Int. J. Dev. Biol. 46: 633-638 (2002)

Homeotic genes influence the axonal pathway of a *Drosophila* embryonic sensory neuron

DAVID J. MERRITT* and PAUL M. WHITINGTON[1]

School of Life Sciences, The University of Queensland, Brisbane, Queensland and [1]Department of Anatomy and Cell Biology, University of Melbourne, Victoria, Australia

ABSTRACT Each abdominal hemisegment of the *Drosophila* embryo has two sensory neurons intimately associated with a tracheal branch. During embryogenesis, the axons of these sensory neurons, termed the v'td2 neurons, enter the CNS and grow toward the brain with a distinctive pathway change in the third thoracic neuromere. We show that the axons use guidance cues that are under control of the *bithorax* gene complex (BX-C). Pathway defects in mutants suggest that a drop in *Ultrabithorax* expression permits the pathway change in the T3 neuromere, while combined *Ultrabithorax* and *abdominal-A* expression represses it in the abdominal neuromeres. We propose that the axons do not respond to a particular segmental identity in forming the pathway change; rather they respond to pathfinding cues that come about as a result of a drop in BX-C expression along the antero-posterior axis of the CNS.

KEY WORDS: *neuronal identity, axonal guidance, homeosis, Drosophila, PNS*

Introduction

The segment-specific characteristics of the epidermis of the insect trunk are determined by the action of the homeotic genes of the Antennapedia complex (ANT-C) and Bithorax complex (BX-C). These genes are also strongly expressed in the CNS (Doe and Scott, 1988) where they are involved in regulating segmental differences in the complement of neurons comprising the thoracic and abdominal neuromeres of the ventral nerve cord (VNC) (Green, 1981; Jimenez and Campos-Ortega, 1981; Teugels and Ghysen, 1985; Ghysen and Lewis, 1986; Gould *et al.*, 1990; Graba *et al.*, 1992; Jijakli and Ghysen, 1992; Prokop and Technau, 1994; Prokop *et al.*, 1998). A key aspect of neuronal identity, axon morphology, often shows segmental specificity: serially homologous neurons or axons that run inter-segmentally can display distinct patterns of axonal arborisation in different neuromeres. Given their roles in determining segment-specific neuron identity, one would also expect homeotic genes to be involved in specifying segment-specific patterns of axon growth in the CNS. Indeed, Thomas and Wyman (1984) have reported that in a semi-viable BX-C mutant of *Drosophila*, in which the metathorax takes on a mesothoracic identity, the adult giant fibre interneuron shows a mesothoracic-like axon branching pattern within the metathoracic ganglion. This finding strongly suggests that axon guidance cues in the CNS are under the control of homeotic genes. However the mechanism by which homeotic genes specify segment-specific axon morphologies remains unclear, in large part because earlier mutant analyses have been restricted to partial loss-of-function alleles of the homeotic genes.

We have readdressed this question, using as our model a pair of stretch receptive sensory neurons, termed v'td2 (Bodmer *et al.*, 1989), found in the abdominal segments of *Drosophila* embryos and larvae. In wild type individuals, these axons project anteriorly to the 3rd thoracic (T3) neuromere where they show a marked pathway change, crossing from a lateral to a medial position, before continuing anteriorly toward the brain. Irrespective of their point of entry into the CNS, all axons originating in abdominal segments 1-7 (A1-7) form this crossover in neuromere T3 (Merritt and Whitington, 1995).

In this study, we have examined the role that homeotic genes play in specifying the location of this axonal crossover. We have used single neuron staining methods to reveal the arborizations of the v'td2 axons in the CNS of embryos (Merritt and Whitington, 1995). This has enabled us to examine the phenotype of null mutants rather than adult-viable mutants in which homeosis is incomplete.

Abbreviations used in this paper: BX-C, bithorax gene complex; CNS, central nervous system; PNS, peripheral nervous system.

***Address correspondence to:** Dr. David J. Merritt. School of Life Sciences, The University of Queensland, Brisbane, Qld 4072, Australia. Fax: +61-7-3365-1922. e-mail: dmerritt@zen.uq.edu.au

0214-6282/2002/$25.00

Printed in Spain
www.ijdb.ehu.es

Our results show that the trajectory followed by the v'td2 axons within the CNS is influenced by the combined activity of the BX-C genes *Ubx* and *abd-A*. Furthermore, they suggest a model in which these genes act by repressing medial turning of the v'td2 axons in segments posterior to T3. When BX-C gene activity drops to a low level, in the middle of neuromere T3, this repression is lifted, allowing the axons to grow medially. This model therefore proposes that homeotic genes influence axon growth by defining the anterior limit of a broadly distributed repulsive zone within the CNS.

