Hedgehog and Wingless Induce Metameric Pattern in the Drosophila Visceral Mesoderm

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The Drosophila visceral mesoderm (VM) is a favorite system for studying the regulation of target genes by Hox proteins. The VM is formed by cells from only the anterior subdivision of each mesodermal parasegment (PS). We show here that the VM itself acquires modular anterior-posterior subdivisions similar to those found in the ectoderm. As VM progenitors merge to form a continuous band running anterior to posterior along the embryo, expression of connectin (con) in 11 metameric patches within the VM reveals VM subdivisions analogous to ectodermal compartments. The VM subdivisions form in response to ectodermal production of secreted signals encoded by the segment polarity genes hedgehog (hh) and wingless (wg) and are independent of Hox gene activity. A cascade of induction from ectoderm to mesoderm to endoderm thus subdivides the gut tissues along the A–P axis. Induction of VM subdivisions may converge with Hox-mediated information to refine spatial patterning in the VM. Con patches align with ectodermal engrailed stripes, so the VM subdivisions correspond to PS 2–12 boundaries in the VM. The PS boundaries demarcated by Con in the VM can be used to map expression domains of Hox genes and their targets with high resolution. The resultant map suggests a model for the origins of VM-specific Hox expression in which Hox domains clonally inherited from blastoderm ancestors are modified by diffusible signals acting on VM-specific enhancers. © 1998 Academic Press

Key Words: Hox; homeotic genes; connectin; segmentation; parasegment; visceral mesoderm; anterior-posterior; Drosophila.

INTRODUCTION

Patterning of the anterior-posterior (A–P) axis is a fundamental process in animal development. In most metazoans, the A–P axis is defined by the locations of the mouth and anus, two structures involved in digestion. Between the mouth and anus, different regions of the gastrointestinal tract are specialized to perform distinct digestive functions. In vertebrates, for example, the stomach, pancreas, liver, gall bladder, and small and large intestines develop at specific sites along the initially undifferentiated gut tube.

Insect guts, like vertebrate guts, exhibit considerable A–P diversity in both organ structure and cellular function. The generation of this diversity has been particularly well studied in the Drosophila embryo and larva, where cell types with distinct histological and functional properties develop at specific sites along the midgut endoderm (reviewed in Skaer, 1993). Gut endoderm derives from primordia located in the unsegmented terminalia of the embryo, so the genetic hierarchies that provide A–P patterning information to segmentally derived tissues such as the epidermis and nervous system do not pattern the early endoderm. Instead, endoderm A–P patterning is induced at midembryogenesis by the surrounding visceral mesoderm (VM), a tissue that originates from the segmented blastoderm (reviewed in Bienz, 1994). Each of four regions of the VM along the A–P axis transcribes a characteristic Hox gene, and each Hox gene is required to direct morphogenesis events in its region. Hox genes also direct production of secreted signals in the VM that induce specific cell fates in underlying endoderm cells. The VM domains of Hox gene expression are crucial for providing appropriate A–P patterning information to the unsegmented gut and to create its highly regular structure. How is A–P pattern, including the discrete domains of Hox gene expression, established in the VM?
The initial blastoderm patterns of Hox gene expression in the ectoderm and early mesoderm are established by the gap and pair-rule gene hierarchies, which activate Hox genes in domains based on parasegments (PSs), metameric units in the early embryo that are offset from segments (reviewed in Lawrence and Morata, 1994). These domains are maintained in the ectoderm by the Polycomb and Trithorax gene families, which are necessary for Hox activity to be stably inherited through cell divisions (Paro, 1993). However, the complex patterns of Hox expression in tissues such as the VM and nervous system cannot be inherited in any simple way from the patterns initiated at the blastoderm stage. In particular, the domains of expression of the four Hox genes active in the midgut VM—Sex combs reduced (Scr), Antennapedia (Antp), Ultrathorax (Ubx), and abdominal-A (abdA)—seem to be shifted posteriorly by one PS compared to their domains in ectoderm and early mesoderm (Lawrence and Morata, 1994; Tremml and Bienz, 1989).

