

***cyk-1*: a *C. elegans* FH gene required for a late step in embryonic cytokinesis**

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SUMMARY

A maternally expressed *Caenorhabditis elegans* gene called *cyk-1* is required for polar body extrusion during meiosis and for a late step in cytokinesis during embryonic mitosis. Other microfilament- and microtubule-dependent processes appear normal in *cyk-1* mutant embryos, indicating that *cyk-1* regulates a specific subset of cytoskeletal functions. Because cytokinesis initiates normally and cleavage furrows ingress extensively in *cyk-1* mutant embryos, we propose that the wild-type *cyk-1* gene is required for a late step in cytokinesis. Cleavage furrows regress after completion of mitosis in *cyk-1* mutants, leaving multiple nuclei in a single cell. Positional cloning and sequence analysis of the *cyk-1* gene reveal that it

encodes an FH protein, a newly defined family of proteins that appear to interact with the cytoskeleton during cytokinesis and in the regulation of cell polarity. Consistent with *cyk-1* function being required for a late step in embryonic cytokinesis, we show that the CYK-1 protein co-localizes with actin microfilaments as a ring at the leading edge of the cleavage furrow, but only after extensive furrow ingression. We discuss our findings in the context of other studies suggesting that FH genes in yeast and insects function early in cytokinesis to assemble a cleavage furrow.

Key words: Cytokinesis, FH gene, Microfilaments, Microtubules, Embryogenesis, *Caenorhabditis elegans*

INTRODUCTION

Cytokinesis in animal cells involves a circumferential activation of the cortical actin-myosin cytoskeleton to form an ingressing furrow in the plasma membrane. The cleavage furrow ultimately bisects the mitotic spindle and resolves to yield two separate daughter cells, in a process that appears to involve a sequence of discrete steps (for a review see Glotzer, 1997). These steps include positioning of a cleavage furrow, assembly of an actin and myosin contractile ring, invagination of the plasma membrane, and finally a termination step that partitions the two daughter nuclei into separate cells. Although several genes required for cytokinesis have been identified (see below), evidence that their corresponding gene products mediate a sequence of discrete steps remains minimal.

Genetic studies in several organisms show that members of the recently defined FH family of genes are required for cytokinesis, the regulation of cell polarity, or both (Frazier and Field, 1997; Wasserman, 1998). The FH family is named for two 'formin homology' regions, FH1 and FH2, which are conserved in sequence and, to some extent position, in all family members (see below). FH genes include *fusD* in *Drosophila*

melanogaster (Castrillon et al., 1994; Emmons et al., 1995), the *limb deformity* (*ld*) locus and *mDia* in *Mus musculus* (Woychik et al., 1990; Watanabe et al., 1997) and the human deafness gene *DFNA1* (Lynch et al., 1997).

FH proteins are predicted to range in size from about 100 to 200 kDa. In addition to the shared FH1 and FH2 regions, FH proteins exhibit lower levels of sequence similarity throughout their length, with the C-terminal region showing the highest sequence conservation (Frazier and Field, 1997; Wasserman

cytoskeleton through their FH1 domains. No proteins have been found that bind only the FH2 region. Cdc42p in *S. cerevisiae*, however, interacts with the N-terminal region of Bni1p, while Bnr1p binds a different Rho family member, Rho4p (Evangelista et al., 1997; Imamura et al., 1997). Thus, FH proteins interact with the actin cytoskeleton and may be effectors of small G-proteins.

Because actin microfilaments are required for the formation and ingression of a cleavage furrow during cytokinesis, and because FH1 domains interact with proteins that regulate or associate with actin microfilaments, FH proteins may function in cytokinesis by a localized modification of the membrane-associated actin cytoskeleton (Frazier and Field, 1997; Wasserman, 1998). For example, Cdc12p in *S. pombe* is found in the contractile ring during cytokinesis and appears to be required for actin ring assembly (Chang et al., 1997). In contrast, the mouse FH proteins encoded by the *ld* locus are found in the cytoplasm and nucleus, and the severity of mutations in the *ld* locus correlates with the loss of nuclear localization (Chan and Leder, 1996). Mutational inactivation of the mouse *ld* locus perturbs limb and kidney development, but the cellular functions of the encoded formin isoforms remain unknown (Woychik et al., 1990; Chan and Leder, 1996). The different phenotypes that result from mutational inactivation of different FH genes, and the varied protein localization patterns of the FH proteins, indicate that they participate in multiple processes, perhaps united only by a conserved interaction with the cytoskeleton.

To investigate the mechanism of FH function, we have examined the requirements for a maternally expressed FH gene in *C. elegans* called *cyk-1*, for cytokinesis-defective. Our genetic studies suggest that *cyk-1* is required for a late step in embryonic cytokinesis. Consistent with this hypothesis, we show that the CYK-1 protein localizes to the leading edge of the cleavage furrow near the end of cytokinesis.

