Mutations in the *bli-4* (*I*) Locus of *Caenorhabditis elegans* Disrupt Both Adult Cuticle and Early Larval Development

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ABSTRACT

The *bli-4* (*I*) gene of *Caenorhabditis elegans* had been previously defined by a single recessive mutation, *e937*, which disrupts the structure of adult-stage cuticle causing the formation of fluid-filled separations of the cuticle layers, or blisters. We report the identification of 11 new alleles of *bli-4*, all early larval lethals, including an allele induced by transposon mutagenesis. Nine of the lethal alleles failed to complement the blistered phenotype of *e937*; two alleles, *s90* and *h754*, complement *e937*. The complementing alleles arrested development somewhat later than the noncomplementing alleles, which blocked just prior to hatching. We conclude that *bli-4* is a complex locus with an essential function late in embryogenesis. We investigated the blistered phenotype of *e937* through interactions with other mutations that alter worm morphology or cuticle structure. Recessive and dominant epistasis of several dumpy mutations over the blistered phenotype was observed. Using two heterochronic mutations that alter the developmental stage at which adult cuticle is expressed, we observed that adult worms that lack an adult-stage cuticle could not express blisters. However, late larval worms that expressed the adult cuticle did not express blisters either. It seems likely that the presence of the adult cuticle is necessary, but not sufficient, for blister expression. Blistering resulting from *e937* is more severe in *trans* to null alleles, indicating that *e937* is hypomorphic. We postulate that the adult-specific blistering is due to an altered or reduced function of *bli-4* gene product in the adult cuticle. In addition to its essential role in development, the *bli-4* gene product is involved in the structure of the cuticle, possibly in a function required for the processing or assembly of structural components.

THE nematode *Caenorhabditis elegans* is a well established genetic model system that has a complex, developmentally regulated extracellular cuticle. For this reason, the cuticle of *C. elegans* has been proposed as a model system for the study of the assembly, architecture and function of extracellular matrices (HIGGINS and HIRSH 1977; COX *et al.* 1980). Studies of *C. elegans* cuticle have focused on three areas: ultrastructural and biochemical analysis (COX, KUSCH and EDGAR 1981; COX, STAPRANS and EDGAR 1981); characterization of collagen genes (COX, KRAMER and HIRSH 1984); and isolation and characterization of mutations affecting cuticular morphology (BRENNER 1974; HIGGINS and HIRSH 1977; COX *et al.* 1980; KUSCH and EDGAR 1986). The first approach to the dissection of the cuticle, biochemical and ultrastructural analyses, has revealed that the cuticle is arranged in two layers, a basal layer and a cortical layer, and is primarily composed of collagenous proteins that are extensively cross-linked by disulfide bonds (COX, KUSCH and EDGAR 1981). The structures of the layers vary with developmental stage (COX, STAPRANS and EDGAR 1981). The adult cuticle has an additional layer consisting of a fluid-filled space spanned by columnar structures termed struts connecting the basal and cortical layers (COX, KUSCH and EDGAR 1981). The second area of study of the cuticle has been the analysis of the *C. elegans* collagen gene family, which consists of 50–150 members, encoding small collagens of 30–40 kilodaltons (kD) that are covalently cross-linked in the cuticle (KRAMER, COX and HIRSH 1982; COX, KRAMER and HIRSH 1984). Collagen gene expression varies with developmental stage (COX and HIRSH 1985; KRAMER, COX and HIRSH 1985). Recently three genes that affect cuticle morphology, *dpy-13* (VON MENDE *et al.* 1988), *sqt-1* (KRAMER *et al.* 1988), and *rol-6* (J. M. KRAMER, personal communication) have been cloned and shown to be collagen genes. The third approach to the study of *C. elegans* cuticle has been the isolation and characterization of mutants that alter the shape of the worm, many of which are likely to alter the cuticle structure. These include *dum*py (*dpy*), *rol*ler (*rol*), *sq*uat (*sqt*), *long* (*lon*), *sma*ll (*sma*), and *bli*ster (*bli*) (BRENNER 1974; HIGGINS and HIRSH 1977; COX *et al.* 1980; KUSCH and EDGAR 1986). Dpy worms are short and fat (BRENNER 1974); Rol worms roll to the right or left due to the helical twisting of their cuticle (BRENNER 1974; HIGGINS and HIRSH 1977); Sqt mutants are dominant Rol and recessive Dpy (COX *et al.* 1980; KUSCH and EDGAR 1986); Lon worms are longer than wild type (BRENNER 1974); Sma are smaller; and Bli have fluid-filled cuticular swellings (BRENNER 1974) resulting from the separation of the basal and cortical layers.