Although patterning of the midgut relies on the Hox genes, division of the VM into four Hox domains is inadequate to supply the fine A–P pattern information that is reflected both in gut morphology and in the varied and highly restricted midgut expression of Hox target genes such as decapentaplegic (dpp), wingless (wg), teashirt (tsh), odd-paired (opa), pointed (pnt), labial (lab), and pdm-1 (Reuter et al., 1990; Immergäück et al., 1990; Mathies et al., 1994; Cimbora and Sanoniu, 1995; Affolter et al., 1993; Bilder et al., 1998). None of the target gene transcription patterns exactly matches the size or boundary of a Hox domain. The refinement in midgut patterning from Hox genes to targets clearly involves spatial information from other sources.

In this work we present evidence that the ectoderm provides spatial information to the VM that is independent of that provided by the Hox genes. Ectodermal induction creates metameric subdivisions with each visceral mesoderm segment. This information may converge with Hox regulation to finely subdivide the A–P axis and determine cell fates.

MATERIALS AND METHODS

Fly stocks. The following mutant alleles and GAL4-UAS lines were used: hh9K, hhG51, wg CX4, nkd 7E89, Scr w17, Antp w10, Df(3R)P109, UAS-wg, UAS-hh, UAS-dpp, and UAS-abdA. The mesodermal driver GAL-SG30 is described in Azpiazu et al. (1996) and the ectodermal driver GAL-e22c in Lawrence et al. (1996). Note that GAL-e22c shows no detectable GAL4 production in the mesoderm (Azpiazu et al., 1996). Flies carrying the enhancer fusion lines dppP/X and wgU/C were provided by L. Mathies, and ScrH/X by T. Kaufman. The null Con alleles fux14 and D1 were provided by C. Goodman, wg and nkd embryos were identified by the absence of lacZ balancers, hh embryos by the absence of en stripes, ScrAntpDTP109 embryos by the absence of Ubx, and con embryos by the absence of Con. For restrictive-permissive temperature shifts, 1-h collections of hh9K/hhG51 embryos at 25°C were held for 3.5 h further at 25°C and then aged for 10 h at 18°C. For permissive-restrictive temperature shifts, 1-h collections were held for 8.5 h at 18°C and then aged for 4 h at 25°C.

Antibody staining. Embryos were fixed and stained as previously described (Bilder et al., 1998), except for the following modifications. Fixation was in 1% formaldehyde (Polysciences) diluted 1:4 in PBS. For Con staining, embryos were fixed 60 min or more. Monoclonal supernatant 1D4-g9 (anti-Con), generously provided by R. White, was used at 1:5. Anti-Antp and anti-Fpl antibodies were used at 1:1000, while anti-en was used at 1:10.

Riboprobe preparation. Antisense riboprobes were produced using standard methods (Boehringer-Mannheim Genius kit). One microgram of template was used in a 20-μl synthesis reaction, which, following ethanol precipitation with tRNA and 40 min hydrolysis with carbonate buffer, was resuspended in 500 μl of dH₂O and stored at −20°C. Ten percent of the reaction was added to 500 μl of HB to add to embryos. Plasmids, polymerases, and restriction enzymes were as follows: N Bopa, kindly provided by D. Cimbora, and PTPrntP1, kindly provided by E. O’Neill, were linearized with BamHI and transcribed with T7. BS wgCV, kindly provided by K. Cadigan, was linearized with Xbal and transcribed with T3. BS dpp, provided by L. Mathies, was linearized with BamHI and transcribed with T3. N BabA, provided by Y. Graba, PTZabdB, kindly provided by W. Bender, and PGembap, kindly provided by M. Frasch, were linearized with HinDIII and transcribed with T7.

In situ hybridization. Detection of RNA in embryos generally followed the protocol of Lehmann and Tautz (1994). Briefly, embryos were dechorionated in bleach, fixed for 45 min in 4% formaldehyde (Polysciences) in PBS, devitellinized with a heptane/methanol interface, and stored in methanol. Following dehydration into PBT (PBS + 0.1% Tween 20), embryos were postfixed 20 min in 4% formaldehyde in PBT. Omission of proteinase K digestion, which was possible due to previous optimization of reaction conditions and probe hydrolysis, allowed subsequent antibody staining. Embryos were prehybridized for 1 h at 65°C and were hybridized overnight with probe. Embryos were rehydrated into PBT and incubated 60 min with anti-DIG-AP (Boehringer-Mannheim: 1:10,000). Following washes in PBT and AP buffer, the staining reaction was developed using BCIP/NBT. Stained embryos were either dehydrated in ethanol and mounted in Gary’s Magic Mountant (3 mg/ml Canada balsam in methyl salicylate) or washed in PBT before proceeding to antibody staining as above.

