The Generation and Genetic Analysis of Suppressors of Lethal Mutations in the *Caenorhabditis elegans rol-3(V)* Gene

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ABSTRACT

The *Caenorhabditis elegans rol-3(e754)* mutation is a member of a general class of mutations affecting gross morphology, presumably through disruption of the nematode cuticle. Adult worms homozygous for *rol-3(e754)* exhibit rotation about their long axis associated with a left-hand twisted cuticle, musculature, gut and ventral nerve cord. Our laboratory previously isolated 12 recessive lethal alleles of *rol-3*. All these lethal alleles cause an arrest in development at either early or mid-larval stages, suggesting that the *rol-3* gene product performs an essential developmental function. Furthermore, through the use of the heterochronic mutants *lin-14* and *lin-29*, we have established that the expression of *rol-3(e754)*'s adult specific visible function is not dependent on the presence of an adult cuticle. In an attempt to understand *rol-3*'s developmental role we sought to identify other genes whose products interact with that of *rol-3*. Toward this end, we generated eight EMS induced and two gamma irradiation-induced recessive suppressors of the temperature sensitive (ts) mid-larval lethal phenotype of *rol-3(s1040*ts). These suppressors define two complementation groups *srl-1 II* and *srl-2 III*; and, while they suppress the *rol-3(s1040)* lethality, they do not suppress the adult specific visible rolling phenotype. Furthermore, there is a complex genetic interaction between *srl-2* and *srl-1* such that *srl-2(s2506)* fails to complement all *srl* alleles tested. These results suggest that *srl-1* and *srl-2* may share a common function and, thus, possibly constitute members of the same gene family. Mutations in both *srl-1* and *srl-2* produce no obvious hermaphrodite phenotypes in the absence of *rol-3(s1040*ts); however, males homozygous for either *srl-1* or *srl-2* display aberrant tail morphology. We present evidence suggesting that the members of *srl-2* are not allele specific with respect to their suppression of *rol-3* lethality, and that *rol-3* may act in some way to influence proper posterior morphogenesis. Finally, based on our genetic analysis of *rol-3* and the *srl* mutations, we present a model whereby the wild-type products of the *srl* loci act in a concerted manner to negatively regulate the *rol-3* gene.

IN the nematode *Caenorhabditis elegans*, a developmentally regulated proteinaceous extracellular structure surrounds and encloses the animal. This structure, the nematode cuticle, is composed primarily of collagens and is thought to play a role in the definition and maintenance of *C. elegans* body morphology (COX, KUSCH and EDGAR 1981; COX, STAPRANS and EDGAR 1981; COX and HIRSH 1985). Therefore, mutations in genes encoding cuticle components should affect gross morphology (KUSCH and EDGAR 1986). Several morphology mutations have been isolated and analyzed genetically in *C. elegans*, they include Dumpy, Blister, Long and Squat mutants. Recent molecular analysis of some of these morphological mutants have confirmed their role in cuticle formation. For example, *sqt-1* (KRAMER *et al.* 1988), *dpy-13* (VON MENDE *et al.* 1988) and *rol-6* (KRAMER *et al.* 1990) are known to encode collagens. Roller mutants display abnormally twisted cuticles and rotate about their long axis as they crawl. Previous theories suggest that roller mutants are the result of mutations affecting cuticle structure (HIGGINS and HIRSH 1977; COX *et al.* 1980; GREENWALD and MOERMAN 1989). Our laboratory is analyzing mutations of a particular roller mutant: *rol-3*. *rol-3* is on the left half of LGV and was initially defined by the recessive visible allele *e754* (BRENNER 1974). Worms homozygous for *rol-3(e754)* display abnormally left-hand twisted cuticles, body musculature, gut and ventral nerve cords, as well as an aberrant left-handed rotation during locomotion. The *rol-3* phenotype appears after the final cuticle molt, which suggests that *rol-3* may act to establish adult cuticle (HIGGINS and HIRSH 1977; COX *et al.* 1980; KUSCH and EDGAR 1986).

Here we report the characterization of 12 new alleles of *rol-3* that are recessive for an early- to mid-larval lethal phenotype. The existence of alleles causing arrest during larval development and those resulting in an adult-specific phenotype suggest that *rol-3* may function at more than one developmental stage. Furthermore, we report the isolation and genetic characterization of suppressors of the temperature

sensitive (ts) mid-larval lethal phenotype of *rol-3(s1040*ts). These suppressor mutations have been named *srl* for *s*uppressor of *r*oller *l*ethal and define two complementation groups. We provide evidence suggesting that the *srl* gene products are involved in some aspect of *C. elegans* posterior pattern formation and that this includes, but may not be restricted to, the male sex-specific lineages. Although these suppressor mutations have been induced in a *rol-3(s1040)* background, alleles of *srl-2* are able to suppress other *rol-3* lethal alleles, suggesting that it is not an allele-specific suppressor. The results from experiments designed to determine *srl-2*'s ability to suppress *rol-3* lethal alleles, as well as studies directed at determining the cuticle requirements for expression of the roller phenotype, have led us to argue that *rol-3* influences morphology in a much more global manner than would be expected for a simple cuticle collagen. In fact, we suspect that *rol-3* functions either directly or indirectly in the developmental establishment of *C. elegans* posterior morphology. Finally, based on our genetic data, we present a model in which the wild-type *srl-1* and *srl-2* gene products normally function as down-regulators of *rol-3* gene activity.

MATERIALS AND METHODS

General: The nomenclature follows the uniform system adopted for *C. elegans* (HORVITZ *et al.* 1979). The nematodes were cultured in petri dishes on a simple agar nematode growth medium streaked with *Escherichia coli* strain OP50. For details of maintenance, as well as procedures for observing and handling of the nematodes, refer to BRENNER (1974). All manipulations were performed at 20° except where otherwise noted.

Mutations: The wild-type *C. elegans* (var. Bristol) N2 strain and strains carrying the following mutations were obtained from the MRC, Cambridge, England, from the Caenorhabditis Genetics Center at the University of Missouri, Columbia, Missouri, or as cited: LGI *dpy-5(e61)*; LGII *dpy-10(e128), lin-29(n1440)* (AMBROS and HORVITZ 1984) received from A. M. ROSE; *mab-9(e1243)* (CHISHOLM and HODGKIN 1989) LGIII *dpy-1(e1), dpy17(e164), sma-2(e502), dpy-18(e364)*; *pal-1(e2091)* (WARING and KENYON 1991) received from C. KENYON; *mab-5(e2088)* (KENYON 1986) received from C. KENYON; *smg-6(r896)* (HODGKIN *et al.* 1989); *mab-10(e1248)* (HODGKIN 1983); LGIV *dpy-4(e1166), dpy-13(e184)*; LGV *dpy-11(e224), unc-46(e177), let-456(s1479)* (JOHNSEN and BAILLIE 1991), *rol-3(e754), rol-3(s126, s422, s502, s742, s833, s1030)* (ROSENBLUTH *et al.* 1988); *rol-3(s1040*ts, *s1408, s1409, s1494, s1519)* (JOHNSEN and BAILLIE 1991); *unc-42(e270)*; LGX *lin-14(n179*ts) (AMBROS and HORVITZ 1984, 1987) received from A. M. ROSE. The LGV deficiency *mDf3* (BROWN 1984) was received from D. L. RIDDLE; and the reciprocal translocation *eT1* was characterized previously by ROSENBLUTH and BAILLIE (1981). All mutations denoted with the *s* prefix arose in this laboratory. All mutations and genes have been named according to the conventions of HORVITZ *et al.* (1982).

Characteristics of *eT1(III;V)*: *eT1*(III;V) is a reciprocal translocation that recombinationally balances the right half of LGIII and the left half of LGV. A total of about 43 m.u. (14% of the genome) is balanced (ROSENBLUTH and BAILLIE 1981). The balanced regions of each chromosome are approximately the same size recombinationally. On LGV, recombination appears to be completely suppressed from the left end to a region between *dpy-11* and *unc-42* near the center of the chromosome. Ten-sixteenths of the progeny of *+/eT1(III);+/eT1(V)* hermaphrodites stop maturing early in development, this is considered to be the result of those animals having aneuploid genomes. Because of the aneuploidy and no crossing over between markers and *eT1* breakpoints, markers on LGIII(right) and LGV(left) are pseudolinked. The breakpoint of *eT1* on LG(III) had been mapped close to and may be within *unc-36*. Worms homozygous for *eT1* are viable and exhibit an Unc-36 phenotype.

Developmental blocking stage: Developmental blocking stages of the *rol-3* lethal alleles were determined as described by JOHNSEN and BAILLIE (1991) and were further confirmed by examining the extent of hermaphrodite gonad development in *rol-3* homozygotes (KIMBLE and HIRSH 1979).

***rol-3(s1040*ts) the mutation to which suppressors were generated:** Worms homozygous for *rol-3(s1040)* arrest during mid-larval development at 20–25°. However, at 15°, homozygous *rol-3(s1040)* animals develop to adulthood, are fertile and display a weak left-handed roller phenotype. BC1941 *dpy-18(e364)/eT1(III);unc-46(e177)rol-3(s1040*ts)*/eT1(V)* hermaphrodites were placed at 15° and individual F_1 Dumpy-Uncs were collected and maintained at 15°. A single line was retained and named BC3129. All progeny of adult BC3129 worms shifted to 20–25° display terminal lengths of approximately 0.35 mm, indicative of mid-larval arrest. The BC3129 strain [*dpy-18(e364)/eT1(III);unc-46(e117)rol-3(s1040*ts)*/eT1(V)*] was used as the background for the generation of suppressors of Rol-3 lethality.