MATERIALS AND METHODS

Strains and alleles

Standard procedures for nematode culture and genetics were followed

embryos, the pseudocleavage furrow is a prominent but transient ingression in the plasma membrane that forms early in the first zygotic cell cycle and then regresses as the maternal and paternal pronuclei meet near the center of the embryo. As

furrow into the cell (Fig. 1A), the first cleavage furrow is difficult to preserve during fixation of these mutant embryos. A cleavage furrow is detectable but does not extend deeply into the interior (Fig. 3B).

To further assess cleavage furrow formation and function in *cyk-1* mutant embryos, we also examined the distribution of NMY-2, a non-muscle myosin II required for both polarity and embryonic cytokinesis (Guo and Kemphues, 1996). In early stage wild-type embryos, NMY-2 localizes to the cortex of blastomeres (Guo and Kemphues, 1996), and is enriched in the cleavage furrow during cytokinesis (Fig. 3C). We found that NMY-2 is present in the cleavage furrows of *cyk-1* mutant embryos (Fig. 3C). In summary, actin and myosin are present in the cleavage furrow and mediate extensive ingression in most *cyk-1* mutants.

The *cyk-1* sequence predicts an FH protein

By positional cloning and a gene candidate approach, we determined that *cyk-1* corresponds to the predicted gene F11H8.4, identified by the

embryos when the contractile rings were 49%, 29%, 22%, 22% and 18% of the cell diameter. Thus only after the cleavage furrows close down substantially around the midzone of the mitotic spindle do detectable amounts of CYK-1 co-localize with the actin contractile ring. We see a similar pattern of CYK-1 localization to the leading edge of cleavage furrows late in cytokinesis during subsequent embryonic cell divisions (data not shown). Earlier in development we also detect CYK-1 near the site of polar body extrusion during meiosis (Fig. 6B), consistent with a meiotic requirement for *cyk-1* function (Fig. 2).

While we do not detect CYK-1 antibody staining in *t1568* and most *t1611* mutant embryos (Materials and methods), we do see localization of CYK-1 to the leading edge of abortive cleavage furrows in *or36* embryos (Fig. 6C). Thus truncation of CYK-1 after the FH2 sequence and the putative coiled-coil region near the C terminus (Fig. 4C) does not appear to preclude CYK-1 localization to the leading edge of the cleavage furrow, and sequences in the C-terminal 10% of the predicted CYK-1 protein may be important for the termination of cytokinesis.

Maternal expression of *cyk-1* mRNA during oogenesis

Using in situ hybridization (Materials and methods), we found that *cyk-1* mRNA is detectable in the germline and throughout all blastomeres in early stage embryos, fading rapidly to background levels after the 28-cell stage in embryogenesis (Fig. 7), with persistence of mRNA seen in the germline lineage (Fig. 7D). This pattern of accumulation is typical of many maternally expressed genes in *C. elegans* (Seydoux and Fire, 1994), indicating that both by genetic criteria and by in situ hybridization, *cyk-1* appears to be maternally expressed. Although our data do not rule it out, we see no evidence for zygotic expression: *cyk-1* mRNA levels fade throughout the embryo. Early in oogenesis, we see *cyk-1* mRNA in the oocyte and in the early cleavage furrows, and in the germline lineage throughout embryogenesis.

midzone microtubules. In support of the necessity of such a function in cytokinesis, several groups have recently reported a requirement for midzone microtubules throughout cytokinesis (Giansanti et al., 1998; Wheatley and Wang, 1996; Eckley et al., 1997). In this model of CYK-1 function, the FH1 domain of the predicted CYK-1 protein would bind to plasma membrane-associated microfilaments, perhaps through a nematode profilin or through an SH3 or WW domain protein (see Introduction). We further propose that CYK-1 might bind, directly or indirectly, through a different domain to midzone microtubules. This simple model does not exclude similar bridging functions for other proteins, or interactions of CYK-1 with additional proteins associated with the cleavage furrow.

While speculative, this model for CYK-1 function is consistent with a late defect in cytokinesis, and with the late localization of CYK-1 to the leading edge of the cleavage furrow. Perhaps CYK-1 must associate with both microfilaments and microtubules to concentrate at the leading edge of the cleavage furrow late in cytokinesis, once the actin-myosin contractile ring and the microtubule spindle are tightly apposed to one another. We also found that formation of the pseudocleavage furrow after fertilization is defective in *cyk-1* mutant embryos, another process that requires both microfilaments and microtubules (Hill and Strome, 1990; Hird and White, 1993). Finally, we note that inactivation of the *D. melanogaster* FH gene *cappuccino* results in a disorganization of the microtubule cytoskeleton during oogenesis (Manseau et al., 1996). Perhaps *cappuccino* organizes or polarizes microtubules by mediating an interaction between microfilaments and microtubules during insect oogenesis. It is intriguing to speculate that enabling concerted action between the actin- and microtubule-based cytoskeletons is a general property of FH proteins.

