Proteolytic Processing of *Caenorhabditis elegans* SQT-1 Cuticle Collagen Is Inhibited in Right Roller Mutants whereas Cross-linking Is Inhibited in Left Roller Mutants*

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The *sqt-1* gene encodes a *C. elegans* cuticle collagen that when defective can cause dramatic alterations of organismal morphology. Specific antisera were used to examine the assembly of wild-type and mutant SQT-1 in the cuticle. Wild-type SQT-1 chains associate into dimer, tetramer, and higher oligomers that are cross-linked by non-reducible, presumably tyrosine-derived, covalent bonds. The SQT-1 pattern differs from the bulk of cuticle collagens which are found in trimer and larger forms. *sqt-1* mutations that cause left-handed helical twisting of animals remove a conserved carboxyl-domain cysteine and inhibit formation of these non-reducible bonds. SQT-1 monomers accumulate and novel trimer-sized products form. A conserved tyrosine immediately adjacent to the affected cysteine suggests that disulfide bond formation is required for this tyrosine to form a cross-link. *sqt-1* mutations that cause right-handed helical twisting affect conserved arginines in a predicted cleavage site for a subtilisin-like protease. These mutant SQT-1 molecules retain residues on the amino side of the predicted cleavage site and are larger than wild-type by the amount expected if cleavage failed to occur. The conservation of this site in all nematode cuticle collagens indicates that they are all synthesized as procollagens that are processed by subtilisin-like proteases.

The cuticle (exoskeleton) of the nematode *Caenorhabditis elegans* is a complex, multilayered extracellular structure composed primarily of collagens encoded by a family of approximately 150 genes (1, 2). The cuticle collagens all have similar domain structures (3) (Fig. 1). The central Gly-X-Y repeat domain contains two to four interruptions of non-Gly-X-Y sequence and is flanked by three cysteines on the amino side and two cysteines on the carboxyl side. The precise positions of these cysteine residues differs in different subfamilies of the cuticle collagen genes. The amino-terminal domain contains four short conserved stretches of amino acids that are referred to as homology blocks A-D. The carboxyl-domain is generally short and contains cysteine and tyrosine residues, the positions of which are generally conserved within but not between subfamilies.

With a few exceptions, the cuticle collagen genes are predicted to encode polypeptides of about 30 kDa. However, reductive extraction of cuticles results in collagen molecules that migrate with apparent molecular masses from 90 to over 200 kDa (4, 5). The unexpectedly low mobility of the cuticle collagens is due to cross-linking between chains by di-, tri-, and/or isotri-tyrosine residues (6, 7). These cross-links are non-reducible and result in the formation of multimers of the collagen chains.

Mutations in *C. elegans* cuticle collagens can cause a variety of gross morphological defects, including dumpy (Dpy, short and fat), long (Lon, long and thin), roller (Rol, helically twisted), and abnormal tail structure (Tal, defective tail structure) (1, 2). Roller mutants can be twisted in either a left-handed (LRol) or right-handed (RRol) helix, but all animals carrying a particular mutation show the same handedness. Different mutations in the *sqt-1* and *rol-6* cuticle collagen genes can cause Dpy, Tal, RRol, or LRol phenotypes (8–10). *sqt-1* and *rol-6* are members of the same cuticle collagen subfamily (3), are expressed at the same developmental stages (11), and equivalent mutations in them cause similar phenotypes (10, 12). Genetic interactions between *sqt-1* and *rol-6* have been described which suggest that the SQT-1 and ROL-6 collagen chains may interact in some manner (10, 13).