Temperature-sensitive period of *rol-3(s1040ts)*: Several plates, each with 10 gravid BC3129 hermaphrodites were placed at permissive temperature (15°) for 1 hr. After 1 hr at 15° (this point is time zero, t_0), the hermaphrodites were removed and the plates containing eggs were transferred to 25° (restrictive temperature) at recorded times. The plates were kept at 25° for a minimum of 4 days after which five worms were picked at random and their terminal lengths were measured. A second set of plates were placed at 25° immediately following removal of the adult hermaphrodites. These plates were treated in an identical manner to those above, though they were shifted down to 15° at recorded times. All data were normalized to 15°.

Mutagenesis: Two suppressor screens were performed, one using gamma irradiation and one using EMS. In both cases 100,000 chromosomes were treated.

EMS mutagenesis: Mutagenesis of BC3129 was carried out according to BRENNER (1974) except that the dose was decreased to 0.012 M EMS to minimize the frequency of second hits (ROSENBLUTH, CUDDEFORD and BAILLIE 1985). Hermaphrodites were treated for 4 hr at room temperature. After treatment a total of 10 plates were set up with 50 worms per 10-cm petri plate and incubated at 15° for 7–10 days (two generations). The plates were then shifted to 20° and monitored for the presence of developing and fertile Dpy Uncs. Such individuals were maintained (one per mutagenesis plate) and the nature of the suppression was analyzed.

Gamma irradiation mutagenesis: The mechanics of the gamma irradiation screen were the same as the EMS screen described above except BC3129 Po hermaphrodites were treated with 1500 R of Gamma radiation emitted from a 60 Co radiation unit (Gamma Cell 200, Atomic Energy of Canada). The suppressor mutations were named *srl* for *s*uppressor of *r*oller *l*ethal.

Mapping suppressor mutations to linkage groups: *Test-*

ing for linkage to LGs III, V or X: All recombination experiments were carried out at 20° (ROSE and BAILLIE 1979). Advantage was taken of the fact that *dpy-18 III, unc-46 V* lie in the regions recombinationally balanced by *eT1 (III;V)* and therefore appear pseudolinked in the progeny of *eT1* heterozygotes. Hermaphrodites from each suppressor (*srl*) mutant strain of the genotype *srl-(sx). . .dpy-18/eT1 (III);unc-46 rol-3(s1040*ts)*/eT1(V)* males. The F_1s were scored for the presence of Dpy Unc-46 males. Absence of such males indicated that the suppressor is recessive and that it is not on LGX. Wild-type F_1 hermaphrodites were picked and the F_2s were scored. Due to aneuploidy effects, only 6/16 of an *eT1* heterozygote's progeny are normally viable (ROSENBLUTH and BAILLIE 1981). If the *srl* mutation was within the *eT1* balanced region of LGIII or LGV, and thus pseudolinked to *dpy-18 III, unc-46 V* and *rol-3 V*, we expected to see Dpy Unc-46 Rol:wild-type:Unc-36 progeny in a 1:4:1 ratio. If the *srl* assorted independently of LGIII and LGV, then we expected the ratio to be 1:16:4.

Distinguishing between linkage to LGIII or LGV: Homozygous *srl-(sx). . .dpy-18 III;unc-46 rol-3(s1040*ts) *V* hermaphrodites were mated to N2 males and the F_2 progeny scored. The Dpy Unc-46 Rol-3/Unc-46 ratio was used to distinguish between the *srl*'s linkage to LGIII or to LGV. Taking into account the map distance of approximately 3.6 map units between *unc-46* and *rol-3* (M. L. EDGLEY and D. L. RIDDLE, unpublished data), tight linkage of the *srl* mutation to *dpy-18 III* would give a ratio of 4.2:1, while in the case of tight linkage to *unc-46 V*, the ratio would be 0.33:1.

Testing for linkage to markers "m" on LGI, II, III or IV: Hermaphrodites, homozygous for *srl-(sx)*, marker "*m*," *unc-46* and *rol-3(s1040*ts), were mated to N2 males and the F_2 progeny were scored. The ratio *M* Unc-46:Unc-46 was used to determine whether the *srl* mutation was linked to the marker. Tight linkage to the marker would give a ratio of 4.2:1. Linkage to neither the marker nor *unc-46* would give a ratio of 0.31:1.

Two-factor mapping *srl-2* relative to LGIII markers: srl-2(s2507) was two-factor mapped relative to *dpy-1 (III), dpy-17 (III)* and *sma-2 (III)*. Hermaphrodite strains of the genotype *M srl-2(s2507);unc-46 rol-3(s1040*ts) (where *M* = *dpy-1, dpy-17* or *sma-2*) were mated to N2 males. Individual wild-type F_1 hermaphrodites were allowed to self-fertilize at the standardized mapping temperature of 20° (ROSE and BAILLIE 1979) and the F_2s scored for the presence of recombinant Unc progeny. Given that *rol-3* is separated from *unc-46* by 3.6 map units (M. L. EDGLEY and D. L. RIDDLE, unpublished data), the number of Unc progeny expected due to recombination between *unc-46* and *rol-3* rather than due to recombination between *M* and *srl-2(s2507)* was determined from the formula $U = T[1 - (1 - P)^2]/3$ where *U* is the number of Uncs, *T* is the total number of worms and *P* is the frequency of recombination between *unc-46–rol-3*. Once determined, the value *U* was subtracted from the total number of Unc-46 recombinants scored, and the corrected value for Unc-46 recombinant progeny was used to calculate the recombination distance between the marker and *srl-2*. The formula used to determine the recombination distance between *M* and *srl-2* was $P = 1 - [1 - (13R/T)]^{1/2}$, where *P* is the recombination frequency between *M* and *srl-2*, *R* is the corrected number of Unc recombinants and *T* represents the total number of worms; 95% confidence limits are based on the limits of Unc-46 recombinants. These limits were taken from Table 1 of CROW and GARDENER (1959).

***srl-inter se* complementation:** All complementation experiments were conducted at 20°. Hermaphrodites carrying one *srl* allele to be tested (designated srl-A in Figure 1) were crossed to *dpy-18/eT1(III);unc-46 rol-3(s1040*ts)*/eT1(V)* males and the resulting F_1 males were picked. These males were mated to a second hermaphrodite carrying a different *srl* allele. The presence of Dpy Unc males indicated a failure to complement. If the *srl* alleles tested were linked to either LGIII or LGV then failure to complement would result in a ratio of Dpy Unc to wild-type males approximately equal to 1:1. Conversely, if the *srl* mutants are not linked to LGIII or LGV, then a ratio of Dpy Unc to wild-type males of 1:2 would occur. In some cases *srl-;unc-46 rol-3(s1040*ts) was used rather than *srl-;dpy-18;unc-46 rol-3(s1040*ts). In these instances the ratios discussed above are applicable although the frequency of Unc to wild-type males was scored rather than Dpy Unc to wild-type males.

Test of *srl-2*'s ability to suppress *rol-3* lethal alleles other than *s1040*ts: *srl-2 dpy-18/++* males were mated to *eT1* balanced *rol-3* lethal allele bearing strains of the type *dpy-18/eT1(III);unc-46 rol-3/eT1(V)*, or *dpy-18/eT1(III);dpy-11 rol-3/eT1(V)* [in the case of *rol-3(s833)*, or *dpy-18/eT1(III);dpy-11 rol-3 unc-42/eT1(V)* (in the case of *rol-3(s126)*.] F_1 Dpy hermaphrodites were picked and allowed to self. F_2 Dpy Unc-46s were scored and picked up (or Double Dpys in the case of *s833*, or Double Dpy-Unc-46s in the case of *s126*). For suppression we expected a Dpy Unc F_2 frequency of approximately 1/16. Furthermore, we expected Dpy Unc F_2s at a frequency of 2/100 due to recombination between *unc-46* and the *rol-3* allele being tested. Therefore, progeny of the putative *srl-2 dpy-18;unc-46 rol-3* were mated to N2 males and F_1 hermaphrodites were picked. The F_2s were observed for the presence of arresting larvae confirming that the *rol-3* lethal was present.

Microscopy: Posterior and male specific alterations were observed and photographed using differential interference contrast (Nomarski) optics (SULSTON and HORVITZ 1977). Worms were mounted on 5% agar pads containing 2-μl S Buffer (BRENNER 1974). Photographs were taken with Kodak Technical Pan film, ASA 64. To avoid movement during long exposure times, worms were anaesthetized with sodium azide (final concentration of 5 mM). A cover slip was then added, and photographs were taken with as little time delay as possible to avoid desiccation. All Nomarski microscopy was performed on an Olympus AHBS3 Research photomicrographic microscope system equipped with dual Olympus C35AD-4 35-mm cameras.

