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Early tagma-specific commitment of *Drosophila* CNS progenitor NB1-1

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SUMMARY

The developing central nervous system of many species expresses distinct segment-specific characteristics. We recently described the entire embryonic lineage of *Drosophila* neuroblast NB1-1 and showed that the composition of this lineage differs between the thoracic and abdominal tagmata with respect to the presence or absence of specific glial and neuronal components (Udolph, G., Prokop, A., Bossing, T. and Technau, G. M. (1993) *Development* 118, 765-775). Here, we demonstrate by heterotopic transplantations that tagma specificity of NB1-1 is determined in the neuroectoderm at the early gastrula stage (stage 7). Heterogenetic transplantation and mutant analysis show that the activity of the homeotic genes *Ubx* or *abd-A* is required for the expression of the abdominal variant of the lineage. Heat induction of *Ubx* or *abd-A* expression or their derepression in *Polycomb* mutant embryos can override thoracic determination several hours after gastrulation (stage 10/11). At that stage antibody stainings reveal both proteins to be present in NB1-1 during normal development. Possible mechanisms conferring the early tagma-specific determination are discussed.

Key words: cell lineage, CNS, glia, homeotic genes, determination, *Drosophila*

INTRODUCTION

The ventral nerve cord (vNC) of *Drosophila* derives from neural stem cells, the neuroblasts (NB) (Poulson, 1950), which delaminate from the ventral neurogenic region of the ectoderm (vNR) in a reproducible spatiotemporal segmental pattern (Hartenstein and Campos-Ortega, 1984; Hartenstein et al., 1987; Doe, 1992b). In the trunk, this pattern of segregating NB does not show any obvious differences between segments (Doe, 1992b). However, significant segmental-specificities in the vNC arise gradually during further development: in the course of embryogenesis segment-specific expression of several markers and genes arises (e.g. Jiménez and Campos-Ortega, 1981; Gould et al., 1990; Bourgoin et al., 1992; Graba et al., 1992; Mellerick et al., 1992; Hoshino et al., 1993), and proliferation patterns in the thorax and abdomen diverge gradually (Prokop and Technau, 1991) and become even more distinct during postembryonic stages (Truman and Bate, 1988). Eventually, in the imago, the volume of the thoracic neuromeres clearly predominates over that of the abdominal ones (Hertweck, 1931).

In the early embryo a cascade of patterning genes provides the blastoderm with positional cues along the anterior-posterior as well as the dorsal-ventral axis. These coordinates finally define polarized metameric units, the segments (for review see e.g. Pankratz and Jäckle, 1993). Specification of these segments according to their location in the anterior-posterior axis is mediated by the homeotic selector genes (for reviews see e.g. Lewis, 1978; Duncan, 1987; Peifer et al., 1987; Beachy, 1990; McGinnis and Krumlauf, 1992; Morata, 1993). For the vNC of *Drosophila* it has been demonstrated that mutations in the *bithorax*-complex alter segment-specific patterns of markers and gene expression (Jiménez and Campos-Ortega, 1981; Graba et al., 1992; Gould et al., 1990) and eventually interfere with neuromere-specific morphology (reviewed by Doe and Scott, 1988). However, the mechanisms of segment-specific regulation in the CNS are not yet understood.

In order to address such mechanisms at the cellular level we made use of the previously described embryonic lineage of *Drosophila* neuroblast NB1-1 (Udolph et al., 1993). NB1-1 delaminates from abdominal and thoracic segmental domains of the neuroectoderm and gives rise to the aCC and pCC neurons in all of these segments (Doe et al., 1988a and b; Doe, 1992a). However, further components of the NB1-1 lineage express specific differences between thoracic segments (referred to as thoracic tagma) and abdominal ones (abdominal tagma) (Udolph et al., 1993). We show that tagma specificity of this lineage is determined at the early gastrula stage and is autonomously expressed by the neuroectodermal progenitors upon heterotopic transplantation. Activity of the homeotic genes *Ubx* or *abd-A* is autonomously required for the abdominal pathway of the NB1-1 lineage. By ectopic induction of *Ubx*- or *abd-A* expression until several hours after gastrulation or by homeotic derepression in *Polycomb* mutants, thoracic determination of NB1-1 can be overridden. About the same time, during normal development, UBX and ABD-A are detectable in NB1-1. We suggest that tagma-specific commit-

ment of NB1-1 is induced by an early impact of segmentation genes on later homeotic gene expression.

MATERIALS AND METHODS

Fly stocks

For genetic analysis we used the following mutants: *pb*^{5} is a protein null allele of the *proboscipedia* gene (Pultz et al., 1988; kindly provided by T. Kaufman, Bloomington). Homozygous mutants were identified by the absence of a *TM3* blue balancer chromosome carrying the *lacZ* gene as a marker (courtesy of M. González-Gaitán, Göttingen). *Antp*^{W10} (Wakimoto et al., 1984; kindly provided by J. Castelli-Gair, Cambridge UK and W. Gehring, Basel) is a protein null mutation of the *Antennapedia* gene. Embryos mutant for *Antp* could be detected by the lack of the first midgut constriction (Tremml and Bienz, 1989). *Ubx*$^{6.28}$ (Kerridge and Morata, 1982; kindly provided by R. Reuter, Tübingen) is a deletion in the 5′exon region of the *Ultrabithorax* gene (Beachy et al., 1985). *Ubx* mutants could be identified by the lack of the second midgut constriction (Tremml and Bienz, 1989) and by altered patterns of exit glia cells labeled in *M84* (Klämbt and Goodman, 1991). The *abd-A*^{MX1} mutation (Sánchez-Herrero et al., 1985; kindly provided by R. Reuter) is a deletion in the 5′ exon region of the *abdominal-A* gene (*abd-A*; Karch et al., 1985). Embryos mutant for *abd-A* could be identified by the lack of the second and third midgut constrictions (Tremml and Bienz, 1989) and by the lack of gonads (Cumberledge et al., 1992). *Df(3R)Ubx*^{109} (Lewis, 1978; courtesy of R. Paro, Heidelberg; here referred to as *Df109*) deletes the *Ubx* and *abd-A* genes (Karch et al., 1985; Sánchez-Herrero et al., 1985). *Df109* embryos could clearly be identified by the lack of gonads (see above), even following heat induction of UBX. To identify *Df109* homozygotes in transplantation experiments the stock was balanced with a *TM3* blue balancer chromosome (see above). *Pc*^{3} (Lewis, 1978; kindly provided by R. Paro) is an antimorphic allele of *Polycomb*, that produces a phenotype stronger than the null (Duncan and Lewis, 1982; Haynie, 1983). Homozygous embryos were detected by the use of a blue balancer chromosome (see above). For heatshock induction of *Ubx* we used the *HSU-42* transformant line in a wild-type background or recombined into *Df109* (González-Reyes et al., 1990; González-Reyes and Morata, 1990; both kindly provided by R. Reuter). This *hsp*^{70}*-Ubx* construct is based on the *Ubx Ia* splice variant. For induction of ABD-A we made use of a *hsp*^{70}*-abd-A* stock (González-Reyes et al., 1992; courtesy of E. Sánchez-Herrero, Madrid). Heat induced *HSU-42* and *hsp*^{70}*-abd-A* embryos were clearly distinguishable by different effects on the thoracic cuticle according to previous descriptions. For labeling of the A- and B-glia cells we used the enhancer trap lines *M84* (P-element insertion on the 2nd chromosome) and *P101* (3rd chromosome) (Klämbt et al., 1991; Klämbt and Goodman, 1991; both kindly provided by C. Klämbt, Köln). To aid in NB identification we made use of the enhancer trap line *5953* (Doe, 1992b; courtesy of C. Doe, Urbana).

Cell transplantation

Transplantations were carried out according to Technau (1986) and Prokop and Technau (1993a). Donor and host embryos were of the same stage, approximately 10 minutes after the onset of gastrulation (stage 7). Using a pulled and ground glass capillary, with an inner diameter of about 10 μm, cells were removed from the vNR of the donors at about 0-20% ventrodorsal diameter and at either 30% egg length for abdominal or at 60% egg length for thoracic transplantations (according to the early gastrula fate map; Technau and Campos-Ortega, 1985; see Fig. 1B in Technau, 1987). In about one third of the cases the cells were washed in a drop of Ringer's or 0.2 M KCl before implantation into unlabeled hosts (see also Meise and Janning, 1993). This treatment was to clean cells from attached material of their microenvironment and to obtain a more stringent control on cell autonomy. In all cases single cell implantation into hosts was inspected with Nomarski optics. Hosts were reared into the embryonic stage 16/17.