Results

In *Drosophila* embryos and larvae, single cell staining with fluorescent tracers shows that the v'td2 sensory neuron sends its axon on a long traverse through the CNS (Merritt and Whitington, 1995). These neurons occur in pairs on the ventro-lateral body wall of A1-7 (Bodmer and Jan, 1987). On entering the CNS late in embryogenesis, the axons run anteriorly in a very lateral tract. On reaching the third thoracic neuromere all ipsilateral axons from this family of neurons follow a characteristic path within the neuropil of the T3 neuromere. They cross under the connective, turn dorsally, and then turn anteriorly once more to extend towards the brain along a dorsal, medial tract (Fig. 1 A,B). Relating the growth behaviour of v'td2 axons to homeotic gene expression requires knowledge of the normal domain of expression of the homeotic genes in the CNS, and the altered expression domain in homeotic mutants. The CNS expression domains of the Antennapedia (Antp), Ubx and Abd-A proteins in the wild type and mutants have been previously described (White and Wilcox, 1984; White and Wilcox, 1985a,b; Carroll *et al.*, 1986; Karch *et al.*, 1990). They are represented diagrammatically as coloured blocks in Fig. 2. Null mutations of one gene derepress the more anteriorly expressed genes, altering their expression patterns and resulting in homeotic transformation of epidermal structures (Sanchez-Herrero *et al.*, 1985; Hayes *et al.*, 1984). Note that the subfunction alleles of *Ubx*, i.e. *abx* and *bxd*, result in a decreased intensity of expression of Ubx in part of the expression domain (Fig. 2 E,F).

To determine more precisely the relationship between the position of the v'td2 axon crossover and regions of expression of homeotic genes in wild type embryos, we dye-filled v'td2 axons and then subsequently immuno-stained the embryo with anti-Ubx or anti-Antp antibodies. We found that the crossover coincides with the anterior border of strong Ubx expression and the posterior border of Antp expression in T3 (Figs. 1C, 2A), a location that represents the parasegment (PS) 5-6 boundary (White and Wilcox, 1985a). To test whether *Antp* or either of the BX-C genes *Ubx* or *abd-A* play a role in determining the location of the axon crossover, we examined the pathway in null mutations.

In *Antp*$^{-}$ embryos, where no Antp protein is present and Ubx protein expression remains normal, the v'td2 axon pathways appear completely normal (Fig. 2D), indicating that *Antp* is not necessary for formation of the normal pathway in the thoracic and abdominal neuromeres.

In the *Ubx* null, *Ubx*^{101}, the disruption of axonal growth is extreme (Fig. 2C): no axons follow the normal path. More than half

