Increased or Decreased Levels of *Caenorhabditis elegans lon-3*, a Gene Encoding a Collagen, Cause Reciprocal Changes in Body Length

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ABSTRACT

Body length in *C. elegans* is regulated by a member of the TGFβ family, DBL-1. Loss-of-function mutations in *dbl-1*, or in genes encoding components of the signaling pathway it activates, cause worms to be shorter than wild type and slightly thinner (Sma). Overexpression of *dbl-1* confers the Lon phenotype characterized by an increase in body length. We show here that loss-of-function mutations in *dbl-1* and *lon-1*, respectively, cause a decrease or increase in the ploidy of nuclei in the hypodermal syncytial cell, hyp7. To learn more about the regulation of body length in *C. elegans* we carried out a genetic screen for new mutations causing a Lon phenotype. We report here the cloning and characterization of *lon-3*. *lon-3* is shown to encode a putative cuticle collagen that is expressed in hypodermal cells. We show that, whereas putative null mutations in *lon-3* (or reduction of *lon-3* activity by RNAi) causes a Lon phenotype, increasing *lon-3* gene copy number causes a marked reduction in body length. Morphometric analyses indicate that the *lon-3* loss-of-function phenotype resembles that caused by overexpression of *dbl-1*. Furthermore, phenotypes caused by defects in *dbl-1* or *lon-3* expression are in both cases suppressed by a null mutation in *sqt-1*, a second cuticle collagen gene. However, whereas loss of *dbl-1* activity causes a reduction in hypodermal endoreduplication, the reduction in body length associated with overexpression of *lon-3* occurs in the absence of defects in hypodermal ploidy.

THE regulation of body length and body size are poorly understood aspects of animal development (Conlon and Raff 1999). The nematode *Caenorhabditis elegans* represents an excellent model for the study of how these features are controlled because mutations have been isolated that have marked effects on organismal morphology (Kramer *et al.* 1988). Recent work has shown that wild-type body size in *C. elegans* requires a highly conserved signal transduction pathway activated by a member of the TGFβ superfamily, DBL-1 (Savage *et al.* 1996; Morita *et al.* 1999; Suzuki *et al.* 1999). Wild-type *C. elegans* hermaphrodites grow to a length of just over 1 mm. Worms lacking *dbl-1* activity, however, are smaller (Sma): They grow to only about two-thirds the length of wild type and are slightly thinner (Morita *et al.* 1999; Suzuki *et al.* 1999). *dbl-1* is a dose-dependent regulator of body length: Worms overexpressing *dbl-1* can grow to a length of one and one-half times or more that of wild type (they display the Lon phenotype; Morita *et al.* 1999; Suzuki *et al.* 1999). DBL-1 is thought to function by activating the type I and type II TGFβ receptors SMA-6 and DAF-4 (Estevez *et al.* 1993; Krishna *et al.* 1999). In turn, the receptors are thought to activate a complex of SMAD transcription factors containing the proteins, SMA-2, SMA-3, and SMA-4 (Savage *et al.* 1996). Loss-of-function mutations in genes encoding any components of this pathway cause a Sma phenotype identical to that shown by worms lacking *dbl-1* activity (Estevez *et al.* 1993; Savage *et al.* 1996; Krishna *et al.* 1999).

Presently it is not known which genes SMA-2, SMA-3, and SMA-4 regulate in order to affect body length. Differential hybridization analysis with 3390 independent cDNAs has led to the identification of 21 genes whose expression is affected in worms mutant for components of the TGFβ pathway regulating body length (Mochii *et al.* 1999). One of these genes encodes SMA-6. Activation of the pathway thus leads to an increase in the transcription of at least one of the genes in the pathway, suggesting that a positive autoregulatory loop may exist. The functions of the other 20 genes whose transcription is differentially regulated are not presently known. The genes are expressed in a variety of tissues including the intestine, the hypodermis, the head, and the vulva (Mochii *et al.* 1999).

Worms displaying the Lon or Sma phenotypes caused by defects in TGFβ signaling appear to have the same number of somatic cells as wild-type worms (Suzuki *et al.* 1999). This observation suggests that the pathway may affect body length not by altering cell proliferation but rather by directly or indirectly altering the size or

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shapes of some or all cells. However, the molecular mechanisms by which the TGFβ pathway affects body size and body length are not yet known. Recent work has suggested that mutants lacking TGFβ pathway activity may be smaller than wild type in part because of reduced endoreduplication of nuclei in the hypodermal syncytium (Flemming *et al.* 2000). When measured in terms of organismal volume, a major period of growth in nematodes occurs during the adult stage (when cell division has ceased). It is therefore thought that final body size is determined to a significant extent by changes in the size of certain cells and not by increases in cell number (Flemming *et al.* 2000). The syncytial hypodermal cell hyp7 (and its counterpart in other nematodes) is by far the largest cell in the worm and it has been suggested that final body size in nematodes is largely determined by changes in size of the hypodermis (Flemming *et al.* 2000). Nuclei in the syncytial hypoderm in *C. elegans* and a number of other nematode species have been found to undergo rounds of endoreduplication during the adult stage (Flemming *et al.* 2000). Since in some organisms the size of mononucleate cells correlates with ploidy (Nurse 1985), it has been suggested that body size in *C. elegans* (and other nematodes) is determined in part by the degree to which hypodermal nuclei become endoreduplicated. In support of this model, a survey of 12 different nematode species revealed that body size correlated not simply with the number of hypodermal nuclei but with the product of this number and the extent of endoreduplication (Flemming *et al.* 2000). Furthermore, in *C. elegans* the hypodermal nuclei in mutants lacking *daf-4* or *sma-2* activity were found to have reduced ploidy compared to wild type (Flemming *et al.* 2000). These studies, however, have not revealed whether reduced endoreduplication is a consequence of reduced body size or vice versa. It is noteworthy in this regard that young adult *sma-2* or *daf-4* mutant hermaphrodites have wild-type hypodermal ploidy (A. Leroi, unpublished results), suggesting that the Sma phenotype displayed by worms at earlier larval stages is not caused by defects in hypodermal endoreduplication.

Mutations in genes encoding components of the cuticle, the exoskeleton of the worm, can also affect body length in *C. elegans* (Johnstone 2000). For example, mutations that affect body length have been identified in *dpy-2*, *dpy-7*, *dpy-10*, *dpy-13*, *sqt-1*, and *sqt-3*, all of which encode cuticle collagens (Kramer *et al.* 1988; von Mende *et al.* 1988; Johnstone *et al.* 1992; Levy *et al.* 1993; van der Keyl *et al.* 1994). Worms carrying certain mutations in these genes are shorter and fatter than wild type: They display the Dpy phenotype. Cuticle collagens are synthesized and secreted by hypodermal cells and they polymerize on the apical surface of the epithelium to form a complex structure consisting of six definable layers (Cox *et al.* 1981b,c; Peixoto and De Souza 1992). New cuticle is synthesized five times during development, once in the embryo before hatching and then during each of the larval molts. During each molt, different collagen genes are expressed in discrete temporal periods (Cox *et al.* 1981a; Johnstone and Barry 1996). However, the mechanism by which proteins polymerize to form the ordered layers that make up the cuticle is currently unknown. Some collagens are known to be required for a cuticle of the correct shape to be generated. For example, mutations that are thought to reduce or eliminate activity in *dpy-2*, *dpy-7*, *dpy-10*, and *dpy-13* all cause a Dpy phenotype, implying that these genes have specific roles in the formation of the exoskeleton (von Mende *et al.* 1988; Johnstone *et al.* 1992; Levy *et al.* 1993). No evidence exists at the present time, however, that these genes actively regulate body length. The *sqt-1* gene, which also encodes a cuticle collagen, has interesting genetic properties in that it can mutate to give alleles that affect the morphology of the worm in different ways (Kramer *et al.* 1988). Alleles of *sqt-1* have been isolated that can cause, respectively, Rol (Roller) or Dpy (Dumpy) phenotypes. In Roller mutants the entire cuticle has a helical twist to it but the worms are neither shorter nor longer than wild type. Worms mutant for Dpy mutations, on the other hand, are shorter and fatter than wild type. Certain heteroallelic combinations of *sqt-1* mutations can cause a Lon phenotype (Kramer *et al.* 1988). *sqt-1* does not seem to be a prime regulator of body length, however: Null mutations in *sqt-1* cause only a slight decrease in body length. Mutations in *sqt-1* that cause Dpy, Rol, or Lon phenotypes are thought to be neomorphic (Kramer and Johnson 1993).

Here we describe a study of the role of *lon-3* in the control of body length and body size in *C. elegans*. We have cloned the gene, analyzed the pattern of its expression, and investigated genetic interactions between mutations in *lon-3* and in other genes affecting body length. To understand the relationship between the extent of endoreduplication of hypodermal nuclei and body length, we have measured the ploidy of hypodermal nuclei in a variety of Sma and Lon mutants.

MATERIALS AND METHODS

Nematode strains and culture conditions: Maintenance and handling of *C. elegans* strains were as described by Brenner (1974). Experiments were carried out at 20° unless otherwise noted. *C. elegans* Bristol strain N2 is the wild-type parent for all strains used in this study. All strains were constructed by standard genetic methods. The mutations used are listed below. Mutations are described by Brenner (1974) unless otherwise stated.

LG I: *dpy-5(e61)*
LG II: *rol-6(su1006)* (Cox *et al.* 1980), *rol-6(n1178)* and *(e187n1268)* (Park and Horvitz 1986), *sqt-1(sc103)* (Kusch and Edgar 1986), and *sqt-1(sc13)* (Cox *et al.* 1980)
LG III: *daf-7(e1372)* (Riddle and Brenner 1978), *dpy-17 (e164)*, *lon-1(e185)*, *daf-4(m63)* (Estevez *et al.* 1993), *ncl-1*

(e1865) (Hedgecock and White 1985), *unc-36(e251)*, *dpy-19(e1259)*, *sma-2(e502)* (Savage *et al.* 1996), and *dpy-18(e304)*

LG IV: *dpy-9(e12)*, *dpy-13(e184)*, *dpy-4(e1166)*, and *him-8(e1489)* (Hodgkin *et al.* 1979)

LG V: *dpy-11(e224)*, *dbl-1(nk3)* (previously called *kk3*; Morita *et al.* 1999), *sma-1(e30)*, *vab-8(e1017)* (Hedgecock *et al.* 1987), *myo-3(st386)* (Dibb *et al.* 1989; Maruyama *et al.* 1989), *lon-3(e2175)* (Riddle *et al.* 1997), *lon-3(sp5, sp6, sp23, sv18)* (this study), and *unc-42(e270)*

LG X: *dpy-8(e130)*, *dpy-7(e88)*, and *dpy-6(e14)*

ctDp8 is a chromosomal duplication covering much of the right arm of LGV, including *lon-3* (Hunter and Wood 1992). *arDf1* is a deficiency (Tuck and Greenwald 1995). *ctIs40* is an integrated array containing multiple copies of *dbl-1(+)* (Suzuki *et al.* 1999).

Isolation of new *lon-3* alleles: Wild-type (N2) hermaphrodites were treated with ethyl methanesulfonate (EMS) and their F_2 progeny screened for Lon mutants. From a screen of 50,000 haploid genomes three new *lon* alleles were isolated, *sp5*, *sp6*, and *sp23*, which mapped to the right arm of chromosome V. All three mutations failed to complement *lon-3(e2175)* for the Lon phenotype. *sv18* was isolated in an unrelated screen in which the mutagen was EMS.

