Analysis of Mutations in the sqt-1 and rol-6 Collagen Genes of Caenorhabditis elegans

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ABSTRACT

Different mutations in the sqt-1 and rol-6 collagen genes of Caenorhabditis elegans can cause diverse changes in body morphology and display different genetic attributes. We have determined the nucleotide alterations in 15 mutant alleles of these genes. Three mutations in sqt-1 and one in rol-6 that cause dominant right-handed helical twisting (RRol) of animals are arginine to cysteine replacements. These mutations are all within a short conserved sequence, on the amino terminal side of the Gly-X-Y repeats, that is found in all C. elegans cuticle collagens. A recessive RRol mutation of rol-6 is a replacement of one of the same conserved arginines by histidine. In contrast, three sqt-1 mutations that cause recessive left-handed helical twisting (LRol) are replacements of a conserved carboxy-terminal cysteine residue with either tyrosine or serine. These results suggest that disulfide bonding is important in collagen organization and that a deficit or surplus of disulfides may cause cuticle alterations of opposite handedness. In contrast to other collagens, glycine replacement mutations in the Gly-X-Y repeats of sqt-1 cause very mild phenotypes. Nonsense mutations of both sqt-1 and rol-6 cause nearly, but not totally, wild-type phenotypes. A nonsense mutation in sqt-1 suppresses the phenotype of rol-6 RRol mutations, suggesting that rol-6 collagen function is dependent on the presence of sqt-1 collagen. Mutations of sqt-1 are not suppressed by a rol-6 nonsense mutation, however, indicating that sqt-1 collagen can function independently of rol-6.

C. elegans normally has an elongate, vermiform body morphology that is typical of all nematodes. More than 50 genes have been identified that appear to primarily affect the body morphology of C. elegans (Brenner 1974; Higgins and Hirsh 1977; Cox et al. 1980; Hosono 1980; Kusch and Edgar 1986). The phenotypes produced by mutations in these genes include; blister (Bli), dumpy (Dpy, short and fat), long (Lon, long and thin), right roller (RRol, twisted in a right-handed helix) and left roller (LRol, twisted in a left-handed helix). Many of these genes appear to affect the organization of the cuticle (exoskeleton), which is a complex, multilayered extracellular structure. Five cuticles are produced during the C. elegans life cycle, and each can be shown to be distinct by structural, biochemical and/or genetic criteria. Cuticles are composed primarily of a large number of distinct collagen proteins (Cox, Kusch and Edgar 1981; Cox, Staprans and Edgar 1981; Politz, Politz and Edgar 1986).

The collagen gene family of C. elegans contains between 50 and 150 members that are dispersed throughout the genome (Cox, Kusch and Hirsh 1982; Cox, Kramer and Hirsh 1984; Cox et al. 1985, 1989). Two distinct types of collagen genes have been characterized; two genes that encode basement membrane type IV collagen chains (Guo and Kramer 1989; Guo, Johnson and Kramer 1991; Sibley et al. 1993) and more than 30 genes that encode putative cuticle collagens (Kramer, Cox and Hirsh 1982; von Mendel et al. 1988; Kramer et al. 1988, 1990; Johnstone, Shab and Barry 1992; Bird 1992; Waterston et al. 1992; Levy, Yang and Kramer 1993; A. D. Levy and J. M. Kramer, unpublished data). Although in most cases the putative cuticle collagens have not been directly localized to the cuticle, for ease of reference we denote them cuticle collagens to distinguish them from the basement membrane collagens. All of the cuticle collagens have similar domain structures; an amino non(Gly-X-Y) domain of approximately 100 amino acids, a central domain of approximately 50 Gly-X-Y repeats with 2-4 interruptions, and a carboxyl non(Gly-X-Y) domain of from 9-60 amino acids. There are three conserved cysteines preceding and two following the Gly-X-Y repeats, and two or three cysteines within the first interruption of the Gly-X-Y domain. Different sets of cuticle collagen genes are expressed at different developmental stages (Politit and Edgar 1984; Cox and Hirsh 1985; Kramer, Cox and Hirsh 1985; Liu and Ambros 1991; A. D. Levy and J. M. Kramer, unpublished data) presumably giving rise to the distinct stage-specific cuticles.

Several of the genes that affect organismal morphology have been shown to encode collagens: dpy-13 (von Mendel et al. 1988), sqt-1 (Kramer et al. 1988),
rol-6 (**KRAMER** et al. 1990), dpy-7 (**JOHNSTONE, SHAFI** and **BARRY** 1992), dpy-2 and dpy-10 (**LEYV, YANG** and **KRAMER** 1993). All six of these collagens have the structural features common to the cuticle collagen family. Sequence analysis of mutant alleles has shown that replacements of glycine residues in the Gly-X-Y repeats of dpy-7 cause a recessive Dpy phenotype (**JOHNSTONE, SHAFI** and **BARRY** 1992), while glycine replacements in dpy-2 and dpy-10 cause Dpy or DpyLRol phenotypes (**LEYV, YANG** and **KRAMER** 1993). A dpy-10 allele that causes dominant LRol and recessive temperature-sensitive DpyLRol phenotypes was found to be a replacement of a conserved arginine by cysteine on the amino side of the Gly-X-Y repeats (**LEYV, YANG** and **KRAMER** 1993). Different molecular alterations in these collagens can cause different morphological phenotypes and different phenotypes can arise from similar mutations in different collagen genes.

