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Antagonistic roles for *Ultrabithorax* and *Antennapedia* in regulating segment-specific apoptosis of differentiated motoneurons in the *Drosophila* embryonic central nervous system

Ana Rogulja-Ortmann, Simone Renner and Gerhard M. Technau*

The generation of morphological diversity among segmental units of the nervous system is crucial for correct matching of neurons with their targets and for formation of functional neuromuscular networks. However, the mechanisms leading to segment diversity remain largely unknown. We report here that the Hox genes *Ultrabithorax* (*Ubx*) and *Antennapedia* (*Antp*) regulate segment-specific survival of differentiated motoneurons in the ventral nerve cord of *Drosophila* embryos. We show that *Ubx* is required to activate segment-specific apoptosis in these cells, and that their survival depends on *Antp*. Expression of the Ubx protein is strongly upregulated in the motoneurons shortly before they undergo apoptosis, and our results indicate that this late upregulation is required to activate *reaper*-dependent cell death. We further demonstrate that *Ubx* executes this role by counteracting the function of *Antp* in promoting cell survival. Thus, two Hox genes contribute to segment patterning and diversity in the embryonic CNS by carrying out opposing roles in the survival of specific differentiated motoneurons.

KEY WORDS: Programmed cell death, Motoneurons, Segment specificity, Hox genes, CNS, *Drosophila*

INTRODUCTION

The body plan of many animals is composed of groups of homologous and morphologically diverse structures, such as comprise the body segments of *Drosophila*. Many studies over the years have shown that the Hox proteins, a conserved group of homeodomain transcription factors, function in the morphological diversification of segments along the anteroposterior body axis (Mann and Morata, 2000; McGinnis and Krumlauf, 1992). In *Drosophila* larvae, Hox genes have also been suggested to orchestrate the assembly of neuromuscular networks that control region-specific peristaltic movements in crawling (Dixit et al., 2008). *Drosophila* Hox genes are grouped into two complexes that specify the different segment identities: Antennapedia-Complex genes that specify segments in the head and anterior thorax, and Bithorax-Complex genes that specify segments of the posterior thorax and abdomen (Kaufman et al., 1990; Lewis, 1978). In addition to their role as regulators of segment identity, Hox genes can directly affect expression of the so-called 'realizator' genes, which regulate cell behavior, e.g. cell adhesion, migration, proliferation and apoptosis (Garcia-Bellido, 1975; Hombria and Lovegrove, 2003; Pearson et al., 2005). Within the last decade, several reports have emerged describing the involvement of Hox genes in regulating developmental programmed cell death (Economides et al., 2003; Liu et al., 2006; Lohmann et al., 2002). They also regulate apoptosis in the developing *Drosophila* central nervous system (CNS), where *abdominal A* (*abdA*) is required to induce apoptosis of larval abdominal neuroblasts, thus limiting neuronal numbers in abdominal segments (Bello et al., 2003), and where *Abdominal B* (*AbdB*) expression ensures survival of differentiated pioneer neurons in the posterior segments of the embryo (Miguel-Aliaga and Thor, 2004). These studies also underscore the importance of apoptosis in patterning the nervous system, as it enables the selective removal of cells that might otherwise disturb the highly refined circuitry required for control of locomotion.

Generally, apoptotic cell death is abundant in the *Drosophila* embryonic CNS and occurs both in apparently random cells and in a regular, segmentally repeated pattern (Abrams et al., 1993; Rogulja-Ortmann et al., 2007) that implies strict spatio-temporal regulation. The mechanisms of this regulation are largely unknown. The identification of the developmental signals involved is crucial to understanding how apoptosis is integrated into tissue and organ patterning during development. We report here that the Hox genes *Ultrabithorax* (*Ubx*) and *Antennapedia* (*Antp*) have antagonistic functions in motoneuron survival in the embryo. We show that *Ubx* expression in the CNS is strongly upregulated at a late point in development, when most cells have begun to differentiate. This upregulation shortly precedes *Ubx*-dependent segment-specific apoptosis of two differentiated motoneurons. In addition, the proapoptotic gene *reaper* (*rpr*) (White et al., 1996) is transcriptionally activated following *Ubx* upregulation in these motoneurons. Furthermore, we demonstrate that *Antp* is required for the survival of these cells. In segments where these two Hox genes are coexpressed, *Ubx* counteracts the anti-apoptotic function of *Antp*, resulting in cell death. Taken together, our results demonstrate that Hox genes contribute to segment diversity at the cellular level by regulating cell numbers in a segment-specific manner, and that they do so by exerting opposing effects on the survival of individual cells. They may thus contribute to the diversification of neural circuits along the anteroposterior body axis.

MATERIALS AND METHODS

Drosophila stocks

OregonR was used as the wild-type strain. The following mutant fly strains were used: UAS-*mCD8::GFP* (second and third chromosomal insertion), UAS-*eGFP* (second and third chromosomal insertion), UAS-*Antp*, $Antp^{W10}$,Ubx^{1}/*TM6B,Tb,Sb* (all from The Bloomington Stock Center),

Institute of Genetics, University of Mainz, 55099 Mainz, Germany.

*Author for correspondence (e-mail: technau@uni-mainz.de)

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Antp^{W10}/TM6,abdA-lacZ (Wakimoto et al., 1984), *Def(3R)109/TM6,abdA*-lacZ (Lewis, 1978), *abdA^{MX1}/TM3,Sb,Kr*-Gal4,UAS-*GFP* (Sanchez-Herrero et al., 1985), *hth^{P2}/TM6B,abdA*-lacZ (Sun et al., 1995), *exd^{B108}/FM7,ftz*-lacZ (Rauskolb et al., 1993), *numb^{1}/CyO,wg*-lacZ (Uemura et al., 1989), *zfh1^{5}*/TM3,*Kr*-Gal4,UAS-*GFP* (kindly provided by Z. C. Lai, Pennsylvania State University, University Park, PA), *Ubx^{1}/TM6B,Tb,Sb,Dfd*-lacZ, UAS-*Ubx* (both kindly provided by L. Shashidara, Centre for Cellular and Molecular Biology, Hyderabad, India), *eagle*-Gal4 (Mz-360) (Dittrich et al., 1997), *engrailed*-Gal4 (Tabata et al., 1995), *poxN*-Gal4,UAS-*GFP* (*Pox neuro* – FlyBase) (Boll and Noll, 2002), UAS-*hb* (second and third chromosomal insertion) (Wimmer et al., 2000) and *hs-Ubx/TM6B,Tb,Sb,Dfd*-lacZ (Mann and Hogness, 1990).

Immunohistochemistry and in situ hybridization

Antibody stainings were performed as described (Rogulja-Ortmann et al., 2007). Primary antibodies used were: mouse anti-GFP (1:250, Promega), rabbit anti-human activated caspase 3 (1:50, Cell Signaling Technology), mouse anti-Eg (1:100, C. Doe, University of Oregon, Eugene, OR), rabbit anti-Eg (1:500) (Dittrich et al., 1997), guinea pig anti-Hb (1:500, J. Urban, University of Mainz, Mainz, Germany), rabbit anti-β-gal (1:2000, Cappel), mouse anti-β-gal (1:750, Promega), mouse anti-Ubx (1:20, Developmental Studies Hybridoma Bank), mouse anti-Antp (1:20, Developmental Studies Hybridoma Bank), guinea pig anti-Zfh1 (1:500, J. Skeath, Washington University School of Medicine, St Louis, MO), rabbit anti-Hth (1:500) (Kurant et al., 1998) and mouse anti-Exd (1:5) (Aspland and White, 1997). The secondary antibodies used were: Cy3-conjugated anti-mouse and anti-rabbit, Cy5-conjugated anti-guinea pig and FITC-conjugated anti-rabbit (1:500, all from donkey, all Jackson ImmunoResearch) and donkey Alexa488-conjugated anti-mouse (1:500, Molecular Probes).