Null alleles, that result in the complete loss of SQT-1 or ROL-6 collagen from the cuticle, have only small effects, resulting in Tal or slight Dpy phenotypes, respectively (10). However, the presence of abnormal *Sqt-1* or *ROL-6* can cause severe morphological defects. Alterations of conserved amino acids in the amino or carboxyl non-Gly-X-Y domains of *sqt-1* or *rol-6* result in strong Rol or Dpy phenotypes. Several mutations were identified in Homology Block A, which has a consensus of Arg-X-Y-Arg-X-Gln. Substitution of Arg with His in *rol-6* causes a recessive RRol phenotype, and cysteine substitutions for Arg or Arg of *sqt-1* and Arg of *rol-6* cause dominant or semi-dominant RRol phenotypes (10). The same Arg to Cys substitution in the *dpv-10* cuticle collagen gene causes a dominant LRol phenotype (14). Site-directed mutagenesis of HBA and phenotypic analysis in transgenic animals (12) showed that Arg and Arg were the critical residues in this region. Substitution with Lys allowed for apparently normal function, but other substitutions resulted in RRol phenotypes. The spacing of these required basic residues suggested that HBA could function as the cleavage site for a subtilisin-like protease(s) (15).

A second class of mutations were identified in the carboxyl non-Gly-X-Y domain of *sqt-1*, where the loss of one of the two conserved cysteine residues resulted in a recessive LRol phenotype (10). Site-directed mutagenesis of these two cysteines in

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[^1]: The abbreviations used are: Dpy, dumpy; Lon, long; Rol, roller; Tal, abnormal tail structure; PAGE, polyacrylamide gel electrophoresis; HBA, homology domain A.
sqt-1 and rol-6 showed that alteration of either cysteine results in a LRol phenotype, although the phenotype is weaker for rol-6 than sqt-1.

To analyze the effects of these mutations on the SQT-1 polypeptide we have generated antibodies specific for the SQT-1 collagen chain. Here we present evidence supporting the hypothesis that HBA is a protease cleavage site and that the SQT-1 collagen chain. Here we present evidence supporting the polypeptide we have generated antibodies specific for the 70% of the total at harvest. A low percentage of adult animals in the watched closely to ensure that larval stage animals represented at least 70% of the total at harvest. A low percentage of adult animals in the population was critical because SQT-1 becomes highly cross-linked in adult cuticle and cannot be efficiently extracted.

To isolate cuticles, animals were washed with M9 buffer and resuspended in 5 volumes of sonication buffer (10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM phenylmethylsulfonil fluoride). The suspension was sonicated until the bodies of most animals were broken. Animal fragments were collected by centrifugation at 3000 g for 4 min and washed once with sonication buffer. The pelletted fragments were resuspended in 1 ml of ST buffer (1% SDS, 0.125M Tris, pH 6.8), boiled for 2 min, and incubated for 5 min at room temperature. The cuticle fragments were pelletted and washed again with ST buffer. Cuticle proteins were extracted by boiling the cleaned cuticles in ST buffer containing 5% freshly added β-mercaptoethanol for 2 min followed by several hours of agitation at room temperature. Soluble proteins were transferred to fresh tubes after centrifugation and stored at −20°C.

EXPERIMENTAL PROCEDURES

C. elegans Strains—General C. elegans maintenance and handling were performed as described (16). The following strains were used in these experiments: sqt-1(e1350,sc1,sc13,sc113,sc103); rol-6(su1006, e187,n1718); rol-6(n1718)sqt-1(e1350); rol-6(n1718)sqt-1(sc13); daf-2(e1368) (10, 13, 17).

Cuticle Isolation and Protein Extraction—Animals of mixed developmental stages were grown on SNGM plates at 20 °C. Populations were watched closely to ensure that larval stage animals represented at least 70% of the total at harvest. A low percentage of adult animals in the population was critical because SQT-1 becomes highly cross-linked in adult cuticle and cannot be efficiently extracted.

To isolate cuticles, animals were washed with M9 buffer and resuspended in 5 volumes of sonication buffer (10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM phenylmethylsulfonil fluoride). The suspension was sonicated until the bodies of most animals were broken. Animal fragments were collected by centrifugation at 3000 g for 4 min and washed once with sonication buffer. The pelletted fragments were resuspended in 1 ml of ST buffer (1% SDS, 0.125M Tris, pH 6.8), boiled for 2 min, and incubated for 5 min at room temperature. The cuticle fragments were pelletted and washed again with ST buffer. Cuticle proteins were extracted by boiling the cleaned cuticles in ST buffer containing 5% freshly added β-mercaptoethanol for 2 min followed by several hours of agitation at room temperature. Soluble proteins were transferred to fresh tubes after centrifugation and stored at −20°C.

