Remodeling of the extracellular matrix (ECM) is pivotal for various biological processes, including organ morphology and development. The *Caenorhabditis elegans* male tail has male-specific copulatory organs, the rays and the fan. Ray morphogenesis, which involves a rapid remodeling of the ECM, is an important model of morphogenesis, although its mechanism is poorly understood. ADAMTS (a disintegrin-like and metalloprotease with thrombospondin type I motifs) is a novel metalloproteinase family that is thought to be an important regulator for ECM remodeling during development and pathological states. We report here that a new *C. elegans* ADAMTS family gene, *adt-1*, plays an important regulatory role in ray morphogenesis. Inactivation of the *adt-1* gene resulted in morphological changes in the rays as well as the appearance of abnormal protuberances around the rays. In addition, mating ability was remarkably impaired in *adt-1* deletion mutant males. Furthermore, we found that the green fluorescent protein reporter driven by the *adt-1* promoter was specifically expressed throughout the rays in the male tail. We hypothesize that ADT-1 controls the ray extension process via remodeling of the ECM in the cuticle.

ADAMs is a family of genes with structural homology to snake metalloproteinases and disintegrins (1–3). Typical ADAMs are membrane-anchored glycoproteins that are composed of a prodomain, a metzincin-like domain, a disintegrin domain, a cysteine-rich region, an epidermal growth factor repeat, a transmembrane region, and a cytoplasmic domain. In initial functional studies, ADAMs were shown to serve as adhesion molecules involved in cell-cell interaction (4, 5) as well as membrane-anchored metalloproteinases, which are involved in the processing of the membrane-anchored precursors of cytokines and growth factors, adhesion molecules, and cytokine receptors (6–9).

ADAM-1 is an ADAM family protein with thrombospondin (TSP) type I motifs that was originally identified as a gene highly expressed in vivo in the colon 26 cachexigenic tumor (10). The mouse ADAMTS-1 gene is mapped to chromosome 16 (11). ADAMTS-1 is distinguished from typical ADAMs by its lack of a transmembrane region and by its three TSP type I motifs and an interposed spacer region in the C-terminal half. As expected from a structure containing TSP type I motifs, ADAMTS-1 is incorporated into the extracellular matrix (ECM) after secretion from cells (12). A competition experiment with soluble heparin suggested that ADAMTS-1 binds to sulfated glycosaminoglycans of the ECM. Analyses of deletion mutants demonstrated that the spacing region of the C-terminal half, as well as the three TSP type I motifs, is responsible for the tight interaction of ADAMTS-1 with the ECM (12). In addition, our data demonstrating that ADAMTS-1 is able to form a covalent binding complex with α2-macroglobulin indicate that ADAMTS-1 is an active metalloproteinase (13). These observations led us to predict that ADAMTS-1 functions through proteolysis of ECM molecules. On the other hand, it is also known that the anti-angiogenic activity of thrombospondin-1 can be attributed to its three type I motifs (14, 15), and the ability of human ADAMTS-1 (METH-1) to inhibit angiogenesis has been reported (16).

In the last 3 years, several mammalian genes that are structurally related to ADAMTS-1 have been identified, and they make up the ADAMTS family (17–22). This novel family includes procollagen II N-proteinase (ADAMTS-2) (17) and aggrecanase-1 and -2 (ADAMTS-4 and -5) (18, 19). Our recent study demonstrated that ADAMTS-1 has aggrecan-cleaving activity (23). Versican proteolysis by ADAMTS-1 and -4 was also reported (24). It therefore seems that ADAMTS family members may be involved in proteolysis of ECM molecules and may play a role in establishing tissue architecture and tissue degradation in various diseases.

We have recently observed that disruption of the mouse *adams-1* gene results in renal anomalies involving enlargement of the calyx (25). Intravenous pyelography revealed a partial obstruction in the ureteropelvic junction of ADAMTS-1<sup>–/–</sup> mice, demonstrating that ADAMTS-1 plays an important regulatory role in normal development of the ureteropelvic junction. In addition, an abnormal adrenal medullary architecture was observed in ADAMTS-1<sup>–/–</sup> mice. Rohrer et al. (26) have reported that ADAMTS-1 mRNA expression is induced after luteinizing hormone stimulation in preovulatory follicles. We found that fertilization is impaired in ADAMTS-1<sup>–/–</sup> females. These observations demonstrate that ADAMTS-1 is necessary for proper function of the female genital organs (25).
### Amino Acid Sequence of C. elegans ADT-1

