Regulation of Fertility, Survival, and Cuticle Collagen Function by the Caenorhabditis elegans eaf-1 and ell-1 Genes

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EAF2, an androgen-regulated protein, interacts with members of the ELL (eleven-nineteen lysine-rich leukemia) transscription factor family and also acts as a tumor suppressor. Although these proteins control transcriptional elongation and perhaps modulate the effects of other transcription factors, the mechanisms of their actions remain largely unknown. To gain new insights into the biology of the EAF2 and ELL family proteins, we used Caenorhabditis elegans as a model to explore the in vivo roles of their worm orthologs. Through the use of transgenic worms, RNAi, and an eaf-1 mutant, we found that both genes are expressed in multiple cell types throughout the worm life cycle and that they play important roles in fertility, survival, and body size regulation. ELL-1 and EAF-1 likely contribute to these activities in part through modulating cuticle synthesis, given that we observed a disrupted cuticle structure in ell-1 RNAi-treated or eaf-1 mutant worms. Consistent with disruption of cuticle structure, loss of either ELL-1 or EAF-1 suppressed the rol phenotype of specific collagen mutants, possibly through the control of dpy-3, dpy-13, and sqt-3 collagen gene expression. Furthermore, we also noted the regulation of collagen expression by ELL overexpression in PC3 human prostate cancer cells. Together, these results reveal important roles for the eaf-1 and ell-1 genes in the regulation of extracellular matrix components.

Androgens play a key role in prostate development, prostate cancer, and benign prostatic hyperplasia. Thus, identification and characterization of androgen-responsive genes could significantly contribute to the prevention and treatment of prostate cancer and benign prostatic hyperplasia. One such androgen-responsive gene is EAF2 (ELL-associated factor 2), which may serve as a tumor suppressor (1). The EAF2 locus exhibits frequent allelic loss in ~80% of advanced clinical prostate cancer specimens, and evidence for homozgyous deletion also exists (1). EAF2 deficiency in mice leads to carcinogenesis in multiple tissues (2) as well as aspermatogenesis and reduced survival (3). In addition to EAF2, mammals also express EAF1, a protein that shares 58% identity and 74% similarity with EAF2. Both EAF proteins interact with the ELL family of transcription elongation factors, including ELL, ELL2, and ELL3 (4–9). ELL has been identified in an array of species, including yeast (10), Drosophila (11), zebrafish (12), mouse (13), and human (14). This widely expressed gene is essential for embryonic development because deletion of ELL in mouse or Drosophila causes embryonic lethality (15, 16). ELL also plays an important role in leukemia (8, 17). Chromosomal translocation can lead to fusion of ELL with the MLL (multiple lineage leukemia) gene, and the MLL-ELL fusion protein can cause acute myeloid leukemia (8, 18).

The mechanisms of EAF/ELL action appear to be complex and involve multiple signaling pathways. ELL family proteins interact with RNA polymerase II and act as a transcription elongation factor (4, 19). Eissenberg et al. (16) reported that ELL controls the transcription of some larger mRNA transcripts, such as those for the notch and ras genes. Hence, loss of ELL could lead to alterations in gene expression. Other studies demonstrated that the ELL gene might regulate cell death via the p53 pathway (20, 21). EAF2 and ELL can also physically associate with the von Hippel-Lindau (3) and hypoxia-inducible factor 1α (22) proteins, respectively. This would provide a potential mechanism for EAF2 to regulate angiogenesis.

Although the literature supports important biological roles for EAF and ELL, their specific in vivo actions remain unclear. Therefore, we explored the in vivo activities of these genes using Caenorhabditis elegans as a model due to its powerful genetics and the ease of manipulating gene expression via RNAi. We found that the worm orthologs of EAF and ELL, eaf-1 and ell-1, have overlapping functions in regulating worm fertility, survival, and cuticle formation. Consistently, transgenic worms express ELL-1 and EAF-1 in overlapping patterns, and the two proteins interact physically. Many of these phenotypes may be due to regulation of cuticle collagens by eaf-1 and ell-1. We also observed the regulation of collagen expression by EAF1/ELL1 in human cells. These findings point to the conserved regulation of collagen expression by EAF2 and ELL, which could provide a new mechanism for understanding prostate cancer formation.

