An Essential Role in Molting and Morphogenesis of Caenorhabditis elegans for ACN-1, a Novel Member of the Angiotensin-converting Enzyme Family That Lacks a Metallopeptidase Active Site

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Genome sequence analyses predict many proteins that are structurally related to proteases but lack catalytic residues, thus making functional assignment difficult. We show that one of these proteins (ACN-1), a unique multi-domain angiotensin-converting enzyme (ACE)-like protein from Caenorhabditis elegans, is essential for larval development and adult morphogenesis. Green fluorescent protein-tagged ACN-1 is expressed in hypodermal cells, the developing vulva, and the ray papillae of the male tail. The hypodermal expression of acn-1 appears to be controlled by nhr-23 and nhr-25, two nuclear hormone receptors known to regulate molting in C. elegans. acn-1 (RNAi) causes arrest of larval development because of a molting defect, a protruding vulva in adult hermaphrodites, severely disrupted alae, and an incomplete seam syncytium. Adult males also have multiple tail defects. The failure of the larval seam cells to undergo normal cell fusion is the likely reason for the severe disruption of the adult alae. We propose that alteration of the ancestral ACE during evolution, by loss of the metallopeptidase active site and yet having acquired important roles in development (3). Surprisingly, a significant proportion (12–25%) of the total number of the predicted protease-like genes in the human, mouse, D. melanogaster, A. gambiae, and C. elegans genomes encode for proteins that lack one or more catalytic residues and are therefore classified as “non-peptidase” family members. Some of these genes may be nonfunctional pseudogenes, but many have probably lost the catalytic activity of an ancestral protein while acquiring new functions. These non-peptidase proteins are structurally related to proteases distributed across all classes, but homologues of metallopeptidases are particularly well represented. One such protein is UNC-71, a C. elegans member of the ADAMs family, which lacks a zinc binding site and yet has acquired important roles in development (3).

Our attention has recently been drawn to the fact that several invertebrate members of the angiotensin-converting enzyme (ACE)1 (EC 3.4.15.1, peptidyl dipeptidase A) family from D. melanogaster and C. elegans lack crucial active site residues and are therefore predicted not to function as peptidases (4).

Mammalian ACE is a cell surface zinc metallopeptidase that cleaves a C-terminal dipeptide from angiotensin I to generate the vasoconstrictor, angiotensin II (for reviews see Refs. 5 and 6). Bradykinin, a vasodilator, and the hemoregulatory peptide N-acetyl-SDKP are also substrates for mammalian ACE. Somatic ACE comprises two catalytic units in tandem, which are anchored to the cell surface by a transmembrane region and a short C-terminal cytoplasmic domain. ACE knockout mice have, as expected, lower blood pressure but also have severe renal abnormalities and suffer from anemia (7, 8). In mammalian testes, a single-domain ACE (germinale ACE, 100 kDa) is expressed in developing spermatids, and the lack of this isoform results in male infertility (7). Sperm from ACE (−/−) mice appear normal but fail to migrate beyond the extramural uterotubal junction of the oviduct of mated wild-type females, and in vitro experiments demonstrate that they have reduced binding to zona pellucidae. The peptide substrate for the germinal enzyme has not been identified, and it has been suggested that the role of the gACE in reproduction might depend on direct molecular interactions at the cell surface rather than the hydrolysis of a peptide (7).

A D. melanogaster ACE homologue, known as AncE, has very...
similar enzymatic properties to those of mammalian ACE and is required for completion of embryogenesis and for spermatogenesis in adult flies (12, 13). Acer, a second D. melanogaster ACE homologue, is also catalytically active toward peptides but does not convert angiotensin I to angiotensin II (14, 15). Four other ACE-like genes (Ance-2, -3, -4, -5) are present in the D. melanogaster genome, but all four conceptual proteins are missing one or more of the active site residues required for catalysis by human ACE (16). In contrast to flies and mammals, the nematode C. elegans, has only one ACE-like gene (C42D8.5). N-terminal sequencing does not elucidate the ACE-like protein lack the N-terminal signal peptide and its functional metallopeptidase active site. Therefore, the GPI modification to the protein (18). The prediction of the signal peptide sequence and its functional metallopeptidase active site was used to predict a potential GPI modification to the protein (18).