Mutations in the sqt-1 collagen gene can cause the diverse and even contrary phenotypes: Lon, Dpy, LRol and RRol. sqt-1 also shows genetic interactions with several other genes that affect body morphology, including the dpy-2, dpy-10 and rol-6 collagen genes (**COX** et al. 1980; **KUSCH** and **EDGAR** 1986; **LEYV, YANG** and **KRAMER** 1993). For example, a single copy of any non-null sqt-1 allele, even a dominant RRol allele, transforms the Dpy phenotype of dpy-10(e128) to DpyLRol (**KUSCH** and **EDGAR** 1986; **LEYV, YANG** and **KRAMER** 1993), indicating that dpy-10 is sensitive to the presence of any abnormal sqt-1 collagen. The recessive LRol allele sqt-l(sc13) was found to be a dominant suppressor of the recessive RRol allele rol-6(e187) (**COX** et al. 1980). Genetic interactions such as these may reflect interactions between the collagens encoded by these genes. The sequence similarity of the sqt-1 and rol-6 collagens suggests that they could even function within the same triple-helical molecule (**KRAMER** et al. 1990).

In this paper we describe the molecular bases for the diverse morphological phenotypes generated by mutations in the sqt-1 and rol-6 collagen genes. In contrast to the findings with other collagens, the strongest mutations are not replacements of glycines within the Gly-X-Y repeats, but rather occur in conserved sites flanking the repeats. We further describe genetic interactions between these two genes and discuss them in light of their particular molecular alterations.

**MATERIALS AND METHODS**

**Nematode strains:** Maintenance and handling of *C. elegans* strains were as described by **BRENNER** (1974). All strains were maintained at 20° for these studies. The following rol-6 alleles were used: e187, su1006 (**COX** et al. 1980); n1177, n1178, e187n1268, e187n1269, e187n1270, e187n1271, e187n1272, e187n1280 (**PARK** and **HORVITZ** 1986). The following sqt-1 alleles were used: e1350, sc1, sc13 (**COX** et al. 1980; sc99, sc101, sc103, sc104, sc107 (**KUSCH** and **EDGAR** 1986); sc112, sc113 (**KRAMER** et al. 1988). Strains carrying the following chromosomal deficiencies were utilized: mnDf45, mnDf80 (**SUGIRSDON, SPANIER** and **HERMAN** 1984).

**Analysis of interactions between rol-6 and sqt-1:** The rol-6 sqt-1 double mutant strains and various allelic combinations were constructed by standard genetic methods. The closely linked, uncoordinated mutation, unc-4(e120), was used to mark chromosomes in some cases. To directly demonstrate the presence of the appropriate sqt-1 and rol-6 alleles in double mutant strains, Dpy nonUnc and Unc nonDpy recombinants were isolated from + rol-6 + sqt-1/ dpy-10(e128) + unc-4(e120) + hermaphrodites. These recombinant animals were tested with heterozygous males carrying appropriate sqt-1 or rol-6 alleles. To generate the double dominant RRol combination rol-6(su1006) +/+ uncer-4 sqt-1(e1350), unc-4 sqt-1(e1350)/+ sqt-l(sc13) males were used, since sc13 suppresses the dominant RRol phenotype of e1350, allowing males to mate. Phenotypes were analyzed by observation of morphology and movement under a dissecting microscope and by polarized light microscopy (**WATERSTON, THOMSON** and **BRENNER** 1980). In all cases, the phenotype determined with the dissecting microscope was concordant with the organization of muscle bundles as determined with polarized light microscopy. Weak phenotypes were confirmed under a dissecting microscope by comparing wild-type and mutant young adult animals picked onto the same plate. All photomicrographs were taken with TechPan film at ASA100 on a Zeiss Axiopt microscope.

**Generation of a rol-6 sqt-1 double null strain:** rol-6(n1178) sqt-l(sc13)/+ + males were mated to unc-4 sc103 hermaphrodites. LRol F1s were picked and their F2 progeny were screened for non-Rol, non-Unc animals. Several such animals were recovered that segregated 1/4 Unc, 3/4 Tal (abnormal tail) progeny, and homozygous Tal strains were isolated from these. The presence of both null alleles in the strain was confirmed by observing Rol progeny when mated with Rol(sc13)/+ and rol-6(e187)/+ males.

**Determination of mutant gene sequences:** Clopes of the sqt-l(1350), sqt-l(sc13), rol-6(e187) and rol-6(su1006) mutant genes were generated by ligation of size fractionated genomic DNAs into the Bluescribe plasmid vector, as previously described (**KRAMER** et al. 1988). Other mutant sequences were determined by direct sequencing of PCR amplification products or sequencing of multiple clones derived from PCR amplification (**LEYV, YANG** and **KRAMER** 1993). Sequences were generated utilizing sets of genespecific oligonucleotide primers. In all cases, the sequence of the complete coding region of the mutant gene was determined.