Growth curves: A total of 20 adult worms were placed onto a plate with OP50 bacteria and allowed to lay ~100 eggs. After 12 hr, when the majority of worms had hatched, 20 worms (chosen randomly) were photographed by using a video camera connected to the microscope. Their lengths were calculated from images obtained using the software application Object-Image 1.62. A total of 20 worms (chosen randomly) were photographed every 12 hr until 120 hr after hatching.

Mapping and cloning of *lon-3*: We localized *lon-3* to within 0.1 map units of *myo-3* by three-factor mapping. Cosmids from this region were injected at a concentration of 10 μg/ml into hermaphrodites of the genotype *unc-36(e251); lon-3(e2175)* together with 50 μg/ml of RIp16 plasmid DNA [which encodes *unc-36(+)*; Herman 1995]. C35G11 rescued *lon-3(e2175)* when injected at a concentration of 2 μg/ml and caused the majority of F_1 worms to be considerably shorter than wild type when injected at a concentration of 10 μg/ml. From one set of injections in which C35G11 was injected at a concentration of 10 μg/ml into *lon-3(e2175)* hermaphrodites, four transformed lines were generated containing, respectively, the extrachromosomal arrays *svEx50*, *svEx51*, *svEx52*, and *svEx53*. In three of the lines (those containing *svEx50*, *svEx52*, and *svEx53*) the majority of worms carrying the array were Dpy and all worms were rescued for the Lon phenotype. In the fourth (that containing *svEx52*), all worms were partially or completely rescued for the Lon phenotype and ~5% were Dpy. A 5.5-kb *Nco*I-to-*Kpn*I fragment from C35G11 that spans the predicted gene, ZK836.1, was subcloned into pBluescript II KS(+) to generate pVB52JN. pVB52JN both rescued *lon-3(e2175)* (when injected at 2 μg/ml) and caused worms to be shorter than wild type when injected at concentrations of 5 μg/ml or above. The extrachromosomal array, *svEx57*, was generated by injecting pVB52JN at a concentration of 50 μg/ml into hermaphrodites of the genotype *unc-36(e251)* together with 50 μg/ml of RIp16.

Determination of sequence changes associated with *lon-3* mutant alleles: To identify mutations associated with *lon-3* alleles we first used the method of RNAse cleavage mismatch detection (Ambion, Austin, TX) to determine the regions of the gene in which the changes resided. The regions containing the differences were amplified and sequenced. The sequence changes were confirmed by sequencing two independent PCR products. It proved impossible to generate PCR products from genomic DNA isolated from worms homozygous for *lon-3 (e2175)*. Southern blot analysis indicated that this allele is associated with a DNA rearrangement at the *lon-3* locus (data not shown).

***lon-3* reporter genes:** To generate pVB54JN, which contains *lacZ* under the control of *lon-3* promoter sequences, pVB52JN was digested with *Eae*I, blunt ended by filling in using Klenow, and then digested with *Bam*HI. The fragment generated was inserted into pPD95.07 (Fire *et al.* 1990) digested with *Sma*I and *Bam*HI. pVB54JN was injected at a concentration of 100 μg/ml into worms of the genotype *unc-36(e251)* together with 50 μg/ml RIp16 (Herman 1995) to generate the extrachromosomal arrays *svEx77*, *svEx78*, and *svEx79*. *lacZ* expression was analyzed by using a β-galactosidase assay as described elsewhere (Fire *et al.* 1990). The temporal and spatial patterns of expression were the same for all three arrays. To generate pVB82JN, which contains green fluorescent protein (GFP) under the control of *lon-3* promoter sequences, pVB52JN was digested as described for pVB54JN. The fragment generated was inserted into pPD95.67 digested with *Bam*HI and *Bal*I. pVB82JN was injected at a concentration of 50 μg/ml as described for pVB54JN to generate the extrachromosomal array *svEx130*.

Body length measurements: A total of 10–20 hermaphrodites of the appropriate genotype were placed onto seeded plates and allowed to lay eggs for ~1 hr. The adults were removed and the eggs allowed to develop. Worm lengths were measured 96 hr after hatching with a Leica MZ6 dissecting microscope connected to a digital video camera. To avoid bias, the lengths of all progeny on a given plate were measured. A Zeiss Axioplan 2 microscope was used to analyze worms, and the software application Openlab 2.0.7 (Improvision) was used for all micrographs.

Endoreduplication: DNA content was determined by microdensitometry as described elsewhere (Flemming *et al.* 2000). In many instances, the measurements of ploidy did not fall into multiples of two as might be expected. As discussed in Flemming *et al.* (2000), this is explained by the fact that different nuclei endoreduplicate to different extents.

RESULTS

***lon-1* is a negative regulator of endoreduplication:** Mutations that reduce or eliminate *daf-4* or *sma-2* activity cause a reduction in the extent of endoreduplication of hypodermal nuclei (Flemming *et al.* 2000). To determine whether *dbl-1* is also required for wild-type hypodermal ploidy we measured the extent of endoreduplication in hermaphrodites homozygous for a *dbl-1* null mutation, *nk3*. Results presented in Table 1 show that hypodermal nuclei in *dbl-1(0)* mutants have an average ploidy considerably less than those in wild-type hermaphrodites. Thus, as for other genes required for wild-type body size, *dbl-1* is required for hypodermal nuclei to undergo the appropriate number of rounds of endoreduplication.

Since the Sma phenotype is associated with reduced hypodermal endoreduplication, we investigated whether hypodermal nuclei in worms displaying the Lon phenotype were hyperendoreduplicated. Hermaphrodites homozygous for mutations in *lon-1* can be as much as 50% longer than wild type (Brenner 1974). We measured hypodermal ploidy in hermaphrodites homozygous for *lon-1(e185)*, which is thought to be a null mutation (N.

TABLE 1

Extent of endoreduplication of hypodermal nuclei

Genotype	Ploidy[a]	*n*
Wild type	12.66 ± 0.22	65
lon-3(sp23)	12.41 ± 0.40	30
lon-3(e2175)	11.93 ± 0.37	18
lon-3(++)[b]	11.73 ± 0.36	33
dbl-1(nk3)	8.42 ± 0.31	27
dbl-1(++)[c]	13.29 ± 0.45	29
dbl-1(nk3); lon-3(sp23)	7.08 ± 0.67	9
lon-1(e185)	15.31 ± 0.66	19
dbl-1(nk3); lon-1(e185)	9.52 ± 0.46	17

Hypodermal ploidy was measured in adult worms (≥120 hr after hatching) as described elsewhere (Flemming *et al.* 2000).

[a] Sample mean ±SE.

[b] Complete genotype is *unc-36(e251); svEx57[unc-36(+)-lon-3 (+)]*.

[c] Complete genotype is *ctIs40*, which is an integrated array containing multiple copies of *dbl-1(+)* (Suzuki *et al.* 1999).

TABLE 2

Gene dosage studies with *lon-3*

Genotype	Length (mm)[a]	*n*
Wild type	1.35 ± 0.008	71
sp23	1.65 ± 0.009	84
sp23/+[b]	1.41 ± 0.01	41
sp23/sp23/+[c]	1.37 ± 0.01	28
sp23/Df[d]	1.59 ± 0.009	32
svEx57[e]	1.27 ± 0.02	34
e120/+	1.37 ± 0.01	46
e30/+	1.37 ± 0.01	29

[a] Sample mean ±SE.

[b] Complete genotype is *unc-4(e120)/+; lon-3(sp23)/+*.

[c] Complete genotype is *sma-1(e30) lon-3(sp23); ctDp8. ctDp8* is a chromosomal duplication covering much of the right arm of LGV, including *lon-3* and *sma-1* (Hunter and Wood 1992).

[d] Complete genotype is *sma-1(e30) lon-3(sp23)/ unc-42(e270) arDf1. arDf1* is a deficiency that deletes *lon-3* but not *sma-1* (Tuck and Greenwald 1995).

[e] Complete genotype is *unc-36(e251); svEx57[unc-36(+)-lon-3 (+)]*.

Ueno, personal communication), and found that the average ploidy was 20% higher than wild-type hermaphrodites ($P < 0.0001$; Table 1). Thus *lon-1* is a negative regulator of both body length and hypodermal endoreduplication.

To determine whether the Lon phenotype was invariably associated with hyperendoreduplication or whether the effect occurred in only certain Lon mutants, we measured hypodermal ploidy in worms overexpressing *dbl-1*. No significant difference was detected in the extent of endoreduplication in *ctIs40* worms (which overexpress *dbl-1*; Morita *et al.* 1999; Suzuki *et al.* 1999) compared to wild type ($P = 0.3$). Although we cannot conclude definitely that *dbl-1* overexpression does not cause an increase in endoreduplication, power calculations indicate that we would have had a 99% chance of detecting a difference in ploidy of 18%, which is comparable to the difference between N2 and *lon-1(0)*. Our results suggest therefore that if *dbl-1* can cause an increase in endoreduplication when overexpressed, it does not do so very efficiently.

Isolation and characterization of *lon-3* mutant alleles: To learn more about how body length and body size are regulated in *C. elegans* we carried out a genetic screen for new mutations causing a Lon phenotype. From a screen of 50,000 haploid genomes, we isolated 18 new Lon alleles. Genetic mapping and complementation tests (materials and methods) revealed that three of these new mutations were allelic to *lon-3*. While we were carrying out this screen, another allele of *lon-3*, *sv18*, was isolated in an unrelated genetic screen (H. Fares and I. Greenwald, personal communication). Both the new *lon-3* alleles reported here and the previously existing allele, *e2175*, are recessive to wild type (Table 2 and data not shown). By genetic criteria they appear to reduce or eliminate activity. For example, hermaphrodites homozygous for *lon-3(sp23)* but carrying a wild-type allele on a chromosomal duplication are non-Lon, whereas hermaphrodites of the genotype *lon-3(sp23)/Df* are Lon (Table 2).

To determine when *lon-3* mutants become longer than wild type, we measured the lengths of *lon-3* mutant hermaphrodites at different times after hatching. For comparison, wild-type hermaphrodites were measured under identical growth conditions. Two different *lon-3* alleles, *lon-3(sp23)* and *lon-3(e2175)*, were used in the analysis. Results described below suggest that *sp23* is a null allele. Figure 1 shows that *lon-3* mutants first begin to express the Lon phenotype ~36 hr after hatching. After this time they become progressively longer than wild type until, when they are adults, they are ~22% longer than wild type (Figures 1 and 2; Table 3).

To determine whether *lon-3* mutant hermaphrodites are longer than wild type because particular regions of the worm are longer or because all organs are proportionately longer, we carried out a morphometric analysis. Results presented in Table 4 show that, in *lon-3* mutants, the regions between the pharynx and the anterior bend of the gonad and between the posterior bend of the gonad and the rectum are expanded compared to wild type. The gonad is on average 11% longer than in wild type but constitutes a lower percentage of the total worm length.

To determine whether *lon-3* mutations, like the mutation in *lon-1*, cause an increase in endoreduplication of hypodermal nuclei, we measured hypodermal ploidy in *lon-3(sp23)* mutant hermaphrodites. Results presented in Table 1 show that loss of *lon-3* activity causes an increase of body length without increasing hypodermal ploidy [$P = 0.6$ for *lon-3(sp23) vs.* wild type; $P = 0.1$ for *lon-3(e2175) vs.* wild type].