| Protein | Sequence |
|---------|----------|
| Ce ADT-1 | MPEPITY ITLSTFVR ISQSVHHR NEELKQFGV SNKHDFEEY GEGFRPLK NGM KFPA WHITYHNL 77 |
| m ADAMTS-1 | MGVORRARS EGSSAHGMILLILL VARGAFRPTC RGTSTPT TTHLIAAAG CQLLHKEK PD 80 |
| Ce ADT-1 | KSNRVSFH ISQVGDDE VQTVAGKER EQCNRQGQK SHGNSSQK DUALMQHV MEEHYVLK ELKQVHLQK 157 |
| m ADAMTS-1 | SGFLAPFGT QSSPFSG AIQHLDFTN AHPFGQS QGIAVQF YEQEFTFPHI AGV 158 |

**Metallolysin-like**

| Protein | Sequence |
|---------|----------|
| Ce ADT-1 | ERIHNREKSA GLITNAEIS KEEITLQRE QSFQQSEQ LIFAMTIPA HLHNYTIPT QALES---- --226 |
| m ADAMTS-1 | IAFAPEES SAROGQHLA RQKSGSGK CQGMLFLTFSPFQKHFFR TNWHRVPDD RHTAGKPSG BISIK 238 |

**Role of ADT-1 in Ray Morphogenesis**

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**Fig. 1. Deduced amino acid sequence of C. elegans ADT-1.** A, the amino acid sequence of C. elegans (Ce) ADT-1 is aligned with that of mouse (m) ADAMTS-1 (10). The amino acid residues conserved in these two proteins are boxed. The predicted signal sequences at the N terminus are double-underlined. The position of potential zinc-binding motifs is indicated. B, the catalytic zinc-binding site of both proteins are underlined. The potential zinc-binding motif (HEXXHXXGXXHD) is indicated by asterisks. The methionine residue of the Met turn is indicated by the black circle.
ADAM family genes exist in the small nematode Caenorhabditis elegans (27–30), which is the model animal for the genetic investigation of behavior and development. It has been shown that SUP-17, a protein related to that SUP-17, a investigation of behavior and development. It has been shown (27)


ditis elegans each other on the genome. A line of mutant animals with an

tcgaactcgcaag), and CB3D (gttgctcgacaagtgctttg), which are

cagagatgaaggacagatgg), CB3C (ccattggtgaacactacgtc), CB0B (aatggct-


type animals to establish the TN145 strain (32). These mutant animals were out-crossed three times with wild-


transformed mutants (TN281, Tc1 insertion strain TN281 (32) was generated by insertion and imprecise excision of the transposon Tc1, and found that the TN281 deletion mutant exhibits abnormal morphology of the male copulatory organs.

MATTERIALS AND METHODS

Nematode Strains and Cultures—Nematodes were cultured on NGM agar plates seeded with Escherichia coli strain OP50 at 20 °C according to a routine procedure (31). The strains used in this work were as follows: MT3126 (mut-2(e1459) IV, dpy-19(e1347) III, him-8(e1489) IV, and lon-2(e678).

5′-RACE Reaction and RT-PCR—Total RNA was isolated from N2 animals using ISOGEN (Nippon Gene, Tokyo, Japan). The 5′-end of the adt-1 cDNA was amplified using the Marathon cDNA amplification kit (CLONTECH). A 1-μg sample of total RNA from N2 animals was reverse-transcribed with primer 2B5RPR-1 (5′-agccagctacaatggctttc) and TC31 (gatgcaaacggatacgcgac). After ligation of the adapter, the first PCR was carried out using primer 2B5RPR-1 and the primer within the adapter. Subsequently, nested PCR was performed using the first PCR product as a template with the internal primer of the adt-1 gene, 2B5RPR-2 (5′-gtgacatcagctgctcat) and another nested primer within the adapter. Next, the 600-bp PCR product was subcloned into the pGem vector (Promega).

