A Genetic Screen of the Drosophila X Chromosome for Mutations That Modify Deformed Function

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ABSTRACT
We have screened the Drosophila X chromosome for genes whose dosage affects the function of the homeotic gene Deformed. One of these genes, extradenticle, encodes a homeodomain transcription factor that heterodimerizes with Deformed and other homeotic Hox proteins. Mutations in the nejire gene, which encodes a transcriptional adaptor protein belonging to the CBP/p300 family, also interact with Deformed. The other previously characterized gene identified as a Deformed interactor is Notch, which encodes a transmembrane receptor. These three genes underscore the importance of transcriptional regulation and cell–cell signaling in Hox function. Four novel genes were also identified in the screen. One of these, rancor, is required for appropriate embryonic expression of Deformed and another homeotic gene, labial. Both Notch and nejire affect the function of another Hox gene, Ultrabithorax, indicating they may be required for homeotic activity in general.

HOMEOPTIC (Hox) genes of the Antennapedia and Bithorax Complexes of Drosophila encode transcriptional regulators that bind DNA through a 60-amino-acid homeodomain (Scott et al. 1989). They provide positional cues along the anterior-posterior axis of developing embryos, as do their homologs in other animals (McGinnis and Krumlauf 1992; Manak and Scott 1994). Hox mutations often result in homeotic transformations where one body part takes on the appearance of another (Garcia-Bellido 1977; Lewis 1978). This is usually caused by the inappropriate activity of an underlying or ectopically expressed homeotic gene (Struhl 1982; Struhl and White 1985). For example, the absence of the Ultrabithorax (Ubx) gene causes the third thoracic leg to have the morphological characteristics of a second thoracic leg because of inappropriate activity of the Hox gene Antennapedia (Morata and Kerridge 1981; Struhl 1982). The Hox proteins specify structures in concert with dorsal-ventral and anterior-posterior signals that position those structures within a segment. For example, in the Drosophila embryo, the signaling proteins Hedgehog, Wingless, and Decapentaplegic (Dpp) determine the position of cuticular features, such as sensory organs, tracheae, and ventral denticles (Irish and Galbraith 1987; Bejsovec and Wieschaus 1993; Affolter et al. 1994; Hemesker and Dinardo 1994; Wapner et al. 1997). The segmentally unique patterns of these structures are controlled by the Hox proteins (Lewis 1978; Kaufman and Abbott 1984; Castelli-Gair et al. 1994). The involvement of Hox specification in the many disparate developmental pathways required to pattern a metamer animal suggests the Hox proteins integrate their segmental identity functions with many different cofactors.

Understanding how the Hox proteins differentially specify regulatory targets and, thus, determine unique segmental identity has been problematic. The Hox homeodomains are highly related, causing different Hox proteins to have similar or identical monomeric DNA-binding specificity (Ekker et al. 1991, 1992, 1994; Laughon 1991). These proteins can also bind a wide range of sequences in vitro and in vivo, allowing them to interact with the regulatory regions of a great many potential downstream genes (Desplan et al. 1988; Walter and Biggin 1994, 1996). Auto- and cross-regulation of Hox genes further complicate the issue, as factors affecting Hox expression may also affect the ability of Hox proteins to differentially regulate other targets. An alteration in Hox DNA-binding specificity is accomplished by dimerization of Hox proteins with the Extra-denticle (Exd) protein (van Dijk and Murre 1994; Knoepfler and Kamps 1995; van Dijk et al. 1995; Chan et al. 1997). However, because most Hox proteins can dimerize with Exd on similar sites (van Dijk et al. 1995; Chan et al. 1997; Neuteboom and Murre 1997), this is unlikely to explain entirely the distinct morphologies controlled by the homeotics. The appropriate regulation of target genes by Hox proteins is likely to require interactions with many other regulatory factors. Screens for genetic modifiers of homeotic functions have constituted one method for identifying regulatory factors that interact with the Hox system. Previous Hox

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modifier screens have identified many members of the 
repressive Polycomb Group (PcG) or activating trithorax 
Group (trxG) genes (reviewed in Kennison 1995; Pi r r ot ta 1998). PcG genes are required to prevent the 
spread of Hox expression after the establishment of their 
initial expression patterns, and they encode proteins 
involved in the formation of suppressive chromatin 
structure. Genes of the trxG are required for the mainte 
nance of Hox expression and have been proposed to be 
a more diversified group than PcG (Kennison 1995). 
As might be expected, some of the TrxG factors, such 
as Brahma, a likely chromatin-remodeling factor (Tam 
kun et al. 1992; Pazin and Kadonaga 1997; Elfr ing et 
al. 1998), may function by directly reversing the effects 
of PcG proteins. Others, such as Zeste (reviewed in 
Kennison 1995) and Trl (Croston et al. 1991; Farkas 
et al. 1994), are transcription factors that function in 
part by interacting with chromatin components. At least 
one TrxG protein, vacuolar H+-ATPase 55-kD B subunit 
(encoded by Vha55, Davies et al. 1996), does not have 
an obvious role in modifying chromatin structure nor 
in transcription, emphasizing the diversity of the factors 
that influence Hox function.

Another class of Hox-interacting genes (extradenticle, 
tashirt, homothorax, and cap’ncollar) fall into a class that 
we call the Hox modulators. These genes can mutate to 
give homeotic transformations, but they appear to act 
in parallel to Hox proteins, in contrast to the upstream 
regulatory functions of the PcG and trxG (Pfeifer and 
Wieschaus 1990; Roder and Kerridge 1992; Mohler 
et al. 1995; Rieckhoff et al. 1997). Because of the diversity 
of the trxG, there may be some overlap between the 
genesis of the Hox modulator and trxG classes. The modu 
lator proteins that have been characterized perform a 
variety of biochemical roles, which include enhancing the 
DNA-binding affinity of Hox proteins (Mann and 
Chan 1996), regulating their transcriptional activities 
(Pinsonneault et al. 1997), and regulating the nuclear 
entry of Hox cofactors (Rieckhoff et al. 1997).