**RESULTS**

Right roller phenotypes result from alterations in conserved arginine residues: Three existing sqt-1 alleles, e1350, sc1 and sc104, cause a Dpy phenotype when homozygous (recessive Dpy), but have a RRol phenotype when heterozygous to a wild-type allele (dominant RRol). The two existing rol-6 Rol alleles are e187, a recessive RRol mutation, and su1006, a semidominant RRol mutation. su1006 homozygotes have a stronger RRol phenotype than su1006+/+ animals. The nucleotide alterations in these five RRol alleles were determined and are shown in Table 1 and
C. elegans *sqt-1* and *rol-6* genes

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele</th>
<th>Phenotypea</th>
<th>Nucleotide</th>
<th>Amino acid</th>
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<tbody>
<tr>
<td><em>sqt-1</em></td>
<td>sc103</td>
<td>Tal</td>
<td>339</td>
<td>C &gt; T</td>
</tr>
<tr>
<td></td>
<td>sc1</td>
<td>Dominant R Rol, Tal</td>
<td>395</td>
<td>C &gt; T</td>
</tr>
<tr>
<td></td>
<td>sc104</td>
<td>Dominant R Rol, Tal</td>
<td>402</td>
<td>C &gt; T</td>
</tr>
<tr>
<td></td>
<td>e1350</td>
<td>Dominant R Rol, Tal</td>
<td>402</td>
<td>C &gt; T</td>
</tr>
<tr>
<td></td>
<td>sc107</td>
<td>Tal, wLon, wLRol</td>
<td>604</td>
<td>G &gt; A</td>
</tr>
<tr>
<td></td>
<td>sc99</td>
<td>Tal, wLon</td>
<td>649</td>
<td>G &gt; A</td>
</tr>
<tr>
<td></td>
<td>sc101</td>
<td>Lon, Tal</td>
<td>945</td>
<td>G &gt; A</td>
</tr>
<tr>
<td></td>
<td>sc13</td>
<td>sLRol, Tal</td>
<td>1105</td>
<td>G &gt; A</td>
</tr>
<tr>
<td></td>
<td>sc112</td>
<td>sLRol, Tal</td>
<td>1105</td>
<td>G &gt; A</td>
</tr>
<tr>
<td></td>
<td>sc113</td>
<td>sLRol, Tal</td>
<td>1105</td>
<td>G &gt; C</td>
</tr>
<tr>
<td><em>rol-6</em></td>
<td>n1268</td>
<td>wDpy</td>
<td>258</td>
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</tr>
<tr>
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<td>258</td>
<td>G &gt; A</td>
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<tr>
<td></td>
<td>su1606</td>
<td>Semidominant R Rol</td>
<td>415</td>
<td>C &gt; T</td>
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<td>R Rol</td>
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<td></td>
<td>n1178</td>
<td>wDpy</td>
<td>1034</td>
<td>C &gt; T</td>
</tr>
</tbody>
</table>

*a* Phenotypes are: Dpy, dumpy; Lon, long; LRol, left roller; RRol, right roller; Tal, abnormal tail; WT, wild type. Modifiers: s, strong; w, weak.

*Numbering of nucleotides and amino acids is based on published sequences (Kramer et al. 1988, 1990).*

**rol-6 mutations**

Figure 1. All five of these mutations cause replacement of one or the other of two arginine residues in a short conserved sequence found in all *C. elegans* cuticle collagens. We had previously identified this region of amino acid sequence conservation by sequence inspection and named it Homology Block A (HBA). The sequences of HBA in 17 *C. elegans* cuticle collagens are shown in Figure 2. HBA is located between 23 and 85 amino acids to the amino side of the start of the Gly-X-Y repeats in these collagens. One of its most highly conserved features is the presence of two arginines spaced three residues apart. The four mutations that cause dominant RRol phenotypes are replacements of one or the other of these arginines with cysteine, while the recessive RRol mutation is an arginine to histidine change. The identification of five RRol mutations in HBA demonstrates that it has an important role in normal *sqt-1* and *rol-6* collagen function. The fact that all four dominant mutations introduce a cysteine into HBA suggests that dominance may be due to abnormal disulfide bond formation.

**Strong left roller phenotypes result from loss of a conserved cysteine**: The nucleotide alterations in three recessive LRol alleles of *sqt-1* were identified (Table 1; Figure 1). All three mutations result in the replacement of a cysteine residue in the carboxyl terminal non-triple-helical region of the molecule with either tyrosine or serine. All of the *C. elegans* cuticle collagens characterized have two cysteine residues in
Homology Block A

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>dpy-7</td>
<td>-32</td>
<td>G R I K R Q Y G</td>
</tr>
<tr>
<td>col-7</td>
<td>-23</td>
<td>F G R R R Q K K</td>
</tr>
<tr>
<td>col-8</td>
<td>-23</td>
<td>T R K A R Q A Y</td>
</tr>
<tr>
<td>col-19</td>
<td>-23</td>
<td>A R T A R Q A G</td>
</tr>
<tr>
<td>col-1</td>
<td>-27</td>
<td>N R T T R Q A Y</td>
</tr>
<tr>
<td>dpy-13</td>
<td>-32</td>
<td>N R T A R A V R</td>
</tr>
<tr>
<td>col-2</td>
<td>-30</td>
<td>T R V A R Q A Y</td>
</tr>
<tr>
<td>col-14</td>
<td>-47</td>
<td>K R D T L Q D F</td>
</tr>
<tr>
<td>col-6</td>
<td>-65</td>
<td>H R M K R A W L</td>
</tr>
<tr>
<td>col-12,13</td>
<td>-30</td>
<td>R K A R R Q S Y</td>
</tr>
<tr>
<td>col-36</td>
<td>-42</td>
<td>T R S R R D A G</td>
</tr>
<tr>
<td>col-40</td>
<td>-30</td>
<td>T R S R R G G Y</td>
</tr>
<tr>
<td>dpy-10</td>
<td>-40</td>
<td>N R T A R G A Y</td>
</tr>
<tr>
<td>dpy-2</td>
<td>-33</td>
<td>N V T K R A A G</td>
</tr>
<tr>
<td>sqt-1</td>
<td>-62</td>
<td>K R V R R Q Y E</td>
</tr>
<tr>
<td>rol-6</td>
<td>-85</td>
<td>N R V R R Q Q Y</td>
</tr>
</tbody>
</table>