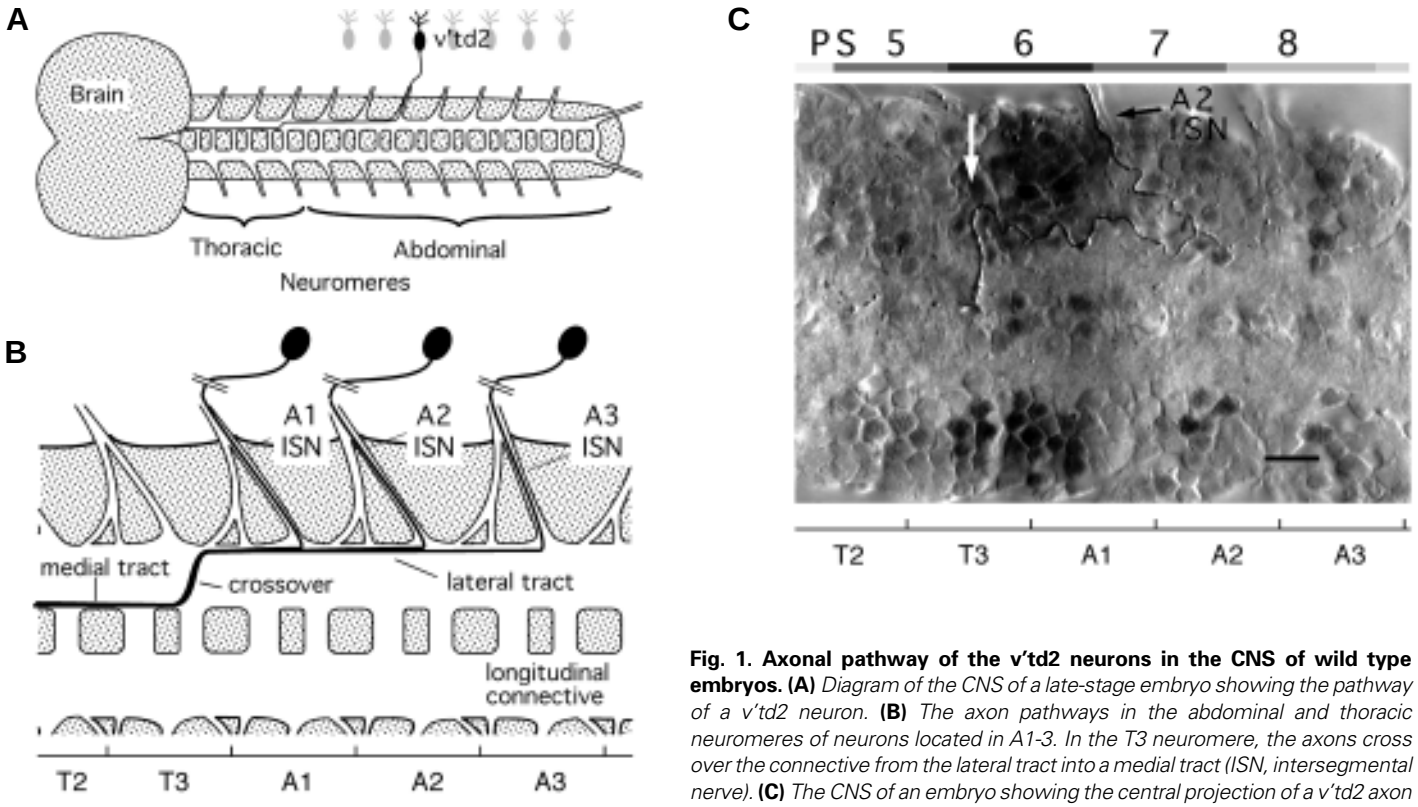

Fig. 1. Axonal pathway of the v'td2 neurons in the CNS of wild type embryos. (A) *Diagram of the CNS of a late-stage embryo showing the pathway of a v'td2 neuron.* **(B)** *The axon pathways in the abdominal and thoracic neuromeres of neurons located in A1-3. In the T3 neuromere, the axons cross over the connective from the lateral tract into a medial tract (ISN, intersegmental nerve).* **(C)** *The CNS of an embryo showing the central projection of a v'td2 axon arising from abdominal segment A2. The CNS is labelled with anti-*Ubx *antibody. The crossover (white arrow) occurs near the boundary between PS 5 and 6 in the T3 neuromere. Image collated from multiple focal planes using Adobe Photoshop. Anterior is to the left. Scale bar, 10 μm.*