RESULTS

Generation and Identification of lethal alleles of *rol-3V*: Our laboratory has undertaken an extensive mutagenic dissection of a 23 m.u. portion of LGV(left) balanced by the reciprocal translocation *eT1* in an attempt to saturate this region for essential genes (JOHNSEN and BAILLIE 1991). Toward this end, gamma irradiation, EMS (JOHNSEN, ROSENBLUTH and BAILLIE 1986; ROSENBLUTH *et al.* 1988; JOHNSEN 1990; JOHNSEN and BAILLIE 1991), formaldehyde (JOHNSEN and BAILLIE 1988), *Tc1* transposon (CLARK *et al.* 1990) and UV (STEWART, ROSENBLUTH and BAILLIE 1991) mutagenesis screens have been instrumental in generating a total of 242 mutations defining 101 essential genes to date within this region. These mutations were complementation tested against known deficiencies and subsequently tested against known genes in the appropriate regions. These analyses resulted in the identification of 12 alleles of *rol-*

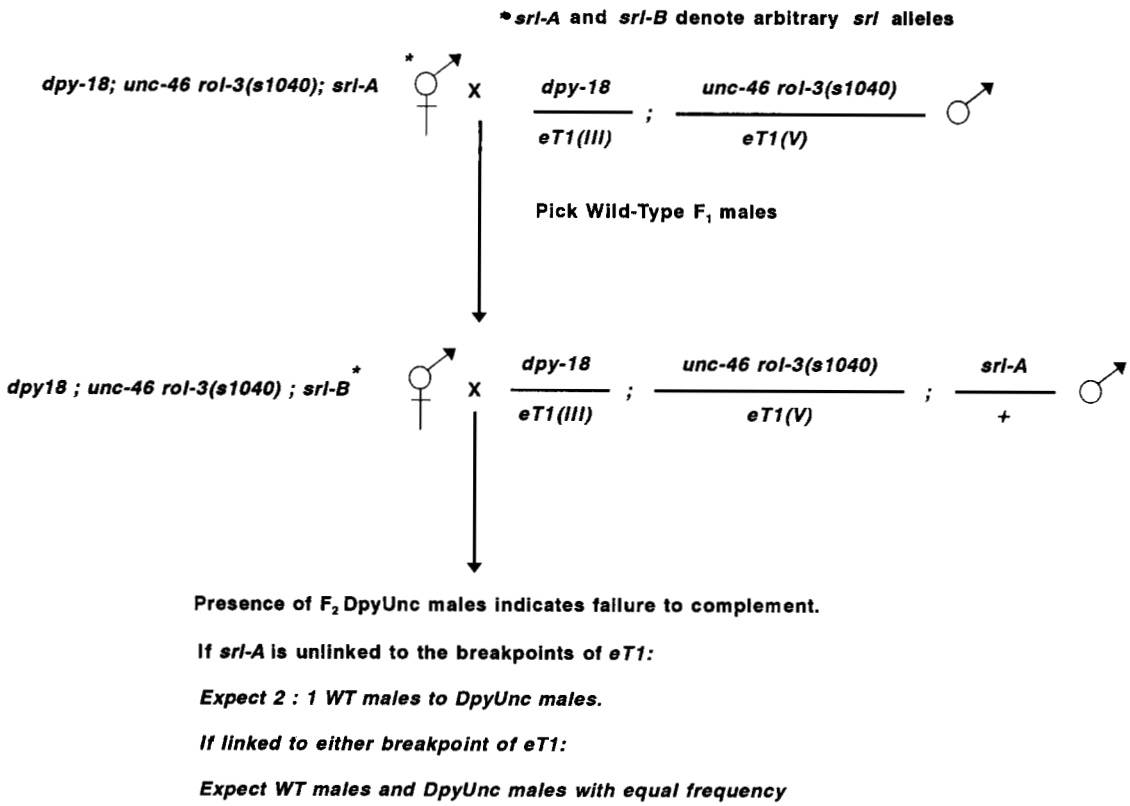

FIGURE 1.—Complementation scheme for determination of *srl* alleleism. The presence of F_2 Dpy Unc males indicates that the heteroallelic combination of *srl-A* and *srl-B* are able to suppress *rol-3(s1040*ts), indicating failure to complement. Complementation data are presented in Figure 4.

3, all of which have recessive lethal phenotypes (JOHNSEN and BAILLIE 1991). Two of these alleles (*s501* and *s742*) had been induced with gamma irradiation, and we have subsequently determined *s501* to be a small deficiency. This deficiency, now known as *sDf57*, either removes or disrupts *rol-3*, as well as *let-456* (Figure 2). The remaining ten alleles had all been isolated following EMS treatment.

The stages of developmental arrest for each of the *rol-3* lethal alleles were determined previously (JOHNSEN and BAILLIE 1991) and are presented in Table 1. It is evident that all but 4 of the 11 lethal *rol-3* alleles are recessive for an early larval lethal phenotype. *s422*, *s742* and *s833* are recessive for a mid-larval phenotype, while *s1040* is a conditional lethal: at 20° worms homozygous for *rol-3(s1040)* display a mid-larval lethal phenotype but at 15° develop as viable weak adult rollers. Nomarski optics were used to examine the extent of gonad development in dying hermaphrodite larvae homozygous for *rol-3* lethal alleles. By this criterion we determined that those alleles designated as early blockers by JOHNSEN and BAILLIE have L1 stage gonads while those designated as mid-larval blockers have L2–L3 stage gonads (KIMBLE and HIRSH 1979). JOHNSEN (1990) calculated the average EMS hit frequency for essential genes within the *eT1* balanced region to be 1.25 mutations per essential gene. EMS-induced recessive lethal mutations of the

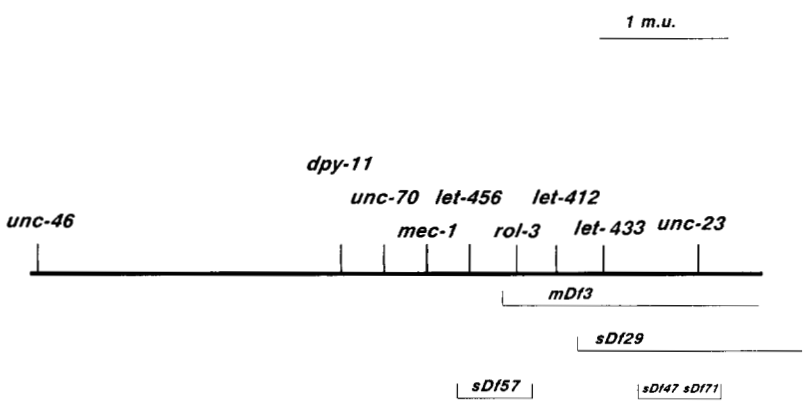

FIGURE 2.—Partial map of LGV left illustrating the positions of *unc-46*, *dpy-11* and *rol-3*, as well as those deficencies defining the location of *rol-3*.

TABLE 1

Alleles of *rol-3* and their phenotypes

Arrest at early larval stage	Arrest at mid-larval stage	Fertile, left-hand rollers
s126	*s422*	*e202*
s1408	*s742*	*e754*
s1409	*s833*	
s1494	*s1040*ts[a]	
s1519		

[a] Arrest during mid-larval development at 20°C–25°C.
Fertile, weak left-hand roller at 15°.
Data from JOHNSEN and BAILLIE (1991).

rol-3 locus occur at a frequency six times that of the average essential gene. This suggests two possibilities. First, it is possible that the high EMS mutability associated with *rol-3* reflects the presence of a physically large coding region. Indeed, *unc-22* (MOERMAN, BENIAN and WATERSTON 1986) and *unc-54* (MACLEOD, KARN and BRENNER 1981) are two highly mutable loci which also constitute large physical targets. Alteratively, *rol-3* may contain one or more hot spots for EMS-induced mutation.

That *rol-3* may code for a large gene product, as well as the fact that *rol-3* lethal alleles display differential blocking stages suggests that the *rol-3* product may be organized into discrete functional domains, and that these may play separate roles during *C. elegans* development. Independently functioning domains can also be considered independently mutable (RAND 1989) and, as such, may elicit intragenic mutations that complement in *trans*. To test the possibility that *rol-3* might represent a complex locus of this type, we performed *rol-3 inter se* complementation tests. We observed no complementation between *rol-3* alleles, suggesting that *rol-3* defines a genetically simple locus and that the *rol-3* protein does not function as a homo-multimer. The presence of a temperature sensitive lethal allele that rolls at permissive temperature, and a genetically simple complementation pattern implies that weak alleles cause rolling, whereas stronger alleles affect viability.

Analysis of *s1040*ts, a temperature sensitive lethal allele of *rol-3*: As previously mentioned, *s1040* is a recessive lethal allele of *rol-3* that displays a temperature dependent phenotype. When subject to a temperature of 20–25°, worms homozygous for *rol-3(s1040)* arrest development at a mid-larval stage. However, when grown at 15°, homozygous *s1040* worms develop to adulthood, are fertile and display a weak left-handed roller phenotype. We studied the temperature sensitive period (TSP) for *rol-3(s1040*ts) in an effort to determine when in development functionally active *rol-3* gene product is required. As evidenced by the TSP for *s1040* (Figure 3), we propose that a functional *rol-3* gene product is first required at approximately 30 hr after egg lay at 15°, and this requirement ceases at 70 hr. With respect to *C. elegans* growth at 25°, the *rol-3* gene product is required from 15–35 hr after egg lay. Correlation of this time with the *C. elegans* developmental profile suggests that *rol-3(+)* is required mid-L1 to mid-L3 stage (WOOD *et al.* 1980). It should be noted that this TSP suggests the stage at which active *rol-3* gene product is required but does not yield any information regarding the stage at which gene activation or protein synthesis occurs (HIRSH and VANDERSLICE 1976).