RESULTS

Wild-type SQT-1 Collagen in Cuticles—Polycarboxylic rabbit antisera were generated against a peptide, s1c, derived from a sequence in the first interruption of the Gly-X-Y domain of SQT-1 (Fig. 1). Affinity purified anti-s1c antisera reacted with several bands in cuticle extracts of wild-type (N2) animals, but showed no reactivity to cuticle extracts from sqt-1(sc103) null mutant animals (Fig. 2A, lane 1; Fig. 4, lane 1). The sc103 mutation generates a nonsense codon near the beginning of the sqt-1 gene that would block production of the SQT-1 protein (10). These results demonstrate that the anti-s1c antisera is specific for SQT-1, and does not cross-react with other cuticle collagens.

The pattern of the SQT-1 collagen in cuticle extracts from wild-type animals is shown in Fig. 2A, lane 2. The major antibody reactive SQT-1 products migrate as single bands at 39 and 82 kDa, and three bands between 160 and 175 kDa (seen more clearly in Fig. 5). There is also a smear of unresolved, high molecular weight material at the top of the gel. The positions of these bands relative to the size standards can change on different percentage polyacrylamide gels. For example, the 82-kDa band runs at 70 kDa on a 15% gel and at 90 kDa on a 9% gel (data not shown). This is presumably due to the abnormal electrophoretic mobility of collagens on SDS-polyacrylamide gels (21), as was previously noted for C. elegans-expressed collagen (22). The 39- and 82-kDa bands are seen on the 10% polyacrylamide gel, and are likely to be monomer and dimer forms of SQT-1, based on the expected size of SQT-1 after the predicted cleavage at HBA (25 kDa) and the fact that collagens migrate approximately 40% slower in SDS-PAGE than expected by their molecular masses (22, 23). Also, the 39-kDa band is stronger in extracts from animals isolated during the molting period and no smaller products are detected (Fig. 5, lane 7). Whether the 82-kDa dimer band consists of two

![Diagram of the SQT-1 cuticle collagen polypeptide](image)
SQT-1 Collagen Mutations Affect Processing

SQT-1 molecules or SQT-1 cross-linked with another molecule is unclear. The higher molecular weight materials are likely to be higher-order oligomers that contain SQT-1. Since all samples were extracted in the presence of reducing agent, these dimers and oligomers must be associated by nonreducible covalent bonds, presumably tyrosine-derived cross-links.

A Coomassie-stained gel of the same wild-type cuticle extract is shown in Fig. 2B. The two major bands run at between 100 and 110 kDa apparent molecular mass. These same bands can also be detected by Ponceau S staining of Western blots (data not shown). The SQT-1 bands do not co-migrate with these major collagen bands, indicating that the cross-linking of SQT-1 differs from that of the majority of cuticle collagens. Since all of the cuticle collagen genes predict protein products of approximately the same molecular mass, the major collagen bands of 100–110 kDa are likely to be tyrosine cross-linked trimers.

Carboxyl Cysteine Substitutions in SQT-1 Interfere with Cross-linking—The sqt-1 LRol mutants sc13 and sc113 have alterations of one of the two conserved cysteine residues in the carboxyl-terminal domain of the protein (Fig. 1) (10). The SQT-1 Western blot patterns of cuticle extracts from sc13 and sc113 animals are essentially identical (Fig. 2A, lanes 5 and 6), consistent with the identical phenotypes of the two mutants (10). However, cross-linking of these mutant SQT-1 collagens is altered. Relative to wild-type, the mutant monomer band is much stronger, the dimer band has slightly higher mobility, and a novel band at the size expected for a SQT-1 trimer is detected. These differences were consistently seen in multiple separately prepared cuticle extracts. The total amount of SQT-1 in sc13 and sc113 cuticle extracts is approximately the same as in wild-type, indicating that the loss of one carboxyl domain cysteine does not significantly alter the efficiency of SQT-1 assembly into the cuticle. However, we noted that in sc13 and sc113 mutant extracts the amounts of the dimer and the high molecular weight smear were consistently decreased (better seen in shorter exposures). This suggests that the extra monomer and trimer size molecules in these mutants represent SQT-1 that normally would be associated into dimers, tetramers, and higher oligomers. Since all samples were extracted with reducing agent, there is a difference in non-reducible, presumably tyrosine-derived, cross-linking in these mutant proteins.

