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cdh-3, a gene encoding a member of the cadherin superfamily, functions in epithelial cell morphogenesis in *Caenorhabditis elegans*

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SUMMARY

Several genes that encode members of the cadherin superfamily have been identified in *Caenorhabditis elegans*. Based on the roles of cadherins in vertebrates and *Drosophila*, it is expected that they function in the control of epithelial morphogenesis, an event which is poorly understood at the molecular level in *C. elegans*. Reporter genes under the control of upstream sequences from one of these genes, *cdh-3*, are expressed in developing epithelial cells, but also in a number of neuroectodermal cells that extend processes along some of these epithelial cells. We generated a loss-of-function mutation in *cdh-3* by transposon-mediated deletion mutagenesis. This mutation affects the morphogenesis of a single cell, hyp10, which forms the tip of the nematode tail. The lack of detectable defects associated with the other cells expressing *cdh-3* reporter constructs hints at the existence of other genes that can compensate for *cdh-3* loss of function.

Key words: cadherins, epithelial morphogenesis, *C. elegans*

INTRODUCTION

Epithelial morphogenesis is an important event during metazoan development and is dependent upon the coordinated control of changes in cell shape, proliferation, and recognition and adhesion (Gumbiner, 1996). In both vertebrates and *Drosophila*, members of the cadherin superfamily of cell surface proteins function in many of these processes (Mahoney et al., 1991; Clark et al., 1995; Takeichi, 1995; Uemura et al., 1996; Tepass et al., 1996). However, little is known about the molecules involved in epithelial morphogenesis in nematodes. We report here that one of the recently identified members of the cadherin superfamily does indeed play a role in this process.

Cadherins were first identified in vertebrates as Ca^{2+}-dependent adhesion molecules, which localized to the adherens junctions of epithelial cells. Most work since then has focused upon these so-called classical cadherins (Takeichi, 1995). The extracellular portions of these molecules can be divided into five tandemly repeated 'cadherin domains' which are approximately 30% homologous to each other. It is the presence of cadherin domains that classifies a protein as being a member of the cadherin superfamily. The Ca^{2+}-dependent adhesion displayed by the classical cadherins is mediated by the cadherin domains via the formation of supramolecular adhesion interfaces (Overduin et al., 1995; Shapiro et al., 1995). These extracellular adhesion interfaces are anchored to the actin cytoskeleton by the interactions between the highly conserved cytoplasmic domains of the classical cadherins and the catenin family of proteins (Ranscht, 1994).

In addition to the classical cadherins, other vertebrate members of the cadherin superfamily have been identified. These include the desmogleins and desmocollins which are components of the desmosomes and are anchored to cytokeratins rather than the actin cytoskeleton (Koch and Franke, 1994). These molecules have a characteristic cytoplasmic domain that is distinct from that of the classical cadherins. One member of the superfamily, T-cadherin, completely lacks a cytoplasmic domain; it is linked to the cell membrane via a GPI linkage (Ranscht and Dours-Zimmermann, 1991). The desmogleins, desmocollins and T-cadherin all have similar extracellular domain structures. More divergent members of the superfamily include the protocadherins (Sano et al., 1993), which are highly expressed in brain tissue, though their functions have yet to be elucidated, and the human homologue of the *Drosophila fat* gene product, FAT (Dunne et al., 1995).

In *Drosophila*, two loci that play a role in epithelial morphogenesis, *fat* (*ft*) and *dachsous* (*ds*), have been shown to encode novel members of the cadherin superfamily (Mahoney et al., 1991; Clark et al., 1995). *ft* and *ds* encode large proteins containing 34 and 27 cadherin domains respectively, in their extracellular portions. The proximal part of the extracellular domain of Ft also contains five EGF-like repeats and two laminin-A G-domain like repeats (Mahoney et al., 1991; Patthy, 1992). The existence of a human homologue of *fat* (Dunne et al., 1995), suggests that this subfamily of large cadherins evolved before the divergence of the protostome and deuterostome lineages. The functions of these very large cadherins is not completely understood (they have not been

tested in adhesion assays); however, mutations in both *ft* and *ds* affect the morphogenesis of the larval imaginal discs.

In addition to *ft* and *ds*, a gene encoding a classical cadherin (*D*E-cadherin) has been identified in *Drosophila* (Oda et al., 1994), based primarily upon its demonstrated interactions with the catenins *D*α-catenin and Armadillo (the *Drosophila* β-catenin homologue). Subsequently, it was shown that *D*E-cadherin is encoded by the *shotgun* gene, mutations in which cause severe defects in embryonic epithelia. These findings are consistent with a role for *D*E-cadherin in the rearrangement and maintenance of epithelia, much like its vertebrate homologues (Uemura et al., 1996; Tepass et al., 1996).

Genes that encode cadherin domains have been identified in *C. elegans* in degenerate PCR-based searches (Sano et al., 1993), and at the time of writing the *Caenorhabditis elegans* Genome Sequencing Consortium (Wilson et al., 1994) has identified four putative genes that encode cadherin-related proteins (sequences are available at http://www.sanger.ac.uk/~sjj/C.elegans_Home.html). We describe here the characterization of one of these genes, *cdh-3*. Like *ft* and *ds* it encodes a large protein (CDH-3) with multiple cadherin domains; like Ft this protein contains EGF-like and laminin-A G-domain repeats. *cdh-3* reporter gene constructs are expressed in several groups of ectodermally derived epithelial cells. In addition, we observe expression in the developing epithelia and neurons of the hermaphrodite egg-laying system. We have isolated a probable null allele of *cdh-3* and shown that it affects the morphogenesis of a single cell that forms the tip of the nematode tail. Other cells that express *cdh-3* reporter constructs appear to be unaffected by this mutation, raising the possibility that other genes can compensate for loss of *cdh-3* function.

MATERIALS AND METHODS

Nematode strains and culturing

All nematode strains used were derived from the N2 (Bristol) strain except as noted. They were cultured as outlined by Sulston and Hodgkin (1988), at 20°C unless indicated otherwise.

Strains bearing the following mutations were used:

Linkage group I: *mut-2(r459)*

Linkage group II: *rol-6(su1006)*

Linkage group III: *unc-36(e251), dpy-19(e1259), nDf16*

Linkage group IV: *dpy-20(e1282), dpy-20(e1362), spe-26(hc138), him-8(e1489).*

The *cdh-3* Tc1 transposon insertion allele (*pk77*) was isolated as described previously (Zwaal et al., 1993; Plasterk, 1995), from cultures of strain MT3126 [*mut-2(r459)* I; *dpy-19(e1259)* III] using nested primers located in exon 4 (JOP1: 5′-CAGAAACCGCTC-CAATAGGTTGG-3′ and JOP2: 5′-TTGGCTACTGTAGACAC-CATGC-3′) and Tc1-specific nested primers (Zwaal et al., 1993; Plasterk, 1995). The deletion allele (*pk87*) was isolated from strain NL721 [*mut-2(r459)* I; *cdh-3(pk77) dpy-19(e1259)* III] as described by Zwaal et al. (1993) and Plasterk (1995), using the same primer set from exon 4 together with nested primers in exon 10 (JOP3: 5′-AATCTTGGATCCAGTGCTCC-3′ and JOP4: 5′-ACAGC-CAATTTGCTCGAGGATG-3′). *pk87* homozygotes were outcrossed six times with N2 prior to further analysis and the deletion allele was verified by Southern analysis.