All previous analyses concerning *rol-3* have been focused upon *e754*, the original recessive visible allele first isolated by BRENNER (1974). COX *et al.* (1980) determined that the visible roller phenotype associated with *rol-3(e754)* is not manifest until after the L4 molt, subsequent to adult cuticle formation. However, the TSP for *s1040* demonstrated that the *rol-3* gene performs an essential function at a stage much earlier than this. The observation that *rol-3* not only plays an essential role during early development but also disrupts adult cuticle morphology suggests that the developmentally essential function may be separable from a second function that is required to establish or maintain the integrity of the adult cuticle.

Disruption of adult cuticle: To further illuminate the association of *rol-3* function with the adult cuticle we studied the interaction of *rol-3(e754)* with mutations of the heterochronic genes *lin-14(X)* and *lin-29 (III). lin-29(n1440)* and *lin-14(n179*ts) demonstrate reciprocal effects on the timing of expression of the adult cuticle. *lin-29* loss of function alleles fail to make the L4 to adult cuticle switch and reiterate the L4-stage cuticle (AMBROS and HORVITZ 1984). This is the only known effect of *lin-29* mutations. We therefore expected that if the rolling phenotype associated with *rol-3(e754)* was dependent on the presence of an adult cuticle, rolling should be suppressed in worms doubly mutant for *rol-3(e754)* and loss of function mutations in *lin-29*. Double mutants for *rol-3(e754)* and *lin-29(n1440)* mutations were constructed and scored for the presence of adult rollers. All *lin-29(n1440);rol-3(e754)* double mutants roll as adults. This result suggests that the expression of *rol-3(e754)* is temporally constrained to the adult stage but does not require the presence of an adult cuticle. In contrast to *lin-29(n1440)*, the *lin-14(n179*ts) allele results in advancement of cuticle expression such that an adult cuticle is formed after the third molt rather then the fourth at the restrictive temperature of 25° (AMBROS and HORVITZ 1984, 1987). If the expression of the rolling phenotype requires an adult cuticle than *rol-3(e754);lin-14(nt179)*, doubles are expected to roll one molt earlier than wild type. All such double mutants constructed do roll, but only as gravid adults, demonstrating that the precocious expression of an adult

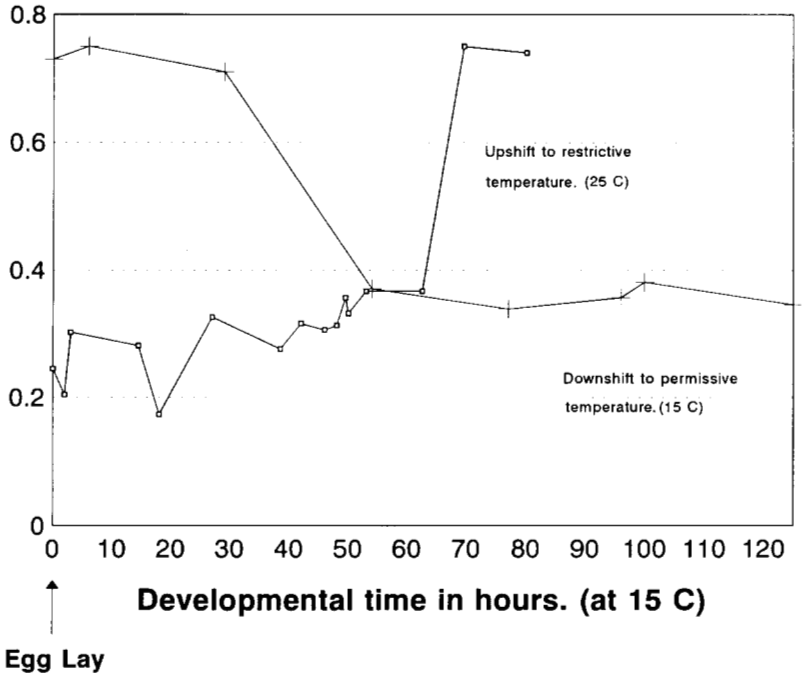

FIGURE 3.—Determination of the temperature sensitivity period for *rol-3(s1040)ts*. Worms homozygous for *rol-3(s1040*ts) were upshifted (O) to restrictive temperature 25° or downshifted (+) to permissive temperature (15°) at the times indicated along the X axis. These times were correlated with the terminal length of the worms shifted. Adults were expected to reach ~0.8 mm in length, while those which arrested development at a mid-larval stage (*rol-3(s1040*ts) phenotype) were only expected to reach ~0.35 mm. All time points were normalized to 15°. This experiment suggests that the requirement for rol-3's essential function may occur as early as 30 hr after egg lay at 15° (15 hr at 25°) and may continue until 70 hr after egg lay at 15° (35 hr at 25°).

cuticle does not alter the expression of the *rol-3(e754)*'s visible phenotype.

Generation of suppressors of *rol-3(s1040*ts): In an effort to elucidate the essential developmental role performed by the *rol-3* gene product we attempted to generate suppressors of the recessive temperature sensitive mid-larval lethal phenotype of *rol-3(s1040*ts). Both gamma irradiation and chemical (EMS) mutagenesis were performed and a total of 100,000 treated F_1 chromosomes were examined for each mutagen. We recovered two gamma irradiation induced suppressors (*s2500* and *s2501*) and eight EMS induced suppressors (*s2502-s2509*). Difficulties associated with outcrossing *s2505* and *s2509* prevented their further analysis. The eight suppressors analyzed function as recessive suppressors of *s1040*ts's temperature-sensitive lethal phenotype, yet do not suppress the rolling phenotype. In fact, it appears that in most cases worms homozygous for both the suppressors and *rol-3(s1040*ts) roll more vigorously than is observed for those homozygous for *s1040*ts alone. We have named these suppressors *srl* for *s*uppressor of *r*oller *l*ethal.

Linkage mapping *srl* mutations: Linkage of the *srl* mutations was first tested with respect to LGs III, V and X (MATERIALS AND METHODS). All *srl* mutations were found to be recessive and none were on LGX. Table 2 presents the progenies from *srl-(sx)/+. . .dpy-18/eT1;unc-46 rol-3(s1040*ts)*/eT1(V)* hermaphrodites. We interpret the data to show that the mutations fall into two classes 1 and 2. Class 1 (*s2500-s2503*) comprises those mutations that are linked to neither LGIII nor LGV, while class 2 mutations (*s2504, s2506-s2508*) are linked to either LGII or LGV but outside the region balanced by *eT1(III;V)*.

TABLE 2

Testing for linkage of *srl-(sx)* to either LGIII or LGV: progeny from *srl-(sx)/+ . . . dpy-18/eT1(III);unc-46 rol-3(s1040*ts)*/eT1(V)*

srl mutation	Viable phenotypes				
	Dpy-Unc-46	Wild type	Unc-36[a]	Ratio[b]	Group
s2500	4	221	33	1:55:8	1
s2501	3	225	25	1:75:8	1
s2502	2	87	14	1:43:7	1
s2503	9	272	49	1:30:5	1
s2504	11	183	26	1:9:2	2
s2506	11	178	27	1:16:2	2
s2507	33	596	99	1:18:3	2
s2508	9	92	17	1:10:2	2

[a] Phenotype of homozygous *eT1*.

[b] If *srl-(sx)* is on LGIII or LGV, within the *eT1* balanced region, expected ratio = 1:4:1. If *srl-(sx)* is linked to neither LGIII nor LGV, expected ratio = 1:16:4.

The mutations of class 2 were tested to determine whether they are linked to LGIII or LGV (MATERIALS AND METHODS). The segregation patterns obtained in the absence of *eT1* (Table 3) demonstrate that all four mutations are linked to LGIII rather than to LGV. Further support for class 2 mutation linkage to LGIII was obtained when *s2507* was tested with other LGIII markers: *dpy-1(e61), dpy-17(e164)* and *sma-2(e502)* (Table 4). Our data suggest that *s2507* is tightly linked to the *sma-2–dpy-17* interval of LGIII.

Two of the class 1 mutations (*s2501* and *s2503*) were tested for linkage to *dpy* markers on LGs I, II, III, IV (MATERIALS AND METHODS). The Dpy Unc-46/Unc-46 ratios (Table 5) suggest that both *s2501* and *s2503* are loosely linked to *dpy-10* on LGII. The average brood size and lengths (based on five individuals) of the *dpy-18;unc-46 rol-3(s1040*ts) strains con-

TABLE 3

Testing class 2 *srl* mutations for linkage to LGIII or LGV: Progeny from *srl-(sx)/+ . . . dpy-18 III/+;unc-46rol-3(s1040ts) V/++*

srl mutation	Viable phenotypes				Ratio[a]	Location
	Dpy Unc-46	Unc-46	Wild type	Dpy	Dpy Unc46:unc-46	
s2504	32	29	215	704	1:1	LGIII
s2506	27	12	140	439	2.2:1	LGIII
s2507	24	14	244	739	1.7:1	LGIII
s2508	22	10	120	433	2.2:1	LGIII

[a] If *srl-(sx)* is tightly linked to *dpy-18*, expected ratio = 4.2:1.
If *srl-(sx)* is tightly linked to *unc-46*, expected ratio = 0.33:14.