EXPERIMENTAL PROCEDURES

Worm Strains—NR222 (rde-1(ne219); kls9[pKK1260 (lin-12p::nls::GFP) + pKK1253 (lin-26p::rde-1) + pRF4 (rol-6 (su1006))], WM118 ((rde-1(ne300) nls9 (myo-3::HA::RDE-1 + pRF4 (rol-6(su1006)))), CB91 (rol-1(e91) II), CB187 (rol-
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6(e187 II), BE98 (rol-8(sc98) II), BE148 (rol-9(sc148) V), BE13 (sqt-1(sc13) II), BE8 (sqt-3(sc8) V), CB4123 (lon-3(e2175) V), and RB1671 (lon-3(ok2076) V) were obtained from the C. elegans Genetics Center. ALF50 (eaf-1(tm3976)) was provided by Dr. Shohei Mitani through the National BioResource Project for the nematode and out-crossed three times with the wild-type N2 strain. OLB11 (rode-1(ne219);pOLB11(elt-2p:rode-1) + pRF4(rol-6(sc1006))), an intestine-specific RNAi strain (23), was a gift from Dr. James D. McGhee (Department of Medical Genetics, University of Calgary, Calgary, Alberta, Canada) and Dr. Olaf Bossinger (Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Aachen, Germany).

ALF51 (eaf-1(tm3976); rol-6(e187)) was generated via standard crosses. The rol-6 gene was genotyped using ZraI restriction fragment length polymorphism, and the eaf-1 gene was genotyped using allele-specific PCR primers. Oligonucleotide sequences are provided in supplemental Table 2.

RNAi Feeding Clones—The elt-1 RNAi clone was ordered from Open Biosystems (Huntsville, AL). Clones for dpy-3, dpy-13, and sqt-3 were retrieved from the Ahinger RNAi library (24). All RNAi clones were validated by DNA sequencing.

Plasmid Construction, Co-immunoprecipitation, and Western Blotting—The detailed procedures were described previously (22). Using RT-PCR, C. elegans ell-1 and eaf-1 ORF regions were amplified and cloned into pEGFP-C1 and pCMV-Myc vectors. Human T293 cells were transiently transfected with 5 μg each of vectors expressing EGFP-ell-1,4 Myc-eaf-1, or a combination of Cell extracts were incubated with anti-GFP antibody beads (clone ROQ2, code D153-8, MBL International), followed by washing with radioimmune precipitation assay buffer. Proteins were then separated by PAGE, and Western blotting was performed with either anti-Myc antibody (clone 9E10, Thermo Scientific) or anti-GFP antibody (Torrey Pines Biolabs).

Generation of DNA Constructs and Transgenic Animals—An elt-1 fosmid clone (WRM0635cB04) was purchased from Source BioScience (Nottingham, United Kingdom). To generate a GFP-ELL-1 fusion protein, we used homologous recombination in the SW106 bacterial strain (25) to insert the GFP sequence after the initial ATG codon of the elt-1 gene. The SW106 strain was also used to integrate the unc-119 gene onto the fosmid backbone using the pLoxP unc-119 plasmid (25).

For the eaf-1 gene, we amplified a 1455-bp nucleotide region from genomic DNA by PCR. The amplified region covers the entire C. elegans eaf-1 gene and putative promoter sequence. The eaf-1 PCR product was then inserted into pPD95.79 (a gift of Dr. Andy Fire and available at Addgene (Cambridge, MA)) to generate a C-terminal eaf-1-GFP transgene. The unc-119 gene was added to the vector backbone using punc-119c as described previously (26).

Transgenic worms were generated from the DP38 (unc-119(ed3)) strain using microparticle bombardment as described previously (27). Transgenic animals were identified via rescue of the unc-119 mutation.

RNAi Treatment—RNAi treatment was performed using the feeding method as described previously (24). Briefly, adult worms were treated with hypochloride solution. Eggs were then collected and seeded onto the RNAi plates. After several days, we observed and analyzed the phenotypes by counting the dead worms or worms with a ruptured vulva or a helically twisted body when moving (rol). For the fertility assay, we put single L2/L3 worms on individual plates and transferred these worms daily to a fresh plate until no further eggs were produced. Comparison analysis was carried out by Student’s t test. The variances of value are shown by S.E. p < 0.05 was considered a significant change.

Reverse Transcription and Real-time PCR—We used TRizol reagent to isolate total RNA from either worm tissues or human cells, and 1 μg of total RNA was subjected to reverse transcription, followed by real-time PCR as described previously (28). Primer information is provided in supplemental Table 2.