Verification of *cdh-3* gene structure

To verify the intron-exon boundaries of *cdh-3* we sequenced across exon junctions of *cdh-3* cDNAs. cDNAs were prepared by reverse transcription of wild-type RNA using three different primers: JPDEL (5′-GTGACGTCATCACGATCAATTG-3′); JOP16 (5′-GTTGAGA-TGGCAGGATGAAC-3′); and CDH-12 (5′-CCGAATTCGATTAA-CCAGGCTCCATC-3′); followed by 30 cycles of PCR using the following primers. The reaction primed with JPDEL was amplified with two sets of primer pairs: JOP2/JOP4; and JOP13 (5′-CGAACG-ATCAGAAGACGTAG-3′)/JPDEL. The reverse transcription reaction primed with JOP16 was amplified with the primer pair JOP17 (5′-GGGGTACCATGTTTCACCTCTTCATCAACTTTACC-3′)/JOP16 and the reverse transcription reaction primed with CDH3-12 was amplified with the primer pair CDH3-10 (5′-GAGATTCGA-GAGAATGAGCTG-3′)/CDH3-12. The resultant cDNAs in each case were sequenced by linear amplification PCR (Craxton, 1993). The remaining exon junctions were verified by sequencing a *cdh-3* cDNA clone, yk153a4, kindly provided by Yuji Kohara.

Sequencing the *cdh-3* alleles

The genomic sequence of *pk77* and *pk87* alleles was determined using linear amplification of specific PCR products (Craxton, 1993). To examine the transcripts produced by *pk87* homozygotes, cDNA was prepared by priming reverse transcription of total RNA from a mixed population of *pk87* homozygotes with the JPDEL primer, located 1786 nucleotides downstream of the deletion breakpoint. This cDNA was then PCR amplified using the nested primers JOP1 and JOP3 in the first round of PCR and primers JOP2 and JOP4 in the second round. The PCR products were isolated from low melting point agarose gels and sequenced using linear amplification sequencing (Craxton, 1993).

lacZ and GFP reporter constructs

The promoter region of *cdh-3* was cloned as a 10 kb *Nco*I-*Sac*I fragment from ZK112 (Fig. 5) into pPD49.83 (Mello and Fire, 1995) to produce pJP#11. To generate the *cdh-3*::*lacZ* construct pJP#19, a 5 kb *Cla*I fragment was cloned into the *Bst*B1 site of pPD22.11 (Fire et al., 1990).

The *lacZ* transmembrane fusion construct pJP#30 was constructed by cloning a 7 kb *Bam*HI fragment from pJP#11 into the vector pPD34.110 (Fire et al., 1990). This resulted in a fusion of the fourth exon of *cdh-3* to an open-reading frame consisting of an artificial transmembrane domain followed by the *lacZ* gene. The derivative of this construct, pJP#30ΔTM, was made by removing the *Kpn*I artificial transmembrane domain cassette (Fire et al., 1990).

To generate the GFP construct pJP#38, a fusion between the GFP gene of the vector pPD95.81 (A. Fire, S. Xu, J. Ahnn and G. Seydoux, personal communication) and the first six codons of *cdh-3* was created. A *Kpn*I site was introduced into the fifth codon of *cdh-3* by amplification of genomic DNA with primers CDH3-2 (5′-TACTTTCAAGGATCTCGACTG-3′, located 270 bp upstream of the start codon) and CDH3-5 (5′-GGTACCCGTATTGTCATC-TATTCAG-3′, which contains the first four codons of *cdh-3*). The amplification product was used to replace the corresponding region of *cdh-3* and together with the 6 kb upstream presumptive promoter region contained in pJP#30, cloned into the *Kpn*I site of pPD95.81.

Generation and analysis of transgenic strains

Transgenic strains were generated by microinjection of DNA into the gonad arms of adult hermaphrodites (Mello et al., 1991). Three different marker gene strategies were used to identify transgenic animals. In each case, several transmitting lines were isolated that segregated approximately 50-60% transgenic progeny at each generation. Injections into N2 and *dpy-20(e1362)* hermaphrodites were carried out as described previously (Mello et al., 1991; Han and Sternberg, 1991). Injections into *spe-26(hc138)* animals were carried out using the plasmid pJV145 (Varkey et al., 1995). *spe-26(hc138)* homozygotes are sterile at 25ºC, but are rescued to normal fertility by the wild-type *spe-26* gene present on pJV145.

To rescue *cdh-3*(*pk87*) homozygotes, ZK112 cosmid DNA was injected at 10 μmg/ml together with 100 μg/ml of plasmid pRF4

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MTIRIFFSIFLLNHLIFFHLFNFTHQ                                                                                                  26

cadherin repeats
 1: FSEETIKFSVSEDAKLNTI....IGHLEAEIG.........YTYRLSRGN.........SKIKFDEQTLELSVSSP...LDRESEN..........AIDMLIITS..PPSIIH.............ILIDVLDVNDNSPIF            117
 2:  PIDVQRVEIPETAPIGWR....VQISGATDPDEGKNGTI..GKYELVDSLATVDTMSP.FGIVQSDGFLFLEVTGK...LDRETRDL...YSMRLT.AIDQGVPEL....SSSCH.............LNILILDINDNPPNF            229
 3:  GIRSLTLNWNGLPNTK......LFSLNATDLDSNENSL...LTYRILPSGPTSEM....FSISDENILVTQNNTEC...LQRCEFVVE.........ARDSGV.....PPLSTTLN.............IVVNMEYGNEHEPNINIRF        346
 4:  YPSDYPFIIVQPEDVNGKTLA.ILSITDSDGPLGANST...IWIENGNEQSI.......FSLISRQSINILTVKHVENANQE........YILEFR.ANDGQS......PADRITRKELKIFFKKYVKSQIH                          442
 5:  VERESHVTVEKDTVPGSF....VAHVETNCTDM........CSFELANSDV.........FKIDPFNGIIVTSSILPEGVTS.........YHLPIRIHLP.........PPSTQLVEAD..........VFVKVIQESVPKNLI            548
                                  RSSESPIHLKRAYTFTTWQDVSLGTVIGRLPKAQIYSTIDTVSELGVFPDGSVFVGKTITSDFVTLPVTLVNRNTTQTSI.........ITLIVKPLNQHSPIC            633
 6:    QITEIHVLENAPIGTI....FGRIQARDEDSGLSGV...VSYKILTKSDDYDGI...FHLDSTSGSLRSLKA.....FDAEKKRS...YTFEYE.AKDLGT......PSKTTNCP...........ATIFIEDVNDNVPKF            738
 7:  GSRYYTATISGKSNET......VAIVQANDNDVDVKNQK..LQYHLLNYHDF.......FQLDKETGKVTTIQDVPMTWQR.........LNISIS AVNMDSERFLQ..SKTFLLVTVTSSSKLA....VQLNSGNLIRIFK            850

