교육학석사 학위논문

Regulation of Thoracic Segment-Specific *zfh1* Expression by *Ultrabithorax* and *Abdominal A* in *Drosophila melanogaster*

초파리의 *Ultrabithorax*와 *Abdominal A*에 의한 *zfh1*의 가슴 체절 특이적 발현 조절

2017년 8월

서울대학교 대학원

과학교육과 생물전공

송 세 이

ABSTRACT

Regulation of Thoracic Segment-Specific *zfh1* Expression by *Ultrabithorax* and *Abdominal A* in *Drosophila melanogaster*

Song, Sae-e

Major in Biology Education

Dept. of Science Education

The Graduate School

Seoul National University

Zinc finger homeodomain1 (zfh1) encodes a transcription factor containing zinc fingers and is expressed in the central nervous system (CNS) and in various mesodermal lineages, suggesting its pleiotropic functions. However, regulation of *zfh1* expression specifically in the thorax remains unknown. In this study, we examined how the embryonic spatial expression pattern of *zfh1* is established and regulated. Although *zfh1* was co-expressed in many neurons and glia, *zfh1* that was specifically expressed in the thorax was not overlapped with the marker proteins that label neurons or glia, suggesting that the thorax-specific cells showing *zfh1* expression are not neurons or glia. When *twist* was knocked down, thorax-specific *zfh1* expression was eliminated, suggesting that thorax-specific cells are mesodermally derived. *zfh1* was ectopically expressed in *Ultrabithorax* (*Ubx*) or

Ubx and *abdominal-A* (*abd-A*) double mutant embryos, suggesting that the normal UBX and ABD-A repress *zfh1* in the abdominal segments. These results indicate that *Twist* activates thorax-specific expression of *zfh1*, and that UBX and ABD-A repress *zfh1* expression in the abdomen, allowing the thorax-specific expression of *zfh1*.

Keywords: *Ubx, abdA, zfh1*, DNA-binding motif

Student number. 2015-21631

CONTENTS

CONTENTS OF FIGURES

I. Introduction

Genetic screens have led to the isolation of many genes required for the proper establishment of pattern formation in *Drosophila melanogaster* (Nusslein-Volhard et al., 1984; Jurgens et al., 1984). Many of these genes are transcriptional factors containing homeodomain or zinc-finger DNA-binding motifs. One of these genes is *Zinc finger homeodomain1* (*zfh1*). *zfh1* encodes a transcription factor that contains nine Cys_2His_2 type zinc fingers and one homeodomain, which was the first case with both zinc finger motifs and a homeodomain (Fortini et al., 1991; Lai et al., 1991).

zfh1 is expressed in most embryonic motor neurons of the developing CNS (Fortini et al., 1991). Ubiquitous expression of *zfh1* caused CNS defects as well as adult eye and bristle abnormalities (Lai et al., 1991). *zfh1* promotes ventral motor axon exit from the CNS to innervate somatic or visceral mesodermal structures (Layden et al., 2006). *zfh1* was also detected in glia associated with the CNS surface, in motor axons, and in some interneurons (Layden et al., 2006). *zfh1* is also expressed in the mesoderm of early embryos and in a number of mesodermal structures in late embryos, including the dorsal vessel, support cells of the gonads, and segment-specific arrays of adult muscle precursors (Lai et al., 1991; Lai et al., 1993). In *twist* and *snail* mutant embryos, *zfh1* expression in mesoderm-derived structures was absent, suggesting that these cells are mesodermal in origin and that *zfh1* is downstream of *twist* and *snail*. *zfh1* is required for germ cell migration and gonadal mesoderm development (Broihier et al., 1998). Loss of *zfh1* activity disrupts development of the caudal visceral mesoderm and the gonadal mesoderm (Broihier et al., 1998).

One of the interesting aspects of *zfh1* expression is its thorax-specific expression at one spot in the left and right sides of each thoracic segment. During

embryogenesis, segment-specific expression of many genes arises and gradually becomes diverse along the anterior/posterior (A/P) and dorsal/ventral (D/V) axes (Truman and Bate, 1988; Gummalla, 2014). For example, neuroblast 1-1 (NB1-1) shows specific differences between the thoracic and abdominal segments (Udolph et al., 1993). This segment specificity is determined by homeotic genes. The activity of homeotic genes *Ubx* or *abd-A* is autonomously required for the abdominal pathway of the NB1-1 lineage (Prokop and Technau, 1994).

The positional information for segment-specific gene expression is determined by homeotic genes. In *Drosophila*, segment identity along A/P axis is defined by the spatial expression patterns of homeotic genes in the Antennapedia complex (ANT-C) and Bithorax complex (BX-C) (Kaufman et al., 1990; Duncan, 1987). The ANT-C genes specify fates within the head and anterior thoracic segments, whereas the BX-C genes determine the identity of the posterior thoracic and abdominal segments. The ANT-C genes include *labial* (*lab*), *proboscipedia* (*pb*), *Deformed* (*Dfd*), *Sex combs reduced* (Scr), and *Antennapedia* (*Antp*). The BX-C genes include *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*). *Ubx* is expressed in the parasegment (PS) 5-13 with a peak in PS6 (T3p/A1a) (Beachy et al., 1985), *abd-A* in PS7-13 (Celinker et al., 1989), and *Abd-B* in PS10-14 (Karch et al., 1990).

In this study, we examined the detailed expression pattern of *zfh1* during embryogenesis and the regulation of *zfh1* expression in thoracic segments, and the fate of these cell groups.

