A Screen for Modifiers of *Deformed* Function in Drosophila

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ABSTRACT

Proteins produced by the homeotic genes of the Hox family assign different identities to cells on the anterior/posterior axis. Relatively little is known about the signalling pathways that modulate their activities or the factors with which they interact to assign specific segmental identities. To identify genes that might encode such functions, we performed a screen for second site mutations that reduce the viability of animals carrying hypomorphic mutant alleles of the Drosophila homeotic locus, *Deformed*. Genes mapping to six complementation groups on the third chromosome were isolated as modifiers of *Deformed* function. Products of two of these genes, *sallimus* and *moira*, have been previously proposed as homeotic activators since they suppress the dominant adult phenotype of *Polycomb* mutants. Mutations in *hedgehog*, which encodes secreted signalling proteins, were also isolated as *Deformed* loss-of-function enhancers. *Hedgehog* mutant alleles also suppress the *Polycomb* phenotype. Mutations were also isolated in a few genes that interact with *Deformed* but not with *Polycomb*, indicating that the screen identified genes that are not general homeotic activators. Two of these genes, *cap 'n' collar* and *defaced*, have defects in embryonic head development that are similar to defects seen in loss of function *Deformed* mutants.

HOMEOTIC proteins encoded in the Antennapedia and bithorax complexes of Drosophila, like their Hox homologues in other animals, determine anterior-posterior identities in developing the embryonic body plan (GARCÍA-BELLIDO 1977; LEWIS 1978; KAUFMAN *et al.* 1980, 1990; BENDER *et al.* 1983; MCGINNIS and KRUMLAUF 1992). Homeotic proteins are transcription factors that bind DNA through their 60 amino acid homeodomain regions (SCOTT *et al.* 1989; AFFOLTER *et al.* 1990; HAYASHI and SCOTT 1990; LAUGHON 1991; DESSAIN and MCGINNIS 1993); their abilities to instruct cells to develop as head, thorax, or abdomen are apparently mediated by the differential regulation of diverse sets of target genes (GARCÍA-BELLIDO 1977; BOTAS 1993). Little is known about the underlying mechanisms responsible for differential target selection, but the modest differences in *in vitro* DNA binding specificity between different homeodomains do not appear to determine target selection *in vivo* (HAYASHI and SCOTT 1990).

Homeotic proteins are required in a diverse set of cell types at successive stages of development; these proteins must therefore be able to coordinate their anterior/posterior identity function with many different cellular regulatory pathways. The requirement to function in many developmental environments makes it seem likely that a variety of different cofactors contribute both to the activity and to the specificity of homeotic proteins in embryos, and some evidence exists to support this view. One breakthrough has come from the characterization of a gene called *extradenticle* (*exd*), which seems to modulate homeotic protein specificity in a dose-dependent manner (PEIFER and WIESCHAUS 1990). Recent results have shown that *exd* encodes a homeodomain protein that enhances the DNA binding affinity of some, but not all, homeotic proteins (RAUSKOLB *et al.* 1993; CHAN *et al.* 1994; VAN DIJK and MURRE 1994).

Previous genetic screens for factors required for homeotic gene function have identified genes that can be divided into two broad classes: the trithorax group (LEWIS 1968; CAPDEVILA and GARCÍA-BELLIDO 1981; INGHAM 1981, 1983; SHEARN *et al.* 1987; KENNISON and TAMKUN 1988; SHEARN 1989; TAMKUN *et al.* 1992; FARKAS *et al.* 1994) and the Polycomb group (LEWIS 1978; STRUHL 1981, 1983; DUNCAN and LEWIS 1982; DUNCAN 1982; SATO *et al.* 1983; INGHAM 1984; DURA *et al.* 1985; JURGENS 1985; JONES and GELBART 1990; PHILLIPS and SHEARN 1990). Polycomb group functions are required to maintain the normal boundaries of homeotic gene expression by repressing homeotic gene transcription in regions outside these boundaries (STRUHL and AKAM 1985; WEDEEN *et al.* 1986; KUZIORA and MCGINNIS 1988b; MCKEON and BROCK 1991; SIMON *et al.* 1992). Few trithorax group functions are well understood, but *trithorax* (*trx*) and *brahma* (*brm*) activities are required to maintain wild-type levels of homeotic gene expression (TAMKUN *et al.* 1992; BREEN and HARTE 1993; SEDKOV *et al.* 1994). The repressive function of the Polycomb group proteins is believed to be exerted by their participation in large multimeric complexes that alter chro-

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matin structure (PARO 1990; PARO and HOGNESS 1991; FRANKE *et al.* 1992; MARTIN and ADLER 1993; RASTELLI *et al.* 1993). Trithorax group proteins, often defined by their ability to suppress the effects of *Polycomb (Pc)* mutations (SHEARN *et al.* 1987; KENNISON 1993), are a heterogeneous group. Some promote transcription of homeotic genes by contributing to a multimeric complex that could antagonize the repressive effects of Polycomb group proteins (TAMKUN *et al.* 1992; JONES and GELBART 1993). Other trithorax group genes such as *Trl* (encoding GAGA factor) (BIGGIN and TJIAN 1988; FARKAS *et al.* 1994) probably act more directly to influence homeotic gene function, while the function of most others is not understood. To generalize the current understanding of their function, the trithorax and Polycomb group proteins are required to maintain the wild-type levels and boundaries of homeotic selector gene expression.

We were interested in using a genetic screen to identify factors required for the function of the homeotic gene *Deformed* (*Dfd*), especially those influencing *Dfd* functional specificity. Such factors are expected to include members of the trithorax/Polycomb groups, which are required for homeotic expression and may be involved in functional specificity. Such a screen could also identify other factors acting in parallel and/or more specifically with *Dfd* as well as those that act downstream of *Dfd* in head developmental pathways. In contrast to the Drosophila homeotic genes controlling abdominal development, *Dfd* has a relatively weak requirement for *trithorax* (*trx*) activity for maintenance of its transcription. *Dfd* relies on an autoregulation circuit that involves DFD protein persistently activating the transcription of its own gene in the embryonic CNS and epidermis through upstream and intronic enhancers (KUZIORA and MCGINNIS 1988a; BERGSON and MCGINNIS 1990; MCGINNIS *et al.* 1990; REGULSKI *et al.* 1991; ZENG *et al.* 1994).

Dfd is required for the specification of posterior head segment identity. *Dfd* null mutants die at the end of embryogenesis due to the loss of structures derived from the maxillary and mandibular segments, dorsal ridge and optic lobe (MERRILL *et al.* 1987; REGULSKI *et al.* 1987). Ectopic *Dfd* expression in embryos induces the development of maxillary cuticular structures in the labial and thoracic segments (KUZIORA and MCGINNIS 1988a). Animals carrying partial loss-of-function *Dfd* alleles can survive to adulthood but often lack maxillary palps, have malformed rostral membranes, and show apparent transformations of dorsal head to dorsal thorax (MERRILL *et al.* 1987). Some of the mouse homologues of *Dfd* have been shown to be required for the normal development of structures in the cervical region of the embryos (RAMÍREZ-SOLÍS *et al.* 1993; KOSTIC and CAPECCHI 1994) and sufficient to induce cervical-like vertebral structures in more anterior regions of the head (LUFKIN *et al.* 1992).

To identify genes in the *Dfd*-dependent posterior head specification pathway, we screened for mutations that, when heterozygous, cause a striking reduction in the viability of adults carrying two hypomorphic *Dfd* alleles. Analogous genetic screens for haplo-insufficient enhancers have provided insight into signal transduction pathways used in Drosophila development (GERTLER *et al.* 1989; SIMON *et al.* 1991; DOYLE and BISHOP 1992; DICKSON and HAFEN 1993; RAFTERY *et al.* 1995). In this paper we report the *Dfd* enhancers we have identified in a screen of third chromosome mutations. More than 12,000 mutagenized chromosomes were tested for *Dfd* modifiers, and 49 chromosomes were found to carry mutations that enhance the lethality of *Dfd* hypomorphs. We have concentrated on the loci for which we isolated multiple alleles; these mutations correspond to six lethal complementation groups. Analysis of these groups indicates that we isolated new alleles of two trithorax group genes: *sallimus* (*sls*) and *moira* (*mor*). We also identified *hedgehog* (*hh*) as a gene critical for *Dfd* function; surprisingly, *hh* mutations also satisfy the criteria for inclusion in the trithorax group. Three alleles of a gene recently shown to modulate *Dfd* function, *cap 'n' collar* (*cnc*), were isolated, as were two alleles of a novel gene, *defaced* (*dfc*), that might function as a *Dfd* coactivator. The phenotypes and behavior of the mutations recovered in the screen suggest that a variety of mechanisms contribute to *Dfd* activity and targeting specificity in developing Drosophila.