Six genes have been identified that can be mutated to cause fluid-filled blisters in the cuticle of adult worms (BRENNER 1974; PARK and HORVITZ 1986). The blister genes are all incompletely penetrant and variably expressed, despite being in virtually homozygous backgrounds. This paper describes a genetic analysis of a member of the blister class of genes, *bli-4*. The only previously reported allele of *bli-4* was the recessive allele *e937*, which was recovered after mutagenesis with ^{32}P (BRENNER 1974). *e937* results in a visible phenotype, the formation of blisters in homozygous adults. Preliminary results of ours (K. PETERS, cited in HOWELL *et al.* 1987) had shown that a recessive lethal mutation, *h42*, failed to complement both *bli-4(e937)* and the lethal mutation, *let-77(s90)*. Since *e937* and *s90* complemented each other, it was suggested that *h42* could be a small deletion or a two-hit event, or that *e937* and *s90* could be complementing alleles of the same gene. In this study we have investigated the possibility that *bli-4* has an essential function in the development of *C. elegans*. We tested *e937* for complementation with a set of EMS-induced lethal mutations that had been previously mapped to a 0.5 map unit (m.u.) region around *bli-4* (HOWELL *et al.* 1987; MCDOWALL 1990). We report the identification and characterization of 11 new lethal alleles of *bli-4*, including an allele induced by Tc1 mutagenesis (reviewed by MOERMAN and WATERSTON 1989).

MATERIALS AND METHODS

General: Worms were maintained and mated on 10 × 35 mm Petri plates containing strain OP50 *Escherichia coli* streaked on nematode growth media (NGM) (BRENNER 1974) at 20° except where noted otherwise. Nomenclature used conforms to the uniform system for *C. elegans* (HORVITZ *et al.* 1979). Nomenclature for translocations conforms to that used by MCKIM, HOWELL and ROSE (1988).

Strains: The wild-type strain N2 (Bristol) and some mutant strains used in this study were obtained from D. L. BAILLIE, Simon Fraser University, Burnaby, British Columbia, and others from the Caenorhabditis Genetics Center, University of Missouri, Columbia. *bli-4(e937)* was recovered by BRENNER (1974) in an F_2 screen following mutagenesis with ^{32}P. *e937* is not fully penetrant. Ninety-five percent of homozygous worms blister, while the remaining 5% do not express blisters. *bli-4* was first mapped in the *dpy-5* cluster on linkage group (LG) I between *dpy-5* and *dpy-14* by ROSE (1980), and was subsequently positioned between the right-end breakpoints of the duplications *hDp16* and *hDp19* (MCKIM and ROSE 1990). A partial genetic map of the region of LGI around *bli-4* is presented in Figure 1. Mutations used in this study were: LGI *unc-63(e384), dpy-5(e61), unc-40(e1450), bl-4(e937), dpy-14(el88), unc-(3(e51, e450)*; LGII *dpy-10(e128), lin-29(n1440)* LGIII *dpy1(e1), dpy-17(e164), dpy-18(e364)*; LGIV *dpy-9(e424), dpy-13(e184), mut-6(st702)*; LGV *sqt-3(e24)*; LGX *lon-2(e678), dpy-3(e27), dpy-6(e14), lin-14(n179ts)*.

LGI lethal mutations: *s90* was isolated *cis*-linked to *unc-13(e51)* and identified the gene *let-77* (ROSE and BAILLIE 1980). *s90* was maintained in a strain of the genotype *let-77 unc-13+/++unc-15*. *h1010* was isolated as described below, and was maintained in a strain of the genotype *szT1(I;X)[lon-2]/unc-63 bli-4(h1010) unc-13*. The remaining lethal alleles of *bli-4* were isolated on *dpy-5(e61) unc-13(e450)* marked chromosomes in screens using the balancer *sDp2* (HOWELL *et al.* 1987; MCDOWALL 1990). These *bli-4* lethal alleles were maintained in strains having the genotype *sDp2/dpy-5 let-X unc-13/dpy-5 let-X unc-13*.

Chromosomal rearrangements: *sDp2(I)* is a free duplication of the right-third of LGI. *sDp2* is not transmitted through the male (ROSE, BAILLIE and CURRAN 1984). *szT1(I;X)* is a reciprocal translocation involving LGI and LGX. *szT1* completely suppresses recombination on the left half of LGI and spontaneously segregates 3% *lon-2* males due to *X* chromosome nondisjunction. The *lon-2* males are heterozygous for the LGI markers and can be used for mating (FODOR and DEAK 1985; MCKIM, HOWELL and ROSE 1988).

Screen for mutator-induced alleles of *bli-4*: *mut-6* causes high levels of transposition of the transposable genetic element Tc1 (MORI, MOERMAN and WATERSON 1988). A mutator strain (KR1822) of the genotype *unc-63(e384) unc-13(e450); mut-6(st702)* was constructed (*mut-6* was from RW7097, a strain obtained from D. G. MOERMAN and R. H. WATERSTON). Mutator activity in KR1822 was confirmed by screening in 1% nicotine for twitcher worms resulting from the insertion of Tc1 into the *unc-22* gene as described by MORI, MOERMAN and WATERSON (1988). KR1822 segregates spontaneous twitchers at a rate of 3×10^{-4}. KR1822 was screened for spontaneous *bli-4* alleles by mating KR1822 hermaphrodites to *dpy-5(e61) bli-4(e937)/++* heterozygous males, and screening the progeny for blisters. Three Bli worms, two hermaphrodites and one male, were identified after screening 82,300 chromosomes, an induction frequency of 3.6×10^{-5}. Of the three spontaneous blistered animals recovered, one survived. The surviving hermaphrodite carried a *bli-4* lethal allele designated *h1010* and was maintained using the translocation *szT1(I;X)* in the strain KR1858.