Preparation and staining of donors and hosts

Stage 16/17 embryos were fixed with glutaraldehyde saturated heptane according to Zalokar and Erk (1977). During fixation, embryos remained stuck to the cover-slip. After several washes with phosphate buffer (PB) the embryos were mechanically removed from the vitelline membrane with a pulled and broken glass capillary. Embryos were stained in a solution of 0.1% diaminobenzidine in PB for about 5 minutes. For double labeling experiments we used the enhancer-trap line *P101* as donors and hosts. Following diaminobenzidine treatment donors were stained for β-galactosidase activity in X-Gal solution (adding one part of 20% X-Gal in DMSO to 99 parts of a warm (approx. 60˚C) solution of 150 mM NaCl, 1 mM $MgCl_2$, 3.3 mM $K_3[Fe(CN)_6]$ in PB) at 37˚C for several hours. For determination of the genotype of donors carrying a blue balancer chromosome, the embryos were raised to stage 14/15, fixed and washed as mentioned above (still stuck to the cover-slip), the vitelline membrane was torn with a broken capillary and the whole cover slip covered with X-Gal solution. Embryos that did not stain blue were homozygous for the mutation. Hosts that received cells from such embryos were treated separately. Embryos were dehydrated in alcohol, cleared in xylene, embedded in Araldite (Serva) and sucked into glass capillaries of 0.2 mm inner diameter (Hilgenberg), to allow inspection from all sides (for details see Prokop and Technau, 1993a).

Immunohistochemistry

Staged embryos were stained with antibodies as described elsewhere (e.g. Schmidt-Ott and Technau, 1992). The final dilutions of primary antibodies were 1:1500 for anti-abd-A (Macías et al., 1990; kindly provided by E. Sánchez-Herrero, Madrid), 1:3 for anti-eve (Frasch et al., 1987; kindly provided by S. Romani, Cambridge), 1:5000 for anti-β-galalactosidase (Cappel) and 1:20 for anti-Ubx (White and Wilcox, 1984 and 1985; kindly provided by R. White, Cambridge). Following the diaminobenzidine reaction embryos were washed in PB. Specimens were dehydrated in ethanol, cleared in xylene and embedded in Araldite (Serva). Single embryos were selected and transferred to a slide with a small drop of Araldite. Whole ventral nerve cords were dissected out using forceps and sucked into borosilicate capillaries with an outer diameter of less than 0.2 mm (Hilgenberg).

Heatshock procedures

Flies were allowed to lay eggs for 30 minutes onto a filter paper soaked with yeast suspension. Egg laying and further development was carried out at 25˚C. For heat shocks, the filter paper was transiently transferred to a 37˚C wet chamber, so that the embryos directly contacted the warm water. After a heatshock period of 30, 45 or 60 minutes the filter paper with the embryos was kept in a humid chamber at 18˚C. In combined transplantation- and heatshock-experiments the transplantations were carried out at room temperature throughout a period of approx. 1 hour, the coverslip with the hosts was transferred for 30 or 45 minutes to a humid chamber at 25˚C and subsequently for 30 or 45 minutes to a 37˚C weighing dish with 3S fluorocarbonoil. Further incubation took place at 18˚C. In most cases donors were heat-shocked in parallel, subsequently incubated at 25˚C and analyzed for the cuticular phenotype under the dissecting microscope prior to preparation of the hosts.

Cuticle preparations

In all experiments, the genotype of embryos was verified by cuticle preparations (Wieschaus and Nüsslein-Volhard, 1986). Embryos were kept at 25˚C until viable larvae hatched. The remaining eggs were collected with a needle, directly transferred to Hoyer's medium, incubated at about 60˚C for several hours and analyzed with Nomarski optics.

RESULTS

Tagma specificity of the NB1-1 lineage is determined at the early gastrula stage

During embryogenesis *Drosophila* NB1-1 gives rise to a unique lineage. The sibling neurons aCC and pCC represent its first progeny in the thorax as well as in the abdomen (Doe and Scott, 1988). However, among further components of the NB1-1 lineage there are characteristic differences between these tagmata (Udolph et al., 1993): 2-3 subperineural glia cells (SuPn-cells) are part of the lineage only in the abdomen; the thoracic lineage, however, has a higher number of neurons, among them 1-2 motoneurons, that are not present in the abdomen (Fig. 1). The composition of thoracic and abdominal NB1-1 lineages was disclosed by DiI labeling, HRP-injections and isotopic transplantations of HRP-labeled neuroectodermal progenitor cells at the onset of gastrulation (stage 7; stages according to Campos-Ortega and Hartenstein, 1985). We then transplanted neuroectodermal cells heterotopically to test for determination of tagma specificity at the early gastrula stage. Those transplants that resulted in NB1-1 lineages were analyzed at stage 16/17.

(1) Cells were randomly taken from ventral sites of the thoracic vNR and transplanted into the ventral vNR of the abdomen. 8 NB1-1 lineages were obtained from these experiments. All of them showed the typical thoracic characteristics (Table 1, Fig. 2), indicating that the neuroectodermal progenitors developed according to their origin. The ectopic motoneuronal projection of the cluster cells (see Fig. 2), which does not normally exist in the abdomen, projected into the region between the ventral oblique muscles 14.1 and 14.2 and the ventral tip of the lateral transverse muscles 21 to 23 (descrip-