    NDKIGEKVGHLDIASSETVYWSTLDPRLHVDSSGNIILIRRNAKQASTGFDIILTSENGEKTEKVNFEVEFVDSERSEDVEKVMDIVLNENTTEVSNLMNDWKNWKISRVILENANNSGNNTFFLE                          979
    HKKLWRTKNATVSNA IILESEDQEGSPKSFKLLHVTTSPSPSSESSCISPAHLISPPSTVPLPSNCSNVKLQNLKTSLQIHENNLLIPTQSELINHVDLVSTQNSDMKPFMMTLIKDYLSEDVRF                        1107

 8:  STNNVLMLLSSIHPIGTS....FGRVTAESGYR.......  RYYIVGTDK........ISIDADTGELILKER.....FYRNLNDI...LIVAVIPKGIAKAKITIEVIEDRLILPQS
                                                                                                                NFFIPSPPSFNSKSKIGKIPIDRDD.VTIDVIDEHFYVRNF            1233
    EIFVKRHFIPNSNFYDLKGTVKKGKLSAPISVTLFFGEKMKSREIR
 9:   ENELMFEIEENSPIGTV....VGVVPNSDTTK........YRLVDPTCG.........LLIDQE.GIIRITTV.....FDRENTSLLKTKMIE..............PSENRIWN..........LLIFIADVNDNKPKI            1368
10:           LNAPGRIIVYDDLNYKLEWEDLDAIASDVS...................FSIVDGDVFGN...................LEIE.....DSGVISLNSIPNES..............FNATIRIYDNRPPFKVHFDDVTI  1450
11:  EFQVTQKLRAVTCEDAEFWMFFGNEDVGMLIASEIVTWRIVPQIGSDS...........FKIDPITGIIQSTPN.....TKPTSDIAK..LKIQ...AISYDGER.....................VGFCDVKIHIDKAAFVENVVL    1550
12:  SNGTFEFNISETADRFTE....VGKIVILGAGLEGSVFR.IQDNDYN............FTISPFDGTIFINSP.....LDFENIKT...YRFNIT.AGKSTSQ.....................VIIHVTDENDEAPRF            1648
13:  ITGDVVNLKVLEELDTVSYPLIIGSSIAEDLDEGQNGL..VTYSILSGNTSL.......FAVNSTTGDILSLIP.....LDREESSLHE...LLIE.AKDAGI......PSLSATSK..........ILIHVGDINDNTPEF            1756
14:  ELSSYFIKISENSKIGSK....IIRILATDKDKDAE....LQYSLESNDEITIP.....FRINVATGWITVAGK.....VNREENEE...FRFFVK.VTDGEK.......SSKVI.............VEIHVEDFNDNHPMI            1857
15:  NDRNSDIFVPDPTRSVEI....IHVINVHDLDKSDH....LKFSLNNSN..........LNLSEN.GEITLKSP.....LQTAVP.....VRVTV..SDDAGHVAF.....................MEYLFHPHSRKHFPVF            1948
16:  VEKLDTVSVREH..........DEQELAVFKANGDS....IRYSIVSRCSDH.......LEMEKSTGILKTKSS.....LDAEEYSE...CLVFII.ATTYFDNK...PLSTITK.............ATIKIVDINDNSPRF            2055
17:  DQQLYRFNVTENSGPKL.....IGHVIARDIDRSSR....VFYEIVGGDANHE......FMVTES.GQIESVRD.....LDRETKSE...YHLIVE.AIDDGK.....PRRRGNTT.............VIVTVLDEDDNAPRF            2146
18:   SRIFHVEVPEDVRIGEP....VIQLSASDADEHSNHR..FELDGGGEGIP........FRVDENTGMVFVNDS.....LDFEKKQ....YRIKVK.LTDGAWLI...ETS..................LFVNVKDVNDNAPIF            2245
19:  EKPEYLFISEENSAE.......IGQFHASDMDSENNGK.. RYSVTSPY..........FKIEPSTGVLSRFRQ.....QLPQPL.....MSLKVT.ATDHGVPRLQKT..................VLAHLVDKS                2333

SFGKIKQRRIRETTKVGDVIGKKIDSGATIFPLDVATVTRDGDVVLKKNATQFWILENDTIYEFVKTDAMESTKNENITLNITSDISMNSDNFKVLRNGSLIVFGFSGNQAHLKIQCDDGFWPKQDRKIINLVVNNLDADRNSFPL            2479
ARQPTIRKSMSLPKTMILNIPFDSPTGTIIWKNLENAVQYMENQKNVNFSNGSKNLILKTPLEETMQIDIFGQNFERSALTITPNRSLMACPVFQKNFYFFESVANLDSKHPTEIHNFGWSSDEIKGCQIDIFDKTHLFYQNGSSL            2624
IFLKPLLPGTYQFSLQIKSQSDSKIRSACHVVVTVIPPTNLTTWNIPSVIFATRNYNIPNLFHLPSGYSLSSDQRTFSLIGSGTGKNISKLSSGVYQVNVVGKDEKKEIVRILLDDVADDVTSKDIEYHVVSSTLSNLKIPTPIDV            2761
ECFPRTEENLYEITKDCRLLFNSDVINTTIPVVTSPANSTWNLRIINESPETVKSLENNAVSLEIITQKSSIPRLITDLRVTYSDMKIYCLGTWQTSEDIKYHITFVIVDRNGVVIEESEARQTLTSFLKKHRPGYLDFVDFDKDP            2917

EGF-like domains
CDGVTCIQKNSTCQPTLVGDSASRLVSRSSSVIFDLPLKKLTARCFCSSGID....CYDDTTNETIQKTQKINVITT......                                                                          2990
CDDIDCGPRGK.C.....................FMEESSQPICRCGQGFESMYSCERADDVFSMSTGGSVEISVRNGTSHL                                                                          3050

laminin-A G-domain
LKCSENCDGRDIQKIEFDFRTVQLEKSELFRVDFGKQVALIELIGGSLTFSITDAYARPIETRIEKRVNDGRWHRLLFQMSEDGRRISIQVNGRGKEVKSRVPLQMLFTAKKIQLMTPAAFCFRRLLAQNQFVHPILNRNKFFEIS            3196

STGTSRNECQFDSIQSGSGGGFRLFSNFSNTTTLI LLITLALISL IGFSVCLLAI                                                                                                    3250

cytoplasmic domain
RRRWRQKSPGDQKQTERSNGWTGHVMPRRRGHINRSMVKSPDDDTYDVATVYGMKSTSTDDITHIYTSSSSRRYQPPTAPSYRRDGHINMAYL                                                              3343
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Fig. 1. *cdh-3* encodes a member of the cadherin superfamily. The amino acid sequence of the putative CDH-3 protein is shown. The signal sequence and the transmembrane domain are underlined twice. The cadherin domains are numbered and aligned for optimal similarity with each other. The amino acid coordinates are indicated on the right of each line. Those residues that appear frequently at similar positions in these domains and in cadherin domains found in other members of the superfamily are underlined once. The amino acid sequence was derived from Genefinder analysis of cosmid sequence ZK112 as well as visual inspection of the sequence.

(Mello et al., 1991). Transgenic arrays were chromosomally integrated as described previously (Way et al., 1991; Mello and Fire, 1995) using a dose of 4000 rads delivered by a ^{137}Cs X-ray source.