Consensus N R T R Q A Y A

**Figure 2.**—Homology Block A sequences of 17 C. elegans cuticle collagens. The most highly conserved residues are shaded. Location numbering is for the first amino acid shown and indicates its position counting back from the start of the Gly-X-Y repeats. Sequences were taken from the following references: KRAMER, COX and HIRSH 1982; VON MENDE et al. 1988; KRAMER et al. 1988, 1990; COX et al. 1989; JOHNSTONE, SHAFI and BARRY 1992; LEVY, YANG and KRAMER 1993; A. D. LEVY and J. M. KRAMER, unpublished data.

This region, although their precise spacing varies in different collagens. All three of the LRol mutations affect the cysteine located closest to the end of the Gly-X-Y repeat domain. These mutations show that this cysteine is important for normal function of the sqt-1 collagen.

One of these LRol alleles, sc13, was EMS induced (COX et al. 1980), while the other two, sc112 and sc113, arose in a mutator strain background (KRAMER et al. 1988). Mutator strains are known to exhibit a high frequency of transposition of C. elegans transposable elements, but have not been shown to cause point mutations (COLLINS, SAARI and ANDERSON 1987). Only the single nucleotide changes indicated were found in the sequences of sc112 and sc113; there was no indication that a transposable element had inserted and subsequently excised from the gene. The G to C nucleotide change of sc113 differs from the almost exclusive G to A changes found with EMS mutagenesis, supporting the notion that it arose by some other mechanism.

**Glycine replacement mutations in sqt-1 cause mild phenotypes:** Three alleles of sqt-1 were found to cause glycine to glutamate replacements in the Gly-X-Y repeat region (Table 1; Figure 1). Each of these mutations affects a different glycine residue and causes a different phenotype: sc107 is Tal, wLon, wLRol; sc99 is Tal and wLon; sc101 is Lon and Tal. The Tal phenotype indicates a shortened, abnormally shaped hermaphrodite tail (see below). The phenotypes of sc99 and sc107 were previously reported to be approximately wild-type (KUSCH and EDGAR 1986), but they clearly display the abnormal phenotypes indicated. These three glycine replacement mutations cause much less severe morphological changes than the strong RRol and LRol mutations described above.

A **nonsense mutation of sqt-1:** sqt-1(sc103) was proposed to be a null allele based on the fact that it acted similar to a deficiency in complementation tests (KUSCH and EDGAR 1986). Sequence analysis shows that sc103 is an ochre mutation in codon 48, well upstream of the Gly-X-Y repeats of the sqt-1 gene (Table 1; Figure 1). This mutation would result in a severely truncated, presumably nonfunctional polypeptide product and therefore represents a true null, as predicted.

**Identification of nonsense mutations of rol-6:** The null phenotype of rol-6 has been reported to be essentially wild-type (SIGURDSON, SPANIER and HERMAN 1984; PARK and HORVITZ 1986). Eight putative null mutations of the rol-6 gene were identified by PARK and HORVITZ (1986), six by reverting the RRol phenotype of rol-6(e187) and two by their failure to complement e187. Our examination of these putative rol-6 null strains indicated that none of them had completely wild-type phenotypes, and in some cases their phenotypes differed from each other (Table 2). Five of the strains had a very weak Dpy phenotype that was readily discernible only when compared side-by-side with wild-type animals. Two strains displayed head catching (a very weak roller phenotype), and one was a dauer-specific roller. Since there can only be one null phenotype, not all of these strains could be rol-6 nulls. We examined six of the strains further to determine which were most likely to be true nulls. When placed in trans to a deficiency that deletes rol-6, e187n1269 and e187n1271 produce a RRol phenotype, indicating that they are not rol-6 nulls. After mating to wild-type males, strains carrying e187n1269, e187n1271 and e187n1280 segregated a recessive RRol allele that was indistinguishable from e187. These results indicate that these three strains are not rol-6 nulls, but rather they carry linked suppressors of the original e187 mutation. In contrast, the three strains carrying n1178, e187n1268 and e187n1270 exhibited the same weak Dpy phenotype in the homozygous state as when placed in trans to a deficiency. These three strains also failed to segregate e187. We chose to examine these three strains further to determine whether they represented true rol-6 nulls.

Sequence analysis showed that n1178, n1268 and n1270 are nonsense mutations in the rol-6 gene (Table 1; Figure 1). n1268 and n1270 are the same nucleotide
C. elegans sqt-1 and rol-6 genes

