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Screening and Characterization of *Drosophila* Development Mutants Using Single P[en-lacZ] Element Mutagenesis

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Abstract

Single P[en-lacZ] element including 5.7 Kb of *engrailed* upstream sequences and the *E. coli* lacZ fusion gene, localized on 48A in *ryxho25* strain was transposed to different sites in the *Drosophila* genome by the jumpstart technique. From 3315 individual genetic crosses, 113 new insertion lines carrying P[en-lacZ] inserted at different sites were obtained. β-Galactosidase expression in larval tissues of 113 insertion lines were detected by X-gal staining. 7 among 113 lines have been identified to be for recessive lethal mutations. Among 7 lines, the #1119 line being lethal during embryogenesis was examined about the β-galactosidase expression, nuclear behavior and cellularization pattern during embryogenesis. The P[en-lacZ] insertion lines obtained in this study could be utilized for studying structure and function of the *Drosophila* development-related genes.

Key words : *Drosophila development related gene, P element mutagenesis*

Introduction

One approach to studying development and its related genes is to obtain genetic variants that are defective in critical step. For making a mutant, there are some methods. In *Drosophila* system, P element has been used for induction of new mutation to study structure and function of genes and to characterize gene product[1]. A scheme wherein single P element are mobilized to new chromosomal locations has been carried out successfully [2]. The use of P element-mediated enhancer detection system is able to approach identifying and isolating genes involved in *Drosophila* development[3,4,5,6].

The *engrailed* gene plays a unique and critical role in organizing the segmented body plan of *Drosophila*[7,8,9]. Genetic evidences have suggested that the *engrailed* function itself selects a posterior compartment developmental pathway by its presence, or an anterior compartmental pathway by its absence[10,11]. The *engrailed* mutation increases the size, changes the shape of the posterior compartment and causes local wing enlargement only if they are dorsal and include the posterior margin of the wing[10]. The engrailed protein is phosphorylated by a serine specific protein kinase[12].

The *engrailed* gene is expressed early in *Drosophila* development in a series of stripes that transect the anterior-posterior axis of the ectoderm[9,13,14]. Genetic experiments have indicated that different genes are required for *en* stripes at different times in development[15,16]. Different regulatory sequences are required for *en* stripes

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at different times in development[17].

To investigate how the *engrailed* gene is regulated, Hama *et al.*[18] incorporated chimeric gene consisting of fragments of the *engrailed* promoter and *E. coli* lacZ into the *Drosophila* germ line by P element-mediated recombination. In the study, 7 of 20 independent transformants with constructs containing more than 1 Kb of 5'-flanking DNA, integrated in or around the *engrailed* locus. Among these transformants, *ryXho25* strain carrying P[en-lacZ] vector constructed with 5.7 Kb of the *engrailed* sequences immediately upstream of the translation start and the *lacZ* gene on 48A shows expression of β-galactosidase similar to *engrailed* expression patterns in normal animals, while other strains carrying the P[en-lacZ] at other sites did not[18].

In this study, a P[en-lacZ] element integrated at 48A have been mobilized by the jumpstart technique[2]. 113 new insertion lines were obtained from 3315 individual crosses and expression patterns of β-galactosidase in larval tissues of these lines were analyzed. The 7 lines for recessive lethal mutations were screened and the embryonic phenotypes of the #1119 line being lethal during embryogenesis were examined.

Materials and Methods

P[en-lacZ] element-mediated mutagenesis

ryXho25 strain[17] was used for the jumpstart experiment. In *ryXho25* strain, a P[en-lacZ] element containing 5.7 Kb *engrailed* promoter fused to *E. coli* lacZ gene and ry^+ gene as a marker was located on the second chromosome(48A), and balanced with *CyO*. Females of this strain were crossed to males carrying a P[$ry^+\Delta2-3$] element which is inserted at 99B and immobilized[19]. The P[$ry^+\Delta2-3$] transposase-producing chromosome was marked with dominant *Sb*. From this cross, males carrying a P[en-lacZ] on the second chromosome (maternally driven) and a P[$ry^+\Delta2-3$] on the third chromosome were recovered. These males are called jumpstartmale. Single jumpstart male was mated to three females of ry^{506} strain.