C. elegans Microscopy—We used ImageJ to quantify the intensity of col-19-GFP fluorescence in digital images captured with a ×10 objective (29). Fluorescent images for the eaf-1::eaf-1-GFP and ell-1::GFP-ell-1 transgenic worms were captured as described previously (25). The videos of worm movement were recorded using a Zeiss Stemi dissecting microscope connected with a digital camera. Each worm was video-taped for 10 s.

RESULTS

Identification of C. elegans Homologs for Human EAF and ELL Genes—We performed a BLAST search of the C. elegans genome using human EAF (EAF1 and EAF2) and ELL (ELL and ELL2) family protein sequences and identified a single C. elegans ortholog for each family. The worm genes Y24D9A.1 and D1007.16 (named eaf-1 and eaf-1 here) encode proteins with ~40 and ~63% similarity to the human ELL and EAF family proteins, respectively (supplemental Figs. 1 and 2). Notably, the C. elegans orthologs retain the functional domains of both the ELL and EAF2 proteins. For ELL, the N-terminal p53 inhibitory domain (positions 1–60), the middle region related to transcriptional elongation activity (positions 60–373), and the C-terminal occludin homology domain (positions 521–616) are all present in C. elegans ELL-1 (supplemental Fig. 1) (20). For EAF2, the transactivation domain (positions 168–251) and the domain (positions 68–113) required for interaction with ELL and essential for apoptosis and growth suppression are present in C. elegans EAF-1 (supplemental Fig. 2) (30).

Mammalian ELL and EAF proteins form a complex in vivo, so we tested C. elegans ELL-1 and EAF-1 interaction via co-immunoprecipitation. EGGF-ell-1 and Myc-eaf-1 expression constructs were transfected into T293 cells. EGGF-ELL-1 was pulled down by anti-GFP antibody beads and then subjected to PAGE and blotted with anti-Myc antibody or anti-GFP antibody. We found that C. elegans ELL-1 and EAF-1 proteins tightly bound to each other (Fig. 1), similar to their mammalian counterparts.

eaf-1 and ell-1 Are Widely Expressed in Overlapping Patterns—We studied the pattern of eaf-1 and ell-1 gene expression using transgenic worms. An ell-1::GFP-ell-1 transgene was generated by inserting GFP into a fosmid carrying the genomic DNA for

4The abbreviation used is: EGFP, enhanced GFP.
the entire ell-1 gene. Because no fosmid clone contains the eaf-1 gene, we cloned a 1.5-kb genomic DNA PCR fragment that includes the C. elegans eaf-1 gene and promoter in front of GFP to generate an eaf-1-GFP transgene. The transgenic worms expressed GFP in all developmental stages and in the nuclei of multiple cell types in the hypodermis, pharynx, intestine, tail, and vulva (Fig. 2 and supplemental Fig. 3). The overlap in ELL-1 and EAF-1 expression patterns suggests that the proteins work together in the nucleus to control downstream gene expression in C. elegans.

Worms Require eaf-1 and ell-1 for Normal Development and Longevity—We then studied ell-1 function by knocking down the gene through RNAi feeding. Larval worms treated with ell-1 RNAi grew in a manner similar to control worms, albeit at a somewhat slower rate (data not shown). However, adult ell-1 RNAi-treated worms displayed a slightly dumpy phenotype and often had a rupture of the intestines through the vulva (Fig. 3, A, F, and G). Moreover, about half of the worms treated with ell-1 RNAi died by day 4 of treatment (Fig. 3B), suggesting a critical role for the ell-1 gene in worm development and normal longevity. The abnormal vulval structure and subsequent rupturing phenotype likely contributed to the worm death.

We also found that ell-1 RNAi significantly reduced the number of progeny, as ell-1 RNAi-treated worms laid half as many eggs as control RNAi-treated worms (Fig. 3C). Additionally, ell-1 RNAi treatment of the rol-3 strain, which has enhanced sensitivity to RNAi, resulted in a more significant reduction in fertility (supplemental Fig. 4) as well as in survival and body size (data not shown).

