A Putative Catenin–Cadherin System Mediates Morphogenesis of the *Caenorhabditis elegans* Embryo

Michael Costa,* William Raich,‡ Cristina Agbunag,*‖ Ben Leung,*‖¶ Jeff Hardin,‡§ and James R. Priess*‖¶**

*Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109; ‡Program in Cellular and Molecular Biology, §Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706; ‖Howard Hughes Medical Institute; and ¶Molecular and Cellular Biology Program, **Department of Zoology, University of Washington, Seattle, Washington 98195

Abstract. During morphogenesis of the *Caenorhabditis elegans* embryo, hypodermal (or epidermal) cells migrate to enclose the embryo in an epithelium and, subsequently, change shape coordinately to elongate the body (Priess, J.R., and D.I. Hirsh. 1986. *Dev. Biol.* 117:156–173; Williams-Masson, E.M., A.N. Malik, and J. Hardin. 1997. *Development [Camb.].* 124:2889–2901). We have isolated mutants defective in morphogenesis that identify three genes required for both cell migration during body enclosure and cell shape change during body elongation. Analyses of *hmp-1*, *hmp-2*, and *hmr-1* mutants suggest that products of these genes anchor contractile actin filament bundles at the adherens junctions between hypodermal cells and, thereby, transmit the force of bundle contraction into cell shape change. The protein products of all three genes localize to hypodermal adherens junctions in embryos. The sequences of the predicted HMP-1, HMP-2, and HMR-1 proteins are related to the cell adhesion proteins α-catenin, β-catenin/Armadillo, and classical cadherin, respectively. This putative catenin–cadherin system is not essential for general cell adhesion in the *C. elegans* embryo, but rather mediates specific aspects of morphogenetic cell shape change and cytoskeletal organization.

T HE morphology of the animal body and its tissues arise as embryonic cells change their shapes and/or positions (Mittenthal and Jacobson, 1990). Many of these changes are mediated by dynamic rearrangements of cytoskeletal components (Wessells et al., 1971). Cells can organize diverse patterns of microtubules and actin filaments, and movement of actin filaments by myosin proteins is thought to generate the force that drives many morphogenetic processes. An important step toward understanding the mechanical basis of morphogenesis is the identification and characterization of molecules that pattern the cytoskeleton and translate force into concerted cell movements.

For cells to change shape coordinately or move relative to each other, forces generated within an individual cell must be transmitted to adhesive junctions at the plasma membrane and exerted on neighboring cells or the extracellular matrix (Gumbiner, 1996). The best characterized cell–cell junction is the adherens junction. This type of junction usually forms a subapical, beltlike structure that mechanically links the lateral surfaces of adjacent epithelial cells. Adherens junctions contain transmembrane proteins of the cadherin family that mediate homotypic adhesion. Cadherins are thought to connect to the actin cytoskeleton indirectly through the proteins α-catenin and β-catenin. Catenin–cadherin complexes also are associated with sites of contact between blastomeres in vertebrate and invertebrate embryos. In *Drosophila*, mice, and *Xenopus*, gene inactivation of catenins or cadherins disrupts general cell adhesion and apicobasal polarity of blastomeres and epithelial cells (Heasman et al., 1994; Larue et al., 1994; Haegel et al., 1995; Cox et al., 1996; Müller and Wieschaus, 1996; Kafron et al., 1997; Torres et al., 1997). Thus, it has been difficult to define direct requirements for these proteins in cytoskeletal organization and morphogenesis, although there is evidence for specific roles in tracheal cell migration (Tanaka-Matakatsu et al., 1996) and axon outgrowth (Iwai et al., 1997) in *Drosophila*.

The *Caenorhabditis elegans* embryo provides a model system for studying how cells move and change shape to generate body and tissue morphologies. At hatching, the outermost cellular layer of the body consists of a monolayer of 85 epithelial cells called hypodermal cells that are linked together by adherens junctions (White, 1988). During embryogenesis, hypodermal cells are involved in two distinct processes that transform the initially ellipsoidal

mass of embryonic cells into a long, thin worm; these processes are called body enclosure and body elongation (Sulston et al., 1983; Priess and Hirsh, 1986; Williams-Masson et al., 1997). The hypodermal cells are born on the dorsal surface of the embryo. As the hypodermal cells develop adherens junction connections, they begin to spread as a sheet across the embryo until the contralateral edges of the sheet meet at the ventral midline. In the anterior of the embryo, ventral hypodermal cells on the periphery of the spreading sheet develop filopodial extensions that may function to draw the contralateral edges of the sheet together (Williams-Masson et al., 1997). In the posterior of the embryo, the contralateral edges appear to be drawn together by a purse-string–like contraction that completes the enclosure process (Williams-Masson et al., 1997). In several respects, these processes are similar to epithelial cell movements described in a variety of systems, such as wound healing in vertebrates (Martin and Lewis, 1992) and dorsal closure in *Drosophila* (Young et al., 1993).

At the completion of body enclosure in *C. elegans*, the apical surfaces of the hypodermal cells resemble rectangles that are elongated along the circumferential contour of the embryo's body. These apical surfaces begin to change shape, constricting along the circumferential contour of the body and elongating along the anterior–posterior (longitudinal) axis. The coordinate changes in the shapes of the hypodermal cells appear to cause the body to decrease in circumference and to elongate about fourfold along its longitudinal axis (Sulston et al., 1983; Priess and Hirsh, 1986). Before body elongation, the apical cytoskeleton of each hypodermal cell reorganizes to form an array of parallel actin filament bundles oriented along the circumferential contour of the body (Priess and Hirsh, 1986; Costa et al., 1997). The parallel filament bundles bridge two opposing sides of each hypodermal cell, apparently connecting to the subapical adherens junction. Contraction of the filament bundles has been proposed as the force that elongates the embryo; the bundles become shorter and thicker during elongation, and drugs that disrupt actin filament organization prevent elongation. Apical constriction of cells has been shown in other systems to drive the invagination of epithelial sheets; because of the closed, cylindrical geometry of the hypodermal sheet in *C. elegans*, an analogous apical constriction might instead drive body elongation (Priess and Hirsh, 1986).

Although the morphology and properties of the hypodermal cells strongly suggest that they mediate body elongation, almost all of the elongation-defective mutants described thus far have mutations in genes encoding muscle or basement membrane components. Body-wall muscles underlie the hypodermis, separated by a basement membrane (Hresko et al., 1994; diagram in Fig. 8 *a*). Mutations in any of several genes that eliminate embryonic muscle contraction prevent elongation beyond a twofold increase in body length; this phenotype is called Pat[1] (paralyzed, arrested elongation at twofold; Williams and Waterston,

1994). Some of the genes of the Pat class have been shown to encode muscle-specific proteins. Because the muscles and myofilaments are oriented longitudinally, muscle contraction would be expected to oppose body elongation; thus, it is not yet understood why muscle function is required for complete elongation. The genes *let-2* and *emb-9* encode basement membrane collagens, and mutations in these genes produce elongation defects similar to those of Pat mutants (Guo et al., 1991; Sibley et al., 1993; Williams and Waterston, 1994). The only gene identified that is both required for proper body elongation and apparently expressed in hypodermal cells is *let-502* (Wissmann et al., 1997). The predicted LET-502 protein is related to Rho-binding kinases, which can activate myosin light chain kinase, suggesting that LET-502 could have a role in hypodermal cells for the contraction of the array of actin filament bundles.

To expand our understanding of the molecular basis for morphogenesis, we have isolated and characterized a group of mutants that display similar defects in embryo morphogenesis. In this paper, we present evidence that a *C. elegans* catenin–cadherin system mediates morphogenetic cell shape changes and specific aspects of cytoskeletal organization. We show that the genes *hmp-1*, *hmp-2*, and *hmr-1* are required for the proper migration of hypodermal cells during body enclosure and for body elongation. We demonstrate that *hmp-1*, *hmp-2*, and *hmr-1* can encode proteins related to α-catenin, β-catenin, and cadherin, respectively. We show that the protein products of these genes are localized to adherens junctions in the hypodermis. Our results indicate that these proteins anchor the parallel actin filament bundles to the adherens junctions in hypodermal cells and that this coupling translates the force of bundle contraction into cell shape change.

Materials and Methods

C. elegans Strains

Bristol strain N2 was used as wild type. Listed below are the marker mutations and genetic deficiencies obtained from the *C. elegans* Genetic Stock Center (Bristol, England) and used in this study: Chromosome *I*: *dpy-5 (e61)*, *eDf3*, *eDf4*, *eDf6*, *eDf9*, *eDf11*, *eDf12*, *eDf13*, *eDf14*, *eDf15*, *eDf16*, *hIn1 (unc-54 [h1040])*, *lin-11 (n566)*, *unc-54 (e190)*, *unc-75 (e950)*; chromosome *IV*: *him-3 (e1147)*; chromosome *V*: *ctDf1*, *daf-11 (m84ts)*, *dpy-11 (e244)*, *sma-1 (e30)*, *unc-42 (e270)*; and chromosome *X*: *lin-2 (e1309)*. The *hmp-1(ca202)* allele was provided by Andreas Wissmann (University of Calgary, Calgary, Canada).

Genetics

Hmp alleles were isolated using the method described by Priess et al. (1987) with the following modifications for a nonclonal, F2 mutagenesis screen: Individual F1 progeny were not separated, F2 embryos were screened by Nomarski microscopy while inside the carcass of their mothers, and recessive zygotic-lethal mutations were recovered through the heterozygous siblings in the carcass. Standard genetic complementation tests and mapping ordered the Hmp mutations into the following three complementation groups. *hmp-1*: *zu242*, *zu244*, *zu278*, *zu349*, *zu365*, *zu399*, *zu402*, *zu403*, *zu404*, *zu406*, and *ca202*; *hmp-2*: *zu364*; and *hmr-1*: *zu248*, *zu389*.