We have previously screened the second and third 
chromosomes of Drosophila for genes that show dose 
sensitive interactions with the Hox gene Deformed (Dfd; 
Harding et al. 1995; Gellon et al. 1997). The Dfd gene 
is required for the identity of two head segments (maxi 
mary and mandibular), and it is required for larval viabil 
ity (Merrill et al. 1987). Dfd is distinct from the trunk 
homeotic in that its embryonic expression pattern does 
not overlap that of other Hox genes (with the exception 
of proboscipedia, which has no obvious morphogenetic 
function in embryos, Pul tz et al. 1988). Without under 
lying homeotic expression, loss-of-function Dfd muta 
tions result in deletions of embryonic structures rather 
than the homeotic transformations caused by mutations 
in trunk Hox genes (Garcia-Bellido 1977). Therefore, 
underlying homeotic expression cannot compensate for 
mutations in genes that limit Dfd function. In general, 
the genes isolated as Dfd interactors that have been also 
molecularly characterized fall into two categories: those 
involved in cell-cell signaling (Collagen type IV, viking, 
davenir, Laminin A; Vha55, hedghog, and Serrate) and 
those involved in transcriptional regulation (Ecdysone 
Receptor, apontic, cap’ncollar, Pc, and trx). Mutations 
in many of the Dfd-interacting genes suppress the domi 
nant Pc phenotype (kismet, Ecdysone Receptor, sallimus, 
davenir, moira, Vha55, and hedghog), indicating that they 
are likely to have general roles as regulators of homeotic 
expression patterns (Kennison and Tamkun 1988; Har d ing et al. 1995; Gellon et al. 1997). However, some 
Dfd-interacting genes do not play a role in regulating 
homeotic expression (Gellon et al. 1997; Harding, 
N. McGinnis, E. Wiellette and W. McGinnis, unpub 
lished data) and, therefore, are likely to act in parallel 
or downstream of Hox proteins.

The X chromosome presents practical difficulties in 
modifier screens, and it has been relatively neglected 
when screening for mutations that interact with homeo 
tic genes. We present here a screen of the X chromo 
some of Drosophila for genes involved in Dfd function. 
We have screened >2000 lethal chromosomes, isolating 
14 alleles that show dose-sensitive interactions with Dfd 
hypermorphic alleles. These alleles map in seven differ 
ent complementation groups. Three of these groups 
correspond to previously characterized X-chromosome 
genes involved in either cell-cell signaling (Notch) or 
transcriptional regulation (nejire and extradenticle). An 
other gene identified in the X-chromosome screen 
corresponds to a previously isolated but uncharacterized 
locus, 1(1)6Ee (Nickl as and Cline 1983), which we 
have renamed strung out (stout) based on its embryonic 
phenotype (see text). Mutations in stout and three other 
interacting genes cause larval head defects in Dfd-depend 
ent structures, indicating that they are required for 
Dfd embryonic function. However, only two of these 
mutants exhibit changes in Dfd expression. One of these, 
rancor (rnc), is a novel gene that is required for the 
proper regulation of both Dfd and labial. In addition, 
mutations in Notch and nejire modify a dominant Ubx 
phenotype, indicating that they may be generally in 
olved in homeotic function.

MATERIALS AND METHODS

Drosophila strains: P{cosG479BE}7-20, N+ was obtained from S. Artavans-Tsakonas (Ramos et al. 1989). l(1)6Ee, 
Df(1)Sxl6A, and Dp(1;Y)ct’y+ were obtained from T. Cline 
(Johnson and Judd 1979; Nickl as and Cline 1983). 
Df(1)Den1 was obtained from A. Christensen (Dorer et al. 
1993). Df(1)C52 and Dp(1;Y)l2’y+ were obtained from A. Sch a 
el, and Dp(1;Y)FF1 was obtained from P. Santamaria 
(San tamaria and Randsholt 1995). Tp(1;Y)l and l(1)11Ed were 
obtained from B. Ganetsky (Banerjee et al. 1995). Dp(1;Y)g+ 
and ma were obtained from D. Brower (Brower et al. 1995). 
g+ was obtained from S. Smolik (Aki 
mar u et al. 1997). Ubx6+ was obtained from G. Morata. 
All other strains were obtained from the Bloomington Stock 
Center. y2;YL is C(1;Y)1 marked with y2 and YS is C(YS)1.
**Mutagenesis:** EMS mutagenesis was performed according to standard protocols (Grigliatti et al. 1986). The screen is outlined in Figure 1. Briefly, isogenic y^2^YL/YS males were fed mutagen and individually crossed to virgin vinscy (inscy, v^0^) females for 2 days. The females were then transferred to fresh vials, and the males were discarded. Single F1 females carrying a uniquely mutagenized X chromosome were mated to v^+^ eye color. F2 from progeny were also used to establish lines (see materials and methods). Asterisks indicate mutagenized chromosomes; asterisk in parentheses indicates a possibly mutagenized chromosome.

**Dfd function on the X chromosome:** For the F2, F3, and F4 generations, males are on the left, and females are on the right. Lethal X chromosomes were screened for enhanced lethality of Dfd^12^/Dfd^1^ hypomorphs at 29°C in an F2 screen. Dfd females were identified by the presence of a closely linked Ki mutation (Harding et al. 1995) and as being Tb^+. The presence of the mutagenized X chromosome was determined by v^+^ eye color. F2 from progeny were also used to establish lines (see materials and methods). Asterisks indicate mutagenized chromosomes; asterisk in parentheses indicates a possibly mutagenized chromosome. y^2^YL/YS males were used to facilitate the collection of virgins in the F1 generation.