For fluorescent in situ hybridization, digoxigenin-labeled RNA probes for *rpr* and *hid* were made from cDNAs obtained from H. Steller (Rockefeller University, New York, NY) and J. Abrams (University of Texas Southwestern Medical Center, Dallas, TX), and for *grim* from cDNA RE28551 (Stapleton et al., 2002), using the DIG-RNA Labeling Kit (Roche Applied Science). In situ hybridizations were performed according to standard procedures (Tautz and Pfeifle, 1989), using a 40% formamide hybridization solution. A Leica TCS SPII confocal microscope was used for fluorescent imaging, and the images were processed using Leica Confocal software and Adobe Photoshop.

Heat-shock procedure

For the 3.5-hour heat-shock (HS) experiment, 30-minute collections were made. At 3.5 hours after egg laying (AEL), embryos were dechorionated in 6% bleach and rinsed into a mesh. The HS was administered by placing the mesh into a beaker of PBS in a 35°C water bath for 30 minutes. For the 12-hour HS, 60-minute collections were made. At 12 hours AEL, embryos were dechorionated as above and the HS performed for 60 minutes at 35°C. In both experiments, after the HS embryos were allowed to develop at 25°C until late stage 16, when they were fixed as described by Rogulja-Ortmann et al. (Rogulja-Ortmann et al., 2007).

RESULTS

Segment-specific apoptosis of two differentiated motoneurons

Two neuroblast (NB) lineages, NB7-3 and NB2-4t, in which segment-specific cell death has been observed (Rogulja-Ortmann et al., 2007), were analyzed. These two lineages, along with NB3-3 and NB6-4, express the transcription factor *eagle* (*eg*) (Dittrich et al., 1997; Higashijima et al., 1996), which we used as an identification marker. In NB7-3 and NB2-4, *eg* is expressed in all progeny cells throughout development, although expression in NB2-4 is weaker. NB7-3 comprises three interneurons (EW1-3) and one motoneuron (GW) (Fig. 1A) (Bossing et al., 1996; Dittrich et al., 1997; Higashijima et al., 1996; Novotny et al., 2002; Schmid et al., 1999). In the anterior thoracic segments (T1 and T2), the cluster often comprises five cells in late embryos, the fifth cell probably being one of the progeny that dies immediately after birth in abdominal segments (Lundell et al., 2003). This cell also undergoes apoptosis in T1 and T2, but this takes place at the end of embryogenesis (A.R.-O., unpublished). The individual NB7-3 neurons can be identified based on marker gene expression and their position in the cluster. The GW motoneuron and the fifth cell in T1 and T2 are positive for the transcription factor *zinc-finger homeodomain protein 1* (*zfh1*) (Isshiki et al., 2001; Novotny et al., 2002). In addition, GW and EW1 both express the transcription factor *hunchback* (*hb*), but are easily distinguished from one another based on their size and position: GW is smaller and lies posterior to the three interneurons (Bossing et al., 1996; Dittrich et al., 1997; Higashijima et al., 1996; Isshiki et al., 2001; Novotny et al., 2002; Schmid et al., 1999). We used anti-Hb and anti-Zfh1 antibodies to identify the GW motoneuron.

As we reported previously, GW undergoes Caspase-dependent cell death specifically in segments T3 to A8 (Fig. 1B) (Rogulja-Ortmann et al., 2007). We restricted further analyses to the thorax and abdominal segments A1-A7, as NB7-3 appears to develop differently in A8 and comprises only two to three cells. At late stage 16, GW was either positive for activated Caspase 3 (a marker for apoptosis activation) or had already been removed from the CNS in 55.6% of analyzed T3 hemisegments (hs) (n=27) and 81.2% of analyzed abdominal hs (A1-A7, n=69) (Table 1). The considerable difference in the percentage of apoptotic GWs between T3 and the abdomen appears to be a consequence of differential apoptosis timing, as wild-type embryos at mid stage 16 showed Caspase 3-positive GWs in 12.5% of analyzed A1-A7 hs (n=112) and we did not observe dying T3 GWs at this stage (n=16). In addition, Caspase 3 activation did not occur simultaneously in the GWs of all abdominal segments: late stage 16 embryos showed hemisegments where GW appeared intact, others where GW showed Caspase 3 activation, and a third group where GW could no longer be visualized with the Eg marker. These observations are indicative of the dynamics of apoptosis, and show that the analyzed specimens can only be regarded as snapshots taken at the time of embryo fixation. In addition, they suggest that GW apoptosis in T3 is initiated slightly later than in the abdomen. TUNEL staining on late stage 16 embryos revealed TUNEL-positive GWs, confirming GW apoptosis. By the time of hatching (L1), GW had been cleared from the ventral nerve cord in practically all T3 and A1-A7 hs examined (see Fig. S1C in the supplementary material).

The NB2-4t lineage comprises 8 to 12 neurons, two of which are motoneurons (Fig. 1D) (Schmid et al., 1999; Schmidt et al., 1997). Later in development, these two motoneurons lie in a dorsomedial position, separate from the other NB2-4 neurons. In the abdomen, the motoneurons lie slightly apart from one another, whereas in the thorax they occupy the same dorsoventral position, with one lying anterior to the other (Fig. 1D,E). In segment T3, and only in this segment, the anterior NB2-4t motoneuron (from here on referred to as MNa) underwent Caspase-dependent apoptosis at late stage 16 (Fig. 1E). As *eg* expression is low in the NB2-4 lineage, we used the *eg*-Gal4 driver combined with UAS-*mCD8::GFP* for easier identification of NB2-4 cells. In 64.7% of analyzed T3 hs (n=17), MNa either showed Caspase 3 activation or had already been removed from the CNS (Table 1). Apoptosis of this motoneuron was confirmed by TUNEL staining (see Fig. S1 in the supplementary material).

We also examined which of the three *Drosophila* proapoptotic genes is involved in GW and MNa apoptosis. Fluorescent in situ hybridization revealed transcriptional activation of *rpr* (White et al., 1996) in both GW (Fig. 1C) and MNa (Fig. 1F), but not of *head*