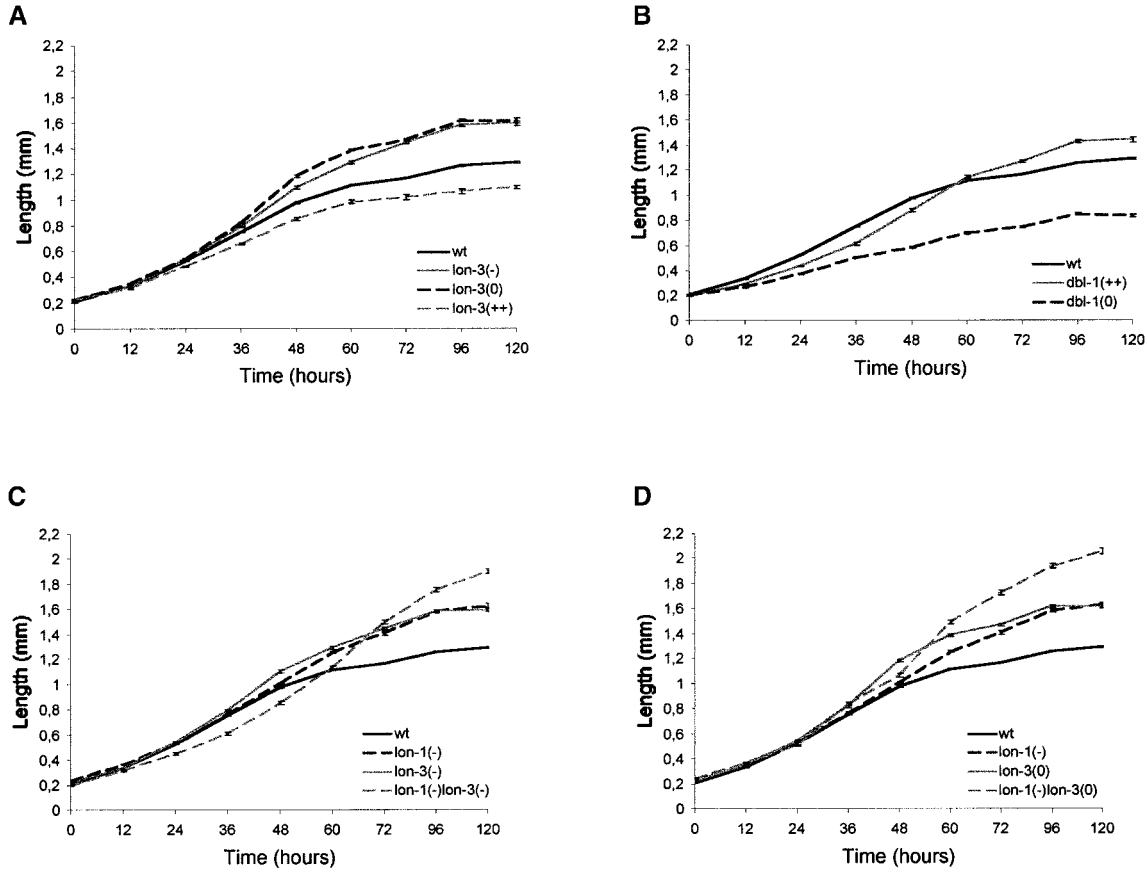

FIGURE 1.—Growth curves. (A) *lon-3(−)* denotes *lon-3(e2175)*, *lon-3(0)* denotes *lon-3(sp23)*, and *lon-3(++)* denotes *unc-36 (e251);svEx57*. *svEx57* is an extrachromosomal array containing multiple copies of *lon-3(+)*. (B) *dbl-1(++)* denotes *ctIs40*, an integrated array containing multiple copies of *dbl-1(+)* (SUZUKI *et al.* 1999), and *dbl-1(0)* denotes *dbl-1(nk3)* (MORITA *et al.* 1999). (C) *lon-1(−)* denotes *lon-1(e185)* and *lon-3(−)* denotes *lon-3(e2175)*. (D) *lon-1(−)* denotes *lon-1(e185)* and *lon-3(0)* denotes *lon-3(sp23)*.

Cloning of *lon-3*: To investigate further how *lon-3* functions we cloned the gene (Figure 3A and MATERIALS AND METHODS). A 5.5-kb fragment from the cosmid C35G11, spanning the predicted gene ZK836.1, rescued the Lon phenotype caused by *lon-3(e2175)*. We found that the *lon-3* alleles *sp6, sp23*, and *sv18* are associated with mutations that introduce premature stop codons into ZK836.1, indicating that this predicted gene does indeed correspond to *lon-3* (Figure 3B). The splicing pattern predicted by the Genefinder program for ZK836.1 was confirmed by sequencing the cDNA clone cm06a10.

lon-3 is predicted to encode a collagen (Figure 3B). The predicted protein sequence contains a central region containing four closely spaced domains consisting of Gly-X-Y repeat sequences (in which X and Y frequently are prolines), characteristic of collagens in both vertebrates and invertebrates (VAN DER REST and GARRONE 1991; KRAMER 1994). The primary structure of LON-3 is typical of *C. elegans* cuticle collagens (KRAMER 1994). First, at the amino terminus there are four short sequence motifs named homology blocks D-A that are conserved in most cuticle collagens. Second, the predicted sequence contains a number of cysteine residues that show spacing typical of that seen in cuticle collagens: Three conserved cysteine residues lie immediately N terminal to the first Gly-X-Y domain, two lie immediately after, and two lie after the fourth and last Gly-X-Y domain (Figure 3, B and C). *C. elegans* cuticle collagens have been divided into nine subfamilies on the basis of the precise spacing of the cysteine residues (KRAMER 1997). It is thought that collagens belonging to the same subfamily may be able to form heterotrimers (KRAMER 1994). The spacing of the cysteines places LON-3 in the SQT-1 subfamily of cuticle collagens (KRAMER *et al.* 1988; KRAMER 1994; Figure 3C).

Increasing or decreasing *lon-3* activity causes reciprocal changes in body length: It is known that neomorphic mutations in certain *C. elegans* cuticle collagen genes can affect the morphology of the worm (KRAMER and JOHNSON 1993). In some cases null mutations in these genes have a wild-type phenotype suggesting that, in an otherwise wild-type background, the genes are not necessary for the formation of a cuticle of the correct shape. To help elucidate the nature of the mutations in *lon-3* causing a Lon phenotype, we determined the

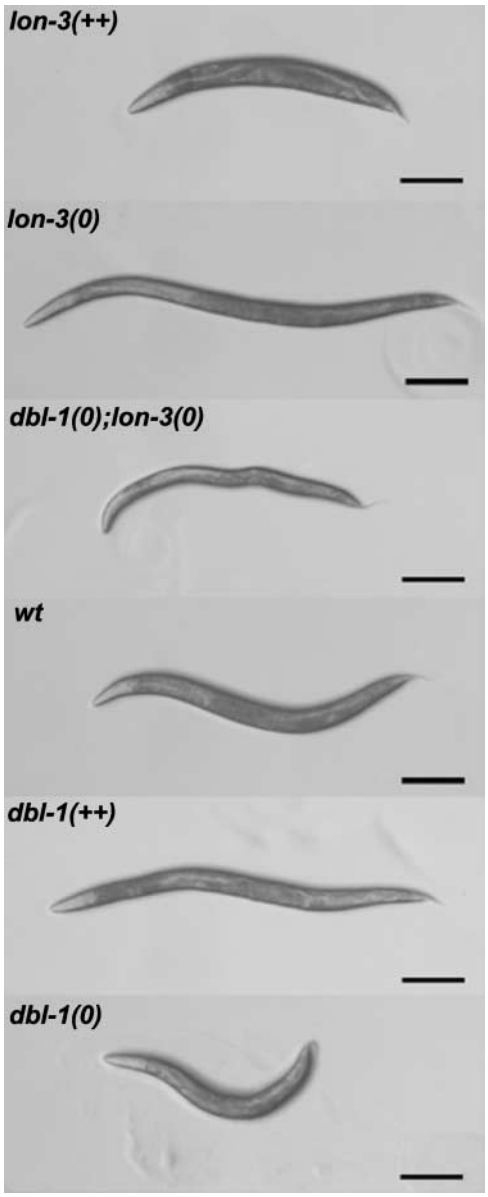

FIGURE 2.—Defects in *lon-3* expression affect body length. *dbl-1(0)* denotes *dbl-1(nk3)* (MORITA *et al.* 1999), *lon-3(0)* denotes *lon-3(sp23)*, and *lon-3(++)* denotes worms carrying an extrachromosomal array, *svEx57*, containing multiple copies of *lon-3(+)*. *dbl-1(++)* denotes *ctIs40*, an integrated array containing multiple copies of *dbl-1(+)* (SUZUKI *et al.* 1999). Bar, 0.2 mm.

sequence changes associated with three mutant *lon-3* alleles. *lon-3(sp23)* was found to be associated with a G-to-A transition in codon 34 that leads to the generation of a stop codon (Figure 3B). *sp23* is therefore predicted to encode a severely truncated protein lacking eight-ninths of the full-length protein. Two other *lon-3* alleles were also found to be associated with sequence changes that introduce stop codons into the *lon-3* open reading frame (Figure 3B). *sp6* is associated with a C-to-T transition that introduces an ochre stop codon into position 112, and *sv18* is associated with a C-to-T transition that introduces an ochre stop at position 237. Two alleles of *rol-6*, *e187n1268* and *n1178*, are predicted to encode truncated proteins similar in length to those predicted to be encoded by *lon-3(sp23)* and *lon-3(sv18)*, respectively (KRAMER and JOHNSON 1993). *rol-6(n1178)* and *rol-6(e187n1268)* are both thought to be null alleles (KRAMER and JOHNSON 1993). We were unable to identify any lesions in the *lon-3* open reading frame associated with *sp5*. It is possible that the *sp5* mutation lies in a site in noncoding DNA that is required for the transcriptional regulation of *lon-3*.

TABLE 3

Comparison of *lon-3* mutant alleles

Allele	Length (mm)[a]	*n*
Wild type	1.37 ± 0.008	60
sp23	1.72 ± 0.01	33
e2175[b]	1.58 ± 0.01	30
sp6	1.63 ± 0.01	29
sv18	1.45 ± 0.01	35

[a] Sample mean ±SE.
[b] *e2175* arose spontaneously (RIDDLE *et al.* 1997).

The nature of the sequence changes associated with the *sp6*, *sp23*, and *sv18* alleles suggests that these alleles reduce or eliminate gene activity and therefore that the loss-of-function phenotype of *lon-3* is Lon. Consistent with this idea, *lon-3(RNAi)* hermaphrodites were Lon (Figure 4 and data not shown). No *C. elegans* collagen gene has previously been identified for which the loss-of-function phenotype is Lon. The null phenotypes of cuticle collagen genes that have been characterized genetically to date are Dpy, wild type, lethal, or Tal (KRAMER 1997).

In the course of cloning *lon-3* we noticed that DNA encoding *lon-3* was not only able to rescue the *lon-3* mutant phenotype but could also cause worms to be shorter than wild type. For example, whereas injection of 2 μg/ml of C35G11 cosmid DNA into *lon-3(e2175)*

TABLE 4

Morphometric analysis of Lon worms

Genotype	Pharynx[a]	Pharynx-gonad[a,b]	Gonad-rectum[a,c]	Gonad[a,d]
Wild type	14	6	10	60
lon-3(sp23)	11	13	14	54
dbl-1(++)	11	19	14	48

[a] Percentage of total body length.
[b] The distance between the end of the pharynx and the anterior bend of the gonad.
[c] The distance between the posterior bend of the gonad and the rectum.
[d] The distance from the anterior bend to the posterior bend of the gonad.