Mobilization of the P[en-lacZ] element takes place in the germ-line cell of the jumpstart males, and some progeny having a P[en-lacZ] insertion at new sites will be produced. The new transposants were selected by screening individuals carrying neither the P[en-lacZ] transposants 48A chromosome nor the *Sb*-marked chromosome containing the transposase gene, but a new P[en-lacZ] insertion, which are ry^+ marker (Fig. 1).

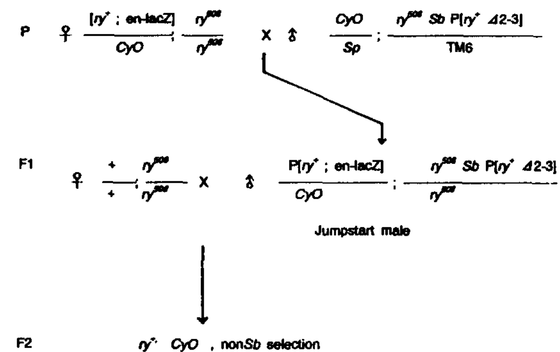

Fig. 1. Mating scheme of P[en-lacZ] mediated mutagenesis.

Viability of insertion line

Ten pairs of flies from each line were mated in individual vial containing standard corn-meal media. They were transferred, and fed fresh media at 12 hr intervals. The number of embryos were counted at 12 hrs after egg laying. Embryos were allowed to develop for 24 hrs and the hatch rate were checked. The viability were examined by counting the adults until 17 days after egg laying.

X-gal and DAPI staining

Embryos were dechorionated with diluted sodium hyperchlorite, washed in water, and fixed in 3.7% paraformaldehyde in PBS (130 mM NaCl, 7 mM $Na_2HPO_4 \cdot H_2O$, and 3 mM $NaH_2PO_4 \cdot 2H_2O$, pH7.0) and equal volume of n-heptan, vigorously shaking for 15 min. Then embryos were recovered and washed in PBS con-

taining 0.2% Triton-X-100 (PBST). Embryos were incubated for 1 hr to overnight at 37℃ in staining solution [100mM NaCl, 50 mM $MgCl_2$, 100mM Tris-HCl (pH9.0)] containing 0.2% X-gal (5-bromo-4-chloro-3-indolyl-β-D-galatopyranoside).

Third instar larvae were dissected with forceps in the *Drosophila* Ringer solution [182 mM KCl, 46 mM NaCl, 3 mM $CaCl_2$, 10 mM Tris-HCl (pH7.2)]. The tissues were fixed in 0.2% glutaraldehyde in PBS for 2 min. The sample was washed in PBS and incubated for 1 hr to overnight at 37℃ in staining solution containing 0.2 %X-gal.

In case of DAPI (4',6-diamidine-2-phenylindole) staining, embryos were dechorionated, fixed and devitellinated described above. The embryos were rinsed by PBST, and stained in 1 μg/ml DAPI for 5 min. Embryos rinsed with PBS were mounted under coverslips in 60% glycerol and examined with fluorescence microscopy.

Cuticle preparation

Cuticle preparation was modified from Wieschaus and Nüsslein-Volhard[20]. Embryos were collected and allowed to develop at 25℃ for 24 hrs. The unhatched embryos were collected and dechorionated. They were incubated in a volume of glycerol and 4 volumes of acetic acid at 60℃ for 1 hr. Embryos were mounted in Hoyer's mountant, and incubated for overnight. Embryos were examined by light microscopy for dark-field or phase contrast optics.

Results

P[en-lacZ] element mediated mutagenesis

ryXho25 strain carries P[en-lacZ] element containing 5.7 Kb of *engrailed* upstream sequence and *E. coli* lacZ fusion gene on the second chromosome (48A)[17]. For transposing the P[en-lacZ] element to a new genomic site, females of *ryXho25* strain were crossed to males of the helper strain containing P[$ry^+\Delta2-3$] element which provides the strong transposase, but can not mobilize by itself (Fig. 1). Male progeny among F1 generation having both P[en-lacZ] and P[$ry^+\Delta2-3$] element, which is called jumpstart male, were individually mated to females of ry^{506} strain. In F2 generation, individuals having P[en-lacZ] element inserted to new sites but no P[$ry^+\Delta2-3$] element were selected by using appropriate genetic marker such as ry^+ eye color, the cury wing shape and normal bristles (Fig. 1). The individuals were mated with strains having balancer chromosome. From 3315 matings, 113 new insertion lines were obtained. The rate of mobilization of P[en-lacZ] elements to new genomic site were calculated by 3.4% (Table 1). We obtained 7 insertions on the X chromosome, 40 on the second chromosome, and 66 on the third chromosome, respectively. The second and third chromosome integration rate was higher than X chromosome.