TABLE 2
Analysis of putative rol-6 null strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>m/f</th>
<th>m/Df</th>
<th>Recovery e187d</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT2957</td>
<td>rol-6(n1178)</td>
<td>wDpy</td>
<td>WT</td>
<td>wDpy</td>
<td>No</td>
</tr>
<tr>
<td>MT2707</td>
<td>rol-6(e187n1268)</td>
<td>wDpy</td>
<td>WT</td>
<td>wDpy</td>
<td>No</td>
</tr>
<tr>
<td>MT2709</td>
<td>rol-6(e187n1270)</td>
<td>wDpy</td>
<td>WT</td>
<td>wDpy</td>
<td>No</td>
</tr>
<tr>
<td>MT2708</td>
<td>rol-6(e187n1269)</td>
<td>Tal, wLon dauer, RRol</td>
<td>wDal</td>
<td>RRol</td>
<td>Yes</td>
</tr>
<tr>
<td>MT2710</td>
<td>rol-6(e187n1271)</td>
<td>Tal, wLon, Hdc</td>
<td>WT</td>
<td>RRol</td>
<td>Yes</td>
</tr>
<tr>
<td>MT2719</td>
<td>rol-6(e187n1280)</td>
<td>wDpy</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
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<td>rol-6(e1177)</td>
<td>wDpy</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
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<td>MT2711</td>
<td>rol-6(e187n1272)</td>
<td>wDpy</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Phenotypes are defined in Table 1. Hdc: head catching, a very weak roller phenotype.
b Mutant strains were mated with unc-4(e120)/+ males and F1s that segregated Uncs were examined.
c m/+ males were mated to mnDf45 unc-4/mnCl[dpy-10(e128) unc-52(e444)] hermaphrodites and individual F1s that failed to segregate Dpy Uncs were examined.
d Offspring of m/+ hermaphrodites were examined for the RRol phenotype. No: no RRol animals were observed among approximately 500 progeny examined. Yes: approximately 1% of offspring were RRol and carried a recessive rol-6 allele indistinguishable from e187. ND: not determined.

change located near the amino end of the collagen, while n1178 is located closer to the carboxyl end. The original e187 mutation was also identified in n1268 and n1270, as expected. All three of these mutations would truncate the collagen polypeptide and be expected to produce a nonfunctional product, confirming that they are true nulls.

Phenotypes of rol-6 and sqt-1 null mutants: Previous studies had indicated that the phenotypes of putative sqt-1 and rol-6 null mutant strains were approximately wild type. Careful examination of strains shown to carry nonsense mutations has identified slightly abnormal phenotypes. sqt-1(scl03) hermaphrodites have normal overall body morphology (Figure 3F), but have abnormal tails (Figure 4E). The tail whip is much shorter than normal and often has balls or lumps of apparent cuticular material. scl03 male tails have normal morphology, an observation also made by Baird and Emons (1990). Thus, normal hermaphrodite tail morphogenesis requires sqt-1 collagen.

rol-6(n1178) hermaphrodites and males have normal tails (Figure 4D), but are slightly shorter and fatter (wDpy) than wild-type animals (Figure 3I). The wDpy phenotype is difficult to recognize unless wild-type animals of the same age are present on the same plate. As a test of our ability to recognize this phenotype, 10 wild-type and 10 wDpy offspring from a n1178/+ unc-4 parent were picked to individual plates and their offspring examined. All 10 wild-type animals segregated Unc progeny and were therefore n1178/+ unc-4, while all ten wDpy animals failed to segregate Unc progeny and were n1178 homozygotes. Thus, rol-6(null) animals do display a weak, but discernible Dpy phenotype.

Effects of null backgrounds on Rol mutations of sqt-1 and rol-6: rol-6 sqt-1 double mutant animals carrying a visible allele of one gene and a null allele of the other were analyzed. In the sqt-1(e1350) null background neither rol-6(e187) nor rol-6(su1006) cause a roller phenotype (Table 3; Figure 3H). The double mutant animals look similar to scl03 alone. Therefore, expression of the rol-6 RRol phenotype requires the presence of sqt-1 collagen.

In contrast, sqt-1 visible phenotypes are apparent in the rol-6 null background. rol-6(n1178) sqt-1(scl13) animals have a LRol phenotype that is somewhat weaker than scl13 alone (Table 3). rol-6(n1178) sqt-1(e1350) animals do not have the same Dpy phenotype as e1350 (Table 3; Figure 3, B and G), but their phenotype is different from n1178 (Figure 31), indicating that the sqt-1(e1350) collagen does function in the absence of rol-6. The dominant phenotype of e1350 is expressed in the rol-6 null background since rol-6(n1178) sqt-1(e1350)/rol-6(n1178) + animals are RRol (Table 3). Thus, sqt-1 collagen can function in the absence of rol-6 collagen.

rol-6 RRol phenotypes are indistinguishable whether there are one (scl13/+ ) or two (sqt-1+) functional copies of wild-type sqt-1 (Table 3). Similarly, sqt-1(scl13) displays the same sLRol phenotype when either one or two rol-6+ alleles are present. In these cases, mutations in one gene show the same phenotype whether there are one or two wild-type copies of the other gene. The sqt-1(e1350) mutation, however, is sensitive to the number of functional copies of rol-6. rol-6(n1178) sqt-1(e1350)/+ sqt-1(e1350) animals are much less Dpy than e1350, and n1178 e1350 animals are actually wLon (Table 3; Figure 3G). Thus, the phenotype of e1350 animals is dependent on the rol-6 dosage, but the phenotype of e1350/+ animals is not (see above, and Table 3).

Double null mutant animals: The double null mutant strain rol-6(n1178) sqt-1(scl103) has abnormal tail
FIGURE 3.—Polarized light micrographs of wild-type, sqt-1 and rol-6 mutant adult animals. (A) N2, wild-type; (B) sqt-1(el350); (C) rol-6(su1006); (D) rol-6(su1006) +/+ sqt-1(sc13); (E) rol-6(su1006) sqt-1(sc13); (F) sqt-1(sc103); (G) rol-6(n1178) sqt-1(el350); (H) rol-6(su1006) sqt-1(sc103); (I) rol-6(n1178); (J) rol-6(n1178) sqt-1(sc103). The scale bar in A represents 0.1 mm.