In mammals, EAF2 interacts both physically and functionally with ELL. The overlapping expression patterns of EAF-1 and ELL-1, as well as the physical interaction between two proteins, suggest that EAF-1 and ELL-1 may also function as a complex in the worm. To evaluate this possibility, we used a recently identified C. elegans eaf-1 knock-out allele, eaf-1(tm3976), which has a 257-bp deletion in the second exon of eaf-1 and an altered reading frame. We found that eaf-1(tm3976) has a similar phenotype as ell-1 RNAi in terms of fertility, mortality, and vulval morphology, consistent with eaf-1 and ell-1 acting together in the same pathways (see Fig. 6). RNAi knockdown of eaf-1 also produced similar, although weaker, phenotypes (data not shown).

The mechanisms leading to the reduction in fertility are not clear, as we did not detect any obvious changes in the germ line structure using an H2B-GFP transgene expressed in the germ line (data not shown). Also, the eggs from eaf-1(tm3976) mutants had a normal cobblestone shape, in contrast to the dark granular unfertilized eggs produced by TJ1060 (spe-9[hc88]; fer-15[b26]) worms raised at 25 °C (supplemental Fig. 5). The TJ1060 mutants are defective in spermatogenesis and lay unfertilized eggs at 25 °C, so our findings suggest that the reduced fertility of eaf-1(tm3976) is not due to defects in spermatogenesis.

**Genes eaf-1 and ell-1 Are Required for C. elegans Cuticle Formation and Function**—Collagens regulate worm body size and structure, and mutations in cuticle collagen genes can cause the dumpy phenotype, with worms exhibiting a shorter body size (31, 32). In addition to the dumpy phenotype, collagen gene mutations can also lead to blister and roller phenotypes, in which worms display a blistered cuticle and twisted shape, respectively (31). To evaluate if the mildly dumpy phenotype produced by ell-1 and eaf-1 knockdown results from altered collagen function, we used rol-6 mutants, which move in a rolling fashion due to a helically twisted cuticle structure produced by a point mutation in the rol-6 cuticular collagen (33). Strikingly, the eaf-1 RNAi-treated rol-6 mutants no longer displayed a rol phenotype (Fig. 4). The rol phenotype of rol-6 mutants depends on the expression of multiple collagens, suggesting that eaf-1 RNAi may have suppressed the rol phenotype by modulating the expression, processing, or secretion of one or more collagen genes (33–35). Thus, the possibility exists that eaf-1 RNAi alters the phenotype of some but not all other roller mutants, depending on which collagen genes the eaf-1 RNAi targets. Indeed, eaf-1 RNAi suppressed the roller phenotype of the rol-6, sqt-3, and sqt-1 mutants but did not affect the roller phenotype of the rol-1, rol-8, and rol-9 mutants (Fig. 4 and supplemental Videos 1–4). The same phenomena were observed after continuous RNAi treatment for several generations (data not shown). Furthermore, eaf-1 RNAi treatment of the lon-3 mutants, which show a longer body length due to the loss of the lon-3 collagen gene, resulted in a shortening of worm body length (Fig. 5). The increased length of the lon-3 mutants also depends on the function of multiple collagen genes (36). Together, these results suggest a role for eaf-1 in regulating cuticle collagen function at either the gene or protein expression level.

The C. elegans cuticle is produced by hypodermal cells (31, 32), and thus, the hypodermal localization of ELL-1 and EAF-1 (Fig. 2) suggests that they could act in a cell-autonomous manner to control the production of cuticular collagen. To address this hypothesis, we tested three rol mutant strains that respond to RNAi in only a single tissue: the hypodermis for the NR222 strain, muscle for the WM118 strain, and the intestine for the OLB11 strain (23, 37, 38). These C. elegans strains all have a mutation in the rde-1 gene that renders them resistant to RNAi. Tissue-specific RNAi sensitivity is achieved with the use of an rde-1 RNAi target. Indeed, eaf-1 RNAi suppressed the roller phenotype in only NR222, which responds to RNAi in the hypodermis (Fig. 4). This implicates the cell-autonomous action of eaf-1 in hypodermal cells.

To study the effect of eaf-1 on collagen genes, we generated a rol-6; eaf-1(tm3976) double mutant by crossing. Remarkably,
**FIGURE 3.** *ell-1* RNAi affects *C. elegans* survival, fertility, and body shape. Eggs from N2 adult worms were seeded on RNAi plates, and after 4 and 6 days, the treated worms were scored for a ruptured vulva phenotype or death. *ell-1* RNAi treatment resulted in a ruptured vulva (A, F, and G), death (B), and reduced fertility (C) compared with control RNAi treatment. D and E, control RNAi-treated worms. F and G, *ell-1* RNAi-treated worms. Scale bars = 1 mm (D and F) and 100 μm (E and G), respectively. The arrowhead in G points to protrusion through the vulva. *****, p < 0.001.

