genesis 33:67–76 (2002)

ARTICLE

DEAF-1 Function Is Essential for the Early Embryonic Development of *Drosophila*

Alexey Veraksa,[1]† James Kennison,[2] and William McGinnis[1]*

[1]Division of Biology, University of California, San Diego, La Jolla, California
[2]Laboratory of Molecular Genetics, National Institutes of Health, Bethesda, Maryland

Received 8 February 2002; Accepted 19 March 2002

Summary: The *Drosophila* protein DEAF-1 is a sequence-specific DNA binding protein that was isolated as a putative cofactor of the Hox protein Deformed (Dfd). In this study, we analyze the effects of loss or gain of DEAF-1 function on *Drosophila* development. Maternal/zygotic mutations of *DEAF-1* largely result in early embryonic arrest prior to the expression of zygotic segmentation genes, although a few embryos develop into larvae with segmentation defects of variable severity. Overexpression of DEAF-1 protein in embryos can induce defects in migration/closure of the dorsal epidermis, and overexpression in adult primordia can strongly disrupt the development of eye or wing. The DEAF-1 protein associates with many discrete sites on polytene chromosomes, suggesting that DEAF-1 is a rather general regulator of gene expression. genesis 33:67–76, 2002. © 2002 Wiley-Liss, Inc.

Key words: *Drosophila*; DEAF-1; *Deformed*; Hox; segmentation

INTRODUCTION

The DEAF-1 protein was identified as protein that bound to DNA sites containing TTCG nucleotides in an autoregulatory enhancer that is transcriptionally activated by the *Drosophila* Hox protein Deformed (Dfd) (Gross and McGinnis, 1995; Li *et al.*, 1999b; Zeng *et al.*, 1994). Initially, DEAF-1 was proposed to be a cofactor that assisted in the maxillary-specific activation of this small Dfd response element (Gross and McGinnis, 1995). However, further mutagenesis of the module E Dfd response element, as well as a Dfd response element from the *Drosophila* 1.28 gene, has indicated that the TTCG DEAF-1 binding motifs are not required for the activation of either element (Li *et al.*, 1999; Pederson *et al.*, 2000).

In *Drosophila* embryos, DEAF-1 protein is ubiquitously expressed and appears to be constitutively localized in nuclei. The DEAF-1 protein contains two conserved domains, SAND and MYND, which are present in several transcription factors. The SAND domain (for SP100, AIRE-1, NucP41/75 and DEAF-1; Gibson *et al.*, 1998) was originally named "KDWK" after a conserved core of amino acid residues in DEAF-1 and other proteins (Gross and McGinnis, 1995). NUDR (nuclear DEAF-1-related factor, also known as Suppressin or mDEAF-1) is an apparent mammalian ortholog of DEAF-1 and is the only non-*Drosophila* protein containing both the SAND and the carboxy-terminal MYND domain (Huggenvik *et al.*, 1998; LeBoeuf *et al.*, 1998; Sugihara *et al.*, 1998; Michelson *et al.*, 1999; Bottomley *et al.*, 2001). NUDR was characterized as a nuclear DNA binding protein that also preferentially binds DNA sites containing TTCG motifs, with a preference for clustered TTCG sequences. Experiments on NUDR suggest that it is required to activate the proenkephalin promoter (Huggenvik *et al.*, 1998), but in a manner that does not involve DNA binding. In contrast, NUDR protein, as well as NUDR DNA binding sites, are required for the repression of the hnRNP A2/B1 promoter in tissue culture cells (Michelson *et al.*, 1999).

Unpaired copies of the SAND and MYND domains are also found in other proteins. A divergent SAND domain is present in the SP100 family of proteins, which are localized to subnuclear structures (PML bodies), and are thought to play a role in the etiology of acute promyelocytic leukemia (reviewed in Zhong *et al.*, 2000). Different SP100 isoforms associate with chromatin components, and when bound to a promoter, behave as transcriptional activators or repressors (reviewed in Bottomley *et al.*, 2001). Other well-studied SAND domain proteins include GMEB-1 and GMEB-2 (glucocorticoid modulatory element binding factors; Oshima *et al.*, 1995; Thériault *et al.*, 1999; Zeng *et al.*, 1998). Their heterooligomeric complex was shown to bind variably spaced PuCGPy motifs. In cell transfection assays, GMEB-1 and

Grant sponsor: National Institute of Child Health/Human Development.
† Current address: MGH Cancer Center, Harvard Medical School, Charlestown, MA 02129.
* Correspondence to: William McGinnis, Division of Biology, 0349, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0349. E-mail: mcginnis@biomail.ucsd.edu

Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/gene.10090

GMEB-2 have been shown to function as transcriptional activators, repressors, or modulators (Christensen *et al.*, 1999; Jimenez-Lara *et al.*, 2000; Kaul *et al.*, 2000). The three-dimensional structure of a SAND domain has been recently determined (Bottomley *et al.*, 2001). The authors proposed that the SAND domain represents a novel DNA binding module characteristic for chromatin-dependent transcriptional regulation.

The MYND domain (myeloid, Nervy, and DEAF-1) is present in a large group of proteins that includes RP-8 (PDCD2), Nervy, and several ESTs and predicted proteins from *Drosophila*, mammals, *C. elegans*, yeast, and plants (Feinstein *et al.*, 1995; Gross and McGinnis, 1995; Owens *et al.*, 1991). The MYND domain consists of a cluster of cysteine and histidine residues, arranged with an invariant spacing to form a potential zinc-binding motif (Gross and McGinnis, 1995). Mutating conserved cysteine residues in the DEAF-1 MYND domain does not abolish DNA binding, which suggests that the MYND domain might be involved in protein-protein interactions (Gross and McGinnis, 1995). Indeed, the MYND domain of ETO/MTG8 interacts directly with the N-CoR and SMRT corepressors (Gelmetti *et al.*, 1998; Lutterbach *et al.*, 1998a, 1998b; Wang *et al.*, 1998). Aberrant recruitment of corepressor complexes and inappropriate transcriptional repression is believed to be a general mechanism of leukemogenesis caused by the t(8;21) translocations that fuse ETO with the acute myelogenous leukemia 1 (AML1) protein. Recently, ETO was shown to be a corepressor recruited by the promyelocytic leukemia zinc finger (PLZF) protein (Melnick *et al.*, 2000). A divergent MYND domain present in the adenovirus E1A binding protein BS69 was also shown to interact with N-CoR and mediate transcriptional repression (Masselink and Bernards, 2000). The current evidence suggests that the MYND motif in mammalian proteins constitutes a protein-protein interaction domain that functions as a corepressor-recruiting interface.