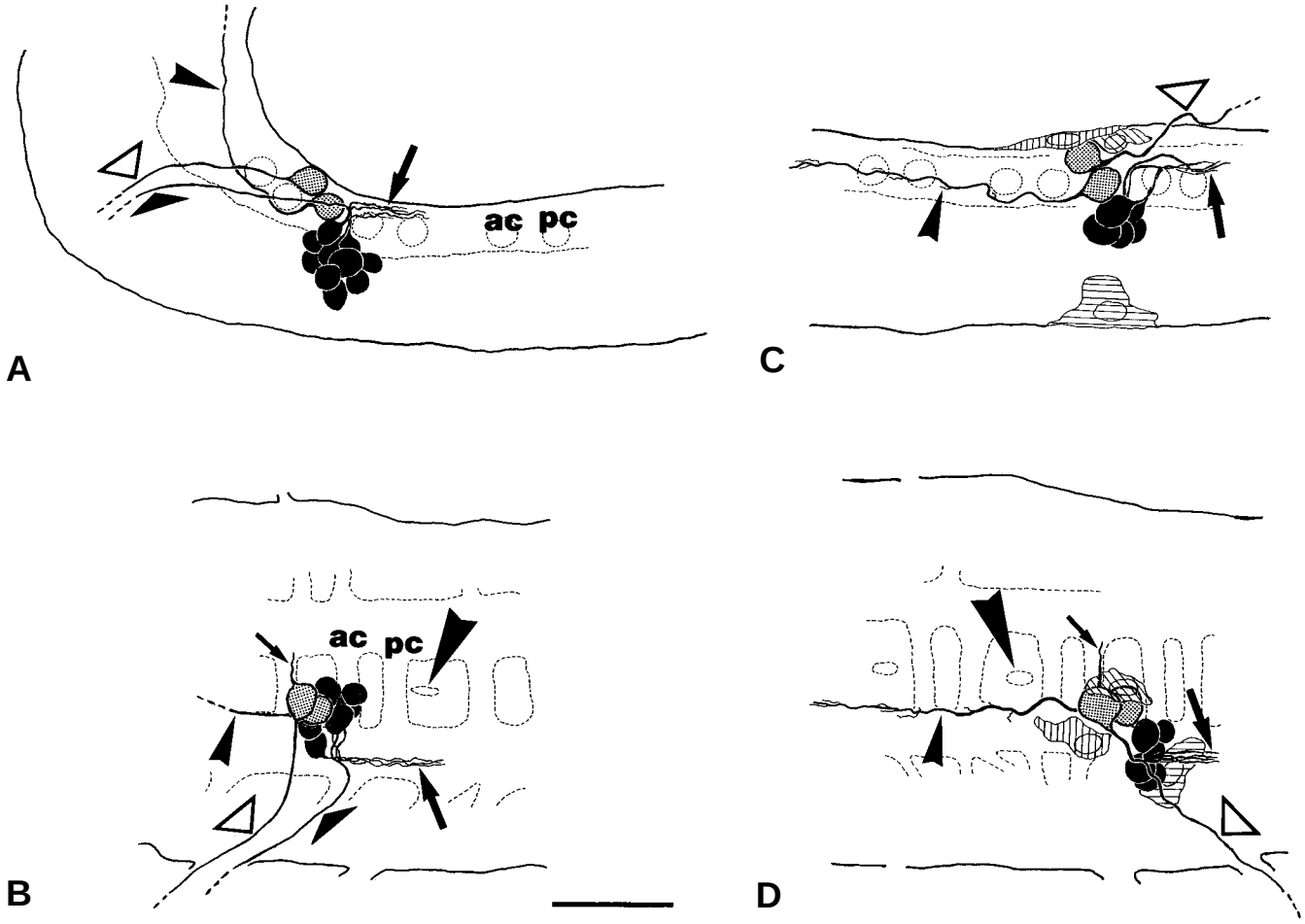


Fig. 1. Camera lucida drawings of the thoracic (A,B) and abdominal variant (C,D) of the NB1-1 lineage derived from isotopic transplantations. (A,C), Lateral views; (B,D), horizontal views, anterior points to the left. The aCC motoneuron and the pCC interneuron (shaded) show no obvious differences between thoracic and abdominal lineages with regard to their intrasegmental position and projections. In thorax and abdomen a cluster of neuronal cells (black) is located in a comparable position and forms a similar ipsilateral fascicle (large arrow) directed posteriorly. However, the number of cells in the cluster is significantly higher in the thorax (8-15 cells) than in the abdomen (4-6 cells; see also Table 1). Furthermore, only the thoracic cluster forms a motoneuronal projection (flattened black triangle), consisting of 1-2 fibers, which leave the vNC via the segmental nerve. Only the abdominal lineage contains three SuPn-glia cells: one ventral glia cell (horizontally hatched) one dorsal B-glia cell anterior to aCC/pCC (vertically hatched) and one dorsal A-glia cell posterior to aCC/pCC (diagonally hatched). For more detailed descriptions see Udolph et al. (1993). Large arrowheads, dorsoventral channels at the neuromere border; solid lines, outline of the CNS; broken lines, outline of the neuropil; open triangle, axon of the aCC motoneuron; ac, anterior commissure; pc, posterior commissure; small arrow, contralateral projection of the aCC; small arrowhead, interneuronal ipsilateral projection of the pCC. Scale bar, 20 μm.

Table 1. Composition of NB1-1 clones derived from different transplantation experiments

No	NM	aCC	pCC	vG	dG	Cl	MN
Isotopic transplantations : A to A							
1	A1	1	1	1	1?	5*	–
2	A1/A2	1	1	1	1 - 2	5	–
3	A4?	1	1	–	1	6	–
4	A5	1	1	1	1 - 2	5*	–
5	A5	1	1	1	2	5	–
6	A5	1	1	1?	-	5 - 6	–
7	A5?	1	1	1	1 - 2	5	1?
8	A5/A6	1	1	1	1	4 - 5*	–
9	A6?	1	1	1	1 - 2	5 - 6	–
10	A6	1	1	1	1	5	–
11	A7/A8	1	1	1	2	5	–
12	A8	1	1	1	1 - 2	5	–
Isotopic transplantations : T to T							
13	T2	1	1	–	–	9 - 11*	1 - 2
14	T2	1	1	–	–	11 - 12	2
15	T2	1	1	–	–	13 - 14	1
16	T2	1	1	–	–	12*	–
17	T2	1	1	–	–	8 - 10*	1?
18	T2	1	1	–	–	10 - 11	1 - 2
19	T3	1	1	–	–	10 - 12	1
20	T3	1	1	–	–	8 - 9*	1
Heterotopic transplantations : T to A							
21	A4	1	1	–	–	10 - 11*	–
22	A4	1	1	–	–	13 - 14	1 - 2
23	A5	1	1	–	–	10 - 12	1 - 2
24	A5	1	1	–	–	10 - 12	2
25	A5	1	1	–	–	12 - 13	2
26	A6	1	1	–	–	14 - 15*	2
27	A6	1	1	–	–	8 - 9	1
28	A6	1	1	–	–	12 - 13	2
Heterotopic transplantations : A to T							
29	T1?	1	1	1	1 - 2	4 - 5*	–
30	T2	1	1	1	1 - 2	4	–
31	T2	1	1	1?	1	5	–
32	T2	1	1	1	1 - 2	5*	–
33	T2	1	1	1	- ?	4 - 5	–
34	T2	1	1	1	1	5	1
35	T2/3	1	1	1	2	5	–
36	T2/3	1	1	1	1	5	–
37	T3	1	1	1	2 - 3	4	–
38	T3	1	1	1	1	6	–
39	T3	1	1	1	1 - 2	3	–
40	T3/A1	1	1	1 - 2	1 - 2	5 - 6*	–
Abdominal transplantations: Df 109 into wild type							
41	A4	1	1	–	–	11 - 12	1
42	A4	1	1	–	–	14 - 15	1
43	A4	1	1	–	–	11 - 12	1
44	A5	1	1	–	–	11 - 12	1
45	A5	1	1	–	–	10 - 11	1
46	A6?	1	1	–	–	12 - 13	1
47	A6	1	1	–	–	10 - 11	1
Thoracic transplantations: HSU-42 into wild type							
48	–	1	1	**1**	**2**	**5**	**–**
49	–	1	1	–	**1 - 2**	**5 - 6***	**–**
50	–	1	1	**1**	**2**	**6**	1
51	–	1	1	–	**1 - 2**	**5 - 7**	1
52	–	1	1	–	?	8	1
53	–	1	1	–	–	10	1
54	–	1	1	–	–	9 - 11	1 - 2
55	–	1	1	–	–	10 - 12	1

Numbered (No) horizontal lines represent individual clones. Cases are arranged according to the neuromere (NM) they are located in (T, thoracic; A, abdominal) and to the kind of experiment they originate from (see text; isotopic transplantations were published in Udolph et al., 1993 and are listed for reasons of comparison). Cell numbers of the various clonal components are listed in subsequent columns: aCC motoneuron (aCC), pCC interneuron (pCC), ventral glia (vG), dorsal A- and B-glia (dG), cells in the ventral cluster (Cl) and motoneuronal fibers of the cluster labeled in addition to the aCC motoaxon (MN). Cases in which cells of the cluster are shifted from their normal position towards more dorsal or ventral sites are marked (*). Those components of thoracically derived lineages (last experiment) that express abdominal characteristics following heat induction of *Ubx* are emphasized by bold type.

tion of muscles according to Bate, 1993). These regions are comparable to the target sites of these motoneurons in thoracic segments (Udolph et al., 1993). In the single case where this motoneuronal projection was missing (No. 21 in Table 1), further criteria (10 cluster cells, no glia) pointed towards a thoracic identity of that lineage.

(2) Transplantations from the ventral abdominal vNR into the ventral thoracic vNR resulted in 12 NB1-1 lineages. All of them expressed the abdominal characteristics (Table 1; Fig. 2). One of these clones derived from a cell taken from the enhancer-trap line *P101* (Klämbt et al., 1991; see Materials and methods), which expresses β-galactosidase (β-gal) in the A- and B-glia cells (Klämbt and Goodman, 1991). These cells correspond to the dorsal SuPn glia cells in the abdominal NB1-1 clone (Udolph et al., 1993). Although these cells are normally not found in the thorax of *P101*, at least the anterior dorsal glia cell in the transplant was double labeled for β-gal and HRP, demonstrating its identity as B-glia (not shown). The fact that we could not detect β-gal in the A-glia of this clone is most likely due to the limits of the double labeling technique (see Udolph et al., 1993). Taken together, these results indicate that neuroectodermal progenitors are committed with respect to thoracic versus abdominal specificity at the onset of gastrulation (stage 7), 40-50 minutes before the delamination of NB1-1 (see Doe, 1992b; Udolph et al., 1993). Cells at this stage autonomously express the thoracic or the abdominal NB1-1 lineage pathway.