To examine the expression of the *cdh-3*::*lacZ* reporter construct, transgenic animals were histochemically stained as described by Perry et al. (1993) and observed under Nomarski optics. The expression of the *cdh-3*::GFP construct was examined by placing transgenic animals on a 5% agar pad containing 0.1% 1-phenoxy-2-propanol and observing them under ultra-violet illumination.

RESULTS

cdh-3 encodes a cadherin-related protein with similarity to Ft and Ds

The sequencing of chromosome III by the *C. elegans* Genome Sequencing Consortium (Wilson et al., 1994) revealed a gene, ZK112.7 (EMBL accession number, L14324), whose predicted translation product showed similarity to members of the cadherin superfamily. Since two other putative cadherin-related genes had already been identified in *C. elegans* we named this gene *cdh-3* (*cadh*erin-related).

In order to verify that the predicted *cdh-3* gene structure, and hence the predicted translation product, was correct we sequenced the putative exon boundaries of *cdh-3* cDNAs. This analysis confirmed the exon-intron structure as predicted by the Genome Consortium. The CDH-3 protein (Fig. 1) begins with a 26 residue signal peptide (von Heijne, 1986) followed by the first cadherin domain. A putative transmembrane domain is located at residues 3226-3250. *cdh-3* is, therefore, predicted to encode a single-pass, transmembrane protein which is shuttled to the membrane via the function of the signal peptide. To confirm this function of the predicted N terminus of CDH-3, we took advantage of the fact that extracellular β-galactosidase has no activity (Fire et al., 1990). The construct pJP#30 (Materials and Methods) was expected to produce a fusion protein consisting of the first 143 amino acids of CDH-3 fused to a synthetic transmembrane domain followed by β-galactosidase. The fusion protein produced by this construct is expressed in a punctate fashion, as was found for other fusion proteins expected to be shuttled to cell membranes (Fire et al., 1990), and appeared to localize to the nuclear membranes and plasma membranes of the cells that

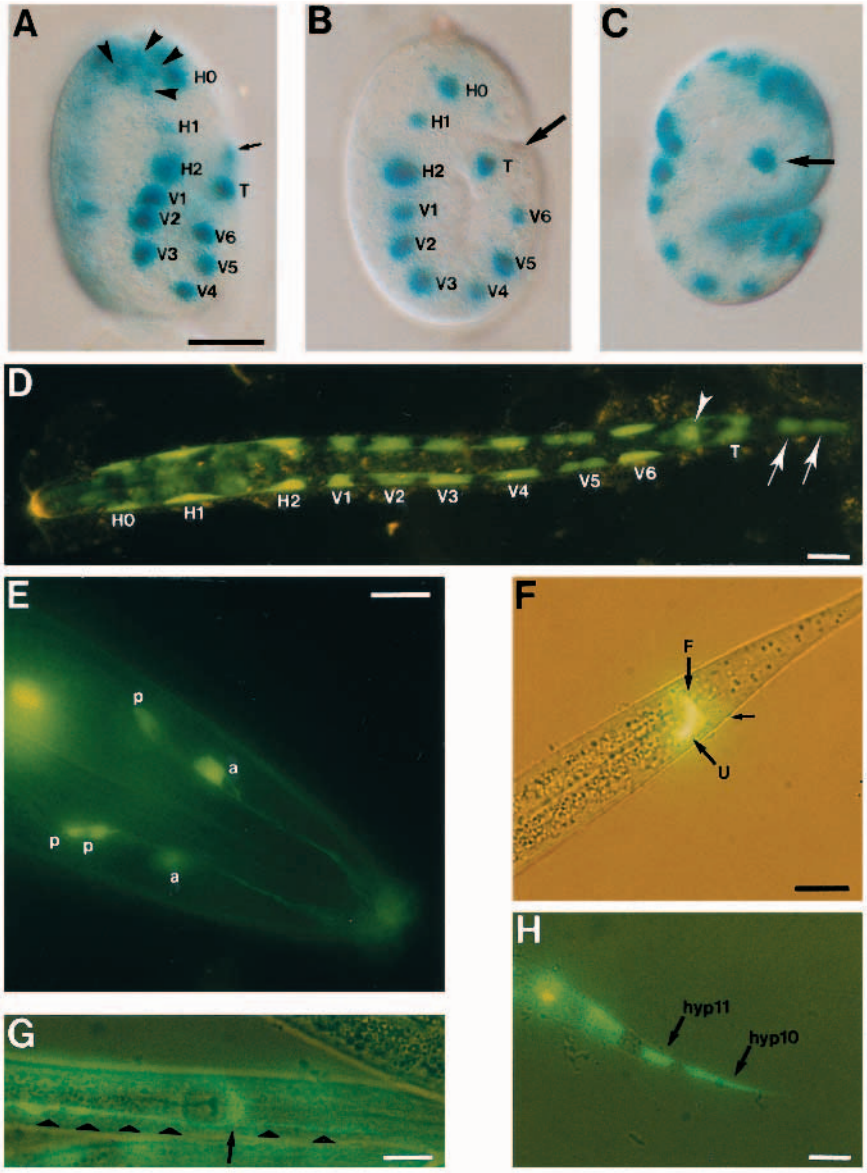


Fig. 2. Cells that express *cdh-3* reporter constructs during embryonic morphogenesis and postembryonic development. Two different reporter constructs were used (see Materials and Methods): (A-C) animals carrying extrachromosomal copies of the pJP#19 reporter construct; (D-H) animals carrying extrachromosomal copies of the reporter construct pJP#38. In each case the bar indicates 10 μm. (A) Right lateral view of a 1.5-fold embryo. Dorsal is to the left and anterior is to the top. The *lacZ*-expressing nuclei of the right seam cells are labelled. The small arrow indicates the position of the *lacZ*-expressing hyp10 cell. The arrowheads indicate the positions of four *lacZ*-expressing nuclei whose positions are consistent with those of arcade cells. (B) A later stage embryo (2-fold) showing continued *lacZ* expression in the seam cells. The arrow indicates the position of the rectum. (C) A ventral view of a 1.5-fold embryo, showing the large *lacZ*-expressing excretory cell nucleus (arrow). (D) Dorsal view of a newly hatched L1 larva. The GFP-expressing seam cells on the left side are labelled; the numbering corresponds to those of their right homologues. Note that V5L and R have divided, but the other seam cells have not, indicating that this animal was photographed approximately 5 hours after hatching. The F and U cells (just out of the focal plane) are indicated with an arrowhead and the hyp11 and hyp10 cells by arrows. (E) A right lateral view of an adult hermaphrodite showing GFP expression in the arcade cells. In earlier stages it is more difficult to resolve these cells. The anterior (a) and posterior (p) arcade cell bodies are indicated. The other arcade cell bodies (three from the posterior arcade and one from the anterior arcade) are not visible in this focal plane. (F) Lateral view of the rectum of an L2 larva. The GFP-expressing F and U cells are indicated by large arrows. The small arrow shows the position of the anus. (G) Residual GFP expression in the excretory cell of a newly hatched L1 larva (right lateral view, anterior is to the right). The arrow indicates the cell body. Triangles indicate the anteriorly and posteriorly extended lateral processes; only the right lateral processes are visible in this focal plane. (H) GFP expression in the hyp10 and hyp11 cells of the tail of an L1 larva.

express it (data not shown). If the transmembrane domain is removed, without altering the reading frame (pJP#30ΔTM), no activity is detectable (though the transgene is still present, as determined by PCR amplification of pJP#30 DNA). This indicates that the fusion protein is now secreted extracellularly and is hence inactive. The GFP construct pJP#38 (see Materials and Methods) produces a fusion protein consisting of the first four predicted amino acids of *cdh-3* fused to GFP and thus does not contain the predicted secretion signal. This construct produces only cytoplasmically localized GFP (Figs 2, 3), suggesting that there is not another secretion signal upstream of the one identified. Thus, the N terminus of CDH-3 is as predicted in Fig. 1 and is sufficient to shuttle the protein to the plasma membrane.