**Figure 1.—Screen to isolate dominant enhancers of Dfd function on the X chromosome.** For the F2, F3, and F4 generations, males are on the left, and females are on the right. Lethal X chromosomes were screened for enhanced lethality of Dfd^12^/Dfd^1^ hypomorphs at 29°C in an F2 screen. Dfd females were identified by the presence of a closely linked Ki mutation (Harding et al. 1995) and as being Tb^+. The presence of the mutagenized X chromosome was determined by v^+^ eye color. F2 from progeny were also used to establish lines (see materials and methods). Asterisks indicate mutagenized chromosomes; asterisk in parentheses indicates a possibly mutagenized chromosome. y^2^YL/YS males were used to facilitate the collection of virgins in the F1 generation.

**RESULTS**

We have screened the X chromosome of Drosophila for mutations that show dominant interactions with the homeotic gene Dfd. This was done using a slightly modified version of earlier screens (Harding et al. 1995; Gellon et al. 1997; see materials and methods). Briefly, males carrying an isogenic X chromosome were treated with EMS by standard methods. The resulting lethal X chromosomes were placed over a wild-type chromosome in a semilethal Dfd^12^/Dfd^1^ hypomorphic background and tested for a dominant reduction in viability (see Figure 1; materials and methods). An interaction was considered positive if 65% of the expected Dfd adult female hypomorphs survived. Potentially interacting chromosomes were re-screened at least twice to confirm the interaction. In all, 2048 lethal chromosomes were screened, resulting in 17 chromosomes that consistently showed an interaction with Dfd. Lethals on
TABLE 1

Cytological positions of Dfd-interacting genes

<table>
<thead>
<tr>
<th>Locus</th>
<th>Included in</th>
<th>Excluded from</th>
<th>Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>P[cosP479BE]7-20(N +), Dp(1;2;Y)w +</td>
<td>Df(1)H C244, Dp(1;3)w +, In(1)dm75e19</td>
<td>3C7+</td>
</tr>
<tr>
<td>WC1</td>
<td>Df(1)A113, Dp(1;3)w +, 67k</td>
<td>Df(1)ctj4, Df(1)5xh +</td>
<td>3E4-3E8</td>
</tr>
<tr>
<td>stout</td>
<td>Df(1)HA32, Dp(1;Y)ct+ 72d</td>
<td>Df(1)C128, Df(1)KA14, Df(1)RA2, Df(1)GE202, Df(1)HA11, Df(1)ctj4, Dp(1;3)sn +</td>
<td>6E1-6E4</td>
</tr>
<tr>
<td>mc</td>
<td>Df(1)Dex/Dp(1;2;SN + 72d</td>
<td>Df(1)z90b24, Df(1)9a4-5, Df(1)v-L15, Dp(1;2)v + 75d</td>
<td>7D6-7D10</td>
</tr>
<tr>
<td>nj</td>
<td>Df(1)C52, Dp(1;Y)iz+ Dp(1;Y)FF1</td>
<td>Df(1)g, Dp(1;f)L9, Dp(1;Y)gt, Tp(1;Y)1, Dp(1;fly+)</td>
<td>8F3-9A1</td>
</tr>
<tr>
<td>UC119</td>
<td>Dp(1;Y)shi</td>
<td>Dp(1;Y)sh1</td>
<td>14A1-9+</td>
</tr>
</tbody>
</table>

The tentative cytological position for UC119 is estimated on the basis of its map position of 44 map units, as well as its failure to be included in the deficiencies and duplications for the region. UC119 also complements l(1)Ed and mew.

* Data obtained from FlyBase.

TABLE 2

X chromosome loci showing a dominant interaction with Dfd

<table>
<thead>
<tr>
<th>Locus</th>
<th>Alleles</th>
<th>I5</th>
<th>Lethal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>SI77</td>
<td>3</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td>WB34</td>
<td>49</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>UB53</td>
<td>57</td>
<td>E</td>
</tr>
<tr>
<td>WC1</td>
<td>WC1</td>
<td>30</td>
<td>L</td>
</tr>
<tr>
<td>stout</td>
<td>UA104</td>
<td>0</td>
<td>N.D.</td>
</tr>
<tr>
<td>mc</td>
<td>TA181</td>
<td>21</td>
<td>E</td>
</tr>
<tr>
<td>nj</td>
<td>TA54</td>
<td>44</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td>WA69</td>
<td>0</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>S542</td>
<td>3</td>
<td>EL S542/ Q7</td>
</tr>
<tr>
<td></td>
<td>S703</td>
<td>52</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td>TA57</td>
<td>52</td>
<td>E TA57/ Q7</td>
</tr>
<tr>
<td>UC119</td>
<td>UC119</td>
<td>54</td>
<td>EL</td>
</tr>
<tr>
<td>exd*</td>
<td>SI36</td>
<td>21</td>
<td>EL SI36/1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>52</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>63</td>
<td>N.D.</td>
</tr>
<tr>
<td>y2-YL control</td>
<td>81</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

Alleles, allele designation—extant alleles and associated data are in italics (exd, Wieschaus et al. 1984; nj, Akimaru et al. 1997). I5 interaction determined per material s and methods; the smaller the number, the stronger the interaction. Lethal phase indicates the stage at which lethality occurs in males. If females were tested, the allelic combination is listed with the maternally contributed allele first: E, embryonic; EL, embryonic/ larval; L, larval (definitions are in materials and methods); N.D., not determined.

* Named after an animal from Tatooine with a similar phenotype.