For the RT-PCR experiment, total RNA (1.5 μg) from the wild-type or mutant animals was reverse-transcribed using random primers and Superscript II (Invitrogen Life Technologies). Specific primers for the adt-1 gene were used to amplify the cDNA. DNA sequencing analysis was performed by PCR employing fluorescent dideoxynucleotides and a Model 373A automated sequencer (Applied Biosystems).

adt-1-GFP Reporter Construct—The GFP reporter plasmid pPD95-75 was provided by Dr. A. Fire. To construct the GFP expression vector, a HindIII-HindIII fragment (3.7 kb) containing both the promoter region and exons 1 and 2 of the adt-1 gene from cosmid C02B4 (obtained from Dr. Alan Coulson, Sanger Center, Hinxton, United Kingdom) was subcloned into pPD95-75. Subsequently, the promoter region and exons 1 and 2 of the adt-1 gene were amplified by PCR employing fluorescent dideoxynucleotides and a Model 373A automated sequencer (Applied Biosystems). The resultant GFP expression vector (pGFP75-adt-1-St) contains a complete zinc-binding motif (HEXXHXGXXHD) and the subsequent Met turn, which comprises the zinc binding environment of the reprolisin family, including ADAM and snake metalloproteinases. This suggests the possibility that ADT-1 is an active metalloproteinase. The C-terminal region of ADT-1 is remarkably longer than these regions of ADAMTS-1 and other mammalian ADAMTS family members, and C. elegans ADT-1 has multiple repeats of TSP type I motifs (Fig. 2).

RESULTS

Identification of a Novel C. elegans ADAMTS Family Gene—To examine the roles of the ADAMTS family during various developmental processes, we first searched for novel ADAMTS family genes of C. elegans in the GenBank (32) Data Bank. A homology search analysis disclosed that cosmid C02B4 (accession number Z50004) contains the ADAMTS family gene (designated C02B4.1 by the C. elegans Sequencing Consortium) (33). We designated this gene as adt-1 (ADAMTS in C. elegans). As shown in Fig. 1A, C. elegans ADT-1 shows overall homology to mouse ADAMTS-1 without any large gap, indicating that the domain organization of C. elegans ADT-1 is very similar to that of mouse ADAMTS-1. This sequence alignment revealed that, like mammalian ADAMTS family proteins, ADT-1 is composed of multiple functional domains, including proprotein, metalloproteinase, and disintegrin-like domains and TSP type I motifs (Fig. 2).

Mating Efficiency—Six males and six lon-2 hermaphrodites at late L4 stage were placed on a 3.5-cm dish, and males were removed after 24 h. Total cross-progeny (non-Lon F1) were counted.

Transgenic Animals—C. elegans transformation was carried out as described by Mello et al. (32). The pRF4 plasmid, carrying rol-6su1006dm), was used as a transformation marker. Hermaphrodites of either strain N2 or him-8 were injected with a DNA mixture containing plasmid pRF4 (80 μg/ml) and the GFP expression vector (20 μg/ml). Transgenic lines carrying the extrachromosomal array of injected DNA were established from F2 Rol progeny and were observed under fluorescent microscopy to examine GFP expression. For the rescued experiments, cosmid C02B4 (1–2 μg/ml) was injected with the pRF4 marker plasmid (100 μg/ml) into adt-1 deletion mutants. The F2 Rol progeny derived from the injected animals were observed under Nomarski optics.

Role of ADT-1 in Ray Morphogenesis

In this study, we have identified a novel C. elegans ADAMTS family gene, adt-1 (ADAMTS in C. elegans). To better understand the roles of the ADAMTS family in organogenesis of C. elegans, we isolated the deletion mutant for the adt-1 gene using Tc1 and found that the adt-1 deletion mutant exhibits abnormal morphology of the male copulatory organs.

ADT-1 is composed of multiple functional domains, including proprotein, metalloproteinase, and disintegrin-like domains and TSP type I motifs (Fig. 2). As shown in Fig. 1B, ADT-1 contains a complete zinc-binding motif (HEXXHXGXXHD) and the subsequent Met turn, which comprises the zinc binding environment of the reprolisin family, including ADAM and snake metalloproteinases. This suggests the possibility that ADT-1 is an active metalloproteinase. The C-terminal region of ADT-1 is remarkably longer than these regions of ADAMTS-1 and other mammalian ADAMTS family members, and C. elegans ADT-1 has multiple repeats of TSP type I motifs (total of 13 copies) in its C-terminal half-region. It is known that two elements (W/S-G-X-W and CSVTCG) of the TSP type I motif of thrombospondin-1 are functional with regard to the binding to sulfated glycosconjugates (35, 36). As for ADT-1, nine of thirteen repeats have both elements, but repeats 1, 6, 9, and 11 of ADT-1 seem to be incomplete.