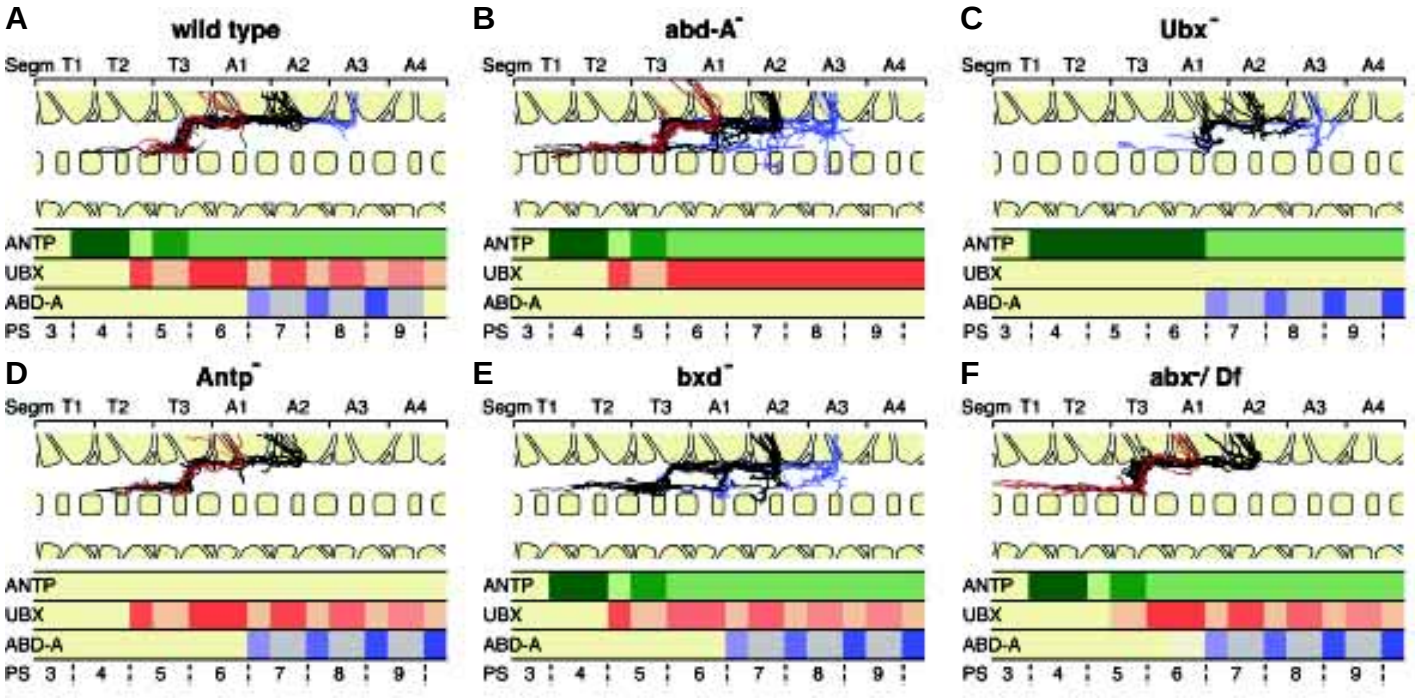

Fig. 2. Diagrams of axonal pathways and homeotic gene expression in wild type and mutant embryos. *The genotypes are: wild type* **(A)**, abd-A^{MX1} **(B)**, Ubx^{101} **(C)**, Antp^{w10} **(D)**, bxd^{55i} **(E)** *and* abx^{1}*/Df* **(F)**. *The superimposed pathways of a number of individual axons arising in different segments are shown at the top of each panel, based on a CNS template derived from Fig. 1B. Axons are color-coded according to their segment of origin. The bottom of each panel shows the homeotic protein expression patterns in the CNS of each genotype. The intensity of shading represents the intensity of antibody staining for homeotic proteins within parasegments. The segmental (Segm) borders are shown above each panel, and parasegmental (PS) borders below.*

(56%) of axons initially project normally along a lateral tract, but then form a crossover in A1, rather than T3. The trajectory of this axon crossover corresponds to the path normally followed in T3 and coincides with the altered anterior limit of Abd-A expression in the *Ubx* null mutant (Fig. 2C). This class of axons subsequently projects anteriorly as in normal embryos, although in one case this anterior branch was located in the middle of the connective, rather than in a medial position. The other 44% of axons show abnormal projections in neuromeres posterior to A1. Some of these run medially, although along novel trajectories that do not correspond to the normal T3 projection. Others course anteriorly in tracts that lie medial to the normal lateral position of the v'td2 axons. Of the *Ubx* subfunction alleles, *abx* has no effect on the axonal pathway (Fig. 2F; Table 1). Phenotypes of *bxd* alleles include a proportion of axons with a normal morphology (25-57%, depending on the allele), while the remainder display a crossover in A1 or are otherwise misrouted in A2 and A3, similar to the pattern seen in *Ubx* mutants (Figs. 2E, 3C; Table 1).

In *abd-A* mutants, 7 out of 8 v'td2 axons arising from A1 show a normal morphology. In the other case, the v'td2 axon began to grow out of the CNS along the T3 intersegmental nerve (Fig. 2B). Axons arising from A2 and A3 are either normal (48%) or show a range of pathfinding abnormalities. Most of these axons initially follow a lateral tract then project medially in A1 along a path corresponding to the normal T3 path before turning anteriorly along a normal medial tract. Other axons become misrouted after entering the lateral tract, while others do not enter the lateral tract and cross medially along a variety of aberrant routes (Fig. 2B). In one case, such a branch coursed across the midline. A range of

TABLE 1

PATHWAY DISRUPTION RECORDED IN THE V'TD2 NEURON OF HOMEOTIC MUTANT EMBRYOS

Genotype	Segment	Axonal projection type: Normal %	Axonal projection type: Misrouted %	Number Scored (n)	
Wild type	all	100	0	21	
	A2	100	0	5	
	A3	100	0	11	
	A4	100	0	5	
abd-A^{mx1}	al$_i$	65	35	31	
	Al	88	12	8	
	A2	83	17	12	
	A3	27	73	11	
Ubx^{101}	all	0	100	18	†
	A2	0	100	11	
	A3	0	100	5	
	A4	0	100	2	
bxd^{55i}	all	57	36	14	†
	A2	50	50	8	
	A3	66	33	6	
bxd^{113}	all	25	63	16	†
	A2	38	62	8	
	A3	13	87	8	
bxd ^{100}	all	31	69	16	†
	A2	42	58	12	
	A3	0	100	4	
Abx^{1}Df	all	100	0	13	
	A1	100	0	5	
	A2	100	0	8	
Antp^{w10}	all	100	0	8	
	A1	100	0	4	
	A2	100	0	4	

† The v'td2 neurons are not present in A1 due to homeosis of the PNS (DM, personal observations). All wild type axons form a crossover in the T3 neuromere. Mutants may form a normal axon projection or show some form of misrouting. For each mutation data from all segments are summed (all) and are then shown according to segment of origin of the v'td2 neuron.

phenotypes can be seen in an individual: for example, in the embryos illustrated in Fig. 3 A,B, the left and right side neurons from the same segment show different phenotypes.

Discussion

We have found that the central projections of v'td2 sensory axons in the *Drosophila* embryo are significantly affected by loss of function of the BX-C genes *Ubx* and *abd-A*: in null mutants for these genes, the axons either project into foreign medial regions of the neuropile posterior to T3 or make a normal lateral-medial crossover, but in A1 rather than T3. This finding, combined with the correspondence between the normal location of the v'td2 axon crossover and the anterior limit of *Ubx* expression in wild type embryos, suggests that the BX-C genes are involved in determining this segment-specific axon projection. Similarly, Thomas and Wyman (1984) concluded that BX-C genes specify central cues that cause the adult giant fibre neuron to arborize in a specific thoracic segment. By comparison, our results indicate that the homeotic gene *Antp* plays no role in the v'td2 projection.

