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Homeotic Genes Autonomously Specify the Anteroposterior Subdivision of the *Drosophila* Dorsal Vessel into Aorta and Heart

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The embryonic dorsal vessel in *Drosophila* possesses anteroposterior polarity and is subdivided into two chamber-like portions, the aorta in the anterior and the heart in the posterior. The heart portion features a wider bore as compared with the aorta and develops inflow valves (ostia) that allow the pumping of hemolymph from posterior toward the anterior. Here, we demonstrate that homeotic selector genes provide positional information that determines the anteroposterior subdivision of the dorsal vessel. *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*) are expressed in distinct domains along the anteroposterior axis within the dorsal vessel, and, in particular, the domain of *abd-A* expression in cardioblasts and pericardial cells coincides with the heart portion. We provide evidence that loss of *abd-A* function causes a transformation of the heart into aorta, whereas ectopic expression of *abd-A* in more anterior cardioblasts causes the aorta to assume heart-like features. These observations suggest that the spatially restricted expression and activity of *abd-A* determine heart identities in cells of the posterior portion of the dorsal vessel. We also show that *Abd-B,* which at earlier stages is expressed posteriorly to the cardiogenic mesoderm, represses cardiogenesis. In light of the developmental and morphological similarities between the *Drosophila* dorsal vessel and the primitive heart tube in early vertebrate embryos, these data suggest that Hox genes may also provide important anteroposterior cues during chamber specification in the developing vertebrate heart.

Key Words: **cardiogenesis; heart patterning; dorsal vessel; homeotic genes; *abd-A*; *Abd-B*; Hox genes.**

INTRODUCTION

The development of the chambered vertebrate heart with inflow and outflow tracts depends on the establishment of a defined anteroposterior (A-P) polarity within the primitive linear heart tube. In particular, the prospective tissues of the aortic sac, outflow tract (conotruncus), right ventricle, left ventricle, and atria are specified in an anterior-to-posterior order along the tube. The regional subdivision of the cardiac tube is also reflected in the spatially restricted expression of a number of differentiation markers in distinct domains along the A-P axis, including ventricular myosin light chain 2 (MLC2V) and atrial myosin light and heavy chains (MLC2A and AMHC1) (reviewed in Yutzey and Bader, 1995; Kelly *et al.*, 1999). The spatially restricted expression and activity of particular transcription factors, including *Irx4* in prospective ventricular and *Tbx5* in prospective sinoatrial domains, appear to be crucial for regional specification and gene expression patterns within the heart tube (Bao *et al.*, 1999; Liberatore *et al.*, 2000; Bruneau *et al.*, 2001a,b). However, in spite of the fundamental importance of proper anteroposterior organization of the cardiac tube for normal heart morphogenesis, we have very little insight into the positional cues and molecular processes that generate this polarity and define the restricted expression domains of early cardiac regulators.

The dorsal vessel of *Drosophila* resembles the primitive heart tube of early vertebrate embryos and has become a valuable model for early heart development. Like the ver-

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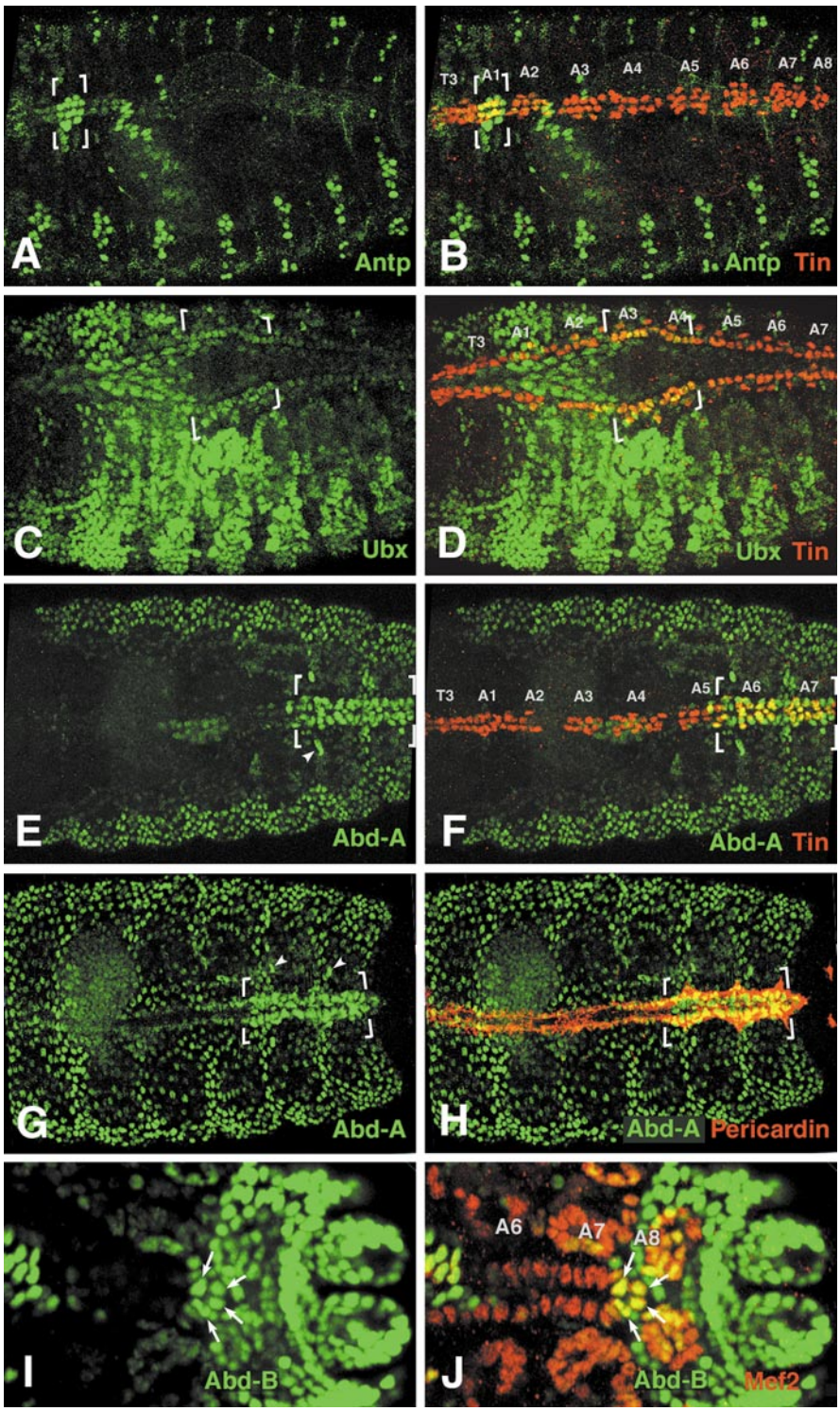

FIG. 1. Expression pattern of homeotic genes in the dorsal vessel of late stage embryos. (A–J) Confocal laser scans of embryos flourescently double-stained for a specific homeotic gene product (green) and a dorsal vessel marker (anti-Tin or anti-Pericardin; red). These are dorsal scans with anterior to the left. (A, C, E, G, and I) The single-channel scans of a homeotic gene product (green), while the respective panel to the right is the corresponding two-channel overlay of the same embryo with anti-Tin (B, D, and F), anti-Pericardin (H), or anti-Mef2 (J) shown in red. Overlapping expression in the two-channel scan is seen as yellow. The domains of peak expression of homeotic genes in the cardioblasts are marked by angles. (A, B) Antennapedia protein expression in the dorsal vessel of a late stage 16 embryo. (C, D) Ultrabithorax