Table 1. Jumping frequency of P[en-lacZ] element

Chromosome	Number of novel insertion lines	Integration frequency (%)
X	7	0.2
II	40	1.2
III	66	2.0
Total	113	3.4

Table 2. Lethal stages of recessive lethal lines

Recessive lethal lines	Insertion chromosome	Lethal stage
#151	X	larva to pupa
#370	III	pupa to adult
#535-1	III	embryogenesis
#603	III	embryogenesis
#1023	III	embryogenesis
#1085	X	embryogenesis
#1119	II	embryogenesis

The viability from embryo to adults of 113 independent lines were examined. 7 of 113 new insertion lines were identified to be for recessive lethal mutations and

lethal stages of these lines were characterized (Table 2).

β-Galactosidase expression pattern of P[en-lacZ] transposants

Expression of β-galactosidase in larval tissues of the P[en-lacZ] insertion lines were examined (Table 3). β-Galactosidase expression of *ryXho25* strain was similar to posterior compartment specific *engrailed* expression in normal development of *Drosophila*. β-Galactosidase expression of 113 transposant strains exhibit a great variety. Among 113 strains, the expression patterns of some lethal strains are shown in Fig. 2. The #535-1 line showed a reduced β-galactosidase activity and the #603 line lost specificity of restriction in posterior compartment, but the #1119 line had similar pattern to *ryXho25* throughout embryonic and larval development. It is assumed that the P[en-lacZ] element is also influenced by transcriptional regulatory elements of nearby genomic genes of insertion site.

Characterization of the #1119 line

Of 7 lethal lines, the #1119 line have been characterized since heterozygous #1119 larvae showed the pattern indistinguishable from the β-galactosidase expression of the *ryXho25*.

Embryos from heterozygous #1119 parents were collected, dechorionated, and stained with X-gal. From stage 9 to 13 of embryogenesis (AEL 3-9 hr) about 23% of embryos showed disruption of the striped pattern (Fig. 3).

To investigate the #1119 strain phenotype on precellular embryos, the DAPI staining developed to examine nuclei was used. Embryos were dechorionated, fixed, and stained with a DNA specific dye DAPI. In *ryXho25* strain, the relative spacing of blastoderm nuclei over the surface is remarkably even and uniform and the variation in the stage of mitosis between adjacent nuclei is small, i. e., abrupt regional changes in mitotic state were not observed. In homozygous #1119 embryos, both the nuclear spacing and mitotic patterns were consistently altered (Fig. 4). Fewer nuclei have been seen in the middle region and posterior of mutant embryos (Fig. 4). Abnormal expression pattern of #1119 mutant embryos is consistent with the nuclear spacing and mitotic patterns.

Cuticles of embryos from heterozygous #1119 parents were examined (Fig. 5), only 25% of the #1119 embryos were able to produce the cuticle of the first instar larvae. Some of them had disturbed their head skeletons and missed dentical bands (Fig. 5). The large proportion of unhatched embryos of #1119 line were detected as shrunk form. At the same condition, 70% of the *ryXho25* embryos were able to develop the cuticle.

Discussion

In this study, single P[en-lacZ] element containing a 5.7 Kb of *engrailed* upstream sequences and *E. coli* lacZ fusion gene was mobilized by the jumpstart technique[2].

