Embryonic Head Involution and Rotation of Male Terminalia Require the Drosophila Locus *head involution defective*

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ABSTRACT

We have characterized the *head involution defective* (*hid*) locus which is located within the chromosomal region 75B8-C1,2. During the morphogenetic reorganization of the embryonic head region, hid^+ function is necessary for the movement of the dorsal fold across the procephalon and clypeolabrum, a process that forms the frontal sac. The absence of the frontal sac in the *hid* mutant embryos affects the formation of the dorsal bridge and disrupts the development of the larval cephalopharyngeal skeleton. In addition to its embryonic role, this same *hid* function is also required during pupal development for the 360° rotation of the male terminalia about the anterior-posterior body axis, and for a late step of wing blade morphogenesis. Although the abnormal wing phenotype caused by the *Wrinkled* (*W*) mutation is quite different from the one resulting from the loss-of-function *hid* mutations, the characterization of EMS-induced *W* revertants reveals that *W* is actually an antimorphic allele of *hid*.

THE polytene region 75B8-C1,2 is defined genetically by the overlap of the deficiencies *Df(3L)Cat* and $Df(3L)W^{R+10}$ (MACKAY and BEWLEY 1989; SEGRAVES and HOGNESS 1990). One gene located within this region is *terminus* (*ter*), which was identified molecularly in a screen for blastoderm-specific genes (ROARK *et al.* 1985; ROARK 1985; LENGYEL *et al.* 1985). The *ter* gene encodes a conceptual protein with a putative Zn-binding, DNA-binding finger and produces a transcript that is present uniformly at the cellular blastoderm stage, becoming restricted during gastrulation to regions where cells are invaginating or ingressing before rapidly disappearing later in embryogenesis (BALDARELLI *et al.* 1988).

Genetic studies have shown that the gene responsible for the Wrinkled wing phenotype is located in the same region as *ter*. The wings of flies carrying the *W* mutation remain folded like those of the late pupa; their inflation by hemolymph during the period immediately following eclosion does not occur properly, perhaps because of constrictions of the wing veins (WADDINGTON 1940). The generation of radiation-induced revertants of *W* showed that the mutant phenotype is caused by a gain-of-function mutation and it was possible to localize the gene responsible for this trait within the region 75C-D since these revertants were associated with chromosome rearrangements (ASHBURNER *et al.* 1980; ASHBURNER, RICHARDS and VELISSARIOU 1980). This localization was subsequently confirmed by the isolation of the *W* revertants $Df\,W^{R+4}$ and $Df\,W^{R+10}$ (SEGRAVES and HOGNESS 1990).

Both the W^{R+4} and W^{R+10} deficiencies eliminate a genetic function responsible for the normal development of the larval cephalopharyngeal skeleton (CPS) because an abnormally formed CPS is observed in embryos heterozygous for these deficencies (ROARK *et al.* 1985; LENGYEL *et al.* 1985). To clarify the genetic relationship between this function, the *ter* gene, and the *W* mutation, we have isolated and studied recessive lethal mutations affecting genes in the 75B8-C1,2 region. We describe here genetic studies on twelve recessive lethal alleles of a locus lying in this region that we have named *head involution defective* (*hid*). The dorsal apects of embryonic head involution do not occur in the absence of hid^+ function and the resulting unhatched larvae possess CPS defects that are indistinguishable from those exhibited by $Df\,W^{R+4}/Df\,W^{R+10}$ embryos. In addition to its embryonic role, hid^+ function is also required during pupation for the normal 360° rotation of the developing male terminalia and for an aspect of wing blade morphogenesis. The role of a hid^+ product in wing development is also consistent with genetic results indicating that an antimorphic *hid* allele causes the Wrinkled phenotype.