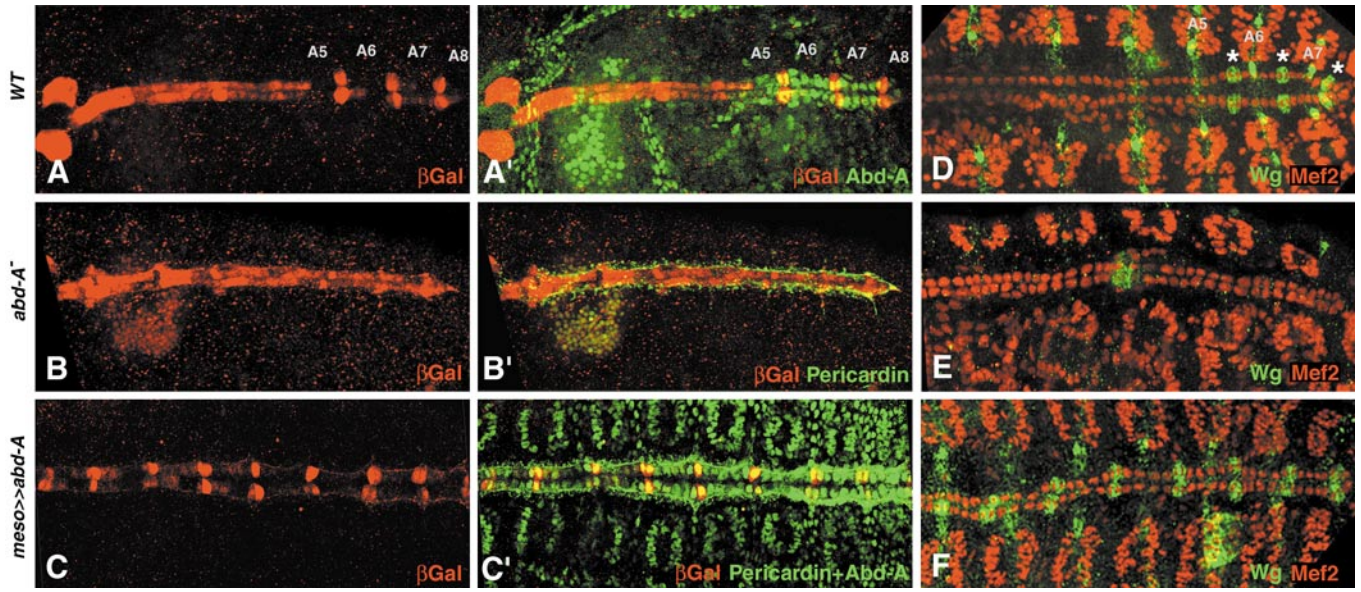

FIG. 2. The *abdominal-A* gene specifies the heart portion of the dorsal vessel. (A–F) Confocal laser scans of flourescently double-stained embryos. These are dorsal scans with anterior to the left. (A–C) Single-channel scans of the β-Gal expression derived from the tinCΔ5-*lacZ* transgene (red), while the respective panel to the right (A′–C′) is the corresponding two-channel scan of the same embryo with an additional dorsal vessel marker shown in green. Overlapping expression in the two-channel scan is seen as yellow. (D–F) Two-channel scans for Wg (green) and Mef2 (red) expression. (A, A′, D) Stage 16 wildtype embryos. (A, A′) The normal pattern of β-Gal expression in the dorsal vessel derived from the tinCΔ5-*lacZ* transgene (A) is continuous in the aorta, while in the heart it is restricted to three separated double pairs of cardioblasts. Double-staining for Abd-A (A′) demonstrates that this posterior pattern of β-Gal expression corresponds to the heart, where *abd-A* is expressed. (D) The normal pattern of Wg expression (green) in the cardioblasts (stained for Mef2 in red) of the dorsal vessel, where only three double pairs of cardioblasts in the heart express Wg. (B, B′, E) Stage 16 homozygous *abd-A* null mutant embryos. (B, B′) The pattern of β-Gal expression derived from the tinCΔ5-*lacZ* transgene (B) is continuous in the entire dorsal vessel. Double-staining with the EC11 antibody for Pericardin (B′) outlines the morphology of the dorsal vessel and demonstrates that the posterior region has the same narrower width as the aorta. (E) Wg expression is absent from the posterior of the dorsal vessel in homozygous *abd-A* null mutant embryos. (C, C′, F) Stage 16 embryos with the expression of *abd-A* driven in the entire dorsal vessel by either the 24B (C, C′) or twist- (F) Gal4 driver (see Materials and Methods). (C, C′) The β-Gal expression pattern derived from the tinCΔ5-*lacZ* transgene (C) is now discontinuous throughout the entire dorsal vessel such that there are segmentally spaced double pairs of β-Gal-expressing cardioblasts amid nonexpressing cardioblasts in the aorta as well as in the heart. These embryos have been double-stained for both Abd-A and Pericardin (C′; both in green); the former to demonstrate that abd-A is being driven in all the cardioblasts and the latter to outline the shape of the dorsal vessel, where the anterior appears to have the same broader width and larger lumen characteristic of the heart. (F) Wg expression in segmentally spaced double pairs along the entire dorsal vessel in these embryos.

tebrate heart tube, the dorsal vessel is formed by the fusion of bilateral primordia at the embryonic midline. Mechanistically, common features between vertebrate and *Drosophila* cardiogenesis include the induction of cardiac primordia in the lateral mesoderm via BMP/Dpp signals, which results in the expression of related cardiogenic transcription factors, particularly Nkx2-5/Tinman and GATA4,5,6/Pannier (reviewed in Bodmer and Frasch, 1999; Cripps and Olson, 2002).

Like the primitive heart tube in vertebrates, the dorsal vessel features a distinct A-P polarity. This polarity can be observed at two different levels; first, as a metamerically

protein expression in the dorsal vessel of a stage 15 embryo. (E–H) Abdominal-A protein expression in the dorsal vessel of late stage 16 embryos double-stained with anti-Tin (E, F) or anti-Pericardin (G, H). Pericardin is an ECM protein secreted by pericardial cells adjacent to the cardioblasts (Chartier et al., 2002), and antibody staining for this protein clearly outlines the morphology of the dorsal vessel. Examples of alary muscle nuclei which express Abd-A are marked by arrowheads. (I, J) Abdominal-B expression in the dorsal vessel of a stage 15 embryo double-stained with anti-Mef2 (high magnification view of posterior segments). All cardioblasts with peak Abd-B expression are marked by arrows. High and intermediate levels of Abd-B are also seen in the somatic muscles of A8 and A7, respectively.

repeated pattern along the A-P axis within each segment; and second, as a broad subdivision along the A-P axis along the entire dorsal vessel. The intrasegmental polarity of the dorsal vessel has been defined through the expression patterns of several genes in the two rows of cardiomyocytes (cardioblasts) and surrounding pericardial cells, most notably the homeodomain proteins Tinman (Tin) and Ladybird (Lb), the nuclear orphan receptor Seven-up (Svp), and the T-box gene product Dorsocross (Doc) (Jagla *et al.*, 1997; Gajewski *et al.*, 2000; Lo and Frasch, 2001; Ward and Skeath, 2000). These studies demonstrated that, among the six bilateral pairs of cardioblasts in each segment, two bilateral pairs lack Tinman and express both Svp and Doc, another two pairs express both Tin and Lb, and the remaining two pairs express only Tin. Based on these position-dependent patterns, the cardioblasts are thought to acquire at least three different identities within each segment, although it is not completely clear whether these identities correlate with any functional differences. However, the $Svp^+/Doc^+/Tin^-$ cells in the posterior three segments of the dorsal vessel do become different from the remaining cardioblasts morphologically and functionally, as these are the cells that form the inflow valves (ostia) of the organ (Molina and Cripps, 2001).

A broad subdivision along the A-P axis divides the dorsal vessel into two major portions, an anterior one termed aorta and a posterior one termed heart (Rizki, 1978; Rugendorff *et al.*, 1994). The heart, which is located in the abdominal segments A6–A8, features a wider diameter and lumen as compared with the aorta, which is positioned in the thoracic and abdominal segments T3–A5. In addition, the heart portion contains the ostia and is attached to the body wall with three pairs of alary muscles that are significantly larger than those of the aorta. This subdivision of the dorsal vessel into two morphologically different chamber-like portions as well as the flow of hemolymph from posterior to anterior are reminiscent of the developing vertebrate heart. Taken together, these observations lead us to anticipate that some aspects of A-P axis specification during early cardiogenesis are evolutionarily conserved and that studies in *Drosophila* may provide important clues to the understanding of the mechanisms that define A-P polarity within the developing vertebrate heart.

For the ectoderm, it has been well established that homeotic selector genes of the Antennapedia and Bithorax Complexes play key roles in conferring segment-specific identities along the anteroposterior axis (reviewed in Gellon and McGinnis, 1998). Additional evidence suggests that these homeotic genes also act within the mesoderm to determine segment-specific identities of cells of the somatic and visceral mesoderm, thereby controlling the variation of the body wall muscle pattern and midgut morphology along the A-P axis (Hooper, 1986; Reuter *et al.*, 1990; Greig and Akam, 1993; Bienz, 1994; Michelson, 1994). In the present study, we demonstrate that homeotic genes from these two complexes also have major roles in establishing the broad anteroposterior polarity of the dorsal vessel. We show that *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*) are expressed in distinct domains from anterior to posterior within the dorsal vessel. Importantly, we demonstrate that the expression of *abd-A* coincides with the heart portion and provide evidence using loss- and gain-of-function experiments that *abd-A* determines heart vs aorta identities within the dorsal vessel. Moreover, we demonstrate that at earlier stages *Abdominal-B (Abd-B)* is expressed just posteriorly to the cardiogenic mesoderm and suppresses cardiogenesis in this region.