To isolate germline mosaic animals, we constructed *hmp-1* strains carrying a wild-type *hmp-1* transgene on an extrachromosomal array. *hmp-1* mutants that produced only inviable progeny were assumed to have spontaneously lost the extrachromosomal array from the germline. Germline mosaics were recovered for all three alleles tested (*zu242*, *zu278*, and *ca202*) at a frequency of 1–2%, and all produced embryos with the Hmr

---

1. *Abbreviations used in this paper*: AJ, adherens junction; AJF, adherens junction filament; CF, circumferential filament; CFB, circumferential filament bundle; GST, glutathione S-transferase; Hmp, humpback; Hmr, hammerhead; Pat, paralyzed, arrested elongation at twofold; RNAi, RNA-mediated interference.

phenotype. For the seven *hmp-1* alleles tested (*zu244*, *zu278*, *zu349*, *zu365*, *zu399*, *zu404*, and *ca202*), transgenic animals that are not germline mosaics produce some Hmr embryos. These embryos probably result from low or no maternal expression of the *hmp-1* transgene, typical of transgenes generated by the method used here, and meiotic loss of the transgenic extrachromosomal array. Consistent with this hypothesis, many cleavage-stage embryos from the *hmp-1 (zu278) V; zuEx5* strain show a lack of HMP-1 antiserum staining, as do many differentiating embryos. Thus we have used *hmp-1* transgenic strains to examine the phenotype of loss of both maternal and zygotic *hmp-1* activity in fixed, stained embryos.

Molecular Biology

We mapped *hmp-1* between the cloned genes *daf-11* and *sma-1* on chromosome V. Germline transformation with genomic cosmid DNA from this region, provided by the *C. elegans* Genome Project, revealed that cosmid R13H4 completely rescued the *hmp-1* mutant phenotype. Subcloning of this genomic DNA delimited the *hmp-1* gene to a 7.6-kb rescuing fragment. We identified a cDNA (yk36d4) from the *C. elegans* cDNA Project (Y. Kohara, National Institute of Genetics, Mishima, Japan) that maps to the *hmp-1* rescuing genomic fragment. RNA-mediated interference (RNAi) was performed as described previously (Guo and Kemphues, 1995; Rocheleau et al., 1997); when RNA made from the yk36d4 cDNA clone was injected into wild-type gonads, the resulting embryos showed the Hmp phenotype. We isolated and sequenced *hmp-1* cDNA clones from cDNA libraries (provided by R. Barstead and R. Waterston [Washington University, St. Louis, MO], J. Zhou, and J. Rothman [University of California, Santa Barbara, CA]) and from cDNA amplified by PCR. Together, these cDNAs span 3.2-kb and contain a single long open reading frame predicted to encode a protein of 927 amino acids. Genomic sequence of the cosmid R13H4, available from the *C. elegans* Genome Project under accession number Z81579, agrees with our cDNA sequence and indicates that HMP-1 is encoded by eight exons included within the 7.6-kb rescuing fragment. The *hmp-1* cDNA hybridizes to a single, ~3.0-kb mRNA species in RNA isolated from either embryonic or mixed larval and adult stages.

hmp-2 maps genetically to chromosome I, ~1.5 map units to the left of the *unc-54* gene. We found that the cDNA clone yk126e3, which was identified by the *C. elegans* cDNA Project (Y. Kohara) and shows homology to β-catenin/armadillo, maps near *hmp-2* on the physical map constructed by the *C. elegans* Genome Project (yeast artificial chromosome clones Y27H10, Y54E5, and Y53C6). Injection of RNA made from the cDNA clone into the gonads of wild-type worms generated embryos showing the *hmp-2* mutant phenotype. We used the cDNA to isolate a 16.5-kb genomic DNA fragment from a phage library (Stratagene, La Jolla, CA). This genomic fragment displayed transgenic rescue of the *hmp-2* mutant. Our complete cDNA sequence of *hmp-2* reveals a 2.2-kb transcript containing a single long open reading frame that can encode a 678–amino acid protein. The sequence of our genomic clone and cosmids from the *C. elegans* Genome Project indicates that HMP-2 is encoded by five exons located on the transgenic rescuing fragment.

The *hmr-1* gene maps 1.3 map units to the right of *lin-11* on chromosome I. From the *C. elegans* Genome Project, we obtained a genomic cosmid clone (W02B9) that maps near this region and contains sequences homologous to classical cadherins. Germline transformation of this cosmid fully rescued all *hmr-1* mutant phenotypes. We subcloned a 21.2-kb fragment of the cosmid that is predicted to contain only the cadherin-related gene and found that this DNA fragment also exhibits transgenic rescue of *hmr-1*. Genomic DNA sequence from the *C. elegans* Genome Project and our sequence of *hmr-1* partial cDNA clones suggest that the *hmr-1* gene contains 10 exons that generate a 4.7-kb mRNA transcript and a 1,223–amino acid protein.

Sequence data are available from GenBank/EMBL/DDBJ under accession numbers AF016852 (*hmp-1*), AF016853 (*hmp-2*), and AF016854 (*hmr-1*).

Antibody Production and Morphological Analysis

To generate antisera against HMP-1, a plasmid construct designed to produce a glutathione S-transferase (GST)–HMP-1 fusion protein was made by subcloning a 0.8-kb cDNA fragment from the 3′ end of the *hmp-1* transcript into the vector pGEX-4T2 (Pharmacia Biotech, Piscataway, NJ). Fusion protein was purified according to the instructions of Pharmacia Biotech. Rabbits were immunized with purified fusion protein by the Uni-

versity of Wisconsin Antibody Production Services Animal Care Unit. Immune serum was affinity purified against GST–HMP-1 fusion protein cross-linked to Actigel A resin (Sterogene Bioseparations, Inc., Carlsbad, CA) according to the instructions of the manufacturer. To remove anti-GST antibodies, the process was repeated with a GST column. *hmp-1 (zu278)* mutant embryos lack detectable HMP-1 antiserum staining after the start of hypodermal enclosure, indicating that this antiserum specifically recognizes HMP-1 protein. Before hypodermal enclosure, *hmp-1 (zu278)* embryos appear to express HMP-1; this is presumably maternally supplied gene product.

For HMP-2 and HMR-1 antisera production, specific peptides were synthesized: NH$_2$-terminal HMP-2 sequence DHEVETRTSRIRSAC and COOH-terminal HMR-1 sequence CAPYDELRIYDDERDN (by Quality Controlled Biochemicals Inc., Hopkinton, MA). Rabbits were immunized by the Fred Hutchinson Cancer Research Center Animal Care Facility. The HMP-2 and HMR-1 antisera were affinity purified using BSA-coupled peptide attached to nitrocellulose membrane (ProBlott; Applied Biosystems, Inc., Foster City, CA) as described previously (Robinson et al., 1988). Specificity of the antisera was tested on *hmp-2(RNAi)* and *hmr-1(RNAi)* embryos generated as described previously (Guo and Kemphues, 1995; Rocheleau et al., 1997) using the cDNAs yk126e3 and yk105b7 as templates for RNA.

Immunostaining of embryos with affinity-purified HMP-1 antiserum, mAbMH27, and β-tubulin antibody (Boehringer-Mannheim Corp., Indianapolis, IN) was performed by both of the protocols described by Costa et al. (1997) with identical results. Staining with the affinity-purified HMP-2 and HMR-1 antisera was as described by Albertson (1984). For double labeling, fluorescein- and either rhodamine- or Texas Red–conjugated secondary antibodies were used. Phalloidin staining was done according to Costa et al. (1997). Fluorescent images were obtained using either a confocal laser scanning microscope (model MRC1024 [laboratory of Judith Kimble, University of Wisconsin, Madison, WI] or MRC600; Bio-Rad Laboratories, Hercules, CA) or a Dage cooled CCD camera (Michigan City, IN) and DSP2000 processor.

For Nomarski time-lapse videomicroscopy, embryos were mounted on agarose pads in M9 solution and sealed under a coverslip with Vaseline. Single focal plane recordings were made on a Panasonic (Bangkok) time-lapse video recorder using a Hammamatsu VE2000 camera (Bridgewater, NJ). Four-dimensional recordings were made as described by Draper et al. (1996).

Results

hmp-1, hmp-2, and hmr-1 Genes Are Required for Elongation of the Embryo

The wild-type *C. elegans* embryo increases fourfold in body length during morphogenesis (see Fig. 1 *A*). After standard ethylmethane sulfonate mutagenesis, we screened directly for mutant embryos that are defective in body elongation. We isolated 13 recessive, zygotic-lethal mutants that, in addition to failing to elongate properly, show abnormally bulged dorsal surfaces (Fig. 1 *B*). We call this novel phenotype Hmp (humpback); this phenotype contrasts with previously described elongation-defective Pat mutants that display bulges at variable positions (Williams and Waterston, 1994). The 13 Hmp mutants identify the genes *hmp-1*, *hmp-2*, and a third gene that we name *hmr-1* for an additional phenotype described below.