Genes of the *bithorax*-complex confer tagma-specific differences to the NB1-1 lineage

Homeotic selector genes of the *Antennapedia*- and *bithorax*-complex (Lewis, 1978; Kaufmann et al., 1980) have been shown to coordinate segment specificity in the CNS of *Drosophila* (for review see Doe and Scott, 1988). In order to test their influence on the tagma specificity of the NB1-1 lineage, we analyzed the pattern of NB1-1 lineage components in embryos mutant for those homeotic genes that are expressed in most or all thoracic and abdominal neuromeres. As a marker we used the enhancer-trap line *M84* (Klämbt et al., 1991; see Materials and methods) which, like *P101*, expresses β-gal in the A- and B-glia, arising from abdominal NB1-1 only. In addition, some of the mutants were stained with anti even-skipped antibodies (eve; see Materials and methods), which at stage 16 detect the aCC/pCC neurons in the neuromeres of T1 to at least A7 (Doe et al., 1988a and b; Doe and Scott, 1988; Patel et al., 1989).

The homeotic gene *proboscipedia* (*pb*) is expressed in all thoracic and abdominal segments, but is most strongly expressed in head segments (Pultz et al., 1988; Mahaffey et al., 1989). Embryos mutant for the null allele pb^5 (see Materials and methods) did not reveal any pattern defects in the vNC when tested for *M84*- or *eve*-expression (data not shown).

Antennapedia (*Antp*) is expressed in all thoracic and abdominal segments with highest concentrations in the thorax (e.g. Levine et al., 1983; Martínez Arias, 1986; Bermingham et al., 1990). Inspection of embryos mutant for the null allele $Antp^{W10}$ (see Materials and methods) did not reveal any pattern defects in *M84*- and *eve*-expression.

Ultrabithorax (*Ubx*) is expressed from T2p (p, posterior) to A9 with a peak in T3p/A1a (a, anterior) (reviewed by Beachy, 1990). Embryos mutant for the $Ubx^{6.28}$ allele (see Materials

and methods) lacked the A- and B-cells in A1, where instead thorax-specific A/B-like glia cells could be detected; these do not derive from NB1-1 (Fig.3; Udolph et al., 1993). Thus, normal function of *Ubx* is required for the expression of abdominal characteristics of the NB1-1 lineage in A1.

The *abdominal-A* gene (*abd-A*) is expressed from A1p to A8 (Harding et al., 1985; Karch et al., 1990; Macías et al., 1990). Embryos mutant for the null allele *abd-A*^{MX1} (see Materials and methods) did not show any defect of the A- and B- glia pattern. However, in embryos homozygous for *Df109* (a deletion covering the genes *Ubx* and *abd-A*; see Materials and methods), the A- and B-cells were clearly substituted with A/B-like glia in A1-7, whereas the pattern appeared unaffected in A8 (Fig. 3). These observations suggest that *abd-A* regulates abdominal development of the NB1-1 lineage in A2-7. However, this function seems to be replacable by *Ubx* (see Discussion).

To test whether homeotic genes regulate further tagma-specific components of the NB1-1 lineage, we made use of the significant phenotype of *Df109*. We transplanted cells at the early gastrula stage from the ventral abdominal vNR of *Df109* into the same region of wild-type embryos. 7 NB1-1 lineages were obtained from donors homozygous for *Df109* (as identified by the absence of a blue balancer, see Materials and methods). All of them expressed the thoracic characteristics: no glial cells, additional motoneuronal projection and 10-15 cells in the ventral cluster (Table 1). All NB1-1 from blue balancer-carrying donors expressed abdominal characteristics. These heterogenetic transplantations show that, independent from the genotype of surrounding tissue, the absence of *Ubx* and *abd-A* is sufficient to allow thoracic development of the complete NB1-1 lineage.

Taken together, the expression of *Ubx* or *abd-A* is not required for NB1-1 development per se but is essential for the expression of the abdominal variant of the NB1-1 lineage, whereas *Antp* and *pb* seem to play no role in NB1-1 development.

Ectopic expression of *Ubx* or *abd-A* can override thoracic determination of NB1-1

The heterotopic transplantations revealed that NB1-1 progenitors are committed to thoracic or abdominal development at the early gastrula stage (about 3 hours AEL, stage 7). To determine the temporal profile in which *Ubx* expression exerts regulatory effects on the NB1-1 lineage, we crossed flies, carrying an *hsp*^{70}*-Ubx* construct (*HSU-42*; González-Reyes et

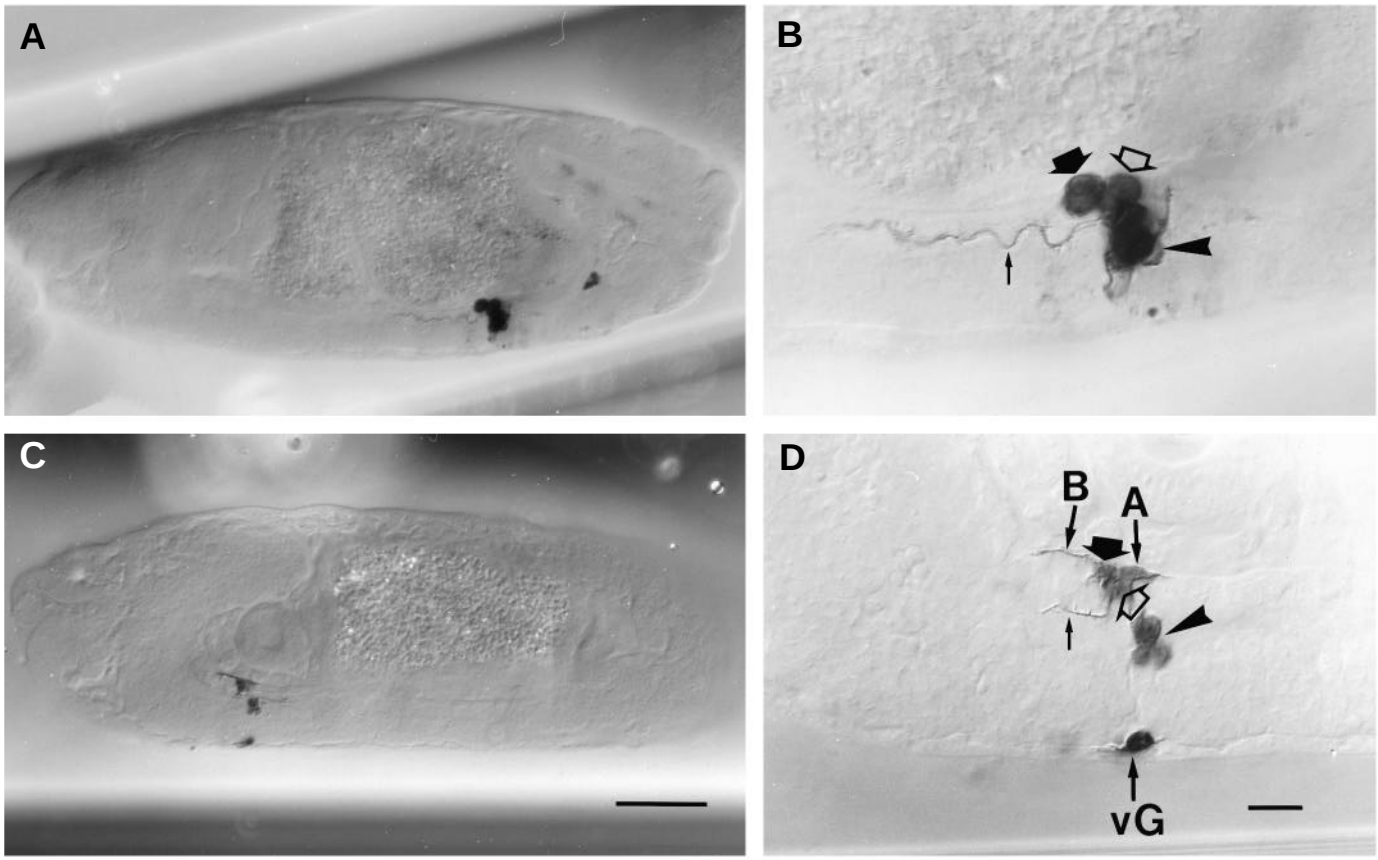
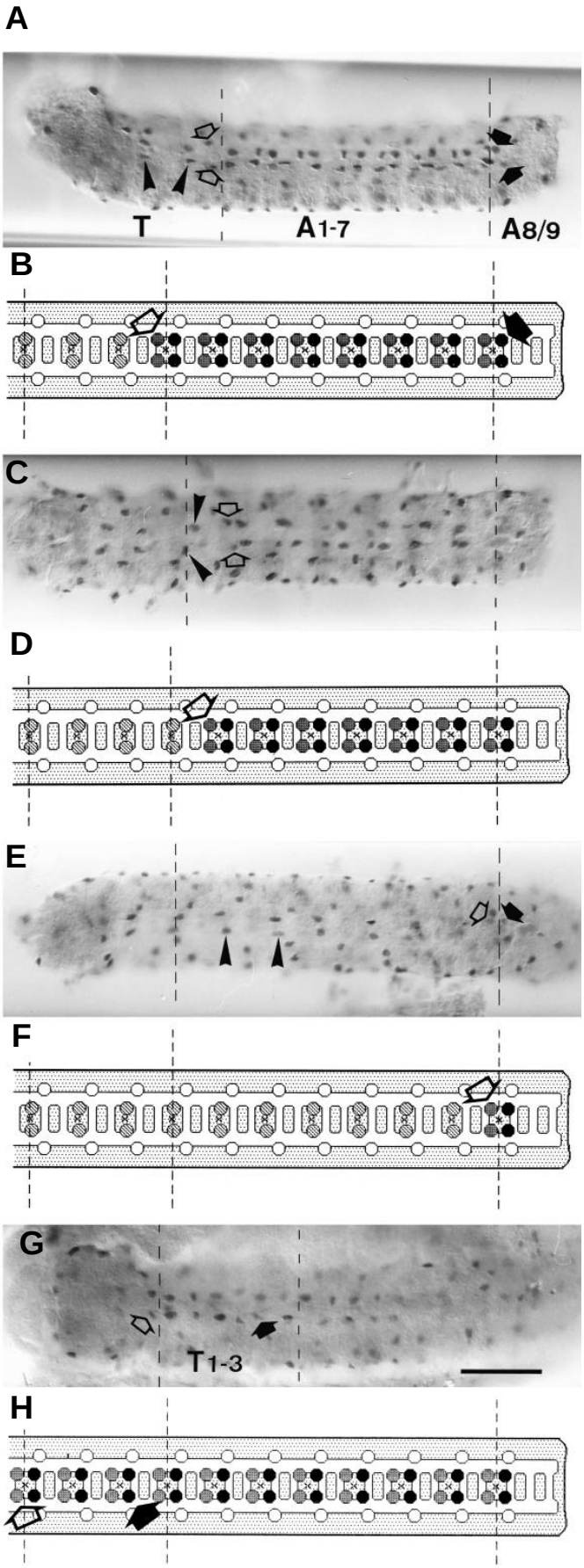