EXPERIMENTAL PROCEDURES

Nematode Culture and Transformation—The C. elegans Bristol strain (N2) was grown at 20 °C on NGM agar (1.7% (w/v) agar, 25 mM potassium phosphate, pH 6.0, 50 mM NaCl, 2.5 μg ml⁻¹ peptone, 5 μg ml⁻¹ cholesterol, 1 mM MgCl₂, 1 mM CaCl₂) supplemented with Escherichia coli OP50.

cDNA Cloning and Sequence Analysis—cDNA clones yk1153 and yk6315, derived from a C. elegans embryonic library, and yk759d02, from an L2 stage hermaphrodite larval library, were obtained from the laboratory of Professor Y. Kohara, National Institute of Genetics, Japan. The cDNA clones were sequenced by primer walking using Taq DyeDeoxy terminator cycle sequencing and the data analyzed with respect to DNA sequence present in the C. elegans data base. BLAST® similarity searching was performed from the NCBI suite of programs (www.ncbi.nlm.nih.gov/). The prediction of the signal peptide sequence and its cleavage site was performed using the SignalP V1.1 World Wide Web Prediction Server (www.cbs.dtu.dk/services/SignalP/ (17)). The GPI Prediction Server (mendel.imp.univie.ac.at/sat/gpi/gpi_server.html) was used to predict a potential GPI modification to the protein (18).

Glycosylation sites were predicted using NetOGlyc 2.0 (www.cbs.dtu.dk/services/NetOGlyc) and NetNGlyc 1.0 (www.cbs.dtu.dk/services/NetNGlyc) prediction servers (19).

Construction and Expression of the acn-1::gfp Transgene—Green fluorescent protein (GFP) reporter genes were generated for ACN-1 using a polymerase chain reaction (PCR)-based technique (20). The entire acn-1 gene and 4.9 kb of sequence immediately upstream was PCR-amplified using Expand High Fidelity Taq (Roche Diagnostics GmbH) from the cosmid C42D8 (obtained from the Sanger Centre, Hinxton, Cambridgeshire, UK) using oligonucleotides (5′ to 3′) GAT-CACGTGATCCTTGTGTCG (C42D6, 23153–23132) and ggacctgcag-cgatcacgatcAgCAAAGCAAAATAGATAATTAAC (C42D8, 13896–13916) of sense and antisense RNAs with T3 promoter sequences. The reporter construct were examined on a Zeiss Axioplan microscope (Carl Zeiss, Germany) at an excitation wavelength of 475 nm. Images were captured using a CCD camera and Openlab 3.0.4 image capture software (Improvision Inc., Lexington, MA).

dsRNA Preparation and Delivery—Exon 5 of acn-1 was PCR-amplified (initial denaturation of 94 °C for 5 min followed by 25 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s) from cosmid C42D8 using Taq DNA polymerase (Stratagene, La Jolla, CA) and oligonucleotides (5′ to 3′) GAT-CACGTGATCCTTGTGTCG (C42D6, 23153–23132) and ggacctgcag-cgatcacgatcAgCAAAGCAAAATAGATAATTAAC (C42D8.5) PCR cycling parameters were as above with the extension time increased to 11 min. The products of this final PCR were directly used as the template for PCR amplification with Taq DNA polymerase (Stratagene, La Jolla, CA) and oligonucleotides (5′ to 3′) GAT-CACGTGATCCTTGTGTCG (C42D6, 23058–23038) and a nested 3′ primer, GGAAGAGTAATTTTCTTATGG (lowercase represents T7 promoter sequence) and aataaacctcgaaga- CACAACTATTCTTGCTGTTGATG (lowercase represents T3 promoter sequence). The resulting 524-bp PCR product was purified using QIAquick PCR purification columns (Qiagen GmbH, Hilden, Germany) and used as the template to transcribe sense and antisense RNAs with T3 transcribers using PhyScript™ in vitro transcription kits (Ambion, Austin, TX). Double-stranded RNA (dsRNA) was assembled by mixing equal amounts (5 μl) of sense and antisense RNAs followed by incubation at 68 °C for 10 min and then at 37 °C for 30 min. Annealed acn-1 dsRNA was directly injected into the syncytiotum gondai of young adult hermaphrodites (Bristol strain, N2). Injected animals were allowed to recover for 16 h at 20 °C in order to lay any eggs present in utero prior to injection and were then transferred onto fresh NGM agar plates. The resultant F1 progeny were assessed visually for changes in phenotype.