We isolated 10 alleles of the gene *hmp-1* and received another from A. Wissmann. All alleles show the same phenotype. Embryogenesis proceeds normally in *hmp-1* mutants through the start of elongation. However elongation arrests at the 1.25× to 1.5× stage, and the mutant embryos retract to their original 1× length as abnormal bulges form on the dorsal side (Fig. 1 *B*). Although *hmp-1* mutants have an early defect in elongation, cells appear to differentiate fully. For example, muscles, neurons, and pharyngeal cells in *hmp-1* embryos express markers for terminal dif-
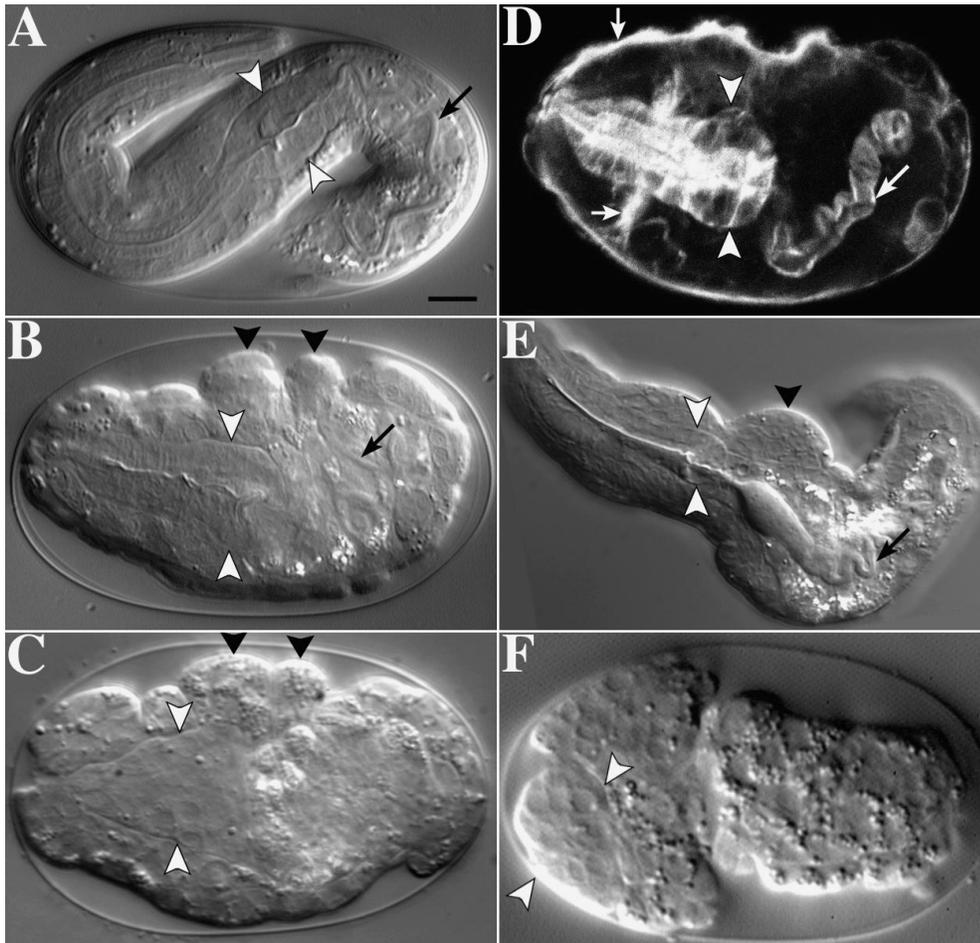
Figure 1. Body morphology of wild-type, Hmp, and Hmr embryos. (*A*) Nomarski photomicrograph of a fully elongated wild-type embryo; the posterior half of this embryo is in a lower focal plane and not visible here. In this and all other panels, the pharynx is demarcated by two white arrowheads. Where visible, the intestine brush border is indicated by a diagonal arrow. (*B*) Terminal stage *hmp-1* mutant embryo with abnormal dorsal bulges (*black arrowheads*). Unless otherwise indicated, embryos in all figures are oriented with dorsal toward the top of the page and anterior to the left. (*C*) *ctDf1* homozygote. (*D*) Fluorescence micrograph of a *hmp-1* mutant embryo as in *B* after staining with phalloidin to visualize internal actin-containing structures. Visible structures include a well-formed nerve ring (*horizontal arrow*), body-wall muscles (*vertical arrow*), pharynx (*arrowheads*), and intestine (*diagonal arrow*). (*E*) Hatched *hmp-2* mutant showing partial elongation and a prominent dorsal bulge (*black arrowhead*). (*F*) Embryo from a *hmp-1* germline mosaic animal displaying the Hmr phenotype. The posterior of the embryo has enclosed in hypodermis and constricted in circumference while the anterior has not. The pharynx lies on the exterior of the embryo. Bar, 5 μm.

ferentiation (Fig. 1 *D* and data not shown). The *hmp-1* mutants may completely lack *hmp-1* gene activity, at least with respect to the zygotic requirement for elongation, since embryos homozygous for the *hmp-1* deficiency *ctDf1* show a similar Hmp phenotype (Fig. 1 *C*).

Our mutant screen identified a single allele of the *hmp-2* gene. Approximately 40% (*n* = 251) of *hmp-2 (zu364)* mutant embryos appear indistinguishable from *hmp-1* mutants. The other 60% of *hmp-2* embryos elongate to variable extents with less bulging of the dorsal hypodermis than *hmp-1* mutants (Fig. 1 *E*). About 1% of *hmp-2* embryos hatch to form viable dumpy or lumpy larvae. Embryos hemizygous for *hmp-2 (zu364)* or homozygous for a *hmp-2* deficiency (either *eDf4* or *eDf15*), display variable Hmp phenotypes similar to *hmp-2* homozygotes, suggesting *hmp-2 (zu364)* is a null allele (data not shown).

We isolated two alleles of the *hmr-1* gene. For both mutations, ∼2% (*n* = 450) of the homozygous embryos show a Hmp elongation phenotype identical to that of *hmp-1* or *hmp-2* mutants. However, in the remaining *hmr-1* mutants the hypodermis fails to fully enclose the embryo. We call this phenotype Hmr (hammerhead; see Fig. 1 *F*). Hmr embryos, like Hmp embryos, show terminal differentiation of all tissues (data not shown). Before presenting further phenotypic characterization of Hmr and Hmp mutants, we

describe here the cloning and molecular nature of the *hmp-1*, *hmp-2*, and *hmr-1* genes.

hmp-1, hmp-2, and hmr-1 Encode Homologues of α-Catenin, β-Catenin/Armadillo, and Classical Cadherin

We cloned the *hmp-1*, *hmp-2*, and *hmr-1* genes by positional cloning strategies using genetic mapping and transgenic rescue experiments (see Materials and Methods). For *hmp-1* , *hmp-2*, and *hmr-1*, we demonstrated that injection of in vitro–synthesized RNA into the gonads of wild-type adults produced Hmp or Hmr embryos (data not shown).

Sequence analysis of *hmp-1* cDNAs revealed that *hmp-1* can encode a protein, HMP-1, with 35–38% identity to α-catenins from vertebrates and invertebrates (Fig. 2 *A*). α-Catenin is a component of adherens junctions that appears to bind directly to F-actin (Rimm et al., 1995) and indirectly to classical cadherins (transmembrane cell adhesion proteins) through β-catenin (Pai et al., 1996). The homology extends throughout almost the entire length of HMP-1 and α-catenins, including putative binding domains for actin and β-catenin. However, the HMP-1 sequence is more diverged than that of previously isolated α-catenins, which show greater than 55% identity to each other.
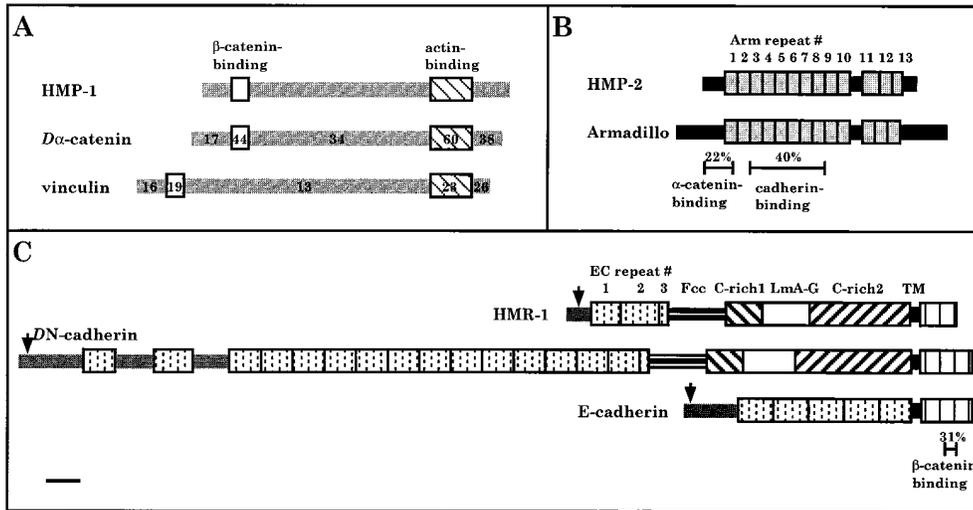
Figure 2. Sequence similarity of HMP-1, HMP-2, and HMR-1 proteins to catenins and cadherins. Schematic diagrams comparing the primary amino acid sequences of HMP-1 (*A*), HMP-2 (*B*), and HMR-1 (*C*) proteins from *C. elegans* with their *Drosophila* (labeled *D*) or vertebrate homologues. Domains that have been described for α-catenin, β-catenin, and classical cadherins are labeled. The percentage of amino acid identity with the corresponding regions of HMP-1, HMP-2, or HMR-1 is shown. (*A*) The β-catenin–binding domain identified in chicken αE-catenin (Nieset et al., 1997) appears to correspond to amino acids 92–143 in HMP-1. α-catenin seems to contain two F-actin–binding regions (Rimm et al., 1995). Although neither has been mapped extensively, the COOH-terminal region includes the portion homologous to the more precisely mapped actin-binding domain in the related protein vinculin (Menkel et al., 1994). This portion is depicted and corresponds to amino acids 690–817 of HMP-1. (*B*) The α-catenin–binding and cadherin-binding domains of Armadillo/β-catenin (Pai et al., 1996) are homologous to HMP-2 amino acids 19–84 and 146–397, respectively. (*C*) The β-catenin–binding domain identified in the intracellular region of *D*E-cadherin (Pai et al., 1996) corresponds to amino acids 1184–1223 of HMR-1. Arrows indicate predicted signal sequence cleavage sites. Bar, the length of 100 amino acids for all panels.

The complete cDNA sequence of *hmp-2* indicates that the predicted HMP-2 protein shows ~26% amino acid identity to β-catenin/Armadillo from vertebrates and invertebrates (Fig. 2 *B*). This homology extends throughout the entire length of the proteins and includes the region of β-catenin/Armadillo that binds to α-catenin. HMP-2, like β-catenin, contains 13 imperfect repeats, called Arm repeats (Peifer and Wieschaus, 1990), of ~42 amino acids each that are arranged in tandem with an insertion between the 10th and 11th repeats. This arrangement is also found in plakoglobin, a component of both adherens and desmosome junctions; however, plakoglobin lacks a COOH-terminal hexapeptide conserved between HMP-2 and β-catenins.