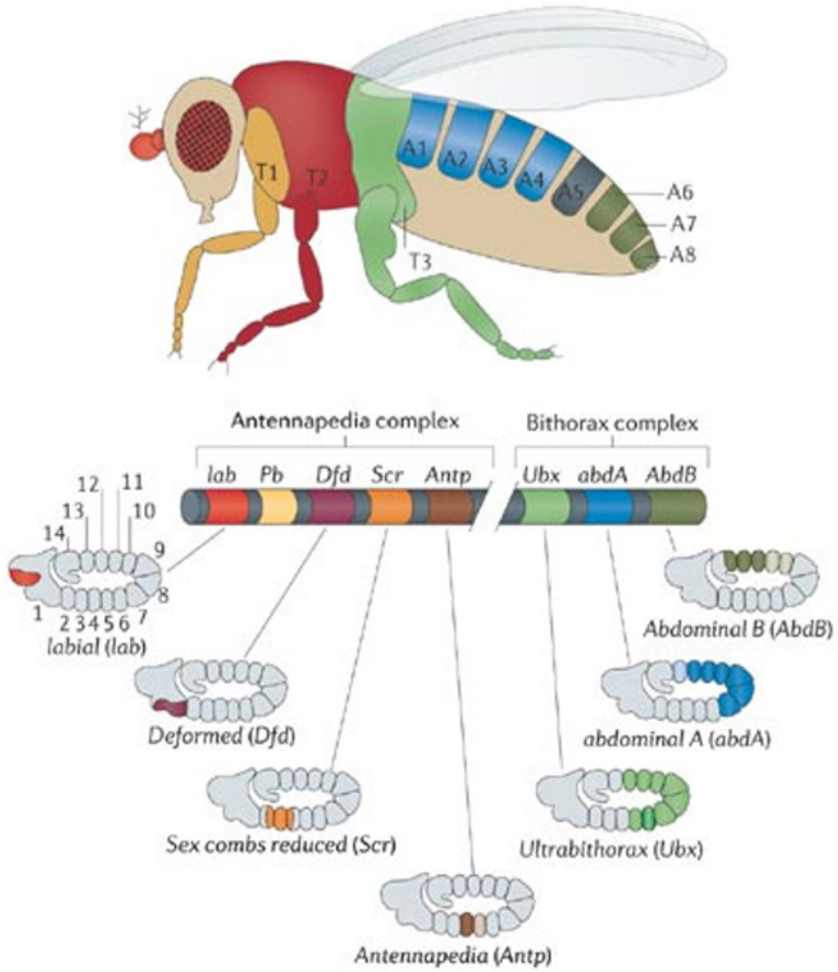

Figure 1. Hox gene expression in *Drosophila* (Kaufman et al., 1990; Dessain et al., 1992; Sparmann et al., 2006).

There are two homeotic gene complexes, ANT-C and BX-C. The ANT-C specifies structures from head to part of the third thoracic segments, while the BX-C determines structures from part of the third thoracic to the rest of segments.

II. Materials and Methods

1. Fly Strains

Oregon R was used as the wild-type strain. The mutant fruit flies used in this experiments are as follows: *Ubx9.22* (Capdevila and García-Bellido, 1981), *abdAMX1, Df(3R) Ubx109/Dp3;3(P5)* (*Ubx abdA* double mutant) (Bloomington Drosophila stock center), and *Df(3R)P115,e[11]/TM1;Dp(3;1)P115/+* (BX-C triple mutant) (Bloomington Drosophila stock center). Ectopic expression induced using the UAS/GAL4-system as described by Brand and Perrimon (1993). *twist*-GAL4 (*twi*-GAL4) was crossed to UAS-*Ubx* and UAS-*abdA* (Castelli-Gair et al., 1994) for the ectopic expression of *Ubx* and *abdA*, respectively. Such embryos were named *twi;;Ubx* and *twi;;abdA*.

2. Antibody Staining

Antibody staining was performed under standard conditions as described elsewhere (Patel, 1994; Jung et al., 2008). Embryos were collected, dechorionated, fixed, devitellinized, and then stained with antibodies. After embryos were preincubated with 5% goat serum, they were treated with the primary antibody. The primary antibodies used were: rabbit anti-zfh1 (1:1,000, kindly provided by professor Siuk, Yoo), mouse anti-repo and anti-elav (1:200, Hybridoma Bank, University of Iowa, USA). The primary signal was amplified and detected with Tyramide 488 and 594 conjugated anti-IgG. Embryos were viewed and photographed with an Olympus microscope BX51 and Carl Zeiss-LSM780 confocal microscope.

3. Embryonic Cuticle Preparation

Wild-type and mutant embryos were collected for several hours and were incubated for one day further at 25 ºC. Wild type embryos were collected just before hatching and transferred to a double-sided cellophane tape for manual dechorionation using forceps. The dechorionated embryos were transferred to a new slide glass with a drop of a 1:1 mixture of Hoyer's mounting solution and lactic acid (Kwon et al., 2004). Then the vitelline envelope was peeled off by tapping around the embryo with forceps. The slides were cover-slipped and placed at room temperature for one day and then on the slide warmer on the next day. Loss-of-function mutant embryos were collected when they showed brown-colored lethal embryos with a pharyngeal skeleton. The subsequent method is the same as that used for wild-type embryos. The embryos were viewed and photographed using an Olympus BX51 with dark-field.

III. Results

1. Expression of ZFH1 in Wild-Type Embryos

zfh1 was originally known to be expressed in the mesoderm of early embryos and in a number of mesodermally derived structures in late embryos, including the dorsal vessel, support cells of the gonads, and segment-specific arrays of adult muscle precursors (Lai et al., 1991). However, further analysis showed that *zfh1* was expressed in embryonic somatic motor neurons and in glia associated with the CNS (Layden et al., 2006).

We found that *zfh1* was expressed in numerous cells across the entire body with repetitive pattern per segment. However, some of the expression was segment-specific (Fig. 1A, arrowheads). In order to determine whether these cells are neurons or glia, we stained the embryos with anti-elav antibody, which is a neuronal marker, or anti-repo antibody, which is a glial marker. Although *elav* or *repo* were co-expressed with *zfh1* in many cells, neuronal or glial markers were not co-expressed in cells where *zfh1* is specifically expressed in the thorax (Fig. 2C, D, arrowheads in C and D). These results suggest that the thorax-specific *zfh1*-expressing cells are not neurons or glia.

Although the thorax-specific expression of *zfh1* is not a neuron of glia, *zfh1* can be co-expressed with *elav* and *repo* in other cells. In the results obtained by double antibody staining *elav* and *zfh1*, it is presumed that cells in circle are ventral unpaired median motor neurons. (Fig. 2C'') (Laden et al., 2006; Wheeler et al., 2006). In the results of double antibody staining of *repo* and *zfh1*, it appears that cells in circle and marked with asterisk are surface-associated glia which enwrap the motor nerves (Fig. 2D'') (Layden et al., 2006; Beckervordersandforth et al., 2008).