For feeding acn-1 dsRNA, a 387-bp HindIII fragment, encompassing exon 7 to exon 9, was excised from the EST yk6315 and subcloned into the corresponding site of plasmid pPD129.36 to generate plpL543. This construct was transformed into E. coli HT115(DE3), and expression of dsRNA was induced by adding 0.4 mM isopropyl-1-thio-β-D-galactopyranoside to a mid-log phase culture (22). At 4 h post-induction a further 0.4 mM isopropyl-1-thio-β-D-galactopyranoside was added to the culture, and the bacteria were then seeded onto NGM agar plates containing 1 mM isopropyl-1-thio-β-D-galactopyranoside. The C. elegans RNAi-sensitizing mutant rrf-3 (23) was allowed to feed on the dsRNA induced bacteria, and phenotypic effects were observed. Controls were carried out in parallel using HT115(DE3) lacking pL543 as the food source. The effect of RNAi on males was assessed by allowing rrf-3 mutant males and hermaphrodites to mate on NGM agar plates containing OP50 bacteria and then transferring the L1/L2 progeny to dsRNA feeding plates as described above. To assess the effect of acn-1 RNAi on seam cell adhesions junctions, the strain SU93 (24) was fed on bacteria expressing acn-1 dsRNA as described above.

Bacterial strains used to express dsRNA targeting nhr-23 and nhr-25 were obtained from Dr. J. Ahringer (University of Oxford) (22). Induction of dsRNA in these strains was as described above. To study the effect of silencing the nhr genes on acn-1 and ltl-1 expression in seam cells, six young adult hermaphrodites from acn-1::gfp and ltl-1::gfp transgenic lines (25) were transferred to individual culture plates seeded with E. coli HT115 (DE3) either expressing nhr dsRNA or carrying no plasmid. After 3 days at 20 °C, larvae were scored for the defective cuticle phenotype and seam cell fluorescence. Because of mosiacism, not all seam cells of individual animals expressed the GFP reporter. Therefore, larvae were scored even if GFP-induced fluorescence was observed in only one seam cell.

Scanning Electron Microscopy—Nematodes were fixed for 2 h in 2.5% (v/v) glutaraldehyde, 0.1 M phosphate buffer, pH 6.9, and then washed twice with 0.1 M phosphate buffer prior to overnight fixation in 1% (w/v) osmium tetroxide, 0.1 M phosphate buffer. Nematodes were washed with distilled water and then dehydrated by 1 h in an increasing concentration of acetone (20–100%). Samples were critical point dried with a Polaron E3000 critical point drying apparatus using CO₂ as the transition fluid and then mounted on 15-mm diameter pin stubs. Nematodes on stubs were coated with gold to a thickness of 50 nm using a Polaron freeze dryer sputter-coating unit and observed and imaged with a CamScan 3-30BM scanning electron microscope.

RESULTS

The Primary Structure of C. elegans ACN-1—The sequencing of the full-length EST clone yk759d02 encoding ACN-1 (C42D8.5) confirmed the open reading frame predicted for this gene by the C. elegans sequencing consortium (GenBank™ accession number 25151554) (Fig. 1). The translated protein (CE30627) is 906 amino acids long with an N-terminal signal peptide that is predicted to be removed by cleavage between amino acids 19 and 20 (VFP | QE). In comparison with human germinal ACE and Drosophila ANCE, it is evident that conserved residues (25% common identity and 46% similarity) extend along a central 600-amino acid region of ACN-1 (Fig. 1).
FIG. 1. Alignment of the amino acid sequences of D. melanogaster ACE (DrmANCE), germinal isoform of human ACE (gHumACE) and C. elegans ACN-1 (ACN-1). —, signal peptide; —, Pro/Glu-rich domain; =, conserved active site residues of DrmANCE and gHumACE; —, Cys-rich; ■, Thr/Pro-rich domain; |, potential N-glycosylation sites; †, potential O-glycosylation sites; boxed letters, predicted point of attachment for a GPI anchor.
potential sites for four extension that contains a cysteine-rich region juxtaposed to a N-terminal extension rich in Pro and Glu (24 residues across a region of 68 amino acids). There is also a C-terminal O-linked glycan sites distributed throughout the entire ACN-1 sequence and four potential N-glycosylation sites. The GPI Modification Site Prediction Server indicates that ACN-1 is probably attached to a GPI anchor at Ala-898 (18).