Fig. 2. NB1-1 lineages derived from ventral vNR cells upon heterotopic transplantations along the a-p-axis. (A,C) Whole mounts of stage 16/17 embryos (anterior to the left); (B,D) close-ups of the HRP-labeled lineages in A and C. (A,B) NB1-1 lineage obtained from a thoracic vNR cell upon transplantation into the abdominal vNR. The lineage expresses thoracic characteristics, although it developed in the abdominal neuromer A4 (see location in A): the cell cluster (large arrowhead) comprises about 10 cells, the glial cells are missing (compare D). Conversely, the progenitor for the NB1-1 lineage in C and D derived from the abdominal vNR. Although it developed in T2, it expresses abdominal features like the A-, B- and ventral glia (A,B, vG) and a small cluster of only 4 cells (arrowhead). Broad black arrow, aCC; broad open arrow, pCC; small arrow, axon of the pCC; Scale bars, 50 μm in A,C and 10 μm in B,D.



al., 1990; see Materials and methods), into the enhancer-trap line *P101*. *Ubx* was induced at different time periods and the A- and B-glia pattern was analyzed in thoracic neuromeres. *P101* control embryos without the *HSU-42* construct were insensitive to heat induction and no glial cells near the dorsal midline of the thorax were labeled (data not shown; see Udolph et al., 1993). However, *P101*;*HSU-42* embryos heat shocked 4 hours AEL showed a complete or almost complete pattern of ectopic A- and B-glia cells in T1-3 (Fig. 3). Also *Df109*, *HSU-42* embryos (carrying the *M84* insertion; see Materials and methods), which at 25˚C lacked A- and B-glia cells in A1-7 (see above), expressed these cells throughout the vNC when *Ubx* was heat induced 4 hours AEL (33 out of 37 analyzed *Df109* embryos). Induction of *Ubx* later than 4 hours AEL led to a gradually decreasing number of ectopically induced A- and B-glia cells in the thorax (Fig. 4), although there was still an effect when heat induction started at 7 hours AEL (stage 11).

Similar results were obtained upon heat induction of *abd-A* (see Materials and methods): 60 minute heat shock starting at 4 hours AEL led to ectopic A- and B-glia cells in the thorax, but almost no effect was seen when the heat shock started at 7 hours AEL. Thus, the competence of NB1-1 to respond to these proteins follows a comparable time course.

To test whether heat induction of *Ubx* influences other segment-specific features of the NB1-1 lineage, HRP-labeled cells were transplanted from the presumptive thoracic region of *HSU-42* embryos into wild-type hosts. 30-105 minutes after the transplantation, hosts were heat induced for 30 or 45 minutes (see Materials and methods) and then raised to embryonic stage 16/17 (Table 1). Two of the eight resulting NB1-1 clones expressed all abdominal characteristics (glial cells, no additional motoaxon, a small cell cluster), and two further lineages expressed abdominal features (glial cells, small cell cluster) as well as thoracic ones (an additional motoaxon). However, four of the clones were completely thoracic, likely due to the fact that *Ubx* was induced too late,

Fig. 3. Effects of mutations or overexpression of *Ubx* and/or *abd-A* on the pattern of A- and B- glia. Dorsal views of isolated vNC of stage 16/17 embryos carrying the *M84* (a-f) or *P101* (g,h) P-element insertion and stained with antibodies against β-gal. (B, D, F, H) show schematic drawings of (A, C, E, G). Dashed lines indicate the border between subesophageal ganglion and T1, between T3 and A1 and between A7 and the terminal neuromer A8/9 (from left to right). (A,B) The wild-type situation: the most anterior B-glia cells (darkly shaded circles; open arrows in a and b) derive from the NB1-1 in A1, the most posterior A-glia cells (black circles; black arrows in a and b) seem to originate from NB1-1 in A8. A pair of A/B-like glia cells (hatched circles; arrowheads) is located most anteriorly in each thoracic neuromere. These cells do not derive from NB1-1. (C,D) In $Ubx^{6.28}$ the A- and B-glia cells at the T3/A1 border are replaced by A/B-like cells (arrowheads). (E,F) *Df109* embryos show A- and B-glia only at the border of A7 and A8/9, whereas all the other neuromeres contain A/B-like glia. (G,H) Embryos of the enhancer trap line *P101* do not stain any glial cells near the dorsal midline of the thorax (data not shown; see Udolph et al., 1993). However, following a 45 minute heat shock starting at 4 hours AEL, ectopic A- and B-glia cells are detectable in the neuromeres T1-T3 of *P101*;*HSU-42* embryos (demarcated by an open and black arrow). Shaded area, cortex region; white circles, dorsal SuPn-glia cells flanking the A-, B, and A/B-like glia in the abdomen and thorax of *M84* embryos; white area, neuropil. Scale bar, 50 μm.

suggesting that regulation of other segment-specific components of the NB1-1 lineage follow a time course similar to the A- and B-glia (compare variable effects in Fig. 4). Taken together, these experiments show that the induction of *Ubx* or *abd-A* expression about 4-6 hours AEL (stage 10/11) is sufficient to induce abdominal development of the complete NB1-1 lineage and to override thoracic commitment.