From 3315 crosses for transposition of the P[en-lacZ] element, 113 individual insertion lines were obtained. The jumping rate of P[en-lacZ] element to new genomic sites was 3.4%. In case of the other groups, the jumping

Table 3. Summary of the staining pattern of β-galactosidase in larval tissues of the insertion lines

Chromosome	Total number of lines	β-Galactosidase expression in larval tissues		
		Brain & Ganglion	Imaginal discs	Salivary gland
I	7	5	4	2
II	40	34	28	3
III	66	19	11	1
Total	113	58	43	6

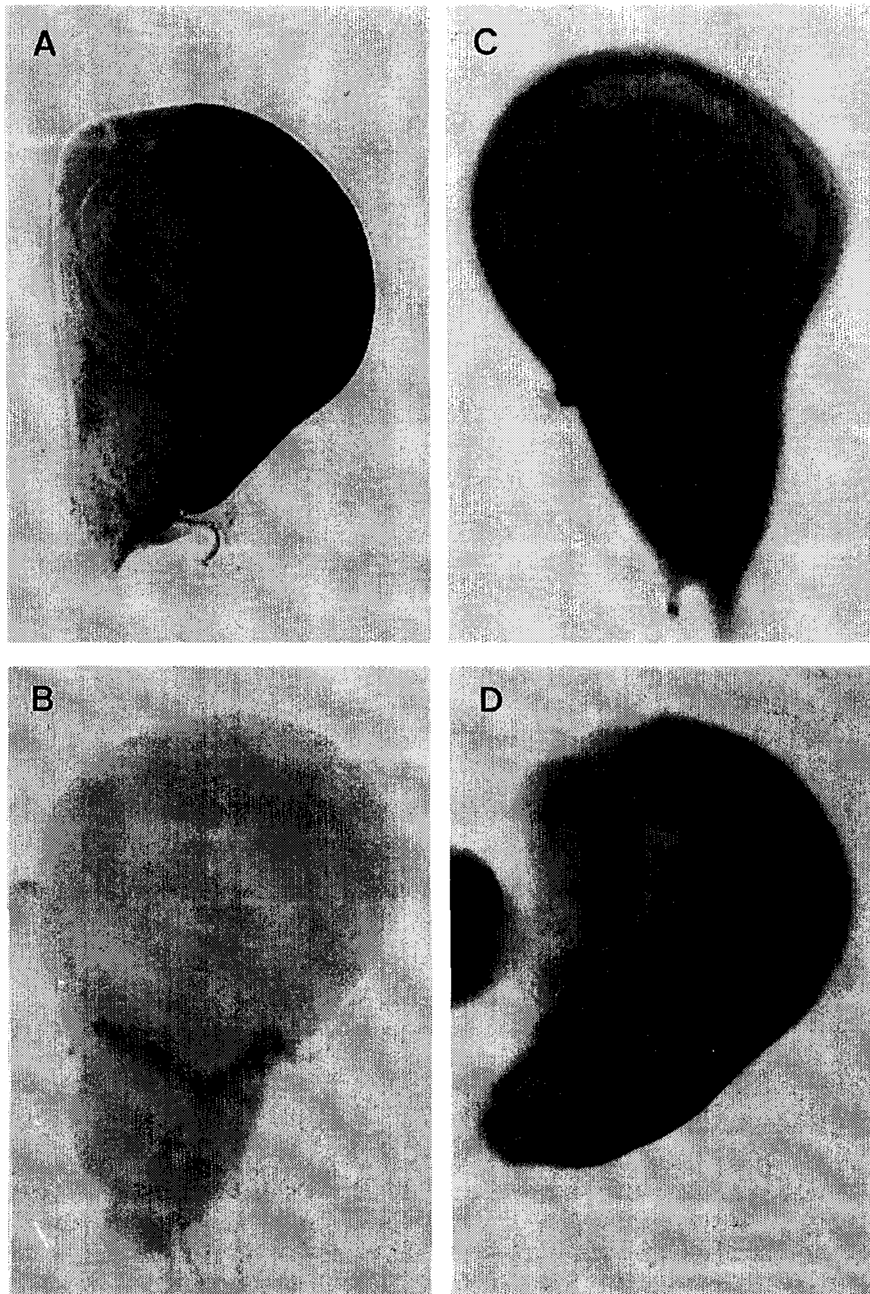

Fig. 2. Expression pattern of β-galactosidase on the third instar larval wing imaginal disc of some obtained P[en-lacZ] insertion lines. The third insta larvae were dissected, fixed, and stained for β-galactosidase activity using X-gal. *ryXho25* strain of which β-galactosidase expression was restricted in posterior compartment (A). #535-1 was shown a reduced β-galactosidase activity (B). #603 was lost specificity of restriction in posterior compartment (C). #1119 recessive lethal line had similar pattern to *ryXho25* strain (D).