MATERIALS AND METHODS

Drosophila Stocks

The *svp-lacZ* line AE127 (svp^{AE127}/*TM3*) was a gift of Y. Hiromi (National Institute of Genetics, Japan) and *UAS-svpI* from M. Hoch (Bonn University, Germany). *tinCΔ5-lacZ*, *tinCΔ4-GAL4-12a*, and *mef2-GAL4* were generated as described previously (Lo and Frasch, 2001; Gajewski *et al.*, 2000). $abd\text{-}A^{MX1}$/*TM1*, *w*; L^2 Pin^1/*CyO*, *P{GAL4-Kr.C} DC3*, *P{UAS-GFP.S65T}DC7*, and *w; Sco/Cyo, wg-lacZ* ($=wg^{en11}$) were obtained from the Bloomington Stock Center. wg^{en11} expresses lacZ in the endogenous wingless pattern (N. Perrimon; http://flybase.bio.indiana.edu/.bin/fbpcq.html?FBrf0086244).

wg^{IL}/*SMΛTM6B* was a gift from M. Mlodzik (Mount Sinai Medical School), wg^{DE}/CyO from A. Bejsovec (Duke University), *24B-Gal4* ($=how^{24B}$) from N. Perrimon (Harvard University), *SG30-GAL4 (twi-GAL4 + 24B-GAL4)* from A. Michelson (Harvard University), *twi.G-GAL4* from M. Baylies (Memorial Sloan-Kettering Cancer Center), *twi.2xPE-GAL4* from G. Schubiger (University of Washington), *UAS-abd-A.G* from J. Botas (Baylor College of Medicine), and *UAS-Abd-B.m.C* and $Abd\text{-}B^{D18}$ from I. Duncan (Washington University).

Antibody Stainings

Fluorescent antibody stainings were done as described in Knirr and Frasch (1999), while immunochemical stainings were carried out as described in Gajewski *et al.* (1999). The *yw* strain was used as the source for wildtype embryos. The following primary antibodies were used: rabbit anti-Lab (gift from M. Bienz), guinea pig anti-Dfd (gift of W. McGinnis), rat anti-Abd-A (gift from G. Morata), rabbit anti-Tin, rabbit anti-Mef2 (gift from H. Nguyen), rabbit anti-β-gal (Cappel); mouse monoclonal antibodies were anti-Sex combs reduced, anti-Antp (both gifts from T. Kaufman), anti-Ubx (gift from Rob White), anti-Abd-B (gift of S. Celniker), anti-Wg (Developmental Hybridoma Bank), and anti-Pericardin (EC11; gift from S. Zaffran).

Dissections of third instar *w; Sco/Cyo,wg-lacZ* larvae and staining of larval dorsal vessels were carried out as described in Molina and Cripps (2001). In the *wg*-ts experiments, embryos from a wg^{IL}/*SM6B, eve-lacZ* line were shifted from 18 to 29°C after 24 h postfertilization (i.e., subsequent to the requirement for *wg* in early cardioblast specification; Wu *et al.*, 1995) and fixed upon aging to stage 16. For the dissection of larval dorsal vessels, embryos from a cross wg^{IL}/*CyO, Kr-GAL4, UAS-GFP* × wg^{DE}/*CyO, Kr-GAL4, UAS-GFP* were temperature-shifted as above and aged to third

instar at 29°C. wg^{IL}/wg^{DE} larvae were selected based upon the absence of GFP fluorescence (third instar wg^{IL}/wg^{IL} or wg^{IL}/wg-null animals could not be analyzed due to early larval lethality under these conditions).

RESULTS

The Homeotic Genes Antp, Ubx, abd-A, and Abd-B Are Expressed in Distinct Regions of the Dorsal Vessel

Preliminary data from previous studies indicated that the homeotic genes *abd-A* and *Ubx* are expressed in subpopulations of pericardial cells and/or cardioblasts (Karch *et al.*, 1990; Bate, 1993). In the present study, we sought to more precisely define the expression domains of *abd-A* and *Ubx* in the dorsal vessel and to determine whether additional homeotic genes are also expressed within this organ. *labial* (*lab*), *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*), *Antp, Ubx, abd-A,* and *Abdominal-B* (*Abd-B)* were each examined for their expression in the dorsal vessel of late stage embryos in double antibody stainings with anti-Tin antibody. The segmental interruptions of Tin expression in cardioblasts by non-Tin-expressing svp cardioblasts facilitates the identification of the segmental register of homeotic gene expression in the DV.

Antp is strongly expressed in four consecutive pairs of cardioblasts in the anterior of the dorsal vessel (Fig. 1A). The three anterior cardioblast pairs of this domain of strong *Antp* expression are the posterior three tin cardioblast pairs of segment A1, while the fourth pair corresponds to the anterior pair of the two svp cardioblast pairs located between A1 and A2 (Fig. 1B). There is also strong expression in at least six pericardial cells flanking the domain of strong cardioblast expression, all of which are non-Tin expressing pericardial cells. Weaker *Antp* expression is seen in a row of three or four consecutive cardioblast pairs in T3 immediately anterior to the domain of strong *Antp,* and also in the four tin cardioblast pairs of segment A2 (Figs. 1A and 1B; see Fig. 6).

Located posterior to the domain of *Antp* expression is a domain of *Ubx* expression in the midsection of the dorsal vessel (Fig. 1C). The highest levels of *Ubx* are observed in the tin cardioblasts of segments A3 and A4, while lower levels are seen in the svp cardioblasts at the A3/A4 border and in the cardioblasts of segments A2 and A5. In addition, the cardioblasts in the heart segments contain barely detectable levels of Ubx (Fig. 1D; see Fig. 6). There also appears to be *Ubx* expression in some of the pericardial cells within A2 to A5, but due to the low expression levels, it is difficult to determine their exact number and whether any of these are tin pericardial cells.

As previously observed by Karch *et al.* (1990), expression of *abd-A* is found in the posterior of the dorsal vessel; however, it is present not only in the pericardial cells but also in the cardioblasts of this region (Figs. 1E and 1G). Strong *abd-A* expression is present in all the cardioblasts of segments A6 and A7 as well as the pericardial cells of these segments (Fig. 1F). Weaker expression is observed in the posterior-most pair of A5 tin cardioblasts and in the cardioblasts of segment A8. The entire domain of *abd-A* expression corresponds exactly to the heart portion of the dorsal vessel (Fig. 1H; see Fig. 6). In addition to the *abd-A* expression in the dorsal vessel proper, we observe expression in the four posterior pairs of the seven pairs of alary muscles, which attach the dorsal vessel to the dorsal underside of the body wall (Fig. 1E; see also Karch *et al.*, 1990).

High levels of Abd-B expression are detected only in two bilateral pairs of cardioblasts at the posterior end of the dorsal vessel and low levels are present in one additional pair abutting them anteriorly (Figs. 1I and 1J; see Fig. 6). In contrast to *Antp, Ubx, abd-A,* and *Abd-B,* the homeotic genes *lab, Dfd,* and *Scr* appear not to be expressed in the embryonic dorsal vessel (data not shown).

abd-A Specifies the Heart Portion of the Dorsal Vessel

Since *abd-A* expression coincides with the heart portion of the dorsal vessel, we examined whether it acts to specify the cardioblasts in which it is expressed to eventually form the heart. In order to distinguish aorta cardioblasts from heart cardioblasts, two different molecular markers were utilized. The first marker was the pattern of β-Gal derived from the tinCΔ5-*lacZ* transgene, where the expression of a *lacZ* gene is controlled by an internally deleted *tinman* cardiac enhancer element, tinCΔ5 (Lo and Frasch, 2001). This element drives β-Gal expression in all the cardioblasts of the aorta, whereas in the heart it is only expressed in three segmentally-spaced double pairs of cardioblasts (Figs. 2A and 2A′). These particular cardioblasts correspond to the svp cardioblasts of the heart (see below). The second marker is *wingless (wg),* which is expressed in these same three double pairs of svp cardioblasts within the heart of the late embryonic dorsal vessel (Fig. 2D).

In *abd-A* null mutant embryos, the pattern of tinCΔ5-*lacZ*-derived β-Gal is continuous in the heart as well as in the aorta of the dorsal vessel (Figs. 2B and 2B′). In addition, it appears that the width of the heart is now the same as that of the aorta when compared with a wildtype embryonic dorsal vessel (cf. Figs. 2A and 2A′). Similarly, the late expression of Wg in the svp cardioblasts of the heart is not detectable in these mutant embryos (Fig. 2E). The alterations in the pattern of these two markers strongly suggest that heart cardioblasts have not been specified in the posterior of the dorsal vessel of *abd-A* null mutant embryos and that these posterior cardioblasts have been transformed instead into aorta cardioblasts. This would indicate that *abd-A* is necessary for the specification of heart cardioblasts in the posterior portion of the dorsal vessel where it is normally expressed.