The extracellular portion of the protein contains 19 cadherin domains, which like those found in Ft and Ds, contain most of the key amino acids contained in the cadherin domain consensus sequence, including the Ca^{2+}-binding sites (Overduin et al., 1995; Shapiro et al., 1995). The extracellular portion of CDH-3 also contains two EGF-like repeats followed by a domain that shows similarity to the laminin-A G-domain repeat which is also found in neurexins, agrin, perlecan and the *Drosophila* Slit protein (Fig. 1). Both domains are also found in similar positions in Ft and FAT, although these proteins have five EGF-like repeats and two laminin-A G-domain repeats. The cytoplasmic domain of CDH-3 is short (only 93 amino acids) and does not significantly resemble the Ft, Ds, or classical cadherin cytoplasmic domains.

cdh-3 reporter constructs are expressed in ectodermal cells throughout development

To determine where *cdh-3* is expressed we made constructs that placed *lacZ* and GFP reporter genes under the control of 6.0 kb of *cdh-3* upstream sequences. A number of independent

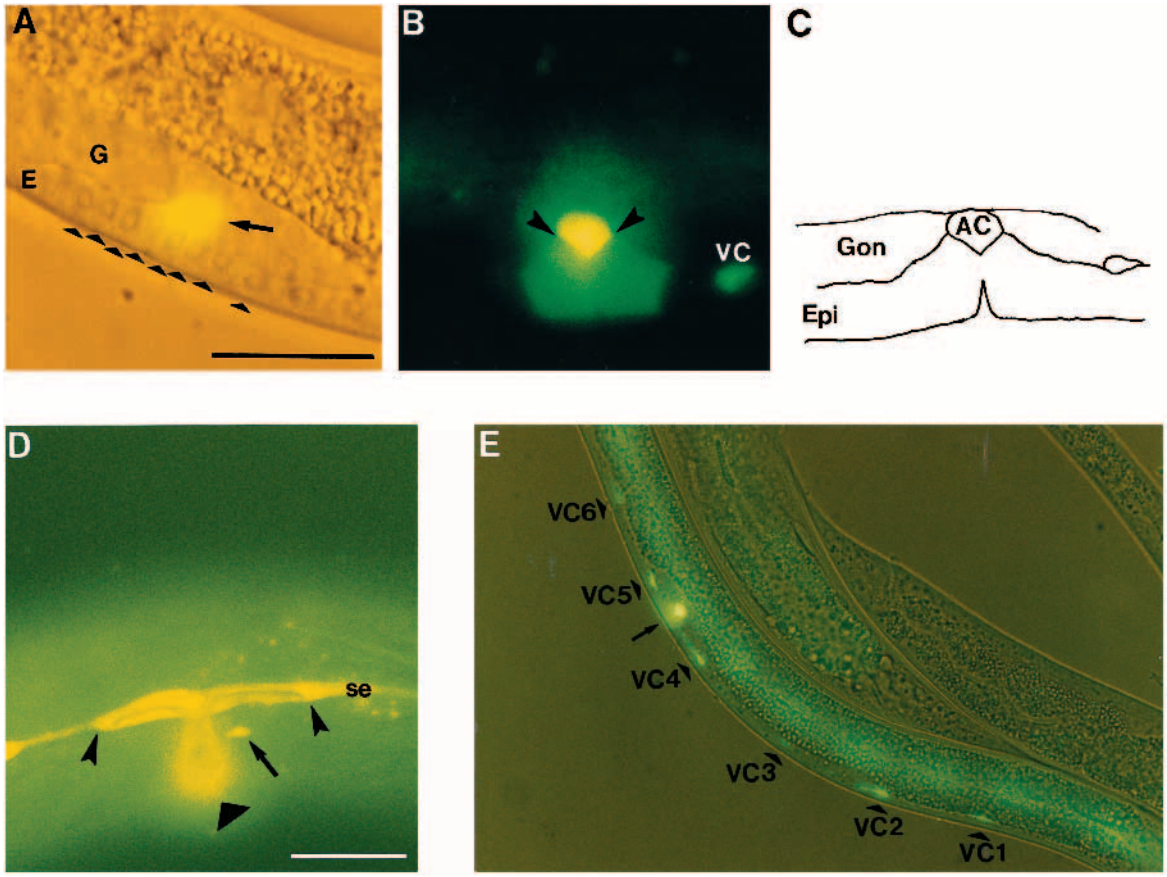


Fig. 3. *cdh-3*::GFP expression in the egg-laying system of animals carrying extrachromosomal copies of the pJP#38 reporter construct. In each case the bar indicates 10 μm. (A) An L3 larva. The developing gonad (G) and ventral epidermis (E) are indicated. The arrow indicates the GFP-expressing anchor cell. The triangles indicate those nuclei of the developing vulva that are visible in this focal plane. (B,C) GFP expression beginning in the invaginating vulval epidermal cells of an L3 larva. Note the anchor cell (AC) between the two nearest vulval cells (arrowheads). VC indicates the GFP-expressing VC5 neuron (see E). The developing gonad (Gon) and ventral epidermis (Epi) are indicated. (D) Left lateral view showing GFP expression in the utse cell of an L4 stage larva. Arrowheads indicate the positions of attachments to the seam cells (se). The GFP-expressing vulval epidermis (out of the focal plane) is indicated by the triangle. The left HSN is visible (arrow), though its anterior process is not. (E) GFP expression in the VC neurons of an L3 larva. Anterior is to the right. GFP expression in the developing vulva is indicated by an arrow.

transgenic lines were generated for each construct; all gave identical patterns of expression, differing only in their intracellular localization. Sequences placed between the *cdh-3*-derived part of each construct and the reporter gene determined the intracellular localization of the reporter protein (Fire et al., 1990; see below). To control for the possible influence of the marker genes used to identify transgenic lines, we used three different marker genes (see Materials and Methods). We did not observe any effect of the marker genes upon the expression pattern. To determine the expression pattern in embryos we used a construct, pJP#19, which produces a nuclear localized *cdh-3*-β-galactosidase fusion protein, since it was easier to identify cells within the restricted space of the eggshell. Throughout the rest of postembryonic development we used a *cdh-3*::GFP construct, pJP#38.