Complementation testing: *sDp2 lethal alleles:* Lethal alleles rescued by sDp2 were complementation tested *inter se* as described by HOWELL *et al.* (1987). Heterozygous males of the genotype *dpy-5 let-X unc-13/+++* were mated to hermaphrodites of the genotype *sDp2/dpy-5 let-Y unc-13/dpy-5 let-Y unc-13*. The absence of fertile Dpy-5 Unc-13 in the cross progeny indicated failure to complement.

sDp2 lethal alleles and e937: Complementation tests were done in both of the following ways. Heterozygous males of the genotype *dpy-5 let-X unc-13/+++* were mated to (1) hermaphrodites of the genotype *bli-4(e937) unc-13/bli-4(e937) unc-13*; the presence of Bli-4 Unc-13 males indicated failure to complement. (2) Hermaphrodites of the genotype *bli-4(e937)/bli-4(e937)*; the presence of Bli-4 males in the cross progeny indicated failure to complement. In reciprocal crosses, both *bli-4 unc-13/++* males and *bli-4/+* males were mated to *sDp2/dpy-5 let-X unc-13/dpy-5 let-X unc-13* hermaphrodites. The presence of Bli-4 Unc-13 or Bli-4 hermaphrodites and males indicated failure to complement.

let-77(s90) and bli-4(h1010): let-77(s90) and *bli-4(h1010)* were not linked to *dpy-5*, making it necessary to use a different complementation testing protocol from that used for the *sDp2* balanced lethal alleles. *s90* and *h1010* were each balanced by the translocation *szT1(I;X)*, and complementation tests performed as follows: (1) *dpy-5 let-X unc-13/+++* males were crossed to *szT1(I;X)[lon-2]/let-77 unc13* or *szT1(I;X) [lon-2]/unc-63 bli-4(h1010) unc-13* hermaphrodites. The absence of Unc-13 progeny indicated failure to complement. Successful mating was indicated by the presence of wild-type males. (2) Spontaneous Lon-2 males of the genotype *szT1(I;X) [lon-2]/let-77 unc13; 0* or *szT1(I;X)[lon-2]/unc-63 bli-4(h1010) unc-13; 0* were crossed to *bli-4(e937)*

TABLE 1

***bli-4* map data**

Allele	Maternal genotype	Wild type	Dpy	Unc	Recombination frequency (m.u.)[a]	
					dpy-5 to *bli-4*	*bli-4* to *unc-13*
h42	*dpy-5 h42 unc-13* / + + +	1376	10	8	0.5 m.u. (0.3–0.9)[b]	0.4 m.u. (0.2–0.8)
h199	*dpy-5 h199 unc-13* / + + +	1781	0	10	0.0 m.u. (0.0–0.1)	0.4 m.u. (0.2–0.8)
h254	*dpy-5 h254 unc-13* / + + +	1033	9	3	0.7 m.u. (0.3–1.2)	0.2 m.u. (0.1–0.5)
s90	*s90 unc-13* / + +	1896	N/A	14	N/A	0.6 m.u. (0.3–0.9)

[a] Recombination frequency of lethal alleles was calculated using the mapping function $p = 1 - (1-2R)^{1/2}$ where R = (number of recombinant progeny in one class)/(4/3) number wild-type progeny + one recombinant class (ROSE and BAILLIE 1979).

[b] 95% confidence intervals are given in parentheses. Confidence intervals were calculated using the statistics of CROW and GARDENER (1959).

unc-13 hermaphrodites. The presence of non-Bli-4 Unc-13 male progeny and the absence of Bli Unc-13 male progeny indicated complementation (Bli Unc hermaphrodites in this experiment could have resulted from self-fertilization). In reciprocal crosses, the presence of non-Bli-4 Unc-13 and the absence of Bli-4 Unc-13 hermaphrodite and male progeny indicated complementation.

Determination of penetrance: Penetrance was defined as the percentage of blistered animals out of the total number of *e937* homozygotes. Penetrance was determined by scoring adult hermaphrodites for blisters. These were either Bli-4 Dpy-X homozygotes or non-Dpy F_1 progeny from the cross *bli-4(e937)/+* heterozygous males by *bli-4(e937); dpy-X* hermaphrodites. Because the males were *bli-4* heterozygotes, a maximum of 50% of the worms were expected to blister if penetrance was 100%. Therefore penetrance in the cross experiment was defined as the percentage of blistered worms out of one-half the total number of progeny.

Mapping *bli-4* alleles: *h42*, *h199*, *h254* and *e937* were three-factor mapped by scoring segregation from strains bearing *cis*-linked flanking markers *dpy-5* and *unc-13* in *trans* to an unmarked chromosome (Table 1). Each allele mapped near the center of the interval, with the exception of *h199* (see below). Recombinants were picked as Dpy-5 or Unc-13 worms and progeny tested for the presence of *bli-4*. *s90*, which was not induced on a *dpy-5* chromosome, was two-factor mapped to 1.1 m.u. from *unc-13(e51)*, consistent with a position between *dpy-5 unc-13*. Recombination frequency was calculated using the mapping function $p = 1 - (1 - 2R)^{1/2}$ where R is the fraction of recombinant progeny over total progeny (BRENNER 1974), and total progeny is calculated as 4/3 (the number of wild type plus one recombinant class) (ROSE and BAILLIE 1979).