Figures. Images were photographed on a Zeiss Axiophot onto Kodak Ektachrome 64T slide film and scanned using a Kodak RFS 1035 scanner. Composites were assembled in Adobe Photoshop 3.0. In all panels embryos are oriented with the anterior to the left. Text in the upper right-hand corner of the panel indicates gene products detected, with Roman font for protein and italics for RNA. Text in the lower right-hand corner indicates the genotype of the embryo.

RESULTS

Metameric expression of Con in the VM is congruent with ectodermal parasegments. The midgut VM is formed from progenitors in the mesoderm primordium of each segment. The mesoderm primordia are formed during gastrulation from sheets of cells that invaginate through the ventral furrow and travel dorsally along the interior of each side of the embryo. At this stage, periodic dorsal crests are evident in the mesoderm, revealing the segmented organi-
zation of this tissue. Ectodermal engrailed (en) expression reveals that parasegments are subdivided into two "compartments," a term originally used to describe the units of cell lineage in imaginal discs that are revealed by en expression. Mesodermal cells located just under the en-expressing cells in the posterior ectodermal compartment have been called the mesodermal "P domain" (Azpiazu et al., 1996). The dorsal-most cells of the mesoderm P domain in each PS express the homeobox gene bagpipe (bap), detach from the mesodermal fold, and move inward toward the center of the embryo. These bap-expressing cells are the VM progenitor groups. The VM cells initiate expression of Fasciclin III (Fas III) as they migrate to join each other, forming a continuous band of VM running along each side of the embryo (Fig. 1A). Thus, all of the VM is derived from the posterior parts of the initial mesoderm metameres (Azpiazu et al., 1996).

As the VM progenitors merge during stage 11 (stages according to Campos-Ortega and Hartenstein, 1997), expression of con initiates in the VM. Con encodes a transmembrane protein that is distributed throughout the cell surface (Gould and White, 1992). Con is found in 11 patches equally spaced along the midgut VM (Fig. 1C; Gould and White, 1992; Bate, 1993). Con is also found on all cells of the visceral musculature that ensheathes the foregut and hindgut. Con is particularly distinct at stage 13, immedi-
ately before the VM cells divide and split to migrate dorsally and ventrally (Fig. 1B). By stage 15, con expression in the VM can no longer be detected.

In the stage 11 embryo, Con patches in the VM align with the overlying ectodermal stripes of En (Fig. 1D). Con patches 1-11 are in register with En stripes 2-12; both the anterior and posterior borders of Con and En are extremely similar. As the germ band retracts, VM and the ectoderm move at different rates, and Con patches and En stripes fall out of register. To evaluate the stability of Con expression in individual VM cells, Con was compared to expression of a lacZ reporter gene (dpp P/X: Manak et al., 1994) that is transcribed in a subset of VM cells. No changes in the relative expression of Con and βgal were seen from stages 11-14 (data not shown), demonstrating that the VM cells that produce Con at stage 14 are the same cells in which Con production was initiated. The long-term stability of βgal excludes the possibility that Con and the reporter gene change expression congruently. Thus, Con patches at stage 13 identify VM cells that lie adjacent to ectoderm posterior compartments at stage 11.

Several genes in addition to con are active in segmented patterns in the early VM, in register with ectodermal compartments, bap, initially activated in all cells that form the VM (Fig. 1E; Azpiazu et al., 1996), is later repressed in patches that align with posterior compartments. Staining of stage 11 embryos with Con and bap probes reveals that bap is lost from the same cells in which con is activated (Fig. 1F). ota, a target of Antp and abdA in the VM (Cimbora and Sakonju, 1995), is initially limited to six strong patches in the newly formed VM. Staining with anti-Con reveals that ota transcript shares a boundary with Con patches (Fig. 1G).

Visceral mesoderm con stripes are regulated by wg and hh. The striped expression of con, bap, and ota in the early VM suggests a metameric organization of the VM similar to ectodermal compartments. How are modular subdivisions established in the VM, a tissue derived exclusively from cells of a single (posterior) PS subdivision? Unlike all other genes with spatially restricted VM expression (reviewed in Bienz, 1994), the restricted expression of con does not depend on Hox genes. Homozygous Scr Antp Df(3R)P109 embryos, which are deficient for all Hox genes expressed in the VM, have wild-type expression of con (Fig. 2B). Therefore, con regulation requires an input of patterning information to the VM from an unappreciated source.