MATERIALS AND METHODS

Stocks: Flies were reared on standard yeast-agar-cornmeal-molasses medium at 25°. In all experimental crosses, care was taken not to overcrowd the vials and food was periodically supplemented with dry yeast; unless otherwise indicated, experimental crosses were performed at 25°. All mutations, aberrations and abbreviations are described either in LINDSLEY and ZIMM (1992) or as indicated. hh^2 was obtained from the Bowling Green stock center. Pc^3 and Pc^4 were provided by J. TAMKUN. trx^3, *sls*, mor^2 and $l(3)87Ca^{12}$ were provided by J. KENNISON. cnc^{VL110} is a *P*-induced null *cnc* allele and was provided by J. MOHLER. *Ki* p^p and a set of third chromosome deficiencies (Df(3)kit) were obtained from the Bloomington stock center. *rucuca* is the designation for a multiply marked chromosome bearing the markers *ru h th st cu sr* e^s *ca* and was provided by E. WIESCHAUS. *ve st ca* flies were obtained by outcrossing a *sc f; al b sp; ve st ca* stock provided by the Bowling Green stock center.

Two *Dfd* alleles in several different genetic backgrounds were used in this study. Dfd^{rV8} (LINDSLEY and ZIMM, Dfd^3) is a weak hypomorphic allele and Dfd^{rC11} (LINDSLEY and ZIMM, Dfd^{13}) is a temperature sensitive allele (MERRILL *et al.* 1987). Dfd^{rV8} *red e*, Dfd^{rC11}*red e* and the parental *red e* chromosome were provided by T. KAUFMAN. The *Ki* Dfd^{rV8} *red* chromosome used throughout the screen was constructed from isogenic Dfd^{rV8} *red e* and *Ki* p^p lines. Two separate *Ki* Dfd^{rV8} *red* lines were tested for viability over an isogenized Dfd^{rC11}*red e* chromosome at 25, 27 and 29.2°, and the one with the higher viability was used in the screen.

To identify the mutations resulting from the screen as enhancers of the *Dfd* phenotype, it was necessary to test their

interaction with *Dfd* in another genetic background (see below). The *Dfd* chromosomes used in these tests were made as follows. The *ve st* Dfd^{rC11} p^{p} *ca* chromosome was obtained by first making a Dfd^{rC11} p^{p} chromosome from Dfd^{rC11} *red e* and *Ki* p^{p} and then recombining Dfd^{rC11} p^{p} onto an isogenized *ve st ca* chromosome. The *ve st* Dfd^{rC11} p^{p} *ca* chromosome is homozygous viable at 25°. The Dfd^{rV8} p^{p} chromosome was constructed from isogenic Dfd^{rV8} *red e* and *Ki* p^{p} lines; it is homozygous viable.

Sequencing of mutant alleles: DNA was isolated from homozygous *Ki* Dfd^{rV8} *red* larvae, homozygous *ve st* Dfd^{rC11} p^{p} *ca* adults, or control adults (*red e*). The DNA sequence of all five exons from the *Dfd* loci on these chromosomes was determined by asymmetric PCR amplification and chain termination sequencing, as described in detail in ZENG *et al.* 1994.

The enhancer screen: We wished to isolate mutations in genes whose products are required for the wild-type function of *Dfd*. To do this, we performed an F2 screen for second site mutations that, when heterozygous, dramatically decrease the viability of *Ki* Dfd^{rV8} *red*/Dfd^{rC11} *red e* individuals. The screen is diagrammed in Figure 1. *Ki* Dfd^{rV8} *red*/*TM3Sb* males were mutagenized with EMS as described in GRIGLIATTI (1986). The mutagenized males were mass mated to *TM1*/*TM3Ser* virgin females and removed after 3 days. The females were transferred to fresh vials and allowed to lay eggs for 2–3 more days before being discarded. *TM1* is lethal in combination with *TM3Sb*, so all surviving F1 progeny carried the mutagenized *Ki* Dfd^{rV8} *red* chromosome (* *Ki* Dfd^{rV8} *red*, where * denotes a mutation of potential interest) and were balanced with either *TM1* or *TM3Ser* (*TM*).

**Ki* Dfd^{rV8} *red*/*TM* males (or virgin females) were individually crossed to Dfd^{rC11} *red e*/*TM* virgin females (or males), left at room temperature for 4–6 hr and then placed at 29.2°. Four to six females were used for each F1 male and three males for each F1 female. Parents were removed from the vials after 4–5 days. Vials were scored daily on the 11th–14th days after mating for the presence of **Ki* Dfd^{rV8} *red*/Dfd^{rC11} *red e* adult progeny. **Ki* Dfd^{rV8} *red* lines giving zero to two **Ki* Dfd^{rV8} *red*/Dfd^{rC11} *red e* adults per vial were recovered as **Ki* Dfd^{rV8} *red*/*TM* males and immediately rescreened. Sibling **Ki* Dfd^{rV8} *red*/*TM* males were outcrossed to *TM1*/*TM3Ser* and the progeny was used for the final rescreen. In this second rescreen, **Ki* Dfd^{rV8} *red*/*TM1* males were crossed to Dfd^{rC11} *red e*/*TM1* virgin females and 100–300 progeny counted per cross. Lines where **Ki* Dfd^{rV8} *red*/Dfd^{rC11} *red e* showed a viability ≤33% that of *Ki* Dfd^{rV8} *red*/Dfd^{rC11} *red e* at 29.2° were kept for further study.

Calculation of *Dfd* interaction strength: The degree of interaction with *Dfd* for a given **Ki* Dfd^{rV8} *red* chromosome was determined by expressing the viability of **Ki* Dfd^{rV8} *red*/Dfd^{rC11} *red e* as a fraction of the viability of *Ki* Dfd^{rV8} *red*/Dfd^{rC11} *red e* at 29.2°. **Ki* Dfd^{rV8} *red* chromosomes were placed into different categories of interaction strength based on their relative viability: strong (relative viability = 0–0.11), moderate (relative viability = 0.12–0.33) or weak (relative viability >0.33).

The degree of *Dfd* interaction for $*^{E}$ Dfd^{rC11} p^{p} lines (where $*^{E}$ represents an apparent *Dfd* enhancer, see below) was calculated as the viability of $*^{E}$ Dfd^{rC11} p^{p}/*Ki* Dfd^{rV8} *red* relative to that of *ve st* Dfd^{rC11} p^{p} *ca*/*Ki* Dfd^{rV8} *red* at 29.2°.

The degree of *Dfd* interaction for hh^{G50} was calculated as the viability of *st* Dfd^{rC11} p^{p} hh^{G50}/Dfd^{rV8} p^{p} relative to that of *ve st* Dfd^{rC11} p^{p} *ca*/Dfd^{rV8} p^{p}.

Identification of mutations at known loci: We used two methods to test whether the mutants isolated from our screen corresponded to previously identified loci: embryonic cuticular phenotype and lethal complementation behavior.

We analyzed the homozygous cuticular phenotype of each **Ki* Dfd^{rV8} *red* line (see below); for this analysis, the balancer chromosome was not removed. If the **Ki* Dfd^{rV8}*red*/**Ki* Dfd^{rV8} *red* phenotype resembled that of a previously described mutation, we tested the **Ki* Dfd^{rV8} *red* line for allelism with that locus. **Ki* Dfd^{rV8} *red* lines with a *hh* or *Polycomb* phenotype were tested against hh^{2} or Pc^{3}.

Previous screens for suppressors of *Pc* resulted in the identification of a number of loci required for homeotic gene function (KENNISON and RUSSELL 1987; KENNISON and TAMKUN 1988). To see whether we had isolated mutations at any of these loci, each **Ki* Dfd^{rV8} *red* line not yet assigned to a complementation group was tested for lethal complementation behavior against brm^{5}, $ash1^{6}$, mor^{2}, osa^{1}, trx^{3}, kto^{3}, $l(3)87Ca^{12}$, $ash2^{2}$, dev^{2}, *sls*, skd^{2}, urd^{2} and vtd^{3} (all provided by J. KENNISON).

Separation of mutations from *Ki* Dfd^{rV8} *red*: The mutations isolated in the screen had to be separated from the parental *Ki* Dfd^{rV8} *red* background before further analysis was possible. To do this, it was necessary not only to remove the mutation from the **Ki* Dfd^{rV8} *red* chromosome but also to show that the mutation in question was responsible for the observed interaction with *Dfd*. We performed two series of crosses for each relevant **Ki* Dfd^{rV8} *red* line. In the first series, we recombined apparent *Dfd* enhancers ($*^{E}$) off **Ki* Dfd^{rV8} *red* onto a Dfd^{rC11} chromosome and tested each for the ability to enhance the Dfd^{rV8}/Dfd^{rC11} phenotype. Those having an effect similar to that seen with the original **Ki* Dfd^{rV8} *red* line were balanced and retained as $*^{E}$ Dfd^{rC11} p^{p} stocks. In the second series of crosses, we recombined lethal mutations ($*^{L}$) off **Ki* Dfd^{rV8} *red* onto a Dfd^{+} chromosome ($*^{L}$+). We then tested the $*^{L}$+ lines for failure to complement the $*^{E}$ Dfd^{rC11} p^{p} lines. This procedure is outlined in Figure 2.