Despite all of the molecular and biochemical information concerning DEAF-1 and related proteins, the lack of conventional genetic studies has hampered the understanding of this family of transcription factors. In this study, we have identified and characterized mutations in the *Drosophila DEAF-1* gene. DEAF-1 maternal function is essential for early embryonic development, as most maternal mutant embryos arrest before reaching the stage at which the zygotic segmentation genes are activated.

RESULTS

Identification of Mutations in the *DEAF-1* Gene

To gain insight into the biological functions of *DEAF-1*, we first identified mutations in the gene using (1) local P element transpositions and (2) rescue of lethality of potential mutants with a genomic rescue construct. *DEAF-1* coding sequences map to polytene division 76D3-D4 (Adams *et al.*, 2000; Gross and McGinnis, 1995). Because P element transpositions or deletions are frequent in nearby chromosomal regions (Tower *et al.*, 1993), we mobilized the transposition of the P element *P{lacW}l(3)L0189*, which is inserted approximately 32 kb from the 3′ end of the *DEAF-1* transcription unit. In progeny from flies with potential transpositions, we used an inverse polymerase chain reaction (PCR) method (modified from Dalby *et al.* 1995, see Materials and Methods) to screen for insertions or deletions in a cloned genomic interval containing *DEAF-1* (cosmid 104C6 and P1 clone DS00027). A map of the genomic region containing *DEAF-1* and adjacent genes is shown in Figure 1a. No simple transpositions into *DEAF-1* were recovered. However, after screening 2000 lines, we obtained a deletion, *Df(3L)25-21*, that removes a genomic region flanking the left side of the *L0189* P element, including the entire *DEAF-1* locus (Fig. 1a, black rectangle). The absence of *DEAF-1* sequences on the *Df(3L)25-21* chromosome was confirmed by Southern blot analysis (data not shown).

This region of the third chromosome is also deleted in $Df(3L)kto^2$, and a large screen has been done to identify EMS-induced lethal complementation groups that map within this deficiency (J. Kennison, unpublished results). Further genetic tests have shown that *Df(3L)25-21* and $Df(3L)kto^2$ both uncover five lethal complementation groups including *tricorner* (*trc*) and *kohtalo* (*kto*). To determine whether one of these complementation groups corresponded to *DEAF-1*, we used P element transformation to make transgenic lines containing a genomic rescue construct that included all of the *DEAF-1* transcription unit plus approximately 4 kb on each side (Fig. 1a, gray rectangle).

Only the *k3* and *S10B* alleles, in the same complementation group, were adult viable in combination with the *DEAF-1* genomic rescue construct. PCR amplification and sequencing of the *DEAF-1* open reading frame from these mutant chromosomes revealed that *k3* has a G to A transition that changes a cysteine codon TGT (amino acid 262) to a tyrosine codon TAT. In *S10B*, a G to A transition changes a tryptophan codon TGG (amino acid 400) to a stop codon TGA. No other codon changes were detected in the rest of the *DEAF-1* open reading frame in these mutants. Based on the genetic rescue, complementation results, and identification of mutations in the open reading frame of *DEAF-1*, we conclude that *k3* and *S10B* are mutant alleles of *DEAF-1* ($DEAF\text{-}1^{k3}$ and $DEAF\text{-}1^{S10B}$). The locations of these mutations in the DEAF-1 protein sequence are shown in Figure 1b.

Phenotypic Analysis of $DEAF\text{-}1^{k3}$ and $DEAF\text{-}1^{S10B}$ Mutants

The effects on embryos of zygotic loss of *DEAF-1* function were assayed by analysis of transheterozygous combinations of $DEAF\text{-}1^{k3}$ and $DEAF\text{-}1^{S10B}$ over each other and over *Df(3L)25-21*. The zygotic *DEAF-1* mutants developed normal first instar larval cuticles (Fig. 2c). In fact, most the zygotic mutant embryos hatch, develop to late third instar larvae, and form pupae, but never eclose as adults. This is correlated with a large