We noticed that the *zfh1* thorax-specific pattern is very similar to that of *Distaless* (*Dll*) (Panganiban et al., 1994). Therefore, we double-stained the embryos using anti-zfh1 and anti-Dll antibodies. However, *Dll* and *zfh1* were not co-expressed in the thorax-specific *zfh1*-expressing cells (Fig. 3A and B). As *Dll* is involved in all the ventral appendages in *Drosophila*, including the leg, clypedolabrum, maxillary and labial palps, antennae, and legs, thorax-specific *zfh1*-expressing cells may not be involved in ventral appendage formation (Vachon et al., 1992; Panganiban et al., 1994; Galindo et al., 2011).

We then examined the possibility that these thorax-specific *zfh1*-expressing cells might be mesodermal cells because *zfh1* is expressed in numerous mesodermal cells (Lai et al., 1991). Embryos obtained from a cross of *twist*-Gal4 and *UAS-lacZ* flies were double-stained with anti-zfh1 antibody and anti-β-galactosidase antibody. As *zfh1* was co-expressed with *twist* (Fig. 3C and D), these thorax-specific *zfh1* cells appear to be mesodermal cells.

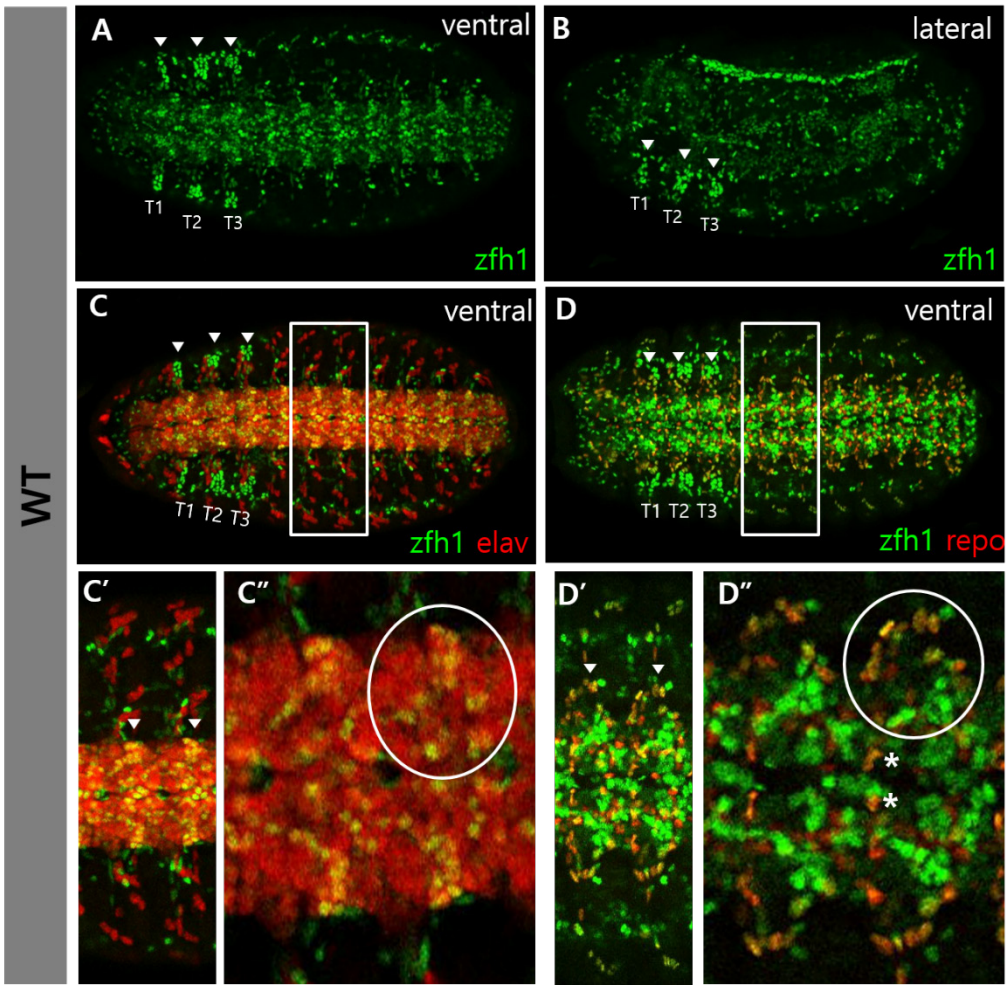

Figure 2. Expression pattern of ZFH1, ELAV and REPO. Embryos were stained with *zfh1*, *elav* and *repo* anti-body.

Embryos were stained with anti-zfh1 antibody (A and B), and double staining with anti-Elav antibody and anti-zfh1 antibody or double staining with anti-Repo antibody and anti-zfh1 antibody (C and D). *zfh1* is co-expressed in many neurons and glia at stage 15. However, thorax-specific *zfh1* expression (arrowheads in C and D) was not overlapped with that of *elav* or *repo*.

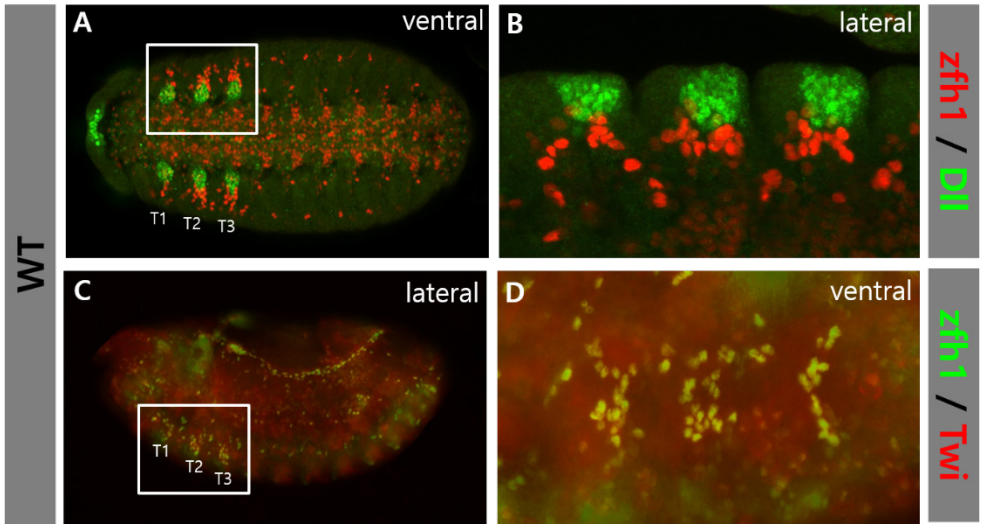

Figure 3. Expression pattern of DLL and TWI with ZFH1. Embryos were stained with *zfh1*, *Dll* and β-gal anti-body.