How then do the BX-C genes determine the v'td2 axon projection? One possibility is that they specify a T3-specific feature that causes these axons to make a lateral-medial crossover only in that segment. This hypothesis would predict that in a *Ubx* mutant, the v'td2 axons should not crossover at all, since in that mutant there is no segment with an identity of T3: A1 takes on a mixed T2 /A1 identity, while T3 takes on a mixed T1/T2 identity (Hayes *et al.*, 1984; Sanchez-Herrero *et al.*, 1985; Heuer and Kaufman, 1992). An *abd-A* null mutant, on the other hand, would be expected to show a normal v'td2 axon morphology with a crossover restricted to T3, since this gene is normally not expressed in T3 and the identity of T3 is unaffected in the mutant. However, neither of these predictions is met. We find that in both *Ubx* and *abd-A* null mutant embryos, v'td2 axons crossover in either T3 or A1 or project medially into foreign neuropile regions in A2 and A3.

An alternative hypothesis, consistent with the results of this study, is that the *Ubx* and *abd-A* genes act cooperatively to activate expression of a factor (or factors) that repels v'td2 axons in regions of the CNS posterior to T3. This repulsion would keep the v'td2 axons in the lateral-most region of the neuropile until they have reached the anterior limit of high BX-C (combined *Ubx* and *abd-A*) expression in the middle of the T3 neuromere. At this point repulsion would be lifted, allowing the v'td2 axons to turn medially.

This hypothesis is supported by the following observations made in the present study. (1) The location of the v'td2 crossover in wild-type embryos coincides with the point in the T3 neuromere where combined BX-C gene expression drops to a low level. In neuromeres posterior to that point, either *Ubx* or *abd-A* is expressed at high levels. (2) The anterior limit of v'td2 crossovers coincides with the anterior limit of high BX-C expression in *Antp, abd-A* and *Ubx* mutants: this point is mid T3, mid T3 and mid A1 for the *Antp*, *abd-A*, and *Ubx* mutants respectively. (3) Eliminating either *abd-A* or *Ubx* expression results in v'td2 axons exploring medial regions of the neuropile in neuromeres posterior to T3, where the overall BX-C activity is lower than normal. The few examples in *abd-A* mutants of a v'td2 axon crossing the midline, which is normally strongly repulsive to ipsilaterally coursing axons, may be taken as further evidence of a breakdown in axon repulsive signalling mechanisms in this mutant. (4) The extent of