**FIGURE 2.** Overlapping expression of *ell-1* and *eaf-1* genes in *C. elegans*. GFP-*ell-1* and *eaf-1*-GFP fusion genes were generated and used to create transgenic worms. GFP expression was visualized by fluorescent microscopy. A, E, and I, GFP expression in *ell-1::GFP-ell-1* adult transgenic worms was located in the pharynx, hypodermis, intestine, and tail neurons. C, G, and K, GFP in *eaf-1::eaf-1-GFP* transgenic worms overlapped with *ell-1* and was localized in the hypodermis, vulva, and tail. B, F, J, D, H, and L are the corresponding Nomarski images. Arrowheads highlight the GFP signals in the hypodermis (Hyp), tail neurons (T), vulva (V), and intestinal cells (I). Scale bar = 100 μm.
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...suppressed the roller phenotype in the rol-6, eaf-1 double mutants even more strongly than the ell-1 RNAi (Fig. 6). We checked ∼3000 F1 progeny of the rol-6:eaf-1(tm3976) double mutant worms and found only 10 rollers that became non-rollers in the next generation. Moreover, these rol-6:eaf-1(tm3976) worms also had reduced fertility and increased mortality (Fig. 6), which are similar phenotypes resulting from ell-1 RNAi treatment. To validate the specificity of this knock-out, we performed rescue experiments in the rol-6:eaf-1(tm3976) mutants using a transgene of C. elegans eaf-1. We found that the eaf-1-GFP transgene completely corrected all of the eaf-1(tm3976) phenotypes and turned the rol-6:eaf-1(tm3976) mutants into rol-6-like rollers with normal fertility, mortality, and vulval structure (supplemental Fig. 6). The above observations suggest that ELL-1 and EAF-1 likely work together as a complex.

Our findings that ell-1 and eaf-1 altered body size and vulval structure and modified phenotypes produced by mutations of cuticle collagens genes, such as lon and rol, suggest that ell-1 and eaf-1 affect the synthesis or structure of the cuticle. To test this hypothesis, we examined the cuticle structure using the TP12 transgenic worm line, which expresses the cuticle collagen col-19 gene fused to GFP. This results in COL-19-GFP being incorporated into the collagen structure during cuticle formation (40). Both ell-1 RNAi treatment and eaf-1(tm3976) dramatically decreased GFP expression in the cuticle (Fig. 7), indicating that ell-1 and eaf-1 directly or indirectly control col-19 expression. To view the cuticle structure in more detail, we used a high-power objective and adjusted the camera exposure time to produce similar fluorescence levels in the cuticles of both ell-1 RNAi-treated and eaf-1(tm3976) mutant worms (Fig. 7). These results suggest that both ell-1 and eaf-1 are involved in cuticle formation and that the changes in cuticle structure may unite multiple ell-1 and eaf-1 phenotypes.

Regulation of Cuticle Collagen Gene Expression by eaf-1 and ell-1 Genes—It appears that multiple cuticle collagen genes contribute to cuticle structure and rol phenotypes; thus, we tested if ell-1 and eaf-1 affect expression of a collection of collagen genes. We carried out two pilot microarray assays to compare gene expression patterns between ell-1 RNAi-treated versus control RNAi-treated worms, as well as eaf-1(tm3976) versus wild-type N2 worms. We found that, in both microarrays, multiple collagen genes were down-regulated in both ell-1 RNAi-treated and eaf-1(tm3976) worms (data not shown). To determine whether these collagen genes could mediate the effects of ell-1 and eaf-1 on cuticle formation, a total of 29 collagen genes (supplemental Table 1) were tested via RNAi; dpy-3, dpy-13, and sqrt-3 suppressed the rol-6 roller phenotype when inhibited by RNAi (Fig. 8). Additionally, real-time PCR analysis confirmed the down-regulation of dpy-3, dpy-13, and sqrt-3 in both ell-1 RNAi-treated and eaf-1(tm3976) worms (Fig. 8). Taken together, these observations suggest a role for ell-1 and eaf-1 in the regulation of at least these specific collagen genes.

