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*.

[Key Words: extradenticle; homeo domain protein; segmental identity; 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-
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revealed 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, engramed (en). These results are used to present a model for exd function.

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)sq72R and Df(1)L9]. Two weaker exd alleles are postembryonic lethals. Homozygotes for one of these weak alleles, exdRMS, 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

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 exdRMS. 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 exdSP11 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).
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 aT1 = 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 antisera 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^{P11} 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:
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| Genotype  | Segment |
|-----------|---------|
| wild-type | T2 T3 A1 |
| Ubx       | T2 T3 A1 |
| exd       | T1 T1/A3 A3 |
| exd Ubx   | 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.

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**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. [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.

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**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. 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
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 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
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 mutations [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<sup>em5</sup>. (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<sup>aus</sup>. Whereas A1 is nearly normal, the dentine 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<sup>em5</sup>. 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<sup>em5</sup>. 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 mutants have normal en expression, data not shown]. We assayed en expression in two ways: by use of antibody to the *inverted* 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

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**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 antiserum to horseradish peroxidase [Ian 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, ap-
parently 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 asymme-
tries in cuticular phenotype of maternal effect exd em-
bryos.

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 ex-
pression 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 re-
main. wg seems to be lost slightly later than en but with 
a similar dorsal/ventral asymmetry. An effect on wg ex-
pression is expected, because en is required for wg main-
tenance [Martinez-Arias et al. 1988]. The effect on wg 
could be a secondary consequence of the loss of en ex-
pression.

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 em-
bryos [e.g., Klingensmith et al. 1989]. Thus, changes in 
en promoter activity may not provide the entire explana-
tion 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 expres-
sion 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 [Di-
Nardo 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 ef-
fect phenotype, no difference in ftz expression was seen 
in any of the embryos [data not shown]. In fact, the cor-
rect initiation of en expression is evidence that a num-
ero 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 ecto-
dermal 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 or-
ganized, but some commissures and longitudinal 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 spe-
cific 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 morpho-
logical consequences of normal combinations of ho-
meotic 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 pheno-
type 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 Anten-
apedia 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 do-
main 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 do-
main 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 affi-
nities [Beachy et al. 1988, Desplan et al. 1988; Hoye 
and Levine 1988]. Despite this, homeotic proteins likely re-
cognize 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 homeootic 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, 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, \( \text{exd} \) does not affect initiation of \( \text{en} \) expression but is required for its maintenance. A number of genes are required for proper maintenance of \( \text{en} \) (DiNardo et al. 1988), prominent among which are the segment polarity genes, such as \( \text{wg} \). In the absence of \( \text{wg} \), \( \text{en} \) expression decays soon after its activation. Is the effect of \( \text{exd} \) on \( \text{en} \) mediated through \( \text{wg} \)? We believe not, for three reasons. (1) The cuticle phenotype of \( \text{exd} \) maternal effect mutants is similar to that of \( \text{en} \), not \( \text{wg} \); (2) although both \( \text{wg} \) and \( \text{exd} \) show asymmetries in their effects on \( \text{en} \) maintenance, these asymmetries are opposite from each other; (3) the effects on \( \text{wg} \) expression seen in \( \text{exd} \) germ line clone embryos occur after the first effects on \( \text{en} \) expression.

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

The specificity of the effect of \( \text{exd} \)

The aspects of development left unaltered by the mutations in \( \text{exd} \) tell us a number of things about the role of \( \text{exd} \) in the embryo. \( \text{exd} \) 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 \( \text{exd} \), as demonstrated by the appropriate activation of both the homeotic genes and \( \text{en} \). \( \text{exd} \) is quite specific in which genes it affects.

There are certain similarities between \( \text{en} \) and the homeotic genes, not shared by many other genes in the fly which encode homeo domain proteins. \( \text{en} \) 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 \( \text{en} \) 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 \( \text{exd} \) 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 \( \text{exd} \). \( \text{exd} \) 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 \( \text{exd} \) could be involved in the modification process. All of these possibilities are completely theoretical. The molecular analysis of \( \text{exd} \) may shed some light on the function of the \( \text{exd} \) protein.