MATERIALS AND METHODS

Isolation of recessive lethal *hid* mutations: Six *hid* mutations were recovered as recessive lethals from mutant screens utilizing the third chromosome deletions $Df(3L)W^{R+4}$ or *Df(3L)Cat*; the former deficiency is described by SEGRAVES and HOGNESS (1990) and the latter by MACKAY and BEWLEY (1989). The hid^{A22} mutation was induced on a *red e* chromosome by X-irradiation (4000 R); the hid^{A206} and

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hid^{A329} mutations were induced on a *ru h th ri e* chromosome by ethyl methanesulfonate (EMS) according to the method of LEWIS and BACHER (1968). Subsequent to their recovery, portions of the original chromosomes carrying the *hid*^{A206} and *hid*^{A329} mutations were exchanged to yield the chromosomes *th hid*^{A206} *ri red* and *th hid*^{A329} *ri*. The *hid* mutant alleles *H89*, *H99* and *H109* were originally recovered by MACKAY and BEWLEY (1989) as the result of γ-irradiation of flies carrying a *ri sbd e* chromosome. All other third chromosome mutations are described by LINDSLEY and GRELL (1968).

Six other *hid* mutations were recovered as EMS-induced *W* revertants. Males heterozygous for *ru W p red* and *TM3, Sb* were treated overnight with EMS and then mated with *Oregon R* (Ore R) virgin females. Putative *W*-revertant bearing single F_1 males (W^+, Sb^+), were crossed with *Df(3L) Cat, ri red/TM6B* females to verify that the original *ru red p* chromosome was present (*ru W red p/TM6B* flies have orangish-pink eyes) and that the W phenotype had been lost; in addition, the absence of F_1 progeny with red eyes (*Df Cat* heterozygotes) indicated that a newly induced recessive lethal mutation had been recovered. A stock of each confirmed revertant was established using the *ru* W^{R+} *p red/TM6B* progeny resulting from the test cross.

Determination of embryonic lethality and abnormal CPS development: Crosses between *hid*$^-$*/TM3, Ser* males and *Df* W^{R+4}*/TM3, Ser* females were set up and the resulting eggs were collected over 12 hr intervals. The eggs that were collected were held for 24–36 hr to permit the completion of embryogenesis and all of those which failed to hatch during this period were dechorionated with bleach for microscopic examination. The numbers of unfertilized eggs (these are pearly white, in contrast to an egg containing a dead embryo or larva which exhibits a yellow discoloration), of eggs containing unhatched larvae with a normal CPS, and of unhatched larvae with an abnormal CPS were determined under low magnification (8–10×).

Preparation of cuticle from unhatched larvae: Eggs which were 22–24 hr old were collected, dechorionated, and devitellinized using the method of ZALOKAR and ERK (1977). Their internal tissues were cleared in a solution of glycerol and acetic acid (3:1) overnight at 65°. The cuticles were mounted in polyvinyllactophenol, incubated at 65° overnight, and examined using a Zeiss phase contrast microscope.

Preparation of embryos for scanning electron microscopy (SEM): After removal of the chorion, embryos were fixed and devitellinized according to the phase partition method of ZALOKAR and ERK (1977), then treated with 1% OsO_4 in a 0.1 M sodium cacodylate buffer for several hours at 4°. Following fixation, the embryos were rinsed in distilled water, dehydrated in a graded ethanol series, placed in hexamethyldisilazane (Sigma Chemical Co.) for 30 min and subsequently air dried. The embryos were mounted on SEM stubs, coated with approximately 400 Å of gold using a Polaron ISI-5400 sputter-coater and examined with an ISI DS-130 scanning electron microscope.

Preparation of whole mounts of male terminalia: Males were stored in 95% ethanol prior to preparation. Their carcasses were treated to remove the internal tissues using the method of SZABAD (1978) and then the posterior third of the abdomen was removed from each carcass with a razor blade. These pieces of cuticle were placed into a drop of Poly/Bed 812 embedding media (Polysciences, Inc.) on a concavity microscope slide; after the addition of a coverslip, these slides were incubated at 65° overnight to promote hardening of the media.

TABLE 1

Results of complementation tests between the *hid* mutations

	H89	*A329*	*A206*	*A22*	*H109*	*H99*	*Df Cat*	*Df* W^{R+4}
H89	—							
A329	100	—						
A206	100	67	—					
A22	90	83	71	—				
H109	0	46	41	21	—			
H99	0	37	37	12	0	—		
Df Cat	0	24	18	5	0	0	—	
Df W^{R+4}	0	4	3	0	0	0	0	—

Each value represents the survival of the mutant heterozygotes, which is expressed as the percent of these flies which were recovered relative to the most numerous sibling progeny class. These results are based on scoring at least 200 F_1 progeny from each cross.