TABLE 4

Two-factor mapping of class 2 *srl* mutations to LGIII markers: Progeny from *M srl-2(s2507)/++;unc-46rol-3(s1040ts)/++*

LGIII marker	Viable phenotypes				Adjusted Unc-46 total[a]	Distance (95% confidence) m.u.[b]
	Dpy Unc-46 or Sma Unc-46	Unc-46	Dpy or Sma	Wild type		
dpy-1	111	36	388	1360	15	6.59–20.87
dpy-17	55	7	200	671	0	0–2.6
sma-2	59	35	212	520	0	0–2.6

[a] Number of Unc-46 worms expected due to loss of rol-3(s1040ts) was determined by the formula $U = T[1 - (1 - P)^2]/3$, where U is the number of Unc-46, T is the total number of worms, and P is the unc-46 − rol-3 distance in mu. divided by 100. This value was subtracted from the observed number of Unc-46 animals to yield the adjusted total.

[b] Recombination distance between srl- and the LGIII marker in question was determined by the formula $p = 1 - [1\text{-}13R/T]^{1/2}$ where p is the recombination frequency between M and srl-2, R is the adjusted Unc-46 total and T is the total number of worms.

95% confidence limits are based on the limits of Unc-46 recombinants.

These limits are from Table 1 of CROW and GARDNER (1959).

TABLE 5

Testing class 1 *srl* mutations for linkage to *dpy* markers on LGs I, II, III and IV: progeny from *srl-(sx)/+ . . . dpy/+;unc-46rol-3(s1040ts)/++*

srl mutation	Dpy marker	Viable phenotypes				Ratio[a]	Linkage
		Dpy Unc-46	Unc-46	Dpy	Wild type	Dpy Unc-46:Unc-46	
s2501	*dpy-5 I*	16	58	302	764	0.3:1	Unlinked
	dpy-10 II	51	29	352	1140	1.8:1	Loose linkage
	dpy-18 III	3	7	40	126	0.4:1	Unlinked
	dpy-13 IV	32	45	258	549	0.7:1	Unlinked
s2503	*dpy-5 I*	8	30	160	385	0.3:1	Unlinked
	dpy-10 II	55	31	337	1039	1.8:1	Loose linkage
	dpy-18 III	3	6	49	220	0.5:1	Unlinked
	dpy-4 IV	30	55	337	628	0.5:1	Unlinked
	dpy-13 IV	21	28	251	459	0.7:1	Unlinked

[a] If srl-(sx) is tightly linked to *dpy* marker, expected ratio = 4.2:1.
If srl-(sx) is linked to neither the marker nor *unc-46*, expected ratio = 0.3:1.

taining each of the eight suppressor mutations are displayed in Table 6. Worms triple homozygous for *dpy-18*, *unc-46* and *rol-3(s1040ts)* are inviable at restrictive temperature but have an average brood size of 100 at permissive temperature. This is a 50% decrease in brood size relative to worms doubly homozygous for *dpy-18;unc-46* alone. The presence of a homozygous suppressor mutation renders worms homozygous for *rol-3(s1040ts)* viable at restrictive temperature and also increases brood sizes relative to the *rol-3(s1040ts)* animals grown at permissive temperature. However, all Srl Dpy Unc Roller strains display brood sizes that are lower than those seen in *dpy-18;unc-46* mutant strains. The average length of a Srl Dpy Unc-46 Roller individual remains slightly shorter than either a *dpy-18;unc-46* or a *dpy-18;unc-46 rol-3(s1040)* homozygous strain.

The *srl* mutations fall into two complementation groups, *srl-1 II* and *srl-2 III*: Figure 4 displays the complementation results observed between the various *srl* mutations. It is immediately obvious that the eight mutations fall into only two complementation groups, designated *srl-1* and *srl-2*. Furthermore, the complementation results are consistent with the link-

TABLE 6

srl mutations: mutagen, linkage group, fecundity and terminal length

	Gene and mutation	Mutagen	Linkage group	Brood size	Length (mm)
srl-1[a]					
	s2500	Gamma	II	187	0.59
	s2501	Gamma	II	121	0.57
	s2502	EMS	II	203	0.63
	s2503	EMS	II	110	0.60
srl-2[a]					
	s2504	EMS	III	181	0.62
	s2506	EMS	III	121	0.61
	s2507	EMS	III	137	0.70
	s2508	EMS	III	155	0.63
Controls					
dpy-18;unc-46				212	0.78
dpy-18;unc-46 rol-3(s1040ts)[b]				100	0.72

[a] *dpy-18;unc-46 rol-3(s1040ts)* background.
[b] Data taken at permissive temperature.

age data. All mutations that were found to be unlinked to either LGIII, LGV or LGX (class 1) failed to complement one another and are the alleles of *srl-1*. Since two of these mutations (*s2501* and *s2503*) were shown to be on LGII, *srl-1* is located on LGII. All mutations that were found to be linked to LGIII outside the *eT1* balanced region (class 2) failed to complement each other and are the alleles of *srl-2*. Thus, *srl-2* is located on the left half of LGIII, tightly linked to the *sma-2–dpy-17* interval. While the combined linkage and complementation data show that the mutations are in two distinct loci, the complementation pattern displayed by *srl-2(s2506) III* was unexpected. All alleles of both *srl-1* and *srl-2* failed to complement *s2506* indicating the existence of a complex genetic interaction between the *srl* loci.

Although the frequency at which these *srl* alleles were generated suggests that they probably represent loss of function alleles (~1 in 12,000 for the 0.012 M EMS induced mutations), there are small differences within the complementation pattern which suggests that some of these alleles are not null alleles. For instance, *srl-1(s2502)* by *srl-2(s2507)* clearly complements. Although we do observe some exceptional males, they only represent 20% of the number expected if these alleles were to fail to complement. Evidently, the doubly heterozygous combination of these alleles result in weak suppression. This interac-

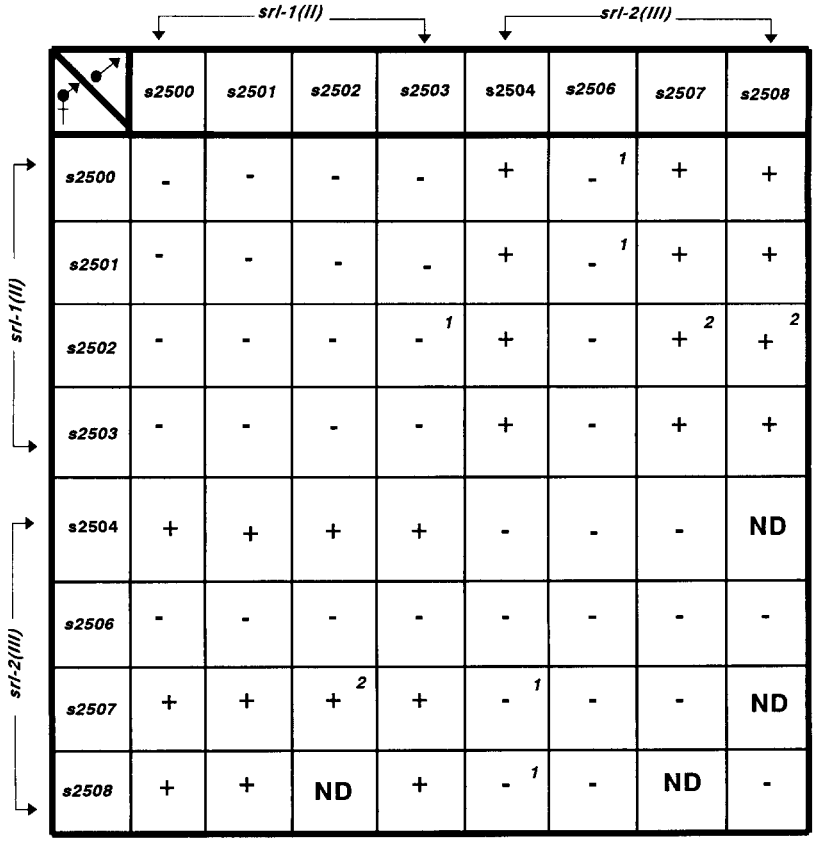

FIGURE 4.—*Inter se* complementation results for the *srl* mutations.

TABLE 7

rol-3 lethal alleles tested for suppression by srl-2

rol-3 lethal allele	srl-2 allele		
	s2506	s2507	s2508
s126	−	−	−
s422	+	ND	+
s742	+	+[a]	+
s833	+	+	ND
s1040	+	+	+
s1409	+	ND	+
s1494	−	−	−
s1519	−	−	−

+ indicates suppression; − indicates failure to suppress.
[a] Weak suppression.

tion occurs for these alleles regardless of which parent contributes which allele.

Another unusual feature of the data in Figure 4 concerns the complementation results of *srl-1(s2500* and *s2501)* hermaphrodites by *srl-2(s2506)* males. As previously mentioned, *srl-2(s2506)* fails to complement all analyzed *srl* mutations; however, in these two doubly heterzygous combinations only one half of the expected exceptional males that indicate failure to complement are observed. Furthermore, these are clearly maternal effects since *srl-2(s2506)* hermaphrodite *vs. srl-1(s2500)* male generates the expected number of exceptional males.