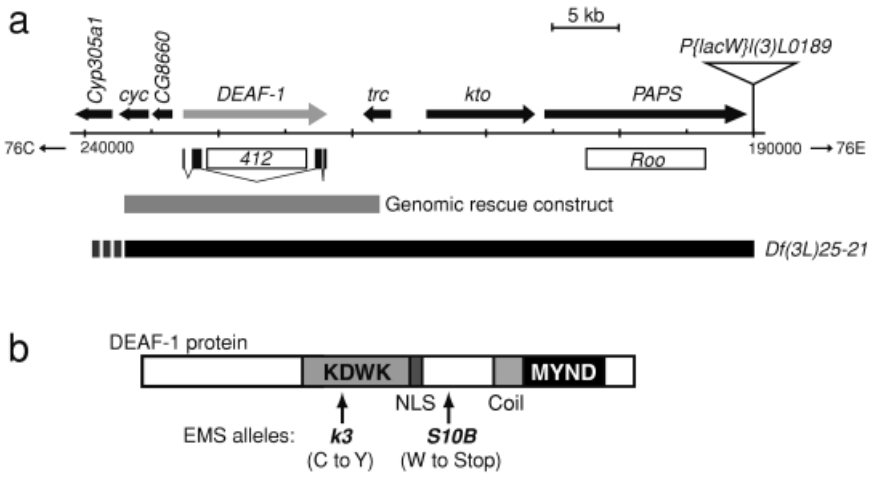

FIG. 1. *DEAF-1* genomic locus and locations of two mutations in *DEAF-1*. (**a**) A summary of genomic structure around the *DEAF-1* gene. Known and predicted genetic loci are indicated by arrows (based on GadFly annotations, http://flybase.bio.indiana.edu/annot/). Black boxes underneath *DEAF-1* represent spliced exons. Transposon insertions (*412* and *Roo*) are indicated by open rectangles. Note that these are present in the *iso-1* line used for genome sequencing, but absent from the Oregon R line maintained in our lab (data not shown). Location of *P{lacW}l(3)L0189* is indicated, as well as the approximate extent of the *Df(3L)25-21* deletion (black bar). The genomic rescue construct (gray bar) includes all of the *DEAF-1* and *CG8660* genes but only portions of *cyc* and *trc*. (**b**) Schematic locations of the *k3* and *S10B* mutations in the DEAF-1 protein. KDWK (SAND) and MYND are conserved domains in the DEAF-1 protein, NLS is a putative nuclear localization signal, Coil refers to a predicted coiled coil region (see Fig. 5b).

contribution of DEAF-1 transcript and protein from maternal stores (Gross and McGinnis, 1995) and a persistence of abundant DEAF-1 protein until late stages of embryogenesis (data not shown). Dissected pharate adults from late *DEAF-1* mutant pupae show no obvious morphological abnormalities, so the cause of the developmental arrest in zygotic *DEAF-1* mutants is currently unknown. Zygotic mutants that are homozygous for *Df(3L)25-21* arrest at the end of embryogenesis are smaller in size than wild-type controls and have defects in patterning of dorsal hairs of the cuticle (data not shown). This phenotype could not be attributed to *DEAF-1* because *Df(3L)25-21* uncovers four other essential genes.

To test whether elimination of the maternal contribution of DEAF-1 would result in visible embryonic phenotypes, we used an FLP-DFS system (Chou *et al.*, 1993) to generate *DEAF-1*^{k3} and *DEAF-1*^{S10B} mutant maternal germline clones (see Experimental Procedures). We obtained similar phenotypic results from several recombinant chromosomes bearing FRT-*ovo*^{D1} chromosomes and either of the two *DEAF-1* point mutations. For both alleles, large numbers of maternal$^-$/zygotic$^-$ or maternal$^-$/zygotic$^+$ *DEAF-1* mutant embryos were laid, but approximately 70% of these embryos show developmental arrest at early stages of embryogenesis, prior to the onset of gastrulation and germ band extension. We stained maternal mutant embryos to detect the transcript patterns of the zygotic segmentation genes *Kruppel*, *knirps*, and *fushi tarazu*. In all cases, more than half of the maternal mutants did not show any segmentation gene transcripts. Almost all of the remaining mutant embryos showed variable loss of pattern elements from the expression patterns of these three early zygotic patterning genes (data not shown).

The few maternal$^-$/zygotic$^-$ or maternal$^-$/zygotic$^+$ *DEAF-1*^{k3} embryos that complete embryogenesis reveal segmental pattern defects that range from mild to very severe (Fig. 2e, g). The range of cuticular abnormalities in maternal$^-$/zygotic$^-$ mutant embryos was similar to those in maternal$^-$/zygotic$^+$ embryos. Similar results were obtained with the *DEAF-1*^{S10B} maternal mutants (Fig. 2f, h). The defects consist of loss and fusions of segments, affecting all parts of the embryonic anterior/posterior axis. In the least affected embryos, segmental abnormalities were more frequently observed in the area of A5–A7 abdominal segments (Fig. 2e, f). In a few rare cases, maternal$^-$/zygotic$^+$ embryos hatched and developed into adults that displayed no morphological defects, indicating that the loss of maternal *DEAF-1* can occasionally be rescued by the paternal zygotic contribution. No maternal$^-$/zygotic$^-$ mutants survived to adulthood.

We analyzed DEAF-1 protein levels in well developed stage 14 embryos that were maternal mutants for the *DEAF-1*^{S10B} allele and zygotic mutants for *DEAF-1*^{S10B}/*Df(3L)25-21*. In such embryos, little or no protein was detected (Fig. 2b), suggesting that the *S10B* nonsense mutation leads to a striking decrease in DEAF-1 protein stability and accumulation. Residual DEAF-1 protein levels in maternal$^-$/zygotic$^-$ *DEAF-1*^{k3} mutant embryos were higher than for *S10B* and approached those in *Df(3L)25-21* heterozygotes (data not shown). This suggests that though the *k3* missense mutation somewhat reduces DEAF-1 protein levels, it is likely to affect DEAF-1 function by interfering with a critical molecular interaction (see Discussion).