FIGURE 4.—Nomarski images of wild-type, sqt-1 and rol-6 mutant adult hermaphrodite tails. (A) N2, wild-type; (B) sqt-1(el350); (C) rol-6(su1006); (D) rol-6(n1178); (E) sqt-1(sc103); (F) rol-6(n1178) sqt-1(sc103); (G) sqt-1(sc13); (H) rol-6(su1006) +/+ sqt-1(sc13). The scale bar in (A) represents 45 μm.
cessive Lon, Tal alleles a wild-type allele, the recessive LRol allele dominant to disrupt tail morphology. The homozygotes do not display the wDpy phenotype seen in RRol mutations, indicating that e187 is a dominant suppressor of the RRol phenotype of e187. Essentially the same result was reported by Cox et al. (1980). One or two copies of e187, however, only slightly reduce the severity of the LRol phenotype of sc13. One copy of sc13 can suppress one copy of su1006 (Figure 3D). However, two copies of su1006 are not fully suppressed since su1006 sc13/su1006 + animals are wRRol (Table 3). Two copies of sc13 override the su1006 RRol phenotype such that su1006 sc13 animals are wLRol (Figure 3E). All of the nonRRol combinations of e187 or su1006 with sc13/+ have the same wTal phenotype seen in sc13/+ alone (Figure 4H), indicating that the effect of sc13 on the hermaphrodite tail is not suppressed by these rol-6 mutations. su1006 e1350 animals are wDpy indicating that the e1350 Dpy phenotype is partially suppressed by two copies of su1006 and the sRRol phenotype of su1006 is completely suppressed.

**Dominant effects on hermaphrodite tail morphology:** Hermaphrodite tail morphology is disrupted in all homozygous sqt-1 mutants (Figure 4B & E, for example) and in animals heterozygous for the dominant RRol mutations. We looked for dominant tail defects in sqt-1 mutations that have recessive effects on overall body morphology. When placed in trans to a wild-type allele, the recessive LRol allele e1350 and the recessive Lon allele sc101 (Table 4) cause slightly shortened, abnormally shaped tails. The recessive Lon, Tal alleles sc99 and sc107, and the null allele, sc103, display wild-type tail morphology as heterozygotes. One copy of sqt-1 is, therefore, sufficient for normal tail morphology. The sc13 and sc101 mutant collagens can dominantly disrupt tail morphology, but have no evident dominant effect on overall body shape. rol-6 mutants have apparently wild-type tail morphology (Figure 4, C and D).

**Genetic interactions between rol-6 and sqt-1 roller alleles:** To further analyze possible genetic interactions between the rol-6 and sqt-1 genes the phenotypes of animals carrying various combinations of mutant alleles of the two genes were examined (Table 3). e187 sc13/e187 + animals have the same wTal phenotype as sc13/+, indicating that sc13 is a dominant suppressor of the RRol phenotype of e187. Essentially the same result was reported by Cox et al. (1980). One or two copies of e187, however, only slightly reduce the severity of the LRol phenotype of sc13. One copy of sc13 can suppress one copy of su1006 (Figure 3D). However, two copies of su1006 are not fully suppressed since su1006 sc13/su1006 + animals are wRRol (Table 3). Two copies of sc13 override the su1006 RRol phenotype such that su1006 sc13 animals are wLRol (Figure 3E). All of the nonRRol combinations of e187 or su1006 with sc13/+ have the same wTal phenotype seen in sc13/+ alone (Figure 4H), indicating that the effect of sc13 on the hermaphrodite tail is not suppressed by these rol-6 mutations. su1006 e1350 animals are wDpy indicating that the e1350 Dpy phenotype is partially suppressed by two copies of su1006 and the sRRol phenotype of su1006 is completely suppressed.

**Interactions of sqt-1 glycine replacement alleles:** The sqt-1 glycine replacement mutations sc99, sc101 and sc107 fail to complement the LRol allele sc13 and, like sc13, suppress the dominant RRol phenotype of e1350 (Table 4). Also similar to sc13, one copy of any of these three alleles can suppress one copy of the semidominant RRol mutation rol-6(su1006). Thus,
these *sqt-1* glycine replacement mutations and the
sLRol cysteine replacement mutation show similar
interactions with RRol mutations of both *sqt-1* and
*rol-6*. The *sc101* allele also shows a weak dominant
hermaphrodite tail defect similar to that seen with
*sc13* heterozygotes. In general, the glycine and cy-
steine replacement mutations of *sqt-1* act similarly,
except that cysteine replacements cause a sLRol ph-
notype while glycine replacements cause weak ph-
notypes.

**DISCUSSION**

The *sqt-1* and *rol-6* mutations described here are
unusual in comparison to mutations that have been
classified in other collagens. Glycine replace-
ments within the triple-helical domains of the human
*a1(I), a2(I), a1(II), a1(III)* (BEIGHTON et al. 1992) and
*a5(IV)* (TRYGGVASON et al. 1993) collagen chains, and
the *C. elegans* emb-9 (GUO, JOHNSON and KRAMER
1991), *dpy-7* (JOHNSTONE, SHAFI and BARRY 1992),
dpy-2 and dpy-10 (LEVY, YANG and KRAMER 1993)
collagen chains cause severe phenotypes. In contrast,
glycine replacement mutations in *sqt-1* cause very mild
phenotypes. This difference may be due to the fact
that the null phenotype of *sqt-1* is nearly wild type.
Among the other collagens, the null phenotypes are
only known with certainty for the *a5(IV)* collagen
*gene* (NETZER et al. 1992) and dpy-10 (LEVY, YANG
and KRAMER 1993). In both cases the null phenotype
is strong; juvenile onset Alport’s syndrome (renal fail-
ure, deafness and ocular defects) for *a5(IV)* and
DpyLRol for dpy-10.

