Mutations in the *Drosophila* gene *extradenticle* affect the way specific homeo domain proteins regulate segmental identity

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We characterized a gene, *extradenticle*, which seems to interact with a specific subset of *Drosophila* homeo domain proteins, possibly affecting their target specificity. This interpretation is based on an examination of the zygotic and maternal effect phenotypes of *extradenticle* mutations. In embryos with reduced levels of *extradenticle* gene product, anterior and posterior segmental transformations occur. Segmental identity in *Drosophila* is mediated by the products of the Antennapedia and bithorax complexes. These homeo domain proteins are thought to regulate different target genes specifically in each segment, resulting in different morphologies. *extradenticle* alters segmental identity without affecting the pattern of expression of homeotic genes. Genetic tests demonstrate that in *extradenticle* mutants, the homeotic proteins are functional and act in their normal segmental domains, yet segmental identities are altered. Even when homeotic proteins are ectopically expressed under the control of a heterologous promoter, *extradenticle* mutations affect their consequences. Thus, in the absence of sufficient *extradenticle* product, altered segmental morphology results from alteration of the functional consequences of specific homeo domain proteins, possibly through alterations in their target gene specificity. *extradenticle* is also expressed maternally. Complete removal of *extradenticle*, maternally and zygotically, leads to specific alterations in segmentation, many of which result from failure to maintain the expression of the homeo domain protein *engrailed*.

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The establishment of pattern in a *Drosophila melanogaster* embryo begins before fertilization when maternal gene products set up the axes of polarity (Nüsslein-Volhard et al. 1987). By the cellular blastoderm, these maternal cues activate numerous zygotic genes. The overlapping patterns of expression of the products of these genes provide blastoderm cells with positional identities. The zygotic gap and pair-rule genes then activate the segment polarity genes, which are responsible for generating pattern within each segment (for review, see Akam 1987; Ingham 1988).

The action of these gene products alone would result in an embryo with identical segments; however, each segment has its own unique morphology. The process of assigning segmental identity is controlled by the genes of the Antennapedia and bithorax complexes (Lewis 1978; Kaufman 1983; for review, see Duncan 1987). These genes receive an extremely complex set of cues from the segmentation genes (e.g., Ingham and Martinez-Arias 1986; Ingham et al. 1986; White and Lehmann 1986), which results in complicated patterns of expression of the individual homeotic genes in the two complexes (e.g., Beachy et al. 1985; White and Wilcox

1985; Carroll et al. 1986). In the absence of a given homeotic gene, the morphology of certain segments is altered to reiterate the morphology of other segments. This role extends throughout the life of the fly (Garcia-Bellido and Lewis 1976; Morata and Garcia-Bellido 1976).

Molecular analysis of the homeotic genes demonstrated that they encode proteins with certain similarities. Each contains a homeo domain, a conserved set of amino acids resembling those found in the DNA-binding domain of certain bacterial regulatory genes (Laughon and Scott 1984; McGinnis et al. 1984). The homeo domain is responsible for DNA binding (e.g., Desplan et al. 1985; Beachy et al. 1988; Hoey and Levine 1988; Muller et al. 1988), and evidence is mounting that homeo domain proteins are transcription factors that interact with specific targets (Jaynes and O'Farrell 1988; Thali et al. 1988; Han et al. 1989; for review, see Levine and Hoey 1988). These observations led to the suggestion that by activating and repressing unique combinations of downstream genes, the homeotic genes confer on each segment its unique morphology.

These same molecular analyses, however, have re-

vealed a paradox about the role of the homeotic genes. Often the homeo domains, particularly those amino acids presumed to interact with DNA, are highly conserved between homeotic genes (Gehring 1987; Scott et al. 1989). In addition, in vitro DNA binding studies reveal that the different homeo domain proteins bind in vitro to similar or identical target sequences (Beachy et al. 1988; Desplan et al. 1988; Hoey and Levine 1988). These in vitro results cannot reflect the state of affairs in vivo, where each homeotic gene product confers a unique segmental identity. How do the homeotic gene products recognize their presumed unique set of target genes, in spite of these similarities?

One approach to understanding this specificity is the search for mutations that alter it. We have examined mutations in the gene *extradenticle* (*exd*), in which the target specificity of homeotic genes appears to be altered. *exd* is required both for proper segmentation and for appropriate segmental identity. We used molecular and genetic probes to dissect the role of *exd* in these two processes. We present evidence that the alterations in segmental identity seen in embryos with reduced levels of *exd* gene product are not due to alterations in the expression patterns or in the domain of action of the homeotic genes. Reduced *exd* levels result in segmental transformations that reflect changes in the morphological consequences of the action of a small subset of the homeo domain proteins of the fly. We also show that *exd* is required for the maintenance of expression of another homeo domain protein, *engrailed* (*en*). These results are used to present a model for *exd* function.

Figure 1. The zygotic phenotype of *exd*. (A) Ventral side of a wild-type embryo. Certain segments are numbered for comparison to the *exd* mutant in B. Each segment has a denticle belt of a characteristic shape. (B) Ventral side of an embryo hemizygous for *exd*^{Y012}. This null *exd* mutation alters most segments. Certain head derivatives, such as the H piece, are often severely disrupted, whereas other structures, including the antennal-maxillary complex, mouth hooks, and dorsal tooth, are often nearly normal. T1 is normal in pattern; T2 is partially transformed to T1. Note the increased denticle size and the appearance of a rudimentary beard—the group of denticles normally found posterior to the T1 denticle belt. T3 has an ambiguous morphology, with some characteristics of both T1 and A2. The Keilin's organs and ventral pits are unchanged. Abdominal segments A1–A5 are transformed posteriorly, assuming the morphology of the segment two or three segments posterior. A6–A8 are normal or near normal. A8 sense organs are normal, as are anterior and posterior terminal structures of the embryo, including the esophagus, proventriculus, posterior spiracles, anal pads, and tuft. (C) Dorsal side of a wild-type animal, showing segments T2, T3, and A1. Abdominal segments differ from thoracic segments in that they have large, denticle-like dorsal hairs. T2 and T3 (small arrowhead) have none, A1 has only a few (large arrowhead), and A8 has many. (D) The dorsal side of an *exd*^{XP11} animal. Note the posterior transformations of all three segments, indicated by the appearance of an occasional denticle-like hair in T2 (arrow), a number of such hairs in T3 (small arrowhead), and many such hairs in A1 (large arrowhead).

Results

Embryos lacking exd have segmental transformations

exd (Wieschaus et al. 1984; Wieschaus and Noell 1986) is a zygotic, X-linked embryonic lethal mutation that causes segmental transformations. Five *exd* alleles share this phenotype (Fig. 1B). These five are null or nearly null, as a similar phenotype is seen in embryos heterozygous for overlapping deficiencies removing *exd* [*Df(1)sd*^{72b} and *Df(1)L9*]. Two weaker *exd* alleles are postembryonic lethals. Homozygotes for one of these weak alleles, *exd*^{EM5}, die as pharate adults with specific cuticular defects, suggesting a postembryonic role for *exd*.