As ectopic expression of homeotic genes is genetically induced in mutants of *Polycomb* group genes (reviewed by Paro, 1990), we also analyzed the effect of Pc^3 (see Materials and methods) on the NB1-1 lineage by looking at *M84* and *eve* expression. In the abdomen, Pc^3 embryos showed quite normal patterns of *eve*- and *M84*-positive cells, although cells were sometimes dislocated, and single cells, including A- and B-glia and aCC/pCC, were missing at random. For the thoracic vNC of Pc^3 tagma-specific defects had been described before (Jiménez and Campos-Ortega, 1981) and were also detectable in our preparations: the number of anti-eve positive EL neurons (Patel et al., 1989; normally about 4 in the thorax) was increased to the abdominal numbers of 5-7 in all thoracic neuromeres. Also ectopic A- and B-glia cells were detected in most embryos, however, they were always restricted to T3, whereas T1 and T2 contained the normal set of A/B-like glia (not shown). Thus, although Pc^3 in principle interferes with all thoracic neuromeres, it affects the regulation of tagma-specific NB1-1 components only in part of the thorax (see also below).

UBX and ABD-A are localized in NB1-1 during normal development

Referring to existing NB maps (Doe, 1992) we examined NB1-1 for the presence of UBX and ABD-A in the wild type. During stage 10, antibody staining for both proteins can be detected in the NB-layer. However, in our preparations strong staining of either protein in the nucleus of NB1-1 was first detected at early to mid stage 11 (Fig. 5). UBX was clearly detected in A1, but not in T3. ABD-A in NB1-1 was found in variable numbers of neuromeres within the *abd-A* expression domain A1p to A8a. Thus, UBX and ABD-A are first detectable in NB1-1 of the wild type at least 2-3 hours after its segment-specific commitment, at a time when heat induction still revealed regulative response of the NB1-1 lineage (see Discussion). Similar staining can also be detected in neighbouring NB such as NB6-1 or NB7-1.

In Pc^3 mutant embryos, at stage 16, the UBX and ABD-A pattern is similar in all neuromeres of the vNC and matches more or less the wild-type pattern of A7/8 (Beachy et al., 1985; Simon et al., 1992; own observations). However, these patterns are built up gradually (Simon et al., 1992; Struhl and Akam, 1985). Ectopic expression of both proteins in the NB layer starts at stage 10/11. UBX expressing NB are clearly more abundant in T3 than in more anterior locations (Fig. 6), although we did not detect nuclear UBX in cells likely to be thoracic NB1-1. Ectopic ABD-A could be clearly detected in NB near the border of T2/T3, but not, or only very weakly, in more anterior positions. In some specimens NB1-1 appeared to be among the ABD-A-positive NB in T3 as judged from morphological markers (see Fig. 6). Thus the staining pattern of UBX and ABD-A is consistent with the fact that A- and B-glia are ectopically induced in *Pc3* only in T3 (see above).

Taken together, effects of heat induced homeotic gene expression, and of ectopic expression in Pc^3, as well as the time

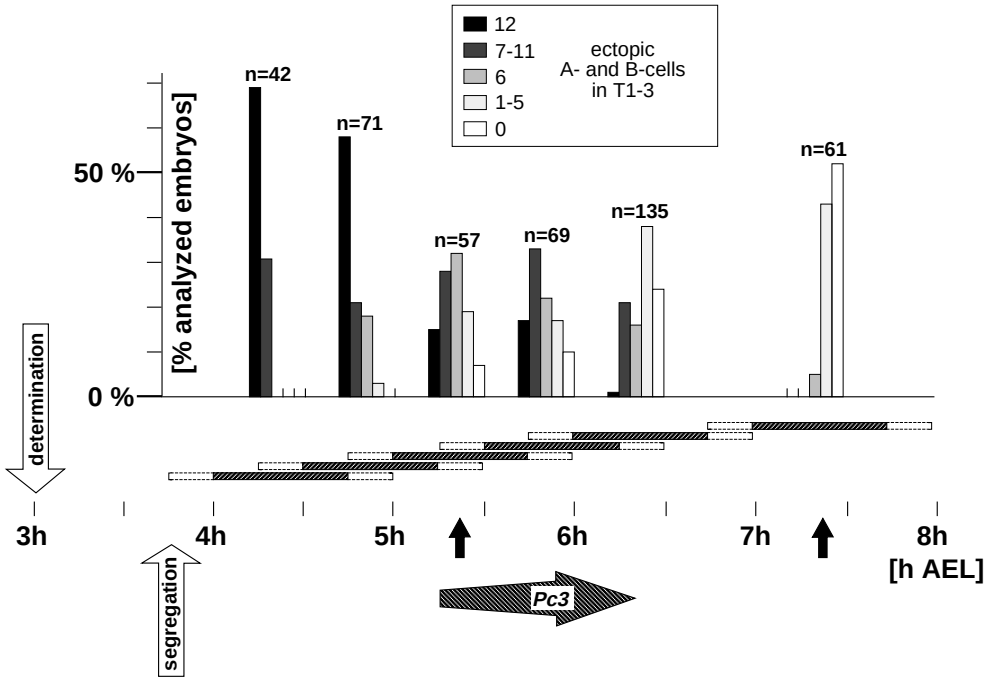


Fig. 4. Effect of heat induced UBX on the thoracic A- and B-glia pattern in *HSU-42*. The abscissa marks the time of development in hours after egg laying (h AEL), black arrows label the beginning and end of stage 11. Eggs were collected over 30 minutes and were heat shocked for 45 minutes starting at 4, 4.5, 5, 5.5, 6 and 7 hours AEL. Embryos heat shocked earlier than 4 hours AEL were not analyzed because of the resultant alterations in morphology, but clearly expressed ectopic A- and B-glia cells in the thorax. Darkly hatched horizontal bars indicate the periods of *Ubx* induction with their open dashed ends indicating variabilities between eggs taking into account the period of egg collection. White, shaded and black vertical columns above each bar represent the respective number of heat-shocked embryos carrying 0, 1-5, 6, 7-11 or 12 (4 per neuromere) ectopically induced A- and B-glia cells in T1-3 (see frame). The number of analyzed embryos per experiment is indicated (n). The time of segment-specific determination of neuroectodermal progenitors (as deduced from heterotopic transplantations; see Table 1) and the time of segregation of NB1-1 from the vNR (according to Doe, 1992b; Udolph et al., 1993) are indicated by open arrows. The darkly hatched arrow indicates the approximate time at which UBX and ABD-A become detectable in the thorax of Pc^3 (see Fig. 6).

when the proteins become detectable in NB1-1 suggest that *Ubx* and *abd-A* mediate tagma-specific differentiation of the NB1-1 lineage at stage 11, i.e. 2-3 hours after the progenitor becomes committed for tagma specificity.

DISCUSSION

Early commitment for tagma specificty of neuroectodermal cells

NB1-1 provides a good model system for the study of mechanisms underlying tagma specificity in the developing CNS of *Drosophila*. It expresses tagma-specific lineage differences during embryogenesis and is readily amenable to detailed analysis by independent markers for specific lineage components and by experimental manipulations like cell transplantation (Udolph et al., 1993).

The transplantation experiments described here revealed that early gastrula (stage 7) CNS progenitor cells develop according to the tagma of their origin and autonomously express the thoracic or abdominal variant of the NB1-1 lineage. One could argue that the autonomous behavior of the cells is an artefact due to the transplantation procedure, which might render the cells insensitive to external cues. This, however, is very unlikely, since studies on NB1-1 and other lineages revealed that transplanted progenitors react to and interact with the host tissue. For example, the isotopic implantation of a cell is regulated in the neuroectoderm of the host, as it does not independently produce an additional NB1-1 lineage (Udolph et al., 1993). Furthermore, single progenitor cells transplanted from lateral to ventral sites of the neuroectoderm are amenable to signals inducing ventral cell fates. (Doe and Technau, 1993; Udolph and Technau, unpublished results; for further examples see Technau and Campos-Ortega, 1986; Stüttem and Campos-Ortega, 1991). Therefore, our transplantations strongly suggest that NB1-1 progenitors are normally already committed to thoracic or abdominal development in the neuroectoderm, about 40-50 minutes before they delaminate. It is