When the expression of *abd-A* is ectopically driven in the

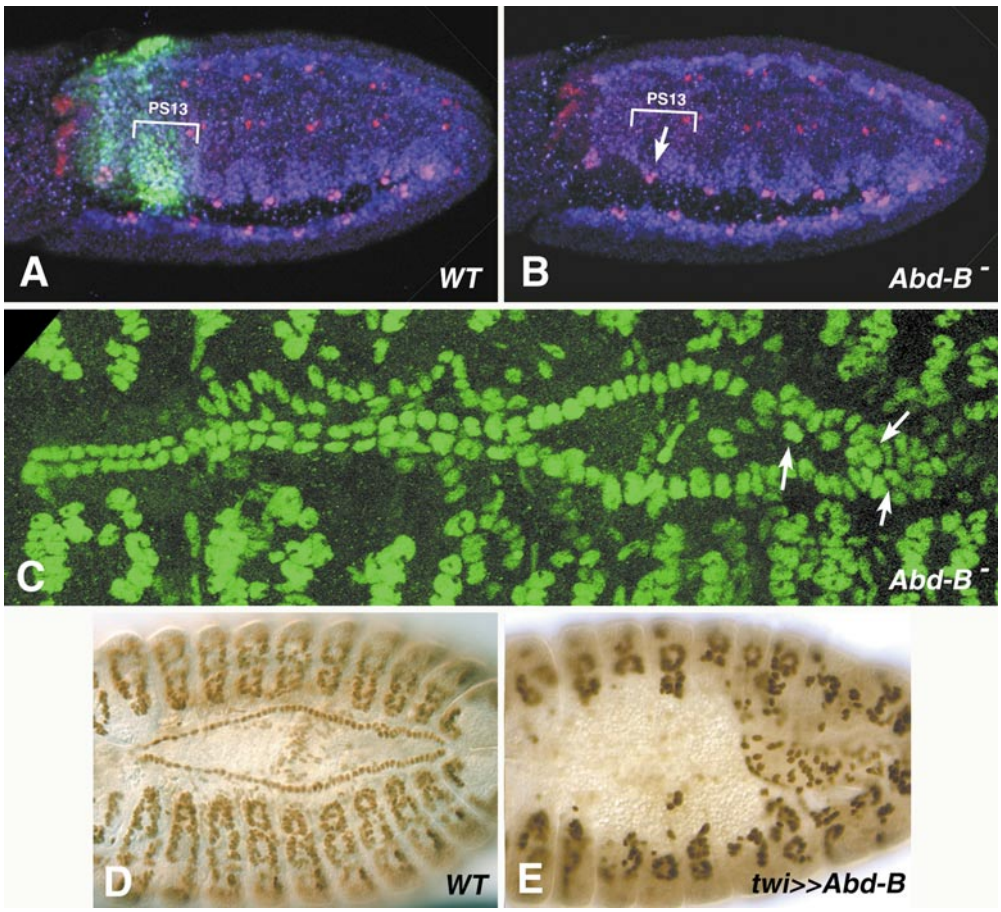

FIG. 3. *Abdominal-B* suppresses dorsal vessel formation. (A) Stage 11 wildtype and (B) *Abd-B* mutant embryo stained for Abd-B (green), Eve (red), and Tin (blue). Arrow indicates an ectopic cluster of Eve-positive pericardial cell and dorsal muscle progenitors in parasegment 13 (PS13) of the *Abd-B* mutant embryo. Note that the *dpp*-dependent expression of Tin in the dorsal mesoderm is not affected by the loss of *Abd-B* function at this stage, although there are extra Tin-positive heart cells at later stages (data not shown). (C) Dorsal vessel and dorsal somatic muscle nuclei of an *Abd-B* mutant embryo stained for Mef2. Examples of supernumerary cardioblasts in the heart portion are highlighted by arrows. (D) Dorsal view of stage 15 Mef-2-stained wildtype embryo showing the two rows of cardioblasts during dorsal closure. (E) Mef-2-stained embryo with *twist-GAL4*-driven expression of *Abd-B* in the mesoderm showing a complete absence of cardioblasts and a reduction of somatic muscle nuclei.

entire dorsal vessel, the pattern of tinCΔ5-*lacZ*-derived β-Gal in the anterior of the dorsal vessel resembles that of the heart, i.e., only segmentally repeated double pairs of cardioblasts which appear to be svp cardioblasts express β-Gal (Figs. 2C and 2C′). In addition, the anterior portion of these dorsal vessels has the greater width and wider lumen characteristic of the heart in wildtype dorsal vessels (cf. Figs. 2A and 2A′). Expression of Wg in late stage dorsal vessels of these embryos is now also present in the svp cardioblasts of the anterior portion of the dorsal vessel, in addition to the normal heart svp cardioblasts (Fig. 2F). These results indicate that ectopic expression of *abd-A* in anterior cardioblasts that normally develop into the aorta is sufficient to specify them as heart cardioblasts instead.

Abd-B Negatively Regulates Embryonic Cardioblast Development

During early cardiogenesis at embryonic stages 10–11, peak levels of *Abd-B* are observed in parasegments (PS) 13 and 14, which express the m and r proteins of *Abd-B*, respectively (Kuziora and McGinnis, 1988; Sanchez-Herrero and Crosby, 1988; Celniker *et al.*, 1989; Boulet *et al.*, 1991). These two parasegments abut the region of PS 2–12 from which heart progenitors arise (Azpiazu *et al.*, 1996; Riechmann *et al.*, 1998). Indeed, double stainings of stage 11 embryos for Abd-B (combined m+r variants) and Even-skipped (Eve), an early marker for pericardial cell and dorsal muscle progenitors, confirm that the previously known absence of mesodermal eve cells in PS 13 (Azpiazu and