Our sequence of *hmr-1* partial cDNA clones and the sequence of the corresponding genomic region provided by the *C. elegans* Genome Project reveals that the predicted HMR-1 protein contains a putative signal peptide and a transmembrane domain (Fig. 2 *C*, *TM*). The putative intracellular domain of HMR-1 shows 20–25% amino acid identity to the intracellular region of classical cadherins; this domain of classical cadherins binds to β-catenin/Armadillo (Pai et al., 1996). The putative extracellular portion of HMR-1 contains two and one-half repeats of an ~100–amino acid domain, called the EC repeat, believed to mediate homotypic binding (Shapiro et al., 1995). The HMR-1 extracellular domain also possesses a region of 733 amino acids (divided into segments called Fcc, C-rich1, LmA-G, and C-rich2) that is homologous to a region found in *Drosophila* N- and E-cadherin, but not in vertebrate cadherins (Iwai et al., 1997).

Hmp Mutants Are Defective in Cell Shape Changes of the Dorsal Hypodermis

The finding that the *hmp-1*, *hmp-2*, and *hmr-1* genes can encode proteins related to α-catenin, β-catenin, and cadherin, respectively, suggests that mutants in the Hmp and Hmr classes might be defective in cellular adhesion or cytoskeletal function. We present here an analysis of the embryonic Hmp phenotype of *hmp-1* mutants; similar results were obtained from experiments on *hmp-2* and *hmr-1* mutant embryos expressing the Hmp phenotype. Living embryos were analyzed by videomicroscopy. Fixed embryos of various stages were analyzed by staining with phalloidin to detect actin filaments and by staining with the antibody mAbMH27 (Priess and Hirsh, 1986; Francis and Waterston, 1991; Podbilewicz and White, 1994). Immunoelectron microscopic studies have shown that mAbMH27 stains adherens junctions in the hypodermal cells, pharyngeal cells, and intestinal cells (Hall, D., personal communication).

Before body elongation commences, the body has a slight ventral flexure and the hypodermal cells are organized into distinct dorsal, lateral, and ventral rows (Fig. 3 *A*). During body elongation, each hypodermal cell lengthens along the longitudinal axis of the body, and each cell shortens along the circumferential contour of the body (Fig. 3 *C*; see also Priess and Hirsh, 1986). The dorsal and ventral sides of the body elongate at similar rates, and the body decreases in circumference uniformly. The nascent tail extends ventrally and then turns anteriorly toward the head; with further elongation, the body is contorted into a pretzel-like shape within the eggshell (Fig. 3 *C*).

hmp-1 mutant embryos appear identical to wild-type embryos before body elongation (Fig. 3 *A*). However, when elongation commences, only the ventral hypodermal cells elongate appreciably. The ventral flexure of the embryo disappears as the ventral hypodermal cells lengthen; the body straightens (Fig. 3 *B*) and then develops a prominent dorsal flexure (Fig. 3 *D*). During this process, the dorsal hypodermis appears to retract slightly in length and to
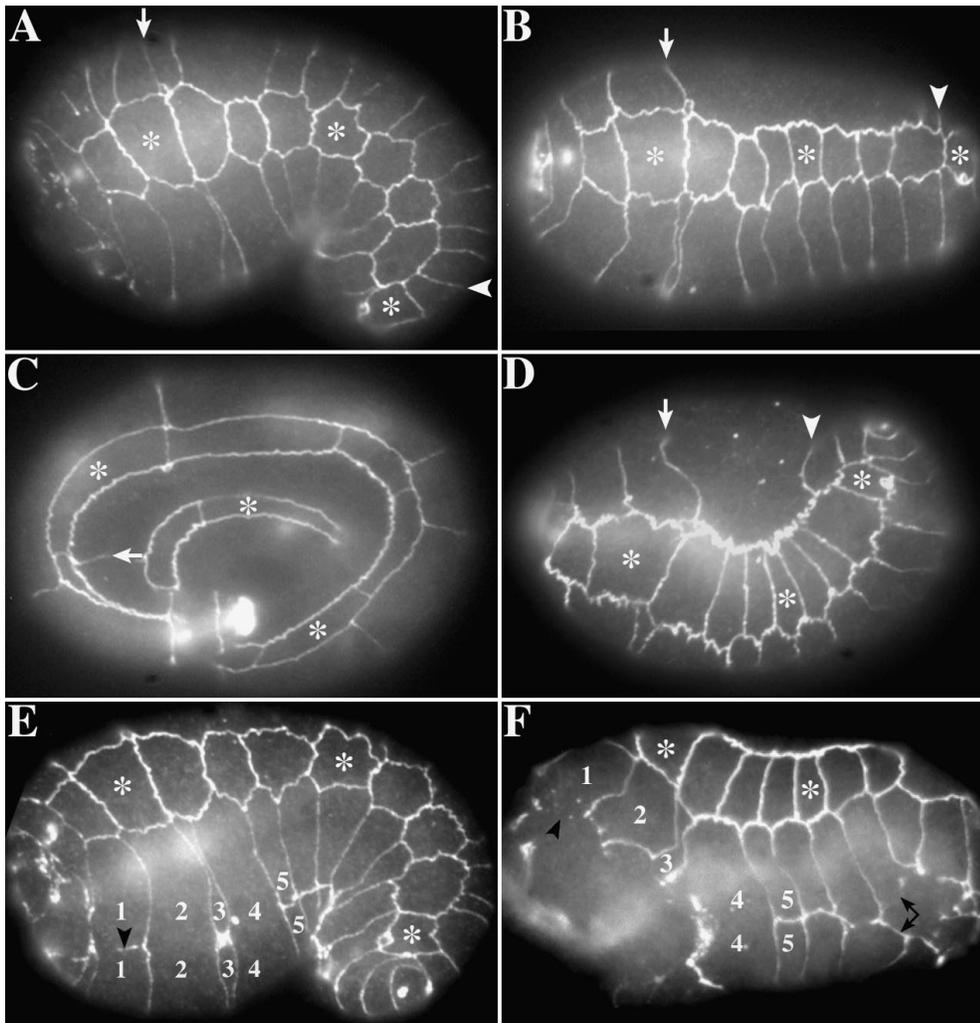
Figure 3. Hypodermal cell shapes and positions in wild-type, Hmp, and Hmr embryos. Embryos stained with mAbMH27 to visualize adherens junctions encircling the hypodermal cells. Wild-type embryos are shown in the left column, mutant embryos in the right column. In all panels, the same three hypodermal cells are indicated by asterisks. (*A*) Wild-type embryo just before body elongation; the dorsal hypodermal cells between the arrow and arrowhead will fuse together into a syncytium (compare with *B* and *D*; embryo in *C* is inverted with dorsal down). (*B*) *hmp-1* mutant embryo beginning to elongate. (*C*) Fully elongated wild-type embryo. (*D*) Terminal stage *hmp-1* mutant embryo. (*E*). Ventrolateral views of an elongating wild-type embryo. Some of the contralateral pairs of ventral hypodermal cells are numbered. Note that some pairs fuse at the ventral midline (*2* and *4*) while others do not (*1*, *3*, and *5*); the *3* pair is called G2/W. (*F*). *hmp-1* mutant embryo displaying the Hmr phenotype. The embryo is from a *hmp-1* germline mosaic mother. The *1–3* pairs of ventral hypodermal cells do not reach the ventral midline. Note the rupture at the ventral midline between ventral hypodermal cells in the posterior of the body (*double arrow*).

increase in circumference, forming the dorsal bulges characteristic of the Hmp class of mutants.

Longitudinally oriented body wall muscles underlie the hypodermis (Fig. 4 *A*) and contract in opposition to the direction of body elongation. To ask if inappropriate dorsal muscle contraction causes the dorsal retraction in *hmp-1* mutants, we examined the development of embryos homozygous for the genetic deficiency *ctDf1*. This deficiency deletes both the *hmp-1* gene and *myo-3*; the *myo-3* gene encodes a body wall muscle myosin that is essential for muscle contraction. Videomicroscopy of *ctDf1* homozygotes (*n = 4*) demonstrates that although these embryos have no visible muscle contractions, they show a pattern of dorsal retraction similar to that of *hmp-1* mutants (Fig. 1 *C*).

Actin Filament Bundles in Hmp Mutants Detach from Adherens Junctions

The cytoskeletal organization of wild-type hypodermal cells during body elongation has been described previously (Priess and Hirsh, 1986; Costa et al., 1997). Briefly, each hypodermal cell contains an array of actin filament bundles in its apical cortex; these bundles are oriented parallel to the circumferential contour of the body, and we refer to them as circumferential filament bundles (CFBs; see Fig. 8 *B*). The CFBs appear to connect with the adherens junction that encircles each hypodermal cell (Figs. 4 *A* and 8, *A* and *B*). Microtubules in the dorsal and ventral hypodermal cells are aligned parallel to the CFBs. However, few if any microtubules appear to contact the adherens junction; most instead terminate before, or run parallel to, the junction.