The segmental transformations in null *exd* alleles are identified by use of cuticular differences between segments. Ventrally, each segment has a characteristic band

of denticles, along with specific sense organs (Fig. 1A; Campos-Ortega and Hartenstein 1985). In null *exd* mutant embryos, abdominal segments A1 (first abdominal) to A5 adopt morphologies usually seen two to three segments more posteriorly (Fig. 1B). For example, A1 looks like a wild-type A3 or A4. The most posterior abdominal segments are altered little, if at all. Some thoracic segments are transformed anteriorly. T1 (first thoracic segment) is unaltered. T2 is transformed partially toward T1, evidenced by the larger denticles and the appearance of a partial "beard," which is a second group of denticles normally found in T1. T3 adopts an ambiguous morphology. A very partial beard is formed, but there is also a rudimentary anterior row of denticles, resembling that in A2 and posterior. Most derivatives of the head are reduced, whereas the terminal, nonsegmented structures of the embryo are normal.

Dorsally, segments have specific patterns of fine dorsal hairs (Fig. 1C; Campos-Ortega and Hartenstein 1985). In *exd* mutants, dorsal hair alterations show that dorsal abdominal segments are transformed posteriorly (Fig. 1D). Posterior T3 (pT3) and pT2 are also transformed posteriorly. Anterior T2 (aT2) and aT3 seem quite normal, as does T1. The transformations are roughly parasegmental (parasegments are adjacent posterior and anterior compartments of different segments, for example, pT3 and aA1 = parasegment 6; Martinez-Arias and Lawrence 1985), but discrepancies between dorsal and ventral transformations and the appearance of a normal Keilin's organ on T3 straddling the parasegment boundary suggest that the rules may be more complex.

Expression patterns of homeotic genes are unaltered in exd mutants

We used genetic and molecular tests to learn how *exd* interacts with the homeotic genes. The homeotic genes of the *Antennapedia* and *bithorax* complexes are the central determinants of segmental identity (Lewis 1978; Kaufman 1983). Mutations in other genes result in homeotic transformations, of which genes in the *Polycomb* class (Lewis 1978; Struhl 1981; Duncan 1982; Jürgens 1985) are best characterized. *Polycomb*-like mutations resemble *exd* superficially, as they transform most segments into copies of A8. *Polycomb*-like transformations result from perturbation of homeotic gene expression. All homeotic genes become active everywhere during mid-embryogenesis (Struhl and Akam 1985; Struhl and White 1985; Wedeen et al. 1986). Because of cross-regulatory interactions between the homeotic products, all segments are transformed into A8.

To address whether *exd* affects homeotic gene expression, we used antisera to *Antennapedia* (*Antp*; Condie et al. 1990), *Sex combs reduced* (*Scr*; Glicksman and Brower 1988), *Ultrabithorax* (*Ubx*; White and Wilcox 1984), and *abdominal-A* (*abd-A*; Karch et al. 1990). Mixed populations of wild-type and mutant embryos were stained with the antisera to *Scr*, *Antp*, *Ubx*, and *abd-A* and carefully examined for any subtle alterations in pattern. All embryos exhibit a wild-type pattern of

staining [data not shown; wild-type patterns are described in Mahaffey and Kaufman 1987; Riley et al. 1987 (*Scr*); Carroll et al. 1986; Wirz et al. 1986 (*Antp*); Beachy et al. 1985; White and Wilcox 1985 (*Ubx*); Karch et al. 1990 (*abd-A*)].

To confirm that *exd* does not alter homeotic gene expression, we used antiserum to *Sex-lethal* product (see Experimental procedures; D. Bopp and P. Schedl, pers. comm.) to identify mutant embryos unambiguously. The spatial and temporal patterns of *Antp*, *Ubx*, and *abd-A* expression in *exd* animals are indistinguishable from wild type (Fig. 2; data not shown). For example, *Ubx* expression has its normal anterior margin at parasegment 5, shows a maximal expression in parasegment 6, switches correctly from epidermal to nervous system expression, and is repressed in the abdominal segments. The fine-scale details of cell-by-cell expression also appear normal. Most importantly, there is absolutely no detectable anterior expansion of any of these homeotic gene products at any stage. This result is in marked contrast to the effects of the *Polycomb*-like genes, in which effects on homeotic gene expression are seen by mid-germ-band extension.

In exd mutants, homeotic protein regulation of segmental identity is altered

Normal homeotic gene expression does not necessarily mean normal activity. To assay whether homeotic genes remain active in *exd* mutants, we combined the null mutation *exd^{XP11}* with null mutations in homeotic genes. Some double and triple mutant combinations are illustrated in Figure 3, and the rest are summarized in Table 1. Although homeotic genes act in parasegmental patterns (Hayes et al. 1984; Struhl 1984), we will discuss mutant phenotypes in terms of segments, which are easier to visualize. In addition, because *exd* mutations cause one wild-type segment to look like another, we will distinguish between the position of a segment on the embryonic body (e.g., "A1"), and the cuticular structures found in a given segment of a wild-type embryo (e.g., the "A1 morphology").

If homeotic genes are still active in *exd* mutants, *exd* homeotic gene double mutants should differ from *exd* single mutants. Observation of double mutants fulfills this expectation. In each case, removal of a homeotic gene, in an *exd* mutant background, results in alterations in the identities of the same segments affected by that homeotic gene in the wild-type animal. Thus, in *exd* mutants, homeotic proteins are not only present in the appropriate segments but are also acting there to confer segmental identity. However, in *exd* mutants, the resultant morphology of each segment is altered, so that it resembles a different wild-type segment. This additivity is illustrated most easily by an example.

We compared the effects on segmental fate of single and double mutant combinations of *exd* and the homeotic gene *Ubx*; the effects of these mutants on the identity of each segment are presented in a simplified form in the following diagram:

Genotype	Segment		
	T2	T3	A1
wild-type	T2	T3	A1
<i>Ubx</i>	T2	T2	T2
<i>exd</i>	T1	T1/A3	A3
<i>exd Ubx</i>	T1	T1	T1

Ubx normally maintains the identity of parasegments 5 and 6 (pT2 to aA1). In an embryo mutant for *Ubx*, T3 and A1 are transformed into T2; thus, all three segments have the fine denticle belts normally found in T2 (cf. Fig. 3A with 3B; Lewis 1978). To determine, in an *exd* mutant, whether removal of *Ubx* has consequences (and if so, in which segments), we examined *exd Ubx* double mutants. Removal of *Ubx* in an *exd* mutant background affects only the identities of T3 and A1, which now resemble a wild-type T1, as would the T2 segment in an *exd* single mutant (cf. Fig. 3C with 3D). Thus, mutations in *exd* and *Ubx* are additive in phenotype, demonstrating that in an *exd* mutant embryo, *Ubx* is active in its normal domain to promote differentiation of T3 and A1 from T2. In the *exd* mutant, however, this active *Ubx* has different consequences from those in a wild-type animal; for example, the A1 segment assumes the

morphology of a wild-type A3. The A3 morphology is usually conferred by a different homeotic gene *abd-A*. *abd-A*, normally active in A2–A6, and *Abd-B*, active in A5–A8, are the “abdominal” genes of the Bithorax Complex (Karch et al. 1985; Sanchez-Herrero et al. 1985). Further double and multiple mutant analyses demonstrate that the A3 morphology of the A1 segment in an *exd* animal (Fig. 3G) is not affected by mutations in *abd-A* or *Abd-B*; it is affected only by *Ubx* mutations (Fig. 3F–L). *Ubx* and *Ubx* alone is responsible for this altered abdominal morphology of A1.

