A putative GDP–GTP exchange factor is required for development of the excretory cell in Caenorhabditis elegans

Norio Suzuki, Matthew Buechner1, Kiyoji Nishiwaki2, David H. Hall3, Hiroyuki Nakanishi4, Yoshihiko Takai4, Naoki Hisamoto & Kunihiro Matsumoto+

Department of Molecular Biology, Graduate school of Science, Nagoya University and CREST, Japan Science and Technology Corporation, Chikusa-ku, Nagoya 464-8602, 2PRESTO, Japan Science and Technology Corporation and Fundamental Research Laboratories, NEC Corporation, Miyukigaoka, Tsukuba 305-8501, 3Department of Molecular Biology and Biochemistry, Osaka University Medical School, Suita 565-0871, Japan, 4Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045 and 3Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Received January 12, 2001; revised April 10, 2001; accepted April 20, 2001

The Caenorhabditis elegans excretory cell extends tubular processes, called canals, along the basolateral surface of the epidermis. Mutations in the exc-5 gene cause tubulocystic defects in this canal. Ultrastructural analysis suggests that exc-5 is required for the proper placement of cytoskeletal elements at the apical epithelial surface. exc-5 encodes a protein homologous to guanine nucleotide exchange factors and contains motif architecture similar to that of FGD1, which is responsible for faciogenital dysplasia. exc-5 interacts genetically with mig-2, which encodes Rho GTPase. These results suggest that EXC-5 controls the structural organization of the excretory canal by regulating Rho family GTPase activities.

INTRODUCTION

One of the central questions in animal development is how the formation of organ structure during development is controlled. Genetic approaches in model organisms such as the nematode Caenorhabditis elegans have been useful in providing new insights into the molecular mechanisms controlling organ structure formation.

The C. elegans excretory system is believed to regulate osmoregulation and waste elimination, analogous to the renal organs of higher animals. The excretory cell, and its associated gland, duct and pore cells, form the C. elegans renal system (Chitwood and Chitwood, 1974; Nelson et al., 1983; Broeks et al., 1995). The excretory cell is a large ectodermal cell located beneath the posterior pharyngeal bulb, and it extends tube-like canals dorsally on both sides during embryogenesis (Hedgecock et al., 1987). Upon reaching the lateral epidermis, these canals bifurcate and grow anteriorly and posteriorly for nearly the length of the animal, creating an H-shaped canal system (Figure 1A). The longer, posterior canal reaches epidermoblast V3 by the time of hatching, and V6 by the end of the first larval stage (L1), thus completing its active outgrowth. Active outgrowth at the tip of the canal accounts for less than one-third of the adult canal length. The remaining growth occurs along the length of the canal, as the hypodermal cells, to which the canal is connected by gap junctions, expand. The C. elegans excretory cell provides a unique system to study the formation of organ structure in a unicellular epithelium.

In this study, we show that the exc-5 gene is required for the formation of the excretory canal structure. exc-5 encodes a protein homologous to guanine nucleotide exchange factors (GEFs) and contains motif architecture similar to that of FGD1, which is responsible for faciogenital dysplasia or Aarskog–Scott syndrome (Pasteris et al., 1994). Our results suggest that EXC-5 controls the structural organization of the excretory canal by regulating activity of Rho family GTPases.

RESULTS AND DISCUSSION

To understand how the formation of the canal structure is determined, mutants with abnormal excretory canals were isolated using the Tc1 mutator strain NL917. One isolate, km508, exhibits extremely large septate cysts, formed predominantly at
We cloned the exc-5 gene by transposon tagging. This gene corresponds to the predicted C33D9.1 gene identified by the C. elegans sequencing consortium. A 13.7-kb genomic DNA fragment that contains both regulatory and coding sequences of C33D9.1 was found to rescue the exc-5(km508) mutant phenotype. Analysis of the exc-5 cDNA indicated that the exc-5 gene has 15 exons (Figure 3A) and encodes a single protein of 826 amino acids (Figure 3B). To confirm that we had cloned the correct gene, we sequenced the genomic DNA of three exc-5 mutant alleles: km508, rh232 and n2672 (Figure 3). km508 has a Tc1 transposon insertion at Cys758. The rh232 mutation is a deletion that removes at least the first 12 exons of exc-5 and thus is presumably a null allele. n2672 is a nonsense mutation occurring at Trp604.

To determine the expression pattern of EXC-5, we generated a transgene, called exc-5 5′::GFP, carrying a predicted exc-5 promoter fused to the coding region for green fluorescent protein (GFP). exc-5 5′::GFP drives GFP expression in the excretory canal (see Figure 4B). We also constructed a gene fusion containing the exc-5 upstream regulatory sequence and its entire coding region fused to the coding region for GFP. This transgene, exc-5::GFP, rescued the exc-5 phenotype, suggesting that it was functional and expressed in all cells that require exc-5 activity. GFP fluorescence was observed in the excretory canal (Figure 4A), consistent with the role this protein plays in the formation of canal structure. In addition, exc-5 expression was also seen in parts of pharyngeal muscles, rectal epithelial cells, and several head and tail neurons.