A major consequence of glycine replacement mu-
tations is inhibition of triple helix assembly and intra-
cellular degradation of much of the abnormal collagen
(PROCKOP et al. 1989). Thus, such mutations generally
result in severely reduced amounts of collagen reach-
ing the extracellular matrix. In this sense, they are
reduction of function mutations and their phenotypes
are similar, though not identical, to the null pheno-
type. The wLon and wLRol phenotypes of *sqt-1* gly-
cine replacement mutations must be due to interfering
effects of the abnormal collagen, since these phen-
otypes are not seen in the null mutant. Interference
could be caused by mutant collagen that is secreted
into the matrix, or could occur at the level of intra-
cellular assembly. For instance, if the *sqt-1* collagen
interacts intracellularly with some other collagen, then
the interfering *sqt-1* protein could affect the function
of that collagen. Glycine replacement mutants of dpy-
10 can have more severe DpyLRol phenotypes than
the null (LEVY, YANG and KRAMER 1993), indicating
that collagens with strong null phenotypes can also
show interfering effects.

Strong *sqt-1* and *rol-6* mutant phenotypes result
from alterations outside of the triple-helical domains
of these collagens. Recessive LRol mutations result
from loss of a cysteine near the carboxyl end of the
*sqt-1* collagen, while dominant RRol mutations of *sqt-
1* or *rol-6* result from addition of a cysteine toward
the amino end. Opposite phenotypes arise from essen-
tially opposite molecular defects in these collagens.
Loss of a normal disulfide bond causes a left-handed
twist of the animal, while the possible formation of
extra or ectopic disulfide bonds causes a right-handed
twist. Ectopic disulfide bonds have been shown to
form in human *a1(I)* collagen containing a Gly to Cys
mutation that causes dominant osteogenesis imper-
feceta (COHN et al. 1988; STEINMANN, NICHOLLS and
POPE 1986). The fact that the recessive RRol mutation
*rol-6(e187)* does not introduce an extra cysteine, how-
ever, indicates that aberrant disulfide bond formation
cannot be the only way the RRol phenotype can be
generated.

The suppression of a *rol-6* RRol, gain of cysteine
mutation by a *sqt-1* LRol, loss of cysteine mutation
could be considered to result from restoration of the
normal number of potential disulfide bonds. How-
ever, *sqt-1* Gly to Glu mutations show the same ability
to suppress these *rol-6* mutations, indicating that
suppression must occur by some other means. In
general, the RRol phenotypes of both *sqt-1* and *rol-6*
can be suppressed by any nonRRol mutation in *sqt-1*,
i.e., wild-type *sqt-1* collagen is required for expres-
sion of the RRol phenotype in adults. In contrast, the *sqt-
1* LRol phenotype is only suppressed by wild-type or
RRol *sqt-1* collagen, not by the *rol-6* mutations.

All of the RRol mutations are alterations of one of
the two highly conserved Homology Block A (HBA)
arginines, *Arg1-X2-X3-Arg4*. A related motif, (*Arg/
Lys1-X2-(Arg/Lys)s-Arg4*, has been shown to function
as a cleavage site for subtilisin-like serine endoprotei-
nases (BARR 1991). Ten of the 17 *C. elegans* collagen
HBA sequences (Figure 2) do have an Arg or Lys at
position three, matching this subtilisin-like protease
cleavage motif. *Arg/Lys1-X2-X3-Arg4* has also been
identified as a protease processing site, although it is
not known whether the same class of endoprotei-
nases is involved. It is possible that HBA represents a
protease processing site in the *C. elegans* cuticle collagens.
If so, then the mutations in HBA may interfere with
this processing event and result in collagens with
abnormal amino-terminal extensions. The fact that an
Arg to His mutation in HBA is recessive, while Arg
to Cys mutations are dominant could be explained if
the Cys blocks cleavage and also forms ectopic disul-
fide bonds. Alternatively, insertion of Cys could in-
hibit cleavage to a greater degree than does insertion
of His.

The apparent limitation of dominant RRol muta-
tions to HBA might be a result of the limited number
of appropriate codons that can be mutated to encode
C. elegans sqt-1 and rol-6 genes

Cys. EMS causes primarily G:C to A:T transitions and occasional small deletions (COULONDRE and MILLER 1977; BEJSOVEC and ANDERSON 1990). The HBA Arg; and Arg, codons in both sqt-1 and rol-6 are CGT and would be expected to mutate only to TGT Cys or CAT His with EMS. Cys (TGT, TGC) and His (CAT, CAC) codons can only be generated from CGT or CGC, both Arg codons. The HBA Arg, codon in sqt-1 is CGT and could mutate to Cys or His, while in rol-6 it is AGA, which would only be expected to generate AAA Lys. There are no other CGT or CGC codons in the amino non(Gly-X-Y) domain of sqt-1 and only one in rol-6, located approximately 70 amino acids to the carboxyl side of HBA. Possibly, the insertion of Cys residues at other positions in the amino domains of these collagens could cause a dominant phenotype, but such mutations would not be expected to occur at high frequency with EMS mutagenesis.