Experimental procedures

Fly stocks and genetics

Balancers and other mutants can be found in Lindsley and Grell (1968). Two \( \text{exd} \) mutants, \( \text{exd}^{P311} \) and \( \text{exd}^{P302} \), along with twisted gastrulation\(^{\text{TMNP}} \), come from the zygotic lethal screen of Wieschaus et al. (1984). The other \( \text{exd} \) alleles were provided by Alissa Katzen as lethals from the region derived from a saturation mutagenesis. The bithorax complex mutants used, \( \text{Ubx} = \text{Ubx}^{\text{e}} \) (Bender et al. 1983), \( \text{abd-A} = \text{abd-A}^{\text{D1}76} \), \( \text{Abd-B} = \text{Abd-B}^{\text{D}20001} \), \( \text{Ubx} \text{abd-A} = \text{D}(\text{3R})\text{P2} \), \( \text{Ubx} \text{abd-A} \text{Abd-B} = \text{D}(\text{3R})\text{P9} \), \( \text{Df}(3\text{R})\text{PS} \) (used to construct all \( \text{Abd-B} \) double mutants; Karch et al. 1985), and \( \text{abd-A} \text{Abd-B} = \text{D}(\text{3R})\text{McB6}^{-1} \), were provided by Welcome Bender, except the last, which was provided by Ian Duncan. The Antennapedia complex mutants used, \( \text{ScrW17} \), \( \text{Antp}^{\text{W10}} \), and \( \text{Scr}^{\text{W71}} \) \( \text{Antp}^{\text{W10}} \) (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 \( \text{Pc} \) allele used was \( \text{Pc}^{\text{D}} \) (Lewis 1980), and the weak \( \text{en} \) mutant was \( \text{en}^{\text{TM90}} \) (Nüsßlein-Volhard et al. 1984). \( \text{Df}(1)\text{sf290} \) and \( \text{Df}(1)\text{L9} \) were provided by Shelagh Campbell. The \( \text{hs}^{\text{--}}\text{Ubx} \) stock was a third chromosome-linked stock provided by Richard Mann (Mann and Hogness 1990). The \( \text{hs}^{\text{--}}\text{Antp} \) stock, the third chromosome-linked line \( \text{P2-3} \), 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 \( \text{exd}^{\text{P311}} \) 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. \( \text{hs}^{\text{--}}\text{Ubx} \)-bearing embryos were heat-shocked for 30 min, and \( \text{hs}^{\text{--}}\text{Antp} \) 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 \( \text{exd} \), \( \text{yellow}^{+} \) attached-X females were mated to \( \text{yellow exd}^{\text{Dupl}} \) \( \text{male} \) progeny were identified by scoring for \( \text{yellow denticles} \).

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 3 × 5 min in PBS + 0.1% Triton X-100 and 0.2% BSA, post-fixed in 37°C for 30 min in 1 : 1 ethanol/Peldri II (Ted Pella, Inc.) and fixed for 15 min in 25% gluteraldehyde/heptane. The fixative was provided by Henry Krause (Krause et al. 1988). Antibody to Sex-lethal was provided by Nipam Patel (Patel et al. 1989). Antibody to Antp (Condie et al. 1990) were provided by Welcome Bender. Antibodies to Sex-lethal were provided by Ian Duncan, Welcome Bender, and Shelagh Carroll. Dari Sweeton and Elaine Lenk were invaluable in discussions about in situ hybridization and antibody staining; Bob Riggeleman provided dioxgeyn-labeled wg probe, and Gordon Gray ably ran the fly kitchen. John Scott assisted in the early stages of this work. We are indebted to Welcome Bender and members of the Wieschaus and Schüpbach laboratories for stimulating discussions and to Iva Greenwald, Bill Morgan, Cordelia Ranskolb, Lesilec Simpson, and Trudi Schüpbach for valuable comments on various drafts of the manuscript. Dari Sweeton and Kate Harding provided timely advice and help with photography. The research was funded by National Institutes of Health [NIH] grant 5R01HD22780 to E.W. and by an NIH postdoctoral fellowship to M.P.

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**exd affects homeo domain protein function**

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