cell migration along the main axis of anterior half of stage-9 and -10 egg chamber is important for border cell migration. In the EDL149, *lacZ* expression also occurred in the posterior polar cells throughout the oogenesis. But they did not migrate. Posterior polar follicle cells are connected to anterior polar follicle cells of next more grown egg chamber by interfollicular stalk. Interfollicular stalk is well developed in early stage of follicle but degenerates in the mature egg chambers. Therefore the possibility can not be excluded that contact with interfollicular stalk attatched to both pole of egg chamber might have suppressed the migration of posterior polar follicle cells. But it is still unclear that what kinds of contact exist between the polar follicle cells and interfollicular stalk.

The incidence of retardation of border cell migration was higher in the egg chambers of homozygotic insertion of P [lArB] (P[lArB]/P[lArB]) than in the heterozygotic one (+/P[lArB]) (Fig. 3). Whether the insertion merely occurred near the enhancer element that controls the cell type specific gene expression among the different set of follicle cell during the oogenesis in *Drosophila* (Jin & Petri, 1993) or occurred within the ORF of the gene responsible for border cell migration is still unclear. However, it is evident that the insertion made change of genomic structure responsible for border cell migration. *lacZ* expression in the meiotic germ cells in testis as well as antenna indicates that this enhancer trapped gene is not ovary-specific (Fig. 3A and B).

β-galactosidase activities increased exponentially during 24 to 96 h after eclosion (Fig. 2). During this period, ovary contained nearly all stages of egg chambers and the number of stage-9 to stage-10 egg chamber was higher than that of the younger. Six to ten border cells differentiate from follicle cells in the anterior pole of egg chamber and persist onwards. Therefore the increase in β-galactosidase activities during the early stage of adult life reflects increased number of border cells accompanying the follicle development. On the other hand, sustained activity of β-galactosidase in ovary of virgin female after 8 days from eclosion suggests inhibition of both ongoing of oogenesis and emergence of new egg chambers expressing the *lacZ* in the border cells without oviposition of mature egg.

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