h199 mapped 0.8 m.u. from *unc-13*, but failed to recombine with *dpy-5*. This apparent crossover suppression could indicate that *h199* is a deficiency spanning *bli-4*, or that *h199* is linked to a second mutation in an essential gene closely linked or to the left of *dpy-5*. *h199* is unlikely to be a deficiency, because it complements alleles of *unc-40*, which is between *bli-4* and *dpy-5*. However, the chromosome carrying *h199* fails to complement *sDf4*, a deficiency of *dpy-5* (HOWELL 1989). *h199* could be an inversion with breakpoints in *bli-4* and an essential gene in *sDf4*. More likely, however, *h199* is a double hit of *bli-4* and a gene to the left of *unc-40* (Figure 1).

We confirmed the position of the *e937* allele of *bli-4* on LGI between the markers *dpy-5* and *unc-13* by three-factor mapping. Recombinant F_1 progeny of hermaphrodites of the genotype *dpy-5 bli-4 unc-13(e450)/+++* were picked. Eleven Bli Unc, six Dpy, ten Unc, and seven Bli Dpy recombinants were recovered. This gives the map position *dpy-5* (17/34) *bli-4* (17/34) *unc-13*, placing *bli-4* at the center of the *dpy-5 unc-13* interval, which is 1.6 m.u. (HOWELL *et al.* 1987).

Determination of lethal blocking stages: The stage at which lethal homozygotes arrested development was determined. Several heterozygous hermaphrodites of the genotype *dpy-5 let-X unc-13/+++* were permitted to lay eggs on an NGM plate for a short period (not more than 2 hr) and then the homozygous lethal progeny were examined by Normarsky differential interference microscopy for time of arrest.

RESULTS

Identification of lethal alleles that failed to complement *bli-4(e937)*: To reduce the number of complementation tests required to identify which of the approximately 500 lethals rescued by *sDp2* were allelic to *bli-4*, the lethals were mapped using duplications of the *bli-4* region described in MCKIM and ROSE 1990. Forty-four of these lethal mutations, representing 16 genes, were mapped to the 0.5-m.u. interval around *bli-4* defined by the breakpoints of *hDp16* and *hDp19*, shown in Figure 1 (MCDOWALL 1990). We tested each lethal in this interval for complementation by *bli-4(e937)*, and identified eight lethal mutations that failed to complement the blistered phenotype produced by *e937*. These eight lethal mutations also failed to complement each other with respect to lethality. An additional noncomplementing lethal allele, *h1010*, was identified as a spontaneous allele in a mutator strain carrying the mutator *mut-6* as described in MATERIALS AND METHODS. The mutator-induced allele failed to complement *e937* for blistering, and all *bli-4* lethal alleles.

Identification of complementing alleles of *bli-4*: The lethal mutation *s90*, which had fully complemented *e937* (ROSE and BAILLIE 1980) failed to complement the lethal alleles of *bli-4*. A second lethal,

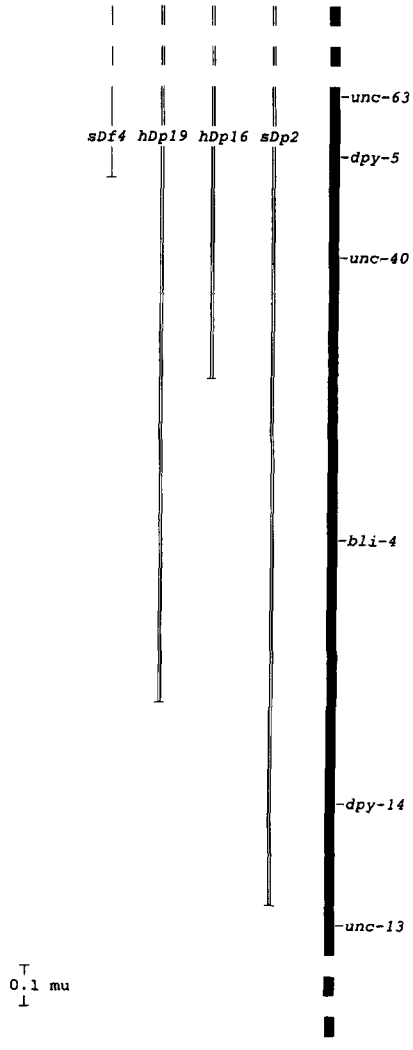

FIGURE 1.—A partial genetic map of the region of LGI around *bli-4* illustrating map positions for closely linked visible markers and duplications. Some map data are from EDGLEY and RIDDLE (1987). The *sDp2* breakpoint was mapped by ROSE, BAILLIE and CURRAN (1984). *hDp16*, *hDp19* and *sDf4* were mapped by MCDOWALL (1990).