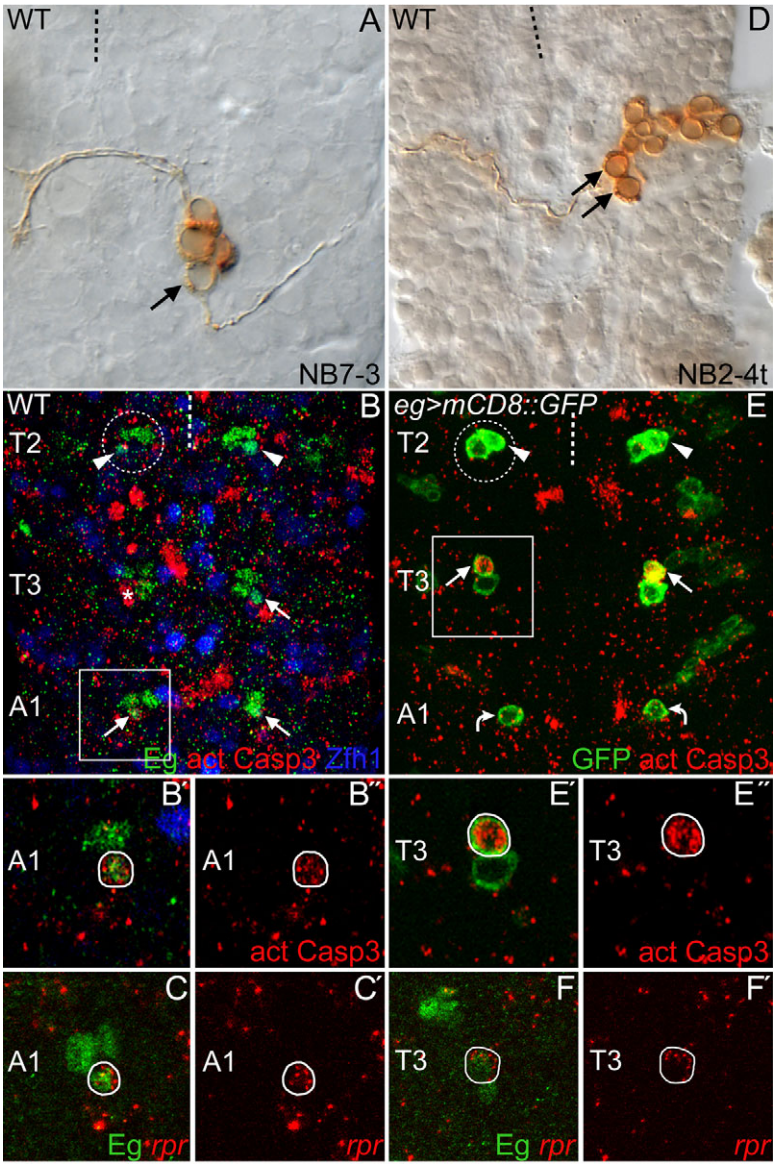

Fig. 1. Two differentiated motoneurons undergo segment-specific *rpr*-dependent cell death. An NB7-3 lineage (A-C) and an NB2-4t lineage (D-F) of wild-type (A-D,F) and *eg*-Gal4,UAS-*mCD8::GFP* (E) *Drosophila* embryos at late stage 16. Dashed lines mark the midline. Dashed circles indicate NB7-3 (B) and NB2-4t (E). B′,B″ and E′,E″ show magnified single scans of one lineage as boxed in B and E, respectively. Anterior is up in all images. (**A**) A DiI-labeled NB7-3 clone. The GW motoneuron (arrow) lies at the posterior end of the cluster and exhibits an ipsilateral outward projection. (**B**) GWs (arrows) undergo apoptosis in segments T3 and A1, but not in T2 (arrowheads). Asterisk indicates that the GW has already been removed. (**B′**) A magnified single scan of the A1 NB7-3 lineage. The apoptotic GW is outlined. (**B″**) Same image as in B′, showing only the activated Caspase 3 (act Casp3) staining. (**C**) A magnified single scan of an A1 NB7-3 lineage. (**C′**) *rpr* is transcriptionally activated in apoptotic GWs (outlined). (**D**) A DiI-labeled NB2-4t clone. The motoneurons (arrows) are the most medial and most dorsal cells of the cluster and show contralateral outward projections. (**E**) The anterior NB2-4t motoneuron in segment T3 undergoes apoptosis (arrows). In A1, only one motoneuron lies in the same position (curved arrows). (**E′**) A magnified single scan of the T3 NB2-4t. The apoptotic MNa is outlined. (**E″**) Same image as in E′, showing only the activated Caspase 3 staining. (**F**) A magnified single scan of a T3 NB2-4t lineage. (**F′**) *rpr* is transcriptionally activated also in apoptotic MNas (outlined).

involution defective (*hid*; *Wrinkled* – FlyBase) (Abbott and Lengyel, 1991; Grether et al., 1995) or *grim* (Chen et al., 1996) (data not shown).

Expression pattern of *Ubx* in the NB7-3 and NB2-4t lineages

The observed pattern of GW apoptosis bears an intriguing resemblance to the expression pattern of the Hox gene *Ubx*. In the embryonic CNS, *Ubx* is expressed from the posterior half of segment T2 to the anterior half of segment A7, with strongest expression in posterior T3 and anterior A1 (Beachy et al., 1985; Carroll et al., 1988; White and Wilcox, 1985). We therefore examined the CNS expression of *Ubx* more closely, and found that it was present in the NB7-3 lineage from segments T2 to A7 (Fig. 2A and see Fig. S2 in the supplementary material). In T2, the early NB7-3 neuroblast did not express *Ubx*. Its progeny showed no, or very low, *Ubx* expression until stage 15, when *Ubx* was strongly upregulated, but only in the EW interneurons. High *Ubx* levels were then maintained in the EWs at least until early stage 17, while the GW remained devoid of *Ubx*. In segments T3 to A7, *Ubx* was expressed already in the newly delaminated NB7-3 neuroblast (Fig. 2A and see Fig. S2 in the supplementary material) and showed weak expression in all progeny cells until stage 15. At stage 15, *Ubx* was strongly upregulated in all cells of the NB7-3 cluster. At late stage 16, *Ubx* was expressed at high levels in all NB7-3 cells of segments T3 to A7 (Fig. 2A, Fig. 3A). Thus, the pattern of *Ubx* expression in GW coincides with the timing and pattern of apoptosis.

Because apoptosis in NB2-4t occurs in segment T3, where Ubx levels are highest, we examined *Ubx* expression in this lineage and found that it is expressed from segments T3 to A8 (Fig. 2B and see Fig. S2 in the supplementary material). In T3, the early NB2-4t neuroblast did not express *Ubx*. The first *Ubx*-positive cells in NB2-4t of T3 probably appeared around stage 12. However, cells of NB2-4t and of the Eg-positive NB3-3 intermingle, making it difficult to distinguish between their progeny. From stage 14 onwards, the NB2-4t motoneurons are identifiable from their dorsal position, and both showed weak *Ubx* expression. At stage 15, *Ubx* was strongly upregulated in MNa, whereas the posterior motoneuron showed low *Ubx* levels. This difference was maintained at least until late stage 16 (Fig. 2B, Fig. 3D), when the motoneuron exhibiting high *Ubx* levels underwent apoptosis. In segments A1 to A7, the young NB2-4 neuroblasts were all *Ubx*-positive (Fig. 2B, see Fig. S2 in the

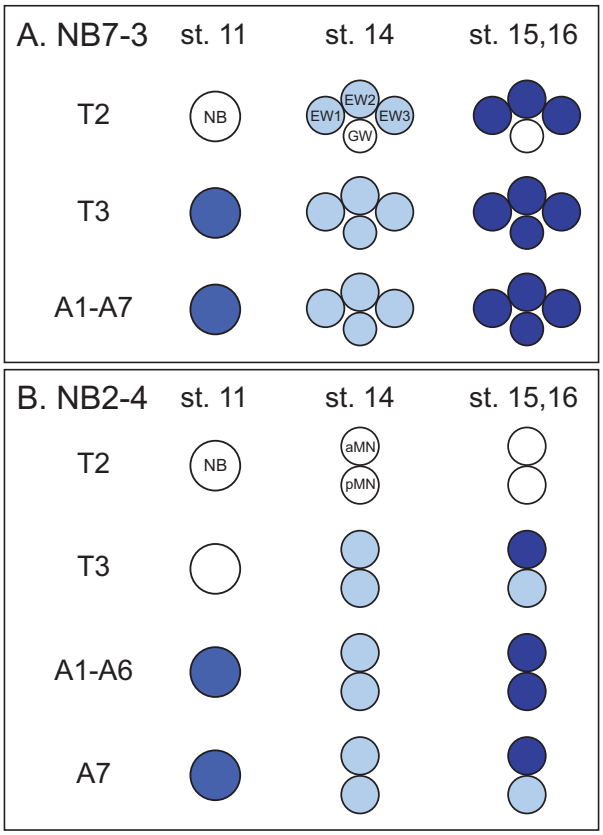

Fig. 2. Summary of *Ubx* expression in the NB7-3 and NB2-4 lineages. The segmental pattern of *Ubx* expression is depicted at the indicated developmental stages. The intensity of the blue shading reflects the *Ubx* expression level; white indicates no expression. (**A**) The neuroblast and progeny of NB7-3. (**B**) The neuroblast and the two motoneurons of NB2-4t.

supplementary material). The progeny cells all appeared to show weak *Ubx* expression (although they could not be distinguished from progeny of NB3-3). At stage 14, when the motoneurons can be identified based on their position, they still maintained weak *Ubx* expression. After this stage, and in contrast to segment T3, *Ubx* was strongly upregulated in both motoneurons in segments A1 to A6 (Fig. 2B and see Fig. 2 in the supplementary material). In A7, the pattern of *Ubx* expression in these cells resembled that in segment T3: it was strong in the anterior cell and weak in the posterior cell. By late stage 16, *Ubx* levels were reduced only in the posterior cell of all abdominal segments, whereas MNa maintained high *Ubx* expression but did not undergo apoptosis (Fig. 2B, Fig. 3D). The pattern of *Ubx* upregulation in MNa of T3 thus correlates with apoptosis induction, although its counterparts in other segments also upregulate *Ubx* but do not undergo apoptosis.