*acn-1* Is Expressed in Embryonic and Larval Hypodermis, in the Vulva during Organogenesis, and in the Ray Papillae of the Male Tail—The spatial and temporal expression pattern of *acn-1* was studied by generating independent lines of transgenic *C. elegans* expressing a C-terminal reporter gene fusion *(acn-1::gfp)* under the control of the 4.9-kb promoter region immediately upstream of *acn-1*. Strong reporter gene expression was first observed in the hypodermal seam cells during embryogenesis and subsequently in the seam cells of all larval stages (Fig. 2, A–D). The main hypodermal syncytium, hyp7, of larvae and young adult nematodes also displayed reporter gene activity (Fig. 2B). The expression in individual seam cells was often highly punctate (Fig. 2C). Strong GFP expression was observed in the toroid cells that form the developing vulva in the L4 larval stage (Fig. 2, D and E), and expression persisted into early adulthood but was not present in the vulva of mature hermaphrodites.

In addition to the hypodermal expression described above for hermaphrodites, intense reporter gene activity was also observed in the developing ray papillae of the L4 male tail (Fig. 2F). Young adult males exhibited weak expression, restricted to the hypodermis just anterior to the tail (not shown). No transgene expression was detected in the dauer larvae, but expression re-appeared ~12 h after dauer larvae had transferred to a fresh lawn of *E. coli*.

**acn-1(RNAi) Causes Morphological Defects in Larvae and Adults**—To investigate the physiological role of ACN-1, we used RNA interference (RNAi) to selectively silence expression of *acn-1* (26). The specificity of *acn-1(RNAi)* was confirmed by abrogation of reporter gene activity in larval hypodermal cells of the transgenic *acn-1::gfp* line. In initial experiments, dsRNA was injected into the gonads of young adult hermaphrodites, and the phenotype of F1 progeny was analyzed 16–40 h post-injection. Larvae arrested at the L2 stage (penetrance of around 85%) and were either trapped within the L1 cuticle or were carrying fragments of unshed cuticle at variable positions along the body of the nematode. This early lethality prevented the detection of any RNAi-induced phenotype in later larval stages and in adults. This was circumvented by feeding *E. coli*-expressing *acn-1* dsRNA to L1/L2 *C. elegans* larvae (A–D, F) as well as their normal appearance (*E* and *G*). A, an adult nematode almost completely encased in an L4 cuticle. The raised cuticle surrounding the anal pore (AP) and the protruding vulva (Vv) are identified. B, higher magnification image of the head of an L4 larva trapped inside the old cuticle. C, protruding vulva of an adult hermaphroditic. The unshed L4 cuticle has rippled as a result of expansion of the adult body trapped inside the larval cuticle. The pressures generated within the constricted adult might be responsible for the protruding vulva. D, severely disorganized sections of the adult alae of *acn-1(RNAi)* nematodes. E, normal alae of a wild-type adult. F, higher magnification image of an *acn-1(RNAi)* nematode showing amorphous alae and circumferential annuli ridges (double-headed arrow). G, normal appearance of alae and annuli ridges of wild-type nematodes.

![Fig. 2. Spatio/temporal expression pattern of acn-1::gfp in C. elegans. A, hypodermal (†) expression in an embryo (1.5-fold stage). B, hyp7 and seam cell expression in an L4 larva. C, punctate fluorescence (†) seen in larval seam cells. D, seam and vulva expression in a late L4 larva; E, expression observed in toroidal cells that surround the vulva invagination space (Is) of a late L4 larva. F, ray precursor cells (papillae) in the tail of a male L4 larva.](image)

![Fig. 3. Scanning electron micrographs (A–F) showing the morphological defects generated by feeding acn-1 dsRNA to L1/L2 C. elegans larvae (A–D, F) as well as their normal appearance (E and G). A, an adult nematode almost completely encased in an L4 cuticle. The raised cuticle surrounding the anal pore (AP) and the protruding vulva (Vv) are identified. B, higher magnification image of the head of an L4 larva trapped inside the old cuticle. C, protruding vulva of an adult hermaphroditic. The unshed L4 cuticle has rippled as a result of expansion of the adult body trapped inside the larval cuticle. The pressures generated within the constricted adult might be responsible for the protruding vulva. D, severely disorganized sections of the adult alae of *acn-1(RNAi)* nematodes. E, normal alae of a wild-type adult. F, higher magnification image of an *acn-1(RNAi)* nematode showing amorphous alae and circumferential annuli ridges (double-headed arrow). G, normal appearance of alae and annuli ridges of wild-type nematodes.](image)
length than wild-type nematodes, and several internal structures, particularly the pharynx, were displaced because of the constriction presumably caused by the attached L4 cuticle. The pharynx, In, distended intestine wall. C, an adult male has failed to form a fan and rays and still has the old cuticle (arrowheads) from previous molts attached to the posterior body wall. D, an adult male that has formed a fan but has failed to make a complete set of sensory rays (arrow).