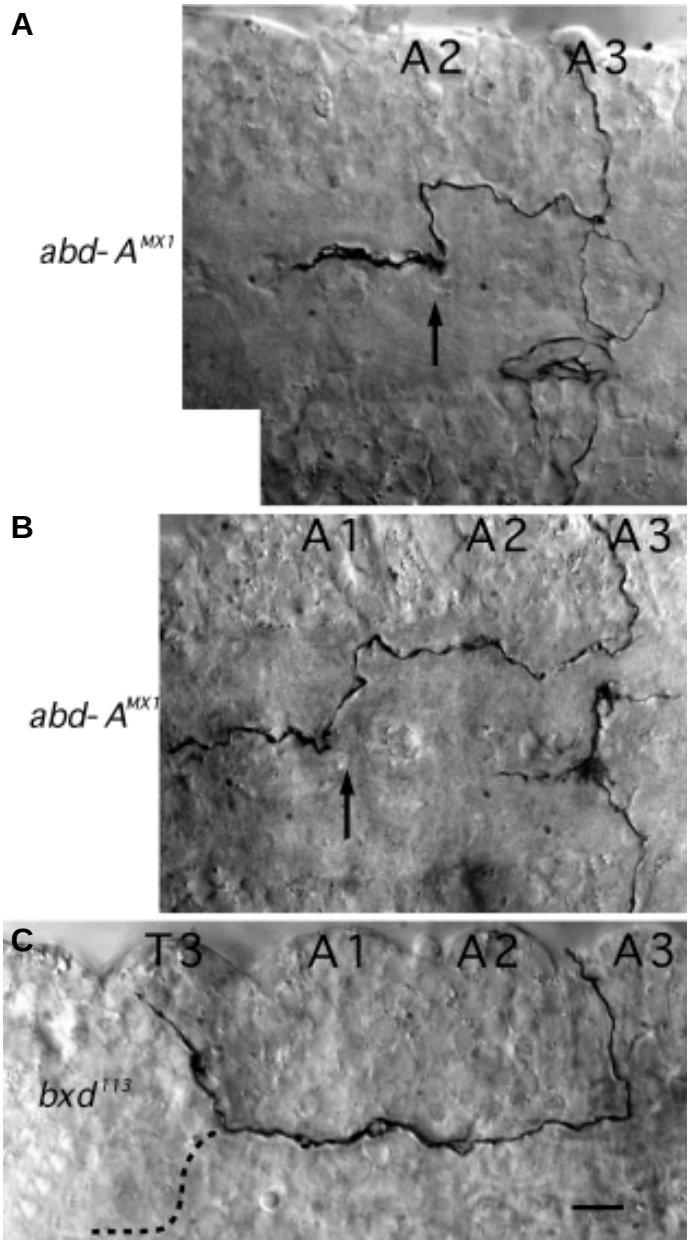

Fig. 3. Representative axonal pathway defects of v'td2 neurons in abd-A$^-$ and bxd$^-$ embryos. *In (A) and (B), a neuron was stained in both hemisegments of A3.* **(A)** *Upper side. The neuron forms an ectopic crossover in A2 (arrow). Lower side. The neuron is severely misrouted on entering the CNS.* **(B)** *Upper side. The axon forms an ectopic crossover in A1 (arrow), while its contralateral homolog is severely misrouted.* **(C)** *The axon enters the lateral tract in A3 though it fails to form a crossover in T3 and exits the CNS through the root of the T3 intersegmental nerve. The approximate pathway of a normal crossover is indicated by a dotted line. Anterior is to the left. Scale bar, 10 μm.*

reduction of *Ubx* expression in mutants correlates with the frequency of axons that explore medial regions of the neuropile in segments posterior to T3 (100% in *Ubx^{101}*, 36-69% in different *bxd* alleles). (5) In *abx^1/Df* all axons show a normal projection. This is expected because *abx* affects *Ubx* expression only in parasegment 5, anterior to the location of the crossover. (6) v'td2 axons that

enter the CNS in A1 in *abd-A* null mutant embryos almost all show a normal projection with a crossover in T3. This is expected as in wild-type embryos there is no expression of *abd-A* in the regions of the CNS through which the A1 v'td2 axons grow.

Our hypothesis assumes that, in the absence of repulsion, v'td2 axons will grow medially. Two alternative explanations could be advanced for this behaviour: the medial region of the neuropile generally is a permissive territory or there is a specific, attractive axon growth substrate leading from lateral to medial regions. The tendency of v'td2 axons in BX-C mutants to take a characteristic pathway in forming the crossover in the more posterior neuromeres favours the latter explanation. An attractive pathway may be present in all neuromeres but unavailable in segments posterior to T3 because of the BX-C regulated repulsive signalling system.

While axon repulsion in the CNS of the *Drosophila* embryo has been most intensively investigated at the midline (Tear *et al.*, 1996), a role for members of the *roundabout* (*robo*) gene family in axon repulsion in regions of the neuropile lateral to the midline has recently been uncovered (Murray and Whitington, 1999; Simpson *et al.*, 2000). Whether the proposed repulsion of v'td2 axons is mediated by Robo/Slit signalling or another receptor/ligand combination (e.g. Hummel *et al.*, 1999) remains to be determined.

Finally, we need to consider the possibility that the defects in v'td2 axon morphology observed in our study are due to homeotic transformation of the sensory neurons themselves, rather than of axon guidance cues in the CNS. The observations that Ubx expression extends posteriorly at least as far as A4 and that *Ubx* functions to repress ventral pit formation in all abdominal segments (Lewis, 1978) are consistent with this hypothesis. Nonetheless, we consider it to be an unlikely explanation for the v'td2 axon growth defects seen in *Ubx* mutants because loss of *Ubx* function does not apparently affect the identity of sensory neurons in abdominal segments posterior to A1 (as assayed by the pattern of external sensory and chordotonal organs; Heuer and Kaufman, 1992). A definitive test of the sensory neuron homeosis hypothesis will require the selective deletion of BX-C gene activity in the PNS. This hypothesis would predict that v'td2 neurons lacking BX-C gene expression should show disrupted central axon projections when growing into a genotypically wild-type CNS.

Our results indicate that homeotic genes influence the crossover point of v'td2 axon projections by defining a transition region along the antero-posterior axis of the CNS rather than by specifying segmental identity *per se*: BX-C gene activity represses the medial turn of the axons in segments posterior to T3 and when BX-C gene activity drops to a low level this repression is lifted. This model is similar to that proposed for BX-C gene regulation of ectodermal features of the trunk segments: within the broad BX-C expression domain in abdominal segments, the formation of thoracic structures such as legs and wings is suppressed (Graba *et al.*, 1992; Heuer and Kaufmann, 1992; Vachon *et al.*, 1992; Appel and Sakonju, 1993; Mann, 1994; Carroll *et al.*, 1995; Prokop and Technau, 1994).

Materials and Methods

Fly Strains

Null alleles utilised in this study are: *abd-A* ^{MX1} (Sanchez-Herrero *et al.*, 1985), *Ubx*^{101} (Hayes *et al.*, 1984), and *Antp*^{w10} (Wakimoto and Kaufman, 1981). The subfunction alleles are *bxd*^{55i}, *bxd*^{100}, *bxd*^{113}, and *abx*^{1}/DfP9 (Lewis, 1978; Bender *et al.*, 1983).