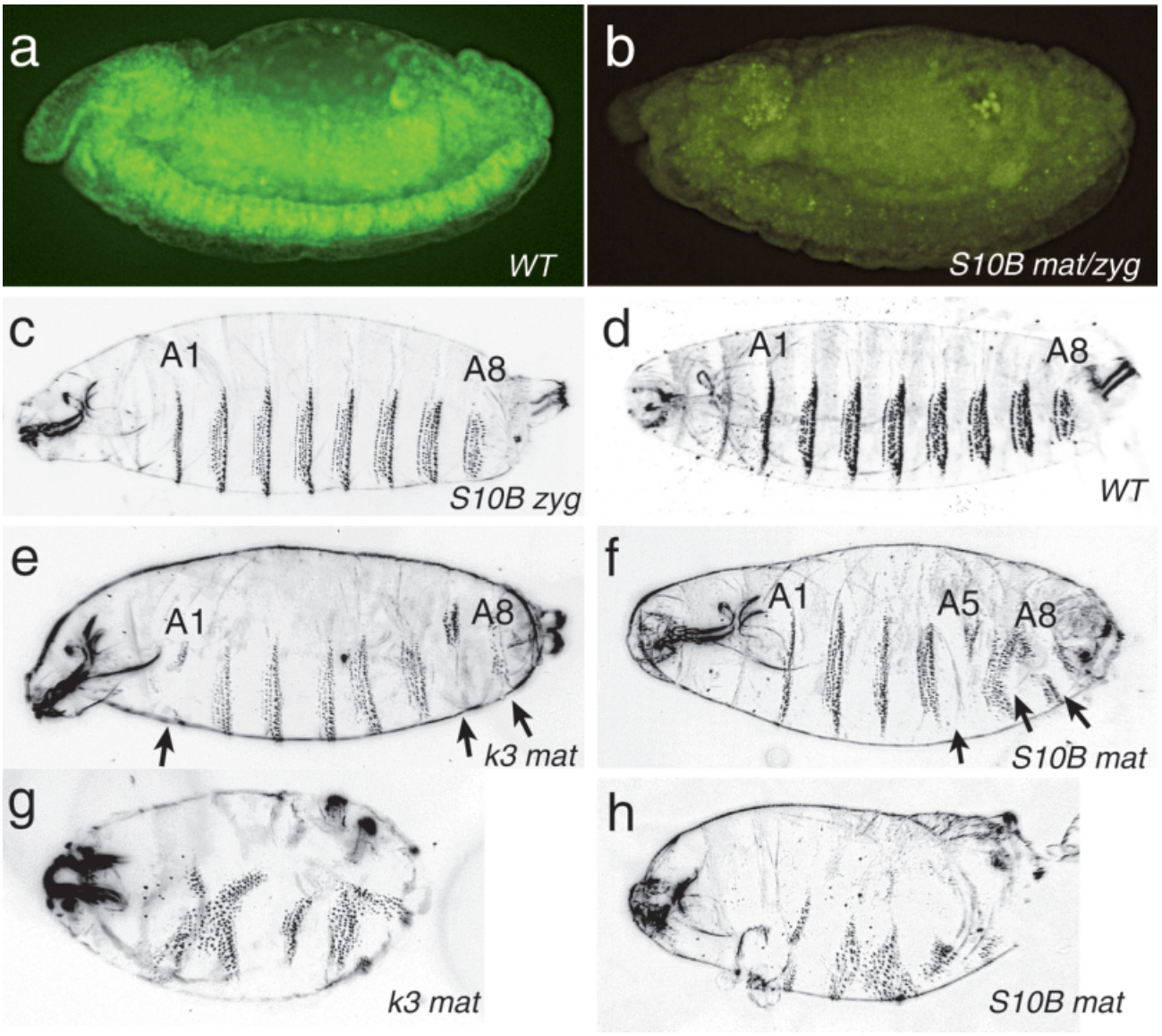

FIG. 2. *DEAF-1* loss of function phenotypes. (**a**) Fluorescence immunolocalization of DEAF-1 protein in a wild-type stage 13 embryo. Note the ubiquitous nuclear staining. (**b**) Immunostain for DEAF-1 protein in a *DEAF-1*^{S10B} maternal/zygotic mutant embryo. (**c**) A cuticular preparation of a *DEAF-1*^{S10B}*/Df(3L)25-21* zygotic mutant embryo. No defects are visible in this zygotic mutant (compare to d). A1 and A8 indicate abdominal segments. (**d**) Cuticle of a wild-type embryo. (**e, g**) Examples of segmentation defects in *DEAF-1*^{k3} maternal mutants. Arrows indicate missing or abnormally developed segments. (**f, h**) Segmentation defects in the *DEAF-1*^{S10B} maternal mutants. Note similarities with the *DEAF-1*^{k3} maternal phenotypes.

DEAF-1 Overexpression Induces Gain-of-Function Defects in Embryonic and Adult Morphology

To complement the data from loss of function analysis, we studied the phenotypic effects of overexpression of DEAF-1 in embryos and adults, using a GAL4-UAS system. Higher levels of DEAF-1 induced at embryonic stages do not result in significant defects, as seen in cuticular preparations of *69B-GAL4/UAS-DEAF-1* and *arm-GAL4*^{4}*/UAS-DEAF-1* embryos, when they are allowed to develop at room temperature or 25°C (data not shown). Many embryos hatch and stop in development at larval or pupal stages. Incubation of *arm-GAL4*^{4}*/UAS-DEAF-1* embryos at 29°C, with *arm-GAL4*^{4} expressed maternally, gives an abnormal phenotype. In such embryos, most axial cuticular elements are normal, and the only observed defect is a failure of dorsal closure and incomplete germband retraction (Fig. 3a). This experiment indicates that high levels of DEAF-1 are tolerated in most cells during early development, consistent with the ubiquitous expression of the protein in embryos.

In contrast, staining of third instar larval imaginal discs showed lower levels of DEAF-1 protein (data not shown). To test whether higher levels of DEAF-1 would cause any phenotypic defects in adults, *UAS-DEAF-1* flies