At the beginning of embryonic morphogenesis, all ten pairs of lateral epidermal cells, the seam cells, express *cdh-3*::*lacZ* (Fig. 2A,B). *cdh-3*::GFP expression in these cells is observed at hatching and throughout subsequent postembryonic development (Fig. 2D). With the exception of H0, the seam cells are blast cells: each divides in a stem-cell-like pattern, generating an anterior (posterior in the case of H1) daughter which fuses to the multinucleate epidermal syncytium, hyp7, and a posterior daughter that becomes a seam cell (Podbilewicz and White, 1994). This general pattern is modified in specific pairs of seam cells, which generate neuroblasts at specific developmental stages, or result in the generation of additional seam cell lineages (Sulston and Horvitz, 1977). Seam cells lose and reform their contacts with each other as they go through each round of cell division. During the last larval stage (L4) the 15 pairs of seam cells generated during larval development fuse to form two continuous lateral syncytia, surrounded by hyp7. *cdh-3*::GFP expression correlates with seam cell identity during these postembryonic divisions; it is not observed in daughters that fuse with hyp7 or adopt other fates.

In embryos undergoing morphogenesis, *lacZ* is expressed in a single large nucleus, whose size and position is consistent with that of the excretory cell (Fig. 2C) (Sulston et al., 1983). This identification is reinforced by the observation that in many newly hatched L1 larvae, low levels of GFP expression are visible in the excretory cell (Fig. 2G). This cell, which is involved in osmoregulation, extends long processes along the

basal faces of the left and right seam cells during embryonic morphogenesis (Nelson et al., 1983). We observe this expression of *cdh-3*-reporter genes only during late embryogenesis and in some newly hatched L1s (the latter may be due to perdurance of the GFP fusion protein), but not at later stages. This is suggestive of a role for CDH-3 in excretory cell process outgrowth, especially given that this outgrowth occurs along the seam cells, which also express *cdh-3*.

Several other cells expressed the *cdh-3* reporter constructs during embryonic morphogenesis; as in the seam cells this expression continued upon hatching. In the tail we see two cells, hyp10 and hyp11 (Fig. 2H), which show strong GFP expression that persists only during the first larval stage. These cells, together with hyp8 and hyp9, form the tail, with the binucleate hyp10 forming the very tip of the tail.

We observed strong expression in cells that form interfacial epithelia between the intestinal epithelium and the epidermis. Two cells that form part of the rectal epithelium, designated F and U, express *cdh-3*::GFP during embryonic morphogenesis (Fig. 2F) and throughout larval development. F and U form two halves of a toroid, which is the second in a series of three toroids that make up the anus. In the anterior of embryos undergoing morphogenesis we see several cells expressing *cdh-3*::*lacZ*, and in larvae and adults, GFP expression is seen in nine cell bodies located just anterior to the first bulb of the pharynx. Processes extend anteriorly from these cell bodies and terminate at the level of the buccal capsule. Based upon the location of the cell bodies and the morphology of the processes, we identified these cells as the anterior and posterior arcade cells (Wright and Thompson, 1981). These multinucleate cells, like those of the anus, form a toroid that interfaces between the pharynx and the epidermis.

cdh-3::GFP expression in the egg-laying system

The egg-laying system of hermaphrodites includes several specialized epithelia. The uterine epithelium is linked to the epidermis via the vulval epidermal cells, and egg-laying is controlled by muscles associated with these two structures. Two sets of neurons synapse onto the vulval muscles: the two hermaphrodite-specific neurons (HSNs) and the six VC motor neurons (which also innervate the body wall muscles). The HSNs are required for egg-laying, but functions have yet to be assigned to the VC neurons (Sulston and Horvitz, 1981; Li and Chalfie, 1990; Garriga et al., 1993).

We observe *cdh-3*::GFP expression in the developing hermaphrodite vulva (Fig. 3A-D). This structure is produced by the invagination of a set of ventral epidermal cells centred around the anchor cell of the somatic gonad (Sternberg and Horvitz, 1986). Morphogenesis of the vulva begins during the L3 larval stage and ends during the L4 moult (Sternberg and Horvitz, 1986). We first observe GFP expression in the anchor cell in L3 larvae (Fig. 3A). A little later we see expression in those vulval cells that are closest to the anchor cell and are beginning to invaginate (Fig. 3B,C). Just prior to this invagination, the anchor cell moves between the two innermost vulval cells and remains positioned between these cells throughout the rest of the L3 stage. As vulval morphogenesis continues all of the cells that invaginate to form the vulva are expressing GFP (Fig. 3D). During this period, the uterine epithelium closest to the invaginating vulval cells begins to express *cdh-3*::GFP and the anchor cell fuses with the multinucleate uterine seam cell (utse), which also begins to express *cdh-3*::GFP (Fig. 3D). During the late L4 stage, this cell appears to form attachments to the seam cells located on either side of the vulva. Expression continues in these cells into the adult stage, though at somewhat reduced levels, which may perhaps be due to perdurance of the fusion protein, since older adults show much reduced fluorescence compared with younger ones (data not shown).

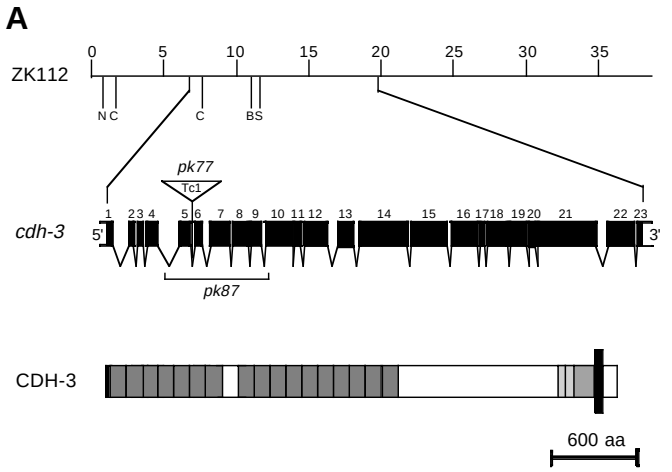
During the time that the vulva is forming, we also see *cdh-3*::GFP expression in the six VC neurons located in the ventral nerve cord (Fig. 3E) and the two HSNs located just posterior and dorsal to the developing vulva (Fig. 3D). These cells begin to extend processes at about this time, and GFP expression continues in these cells and their processes throughout the remainder of larval development and into adulthood. The VC neurons extend processes along the ventral cord to the vulva (Li and Chalfie, 1990), and the HSNs extend processes ventrally, past the vulva, into the ventral cord and ultimately into the nerve ring (Garriga et al., 1993), the site of a dense bundle of synapses that runs circumferentially around the pharynx. The HSNs also form varicosities and a small branch at the vulva, where synapses with the vulval muscles occur (Garriga et al.1993). The VC neurons form branches at a similar point (Li and Chalfie, 1990). We were unable to resolve these branches and varicosities satisfactorily due to the intensity of the vulval fluorescence.

We have also determined the expression pattern of the pJP#38 construct in males. We see expression in the male tail and several male-specific neurons (data not shown), however the expression pattern is complicated and we have yet to determine precisely which cells are responsible for the expression observed.