Double-stained with anti-zfh1 and anti-Dll bodies (A and B) or with anti-zfh1 and anti-β-gal antibodies (C and D*). twi* was expressed from *twi*-gal4 and UAS-lacZ. *zfh1* was not co-expressed with *Dll* (A and B), but co-expressed with *twi.*(C and D), suggesting that thorax-specific *zfh1* expressed cells are mesoderm-derived cells. .

2. *Ubx* and *abd-A* repress *zfh1* expression in abdominal segments during embryogenesis

Homeotic genes provide positional information along the anterior to posterior axis. There are two homeotic gene complexes in *Drosophila*, ANT-C and BX-C. The ANT-C genes include *lab, pb, Dfd, Scr* and *Antp*, whereas the BX-C genes contain *Ubx, abd-A* and *Abd-B* (Carroll et al., 1986).

We confirmed the possibility that *zfh1* could be regulated by SCR and ANTP because SCR and ANTP specify the first and second thoracic segments, respectively. However, we did not see any abnormal expression of *zfh1* in the *Scr* and *Antp* mutant embryos. These results suggest that the positional information of thorax-specific *zfh1*- expressing cells may not be determined by the direct regulation of SCR and ANTP, but may be a result of repression by the BX-C genes, without which, *zfh1* could be expressed in the abdominal segments.

We therefore examined the possibility that BX-C gene products might inhibit *zfh1* expression in the abdominal segments. The BX-C genes determine structures from the posterior half of the thoracic and abdominal segments (Sánchez-Herrero et al., 1985). These genes have been known to repress genes expressed in thoracic segments (Wong and Merritt, 2002). This suggests that the thoracic segment-specific expression pattern of *zfh1* might be due to repression by BX-C genes in the abdominal segments. We examined the expression of *zfh1* in the loss-of-function mutant of *Ubx, abd-A,* and *Abd-B*. *Ubx* is expressed in parasegment (PS) 5-13, *abdA* in PS7-13, and *Abd-B* in PS10-14 (Beachy et al., 1985; Celniker et al., 1989; Karch et al., 1990).

Before using the mutants of BX-C genes, we confirmed them by observing the embryonic cuticle belts (Fig. 4). The wild-type embryo has three thin denticle

belts in the thoracic region and eight denticle belts in the abdomen (Fig. 4A). In *Ubx* mutant embryos, the first abdominal segment is transformed to the third thoracic segment (Fig. 4B), whereas in the *abd-A* mutant embryos, the second to the fifth abdominal denticle belts were transformed to the first abdominal one. In *Ubx abdA* double mutant embryos, the abdominal and third thoracic denticle belts were transformed to the second thoracic denticle belt.

In *Ubx* mutant embryos, *zfh1* was ectopically expressed in the first (A1) and second (A2) abdominal segments (Fig. 5C, D, arrows). Although *Ubx* is expressed in PS5-13, it is expressed at the highest concentration in PS6, which corresponds to the posterior compartment of the third thoracic segment and the anterior compartment of the first abdominal segments, and mainly determines the identity of PS6. Therefore, it was not expected that *zfh1* would be ectopically expressed in the second abdominal segment in the *Ubx* mutant.

In the *abdA, AbdB* mutant embryo, *zfh1* expression in the thoracic segment did not change (data not shown). However, in the *Ubx abdA* double mutant embryo, *zfh1* expression was extended to the fourth (A4) abdominal segment (Fig. 5E, F, arrows). This result suggests that *zfh1* expression is repressed by UBX and ABDA in the abdominal segments.

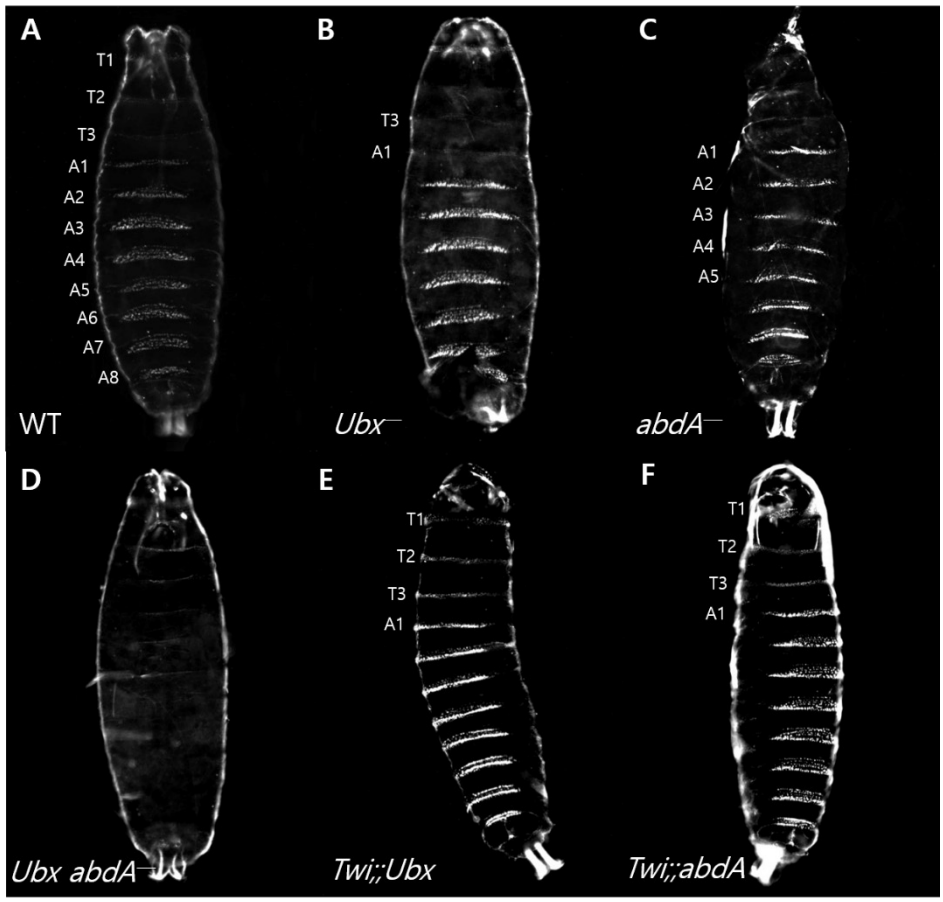

Figure 4. The patterns of embryonic denticle belt.