What are the abdominal genes doing in an *exd* animal? Analyses of double and multiple mutants suggest that *abd-A* acts in its proper domain in an *exd* mutant, leading A2–A6 to differ from A1. In an *exd* mutant, however, *abd-A* has different morphological consequences than in *exd*⁺. For example, in an *exd* embryo, *abd-A* product results in A3 adopting the A5 morphology (cf. Fig. 3G,F,I). In contrast to *abd-A*, *Abd-B* is little affected by *exd*. *Abd-B* remains active in its normal segmental domain in an *exd* mutant, and the morphology of the segments under its control, A6–A8, is affected very little, if at all, by *exd* (Table 1). Further combinations of *exd* and Bithorax Complex mutants (Table 1) confirm that *Ubx*, *abd-A*, and *Abd-B* are all active in their normal domains in *exd* embryos, but the morphological consequences of *Ubx* and *abd-A* are altered.

Figure 2. Homeotic gene expression in *exd* mutants. Embryos were assayed for expression of *Ubx* (left and middle columns), and *abd-A* (right column). Zygotic *exd* mutants are shown in the top row. These were identified as discussed in the text. The background staining in these embryos is higher, as they were double stained with antibody to *Sex-lethal*. Wild-type embryos are shown in the middle row. *exd* maternal effect mutants are in the bottom row (these are discussed later in the text). (A–C) Stage 14 embryos stained with antibody to *Ubx* (anterior is to left and dorsal is up). *Ubx* expression maintains its normal anterior boundary, coming on weakly in parasegment 5 (arrowheads) and strongly in parasegment 6. The *exd* maternal effect mutant has an abnormal head. (D–F) Ventral view of stage 15 embryos stained with antibody to *Ubx* (anterior is to the left). Expression of *Ubx* in the epidermis is declining, whereas CNS expression is strong. The anterior boundary of expression is still maintained, as are intersegmental differences. Note the midline cells in parasegment 4 (arrowheads). (G–I) Embryos stained with antibody to *abd-A* (anterior is left and dorsal is up). *abd-A* is also expressed normally. Note the normal anterior margin at parasegment 7 (arrowheads). The *exd* maternal effect mutant is losing segmental grooves.

Figure 3. Homeotic genes are active in their normal domains in *exd* mutants. (A–D) The role of *Ubx* in *exd* mutants. T1–T3, A1, and A2 are shown. A1 is marked with an arrow for special attention. (A) Wild-type embryo. A1 has a narrow band of large denticles. (B) *Ubx* mutant. A1 and T3 are transformed toward T2, taking on its belt of very fine denticles. (C and D) *exd Ubx* double mutant (C) compared to *exd* single mutant (D). In *exd*, A1 has a trapezoidal belt of large denticles, whereas in the double mutant, this is transformed into a copy of the *exd* T2 segment. (E–L) *exd* and the abdominal genes of the bithorax complex. Segments A1–A4 are shown. (E) Wild-type embryo. A1 has a narrow band of large denticles, the denticle band of A2 is trapezoidal, and as one proceeds posteriorly, the denticle bands become more rectangular. (F) *abd-A* mutant. All segments shown are transformed into copies of A1. (G) *exd* mutant. All segments shown take on the morphology of a more posterior segment. Note the trapezoidal shape of the A1 denticle belt. (H) *exd Ubx* double mutant. The change in A1 is as described in C and D. A2 and posterior are as in the *exd* single mutant. (I) *exd abd-A* double mutant. Whereas A1 still resembles A3 in *exd*, A2 and posterior now are transformed into a copy of the *exd* A1, with less rectangular denticle belts than the *exd* single mutant. (J) *abd-A Abd-B*. In the segments shown, the phenotype is identical to *abd-A*; all denticle belts have the A1 morphology. (K) *exd abd-A Abd-B*. In the segments shown the phenotype is identical to *exd abd-A*, with all denticle bands having the A3 morphology. (L) *exd Ubx abd-A Abd-B*. Upon removal of *Ubx*, all segments now look like the T2 segment of an *exd* animal.

We also combined mutations in *exd* with mutations in some of the homeotic genes of the Antennapedia complex. Combinations of *exd* with mutations in *Scr* suggest that *Scr* functions in its appropriate domain and

is little affected by *exd*, because the morphology of T1, conferred by *Scr*, was not significantly changed by *exd* mutations (Table 1). The effect of *exd* on *Antp* is ambiguous. Both *exd* reduction and *Antp* loss result in similar

Table 1. Tabulation of the phenotypic transformations seen in various combinations of mutations in *exd* and the homeotic genes

Segment identity in wild type	<i>exd</i>	<i>Ubx</i>	<i>exd Ubx</i>	<i>exd abdA</i>	<i>exd AbdB</i>	<i>exd Ubx abdA</i>	<i>exd Ubx AbdB</i>	<i>exd BX-C*</i>	<i>exd BX-C</i>	<i>exd Scr</i>	<i>exd Scr Antp</i>	<i>exd Scr Antp</i>
T1	T1	T1	T1	T1	T1	T1	T1	T1	T1	"T1"	"T1"	"T1"
T2	"T1"	T2	T2	T2	"T1"	T2	"T1"	T2	"T1"	T2	"T1"	"T1"
T3	"T1"/A2	T2	T2	T2	"T1"/A2	T2	"T1"/A2	T2	"T1"/A2	T3	"T1"/T3	"T1"/A2
A1	A3	"T1"	"T1"	A3	A3	A3	A3	T2	"T1"	A1	A1	A3
A2	A4	A2	T2	A4	A4	A4	A4	T2	"T1"	A2	A2	A4
A3	A5	A3	T2	A5	A5	A5	A5	T2	"T1"	A3	A3	A5
A4	A5/A6	A4	T2	A5/A6	A5/A6	A5/A6	A5/A6	T2	"T1"	A4	A4	A5/A6
A5	A6	A5	T2	A6	A6	A6	A6	T2	"T1"	A5	A5	A6
A6	A6/A7	A6	T2/A8	A6/A7	A6/A7	A6/A7	A6/A7	T2	"T1"	A6	A6	A6/A7
A7	A7	A7	T2/A8	A6/A7	A6/A7	A6/A7	A6/A7	T2	"T1"	A7	A7	A7
A8	A8	A8	T2/A8	A6/A7	A6/A7	A6/A7	A6/A7	T2	"T1"	A8	A8	A8

*[BX-C] bithorax complex.