RESULTS

Identification of the *hid* locus: Mutant screens were performed to isolate mutations in genes located within the chromosomal region 75B8-C1,2. Seventy six recessive lethal mutations were recovered on the basis of their failure to complement the lethality of *Df* W^{R+4}. Most of these mutations (72/76) complemented both *Df* W^{R+10} and *Df Cat*; one mutation complemented *Df* W^{R+10}, but failed to complement *Df Cat*. The mutations *A22, A206, A329*, however, did not complement either deficiency showing that they affect genes which are located in the region of interest.

Inter se complementation studies indicate that the *A22, A206* and *A329* mutations all affect the same genetic function. While each mutation partially complements the recessive lethality of the other two (Table 1), all of the surviving mutant heterozygotes possess an abnormal wing phenotype. This mutant phenotype, although difficult to illustrate in photographs, is readily scored using low magnification (8–10×); the mutant wing appears opaque by comparison to wild type and occasionally contains trapped fluid. In addition to a mutant wing phenotype, many of the mutant heterozygous males also possess improperly positioned terminalia (anus, penis and the associated cuticular structures which develop from the genital disc); this mutant phenotype is described in greater detail below. Since the earliest visible abnormality observed in the mutants is the incomplete involution of the embryonic head region, we chose the name *head involution defective* (*hid*) for the locus defined by these mutations.

The mutations *H89, H99* and *H109*, isolated in a separate screen utilizing *Df Cat* (MACKAY and BEWLEY 1989), are also *hid* mutant alleles. These mutations always fail to complement the other *hid* mutations to restore the normal development of the wing, often fail to complement for the positioning of the male terminalia and, in the case of the *H99* and *H109* mutations, also fail to complement the lethal effect (Table 1). This latter result is not true for the *H89*

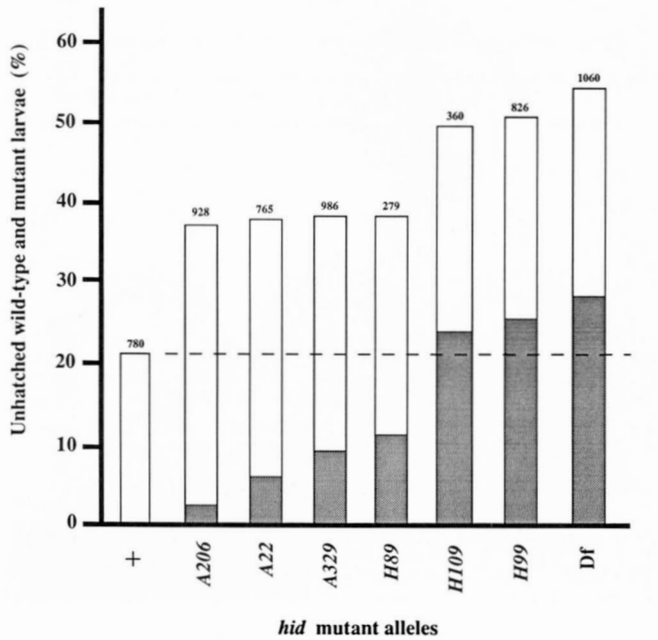

FIGURE 1.--The effect of *hid* mutations on larval viability and CPS development. Each bar represents the percentage of fertilized eggs, collected from the mating of *hid*$^{-}$/*TM3,Ser* and *Df* W^{R+4}/*TM3,Ser* adults, which failed to hatch and contained larvae with either a normal (unshaded portion) or an abnormal (shaded portion) CPS. The total number of fertilized eggs collected from each cross is indicated above the bar. The dashed line shows the fraction of unhatched larvae collected from the control and experimental crosses that are assumed to die because of homozygousity for *TM3*.

allele, however, as it fails to complement the lethal effects of *H99* and *H109*, but complements the lethal effects of *A329* and *A206*. These results may be related to the fact that this mutant allele is associated with the breakpoint of a pericentric inversion located in the 75C1,2 region.

