**enguailed-mediated repression of Ultrabithorax is necessary for the parasegment 6 identity in Drosophila**

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**SUMMARY**

The homeotic genes of *Drosophila* are expressed in overlapping domains along the anterior-to-posterior axis and specify the distinct morphological patterns of each parasegment. Within single parasegments, the levels of homeotic gene expression are often modulated, in part because of cross-regulation by other homeotic gene products. However, the functional significance of different levels of homeotic gene expression is unclear. Here modulations in Ultrabithorax (*Ubx*) expression within parasegment 6 are examined. Specifically, *Ubx* is shown to be down-regulated in the posterior compartment of this parasegment by engrailed (*en*). The significance of *Ubx* repression by *en* was demonstrated by characterizing the expression of the *Ubx* target gene, Distal-less (*Dll*). In the posterior compartment of parasegment 6, *Dll* is normally expressed in a small cluster of cells. If *Ubx* is expressed uniformly via a heat-shock promoter, *Dll* is inappropriately repressed in these posterior compartment cells. In the anterior compartment of parasegment 6, *Dll* is normally repressed by high levels of *Ubx*. However, if *en* is expressed uniformly via a heat-shock promoter, *Ubx* is repressed and *Dll* is derepressed. Because *Dll* is required for the development of larval sensory structures, these results demonstrate that engrailed-mediated repression of *Ubx* in the posterior compartment is necessary for the morphology of parasegment 6. Thus, different levels of homeotic gene expression can be important for their segmental patterning functions.

Key words: bithorax Complex, parasegment, Ultrabithorax, Distal-less, engrailed, Drosophila

**INTRODUCTION**

The homeotic genes of *Drosophila* are responsible for diversifying the identities of each segment in both the embryo and adult (Lewis, 1978; Wakimoto and Kaufman, 1981; Sanchez-Herrero et al., 1985). Most of the homeotic genes are clustered in either the Antennapedia (ANTP-C) or bithorax (BX-C) complexes. The BX-C contains three homeotic genes, Ultrabithorax (*Ubx*), abdominalA (*abdA*) and AbdominalB (*AbdB*), and is required for specifying the identities of parasegments 5 to 14 (Lewis, 1978; Sanchez-Herrero et al., 1985) whereas the homeotic genes of the ANTP-C are required for specifying the identities of thoracic and head segments (Kaufman et al., 1990). As all of the homeotic genes present in the BX-C and ANTP-C encode nuclear proteins that contain a DNA-binding homeodomain these genes are likely to control segmental patterns by differentially regulating the expression of subordinate downstream genes (reviewed in Scott et al., 1989; Hayashi and Scott, 1990; McGinnis and Krumschaft, 1992).

The regulation of homeotic gene expression is complex. However, despite this complexity, two common themes have emerged. First, during embryonic development homeotic expression domains are usually delineated by parasegmental instead of segmental boundaries (see for example Carroll et al., 1988). Second, homeotic gene products often cross-regulate each other’s expression, which results in different levels of expression within individual parasegments. Both of these points apply to *Ubx* expression. Cells within parasegment (ps) 6 contain the highest levels of *Ubx* protein as compared to more posterior parasegments which contain less (Beachy et al., 1985; White and Wilcox, 1985). In embryos mutant for the two more abdinoally acting homeotic genes, *abdA* and *AbdB*, the level of *Ubx* in parasegments 7 to 13 increases to the level normally present in ps 6 (Struhl and White, 1985). The phenotypic consequence of simultaneously losing *abdA* and *AbdB* expression and derepressing *Ubx* expression is that the ps 6 identity is reiterated throughout the abdomen (Lewis, 1978; Struhl, 1984; Casanova et al., 1987). In contrast, expressing uniform and high levels of *Ubx* via the hsp70 promoter (HS-*Ubx*) without the loss of *abdA* and *AbdB* expression does not transform abdominal segments to a ps 6 identity (Gonzalez-Reyes and Morata, 1990; Gonzalez-Reyes et al., 1990; Mann and Hogness, 1990). Thus, the functional significance of *Ubx* repression by *abdA* and *AbdB* is unclear.