Next, we wanted to determine whether a given lethal mutation was, in fact, at a locus required for *Dfd* function. We reasoned that the product of a given locus was likely to be required for *Dfd* function if we had isolated multiple alleles of that locus from our screen and if at least one of these alleles failed to complement the appropriate $*^{E}$ Dfd^{rC11} p^{p} line. To identify such loci, one or more $*^{LE}$+ lines derived from a given **Ki* Dfd^{rV8} *red* chromosome were tested for lethal complementation behavior with all the **Ki* Dfd^{rV8} *red* lines not yet assigned to complementation groups. While performing these tests, we found that a fraction ($^{31}/_{80}$) of the **Ki* Dfd^{rV8} *red* lines carried a lethal mutation in one of three complementation groups. After separation from the **Ki* Dfd^{rV8} *red* chromosome, mutations in any of these three groups showed no significant enhancement of the Dfd^{rV8}/Dfd^{rC11} phenotype. These lines were set aside and not further considered.

Cytogenetic map positions: Mutations corresponding to multiallelic complementation groups were meiotically mapped using the multiply marked *rucuca* chromosome. Once a meiotic map position had been determined, each complementation group was tested for lethality against deficiencies in the relevant region of the chromosome.

Preparation of embryonic cuticles: Unless otherwise indicated, balancer chromosomes were removed by outcrossing mutant stocks. Embryos were collected for 4–6 hr then aged for 24 hr at 25°, harvested, dechorionated in 100% Clorox and placed into 4 ml 1× PBS/4 ml heptane. Hatched larvae sank to the bottom and were removed with the aqueous phase. The aqueous phase was replaced with 4 ml methanol, and embryos were devitillenized by shaking vigorously for 1 min. Devitillenized embryos were placed into microfuge tubes and rinsed three times with methanol and twice with water. Cuticles were fixed in 1:4 glycerol:acetic acid for 1 hr at 65° then overnight at room temperature. Embryos were then mounted in 1:2 lactic acid:Hoyer's medium (WIESCHAUS and NÜSSLEIN-VOLHARD 1986) and allowed to clear at 65° overnight. Prepa-

rations were flattened by placing a 35-g weight on the coverslip for 24–48 hr.

The embryonic cuticular phenotypes presented here are in a Dfd^+ background; mutations were removed from the parental $*Ki\ Dfd^{rV8}\ red$ chromosome as described above. The embryos shown for *sls*, *mor* and *hdln* were obtained by crossing flies heterozygous for the relevant mutant allele to flies heterozygous for the appropriate deficiency. hh^{5A7} embryos were obtained from the cross $shd^2\ hh^{5A7}/+\ +\ \times\ shd^2\ hh^{5A7}/+\ +$; the embryo shown is presumably of the genotype $hh^{5A7}/shd^2\ hh^{5A7}$. *dfc* mutant embryos were obtained from the cross $dfc^{5A54}/+\ \times\ dfc^{C1}/+$; in this case, embryos were collected and aged at 29.2°. *cnc* mutant embryos were obtained from three different crosses: $cnc^{VL110}/TM6B\ \times\ cnc^{VL110}/TM6B$, $C7/+\ \times\ 2E16/+$ and $2E16/TM1$ x $cnc^{VL110}/TM6B$.

Interaction with *Pc*: Previous screens for genes required for homeotic gene function resulted in the isolation of mutations that have been assigned to two broad groups: the *trithorax* group and the *Polycomb* group (KENNISON and TAMKUN 1988). Mutants in the *trx* group suppress the T2 and T3 to T1 leg transformation of $Pc/+$ males; mutants in the *Polycomb* group enhance this phenotype. To see whether any of the loci identified in our screen belong to either of these two groups, we tested them for interaction with Pc^4.

$*Ki\ Dfd^{rV8}\ red/TM1$, $sls/TM6B$, $trx^3/TM3$, $mor^1/TM6C$, $mor^2/TM6C$, $cnc^{VL110}/TM6B$, $Ki\ Dfd^{rV8}\ red/TM1$, $Ki\ Dfd^{rV8}\ red/TM3$ and wild-type males were mated to $Pc^4/TM3\ Sb$ females and cultured at 29.2°. T2 legs were removed from $*/Pc^4$ males and mounted directly in 1:1 lactic acid:Hoyer's medium. The preparations were cleared by overnight incubation at 65°, and the number of sex-combs per leg counted at 150× magnification.

RESULTS

The *Dfd* mutant background: In our genetic screen for *Dfd* modifiers, we tested for an enhancement of the reduced viability of *Dfd* partial loss-of-function alleles. The rationale is to have *Dfd* function at such a low level that the elimination of one of the two doses of gene product from a modifying locus is sufficient to result in lethality. We used two *Dfd* hypomorphic alleles in our screen: Dfd^{rV8} and Dfd^{rC11}. *rV8* is a weak hypomorph and *rC11* is a temperature-sensitive allele (MERRILL *et al.* 1987). Dfd^{rV8} has a G to A transition at position 3 of the first codon, mutating the codon for the initiating methionine; presumably translation is initiated at the next available methionine codon (wild-type codon 6). It is worth noting that the five N-terminal residues eliminated from the presumptive *rV8* protein are evolutionarily conserved between Drosophila and vertebrate DFD-like proteins (REGULSKI *et al.* 1987). *rC11* has a T to A transversion at codon 49 of the homeo domain, resulting in the substitution of isoleucine for phenylalanine. This change results in a smaller hydrophobic group in the core of the homeodomain, which is likely to disrupt its stability and lead to the heat sensitivity of *rC11* homozygotes. Both Dfd^{rV8} and Dfd^{rC11} homozygous embryos accumulate *Dfd* transcripts and protein in the normal maxillary/mandibular pattern, though the levels of protein expression appear to be lower and more variable than in wild-type embryos (data not shown).

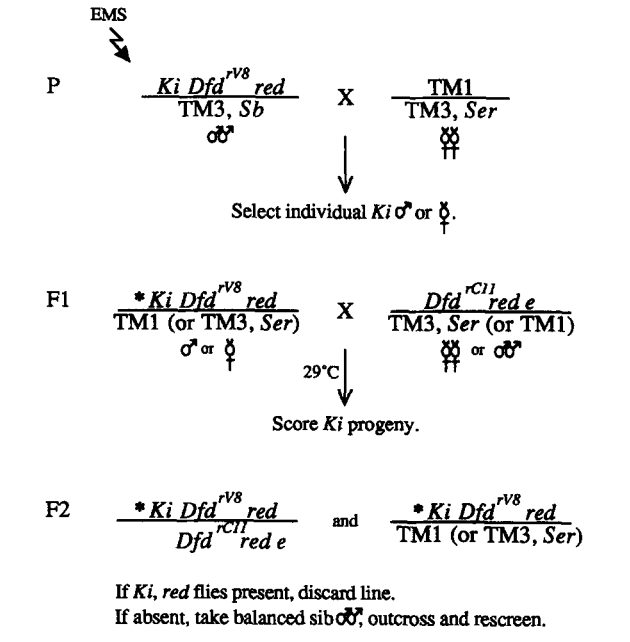

FIGURE 1.—Screen to isolate genes required for Dfd^+ function. Mutagenized $Ki\ Dfd^{rV8}\ red$ chromosomes were screened for lethality in *trans* with $Dfd^{rC11}\ red\ e$ at 29.2°. Dfd^{rV8}/Dfd^{rC11} adults were recognized in the F2 by the presence of *Kinked* bristles and *red* eyes. Mutant lines producing few or no Dfd^{rV8}/Dfd^{rC11} adults in the F2 were recovered as balanced stocks, outcrossed and rescreened. See MATERIALS AND METHODS for details.

F2 screen to identify loci required for Dfd^+ function: At 29.2°, $Ki\ Dfd^{rV8}\ red/Dfd^{rC11}\ red\ e$ adults show an intermediate *Dfd* hypomorphic adult phenotype and have a viability of ~50% that expected. Using this background, we screened for mutations that further decrease the viability of the *Dfd* mutants to near zero (Figure 1). We tested 12,263 mutagenized F1 chromosomes ($*Ki\ Dfd^{rV8}\ red$, where * represents a mutation of potential interest) for a significant reduction in viability when in *trans* with $Dfd^{rC11}\ red\ e$. A total of 49 $*Ki\ Dfd^{rV8}\ red$ lines showed a significant interaction with the $Dfd^{rC11}\ red\ e$ chromosome and were selected for further study.