Before body elongation, the microtubules, CFBs, and adherens junctions in *hmp-1* mutants and wild-type embryos appear identical (Fig. 4 *B* and data not shown). As the hypodermal cells in *hmp-1* mutants begin to change shape, the CFBs in the dorsal hypodermis separate from the adherens junctions (Figs. 4, *C* and *D*). The detached CFBs increase in thickness and retract several μm toward the dorsal midline (Figs. 4, *C* and *D*, and data not shown). These observations suggest that the CFBs in the dorsal hypodermis of *hmp-1*
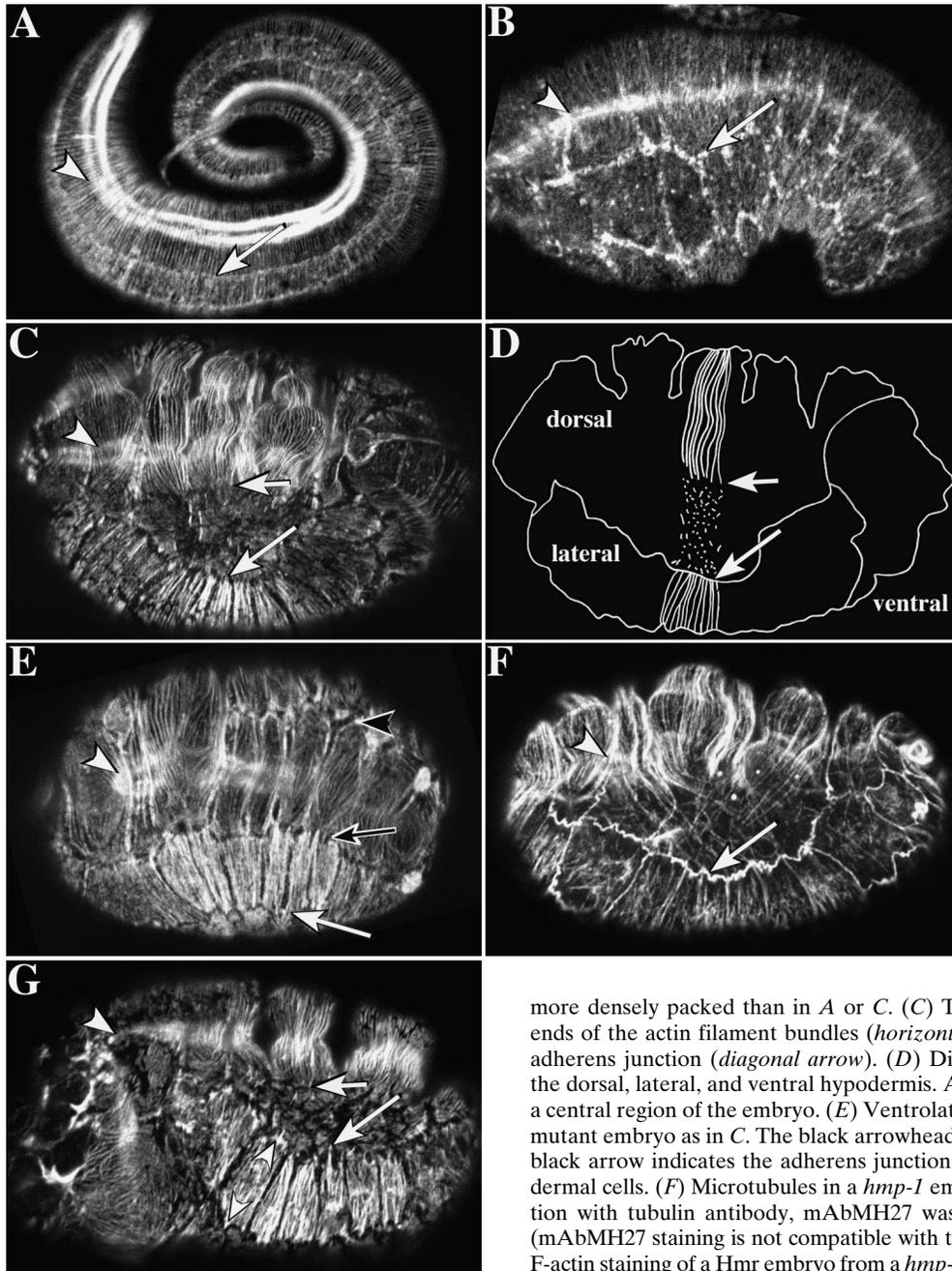
Figure 4. Cytoskeletal organization in the hypodermis of wild-type, Hmp, and Hmr embryos. Embryos stained for F-actin (*A-C, E,* and *G*) or tubulin (*F*). All are viewed dorsolaterally, except for the embryo in *E*, which is oriented ventrolaterally. In each panel, the actin-containing adherens junction at the ventral margin of a dorsal hypodermal cell is indicated by a diagonal white arrow. A white arrowhead marks one of the bands of longitudinally oriented muscle cells that underlie the hypodermis (see Fig. 8 *A* for diagram). (*A*) Fully elongated wild-type embryo. (*B*) *hmp-1* mutant embryo at the onset of elongation. The lateral hypodermal cell row has begun to straighten. Actin filament bundles appear thinner and more densely packed than in *A* or *C*. (*C*) Terminal stage *hmp-1* embryo. The ends of the actin filament bundles (*horizontal arrow*) have separated from the adherens junction (*diagonal arrow*). (*D*) Diagram of embryo in *C* delineating the dorsal, lateral, and ventral hypodermis. Actin filament bundles are traced in a central region of the embryo. (*E*) Ventrolateral view of a terminal stage *hmp-1* mutant embryo as in *C*. The black arrowhead marks the ventral midline, and the black arrow indicates the adherens junction between lateral and ventral hypodermal cells. (*F*) Microtubules in a *hmp-1* embryo oriented as in *C*. In combination with tubulin antibody, mAbMH27 was used to label adherens junctions (mAbMH27 staining is not compatible with the phalloidin staining method). (*G*) F-actin staining of a Hmr embryo from a *hmp-1* germline mosaic; compare with *C*.

embryos contract away from the adherens junction rather than draw together opposing sides of the cell. In contrast, the CFBs in the lateral and ventral hypodermal cells maintain their normal proximity to the adherens junctions (Fig. 4 *E*). The organization of microtubules appears relatively normal in all hypodermal cells (Fig. 4 *F*).

hmp-1, hmp-2, and hmr-1 Are Required for Hypodermal Enclosure of the Embryo

Most *hmr-1* mutant embryos show morphogenetic defects before the start of body elongation In wild type, hypodermal cells are born on the dorsal side of the embryo and migrate ventrally to enclose the body. The hypodermal cells in *hmr-1* mutants initially are positioned normally (data not shown). However, the hypodermis in 98% ($n = 450$) of *hmr-1* mutants fails to fully enclose the anteroventral region of the embryo; we call this the Hmr phenotype (see Fig. 1 *F*). Embryos that are deficient for both maternal and zygotic gene activity of *hmp-1* or *hmp-2* also exhibit the Hmr phenotype: All embryos produced by *hmp-1* germline mosaic animals show the Hmr phenotype ($n > 100$), as do all embryos from rare *hmp-2* homozygous mutants that survive to adulthood ($n > 100$). We have analyzed the cellular defects that prevent body enclosure in these embryos by four-dimensional Nomarski time-lapse videomicroscopy (data not shown) and by staining with mAbMH27 (Fig. 3 *F*).

During body enclosure of the wild-type embryo, the left and right rows of ventral hypodermal cells migrate to the ventral midline (Williams-Masson et al., 1997). The two anterior-most ventral hypodermal cells on each side of the embryo, called the leading cells (Fig. 3 *E*, *1* and *2*), are the first to begin moving and to reach the midline. Some ventral hypodermal cells form adherens junction with contralateral cells as their leading edges meet at the ventral midline, while others fuse together with contralateral cells.

In embryos from *hmp-1* germline mosaic animals, the ventral hypodermal cells initiate ventral movement. However, the anterior three ventral hypodermal cells cease migration prematurely, stopping several micrometers from the ventral midline (Fig. 3 *F* and data not shown). In contrast, the other ventral hypodermal cells complete migration to the midline and either establish adherens junction contacts or fuse with contralateral cells (Fig. 3 *F*).

Circumferential contraction of the partially enclosed embryos seems to force internal cells out through the non-enclosed anterior region, forming the characteristic Hmr phenotype (see Fig. 1 *F*). Dorsal and lateral hypodermal cells contact neighboring cells along their entire margins; however, many embryos fixed after the start of elongation show ruptures at the ventral midline between contralateral pairs of posterior, vental hypodermal cells (Fig. 3 *F*). In these Hmr embryos, as in Hmp embryos, the CFBs in the dorsal but not lateral or ventral hypodermis separate from the adherens junctions (Fig. 4 *G* and data not shown). Thus, both Hmr and Hmp embryos have similar cytoskeletal defects in the dorsal hypodermis. We note that the adherens junction antigen recognized by mAbMH27 is localized properly to the apical margins of hypodermal cells, indicating that at least some aspects of cell polarity and adherens junction structure remain in Hmr and Hmp embryos.

The HMP-1, HMP-2, and HMR-1 Proteins Localize to Adherens Junctions

To examine how the *hmp* and *hmr* gene products might interact with the actin cytoskeleton, we raised polyclonal antibodies against a HMP-1 fusion protein and against peptides specific for the predicted HMP-2 and HMR-1 proteins (see Materials and Methods). Specificity of each affinity-purified antiserum was tested on either mutants or mutant phenocopies generated by RNA-mediated interference (see Materials and Methods). We describe in detail the staining patterns observed with the HMP-1 antiserum; the HMP-2 and HMR-1 antisera gave very similar staining patterns at all stages of embryogenesis. The HMP-1 antiserum recognizes a single protein species of ~105-kD present in *C. elegans* extracts (data not shown).

Before morphogenesis and tissue differentiation, the HMP-1 antiserum stains all blastomeres of embryos (Fig. 5 *A*). HMP-1 antiserum prominently labels all regions of contact between blastomeres, in addition to showing diffuse cytoplasmic staining. At approximately the time adherens junctions begin to form in the hypodermis (Podbilewicz and White, 1994), HMP-1 protein accumulates to high levels along the apical margins of all hypodermal cells (Fig. 5 *B*; all hypodermal staining lies within a 1-μm focal plane near the cell surface). During migration of ventral hypodermal cells, HMP-1 antiserum staining is not detected at the leading edges of these cells (Fig. 5 *C*; these edges also lack mAbMH27 staining [Williams-Masson et al., 1997]). HMP-1 becomes localized to the ventral margins of these cells as they contact contralateral cells at the ventral midline (Fig. 5, *C* and *D*).