A deletion mutation in *cdh-3* affects hyp10 morphogenesis

To determine the role that *cdh-3* plays in the epidermis, we isolated a deletion allele (*pk87*) of *cdh-3* using the transposon-based technique developed by Zwaal et al. (1993). Sequence analysis of the *cdh-3* locus in *pk87* homozygotes shows that the deletion removes 2664 bp (Fig. 4). It removes most of intron 5 and half of exon 10, plus the intervening DNA, and thus removes at least five cadherin domains. The deletion removes the normal acceptor site downstream of the donor site of intron 5; this ‘orphaned’ donor site could splice to the acceptor site of intron 11, resulting in the skipping of all of exon 10 and generating a frameshift. Alternatively, the donor site of intron 5 might splice to cryptic acceptor sites within the sequence of exon 10. To distinguish these possibilities we sequenced cDNA derived from *pk87* homozygotes. This revealed that at least in a substantial proportion of the *cdh-3(pk87)* transcripts, the donor site of intron 5 splices to a cryptic site in exon 10 resulting in a frameshift (Fig. 4B). This should result in the production of a severely truncated protein that would presumably be secreted, since it lacks any transmembrane sequences. Such a mutation might behave as a null mutation, especially given that many transcripts in *C. elegans* that encode severely truncated proteins are actively degraded by an mRNA surveillance system encoded by the *smg* genes (Pulak and Anderson, 1993).

Animals homozygous for *pk87* are viable and have wild-type brood sizes. They do not show any detectable defects associated with abnormalities in the vulval or rectal epithelia. Males appear morphologically normal and are able to mate. We examined the



B

```
        D  R  E  T  R  D  L  Y  S  M  R  L  T  A  I  D
8001    GGATAGAGAGACAAGGGATTTGTACTCAATGAGACTAACTGCTATAGATC

        Q  G  V  P  E  L
8051    AAGGAGTTCCTGAGCTgtaagtctttttattcaatttaaaaattttaaac

8101    gaacattgctttaaaaaaaattttctgacata..................

        ..................AGCAAAAGATCTTGgtgagtcaactgaaaaatg

10651   tttattttcaaaatcgaaacctttccagGAACCCCATCAAAAACGACAAA

10701   TTGCCCTGCAACTATTTTCATAGAAGATGTTAACGACAATGTTCCAAAGT

         F  G  S  R  Y  Y  T  A  T  I  S  G  K  S  N  E  T
10751   TTGGATCCAGATATTATACTGCCACAATATCTGGTAAATCCAATGAAACT

         V  A  I  V  Q  A  N  D  N  D  V  D  V  K  N  Q  K  WT
               D  C  A  S  K  STOP                          pk87
10801   GTAGCGATTGTGCAAGCAAATGATAATGATGTGGATGTCAAAAATCAAAA
```

Fig. 4. (A) The *cdh-3* gene. The top line represents the cosmid ZK112. The coordinates, given in kilobase pairs, are derived from the sequence of ZK112 as determined by the *C. elegans* Genome Sequencing Consortium. The locations of restriction sites used to make reporter constructs are indicated below the line. N, *Nco*I; C, *Cla*I; B, *Bam*HI; S, *Sac*I. The location of *cdh-3* on ZK112 is shown. Exons (boxes) and introns (horizontal lines) are shown as predicted by Genefinder and verified by us (see Materials and Methods). The position of the *pk77* Tc1 insertion and the extent of the *pk87* deletion are indicated. The CDH-3 protein is represented below the gene. Shaded boxes represent cadherin repeats. EGF-like repeats are represented by diagonally shaded boxes and laminin-A G-domain repeats by cross-hatched boxes. (B) The *pk87* allele produces cryptic splicing. The coordinates refer to those of ZK112 (see above). The derived amino acid sequence is shown above the DNA. The wild-type exon sequence is in upper case and the intron sequence in lower case. The region deleted by the *pk87* allele is underlined. The dots represent sequence omitted to save space. The cryptic acceptor site used in *pk87* homozygotes, as detected by RT-PCR, is indicated by double underlining. The frameshifted amino acid sequence derived from this cryptic splice is shown beneath the wild-type sequence. 'STOP' indicates and opal stop codon.

HSN and VC neurons by introducing the *cdh-3*::GFP construct into *pk87* homozygotes. As far as we were able to tell we were unable to detect any defects in the postitions of the cell bodies or processes of these neurons. However, due to the proximity of the fluorescence of the vulva we were unable to determine whether branching of neuronal processes onto the vulval muscles was normal. The excretory cell process outgrowth was similarly examined and we could find no defects associated with this cell.

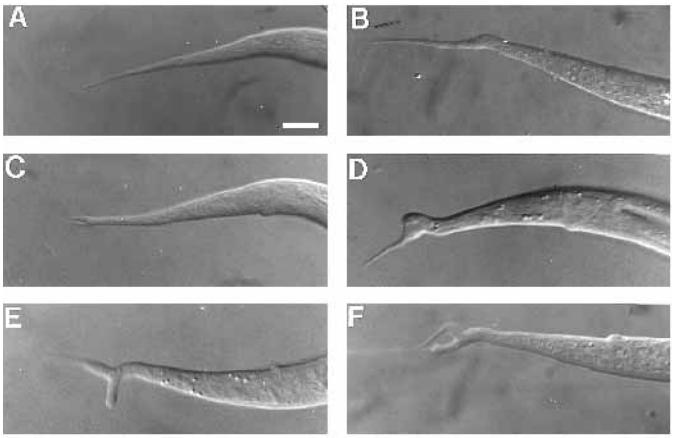


Fig. 5. Defects in hyp10 morphology displayed by *cdh-3(pk87)* homozygotes. (A) Tail of a wild-type L1 larva. (B-F) Examples of the range of defects seen in *cdh-3(pk87)* homozygotes (see Table 1). (B) A 'kinked' tail. (C) A 'forked' tail. (D-F) Variable ectopic protrusions.

Table 1. hyp10 morphology is affected by the *cdh-3(pk87)* mutation

	hyp10 morphology§			
Genotype	Wild-type	Kinked	Forked	Protrusions
cdh-3(+)	100% (115)	0% (0)	0% (0)	0% (0)
cdh-3(pk87)	24% (40)	46% (79)	10% (17)	20% (34)
cdh-3(pk87); pkIs234	97% (109)	0% (0)	1.8% (2)	0.9% (1)

§Examples of the tail morphologies are illustrated in Fig. 5.

We analyzed seam cell morphology using the MH27 antibody, which recognizes a component of the adherens junctions of epithelial cells (Podbilewicz and White, 1994). The seam cell morphology appeared normal, as did the formation of the left and right postdeirids. The postdeirids are neuronal structures derived from the postembryonic lineage of V5L and R (Austin and Kenyon, 1994). The commitment to form the postdeirid is dependent upon signalling between the posterior daughter of V5 and its neighbours, the posterior daughters of V4 and V6. This signalling is contact dependent, suggesting that it occurs via a cell recognition/cell adhesion pathway (Austin and Kenyon, 1994). Since postdeirid formation is unaffected in *pk87* homozygotes, we conclude that *cdh-3* does not play an essential role in this process.

cdh-3(pk87) homozygotes do, however, show variable defects in the morphology of the tail tip, the structure which is formed by hyp10. The defect ranges in severity from animals with approximately normal tails to animals with marked ectopic protrusions (Fig. 5; Table 1). Despite its abnormal morphogenesis, hyp10 appears to form wild-type connections with hyp9 and hyp11 and the abnormal tail morphology does not appear to interfere with the behaviour of affected animals. The phenotype appears recessive and the heterozygous combination of *pk87* with the deficiency *nDf16* (which removes *cdh-3* plus loci surrounding it) results in identical tail defects to *pk87* homozygotes, showing that *pk87* behaves like a null mutation.