* Preliminary data were reported in Pinsonneault et al. (1997).
Chromosome Modifiers of Dfd

**Deformed Interacting Loci**

![Diagram of chromosome loci](image)

**Figure 2**—Summary of Dfd-interacting genes in the Drosohila genome. The X chromosome genes were identified in the current work (see Table 2 and text for details). Genes on the second chromosome were identified by Gellon et al. (1997), and genes on the third chromosome were identified by Harding et al. (1995). E(raf)2A is allelic to poc (E. Wiellette and W. McGinnis, unpublished data), EcR is allelic to snt (E. Wiellette, B. Florence and W. McGinnis, unpublished data), and btl is allelic to dev (Szeged stock center, FlyBase). The fourth chromosome was not screened systematically, but ci^D also showed an interaction with Dfd (M. Martin and W. McGinnis, unpublished data; see text).

alleles do not carry null mutations; other indications of the nature of these mutations will be pointed out below.

The four novel or uncharacterized loci are, proximally to distally, WC1, stout, rnc, and UC119. Only stout has multiple alleles, and these showed a strong interaction with Dfd. The three remaining loci, WC1, rnc, and UC119, showed intermediate interactions with Dfd.

**Genetic interactions with other homeotics**: Although these loci were identified by their ability to interact with the gnathal homeotic Dfd, they might also have a general role in Hox-mediated patterning. We and other researchers have investigated such relationships by observing the genetic modification of the Pc phenotype, particularly the number of ectopic sex combs in adult males (Kennison and Tamkun 1988; Landecker et al. 1994; Harding et al. 1995; Gellon et al. 1997). Because the mutations isolated in this work are male lethal, this test was not practical. Other dominant Pc phenotypes may be scored in females, but their variability in expression and penetrance would make the results difficult to interpret. A screen based on the modification of female Pc phenotypes failed to uncover homeotic interactors on the X chromosome (Landecker et al. 1994). Therefore, we chose to test the ability of the Dfd-interacting mutations to interact with mutations in another homeotic gene, Ubx.

Heterozygotes for Ubx display ectopic bristles on the haltere, an indication of a partial transformation toward wing. Heterozygosity at a locus required for Ubx function could potentially enhance this phenotype. Conversely, heterozygosity for a locus that normally represses Ubx haltere function would be expected to suppress the Ubx phenotype. The Dfd-interacting alleles were placed in a Ubx^M1 heterozygous background, and the number of bristles on ~80 halteres was scored for each line. Only nej mutations showed an enhancement of the Ubx phenotype, while Notch alleles strongly suppressed ectopic bristle formation (Figure 3). The remaining Dfd-interacting alleles showed no effect, nor did the Pc^e and trx^C1 mutations (data not shown).

**Phenotypes**: Dfd is required for the formation of the mouth hook, ectostomal sclerite, H-piece bar, anterior lateralgraten, cirri, ventral organ, and maxillary sense organ (Merrill et al. 1987; Figure 4A). Ablation experiments have provided a rough fate map of the embryonic head for the larval cuticular structures (Jurgens et al. 1986), and they show that these Dfd-dependent structures are derived from the maxillary and mandibular segments where Dfd is expressed (Jack et al. 1988; Jack and McGinnis 1990). The Dfd targets Distalless (Dll; O'Hara et al. 1993), paired (prd; Vanario-Alonso et al. 1995), and Serrate (Ser; Fleming et al. 1990; Speicher et...
al. 1994; E. Wiellette and W. McGinnis, unpublished data) are all expressed in subsets of Dfd-expressing cells. The Dll, prd, and Ser mutant phenotypes in the maxillary segment are strongly correlated with the maxillary subregions in which they are expressed. The effect of the Dfd interactors on Dfd-dependent structures and target genes is discussed below.

Notch: The classical dominant Notch phenotype includes notched wing margins. Although all three Dfd-interacting alleles showed notched wings in adults, their phenotypes were less severe than a Notch null, suggesting that all three alleles are hypomorphic. Mutant embryos lacking Notch have a neurogenic phenotype (reviewed in Artavanis-Tsakonas et al. 1995) where the ventral epidermis is transformed into nervous tissue and, therefore, only the dorsal cuticle is produced. However, approximately half the N^5177 cuticles had laterally derived maxillary cirri, whereas N^UB53 animals did not (data not shown). This suggests that N^5177 is a weaker mutation than N^UB53 even though N^5177 showed the strongest interaction with Dfd.

nejire The extant nej^3 allele has been molecularly characterized as a null (Akimaru et al. 1997). However, in the Dfd interaction test, the viability of Dfd hypomorphs was not affected by heterozygous nej^3 (114% of the expected progeny). The failure of a nej null to interact with Dfd suggests that the Dfd-interacting nej alleles are not amorphic. Consistent with this interpretation, the lethal phases of our nej alleles differ from that of the nej^3. We found that only ~15% of nej^3 males died as embryos, demonstrating that the nej maternal component (Akimaru et al. 1997) is sufficient for embryogenesis. However, 100% of both nej^07 males and nej^TA57/nej^07 females, as well as ~35% of nej^514/nej^07 females, died as embryos (Table 2). The premature lethality of the Dfd-interacting alleles indicates they provided less functional maternal component, perhaps because of an amorphic Nej protein. In nej^TA57/nej^07 or nej^07/Y cuticles,
the maxillary and antennal sense organs often showed a slight disruption in patterning, the mouth hooks and median tooth were reduced, and the proventriculus was sclerotized (data not shown). No other phenotypes were consistently observed in cuticles of any \textit{nej} genotype.