As shown in Table I, the metalloproteinase domain of ADT-1 shows the highest homology to ADAMTS-1, -7, and -12 among mammalian ADAMTS family members. But the homology of ADT-1 to mammalian ADAMTS family members is relatively low compared with that of GON-1. GON-1 is more similar to ADAMTS-1 than is ADT-1. However, GON-1 also shows a higher sequence homology to other ADAMTS family members such as ADAMTS-8, -9, and -12. GON-1 may be similar to mammalian ADAMTS family members in its function, whereas ADT-1 may play a more specific role in C. elegans. The C. elegans genome contains other ADAMTS family genes (F08C6.1 and T19D2.1) in addition to adt-1 and gop-1.

The adt-1 Gene Encodes a Putative Secretory Protein—The adt-1 gene is mapped on the chromosome X (Fig. 3A). In terms of the exon/intron organization of the C. elegans adt-1 gene, the GeneFind program suggests that the adt-1 gene consists of 27 exons and encodes a polypeptide composed of 1444 amino acids (C. elegans Sequencing Consortium (34)). But this predicted amino acid sequence of ADT-1 estimated by the program does not contain the signal sequence at its N terminus, although ADT-1 does belong to the ADAMTS family. Therefore, the 5′-region of the adt-1 cDNA was re-examined through the
sequencing of 5′-RACE reaction products using the wild-type worm. 5′-RACE analysis revealed the existence of an additional exon (exon 1 in Fig. 3) that encodes a signal sequence characterized by stretches of hydrophobic amino acids (Fig. 1A). In addition, sequencing analyses of RT-PCR products confirmed that the remaining exons of the adt-1 gene are absolutely correct. Consequently, the adt-1 gene consists of 28 exons (Fig. 3B) and encodes a polypeptide composed of 1461 amino acids. These data suggest that, like other ADAMTS family members, the adt-1 gene encodes a secretory protein.

Expression Pattern of the adt-1 Gene in Hermaphrodites—The in vivo expression pattern of the adt-1 gene was investigated using a reporter gene encoding GFP driven by 2.9 kb of the 5′-flanking region of the adt-1 gene. Because ADT-1 is a putative secretory protein with a signal peptide at its N terminus, the first ATG codon of the ADT-1 protein was fused in frame with the GFP reporter, followed by the unc-54 3′-untranslated region. Adult hermaphrodites carrying this reporter gene were found to strongly express the GFP signal in a group of cells forming the vulva (Fig. 4, A, C, and G) and in the area surrounding the pharynx, probably in the head ganglia (Fig. 4, E and G). In addition, GFP expression was observed in the ventral nerve cord (Fig. 4C) and in the amphid neurons (Fig. 4E). A weak GFP signal was detected on the body surface (Fig. 4G).

Isolation of the adt-1 Mutant—To address the in vivo function of the adt-1 gene, we inactivated the gene using a transposon-based PCR-sib selection method (33). First, by screening a library of the mutator strain MTS3126, we isolated a line of animals (TN281, adt-1(cn30)) in which Tc1 is inserted into intron 10 of the adt-1 gene (Fig. 3B). As a second step, a mutant line of animals (adt-1(cn30)) harboring a 1-kb deletion upstream of the Tc1 insertion site in the adt-1 gene were obtained (Fig. 3B). This deletion eliminated exons 8–10, which encode most of the metalloproteinase domain of ADT-1. When the adt-1 mRNA of the adt-1(cn30) deletion mutant animals was analyzed by RT-PCR using specific primers near the deleted region, a RT-PCR product that was ~550 bp shorter than that of the wild-type animals was detected in the adt-1(cn30) mutant (Fig. 3C). Sequencing analysis of this RT-PCR product revealed that exon 7 of the adt-1 transcript was linked to exon 11 in frame in the adt-1(cn30) mutant animals (Fig. 3D). This result suggests the possibility that the truncated ADT-1 protein, with the metalloproteinase domain deleted, is expressed in the adt-1(cn30) mutant.