To confirm that this phenotype results from the *pk87* allele and not from some other linked mutation induced during the isolation of *pk87*, we attempted phenotypic rescue of *pk87*

homozygotes with a wild-type copy of *cdh-3* present on cosmid ZK112. Since the tail phenotype is incompletely penetrant, we generated a line of *pk87* homozygotes carrying multiple copies of ZK112 as an integrated array (*pkIs234*) and examined hyp10 morphology in this line compared to the *pk87* parental line. We found that *cdh-3(pk87)*; *pkIs234* animals displayed normal tail morphology compared to the parental *pk87* strain (Table 1), indicating that the tail defect could be rescued by a cosmid carrying a wild-type copy of *cdh-3*.

DISCUSSION

Members of the cadherin superfamily have been shown to function in the morphogenesis of epithelia in *Drosophila* and vertebrates. We have shown that one member of this superfamily in *C. elegans*, *cdh-3*, is involved in this process in a cell that undergoes a change in shape during embryonic development. *cdh-3* encodes a protein containing many cadherin domains in its extracellular portion. On this basis it bears most resemblance to the *Drosophila* cadherins Ft and Ds. These three proteins, together with the human Ft homologue, define a new subfamily of cadherins that clearly existed prior to the radiation of the metazoan phyla. How functionally similar these proteins are remains to be determined, but our results together with those of Mahoney et al. (1991) and Clark et al. (1995), indicate that their function is confined to the morphogenesis of ectodermally derived epithelial cells. This contrasts with the *Drosophila* classical cadherin, which is required in all epithelial cells (Uemura et al, 1996; Tepass et al., 1996). It will be interesting to determine whether the same holds true for the other *cdh* genes, since they too appear to encode similar large cadherins (our unpublished results; *C. elegans* Genome Sequencing Consortium data).

The expression patterns of the *cdh-3* reporter gene constructs suggest a role for *cdh-3* in cell recognition and adhesion. The expression in the seam cells throughout development indicates that *cdh-3* may function in maintaining the integrity of the lateral epidermis. It may do this either by acting as a recognition molecule allowing seam cells to recognize each other after each cycle of division, or by maintaining the interaction between the seam cells once they have contacted one another. Evidence for this latter possibility is provided by considering *cdh-3*::GFP expression in other cells. The arcade cells and rectal epithelial cells do not undergo alterations in their contacts with other cells during postembryonic development, yet they strongly express *cdh-3*::GFP during this period. This result argues that CDH-3 functions to maintain the attachment of cells to one another, though it does not rule out function as a cell recognition molecule that coordinates de novo cell interactions, such as occurs during seam cell development.

The genesis of the egg-laying system requires several sets of cell recognition events, all of which occur during the expression of *cdh-3*::GFP. Firstly, the anchor cell must interact with and invaginate between the two nearest VPCs, an event which takes place soon after we observe GFP expression in the cells involved. Secondly, the vulval epidermal cells invaginate and form a connection with the uterus, and the utse cell makes contacts with the seam cells. Finally, the neurons that innervate the vulval muscles extend processes that are dependent upon the presence of vulval epidermal cells (Li and Chalfie, 1990; Garriga et al., 1993). The timing of expression of *cdh-3*::GFP in all the cells involved in this series of interactions suggest a possible role for CDH-3 in the formation of the egg-laying apparatus. Again it may be acting to coordinate cell interactions and/or to maintain cell interactions during the subsequent morphogenetic movements of the vulval epithelium.

In summary, the expression pattern of *cdh-3* determined by the reporter constructs is consistent with a role in coordinating and maintaining cell interactions. Moreover, since we have not observed cells expressing *cdh-3* surrounded by cells that do not express *cdh-3* these interactions appear to be homophilic in nature.

Despite the roles for *cdh-3* suggested by the expression pattern of the *cdh-3* reporter construct, the expressing cells in *pk87* homozygotes appear unaffected, with the exception of hyp10. This finding raises the possibility that loss of *cdh-3* function in most of the expressing cells can be compensated for by other genes. Redundancy has been observed for many of the genes that encode cell adhesion molecules involved in *Drosophila* neuronal development (Bate and Broadie, 1995), but more relevantly, redundancy also appears to exist for both *ft* and *ds* function. Both genes are expressed in ectodermally-derived tissues throughout embryonic development, but no embryonic phenotype has been observed for any allele or combination of alleles (Mahoney et al., 1991; Clark et al., 1995). This suggests that other genes are able to compensate for *ft* and *ds* loss of function during embryogenesis.

The existence of at least five other cadherin-related genes in *C. elegans* could potentially compensate for loss of *cdh-3* function. Indeed, a recent survey of gene expression patterns based upon fluorescence in situ hybridization revealed that one of these cadherin-related genes (F25F2.2) is expressed in the developing vulva (Birchall et al., 1995). The approach used to generate the *cdh-3(pk87)* homozygotes is currently being applied to these other genes to determine whether any of them can functionally substitute for *cdh-3*. Another possibility is that other, unrelated genes could compensate for *cdh-3* loss of function. Screens for mutations that synergize with the *pk87* mutation should prove fruitful in distinguishing these alternatives.

A function for *cdh-3* in hyp10 morphogenesis

cdh-3 function in hyp10 is indicated both by the expression of *cdh-3*::GFP in this cell and the finding that loss of *cdh-3* function in *pk87* homozygotes results in a variable defect in the morphology of this cell. hyp10 has two neighbours, hyp9 and hyp11. During postembryonic development hyp9 shifts posteriorly (our unpublished observations), so that in the adult both hyp9 and hyp10 form the tip of the tail. Interestingly, the tail defect displayed by *pk87* homozygotes is most severe in early larvae where the tip of the tail is formed only by hyp10, suggesting that in later stages the hyp10 defect is compensated for by the contribution of hyp9 to the tail tip.

The abnormalities associated with hyp10 in *cdh-3(pk87)* homozygotes appear analogous to the defects in *Drosophila* leg imaginal disc morphogenesis caused by mutations in *ds* (the defects caused by mutations in *fat* share some features of *dachsous* mutations, but the interpretation is complicated by the cell proliferation defects associated with many *fat* alleles (Mahoney et al., 1991)). Imaginal disc morphogenesis involves coordinated shape change of the epithelial cells that make up the discs, which results in an elongation and narrowing of the presumptive tissue (von Kalm et al., 1995). Mutations in *ds*

seem to interfere with this process, resulting in malformed legs and other structures, with the severest alleles producing pupal lethality (Clark et al., 1995). hyp10 undergoes an analogous change in shape during embryonic morphogenesis, elongating along the anterior-posterior axis. It is this process which seems to be affected in *cdh-3(pk87)* homozygotes. We postulate that CDH-3 is required to maintain the interaction between hyp10 and hyp11 during tail morphogenesis and that disruption of this interaction in *cdh-3(pk87)* homozygotes prevents normal hyp10 morphogenesis.

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