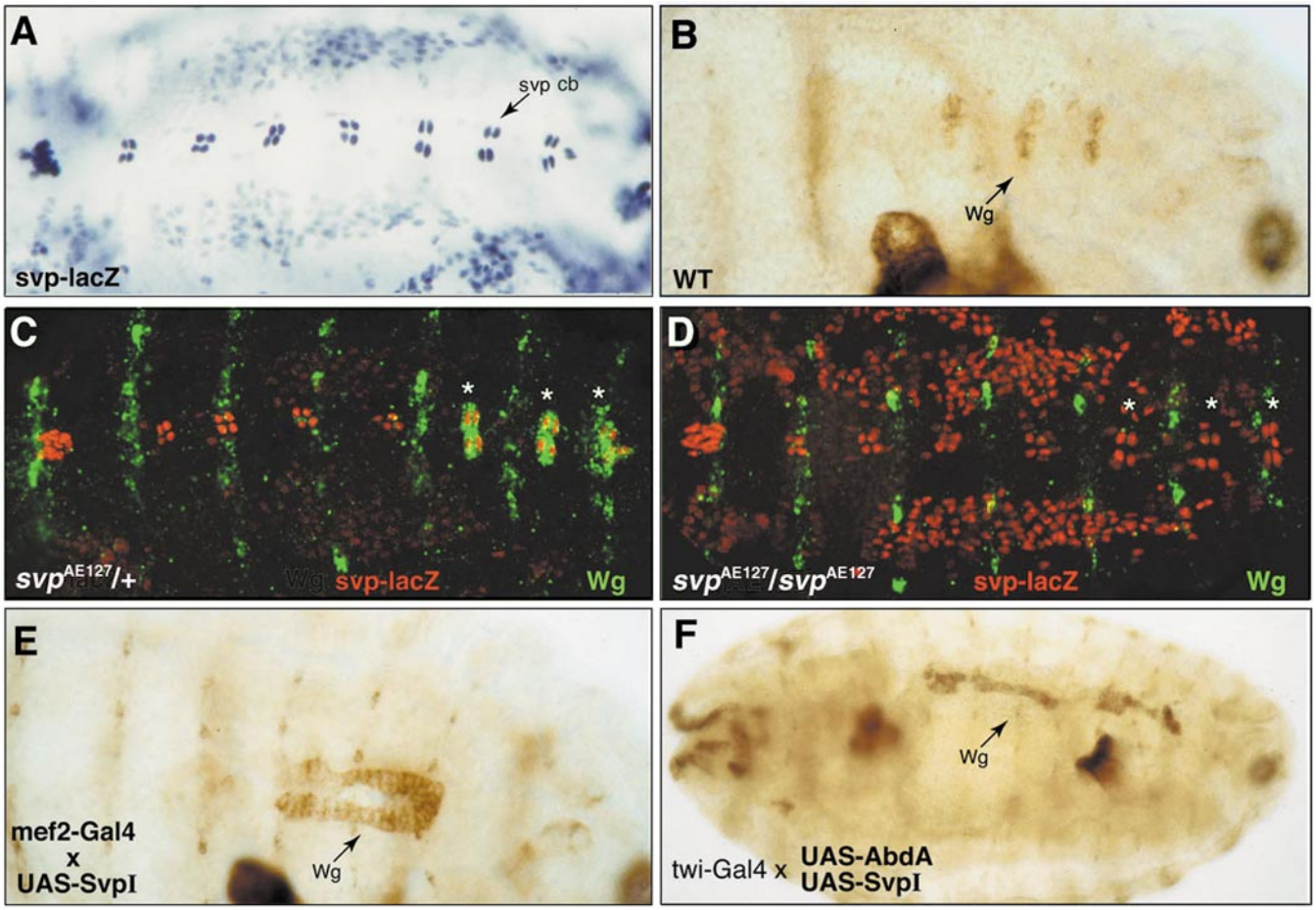

FIG. 4. *wingless* expression and regulation in the svp cardioblasts of the heart. Dorsal views of embryos with anterior to the left. (A) Anti-β-Gal staining of a stage 16 *svp-lacZ* embryo showing the svp cardioblasts. (B) Anti-Wg antibody staining of a wildtype stage 16 embryo. (C) Confocal laser scan of a stage 16 *svp-lacZ* ($svp^{AE127}/+$) embryo double-stained for β-Gal (red) and Wg (green) protein. The three double pairs of heart svp cardioblasts expressing Wg are marked by asterisks. (D) Confocal laser scan of a stage 16 homozygous null mutant *svp-lacZ* (svp^{AE127}) embryo double-stained for β-Gal (red) and Wg (green) protein. Note the absence of Wg expression in the three double pairs of heart svp cardioblasts (asterisks). (E) Anti-Wg antibody staining of a stage 16 embryo with *UAS-SvpI* expression driven by *mef2-GAL4* in all the cardioblasts of the dorsal vessel. Wg expression is seen in all the cardioblasts of the heart. (F) Anti-Wg antibody staining of a stage 16 embryo with *UAS-abd-A* and *UAS-SvpI* expression driven by *twist-GAL4*.

Frasch, 1993) coincides with the domain of peak expression of Abd-B in both ectoderm and mesoderm (Fig. 3A). This observed gap in Eve expression is compatible with the possibility that the Abd-B m variant is able to suppress the formation of eve pericardial and somatic muscle cells in PS 13, whereas Abd-B r is not active in suppressing eve cells (with unknown fates) in PS 14. In agreement with this notion, *Abd-B* mutant embryos generate an additional cluster of mesodermal eve cells in PS 13 (Fig. 3B). This observation suggests that *Abd-B* normally represses cardiogenesis, including the formation of pericardial cells, as well as the formation of somatic muscle #1, which is also derived from Eve-positive progenitor cells, in PS 13. This interpretation is further supported by the presence of supernumerary cardioblasts in the heart portion of the dorsal vessel of late stage embryos, as shown by anti-Mef2 stainings (Fig. 3C; cf. Fig. 2D). In *Abd-B* mutant embryos, we count about 116 cardioblast nuclei as compared with the normal number of 104 in the wildtype. Although the heart does not appear significantly elongated in the mutant embryos, it is frequently much wider and extra cardioblasts are arranged in irregular clusters or double-rows within its posterior portion (Fig. 3C). Similar increases in the number of cardioblasts and pericardial cell within the heart portions were seen in anti-Tin stainings of late stage *Abd-B* mutant embryos (data not shown). In addition to the observed increase in the number of heart cells, the somatic muscles in abdominal segment 8 (A8) in *Abd-B* mutant embryos

show an increase in the number of nuclei and a Mef2 pattern that is more similar to the pattern normally found in A7 (Fig. 3C; cf. Fig. 3D). Together with the Eve expression data at earlier stages and in agreement with the known muscle pattern (Bate, 1993), this observation indicates that *Abd-B* functions also to suppress the formation of the majority of dorsal body wall muscles in A8, including the Eve-expressing muscle #1.

The results of ectopic expression experiments with *Abd-B* are fully consistent with these proposed functions of *Abd-B* in early heart and somatic muscle development. Specifically, ectopic expression of *Abd-B* (*m*) that is driven by the *twist* promoter in the entire mesoderm completely suppresses the formation of cardioblast cells, as determined by anti-Mef2 staining (Fig. 3E). In addition, the number of Mef2-stained somatic muscle nuclei is reduced and more comparable to the number of somatic muscle nuclei normally found in A8 (Fig. 3E; compare with Fig. 3D). It appears therefore that *Abd-B* expression in the early mesoderm of those segments where it is not normally expressed is sufficient to suppress the development of the dorsal vessel as well as the formation of many somatic muscles.

Late Stage Expression of wg in the Heart svp Cardioblasts Depends on svp as Well as abd-A Function

The pattern of Wg expression in three segmentally repeated double pairs of cardioblasts within the late stage heart (Figs. 2D and 4B) is strongly reminiscent of the pattern of *svp* expression (Fig. 4A), which suggests that these are the heart svp cardioblasts. Double antibody staining for Wg and β-Gal in the dorsal vessel of *svp-lacZ* embryos clearly confirmed that the heart svp cardioblasts express the Wg protein (Fig. 4C). Since the heart svp cardioblasts eventually form the ostia (inflow valves) of the larval heart and since Wg is a developmentally significant signaling molecule, we have more closely examined the regulation of Wg expression in the heart svp cardioblasts during late embryogenesis.

As demonstrated above, the Wg expression in heart cardioblasts is dependent on *abd-A* (Fig. 2E). Since these Wg-expressing cardioblasts correspond to svp cardioblasts, we tested whether Wg expression is also dependent on *svp* function. In homozygous null svp^{AE127} mutant embryos, there is no detectable Wg expression in the heart cardioblasts that are marked by svp-lacZ (Fig. 4D). Therefore, the Wg expression seen in the heart svp cardioblasts of late embryonic dorsal vessels requires both *abd-A* and *svp* function. Accordingly, ectopic expression of SvpI in the cardioblasts of the entire dorsal vessel results in *wg* expression in all cardioblasts of the heart (Fig. 4E), and ectopic expression of both SvpI and Abd-A in the whole dorsal vessel causes Wg expression in the majority of the cardioblasts (Fig. 4F) of the entire dorsal vessel. These results demonstrate that the combination of *abd-A* and *svp* is both necessary and sufficient to activate *wg* expression in cardioblasts during late dorsal vessel development.

Since Wg expression in heart svp cardioblasts described above initiates toward the end of embryogenesis (stage 16), we examined whether this expression could also be detected in the dorsal vessel during later larval stages when the corresponding cells have formed the ostia. Because of high levels of unspecific background staining with Wg antibodies in larval preparations, *wg* expression in the dorsal vessel of third instar larvae was indirectly monitored by anti-β-Gal staining of dissected *wg-lacZ* animals. Moderate levels of *wg-lacZ*-derived β-Gal can indeed be detected in the ostia of the heart, although stronger levels are now present in four separated patches in the aorta that correspond to Tin-negative svp cardioblasts (Fig. 5). While this pattern of expression differs from that seen in the late embryonic dorsal vessel, it is clear that *wg* is expressed differentially and in a temporally regulated manner within the heart and aorta, respectively, of late stage embryos and third instar larvae. These observations suggest a yet undefined role for the signaling molecule in larval dorsal vessel development and/or functioning.

DISCUSSION

The key finding of the present paper is that the A-P subdivision of the *Drosophila* dorsal vessel into two distinct chamber-like portions is determined by the homeotic selector (Hox) gene *abd-A* (summarized in Fig. 6). Specifically, we have shown that *abd-A* expression is restricted to the posterior portion of the dorsal vessel that gives rise to the heart and that *abd-A* is genetically required to endow this portion of the dorsal vessel with its particular heart (versus aorta) identity. In the absence of *abd-A* activity, the heart is transformed into aorta, whereas upon ectopic expression of *abd-A* there is a transformation in the opposite direction, i.e., from aorta into heart. These conclusions are based upon the analysis of several distinctive morphological and molecular features of the heart portion, particularly its wider diameter, the expression of Wg in the cells of the heart-associated inflow tracts (ostia), and a *tin-lacZ* marker with a heart-specific pattern of repression. While it is difficult to assess the formation of ostia in late stage embryos by morphological and functional criteria, the observed changes in the ostia-specific Wg patterns in our experiments would suggest that generating the larval ostia is one of the heart-specific features that are determined by *abd-A*. *abd-A* functions as a spatially restricted selector gene in combination with *svp*, which is expressed in the non-Tin cardioblasts throughout the dorsal vessel, during ostia specification. In the remaining cardioblasts of the heart, it is likely that *abd-A* acts in combination with *tin* to activate heart-specific developmental programs.