Segment polarity genes subdivide ectodermal segments, so they are candidates for regulators of con in VM subdivisions. In embryos mutant for hh or wg, Con patches are absent from the midgut VM (Figs. 2C and 2D). VM formation in these embryos, as assayed by fasn III expression, is normal (data not shown), and the VM migrates to cover the gut and form midgut constrictions. By contrast, in naked (nkd) mutant embryos, which produce ectopic hh and wg (Tabata et al., 1992), Con patches are expanded; the breadth of Con expansion parallels that of expanded En. The expansion of Con in nkd embryos is due to Con production in cells throughout the arches of the early VM, rather than in just the apices of these arches (Fig. 2F, compare with Fig. 1F).

Are both hh and wg required for con regulation? Ectodermal transcription of hh and wg is maintained by a positive feedback loop between adjacent rows of cells that express these two genes (reviewed in Perrimon, 1995). Due to the feedback loop, the absence of Con patches in hh and wg embryos, and the expanded Con in nkd embryos, could be explained by changes in either hh or wg expression. To assay the individual roles of the hh and wg signals in con regulation, hh and wg were expressed throughout the mesoderm (including the VM) using the GAL4-UAS system (Brand and Perrimon, 1993), and the effects on Con were examined. In embryos with ubiquitous mesodermal expression of hh (meso-Hh embryos), Con is produced in all cells of the VM (Fig. 3C). In contrast, ubiquitous mesodermal expression of wg results in a severe reduction of Con (Fig. 3B). The role of the wg signaling pathway was confirmed by expressing an activated form of armadillo (arm) throughout the mesoderm. The activated arm produced by the arm10 allele mimics the effects of constitutively active wg signaling in a cell-autonomous manner (van de Wetering et al., 1997). In meso-ArmS10 embryos, Con is absent from the VM (Fig. 3D). These data suggest that both the diffusible signals encoded by wg and hh are necessary and sufficient to establish the Con VM pattern and that hh signaling activates con transcription while wg signaling represses it. The absence of Con in wg embryos is therefore due not to the absence of wg but to the reduced hh function in wg mutant embryos.

The regulation of con by segment polarity genes could occur prior to the segregation of VM precursors from the somatic mesoderm, when segment polarity proteins are present in the mesoderm. Alternatively, secretion of hh or wg from the ectoderm at later stages could induce con expression in the VM. To evaluate these possibilities, hh and wg were expressed throughout the ectoderm, but not mesoderm, using the GAL4-UAS system. Ectopic expression of hh induces Con throughout the VM (Fig. 3E), while ectopic expression of wg again leads to a severe reduction of Con (Fig. 3F). We conclude that either paracrine or autocrine actions of hh and wg can regulate Con.

To distinguish between con regulation by a mesodermal or ectodermal source of hh, the temporal requirement for hh activation of con was determined using a temperature-sensitive hh allele. We find that hh is required for VM Con expression at a time when hh is expressed in the ectoderm but not the mesoderm. hh expression is not detected in the mesoderm in stage 8 or older embryos (Mohler and Vaní, 1992; data not shown). We shifted hh16 embryos between restrictive and permissive temperatures at the beginning of stage 10. If hh activity is eliminated during stage 10, no VM Con expression is seen, as in null hh mutants (Fig. 3G). However, in embryos to which hh activity is restored before stage 10, Con is present in VM patches as in wild type (Fig. 3H). Taken together, these data suggest that ectoder-
mal expression of hh activates con in VM cells near the posterior ectoderm compartments, while ectodermal expression of wg represses con in VM cells near anterior compartments.

**Mapping the boundaries of Hox gene and Hox target transcription in the VM.** Since Con expression marks the imprint of ectodermal PS boundaries on the VM, Con patches can be used to precisely map the domains of Hox gene transcription in the VM. Embryos were stained with antibodies or by RNA in situ hybridization to detect Hox gene products or Hox enhancer–lacZ reporter constructs, and the boundaries of Hox gene expression in relation to Con patches were evaluated. Expression domains of VM patterning genes known to be regulated by Hox genes were also mapped. The results of these studies are shown in Fig. 4 and summarized in Fig. 5.