During body elongation, the HMP-1 antiserum stains predominantly the apical margins of all hypodermal, pha-
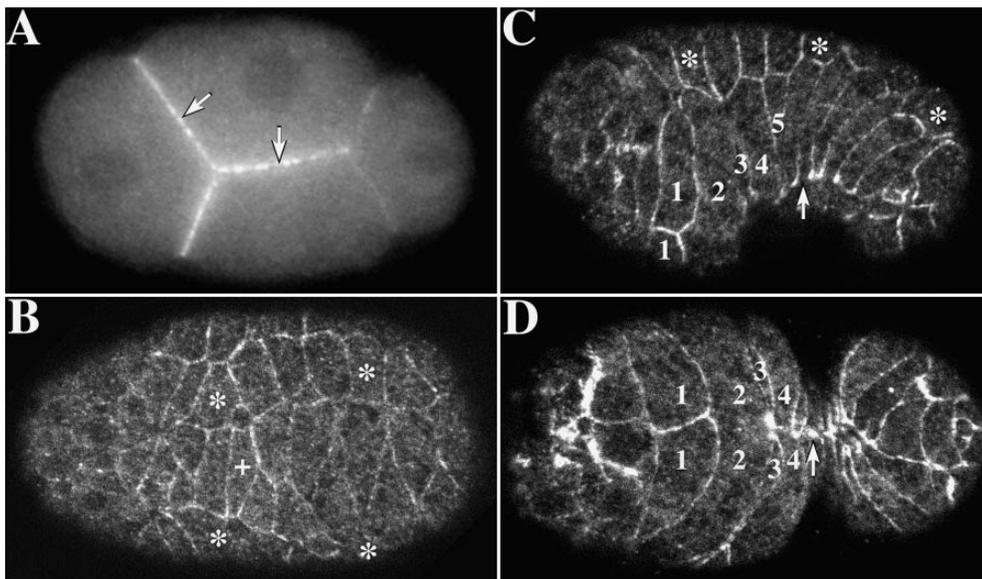
Figure 5. Localization of HMP-1 protein before and during body enclosure of the embryo. Immunofluorescence micrographs of wild-type embryos stained for HMP-1 protein. (*A*) Four-cell stage embryo. HMP-1 protein is concentrated at the sites of contact between blastomeres (*arrows*). (*B*) Dorsolateral view of an embryo at the onset of hypodermal enclosure showing HMP-1 accumulation at developing adherens junctions in hypodermal cells. Left and right rows of lateral hypodermal cells are labeled with asterisks as in Fig. 3; a hypodermal cell in the dorsal row is marked (+). (*C*) Ventrolateral view of an enclosing embryo; labeling as in Fig. 3 *E*. The arrow marks the unstained leading edge of a migrating ventral hypodermal cell. (*D*) Ventral view of an embryo at the end of hypodermal enclosure.
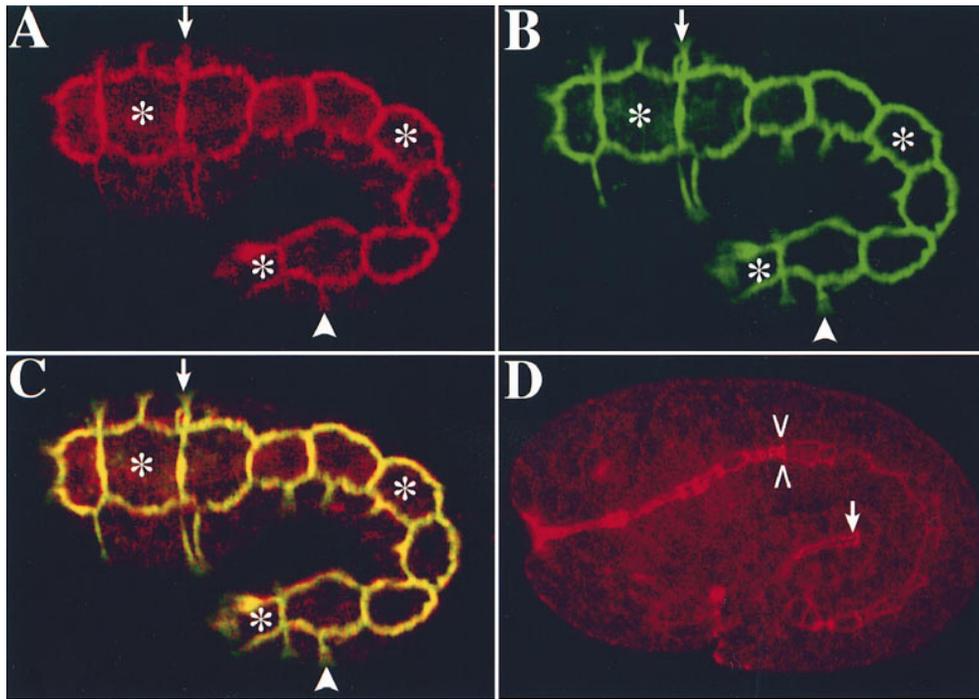
Figure 6. HMP-1 protein localization during elongation in wild-type and *hmp-2* mutant embryos. Labeling of dorsal and lateral hypodermal cells as in Fig. 3. (*A*) HMP-1 protein (*red*) in hypodermal cells of a wild-type elongating embryo at the 1.5× stage. (*B*) mAbMH27 staining (*green*) of hypodermal adherens junctions in the same embryo as in *A*. (*C*) Combined images of *A* and *B* showing the overlapping staining pattern in yellow. (*D*) Internal focal plane of a wild-type embryo as in *A*, revealing HMP-1 at adherens junctions of the pharynx (anterior to *arrowheads*), intestine (posterior to *arrowheads*), and the ventral midline (*arrow*). HMP-1 is also expressed in neurons of the nerve ring during neuronal differentiation (data not shown).

ryngeal, and intestinal cells (Fig. 6, *A* and *D*), as does the mAbMH27 antiserum (Fig. 6 *B*). The staining of these two antisera appears to colocalize (Fig. 6 *C*), strongly suggesting that HMP-1 is a component of the adherens junctions. In later stages, staining appeared most prominent on the margins of the hypodermal cells that abut the two ends of the CFBs. For example, we observed intense staining in ventral hypodermal cells along the ventral midline (Fig. 6 *D*), but staining was faint or undetectable on lateral margins of the ventral cells (data not shown).

To test whether *hmp-2* or *hmr-1* gene activity is required to localize HMP-1 protein to adherens junctions, we stained *hmp-2* and *hmr-1* mutant embryos with the HMP-1 antiserum and with mAbMH27. Both mutants properly localize the adherens junction component recognized by mAbMH27 (Fig. 7 *A*). Although cytoplasmic HMP-1 is visible in these mutants, greatly reduced or undetectable levels of HMP-1 are associated with adherens junctions (Fig. 7 *B*, and data not shown). Similarly, mAbMH27 staining was apparent in *hmr-1* mutant embryos (Fig. 7 *C*), but HMP-2 staining was not (Fig. 7 *D*). Thus *hmr-1(+)* activity appears to be required to localize both HMP-1 and HMP-2 to the adherens junction, and *hmp-2 (+)* activity is required to localize HMP-1. In contrast, neither *hmr-1(+)*, *hmp-1(+)*, nor *hmp-2(+)* activity appears to be necessary to localize the antigen recognized by MH27 to the adherens junction.

Discussion

A Catenin–Cadherin Complex at Cell Junctions in C. elegans

Our mutant screen identified three genes, *hmp-1*, *hmp-2*, and *hmr-1*, that are required for proper body enclosure and body elongation of *C. elegans* embryos. These two morphogenetic processes appear to be driven by shape changes in the hypodermal cells, and both processes are disrupted by inhibitors of actin filament function (Priess and Hirsh, 1986; Williams-Masson et al., 1997). Hypodermal cells in *C. elegans* have two prominent, actin-containing structures: a subapical adherens junction and an array of actin filament bundles that we call CFBs. Our phenotypic analysis of *hmp-1*, *hmp-2*, and *hmr-1* mutants suggests that they are defective in body elongation because the CFBs in the dorsal hypodermal cells detach from adherens junctions.

The HMP-1, HMP-2, and HMR-1 proteins appear to be genuine members of the α-catenin, β-catenin, and classical cadherin families, respectively, although each is more diverged in primary structure than previously identified family members. More than 80% of the *C. elegans* genome has now been sequenced, and no genes that are more homologous have been identified. Catenins and cadherins are components of adherens junctions in other systems, and we have shown here that the HMP-1, HMP-2, and HMR-1 proteins appear to localize to adherens junctions in *C. elegans* embryos. Catenins and cadherins form a molecular complex and their binding sites for each other have been mapped (Pai et al., 1996; Nieset et al., 1997). Since there is a homologous region for each of these binding sites in HMP-1, HMP-2, or HMR-1, we suggest that these three *C. elegans* proteins may form an analogous complex (Fig. 7 *C*). In support of this model, we have shown that *hmr-1(+)* activity is required for proper localization of both HMP-1 and HMP-2 proteins and that *hmp-2(+)* activity is required for the proper localization of HMP-1.

Transmission of Forces Driving Embryo Elongation

The CFBs shorten and thicken as the hypodermal cells elongate in the orthogonal direction (Costa et al., 1997). This change in cytoskeletal morphology is consistent with,
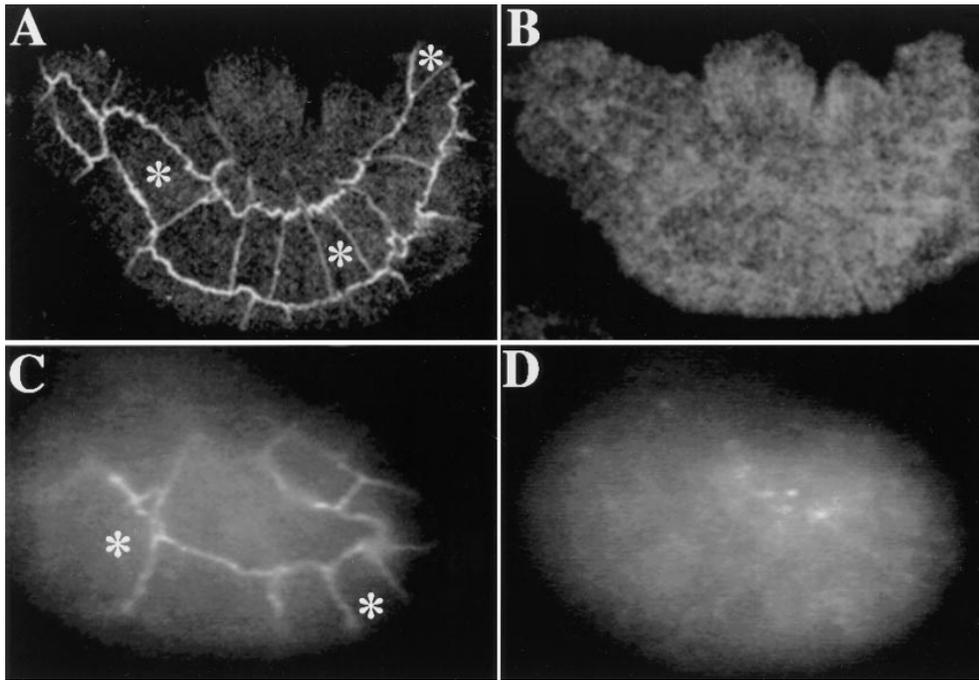
Figure 7. MH27, HMP-1, and HMP-2 localization in mutant embryos. (*A* and *B*) A single, terminal stage *hmp-2* mutant embryo is shown after staining with mAbMH27 (*A*) and with the anti–HMP-1 antiserum (*B*). (*C* and *D*) A single, terminal stage *hmr-1 (RNAi)* embryo is shown after staining with mAbMH27 (*C*) and with the anti–HMP-2 antiserum (*D*). Asterisks label the same hypodermal cells indicated in Figs. 3 and 6.