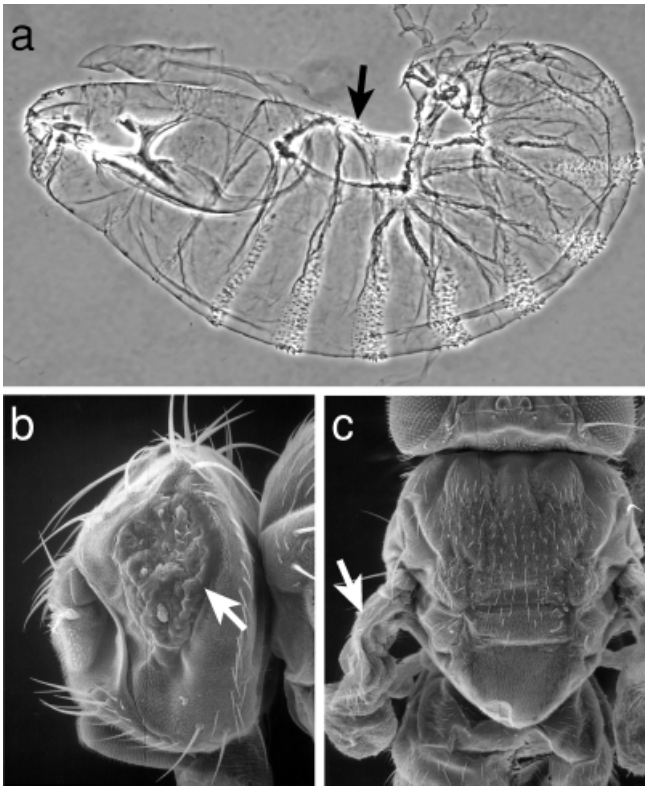

FIG. 3. *DEAF-1* gain of function phenotypes. (**a**) Cuticular preparation of *an arm-GAL4/UAS-DEAF-1* embryo raised at 29°C. Most cuticular structures, including head skeleton, are normal. However, such embryos show incomplete germ band retraction and failure of dorsal closure (arrow). (**b, c**) Scanning electron micrographs of adult flies expressing *DEAF-1* under the control of *pGMR-GAL4* (b) or *MS1096-GAL4* (c). Note a disorganized and severely reduced pattern of eye ommatidia in panel b and rudimentary wing structures in panel c (arrows).

were crossed to the following *GAL4* drivers: *pGMR* (expressed in the eye), *MS1096* (primarily in the wing), dpp^{disc} (anterior/posterior boundary of most discs), *sca* (proneural clusters), Dll^{md23} (distal appendage regions), *A9* (wing plus earlier larval pattern), and *sev* (photoreceptors). Dll^{md23}- and *A9-GAL4/UAS-DEAF-1* combinations were lethal at all temperatures tested. The dpp^{disc}-*GAL4/UAS-DEAF-1* cross gave very few survivors that had curly wings with wider third veins and exhibited a loss of dorsal thoracic and scutellar macrochaete bristles (data not shown). Expression with *pGMR* resulted in loss of ommatidia and a rough eye phenotype (Fig. 3b), whereas *MS1096* produced few survivors with rudimentary wings and defective macrochaete (Fig. 3c). *sev-GAL4/UAS-DEAF-1* flies had a weak rough eye phenotype, with a smooth and lightly colored anterior-central region of the eye (data not shown). These experiments suggest that overexpression of DEAF-1 in adult tissues results in the loss of corresponding cells. At least in the case of *sca-GAL4*, imaginal disc cells overexpressing DEAF-1 show higher uptake of acridine orange, suggesting activation of apoptotic machinery (data not shown).

DEAF-1 Protein Is Bound to Hundreds of Sites on Polytene Chromosomes

DEAF-1 protein is normally expressed in the salivary gland, which allowed us to ask whether the protein bound to polytene chromosomes. Immunolocalization of DEAF-1 on polytenes revealed distinct bands of staining at approximately 200 sites (Fig. 4a), consistent with the in vitro sequence-specific binding function of DEAF-1. One of the sites of DEAF-1 staining corresponds to the cytogenetic location (84A1–2) that contains the *Dfd* locus and other proximal genes of the Antennapedia complex (Fig. 4b). *Dfd* and other Antennapedia complex Hox genes are repressed in salivary cells (data not shown). The number of polytene chromosome binding sites suggests that DEAF-1 regulates transcription of hundreds of *Drosophila* genes.

Localization to a few hundred chromosomal sites is a characteristic feature of Pc/Trx group (PcG/TrxG) proteins, and several of the DEAF-1 polytene localization sites are shared with Pc, Trx, and other members of the two groups (data not shown). We tested $DEAF\text{-}1^{k3}$ and $DEAF\text{-}1^{S10B}$ alleles in standard *Pc* genetic interaction tests (Kennison and Tamkun, 1988) and found no changes in the number of ectopic sex combs on T2 legs in Pc^{4} males (data not shown). Though no genetic interaction was observed between *DEAF-1* mutants and *Pc*, potential DEAF-1-containing protein complexes may be involved in the regulation of chromatin dynamics in a manner analogous to PcG repression complexes.

Domain Architecture of DEAF-1

How does DEAF-1 protein carry out its regulatory functions? Additional insight into this unresolved question can be gained by analyzing the domain architecture of DEAF-1. Conserved structural motifs in the amino acid sequence of DEAF-1 are summarized in Figure 1b. It is noteworthy that DEAF-1 contains both the SAND and MYND domains. In the complete *Drosophila* genome sequence, DEAF-1 encodes the only SAND domain-containing protein (Adams *et al.*, 2000), whereas in mammals and in *C. elegans* the SAND domain has undergone moderate expansion (see Introduction). The SAND domain in NUDR has been shown to form a DNA-binding interface, and given a high degree of conservation at the primary amino acid sequence, the SAND domain in DEAF-1 is likely to mediate sequence-specific binding to TTCG motifs.

A search for *Drosophila* open reading frames encoding MYND domains identified at least 15 known and predicted polypeptides, including DEAF-1 and Nervy (Fig. 5a). The MYND domain is an ancient evolutionary invention present in many divergent copies in mammalian, arthropod, and nematode genomes as well as in many plant proteins and in at least one protein from yeast (see MYND entry in the Pfam database, http://pfam.wustl.edu/). The structure of the MYND domain is unknown, but the conserved spacing of cysteine and histidine residues suggests a zinc-binding motif. As de-