The suppression of lethality produced by *srl-2* is not restricted to *rol-3(s1040*ts): The alleles of *srl-1* and *srl-2* were recovered as suppressors of *rol-3(s1040*ts). To determine whether these suppressors act in an allele general fashion with respect to *rol-3*, seven *rol-3* alleles were tested with three *srl-2* alleles (MATERIALS AND METHODS), and the results are displayed in Table 7. We have been unsuccessful in suppressing the lethal phenotype of either *s126* or *s1519*, both of which cause arrest at an early larval stage. However, we have demonstrated suppression of *rol-3(s422, s742, s833* and *s1409)* by some *srl-2* alleles. Of particular interest is the suppression of *rol-3(s742)* by *srl-2(s2506, s2507* and *s2508).* When either *srl-2(s2506)* or *srl-2(s2508)* is crossed into a strain carrying *rol-3(s742)* yielding Dpy hermaphrodites and these are subsequently selfed, some Dpy Unc F_2s are produced. These are morphologically similar to our *dpy-18;unc-46* reference strain (Figure 5A). However, the F_3s produced by these Dpy Unc F_2s are not morphologically characteristic of the Dpy-18 Unc-46 reference strain. These worms exhibit varying degrees of posterior deformation and some internal disorganization but develop to adulthood and are viable (Figure 5C). If, however, *srl-2(s2507)* is crossed into a *rol-3(s742)* background, we again produce Dpy Unc F_2s that resemble our *dpy-18;unc-46* reference strain. However, *srl-2(s2507) dpy-18;unc-46 rol-3(s1040*ts) F_3s have much different morphological characteristics. These F_3s have severely disrupted posterior morphology, an abnormal cuticle surface, disrupted internal structures and are inviable (Figure 5D and E). It is evident that, although these are inviable, they are morphologically distinct from the arrested *dpy-18;unc-46 rol-3(s742)* homozygotes (B). Therefore, on the basis that viability of the *srl-2(s2507);rol-3(s742)* combination is restricted to first generation while second generation individuals are inviable (clearly a maternal interaction), and that *srl-2(s2508);rol-3(s742)* individuals display no maternal effect on viability; with respect to *rol-3(s742),* we classify *srl-2(s2507)* as a weak suppressor and *srl-2(s2508)* as a strong suppressor. These results not only confirm that suppression by *srl-2* is not restricted to *rol-3(s1040*ts), but indicate that *srl-2* exhibits allele specific variation in the degree of suppression.

Phenotypes associated with *srl-1* and *srl-2:* Hermaphrodites homozygous for any of the suppressor mutations have no readily discernible phenotype in the absence of *rol-3(s1040*ts). However, males homozygous for both suppressor and *rol-3(s1040*ts) display aberrant male tail morphology. The morphology of the *C. elegans* male tail has been well defined (SULSTON, ALBERTSON and THOMSON 1980; BAIRD *et al.* 1991; EMMONS 1992) and is highly specialized for copulation. This specialization entails both the execution of male-specific post-embryonic cell lineages and the morphogenesis of adult body shape (SULSTON and HORVITZ 1977; SULSTON, ALBERTSON and THOMSON 1980). Genes required for male tail development have been identified and studied by HODGKIN (1983), and some such as *mab-3* (SHEN and HODGKIN 1988), *mab-5* (KENYON 1986; COSTA *et al.* 1988; WARING and KENYON 1991; SALSER and KENYON 1992) and *mab-9* (CHISHOLM and HODGKIN 1989) affect cell lineage, thus implying regulatory functions. The *C. elegans* male tail is composed of nine bilateral pairs of sensory rays, each comprising the dendritic ending of two neurons and one structural cell (SULSTON and HORVITZ 1977; SULSTON, ALBERTSON and THOMSON 1980). These three processes are contained within a tube-like extension of hypodermis forming a ray, which is surrounded by a tube of inner layer cuticle. Wild-type rays project radially from the tail and are embedded in the fan composed of outer-layer cuticle (BAIRD and EMMONS 1990; EMMONS 1992). The effects of *srl-1* and *srl-2* on male tail morphology are shown in Figure 6. Figure 6A shows a ventral view of a wild-type *C. elegans* male tail illustrating an orderly ray pattern. Males homozygous for *unc-46 rol-3(s1040*ts) display a similar structure, suggesting that neither *rol-3* nor *unc-46* influence ray or fan morphology (Figure 6B). However, the presence of either *srl-1* (Figure 6C) or *srl-2* (Figures 6D and E) result in a general reduction

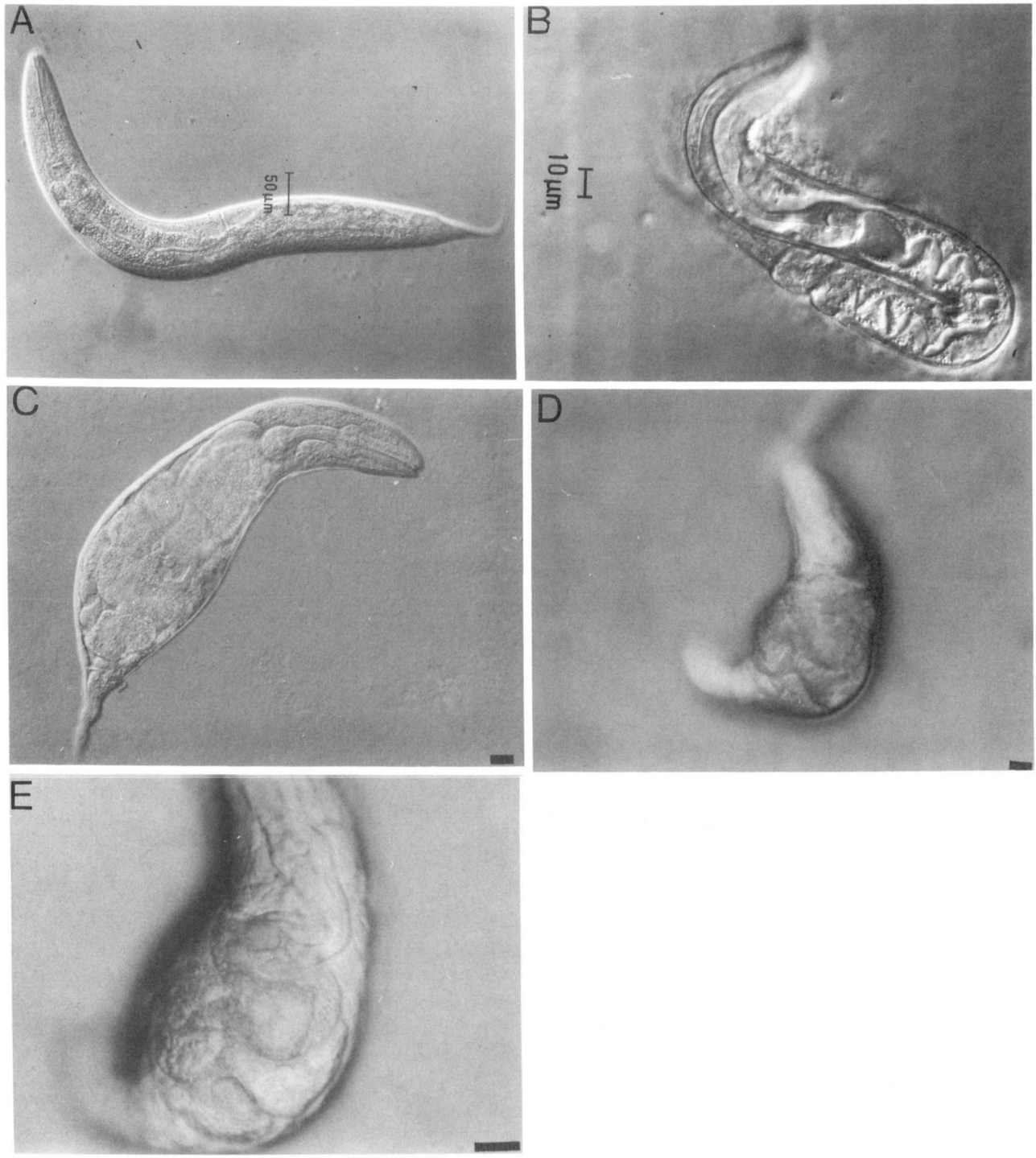

FIGURE 5.—Nomarski micrographs of *srl-2 dpy-18(e364)III;unc-46(e177) rol-3(s742)V* hermaphrodites. (A) *dpy-18(e364)III;unc-46(e177);* (B) dpy-18;unc-46 rol-3(s742) arrested during mid-larval development; (C) *srl-2(s2508) dpy-18(e364);unc-46(e177) rol-3(s742)*: the animals in C exhibit a posterior rounding followed by an obvious cuticle taper. Despite the internal disorganization these animals develop to fertile adults. (D and E) *srl-2(s2507) dpy-18;unc-46 rol-3(s742)*: posterior development appears incomplete lending a croissant-like appearance to animals of this genotype. These animals exhibit aberrant cuticle surface and severe internal disorganization. Note that immediate anterior development appears normal as evidenced by a morphologically wild-type pharynx, although hyper-contraction of the body has imposed a kink between the pharyngeal bulbs. Both of the animals displayed in C and D are 6 days old. Bar in C, D and E = 20 µm.