The two lateral alae of adult C. elegans are formed late in the L4 stage of development from cuticle secreted from the two sets of seam cells after they have undergone cell fusion to form a continuous lateral cord running along both sides of the nematode (27, 28). The integrity and fate of the L4 seam cells was studied by feeding acn-1 dsRNA to the transgenic C. elegans strain SU93. These nematodes express a GFP-tagged adherens junction protein (AJM-1), highlighting adherens junctions and thus marking the boundaries of the hypodermal cells (24). Adult SU93 nematodes displayed the same cuticle and alae defects as described above for the rrf-3 mutant strain but with reduced penetrance (around 50%). Nematodes unaffected by acn-1(RNAi) shed the L4 cuticle during the larval/adult molt to reveal alae of normal appearance. The seam cells of the unaffected adults showed normal alae, a result that is consistent with a low-resolution expression pattern obtained independently by using an in situ hybridization protocol (33).

DISCUSSION

ACN-1 Is Required for Larval Molting, Male Tail Development, and Formation of Adult Alae—The embryonic and larval hypodermis displays strong expression of the GFP-tagged ACN-1 transgenic C. elegans, a result that is consistent with a low-resolution expression pattern obtained independently by using an in situ hybridization protocol (33). The expression is temporally regulated, as fluorescence was not detected in the hypodermis of mature adult hermaphrodites. Hypodermal cells are responsible for the synthesis and secretion of the acellular cuticle, which is shed periodically and replaced during larval development (27, 34). The acn-1 expression pattern therefore suggested that ACN-1 may have a specific role in larval molting. Indeed our results, obtained by using RNAi to specifically silence expression of acn-1, provide conclusive evidence that ACN-1 is essential for the successful replacement of the old cuticle with new cuticle. We also report the lack of acn-1:gfp expression in the hypodermis of dauer larvae, which are alternative third-stage animals that are developmentally arrested as the result of overcrowding and shortage of food (35). When the environment becomes more favorable, dauers resume development by undergoing a molt to the L4 stage. The induction of acn-1:gfp

**Fig. 4. Light micrographs of adult C. elegans following acn-1(RNAi).** A, old cuticle (arrow) that has not been shed remains attached to the nematode and appears as cuticular “horns.” The old and new cuticle appear to jointly form folds (arrowheads). B, a severely constipated nematode with a swollen intestine (*), resulting from blockage to the anal pore by unshed cuticle. P, terminal bulb of the pharynx; In, distended intestine wall. C, an adult male has failed to form a fan and rays and still has the old cuticle (arrowheads) from previous molts attached to the posterior body wall. D, an adult male that has formed a fan but has failed to make a complete set of sensory rays (arrow).
expression around 12 h after introducing dauers to a fresh food source coincides with the synthesis of the new L4 cuticle. This result confirms data generated in a recent global analysis of dauer gene expression, which identify acn-1 as one of 325 genes in which expression is induced late (8–12 h) in the dauer exit time course, just prior to the dauer/L4 molt (36). This expression is coincident with the induction of genes for collagens and collagen processing enzymes and is therefore consistent with a role for ACN-1 in the molting process.

During larval development, all seam cells, apart from H0, undergo a series of cell divisions at the beginning of each larval stage. Each division generates an anterior daughter cell, which fuses to the hyp7 syncytium, and a posterior daughter cell, which remains in the seam and is capable of further division (28). Hyp7 covers 90% of the nematode surface area beneath the cuticle and is the major site of cuticle synthesis (37). Before the L4/adult molt, the two rows of L4 seam cells fuse to form two continuous lateral cords, each of which secretes the cuticular alae that run along the lateral lines of the nematode in the cuticle and is the major site of cuticle synthesis (37). Before the L4/adult molt, the two rows of L4 seam cells fuse to form two continuous lateral cords, each of which secretes the cuticular alae that run along the lateral lines of the nematode in the cuticle and is the major site of cuticle synthesis (37). Before the L4/adult molt, the two rows of L4 seam cells fuse to form two continuous lateral cords, each of which secretes the cuticular alae that run along the lateral lines of the nematode in the cuticle and is the major site of cuticle synthesis (37). Before the L4/adult molt, the two rows of L4 seam cells fuse to form two continuous lateral cords, each of which secretes the cuticular alae that run along the lateral lines of the nematode in the cuticle and is the major site of cuticle synthesis (37). Before the L4/adult molt, the two rows of L4 seam cells fuse to form two continuous lateral cords, each of which secretes the cuticular alae that run along the lateral lines of the nematode in the cuticle and is the major site of cuticle synthesis (37).