(A) Wild type embryo. It has three thoracic denticle belts and eight abdominal denticle belts. (B) *Ubx* mutant embryo. The first abdominal denticle belt is transformed to the third thoracic denticle belt. (C) *abdA* mutant embryo. From the first to the fifth abdominal denticle are transformed to the first abdominal denticle belt. (D) *Ubx abdA* double mutant embryo. The abdominal denticle belt and third thoracic denticle belt transform to the second thoracic denticle belt. (E) (F) *Ubx* and *abdA* Gain-of-function mutant embryo. The thoracic denticle belts are transformed to the first abdominal denticle belt.

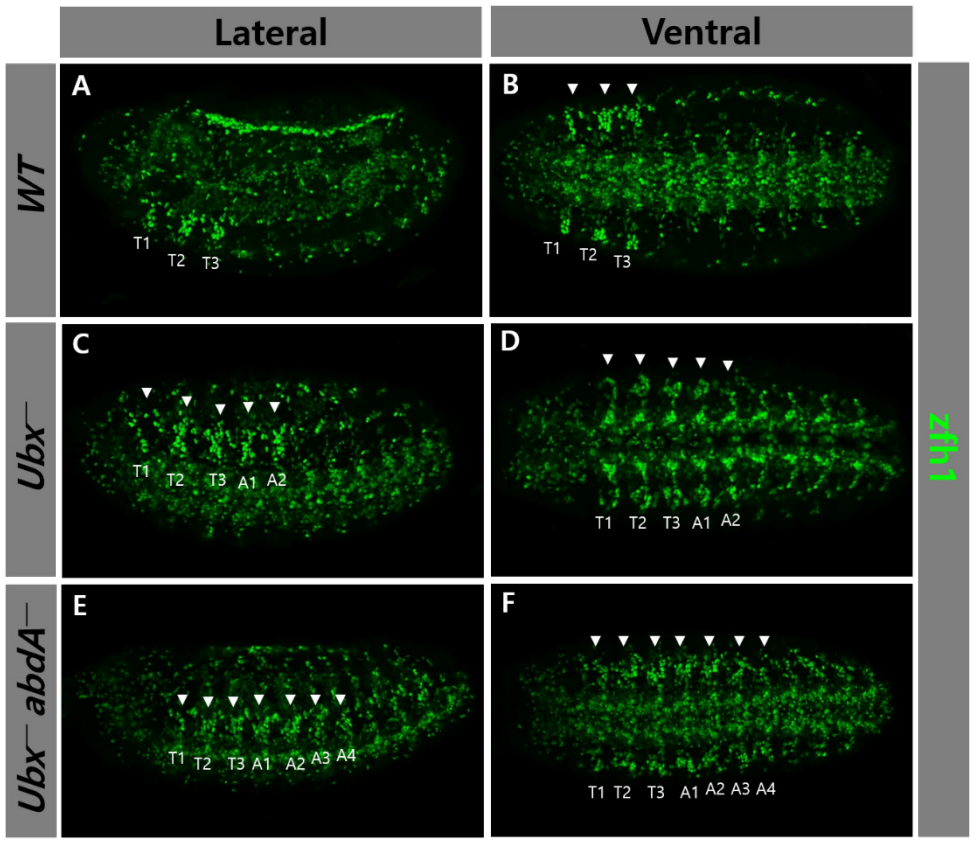

Figure 5. Expression pattern of ZFH1 in *Ubx* and *Ubx abdA* double mutant embryos.

(A and B) Wild type embryo. Thorax-specific expression of *zfh1* is shown (arrowheads). (C and D) *Ubx* mutant embryo. *zfh1* expression was extended to the second abdominal segment (arrowheads). (E and F) *Ubx abdA* Double mutant embryo. *zfh1* is ectopically expressed from first abdominal segment to fourth abdominal segment (A1 to A4).

3. Aberrant expression of *Ubx* and *abdA* genes represses *zfh1* expression in the thorax

In order to confirm that BX-C genes repress the expression of *zfh1*, the *Ubx* and *abdA* were ectopically expressed in the thorax with the GAL4/UAS system. GAL4 protein binds to the UAS regulatory region and activates the expression of genes adjacent to UAS. In this study, we first crossed *Scr*-GAL4 or *Antp*-GAL4 to UAS-*Ubx* to drive *Ubx* expression in the thorax. Embryos obtained from with ectopically expressed *Ubx* did not show elimination of thorax-specific *zfh1* expression. These results indirectly suggest that SCR or ANTP are not involved in the regulation of *zfh1* expression in these specific cells.

As *zfh1* was co-expressed with *twi*, we examined the expression of *zfh1* in *twi* mutants. We could not examine *zfh1* expression in the loss-of-function mutant of twist because *twi* mutant embryos were lethal at an early embryonic stage (Thisse et al., 1987). Instead, we ectopically expressed *zfh1* by crossing *Ubx* to twi-Gal4. In the *Ubx* gain-of-function mutant embryos, the specific expression of *zfh1* in the thorax disappeared and *zfh1* expression in other cells was decreased (Fig. 6). However, when *abd-A* was ectopically expressed in the thorax, unlike *Ubx, zfh1* expression was not reduced in the thorax.

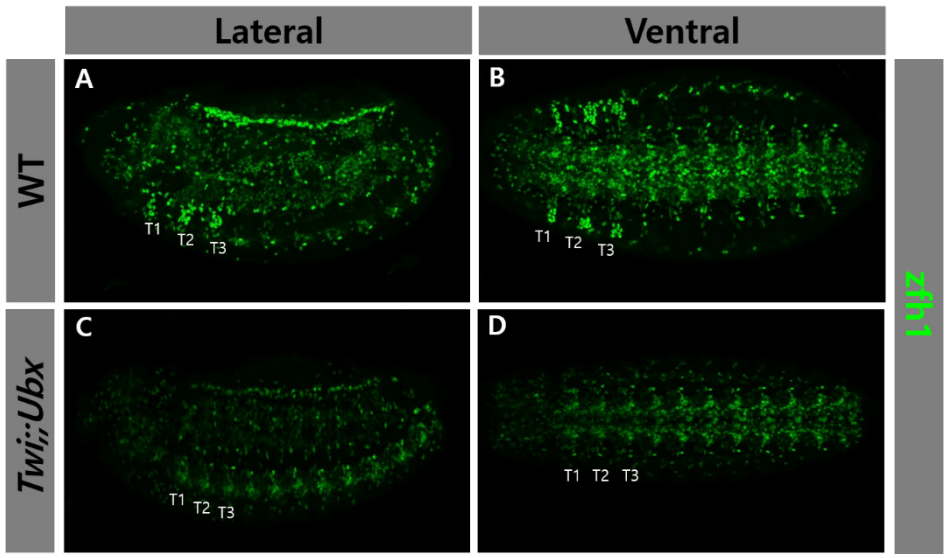

Figure 6. The expression of ZFH1 in the *Ubx* gain-of-function mutant embryos.