Alteration of the HBA Arg, to Cys in the dpy-10 collagen has been shown to cause a dominant f Rol phenotype (LEVY, YANG and KRAMER 1993). Thus, in a third cuticle collagen insertion of a Cys into HBA causes a dominant phenotype. However, in this case a left-handed twist results while the same alteration in sqt-1 or rol-6 causes a right-handed twist. The equivalent molecular alterations in different collagens can cause twisting of opposite handedness. This difference could result if the dpy-10 collagen were organized within the cuticle in a manner that was the mirror image of the sqt-1 and rol-6 collagens. For example, two apposed layers of organized fibers in the adult cuticle spiral around the animal in opposite directions, one clockwise, the other counterclockwise (COX, KUSCH and EDGAR 1981). Because the fiber layers are in opposite orientations, a defect in the clockwise layer could cause the opposite effect as the same defect in the counterclockwise layer. Possibly the dpy-10 collagen is localized to one of these fiber layers and the sqt-1 and rol-6 collagens are in the other.

The null phenotypes of sqt-1 and rol-6 are almost, but not quite, wild type. The functions of these genes are not therefore completely redundant, or replaceable by other collagen genes. The null phenotypes of the two genes are different, indicating that their required functions are not identical. The weak Dpy phenotype of the rol-6 null is suppressed in the sqt-1 null background, such that the double null mutant is nonDpy, but has the abnormal tail phenotype of the sqt-1 null. Also, the RRol mutations of rol-6 are suppressed by the sqt-1 null. All rol-6 phenotypes are abolished in the absence of the sqt-1 collagen, suggesting that rol-6 function requires the presence of sqt-1. A plausible model to explain this requirement is that sqt-1 and rol-6 assemble into a heterotrimeric collagen molecule containing two sqt-1 chains and one rol-6 chain. Studies of human type I collagen have shown that although it is normally found as a heterotrimer of two α1 and one α2 chains, alteration of the α2 chain can result in α1 homotrimer being assembled and secreted (MCBRIDE et al. 1992). There is, however, no evidence that type I collagen α2 chains can form homotrimers. In the absence of sqt-1 (α1-like), the rol-6 (α2-like) chains could not assemble and would therefore not be able to affect cuticle structure. sqt-1 chains, however, could form homotrimers in the absence of rol-6, and these homotrimer molecules could affect cuticle structure. If this were the case, the weak Dpy phenotype of the rol-6 null might be due to abnormal function of sqt-1 homotrimeric collagen molecules, rather than the absence of rol-6 from the cuticle.

Mutations in the sqt-1, dpy-2 and dpy-10 collagen genes have been found to suppress a temperature-sensitive (ts) mutation in the glp-1 gene (MAINE and KIMBLE 1989). glp-1 encodes a transmembrane receptor that is required for control of germline proliferation, embryonic hypodermal development and induction of pharyngeal fate in certain embryonic blastomeres (AUSTIN and KIMBLE 1989; PRIESS, SCHNABEL and SCHNABEL 1987; YOCHEM and GREENWALD 1989). All of the glp-1 functions are partially rescued by certain mutations in these collagens. The collagen suppressor mutations are of three types: Gly to Arg replacements within the triple-helical domain of dpy-10(e8, q292) and dpy-10(q291), a splice acceptor mutation in dpy-10(e128), and an Arg4 to Cys replacement within HBA in sqt-1(e1350). Suprisingly, sqt-1(sc1), Arg4 to Cys in HBA, causes a molecular alteration and phenotype very similar to e1350 but does not suppress glp-1. sqt-1(sc13) and rol-6(e187) were also tested and not found to suppress glp-1. Thus, suppression is allele specific; however, a variety of different mutations in different collagens can act as suppressors of glp-1. dpy-2(e8) and dpy-10(e128) have also been show to suppress certain ts alleles of mup-1, a gene involved in muscle positioning and attachment (GOH and BOGAERT 1991). sqt-1(e1350) partially suppresses ts alleles of the dauer constitutive gene daf-2 (J. KRAMER, unpublished data). So, the same collagen mutations can suppress mutations in several apparently unrelated genes. The collagen mutants themselves do not display any of the phenotypes seen in glp-1, mup-1 or daf-2 mutants, suggesting they are not involved in the same processes.

All of the properties of these suppressions, except the allele specificity of sqt-1, suggest that they are indirect. The mutant collagens most likely do not directly interact with the mutant gene products being suppressed. We suggest that suppression may result from heat shock-like chaperone functions induced by accumulation of the mutant collagens. Mutations in the Drosophila actin 88F gene were shown to induce...
the heat shock response in an allele-specific manner (Parker-Thornburg and Bonner 1987). Presumably heat shock is induced by accumulation of aberrant protein and only certain mutations allow the abnormal protein to accumulate rather than be rapidly degraded. Overexpression of the Escherichia coli GroEL and GroES heat shock chaperones has been shown to suppress ts mutations in unrelated gene products (van Dyk, Gatenby and LaRossa 1989). Thus, the function of ts mutant proteins can be at least partially rescued by the induction of molecular chaperones. We suggest that the C. elegans suppressor mutant collagen genes cause a physiological stress which induces chaperones that can partially rescue the ts mutant glp-1, mup-1 or daf-2 proteins.

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LITERATURE CITED


C. elegans sqt-1 and rol-6 genes


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