A genetic interaction has been previously noted between \textit{nej} and a dominant mutation of \textit{cubitus interruptus} (\textit{cubitus interruptus} \textit{Df(1)DesiS3} ciD function. The \textit{nej} \textit{ciD} allele causes \textit{Ci} to be ectopically expressed in the posterior of the wing, causing defects in vein, bristle, and margin formation (Slusarski et al. 1995). In \textit{ciD} animals, the L4 wing vein is truncated, and small amounts of ectopic wing vein are occasionally present. The posterior wing margin is fused to the alula, has larger bristles, and is often nicked (Figure 5, arrows). This latter defect can be partially suppressed by the \textit{nej} \textit{null allele} (Akimaru et al. 1997). The \textit{nej} \textit{ciD} allele isolated in our screen also suppressed this phenotype (compare black arrows in Figure 5). Paradoxically, \textit{nej} \textit{ciD} also strongly enhanced the formation of ectopic wing veins in the \textit{ciD} background (Figure 5, white arrows). The lack of a similar enhancement in \textit{ciD} animals that are heterozygous for the \textit{nej} \textit{null mutation} supports the idea that \textit{nej} \textit{ciD} is antimorphic.

\textit{rnc}: Cuticles of \textit{rnc} \textit{Df(1)fg153} and \textit{Dfd(1)DesiS3} \textit{Df(1)H-pc} embryos showed defects in derivatives of the antennal and ventral gnathal lobes, including Dfd-dependent structures. Specifically, the hypostomal sclerite and the H-piece arm were present but malformed, the base of the mouth hook was reduced, the mouth hooks were multiply serrated (Figure 4A, arrowhead), and head involution failed. The involution defect caused the maxillary and antennal sense organs to develop on the lateral aspect of the head instead of at the normal anterior-dorsal location. Less often, the H-piece crossbar was reduced or failed to fuse medially, the dorsal-lateral or medial papillus was missing or misplaced (Figure 4A, arrow, DLP, or DMP), the ventral organ was disrupted, and the median tooth was translocated dorsally. The head involution defect is similar to that caused by mutations in another homeotic gene, labial (lab; Merrill et al. 1989). In addition, both lab and \textit{rnc} mutations affect many of the same head structures, such as mouth hooks, hypostomal sclerite, and H-piece.

\textit{strung out}: Cuticles of \textit{stout} \textit{Df(1)HA32} and \textit{stout} \textit{Df(1)H-pc} male and \textit{stout} \textit{Df(1)H-pc} female embryos had very similar phenotypes, although the \textit{stout} \textit{Df(1)H-pc} phenotype was somewhat milder. Most striking in these mutants were the defects surrounding the dorsal sac. The dorsal bridge was diffuse and occasionally unfused. Strands of sclerotic material were “strung out” from the anterior vertical plate and ventral side of the dorsal bridge in the area between the dorsal sac and lateral gräten (white arrow in Figure 4A). Also, the H-piece lateral arm was bifurcated or widened, and the H-piece crossbar was widened or broken. The mouth hooks were of normal length, but thin, and the mouth hook bases were often reduced. The trunk of stout animals also showed narrow third thoracic denticle belts (Figure 4B, white and black arrowheads). A similar denticle phenotype was seen in mutants of another Dfd interactor, EcR (Bender et al. 1997; called \textit{snt} in Cline et al. 1997). The \textit{stout} gene appears to correspond to a previously identified lethal complementation group, \textit{l(1)6Ee} (Nicklas and Cline 1983), as our \textit{stout} alleles were lethal when placed over the \textit{l(1)6Ee} chromosome.

Heterozygous \textit{stout} \textit{Df(1)HA32} and \textit{stout} \textit{Df(1)H-pc} adults have thick aristae (Figure 6, arrows) and arced wings, and many have one or more kinked macrochaete, phenotypes that are not seen in deficiency heterozygotes for the \textit{stout} locus. In addition, males carrying these alleles over two duplications that cover the \textit{stout} locus [\textit{Dp(1;2)sn134} or \textit{Dp(1;Y)snt1}] were sterile. The presence of these dominant phenotypes indicates that the \textit{stout} \textit{Df(1)HA32} and \textit{stout} \textit{Df(1)H-pc} alleles are either neomorphic or antimorphic. Another extant allele of \textit{stout}, \textit{l(1)6Ee}, does not exhibit these dominant phenotypes, nor does this mutant allele exhibit an interaction with Dfd in our assay.

\textit{extradenticle}: The \textit{exd} \textit{Df(1)HA32} allele is embryonic-larval lethal (~40\% die as embryos), while \textit{exd} nulls are embryonic lethal (Table 2; Wieschaus et al. 1984; Peifer and Wieschaus 1990). In addition, homeotic transformations of abdominal and thoracic cuticle (Wieschaus et
notypes in all segments. These variable phenotypes in-illary identity.

Animals that died as embryos showed inconsistent phe-ntial transformation of the mandibular lobe toward max-

These data are consistent with a par-
exdS136

et al.

exd

lethal phase and milder cuticular phenotype indicates indirectly caused by a loss of

and not described in detail. Hemizygous

Peifer

1986). topically activated, and

Figure 6.—Dominant adult phenotype of stout(313). Arrows point to the aristae, which are enlarged in stout heterozygotes.

al. 1984; Peifer and Wieschaus 1990) were absent in exd513 animals at 25° and only slightly apparent at 29° (Figure 4B, white and black arrowheads). The different lethal phase and milder cuticular phenotype indicates that exd513 is hypomorphic and slightly temperature sen-
sitive. The exd cuticular phenotype in the trunk has been described previously (Peifer and Wieschaus 1990), but the zygotic mutant head phenotype has been only noted and not described in detail. Hemizygous exd cuticles showed perturbations in structures derived from all head segments, except the antennal and hypopharyn-
geal lobes. All cuticular structures derived from the max-