Hypodermal cells are also involved in the formation of the male tail (38). Nine pairs of sensory rays embedded in the cuticular fan of the male are generated from the posterior seam cells (V5, V6, and T). Ray development depends upon a series of complex cellular interactions that occurs in the hypodermis during remodeling of the male tail (39). The formation of the adult male tail involves retraction, hypodermal cell fusion, formation of the fan, and extension of the rays. The strong ACN-1:GFP expression in the male hypodermis and ray precursor cells (papillae) and the detrimental effect of acn-1 dsRNA on male tail morphogenesis provide compelling evidence that ACN-1 is involved in the complex cellular interactions that occur during male tail morphogenesis.

The expression of acn-1:gfp in the vulva cells of L4 larvae suggests that this protein might also be required in the formation of the vulva. It is tempting to speculate that ACN-1 is required for cell interactions during vulva development; however, interpretation is difficult due the constricted body form. The commonly observed protruding vulva might, therefore, result from unusually strong internal pressures generated in acn-1(RNAi) nematodes. The role, if any, of acn-1 in vulva development requires further investigation.

Expression of ACN-1 Is Regulated by nhr-23 and nhr-25—Failure to ecdyse the larval cuticle during a molt is also one of the consequences of interfering with the hypodermal expression of the nuclear hormone receptors, nhr-23 and nhr-25 (29–32). Around 50% of male C. elegans fed nhr-23 dsRNA also displayed defects in fan and sensory ray development, some resulting from the failure of the hypodermis to retract during morphogenesis of the adult male tail (32). NHR-23 and NHR-25 are orthologues of D. melanogaster DHR-3 and βPtz-F1, respectively (40). Because DHR-3 is required for βPtz-F1 expression during metamorphosis in the ecdysone (molting hormone) response pathway in flies (41–43), it has been suggested that a similar regulatory cascade comprising nhr-23 and nhr-25 might control molting in C. elegans (30). Such conservation of the regulation of periodic molting between insects and nematodes would be supportive of the proposed, and generally accepted, new clade called the Ecdysozoa that groups nematodes with arthropods and other molting invertebrates (44). The down-regulation of acn-1 promoter activity in seam cells of both nhr-23(RNAi) and nhr-25(RNAi) nematodes identifies acn-1 as a candidate gene positioned downstream of nhr-23 and nhr-25 in the proposed genetic pathway for the control of molting in C. elegans. Because the larval expression of nhr-23 is cyclical with peaks of expression occurring between molts, it is possible that acn-1 expression, under the control of nhr-23, is also periodic and coordinated with the molting cycle. If, during exit from the dauer state, nhr-25 and nhr-23 control the late induction of acn-1, then we would predict that the nuclear hormone receptor genes would be expressed earlier in the dauer exit time course. Therefore, it is interesting to note that nhr-25 and nhr-23 are classed as “early” (2 h) and “climbing” (6–12 h) genes, respectively, in the dauer exit time course of gene expression (36).

The Unusual Structure of ACN-1—The dipeptidyl carboxypeptidase activity of ACE is absolutely dependent upon the presence of an active site zinc, which in human gACE is coordinated to His-383, His-387, and Glu-411 (45). The substitution of all three coordinating residues in ACN-1 means that ACN-1 does not have a canonical zinc binding domain and, therefore, is classed as a “non-metallopeptidase” homologue. Nevertheless, it is possible that the ancestral active site, minus zinc, may function to bind peptides or to interact with exposed peptide sequences of other proteins.

C. elegans ACN-1 has several structural features in addition to the absence of a catalytic active site, which distinguishes it from other members of the ACE family. No other family member has an N-terminal Pro-/Glu-rich domain or a mucin-like C-terminal domain. These unusual structural features might be sites for new molecular interactions that have either a direct impact on the molting process (e.g. by promoting cell recognition and cell fusion of hypodermal cells) or an indirect effect by regulating genes required for molting.

All nematodes, including those responsible for debilitating diseases and for economic losses to agriculture, are covered by a cuticular cuticle, which is periodically replaced to allow for growth and adaptation to different environments (46). The cuticle may vary in composition and fine structure depending upon nematode species and stage of development. However, it is highly likely that basic mechanisms of regulating its construction and subsequent shedding will be conserved. A BLAST search of nematode DNA sequences has identified orthologues of acn-1 in other nematode species including animal and plant
parasites,

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