The EXC-5 protein contains, in order, a Dbl-pleckstrin homology (DH-PH) domain, a cysteine-rich evolutionarily conserved zinc-finger motif termed a FYVE domain, and a second PH domain (Figure 3C). This type of tandem DH-PH domain is conserved among GEFs that act on members of the Rho family. FGD1 and Frabin act as GEFs for the Rho family GTPase Cdc42 (Olson et al., 1996; Zheng et al., 1996). The structural organization of EXC-5 is strikingly similar to that of mammalian FGD1 and Frabin (Figure 3C). FGD1 was determined to be the locus responsible for faciogenital dysplasia or Aarskog–Scott syndrome, a multi-systemic developmental disease affecting skeletal and urogenital systems (Pasteris et al., 1994). Frabin was identified as an F-actin-binding protein (Obaishi et al., 1998). FGD1 and Frabin act as GEFs for the Rho family GTPase Cdc42 (Olson et al., 1996; Zheng et al., 1996; Nagata et al., 1998; Obaishi et al., 1998; Umikawa et al., 1999). Taken together, these results suggest that EXC-5 may function as a GEF for the C. elegans Rho family GTPases.

A number of Rho family members have been identified in C. elegans, including MIG-2, CED-10/RAC-1, RAC-2, CDC-42 and RHO-1 (Chen et al., 1996a,b; Zipkin et al., 1997; Reddien and Horvitz, 2000). Among them, activated and null mutations in the mig-2 gene have been identified (Zipkin et al., 1997). To test whether EXC-5 could act as a GEF for GTPases, we examined the genetic interaction between exc-5 and mig-2. Animals with the activated gm38 allele of mig-2 exhibit normal canal structure (data not shown). We constructed exc-5(rh232); mig-2(gm38) double mutants. Activated Mig-2 partially suppressed the Exc phenotype; these animals have longer posterior canals and much smaller cysts than do exc-5(rh232) single mutants (Figure 1D and E). This supports the possibility that EXC-5 functions as a GEF for Rho family GTPases in the formation of canal structure. However, unlike exc-5 loss-of-function mutants, the mig-2(mu28)/null mutant

Fig. 1. The exc-5 gene is required for formation of excretory canals. (A) Diagram showing the position of excretory canals. The apical surface is shown in pink, the basolateral in blue. (B–E) The excretory canal (arrowheads) is much smaller than that in the termini of shortened canals (Figure 1C). We mapped the km508 allele to the right arm of linkage group IV using STS mapping (Williams et al., 1992). Among the genes in this region lies exc-5, previously isolated in a screen for mutations with tubulocystic defects in the excretory canal (Buechner et al., 1999). The similarity in phenotype and map position suggested that the km508 mutation might be located in the exc-5 gene. Consistent with this observation, km508 failed to complement exc-5(rh232).

Electron microscopy analysis revealed that the canals in wild-type animals are embedded in the hypodermis and are spread extensively along the basement membrane that separates the hypodermis from the pseudocoelomic space (Figure 2A). The central lumen is surrounded by electron-dense material, presumably actin-based cytoskeleton (Figure 2B) (Nelson et al., 1983). In a large exc-5 cyst, the electron-dense cytoskeletal material appears disrupted, and no longer evenly surrounds the lumen. In addition, the lumen swells immensely (Figure 2C). These defects are consistent with a defect of the cytoskeleton at the apical membrane required for organizing cell shape, and raise the possibility that the EXC-5 protein is required for the proper placement of cytoskeletal elements at the apical epithelial surface.
animals have normal excretory canals (data not shown), suggesting that there are additional GTPase targets for EXC-5 activity that control the structural organization of the excretory canal.

To examine the effect of EXC-5 overexpression on canal structure formation, we generated transgenic animals carrying a high copy array of the exc-5 5′::GFP or exc-5::GFP genes. Whereas wild-type animals carrying exc-5 5′::GFP have longer posterior canals that reach a point in V6 at the L1 stage (Figure 4B), the canal in animals overexpressing exc-5::GFP stops at a point between V1 and V2 (Figure 4C). Even at the L4 stage, animals overexpressing exc-5::GFP have drastically shortened canal extension, which contains a convoluted tubule. Furthermore, exc-5::GFP was found to be expressed at the apical surface of the cell (Figure 4D). Mosaic animals expressing exc-5::GFP only in the excretory cells show a shortened canal phenotype also seen in mosaic animals deficient in canal expression of the basement membrane-binding β1 integrin (data not shown). GFP fluorescence was visible only in the center of the cell body as streaks representing convolutions of the lumen (Figure 4E). Mosaic animals that express EXC-5::GFP in cells other than the excretory canal show normal canal structure. This suggests that overexpression of EXC-5 acts in a cell-autonomous manner in the formation of abnormal canal structure.
A putative GDP–GTP exchange factor in *C. elegans*

**METHODS**

**Mutant isolation and genetic mapping.** *exc-5(km508)* was isolated as a mutant with abnormal excretory canals by visual screening of ~12 000 individuals of the mut-7(pk204) strain NL917. *km508* mapped between *stf44* and *stf4* on linkage group IV by PCR STS mapping (Williams et al., 1992).