One example of the diversification of segment identities by homeotic genes is that each thoracic segment, but none of the abdominal segments, contains a pair of Keilin’s organs, which are sensory structures containing three small hairs. Keilin’s organs are thought to be the evolutionary remnants of larval legs (Keilin, 1915; Cohen and Jurgens, 1989). The cells that generate these structures are closely associated with the imaginal disc primordia, which are the precursors to adult struc-
tures, including the adult legs (Keilin, 1915; Cohen, 1990). The homeobox-containing gene, Distal-less (Dll, also called Brista, Ba) is required for Keilin’s organ formation and is expressed in the cells that eventually give rise to these sensory structures (Cohen et al., 1989; Cohen and Jurgens, 1989). Moreover, expression of Dll is a marker for the cells that eventually give rise to the imaginal discs (Cohen et al., 1993).

The ps 6 pattern that is repeated in abdA AbdB mutant embryos often includes a partial Keilin’s organ, as evidenced by one of the three hairs being present in the anterior portion of these parasegments (Lewis, 1978; Struhl, 1984; Hartenstein, 1987). In Ubx mutant embryos parasegments 5 and 6 are transformed into copies of ps 4. In these embryos, the reciprocal phenotype, partial Keilin’s organs containing two of the three hairs, is usually observed in the posterior portion of the transformed parasegments. These phenotypes suggest that Keilin’s organs mark parasegmental boundaries and, more specifically, that the Keilin’s organs present in the third thoracic segment straddle the boundary between parasegments 5 and 6.

Uniform and high levels of Ubx expression from a HS-Ubx gene transforms thoracic and head segments to a parasegment 6-like identity (Gonzalez-Reyes and Morata, 1990; Mann and Hogness, 1990). However, the ps 6-like identity generated in this way is devoid of all Keilin’s organs. Significantly, this identity can be generated in the absence of all other homeotic gene expression normally present in thoracic and abdominal segments (Mann and Hogness, 1990; Chan and Mann, 1993). This demonstrates that uniform levels of Ubx can directly modify the ‘ground state’ segment identity (Lewis, 1978) to generate this ps 6-like pattern.

In this report, the reasons for the differences in the ps 6 identities present in abdA AbdB and HS-Ubx embryos are investigated. Whereas heat-shock-induced Ubx expression generates uniform levels of Ubx, evidence is presented that extends previous work (Carroll et al., 1988; Martinez-Arias and White, 1988), demonstrating that Ubx is expressed at different levels within a wild-type parasegment 6. The different levels of Ubx are, at least in part, due to repression by another homeodomain-containing protein, engrailed (en). Moreover, engrailed-mediated repression of Ubx in the posterior (P) compartment is relevant to the parasegment 6 morphology because inappropriately high levels of Ubx repress the homeotic target gene, Dll, which is necessary for Keilin’s organ formation. Therefore, modulations in the levels of homeotic gene expression can be important for correct segmental patterning.

**RESULTS AND DISCUSSION**

**Engrailed represses Ubx in parasegment 6**

Each parasegment in Drosophila is composed of the posterior (P) compartment of one segment plus the anterior (A) compartment of the next more posterior segment (Martinez-Arias and Lawrence, 1985). Thus, the anterior-most portion of any parasegment is a P compartment and the posterior-most portion is an A compartment. Previous experiments suggested that the P compartment of parasegment 6 (ps 6) contains lower levels of Ubx than the A compartment (Carroll et al., 1988; Martinez-Arias and White, 1988). The lower expression levels may be due to repression by engrailed (en) which is expressed in the P compartments of all parasegments (Kornberg et al., 1985). Moreover, repression of Ubx by en could be an important difference between a wild-type ps 6 and the ps 6-like metamere generated by heat-shock-Ubx expression. To investigate these questions further, the levels of Ubx expression in ps 6 were examined using confocal microscopy. For most of the experiments described here, the abdA AbdB genotype was studied because it repeats the ps 6 identity throughout the abdomen and because it eliminates any potential for cross-regulation by these two more posteriorly acting homeotic genes.

In wild-type embryos, Ubx is highly expressed in ps 6 and expression was directly visualized with a mouse polyclonal anti-Dll antibody or by staining for β-galactosidase in embryos containing a Dll-lacZ reporter gene (construct 304 in Vachon et al., 1992). lacZ expression in this transformant faithfully represents thoracic and abdominal Dll expression (Vachon et al., 1992 and these results).