The target genes of *abd-A* that are required for generating functional ostia and for the other heart cells to adopt their characteristic morphology are not yet known. Based on its

ostia-specific expression in late stage embryos, *wg* is a candidate target of *abd-A* that may function either in an autocrine fashion during ostia differentiation or in a paracrine fashion during the differentiation of the adjacent heart cardioblasts. The activation of the *wg* gene in the svp cells of the aorta during third instar also precedes ostia formation, in this case of the adult ostia, from these cells (our present observations; Miller, 1950; Curtis *et al.,* 1999; Molina and Cripps, 2001). Hence, there is a strong correlation between the initiation of *wg* expression in svp cardioblasts and their subsequent differentiation into functional ostia. In order to establish a functional correlation, we have performed controlled temperature-shift experiments with *wg*-ts mutant embryos and larvae (see Materials and Methods). However, dorsal vessels in stage 17 embryos and third instar larvae that were appropriately shifted to restrictive temperature did not show any noticeable morphological phenotypes in morphology or abnormal staining patterns for F-actin and spectrin in the ostia and other cardioblasts (P.C.H.L., M.F., and J.B.S., unpublished data).

In addition to the heart, *abd-A* is also expressed in the ectoderm as well as in the somatic and visceral mesoderm, which raises the question of whether the observed heart phenotypes could be due to non-autonomous functions of *abd-A*. While our loss-of-function data do not distinguish between heart-autonomous and non-autonomous functions of *abd-A*, it is interesting to note that ectopic expression within the mesoderm and even in a cardioblast-restricted fashion using a *tinCΔ4-GAL4* driver (data not shown) is sufficient to transform the aorta into a heart-like structure. These observations suggest that it is the cardioblasts and the function of *abd-A* within these cells that make the major contribution to the morphology and differentiation of the heart portion of the dorsal vessel. In addition, the expression of *abd-A* within pericardial cells of the heart portion would allow for the possibility that pericardial cells also contribute to some aspects of heart vs aorta development. Altogether, these observations suggest that A-P patterning of the cardioblasts by homeotic genes, and in particular *abd-A*, occurs within the dorsal vessel and that the temporal window of the plasticity of differential cardioblast identities lasts until relatively late in embryogenesis.

Misexpression of *Abd-B* only within cardioblasts using *tinCΔ4-GAL4* neither represses cardioblast development nor causes an expansion of the heart portion (data not shown). However, based on its expression in the posteriormost heart cells, it is conceivable that *Abd-B* is responsible for modifying the morphology of cardioblasts to allow the formation of a proper terminus of the heart. In contrast to this presumed function in heart patterning, the ability of *Abd-B* to repress cardiogenesis must be required prior to stage 13 (when *tinCΔ4-GAL4*-driven expression initiates) since it can be evoked with the early-active *twi-GAL4* driver but not with *tinCΔ4-GAL4*. Once the DV precursor cells have been specified at around stage 11 *Abd-B* is apparently no longer able to prevent these cells from contributing to the dorsal vessel, which suggests the differential presence of necessary cofactors for this repression in the early mesoderm versus the heart itself.

The spatially restricted expression of *Antp* and *Ubx* in portions of the aorta indicates that these two Hox genes function in the regulation of the A-P polarity of the dorsal vessel as well. Based upon our loss- and gain-of-function experiments with *Antp* and *Ubx*, these two genes do not appear to be involved in the subdivision into aorta and heart (P.C.H.L. and M.F., unpublished data). However, it is conceivable that *Antp* and *Ubx* are involved in the later subdivision of this anterior portion of the dorsal vessel into additional chambers that are seen in the adult stage after the remodeling of the dorsal vessel (Miller, 1950; Curtis *et al.,* 1999). In addition, previous observations showed that *Ubx* has a role in the A-P patterning of the larval dorsal vessel that appears to be due to its expression in pericardial progenitors. It has been proposed that lymph glands and pericardial cells descend from a common type of progenitor cell, which form the lymph glands in T3/A1 and pericardial cells in more posterior segments (Campos-Ortega and Hartenstein, 1997). By contrast, in *Ubx* mutant embryos, the lymph gland is strongly expanded toward more posterior abdominal segments (Mastick *et al.,* 1995; Rodriguez *et al.,* 1996). Together, these observations suggest that during normal development the activity of *Ubx* within pericardial progenitors of the posterior portion of the aorta acts to suppress lymph gland formation from these cells.

Although the order of Hox gene expression along the A-P axis in the dorsal vessel is the same as in the ectoderm and visceral mesoderm, there are considerable differences with respect to the anterior borders of their expression among these three tissues. For example, in the dorsal vessel, *abd-A* expression starts in A5, whereas in the visceral mesoderm, it starts at in A2/A3, and in the ectoderm, in the P-compartment of A1 (Tremml and Bienz, 1989; Karch *et al.,* 1990; Macias *et al.,* 1990). In addition, the dorsal vessel lacks *Sex combs reduced* (*Scr*) expression anterior to the *Antp* domain. The spatial expression of Hox genes in the ectoderm is known to be controlled largely by transcription factors that are encoded by segmentation genes of the gap, pair-rule, and segment polarity classes, and Hox gene expression in the early mesoderm is likely established by related mechanisms (reviewed in Gellon and McGinnis, 1998). For a more complete understanding of the A-P patterning mechanisms in the dorsal vessel, it will be important to obtain insight into the processes that determine the modified spatial domains of Hox gene expression within the developing dorsal vessel.

In light of the abundant similarities in the regulation of early cardiac development in *Drosophila* and vertebrates, it is likely that our present findings are also relevant for the understanding of the regulation of A-P polarity and chamber specification of the vertebrate heart. Although there are examples of Hox genes that are known to be expressed during early cardiogenesis (Searcy and Yutzey, 1998), the expression patterns of Hox genes in the developing vertebrate heart have not yet been analyzed systematically.