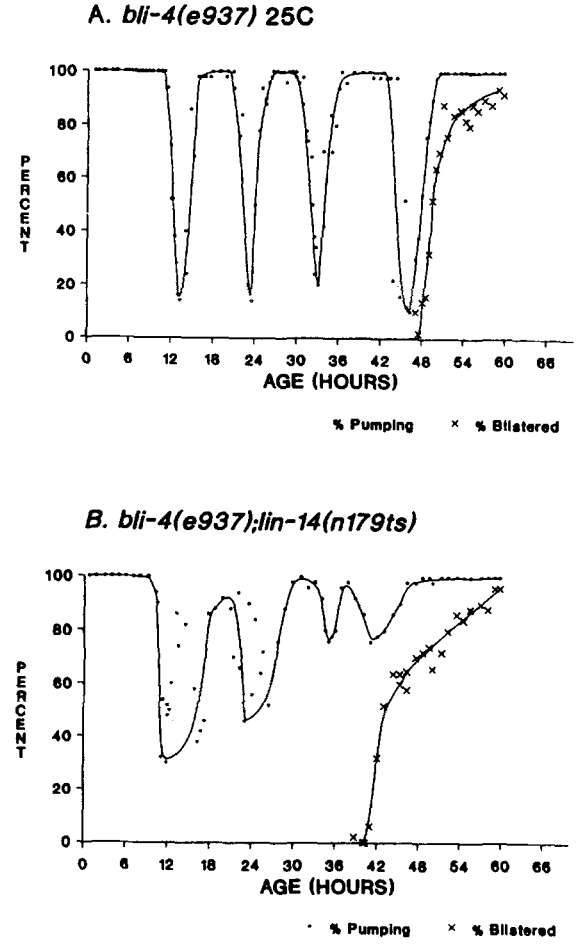

FIGURE 2.—Lethargus periods and expression of blistering in (A) Bli-4 and (B) Bli-4; Lin-14 hermaphrodites. Synchronous populations of several hundred animals were hatched at time zero (±1 hr) and grown on NGM plates at 25° using the method of CASSADA and RUSSELL (1975). At frequent intervals, 50 animals were observed for 5 sec and the percentage with pharyngeal pumping recorded.

h754 (rescued by *sDp2*) also complemented *e937* fully but failed to complement the *bli-4* lethal alleles. Either all of the lethal mutations are deletions, or *s90*, *h754* and *e937* are complementing alleles of *bli-4*. The eight *sDp2* rescued lethals complemented all other lethal complementation groups in the *hDp16* to *hDp19* interval, and there is no other evidence that they are deletions. On this basis, we conclude that, in addition to its function in the adult cuticle, *bli-4* has a function essential in early development, and that the early function and adult cuticle function are independently mutable.

Determination of developmental arrest stage: The complementing alleles (*s90* and *h754*) arrest development during the L1 stage, later than the noncomplementing alleles. Some residual *bli-4* gene product may be functioning in larvae mutant for the complementing alleles. All of the noncomplementing alleles of *bli-4*, including the mutator allele *h1010*, arrest development at or just before hatching. Developmental arrest immediately prior to hatching is the most severe and the most common phenotype of *bli-4* alleles, and is therefore likely to be the null phenotype. This null phenotype indicates an essential function late in embryogenesis.

Disruption of adult cuticle: To determine exactly when during development *e937* worms first blister, we used the method of CASSADA and RUSSELL (1975). Wild-type worms go through four larval stages prior to maturing to adulthood and express the adult cuticle after the fourth molt. The larval to adult molt occurs at 35.5 hr after hatching at 25° (WOOD *et al.* 1980). *C. elegans* undergoes a period of reduced activity prior to each molt, termed the lethargus period. During this period, movement is reduced and pharyngeal pumping ceases. Lethargus periods in *bli-4(e937)* hermaphrodites were monitored at 25° by plotting the percentage of worms that were pumping in synchronous populations with respect to time (Figure 2). The blistered phenotype is adult specific: larval stage

worms did not blister. *e937* hermaphrodites first expressed blisters about 2 hr after the adult molt at 25°. We observed that *e937* worms reached the adult molt at 46 hr after hatching, 11 hr later than wild-type worms. Thus, although *e937* lacks visible effects on larval-stage worms, it slows growth by about 30%.

To determine if the adult specificity of blistering in *e937* worms is due to a requirement for the expression of adult cuticle or the expression of other adult-specific structures, we studied the interaction of *e937* with mutations of the heterochronic genes *lin-29 (III)* and *lin-14 (X)*. *lin-29(nt440)* and *lin-14(nt79ts)* have reciprocal effects on the timing of the expression of the adult cuticle causing retarded and precocious expression, respectively.

lin-29 loss-of-function alleles fail to make the L4 to adult cuticle switch, and reiterate the L4 stage cuticle, causing the animals to undergo extra molts (AMBROS and HORVITZ 1984). This is the only known effect of *lin-29* mutations. We predicted that mutations in *lin-29* would suppress blistering if expression of the *e937* phenotype requires an adult cuticle. A *bli-4(e937); lin-29(n1440)* double mutant was constructed and screened for the expression of blisters. *bli-4(e937); lin-29(n1440)* worms did not express blisters: 0/1160 F_1 progeny of *bli-4; lin-29* hermaphrodites expressed blisters at any age. While the possibility that *lin-29* has effects other that the simple reiteration of L4 cuticle cannot be ruled out, it is most likely that blisters could not form in Lin-29 hermaphrodites because they lacked an adult cuticle. On this basis, we suggest that blisters cannot form in adult worms not expressing the adult cuticle.