of the rays and fan. Furthermore, the presence of *srl* mutations results in the extrusion of the copulatory spicules. This may reflect a role for the *srl* gene products within the structures responsible for the extension/retraction of the copulatory spicules. It is unclear whether the spicules in srl mutants are lengthened relative to those in wild type.

dpy-18 homozygotes display disorganized tail morphology concomitant with a thickening and shortening of the rays (Figure 6F). BAIRD and EMMONS (1990)

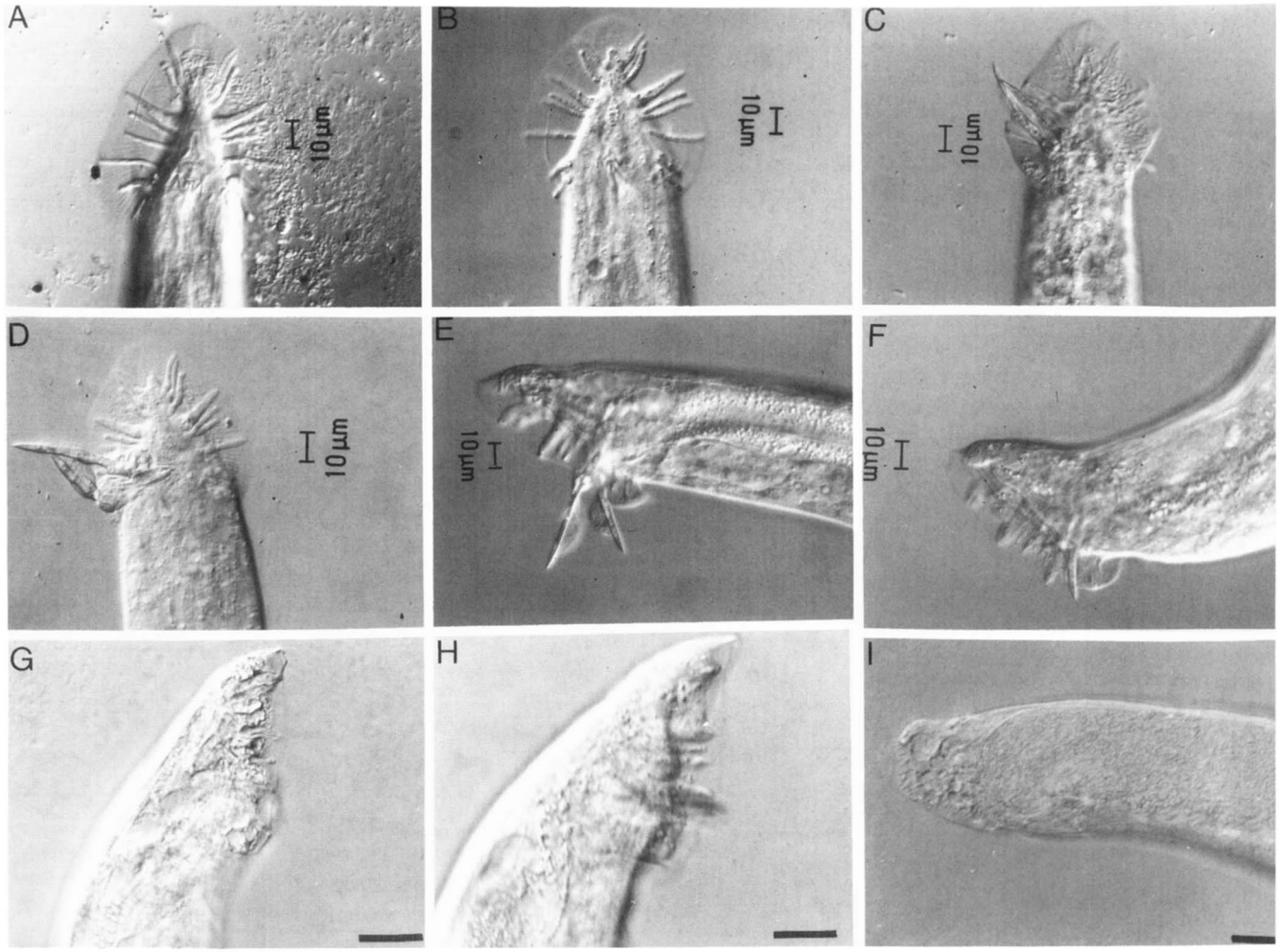

FIGURE 6.—Nomarski micrographs illustrating adult male posterior morphology. (A) Wild type (*N2*); (B) *unc-46 rol-3(s1040*ts) at 15°. Worms homozygous for *rol-3(s1040*ts) at 20–25° are inviable. Based on the comparison between A and B, there is no apparent male tail defect associated with either *rol-3* or *unc-46*. (C) *srl-1(s2501);unc-46 rol-3(s1040*ts); (D) *srl-2(s2507);unc-46 rol-3(s1040*ts). Note a general reduction in tail structures in worms homozygous for *srl* mutations, as well as fully irreversibly extended spicules. (E) *srl-2(s2507);unc-46 rol-3(s1040*ts), the abnormally extended spicules are conspicuous in this lateral view of a male homozygous for *srl-2(s2507)*; (F) *dpy-18;unc-46* male. Tail defects present are associated solely with *dpy-18* since *unc-46* does not influence male tail morphology. (G) *srl-2(s2506) dpy-18* male; (H) *srl-2(s2507) dpy-18* male. In both G and H the defects observed are likely the additive effect of *dpy-18*'s male tail defect and those attributed to *srl-2*. (I) *srl-1(s2501);dpy-18;unc-46 rol-3(s1040*ts). Note that the *srl-1-dpy-18* combination results in a severe dissorganization of the male posterior and lack of recognizable male copulatory apparatus. Bar in G, H and I = 20 μm.

identified and characterized five genes comprising a class of mutations known as *ram* mutants for their effect on ray morphology. They stated that *dpy-18*'s Ram phenotype is presumably due to cuticle effects on ray morphology. Figures 6G and 5H illustrate the effects of *srl-2(s2506)* and *srl-2(s2507)*, respectively, in a *dpy-18(e364)* background. It is evident that *srl-2* has an additional effect on the morphology of the male tail as these worms display increased tail disorganization including a further shortening and/or absence of rays, as well as an obvious decrease in fan size. The effect of *srl-1* on male tail development is even more pronounced. Figure 6I shows a lateral view of a *srl-1(s2501);dpy-18(e364);unc-46(e177) rol-3(s1040*ts) adult male. In this case the male posterior structures are so deformed that there no recognizable male tail structures present. We believe that with respect to overall male tail morphology, the mutations within the *srl-1* locus behave synergistically with mutations within the *dpy-18 (III)* locus.

We tested the *srl-1* and *srl-2* mutations to determine whether they represent alleles of previously identified genes that map in the same vicinity and exhibit male tail defects. We suspected that *srl-1* mutations might represent alleles of *mab-9 II*, (CHISHOLM and HODGKIN 1989) and *srl-2* mutations might represent alleles of *mab-10 III* (HODGKIN 1983), *mab-5 III* (KENYON 1986) or *pal-1 III* (WARING and KENYON 1991). However, loss of function alleles of the above genes both fail to suppress *rol-3(s1040*ts), and complement the tail phenotypes of the *srl-1* mutations tested against. *smg-6* is a recessive suppressor of mutations within a variety of different genes and exhibits a male abnormal phenotype (HODGKIN *et al.* 1989). The *smg-6* allele (r896)

also fails to suppress *rol-3(s1040ts)*, and complements the tail phenotype of *srl-2* alleles; and alleles of *srl-2* do not suppress the *smg* suppressible *dpy-10* allele *e61*.

DISCUSSION

In this paper we describe the genetic characterization of *rol-3* and the isolation and analysis of its intergenic suppressors. *rol-3* has at least two distinct developmental functions. First, *rol-3* has an essential function during early to mid-larval development. Second, *rol-3*'s adult-specific visible phenotype is rotation about the nematode long axis called rolling. Roller mutants have long been considered to be a subset of the morphological mutants (KUSCH and EDGAR 1986). Roller mutations, grouped together with Squat, Long, Blister and Dumpy mutants, are thought to exert their effects on overall nematode morphology by disrupting functions or components that normally act to establish and/or maintain cuticle integrity. Indeed, many of these morphological mutants are now known to result from mutations in genes encoding collagens or other cuticle components (KRAMER *et al.* 1988; VONMENDE *et al.* 1988; KRAMER *et al.* 1990). Worms homozygous for *rol-3(e754)* not only display aberrant adult cuticles but also display epistatic interactions with other cuticle mutants (HIGGINS and HIRSH 1977; COX *et al.* 1980; KUSCH and EDGAR 1986). Until this report it was assumed that the *rol-3* gene would simply encode a cuticle component.