**Heat shocks and immunohistochemistry**

Immunohistochemistry was performed as described (Carroll et al., 1988; Chan and Mann, 1993) using the following primary antibodies: polyclonal mouse anti-Dll antibody (Vachon et al., 1992), the mouse anti-Ubx monoclonal antibody FP3.38 (White and Wilcox, 1985), and a rabbit anti-β-galactosidase antibody (Cappel). The secondary antibodies used were, for Figs 2, 4, and 5, an avidin-conjugated rat anti-mouse antibody followed by the Vectastain ABC Kit (Vector Labs); and, for Figs 1 and 3 a TexasRed-conjugated goat anti-mouse antibody (Jackson Labs) and an FITC-conjugated goat anti-rabbit antibody (Jackson Labs). The embryos in Figs 1 and 3 were fixed 4 to 8 hours AEL (after egg laying) and the embryos in Fig. 5 were fixed 7 to 10 hours AEL. By the pattern of Ubx staining, the embryos in Figs 1 and 3 are approximately late stage 11 to early stage 12 (Irvine et al., 1991; Campos-Ortega and Hartenstein, 1985). Because of the age of these embryos, it is possible that the observed effect on Ubx levels in the en; abdA AbdB embryos (Fig. 1) is in part due to a loss of wingless (wg) expression (DiNardo et al., 1988). abdA AbdB embryos were unambiguously identified by their Ubx staining pattern. en mutant embryos were unambiguously identified by staining for engrailed protein using mAbD9 (Patel et al., 1989). To unambiguously identify Dll, abd-A Abd-B embryos Dll was balanced by an SM6eve-lacZ balancer provided by S. Panzer and S. Beckendorf and the embryos were counter stained with a rabbit anti-β-galactosidase antibody followed by an alkaline phosphatase-conjugated goat anti-rabbit antibody (Cappel). Heat-shocked embryos from the HS-en; abdA AbdB-stock (Fig. 4) were prepared by collecting embryos for 3 hours, aging them for 2 hours, heat shocking at 37°C for 1 hour, and aging a further 2 hours before fixation. For Fig. 1D, ll-lacZ embryos (Liaw and Lengyel, 1992) were collected in parallel to HS-en; abdA AbdB embryos and the two collections of embryos were mixed together before heat shocking and immunostaining.

**MATERIALS AND METHODS**

**Fly stocks**

Wild type (w1118) and Ubx130 (present on TM2) (Lindsley and Zimm, 1992), Sc1Narc1;UbxM12, abdAM1 AbdBMB8 (Chan and Mann, 1993), HS-Ubx-lacZ (Mann and Hogness, 1990), abdAM1 AbdBMB8 (Casanova et al., 1987) and DllP (also referred to as B3) (Cohen and Jurgens, 1989) have all been previously described. The engrailed mutation, Dll(2R)serP (Lindsley and Zimm, 1992), which deletes both engrailed and inverted, was crossed into the abdA AbdB stock to generate en; abdA AbdB embryos. The HS-en containing P element (Poole and Kornberg, 1988) was crossed into the abdA AbdB stock to generate HS-en; abdA AbdB flies. en expression was visualized by staining for β-galactosidase in embryos containing an en-lacZ reporter gene inserted at the en gene (Hama et al., 1990). The expression of lacZ in this transformant faithfully mimics the expression of en. Dll expression was directly visualized with a mouse polyclonal anti-Dll antibody or by staining for β-galactosidase in embryos containing a Dll-lacZ reporter gene (construct 304 in Vachon et al., 1992). lacZ expression in this transformant faithfully represents thoracic and abdominal Dll expression (Vachon et al., 1992 and these results).
more weakly in parasegments 7 to 13 (Beachy et al., 1985; White and Wilcox, 1985; Irvine et al., 1991). In contrast, in \textit{abdA AbdB} mutant embryos, \textit{Ubx} expression appears identical in parasegments 6 to 13 (Struhl and White, 1985) (Fig. 1A). However, the nuclei present in the anterior portion of each of these parasegments have lower levels of \textit{Ubx}. In contrast, in embryos where \textit{Ubx} was ubiquitously expressed via a heat-shock promoter, all nuclei stained equivalently (Fig. 1C). Because of the anterior position of these cells within these parasegments and because they also express \textit{engrailed (en)} (see below), the cells expressing lower \textit{Ubx} levels probably represent the P compartments of these parasegments.