Formation of the frontal sac requires a *hid*$^{+}$ function: To assess the relative effect of each *hid* mutation on embryonic development, crosses were performed in which one-quarter of the resulting embryos were hemizygous for these mutant alleles. All of the eggs resulting from these crosses were dechorionated and then examined with low magnification (8–10×) to determine the number which contained unhatched larvae with either a normal or an abnormal CPS (Figure 1). In the control cross between *Ore R/TM3* flies, approximately 21% of the fertilized eggs failed to hatch, presumably due to the lethality caused by *TM3* homozygosity. The percentage of embryonic lethality resulting from the experimental crosses was always larger than this control value, showing that each *hid* mutation affects normal embryonic development.

The results presented in Figure 1 suggest that all of the larvae which are hemizygous for the *H99* and *H109* mutations die before hatching and possess an improperly formed CPS; therefore, these are amorphic *hid* mutant alleles. The other four mutant

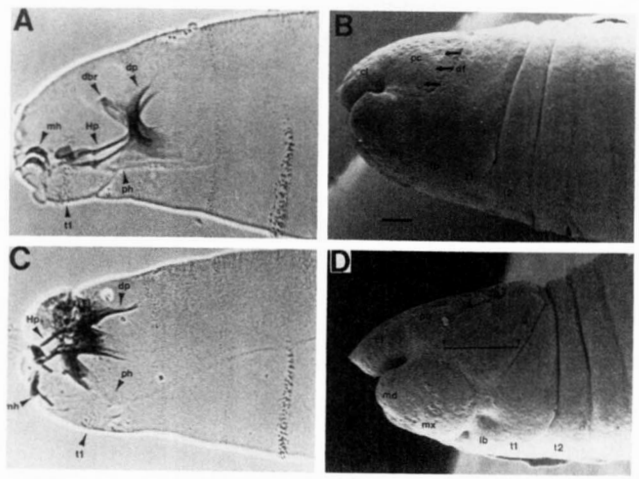

FIGURE 2.—The abnormal development of the CPS in *hid* mutant larvae results from the failure of head involution. Cuticle preparations of unhatched larvae and scanning electron micrographs of developing embryos which are either wild-type (A & B), *hid*^{H99}/*Df* W^{R+4} (C), or *hid*^{H99}/*hid*^{H99} (D). Almost all of the individual components of the wild-type CPS are present in unhatched *hid* mutant larvae, but these components do not show their normal spatial relationship to each other (compare A and C) and the dorsal bridge is frequently missing. During normal embryogenesis, the dorsal aspect of head involution is in progress by early stage 14, as indicated by the presence of the dorsal fold in the wild-type embryo shown in B (arrows show direction of movement of the dorsal fold over the procephalon). In a *hid* mutant embryo of approximately the same stage (as indicated by the completion of the dorsal closure of thoracic segments t1 and t2), a dorsal fold is not present; in contrast to the embryo shown in B, the region lying between the arrowheads in the mutant embryo has not been enclosed within a developing frontal sac. Abbreviations: cl = clypeolabrum, dbr = dorsal bridge, df = dorsal fold, dp = dorsal process, Hp = H-piece, lb = labial segment, md = mandibular segment, mh = mouth hook, mx = maxillary segment, t1 = prothoracic segment, t2 = mesothoracic segment. Anterior is to the left and dorsal is up; the magnification factor for the cuticle preparations is 200×; the bar in panel B indicates 15 μm.

alleles, in comparison, are hypomorphic because they result in lower levels of lethality than the amorphs and far fewer of the unhatched larvae possess an abnormal CPS. The fact that some of the hypomorphic larvae form an apparently normal CPS but still fail to hatch suggests that *hid* may play more than a single role in embryonic development.

Cuticles from the unhatched *H99/Df* W^{R+4} larvae were analyzed using the detailed description made by CAMPOS-ORTEGA and HARTENSTEIN (1985) of the sense organs and cuticular structures present in the anterior region of the wild-type, first instar larva. This examination revealed that although the CPS is not properly formed during the development of the mutant embryos, most of the cuticular components which make up the CPS, as well as the other structures that are located in the anterior region of a wild-type larva, are present in the unhatched mutant larvae (Figure 2). A notable exception to this conclusion, however, is the frequent absence of a well-formed dorsal bridge. The dorsal bridge forms within the frontal sac as the

result of head involution and its absence from the mutant larvae, as well as the abnormal morphology of the CPS, indicates that embryonic head involution requires a *hid*$^+$ function.