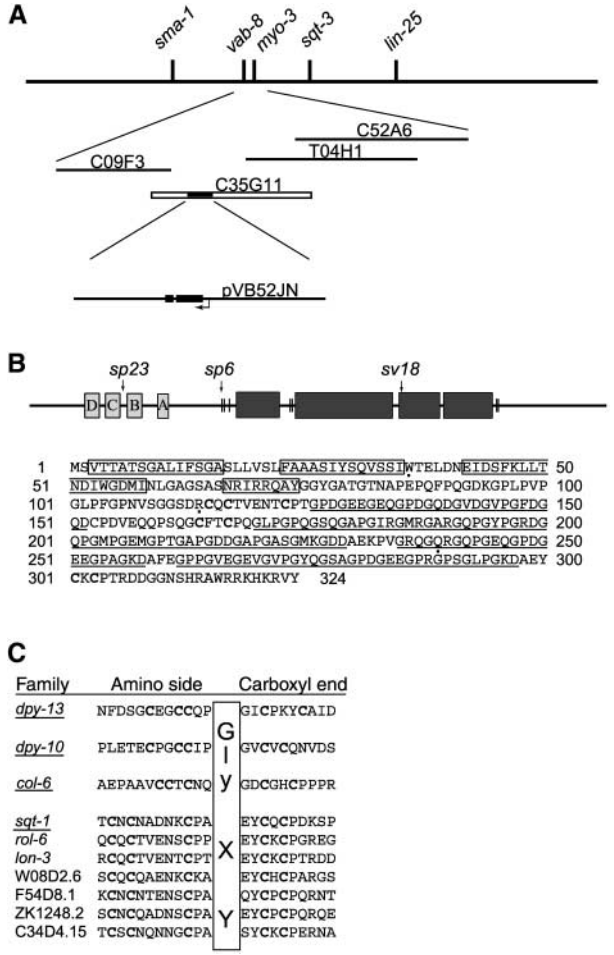

FIGURE 3.—*lon-3* encodes a collagen. (A) Top, genetical and physical maps of the *lon-3* region, bottom, exon/intron structure of *lon-3*, where solid boxes in pVB52JN represent exons. (B) The primary structure of LON-3 protein. The darkly shaded boxes represent Gly-X-Y repeat domains. The lightly shaded boxes, labeled D to A, represent homology blocks found in many cuticle collagens (KRAMER 1994). The sequences contained in the homology blocks have been shown to be important for the processing of collagen chains (YANG and KRAMER 1999). Thin vertical lines represent the positions of highly conserved cysteine residues, important for disulfide bonding during cuticle assembly. The positions of stop codons introduced by the mutations *sp23*, *sp6*, and *sv18* are indicated both by arrows (top) and solid squares below the affected amino acid in the predicted protein sequence of LON-3 (bottom). The Gly-X-Y repeat sequences are underlined, the homology blocks D, C, B, and A are boxed, and the conserved cysteines are in boldface type. (C) The spacing of the cysteine residues in different cuticle collagen families (KRAMER 1994). "Amino side" indicates cysteine residues lying amino terminal to the first Gly-X-Y domain. "Carboxyl end" indicates residues lying immediately after the last Gly-X-Y domain. These sequences have been submitted to the GenBank database under accession no. AF262406.

hermaphrodites rescued the Lon phenotype of transformed progeny to wild type, injection of 10 μg/ml caused the F_1 transformants to be shorter than wild type (MATERIALS AND METHODS). To investigate more closely the effect of multiple copies of *lon-3* on body length, we

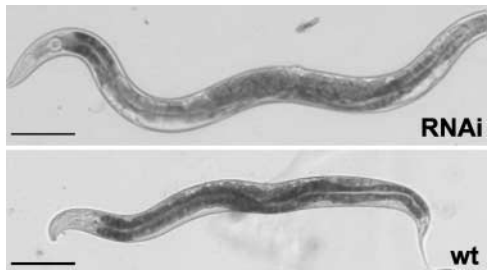

FIGURE 4.—RNA interference experiment with *lon-3* results in worms that are longer than wild type. (Top) The progeny of wild-type worms injected with dsRNA corresponding to *lon-3* cDNA (cm06a10) are much longer than wild type (bottom). Bar, 0.2 mm.

generated worms of the genotype *unc-36(e251); svEx57*. *svEx57* is an extrachromosomal array containing *lon-3(+)* (encoded by the plasmid pVB52JN). Worms carrying the array were Dpy (Figure 2). pVB52JN contains *lon-3*, 3.8 kb of DNA upstream from *lon-3*, and part of the gene ZK836.2 (which encodes 2-oxoglutarate dehydrogenase), in whose third intron *lon-3* lies. Disruption of the *lon-3* open reading frame by insertion of *lacZ* sequences abolished the ability of the 5.5-kb fragment in pVB52JN to confer the Dpy phenotype on transformed progeny (data not shown). Thus the phenotype observed requires LON-3 protein activity and is not caused by other sequences on the rescuing fragment.

Worms carrying the *svEx57* array were first noticeably shorter than wild type during the L3 stage. They remained shorter throughout the period of measurement and as older adults (data not shown). On average, worms carrying the array were 15% shorter than wild type (Table 2; Figures 1 and 2). Many animals, however, were considerably shorter than this: Some were as much as 30% shorter than wild-type hermaphrodites of the same age (data not shown). Animals carrying the array were otherwise wild type and healthy: They did not show defects in locomotion or viability. Furthermore, as described below, the array did not cause synthetic lethality in combination with a variety of other mutations. Together, our results suggest that reducing or eliminating *lon-3* activity results in increased length whereas increasing gene activity decreases length. No other *C. elegans* collagen gene that causes these reciprocal effects on body length has been described previously.

Defects in *lon-3* expression have no effect on dauer development or on development of the male tail: The similarity of the *lon-3* loss-of-function phenotype with the phenotype caused by overexpression of *dbl-1* suggested to us that the TGFβ pathway might regulate LON-3 activity or be regulated by it. For example, one possibility might be that LON-3 functions as a negative regulator of TGFβ ligands in *C. elegans*. To investigate this possibility we first analyzed the effects of defects in *lon-3* expression on processes (other than the control of body length) that are known to require TGFβ-mediated sig-

naling. Besides regulating body length, *dbl-1* is also required for correct dorso-ventral patterning of a group of sensory neurons on the fan, a structure used during mating (Morita *et al.* 1999; Suzuki *et al.* 1999). In wild type, three of the sensory rays open out onto the dorsal surface of the fan while four open out onto the ventral surface (Sulston and Horvitz 1977). In *dbl-1* mutants, the rays that normally open out onto the dorsal surface lose their dorsal identity and instead open out onto the ventral surface (Suzuki *et al.* 1999). Neither *lon-3(0)* mutant males nor males of the genotype *him-8(e1498); svEx57* [which harbor multiple copies of *lon-3(+)*] showed defects in patterning of the sensory rays (data not shown), suggesting that LON-3 has no effect on DBL-1 activity in ray patterning.

A second TGFβ signaling pathway in *C. elegans* regulates the decision between entering the dauer developmental state and undergoing the reproductive life cycle. Larvae mutant for *daf-7*, which encodes the ligand activating this pathway, are dauer constitutive: They enter the dauer state under conditions in which wild-type worms remain in the reproductive life cycle, when the population density is low and the food source plentiful (Ren *et al.* 1996). Neither *lon-3(lf)* mutants nor worms of the genotype *lon-3(+), svEx57[lon-3(+)]*, show defects in dauer development. Furthermore, overexpression of *lon-3* does not enhance the dauer constitutive phenotype of a temperature-sensitive *daf-7* mutation at the semipermissive temperature. Thirty-six percent of both *daf-7(e1372*ts*)* ($n = 50$) mutant hermaphrodites and hermaphrodites of the genotype *daf-7(e1372*ts*); svEx57* ($n = 50$) become dauers when raised at 20° (the semipermissive temperature).

***lon-3* is expressed in hypodermal cells and shows strong genetic interactions with *sqt-1* and *rol-6*:** To determine in which tissues *lon-3* is transcribed, we generated worms carrying either a *gfp* or *lacZ* reporter gene under the control of *lon-3* promoter sequences (materials and methods). The pattern of *gfp* expression in hermaphrodites carrying the *gfp* reporter was dynamic but at all stages restricted to hypodermal cells. Early in the L1 stage, expression was seen in H0, H1, and H2, in the anterior V cells, and in the T cells. Weak expression was also seen in hypodermal nuclei in the head and the tail, including those in hyp5, hyp6, hyp8, hyp9, and hyp10. Expression was not seen at this stage, however, in the P cells or in nuclei in the hyp7 syncytium. After division of H1, both H1.a, a seam cell, and H1.p, which joins hyp7, expressed *gfp*. Likewise, after division of V1–V4 both the anterior daughters (which join hyp7) and the posterior daughters (which remain seam cells) expressed *gfp*. This pattern was repeated at each of the larval molts with the result that in adult worms, all descendents of H1, H2, and V1–V4 expressed *gfp*. At the end of the L1 stage, V5.p could be seen to express *gfp* but not V5.a, which is a neuroblast. In the P cell lineages, expression was first seen toward the end of the L1 stage in P1.p, P2.p, P9.p, P10.p, and P11.p nuclei. These cells fuse with hyp7 during the L1 stage (Sulston and Horvitz 1977). Expression of *gfp* was not seen, on the other hand, in the daughters of P3–P8 or P12 (all of which remain separate from hyp7). By the end of the L1 stage most nuclei in hyp7 expressed *gfp*, as did those in hyp5, hyp6, hyp8, hyp9, hyp10, and hyp11. After division of P3.p, P4.p, and P8.p during the L3 stage, all six daughters started to express *gfp* concomitant with their fusion with hyp7. The daughters of P5.p, P6.p, and P7.p, the progenitors of the vulva, remained negative for *gfp* expression as did all the cells they subsequently gave rise to. In adult worms, no hypodermal nuclei that failed to express *gfp* could be identified. Conversely, no nonhypodermal nuclei were observed at any stage that expressed the *gfp* marker. Examples of the expression of the *lon-3::gfp* marker are shown in Figure 5.

Although we did not analyze worms carrying the *lon-3::lacZ* marker gene in as much detail, the pattern of expression appeared to be the same as that for the *gfp* reporter. In adult worms carrying the *lacZ* reporter gene, expression of *lacZ* was seen in many hypodermal nuclei including those in hyp5, hyp6, hyp7, hyp8, hyp9, hyp10, and hyp11 (Figure 5). Expression of *lacZ* was first seen during the L1 stage (data not shown) and then persisted through the subsequent larval stages and into the adult stage. The sequence of the predicted LON-3 protein—together with the fact that reporter genes under the control of *lon-3* promoter sequences are transcribed in hypodermal cells, cells that synthesize and secrete cuticle—suggested that LON-3 might be a cuticle collagen. Further evidence in favor of this hypothesis is that the plasmids pVB61JN and pVB71JN encoding LON-3::GFP fusion proteins, in which GFP is fused in frame close to the carboxy terminus of LON-3, conferred a Rol phenotype on transformed progeny (Table 5). (Transgenic worms containing these plasmids did not fluoresce green.)