SQT-1 collagen, like most cuticle collagens, cannot be extracted from cuticles without the use of reducing agents because of the extensive disulfide bonding in cuticles. Although the LRol mutant SQT-1 collagens would be expected to lack a carboxyl-domain disulfide bond, their extractability from cuticles without BME is not improved (data not shown), presumably due to the presence of other disulfide bonds in the collagens.

Mutant SQT-1 Collagens with Alterations in the Putative Protease Cleavage Site—Based on site-directed mutagenesis studies, the conserved homology block A (HBA) motif of cuticle collagens has been proposed to be a protease cleavage site for collagen processing (12). This model predicts that mutations that alter the putative cleavage site will block cleavage and leave the collagen unprocessed. Both the e1350 and sc1 alleles of sqt-1 cause dominant RRol and recessive Dpy phenotypes, and each alters one of the conserved arginine residues in HBA (Fig. 1). In multiple independent cuticle extracts made from e1350 and sc1 mutant animals much less SQT-1 was detectable on Western blots than from wild-type animals (Fig. 2A, lanes 3 and 4), indicating that these mutant SQT-1 collagens are not efficiently assembled into the cuticle. Both mutant extracts show a strong band at 91 kDa and a weak band at 44 kDa. These bands are 9 and 5 kDa larger than the comparable wild-type dimer and monomer bands. Extracts from sc1, but not e1350, animals also show a weak band that comigrates with the wild-type dimer at 82 kDa (Fig. 2, lane 4). Proteolytic cleavage of SQT-1 at HBA would remove 35 amino-terminal residues with a predicted mass of 4.4 kDa, very close to the observed 5-kDa difference between wild-type and the e1350 and sc1 SQT-1 monomers. These data indicate that the mutant SQT-1 molecules carrying an altered protease cleavage site are larger in size than wild-type and provide support for the HBA protease cleavage model.

In sc1 cuticle extracts a weak band at 82 kDa, the same size as the wild-type dimer, was seen suggesting that some sc1 mutant SQT-1 is proteolytically processed at HBA. In addition, more high molecular weight material is seen in sc1 than e1350 extracts, indicating that the sc1 mutant collagen is more efficiently assembled into cuticles. The presence of some wild-type size SQT-1 in sc1 animals is consistent with the fact that sc1 animals are less dominant and healthier than e1350 animals (10). The e1350 mutation is at Arg4 of HBA (consensus Arg3-X2-Arg5-Arg4-Gln5) while the sc1 mutation is at Arg3 (Fig. 1). The residual proteolytic processing of sc1 mutant collagen could result from utilization of the remaining Arg3-Arg4 sequence in HBA as an alternative cleavage site, since Arg-Arg is also a substrate for subtilisin-like proteinases (15, 24).

The dimeric forms seen in both wild-type and SQ-1 mutant animals could be homodimers of two SQT-1 chains or heterodimers between SQT-1 and another molecule. The size difference between the dimer bands from wild-type and the e1350 and sc1 mutant animals is 9 kDa, about twice the difference between their monomers (5 kDa). The fact that the size of the mutant dimers is increased by twice the amount as the monomers is consistent with a homodimer of SQT-1 chains. This observation therefore suggests that the dimer band consists of two cross-linked SQT-1 collagen chains.