Neuronal Staining

Eggs were collected, dechorionated in bleach, and mid stage 17 embryos (before the trachea become air filled) selected. Embryos were glued to double-sided tape on a microscope slide, covered with saline and examined with a 100X water immersion objective and differential interference contrast optics. Mutant individuals were recognised by homeosis of the peripheral nervous system; in particular, the absence of v'td2 neurons in segment A1. Embryos were then dissected from the vitelline membrane, attached under saline solution to glass slides pre-coated with 10% poly-*L*-lysine (Sigma -Aldrich, Sydney, Australia) and the v'td2 neurons injected with the lipid-soluble carbocyanine dye, 1,1'-dioctadecyl-3,3,3',3'-tetramethyl indocarbocyanine perchlorate (dil; Molecular Probes Inc., Eugene, USA). Microelectrode tips were filled with an ethanolic solution of dye and the shaft filled with 0.1 M LiCl. The microelectrode was brought into contact with a cell body and depolarizing current applied for up to 5 seconds. Embryos were dissected along the dorsal midline to expose the CNS, fixed in 3.4% formaldehyde in saline while still attached to the slide, photoconverted in the presence of 0.2% diaminobenzidene to give a permanent dark reaction product in the DiI-stained neuron (Sandell and Masland, 1988) and drawn using a camera lucida. Specimens were washed in phosphate-buffered saline (PBS) and mounted in 75% glycerol.

Axonal arborizations were drawn with the aid of a camera lucida and the drawings digitised with an Apple scanner. Drawings were scaled to fit a CNS template using the positions of the anterior and posterior commissures, the anterior and posterior fascicles and the width of the CNS as landmarks, using Canvas software (Deneba Software, Miami, USA) on a Macintosh computer. Selected specimens were viewed using a Pulnix video camera (Pulnix America Inc., Sunnyvale, USA) mounted on a microscope and a series of focal planes digitised with a Neotech (Eastleigh, Hampshire, UK) or PixelBuffer (Perceptics, Knoxville, USA) image grabber. Montages of axonal projections were made by collating in-focus regions onto a single plane using the NIH-Image program (written by W. Rasband, available from http://rsb.info.nih.gov/nih-image/index.html).

Immunostaining

After photo-conversion of the fluorescently-stained axon, some embryos were immuno-stained to show Ubx expression in the CNS. Embryos were washed in PBS and blocked in PBS with 0.4% Triton X-100, 0.25% bovine serum albumin and 2% normal goat serum (PBT-NGS) for one hour. They were incubated overnight at 5˚C in a 1:7 dilution of FP3.38 antibody (White and Wilcox, 1984) in PBT-NGS, washed, and incubated for three hours at room temperature in a 1:50 dilution of horseradish peroxidase-conjugated sheep anti-mouse IgG (Amersham Australia, Sydney) in PBT-NGS. The secondary antibody was visualised with 0.04% nickel chloride in a 0.025% diaminobenzidine solution to give a blue reaction product.

Acknowledgements

We thank the Bloomington Stock Centre and E. Lewis for flies, E. Ball, R. White and D. Brower for antibodies, and K. Harris, V. Hartenstein, M. Murray, S. Wang, and G. Whitington for comments. The work was supported by grants from the Human Frontier Science Program Organization and the Australian Research Council.

References

APPEL, B. and SAKONJU, S. (1993). Cell-type-specific mechanisms of transcriptional repression by the homeotic gene products UBX and ABD-A in *Drosophila* embryos. *EMBO J.* 12: 1099-1109.

BENDER, W., AKAM, M.E., KARCH, F., BEACHY, P.A., PEIFER, M., SPIERER, P., LEWIS, E.B. and HOGNESS, D.S. (1983). Molecular genetics of the bithorax complex in *Drosophila melanogaster. Science* 221: 23-29.

BODMER, R. and JAN, Y.N. (1987). Morphological differentiation of the embryonic peripheral neurons in *Drosophila. Roux's Arch. Dev. Biol.* 196: 69-77.

BODMER, R., CARETTO, R. and JAN, Y.N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila* embryos: DNA replication patterns and cell lineages. *Neuron* 3: 21-32.

CARROLL, S.B., LAYMAN, R.A., MCCUTCHEON, M.A., RILEY, P.D. and SCOTT, M.P. (1986). The localization and regulation of *Antennapedia* protein expression in *Drosophila* embryos. *Cell* 47: 113-122.

CARROLL, S.B., WEATHERBEE, S.D. and LANGELAND, J.A. (1995). Homeotic genes and the regulation and evolution of insect wing number. *Nature* 375: 58-61.

DOE, C.Q. and SCOTT, M.P. (1988). Segmentation and homeotic gene function in the developing nervous system of *Drosophila*. *Trends Neurosci.* 11: 101-106.

GHYSEN, A. and LEWIS, E.B. (1986). The function of *bithorax* genes in the abdominal central nervous system of *Drosophila*. *Roux's Arch. Dev. Biol.* 195: 203-209.

GOULD, A.P., BROOKMAN, J.J., STRUTT, D. I. and WHITE, R.A. (1990). Targets of homeotic gene control in *Drosophila*. *Nature* 348: 308-312.