The strength of interaction with *Dfd* for each $*Ki\ Dfd^{rV8}\ red$ chromosome was determined and is summarized in Table 1. Thirty-three were classified as strong enhancers, 11 as moderate and five as weak. The weak interactions are associated with a strong decrease in the viability of both $*Ki\ Dfd^{rV8}\ red/Dfd^{rC11}\ red\ e$ and $*Ki\ Dfd^{rV8}\ red/TM1$.

Assignment to lethal complementation groups: We were able to assign 15 mutant alleles to seven previously identified loci. Five $*Ki\ Dfd^{rV8}\ red$ lines had embryonic cuticular phenotypes resembling those of known mutants and were tested for allelism with the appropriate stocks (see MATERIALS AND METHODS). In this way, we identified four *hh* alleles and one *Pc* allele. The remaining 44 $*Ki\ Dfd^{rV8}\ red$ lines were tested for lethal complementation with mutations in 13 *trithorax* group loci (see MATERIALS AND METHODS). We found that we

TABLE 1

Loci potentially required for *Dfd* function

Strength of *Dfd* interaction	Locus	No. of alleles	Cytology (map position)
Strong (0–0.11)	*sallimus*	5	62B8-F2[a]
	headline	4	64C-65C
	cap 'n' collar	3	94E[b]
	defaced	2	(3–4.0)
	trithorax	1	88A12-B5[c]
	Polycomb	1	78D7-8[d]
	l(3)87Ca	1	87C[a]
	Unassigned	16	—
Moderate (0.12–0.33)	*hedgehog*	4	94D10-E5[e]
	moira	2	89A11-B4[a]
	Unassigned	5	—
Weak (>0.33)	*devenir*	1	70C2-D3[a]
	Unassigned	4	—

Strength of *Dfd* interaction was calculated as described in the text. Loci listed as unassigned have not yet been assigned to complementation groups. Meiotic map positions are contained within parentheses.

[a] KENNISON and TAMKUN (1988).
[b] MOHLER et al. (1991).
[c] MOZER and DAWID (1989).
[d] PARO et al. (1984).
[e] JURGENS et al. (1984).

had isolated five new *sallimus* (*sls*) alleles, two *moira* (*mor*) alleles and one allele each of *trx, devenir* (*dev*) and *l(3)87Ca*. Four of our **Ki Dfd^{rV8} red* lines failed to complement *vtd*, and two of these four also failed to complement *brm*. However, as the lethal complementation behavior with *vtd* and *brm* could not be separated from the **Ki Dfd^{rV8} red* chromosome, we were unable to unambiguously assign them to a complementation group.

To assign the remaining 34 mutations to lethal complementation groups, we had to separate at least some of them from *Dfd^{rV8}* (see MATERIALS AND METHODS). *^{LE}+ lines were generated from 16 of the 34 as yet unassigned **Ki Dfd^{rV8} red* chromosomes and tested for lethal complementation against each of those same 34 **Ki Dfd^{rV8} red* lines. We found three additional lethal complementation groups: two corresponding to previously unidentified loci: *headline* (*hdln*, four alleles), *defaced* (*dfc*, two alleles), and three alleles of *cnc*. Eleven of the *^{LE}+ lines complemented all the **Ki Dfd^{rV8} red* stocks tested and were set aside.

Determination of cytogenetic location: At this point, we focused on the lethal complementation groups for which we had isolated more than one allele. The cytological locations of *mor* and *hh* had previously been determined (KENNISON and TAMKUN 1988; MOHLER 1988). The genetic positions of the remaining complementation groups were mapped by meiotic recombination, using *rucuca*. Cytogenetic map locations were then determined by testing each group for lethal complementation with appropriate deficiencies. The map positions of these complementation groups are shown in Table 1. For *dfc*, only the meiotic map position was determined. The location of *2E16* group alleles suggested this group might correspond to the previously identified *cap 'n' collar* locus. Two of the three alleles were tested for complementation with *cnc^{VL110}*; both failed to complement *cnc* lethality and so this group was provisionally assigned to the *cnc* locus (however, see below).

Embryonic cuticular phenotypes: *Dfd^{+}* function is required during embryogenesis for the development of structures arising from the maxillary and mandibular segments, the dorsal ridge, and the optic lobe (MERRILL *et al.* 1987; REGULSKI *et al.* 1987). These include cuticular structures such as the cirri, mouth hooks, ectostomal sclerites, H-piece (all principally derived from the maxillary segment), and the lateralgräten (principally derived from the mandibular segment). To see whether any of the mutations isolated from our screen cause disruptions in these *Dfd*-dependent structures, we examined the cuticular phenotypes of mutant embryos. The phenotypes fall into two general classes: those with morphological defects in every segment, *e.g., hh*, and those with head specific defects, *e.g., cnc* and *dfc*. The mutant phenotypes are summarized in Table 2.

As has been described previously, *hh* is a segment polarity gene (NUSSLEIN-VOLHARD and WIESCHAUS 1980; MOHLER 1988). Early in the genesis of segments, secreted HH protein signals adjacent cells to maintain *wingless* transcription (LEE *et al.* 1992; FORBES 1993; SIEGFRIED *et al.* 1994). At later stages in dorsal cells, the *hh* signal appears to work over many cell diameters to induce differential cellular identities in the anterior region of thoracic and abdominal segments (HEEMSKERK and DINARDO 1994). *hh* null mutant embryos develop with an unsegmented lawn of denticles and lack most head structures (NÜSSLEIN-VOLHARD and WIESCHAUS 1980) (Figure 3).

Embryos mutant for the *headline* (*hdln*) gene show defects both in the gut and head. Portions of the gut of these mutants appear more sclerotized than wild type; in particular, the morphology of the proventriculus is abnormal. In cuticular preparations of wild-type embryos, the proventriculus can usually be distinguished as an inverted T shape at the posterior end of the esophagus. In *hdln* mutants, the proventriculus lacks a well-defined shape, often appearing crumpled or diffuse (Figure 3, B and E). In addition to the gut defects, the heads of *hdln* mutants have a conspicuous fold in the maxillary cuticle that is usually located between the antennal and maxillary sense organs (Figure 4B).

sls mutant embryos develop a structure that resembles an ectopic median tooth in place of the ectostomal sclerites (Figure 4C). This interpretation is based on the shape of the abnormal sclerotized structure and its nonrefractile gray color when viewed under phase-contrast

TABLE 2

Phenotypes of mutations in multiallelic complementation groups

Mutant	Phenotype	*Pc* interaction			
		TS_{mean}	TS_{range}	No. of alleles tested	Suppression or enhancement
sallimus (sls)	Ectopic median tooth	0.45	0.04–0.82	6	Moderate suppressor
headline (hdln)	Proventriculus and gut abnormal; extra fold in head	See text	See text	4	See text
hedgehog (hh)	Segment polarity gene; denticle lawn	0.43	0.06–0.83	5	Moderate suppressor
cap 'n' collar (cnc)	Ectopic mouth hooks and cirri; labral structures unaffected	0.95	0.85–1.1	3	No effect
moira (mor)	T rib and hypopharyngeal defects	0.03	0.01–0.05	4	Strong suppressor
defaced (dfc)	Mouth hooks reduced; lateralgräten short and thick; dorsal bridge abnormal	0.74	0.63–0.84	2	No effect

Phenotype refers to the embryonic cuticular phenotype. *Pc* interaction was calculated as described in the text. TS_{mean} is the average transformation strength of all the alleles tested. TS_{range} is the range of transformation strengths observed for the alleles tested. A low TS value indicates a strong transformation. In the case of *sls, hh, cnc* and *mor,* previously isolated alleles were tested for *Pc* interaction as well as the alleles isolated from our screen. At least 40 legs were scored for each genotype.

optics. However, it is also possible that this median tooth-like structure results from the fusion of enlarged and malformed ectostomal sclerites in *sls* mutants. The ectostomal sclerites are missing in *Dfd* null mutants.

mor and *defaced* (*dfc*) mutant embryos show defects similar to those seen in *Dfd* hypomorphs. In both *mor* and Dfd^{rC11} mutant embryos, the lateralgräten are truncated and the T-ribs of the pharynx appear to be split medially (Figure 4D); in addition, *mor* mutant embryos display defects in the hypopharyngeal region (not shown). *dfc* mutant embryos have a phenotype that closely resembles that of more severely disrupted Dfd^{rC11} embryos. Both *dfc* and Dfd^{rC11} mutants have reduced mouth-hooks and truncated lateralgräten; in both cases, the dorsal bridge is displaced anteriorly so that it is adjacent to the base of the median tooth (compare Figure 4, E and F). The frontal sac is often sclerotized, accounting for the extension of the dorsal bridge. The *dfc* phenotype is temperature sensitive and somewhat variable; some mutant embryos die before secreting cuticle, others show more general patterning defects (not shown). However, *dfc* mutants that develop a properly segmented cuticle consistently display the phenotype described above.