A collection of embryos that included *H99* homozyotes, resulting from the cross of *H99/Ore R* flies, was examined with the scanning electron microscope in an attempt to trace the CPS defects to an earlier stage of embryogenesis. The *H99* mutation was chosen because of its complete penetrance and expressivity for the head involution defect; *i.e.*, approximately 25% of the eggs resulting from the mating of *H99/OreR* flies fail to hatch and contain larvae with the *hid* mutant phenotype (data not shown). No obvious phenotypic defects were observed in collections containing embryos less than 12 hr old. In collections containing older embryos, the dorsal aspect of head involution had not occurred; a typical example of one of these mutant embryos is in shown in Figure 2D [compare to embryo undergoing normal head involution in Figure 2B; see also TURNER and MAHOWALD (1979) for scanning electron micrographs of normal head involution]. These results indicate that a *hid*$^+$ function is necessary in some way to facilitate the movement of the dorsal fold across the dorsal surfaces of the procephalic and clypeolabral regions of the head during stages 14 and 15 of embryogenesis.

The failure to form the frontal sac in the *hid* mutant embryos is most likely the cause of the abnormal development of the CPS. SEM examination of older *H99* embryos indicates that early events in the ventral aspect of head involution, involving the movement of the gnathal segments into and around the stomodeum, occur fairly normally in the absence of *hid*$^+$ function (data not shown). This observation is consistent with the fact that those cuticular components of the CPS which develop exclusively from the gnathal segments (*i.e.*, cirri, mouth hooks, antenno-maxillary sense organ, H-piece, pharynx) are always present in the mutant larvae.

Normal rotation of the developing male terminalia requires *hid*$^+$ function: On the basis of morphological evidence, it has been hypothesized that the male terminalia undergo a complete 360° rotation in a clockwise direction during pupal development (GLEICHAUF 1936; GRIFFITHS 1973). Since the orientation of the terminalia of *hid* mutant males varies considerably from the normal position (Figure 3), *hid*$^+$ function appears to be necessary for some aspect of this rotation. Scoring the position of the penis in males heterozygous for different *hid* mutant alleles (Figure 4) suggests that the extent of the normal rotation which occurs in a particular male is related to the amount of *hid*$^+$ function. For example, the penis of 80–85% of the *A22/H109* males had rotated farther than region II (>270°), but this was true for only

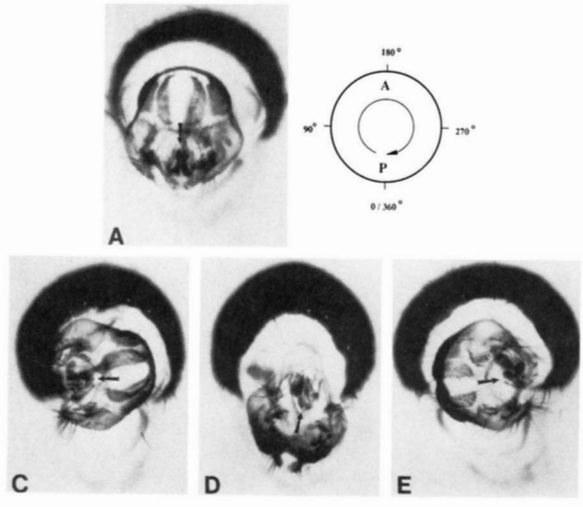

FIGURE 3.—*hid* mutant males have abnormally positioned terminalia. Whole mount preparations of the terminalia taken from wild-type (A) and *hid*^{A22}/*hid*^{H99} (C–E) males; the arrow points to the location of the penis and its base lies in the vicinity of the anus. As illustrated in B, the normal dorsal-ventral relationship of the anus (A) and penis (P) is thought to be established as the result of the 360° clockwise rotation of the terminalia during pupal development; the arrow shows how the position of the penis is affected by this rotation. Dorsal is up; magnification factor 32×.

about 40% of the *A22/H99* males. From these results it is possible to rank the severity of the effect that each *hid* mutation has on the rotation of the terminalia, from the most to the least severe, as follows: *H99* > *H109* > *A22* > *A206* > *A329* > *H89*. This ranking is roughly similar to the one for the degree to which these alleles disrupt embryonic head development, suggesting that the loss of the same *hid*$^+$ function is responsible for both defects. Only the *H89* allele, which has a moderately severe effect on embryonic head morphogenesis but no effect on the rotation of the male terminalia, does not fit this generalization.