To examine whether EAF2/ELL family proteins also regulate collagen gene expression in mammals, we analyzed collagen gene expression in a PC3 prostate cancer cell line stably over-expressing the human ELL gene (22). Among several collagen genes tested, the Col3A1 gene (one of the orthologs of the C. elegans dpy-3, dpy-13, and sqrt-3 genes) was significantly up-regulated by ELL overexpression (Fig. 9). This result suggests that the regulation of collagen genes by ELL family proteins is evolutionarily conserved and may point to a role for collagen genes in prostate cancer.

DISCUSSION

Worm Model of ELL and EAF Genes—In this study, we used C. elegans as an in vivo model system to explore the biological roles of a prostate cancer-related gene, EAF2, and its binding
**eaf-1 and ell-1 in C. elegans**

An advantage of *C. elegans* is that the worms express a single EAF and ELL homolog, EAF-1 and ELL-1, in contrast to their mammalian counterparts. Moreover, in flies and mice, knock-out of *ell-1* results in embryonic lethality, making our knowledge of the *in vivo* roles of these genes incomplete. Thus, the viability of the *eaf-1* mutants and the ease of manipulation of *ell-1* function by RNAi represent additional and important advantages of this model. Further classical mutagenesis or RNAi library screening can be performed in this modern organism to facilitate dissecting of *eaf-1*/*ell-1* function.

**FIGURE 6.** *eaf-1(tm3976)* phenotypes share with *ell-1* RNAi. A genomic deletion of a 257-bp DNA fragment in the *eaf-1* gene resulted in similar phenotypes as *ell-1* RNAi. These included reduced fertility (A) and increased worm death (B). *eaf-1* also suppressed the roller phenotype of the rol-6(e187) mutant (C, D, E, and F). Scale bar = 0.5 mm. ***, p < 0.001.

**FIGURE 7.** *ell-1* and *eaf-1* genes regulate cuticle structure. Cuticle structure was visualized via Col-19-GFP transgenic worms, which express the Col-19-GFP transgene in the cuticle. The cuticle also developed many gaps as a result of *ell-1* RNAi treatment (D) or *eaf-1* RNAi (F) compared with the control RNAi-treated (C) or parental Col-19-GFP worms (E). D and F, images with increased exposure time (100 ms) to show the fine cuticle structure. Arrowheads in D and F point to the cuticle gaps. Scale bar = 50 μm. ***, p < 0.001.

**FIGURE 8.** Collagen genes *dpy-3*, *dpy-13*, and *sqt-3* are targets of both *ell-1* and *eaf-1*. The expression of three collagen genes was down-regulated by either *ell-1* RNAi or *eaf-1* (A and B). Moreover, knockdown of *dpy-3* (D), *dpy-13* (E), or *sqt-3* (not shown) by RNAi suppressed the rol-6 roller phenotype; both *dpy-3* (D) and *dpy-13* (E) RNAi converted rol-6 into a non-roller. *dpy-13* RNAi-treated rol-6 worms were also very short (E). C–E, rol-6 worms treated with the control RNAi, *dpy-3* RNAi, or *dpy-13* RNAi, respectively. Scale bar = 0.5 mm. **, p < 0.01; ***, p < 0.001.

**FIGURE 9.** Up-regulation of collagen gene expression by ELL in prostate cancer cells. The PC3 human prostate cancer cell line was modified to stably express human ELL. Real-time PCR showed that the modified PC3 cells contained higher levels of human ELL and Col3A1 mRNAs but not collagen Col5A1 mRNA relative to the parental PC3 cell line. **, p < 0.01.
when mutated. This cuticle phenotype was confined to the hypodermal expression of the ell-1 gene because knockdown of ell-1 via RNAi in other tissues, such as muscle and intestine, did not affect the roller phenotype. These changes alter the cuticle structure because we found that ell-1 RNAi and eaf-1(tm3976) treatment led to many cavities in the cuticle, as indicated by a COL-19-GFP fusion protein, as well as to lower overall GFP fluorescence, confirming the regulation of collagen expression by ell-1 and eaf-1.