The left-most column indicates the normal position and morphology of the wild-type segments from first thoracic (T1) to eighth abdominal (A8). Across the top are a variety of genotypes, combining probable null mutations of *exd* and the homeotic genes. Beneath each genotype, the subsequent columns indicate the apparent morphology of each segment in the various single and multiple mutant combinations of *exd* and the homeotic genes. "T1" refers to the morphology characteristic of an *exd* T2 segment, which is partially transformed toward the T1 morphology. "T1"/A2 refers to the ambiguous morphology characteristic of the *exd* T3 segment, which has characteristics of both T1 and A2. Likewise, other segments with hybrid morphological features are similarly indicated.

consequences for the segment *Antp* controls, T2. Embryos mutant for both *exd* and *Antp* seem to be identical in phenotype to *exd* single mutants, a conclusion supported by the phenotype of *exd Scr Antp* mutants (Table 1). This result is consistent with two possible conclusions. *exd* may alter the morphological consequences of *Antp* product, such that *Antp* confers a T1 morphology on T2, or reduced *exd* levels may inactivate *Antp* product, with the same phenotypic consequences.

We also examined the effect of *exd* in combination with one unusual homeotic mutation *Ubx^{C1}* (Casanova et al. 1988; Rowe and Akam 1988; Table 1), which produces a fusion protein with its amino terminus derived from *abd-A* and its carboxyl terminus, including the homeo domain, from *Ubx*. *Ubx^{C1}* retains some specificity of both *Ubx* and *abd-A*, giving it weak activity of both genes. The fusion also removes some information required for appropriate regulation of *Ubx* and *abd-A*. These features combine to produce unusual segmental transformations. Despite these peculiarities, double mutants of *Ubx^{C1}* and *exd* are additive in phenotype, suggesting that the hybrid protein is active in its "normal" domain in an *exd* embryo, but that it also has its specificity altered by *exd*.

exd also affects the consequences of ectopic homeotic gene expression

To eliminate the possibility that *exd* acts via effects on homeotic gene transcription, we used constructs that fuse heat-shock regulatory elements to structural genes for homeotic proteins. When embryos carrying these fusion genes are heat-shocked, the fusions produce uniform high levels of homeotic protein throughout the embryo and transform variable numbers of segments into copies of the prototypic segment of that homeotic gene (i.e., the segment in which the homeotic gene is normally maximally expressed; Gibson and Gehring 1988; Mann and Hogness 1990). Embryos carrying a heat-shock (*hs*)–*Ubx* fusion, when heat-shocked and allowed to complete embryogenesis, have head and thoracic segments with the A1 morphology (Fig. 4B). A1 is the segment where *Ubx* expression is normally at its maximum. More posterior segments are unaffected. The effects of the *hs-Ubx* gene do not depend on the endogenous copy of *Ubx* (Mann and Hogness 1990). We also use an *hs-Antp* construct (provided by K. Hill and M. Scott). When embryos carrying this construct are heat-shocked, head involution is prevented and T1 is partially transformed toward T2 (Fig. 4D).

When embryos carrying a *hs-Ubx* construct in an *exd* mutant background are heat-shocked, all segments from A1 anterior adopt the A3, rather than the A1, morphology (Fig. 4C; more posterior segments are unaffected). A3 is the morphology A1 would have in an *exd* mutant in the absence of the heat-shock fusion. Reduction in the level of *exd* thus alters the consequences of *Ubx* expression, regardless of how it is regulated or where it is expressed. The results with the *hs-Antp* fusion were more ambiguous, due to the weaker and more

Figure 4. Effect of *exd* on ectopic homeotic gene expression. (A) Wild-type embryo. (B) *exd*⁺ embryo carrying the *hs-Ubx* construct. Several thoracic and head segments anterior to A1 are transformed to the A1 fate, as demonstrated by the narrow belt of large denticles. (C) *exd* mutant carrying the *hs-Ubx* construct. The transformed head and thoracic segments assume the trapezoidal denticle belt indicative of A3 morphology. Arrows highlight similar transformations dorsally. (D) *exd*⁺ embryo carrying the *hs-Antp* construct. Head involution fails, and T1 is partially transformed toward T2, as evidenced by a reduction in both the beard (arrow) and in denticle size. (E) *exd* mutant carrying the *hs-Antp* construct. *Antp* activity is demonstrated by failure of head involution and partial transformation of T1 to T2 (arrow).

variable nature of the *hs-Antp* transformations. *exd* mutant embryos carrying the *hs-Antp* fusion, when heat-shocked, fail in head involution and show a transformation of T1 toward an intermediate T1/T2 morphology (Fig. 4E). This, together with the double mutant results, supports a model in which *Antp* remains active in an *exd* background, but the consequences of its action are altered.

When *exd* dose is reduced, segmental morphology is altered without altering either homeotic gene expression or the normal domains of action of homeotic genes. The effects of *exd* are on the consequences of homeotic proteins—the morphologies of the segments. The simplest explanation is that each homeotic gene is now regulating different downstream target genes or causing quantitatively different effects on a similar set of target genes; that is, its target specificity or its effects on its target genes are altered by a reduction in the level of *exd*.

Maternal effect of *exd*—similarities and differences with *en*

Segmental transformations result from the reduction in *exd* levels, rather than from the removal of *exd* entirely. In addition to *exd* product produced during embryogenesis, *exd* is also put into the egg by the mother. This maternal contribution can be demonstrated in two ways. Increasing maternal *exd* rescues the homeotic transformations described above, though not the lethality of *exd* (Wieschaus and Noell 1986; data not shown). One can also remove the maternal contribution of *exd* by producing clones of germ cells homozygous for *exd* muta-

tions (Wieschaus and Noell 1986). Embryos that have no maternal contribution of *exd* can have two fates. If they inherit a wild-type copy of *exd* from their father, they survive to be fertile adults; one wild-type *exd* gene zygotically rescues any maternal deficit. In contrast, if they have no maternal or zygotic *exd*, they have a phenotype much more severe than zygotic mutants (cf. Figs. 1 and 5).

We examined the phenotype of embryos lacking both maternal and zygotic *exd* product by use of time-lapse video, scanning electron microscopy (SEM), and cuticle preparations. The phenotype differs drastically between the ventral and dorsal sides (cf. Fig. 5A with 5D). Embryos lacking both maternal and zygotic *exd* product have a dorsal closure defect (Fig. 5D). Many aspects of normal anterior–posterior polarity are still present, however, within each segment, and in the difference between thoracic and abdominal segments. Slight segmental transformations, like those in the zygotic mutant, would be too subtle to detect.