If an altered adult cuticle component is responsible for *rol-3(e754)*'s rolling phenotype, we would expect that heterochronic mutations affecting cuticle development would also affect the expression of the rolling phenotype. We have shown that this is not the case. The heterchronic mutation *lin-14(n179ts)* allows the early expression of an adult cuticle (AMBROS and HORVITZ 1984, 1987) to occur at restrictive temperature (25°). *rol-3(e754);lin-14(n179ts)* roll as gravid adults at the restrictive temperature, suggesting that formation of an adult cuticle one molt earlier then in wild type does not elicit a parallel effect on the onset of rolling. In addition, *lin-29(n1440)* causes reiteration of larval cuticles in adult worms such that an adult cuticle is not produced (AMBROS and HORVITZ 1984). From the fact that all *rol-3(e754);lin-29(n1440)* worms roll as adults we argue that the expression of the rolling phenotype is not dependent on the presence of an adult cuticle. Therefore, *rol-3* itself is unlikely to encode an adult cuticle specific component.

There are an estimated 40–150 collagen genes in the *C. elegans* genome (COX, KRAMER and HIRSH 1984; KINGSTON 1991), suggesting redundancy within members of this gene family. As such, one would predict that mutations in many collagen genes would be nonlethal. *rol-6* and *sqt-1* are good examples of this, both of these genes encode collagens, and both display genetic behavior expected from redundant gene products, including wild-type null alleles (PARK and HORVITZ 1986; KRAMER *et al.* 1988, 1990). *rol-3*, on the other-hand, has 11 recessive lethal alleles and only two viable alleles. In contrast to *rol-6* and *sqt-1*, we suspect that *rol-3* null alleles affect viability. We have two pieces of evidence from which we argue that *rol-3* lethal alleles constitute loss of function alleles. First, from the presence of a temperature sensitive allele that rolls at permissive temperature, it can be argued that the more severe alleles result in lethality. Second, weaker *rol-3* lethal alleles display more severe phenotypes as heterozygotes over a deficiency then as homozygotes, while the strong alleles exhibit no such effect (data not shown). This suggests that those *rol-3* alleles which cause early developmental arrest are likely null mutations and the weaker lethal alleles are hypomorphs. We suspect that the relatively rare visible alleles constitute very weak hypomorphs or represent minor alterations of the *rol-3* gene product.

What function does *rol-3* perform during larval development, and how does this relate to the adult phenotype? It is possible that the *rol-3* gene product is multifunctional and performs an essential developmental role, as well as an unrelated role during adulthood. Alternatively, the rolling phenotype may be a consequential manifestation of altered essential gene function during early larval development and thus only detected in the weakest of alleles. If this is the case then it is possible that *rol-3* may play a role within *C. elegans* basement membrane or basal lamina. Since basement membranes are believed to play roles in a variety of important biological functions, including tissue morphogenesis, maintenance of tissue structure, cell attachment and may also serve as a substrate upon which cells migrate (GOU and KRAMER 1989; GUO, JOHNSON and KRAMER 1991; MCDONALD 1989; HUGHES and BLAU 1990; DISPERSIO, JACKSON and ZARET 1991), mutations affecting such genes would be expected to have repercussions in overall morphology, as well as viability (GUO and KRAMER 1989). For example, *emb-9* encodes the *C. elegans* alpha 1(IV) collagen chain and mutations of this locus cause temperature sensitive lethality during late embryogenesis (GUO, JOHNSON and KRAMER 1991).

Early studies by JARVIK and BOTSTEIN (1973, 1975) showed that intergenic second-site suppressors can identify genes whose products interact with the defective product of the first gene, thus identifying additional genes involved in a particular process and perhaps providing information regarding the function of that system. This approach has also proven effective in *C. elegans* (RIDDLE and BRENNER 1978; WATERSTON *et al.* 1982; HODGKIN, KONDO and WATERSTON 1987; SCHNABEL, BAUER and SCHNABEL 1991). We were

successful in generating recessive mutations within two genes that suppress the mid-larval lethal phenotype of *rol-3(s1040*ts): *srl-1(II)* and *srl-2(III)*. Interestingly, these mutations fail to suppress *s1040*'s weak rolling phenotype. The identification of two loci that in turn affect the lethal phenotype of *rol-3(s1040*ts) suggests that *srl-1* and *srl-2* may act in a common biochemical or regulatory pathway with the *rol-3* gene product.

The complementation data for alleles of *srl-1* and *srl-2* indicate that there exists a complex, allele specific, genetic interaction between these two loci. Specifically, in a *rol-3(s1040*ts) background at restrictive temperature, worms heterozygous for *srl-2(s2506)* and any of the four *srl-1* alleles develop to fertile adulthood. Therefore, *srl-2(s2506)* fails to complement all *srl-1* alleles analyzed to date, despite their residence on different chromosomes.

Intergenic noncomplementation between recessive mutations is indicative of functional interactions between the products of the genes involved (KUSCH and EDGAR 1986; TRICOIRE 1988; DANBLY-CHAUDIERE *et al.* 1988; HOMYK and EMERSON 1988; BAIRD and EMMONS 1990). Our observed intergenic noncomplementation supports *srl-1* and *srl-2*'s participation in a common developmental pathway.

Based on the relatively high forward mutation frequency (1 in 12, 500) at which *srl* mutations are generated, we suspect that the majority of our mutations are gene knockouts. Although the true natures of these mutations are not known, the complementation results of Table 4 suggest that some alleles may retain partial function. In the crosses of *srl-1(s2502)* hermaphrodite by either *srl-2(s2507)* or *srl-2(s2508)* male, and *srl-2(s2507)* hermaphrodite by *srl-1(s2502)* male, we observed a small number of escapees doubly heterozygous for mutations in *srl-1* and *srl-2* indicative of a very weak dominant effect. It is possible that *srl-2(s2507)* and *s2508* each harbor a similar defect to that seen in *s2506* since all three mutations exhibit dominant effects. However, in the case of *srl-2(s2507)* and *s2508*, this dominance is only exhibited in the presence of *srl-1(s2502)*, while *srl-2(s2506)* displays interalleleic noncomplementation with all *srl-1* alleles.

Our observations that males homozygous for either *srl-1* or *srl-2* display aberrant tail structures suggests a possible role for the *srl* gene products. We suspect that these genes normally function in some aspect of *C. elegans* posterior patterning. Mutations in many such genes are conspicuous in the male due to the disruption of the well-defined and prominent male tail. However, most such genes affecting the male tail also show less obvious hermaphrodite effects (HODGKIN 1983). Genetic evidence suggests that many of the genes affecting male tail morphology encode products that function as regulators of downstream gene expression. In the case of *mab-5* (COSTA *et al.* 1988) and *pal-1* (WARING and KENYON 1991), this has been corroborated with molecular data. Both of these genes encode homeo-domains, which are thought to have DNA binding capabilities. It is possible that the *srl* gene products also act to regulate gene expression. Based on our genetic evidence we propose that *srl-1(+)* and *srl-2(+)* are coregulators of the *rol-3* gene. However, since no detectable tail morphological defect has been associated with *rol-3*, the tail phenotype associated with mutations in the *srl-* loci suggests that they may also have roles outside of their association with the *rol-3* gene.

The question remains as to the nature of the relationship between *rol-3* and the *srl* gene products. We propose that the *srl-1*(+) and *srl-2*(+) gene products participate in regulation of the *rol-3*(+) gene product. Specifically, the wild-type *srl* gene products may act in a concerted fashion as negative regulators of *rol-3* expression. In light of this, we expect that subjecting hypomorphic *rol-3* alleles to down-regulation by wild-type *srl* gene products would result in a substantial decrease of functional *rol-3* gene product relative to the levels in wild-type worms. However, in the absence of down regulation, a hypomorphic allele of *rol-3* may produce enough functional *rol-3* product to meet the essential function requirements, thus avoiding developmental arrest. The left-handed rolling phenotype is not suppressed presumably because such a phenotype is the result of a weakly defective *rol-3* product. Up-regulating the expression of this mutant product would not be expected to suppress the rolling phenotype.

This model predicts that mutations in the *srl-1* or *srl-2* loci will suppress other *rol-3* lethal alleles as long as they are not amorphic or null alleles. Indeed, we observe suppression of a number of *rol-3* lethal alleles by mutations at the *srl-2* locus. Worms homozygous for *rol-3* alleles such as *s126* and *s1519* arrest development at an early larval stage. As such, it is expected that these represent severe loss of function or even null alleles of *rol-3*. In no case do any of the *srl-2* alleles tested suppress the lethal phenotype of either of these alleles. However, we have achieved suppression of less severe mid-larval lethal mutations of *rol-3*. In general, the suppressible mutations in *rol-3* constitute weaker lethal alleles, whereas strong alleles are not suppressible.

The suppression of *rol-3(s742)* by *srl-2* gives further insight into the developmental roles played by the *rol-3* and *srl* gene products. As discussed previously, hermaphrodite worms which are homozygous for *rol-3(s742)* and *srl-2(s2507)*, and some that are homozygous for *srl-2(s2506* or *s2508)* and *rol-3(s742)* display abnormal cuticle and internal morphology as well as disrupted posterior morphology. As illustrated in Fig-

ures 5C, D and E, the posteriors are rounded and blunt, while the heads and pharynx appear normal.

In general, we observe *srl-2*-mediated suppression of roller lethality in only the weaker of the *rol-3* alleles. The observation that neither the most severe lethal *rol-3* alleles, nor the rolling defect are suppressible is consistent with the *srl* gene products acting as negative regulators of functional *rol-3* expression. However, the male tail defects associated with *srl-1* and *srl-2* suggest that these gene products are pleiotropic and may regulate the expression of genes other than *rol-3*.

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