but does not prove, the model that the CFBs are contractile, with contraction providing the force that changes the shape of individual hypodermal cells and, consequently, the shape of the embryo. For example, inhibitor studies demonstrate that actin is required not only to initiate body elongation but also to maintain the shape of the body during elongation (Priess and Hirsh, 1996). Thus, the CFBs could respond to, rather than cause, changes in hypodermal cell shape. We have shown here that the CFBs in the dorsal hypodermis of Hmp mutants shorten and thicken even after they have detached from the margins of the hypodermal cells, strongly suggesting that they are contractile. We thus propose that the HMP-1, HMP-2, and HMR-1 proteins normally anchor the CFBs to the adherens junction, allowing the force of CFB contraction to be transmitted into changes in cell shape.

The sequence similarity between HMP-1 and α-catenins suggests that HMP-1 may bind to, and bundle, actin filaments. HMP-1 might directly cross-link actin in the CFBs with actin filaments in the adherens junctions (Fig. 8 *C, upper cell*). Alternatively, since adherens junctions may contain HMP-1 protein along their entire length, rather than localized only to points of contact with CFBs, accessory proteins might provide the actual links to the CFBs (Fig. 8 *C, lower cell*).

The HMP-1, HMP-2, and HMR-1 proteins are present in the dorsal, lateral, and ventral hypodermal cells along the segments of the adherens junction that contact the CFBs. This localization pattern raises the question of why the CFBs detach from the adherens junctions only in the dorsal hypodermis cells. One possible explanation is that lateral and ventral hypodermal cells contain molecules that have redundant functions with HMP-1, HMP-2, and HMR-1. Currently, the *C. elegans* genomic and EST sequences reveal no genes other than *hmr-1* that encode putative classical cadherins; several genes, including *cdh-3* (Pettitt et al., 1996), are similar to cadherins only in the

predicted extracellular domain. There is a second gene (corresponding to the CELK02251 EST) that shows significant homology to α-catenin. However, in preliminary antisense RNA experiments we find no evidence that this gene functions in hypodermal morphogenesis or substitutes for HMP-1 function (our unpublished results). A β-catenin–like gene, *bar-1*, has been shown to function in postembryonic development in *C. elegans*. However, null mutations in this gene have no embryonic phenotype (Eisenmann, D., personal communication). An additional β-catenin gene called *wrm-1* has been shown to be required for a Wnt-like signal transduction pathway in the early embryo, but it is not known whether this gene has a cytoskeletal function (Rocheleau et al., 1997).

Another possibility is that the total mechanical stress on the CFB–adherens junction connections in the lateral and ventral hypodermal cells is less than on connections in the dorsal hypodermis. The force exerted on the connections from contraction of the CFBs within a cell might be similar for all hypodermal cells. However, these same connections must also withstand external stretching forces generated by CFBs contracting in neighboring cells (Fig. 8 *B*). Part of the ability of a hypodermal cell to resist stretching likely derives from the sections of its adherens junction that are oriented parallel to the CFBs. In the dorsal hypodermis, there are relatively few such sections (see Figs. 3 *B* and 8 *A*). This is because the largest dorsal hypodermal "cell" is a multinucleate syncytium formed by the fusion of 23 individual cells. Before fusion, the 23 cells have 46 (two per cell) sections of adherens junction running parallel to the CFBs; after fusion there are only two such sections in the largest dorsal hypodermal cell. Thus, the CFB–adherens junction connections in the dorsal hypodermis may be stretched more forcefully and, consequently, break more easily than connections in the lateral or ventral hypodermal cells in Hmp mutants. It is worth noting that most of the cells in the *C. elegans* embryo are very small; the entire
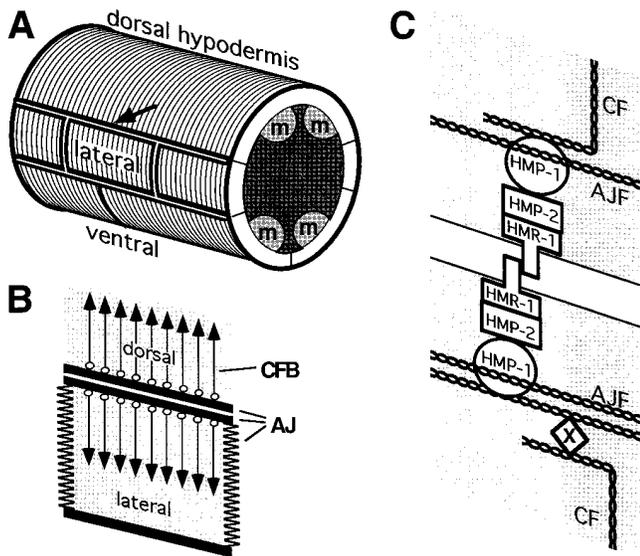
Figure 8. Models of morphogenetic forces and molecular organization at hypodermal cell junctions. (*A*) Oblique view of a schematic cross-section of an embryo after fusion of the dorsal hypodermal cells. CFBs are shown as thin lines, and adherens junctions are shown as thick lines. Bands of longitudinally oriented body wall muscles (*m*) are shown underlying the hypodermal cells. (*B*) Mechanical model of forces between the dorsal hypodermis and a lateral hypodermal cell. The connection between each CFB and the adherens junction (*AJ*) is represented as an open circle. In the lateral hypodermal cell, the connections are pulled downward by contraction of the CFBs within the lateral cell itself and pulled upward as CFBs in the dorsal cell contract. Note that the adherens junction at the two ends of the lateral cell (shown as two springs) are oriented such that they could dissipate some of the force exerted by contractions in the dorsal cell. (*C*) Two molecular models for the linkage between a filament (*CF*) in a CFB to a filament (*AJF*) in the adherens junction. HMR-1 is shown at the membrane contacts between two cells and associated with HMP-2. In the top cell, HMP-1 links a CF and an AJF directly; in the bottom cell, HMP-1 links different AJFs together while another factor (*X*) provides the link between the AJFs and CFs.

egg is only 50 μm in length, which is comparable to the size of a single vertebrate fibroblast. Because of its size, the large dorsal hypodermal cell may be a particularly useful model for studying how vertebrate epithelial cells respond to cytoskeletal stress.

If each CFB in the *C. elegans* hypodermis were attached to the adherens junction at its two ends but was otherwise free to move in the cytoplasm, breakage at one end might be expected to instantly relieve stress at the other end. Thus, a CFB might detach randomly from either side of the hypodermal cell but would not be expected to detach from both sides. Instead, we invariably detect detachment from both sides, and the CFBs appear to contract symmetrically toward the middle of the hypodermal cell surface. This observation suggests that the CFBs may also be linked to the apical cortex of a hypodermal cell. An interesting possibility is that the CFBs may be anchored to the hypodermal cell cortex at sites where body wall muscles contact the hypodermis. Beginning at about the 2× stage of elongation, hemidesmosomes form in the hypodermal cells in regions that overlie the body wall muscles (Hresko

et al., 1994). It is possible that this intermediate filament system is linked to the CFBs. If such a connection was required to maintain the proper organization of the CFBs, it might explain why Pat mutants defective in body wall muscle function show incomplete body elongation.

Cell Migrations Requiring a Catenin–Cadherin System

In *hmp-1*, *hmp-2*, and *hmr-1* mutants, the anterior three pairs of ventral hypodermal cells (the leading cells and G2/W) fail to complete ventral migration, resulting in the Hmr phenotype. In wild type embryogenesis, the leading cells are the first to extend ventrally, and only they project actin-rich filopodia from their leading edges (Williams-Masson et al., 1997). The normal migration behavior of G2/W has not been analyzed. Since initial migration of the leading cells does not appear to be affected in Hmr mutants, we speculate that HMP-1, HMP-2, and HMR-1 may function at the time filopodia from leading cells on either side of the embryo meet at the ventral midline. The HMP-1, HMP-2, and HMR-1 proteins appear to be absent from, or at very low levels in, the leading edge of migrating hypodermal cells. However as hypodermal cells establish contact with contralateral cells and adherens junctions form, these proteins localize to the vental midline. While HMP-1, HMP-2 and HMR-1 apparently are not required in general for adjacent cells to form adherens junctions (see below), they may act to stabilize fine filopodial contacts between contralateral pairs of ventral cells, and expand cell adhesion along these surfaces. This function may be analogous to the role of *Drosophila* E-cadherin in adhesion of filopodia from migrating tracheal cells and expansion of the tracheal lumen (Tanaka-Matakatsu et al., 1996).

Cell Contacts and Epithelial Tissue Integrity

Perhaps as significant as the roles of *hmp-1*, *hmp-2*, and *hmr-1* in specific morphogenetic processes is the apparent lack of requirement for these genes in cell adhesion, apico-basal polarity, and at least some aspects of adherens junction formation in the *C. elegans* embryo. Gene inactivation of *armadillo*/β-catenin in *Drosophila* (Cox et al., 1996; Müller and Wieschaus, 1996), αE-catenin, E-cadherin, and β-catenin in mouse (Larue et al., 1994; Haegel et al., 1995; Torres et al., 1997), and EP-cadherin and α-catenin in *Xenopus* (Heasman et al., 1994; Kafron et al., 1997) demonstrate that some catenin–cadherin systems are essential for the integrity of embryonic epithelia.