Ventrally, however, there are much more dramatic defects (Fig. 5A). The terminal, nonsegmented regions of the embryos are normal. Head involution fails, and the head skeleton is totally absent (Fig. 5D). Posterior and ventral to these head remnants is a plate of smooth, naked cuticle, in place of the ventral head and thorax (Fig. 5A and B). Occasional large denticles seen here may represent remnants of the thoracic denticle belts. The abdomen is more recognizable (Fig. 5A). Posterior to the smooth ventral region is a remnant of A1, followed by rudimentary denticle bands, fused in a pair-rule fashion. These fusions join A2 to A3, A4 to A5, and A6

Figure 5. Maternal effect phenotype of *exd*. All are embryos derived from *exd* germ-line clones, fertilized by Y-chromosome-bearing sperm. (A–D) Phenotype of embryo in which both maternal and zygotic *exd* came from a null *exd* allele, *exd*^{XP11}. (A) Ventral cuticle phenotype (anterior is up). Whereas the esophagus and proventriculus are normal, the head skeleton is completely eliminated. Ventral gnathal and thoracic segments are replaced by smooth cuticle. Abdominal segments are fused in a pair-rule pattern. The denticles are poorly differentiated. The posterior end is relatively normal, with spiracles, filzkörper, anal pads, and sense organs intact. (B and C) A scanning electron micrograph of a stage 14 *exd* maternal effect embryo (B), compared to a similar stage wild type (C) (anterior is left and dorsal is up). By this stage, gross morphological abnormalities are apparent. The segmental grooves in the abdomen are abnormal, whereas those in the thorax are gone (arrow). Already, the ventral thoracic and gnathal segments appear abnormally featureless. This region will give rise to the blank cuticle. The lateral head lobes do not undergo normal fusions or morphological movements, and head involution, just beginning in the wild-type embryo, will never occur in the mutant. (D) Dorsal view of the terminal phenotype of an *exd* germ-line clone embryo. On the dorsal side there are still relatively well-formed thoracic segments, although dorsal closure has not been completed (arrowhead). The mid-dorsal protrusion is internal tissue that is revealed by the failure to close dorsally. The large lobe at the anterior end is the clyprolabrum. Other lobes can be identified with specific head segments, which never fuse or involute but still differentiate some normal structures, such as antennal and maxillary sense organs (arrows). (E) Head and thorax of an embryo in which both maternal and zygotic *exd* product was provided by the weak allele, *exd*^{EM5}. Whereas A1 is nearly normal, the denticle bands of T3 and T2 are reduced and resemble the T1 beard of a wild type. The denticles and dorsal hairs of T1 are gone. The only remnants of T1 are the ventral pit (arrow) and Keilin's organ. The head is reduced, resembling the head of a zygotic, null *exd* mutant. The same phenotype is seen in embryos derived from germ line clones of a null allele, whose zygotic *exd* comes from *exd*^{EM5}.

to A7, with A8 more posterior. The segmental fusions can be seen during development as the disappearance of segmental grooves, but only on the ventral side (Fig. 5B). This disappearance occurs in a pair-rule fashion; grooves within even-numbered parasegments disappear first, matching the pattern of denticle band fusions. The denticle belt fusions are highly reminiscent of those seen in *en* mutants (Nüsslein-Volhard and Wieschaus 1980; Kornberg 1981). *en* mutant embryos lack other defects seen in *exd* maternal effect embryos.

The ability of zygotic *exd* to rescue the lack of maternal *exd* and the ability of increased maternal *exd* to rescue the homeotic phenotype of zygotic *exd* mutations both suggest that maternal and zygotic *exd* product are functionally indistinguishable. We obtained further evidence for this by examining maternal and zygotic combinations of a null allele of *exd* and the weak allele *exd*^{EM5}. All have the same phenotype (Fig. 5E; data not shown), which is similar but not identical to the zygotic phenotype of a null *exd* allele.

Complete loss of *exd* does not disrupt expression of the homeotic genes

Reduction in *exd* levels alters morphology without affecting homeotic gene expression. To determine whether total removal of *exd* has an effect on expression of homeotic genes, we assayed the function and expression of several homeo domain proteins, in animals from germ-line clones of null *exd* alleles in which both the maternal and zygotic *exd* contribution is removed.

The effect of the removal of both maternal and zygotic *exd* on expression of *Antp*, *Scr*, *Ubx*, and *abd-A* is not very dramatic. We can identify germ line clone embryos midway through the extended germ-band stage by their altered morphology. Homeotic gene expression in these embryos, as determined by staining with the respective antibodies, remains relatively unchanged (Fig. 2C, F, and I; data not shown). Each homeotic gene is activated normally and to an approximately normal level in *exd* maternal effect mutants, as measured against their wild-

type siblings. Each homeotic protein maintains an appropriate anterior boundary, undergoes a switch from ectodermal to nervous system expression, and maintains approximately correct differences in expression between segments. Some differences in the pattern within segments were noted; these differences are likely due to alterations in *en* expression described below (Martinez-Arias and White 1988).

This normality suggests that *exd* action is highly specific. Homeotic gene activation requires coordinated action of many maternal and zygotic genes (e.g., Ingham et al. 1986; Ingham and Martinez-Arias 1986; White and Lehmann 1986), activity of which must not be severely disrupted by *exd*. This renders less likely a role for *exd* in general cellular processes, with only indirect effects on homeotic genes, and also makes less likely any essential role for *exd* in the function of all homeo domain proteins, because several segmentation genes are in this class.

en expression is specifically disrupted in *exd* maternal effect embryos

The similarity between the *exd* maternal effect and the zygotic *en* phenotype led us to examine *en* expression. Expression of *en* is significantly altered in embryos lacking both maternal and zygotic *exd* (zygotic *exd* mu-

tants have normal *en* expression; data not shown). We assayed *en* expression in two ways: by use of antibody to the *invected* and *en* proteins (Patel et al. 1989), and by use of a β -galactosidase gene under *en* regulation (C. Hama and T. Kornberg, pers. comm.). Both methods provided very similar results (Fig. 6). *en* is activated normally and switches from its early pair-rule pattern to its 14-stripe pattern. At mid-stage 9 (fully extended germ band), *en* expression deteriorates (Fig. 6A,B). Deterioration of *en* expression is also seen in *wingless* (*wg*) mutant embryos (DiNardo et al. 1988; Martinez-Arias et al. 1988), so we compared patterns of loss.