Table I

| Amino acid identity of C. elegans ADT-1 and GON-1 to mammalian ADAMTS family members |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| ADT-1 MP DIS-like First TSP type 1 motif | GON-1 MP DIS-like First TSP type 1 motif |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mouse ADAMTS-1                         | 27              | 34              | 37              | 49              | 36              | 67              |
| Bovine ADAMTS-2                        | 26              | 26              | 31              | 35              | 27              | 50              |
| Human ADAMTS-4                         | 24              | 26              | 39              | 44              | 34              | 69              |
| Human ADAMTS-5                         | 24              | 31              | 34              | 39              | 34              | 60              |
| Human ADAMTS-7                         | 27              | 26              | 29              | 44              | 26              | 52              |
| Human ADAMTS-8                         | 22              | 22              | 31              | 45              | 28              | 63              |
| Human ADAMTS-9                         | 24              | 24              | 27              | 56              | 41              | 57              |
| Human ADAMTS-10                        | 26              | 26              | 29              | 40              | 28              | 65              |
| Human ADAMTS-12                        | 28              | 24              | 32              | 45              | 27              | 56              |

* MP, metalloproteinase domain; DIS-like, disintegrin-like domain.

FIG. 2 Schematic diagrams of mouse ADAMTS-1 and C. elegans ADT-1. Pro, proprotein region; MP, metalloproteinase domain; DIS-like, disintegrin-like domain; TSP, thrombospondin type I motif; SP, spacer region. Black boxes indicate the signal peptides.
rays 1–3 of adt-1(cn30) mutant males were found frequently bent inside and appeared to be shortened in the lateral view (rays 1–3 in Fig. 5, B and C). In addition, ~10% of adt-1(cn30) mutants formed a disorganized tail with an abnormal mass at the ventral side of the tail (Fig. 5I).

These morphological changes in the tail of adt-1(cn30) males were rescued by reintroduction of cosmid C02B4, containing the full-length adt-1 gene, as an extrachromosomal transgene (Fig. 7). Taken collectively, these data suggest that ADT-1 is necessary for the formation of the overall structure of the male tail as well as for ray morphogenesis.

Because the adt-1 mutants showed an abnormal male tail morphology, we next examined the mating capacity of the adt-1 males. When the adt-1 mutant males were mated with lon-2 hermaphrodites, the number of F1 progeny with normal body length was significantly reduced compared with that of wild-
Role of ADT-1 in Ray Morphogenesis

ADT-1, expressed by ray-forming cells, plays a role in ray morphogenesis. In this study, we have identified adt-1, a C. elegans ADAMTS family gene, and have shown that the deletion mutant of the adt-1 gene exhibits abnormal male tail morphology, including a thickening of ray 6. Our observations clearly demonstrate that ADT-1 plays an important role in male tail morphogenesis.

Development of the male tail of C. elegans, which is specialized for copulation, provides a means of inquiring into the mechanism of morphology. The specific morphology of the male tail portion is generated by post-embryonic development different from that of a hermaphrodite (38, 39). The nine pairs of bilateral rays are derived from the three most posterior seam cells, V5, V6, and T (39). Each ray is composed of three cells, two different types of neurons and the ray structural cell, all of which are generated from one ray precursor cell in the epidermis at the L4 stage. After execution of the ray sublineage, the ray cells undergo a series of changes in shape and cellular fusion (40, 41), resulting in a rearrangement of cell positions. The ray positions are determined by the sites at which the ray structural cells make attachments to the surface (40). The adt-1 mutants showed abnormal ray morphology, especially in ray 6, but the ray positions did not appear to be disturbed in the adt-1 mutant males. Therefore, it seems that ADT-1 is not involved in the process of determining the ray position.

After the ray positions have been decided, the tail region is retracted anteriorly, and simultaneously, the rays extend laterally from the body (38, 39). We found that, in the adt-1 mutant male, the tip of ray 6 had a thin structure, similar to that of the wild-type male. However, at the region proximal to the body, ray 6 of the adt-1 mutant thickened. These findings strongly suggest that ADT-1 is involved in the ray extension process.

When the expression pattern of the adt-1 gene in the male tail was examined using the adt-1::GFP reporter gene construct without a nuclear localization sequence, the GFP signal was observed throughout rays, suggesting the possibility that the adt-1 gene is expressed by the hypodermal cells of the rays. We previously demonstrated that mouse ADAMTS-1 is incorporated into the ECM after secretion from cells and that its C-terminal domain, including TSP type I motifs and the spacer region, is important for binding to the ECM (12). Because ADT-1 shows a domain organization similar to that of ADAMTS-1 and contains multiple copies of TSP type I motifs, it is likely that ADT-1, secreted from hypodermal cells, is incorporated into the ECM around hypodermal cells such as the cuticle.