**Cloning of *exc-5*.** The genomic sequence corresponding to *exc-5* was identified by use of a transposon display method. The mutant-specific Tc1 insertion was found in the region that is deleted in *exc-5(rh232)*, as did a subclone containing only the promoter region of the *exc-5* gene. The 5′-terminus of the *exc-5* mRNA was analyzed by use of the 5′ RACE Strategy for Rapid Amplification of cDNA Ends (Life Technologies). Molecular lesions were identified by direct sequencing of PCR products spanning the *exc-5* gene.

**Analysis of canal defects.** *Caenorhabditis elegans* hermaphrodites were observed using a Zeiss Axioplan microscope equipped with a Plan 63 objective and differential interference contrast optics. Images were captured with a Hamamatsu Photonics C5810 Color Chilled 3-CCD camera connected to a Macintosh G3 computer. To visualize the excretory canal, a construct expressing GFP under control of the *exc-5* promoter was coinjected with pRF4, which contains the rol-6*′* rescue element. The mixture of two cosmids, C33D9 and W07F6, was found to rescue *exc-5* and *Frablin*. Analysis of canal defects was performed by use of the *exc-5* promoter-FRG construct (Williams et al., 1992).

**Electron microscopy.** L4 larvae and young adults were cut through the midbody and fixed immediately in buffered (199 mM HEPES pH 7.5) 3% glutaraldehyde, followed by postfixation in buffered 1% OsO4. After encaement in 1% agar, samples were dehydrated and embedded in Polybed 812 resin.
Serial sections (~70 nm) were post-stained in uranyl acetate followed by lead citrate.

**Transgene experiments.** To construct a plasmid that contains only the C33D9.1 gene, the SalI–PstI fragment was cloned into the SalI–PstI site of the pBSII KS(−) vector. This construct contained 12.5 kb of the upstream sequence, coding regions, and all of the introns of the exc-5 gene, as well as 1.1 kb of its downstream sequence. The plasmid containing only the C33D9.1 gene was co-injected into exc-5(+/+), exc-5(+/−), and exc-5(−/−) to examine the effect of exc-5 overexpression on the structure of the excretory canal. The photo shows the ventral view. The dotted fluorescence results from the autofluorescence of gut granules. (B) L1 unc-119(e2498) larva with exc-5 5′::GFP and pDP#GFP16B (unc-119). The excretory canal is visualized using exc-5 5′::GFP expression. High fluorescence in pharynx (Ph) is overexposed to show expression extending throughout the narrow canal (arrowhead). Go, gonad. (C–E) The excretory canal is visualized using exc-5 5′::GFP expression in unc-119(e2498) with exc-5 5′::GFP and pDP#GFP16B. (C) The photo shows a truncated posterior excretory canal (arrowhead) from a left lateral aspect in L1 larva. (D) The photos show a shortened posterior excretory canal in L4 larva. Upper and lower panels show Nomarski and epifluorescence images, respectively. The animal has a convoluted tubule in the excretory canal. exc-5 5′::GFP is predominantly expressed at the lumenal (apical) surface of the cell. (E) The photos show a large non-cystic cell body in a mosaic L4 larval animal expressing exc-5 5′::GFP in the excretory canal. The middle panel shows the cell body at high magnification. The arrow indicates a pore connecting the excretory canals to the surface of the animal. GFP expression is seen at the apical surface of the cell. The right panel shows a diagram of the middle panel. Scale bars, 20 μm.

Fig. 4. Effect of exc-5 overexpression on structure of the excretory canal. (A) Expression of exc-5 is visualized using exc-5 5′::GFP in exc-5(km508). The photo shows the ventral view. The dotted fluorescence results from the autofluorescence of gut granules. (B) L1 unc-119(e2498) larva with exc-5 5′::GFP and pDP#GFP16B (unc-119). The excretory canal is visualized using exc-5 5′::GFP expression. High fluorescence in pharynx (Ph) is overexposed to show expression extending throughout the narrow canal (arrowhead). Go, gonad. (C–E) The excretory canal is visualized using exc-5 5′::GFP expression in unc-119(e2498) with exc-5 5′::GFP and pDP#GFP16B. (C) The photo shows a truncated posterior excretory canal (arrowhead) from a left lateral aspect in L1 larva. (D) The photos show a shortened posterior excretory canal in L4 larva. Upper and lower panels show Nomarski and epifluorescence images, respectively. The animal has a convoluted tubule in the excretory canal. exc-5 5′::GFP is predominantly expressed at the lumenal (apical) surface of the cell. (E) The photos show a large non-cystic cell body in a mosaic L4 larval animal expressing exc-5 5′::GFP in the excretory canal. The middle panel shows the cell body at high magnification. The arrow indicates a pore connecting the excretory canals to the surface of the animal. GFP expression is seen at the apical surface of the cell. The right panel shows a diagram of the middle panel. Scale bars, 20 μm.

(Philogen).
REFERENCES


DOI: 10.1093/embo-reports/kve110