Ubx is necessary and sufficient to induce apoptosis in two differentiated motoneurons

We next examined the fate of these cells in *Ubx*-null mutants. In the ventral nerve cord of Ubx^{1} homozygous embryos, both motoneurons were rescued in 100% of cases (Fig. 3B,E, Table 1), indicating that *Ubx* is required for apoptosis of GW in segments T3 to A7, and of the NB2-4t MNa in segment T3. To test whether *Ubx* is also sufficient to induce apoptosis of these cells, we made use of the *en*-Gal4 and *poxN*-Gal4,UAS-*GFP* lines to drive *Ubx* expression in the NB7-3 and NB2-4t lineages, respectively, in all segments in the CNS. The *poxN*-Gal4,UAS-*GFP* flies also express GFP in NB2-4, which enabled easier identification of the motoneurons. In segments T3 to A7 of *en*-Gal4/+;UAS-*Ubx*/+ embryos, GW showed Caspase 3 activation already at stage 15 (Fig. 3C). At late stage 16, GW was removed from the CNS in 100% of the analyzed abdominal hs (n=68), and in 90% of all analyzed T3 hs (n=10) (see Table 1). Moreover, GW also died in 80% of analyzed T1 and T2 hs (n=20) (Fig. 3C), suggesting that *Ubx* is sufficient to induce apoptosis in this cell. The earlier onset of apoptosis in *en*-Gal4/+;UAS-*Ubx*/+ embryos is most likely due to the fact that Ubx protein levels are high in NB7-3 from the point of neuroblast delamination on. At late stage 16, we occasionally also observed Caspase 3 activation in an EW interneuron upon ectopic *Ubx* expression; however, such cases were rare. *Ubx*-induced apoptosis appeared cell-specific, as we did not see a general increase in cell death in the posterior segmental stripe of these embryos where the *en*-Gal4 driver is active.

In *poxN*-Gal4,*UAS-GFP/UAS-Ubx* embryos, MNa underwent apoptosis in 75% of analyzed T3 hs (n=16). In addition, segments T1 and T2 exhibited apoptotic MNas in 68.8% of cases (n=32) (Fig. 3F, Table 1). Interestingly, this motoneuron seemed less responsive to *Ubx* in T1 than in T2, as we observed apoptosis in 43.8% (n=16) and 93.8% (n=16) of cases, respectively. Thus, *Ubx* is necessary and sufficient to induce apoptosis of the GW and of the anterior NB2-4t motoneuron.

Ubx-dependent apoptosis is a late function of this gene

Considering that both *en*-Gal4 and *poxN*-Gal4,UAS-*GFP* drive *Ubx* expression from early stages of CNS development through to the end of embryogenesis, there are two ways to explain our results. First, *Ubx* might be required only in the early neuroblast, specifying its segmental identity and developmental program. As such, the apoptotic fate of the progeny would be predetermined, either intrinsically or in response to an extrinsic apoptotic signal, and would not require *Ubx* at a later point in development. In a second scenario, *Ubx* would be required specifically at a later developmental stage to induce the apoptotic program. In support of the second hypothesis, *Ubx* is strongly upregulated at developmental stage 15 in both motoneurons, a few hours before detectable activation of Caspase 3.

To test which of the above possibilities is true, we expressed *Ubx* under the control of a heat-shock promoter. Since the early NB7-3 and NB2-4t neuroblasts and the GW and NB2-4t motoneurons show no *Ubx* expression in wild-type T1 and T2, we reasoned that providing *Ubx* in these cells at relevant developmental timepoints should clarify whether it is required early (at the time of neuroblast determination) or late (in the differentiated neurons) to induce apoptosis. The heat shock (HS) was administered at two different timepoints in separate experiments: at 3.5 hours after egg laying (AEL) (prior to NB7-3 delamination) and at 12 hours AEL (when *Ubx* is normally upregulated in T3 to A7). In the first experiment (early HS), *Ubx* was present in segments T1 and T2 at the time of neuroblast formation, but was degraded by the time it is required to induce apoptosis. This early *Ubx* expression in T1 and T2 might transform the NB7-3 lineage into a T3 identity, as the NB7-3 clusters in T1 and T2 in 26/27 cases contained only four cells instead of five. In the wild type, four cells were observed in 29/36 cases. In addition, we observed a transformation of the thoracic NB6-4 lineage (which contains neurons and glia) into an abdominal lineage (glia only), which indicates that ectopic *Ubx* expression can transform the tagma-specific identity of the neuroblasts. Following early HS, we