The Predicted Procollagen Region Is Retained in SQT-1 HBA Mutants—To further confirm that proteolytic processing of SQT-1 procollagen occurs at the dibasic sequence in HBA, antisera were raised against peptide s1f, which is located im-

**Fig. 2.** A, Western blot analyses of wild-type and sqt-1 mutant cuticle extracts with anti-s1e antisera. Lane 1, sc105; lane 2, N2 wild-type; lane 3, e1350; lane 4, sc1; lane 5, sc13; lane 6, sc113. The positions of molecular weight standards are indicated in kilodaltons to the left. B, Coomassie-stained 10% SDS-PAGE of cuticle extract from wild-type N2 animals. Note that the major protein bands migrate at 100–110 kDa.
anti-s1c reactive bands were seen in any of the mutant cuticle extracts with anti-s1c and anti-s1f antisera. After electrophoresis and transfer to nitrocellulose each lane was divided into two strips. One set of strips was probed with anti-s1c antibodies (A) and the other with anti-s1f antibodies (B). The identities of wild-type (N2) and sqt-1 mutants lanes are indicated at the top. The anti-s1f antisera only shows detectable reaction with the e1350 and sc1 mutant SQT-1 protein. Molecular mass standards are indicated in kilodaltons to the left.

mediately to the amino side of the predicted cleavage site in HBA (Fig. 1). Anti-s1f antibodies should be specific to unprocessed SQT-1 and should recognize the mutant SQT-1 in e1350 and sc1 where cleavage is blocked, but not wild-type SQT-1 or other mutant SQT-1 molecules that have intact HBA cleavage sites.

After electrophoresis and transfer to nitrocellulose, lanes of wild-type and various mutant cuticle extracts were divided in half lengthwise. One set of strips was reacted with anti-s1f antisera (Fig. 3, B lanes) and the other with anti-s1c antisera (Fig. 3, A lanes). The anti-s1f antisera does not react to the sqt-1(sc103) null extract, demonstrating its specificity. The anti-s1f antibodies reacted with e1350 and sc1 mutant SQT-1, but did not react to SQT-1 in either wild-type N2 or sc1,13, a mutant which does not alter the HBA cleavage site. The e1350 and sc1 mutant SQT-1 bands detected with anti-s1f and anti-s1c have the same mobilities. As expected, they have higher apparent molecular weights than the anti-s1c reactive bands seen in wild-type extracts. These results show that residues located on the amino side of the HBA site are not present in mature wild-type SQT-1 collagen, but are retained in mutants that affect the HBA cleavage site. Thus, cleavage of the SQT-1 procollagen normally occurs at or near HBA.

SQT-1 and ROL-6 Are Not Associated by Non-reducible Bonds—Previous genetic and molecular studies suggested that the SQT-1 and ROL-6 collagens might interact with one another (10, 11, 13). To assess this possibility we analyzed the SQT-1 patterns in cuticle extracts from the rol-6 null mutant n1178 and the R Rol mutants su1006 and e187. Both su1006 and e187 are alterations of one of the conserved arginines in the HBA protease cleavage site and would be expected to result in altered size of the ROL-6 collagen. No differences in the sizes of anti-s1c reactive bands were seen in any of the rol-6 mutant extracts (Fig. 4). These results suggest that SQT-1 and ROL-6 chains are not cross-linked by non-reducible bonds, at least as dimers, trimers, or tetramer sized molecules.

We also examined the effect of the rol-6 null background on mutant SQT-1 patterns. The SQT-1 pattern in cuticle extracts from rol-6(n1178)sqt-1(e1350) double mutants is the same as in the e1350 single mutant, but there is significantly less SQT-1 in these cuticle extracts (Fig. 4, lanes 4 and 5). This result may explain why the e1350 Dpy phenotype is suppressed in the rol-6 null background, such that the double mutant phenotype is similar to the sqt-1 null. The pattern of SQT-1 in rol-6(n1178)sqt-1(sc13) and sqt-1(sc13) are similar (Fig. 4, lanes 6 and 7), consistent with the fact that these animals have very similar LRol phenotypes.

SQT-1 in Different Developmental Stages—SQT-1 collagen could have different cross-linking or processing properties at different developmental stages. To assess this possibility cuticle extracts were prepared from synchronized populations of animals at different developmental stages and analyzed on Western blots (Fig. 5). SQT-1 was detected in L2, L3, and L4 larval, and adult extracts (lanes 2–5), but not in L1 or dauer larval extracts (lanes 1 and 6).