Loss of *en* expression in *exd* maternal effect mutants has dorsal/ventral, anterior/posterior, and pair-rule asymmetries (Fig. 6A–F). The deterioration is much more severe ventrally than dorsally. By germ-band shortening, although the dorsal stripes of *en* expression are normal or nearly normal in *exd* maternal effect mutants (cf. Fig. 6C with 6D), the ventral stripes have disappeared (cf. Fig. 6E with 6F). This asymmetry is not an inherent property of *en* decay, as the opposite pattern (stronger ventrally and weaker dorsally) is seen when *en* stripes deteriorate in a *wg* mutant (C. Rauskolb and E. Wieschaus, unpubl.). Second, head and thoracic *en* stripes show an earlier and more severe deterioration than abdominal stripes (Fig. 6B). Once again, this differs from the *wg* mutant, where the maxillary segment is

Figure 6. *en* expression in *exd* maternal effect mutants. (A) A stage 10 *exd* maternal effect mutant embryo, stained with monoclonal antibody to *en/invected*. The ventral *en* stripes are starting to decay, in a pair-rule pattern. Weaker stripes (W) alternate with stronger stripes (S). (B) Stage 13 maternal effect mutant. The ventral *en* stripes are gone in the thorax (arrowhead) and are disappearing in the abdomen. Segmental grooves are also disappearing. (C and D) Dorsal view of stage 15 wild-type (C) and maternal effect *exd* mutant (D) embryos, carrying a β -galactosidase gene under control of the *en* regulatory region. β -Galactosidase was visualized by antibody staining. The *en* stripes of the mutant are nearly normal dorsally, although dorsal closure was not completed. (E and F) Ventral views of the same wild-type (E) and mutant (F) embryos seen in C and D. In the wild type, *en* is active in the CNS (arrow), and the epidermal *en* stripes remain (arrowhead). In the mutant, although CNS expression has been activated (arrow), the ventral epidermal *en* stripes are gone (arrowhead). (G and H) *wg* expression in a stage 12 wild-type (G) and *exd* maternal effect mutant (H). In the wild type, *wg* expression has separated into ventral stripes (arrow) and lateral patches (arrowhead). The mutant retains the lateral patches, but the ventral stripes are nearly gone. (I and J) Nervous systems of a stage 15 wild-type (I) and *exd* maternal effect mutant (J), as revealed by staining with antisera to horseradish peroxidase (Jan and Jan 1982). In the mutant, the CNS is diffuse, but axons have connected different neurons, and some axons of the peripheral nervous system have been sent out (arrowhead), though their pattern is disrupted.

most resistant to decay (DiNardo et al. 1988). Finally, as the stripes deteriorate in an *exd* maternal effect mutant, the odd-numbered *en* stripes remain stronger than the even-numbered ones (Fig. 6A). This aspect of the loss is similar to the pair-rule *en* loss seen in *en* mutants, apparently due to failure of autoregulation, but opposite to the loss seen in *wg* or *armadillo* (DiNardo et al. 1988; J. Heemskerk, S. DiNardo, and P. O'Farrell, pers. comm.; C. Rauskolb and E. Wieschaus, unpubl.). The asymmetries seen in *en* expression closely parallel the asymmetries in cuticular phenotype of maternal effect *exd* embryos.

The asymmetries suggested that the effect of *exd* on *en* expression is not mediated through *wg*. As a further test, we examined *wg* expression in embryos lacking maternal and zygotic *exd* (cf. Fig. 6G with 6H; for wild-type *wg* expression pattern, see Baker 1987, 1988). *wg* comes on normally in *exd* maternal effect mutants; no clear change is seen until stage 10. At this point, *wg* expression normally shifts from uniform segmental stripes to a more complex pattern of ventral stripes and lateral patches. At stage 10, the ventral *wg* stripes fade in *exd* germ line clone embryos, whereas the lateral patches remain. *wg* seems to be lost slightly later than *en* but with a similar dorsal/ventral asymmetry. An effect on *wg* expression is expected, because *en* is required for *wg* maintenance (Martinez-Arias et al. 1988). The effect on *wg* could be a secondary consequence of the loss of *en* expression.

Although *en* protein appears to have a fairly short half-life, as its pattern undergoes very rapid change, β -galactosidase protein may have a longer half-life in embryos (e.g., Klingensmith et al. 1989). Thus, changes in *en* promoter activity may not provide the entire explanation for the loss of expression of β -galactosidase under the control of the *en* promoter in *exd* germ line clone embryos. Instead, death of the *en*-expressing cells may need to be invoked.

To determine whether failure to maintain *en* expression resulted from disruption in an earlier step in the hierarchy of *en* activation, we examined the expression of the pair-rule gene *fushi tarazu* (*ftz*). *ftz* is necessary for the expression of the even-numbered *en* stripes (DiNardo and O'Farrell 1985; Howard and Ingham 1986; Ingham et al. 1988), those most sensitive to *exd* loss. Embryos lacking both maternal and zygotic *exd* were stained with *ftz* antibody (Krause et al. 1988). Whereas half of the embryos have the extreme *exd* maternal effect phenotype, no difference in *ftz* expression was seen in any of the embryos (data not shown). In fact, the correct initiation of *en* expression is evidence that a number of the homeo domain segmentation proteins are still functioning.

Despite dramatic loss of *en* in the epidermis, other tissues expressing *en* are less affected. *en* expression in the hindgut is unaltered in maternal effect *exd* mutants. Nervous system expression of *en* (DiNardo et al. 1985) is activated (Fig. 6F) and maintained after all ventral ectodermal *en* expression is lost. Although *en* expression in the nervous system is roughly normal, the nervous

system as a whole is deranged (cf. Fig. 6I with 6J). The central nervous system is diffuse rather than tightly organized, but some commissures and longitudinals are formed. Some peripheral nerve axons are sent out, but their pattern is disrupted. *ftz* and homeotic proteins are activated in the CNS of germ line clone-derived mutants (e.g., Fig. 2F), although their patterns may be disrupted.

Discussion

We demonstrated that mutations in *exd* disrupt a specific subset of the processes requiring the action of homeo domain proteins during *Drosophila* embryogenesis. One class of proteins affected is certain homeotic gene products. Reduced *exd* levels result in homeotic transformations, which do not result from changes in homeotic gene expression nor from effects on the domain of action of these genes. Rather, the morphological consequences of normal combinations of homeotic proteins are altered, perhaps because of changes in the segment-specific target genes recognized by these homeo domain proteins. Removal of *exd* from both the mother and the embryo results in a more severe phenotype which is explained, in part, by failure to maintain the expression of the homeo domain protein *en*.

Determining segmental identity

Segmental identity is conferred by genes of the Antennapedia and bithorax complexes (Lewis 1978; Kaufman 1983). Work from numerous laboratories has resulted in a model for how homeotic proteins regulate segmental identity. All contain a homeo domain—a protein domain required for DNA binding (for review, see Gehring 1987). The homeotic proteins are transcription factors that bind to downstream genes and turn gene expression on or off (Thali et al. 1988; Krasnow et al. 1989; Winslow et al. 1989). Each is presumed to have its own set of target genes; differential activation of these sets of target genes leads to the different morphologies of the *Drosophila* segments.

One problem with this model is that the homeo domain is highly conserved through evolution. Different homeo domains are often quite similar (Gehring 1987; Scott et al. 1989). For example, *Ubx* and *abd-A* homeo domains differ by only 5 of 61 amino acids (Akam et al. 1988). DNA binding studies of homeo domain proteins have shown that target specificity in vitro is relatively lax. Proteins with divergent homeo domains can bind the same target sequence, and the same protein can bind to two quite different targets, albeit with different affinities (Beachy et al. 1988; Desplan et al. 1988; Hoey and Levine 1988). Despite this, homeotic proteins likely recognize different subsets of target genes and also probably interact in a quantitatively different fashion with common targets. The question is further complicated by the fact that the homeotic genes are not expressed in segment-wide stripes but in complex, overlapping patterns (White and Wilcox 1985; Beachy et al. 1985; Carroll et al. 1986). Different subsets of target genes must be expressed in

response to these different patterns of homeotic gene expression. How is specificity conferred?