GRABA, Y., ARAGNOL, D., LAURENTI, P., GARZINO, V., CHARMOT, D., BERENGER, H. and PRADEL, J. (1992). Homeotic control in *Drosophila*; the scabrous gene is an *in vivo* target of *Ultrabithorax* proteins. *EMBO J.* 11: 3375-3384.

GREEN, S.H. (1981). Segment-specific organization of leg motoneurones is transformed in *bithorax* mutants of *Drosophila*. *Nature* 292: 152-154.

HAYES, P.H., SATO, T. and DENELL, R.E. (1984). Homoeosis in *Drosophila*: The Ultrabithorax larval syndrome. *Proc. Natl. Acad. Sci. USA* 81: 545-549.

HEUER, J.G. and KAUFMAN, T.C. (1992). Homeotic genes have specific functional roles in the establishment of the *Drosophila* embryonic peripheral nervous system. *Development* 115: 35-47.

HUMMEL, T., SCHIMMELPFENG, K. and KLAMBT, C. (1999). Commissure formation in the embryonic CNS of Drosophila I. Identification of the required gene functions. *Dev. Biol.* 209: 381-398.

JIJAKLI, H. and GHYSEN, A. (1992). Segmental determination in Drosophila central nervous system: analysis of the *abdominal-A* region of the bithorax complex. *Int. J. Dev. Biol.* 36: 93-99.

JIMENEZ, F. and CAMPOS-ORTEGA, J.A. (1981). A cell arrangement specific to thoracic ganglia in the central nervous system of the *Drosophila* embryo: its behaviour in homoeotic mutants. *Roux's Arch. Dev. Biol.* 190: 370-373.

KARCH, F., BENDER, W. and WEIFFENBACH, B. (1990). *abdA* expression in *Drosophila* embryos. *Genes Dev.* 4: 1573-1587.

LEWIS, E.B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* 276: 565-570.

MANN, R.S. (1994). *engrailed*-mediated repression of *Ultrabithorax* is necessary for the parasegment 6 identity in *Drosophila*. *Development* 120: 3205-3212.

MERRITT, D.J. and WHITINGTON, P.M. (1995). Central projections of sensory neurons in the *Drosophila* embryo correlate with sensory modality, soma position and proneural gene function. *J. Neurosci.* 15: 1755-1767.

MURRAY, M.J. and WHITINGTON, P.M. (1999). Effects of *roundabout* on growth cone dynamics, filopodial length, and growth cone morphology at the midline and throughout the neuropile. *J. Neurosci.* 19: 7901-7912.

PROKOP, A., BRAY, S., HARRISON, E. and TECHNAU, G.M. (1998). Homeotic regulation of segment-specific differences in neuroblast numbers and proliferation in the *Drosophila* central nervous system. *Mech. Dev.* 74: 99-110.

PROKOP, A. and TECHNAU, G.M. (1994). Early tagma-specific commitment of *Drosophila* CNS progenitor NB1-1. *Development* 120: 2567-2578.

SANCHEZ-HERRERO, E., VERNOS, I., MARCO, R. and MORATA, G. (1985). Genetic organization of *Drosophila* bithorax complex. *Nature* 313: 108-113.

SANDELL, J.H. and MASLAND, R.H. (1988). Photoconversion of some fluorescent markers to a diaminobenzidine product. *J. Histochem. Cytochem.* 36: 555-559.

SIMPSON, J.H., BLAND, K.S., FETTER, R.D. and GOODMAN, C.S. (2000). Short-range and long-range guidance by Slit and its Robo receptors: a combinatorial code of Robo receptors controls lateral position. *Cell* 103: 1019-1032.

TEAR, G., HARRIS, R., SUTARIA, S., KILOMANSKI, K., GOODMAN, C.S. and SEEGER, M.A. (1996). Commissureless controls growth cone guidance across the CNS midline in drosophila and encodes a novel membrane protein. *Neuron* 16: 501-514.

TEUGELS, E. and GHYSEN, A. (1985). Domains of action of bithorax genes in *Drosophila* central nervous system. *Nature* 314: 558-561.

THOMAS, J.B. and WYMAN, R.J. (1984). Duplicated neural structure in bithorax mutant *Drosophila*. *Dev. Biol.* 102: 531-533.

VACHON, G., COHEN, B., PFEIFLE, C., MCGUFFIN, M.E., BOTAS, J. and 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.

WAKIMOTO, B.T. and KAUFMAN, T.C. (1981). Analysis of larval segmentation in lethal genotypes associated with the antennapedia gene complex in *Drosophila melanogaster*. *Dev. Biol.* 81: 51-64.

WHITE, R.A.H. and WILCOX, M. (1984). Protein products of the bithorax complex in Drosophila. *Cell* 39: 163-171.

WHITE, R.A.H. and WILCOX, M. (1985a). Distribution of *Ultrabithorax* proteins in *Drosophila*. *EMBO J.* 4: 2035-2044.

WHITE, R.A.H. and WILCOX, M. (1985b). Regulation of the distribution of *Ultrabithorax* proteins in *Drosophila*. *Nature* 318: 563-567.