The cuticle, the C. elegans exoskeleton, is a major ECM of C. elegans as well as a basement membrane and is important for maintenance of morphology. The cuticle is composed of the ECM molecules such as collagens, which are produced by the hypodermal cells (42). It is thought that remodeling of the cuticle is regulated by the hypodermal cells. As for ray formation, because the rays quickly extend within a few hours at the L4 stage (39, 40), it can be expected that a rapid remodeling of the ECM would occur in the cuticle during the ray extension process. We hypothesize that ADT-1, secreted from hypodermal cells, controls the ray extension process by regulating remodeling of the ECM in the cuticle. Because abnormal rounded protuberances were frequently observed between the sensory rays in the adt-1 mutant male, ADT-1 is also thought to be necessary for the maintenance of the smooth structure of the body surface around the rays.

RT-PCR analysis demonstrated the possibility that the truncated ADT-1 protein, with the metalloproteinase domain deleted, is expressed in the adt-1(cn30) mutant. Therefore, our results for the adt-1(cn30) mutant demonstrate that the metalloproteinase domain of ADT-1 is important for morphogenesis of the male tail, although we do not have direct evidence that ADT-1 is an active metalloproteinase. In mammalian systems, it has recently been shown that proteoglycans such as

DISCUSSION

In this study, we have identified adt-1, a C. elegans ADAMTS family gene, and have shown that the deletion mutant of the adt-1 gene exhibits abnormal male tail morphology, including a thickening of ray 6. Our observations clearly demonstrate that ADT-1 plays an important role in male tail morphogenesis.

Expression of the adt-1 Gene in the Male Tail—To determine whether or not the adt-1 gene is specifically expressed in the male tail region, we generated transgenic lines of him-8 carrying the adt-1::GFP reporter gene containing the 5'-flanking region of the adt-1 gene and subsequently examined the expression patterns of these lines. A GFP signal was detected in ray 6 of the male tail as well as the other rays (Fig. 9, A and C). GFP expression was also observed in the hook (data not shown). These observations demonstrate that the adt-1 gene is specifically expressed in the male tail region, including the rays, and that ADT-1, expressed by ray-forming cells, plays a role in ray morphogenesis in the male tail.

FIG. 4. GFP expression pattern of the adt-1 gene in hermaphrodites. A, C, E, and G, GFP expression in transgenic N2 hermaphrodites carrying the adt-1::GFP reporter gene; B, D, and F, Nomarski micrographs of A, C, and E, respectively. A and B, lateral view of the central body region of an adult; C and D, ventral view of the central body region of an adult; E and F, the head region of an adult; G, a young adult hermaphrodite. The fluorescent portions of the vulva, the head ganglion (hg), the ventral nerve cord (vnc), and amphid neurons (an) are indicated. Scale bars = 20 μm.

Type males (Fig. 8), indicating that the mating ability of the adt-1 mutants is impaired.

RT-PCR analysis demonstrated the possibility that the truncated ADT-1 protein, with the metalloproteinase domain deleted, is expressed in the adt-1(cn30) mutant. Therefore, our results for the adt-1(cn30) mutant demonstrate that the metalloproteinase domain of ADT-1 is important for morphogenesis of the male tail, although we do not have direct evidence that ADT-1 is an active metalloproteinase. In mammalian systems, it has recently been shown that proteoglycans such as
aggrecan and versican in the ECM are target molecules for ADAMTS-1, -4, and -5 (18, 19, 23, 24). In addition, ADAMTS-2 is procollagen I/II N-proteinase, which is known to cause dermatosparaxis in cattle (17). It is therefore thought that ADAMTS family members may be involved in proteolysis of ECM molecules. In C. elegans, GON-1, a member of the ADAMTS family, has been shown to be essential for gonadogenesis (29). Although the substrate for GON-1 has not been identified, it is speculated that GON-1 participates in gonad formation via remodeling of the basement membrane surrounding the developing gonad. The analogy to GON-1 also supports our notion that ADT-1 is involved in the ray extension process via regulation of remodeling of the ECM in the cuticle. In addition, these findings demonstrate that each ADAMTS family member plays a different role in the organogenesis of C. elegans.