As described above, the predicted LON-3 protein sequence is most similar in sequence to the SQT-1 family of cuticle collagens. Certain alleles of *sqt-1* and a gene encoding another member of the family, *rol-6*, have marked effects on organismal morphology. *sqt-1* and *rol-6* show strong genetic interactions with one another and it has been suggested that the two proteins might function together in a physical complex (Kramer *et al.* 1990; Kramer and Johnson 1993; Yang and Kramer 1999). To investigate the possibility that LON-3 might function with either ROL-6 or SQT-1, we examined genetic interactions between *lon-3* mutations and those in *sqt-1* or *rol-6*. Worms homozygous for the *sqt-1* null mutation, *sc103*, or the *rol-6* null mutation, *e187n1268*, are almost wild type in length, being only slightly Dpy (Table 6). The Lon phenotype caused by *lon-3(sp23)* was strongly suppressed by *sqt-1(0)*: *sqt-1(0); lon-3(0)* double mutants were found to be longer than wild type but considerably shorter than *lon-3* single mutants (Table

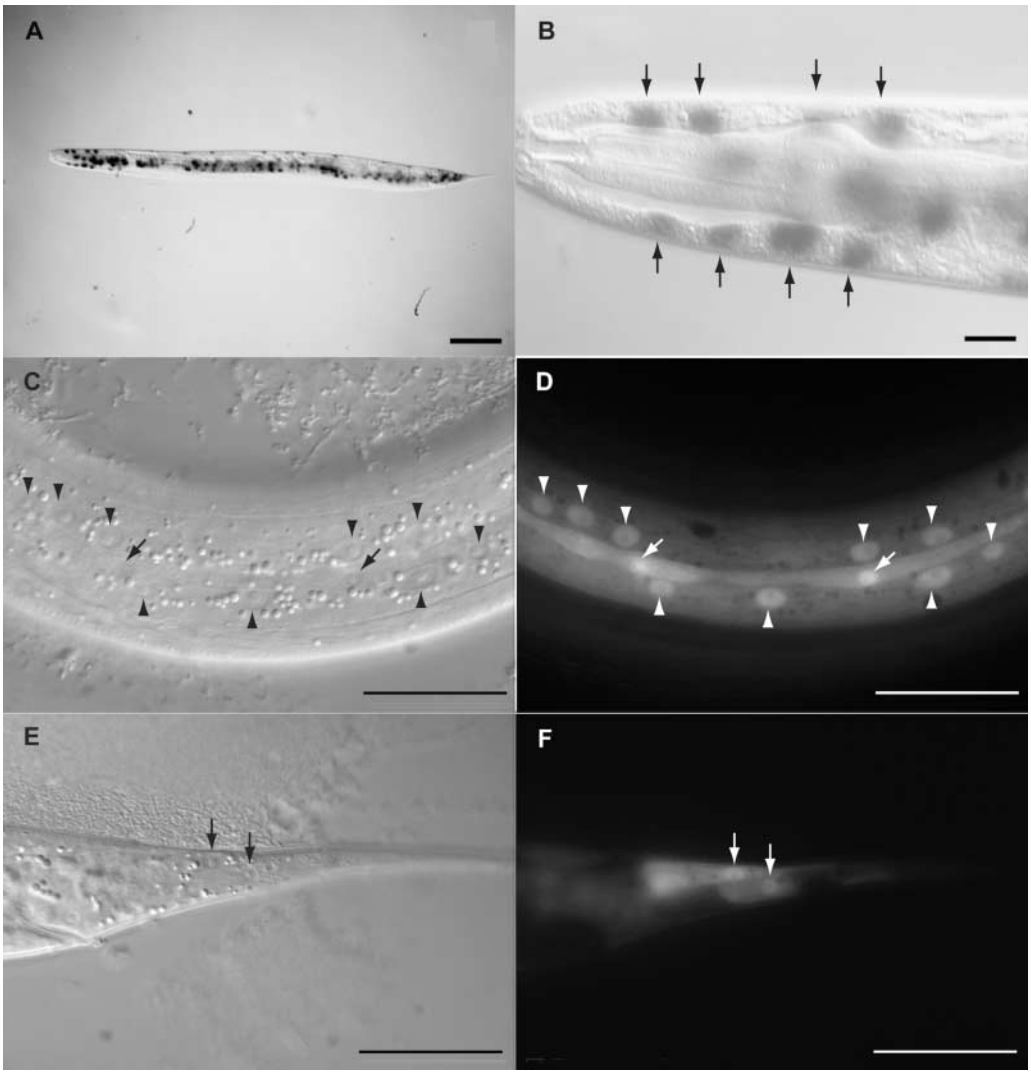

FIGURE 5.—*gfp* and *lacZ* reporter genes under the control of *lon-3* promoter sequences are expressed in epidermal cells throughout the length of the worm. (A) Bright-field photomicrograph of an adult hermaphrodite carrying multiple copies of pVB54JN, a plasmid containing *lacZ* under the control of *lon-3* promoter sequences. Bar, 0.1 mm. (B) Nomarski differential interference contrast photomicrographs of the same worm at higher magnification. Bar, 0.01 mm. Arrows in B indicate nuclei in the hyp5, hyp6, and hyp7 syncytia. (C and E) Nomarski differential interference contrast photomicrographs of an L4 hermaphrodite carrying multiple copies of pVB-82JN, a plasmid containing *gfp* under the control of *lon-3* promoter sequences. (D and F) Fluorescence micrographs of the same views. Bars in C–F, 0.03 mm. (C and D) Arrows indicate nuclei of seam cells and arrowheads indicate hypodermal nuclei in the hyp7 syncytium. (E and F) Arrows indicate nuclei in hyp10 and hyp11.

6). Thus *sqt-1* is required for the expression of the Lon-3 phenotype. To help determine whether the suppression observed represented a significant genetic interaction or simply represented the independent effects of the two genes, we calculated the expected length of the *sqt-1(0); lon-3(0)* double mutant assuming simple additivity of the effects of the individual mutations (Table 6). The double mutant was shorter than would be expected if *sqt-1* and *lon-3* affected length entirely independently of one another ($P < 0.0001$). Similarly, a *rol-6(0)* mutation suppressed the Lon phenotype caused by *lon-3(0)*, and the *rol-6(0); lon-3(0)* double mutant was shorter than expected if the genes acted entirely independently ($P < 0.0001$; Table 6). Interestingly, *sqt-1(0)* also suppressed the phenotype caused by overexpression of *lon-3*: Worms of the genotype *sqt-1(0); svEx57* were longer than either *sqt-1(0)* single mutant worms or worms overexpressing *lon-3* ($P < 0.0001$; Table 6). No *sqt-1(0); svEx57* worms displayed the Dpy phenotype. In contrast to *sqt-1(0)*, *rol-6(0)* did not suppress the phenotype caused by worms overexpressing *lon-3* but rather increased the expressivity of the phenotype: *rol-6(n1178); svEx57* worms were strongly Dpy (Table 5).

To determine whether the interactions observed above were reciprocal, we examined the ability of *lon-3* null mutations to suppress phenotypes caused by neomorphic mutations in *sqt-1* or *rol-6*. We found that *lon-3(0)* almost completely suppressed the Rol phenotypes caused by *sqt-1(sc13)* or *rol-6(su1006)* and the Sqt phenotype caused by *sqt-1(sc1)* (Table 5). Thus not only does LON-3 require *sqt-1* activity to affect body morphology but the reverse is also true: Mutant SQT-1 and ROL-6 proteins require *lon-3* activity to confer Rol or Sqt phenotypes.

***dbl-1* requires *sqt-1* and *rol-6* activity to regulate body length:** Since mutations in both *lon-3* and *dbl-1* can affect body length, and phenotypes caused by defects in *lon-3* expression are modified by null mutations in *sqt-1* or *rol-6*, we investigated whether the Lon phenotype caused by overexpression of *dbl-1* is also modified by *sqt-1* or *rol-6* mutations. A *sqt-1(0)* mutation strongly suppressed the Lon phenotype caused by multiple copies of *dbl-1*

TABLE 5

lon-3* interacts genetically with *sqt-1* and *rol-6

Genotype	Phenotype
unc-36(e251); svEx57[a]	Dpy
unc-36(e251); svEx90[b]	Rol
unc-36(e251); svEx120[c]	Rol
rol-6(su1006)	Rol
rol-6(su1006); lon-3(sp23)	non-Rol, weak Lon
rol-6(n1178)	Weak Dpy
rol-6(n1178); svEx57	Dpy
sqt-1(sc1)	Weak Dpy
sqt-1(sc1); lon-3(sp23)	non-Dpy, non-Lon
sqt-1(sc13)	Rol
sqt-1(sc13); lon-3(sp23)	Weak Rol, Lon

[a] Complete genotype is *unc-36(e251); svEx57[unc-36(+)-lon-3 (+)]*.

[b] Complete genotype is *unc-36(e251); svEx90[unc-36(+)-pVB-61JN]*.

[c] Complete genotype is *unc-36(e251); svEx120[unc-36(+)-pVB-71JN]*.

(Table 6). Worms carrying an integrated array, *ctIs40*, harboring multiple copies of *dbl-1(+)* but homozygous for *sqt-1(0)*, were not longer than wild type. The length of the double mutant was considerably less than the length expected if *db1-1* and *sqt-1* functioned entirely independently ($P < 0.0001$; Table 6). Similarly, *rol-6(0)* suppressed the phenotype caused by *ctIs40* to wild type (data not shown). Thus functional SQT-1 and ROL-6 proteins are required for *dbl-1* to regulate body length.

The fact that both *dbl-1* and *lon-3* require *sqt-1* and *rol-6* to affect body length led us to investigate the relationship between *lon-3* and *dbl-1*. Morphological measurements indicate that the phenotype caused by overexpression of *dbl-1* is similar to that caused by loss of *lon-3* function (Suzuki *et al.* 1999; Table 4). Qualitatively, the regions that are expanded in worms that overexpress *dbl-1* are the same as those in *lon-3(0)* mutant worms. Quantitatively, in the former, the region between the pharynx and the gonad is expanded to a greater extent, and the gonad spans a commensurately smaller percentage of the total worm length.

To investigate further how body length is regulated in *C. elegans*, we carried out genetic epistasis tests with a *dbl-1* null mutation and mutations in *lon-1* or *lon-3*. The Sma phenotype caused by *dbl-1(0)* is characterized by both a reduction in length compared to wild type and also a slight reduction in width (Morita *et al.* 1999; Suzuki *et al.* 1999). *lon-1(e185)* partially suppressed one aspect of the *dbl-1(0)* phenotype: *lon-1(e185); dbl-1(0)* double-mutant hermaphrodites were longer than *dbl-1(0)* single mutants [although not as long as *lon-1(e185)* single mutants (Table 6)]. *lon-1(e185)* also partially suppressed the endoreduplication defect caused by *dbl-1(0)*: Hypodermal ploidy was slightly increased in the *lon-1 (e185); dbl-1(0)* double mutant compared to the *dbl-1(0)* single mutant (Table 1). It is noteworthy, however, that *lon-1(e185)* did not appreciably suppress the thinness aspect of the *dbl-1* phenotype: Double-mutant animals were thinner than both wild type and *lon-1(e185)* single mutants.

lon-3(0) partially suppressed the "shortness" aspect of the *dbl-1(0)* Sma phenotype (Table 6 and Figure 2) but did not suppress the thinness caused by *dbl-1(0)*: *dbl-1(0) lon-3(0)* were thinner even than *dbl-1(0)* single mutants (Figure 2). In addition, *lon-3(0)* did not rescue the endoreduplication defect caused by *dbl-1(0)* (Table 1). The fact that *lon-1*, but not *lon-3* mutations, affect endoreduplication suggests that *lon-3* does not function by regulating *lon-1*. Consistent with this idea, *lon-1(e185); lon-3 (0)* double-mutant hermaphrodites were longer than either single mutant alone (Table 6).