Other loci: The novel WC1 and UC119 alleles displayed larval and embryonic-larval lethality, respectively. Animals that died as embryos showed inconsistent phe-

inappropriately absent (Figure 7A); DII expression was unaffected in rnc mutants (data not shown). These data are consistent with a partial transformation of the mandibular lobe toward max-

Although it was not obvious from the cuticular pheno-
type, the altered expression patterns of Dfd and cnc suggested that the loss of rnc function may result in a partial homeotic transformation of cells in the mandibular segment to maxillary identity. If this hypothesis is correct, maxillary-specific target genes of Dfd should be inappropriately regulated in the mandibular lobe of rnc mutants. We examined the expression of the Dfd targets prd, Ser, and DII in rnc mutants. In most rnc mutant embryos, two of these genes were expressed in maxillary-like patterns in the mandibular segment: prd being ectopically activated, and Ser inappropriately absent (Figure 7A); DII expression was unaffected in rnc mutants (data not shown). These data are consistent with a partial transformation of the mandibular lobe toward max-

As the rnc cuticular phenotype also had similarities
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to the lab phenotype, we examined the lab transcription pattern in rnc mutants. Early phases of lab expression were normal in the rnc mutant embryos (stage 10, Figure 7B). However, by stage 13/14, lab transcript abundance in the intercalary and tritocerebral primordia of the procephalic region was weaker or absent in rnc mutants than in wild-type embryos (Figure 7B). The pattern and amounts of lab transcript in the midgut of rnc mutants was comparable to wild type (Diederich et al. 1989; Figure 7B). However, after stage 15, the second midgut chamber, as marked by lab expression, was smaller and of variable diameter along its length, whereas the other chambers appeared relatively normal (Figure 7B). Therefore, even though both procephalic and midgut lab-expressing tissues are disrupted in rnc mutants, lab expression is affected only in the procephalic region.

DISCUSSION

This is the final paper in a series uncovering genetic requirements for the function of the homeotic gene Dfd (see Figure 2 for a summary). We have isolated mutations in seven loci on the X chromosome that significantly reduced the viability of Dfd hypomorphs. The Notch, exd, and nej genes have been characterized previously, while stout and rnc are either novel or uncharacterized. Many Dfd-interacting alleles we have isolated are either hypomorphic or antimorphic and have stronger interactions with Dfd than nulls. This indicates that the interactions between Dfd and other genes may not always be caused by reduced gene dosage, but by an antagonistic function of the mutant protein on wild-type activity, perhaps by interfering with particular subfunctions of interacting factors. Therefore, molecular characterization of antimorphic or hypomorphic proteins may provide insight as to how the respective wild-type proteins modify Dfd (or Hox) function.

Embryonic lethal Deformed interactors: Mutations in a gene crucial for embryonic Dfd function would be expected to cause defects in or loss of Dfd-dependent cuticular structures, as is seen in Notch, exd, stout, and rnc mutants. As mutations for the other genes identified in our screen do not appreciably affect the formation of the gnathal cuticle, the functions of these genes might be supplied maternally or affect the development of Dfd-dependent tissues not visible in cuticular preparations (e.g., tentorium and subesophageal ganglion). Alternatively, some may interact with Dfd only during post-embryonic development. These results are similar to those from the second and third chromosome screens, where zygotic mutants for one-third of the loci did not exhibit defects in Dfd-dependent embryonic cuticular structures.

Transcription: Only 18 of the 27 genes identified in all three Dfd interactor screens have been sequenced, and their encoded products have been published. Of these 18, 16 may be grouped according to their roles
in either cell-cell signaling (Notch, Collagen type IV, viking, devenir, Vacuolar H^+-ATPase 55-kD B subunit, Laminin A, Serrate, and hedgehog) or transcription (exd, nej, Edysone Receptor, cap’n’collar, apolitc, Polyclom, trithorax, and cubitus interruptus). Because the Dfd protein is a transcription factor, it is not unexpected to have isolated a large number of genes involved in transcription. One of these, exd, is required for many homeotic functions (Peifer and Wieschaus 1990; Gonzalez-Crespo and Morata 1995; Rauskolb et al. 1995; Azpiazu and Morata 1998). Physical interactions between Dfd and most of the genetically interacting transcription factors have not yet been investigated. However, direct interactions have been discovered between Exd and Hox proteins, including Dfd and Dfd homologs (Lu et al. 1995; Phelan et al. 1995; Chan et al. 1997). In vitro, the formation of Hox/Exd heterodimers generates a DNA-binding surface that interacts with a greater number of bases than Hox monomers, resulting in an increase in DNA-binding specificity (Chan et al. 1994; van Dijk and Murre 1994; Chang et al. 1995; Johnson et al. 1995; Kouzarides and Kamps 1995; van Dijk et al. 1995; Chan et al. 1997). From the ~2000 lethal X chromosomes screened, we isolated only one exd allele—the hypomorphic exd(1)16. The interaction strengths of neither exd(1) nor exd vary substantially when placed on different X chromosomes (data not shown), indicating that the interaction with Dfd is not dependent on genetic background. It is not clear why exd(1)16 shows a stronger Dfd interaction than exd nulls. However, as exd(1)16 zygotic mutants often have normal head structures, it is possible the exd(1)16 protein preferentially disrupts Dfd postembryonic activity or functions not apparent in cuticle preparations.