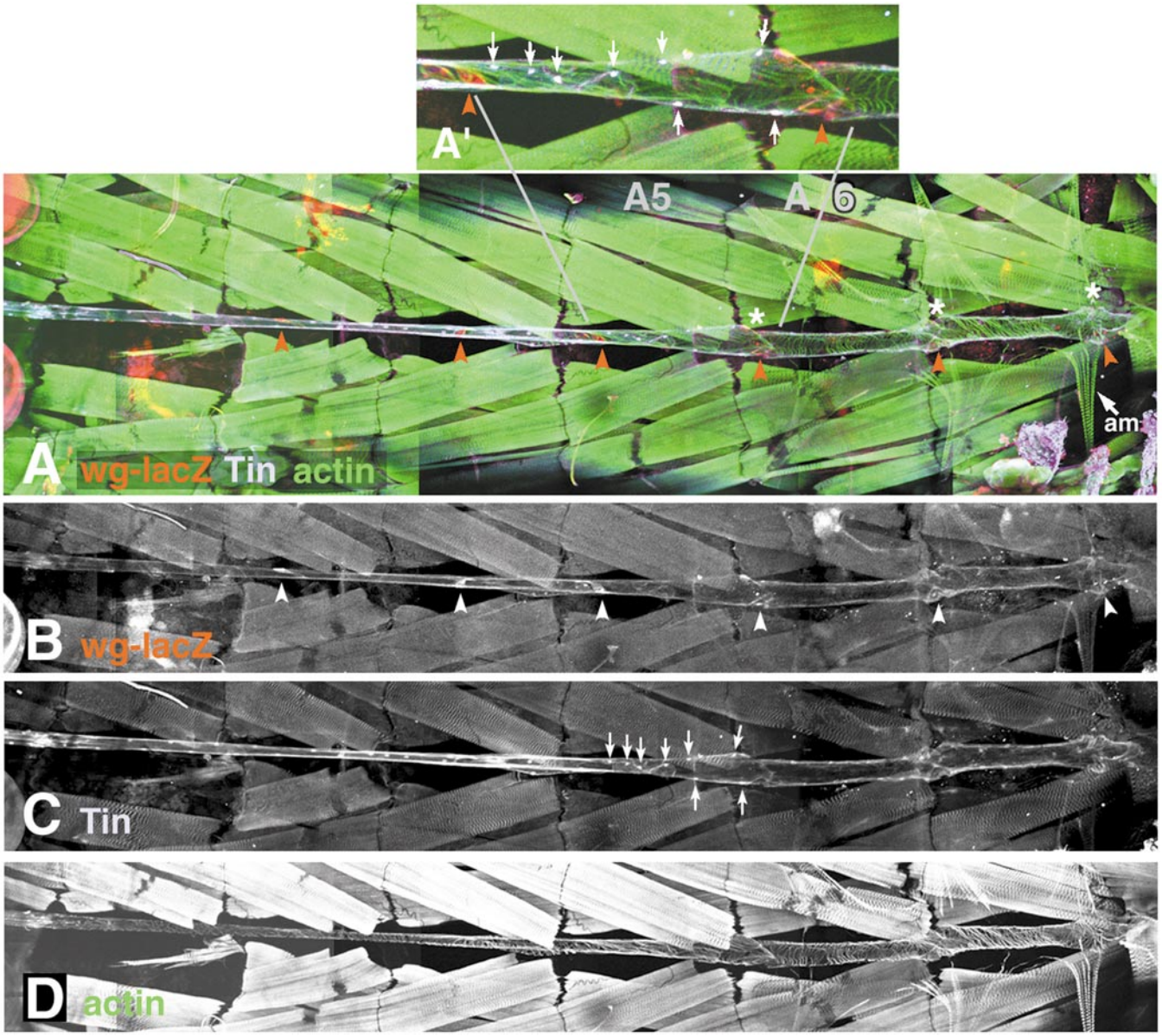

FIG. 5. *wingless-lacZ* expression in the dorsal vessel of 3rd instar larva. (A) Confocal laser scan (view from ventral side; composite) of a dorsal vessel still attached to the body wall of a dissected 3rd instar larval carcass, which has been triple stained for β-Gal (Cy3; red), Tin (Cy5; white), and F-actin (FITC; green). (A′) High magnification view of segment A5 and anterior A6. (B–D) Grayscale images of the single channel confocal scans for *wg-lacZ* (B), Tin (C), and F-actin (D). Ostia are marked with asterisks, *wg-lacZ* signals with arrow heads, and examples of Tin-stained nuclei within one segment (A5) with arrows. am: alary muscle.

Furthermore, functional redundancies of paralogous Hox genes may have largely prevented the detection of heart phenotypes upon genetic inactivation of individual paralogs. Among the few exceptions are *hoxa-3*$^{-/-}$ mouse embryos, which display cardiac neural crest-derived heart defects (Chisaka and Capecchi, 1991). However, there is a large body of evidence that retinoic acid (RA) has a role in the A-P patterning of the vertebrate heart and, in particular, is required and sufficient to promote posterior (atrial and sinus venosa) identities (reviewed in Rosenthal and Xavier-Neto, 2000). In other tissue contexts, such as the central nervous system and axial skeleton, RA is known to cause anterior expansions of Hox gene domains that are accompanied by anterior to posterior transformations (reviewed in Langston and Gudas, 1994). Taken together, these findings have raised the hypothesis that the observed effects of RA

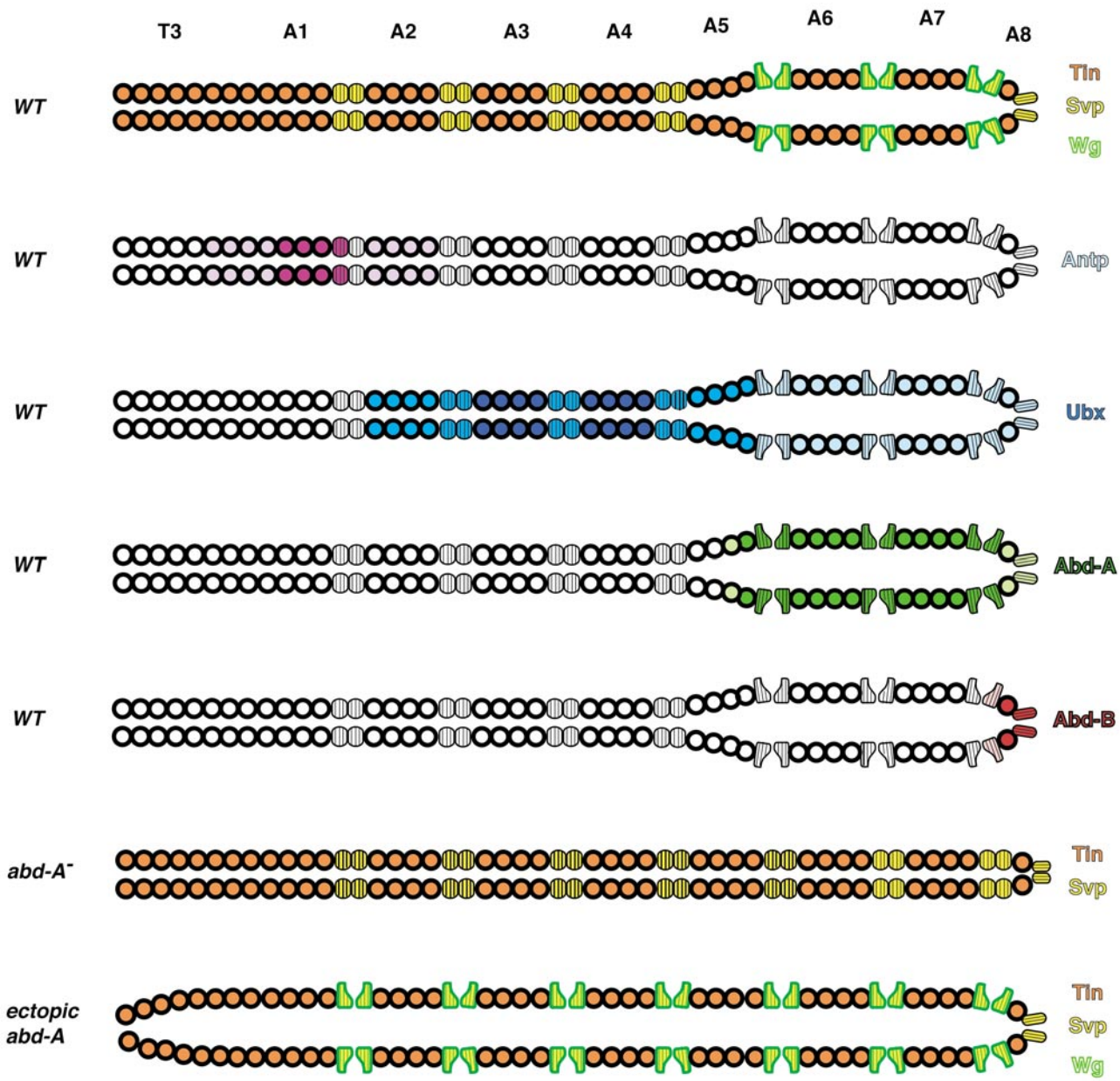

FIG. 6. Schematic diagrams of homeotic gene expression patterns and *abd-A* function in the cardioblasts of the dorsal vessel. A schematic representation of the cardioblasts in a late embryonic (stage 16 on) dorsal vessel is shown at top, with anterior to the left. The svp cardioblasts are hatched, while tin cardioblasts are nonhatched. The expression of Tin, Svp, and Wg is shown respectively as orange, yellow, and light green (outlined). The next three diagrams below show, from top to bottom, the expression pattern of Antp (purple), Ubx (blue), Abd-A (green), and Abd-B (red) with the darker and lighter shade of each color indicating strong and weak expression, respectively. The bottom two diagrams indicate the morphological changes in the dorsal vessel and alterations of Wg expression in cardioblasts associated with loss of *abd-A* expression (*abd-A*$^-$) and overexpression of *abd-A* in the DV (*SG30* $\gg$ *abd-A*). Segmental allocations refer to the position of ectodermal segments above the dorsal vessel at late stages and do not imply segmental origins of cardioblasts.

in heart patterning may be a reflection of the normal cardiac mesoderm-intrinsic activities of Hox genes during A-P patterning of the developing heart (Sundin and Eichele, 1992; Searcy and Yutzey, 1998). Our demonstration that Hox genes, and in particular *abd-A*, have an essential role in the A-P subdivision of the *Drosophila* dorsal vessel provides additional support for this presumed role of vertebrate Hox genes in cardiogenesis.

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REFERENCES

Azpiazu, N., and Frasch, M. (1993). *tinman* and *bagpipe*: Two homeo box genes that determine cell fates in the dorsal mesoderm of *Drosophila*. *Genes Dev.* **7,** 1325–1340.

Azpiazu, N., Lawrence, P., Vincent, J.-P., and Frasch, M. (1996). Segmentation and specification of the *Drosophila* mesoderm. *Genes Dev.* **10,** 3183–3194.

Bao, Z. Z., Bruneau, B. G., Seidman, J. G., Seidman, C. E., and Cepko, C. L. (1999). Regulation of chamber-specific gene expression in the developing heart by *Irx4*. *Science* **283,** 1161–1164.