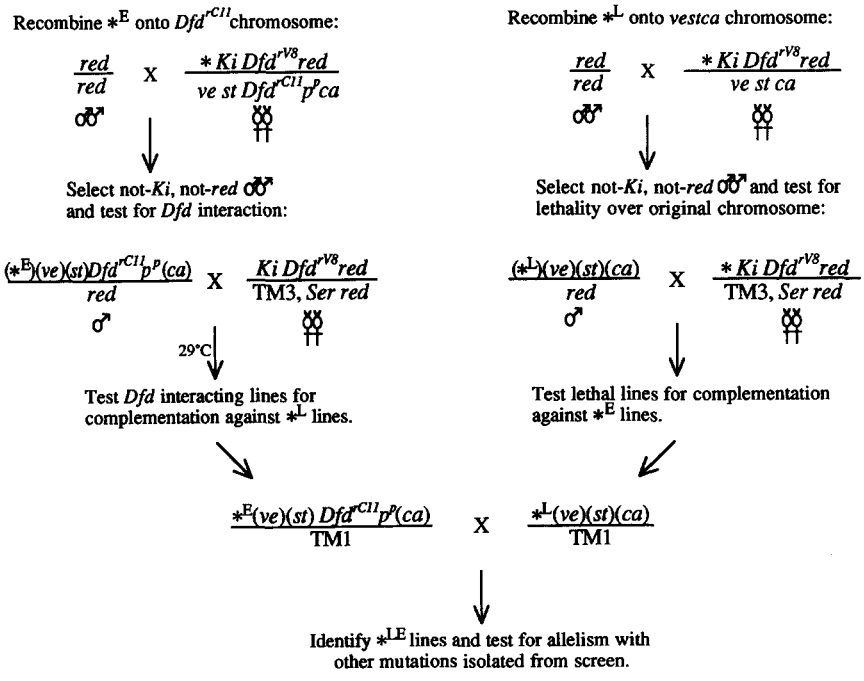

FIGURE 2.—Separation of *Dfd* enhancers from the Dfd^{rV8} mutant background. The scheme to recover *Dfd* enhancers ($*^{E}$) from the mutagenized chromosomes is shown on the left side of the figure; the scheme to recover lethal mutations ($*^{L}$) is shown on the right. Parentheses indicate that the gene in question is present in either wild-type or mutant copy. Potential *Dfd* enhancers were identified by recombining regions of the original $*Ki\ Dfd^{rV8}\ red$ chromosome onto a Dfd^{rC11} chromosome and screening for reduction of *Dfd* function. Lethal mutations were recombined away from Dfd^{rV8} into a Dfd^{+} background. Any $*^{L}$ line carrying a single lethal mutation and failing to complement the corresponding $*^{E}$ lines was considered likely to contain a *Dfd* enhancer in a Dfd^{+} background ($*^{LE}$). $*^{LE}$ lines were tested for lethal complementation against the other $*Ki\ Dfd^{rV8}\ red$ lines isolated in the screen.

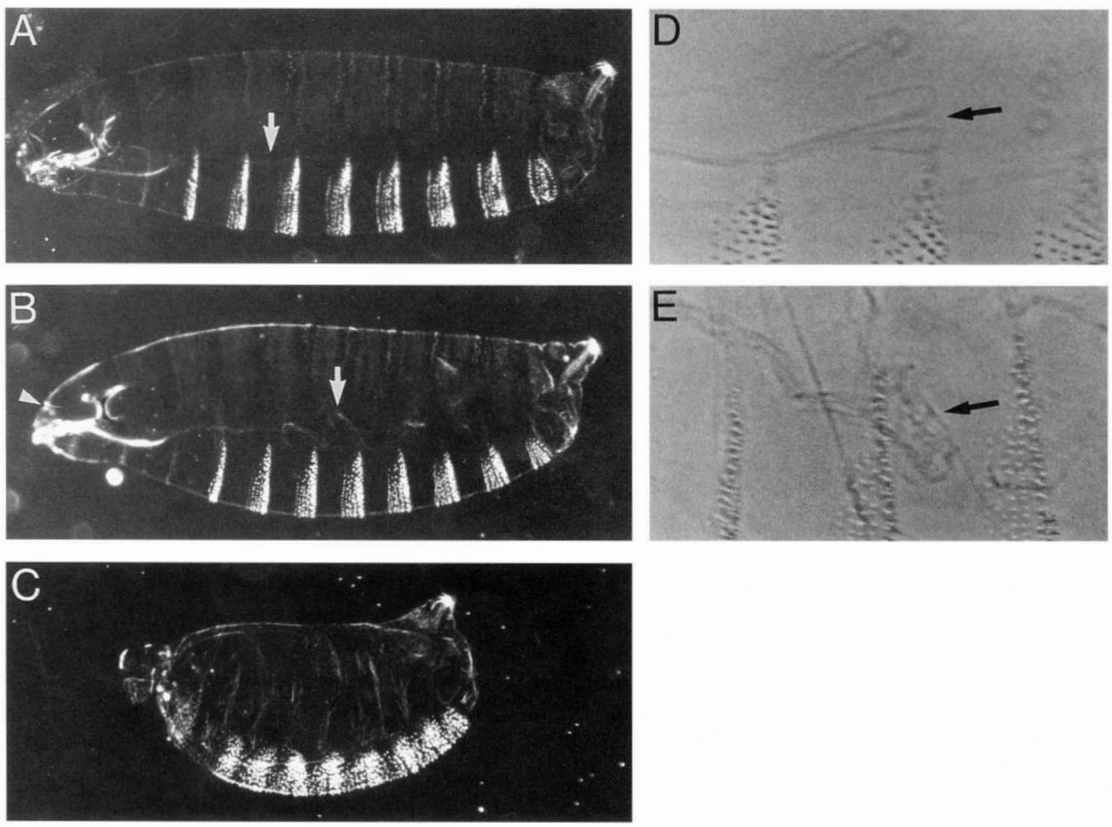

FIGURE 3.—Embryonic cuticular phenotypes of *hdln* and *hh*. Embryos are oriented with anterior to the left and dorsal up. A–C are darkfield photomicrographs of whole embryos. D and E are phase contrast photomicrographs of the proventriculus. (A) Wild type. Eight abdominal and three thoracic segments are clearly visible. Arrow indicates esophagus; the gut is not visible in wild type. (B) $hdln^{6A1}$/Df(3L)*zn-47*. The gut is oversclerotized (arrow) and there is an extra fold in the head (arrowhead). (C) hh^{5A7}/hh^{5A7}. Embryo is short and the ventral denticles are fused to make a continuous lawn. (D) Proventriculus of wild-type embryo (arrow); it has the characteristic inverted T shape. (E) Proventriculus of $hdln^{4A8}$/Df(3L)*zn-47* embryo (arrow); it is disordered and crumpled.

In contrast to *mor* and *dfc*, which are both required for the formation of *Dfd*-dependent structures, two putative mutant alleles of *cnc* (*2E16* and *C7*) result in the loss of some *Dfd*-dependent structures and the ectopic production of others. *cnc* null mutants lack structures derived from the labral segment and show a mandibular to maxillary homeotic transformation (MOHLER *et al.* 1995). The most obvious features of the *cnc* null phenotype are the absence of the median tooth and dorsal bridge, the presence of ectopic mouth-hooks and cirri in the mandibular segment, and the truncation of the lateralgräten (MOHLER *et al.* 1995; Figure 5B). The presence of ectopic maxillary structures in more anterior regions is consistent with an increase in *Dfd* activity, while the truncation of the lateralgräten suggests a decrease.

The phenotype of the *2E16* and *C7* mutant alleles, in either homozygous or *trans*-heterozygous condition, resembles that of *cnc* null mutants. *2E16* and *C7* mutant embryos, like cnc^{VL110}, display ectopic mouth hooks and cirri in the mandibular region, indicating a mandibular to maxillary transformation (Figure 5). However, in contrast to *cnc* nulls, the median tooth, dorsal bridge and other more anteriorly derived structures appear to be unaffected in *2E16* and *C7* mutants. The most likely explanation for the difference in phenotype is that *2E16* and *C7* are hypomorphic alleles of *cnc*. If so, the phenotype of *2E16*/ cnc^{VL110} is expected to be more severe than that of either *2E16* homozygotes or *2E16/C7*. It is not. *2E16*/cnc^{VL110} embryos have a phenotype nearly identical to that of *2E16/2E16* or *2E16/C7* embryos (compare Figures 5C and 6D); the median tooth and dorsal bridge are nearly normal, but the mandibular region has been transformed towards a maxillary identity. Despite the differences in their loss of function phenotypes, we have provisionally classified the *2E16* group as *cnc* based on meiotic map position and failure to complement cnc^{VL110}.