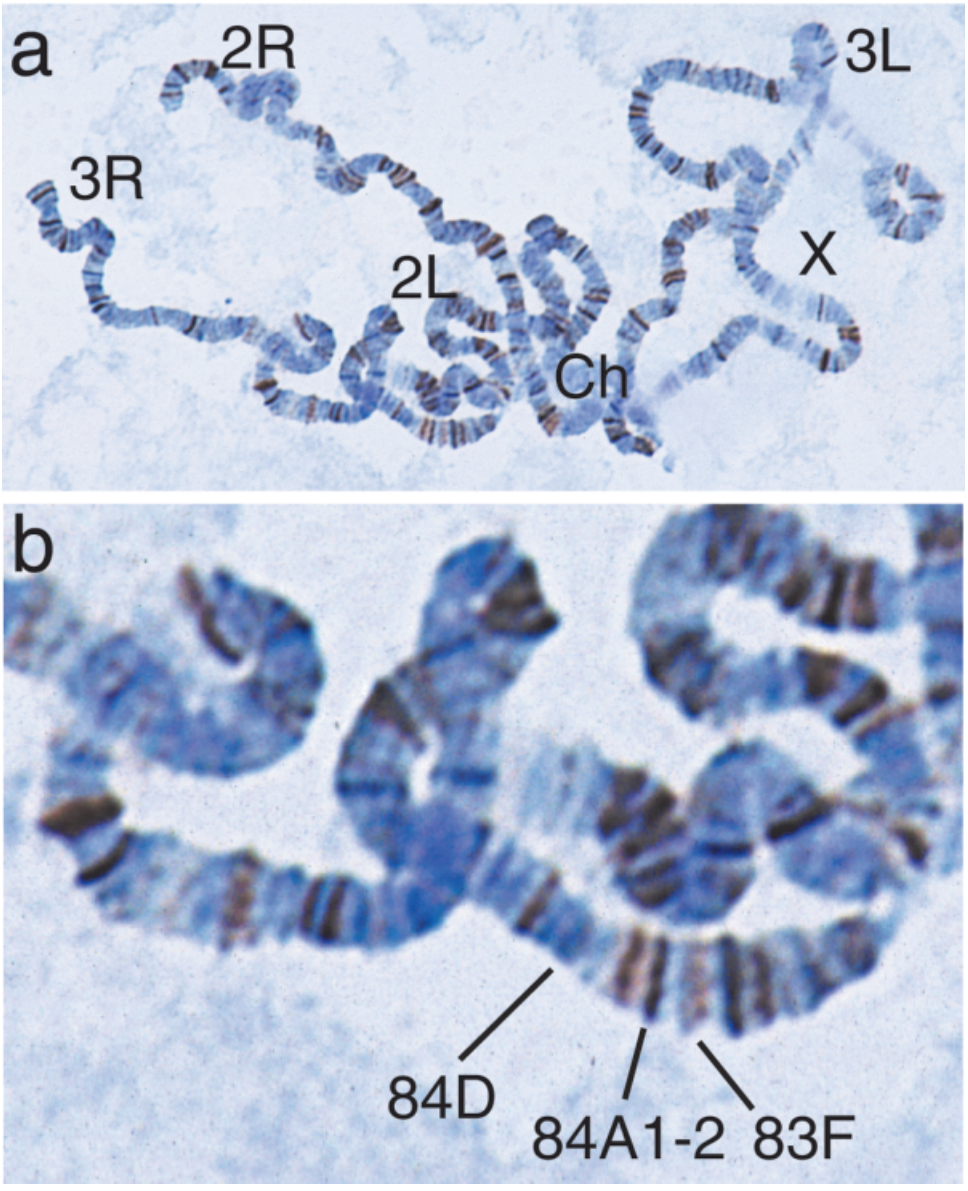

FIG. 4. Immunolocalization of DEAF-1 protein on polytene chromosomes. (**a**) DEAF-1 staining is localized to approximately 200 discrete bands distributed along the lengths of all chromosomal arms (labeled with letters). No staining is observed at the chromocenter (Ch). (**b**) A close-up view of the base of chromosomal arm 3R. The DEAF-1 protein binds to polytene bands 84A1-2, the cytogenetic location of the Antennapedia complex, which includes many homeotic genes.

scribed in the Introduction, one function of the MYND domain is to recruit corepressors of transcription, and perhaps the variations in amino acid sequence allow different family members to recognize different partner proteins.

Secondary structure analysis strongly suggests that both DEAF-1 and NUDR proteins have a coiled-coil structure between amino acids 450 and 500 (Fig. 5b). Nervy and ETO/MTG8 also have predicted coiled coil domains just upstream of their MYND domains, but this is not true of all MYND motif proteins (Fig. 5b and data not shown). Coiled coils are common in proteins involved in homo- and heterodimerization. Peptide J from NUDR was shown to be sufficient for dimeric binding of clustered TTCG sequences, possibly via an additional helix-loop-helix structure predicted to reside in the vicinity of the SAND domain (Bottomley *et al.*, 2001). The slow mobility of DEAF-1/DNA or NUDR complexes in gel shift experiments and extensive DNA regions bound by DEAF-1 or NUDR are consistent with multimer formation (Gross and McGinnis, 1995; Michelson *et al.*, 1999). A coiled coil structure preceding MYND domain in DEAF-1 and NUDR may provide additional framework for oligomerization or for interactions with partner proteins.

DISCUSSION

Properties of Mutations in the DEAF-1 Gene

We isolated two point mutations in the *DEAF-1* gene and have shown that maternal and zygotic reduction of *DEAF-1* function results in many embryos with arrested development during embryogenesis. In those embryos that de-