The Western blot patterns of SQT-1 in L2, L3, and L4 larval stages are very similar to each other, all having the 82-kDa dimer band and three bands between 160 and 175 kDa. The L4 extract also shows the 39-kDa monomer band, which is only variably detected in extracts from non-molting animals. The adult extract shows only the three 160–175-kDa bands and a smear of higher molecular mass material. The absence of smaller SQT-1 forms likely results from a higher degree of cross-linking in the adult cuticle extract. To better assess the nature of SQT-1 cross-linking in the adult stage cuticle, extracts from animals at the L4 to adult molt, when the adult cuticle is being assembled, were analyzed (Fig. 5, lane 7). This extract shows the same monomer, dimer, and three 160–175-kDa bands seen in the L2-L4 extracts. Thus, there are no detectable qualitative differences in the SQT-1 Western blot patterns at different developmental stages.

**DISCUSSION**

The development of antisera specific for the SQT-1 cuticle collagen has allowed the first analyses of the assembly of one representative of the approximately 150 member cuticle collagen family of *C. elegans*. Our results show that SQT-1 chains are rapidly cross-linked by both reducible and non-reducible bonds. The non-reducible bonds are most certainly di-, tri-, and/or isothio-tyrosine residues as have been detected in other nematodes (6, 7) and in *C. elegans* cuticle extracts. The tyrosine cross-linking of SQT-1 is orderly, with the same dimer (82 kDa) and three tetramer (160–175 kDa) sized products being detected in cuticles from different developmental stages. Analysis of sqt-1 mutant proteins indicate that the dimer sized product contains two SQT-1 chains. It is not clear whether dimers form between two SQT-1 chains within a single collagen molecule or between two separate molecules. It is notable that the bulk of cuticle collagen, as detected by Coomassie staining, are primarily trimer and larger sizes. Thus, different cuticle collagens can have distinct patterns of tyrosine-derived cross-linking.

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2 D. Eyre and J. Kramer, unpublished results.
Mutants of sqt-1 that alter a conserved cysteine in the carboxyl-terminal domain of the protein cause inhibition of tyrosine-derived cross-linking. The altered cysteine is immediately adjacent to a tyrosine residue that is conserved in all members of the sqt-1 cuticle collagen subfamily (Fig. 1) and there are similarly located tyrosines in the carboxyl domains of other subfamilies. The ability of this tyrosine to efficiently participate in cross-linking is apparently dependent on formation of an adjacent disulfide bond. The sqt-1 LRol mutants remove this disulfide bond and therefore SQT-1 monomers accumulate. The higher mobility of the LRol mutant dimer-sized product may result from a change in the position of the cross-link connecting monomers and/or a change in which collagen chains are cross-linked together. The appearance of trimer-sized bands in the LRol mutants suggests that normally monomers efficiently form dimers which then associate into tetramers. The high concentration of monomers in the mutants may allow for aberrant trimer formation. The LRol phenotype displayed by these mutants may be a result of the loss of disulfide bonds, loss of non-reducible bonds, and/or the formation of abnormal dimeric, trimeric, or higher oligomeric products.

Previous studies of genetic and in vitro generated mutations showed that RRol mutants of sqt-1 and rol-6 are alterations in homology block A (Fig. 1) and suggested that it is a site for processing by a subtilisin-like protease (10, 12). The results presented here strongly support that suggestion. The portion of the polypeptide on the amino side of HBA is only retained in RRol mutant SQT-1 molecules and the mutant protein is larger by the amount predicted. Thus, SQT-1 is synthesized as a procollagen that is normally cleaved at HBA before incorporation into the cuticle. A reduced amount of the uncleaved pN-SQT-1 assembles into the cuticle, suggesting that this processing is important for the collagen to be efficiently secreted into the cuticle. Nearly all C. elegans cuticle collagenas, as well as those of other nematode species (25), have a well conserved HBA and are therefore likely to be proteolytically processed at this site.