Interaction with Pc^4: Previous genetic analyses have resulted in the identification of two opposing classes of mutations that affect homeotic gene function: the *Polycomb* group and the *trithorax* group (LEWIS 1978; DUNCAN and LEWIS 1982; INGHAM 1983; SATO *et al.* 1983; JURGENS 1985; KENNISON and TAMKUN 1988; KENNISON 1993). *Polycomb* group mutants enhance the *Pc* mutant phenotype, while *trithorax* group mutants suppress it. Complementation analysis indicated we had isolated new alleles for several *trithorax* group genes and

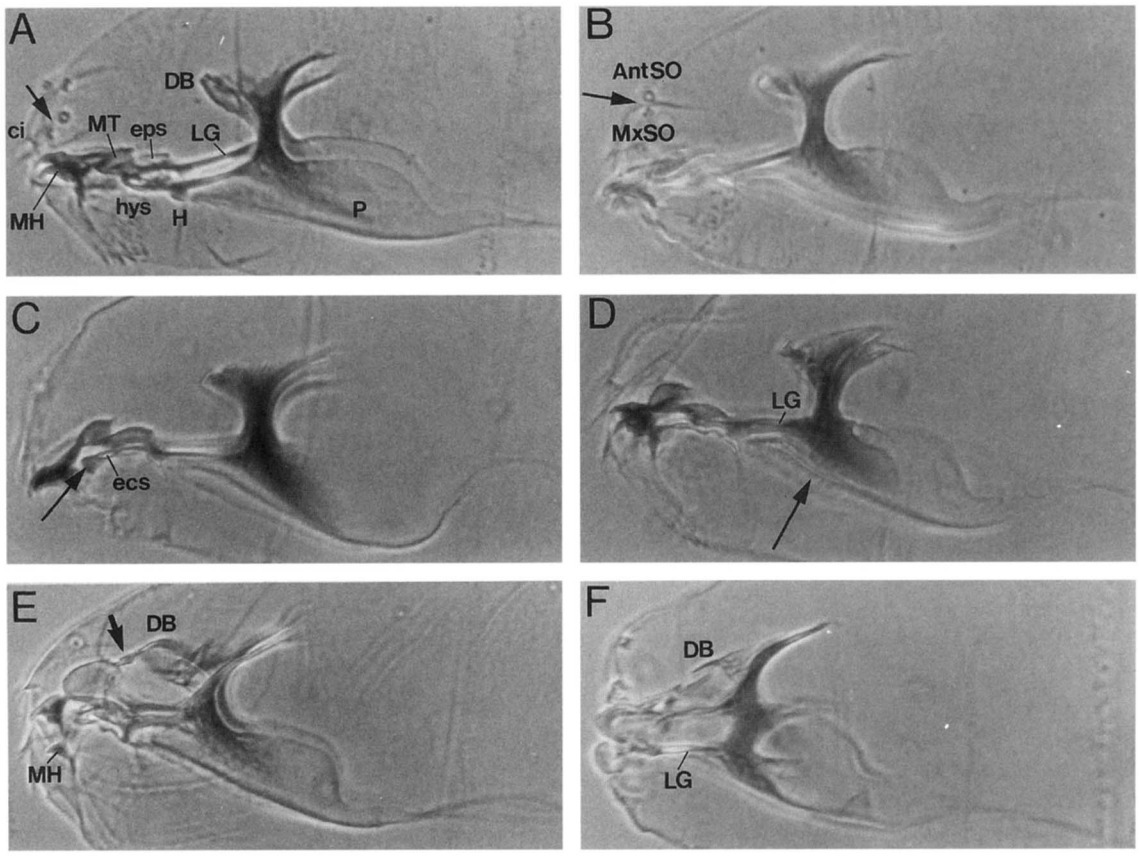

FIGURE 4.—Embryonic cuticular preparations of wild-type and mutant heads. Heads are oriented with anterior to the left and dorsal up. (A) Wild type. Arrow indicates wild-type region between the antennal and maxillary sense organs that develops a extra fold in *headline* mutants. (B) *hdln*^{6A1}/Df(3L)*zn-47*. Arrow indicates extra fold. (C) *sls*^{C138}/Df(3L)*RG-7*. Arrow indicates ectopic median tooth. (D) *mor*^{4A3}/Df(3R)*sbd*. Lateralgräten are truncated; arrow indicates split in T-ribs. (E) *dfc*^{C1}/*dfc*^{5A54}. Mouthhooks are reduced and the lateralgräten truncated; the frontal sac (arrow) appears sclerotized and the dorsal bridge is anteriorly displaced. This phenotype resembles that of *Dfd* mutants. (F) *Dfd*^{rC11}/*Dfd*^{rC11} raised at 29°. Mouth hooks are absent, the lateralgräten truncated and the dorsal bridge displaced. Compare with E. Abbreviations: AntSO, antennal sense organ; ci, cirri; DB, dorsal bridge; ecs, ectostomal sclerites; eps, epistomal sclerite; H, H-piece; hys, hypostomal sclerites; LG, lateralgräten; MH, mouth hook; MT, median tooth; P, pharynx.

one new allele of *Pc*, so we wished to test if any of the other mutants identified in our screen also affect *Pc* function.

Pc^{4} heterozygotes show a partial transformation of T2 and T3 legs toward T1 (HANNAH-ALAVA 1958; DUNCAN 1982). This transformation is easily detected in males by the presence of ectopic sex-combs on the T2 and T3 legs. Since sex-combs are normally found only on the T1 legs, the number of ectopic sex-combs per leg is a rough measure of the degree of transformation.

To determine whether a given locus belonged to the *Polycomb* group, the *trithorax* group or to neither, we first calculated the mean number of sex-combs per T2 leg of **Ki Dfd*^{rV8} *red/ Pc*^{4} males for each allele of the multiallelic complementation groups. To find the relative strength of the T2 to T1 transformation, we then divided this number by the mean number of sex-combs per T2 leg of the appropriate control to give a value called the transformation strength, TS. We assessed the *Pc* interaction of a given complementation group by determining the mean (TS_{mean}) and the range (TS_{range}) of the transformation strengths for all the alleles tested. A TS value of 1.0 indicates no suppression, and a TS value of 0 indicates complete suppression of the *Pc* phenotype. We also tested previously isolated alleles of *sls, mor, hh* and *cnc*. These results are summarized in Table 2.

Mutant alleles for three of the loci identified in our screen clearly suppress *Pc*. *mor* acts as a strong suppressor (TS_{mean} = 0.03, TS_{range} = 0.01–0.05), while the effect of *sls* is more moderate (TS_{mean} = 0.45, TS_{range} = 0.04–0.82). To our surprise, *hh* mutations also acted as moderate supressors of *Pc* (TS_{mean} = 0.43, TS_{range} = 0.06–0.83). The *hdln* group could not be classified: two alleles (*4A8* and *3A1*) had no effect on the *Pc* phenotype while two (*6A1* and *6A52*) appeared to strongly suppress it. Mutations in *dfc* and *cnc* had no discernable effect on *Pc*.

DISCUSSION

To identify *trans*-acting factors responsible for conferring functional specificity onto the *Dfd* protein, we per-