Because \textit{en} is itself a homeodomain-containing transcription factor, it could be responsible for this modulation in \textit{Ubx} protein levels. Therefore, \textit{Ubx} expression was analyzed in \textit{en; abdA AbdB} mutant embryos (Fig. 1B). In these embryos, parasegments 6 to 13 also appeared identical to each other with respect to \textit{Ubx} staining. However, in contrast to \textit{abdA AbdB} embryos, lower levels of \textit{Ubx} were not observed in the anterior portions of these parasegments. Therefore, \textit{en} is directly or indirectly required for the lower levels of \textit{Ubx} normally observed in this portion of parasegment 6.

If \textit{en} is a repressor of \textit{Ubx}, ubiquitous expression of \textit{en} should repress \textit{Ubx} expression in both compartments. To test this, \textit{Ubx} protein was visualized following heat-shock-induced expression of \textit{en}. As predicted, \textit{Ubx} protein levels are greatly reduced in these embryos when compared to the levels observed in control embryos that were heat shocked and stained together with the \textit{heat shock-en (HS-en)} embryos (Fig. 1D). These data support the suggestion that \textit{en} is a repressor of \textit{Ubx}.

**Expression of Distal-less in homeotic mutants**

To address the relevance of \textit{en}-mediated repression of \textit{Ubx} in parasegment 6, expression of the homeotic target gene, \textit{Distal-less (Dll)}, which is a marker for the cells that will generate Keilin’s organs, was characterized. In wild-type embryos, \textit{Dll} is expressed in one cluster of cells in each thoracic hemisegment (Fig. 2A) (Cohen, 1990). The lack of \textit{Dll} expression in abdominal segments is due to repression by the homeotic genes of the bithorax complex, including \textit{Ubx, abdA} and \textit{AbdB} (Cohen et al., 1991; Vachon et al., 1992). In fact, homeotic genes may act only as repressors
of Dll expression because in embryos where all five trunk homeotic genes are absent (Sex combs reduced (Scr), Antennapedia (Antp), Ubx, abdA and AbdB), clusters of Dll-expressing cells can be seen in all thoracic and abdominal segments (Fig. 2B). This demonstrates that Dll expression may be part of the ‘ground state’ segment identity (Lewis, 1978). The fact that Dll is expressed in this genotype is also consistent with the observation that the cuticles secreted by Scr Antp Ubx abdA AbdB embryos have Keilin’s organs in many of their segments (Struhl, 1983; Chan and Mann, 1993).

Heat-shock-induced Ubx expression completely represses Dll expression in the thorax, consistent with the complete suppression of Keilin’s organ formation in this genotype (Fig. 2C) (Vachon et al., 1992). In contrast, despite Ubx derepression in abdA AbdB embryos, Dll is expressed in one small cluster of cells in each abdominal hemisegment (Fig. 2D). Interestingly, the number of cells in each of these clusters is approximately one third the number of cells in a wild-type thoracic cluster. In Ubx mutant embryos only one additional Dll-expressing cluster is observed and this cluster is approximately two thirds the size of a wild-type thoracic cluster (Fig. 2E) (Cohen et al., 1991). The remaining one third of potential Dll-expressing cells is still repressed in these embryos because they are within ps 7, where high levels of abdA are expressed (Karch et al., 1990; Macias et al., 1990). These results demonstrate that the border between ps 5 and ps 6 divides the third thoracic Dll clusters into two unequal parts. Thus, parasegment 6 includes Dll-expressing cells just posterior to its anterior border (in the P compartment), where Ubx levels are low, but prevents Dll expression in cells just anterior to its posterior border (in the A compartment), where Ubx expression is high (Fig. 6).

**In ps 6 there is a three-way correlation between en expression, lower levels of Ubx, and Dll expression**

To investigate further the correlation between the levels of Ubx and Dll expression, double label experiments were performed. Indeed, in abdA AbdB mutant embryos the small clusters of Dll-expressing cells correspond to the cells in which Ubx levels are lowest (Fig. 3A-D). In these photomicrographs, individual cells that co-express Ubx and Dll can be identified; these cells always express lower levels of Ubx protein relative to their anterior neighbors.