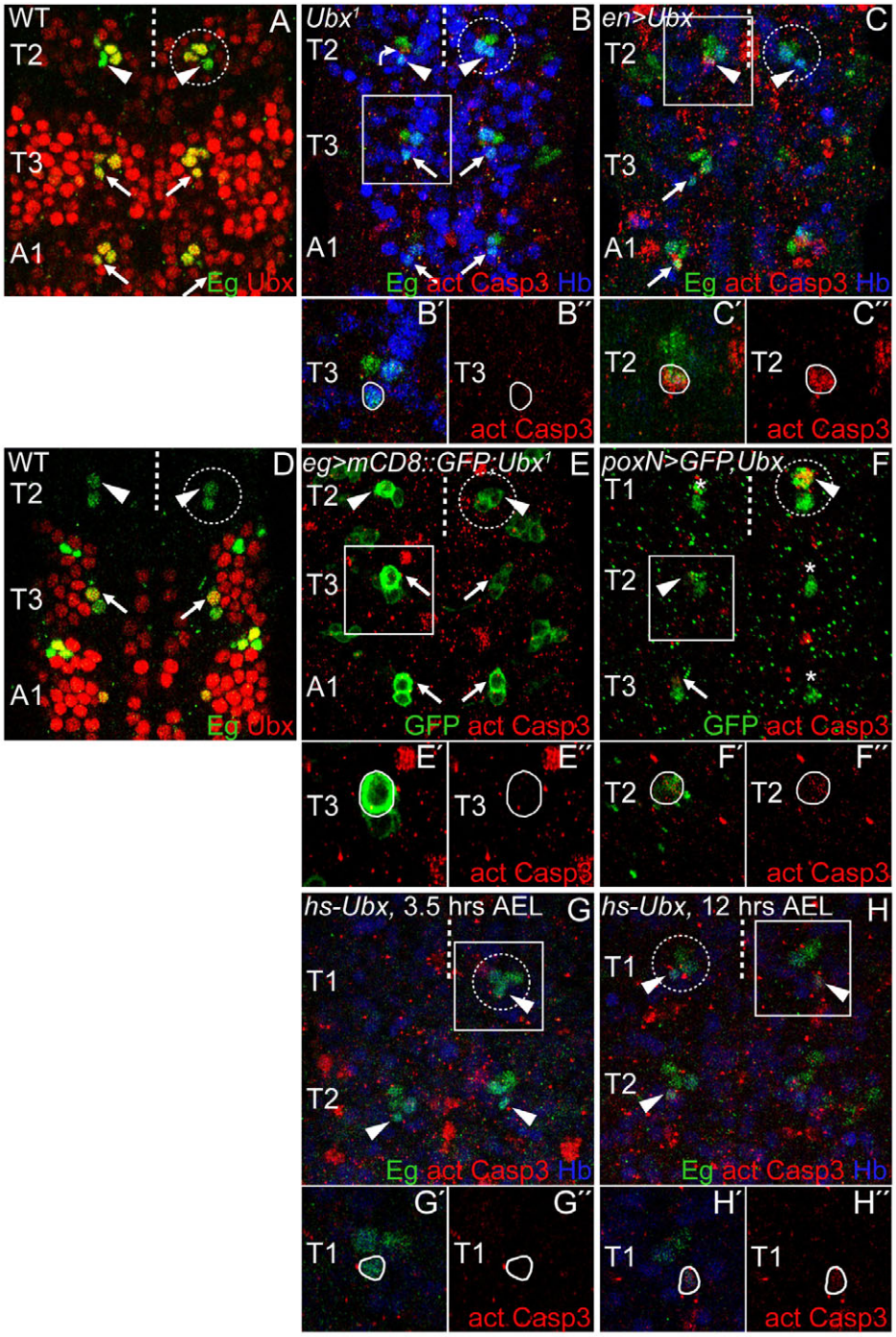

Fig. 3. Apoptosis of both motoneurons requires *Ubx* function at a late developmental stage. NB7-3 neurons in late stage 16 (A,B,G,H) and stage 15 (C) *Drosophila* embryos and NB2-4 (D-F) neurons in late stage 16 embryos. Dashed circles, NB7-3 and NB2-4; dashed lines mark the midline. (B′,C′,G′,H′) Magnified single scans of NB7-3 with GW outlined. (B″,C″,G″,H″) Only the activated is shown; Caspase 3 staining (act Casp3) is shown. (E′,F′) Magnified single scans of NB2-4t with MNa outlined. (E″,F″) Only the activated Caspase 3 staining (act Casp3) is shown. Anterior is up in all images. (**A**) *Ubx* expression levels are high in GWs that undergo apoptosis (arrows) and are barely, if at all, detectable in GWs that survive (arrowheads). (**B-B″**) Apoptosis of GWs is abolished in a *Ubx* loss-of-function mutant (arrows). The apoptotic cell in T2 (curved arrow) is not the GW (arrowhead). (**C-C″**) Ectopic *Ubx* expression induces apoptosis of GWs in anterior thoracic segments (arrowheads). These neurons now die prematurely in T3 and in abdomen as well (arrows). (**D**) *Ubx* is strongly expressed in the anterior, apoptotic NB2-4t motoneuron (arrows). Expression levels are low in the posterior motoneuron. In T2, *Ubx* is not expressed in these neurons (arrowheads). (**E-E″**) In a *Ubx* loss-of-function mutant, apoptosis of the MNa in T3 is abolished (arrows). In A1, these neurons are transformed towards a thoracic phenotype (arrows), and do not undergo apoptosis. T2 is unaffected (arrowheads). (**F-F″**) Ectopic *Ubx* expression induces apoptosis of MNa in anterior thoracic segments (arrowheads), and increases MNa apoptosis in T3 (arrow). Asterisks indicate that MNa has already been removed. (**G-G″**) Early ectopic *Ubx* expression cannot induce GW apoptosis in anterior thoracic segments (arrowheads). (**H-H″**) Late ectopic *Ubx* expression induces GW apoptosis in anterior thoracic segments (arrowheads), and increases it in T3 and abdomen. Genotypes: (A,D) wild type, (B) *Ubx*^{1}, (C) *en*-Gal4/+;UAS-*Ubx*/+, (E) UAS-*CD8::GFP*/+;*eg*-al4,*Ubx*^{1}/*Ubx*^{1}, (F) *poxN*-Gal4,UAS-*GFP*/UAS-*Ubx*, (G,H) *hs-Ubx*.

did not observe apoptotic GWs in T1 and T2 (n=27), which suggests that early *Ubx* expression is not sufficient to induce apoptosis in the NB7-3 lineage (Fig. 3G, Table 1). This conclusion is supported by the fact that the percentage of apoptotic GWs in the abdomen of these embryos (82.4%, n=85) did not differ from that of wild type (81.2%, n=69) (Table 1). In T3, however, we did find an increase in apoptotic GWs (100%, n=12) as compared with the wild type (55.6%, n=27) (Table 1), indicating that in this segment, *Ubx* might be required both early in order to specify an apoptosis-susceptible progeny fate, and late to initiate apoptosis itself.

In the late HS experiment, all NB7-3 progeny cells have been born and have begun to differentiate by the time the HS is given (12 hours AEL). Following such a treatment, we found apoptotic GWs in T1 and T2 in 21.9% of analyzed hs (n=64, Fig. 3H). Moreover, GW apoptosis was increased in T3 and in the abdominal segments (Table 1). We conclude that *Ubx* is required in GW at a late developmental stage to activate programmed cell death.

We were not able to analyze NB2-4t in these experiments because expression of the marker protein Eg was not strong enough to permit reliable evaluation of the data. However, we suggest that *Ubx* is required late in this lineage as well, as it is not expressed in the early NB2-4t neuroblast in T3 and thus probably does not participate in providing this neuroblast with its segment-specific identity. Moreover, early ectopic *Ubx* expression using the *poxN*-Gal4,UAS-*GFP* line was unable to

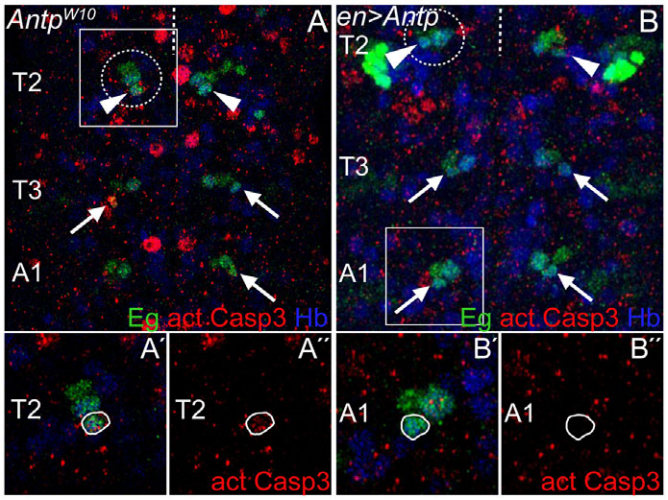

Fig. 4. *Antp* is necessary and sufficient for survival of the GW motoneuron. (**A-B′**) NB7-3 neurons (dashed circles) in late stage 16 *Drosophila* $Antp^{W10}$ (A) and *en*-Gal4/UAS-*Antp* (B) embryos. Dashed lines mark the midline. (A′,B′) Magnified single scans of NB7-3 with GW outlined. (**A″**,**B″**) Only the activated Caspase 3 staining (act Casp3) is shown. Anterior is up in all images. (A) GWs undergo apoptosis in anterior thoracic segments of *Antp* loss-of-function mutants (arrowheads). Apoptosis in T3 and the abdomen is unchanged (arrows). (B) Ectopic *Antp* expression reduces apoptosis of GWs in T3 and abdomen (arrows). T2 is unaffected (arrowheads).

transform the thoracic NB2-4 lineage into the abdominal lineage, but it did induce apoptosis of the MNa in all thoracic segments (Fig. 3F).

Antp is necessary and sufficient for survival of the GW motoneuron

To further explore the differences between GWs in different segments, we analyzed the expression of the Hox gene *Antp* in the NB7-3 lineage. *Antp* is expressed in the embryonic CNS from the posterior half of the labial segment through to the anterior half of A8 (Carroll et al., 1986; Hirth et al., 1998), with strongest expression from the posterior half of T1 to the anterior half of T3. At the end of embryogenesis, *Antp* levels were high in all NB7-3 progeny cells in T1 (Fig. 5A,B). In T2, the EWs had low *Antp* levels, but GW was strongly *Antp*-positive (Fig. 5A,C). In T3 and in the abdomen of late stage 16 embryos, NB7-3 progeny cells exhibited variable, low-level *Antp* expression (Fig. 5A, Fig. 6A). Considering that *Antp* expression is high in surviving GWs of T1 and T2, and is low in GWs of T3 to A7, which undergo apoptosis, we wondered whether *Antp* might be required for GW survival in T1 and T2. Indeed, in $Antp^{W10}$ mutant embryos, we found that GWs underwent cell death in T1 and T2 in 35% of analyzed hs (n=20) (Fig. 4A, Table 1). Additionally, in T3, the frequency of GW apoptosis increased from 55.6% (n=27) in wild type to 90% (n=10) in $Antp^{W10}$. In the abdominal segments, the frequency of GW apoptosis was slightly lower than in the wild type (Table 1). We conclude that *Antp* is necessary for the survival of GW in T1 and T2, and might also be responsible for the slightly later onset of GW apoptosis in T3.