The other X-chromosome Dfd interactor known to participate in transcription in nej (Akimaru et al. 1997), which encodes a Drosophila member of the mammalian CBP/p300 family (Lundblad et al. 1995). These proteins contain an inherent acetyltransferase activity and act as transcriptional adaptor molecules (Bannister and Kouzarides 1996; Gryzko et al. 1996; Gu and Roeder 1997). A plethora of transcription factors physically interact with CBP/p300 proteins, which may act as a bridge to the basal transcriptional machinery (Kwock et al. 1994; Nakajima et al. 1997) or possibly alter chromatin structure (Bannister and Kouzarides 1996; Gryzko et al. 1996) or transcription factor activity (Gu and Roeder 1997) by acetylation. Preliminary experiments have failed to demonstrate an in vitro interaction between either murine CBP and Exd, or CBP and Dfd (B. Florence and W. McGinnis, unpublished data). However, two Dfd-interacting genes encode homologs of mammalian factors that physically contact CBP or p300. The cnc gene encodes a homolog of the p45 subunit of NF-E2 (Cheng et al. 1997), and Edysone Receptor (Ecr) encodes a nuclear hormone receptor (e.g., Kamei et al. 1996). In addition, nej interacts genetically and functionally with cnc (Akimaru et al. 1997), which is downstream of another Dfd interactor, hedgehog. The cnc gene is on the fourth chromosome and, therefore, was not a target of our screens. However, the cnc(1) allele shows a moderate interaction with Dfd in our assay (M. Martin and W. McGinnis, unpublished data). The likely physical interaction of Ecr, Cnc, and Ci proteins with the Nej CBP-like protein raises the possibility that the genetic interaction observed between Dfd and nej is indirect, which is consistent with our preliminary in vitro binding results; i.e., insufficient levels of Nej might simultaneously lower the activity of Ci, Cnc, and Ecr, resulting in a synergism that further lowers Dfd hypomorphic function.

Cell-cell signaling: Nearly half of the molecularly characterized loci from all three Dfd interactor screens (see Figure 2) have functions in cell-cell signaling. Four of the Dfd-interacting loci encode transmembrane receptors (devenir and Notch) or extracellular ligands (hedgehog and Serrate). Three other loci—Laminin A, Collagen type IV, and viking—encode components of the basal lamina that also provide signals that modulate cell differentiation and gene transcription (reviewed in Lafrenie and Yamada 1996). The Vh55 gene codes for the vacuolar H^+-ATPase 55-kD B subunit (Davies et al. 1996), which is likely to be important for the recycling of receptor molecules to the cell surface (Johnson et al. 1993). It is also possible that the calcium channel encoded by Ca-a1D could have a direct effect on Dfd function, as Ca^2+ flux has been shown recently to have a potent effect on the function of some transcriptional activators (Dometsch et al. 1998; Li et al. 1998). Taken together, the isolation of these loci as Dfd interactors indicates that events at the cell membrane can have critical, dosage-sensitive effects on Dfd (and Hox) function in the nucleus.

The interaction of Hox proteins with the transcriptional effectors of signaling cascades has been noted previously. For example, Ubx modulates the ability of Wingless and Dpp signals, allowing appropriate activation of Dll expression in the leg primordia (Cohen et al. 1993; Castelli-Gair and Akam 1995). In other examples, Dpp signaling in the midgut requires lab protein function to refine and amplify the lab autoactivation circuit (Grieder et al. 1997), and signaling by Wingless in the midgut requires abd-A function to activate transcription of the pointed gene (Bilder et al. 1998). In a similar vein, it is possible that the functions of Dfd and of its signaling interactors converge on a subset of targets so that both Dfd and the transcriptional effectors of the signals are required for proper gene expression.

The Notch gene is expressed in every cell (Fehon et al. 1991; Koo et al. 1993) and has a role in many developmental processes: it is required to determine the fate of neurons (reviewed in Artavanis-Tsakonas et al. 1995), muscle cells (Baker and Schubiger 1996; Ruiz-Gomez and Bate 1997), oocytes (Ruo-hola et al. 1991; Cummings and Cronmiller 1994), and probably
molecular functions, are not known. However, the mutant phenotype for another \textit{Dfd} interactor, Serate (E. Wiellet et al. and W. McGinnis, unpublished data), which encodes a Notch ligand. Serate null mutants have reduced mouth hooks (Speicher et al. 1994), which are \textit{Dfd}-dependent structures, suggesting that Notch signaling is required for at least one epidermal \textit{Dfd} function.

The \textit{Notch} alleles that interact with \textit{Dfd} are hypomorphic, with the strongest effect on viability (\textit{I}$_5$) shown by the allele with the least severe cuticular phenotype. The reason for this paradox is unclear, as is the reason why 9 of the 12 \textit{Notch} alleles we tested failed to interact with \textit{Dfd}. One explanation could be that the \textit{Dfd}-interacting hyporphoms have levels of active Notch protein within a critical range required to observe an effect. Alternatively, the aberrations in \textit{Dfd}-interacting Notch proteins could preferentially affect a \textit{Dfd}-(or \textit{Hox}-) specific subfunction.

Notably, no obvious intermediates (e.g., kinases) between transmembrane receptors and the nucleus were isolated in our screens although others have been successful in identifying such factors (e.g., Dickson et al. 1996; Karim et al. 1996). These factors often participate in multiple signaling pathways. For example, protein kinase A transmits signals from Gurken (Lane and Kalderon 1994) and Hedgehog (Jiang and Struhl 1995; Ohl and Kalderon 1997), while Ras functions in the EGF, JAK, IL-IFN, FGF, and integrin pathways (reviewed in Clark and Brugge 1995; Wassarman et al. 1995; Moule and Denton 1997). Mutations in these genes would affect many pathways, some of which may have opposing effects on \textit{Dfd} function, and so a genetic interaction would be difficult to detect. In addition, some individual cell-cell signaling receptors use multiple intracellular signaling cascades, as has been described for integrins (Parson 1996), the FGF receptor (Gisselbrecht et al. 1996; Kanai et al. 1997), and Notch (Axelrod et al. 1996). A defect in any single branch of signal transmission might be insufficient for an observable affect on \textit{Dfd} function.