***Wrinkled* is an allele of *hid*:** When the *W* mutation is reverted to wild-type by radiation, most of these revertants are associated with visible chromosomal rearrangements which alter the 75C region (Table 2). All of these revertants share a common lethal effect with the *hid* mutations (data not shown) and the surviving mutant heterozygotes show the *hid* mutant wing phenotype along with abnormally positioned terminalia in the males. The simplest explanation of these results is that the Wrinkled phenotype is caused by the abnormal expression of a *hid*$^+$ function. Another, more complex, possibility is that the loss of *hid*$^+$ function in a *W* revertant is purely fortuitous as the W phenotype is caused by the altered expression of a gene adjacent to *hid*. If this latter explanation is correct, then the reversion of *W* by point mutation should yield some revertants that are not allelic with the *hid* mutations.

Six EMS-induced *W* revertants (*R+E1* through *R+E6*) were recovered from the approximately

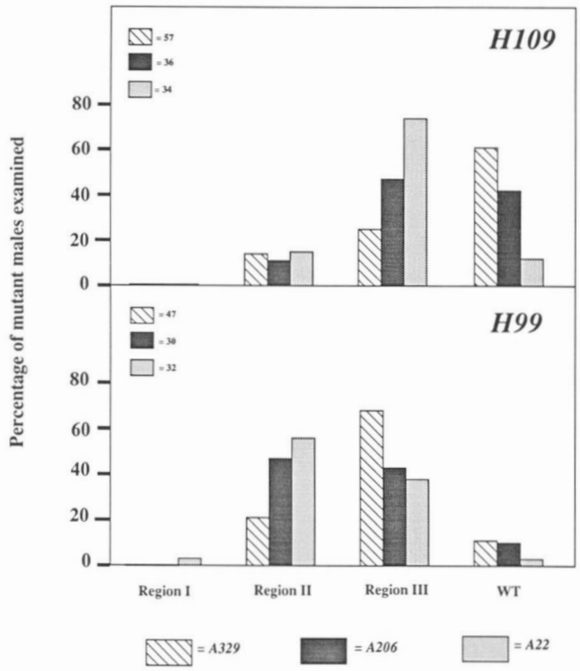

FIGURE 4.—Variation in the position of the penis results from the loss of hid^+ function. Each histogram shows the results obtained from scoring males heterozygous for either hid^{H109} or hid^{H99} and another *hid* mutant allele, which is represented by the type of shading as indicated. The data are expressed as the percentage of mutant males which had a penis located in one of four regions (refer to diagram shown in Figure 3) as follows: the wild-type region (WT) is located directly opposite to the dorsal surface of the abdomen, immediately surrounding the boundary 0°/360°; regions I and III lie on either side of the WT region and include the positions 90° and 270°, respectively; region II includes everything dorsal to regions I and III. The number of males scored for each genotype is shown by the shaded boxes in the the upper left-hand corner of the histograms. Since many of the mutant males die before the effect on the rotation can be assessed (data not shown), it seems reasonable to assume that the effects of each *hid* mutation on the rotation of the terminalia are in fact more severe than is depicted here.

36,000 F_1 progeny which were scored for the loss of the Wrinkled trait. The survival of flies heterozygous for each of these revertants and the amorphic allele *H99* was determined (Figure 5). While essentially all *W/H99* flies survive, many flies heterozygous for *H99* and one of the *W* revertants do not, with the recovery of the mutant heterozygotes ranging between one-quarter to one-half of the numbers expected for full viability depending on which particular revertant is tested. In addition to lethality, all of the surviving mutant heterozygotes exhibit the *hid* mutant wing phenotype and most of the males have abnormally positioned terminalia. These results therefore strongly support the hypothesis that the Wrinkled trait is due to a mutation affecting *hid*.