(A and B) wild type embryo. (C and D) *twi;;Ubx* embryo. The ectopic expression of *Ubx* in thorax repressed the thorax-specific expression of *zfh1*.

IV. Discussion

zfh1 is a transcriptional factor with a homeodomain and nine C_2-H_2 zinc finger DNA binding motif; it is expressed in numerous cells including mesoderm-derived cells, neurons, and glia. *zfh1* is required for mesodermal development and cell migration (Lai et al., 1991; Broihier et al., 1998). *zfh1* also regulates lateral axon growth and CNS exit in ventral-projection motor neurons (Layden et al., 2006). *zfh1* promotes the survival of a peripheral glia subtype by antagonizing a Jun N-terminal kinase-dependent apoptotic pathway (Ohayon et al., 2009). Despite many studies on *zfh1* genes, cell groups in which *zfh1* is expressed specifically in the thorax have not been examined.

As *zfh1* was discovered by screening genes whose expression was affected in the nervous system, we first examined whether these thorax-specific *zfh1*-expressing cells are neurons or glia by immunostaining with the neuronal marker, anti-Elav antibody (Robinow and White, 1988), or the glial marker, anti-Repo antibody (Halter et al., 1995). As *zfh1* was not co-expressed with *elav* or *repo*, these thorax-specific *zfh1*-expressing cells were not neurons or glia.

As *zfh1* is expressed in mesoderm-derived cells (Lai et al., 1991), we examined the co-expression of *zfh1* gene with the mesodermal marker, *twi* (Bate et al., 1991). Embryos from the cross of *twi*-GAL4 and UAS-lacZ were stained with anti-zfh1 and anti-Laz antibodies. As *zfh1* and *twi* were co-expressed, these thorax-specific *zfh1* expressed cells appear to be mesoderm-derived cells. However, these results do not explain why *zfh1* is expressed specifically in the thorax, but not in the abdomen.

During embryogenesis, a cascade of patterning genes provides the blastoderm with positional cues along the anterior-posterior axis, which finally define the

segments (Nasiadka et al., 2002). The homeotic genes mediate the specification of these segments according to their location in the anterior to posterior axis (Ducan, 1987; Beachy 1988; McGinnis and Krumlauf, 1992). For example, the developing central nervous system expresses distinct segment-specific characteristics. Components of the Neuroblast (NB) 1-1 lineage show specific differences between the thoracic and abdominal segments (Udolph et al., 1993). Activity of the homeotic genes is required for the abdominal pathway of the NB1-1 lineage (Prokop and Technau, 1994). NB6-4 also shows a segment-specific pattern between the thorax and abdomen, and this difference was specified by homeotic genes (Kang et al., 2006). Therefore, we first examined *zfh1* expression in *Scr* and *Antp* mutant embryos to determine whether SCR and ANTP might provide positional information for the thorax-specific expression of *zfh1*. We did not observe loss of *zfh1* gene expression in both mutant embryos. These results suggest that thorax-specific expression of *zfh1* is not specified by SCR and ANTP in the thorax. Instead, repression of *zfh1* in the abdomen could explain the thorax-specific expression of *zfh1*. As *zfh1* expression was examined in the *Ubx* or *Ubx abd-A* double mutant embryos, thorax-specific *zfh1*-expressing cells were present in the abdominal segments. These results clearly showed that the thorax-specific pattern of *zfh1* expression was produced by the action of *zfh1* expression by *Twi* and repression of *zfh1* by *Ubx* and *abd-A* in the abdominal segment. The absence of both *Ubx* and *abd-A* function is necessary to allow the abdominal expression of *zfh1*. However, the *Ubx* gain-of function alone appears to be sufficient to repress *zfh1* expression in the thorax.

A peculiar result was that *zfh1* expression was extended to the second abdominal segment, but not to the first abdominal segment. Although *Ubx* is expressed from the posterior of the second thoracic segment to abdominal segments, it is most strongly expressed in PS6 and specifies the posterior compartment of the

third thoracic segment and the anterior compartment of the first abdominal segment (Beachy et al., 1985; McCall et al., 1994; Akam and Martinez-Arias, 1985). This can be proved by the transformation of the first abdominal denticle belt to the third thoracic one in *Ubx* mutant embryos. Since thorax-specific *zfh1*-expressing cells are mesodermal, *zfh1* is presumably under the control of homeotic genes expressed in the mesoderm. *Ubx* and *abd-A* are required for development of the visceral mesoderm (Chauvet et al., 2000). In *Ubx* mutants, the thorax-specific expression of *zfh1* is extended to the second abdominal segment and may be due to the different activity level of UBX between the epidermis and visceral mesoderm (Davis et al., 2007).

In order to determine whether *zfh1* is involved in appendage development, co-expression of *zfh1* and *Dll* was examined. Both genes were not co-expressed and although being indirect evidence, suggested that *zfh1* may not function in appendage formation. The ZFH1 and ZEB proteins in myogenesis are conserved from *Drosophila* to mammals (Postigo et al., 1999). ZEB is also the major target gene of E-cadherin and induces EMT (Epithelial-mesenchymal transition) by inhibiting E-cadherin (Vandewalle et al., 2009). This suggests that ZFH1 might be involved in the migration of thorax-specific *zfh1*-expressing cells for future development. The actual role of these thorax-specific *zfh1*-expressing cells remains to be elucidated.

References

Akam, M. E., & Martinez-Arias, A. (1985). The distribution of *Ultrabithorax* transcripts in *Drosophila* embryos. *The EMBO Journal*, 4(7), 1689

Bate, M., Rushton, E., & Currie, D. A. (1991). Cells with persistent *twist* expression are the embryonic precursors of adult muscles in *Drosophila*. *Development*, 113(1), 79-89.