The possible role of *exd* in homeotic gene specificity

Although the genes of the Antennapedia and bithorax complexes appear to control segmental identity directly, a number of other genes have a homeotic phenotype. The precise role in segmental identity of many of these genes (e.g., Ingham and Whittle 1980; Kennison and Tamkun 1988) remains to be defined. The effects of some of these genes, members of the *Polycomb*-like class (Lewis 1978; Struhl 1981; Duncan 1982; Jürgens 1985), have been examined at a molecular level. All share a similar phenotype—transformation of most segments to A8. The effects on homeotic genes of two of these genes, *Polycomb* and *extra sex combs*, have been examined by use of molecular probes and were shown to affect regulation of the Antennapedia and bithorax complexes; in mutants, all homeotic genes are ectopically expressed (Struhl and Akam 1985; Wedeen et al. 1986). These *Polycomb*-like genes, although homeotic in phenotype, regulate expression, not specificity of action, of the homeotic genes. Other genes in this class are more pleiotropic in their effects, but their homeotic transformations also appear to result from misregulation of the homeotic genes (e.g., *polyhomeotic*; Dura et al. 1985, 1987; Perrimon et al. 1985; Smouse et al. 1988).

In contrast to the *Polycomb*-like genes, mutations in *exd* do not exert their effects through alterations in homeotic gene expression. Genetic tests demonstrate that the homeotic genes are active in their normal domains in *exd* mutants, but this activity leads to different morphologies. Thus, *exd* affects the consequences of homeotic proteins, even when, as in the case of the *heat shock–homeotic protein* fusions, these proteins are expressed ectopically under heterologous regulation.

Having ruled out a role for *exd* in regulating homeotic gene expression, two possible explanations remain for the genetic additivity of mutations in *exd* and in the homeotic genes. One is that *exd* functions downstream of the homeotic genes, acting in most or all segments to turn homeotic gene expression patterns into different segmental morphologies. Such a model would almost certainly require a complicated *exd* expression pattern, presumably regulated by the homeotic genes, to yield different morphological consequences in each segment. Such a model can be ruled out by the interchangeability of the maternal and zygotic contributions of *exd*. The ability of increased maternal *exd* product to rescue the segmental transformations of an *exd* mutant rules out any essential role of the homeotic proteins in creating a complex *exd* expression pattern. Finally, because *exd* has the same effect on *Ubx* when it is expressed ectopically in the head as it does when it is expressed normally, there is probably no underlying pattern of *exd* expression that produces its homeotic effects.

If *exd* does not function downstream of the homeotic genes, it must act in parallel to them. The simplest explanations consistent with these results are that *exd*

alters the target specificity of certain homeotic proteins or that it alters the quantitative effects of a given homeotic protein on a common target gene. Examine, for example, genes activated by *Ubx* in an *exd* mutant (Fig. 7). In the A1 segment of an *exd* mutant, *Ubx* may activate A3-specific genes, rather than A1-specific genes. Likewise, in the A3 segment of an *exd* mutant, *abd-A* may activate A5-specific genes rather than its normal A3-specific targets. This model explains some of the more subtle aspects of the *exd* phenotype. T3, in an *exd* mutant, has an ambiguous morphology, with elements of both T1 and A3. This may be a result of a conflict between *Ubx* and *Antp*, both of which are normally active in T3. In an *exd* mutant, we envision that *Ubx* is trying to confer the A3 morphology, whereas *Antp* is trying to confer the T1 morphology, leading to ambiguity. Not all homeotic proteins are equally affected, however. *Scr* and *Abd-B* seem relatively impervious to reduction in *exd*. It is interesting that the three homeotic genes clearly affected, *Antp*, *abd-A* and *Ubx*, all share very similar homeo domains (Akam et al. 1988).

exd and *en* expression

This apparent role of *exd* in the specificity of the homeotic proteins arises from an analysis of lowering the level of *exd* product by eliminating zygotic activity. Complete removal of *exd*, both from the egg and the embryo, also affects segmentation. This effect seems to be mediated, in part, through alterations in the maintenance of the expression of another homeo domain protein, *en*.

Figure 7. Model for the role of *exd* in homeotic gene specificity. In the wild-type animal, *Ubx* is active in the A1 segment, turning on A1-specific genes and conferring the A1 morphology. Likewise, in A3, *abd-A* acts to turn on A3-specific genes, resulting in an A3 morphology. In contrast, in the *exd* zygotic mutant, each of the homeotics has altered consequences. *Ubx* continues to be active in A1, but it now activates A3-specific genes, leading to an A3 morphology. Likewise, *abd-A* now activates A5-specific genes in the A3 segment, giving an A3 segment with the morphology of a wild-type A5 segment.

As demonstrated above, *exd* does not affect initiation of *en* expression but is required for its maintenance. A number of genes are required for proper maintenance of *en* (DiNardo et al. 1988), prominent among which are the segment polarity genes, such as *wg*. In the absence of *wg*, *en* expression decays soon after its activation. Is the effect of *exd* on *en* mediated through *wg*? We believe not, for three reasons. (1) The cuticle phenotype of *exd* maternal effect mutants is similar to that of *en*, not *wg*; (2) although both *wg* and *exd* show asymmetries in their effects on *en* maintenance, these asymmetries are opposite from each other; (3) the effects on *wg* expression seen in *exd* germ line clone embryos occur after the first effects on *en* expression.

Another gene that affects *en* maintenance is *en* itself (J. Heemskerk, S. DiNardo, and P. O'Farrell, pers. comm.), possibly through autoregulation. The pattern of *en* decay in *en* mutant embryos shares at least one feature with the pattern of decay in *exd* mutants, the pair-rule aspect in which even-numbered *en* stripes are first to decay. One speculative possibility is that *exd* is required for *en* autoregulation. Perhaps in the absence of *exd*, *en* product is no longer functional. The striking difference in the effect of *exd* on *en* between the dorsal and ventral sides is difficult to explain, though it may reflect dorsal/ventral differences in the regulation of late *en* expression (DiNardo et al. 1988). The aspects of the *exd* maternal effect phenotype that are more severe than *en* may reflect loss of *invected* (Coleman et al. 1987); a deletion of both *en* and *invected*, along with a number of other genes, is more severe than a null allele of *en* (Gubb 1985).

The specificity of the effect of exd

The aspects of development left unaltered by the mutations in *exd* tell us a number of things about the role of *exd* in the embryo. *exd* seems to play no part in oogenesis, ruling out an essential function in all cells. Numerous genes involved in segmentation, many of which have homeo domains (Gehring 1987), function properly, even in the absence of *exd*, as demonstrated by the appropriate activation of both the homeotic genes and *en*. *exd* is quite specific in which genes it affects.