In order to test whether *Antp* can also rescue GWs from apoptosis, we overexpressed *Antp* using the *en*-Gal4 driver. In segments T1 and T2 of *en*-Gal4/UAS-*Antp* embryos, no apoptotic GWs were observed (Table 1). In T3, only 30.8% of hs showed

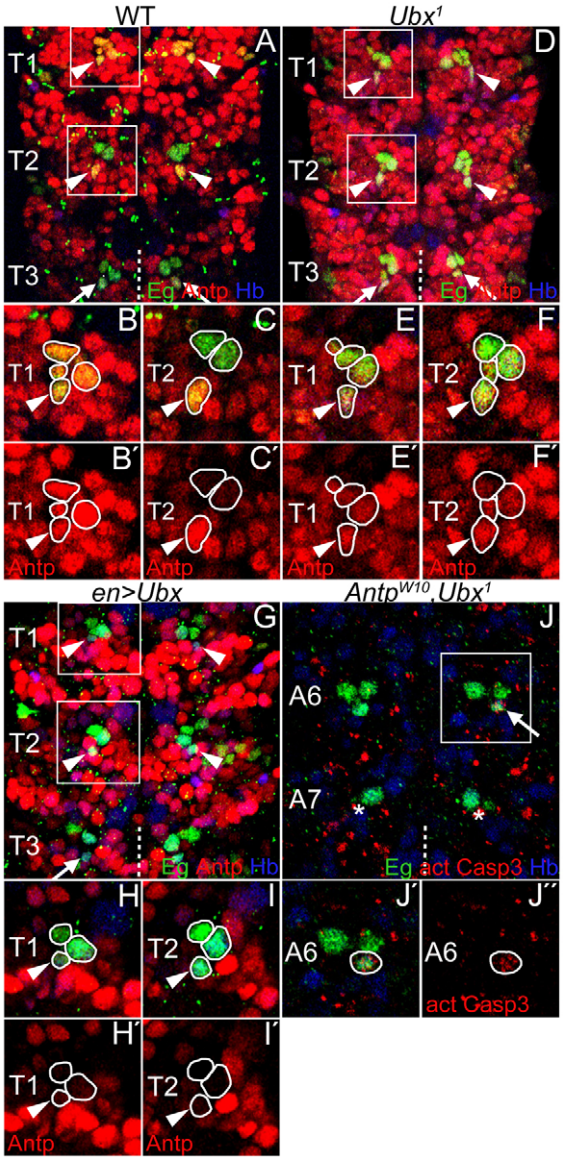

Fig. 5. *Ubx* induces apoptosis by preventing *Antp* from promoting survival of the GW motoneuron. NB7-3 neurons in late stage 16 (A-F,J) or stage 15 (G-I) *Drosophila* embryos. Dashed lines mark the midline. Insets show magnified single scans of NB7-3 as follows: (B,C) the indicated segments from A; (E,F) the indicated segments from D; (H,I) the indicated segments from G. (B′,C′,E′,F′,H′,I′) Only the Antp staining from respective insets is shown. GWs are indicated by arrowheads. Anterior is up in all images. (**A**) GWs show high *Antp* expression levels in segments in which they do not undergo apoptosis (arrowheads). In segment T3, in which they undergo apoptosis, they express *Antp* at low levels (arrows). (**B**,**B′**) In T1, all NB7-3 cells show strong *Antp* expression. (**C**,**C′**) In T2, *Antp* expression is strong in GW (arrowhead) and barely detectable in the EW interneurons. (**D**) In *Ubx* mutants, *Antp* expression is strongly upregulated in all thoracic segments. (**E**,**E′**) In T1, *Antp* expression is unchanged. (**F**,**F′**) In T2, *Antp* expression is equally strong in all NB7-3 cells. (**G**) Ectopic *Ubx* expression suppresses *Antp* in the anterior thoracic segments. (**H**,**H′**) In T1, ectopic *Ubx* strongly suppresses *Antp* expression in all cells of NB7-3. (**I**,**I′**) In T2, GW (arrowhead) also shows reduced *Antp* levels upon ectopic *Ubx* expression. (**J**) In *Antp,Ubx* double mutants, GW apoptosis is restored (arrow). Asterisks indicate that the GW has already been removed. (**J′**) A magnified single scan of the A6 NB7-3. The apoptotic GW is outlined. (**J″**) Same image as in J′, showing only the activated Caspase 3 (act Casp3) staining.

Table 1. Percentages of apoptotic GW and NB2-4t motoneurons in various genetic backgrounds

GW	WT	*Ubx*^{1}	*en>Ubx*	*hs-Ubx* (3.5 hours AEL)	*hs-Ubx* (12 hours AEL)	*Antp*^{W10}	*en>Antp*	*Ubx*^{1}/+	*Antp*^{W10},*Ubx*^{1}/+	*Antp*^{W10},*Ubx*^{1}/ *Antp*^{W10}	*Antp*^{W10},*Ubx*^{1}
T1-T2	0 (*n*=55)	0 (*n*=28)	80 (*n*=20)	0 (*n*=27)	21.9 (*n*=64)	35 (*n*=20)	0 (*n*=28)	0 (*n*=28)	2.8 (*n*=36)	ND	ND
T3	55.6 (*n*=27)	0 (*n*=14)	90 (*n*=10)	100 (*n*=12)	90.6 (*n*=32)	90 (*n*=10)	30.8 (*n*=13)	7.1 (*n*=14)	16.7 (*n*=18)	36.4 (*n*=22)	23.5 (*n*=17)
A1-A7	81.2 (*n*=69)	0 (*n*=98)	100 (*n*=68)	82.4 (*n*=85)	98.8 (*n*=84)	69 (*n*=58)	34.7 (*n*=95)	21.7 (*n*=97)	41.3 (*n*=126)	52.2 (*n*=90)	19.6 (*n*=112)

NB2-4t MNa	WT	*Ubx*^{1}	*poxN>Ubx*
T1-T2	0 (*n*=29)	0 (*n*=20)	68.8 (*n*=32)
T3	64.7 (*n*=17)	0 (*n*=11)	75 (*n*=16)
A1-A7	0 (*n*=70)	0 (*n*=12)*	ND

Shown are percentages of motoneurons that were either positive for activated Caspase 3 or that had already been removed from the CNS.
n, the number of hemisegments counted.
ND, not determined.
*A1 only.

apoptotic GWs (*n*=13) as compared with 55.6% in the wild type (*n*=27). In abdominal segments, only 34.7% of GWs were apoptotic (*n*=95), significantly fewer than in the wild type (81.2%, *n*=69) (Fig. 4B, Table 1). In addition, driving *Antp* expression with *poxN*-Gal4 reduced the percentage of apoptotic NB2-4t MNas in T3 (Fig. 6B). This suggests that *Ubx* might counteract *Antp* in NB2-4t, in a similar way as in NB7-3, to prevent it from promoting survival of this motoneuron. Taken together, our results suggest that *Antp* is necessary and sufficient for GW motoneuron survival and that it can counteract the proapoptotic effect of *Ubx*.

Ubx opposes *Antp* in promoting survival of the GW motoneuron

To examine how *Antp* counteracts the proapoptotic function of *Ubx*, we looked at *Ubx* and *Antp* expression in *Antp* and *Ubx* loss- and gain-of-function mutants, respectively. In *Antp*^{W10} mutants at late stage 16, *Ubx* expression was unaffected. In *en*-Gal4/UAS-*Antp* embryos of the same stage, *Ubx* expression in the NB7-3 cells occasionally appeared somewhat reduced compared with the wild type (summarized in Fig. 6A). This suggests that *Antp* overexpression reduces GW apoptosis, either by downregulating *Ubx* expression or by sustaining cell survival, or both. In the wild type, however, *Antp* is probably unable to suppress *Ubx*, as *Antp* mutants showed no change in *Ubx* expression (Fig. 6A) and no increase in abdominal GW apoptosis (Table 1). In *Ubx*^{1} mutants, high *Antp* expression levels extended to the anterior half of segment A1, as has been shown previously (Carroll et al., 1986), and in all NB7-3 cells *Antp* expression was equally strong from T1 to T3 (Fig. 5D-F). In the abdomen, by contrast, *Antp* levels in GWs did not differ between *Ubx*^{1} mutants and wild type in most segments (Fig. 6A). In *en*-Gal4/+;UAS-*Ubx*/+ embryos, *Antp* was downregulated in the whole NB7-3 cluster, in both thoracic and abdominal segments (Fig. 5G-I). These results show that *Ubx* overexpression represses *Antp*, which contributes to an increase in thoracic and abdominal GW apoptosis. In *Ubx*^{1} mutant embryos, however, NB7-3 *Antp* expression increased only in T2 and T3, and not in the abdominal segments (Fig. 6A), although apoptosis was abolished in both tagmata (Table 1). This suggests that, in the wild type, high Antp levels are required to prevent activation of apoptosis in GWs. In the third thoracic segment and in abdominal segments, low Antp levels were not sufficient to counteract the proapoptotic effect of Ubx. Accordingly, *Antp* overexpression was able to significantly reduce GW apoptosis in all segments (Table 1), without affecting Ubx levels (Fig. 6A). Thus, *Antp* seems to be required to prevent activation of GW apoptosis, and *Ubx* appears to oppose this function of *Antp*.