DISCUSSION

The loss-of-function *hid* alleles do not appear to affect *ter*: One goal for the isolation of point mutations in the 75B8-C1,2 region was to clarify the relationship between the *ter* gene and a genetic function which is necessary for embryonic head involution that is also located in this region. All of the recessive lethal mutations described here affect this function and are therefore alleles of a locus we have named *head involution defective*. Several pieces of evidence resulting from our studies of these mutations argue that ter^+ function is not required for the morphogenetic transformation of the embryonic head region. First, the loss of hid^+ function not only affects head involution, but also the final stages of development of the wings

TABLE 2

Chromosome rearrangements associated with radiation-induced Wrinkled revertants

Revertant	Cytology of rearrangement	Reference
W^{R+8}	In(3LR)75C3-D2;86D1	ASHBURNER *et al* (1980)
W^{R+16}	In(3L)70F1,2;75C3	ASHBURNER *et al.* (1980)
W^{R+19}	T(1;3) 18F3-75C3	ASHBURNER *et al.* (1980)
W^{R+4}	Df(3L) 75B; C4	SEGRAVES and HOGNESS (1990)
W^{R+10}	Df(3L) 75A; C1,2	SEGRAVES and HOGNESS (1990)
W^{R+51}	In(3LR) 75C1,2; 83F + T(2,3) 44F; 84F	SEGRAVES and HOGNESS (1990)
W^{R+X1}	In(3L) 75C1,2; heterochromatin	This investigation
W^{R+X2}	In(3LR) 75C1,2; 85C1,2 + T(2;3) 33B;94A	This investigation

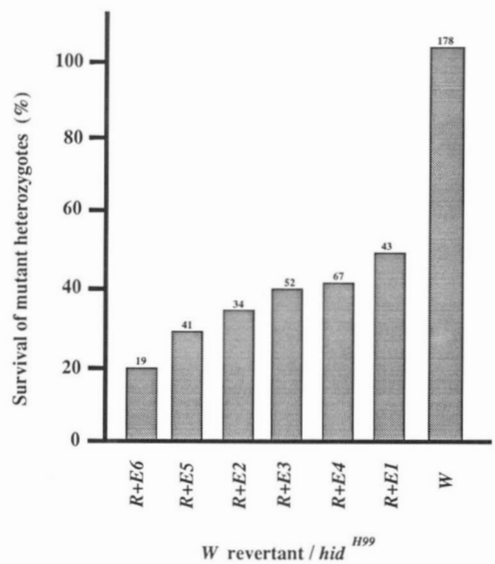

FIGURE 5.—EMS-induced *W* revertants fail to fully complement hid^{H99}. Each bar shows the percentage of flies heterozygous for *H99* and an EMS-induced *W* revertant which survive to adulthood. For comparative purposes, the viability of heterozygotes carrying the parental *W* chromosome and *H99* is also shown. These values represent the survival of the mutant heterozygotes as a percent of the most numerous progeny class, except in the case of the *W*-bearing chromosome; since the *W/H99* class was the most numerous, the survival of these heterozygotes was calculated relative to the next most numerous class.

and male terminalia; these later developmental defects are difficult to reconcile with the early embryonic expression of *ter*. Second, analysis of approximately 80 kb of cloned DNA in the region surrounding *ter* in the *hid* mutants *H89*, *H99* and *H109* reveals no altered restriction fragments, even though at least one of them (*H89*) is associated with a rearrangement breakpoint (M.K. ABBOTT, unpublished results). The proof of the separate identities of *ter* and *hid* will, of course, require the identification of a *hid* transcription unit.

Role of *hid* during embryonic development: SEM examination of the process of head involution in *hid* mutant embryos indicates that the movement of the dorsal fold over the procephalon and clypeolabrum, which normally forms the frontal sac, does not occur in the absence of *hid* function. The failure of the frontal sac to form is most likely responsible for the phenotypic defects observed in the unhatched *hid* mutant larvae, the absence of the dorsal bridge and the abnormal development of the CPS. As CPS development appears to occur normally in many unhatched larvae which are either heterozygous or hemizygous for hypomorphic *hid* alleles, *hid* function may be required for one or more additional aspects of embryogenesis. Since no obvious morphological abnormalities other than the head involution defect were observed in sectioned embryos which lack *hid* function (*i.e.*, $Df W^{R+4}/Df\,Cat$) (MAHONEY 1988; J. A. LENGYEL, unpublished results), a role of *hid* in a subtler aspect of embryonic development remains to be established.