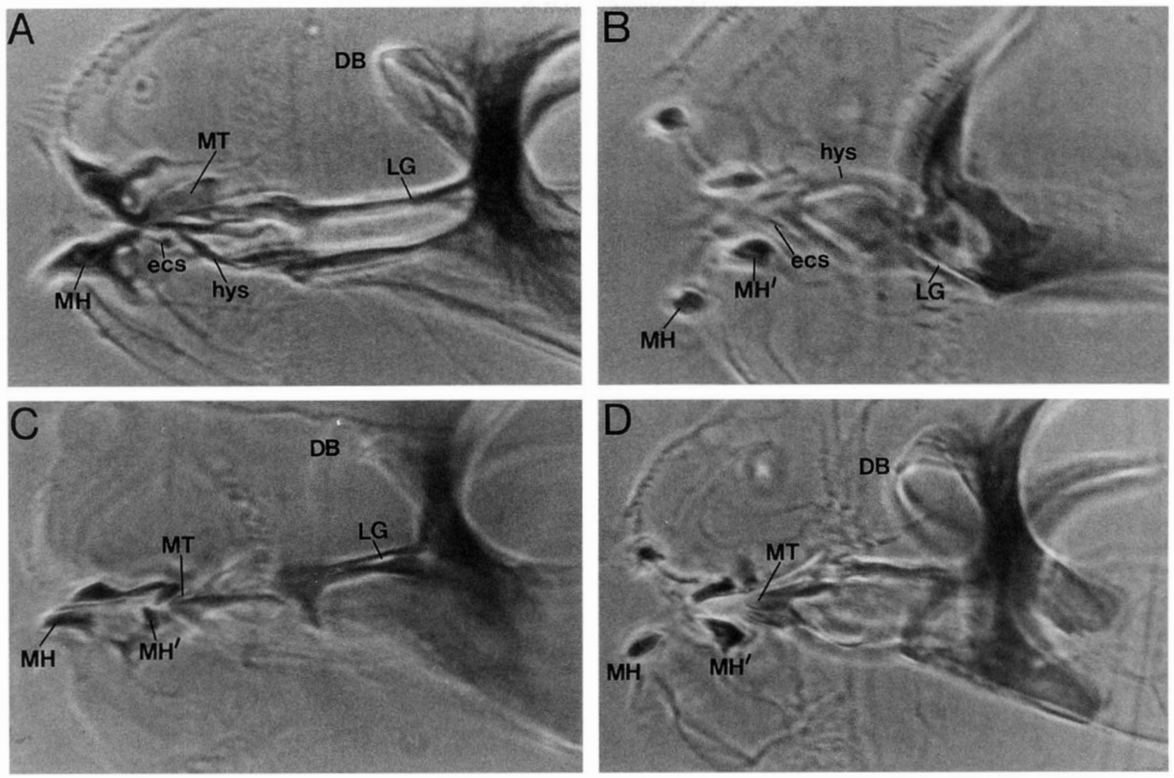

FIGURE 5.—*cap 'n' collar* mutant phenotype. Panels are high magnification phase contrast photomicrographs of the head structures of wild-type and *cnc* mutant embryos. Embryos are oriented with anterior to the left and dorsal up. (A) Wild type. (B) cnc^{VL110}/cnc^{VL110}. A pair of ectopic mouth-hooks (MH′) can be seen in the mandibular region; the lateralgräten are severely reduced. Note the absence of the median tooth and dorsal bridge. (C) cnc^{2E16}/cnc^{C7}. Ectopic mouth-hooks are detected in the mandibular region and the lateralgräten appear truncated. Both the dorsal bridge and median tooth appear relatively unaffected. (D) cnc^{2E16}/cnc^{VL110}. The phenotype of this embryo is nearly identical to that of the one shown in C. Abbreviations: DB, dorsal bridge; ecs, ectostomal sclerites; hys, hypostomal sclerites; LG, lateralgräten; MH, mouth hook; MT, median tooth.

formed a genetic screen to isolate mutations in genes required for Dfd^{+} function. Such genes could affect *Dfd* function at several levels. Upstream genes control *Dfd* activity by regulating the pattern and/or levels of *Dfd* expression. Genes acting in parallel in an additive or synergistic manner might influence *Dfd* target choice and/or the level of activity of DFD on posterior head regulatory elements in a variety of ways. This could be done by transient or stable binding to DFD protein itself (*e.g.,* kinases or coactivators), by the activation of signalling pathways that modify the activity of DFD cofactors, or by regulating target accessibility (*e.g.,* repressors). Mutations might also be isolated in target genes that are responsible for carrying out the program specified by *Dfd* (*e.g., Distal-less, paired*) (E. O'HARA and W. MCGINNIS, unpublished results). These three levels are not mutually exclusive; for example, a *Dfd* target could also be a *Dfd* activator, resulting in a positive feedback loop.

Mutations in genes that interact with *Dfd* are expected to result in the disruption of *Dfd*-dependent structures in the posterior head. In the embryo, these structures include the mouth-hooks, cirri, H-piece, ectostomal sclerites and lateralgräten (MERRILL *et al.* 1987; REGULSKI *et al.* 1987). Most of the mutations isolated in our screen are required for the normal development of these cuticular structures. *dfc, sls, mor* and *cnc* zygotic mutant embryos display relatively specific defects in posterior head structures, whereas mutations in *hh* cause more general defects. In addition, we obtained one allele of *trx,* which has been shown to be required for wild-type levels of *Dfd* expression in the embryo (BREEN and HARTE 1993). The embryonic phenotype of at least two $*Ki\ Dfd^{rV8}\ red$ lines strongly suggests that, as expected, we also obtained mutant alleles in *Dfd* itself (data not shown). At this level of analysis it is not possible to distinguish between upstream, sidestream, or downstream genes; however, we have isolated mutations in a known upstream gene required for maintenance of *Dfd* expression levels (*trx*), and a known gene acting in parallel with *Dfd* (*cnc*).

***Trithorax* group genes that interact with *Dfd*:** The loci identified in our screen can be divided into two types of *Dfd* modifiers: those that interact with other *HOM* genes and those that are more specifically required for *Dfd.* We have isolated new alleles of several *trithorax* group genes and one allele of *Pc;* however, our screen appears to be biased toward a subset of *trithorax* group genes that are different from those isolated in previous screens.

Genetic screens designed to isolate mutations in either *Polycomb* or *trithorax* group genes have principally relied on the enhancement or suppression of either the extra sex-combs or antenna to leg phenotype of adult *Pc* heterozygotes (DUNCAN 1982; SATO *et al.* 1983; KENNISON and RUSSELL 1987; KENNISON and TAMKUN 1988). Since these two phenotypes depend on ectopic expression of *Scr* and/or *Antp* (DUNCAN and LEWIS 1982; PATTATUCCI and KAUFMAN 1991; TAMKUN *et al.* 1992), the mutations isolated from *Pc* suppressor/enhancer screens are expected to correspond to loci required for Scr^+ and $Antp^+$ activity. In contrast our screen was designed to identify loci required for Dfd^+ activity. Based on previous results and evolutionary histories (BOTAS 1993; KENNISON 1993), the regulation and functional activity of *Antp, Scr* and *Dfd* are expected to be influenced by some shared and some different *trans*-acting factors, which is consistent with the spectrum of mutations that we isolated.

It is interesting to compare the results of the KENNISON and TAMKUN (1988) screen for enhancers/suppressors of *Pc* with the results of our screen for *Dfd* modifiers. Their screen resulted in the identification of 11 *trithorax* group genes on the third chromosome. We obtained multiple alleles for only two of these groups (*sls, mor*) and single alleles for three more (*trx, dev, l(3)87Ca*). The *trithorax* group genes identified in both screens are likely to correspond to factors commonly required for *Antp, Scr* and *Dfd* function. However, the distribution of mutations in these *trithorax* group loci was different between the two screens. For example, KENNISON and TAMKUN recovered eight EMS-induced *trx* alleles and one *sls* allele from 11,765 chromosomes, while we recovered only one *trx* allele but five *sls* alleles from 12,263 chromosomes. Additionally, we identified a *trithorax* group gene not recovered in their screen (*hh*). It is also possible that the four mutations that fail to complement *brm* and/or *vtd* correspond to other *trithorax* group loci. The apparent map position of one of these mutations (5A29) indicates that it corresponds to neither *brm* nor *vtd;* however, when in a Dfd^+ background, 5A29/brm^5 adults have held-out wings with serrations along the margins (data not shown). This phenotype suggests that 5A29 interacts with *brm* and so may correspond to a *trithorax* group gene. The map positions of the other three mutations were not determined; it is not clear whether their failure to complement *brm* and/or *vtd* is associated with their effects on *Dfd* function or is due to the presence of other lethal mutations on the chromosome.

The different sets of *trithorax* group genes recovered in the two screens may be explained by the different homeotic genes involved. It is also possible that the criteria of the *Pc* suppressor screens may have been too stringent to allow the isolation of any but strong suppressors. *sls* and *hh* both act as moderate *Pc* suppressors. In fact, the single *sls* allele isolated by KENNISON and TAMKUN acts as a strong *Pc* suppressor (TS_{mean} = 0.11) in our tests, whereas the *sls* alleles isolated in our screen show anywhere from strong to very weak suppression. A recent study (FELSENFELD and KENNISON 1995) has shown that a phenotype conferred by a dominant allele of *hh* (hh^{Mrt}) can be suppressed by mutations in some *trithorax* group genes. This observation is consistent with our identification of *hh* as a *trithorax* group gene; however, it is also possible that the ectopic expression of *hh* seen in *Mrt* mutants results from the insertion of novel *cis* regulatory sequences in the *hh* locus and that these non-*hh* sequences are regulated by the *trithorax* group.