To confirm the opposing roles of *Antp* and *Ubx* in regulating GW survival, we examined *Antp*^{W10},*Ubx*^{1} double mutants and found that GW apoptosis was now partly restored, with 19.6% of GWs being apoptotic in segments A1 to A7 (*n*=112) (Fig. 5J, Table 1). In segment T3, 23.5% of counted hs (*n*=17) contained a cell that was triple-stained for Eg, activated Caspase 3 and Hb. However, thoracic segments of these mutants often showed a disordered pattern of Eg staining, making it difficult to unambiguously identify the GW motoneuron. We also attempted coexpressing *Antp* and *Ubx* using the *en*-Gal4 driver. The NB7-3 cluster was severely affected in these embryos: it was completely missing in 20% of T3 segments (*n*=10) and in 31.4% of abdominal segments (*n*=70), and in most other cases only one to two cells remained at stage 15, making it impossible to analyze GW fate. We therefore examined embryos in which the function of one or both of these genes was partially or completely removed. We made use of the observation that in *Ubx*^{1}/+ heterozygous embryos, GW apoptosis is reduced in T3 from 55.6% (in wild type, *n*=27) to 7.1% (*n*=14), and in the abdomen from 81.2% (in wild type, *n*=69) to 21.7% (*n*=97) (Table 1), which confirms that *Ubx* is required for GW apoptosis and that this effect is dose dependent. Removing one copy of the *Antp* gene in these embryos (*Antp*^{W10},*Ubx*^{1}/+) resulted in an increase in GW apoptosis to 16.7% (*n*=18) in T3 and 41.3% (*n*=126) in the abdomen. Moreover, completely removing *Antp* (*Antp*^{W10},*Ubx*^{1}/*Antp*^{W10}) further increased the proportion of dying GWs to 36.4% (*n*=22) in T3 and to 52.2% (*n*=90) in abdomen (Table 1).

Taken together, our results demonstrate that, in abdominal segments, *Ubx* induces apoptosis of the GW motoneuron late in development, and it does so by counteracting the positive effect of *Antp* on cell survival. In anterior thoracic segments, where *Ubx* expression is repressed by an unknown factor, *Antp* protects these neurons from apoptosis.

DISCUSSION

One of the fundamental questions in biology is how morphological diversity is generated among the homologous body parts (segments). We have addressed this question in the CNS of the *Drosophila*

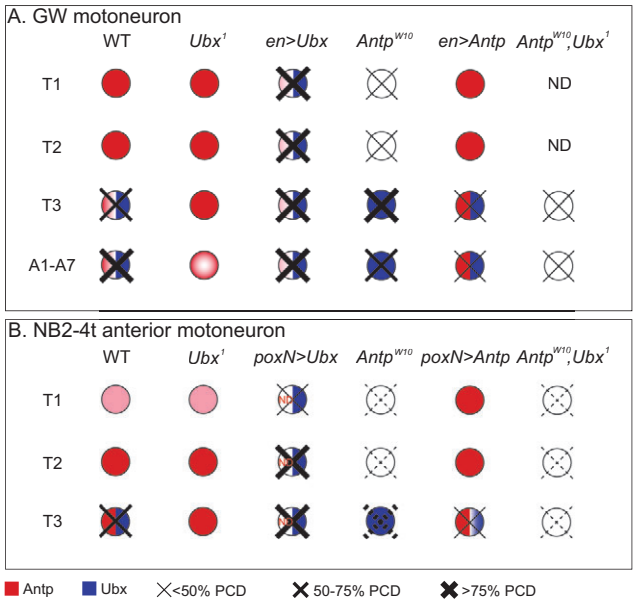

Fig. 6. Summary of GW and NB2-4t motoneuron apoptosis. Expression of *Antp* and *Ubx* as observed at late stage 16. *Antp* expression is indicated in red (strong) or pink (weak) and *Ubx* expression in blue. Crosses indicate apoptotic cell death, with the different line widths reflecting its frequency. Dashed crosses indicate only observed (not evaluated) MNa apoptosis. ND, no data.

embryo. Over the course of development, clear differences emerge between thoracic and abdominal segments in the form of specialized functional networks that support regional locomotion and sensory requirements. These differences are the result of region-specific division patterns of the neural progenitors (neuroblasts) and of the different numbers and types of neural cells that the neuroblasts generate (Technau et al., 2006). Regulation of CNS cell number is achieved through control of cell proliferation and death, and both of these processes have been shown to be orchestrated by Hox genes, in vertebrates and invertebrates (Bello et al., 2003; Economides et al., 2003; Liu et al., 2006; Miguel-Aliaga and Thor, 2004; Peterson et al., 2002; Prokop et al., 1998). It has recently also been reported that mutations in Hox genes affect locomotor behavior in larval crawling (Dixit et al., 2008), suggesting that they provide positional information for neurons and thus regulate the formation of neuromuscular networks that control region-specific peristaltic locomotion. Our studies reveal that the *Drosophila* Hox genes *Ubx* and *Antp* regulate segment-specific survival of two differentiated motoneurons, and may thus pattern neuromuscular networks by regulating the segment-specific presence of individual motoneurons. We show that *Ubx* is necessary and sufficient to induce apoptosis, and that it does so by impeding the positive effect of *Antp* on the survival of these cells.

A dual requirement for *Ubx* in development of the NB7-3 and NB2-4 lineages

The pattern of *Ubx* expression in the GW and NB2-4t motoneurons and their respective lineages is interesting. In early stages, the *Ubx* expression pattern conforms to its role as a homeotic gene: it is expressed from the posterior half of segment T3 to the anterior half of A7. The levels of Ubx protein in NB7-3 and NB2-4 and their progeny are not particularly high at these stages, but removal of both *Ubx* and *abdA*, for example, results in an NB7-3 lineage that is typical for T1 and T2 (A.R.-O., unpublished), suggesting that *Ubx* determines the segmental identity of this neuroblast. At a later developmental stage, *Ubx* expression is strongly upregulated in the NB7-3 and NB2-4 progeny in a segment-specific manner. This dynamic pattern of expression implies two roles of *Ubx* in these lineages: (1) an early role in establishing tagma-specific identity of the neuroblast, such as has already been shown for NB1-1 (Prokop and Technau, 1994); and (2) a late role in inducing apoptosis of the motoneurons. Our heat-shock experiments confirm that, at least in NB7-3, it is this late upregulation of *Ubx* that leads to apoptosis. NB2-4t could not be tested for the proapoptotic function of *Ubx* in the heat-shock experiments. However, we believe that it plays a late role in this lineage as well: as *Ubx* does not play an early role in specifying the T3 identity of the NB2-4t neuroblast, this suggests that the proapoptotic function of *Ubx* must be executed at a later point in development. Also, in *poxN*-Gal4,UAS-*GFP*/UAS-*Ubx* embryos, the NB2-4t lineage does not appear to be transformed into its abdominal counterpart, as we observe two closely positioned dorsal motoneurons in thoracic segments. In these embryos, *Ubx* is still capable of inducing apoptosis of the anterior motoneuron in all thoracic segments. Taken together, these data indicate that, also in this lineage, *Ubx* is needed at a late developmental stage to induce apoptosis. Dual requirements for Hox genes have also been described in determining thoracic bristle patterns (Akam, 1987; Rozowski and Akam, 2002) and in cardiac tube organogenesis (Perrin et al., 2004). It is not clear how the late expression of *Ubx* is regulated. It has been suggested that this might depend on genes that define the differences between cell types (Akam, 1987), and it will be interesting to see whether this is also the case for the NB7-3 neurons.