There are certain similarities between *en* and the homeotic genes, not shared by many other genes in the fly which encode homeo domain proteins. *en* and the homeotic genes are large genes with complex regulatory regions (Drees et al. 1987; Peifer et al. 1987), reflecting, in part, the fact that all of these genes are involved in specifying and maintaining the identity of specific cells. Homeotic genes regulate segmental identity, whereas *en* maintains differences between the anterior and posterior compartments of each segment (Morata and Lawrence 1975). These genes, in contrast to many other patterning genes, function throughout the life of the fly (Morata and Lawrence 1975; Garcia-Bellido and Lewis 1976; Morata and Garcia-Bellido 1976). These complexities and similarities may be reflected in the effect of *exd* on the target specificity and/or activity of all of these genes.

Given our lack of understanding of homeotic gene specificity in the wild-type animal, one can only speculate about possible molecular roles for *exd*. *exd* could, for example, form a complex with homeotic gene products, altering their specificity of DNA binding or changing the proteins with which they interact. Alternatively, because at least some of the homeotic gene products are modified by phosphorylation (Krause et al. 1988; they may also be modified in other ways), modification may modulate specificity, and *exd* could be involved in the modification process. All of these possibilities are completely theoretical. The molecular analysis of *exd* may shed some light on the function of the *exd* protein.

Experimental procedures

Fly stocks and genetics

Balancers and other mutants can be found in Lindsley and Grell (1968). Two *exd* mutants, *exd^{XP11}* and *exd^{YO12}*, along with *twisted gastrulation^{YN97}*, come from the zygotic lethal screen of Wieschaus et al. (1984). The other *exd* alleles were provided by Alissa Katzen as lethals from the region derived from a saturation mutagenesis. The bithorax complex mutants used, *Ubx* = *Ubx¹* (Bender et al. 1983), *abd-A* = *abd-A^{D100.24}*, *Abd-B* = *Abd-B^{D100.16}*, *Ubx abd-A* = *Df(3R)P2*, *Ubx abd-A Abd-B* = *Df(3R)P9*, *DP(3:3)P5 Sb* (used to construct all *Abd-B* double mutants; Karch et al. 1985), and *abd-A Abd-B* = *Df(3R)Mc^{R6-41}*, were provided by Welcome Bender, except the last, which was provided by Ian Duncan. The Antennapedia complex mutants used, *ScrW17*, *Antp^{W10}*, and *Scr^{W17} Antp^{W10}* (Wakimoto and Kaufman 1981), were provided by Ian Duncan. All of these mutants are null or nearly null for the homeotic gene they effect. The *Pc* allele used was *Pc³* (Lewis 1980), and the weak *en* mutant was *en^{IM99}* (Nüsslein-Volhard et al. 1984). *Df(1)sd^{72b}* and *Df(1)L9* were provided by Shelagh Campbell. The *hs-Ubx* stock was a third chromosome-linked stock provided by Richard Mann (Mann and Hogness 1990). The *hs-Antp* stock, the third chromosome-linked line P2-3, was provided by Kevin Hill and Matt Scott. Most crosses were done at 22°C. For the heat-shock experiments, males carrying the heat shock-homeotic gene fusions were mated to *exd^{XP11}* heterozygous females. Three-hour collections of eggs were made at 25°C and aged for 3 hr more. These embryos were then transferred to a prewarmed tube of Voltalef 3S oil in a 37°C water bath. *hs-Ubx*-bearing embryos were heat-shocked for 30 min, and *hs-Antp* embryos were heat-shocked for 1 hr. The heat-shocked embryos were then returned to 22°C, allowed to complete development under oil, and mounted as described below. Germ line clones were produced as described in Wieschaus and Noell (1986). To examine the effects of increased maternal *exd*, *yellow⁺* attached-X females were mated to *yellow exd/Duplication exd⁺* males. The mutant progeny were identified by scoring for *yellow* denticles.

Morphology

Cuticle preparations were made according to the protocol of Struhl (1989). The observation of living embryos is described in Wieschaus and Nüsslein-Volhard (1986). Embryos were prepared for SEM as follows (many steps are described in detail in Wieschaus and Nüsslein-Volhard 1986). Embryos were collected on apple juice agar plates, washed in 0.1% Triton X-100, dechorionated in 50% bleach for 2 min, rinsed in water, and

fixed for 15 min in 25% glutaraldehyde/heptane. The fixative was removed, and embryos were devitellinized by adding methanol and shaking for 15 sec. Devitellinized embryos sink to the bottom. These were rinsed once with methanol, and rinsed 3×5 min in PBS + 0.1% Triton X-100 and 0.2% BSA, post-fixed in 1% OsO₄ at 22°C for 30 min, and dehydrated through 5-min washes in 50%, 70%, 90% and 100% ethanol. After two more washes in 100% ethanol, the embryos were incubated at 37°C for 30 min in 1 : 1 ethanol/Peldri II (Ted Pella, Inc.) and then rinsed twice and incubated for 30 min in Peldri. These embryos were cooled on ice to harden the Peldri, and the Peldri was then allowed to sublime under vacuum for 2–20 hr. The embryos were mounted on double-stick tape, coated with gold–palladium, and viewed under a JOEL JSM840 scanning electron microscope.

Histochemistry

Antibodies to *Ubx* (White and Wilcox 1984) and *abd-A* (Karch et al. 1990) were provided by Welcome Bender. Antibodies to *Scr* (Glicksman and Brower 1988) and *Antp* (Condie et al. 1990) were made by Danny Brower. Antibody to *en/invected* was provided by Nipam Patel (Patel et al. 1989). Antibody to *ftz* was provided by Henry Krause (Krause et al. 1988). Antibody to *Sex-lethal* was provided by Daniel Bopp and Paul Schedl. Antibody to horseradish peroxidase was purchased from Cappel, and antibody to β -galactosidase was purchased from Promega. All embryo fixation and antibody staining was done according to the protocol of Patel et al. (1989). When staining with anti-horseradish peroxidase, the Triton X-100 concentration in all washes was increased to 0.4%. Antibody staining was visualized by use of the Vecta-stain ABC kit (Vector Labs), with the addition of 0.04% nickel chloride. Whole mount in situ hybridization was done according to the protocol of Tautz and Pfeifle (1989).

Use of Sex lethal antiserum to identify exd embryos

Antibody to *Sex lethal* stains female embryos but does not stain male embryos (D. Bopp and P. Schedl, pers. comm.). The progeny of a cross of wild-type males with females heterozygous for *exd*^{Y012} and another closely linked X-chromosomal mutation, *twisted gastrulation* (Wieschaus et al. 1984), were stained with antisera to both *Sex-lethal* and to a homeotic gene. This resulted in three classes of progeny. Females are uniformly stained with *Sex-lethal*. The patterns of homeotic gene expression are visible only in male embryos. Of these, *twisted gastrulation* males are easily distinguished from their *exd* brothers by the dramatic morphological defects in *twisted gastrulation*.

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