To emphasize the relation of expression patterns within the VM to expression patterns in the ectoderm, we adopt the term “visceral mesoderm segment” (VS) to refer to the VM cells between the anterior boundaries of two successive Con patches, in alignment with two successive En stripes. As the alignment of Con patches to En stripes begins with En stripe 2, we will call the cells between Con patch 1 and Con patch 2 “VS 2.” Azpiazu et al. (1996) have introduced the terms “P” and “A” domains to refer to the mesodermal subdivisions adjacent to the anterior and posterior compartments of the ectoderm after gastrulation is completed (these subdivisions are equivalent to, respectively, the
“eve” and “slp” domains of Reichman-Fried et al. (1994) and, later in development, to the A and P domains of Dunin-Borowski et al. (1995); see Reichman-Fried et al. (1994) for a discussion of mesoderm subdivision nomenclature. Following this nomenclature we adopt the terms “VS P” and “VS A” domains to refer to Con-expressing and non-Con-expressing domains of the VM, respectively.

These terms are chosen to emphasize that although both VM domains derive from the mesodermal P domain, hh-induced Con expression in the P domain reveals further differentiation of these cells from the non-Con-expressing A domain cells. One VS thus consists of a VS P domain and the VS A domain immediately posterior to it (Figs. 5 and 7). Hox proteins are found in the VM from the earliest stages.
of VM formation, i.e., from the onset of Con expression. In a close examination of Antp expression, no changes in the boundaries of Antp protein in relation to Con patches were seen from stage 11 through stage 14 (data not shown). While we cannot completely exclude the possibility of subtle changes, we saw no evidence for dynamic patterns of Hox gene expression during these stages.

Hox expression patterns in the VM respect some but not all of the VS boundaries reflected by Con expression. Scr is limited with distinct boundaries to the A domain of VS 3 (Fig. 4A). VS 4 entirely lacks Hox gene expression. The anterior border of Antp expression is coincident with the anterior VS 5 boundary. However, the posterior border of Antp is in the midst of the A domain of VS 6 (Fig. 4C). This is also the anterior boundary of Ubx expression; the posterior boundary of Ubx is in VS 8 (Fig. 4B). Here, Ubx interfaces with abdA expression, which continues posteriorly through at least VS 11 (data not shown). The posterior boundary of abdA expression has not been precisely mapped. AbdB expression was detected only in the hindgut, and not the midgut, VM (data not shown).

The expression of most Hox target genes, like that of Hox
genes, is seen from the earliest stages of VM formation. teashirt (tsh) is expressed in two domains (Mathies et al., 1994). The anterior midgut domain extends from VS 4 to mid-VS 6, where it shares a posterior boundary with Antp; the central midgut domain extends several cells to either side of the VS 8 boundary (data not shown). dpp is also expressed in two domains: at the gastric ceca it is found in the A domain of VS 2 and the P domain of VS 3, while in the central midgut it extends from the A domain of VS 6 to terminate just anterior to the VS 8 boundary (Fig. 4D). wg is expressed just anterior to the VS 8 boundary, with some cells after stage 12 lying in VS 8 (Fig. 4E). pnt is expressed throughout VS 8, although expression is not seen until early stage 13 (Fig. 4F; Bilder et al., 1998). At stage 13, the two domains ofopa expression extend from the P domain of VS 4 to the VS 6 boundary and from VS 9 through VS 11 (Fig. 4G).

PS subdivision-dependent activation of the Hox target wg. The differences evident between VM expression of Hox genes and their targets (Fig. 5) emphasize the increase in patterning information accrued during Hox-regulated subdivision of the midgut. How are target genes activated in only some of the cells that produce the regulating Hox protein?

Several Hox targets appear to respect the PS subdivision organization of the VM. The initial VM expression ofopa is seen only adjacent to Con patches, in A domains of VS 3–5 and 8–11 (Fig. 1F). Similarly, wg is limited to a subset of abdA-expressing cells, those at the border of VS 8 (Figs. 4E and 6A). wg is activated by abdA and dpp (Reuter et al., 1990; Immerglück et al., 1990). Ectopic expression of abdA leads to induction of wg in several patches anterior to its normal expression, while ectopic expression of dpp leads to induction of wg in a single posterior patch. Strikingly, the sites of ectopic wg induction in both genotypes align with the VS boundaries: in cells just anterior to VS 3, 5, and 6 in ectopic AbdA embryos (Fig. 6B) and anterior to VS 9 in ectopic Dpp embryos (Fig. 6C). These results suggest that metameric subdivisions in the VM limit Hox gene activation of VM targets such as wg to restricted areas.