In contrast to *lin-29(n1440)*, the *lin-14* loss-of-function allele *n179ts* results in the precocious expression of the adult cuticle after the third molt at the restrictive temperature of 25° (AMBROS and HORVITZ 1984, 1987). Hermaphrodites undergo a fourth molt producing a second adult cuticle. If the expression of the blistered phenotype requires an adult cuticle, then the precocious expression of the adult cuticle in *n179* worms was predicted to permit blistering one molt earlier than in wild type. A *bli-4(e937); lin-14(n179ts)* double mutant was constructed. Lethargus periods in *bli-4(e937); lin-14(n179ts)* hermaphrodites at 25° were determined by plotting the percentage of worms that were pumping in synchronous populations with respect to time (Figure 2B). The interaction of *bli-4* and *lin-14* was unexpectedly complex. At the restrictive temperature of 25°, most *bli-4; lin-14* animals were sterile. Twenty percent of these worms arrested development prior to reaching the fourth molt. In addition, the rate of growth of the double mutant was variable. Consequently, synchronous populations quickly ceased to be synchronous. This may be seen by comparing the graph in Figure 2A with that in Figure 2B. Sterility, variable growth rates and variable larval arrests are not characteristics of either *lin-14(n179)* or *bli-4(e937)* alone. We conclude that *bli-4(e937)* and *lin-14(n179)* produce an incompletely penetrant synthetic lethality in the double mutant.

From the data presented in Figure 2B, it appears that blistering in the *bli-4; lin-14* double mutant did not occur until after the fourth molt. All of the blistered worms appeared to be adults based on size. Moreover, we did not observe any blistered worm undergoing a lethargus period or molting. Thus, *lin-14(n179)* does not seem to alter the expression of blistering with respect to the number of molts.

The cuticle structures of larval-stage worms are not affected by the *e937* mutation, which requires the presence of the adult cuticle to form blisters. However, the presence of the adult cuticle in *e937* animals does not appear to be sufficient for the expression of blisters. Based on the observed interactions of *bli-4(e937)* with *lin-14* and *lin-29*, we suggest that the adult-specific blistering is due to an altered or reduced function of *bli-4* gene product in the adult cuticle.

Interaction with Dpys: Roller, squat and some dumpy phenotypes are generally epistatic to blistering in *bli-1* and *bli-2* mutants (HIGGINS and HIRSH 1977; COX *et al.* 1980). We observed that *dpy-5* also has a dominant effect on blistering. To determine the effect of Dpys, double mutants of Bli-4 and 11 Dpys were constructed. The penetrance of blistering in *e937* homozygotes was determined in *dpy-X* homozygous and heterozygous backgrounds (Table 2). The average body length for each *dpy3* mutation is presented as a measure of severity. Bli-4 length is about 1.2 mm.

In non-Dpy Bli-4 animals, blister penetrance is 95%. In Dpy homozygotes, blistering was completely or almost completely suppressed by 9 of the 11 Dpy mutations. The exceptions were *dpy-14* and *dpy-17*, in which blister penetrance was reduced to 41 and 54%, respectively. *dpy-14(e188)* and *dpy-17(e164)* are two of the least severe dpy mutations (Table 2). This raises the possibility that BIi-4 penetrance in Dpy worms is related to Dpy severity. However, two other mild *dpy* mutations tested, *dpy-18(e364)* and the *e24* allele of *sqt-3*, did not permit the expression of blisters. In contrast, the *e1* allele of *dpy-1*, one of the most severe *dpy* mutations tested, produced three Dpy Bli worms. Thus, Bli-4 penetrance does not correlate with severity of the Dpy phenotype.

In *dpy* heterozygotes, blistering was completely or almost completely suppressed by 3 of the 11 *dpy* mutations: *dpy-5(e61)* and *dpy-13(e184)* both dominantly suppressed blistering completely in hermaphrodites and nearly completely in males; *dpy-6(e14)*, which is *X*-linked, nearly completely suppressed blistering in hermaphrodites (Table 2). *dpy-13(e184)* is semidominant, but *dpy-5(e61)* and *dpy-6(e14)* are recessive. *dpy-9(e424)* and *dpy-10(e128)* dominantly reduce Bli-4 penetrance to about 20%. Both are fully

TABLE 2

bli-4(e937) penetrance in dpy homozygote and heterozygote backgrounds

dpy-X	Mean length (mm)[a]	Percent penetrance[b] in bli-4; dpy-X / bli-4; dpy-X	Percent penetrance[c] in bli-4; dpy-X / bli-4; +	
		Hermaphrodites	Hermaphrodites	Males
dpy-1	0.50 (0.036)	<1 (761)[d]	58 (214)	51 (203)
dpy-3	0.54 (0.040)	0 (909)	40 (421)	0 (405)[e]
dpy-5	0.49 (0.051)	0 (735)	0 (459)	6 (442)
dpy-6	0.50 (0.051)	0 (245)	2 (346)	0 (337)[e]
dpy-9	0.55 (0.036)	0 (1435)	19 (448)	22 (461)
dpy-10	0.53 (0.050)	0 (885)	19 (446)	12 (427)
dpy-13	0.49 (0.046)	0 (1435)	0 (437)	<1 (427)
dpy-14	0.57 (0.055)	41 (343)	100 (159)	88 (164)
dpy-17	0.72 (0.065)	54 (1564)	68 (361)	81 (370)
dpy-18	0.68 (0.066)	0 (687)	66 (373)	98 (361)
sqt-3	0.81 (0.054)	0 (345)	58 (442)	51 (424)

[a] Average length of 20 *dpy-X unc-13* adult hermaphrodites. Standard error is presented in brackets.