The small clusters of Dll-expressing cells in the abdominal segments of abdA AbdB embryos and the posterior-most cells of the thoracic Dll clusters also express en (Fig. 3G). Moreover, en is expressed where Ubx levels are lowest, confirming that these cells are within the P compartment of parasegment 6 (Fig. 3E,F). Thus, within parasegment 6, there is a three-way correlation between en expression, lower

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**Fig. 2.** Expression of Dll in homeotic mutant embryos. (A) Wild type, lateral view; (B) Scr Antp Ubx abdA AbdB, lateral view; (C) HS-Ubx, lateral view; (D) abdA AbdB, ventral view; (E) Ubx, lateral view. In all photos, anterior is to the left. In wild-type embryos (A), each thoracic hemisegment contains one cluster of Dll-expressing cells (arrows). In embryos mutant for all five trunk homeotic genes (B), an additional nine clusters of Dll-expressing cells are observed (arrows). Ubiquitous Ubx expression represses all thoracic Dll expression (C) and in abdA AbdB embryos (D) an additional nine clusters of Dll-expressing cells (each containing 4 to 7 cells) are observed (arrowheads; only seven pairs of clusters are visible in this photograph because the others are out of the plane of focus). In Ubx embryos (E) only one additional cluster of Dll-expressing cells (containing 12 to 16 cells) is observed (arrowhead) (Cohen et al., 1991).
Fig. 3. In parasegment 6, there is a three-way correlation between en expression, lower levels of Ubx, and Dll expression. All panels are confocal micrographs and ventral views of abdA AbdB embryos. Anterior is to the left. (A,B) Parasegments 11, 12 and 13 (all transformed to ps 6) of the same germ-band elongated embryo doubly stained for Dll-lacZ (green) and Ubx (red). In A only the red fluorescence was imaged. The small clusters of Dll-expressing cells express low levels of Ubx. (C,D) Higher magnifications of the regions indicated by dashed lines in A and B, respectively. Cells that stain for Dll (D) express lower levels of Ubx (C, arrow) than cells that do not express Dll. (E,F) Approximately two abdominal parasegments of a germ-band elongated embryo doubly stained for en-lacZ (green) and Ubx (red). In E only the red fluorescence was imaged. Cells that stain weakly for Ubx (E, arrows) stain strongly for en (F). (G) The parasegment 6 region of a germ-band elongated embryo doubly stained for Dll (red) and en-lacZ (green) (ventral view). Regions of overlap appear yellow. The ps 5-ps 6 and ps 6-ps 7 borders are indicated by vertical lines at the bottom of the photograph. (Because this is an abdA AbdB embryo, ps 7 is transformed to ps 6.) At the ps 5-ps 6 border, a cluster of Dll-staining cells can be seen (small arrow). Some of these Dll+ cells are included within the stripe of en-expressing cells while some are not. At the ps 6-ps 7 boundary, a small cluster of Dll-staining cells can be seen (long arrow). These Dll+ cells are entirely included within the stripe of en-expressing (P compartment) cells.
amounts of Ubx expression andDll expression. Conversely, where en is not expressed (i.e. in the A compartment) higher levels of Ubx are present and Dll expression is repressed.

The most likely explanation for these results is that en blocks Ubx-mediated repression of Dll in the P compartment of ps 6 by repressing Ubx. In the A compartment of ps 6, en is not present and therefore Ubx levels are sufficiently high to repress Dll (Fig. 6). If this is true, then ectopic expression of en should prevent Ubx-mediated repression of Dll in both compartments. Therefore, Dll expression was analyzed following ubiquitous en expression in abdA AbdB mutant embryos (Fig. 4). As predicted, ubiquitous en expression resulted in the derepression of Dll, as evidenced by full-sized clusters of Dll-expressing cells in all segments. This result is consistent with the observation that heat-shock-induced en expression also represses Ubx (Fig 1D). The alternative possibility, that the corepression of en and Ubx in the same cells blocks Ubx repression of Dll, is less likely because Dll is completely repressed in both compartments of all three thoracic segments after heat-shock expression of Ubx, despite the presence of en (Fig. 2C).