DISCUSSION

In this paper we have presented an analysis of the expression and regulation of the connectin gene in the midgut VM. These studies demonstrate the existence of patterning information in the VM that is independent of
the Hox genes. Instead, the information comes from induction by the segmentation genes that establish compartmental divisions in the ectoderm. The visceral mesoderm has been shown to induce cell fates in the underlying endoderm (Hoppler and Bienz, 1994; Immerglück et al., 1990; Reuter et al., 1990). A cascade of induction from ectoderm to mesoderm to endoderm thus subdivides the gut tissues along the A–P axis. The reiterated pattern of Con expression, as well as its independence from Hox gene activity, makes it a useful marker to precisely study morphogenetic and gene regulatory events within the VM. Furthermore, the identifi-
The fate of tissues derived from portions of three neighboring embryonic compartments is diagrammed. Segment polarity genes initially act to establish compartmental pattern in the blastoderm (stage 6) (Martinez Arias, 1993a). At gastrulation (stage 8), compartment-like P and A domains are reformed in the mesoderm under the influence of segment polarity genes active in the ectoderm (Azpiazu et al., 1996). Cells from mesodermal P domains migrate inward (gray arrows) and give rise to VM (pink rectangle) and fat body while A domains remain near the ectoderm and give rise to cardiac mesoderm and somatic muscle (SM; gray rectangle). At the extended germ band (stage 11), as the VM forms a continuous tissue along the anteroposterior axis of the embryo, ectodermal wg and hh again act to restore a modular pattern to the VM, distinguishing Con-expressing VS P domains from VS A domains. The SM does not actually physically interpose between the VM and the ectoderm (Hartenstein, 1993). Not represented in this figure are the patterning influences of wg and hh on the SM (e.g., Baylies et al., 1995; Lawrence et al., 1995; Ranganayakulu et al., 1996).

**FIG. 7.** Model for formation of PS subdivision patterning of the VM. The fate of tissues derived from portions of three neighboring embryonic compartments is diagrammed. Segment polarity genes initially act to establish compartmental pattern in the blastoderm (stage 6) (Martinez Arias, 1993a). At gastrulation (stage 8), compartment-like P and A domains are reformed in the mesoderm under the influence of segment polarity genes active in the ectoderm (Azpiazu et al., 1996). Cells from mesodermal P domains migrate inward (gray arrows) and give rise to VM (pink rectangle) and fat body while A domains remain near the ectoderm and give rise to cardiac mesoderm and somatic muscle (SM; gray rectangle). At the extended germ band (stage 11), as the VM forms a continuous tissue along the anteroposterior axis of the embryo, ectodermal wg and hh again act to restore a modular pattern to the VM, distinguishing Con-expressing VS P domains from VS A domains. The SM does not actually physically interpose between the VM and the ectoderm (Hartenstein, 1993). Not represented in this figure are the patterning influences of wg and hh on the SM (e.g., Baylies et al., 1995; Lawrence et al., 1995; Ranganayakulu et al., 1996).
activation of Ubx, dpp, and pnt and represses Antp and opa (Bilder et al., 1998; Yu et al., 1996). Ectopic wg also disrupts endoderm differentiation: low levels repress development of the copper cell endoderm subtype, while high levels induce ectopic copper cells and prevent iron cells and large flat cells from forming (Hoppler and Bienz, 1995). The derepression of dpp in embryos lacking the Bithorax-complex Hox genes provides another example: dpp is derepressed in a VS subdivision-like fashion (Reuter et al., 1990). These examples provide further evidence for a “ground state” of patterning information in the VM, separate from that provided by the Hox genes, that is organized into both V5s and VS subdivisions.

**Hox and Hox target gene expression domains in the VM.**

Stable Con expression in metamerich patches in the VM provides a convenient landmark for mapping expression domains of midgut patterning genes. The 11 patches of Con expression can serve as a “ruler” for measuring the location and sizes of VM gene expression. A previous description of VM expression domains made use of a fushi tarazu (ftz) promoter which drives lacZ in alternate P5s in the embryonic blastoderm (Tremml and Bienz, 1989). The stability of βgal allows both continued detection long after ftz transcripts disappear and inheritance in cells, such as VM cells, that derive from the marked blastoderm P5s. VM cells marked by βgal in this manner were used to map Hox gene expression boundaries relative to the borders of even-numbered P5s. Con patches, which mark each boundary of VS 2–12 as well as distinguishing between the VS A and P subdivisions, increase the precision of the map. Both Con patches and ftz-βgal stripes align with ectodermal PS borders, suggesting that the two systems—the former using an ectoderm-induced marker, the latter using a marker inherited cell-autonomously from blastoderm to mesoderm to VM—mark identical boundaries. Discrepancies between Hox expression domains mapped in relation to ftz-βgal perdu- and Con patches can largely be accounted for by the differing resolution of the two methods.