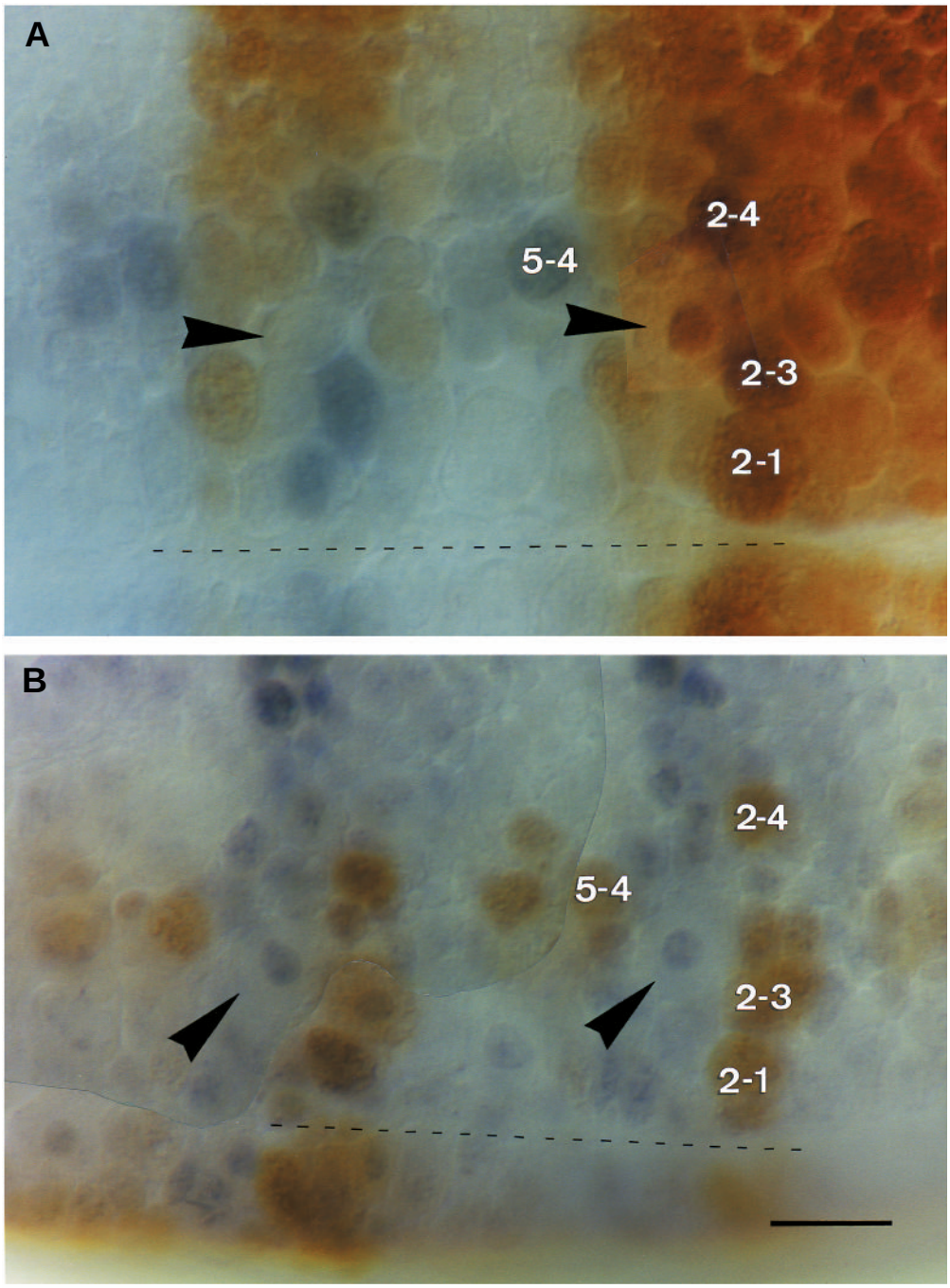


Fig. 5. Nuclear expression of UBX (A) or ABD-A (B) in NB1-1 at early to mid stage 11. Anterior is to the left; a broken line indicates the midline. Stages were determined by the presence of tracheal pits and by the morphology of the alimentary canal primordia (Campos-Ortega and Hartenstein, 1985; Skaer, 1993). Antibody stainings have been carried out in the enhancer trap line *5953* (Doe, 1992b). Numbers indicate those β-gal labeled NB of *5953* (grey in A, and brown in B), that had been used as landmarks for the identification of NB1-1. Also the pattern of further NB around NB1-1 (not all in focus) is in accord with the NB-map of stage 11 (Doe, 1992b). (A) Staining for UBX is brown. Strong nuclear UBX is detected in the NB1-1 of A1 (right arrowhead) but not in T3 (left arrowhead). (B) Staining for ABD-A is grey. Arrowheads indicate nuclear ABD-A in NB1-1 of A4 and A5 respectively. Note that homeotic proteins appear cytoplasmic in several other NB. Scale bar 10 μm.

important to note that these experiments do not address the mechanisms involved in the regulation of the intrasegmental specification of progenitors along the a-p axis.

As shown in other sets of experiments, neuroectodermal cells turned out to be committed also with respect to tagma-specific proliferation behaviour of NB during embryonic and postembryonic development (Prokop, 1993; Prokop and Technau, 1993b). Thus, early commitment may be a common feature of tagma- or *even* segment-specificity during development of the CNS. The same might also be true for other ectodermal derivatives (Simcox and Sang, 1983; Chan and Gehring, 1971).

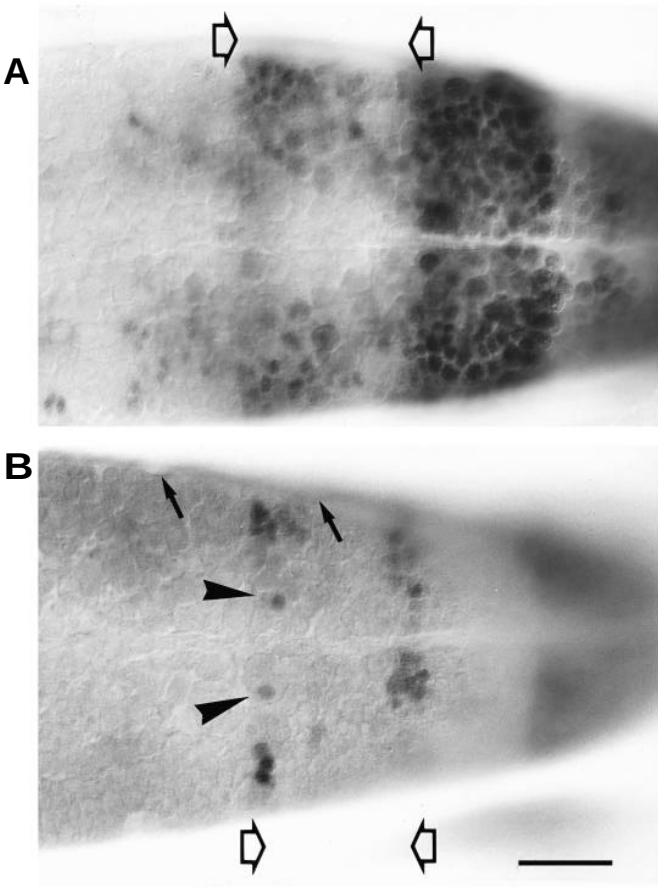


Fig. 6. Ectopic expression of *Ubx* and *abd-A* in Pc^3 embryos. Both pictures present ventral views of Pc^3 embryos at mid stage 11, as judged by the morphology of the alimentary canal primordia (Campos-Ortega and Hartenstein, 1985; Skaer, 1993). Anterior is to the left. The open arrows mark the borders of parasegment 5 (T2p/T3a; Martínez Arias and Lawrence, 1985). (A) At stage 11, strong ectopic expression of UBX is detected in parasegement 5, but is much weaker more anteriorly. UBX is detected in several NB of T3 but is less or absent in NB of T1 or T2 (not completely in focus). (B) During stage 10/11, ectopic ABD-A domains appear immediately behind the parasegmental furrows, with the domains at the T2/T3 and T3/A1 borders being most prominent. Arrowheads point towards strong nuclear label in cells that are likely to be NB1-1 in T3, according to their anterior location in the segment, about 1-2 cell diameters distant from the midline and slightly anterior to the tracheal pits (small arrows). Scale bar, 20 μm.