Possible Roles of Collagen in Prostate Cancer—We found both eaf-1 and ell-1 are associated with the regulation of collagen expression during cuticle morphogenesis. This finding has significant implications because collagens are major components of the extracellular matrix, essential in organogenesis, tissue homeostasis, and carcinogenesis. In fact, a recent study showed down-regulation of many collagen genes in human prostate cancer specimens (42), arguing for a potential role for the EAF/EFL/collagen axis in prostate cancer progression. We have also validated the regulation of collagen gene expression by EFL, implicating the regulation of the extracellular matrix in the prostate by the EAF2/EFL signaling pathway.

In summary, we have reported the identification and characterization of C. elegans homologs of mammalian ELL and EAF family proteins, ELL-1 and EAF-1. Inactivation of ell-1 or eaf-1 resulted in virtually identical phenotypes in C. elegans, reduced fertility and survival rate, as well as suppressing of several roller mutants, suggesting that ELL-1 and EAF-1 form a complex and that such regulation also occurs in mammalian cells, suggesting that a major conserved mechanism of EAF/ELL action is mediated through regulating collagen expression.

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2. Xiao, W., Zhang, Q., Jiang, F., Pins, M., Kozlowski, J. M., and Wang, Z. (2003) Cancer Res. 63, 4698–4704
3. Xiao, W., Zhang, Q., Habermacher, G., Yang, X., Zhang, A. Y., Cai, X., Hahn, J., Liu, J., Pins, M., Doglio, L., Dhir, R., Gingrich, J., and Wang, Z. (2008) Oncogene 27, 1536–1544
4. Xiao, W., Ai, J., Habermacher, G., Volpert, O., Yang, X., Zhang, A. Y., Hahn, J., Cai, X., and Wang, Z. (2009) Cancer Res. 69, 2599–2606
5. Miller, T., Williams, K., Johnstone, R. W., and Shilatifard, A. (2000) J. Biol. Chem. 275, 32502–32506
6. Kong, S. E., Banks, C. A., Shilatifard, A., Conaway, J. W., and Conaway, R. C. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 10094–10098
7. Simone, F., Polak, P. E., Kozlowski, J. M., and Wang, Z. (2003) Blood 101, 2355–2362
8. Simone, F., Luo, R. T., Polak, P. E., Kozlowski, J. M., and Wang, Z. (2003) Blood 101, 2355–2362
9. Simone, F., Habermacher, G., Volpert, O., Yang, X., Zhang, A. Y., Hahn, J., Cai, X., and Wang, Z. (2009) Cancer Res. 69, 2599–2606
10. Miller, T., Williams, K., Johnstone, R. W., and Shilatifard, A. (2000) J. Biol. Chem. 275, 32502–32506
11. Wang, Z. (2003) Cancer Res. 63, 4698–4704
12. Xiao, W., Zhang, Q., Habermacher, G., Yang, X., Zhang, A. Y., Cai, X., Hahn, J., Liu, J., Pins, M., Doglio, L., Dhir, R., Gingrich, J., and Wang, Z. (2008) Oncogene 27, 1536–1544
13. Simone, F., Polak, P. E., Kozlowski, J. M., and Wang, Z. (2003) Blood 101, 2355–2362
14. Simone, F., Luo, R. T., Polak, P. E., Kozlowski, J. M., and Wang, Z. (2003) Blood 101, 2355–2362
15. Simone, F., Habermacher, G., Volpert, O., Yang, X., Zhang, A. Y., Hahn, J., Cai, X., and Wang, Z. (2009) Cancer Res. 69, 2599–2606
16. Miller, T., Williams, K., Johnstone, R. W., and Shilatifard, A. (2000) J. Biol. Chem. 275, 32502–32506
17. Xiao, W., Zhang, Q., Jiang, F., Pins, M., Kozlowski, J. M., and Wang, Z. (2003) Cancer Res. 63, 4698–4704
18. Xiao, W., Zhang, Q., Habermacher, G., Yang, X., Zhang, A. Y., Cai, X., Hahn, J., Liu, J., Pins, M., Doglio, L., Dhir, R., Gingrich, J., and Wang, Z. (2008) Oncogene 27, 1536–1544
19. Xiao, W., Ai, J., Habermacher, G., Volpert, O., Yang, X., Zhang, A. Y., Hahn, J., Cai, X., and Wang, Z. (2009) Cancer Res. 69, 2599–2606
20. Miller, T., Williams, K., Johnstone, R. W., and Shilatifard, A. (2000) J. Biol. Chem. 275, 32502–32506
21. Wang, Z. (2003) Cancer Res. 63, 4698–4704