Comparing midgut gene expression domains with Con patches provides an opportunity to quantify and compare dynamic changes in VM patterning gene expression. Subtle changes of gene expression in mutant and ectopically expressing embryos can be documented or ruled out (Bilder et al., 1998). The four A–P files of VM cells along the midgut tube provide a one-dimensional axis ideal for studying A–P patterning. Furthermore, the midgut becomes a favored system for studying Hox regulation of targets, dpp signaling, and wg signaling (e.g., Manak et al., 1994; Nellen et al., 1994; Riese et al., 1997). The ability to precisely assay gene expression changes in the midgut should contribute to progress in these important fields.

**Derivation of Hox transcription domains: Inheritance or a new program?**

An important previously unanswered question in midgut patterning concerns the establishment of domains of Hox expression in the VM. The relation of VM Hox domains to Hox domains in other tissues has not been clear. It has been thought that most Hox expression in the VM is shifted one PS posterior in relation to its mesodermal and ectodermal precursors (reviewed in Bate, 1993; Lawrence and Morata, 1994); possible mechanisms for such a shift have not been advanced. A reassessment of Hox patterns using Con patches as landmarks, along with knowledge of the origin of the VM from mesodermal PS subdivisions, provides an alternative to a global PS-shift model. The results call attention to departures from the clonal inheritance of Hox expression states from the mesoderm and provide evidence for tissue-specific regulation of Hox genes.

The VM is formed when cells from mesodermal P domains migrate inward and merge to form a continuous band that aligns with PS 2–12 in the ectoderm. In the most simple model, VM cells would migrate in a single direction to fill the space between progenitor groups. This model is supported by the alignment of ftz-βgal stripes, clonally inherited by the VM from the blastoderm stage, with ectodermal PS markers (Tremml and Bienz, 1989). Since no βgal-labeled VM cells are seen anterior to ectodermal ftz-βgal stripes, VM cells appear to migrate only posteriorly during the merging of the VM progenitors from each PS.

In comparing VM Hox domains predicted by this simple inheritance model to the domains actually observed in wild-type embryos (Fig. 8), several discrepancies are apparent. According to the inheritance model, Scr should fill all of VS 3, but instead it is activated only in the A domain of VS 3. VS 6 should contain only Ubx-expressing cells; instead, the P domain and a portion of the A domain express Antp. By contrast, expression of Antp in VS 5, Ubx in VS 6, and the entire domain of abdA expression can be explained by the inheritance of Hox expression states from the mesoderm.

Our analysis refocuses the search for an explanation of divergent Hox expression in the VM, from a PS shift in Hox domains to mechanisms leading to loss of Scr in the P domain of VS 3 and loss of Ubx from much of VS 6. The identification of separable enhancer elements responsible for expression of Scr and Ubx specifically in the VM (Gindhart et al., 1994; Thuringer et al., 1993) suggests the existence of tissue-specific mechanisms responsible for the anomalous VM expression of these genes. A Ubx VM enhancer, which has been carefully studied, is responsive to both dpp and wg (Thuringer and Bienz, 1993). We propose that, following segregation of the VM, Ubx expression in this tissue becomes dependent on the VM enhancer. The restricted range of wg activity from the VS 8 boundary limits the anterior border of Ubx VM expression to within the A domain of VS 6. Ubx is a repressor of Antp transcription in the VM (Reuter and Scott, 1990). Thus, the contraction of Ubx expression results in a derepression of Antp, which expands to fill most of VS 6. The ability of ectopic wg to cause an expansion of Ubx anteriorly by about one VS, at the expense of Antp expression (Thuringer and Bienz, 1993), is consistent with this model. A similar mechanism may
hold for Scr, which is also regulated by dpp. The relevant question for the tissue-specific pattern of Hox expression in the VM thus turns to the unknown regulators that control VM-specific Hox enhancers.

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