The *hid* function required for head involution is also necessary for the 360° rotation of the male terminalia: On the basis of the looping of the ejaculatory duct around the rectum and the crossing over of the lateral tracheal ducts and the posterior peripheral nerves, it has been hypothesized that the developing terminalia of the Drosophila male undergo a 360° rotation in a clockwise direction during pupation (GLEICHAUF 1936; GRIFFITHS 1973). The fact that the rotation of the terminalia in many *hid* mutant males is frequently not complete indicates that hid^+ function is required for this rotation. That this same *hid* function is involved in head involution is suggested by the fact that an almost identical allelic series is generated whether the alleles are examined with regard to rotation of the terminalia or CPS development.

Although other mutations have been identified that affect the morphology of the male terminalia (LINDSLEY and GRELL 1968), their effect on the development of the terminalia have not been described in as much detail as that of *hid*. The roles played by many of these genes, however, are likely to differ significantly from *hid* since mutations in them, unlike *hid*, also affect the development of the female terminalia. Another mutation similar to *hid* in its specific affect on the male terminalia was *rotated penis* (*rp*), described by MORGAN, STURTEVANT and BRIDGES (1929). The gene affected by this mutation mapped to a position on the left arm of chromosome *III* in the same region containing *hid*, but since *rp* is no longer extant it is not possible to test for allelism between it and the *hid* mutations. Since the products of at least three genes located on the *X* chromosome also affect the rotation of the male terminlia (FAHMY 1958; M. K. ABBOTT, unpublished results), it is clear that the rotation process involves the expression of several different genes.

An antimorphic *hid* allele causes the Wrinkled wing phenotype: Complementation studies among the *hid* recessive lethal alleles and both the EMS- and radiation-induced *W* revertants show that reversion of *W* frequently, if not always, results in a loss-of-function *hid* mutation. Since reversion of a dominant mutation can provide insight into the wild-type function of a gene (HAZELRIGG and KAUFMAN 1983; MCGILL *et al.* 1988), the fact that the loss-of-function *W* revertants behave as *hid* mutant alleles indicates that the Wrinkled phenotype is due to a mutation affecting hid^+ function. This finding also corroborates the conclusion that *hid* plays a role in normal wing development, which is based on the observation that essentially all surviving *hid* mutant heterozygotes exhibit a subtle alteration in wing transparency.

A dominant mutant phenotype which results from the misexpression of normal gene function is the result of a neomorphic allele, whereas it is due to an antimorphic allele if it results from the expression of an altered product that in some manner opposes the normal function (MULLER 1932). The addition of extra copies of the wild-type allele will supress the effect of an antimorphic mutation, but not those of a neomorph (MCGILL *et al.*, 1988). Since a duplication of a portion of the third chromosome containing the 75B8-C1,2 (*i.e.*, hid^+) region almost totally suppresses the W phenotype (M. K. ABBOTT, unpublished), the *W* mutation is an antimorphic *hid* allele.

Speculations regarding the cellular and evolutionary basis of multiple *hid* roles: The *hid* locus is required for the seemingly disparate processes of head involution, rotation of the male terminalia, and post-eclosion wing development. Although establishment of correct head segment identity is a prerequisite for normal head involution (JÜRGENS 1985), we believe it likely that the role of *hid* in head involution is closely related to its apparently morphogenetic role in the development of the wing and male terminalia. A unifying feature of these three processes, therefore, is the possibility that the *hid* product(s) play the same role at the cellular level in each one, perhaps by facilitating interactions between cell sheets and/or changes in cell shape.

The morphological traits resulting from embryonic

head involution and the rotation of the male terminalia are distinctive of the muscomorphan flies, a monophyletic group which includes all Drosophila species (GRIFFITHS 1973; MCALPINE 1981). These traits must both have evolved in an ancestral dipteran lineage leading to the muscomorphan common ancestor. We speculate that the role of *hid* in wing development may be a more evolutionarily ancient role and that, during the evolution of the ancestor of the muscomorphan flies, this gene acquired additional developmental roles in the embryonic head region and the male terminalia. Similarly, the acquisition of new developmental roles over time has been proposed to explain the multiple roles now played by the segment polarity gene *engrailed* (PATEL, KORNBERG and GOODMAN 1989).

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