Beachy, P. A., Helfand, S. L., & Hogness, D. S. (1985). Segmental distribution bithorax complex proteins during *Drosophila* development. *Nature*, 313, 545-551.

Beachy, P. A., Krasnow, M. A., Gavis, E. R., & Hogness, D. S. (1988). An *Ultrabiththorax* protein binds sequences near its own and the *Antennapedia* P1 promoters. *Cell*, 55, 1069-1081.

Beckervordersandforth, R. M., Rickert, C., Altenhein, B., & Technau, G. M. (2008). Subtypes of glial cells in the *Drosophila* embryonic ventral nerve cord as related to lineage and gene expression. *Mechanisms of development*, 125(5), 542-557.

Brand, A. H., & Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, 118(2), 401-415.

Broihier, H. T., Moore, L. A., Van Doren, M., Newman, S., & Lehmann, R. (1998). *Zfh-1* is required for germ cell migration and gonadal mesoderm development in *Drosophila*. *Development*, 125(4), 655-666.

Capdevila, M. P., & García-Bellido, A. (1981). Genes involved in the activation of the bithorax complex of *Drosophila*. *Development Genes and Evolution*, 190(6), 339-350.

Carroll, S. B., Laymon, R. A., McCutcheon, M. A., Riley, P. D., & Scott, M. P. (1986). The localization and regulation of *Antennapedia* protein expression in *Drosophila* embryo. *Cell*, 47, 113-122.

Castelli-Gair, J., Greig, S., Micklem, G., & Akam, M. (1994). Dissecting the temporal requirements for homeotic gene function. *Development*, 120(7), 1983-1995.

Celniker, S. E., Keelan, D. J., & Lewis, E. B. (1989). The molecular genetics of the bithorax complex of *Drosophila*: characterization of the products of the *Abdominal-B* domain. *Genes & Development*, 3, 1424-1436.

Chauvet, S., Merabet, S., Bilder, D., Scott, M. P., Pradel, J., & Graba, Y. (2000). Distinct hox protein sequences determine specificity in different tissues. *Proceedings of the National Academy of Sciences*, 97(8), 4064-4069.

Davis, G. K., Srinivasan, D. G., Wittkopp, P. J., & Stern, D. L. (2007) The function

and regulation of *Ultrabithorax* in the legs of *Drosophila melanogaster*. *Dev Biol*. 308: 621-631.

Dessain, S., Gross, C. T., Kuziora, M. A., & McGinnis, W. (1992). Antp-type homeodomains have distinct DNA binding specificities that correlate with their different regulatory functions in embryos. *The EMBO journal*, 11(3), 991.

Duncan, I. (1987). The bithorax complex. *Annual review of genetics*, 21(1), 285-319.

Fortini, M. E., Lai, Z., & Rubin, G. M. (1991). The Drosophila *zfh-1* and *zfh-2* genes encode novel proteins containing both zinc-finger and homeodomain motifs. *Mechanisms of development*, 34(2-3), 113-122.

Galindo, M.., Fernandez-Garza, D., Phillips, R. and Couso, J. P. (2011) Control of *Distal-less* expression in the *Drosophila* appendages by functional 3' enhancers. *Dev Biol*. 353: 396-410.

Gummalla, M., Galetti, S., Maeda, R. K., & Karch, F. (2014). Hox gene regulation in the central nervous system of *Drosophila*.

Halter, D. A., Urban, J., Rickert, C., Ner, S. S., Ito, K., A, A.. Technau, G. M. (1995). The homeobox gene *repo* is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila* melanogaster. *Development*, 121(317-332).

Jung, K. I., & Jeon, S. H. (2008). Temporal regulation of *Distalless* expression by mutations in BX-C and Polycomb genes. *Genes & Genomics* (*구 한국유전학회지*), 30(1), 83-90.

Jürgens, G., Wieschaus, E., Nüsslein-Volhard, C., & Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. *Development Genes and Evolution*, 193(5), 283-295.

Kang, S. Y., Kim, S. N., & Jeon, S. H. (2006). Temporal and Spatial Expression of Homeotic Genes Is Important for Segment-specific Neuroblast 6-4 Lineage Formation in *Drosophila*. *Molecules and Cells*, 21, 436-442.

Karch, F., Bender, W., & Weiffenbach, B. (1990). *abdA* expression in *Drosophila* embryos. *Genes & Development*, 4, 1573-1587.

Kaufman, T. C., Seeger, M. A., & Olsen, G. (1990). Molecular and genetic organization of the *Antennapedia* gene complex of *Drosophila* melanogaster. *Advances in Genetics*, 27, 309-362.

Kwon, S. H., Kim, S. H., Chung, H. M., Girton, J. R., & Jeon, S. H. (2004). The *Drosophila* pleiohomeotic mutation enhances the Polycomblike and Polycomb mutant phenotypes during embryogenesis and in the adult. *International Journal of Developmental Biology*, 47(6), 389-395.

Lai, Z., Fortini, M., & Rubin, G. (1991). The embryonic expression patterns of *zfh-*

1 and *zfh-2*, two *Drosophila* genes encoding novel zinc-finger homeodomain proteins. *Mechanisms of Development*, 34, 123-134.

Lai, Z. C., Rushton, E., Bate, M., & Rubin, G. M. (1993). Loss of function of the Drosophila *zfh-1* gene results in abnormal development of mesodermally derived tissues. *Proceedings of the National Academy of Sciences*, 90(9), 4122-4126.

Layden, M. J., Odden, J. P., Schmid, A., Garces, A., Thor, S., & Doe, C. Q. (2006). *Zfh1*, a somatic motor neuron transcription factor, regulates axon exit from the CNS. *Developmental Biology*, 291(2), 253-263.

McCall, K., O'Connor, M. B., & Bender, W. (1994). Enhancer traps in the *Drosophila* bithorax complex mark parasegmental domains. *Genetics*, 138(2), 387-399.

McGinnis, W., & Krumlauf, R. (1992). Homeobox genes and axial patterning. *Cell*, 68, 283-302.

Nasiadka, A., Dietrich, B. H., & Krause, H. M. (2002). Anterior-posterior patterning in the *Drosophila* embryo. *Advances in developmental biology and biochemistry,* 12, 155-204.

Nüsslein-Volhard, C., Wieschaus, E., & Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. *Development Genes and Evolution*, 193(5), 267-282.