Consistent with the abnormal morphology of the male tail, the mating capacity of adt-1 mutant males was remarkably impaired. It is known that the male-specific organs in the tail such as the rays, the fan, and the spicules are necessary for copulation with hermaphrodites. Male copulatory behavior consists of a series of processes (43). When a male contacts a hermaphrodite, a male mates the ventral side of his tail.

**Fig. 5.** Abnormal male phenotypes of the adt-1 deletion mutant. Lateral views of the tail regions of wild-type (A) and adt-1(cn30) (B and C) males are shown. In B, note that ray 6 is transformed into a thickened shape (arrowhead). In C, in addition to the morphological transformation of rays 4 and 6 (arrowheads), an abnormal rounded protuberance (asterisk) can be seen between rays 3 and 4. Ventral views of the tail regions of wild-type (D and G) and adt-1(cn30) (E, F, and H) males are shown. In E and F, a thick ray 6 is indicated by arrowheads. In F, an abnormal rounded protuberance (asterisk) is also seen between rays 3 and 4. In H, the fan of the adt-1(cn30) male has a closed structure in the anterior region of the tail. A disorganized male tail of adt-1(cn30) is shown in I. A large abnormal mass can be seen at the ventral region of the tail at a frequency of ~10% in adt-1(cn30) males. Scale bars = 10 μm.

**Fig. 6.** Frequency of abnormal thick rays (A) and abnormal rounded protuberances between rays (B) found in adt-1 mutant males. The frequency is represented as a percentage of sides at which each ray is transformed into a thick shape (A) and at which an abnormal rounded protuberance appears between the rays (B) in adt-1(cn30) mutant males. Total number of sides examined = 110.

**Fig. 7.** Cosmid rescue of adt-1(cn30) mutant males. Shown is the tail morphology of adt-1(cn30) mutant males (A) and animals transformed with cosmid C02B4, which contains the full-length adt-1 gene (B). Scale bars = 10 μm.
against her body and moves backward, the so-called “backing.” Just before reaching the end of the hermaphroditic body, a male turns the dorsal side to the ventral side, known as “turning,” and continues backing until he locates the vulva, where the male then inserts its spicules to transfer the sperm. It is thought that sensory rays are required for the backing and turning during the mating process (43). We observed that the \textit{adt-1(cn30)} mutant males are able to respond to contact with hermaphrodites and initiate backing by touching their tails to the hermaphrodites. However, hardly any of these \textit{adt-1} mutant males could accomplish turning near the end of the hermaphrodites and gave up during this stage. In addition, \textit{adt-1} mutant males tend to become detached from hermaphrodites, even during the backing process. Therefore, it is possible that the morphological changes in the rays of \textit{adt-1} mutants affect the ability of their sensory rays to recognize a hermaphrodite’s body. However, because it has been shown that ablation of ray 6 has no effect on mating behavior (43), it is not likely that the thickness of ray 6 fully explains why the mating ability of the \textit{adt-1} mutant was impaired. Transformation of the whole tail morphology, including the bending of rays and the morphological change in the fan in addition to the thickness of ray 6, might be totally responsible for the impaired mating behavior of the \textit{adt-1} mutant.

Recently, Li et al. (44) reported that ADAMTS-2 null mice show male sterility due to their impaired spermatogenesis. In contrast, male sterility of \textit{adt-1(cn30)} results from abnormal mating behavior.

A number of genes, including a group of \textit{mab} genes (45) and \textit{ram} genes (46), have been identified to be necessary for male tail morphogenesis. These genes, which are involved in the specification of cell fates and of ray identities as well as ray morphogenesis, encode transcription factors such as Hox proteins (47–52), components of either a transforming growth factor-\textit{b} (53–55) or Wnt (56) signaling pathway, and a transmembrane protein (57). It is thought that a complex network consisting of these transcription factors and intracellular signaling components leads to a precise regulation of genes essential for tail morphogenesis. A transmembrane protein is postulated to function via cell-cell interactions. In contrast, it is likely that ADT-1 directly modulates the ECM or cell-surface molecules. This is the first report describing the involvement of an extracellular protease in male tail morphogenesis in \textit{C. elegans}. Our findings can be expected to provide new insights into understanding the mechanism of ray morphogenesis.

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