The ability of *Ubx* to induce apoptosis is context dependent

Interestingly, the late *Ubx* expression in NB7-3 extends to the second thoracic segment, but in a cell-specific manner: the GW motoneuron does not activate *Ubx* expression, whereas the EW interneurons do. These findings evoke the following questions: (1) why is it that the EWs in T2 to A7 do not undergo apoptosis although they also upregulate *Ubx*? and (2) what represses *Ubx* expression in the T2 GW?

Regarding the first question, it is likely that the differentiation program of the EWs creates a different cellular context in which *Ubx* is unable to induce apoptosis. The results of our ectopic *Ubx* expression experiments support this assumption: whereas GW undergoes apoptosis, the EWs do so very rarely, although they express *Ubx* at equally high levels. In addition, GW seems to acquire the competence to undergo *Ubx*-dependent apoptosis rather late, as *en*-Gal drives expression strongly from earlier stages but apoptosis does not occur until stage 15. This would suggest that the susceptibility to apoptosis of at least some motoneurons is coupled to differentiation. The context-dependent ability of *Ubx* to activate apoptosis also holds true for the NB2-4t lineage: the anterior motoneuron is susceptible to *Ubx*-induced apoptosis, whereas the posterior one is not, even when *Ubx* is overexpressed.

Our preliminary attempts to determine at least some of the factors contributing to apoptosis-susceptibility were not successful. For the NB7-3 lineage, we examined *abdA* expression and found that, at the onset of GW apoptosis, it is weakly expressed only in the EWs from A1 to A7, but not in GW. However, the survival of the EWs is not impaired in *abdA* mutants (data not shown). We also tested the co-factors *homothorax* (*hth*) (Pai et al., 1998; Rieckhof et al., 1997) and

extradenticle (*exd*) (Peifer and Wieschaus, 1990; Rauskolb et al., 1993), which are known to be required for some functions of homeotic genes, but obtained no compelling evidence for their involvement in the apoptosis of these cells. Mutants of other factors that are differentially expressed in GW and EWs [*numb* (Uemura et al., 1989), *zfh1* (Isshiki et al., 2001)] were also examined, and no indication was found that any of these is involved in the differential effect of *Ubx* on cell survival (data not shown).

Regarding the question of differential *Ubx* expression in NB7-3 cells of T2, this is an intriguing observation that might prove to be key in determining the developmental signal that upregulates *Ubx* late in development. One candidate for repressing *Ubx* expression in GW is the gap gene *hb* (White and Lehmann, 1986). It is known to repress *Ubx* in early embryonic development, and *hb* overexpression can suppress *Ubx* in the NB7-3 lineage (A.R.-O., unpublished). However, *hb* is also necessary to activate *Antp* expression and thereby specify the second thoracic segment (Wu et al., 2001). In *hb* mutants, T2 is not present and we were thus unable to test whether this is the factor repressing *Ubx* expression in GW. Other obvious candidates are the Polycomb group (PcG) proteins, well-known repressors of Hox genes (Ringrose and Paro, 2007). It has recently been shown that, contrary to what had been believed for a long time, target gene repression by these proteins is not necessarily maintained throughout development, but can be reversed in certain developmental contexts (Chen et al., 2005). It is conceivable that repression by PcG proteins could be lifted in some cells (e.g. EWs in T2) and not in others (e.g. GW). However, the question would still remain as to how the difference between the GW and EW neurons is established specifically in this segment. Alternatively, differential *Ubx* regulation might be effected via micro RNAs (Pearson et al., 2005) or non-coding RNAs (Petruk et al., 2006).

Ubx counteracts *Antp* to induce programmed cell death

We also show that *Antp* is required for GW survival in all segments, and that *Ubx* counteracts *Antp* in T3 to A7 to induce apoptosis. Although the lower percentage of dying abdominal GWs in *Antp* mutants (69%) as compared with wild type (81.2%) (Table 1) might indicate a proapoptotic function of *Antp* in the abdomen, we believe that this is not the case because *Antp* overexpression actually reduces the amount of abdominal GW apoptosis more than twofold (see Table 1, *en>Antp*). Moreover, removing *Antp* function in a *Ubx* heterozygous background increases GW apoptosis (both in T3 and in abdomen) in a dose-dependent manner, and removing both *Ubx* and *Antp* results in a recurrence of GW apoptosis, albeit with low penetrance (Table 1), lending further support to a pro-survival function of *Antp*.

It is not clear at which level these two factors interact. GW apoptosis is inhibited both in T3 and in abdominal segments of *Ubx* mutants. However, *Antp* expression in *Ubx* mutants is upregulated only in T3, and remains low in abdominal segments, suggesting that here *Ubx* does not induce apoptosis through *Antp* repression. In addition, the pattern and levels of *Ubx* expression do not change at all in *Antp* mutants, indicating that in this context *Antp* does not promote survival via *Ubx* regulation. We therefore propose that in the wild type, Antp and Ubx might compete for a co-factor or for a target enhancer, rather than cross-regulating each other. The proapoptotic gene *rpr* (White et al., 1996), which is transcriptionally activated in both GW and MNa motoneurons, is a candidate target. In fact, we found several binding sites for both Ubx and Antp in the enhancer of the *rpr* gene. It will be interesting to see whether *Antp* can prevent activation of the apoptotic machinery by affecting an upstream factor or through direct repression of *rpr*. The presence of several Antp binding sites in the *rpr* enhancer permits such speculation, and although it awaits experimental validation, this does suggest a model for the antagonistic effects of Antp and Ubx on cell survival. According to this model, Ubx and Antp compete for sites in the *rpr* enhancer. In cells that express Antp at high level, this would repress *rpr* transcription. Ubx would overcome repression by Antp and activate *rpr* transcription to induce apoptosis. Such positive Hox regulation of *rpr* has already been demonstrated in shaping segment borders in *Drosophila* embryos, where *Deformed* directly activates *rpr* transcription (Lohmann et al., 2002). In addition, antagonistic transcriptional regulation of the P2 *Antp* promoter in the embryonic ventral nerve cord has been demonstrated for Antp and Ubx (Appel and Sakonju, 1993). In this case, Antp positively autoregulates its own P2 promoter in the thoracic segments, and Ubx competes with Antp for the same binding sites and thus prevents high-level expression of *Antp* in the more posterior segments.

Hox-dependent apoptosis as a mechanism for CNS patterning

A requirement for Hox genes in segment-specific cell survival has already been shown for the MP2 and MP1 pioneer neurons (Miguel-Aliaga and Thor, 2004), where *AbdB* expression is necessary for survival of these neurons in the three most-posterior abdominal segments. In the more anterior segments, the dMP2 and MP1 motoneurons undergo apoptosis at the end of embryonic development, after they have completed their role in pioneering axonal tracts. The surviving dMP2 neurons innervate the hindgut and differentiate into insulinergic neurons (Miguel-Aliaga et al., 2008). The exact function and targets of the GW and the anterior NB2-4t motoneurons in the first and second thoracic segment are unclear (Bossing et al., 1996; Schmid et al., 1999; Schmidt et al., 1997), as is the reason for their removal in the relevant segments. The surviving GW and MNa might exert a region-specific neurosecretory function and thus modulate neuronal or muscle activity, as has been described for neurosecretory cells in the larval brain, the processes of which arborize on the wall of the anterior aorta adjacent to the ring gland (Johnson et al., 2003). Alternatively, the elimination of certain outward-projecting neurons in the posterior thoracic and/or abdominal segments might be related to the pattern of muscle fibers, which differs considerably between the thorax and the abdomen (Bate, 1993). We suggest that Hox-regulated segment-specific motoneuron survival is a part of the patterning process that enables formation of region-specific functional neuromuscular networks.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/135/20/3435/DC1

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