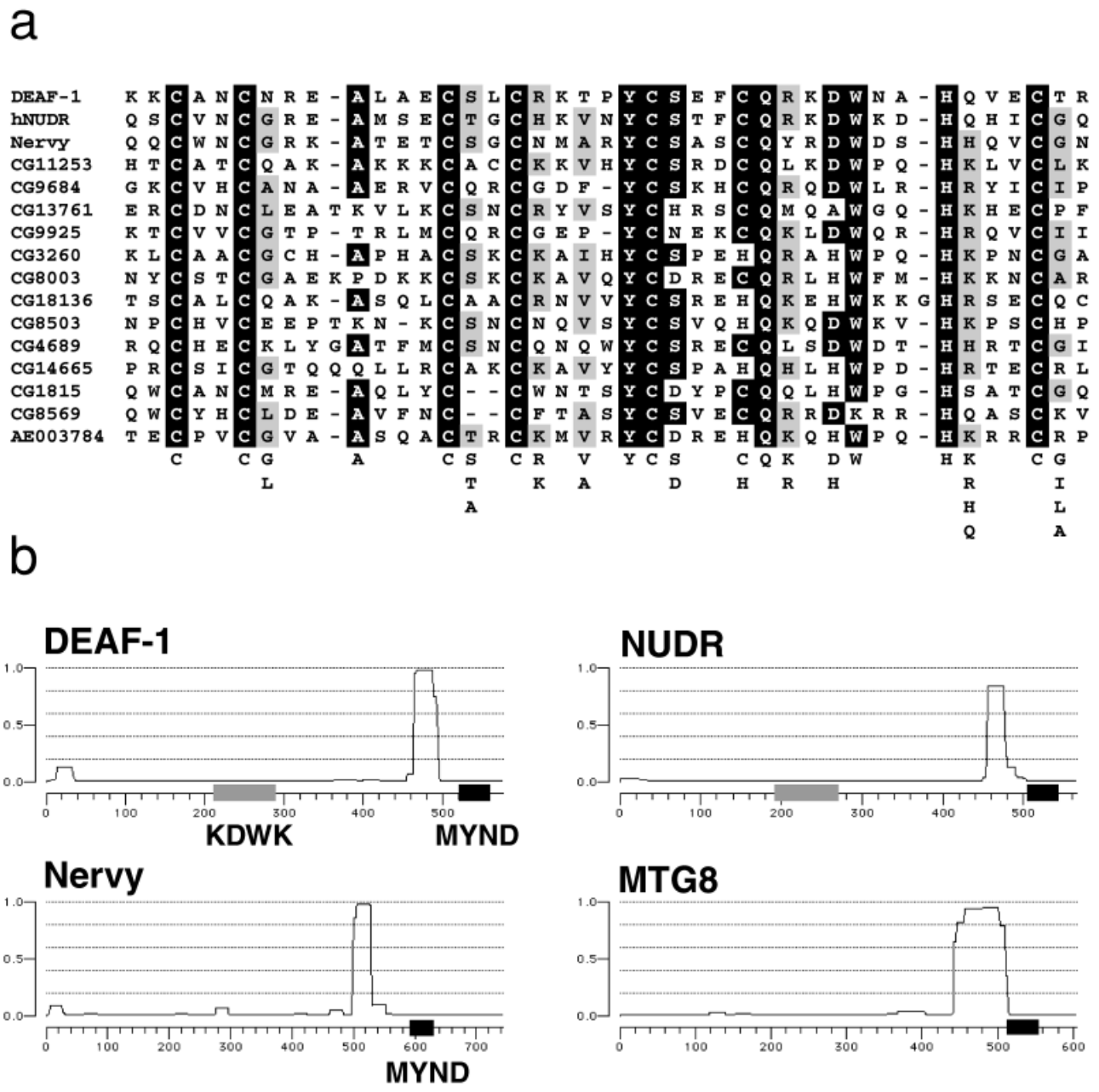

FIG. 5. Domain structure of DEAF-1 and its homologs. (**a**) Alignment of *Drosophila* MYND domain-containing proteins. All known and predicted *Drosophila* proteins with MYND domains are shown, aligned to DEAF-1 and a human DEAF-1 homolog, NUDR. A consensus sequence, including invariantly spaced cysteine and histidine residues forming a putative zinc-binding motif, is indicated at the bottom. Alignment was generated with a CLUSTALW module of MacVector (Oxford Molecular) and manual editing. *Drosophila* proteins are given their CG identifier from GadFly (http://flybase.bio.indiana.edu/annot/). The protein indicated as AE003784 is a putative open reading frame identified by the TBLASTN program and is located between predicted genes CG7843 and CG14590 in the sequence contig AE003784. (**b**) Predicted coiled coil regions in DEAF-1 and other MYND family members. In DEAF-1, NUDR, Nervy, and ETO/MTG8 the MYND domain is preceded by a region with a high probability of coiled coil formation (numbers on the vertical axis), predicted by the MacStripe program (window length = 21, matrix = MTK).

velop to late embryogenesis and secrete cuticule, there are segmental defects of variable severity. In salivary cells, we found that DEAF-1 protein localizes to about 200 sites on the polytene chromosomes. The loss of function phenotypes and in vivo chromosomal localization pattern are consistent with the idea that DEAF-1 functions in the regulation many different genes, especially at the earliest stages of *Drosophila* development, and perhaps also has an important role in the regulation of maternal genes whose products are deposited in the oocyte.

One of the alleles, *DEAF-1^{S10B}*, is predicted to truncate the protein after the SAND domain, thereby eliminating all of the MYND domain and a predicted coiled coil structure. MYND domains in mammalian DEAF-1 homologs have been shown to interact with corepressors of transcription. The *S10B* mutation may compromise the repressive function of DEAF-1 by abolishing such interaction.

Another allele, *DEAF-1^{k3}*, is a missense mutation that changes a cysteine residue to tyrosine. This residue corresponds to the carboxy terminus of the α2 helix in the SAND domain (Bottomley *et al.*, 2001). In other DEAF-1 homologs, only glycine or alanine are encountered at the same position (see alignment in Bottomley *et al.*, 2001). These amino acids and cysteine have small side chains, and introduction of a bulky hydrophobic residue of tyrosine may impair the folding of the SAND domain in this mutant or prevent crucial intermolecular interactions. Without any other point mutations available, it is difficult to assess whether *DEAF-1^{k3}* and *DEAF-1^{S10B}* represent true null alleles. The variability of DEAF-1 mutant phenotypes suggests that these alleles are hypomorphic. However, variable phenotypes resulting from loss of maternal protein have been reported for the TrxG genes *kismet* (Daubresse *et al.*, 1999), *osa* (Vazquez *et al.*, 1999), and the histone deacetylase *Rpd3* (Chen *et al.*, 1999; Mannervik and Levine, 1999).