Tagma specificity of the NB1-1 lineage is regulated by *Ubx* and *abd-A*

Screening homeotic mutants with independent cellular markers revealed that the *bithorax*-complex genes, *Ubx* and *abd-A*, affect segment-specific features of the NB1-1 lineage. In the $Ubx^{6.28}$ null allele the A- and B-glia cells were missing in A1. This observation parallels former descriptions on various ectodermal derivatives where T2p to A1a express characteristics of T1p/T2a (CNS: e.g. Jiménez and Campos-Ortega, 1981; Ghysen and Lewis, 1986; Truman et al., 1993; PNS: Hartenstein, 1987; Heuer and Kaufman, 1992; cuticula: Lewis, 1978; Hayes et al., 1984). As shown by *M84* expression and transplantations, *Df109* embryos express the thoracic NB1-1 variant in all abdominal neuromeres except A8. Again, this phenotype is in accord with defects in other ectodermal tissues (CNS: Jiménez and Campos-Ortega, 1981; PNS: Heuer and Kaufman, 1992; cuticula: Lewis, 1978). The presence of A- and B-cells in A8 of *Df109*, suggests NB1-1 in A8 to be regulated by *Abd-B* (Casanova et al., 1986). Since *Df109* deletes *Ubx* as well as *abd-A* (Karch et al., 1985; Sánchez-Herrero et al., 1985), and since *Ubx* only affects the A- and B-pattern in A1, we conclude that *abd-A* regulates segment-specific expression of the NB1-1 lineage in segments A2-7. The lack of any phenotype in $abd\text{-}A^{MX1}$ (see also Graba et al., 1992) is likely to be due to the derepression of UBX in more posterior segments (Struhl and White, 1985; Macías et al., 1990), resulting in T3p/A1a-like differentiation in ectodermal tissues of A1p to A7/8 (CNS: Jijakli and Ghysen, 1992; PNS: Karch et al., 1990; Heuer and Kaufman, 1992; cuticula: Sánchez-Herrero et al., 1985). Further examples of substitution of *abd-A* function by *Ubx* have been reported previously (Gould et al., 1990; Graba et al., 1992; Röder et al., 1992; Vachon et al., 1992; Appel and Sakonju, 1993; see also Rowe and Akam, 1988).

Taken together, the absence of *Ubx* and *abd-A* is sufficient to allow for lineage autonomous thoracic development of NB1-1 (see also Palka et al., 1979; Morata and García-Bellído, 1985; Teugels and Ghysen, 1983). Conversely, ectopic induction of *Ubx* and *abd-A* leads to abdominal development of NB1-1.

Besides NB1-1, the NB that generates the thoracic A/B-like glia seems to be regulated by homeotic genes. Furthermore, homeotic mutations have been shown to affect other segment-specific features in the *Drosophila* vNC, such as expression of various genes and markers (Jiménez and Campos-Ortega, 1981; Graba et al., 1992; Gould et al., 1990), proliferation patterns in the embryonic and postembryonic vNC (Prokop, 1993; Truman et al., 1993; see also Booker and Truman, 1989; Miles and Booker, 1993), neuropile structures (e.g. Ghysen and Lewis, 1986; Jijakli and Ghysen, 1992), projection patterns (e.g. Palka et al., 1979; Strausfeld and Singh, 1980; Green, 1981; Thomas and Wyman, 1984) and function (Schneiderman et al., 1993). In most of these cases homeotic defects in the CNS more or less paralleled the epidermal phenotypes (for an exception see Ghysen, 1978).

UBX and ABD-A are required during a limited time period for the abdominal development of NB1-1

The aCC/pCC neurons as the earliest components of the NB1-1 lineage derive from a ganglion mother cell born at about stage 9 (Doe et al., 1988; Doe, 1992b). They show no obvious segment specificity and do not suggest the need for early activity of homeotic genes, and it remains uncertain whether

previously reported *Ubx* expression in abdominal aCC and pCC cells later on is of functional relevance (Doe et al., 1988a). Accordingly, antibody staining revealed UBX and ABD-A in NB1-1 not before stage 10/11. As the anti-UBX antibody is directed against all UBX-variants (White and Wilcox, 1984, 1985), positive staining does not tell us whether the detected protein is really functional, with reference to its splice variant and modifications (O'Connor et al., 1988; Kornfeld et al., 1989; Gavis and Hogness, 1991; López and Hogness, 1991). The same might be true for ABD-A. However, a functional relevance of the observed expression is suggested by the heat shock experiments, which still led to induction of A- and B-glia cells at about stage 11 (see also Michelson, 1994). The correlation between the heat shock results and the expression data becomes even more convincing if the delay in UBX-expression is considered. According to González-Reyes and Morata (1990) it takes up to 1 hour following the end of the heat shock for UBX expression to reach its highest level. However, the effects of early induced heat shocks can be easily explained by the fact that the level of ectopically induced UBX will remain high for at least 4 hours (González-Reyes and Morata, 1990). Also the spatial distribution of UBX at stage 11 supports its functional significance: UBX is located in the nuclei of NB1-1 in A1 but is clearly lacking in NB1-1 in the T2p/T3a domain of *Ubx* expression (reviewed by Beachy, 1990), and is thus in accord with the restriction of mutant effects to A1. Similarly, both expression and mutant defects of *abd-A* (as uncovered by *Df109*) coincided in A2-7. Taken together, the expression data argue for a requirement of UBX and ABD-A in NB1-1 starting at stage 10/11. However, the fading effects of later induced heat shocks suggest a requirement for UBX or ABD-A not later than stage 11/12 (see Fig. 4). A requirement for both proteins during stage 11 by the A- and B-glia is supported by the similarity of their transient expression patterns in Pc^3 with the spatially restricted effect on the A- and B-glia pattern to T3 only. In mice also, the type of transformation of vertebrae resulting from ectopically induced Hox gene products is dependent on the time of manipulation (Kessel and Gruss, 1991).

Which mechanisms cause early tagma specific commitment of NB1-1?

As discussed above, UBX and ABD-A seem to be required at about stage 11 for abdominal development of NB1-1. However, as revealed by the heterotopic transplantation experiments, tagma-specific commitment of the NB1-1 progenitors is already achieved in the early gastrula (stage 7). Which factors mediate this early commitment ?

At the early gastrula stage only *Ubx* and *abd-A* transcripts are present and they are not translated before stage 8/9 (Akam and Martínez Arias, 1985; Beachy et al., 1985; Harding et al., 1985; White and Lehmann, 1986; Karch et al., 1990; Macías et al., 1990). Thus, early commitment of the cells might be achieved by the presence or absence of *Ubx* or *abd-A* transcripts at the blastoderm stage mediating homeotic gene expression at later stages e.g. via autoregulation (Christen and Bienz, 1992; Irvine et al., 1993). However, derepression of homeotic genes in *Polycomb* group mutants contradicts this view: although in Pc^3 there were no *Ubx* and *abd-A* transcripts present in most of the thoracic region of the early gastrula stage, during later stages these mutants gradually build up a proper pattern of *Ubx* and *abd-A* expression even in the thorax, including expression in NB1-1 in T3 at stage 11 (Beachy et al., 1985; Struhl and Akam, 1985; Struhl and White, 1985; Wedeen et al., 1986; Simon et al., 1992 and 1993; own results). This suggests the presence of trans-activating mechanisms for *Ubx* and *abd-A* also in the thorax, although during wild-type development both genes are not or only partly activated in that region. Such late trans-activating mechanisms could involve CNS expression of segmentation genes like *ftz* (Doe et al., 1988a) and other gene classes like the *trithorax*-group genes (reviewed by Kennison, 1993). These activators in the thorax might also allow for an induction of *Ubx* or *abd-A* in heterotopic transplants derived from the abdomen.

We suggest that early commitment of thoracic specificity is due to repression of *Ubx* and *abd-A*. A known trans-repressor for *Ubx* in the anterior region of the embryo is the *hunchback* gene (*hb*) (e.g. White and Lehmann, 1986; Quian et al. 1993; Zhang and Bienz, 1992). *hb* represses *Ubx* during early stages and the repressed state of *Ubx* seems to be maintained later on by *Polycomb* group genes (Paro, 1990). Consequently, the *Ubx* promoter is not 'open for business' (Peifer et al., 1987), although activators for *Ubx* are present throughout the vNC. This repressed state of the *Ubx* promoter in the anterior vNC (which can only be overridden by the *Ubx* enhancer element in some cells of parasegment 5, excluding NB1-1; Simon et al, 1990; Müller and Bienz, 1991) is cell autonomously expressed upon heterotopic transplantation (see also Paro, 1990) and could reflect the thoracic commitment from the early gastrula stage on.

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