The HMP-1, HMP-2, and HMR-1 proteins are present on the surfaces of all blastomeres in early embryos. Although aspects of body morphogenesis are abnormal in Hmp and Hmr mutants, tissue-specific cell associations and cell polarities are clearly visible. For example, during early embryogenesis, germ cell precursors apparently must adhere to the intestinal cell precursors to be brought into the body cavity during gastrulation. We find that germ cells are internalized correctly in *hmp-1*, *hmp-2*, and *hmr-1* mutants. Also, if the *hmp* and *hmr* genes were essential for hypodermal cell adhesion, contraction of the CFBs might be expected to cause the rows of hypodermal cells to split apart from each other in the mutant embryos. Instead, we find that contacts between hypodermal cells generally remain intact. The only exception we observed was the sepa-

ration of some contralateral pairs of ventral hypodermal cells late in morphogenesis, which may result from incomplete body enclosure. Hypodermal cells clearly retain apical–basal polarity in Hmp and Hmr mutants since these cells secrete a cuticle on their apical surfaces and show subapical localization of the adherens junction antigen recognized by mAbMH27 antibody.

We do not believe the lack of additional defects in the Hmp or Hmr mutants is likely to be due to residual activities of the HMP or HMR proteins. *hmp-1 (zu278)* behaves genetically as a null mutation and produces no detectable HMP-1 protein, and embryos treated with RNA from the *hmp-2* or *hmr-1* genes do not produce detectable levels of the HMP-2 or HMR-1 proteins. Thus, if the HMP or HMR proteins have important, general roles in cell adhesion or cell polarity, other proteins must provide redundant functions. In any case, the absence of general cell adhesion defects in *hmp-1*, *hmp-2*, and *hmr-1* mutants has allowed us to characterize specific functions for a presumptive catenin–cadherin system in cell shape changes, cell movement, and cytoskeletal integrity.

References

Albertson, D.G. 1984. Formation of the first cleavage spindle in nematode embryos. *Dev. Biol.* 101:61–72.

Cox, R.T., C. Kirkpatrick, and M. Peifer. 1996. Armadillo is required for adherens junction assembly, cell polarity, and morphogenesis during *Drosophila* embryogenesis. *J. Cell Biol.* 134:133–148.

Costa, M., B.W. Draper, and J.R. Priess. 1997. The role of actin filaments in patterning the *Caenorhabditis elegans* cuticle. *Dev. Biol.* 184:373–384.

Draper, B.W., C.C. Mello, B. Bowerman, J. Hardin, and J.R. Priess. 1996. MEX–3 is a KH domain protein that regulates blastomere identity in early *C. elegans* embryos. *Cell.* 87:205–216.

Francis, G.R., and R.H. Waterston. 1991. Muscle cell attachment in *Caenorhabditis elegans*. *J. Cell Biol.* 114:465–479.

Gumbiner, B.M. 1996. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell.* 84:345–357.

Guo, S., and K.J. Kemphues. 1995. *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell.* 81:611-620.

Guo, X., J.J. Johnson, and J.M. Kramer. 1991. Embryonic lethality caused by mutations in basement membrane collagen of *C. elegans*. *Nature.* 349:707–709.

Haegel, H., L.Larue, M. Ohsugi, L. Fedorov, K. Herrenknecht, and R. Kemler. 1995. Lack of β-catenin affects mouse development at gastrulation. *Development (Camb.).* 121:3529–3537.

Heasman, J., D. Ginsberg, B. Geiger, K. Goldstone, T. Pratt, C. Yoshida-Noro, and C. Wylie. 1994. A functional test for maternally inherited cadherin in Xenopus shows its importance in cell adhesion at the blastula stage. *Development (Camb.).* 120:49–57.

Hresko, M.C., B.D. Williams, and R.H. Waterston. 1994. Assembly of body wall muscle and muscle cell attachment structures in *Caenorhabditis elegans*. *J. Cell Biol.* 124:491–506.

Iwai, Y., T. Usui, S. Hirano, R. Steward, M. Takeichi, and T. Uemura. 1997.

Axon patterning requires *D*N-cadherin, a novel neuronal adhesion receptor, in the *Drosophila* embryonic CNS. *Neuron.* 19:77–89.

Kafron, M., A. Spagnuolo, M. Klymkowski, C. Wylie, and J. Haesman. 1997. The roles of maternal α-catenin and plakoglobin in the early Xenopus embryo. *Development (Camb.).* 124:1553–1560.

Larue, L., M. Ohsugi, J. Hirchenhain, and R. Kemler. 1994. E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proc. Natl. Acad. Sci. USA.* 91:8263–8267.

Martin, P., and J. Lewis. 1992. Actin cables and epidermal movement in embryonic wound healing. *Nature.* 360:179–183.

Menkel, A.R., M. Kroemker, P. Bubeck, M. Ronsiek, G. Nikolai, and B.M. Jockusch. 1994. Characterization of an F-actin binding domain in the cytoskeletal protein vinculin. *J. Cell Biol.* 126:1231–1240.

Mittenthal, J.E., and A.G. Jacobson. 1990. The mechanics of morphogenesis in multicellular embryos. *In* Biomechanics of Active Movement and Deformation of Cells. N. Akkas, editor. Springer-Verlag, Berlin. 295–401.

Müller, H.J., and E. Wieschaus. 1996. *armadillo*, *bazooka*, and *stardust* are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in *Drosophila*. *J. Cell Biol.* 134:149–163.

Nieset, J.E., A.R. Redfield, F. Jin, K.A. Knudsen, K.R. Johnson, and M.J. Wheelock. 1997. Characterization of the interactions of α-catenin with α-actinin and β-catenin/plakoglobin. *J. Cell Sci.* 110:1013–1022.

Pai, L., C. Kirkpatrick, J. Blanton, H. Oda, M. Takeichi, and M. Peifer. 1996. *Drosophila* α-catenin and E-cadherin bind to distinct regions of *Drosophila* Armadillo. *J. Biol. Chem.* 271:32411–32432.

Peifer, M., and E. Wieschaus. 1990. The segment polarity gene *armadillo* encodes a functionally modular protein that is the *Drosophila* homolog of human plakoglobin. *Cell.* 63:1167–1178.

Pettitt, J., W.B. Wood, and R.H.A. Plasterk. 1996. *cdh-3*, a gene encoding a member of the cadherin superfamily, functions in epithelial cell morphogenesis in *Caenorhabditis elegans*. *Development (Camb.).* 122:4149–4157.

Podbilewicz, B., and J.G. White. 1994. Cell fusions in the developing epithelia of *C. elegans*. *Dev. Biol.* 161:408–424.

Priess, J.R., and D.I. Hirsh. 1986. *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Dev. Biol.* 117:156–173.

Priess, J.R., H. Schnabel, and R. Schnabel. 1987. The *glp-1* locus and cellular interactions in early *C. elegans* embryos. *Cell.* 51:601–611.

Rimm, D.L., E.R. Koslov, P. Kebriaei, C.D. Cianci, and J.S. Morrow. 1995. α_1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. *Proc. Natl. Acad. Sci. USA.* 92:8813–8817.

Robinson, P.A., B.H. Anderton, and T.L.F. Loviny,. 1988. Nitrocellulose-bound antigen repeatedly used for the affinity purification of specific polyclonal antibodies for screening DNA expression libraries. *J. Immunol. Methods.* 108:115–122.

Rocheleau, C., W.D. Downs, R. Lin, C. Wittmann, Y. Bei, Y. Cha, M. Ali, J.R. Priess, and C.C. Mello. 1997. Wnt signaling and an APC related gene specify endoderm in early *C. elegans* embryos. *Cell.* 90:707–716.

Shapiro, L., Fannon, A.M., P.D. Kwong, A. Thompson, M.S. Lehmann, G. Grübel, J. Legrand, J. Als-Nielsen, D.R. Colman, and W.A. Hendrickson. 1995. Structural basis of cell-cell adhesion by cadherins. *Nature.* 374:327–377.

Sibley, M.H., J.J. Johnson, C.C. Mello, and J.M. Kramer. 1993. Genetic identification, sequence, and alternative splicing of the *Caenorhabditis elegans* α2(IV) collagen gene. *J. Cell Biol.* 123:255–264.

Sulston, J.E., E. Schierenberg, J.G. White, and J.N. Thomson. 1983. The embryonic lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100:64–119.

Tanaka-Mattakatsu, M., T. Uemura, H. Oda, M. Takeichi, and S. Hayashi. 1996. Cadherin-mediated cell adhesion and cell motility in *Drosophila* trachea regulated by the transcription factor Escargot. *Development (Camb.).* 122:3697–3705.

Torres, M., A. Stoykova, O. Huber, K. Chowdhury, P. Bonaldo, A. Mansouri, S. Butz, R. Kemler, and P. Gruss. 1997. An α-*E*-catenin gene trap mutation defines its function in preimplantation development. *Proc. Natl. Acad. Sci. USA.* 94:901–906.

Wessells, N.K., B.S. Spooner, J.F. Ash, M.O. Bradley, M.A. Luduena, E.L. Taylor, J.T. Wrenn, and K.M. Yamada. 1971. Microfilaments in cellular and developmental processes. *Science.* 171:135–143.

White, J. 1988. The anatomy. *In* The Nematode *Caenorhabditis elegans.* W.W. Wood, editor. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 81–122.

Williams, B.D, and R.H. Waterston. 1994. Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. *J. Cell Biol.* 124:475–490.

Williams-Masson, E.M., A.N. Malik, and J. Hardin. 1997. An actin-mediated two-step mechanism is required for ventral enclosure of the *C. elegans* hypodermis. *Development (Camb.).* 124:2889–2901.

Wissmann, A., J. Ingles, J.D. McGhee, and P.E. Mains. 1997. *Caenorhabditis elegans* LET-502 is related to Rho-binding kinases and human myotonic dystrophy kinase and interacts genetically with a homolog of the regulatory subunit of smooth muscle myosin phosphatase to affect cell shape. *Genes Dev.* 11:409–422.

Young, P.E., A.M. Richman, A.S. Ketchum, and D.P. Kiehart. 1993: Morphogenesis in *Drosophila* requires nonmuscle myosin heavy chain function. *Genes Dev.* 7:29–41.