Ohayon, D., Pattyn, A., Venteo, S., Valmier, J., Carroll, P., & Garces, A. (2009). *zfh1* promotes survival of a peripheral glia subtype by antagonizing a Jun N-terminal kinase-dependent apoptotic pathway. *Embo Journal*, 28(20), 3228-3243.

Panganiban, G., Nagy, L., & Carroll, S. B. (1994). The role of the *Distal-less* gene in the development and evolution of insect limbs. *Current Biology*, 4(8), 671-675.

Patel, N. H. (1994). Imaging neuronal subsets and other cell types in whole-mount *Drosophila* embryos and larvae using antibody probes. *Methods in cell biology*, 44, 445-487.

Postigo, A. A., Ward, E., Skeath, J. B., & Dean, D. C. (1999). *zfh-1*, the *Drosophila* homologue of ZEB, is a transcriptional repressor that regulates somatic myogenesis. *Molecular and Cellular Biology*, 19, 7255-7263.

Prokop, A., & Technau, G. M. (1994). Early tagma-specific commitment of *Drosophila* CNS progenitor NB1-1. *Development,* 120(9), 2567-2578.

Robinow, S., & White, K. (1988). The locus elav of *Drosophila melanogaster* is expressed in neurons at all developmental stages. *Developmental biology*, 126(2), 294-303.

Sanchez-Herrero, E., Vernos, I., Marco, R., & Morata, G. (1985). Genetic

organization of *Drosophila* bithorax complex. *Nature*, 313, 108-113.

Sparmann, A., & van Lohuizen, M. (2006). Polycomb silencers control cell fate, development and cancer. *Nature Reviews Cancer*, 6(11), 846-856.

Thisse, B., Messal, M. E., & Perrin-Schmitt, F. (1987). The *twist* gene: isolation of a *Drosophila* zygotle gene necessary for the establishment of dorsoventral pattern. *Nucleic acids research*, 15(8), 3439-3453.

Truman, J. W., & Bate, M. (1988). Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila melanogaster*. *Developmental biology*, 125(1), 145-157.

Udolph, G., Prokop, A., Bossing, T., & Technau, G. M. (1993). A common precursor for glia and neurons in the embryonic CNS of *Drosophila* gives rise to segment-specific lineage variants. *Development,* 118(3), 765-775.

Ueno, K., Ohta, M., Morita, H., Mikuni, Y., Nakajima, S., Yamamoto, K., & Isono, K. (2001). Trehalose sensitivity in Drosophila correlates with mutations in and expression of the gustatory receptor gene Gr5a. *Current Biology*, 11(18), 1451-1455.

Vachon, G., Cohen, B., Pfeifle, C., McGinnis, E., Botas, J., & Cohen, S. M. (1992). Homeotic genes of the bithorax complex repress limb development in the abdomen of the *Drosophila* embryo through the target gene *Distal-less*. *Cell*,

71, 437-450.

Vandewalle, C., Van Roy, F., & Berx, G. (2009). The role of the ZEB family of transcription factors in development and disease. *Cellular and molecular life sciences,* 66(5), 773-787.

White, R. A., & Wilcox, M. (1985). Regulation of the distribution of Ultrabithorax proteins in *Drosophila*. *Nature*, 318(6046), 563-567.

Wheeler, S. R., Kearney, J. B., Guardiola, A. R., & Crews, S. T. (2006). Single-cell mapping of neural and glial gene expression in the developing *Drosophila* CNS midline cells. *Developmental biology*, 294(2), 509-524.

Wong, D. C. C., & Merritt, D. J. (2002). The role of homeotic genes in determining the segmental pattern of chordotonal organs in *Drosophila*. *International Journal of Developmental Biology,* 46, 475-481.

국문초록

초파리의 *Ultrabithorax* 와 *Abdominal A* 에 의한 *zfh1*의 가슴 체절 특이적 발현

*zinc finger homeodomain1(zfh1)*은 다양한 역할을 하는 유전자이다. 이 유전자는 신경계 발생에 관여하며, 특히 중추신경계에서 말초신경계로 운동 뉴런이 뻗어 나가는데 관여하는 유전자이다. 또한 *zfh1* 은 주변적신경교세포(Peripheral glia)의 생존에도 중요한 역할을 한다. *zfh1* 은 근육의 분화에도 관여를 하는데 *zfh1* 와 상동인 zinc finger E box binding protein(ZEB)도 근육의 분화에 역할이 있다는 연구가 되어있다. 본 연구에서는 초파리 배아 발생단계에서 가슴 체절에서 특이적으로 발현하는 *zfh1* 이 호메오틱 유전자의 조절을 받는지 유전학적 분자생물학적 측면에서 살펴보았다. 가슴 체절에서 특이적으로 발현하는 *zfh1* 이 무엇인지 알아보기 위해 *Dll* 와 항체이중염색을 실시하였지만 *zfh1*과 *Dll*는 공동발현하지 않는다는 결과를 얻었다. 그 특이적인 발현이 신경계일 가능성을 두고 전체적인 신경계에서 발현하는 *embryonic lethal abnormal vision(elav)*과 신경교세포에서 발현하는 *revered polarity(repo)*를 *zfh1* 과 각각 이중 염색하였다. 결과적으로 *zfh1* 의 특이적인 발현이 신경계는 아니었으나 *zfh1* 이 주변적신경교세포에서 발현한다는 것을 다시 한 번 확인할 수 있었다. *zfh1* 과 *twist(twi)*를 이중 염색 하였을 때, 두 유전자가 공동 발현을 하는 것을 확인할 수 있었고 결과적으로 *zfh1* 의 특이적 발현은 근육 모세포일 가능성이 크다고 볼 수 있다. 이 특이적 발현은 *Ubx* 와 *Ubx abdA* 의 기능 상실 돌연변이에서 그 발현이 네번째 복부 체절까지 증가하는 것을 볼 수 있었다. *twi*-gal4 를 이용한 *Ubx* 기능 획득 돌연변이에서는 특이적인 발현이 감소하였고 전체적인 발현도 감소한 것을 확인할 수 있었다. 이러한 결과는 UBX 와 ABD-A 가 *zfh1* 의 발현이 가슴체절에서 특이적으로 나타나도록 억제한다는 것을 보여준다.

주요어: *Ubx, abdA, AbdB, zfh1*, 근육 모세포

학 번. 2015-21631