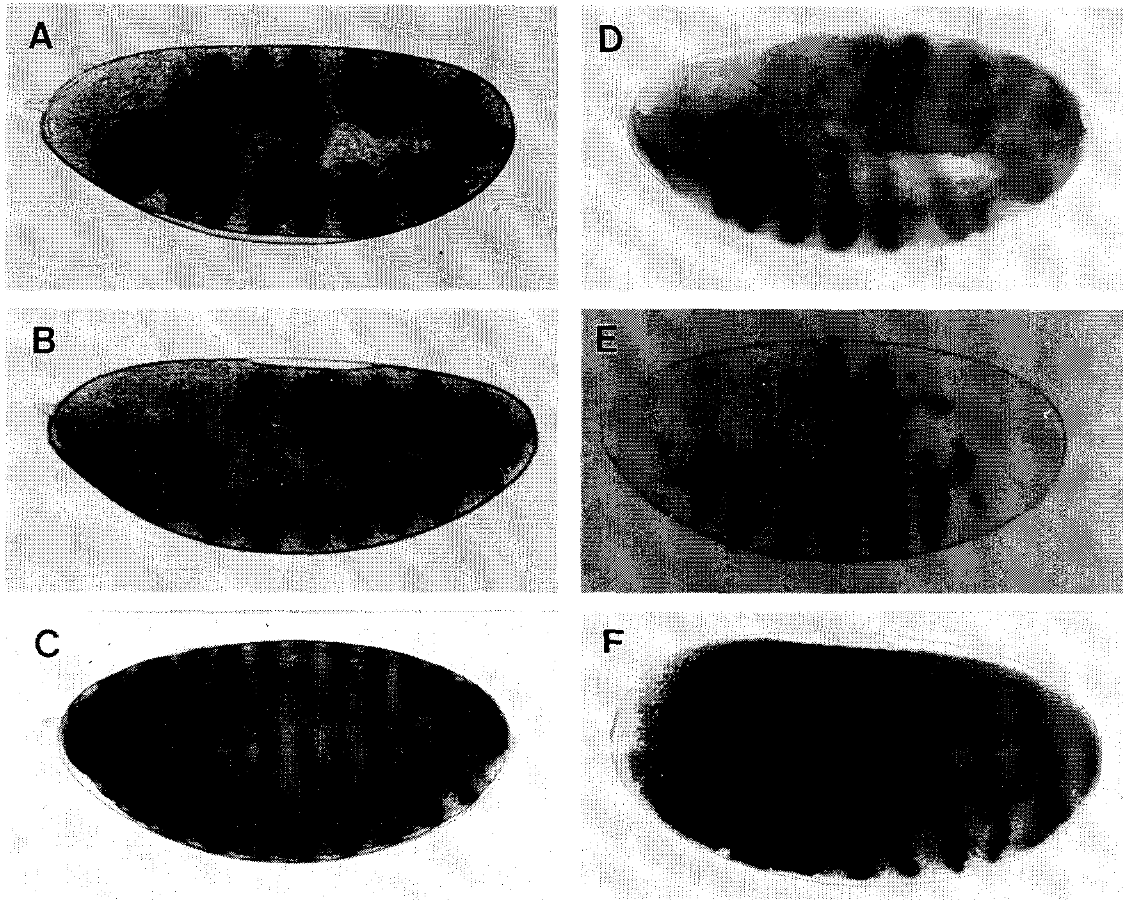

Fig. 3. Expression pattern of β-galactosidase of embryos of the #1119 line.
Embryos of the *ryXho25* stain (A, B, C) or the #1119 line (D, E, F) were fixed and stained for β-galactosidase activity using X-gal. At the gastrulation, the striped pattern was indicated in *ryXho25* strain, but disturbed in #1119 line. The embryos is at stage 7 (A, D), stage 10 (B, E), and stage 13 (C, F).

rate of pUC *hsneo* of Cooly *et al.*[2] was 7.5% and P[lArB] of Bellen *et al.*[5] was 1%.