[b] Penetrance is defined as the percentage of blistered worms out of the total number observed.

[c] Because the *bli-4;dpy-X* hermaphrodites were mated to *bli-4* heterozygous males in this experiment (see MATERIALS AND METHODS), a maximum of 50% of the worms are expected to blister if penetrance is 100%. Therefore, penetrance is defined as the percentage of blistered worms out of one half the total number of observed, or twice the percentage of blistered worms to a maximum of 100%.

[d] Number of worms.

[e] *dpy-3* and *dpy-6* are *X*-linked. Therefore, male progeny resulting from this mating are Dpy.

recessive. *dpy-3(e27)*, *dpy-14(e188)*, *dpy-17(e164)*, *dpy-18(e364)* and *sqt-3(e24)* all had moderate or no dominant effects on Bli-4 penetrance. Of these, only *sqt-3(e24)* is semidominant.

Phenotype of e937: The dominant epistasis of *dpy-5(e61)* over the blistered phenotype of *e937* was reversed when *e937* was heterozygous to a lethal (presumably null) allele. When *bli-4(e937)/bli-4(e937)* hermaphrodites were crossed to *dpy-5 bli-4(h42) unc-13/ +++* males, 46% of hermaphrodites and 50% of males were blistered. The maximum percentage of blistered progeny expected in this cross was 50%. Similar results were obtained with other lethal alleles (data not shown). This result suggests that *e937* is more severe in *trans* to null alleles, indicating that it is hypomorphic according to the definition of MULLER (1937).

DISCUSSION

This paper describes a genetic analysis of the *bli-4* gene, a member of a class of genes that can be mutated to cause the formation of fluid filled cuticular blisters. We have found that mutations in *bli-4* disrupt both the structure of adult-cuticle and development at the time of hatching. These two functions are independently mutable. None of the other five blister genes of *C. elegans* has been shown to be essential to development.

Two observations support a role for the *bli-4* gene product in the adult cuticle. First, *e937* causes an obvious cuticular abnormality, the formation of blisters. Second, blistering is modified by many mutations that affect worm morphology, including some that are known to affect cuticle structures directly. Blistering in *bli-1* and *bli-2*, for example, is reduced in many Dpy, Rol and Sqt mutants (HIGGINS and HIRSH 1977; COX *et al.* 1980). The interaction of *bli-4(e937)* with Dpy mutations is comparable to that of *bli-1* and *bli-2*. All of the mutations tested in our study suppressed blistering to some extent.

COX *et al.* (1980) suggested that interactions between Dpy and Bli phenotypes represent a pattern of structural interdependence rather than a series of enzymatic steps. *dpy-5* disrupts cuticle structure (OUAZANA, GARRONE and GODET 1985), and *dpy-13* (VON MENDE *et al.* 1988) and *dpy-10* (A. LEVY and J. KRAMER, personal communication) have been shown to encode collagens that are likely to be structural components of the cuticle. Thus, it seems likely that the interaction of *bli-4* with these genes results from the disruption of cuticle structure such that the cuticle of these worms is altered to resist blistering. This result does not imply a direct interaction. In contrast, *dpy-14* and *dpy-17* had a small affect on blistering. It is possible that these genes do not encode cuticle structural components, or if they do, that those components do not affect structures involved in blistering.

In addition to recessive epistasis, Dpy phenotypes were found to reduce blistering dominantly or semidominantly. Strong dominant effects were observed for both semidominant (*dpy-13*) and fully recessive (*dpy-5* and *dpy-6*) Dpy phenotypes.

To determine the effect of the timing of adult cuticle expression on blister expression, heterochronic mutants of *lin-29* and *lin-14* were used. *lin-29(n1440)* causes the reiteration of larval cuticle structures in adult worms (AMBROS and HORVITZ 1984). The complete absence of blistering in *bli-4(e937); lin-29(n1440)* animals suggests that the presence of the adult cuticle is a prerequisite to blister formation in *e937* worms. This conclusion is based on the assumption that *n1440* affects cuticle only, and that the reiterated L4 cuticle in adults is a normal larval cuticle.

In contrast to *lin-29(n1440)*, *lin-14(n179)* permits the expression of the adult cuticle one molt earlier than in wild type. The interaction of *lin-14(n179)* with *e937* was predicted to be opposite to that of *lin-29*. However, this was not the case. The fact that *bli-4; lin-14* worms did not blister until after the fourth molt suggested that the expression of the adult cuticle is not, by itself, sufficient to permit the expression of blisters. The interpretation of this experiment is not clear, however, because, unlike *lin-29*, *lin-14* is pleiotropic. In addition to their effects on the hypodermal cell lineages, *lin-14* loss of function alleles cause pre-

cocious expression in cell lineages generating intestine (E cell lineage), neuroblasts (Q cell lineage), and mesodermal structures (M cell lineage) (AMBROS and HORVITZ 1984, 1987). Furthermore, it is possible that Bli-4 Lin-14 L4 larvae do not express a normal adult cuticle.