Results presented in Table 6 show that mutations in *lon-1* or *lon-3* can partially suppress the Sma phenotype caused by null mutation in *daf-4* and a hypomorphic mutation in *sma-2*.

The *lon-3* overexpression phenotype is not caused by indiscriminate inhibition of collagen function: Thirty-three genes that can mutate to give rise to alleles conferring a Dpy phenotype have previously been identified. Seven of these genes (*dpy-2*, *dpy-7*, *dpy-10*, *dpy-13*, *sqt-1*, *sqt-3*, and *rol-6*) are known to encode collagens (Johnstone 2000), six [*dpy-21*, *dpy-26*, *dpy-27*, *dpy-28*, *sdc-3* (previously *dpy-29*), and *dpy-30*] encode genes that are involved in X chromosome dosage compensation (Meyer 2000), one (*dpy-18*) is a prolyl-hydroxylase (Hill *et al.* 2000), one (*dpy-23* or *apm-2*) a clathrin-associated protein (Shim and Lee 2000), and three (*dpy-5, dpy-19*, and *dpy-20*) encode novel proteins (Clark *et al.* 1995; Riddle *et al.* 1997; Honigberg and Kenyon 2000). The products encoded by the remainder are not yet known. We reasoned that *lon-3* might cause a Dpy phenotype by interfering with the function of one or more of the *dpy* genes. To test this possibility we examined a panel of Dpy mutants for their genetic interactions with *sqt-1(0)*. As described above, the *sqt-1* null mutation *sqt-1(sc103)* completely suppresses the Dpy phenotype caused by overexpression of *lon-3(+)* (Table 6). Therefore, if *lon-3* functioned by interfering with *dpy-17* activity, for example, the phenotype caused by *dpy-17* mutations should also be suppressed by *sqt-1(0)*. However, for *dpy-17* and all the other genes we tested, *sqt-1(0)* had the converse effect: It slightly or markedly enhanced the expressivity of the Dpy phenotype. Results presented in Table 7 show that worms homozygous for *sqt-1(0)* and a mutation in *dpy-5*, *dpy-13*, or *dpy-4* were not wild type in length; rather, they displayed an extreme Dpy phenotype. *sqt-1(0)* did not enhance the Dpy phenotype caused by mutations in *dpy-6*, *dpy-7*, *dpy-8*, *dpy-9*, *dpy-11*, *dpy-14*, or *dpy-17* to the same extent but in no case was the Dpy phenotype suppressed. These observations suggest that *lon-3* overexpression does not cause a Dpy phenotype

TABLE 6

Body length measurements of double mutants

Genotype[a] (mm):	Wild type	*a*	*b*	*a* + *b*-wild type[b]	*a*; *b*	*a* × *b*; *P*[c]
	N2	*dbl-1(nk3)*	*lon-3 (sp23)*		*dbl-1(nk3) lon-3(sp23)*	
	1.23 (±0.005)	0.90 (±0.009)	1.46 (±0.007)	1.12	0.97 (±0.0009)	<0.0001
	N2	*dbl-1(nk3)*	*lon-3 (e2175)*		*dbl-1(nk3) lon-3(e2175)*	
	1.23 (±0.005)	0.90 (±0.009)	1.34 (±0.008)	1.00	1.03 (±0.007)	0.06
	N2	*sma-2 (e502)*	*lon-3 (e2175)*		*sma-2(502); lon-3(e2175)*	
	1.23 (±0.001)	0.61 (±0.001)	1.56(±0.01)	0.94	0.76 (±0.015)	<0.0001
	N2	*daf-4 (m63)*	*lon-3 (e2175)*		*daf-4(m63); lon-3(e2175)*	
	1.23 (±0.001)	0.73(±0.009)	1.56(±0.01)	1.03	1.16 (±0.015)	<0.0001
	N2	*dbl-1(nk3)*	*lon-1 (e185)*		*lon-1(e185); dbl-1(nk3)*	
	1.23 (±0.06)	0.90 (±0.08)	1.48 (±0.09)	1.18	1.17 (±0.007)	0.09
	1.26 (±0.005)	0.85 (±0.10)	1.59 (±0.013)	1.17	1.13 (±0.011)	0.03
	1.29 (±0.009)[II]	0.84 (±0.011)	1.63 (±0.018)	1.17	1.18 (±0.010)	0.7 [0.3]
	N2	*sma-2 (e502)*	*lon-1 (e185)*		*lon-1(e185); sma-2(e502)*	
	1.23 (±0.001)	0.61 (±0.001)	1.57 (±0.010)	0.94	0.93 (±0.012)	0.3
	1.26 (±0.005)	0.64 (±0.007)	1.57 (±0.014)	0.95	0.96 (±0.008)	0.8 [0.6]
	N2	*daf-4 (m63)*	*lon-1 (e185)*		*lon-1(e185); daf-4(m63)*	
	1.23 (±0.001)	0.73 (±0.009)	1.57 (±0.010)	1.06	1.16 (±0.015)	<0.0001
	1.26 (±0.005)	0.78 (±0.006)	1.57 (±0.014)	1.09	1.17 (±0.008)	0.0001 [<0.0001]
	N2	*lon-1 (e185)*	*lon-3(sp23)*		*lon-1(e185); lon-3(sp23)*	
	1.27 (±0.005)	1.59 (±0.013)	1.62 (±0.10)	1.94	1.94 (±0.16)	0.10
	1.29 (±0.005)[d]	1.63 (±0.019)[d]	1.62 (±0.02)[d]	1.96	2.06 (±0.025)[d]	0.002 [0.02]
	N2	*lon-1 (e185)*	*lon-3(e2175)*		*lon-1(e185); lon-3(e2175)*	
	1.25 (±0.005)	1.67 (±0.010)	1.61 (±0.01)	2.04	1.99 (±0.018)	0.03
	1.31 (±0.006)[d]	1.64 (±0.008)[d]	1.55 (±0.06)[d]	1.88	1.78 (±0.009)[d]	<0.0001 [0.001]
	N2	*sqt-1(sc103)*	*lon-3 (sp23)*		*sqt-1(sc103); lon-3(sp23)*	
	1.43 (±0.10)	1.29 (±0.011)	1.87 (±0.009)	1.72	1.57 (±0.007)	<0.0001
	N2	*sqt-1(sc103)*	*svEx57*		*sqt-1(sc103); svEx57*	
	1.34 (±0.008)	1.19 (±0.008)	1.21 (±0.016)	1.06	1.31 (±0.007)	<0.0001
	N2	*sqt-1(sc103)*	*dbl-1 (ctls40)*		*sqt-1(sc103); dbl-1(ctls40)*	
	1.34 (±0.01)	1.26 (±0.009)	1.53 (±0.01)	1.45	1.22 (±0.008)	<0.0001
	N2	*rol-6(e187n1268)*	*lon-3 (sp23)*		*rol-6(e187n1268); lon-3(sp23)*	
	1.37 (±0.007)	1.17 (±0.007)	1.69 (±0.007)	1.49	1.34 (±0.005)	<0.0001

Worms in each row represent one set of measurements in which worms of the different genotypes were cultured under as identical as possible growth conditions. Small variations are seen in the length measurements for a given genotype between different experiments. These variations may result from variations in the osmolarity or consistency of the plates used. We have noticed that worms grown on drier plates (or on plates with a higher agar concentration) tend to be slightly longer than worms of the same genotype grown on wetter plates (or plates with lower agar content).

[a] Means (±SE). $N = 27$–70; all worms measured at 96 hr posthatching unless stated otherwise.

[b] *a* + *b*-wild type is the estimated additive value. It represents the phenotypic value of the double homozygous mutant *a*;*b* in the absence of any interaction between *a* and *b*.

[c] *a* × *b*; *P* is the probability of the interaction between *a* and *b* being due to chance alone. It is estimated as the interaction term of a two-factor, fixed effects analysis of variance. Combined probabilities of several experiments given in brackets.

[d] Measured at 120 hr posthatching.

by interfering with the activity of these genes and, in turn, that *lon-3* does not affect length by indiscriminately blocking the function of all cuticle collagens.

DISCUSSION

We show here that increasing or decreasing *lon-3* levels causes reciprocal changes in body length in *C. elegans*. Loss-of-function mutations in *lon-3* cause a 22% increase in body length whereas an increase in *lon-3* activity causes worms to be considerably shorter than wild type. We show that *lon-3* is predicted to encode a collagen that likely is a component of the cuticle and that *lon-3* requires the activity of two cuticle collagen genes, *sqt-1* and *rol-6*, to affect body length. We also demonstrate that defects in *lon-3* expression can affect body length independently of their effects on endoreduplication of hypodermal nuclei. Finally, we have shown that null

TABLE 7

***lon-3* does not affect length by indiscriminately blocking the function of all cuticle collagens**

Genotype	Phenotype
sqt-1(sc103); svEx57	Non-Dpy
dpy-5(e61); sqt-1(sc103)	Extreme Dpy
sqt-1(sc103); dpy-6(e14)	Dpy
sqt-1(sc103); dpy-7(e88)	Dpy
sqt-1(sc103); dpy-8(e130)	Dpy
sqt-1(sc103); dpy-9(e12)	Dpy
sqt-1(sc103); dpy-11(e224)	Dpy
sqt-1(sc103); dpy-13(e184)	Extreme Dpy
sqt-1(sc103); dpy-4(e1166)	Extreme Dpy
sqt-1(sc103); dpy-17(e164)	Dpy
sqt-1(sc103); dpy-18(e304)	Dpy
sqt-1(sc103); dpy-19(e1259)	Dpy

mutations in *dbl-1* or *lon-1* cause, respectively, a decrease or increase in hypodermal endoreduplication.

***lon-3* encodes a collagen that requires *sqt-1* activity to function:** The predicted LON-3 protein sequence is most similar to that of the cuticle collagen ROL-6. Furthermore, the spacing of conserved cysteine residues in LON-3 places it in the SQT-1 subfamily of cuticle collagens of which ROL-6 is also a member (Kramer 1994). Besides these three proteins, four other members of the family are encoded in the *C. elegans* genome (Figure 3C), F54D8.1, W08D2.6, ZK1248.2, and C34D4.15. F54D8.1 may correspond to *dpy-17*. To date *lon-3*, *rol-6*, and *sqt-1* (and possibly *dpy-17*) are the only genes of the family for which mutations have been isolated. W08D2.6, ZK1248.2, and C34D4.15 do not lie in regions to which any of the mutations affecting body morphology map. It appears therefore that mutations in these other genes that affect body morphology are rarer than those in *sqt-1*, *dpy-17*, *rol-6*, or *lon-3*. *lon-3* is the first member of the family for which the loss-of-function phenotype has been shown to be Lon. *lon-3* is also the first collagen gene that has been reported to give rise to a Dpy phenotype when present in multiple copies.