As a complement to the loss of function studies reported here, we also generated an inducible *DEAF-1* transgene using a *DEAF-1* cDNA downstream of UAS sequences. This construct was tested with a variety of imaginal disc specific GAL4 drivers. In all cases (examples shown in Fig. 3), overexpression of DEAF-1 resulted in partial or complete loss of tissues in which the transgene was activated. These abnormalities were likely to be a consequence of apoptosis, as revealed by increased uptake of acridine orange by cells overexpressing DEAF-1 (data not shown).

MATERIALS AND METHODS

Inverse PCR-Based Local P Element Transposition Screen

A protocol used here was a modification of a procedure described in (Dalby *et al.*, 1995). Targets for Southern hybridization were strips of nylon membrane that contained lanes of cosmid 104C6 and P1 clone DS00027 DNA (containing the *DEAF-1* locus) digested with NheI and ApaI, electrophoresed, and transferred. Each strip also contained an ApaI digest of a cloned genomic region encompassing the insertion of the *l(2)k06805* P element used as a positive control. The *P{lacW}l(3)L0189* line was used as a jumpstarter. *l(3)L0189/TM3Ser* flies were crossed to a transposase stock, *w; TMSΔ2-3/TM6B*. 2-3 *l(3)L0189/TMSΔ2-3* virgin females or males were crossed to a double balancer stock, *w; TM3Sb/TM6B*. A total of approximately 1,000 vials were set up. Randomly picked *w^{+}/TM3Sb* progeny males obtained from this cross (two from each vial, with different eye colors) were singly crossed to *w; TM3Sb/TM6B* virgin females, yielding a total of 2,100 individual lines. After mating for 3–4 days, the males were back-collected and used for QiaAmp (QIAGEN) genomic DNA preparation from pools containing 50 individuals plus one *l(2)k06805* male as an internal positive control. Genomic DNA was digested to completion with Sau3A, ligated under dilute conditions to allow self-circularization, and used as a template for PCR with the P31ITR and LACZ primers and hot dNTPs. P31ITR: 5′ CGACGGGACCACCTTATGTTATTTCATCATG, LACZ: 5′ AGCTGGCGTAATAGCGAAGAGGCCCGCA. After PCR, the products were purified using QIAquick PCR Purification kit (QIAGEN) and hybridized to cosmid/P1 strips under standard conditions (Sambrook *et al.*, 1989). One pool of males gave a strong positive hybridization signal, corresponding to a location near the *DEAF-1* gene that was closer than the original insertion. After two pool subdivisions and rescreens, a single positive line was identified. Mapping by Southern hybridization showed that this chromosome contained a deletion on one side of the original P element, *Df(3L)25-21*, which removed all of the *DEAF-1* locus (Fig. 1).

Genomic Rescue Construct and Identification of Locations of Mutations in the *DEAF-1* Gene

To make a *DEAF-1* genomic rescue construct, a 19-kb region from an *iso-1* λ genomic library was assembled from three pieces into the pCaSpeR4 vector to generate the rescue construct pC4D1R (Fig. 1). Transformant lines carrying the rescue construct (*DEAF-1^{RESCUE}*) were established using standard procedures (Spradling, 1986). An insertion on the second chromosome was used to make two rescue stocks: *w; DEAF-1^{RESCUE}/DEAF-1^{RESCUE}; Df(3L)25-21/TM3Sb Ubx-lacZ* and *w; DEAF-1^{RESCUE}/DEAF-1^{RESCUE}; Df(3L)kto^{2}/TM6C*. These lines were used in a test for the rescue of heterozygous lethality of several EMS alleles identified by J. Kennison in the *Df(3L)kto^{2}* region (unpublished observations). Two lines, *k3* and *S10B*, were rescued by each stock to adult viability.

To identify locations of mutations in these EMS alleles, *DEAF-1* coding region was amplified in two parts from genomic DNA isolated from *k3* and *S10B* heterozygous adults, as well as from parental chromosomes: *red e* for *k3* and *iso-1* for *S10B*. The resulting PCR fragments were sequenced using internal primers. A single nucleotide substitution was found in each mutant chromosome, and their exact locations are described in the text and shown in Figure 1.

Generation of *DEAF-1* Germline Clones

The *k3* and *S10B* mutations were recombined onto a chromosome with an *FRT^{2A}* insert on 3L and used to generate maternal homozygous germline clones (Chou *et al.*, 1993). In brief, a strain of *HS-FLP* (Bloomington stock no. 1970) was crossed to a strain of *ovo^{D1} FRT* (Bloomington stock no. 2139). The *FLP FRT*-carrying males from this cross were crossed to *DEAF-1 FRT* chromosomes and to *FRT* without mutations as a control

(Bloomington stock no. 1997). At the L3 stage, the progeny were heat shocked for 2 h at 37°C, then once again after a 24-h period. For maternal^{-}/zygotic^{+} analysis, virgin females carrying germline clones homozygous for the *DEAF-1* mutations were crossed to a wild-type line. For maternal^{-}/zygotic^{-} analysis, females with germline clones were crossed to an *Df(3L)25-21/TM3Sb Ubx-lacZ* males. The number of early arrest maternal mutant embryos that failed to progress was determined by counting the number of early arrest embryos (of 300) in a collection that was stained for DEAF-1 protein.

Cuticular Preparations, Immunolocalization on Polytene Chromosomes

Cuticular preparations were done as previously described (McGinnis *et al.*, 1998). Anti-DEAF-1 guinea pig polyclonal antibody was described in Gross and McGinnis (1995). In situ DEAF-1 protein localization on polytene chromosomes was a modification of a procedure described in Zink and colleagues (1991).

ACKNOWLEDGMENTS

We thank Kelly Hsu for her help with P element screens. DNA sequencing was performed by the UCSD Center for AIDS Research Sequencing Facility. A.V. was an HHMI predoctoral fellow.

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