The predominance of *sls* alleles may indicate that *Dfd* has a stronger requirement for *sls* function than do other homeotic genes; that is, a wider range of defects in *sls* function affect *Dfd* activity. This possibility is supported by the apparent maxillary (ectosomal sclerites) to labral (median tooth) transformation, in the absence of any other obvious homeotic phenotype, seen in *sls* mutant embryos. How this effect is mediated is not clear. Only two members of the *trx* group are known to act by regulating the transcription of homeotic selectors (TAMKUN *et al.* 1992; BREEN and HARTE 1993; SEDKOV *et al.* 1994), so many mechanisms are possible. Some may affect HOM protein activity by contributing to differential regulation of target genes.

It is already known that different homeotic selector genes have different requirements for *trx* function. For example, in embryos homozygous for a presumed null allele, trx^{B11}, bithorax complex gene expression is decreased as early as stage 10–11, but Antennapedia complex gene expression is unaffected until late stages of embryogenesis (stage 16–17) (BREEN and HARTE 1993; SEDKOV *et al.* 1994); however, embryos homozygous for the hypomorphic allele, trx^{E3}, display normal bithorax complex expression throughout embryogenesis but show decreased Antennapedia complex expression at stage 16–17 (SEDKOV *et al.* 1994). The requirements of HOM genes for other *trx* group genes may have similar specificities. Different hypomorphic alleles of *ash-1, ash-2* and *brm* show different homeotic transformations (SHEARN 1989; BRIZUELA *et al.* 1994; TRIPOULAS *et al.* 1994). These differences could be due either to differences in allele strengths or to different degrees of interactions with different *HOM* genes or proteins.

The mechanisms of two trithorax group proteins suggest they are involved in regulating target accessibility in chromatin. The trithorax group protein *brahma* has homology to the yeast transcriptional activator SNF2/SWI2 (TAMKUN *et al.* 1992). SNF2/SWI2 has been shown to act in a multimeric complex comprised of several proteins including SNF5, SNF6, SWI1 and SWI3 (COTE *et al.* 1994). This complex assists in the binding of transactivator proteins like GAL4 to regulatory elements (PETERSON and HERSKOWITZ 1992). The GAGA binding protein is encoded in the *Trithorax-like (Trl)*

gene (FARKAS *et al.* 1994). The GAGA factor has been shown to act as an antirepressor *in vitro* (KERRIGAN *et al.* 1991), capable of antagonizing the formation of nucleosomes in an ATP-dependent manner (TSUKIYAMA *et al.* 1994).

Although we did not isolate any lethal alleles of *brm*, we did identify one locus, *hdln*, that could correspond to a subunit of a multimeric complex. All four alleles of *hdln* fail to complement the lethality of several alleles of a gene identified in a screen for second chromosome enhancers of *Dfd* hypomorphs (G. GELLON, K. HARDING, M. MARTIN, N. MCGINNIS and W. MCGINNIS, unpublished results). Extragenic lethal noncomplementation of recessive mutations has been associated with components of multimeric complexes. For example, specific mutations in nonallelic members of the *Pc* group fail to complement one another (CHENG *et al.* 1994). Although only two of the four *hdln* alleles were capable of suppressing *Pc*, it is possible that *hdln* represents another member of the trithorax group.

In addition to the mutations in *trithorax* group genes, we also isolated one allele of *Pc*. This was unexpected since Pc^+ function represses ANT-C and BX-C gene expression; mutations in *Pc* should therefore suppress, not enhance, the *Dfd* hypomorphic phenotype. However, if other homeotic genes are weakly derepressed in *Pc* heterozygotes (DUNCAN and LEWIS 1982), then the ectopic expression of the homeodomain proteins encoded by these genes might compete with mutant DFD proteins and enhance the lethality of Dfd^{rV8}/Dfd^{rC11}.

***Dfd*-interacting genes required for posterior head development:** In addition to the *trithorax* group genes, we have isolated mutations in loci that appear to be more specifically required for *Dfd* embryonic activity. The phenotype of *dfc* mutant embryos resembles that of strong Dfd^{rC11} mutants. The mouth-hooks are reduced, the lateralgräten truncated and the dorsal bridge displaced anteriorly. The similarity between the *dfc* and *Dfd* mutant phenotypes suggests that *dfc* is required for *Dfd* activity. *dfc* mutants do not show any other obvious homeotic phenotype and do not have a detectable effect on *Pc* function. Therefore, the interaction between *Dfd* and *dfc* appears to be relatively specific. At this level of analysis it is not possible to offer a mechanistic explanation for how the *dfc* product affects *Dfd* function; however, the specific positive requirement of *Dfd* for *dfc* is what one would expect for a coactivator.

In contrast to *dfc*, *cnc* has been molecularly and genetically characterized (MOHLER *et al.* 1991, 1995). The *cnc* protein belongs to the bZIP class of transcription factors; *cnc* has been proposed both to repress *Dfd* activity in the anterior part of the mandibular segment and to act in a combinatorial fashion with *Dfd* in more posterior mandibular cells. In the absence of *cnc* function, some mandibular cells are transformed to maxillary identities, elaborating *Dfd*-dependent maxillary structures; in addition, some mandibular structures are reduced. *cnc* is also required for structures anterior to the *Dfd* domain such as the median tooth and the dorsal bridge. Two of the putative *cnc* alleles isolated from our screen, *2E16* and *C7*, appear to affect only the mandibular function of *cnc;* the third was not analyzed.

2E16 and *C7* mutant embryos show truncated lateralgräten and a strong mandibular to maxillary transformation. This latter phenotype suggests increased, rather than decreased, *Dfd* activity. Such functions would not be expected to be identified in our screen, as it was designed to isolate mutants that decrease *Dfd* activity. One possible explanation is that in *2E16* and *C7* mutants, ectopic maxillary *Dfd* function occurs at the same time as a loss of mandibular *Dfd* function. This explanation is supported by the truncation of lateralgräten seen in both Dfd^{rC11} and *2E16/C7* mutants. Thus, *2E16* and *C7*, like *cnc*, appear to be positively required for mandibular *Dfd* function but have a negative effect on maxillary *Dfd* function. However, unlike cnc^{VL110}, these mutations do not severely disrupt the more anterior *cnc* dependent structures. It seems likely that *2E16* and *C7* are hypomorphic *cnc* alleles that disrupt the *cnc* mandibular function while having little effect on the *cnc* labral function. The meiotic map position and complementation behavior support this possibility, so we have assigned the *2E16* group to the *cnc* locus.

hh is a limiting component in a pathway required for *Dfd* function: *hh* is a signalling molecule involved in the segment polarity pathway (NUSSLEIN-VOLHARD and WIESCHAUS 1980; MOHLER 1988; LEE *et al.* 1992; FORBES 1993; HEEMSKERK and DINARDO 1994; SIEGFRIED *et al.* 1994). Segment polarity genes are required to specify anterior-posterior information within each segment (NUSSLEIN-VOLHARD and WIESCHAUS 1980). This process occurs at the same time as the refinement of homeotic selector gene expression patterns. If the positional information specified by the segment polarity genes is disrupted, the homeotic selectors are unable to specify the correct cell fate. For example, if *en* is ectopically expressed in the anterior compartment of parasegment 6, *Ubx* expression is repressed and the anterior compartment cells inappropriately express the *Ubx* target gene *Dll* (MANN 1994). The concentration of *hh* protein in midstage embryos has been shown to be critical in specifying anterior-posterior information within at least the dorsal regions of segments (HEEMSKERK and DINARDO 1994). It is possible that this concentration dependence allowed us to identify *hh* as a dosage sensitive activator of *Dfd* function. However, whether *hh* patterning functions in the posterior head are similar to, or different from, *hh* functions in the trunk segments is unknown.

Conclusions: The mutations isolated from our screen suggest several ways of conferring functional activity and specificity onto *Dfd*. First, the action of segment polarity signalling pathways may limit or modify home-

otic gene expression and/or activity in specific anterior-posterior positions within a segment. The concentration of *hedgehog* may be of great importance in this manner for regulating *Dfd* function.

Second, more globally active functions from *trithorax* group genes like *sls* and *mor,* provide another pathway through which homeotic gene expression is known to be regulated and through which homeotic gene activity might be differentially modulated. Target choice might depend on appropriate combinations of HOM and trithorax group proteins. These combinations could affect *HOM* gene activity on at least three levels: (1) direct regulation of the expression of the *HOM* gene, (2) direct interaction with the HOM protein to act on a target, and (3) regulation of target accessibility independent of *HOM* gene activity. Although we have no direct evidence for such a combinatorial mechanism, the large number of *trithorax* group genes provides more than enough combinations to make it a possibility. Such a mechanism could provide target specificity simply on the basis of the domains of homeotic gene expression; the *trithorax* group genes would not have to have precisely regulated domains of expression.

Third, the gene products from non-*trithorax* group genes such as *dfc* and *cnc* appear to be crucial for *Dfd* activity in the posterior embryonic head. The phenotypes of these mutations suggest they may be somewhat specialized for modulating *Dfd* function.

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