It has previously been speculated that cuticle collagens in the same subfamily may function together in the generation of the cuticle (Kramer *et al.* 1990). Evidence supporting this idea is that *sqt-1* and *rol-6* show strong genetic interactions with one another: Null mutations in one gene can suppress phenotypes associated with neomorphic mutations in the other (Kramer and Johnson 1993). Although no evidence for a direct covalent interaction between ROL-1 and SQT-1 proteins has been forthcoming, it is thought that the two proteins may be able to form noncovalent heterodimers (Kramer *et al.* 1990; Kramer and Johnson 1993). The genetic interactions that we have observed between *lon-3* and *rol-6* and between *lon-3* and *sqt-1* are similar in one respect to those reported for *rol-6* and *sqt-1*: A null mutation in *lon-3* can suppress phenotypes caused by neomorphic mutations in *rol-6* and *sqt-1* (Table 5). For example, the Rol phenotype of *sqt-1(sc13)* is suppressed by *lon-3(0)*. In the case of Rol alleles of *sqt-1* and *rol-6*, the suppression is not simply at the level of the ability of the worms to roll: *lon-3(0)* suppressed the twisting of the cuticle caused by aberrant SQT-1 or ROL-6 proteins. These observations suggest that these neomorphic forms of SQT-1 and ROL-6 require LON-3 to cause defects in the cuticle. We have not addressed in this article whether LON-3 can bind directly to SQT-1 or ROL-6. It will be interesting in the future to investigate whether the genetic interactions we have observed reflect direct physical interactions between SQT-1 or ROL-6 and LON-3.

The fact that a null mutation in *sqt-1* completely suppresses the phenotype caused by multiple copies of *lon-3(+)* is instructive since it implies that wild-type LON-3 requires wild-type SQT-1 to cause a decrease in body length. Furthermore, our observation reported here that *sqt-1(0)* does not suppress the Dpy phenotype caused by mutations in *dpy-5*, *dpy-6*, *dpy-7*, *dpy-8*, *dpy-9*, *dpy-11*, *dpy-13*, *dpy-14*, *dpy-17*, *dpy-18*, *dpy-19*, or *dpy-20* suggests that overexpression of *lon-3* does not give rise to a Dpy phenotype by causing nonspecific defects in the generation of the cuticle (for example, by binding to and inactivating many different components of the cuticle). It has previously been reported that *sqt-1(0)* strongly enhances the Dpy phenotype caused by mutations in the genes *dpy-3* and *dpy-10* (Kusch and Edgar 1986; Levy *et al.* 1993). Thus LON-3 is unlikely to function by interfering with the activities of these genes. Although LON-3 requires SQT-1 to confer a Dpy phenotype, it is not possible to conclude from our results that LON-3 necessarily functions directly with SQT-1. SQT-1 could be required for the expression or function of a protein together with which LON-3 acts.

The identity of LON-3, its pattern of expression, and the fact that *lon-3::gfp* fusion genes can cause morphological defects suggest that one possible mechanism by which LON-3 might act is to affect directly the elasticity of the cuticle. Confirmation of this model, however, must await the demonstration that LON-3 is indeed a component of the cuticle. Worms homozygous for *lon-3 (0)* do not appear to be longer than wild-type worms because of an increase in cell number. Although we did not determine the exact numbers, no obvious increase in the number of somatic nuclei compared to wild type was seen in *lon-3* mutants stained with 4′6-diamidino-2-phenylindole (J. Nyström and S. Tuck, unpublished results). We also examined *lon-3* mutant worms by Nomarski microscopy but found no extra somatic cells.

***dbl-1* and *lon-3* could function either in the same pathway or in parallel:** We have not determined in this article whether *lon-3* and *dbl-1* function in the same pathway or in parallel pathways. It is noteworthy, however, that

morphometric analyses indicate that both *lon-3(0)* mutants and worms overexpressing *dbl-1* are longer than wild type largely because two particular regions of the worm—that between the pharynx and the anterior arm of the gonad and that between the posterior arm of the gonad and the rectum—are expanded relative to wild type. Although it is presently not clear how *dbl-1* regulates body length, as in the case with *lon-3*, it is not thought that the number of somatic cells in worms expressing different levels of *dbl-1* is different from that in wild type (Suzuki *et al.* 1999).

The expression of the *lon-3::lacZ* fusion gene reported here was not affected by mutations that increase or decrease the activity of the TGFβ pathway affecting body length (J. Nyström and S. Tuck, unpublished data). Thus no evidence exists presently to suggest that *dbl-1* regulates body length by regulating the transcription of *lon-3*. However, since LacZ protein perdures, it is possible that the *lon-3::lacZ* transgene is not a sufficiently sensitive reporter to detect changes in the rate of *lon-3* gene transcription caused by mutations in genes in the TGFβ pathway. Further work will be needed to address this issue. Another possibility is that *dbl-1* does not affect *lon-3* transcription but instead regulates length, in part at least by affecting LON-3 protein levels. It is noteworthy in this regard that the TGFβ pathway regulating body length regulates the expression of a gene predicted to encode a protein similar to collagenase (Mochii *et al.* 1999). It will be interesting in the future to determine whether the product of this gene can affect body length, LON-3 protein stability, or both.

It is also possible that *dbl-1* does not regulate *lon-3* and that the genes affect body length independently. However, our findings that both *lon-3* and *dbl-1* interact genetically with *sqt-1* suggest that if *lon-3* and *dbl-1* do not regulate one another's activity, they may at least have a common target. Further biochemical work will be required to determine more precisely how the two genes function.

Several observations suggest that if *dbl-1* does regulate *lon-3*, then *lon-3* cannot be the only target of the pathway. First, the phenotype caused by overexpression of *lon-3* is Dpy rather than Sma. Second, *lon-3(0)* is not fully epistatic to *dbl-1(0)*. Furthermore, while worms lacking *dbl-1* activity show appreciably reduced endoreduplication of hypodermal nuclei, the ploidy of hypodermal nuclei in worms that overexpress *lon-3* is only slightly less than wild type (Table 1). This observation suggests that the effect of *dbl-1* loss-of-function mutations on the ploidy of hypodermal nuclei is not mediated through LON-3.

The data presented here do not exclude the possibility that LON-3 functions upstream of the TGFβ pathway regulating body length. Studies on vertebrates have shown that the activity of some ligands in the TGFβ superfamily can be inhibited by decorin, a protein that was first isolated by virtue of its ability to bind to collagen (Vogel *et al.* 1984; Yamaguchi *et al.* 1990). One possibility therefore is that LON-3 is required for the function of a negative regulator of DBL-1. However, the fact that the Sma phenotype caused by *dbl-1* null mutants is not fully epistatic to a *lon-3* null mutation argues against a model in which *lon-3* functions solely upstream of the TGFβ pathway, for example, by recruiting an inhibitor of DBL-1. A model for LON-3 as an inhibitor of DBL-1 cannot be excluded, but one in which LON-3 functions exclusively in this way is not consistent with our results: LON-3 may be an inhibitor of DBL-1 but, if so, it must also function either downstream of the pathway or in parallel.

Recent work has shown that *dbl-1* negatively regulates the transcription of *lon-1* (N. Ueno, personal communication). We have shown here that endoreduplication of hypodermal nuclei is increased in a *lon-1* mutant. Therefore, the fact that we failed to detect a significant increase in hypodermal ploidy in worms overexpressing *dbl-1* is surprising. One possible explanation for this paradox could be that even small amounts of LON-1 can prevent hyperendoreduplication and that the worms used in this study that overexpress *dbl-1* do not express high enough levels to reduce LON-1 activity sufficiently to allow extra rounds of endoreduplication to occur. Alternatively, LON-1 might be regulated in more than one way and a second, as yet unidentified, negative regulator of *lon-1* might exist that functions in parallel to *dbl-1*.

Worms that are either mutant for *dbl-1* or overexpress the gene appear to have the same number of somatic cells as wild type (Suzuki *et al.* 1999). Since *dbl-1* mutants are both shorter and thinner than wild type, it is thought that at least some cells in *dbl-1* mutants are smaller than in wild type. It has been proposed that the decrease in body size seen in mutants defective in TGFβ signaling results in part at least from a decrease in the size of the hypodermis caused in turn by reduced ploidy of hypodermal nuclei (Flemming *et al.* 2000). These observations raise the question of whether some or all cells are larger in worms that overexpress *dbl-1(+)* or that are mutant for *lon-3*. We have made estimates of the volumes of such worms on the basis of images obtained by Nomarski microscopy. Within the limits of the accuracy of this technique, neither *lon-3* mutants nor worms that overexpress *dbl-1* have volumes that are obviously greater than wild type (J. Nyström and S. Tuck, unpublished results). Both *lon-3* null mutants and worms that overexpress *dbl-1* are longer than wild type but also thinner. Since the nuclei in the hypodermis of these worms are not markedly hyperendoreduplicated, our results are consistent with a model in which final body size is determined to a significant extent by the extent of endoreduplication. It will be interesting in the future to test this model rigorously by making accurate measurements of the size of the hypodermis, for example, by

electron microscopy. Such measurements would make it possible to determine, first, whether body size is mostly correlated with the size of the hypodermis and, second, whether the size of the hypodermis is determined largely by the degree of endoreduplication of hypodermal nuclei.

A total of 33 genes that can mutate to give rise to alleles conferring Dpy or Sqt phenotypes have been identified in *C. elegans*. Seven of these (*dpy-2*, *dpy-7*, *dpy-10*, *dpy-13*, *rol-6*, *sqt-1*, and *sqt-3*) have been shown to encode cuticle collagens (Kramer *et al.* 1988, 1990; Bird 1992; Johnstone *et al.* 1992; Levy *et al.* 1993; van der Keyl *et al.* 1994). At least 6 of these (*dpy-2*, *dpy-7*, *dpy-10*, *dpy-13*, *sqt-1*, and *rol-6*) do not cause a Lon phenotype when present in multiple copies (Johnstone *et al.* 1992; Yang and Kramer 1994; Gilleard *et al.* 1997; J. Nyström and S. Tuck, unpublished results). (The effect of overexpressing *sqt-3* has not been reported.) Thus while these genes may in some cases be required for wild-type body morphology, they do not appear to be able to regulate body length. The *C. elegans* genome is predicted to encode 150 cuticle collagens. However, the loss-of-function phenotype of the majority of these genes is unlikely to be either Lon or Dpy since screens for mutations conferring these phenotypes appear to be approaching saturation. Multiple alleles of all but two of the *dpy* genes (*dpy-24* and *dpy-25*) have been isolated. In the screen reported here for new Lon mutations, multiple alleles of *lon-1*, *lon-2*, and *lon-3* were isolated, together with rare alleles of four other genes, *lon-4*, *lon-5*, *lon-6*, and *lon-7* (Z.-Z. Shen and A. Leroi, unpublished results). In addition, RNAi surveys have yielded just four loci that give a Lon phenotype out of the ~4500 currently tested (Fraser *et al.* 2000; Maeda *et al.* 2001); none appear to be collagens. Just two collagen genes, C31H2.2 and F38B6.5, were found to give rise to a Dpy phenotype (Maeda *et al.* 2001). C31H2.2 maps very close to *dpy-8* and may therefore correspond to this gene.