The presence of pN-SQT-1 in the cuticle can cause severe morphological abnormalities. Mutant homozygotes are slow growing and have a Dpy phenotype, while heterozygotes display a strong RRol phenotype. The pN-SQT-1 molecules dominantly disrupt the normal cuticle structure. Mutations that alter the procollagen N-proteinase processing site of human type I collagen cause autosomal dominant Ehlers-Danlos syndromes type VIIA and VIIIB (26). These patients have joint hypermobility and subluxations, hip dislocations, scoliosis, and increased bone fractures. The unprocessed type I collagen affects fibrillogenesis, as collagen fibrils in patient’s skin show irregular cross-sections and spacing. The C. elegans sqt-1 HBA mutations represent the cuticle collagen equivalent of human EDS types VIIA and VIIB.

The spacing of conserved basic residues in HBA, Arg-X-X-Arg, suggested that it could be cleaved by a subtilisin-like protease, based on their known substrate specificity (15, 27). The utilization of subtilisin-like enzymes for collagen processing appears to be unusual. Proteolytic processing of procollagen occurs with many vertebrate collagen types, however, the identified N- and C-propeptidases are not subtilisin-related enzymes (28–30). Processing of type XVI collagen, at least when expressed as a recombinant molecule in cultured kidney cells, may occur via a subtilisin-like protease (31), but it is not clear that this represents its normal processing. The bli-4 gene of C. elegans encodes a family of subtilisin-like protease isofoms (32, 33). Most mutations in bli-4 cause early larval lethality, however, one isoform-specific mutation causes blistering of the cuticle suggesting that BLI-4 could process cuticle collagens. Additional genes encoding subtilisin-like proteases are present in the C. elegans genome and they could also be involved processing cuticle collagens.

Genetic interactions between sqt-1 and rol-6 (10, 13) and their equivalent temporal expression patterns (11) led to the suggestion that these collagen chains may physically interact. In these studies we found no evidence for formation of non-reducible cross-links between SQT-1 and ROL-6. However, it is still possible that these two collagen chains could be part of a single heterotrimeric collagen molecule. Because of the conditions necessary to extract collagens from the cuticle, noncovalent or reducible covalent bonds between the chains would have been disrupted.

One genetic interaction, suppression of sqt-1 HBA mutant Dpy phenotypes in the rol-6 null background (10), can be explained from our results. In the rol-6 null background much less of the sqt-1(e1350) mutant protein is present in the cuticle. In some manner, the absence of ROL-6 inhibits the assembly and/or secretion of this class of SQT-1 mutant protein. Since it is the presence of abnormal SQT-1 in the cuticle that causes the Dpy phenotype, the rol-6 null suppresses by removing the abnormal protein. This mechanism does not affect the LRol class of sqt-1 mutants since normal amounts of the LRol SQT-1 are found in rol-6 null background cuticles.

Analyses of sqt-1 and rol-6 mRNA detected expression of both genes at all stages that exhibit mutant phenotypes, except the dauer larva stage (11). Dauer larvae display sqt-1 and rol-6 mutant phenotypes, but no expression of either gene was detected during formation of the dauer cuticle. The Western blot analyses of SQT-1 protein expression reported here are consistent with the mRNA expression data. SQT-1 is detected in cuticles from L2, L3, and L4 larvae, and adults, but not in cuticles from dauer larvae. In sqt-1 mutant animals, the dauer cuticle can be helically twisted even though no SQT-1 protein is detectable in the cuticle.

We have proposed that expression of the Rol phenotype at the dauer stage occurs in the absence of mutant SQT-1 or ROL-6 collagen and results from maintenance of the helical twist generated in the preceding L2 stage animal (11). The hypodermis is attached to the cuticle and becomes helically twisted when the L2 cuticle, which does contain SQT-1, twists. When the dauer cuticle is synthesized no mutant collagen is made, but the new cuticle is assembled by a hypodermis that is already helically twisted. The resulting dauer cuticle is constructed of normal collagen and maintains the twist that was
built into it by the twisted hypodermis. The results showing that SQT-1 protein is not detected in the dauer cuticle provide further support for this model.

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