The β-galactosidase expression in the third instar larvae of 113 independent P[en-lacZ] insertion lines were examined. P[en-lacZ] transposants exhibited a great variety of staining patterns. It must be due to response of the β-galactosidase report gene in the insertion of P[en-lacZ] to nearby transcriptional regulating sequence in the *Drosophila* genome. 7 of 113 independent line (6.2%) were for recessive lethal mutations.

Among 7 recessive lethal lines, the phenotype of #1119 line were characterized. The β-galactosidase expression pattern of heterozygous embryos was similar to that of *ryXho25* embryos showing stripes pattern. However, homozygous embryos at the stage 7−13 showed the fused and disturbed stripes pattern. The phenotype

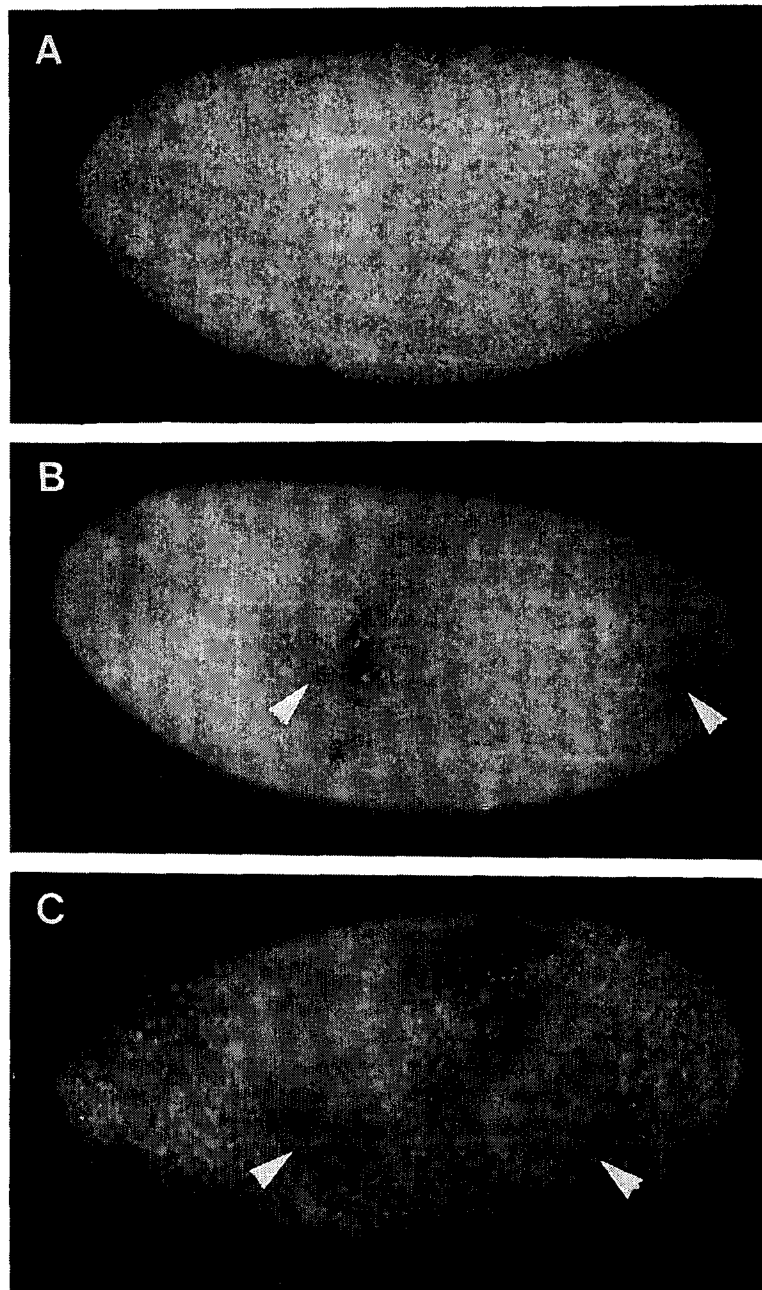

Fig. 4. Fluorescence micrographs of DAPI stained embryos of *ryXho25* strain or #1119 line at embryogenesis stage 8. Embryos were fixed, stained with DAPI, and photographed under fluorescence optics to visualized peripheral surface nuclei. Uniform arrangement of nuclei in *ryXho25* strain (A). In #1119 embryos, fewer nuclei have seen in the middle and posterior region of embryos, indicated with arrowheads (B, C).