Hierarchical relationships of unclassified loci: The \textit{X} chromosomes are not known. However, the morphological and molecular nature of their phenotypes gives some indication of their roles in specification by \textit{Dfd}. Mutants for \textit{rcn} show alterations in \textit{Dfd} expression (see below), while \textit{stout} mutants show no detectable alteration in \textit{Dfd} expression. In addition, extant alleles of \textit{stout} do not have a maternal effect (Perrimon et al. 1989), indicating that stout functions in parallel with or downstream of \textit{Dfd}. Placement of \textit{rcn} function upstream of \textit{Dfd} and \textit{stout} downstream from or in parallel to \textit{Dfd} is evidence for their distinct relationships with \textit{Dfd}.

\textbf{Hierarchical relationships of unclassified loci:} The products encoded by \textit{stout} and \textit{rcn}, and therefore their molecular functions, are not known. However, the morphological and molecular nature of their phenotypes gives some indication of their roles in specification by \textit{Dfd}. Mutants for \textit{rcn} show alterations in \textit{Dfd} expression (see below), while \textit{stout} mutants show no detectable alteration in \textit{Dfd} expression. In addition, extant alleles of \textit{stout} do not have a maternal effect (Perrimon et al. 1989), indicating that stout functions in parallel with or downstream of \textit{Dfd}. Placement of \textit{rcn} function upstream of \textit{Dfd} and \textit{stout} downstream from or in parallel to \textit{Dfd} is evidence for their distinct relationships with \textit{Dfd}.

\textbf{Strong out:} Although the major defects in \textit{stout} mutants are in \textit{Dfd}-independent dorsal structures, these cuticles also have reduced mouth hooks and a malformed H-piece lateral bar, which are \textit{Dfd} dependent. In addition, \textit{stout} mutants have reduced third thoracic denticle belts, indicating a possible role in thoracic \textit{Hox} function as well. A similar phenotype is also seen in \textit{EcR} mutants (Bender et al. 1997), suggesting a relationship between \textit{Hox}, \textit{stout}, and \textit{EcR} in cephalic homeotic regulation and head development. A defect in any single branch of signal transmission might be insufficient for an observable affect on \textit{Dfd} function.

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involution, resulting in the maxillary and antennal lobes being more posterior and lateral. The head involution defect is distinct from and more severe than that seen in Dfd mutants, but it is similar to that observed in mutants for another homeotic gene, lab (Merrill et al. 1989). In addition, rnc and lab mutations disrupt some of the same cuticular structures (the hypostomal sclerite and H-piece), suggesting related biological functions for rnc and lab. Mutants for rnc have lower levels of lab transcripts in the intercalary region of the head. The timing of this requirement for rnc coincides with embryonic stages during which Lab autoactivates its own transcription unit (Chouinard and Kaufman 1991), suggesting that rnc activity is required for lab autoregulation (Figure 8). In the second midgut chamber, lab expression is unaffected by the absence of rnc, but the tissue itself has an altered morphology. Unlike autoregulation of lab in the head, midgut autoregulation of lab is dependent on Dpp signaling (Chouinard and Kaufman 1991; Tremml and Bienz 1992; Grieder et al. 1997), indicating that Dpp signaling may substitute for rnc activity in lab midgut regulation.

**Interactions with other homeotics:** The molecular and phenotypic analyses of our mutants suggest that they play a role in the function of homeotic genes other than Dfd. We tested this possibility by looking at their genetic interactions with Ubx. Unlike the interaction with Dfd, the Ubx test will detect interactions in only a small subset of Ubx-expressing cells (the haltere margin) during a very narrow period of time [early third instar (Kaufman et al. 1973; Sanchez-Herrero and Morata 1983)]. Ubx activity is required to repress the expression of scute (sc), which is necessary for bristle formation on the wing margin (e.g., Neumann and Cohen 1996). Conversely, Notch is required (indirectly) for sc activation (Neumann and Cohen 1996). Therefore, the simplest explanation for the suppression of the Ubx haltere phenotype in Notch mutants is that lowered Notch signaling normalizes relative levels of Ubx and Notch function, resulting in a wild-type haltere.

The enhancement of the Ubx phenotype by rnc mutations suggests that the Nej protein might be involved in either the activation of Ubx transcription or the repression of sc transcription. The role of CBP/p300 family members as transcriptional activators (reviewed in Goldman et al. 1997) would favor the former, if CBP is acting directly. However, our nej alleles also suppress the Notch nicked-wing phenotype (data not shown), suggesting that the Nej protein is indirectly involved in the suppression of sc. Further molecular analyses will be required before any conclusions can be drawn.

The remaining mutant alleles from our screen did not have a significant effect on Ubx function in the haltere. For exd, this is consistent with earlier work that showed that clones of exd in the haltere pouch have no effect on Ubx expression (Azpiazu and Morata 1998) or function (Gonzalez-Crespo and Morata 1995; Rauskow et al. 1995). Both trx and Pc also failed to modify the Ubx phenotype even though they are known to regulate the expression and function of Ubx and other homeotics (Morata and Kerridge 1981; Ingham 1983; Capdevila et al. 1986; Wedeen et al. 1986; Busturia and Morata 1988; Smolik-Utlaat 1990; Mckenzie and Brock 1991; Breen and Harte 1993; Sedkov et al. 1994). Therefore, the Ubx-haltere interaction test appears to reveal only some of the genes required for homeotic function. Indeed, although neither rnc nor stout showed an interaction with Ubx, the data discussed above demonstrate that rnc regulates homeotic genes other than Dfd, while the stout phenotype strongly suggests it interacts with other thoracic homeotic genes.
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