Bate, M. (1993). The mesoderm and its derivatives. In "The Development of *Drosophila melanogaster*" (M. Bate and A. Martinez-Arias, Eds.), pp. 1013–1090. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bienz, M. (1994). Homeotic genes and positional signalling in the *Drosophila* viscera. *Trends Genet.* **10,** 22–26.

Bodmer, R., and Frasch, M. (1999). Genetic determination of *Drosophila* heart development. In "Heart Development" (R. Harvey and N. Rosenthal, Eds.), pp. 65–90. Academic Press, San Diego.

Boulet, A., Lloyd, A., and Sakonju, S. (1991). Molecular definition of the morphogenetic and regulatory functions and the *cis*-regulatory elements of the *Drosophila Abd-B* homeotic gene. *Development* **111,** 393–405.

Bruneau, B. G., Bao, Z. Z., Fatkin, D., Xavier-Neto, J., Georgakopoulos, D., Maguire, C. T., Berul, C. I., Kass, D. A., Kuroski-de Bold, M. L., de Bold, A. J., Conner, D. A., Rosenthal, N., Cepko, C. L., Seidman, C. E., and Seidman, J. G. (2001a). Cardiomyopathy in *Irx4*-deficient mice is preceded by abnormal ventricular gene expression. *Mol. Cell. Biol.* **21,** 1730–1736.

Bruneau, B. G., Nemer, G., Schmitt, J. P., Charron, F., Robitaille, L., Caron, S., Conner, D. A., Gessler, M., Nemer, M., Seidman, C. E., and Seidman, J. G. (2001b). A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. *Cell* **106,** 709–721.

Campos-Ortega, J. A., and Hartenstein, V. (1997). "The Embryonic Development of *Drosophila melanogaster*." Springer Verlag, Berlin.

Celniker, S. E., Keeland, J., and Lewis, E. B. (1989). The molecular genetics of the bithorax complex of *Drosophila*: Characterization of the products of the *Abdominal-B* domain. *Genes Dev.* **3,** 1424–1436.

Chartier, A., Zaffran, S., Astier, M., Semeriva, M., and Gratecos, D. (2002). Pericardin, a *Drosophila* type IV collagen-like protein is involved in the morphogenesis and maintenance of the heart epithelium during dorsal ectoderm closure. *Development* **129,** 3241–3253.

Chisaka, O., and Capecchi, M. (1991). Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene *hox-1.5*. *Nature* **350,** 473–479.

Cripps, R., and Olson, E. (2002). Control of cardiac development by an evolutionarily conserved transcriptional network. *Dev. Biol.* **246,** 14–28.

Curtis, N., Ringo, J., and Dowse, H. (1999). Morphology of the pupal heart, adult heart, and associated tissues in the fruit fly, *Drosophila melanogaster*. *J. Morphol.* **240,** 225–235.

Gajewski, K., Choi, C., Kim, Y., and Schulz, R. (2000). Genetically distinct cardial cells within the *Drosophila* heart. *Genesis* **28,** 36–43.

Gajewski, K., Fossett, N., Molkentin, J., and Schulz, R. A. (1999). The zinc finger proteins Pannier and GATA4 function as cardiogenic factors in *Drosophila*. *Development* **126,** 5679–5688.

Gellon, G., and McGinnis, W. (1998). Shaping animal body plans in development and evolution by modulation of Hox expression patterns. *Bioessays* **20,** 116–125.

Greig, S., and Akam, M. (1993). Homeotic genes autonomously specify one aspect of pattern in the *Drosophila* mesoderm. *Nature* **362,** 630–632.

Hooper, J. E. (1986). Homeotic gene function in the muscles of *Drosophila* larvae. *EMBO J.* **5,** 2321–2329.

Jagla, K., Frasch, M., Jagla, T., Dretzen, G., Bellard, F., and Bellard, M. (1997). *ladybird*, a new component of the cardiogenic pathway in *Drosophila* required for diversification of heart precursors. *Development* **124,** 3471–3479.

Karch, F., Bender, W., and Weiffenbach, B. (1990). *abd-A* expression in *Drosophila* embryos. *Genes Dev.* **4,** 1573–1587.

Kelly, R., Franco, D., Moormann, A., and Buckingham, M. (1999). Regionalization of transcriptional potential in the myocardium. In "Heart Development" (R. Harvey and N. Rosenthal, Eds.), pp. 333–355. Academic Press, San Diego.

Kuziora, M. A., and McGinnis, W. (1988). Different transcripts of the *Drosophila Abd-B* gene correlate with distinct genetic subfunctions. *EMBO J.* **7,** 3233–3244.

Langston, A., and Gudas, L. (1994). Retinoic acid and homeobox gene regulation. *Curr. Opin. Genet. Dev.* **4,** 550–555.

Liberatore, C. M., Searcy-Schrick, R. D., and Yutzey, K. E. (2000). Ventricular expression of *tbx5* inhibits normal heart chamber development. *Dev. Biol.* **223,** 169–180.

Lo, P. C. H., and Frasch, M. (2001). A role for the *COUP-TF*-related gene *seven-up* in the diversification of cardioblast identities in the dorsal vessel of *Drosophila*. *Mech. Dev.* **104,** 49–60.

Macias, A., Casanova, J., and Morata, G. (1990). Expression and regulation of the *abd-A* gene of *Drosophila*. *Development* **110,** 1197–1207.

Mastick, G., McKay, R., Oligino, T., Donovan, K., and Lopez, A. (1995). Identification of target genes regulated by homeotic proteins in *Drosophila melanogaster* through genetic selection of *Ultrabithorax* protein-binding sites in yeast. *Genetics* **139,** 349–363.

Michelson, A. (1994). Muscle pattern diversification in *Drosophila* is determined by the autonomous function of homeotic genes in the embryonic mesoderm. *Development* **120,** 755–768.

Miller, A. (1950). The internal anatomy and histology of the imago of *Drosophila melanogaster.* In "Biology of *Drosophila*" (M. Demerec, Ed.), pp. 420–480. Wiley, New York.

Molina, M., and Cripps, R. (2001). Ostia, the inflow tracts of the *Drosophila* heart, develop from a genetically distinct subset of cardial cells. *Mech. Dev.* **109,** 51–59.

Reuter, R., Panganiban, G. E. F., Hoffmann, F. M., and Scott, M. P. (1990). Homeotic genes regulate the spatial expression of putative growth factors in the visceral mesoderm of *Drosophila* embryos. *Development* **110,** 1031–1040.

Riechmann, V., Rehorn, K., Reuter, R., and Leptin, M. (1998). The genetic control of the distinction between fat body and gonadal mesoderm in *Drosophila. Development* **125,** 713–723.

Rizki, T. M. (1978). The circulatory system and associated cells and tissues. In "The Genetics and Biology of *Drosophila*" (M. Ashburner and T. R. F. Wright, Eds.), Vol. 2b, pp. 397–452. Academic Press, New York.

Rodriguez, A., Zhou, Z., Tang, M., Meller, S., Chen, J., Bellen, H., and Kimbrell, D. (1996). Identification of immune system and response genes, and novel mutations causing melanotic tumor formation in *Drosophila melanogaster. Genetics* **143,** 929–940.

Rosenthal, N., and Xavier-Neto, J. (2000). From the bottom of the heart: Anteroposterior decisions in cardiac muscle differentiation. *Curr. Opin. Cell Biol.* **12,** 742–746.

Rugendorff, A., Younossi-Hartenstein, A., and Hartenstein, V. (1994). Embryonic origin and differentiation of the *Drosophila* heart. *Roux's Arch. Dev. Biol.* **203,** 266–280.

Sanchez-Herrero, E., and Crosby, M. (1988). The *Abdominal-B* gene of *Drosophila melanogaster:* Overlapping transcripts exhibit two different spatial distributions. *EMBO J.* **7,** 2163–2173.

Searcy, R. D., and Yutzey, K. E. (1998). Analysis of *Hox* gene expression during early avian heart development. *Dev. Dyn.* **213,** 82–91.

Sundin, O., and Eichele, G. (1992). An early marker of axial pattern in the chick embryo and its respecification by retinoic acid. *Development* **114,** 841–852.

Tremml, G., and Bienz, M. (1989). Homeotic gene expression in the visceral mesoderm of *Drosophila* embryos. *EMBO J.* **8,** 2677–2685.

Ward, E., and Skeath, J. (2000). Characterization of a novel subset of cardiac cells and their progenitors in the *Drosophila* embryo. *Development* **127,** 4959–4969.

Wu, X., Golden, K., and Bodmer, R. (1995). Heart development in *Drosophila* requires the segment polarity gene *wingless. Dev. Biol.* **169,** 619–628.

Yutzey, K. E., and Bader, D. (1995). Diversification of cardiomyogenic cell lineages during early heart development. *Circ. Res.* **77,** 216–219.

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