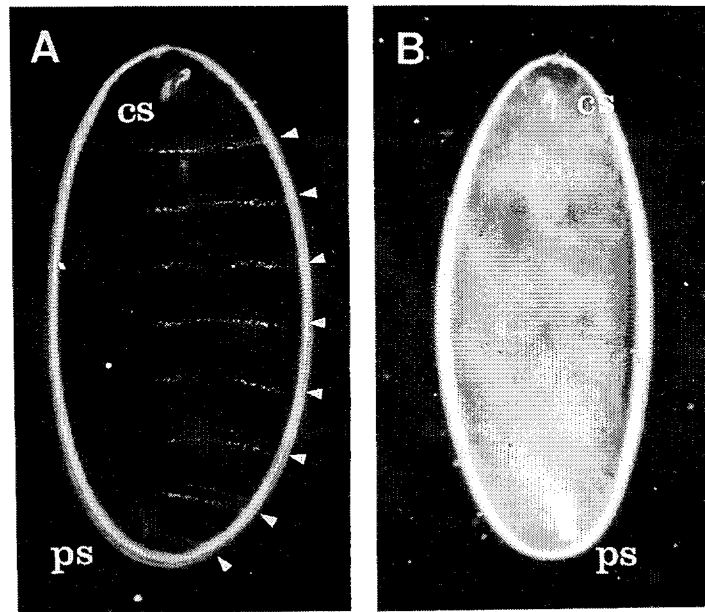

Fig. 5. Phenotypes of cuticle of *ryXho25* strain and of homozygous #1119 embryos. *ryXho25* (A) and #1119 (B) embryos cuticle were prepared as described in Materials and Methods, and photographed under dark-field illumination. The arrowhead indicated normally developed dentical bands of *ryXho25* strain (A). The dentical bands were missed in the homozygous embryo of #1119 line (B). Abbreviations ; cephalopharyngeal skeleton (cs), posterior spiracles (ps).

of homozygous embryos for #1119 line stained by DAPI showed fewer nuclei in the middle region and posterior of mutant embryos than that of wild type embryos (Fig. 4). The nuclear spacing and mitotic patterns observed with DAPI staining of #1119 mutant embryos was consistent with abnormal β-galactosidase expression pattern. Moreover, the cuticle pattern of homozygous #1119 embryos were similar to those known for *engrailed* mutant, which fused and abnormal head morphology[21]. These results suggest that function of P[en-lacZ] insertion locus of #1119 line might be related to that of the *engrailed* gene.

The P[en-lacZ] insertion lines obtained in this study could be utilized for studying structure and function of the *Drosophila* development-related genes.

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초록 : *Drosophila* single P[en-lacZ] element mutagenesis를 이용한 발생 관련 돌연변이체 작성

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engrailed 5.7Kb upstream sequence와 *E. coli* lacZ의 융합 유전자를 가진 P[en-lacZ] 인자를 jumpstart 기법을 이용하여, *ryXho25* strain의 초파리 48A 염색체 위치로부터 새로운 위치로 삽입시켰다. 총 3315의 유전적 교배를 통해서, P[en-lacZ]가 다른 염색체 상으로 삽입된 113 계통을 얻었다. X-gal 염색으로 이들 113 계통의 3령기 유충 조직에서의 β-galactosidase 발현을 조사하였다. 또한 113 계통 중 7계통이 열성치사돌연변이인 것으로 동정되었다. 이들 7 계통 중 초기 배발생 과정에서 치사하는 것으로 조사된 #1119의 초기 배발생 과정에서의 β-galactosidase 발현과 핵의 이동 및 세포화 양상을 조사하였다. 본 연구에서 얻어진 P[en-lacZ] 삽입 돌연변이체들은 앞으로 *Drosophila* 발생에 관련된 유전자들의 구조와 기능을 연구하는데 활용될 수 있을 것이다.