Blister formation is incompletely penetrant and variably expressed, despite the fact that the CB937 strain (*bli-4(e937)* homozygote) has been maintained as a self-fertilizing and presumably isogenic hermaphrodite for hundreds of generations. Thus the variability in expression of the blistered phenotype results from variability inherent in the altered gene product in *e937*, and not from the segregation of phenotypic modifiers. None of the other *bli-4* alleles resulted in incompletely penetrant or variably expressed phenotypes. In the case of *bli-4*, therefore, incomplete penetrance and variable expression are specific to the *e937* allele.

The identification of 11 new lethal alleles of *bli-4* brings the number of alleles of this gene to 12. We have identified two classes of *bli-4* lethal alleles: lethal alleles that fail to complement the original allele, *e937* (nine alleles); and lethal alleles that complement the viable allele (two alleles). One allele, *h1010*, was induced in a screen for a transposon-induced allele. *h1010* fails to complement *e937* and is a recessive lethal that arrests at hatching.

The induction frequency of *bli-4* alleles in the *sDp2* set is nine in 31,600 chromosomes screened, or 3×10^{-4}. The average induction frequency of mutations in essential genes in the *sDp2* set is 5×10^{-5} (HOWELL *et al.* 1987). Thus, *bli-4* lethal allele induction frequency is six times greater than the average. This high frequency of induction indicates that *bli-4* is either a large gene or is mutationally sensitive.

The identification of *bli-4* lethal alleles that arrest at hatching indicates that *bli-4* has an essential function in development. The majority of these alleles (9 out of 11) cause more severe blisters when heterozygous to *e937* than does *e937* alone. Thus, they provide less of the *bli-4* product than *e937* does. This fact, combined with the high frequency of induction of the lethal alleles, and the fact that these alleles block at the same time, suggest that the developmental arrest phenotype of *bli-4* lethal alleles is most likely the null phenotype of this gene. This conclusion is supported by the fact that the mutator allele *h1010* results from the insertion of the 1.6-kb transposable element Tc1 into the *bli-4* coding region (K. PETERS, unpublished results).

Two of the *bli-4* lethal alleles, *s90* and *h754*, complement the visible phenotype of *e937*, and arrest development somewhat later than the other lethal alleles. Thus, these alleles are not null, and can provide wild-type *bli-4* function in adults in *trans* to *e937*. The identification of *bli-4* lethal alleles that complement the viable allele *e937* indicates that the adult cuticle function disrupted by *e937* and the essential function of *bli-4* are independently mutable. This observation may reflect two functions of *bli-4*, separately mutable domains, or separate times of expression.

What types of functions might *bli-4* encode? *A priori*, two classes of genes affecting the morphology of the cuticle might be predicted: genes that encode structural components of the cuticle; and genes that encode enzymatic functions that cross-link or otherwise process the components of the cuticle so that they assemble correctly. The first class are those genes that encode cuticle components. Four genes that encode collagens with mutations that affect cuticle structure have been cloned. These are: *sqt-1* (KRAMER *et al.* 1988); *dpy-13* (VON MENDE *et al.* 1988), *dpy-10* and *rol-6* (J. KRAMER, personal communication). Because cuticle components physically interact with each other, they are predicted to have characteristics unique to complex multimeric structures. *sqt-1* and *rol-6* both possess genetic properties predicted of redundant gene products that physically interact in a multimeric structure, including: frequent dominant alleles; unpredictable intergenic and intragenic interactions affecting cuticle structure; and a wild-type null phenotype (KUSCH and EDGAR 1986).

The second predicted class of genes that affect cuticle morphology are those encoding enzymatic functions that cross-link, or otherwise process, the components of the cuticle so that they assemble correctly. KUSCH and EDGAR (1986) suggest that enzymatic functions required for cross-linking or processing of cuticle components could affect cuticle morphology, but, in contrast to structural cuticle components, phenotypes resulting from mutations in such genes might be expected to differ from each other in severity rather than overall shape. That is, mutations in an enzymatic function would not result in several different morphological phenotypes. There are currently no examples of mutations in genes known to encode enzymatic functions required specifically by the cuticle. An enzymatic function required for the processing might be expected to affect more than one gene product. The fact that *bli-4(e937)* slows the growth of the worm indicates that it affects processes or structures other that the adult cuticle. At least some of these processes or structures are essential to development, as indicated by the early larval lethal null phenotype of *bli-4*.

bli-4 exhibits none of the characteristics associated with the *sqt*, *rol* or *dpy* genes known to encode collagens. It is certainly not a redundant member of the collagen gene family. It is possible that *bli-4* encodes a structural component of the cuticle with an essential role in the early larva. Examples of structural proteins with larval lethal phenotypes have been described, *clb-*

1 and *clb-2* (GUO and KRAMER 1989; GUO, JOHNSON and KRAMER 1991). These genes encode the *C. elegans* alpha2(IV) and alpha1(IV) collagens, which are components of basement membranes. More likely, *bli-4* may encode an enzymatic function required throughout development.

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