Thus while we cannot exclude the possibility that other *C. elegans* cuticle collagens can, like *lon-3*, cause reciprocal changes in body length when over- or underexpressed, it is unlikely that many have this property. While the *C. elegans* cuticle is somewhat unusual in that it contains so many collagens (Cox *et al.* 1984; Kusch and Edgar 1986; Kramer 1994), it nevertheless serves as a useful model for the control of morphology (Johnstone 2000). Many structures within more complicated organisms are surrounded by collagenous membranes. Perhaps some collagens in these membranes might not simply have a passive role in allowing the membrane to adopt the appropriate shape but may instead actively determine the shape of the structures they surround. It will be interesting in the future to determine how changes in the expression of just one collagen gene in *C. elegans* can have such pronounced effects on body length.

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LITERATURE CITED

Bird, D. M., 1992 Sequence comparison of the *Caenorhabditis elegans dpy-13* and *col-34* genes, and their deduced collagen products. Gene **120:** 261–266.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Clark, D. V., D. S. Suleman, K. A. Beckenbach, E. J. Gilchrist and D. L. Baillie, 1995 Molecular cloning and characterization of the *dpy-20* gene of *Caenorhabditis elegans*. Mol. Gen. Genet. **247:** 367–378.

Conlon, I., and M. Raff, 1999 Size control in animal development. Cell **96:** 235–244.

Cox, G. N., J. S. Laufer, M. Kusch and R. S. Edgar, 1980 Genetic and phenotypic characterizations of roller mutants of *Caenorhabditis elegans*. Genetics **95:** 317–339.

Cox, G. N., J. M. Kramer and D. Hirsh, 1984 Number and organization of collagen genes in *Caenorhabditis elegans*. Mol. Cell. Biol. **4:** 2389–2395.

Cox, G. N., M. Kusch, K. DeNevi and R. S. Edgar, 1981a Temporal regulation of cuticle synthesis during development of *Caenorhabditis elegans*. Dev. Biol. **84:** 277–285.

Cox, G. N., M. Kusch and R. S. Edgar, 1981b Cuticle of *Caenorhabditis elegans*: its isolation and partial characterization. J. Cell Biol. **90:** 7–17.

Cox, G. N., S. Staprans and R. S. Edgar, 1981c The cuticle of *Caenorhabditis elegans*. II. Stage-specific changes in ultrastructure and protein composition during postembryonic development. Dev. Biol. **86:** 456–470.

Dibb, N. J., I. N. Maruyama, M. Krause and J. Karn, 1989 Sequence analysis of the complete *Caenorhabditis elegans* myosin heavy chain gene family. J. Mol. Biol. **205:** 603–613.

Estevez, M., L. Attisano, J. L. Wrana, P. S. Albert, J. Massague *et al.*, 1993 The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. Nature **365:** 644–649.

Fire, A., S. W. Harrison and D. Dixon, 1990 A modular set of lacZ fusion vectors for studying gene expression in *Caenorhabditis elegans*. Gene **93:** 189–198.

Flemming, A. J., Z. Z. Shen, A. Cunha, S. W. Emmons and A. M. Leroi, 2000 Somatic polyploidization and cellular proliferation drive body size evolution in nematodes. Proc. Natl. Acad. Sci. USA **97:** 5285–5290.

Fraser, A. G., R. S. Kamath, P. Zipperlen, M. Martinez-Campos, M. Sohrmann *et al.*, 2000 Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. Nature **408:** 325–330.

Gilleard, J. S., J. D. Barry and I. L. Johnstone, 1997 cis regulatory requirements for hypodermal cell-specific expression of the *Caenorhabditis elegans* cuticle collagen gene *dpy-7*. Mol. Cell. Biol. **17:** 2301–2311.

Hedgecock, E. M., and J. G. White, 1985 Polyploid tissues in the nematode *Caenorhabditis elegans*. Dev. Biol. **107:** 128–133.

Hedgecock, E. M., J. G. Culotti, D. H. Hall and B. D. Stern, 1987 Genetics of cell and axon migrations in *Caenorhabditis elegans*. Development **100:** 365–382.

Herman, R. K., 1995 Mosaic analysis, 123–146 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Hill, K. L., B. D. Harfe, C. A. Dobbins and S. W. L'Hernault,

2000 *dpy-18* encodes an alpha-subunit of prolyl-4-hydroxylase in *Caenorhabditis elegans*. Genetics **155:** 1139–1148.

Hodgkin, J. A., H. R. Horvitz and S. Brenner, 1979 Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

Honigberg, L., and C. Kenyon, 2000 Establishment of left/right asymmetry in neuroblast migration by UNC-40/DCC, UNC-73/Trio and DPY-19 proteins in *C. elegans*. Development **127:** 4655–4668.

Hunter, C. P., and W. B. Wood, 1992 Evidence from mosaic analysis of the masculinizing gene *her-1* for cell interactions in *C. elegans* sex determination. Nature **355:** 551–555.

Johnstone, I. L., 2000 Cuticle collagen genes. Expression in *Caenorhabditis elegans*. Trends Genet. **16:** 21–27.

Johnstone, I. L., and J. D. Barry, 1996 Temporal reiteration of a precise gene expression pattern during nematode development. EMBO J. **15:** 3633–3639.

Johnstone, I. L., Y. Shafi and J. D. Barry, 1992 Molecular analysis of mutations in the *Caenorhabditis elegans* collagen gene *dpy-7*. EMBO J. **11:** 3857–3863.

Kramer, J. M., 1994 Structures and functions of collagens in *Caenorhabditis elegans*. FASEB J. **8:** 329–336.

Kramer, J. M., 1997 Extracellular matrix, pp. 471–500 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kramer, J. M., and J. J. Johnson, 1993 Analysis of mutations in the *sqt-1* and *rol-6* collagen genes of *Caenorhabditis elegans*. Genetics **135:** 1035–1045.

Kramer, J. M., J. J. Johnson, R. S. Edgar, C. Basch and S. Roberts, 1988 The *sqt-1* gene of *C. elegans* encodes a collagen critical for organismal morphogenesis. Cell **55:** 555–565.

Kramer, J. M., R. P. French, E. C. Park and J. J. Johnson, 1990 The *Caenorhabditis elegans rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encodes a collagen. Mol. Cell. Biol. **10:** 2081–2089.

Krishna, S., L. L. Maduzia and R. W. Padgett, 1999 Specificity of TGFbeta signaling is conferred by distinct type I receptors and their associated SMAD proteins in *Caenorhabditis elegans*. Development **126:** 251–260.

Kusch, M., and R. S. Edgar, 1986 Genetic studies of unusual loci that affect body shape of the nematode *Caenorhabditis elegans* and may code for cuticle structural proteins. Genetics **113:** 621–639.

Levy, A. D., J. Yang and J. M. Kramer, 1993 Molecular and genetic analyses of the Caenorhabditis elegans *dpy-2* and *dpy-10* collagen genes: A variety of molecular alterations affect organismal morphology. Mol. Biol. Cell **4:** 803–817.

Maeda, I., Y. Kohara, M. Yamamoto and A. Sugimoto, 2001 Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. Curr. Biol. **11:** 171–176.

Maruyama, I. N., D. M. Miller and S. Brenner, 1989 Myosin heavy chain gene amplification as a suppressor mutation in *Caenorhabditis elegans*. Mol. Gen. Genet. **219:** 113–118.

Meyer, B. J., 2000 Sex in the worm-counting and compensating X-chromosome dose. Trends Genet. **16:** 247–253.

Mochii, M., S. Yoshida, K. Morita, Y. Kohara and N. Ueno, 1999 Identification of transforming growth factor-beta-regulated genes in *Caenorhabditis elegans* by differential hybridization of arrayed cDNAs. Proc. Natl. Acad. Sci. USA **96:** 15020–15025.

Morita, K., K. L. Chow and N. Ueno, 1999 Regulation of body length and male tail ray pattern formation of *Caenorhabditis elegans* by a member of TGF-beta family. Development **126:** 1337–1347.

Nurse, P., 1985 The genetic control of cell volume, pp. 185–196 in *The Evolution of Genome Size*, edited by T. Cavalier-Smith. John Wiley & Sons, Chichester, UK.

Park, E. C., and H. R. Horvitz, 1986 Mutations with dominant effects on the behavior and morphology of the nematode *Caenorhabditis elegans*. Genetics **113:** 821–852.

Peixoto, C. A., and W. De Souza, 1992 Cytochemical characterization of the cuticle of *Caenorhabditis elegans* (Nematoda: Rhabditoidea). J. Submicrosc. Cytol. Pathol. **24:** 425–435.

Ren, P., C. S. Lim, R. Johnsen, P. S. Albert, D. Pilgrim *et al.*, 1996 Control of *C. elegans* larval development by neuronal expression of a TGF-beta homolog. Science **274:** 1389–1391.

Riddle, D. L., and S. Brenner, 1978 Indirect suppression in *Caenorhabditis elegans*. Genetics **89:** 299–314.

Riddle, D. L., T. Blumenthal, B. J. Meyer and J. R. Priess, 1997 *C. elegans II*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Savage, C., P. Das, A. L. Finelli, S. R. Townsend, C. Y. Sun *et al.*, 1996 *Caenorhabditis elegans* genes *sma-2*, *sma-3*, and *sma-4* define a conserved family of transforming growth factor beta pathway components. Proc. Natl. Acad. Sci. USA **93:** 790–794.

Shim, J., and J. Lee, 2000 Molecular genetic analysis of *apm-2* and *aps-2*, genes encoding the medium and small chains of the AP-2 clathrin-associated protein complex in the nematode Caenorhabditis elegans. Mol. Cell **10:** 309–316.

Sulston, J., and H. R. Horvitz, 1977 Postembryonic lineages of the nematode *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

Suzuki, Y., M. D. Yandell, P. J. Roy, S. Krishna, C. Savage-Dunn *et al.*, 1999 A BMP homolog acts as a dose-dependent regulator of body size and male tail patterning in *Caenorhabditis elegans*. Development **126:** 241–250.

Tuck, S., and I. Greenwald, 1995 *lin-25*, a gene required for vulval induction in *Caenorhabditis elegans*. Genes Dev. **9:** 341–357.

van der Keyl, H., H. Kim, R. Espey, C. V. Oke and M. K. Edwards, 1994 *Caenorhabditis elegans sqt-3* mutants have mutations in the *col-1* collagen gene. Dev. Dyn. **201:** 86–94.

van der Rest, M., and R. Garrone, 1991 Collagen family of proteins. FASEB J. **5:** 2814–2823.

Vogel, K. G., M. Paulsson and D. Heinegård, 1984 Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. Biochem. J. **223:** 587–597.

von Mende, N., D. M. Bird, P. S. Albert and D. L. Riddle, 1988 *dpy-13*: a nematode collagen gene that affects body shape. Cell **55:** 567–576.

Yamaguchi, Y., D. M. Mann and E. Ruoslahti, 1990 Negative regulation of transforming growth factor-beta by the proteoglycan decorin. Nature **346:** 281–284.

Yang, J., and J. M. Kramer, 1994 In vitro mutagenesis of *Caenorhabditis elegans* cuticle collagens identifies a potential subtilisin-like protease cleavage site and demonstrates that carboxyl domain disulfide bonding is required for normal function but not assembly. Mol. Cell. Biol. **14:** 2722–2730.

Yang, J., and J. M. Kramer, 1999 Proteolytic processing of *Caenorhabditis elegans* SQT-1 cuticle collagen is inhibited in right roller mutants whereas cross-linking is inhibited in left roller mutants. J. Biol. Chem. **274:** 32744–32749.

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