**Dll is not necessary for Ubx repression**

In addition to the down-regulation of Ubx expression in all P compartment cells of ps 6, Ubx levels may be lower in the subset of cells that also express Dll (Fig. 5). In addition, although part of the P compartment, these cells are morphologically distinct (Fig. 5). Because Dll encodes a homeodomain protein, it was possible that Dll directly or indirectly represses Ubx in these cells and that feedback regulation of Ubx by Dll is necessary for maintaining Dll expression. To test this, Ubx expression was analyzed in embryos simultaneously mutant for Dll and abdA AbdB. Low levels of Ubx expression were observed in similar subsets of ps 6 cells in both the presence and absence of Dll demonstrating that Dll is not necessary for Ubx repression in these cells (Fig. 5A,B). Therefore, it is more likely that the particular morphology of these cells is a consequence of the same positional information that activates Dll. This result is consistent with the previous observation that, despite the absence of Kellin’s organs in Dll mutant larvae, the position where they would have formed in the cuticle remains morphologically distinct (Cohen and Jurgens, 1989). Thus, the fate of these cells is in part determined in a Dll-independent manner.

**CONCLUSIONS**

Although the levels of homeotic gene expression often vary within a single parasegment, there are very few examples where this regulation has been shown to be functionally significant. One example is that repression of Scr in the second and third thoracic segments by other homeotic genes is important for the correct patterns of these segments in both the embryo and adult (Struhl, 1982; Gibson et al., 1990). In contrast, repression of Ubx in abdominal segments by abdA and AbdB has little, if any, morphological significance for the larval cuticle (Mann and Hogness, 1990; Gonzalez-Reyes and Morata, 1990; Gonzalez-Reyes et al., 1990; Lamka et al., 1992). The results presented here (summarized in Fig. 6) demonstrate that en, which is a selector gene for the P compartment, modulates the expression levels of the homeotic gene Ubx within parasegment 6. Repression of Ubx levels by en is necessary for the correct spatial expression of the homeotic target gene, Dll which, in turn, is necessary for a specific morphological characteristic of the larval cuticle. These results therefore provide evidence that modulations in homeotic gene expression levels are functionally relevant to segment morphologies. en has also been shown to influence the expression of Scr (Pelaz et al., 1993) suggesting that a common...
mechanism for how *en* controls segmental patterns is by regulating the expression of the homeotic genes.

In general, uniform and high levels of homeotic gene expression using the *hsp70* promoter lead to phenotypic transformations that closely mimic wild-type segment morphologies. The overall accuracy of these transformations indicate that homeotic gene products largely operate on cells that already have a defined positional identity within the segment. However, the work described here provides an exception to this rule. The requirement in parasegment 6 for suppression of *Ubx* by *enlarged* demonstrates that a positionally restricted regulator is modifying homeotic gene expression instead of altering cell fates by acting before or in parallel to the homeotic genes.

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Fig. 6. Modification of the ground state identity by homeotic genes. The ground state is defined as the segment morphology that is generated in the absence of all homeotic gene expression and is probably close to that defined by the quintuple mutant Scm *Antp Ubx abdA AbdB* (Lewis, 1978; Struhl, 1983; Chan and Mann, 1993). In the ground state, *DII* expression is on (indicated by shaded semi-circles) in both the anterior (A) and posterior (P) compartments. Spatial cues that are independent of the homeotic genes (e.g. wingless; Cohen et al., 1993) turn on *DII* in the correct subset of cells in each parasegment. The remaining three diagrams show the proposed compartment-specific regulation of *DII* by homeotic genes in wild-type embryos. In thoracic parasegments (e.g. ps 4 (shown) and ps 5 (not shown)) homeotic genes do not repress *DII* expression in either compartment. It is possible, however, that some homeotic genes (e.g. *Antp*) help to turn on or maintain *DII* expression. In parasegment 6, *DII* expression is repressed in the A compartment by *Ubx* (indicated by unshaded semi-circles). *Ubx*-mediated repression of *DII* is blocked in the P compartment of ps 6 because *en* downregulates *Ubx*. Because *Ubx* expression is non-uniform within both compartments (see Figs 1, 3), there are probably additional positional modulators of *Ubx* expression in addition to *en*. In parasegment 7, *DII* is repressed in both compartments. *abdA* is almost certainly responsible for *DII* repression in the P compartment where *en* may increase *abdA* expression (Karch et al., 1990). In the A compartment, *DII* is repressed by *Ubx*, *abdA* or both. However, in the absence of *Ubx*, *abdA* alone can repress *DII* (Fig 2E). While *Ubx* and *abdA*-mediated repression of *DII* is likely to be direct (Vachon et al., 1992), it is not known if the other proposed interactions are direct or indirect.

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