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REFERENCES

- Adachi, H., Takahashi, Y., Hasebe, T., Shirouzu, M., Yokoyama, S. and Sutoh, K. (1997). Dictyostelium IQGAP-related protein specifically involved in the completion of cytokinesis. *J. Cell. Biol.* **137**, 891-898.
- Albertson, D. G., Rose, A. M. and Villeneuve, A. M. (1997). Chromosome organization, mitosis and meiosis. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 47-78. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Albertson, D. G. and Thomson, J. N. (1993). Segregation of holocentric chromosomes at meiosis in the nematode, *Caenorhabditis elegans*. *Chromosome. Res.* **1**, 15-26.
- Aroian, R. V., Field, C., Pruliere, G., Kenyon, C. and Alberts, B. M. (1997). Isolation of actin-associated proteins from *Caenorhabditis elegans* oocytes and their localization in the early embryo. *EMBO J.* **16**, 1541-1549.
- Bione, S., Sala, C., Manzini, C., Arrigo, G., Zuffardi, O., Banfi, S., Borsani, G., Jonveaux, P., Philippe, C., Zuccotti, M., Ballabio, A. and Toniolo D. (1998). A human homologue of the *Drosophila melanogaster diaphanous* gene is disrupted in a patient with premature ovarian failure: evidence for conserved function in oogenesis and implications for human sterility. *J. Human. Genet.* **62**, 533-541.
- Bowerman, B., Eaton, B. A. and Priess, J. R. (1992). *skn-1*, a maternally expressed gene required to specify the fate of ventral blastomeres in the early *C. elegans* embryo. *Cell* **68**, 1061-1075.
- Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.
- Castrillon, D. H. and Wasserman, S. A. (1994). *Diaphanous* is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the *limb deformity* gene. *Development* **120**, 3367-3377.
- Chan, D. C., Bedford, M. T. and Leder, P. (1996). Formin binding proteins bear WWP/WW domains that bind proline-rich peptides and functionally resemble SH3 domains. *EMBO J.* **15**, 1045-1054.
- Chan, D. C. and Leder, P. (1996). Genetic evidence that formins function within the nucleus. *J. Biol. Chem.* **271**, 23472-23477.
- Chang, F., Drubin, D. and Nurse, P. (1997). *cdc12p*, a protein required for cytokinesis in fission yeast, is a component of the cell division ring and interacts with profilin. *J. Cell. Biol.* **137**, 169-182.
- Chisolm, R. (1997). Cytokinesis: a regulatory role for Ras-related proteins? *Curr. Biol.* **7**, 648-650.
- Draper, B. W., Mello, C. C., Bowerman, B., Hardin, J. and Priess, J. R. (1996). MEX-3 is a KH domain protein that regulates blastomere identity in early *C. elegans* embryos. *Cell* **87**, 205-216.
- Drechsel, D. N., Hyman, A. A., Hall, A. and Glotzer, M. (1997). A requirement for Rho and Cdc42 during cytokinesis in *Xenopus* embryos. *Curr. Biol.* **7**, 12-13.
- Eckley, D. M., Ainsztein, A. M., MacKay, A. M., Goldberg, I. G. and Earnshaw, W. C. (1997). Chromosomal proteins and cytokinesis: Patterns of cleavage furrow formation and inner centromere protein positioning in mitotic heterokaryons and mid-anaphase cells. *J. Cell Biol.* **136**, 1169-1183.
- Emmons, S., Phan, H., Calley, J., Chen, W., James, B. and Manseau, L. (1995). *cappuccino*, a *Drosophila* maternal effect gene required for polarity of the egg and embryo, is related to the vertebrate *limb deformity* locus. *Genes Dev.* **9**, 2482-2494.
- Evangelista, M., Blundell, K., Longtine, M. S., Chow, C. J., Adames, N., Pringle, J. R., Peter, M. and Boone, C. (1997). Bni1p, a yeast formin linking Cdc42p and the actin cytoskeleton during polarized morphogenesis. *Science* **276**, 118-122.
- Fire, A., Xu, S.-Q., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806-811.
- Frazier, J. A. and Field, C. M. (1997). Are FH proteins local organizers? *Curr. Biol.* **7**, 414-417.
- Giansanti, M. G., Bonaccorsi, S., Williams, B., Williams, E. V., Santolamazza, C., Goldberg, M. L. and Gatti, M. (1998). Cooperative interactions between the central spindle and the contractile ring during *drosophila* cytokinesis. *Genes Dev.* **12**, 396-410.
- Glotzer, M. (1997). The mechanism and control of cytokinesis. *Curr. Opin. Cell Biol.* **9**, 815-823.
- Guo, S. and Kemphues, K. J. (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, S. and Kemphues, K. J. (1996). A non-muscle myosin required for embryonic polarity in *Caenorhabditis elegans*. *Nature* **382**, 455-458.
- Harris, S. D., Hamer, L., Sharpless, K. E. and Hamer, J. E. (1997). The *Aspergillus nidulans sepA* gene encodes an FH1/2 protein involved in cytokinesis and the maintenance of cellular polarity. *EMBO J.* **16**, 3474-83.
- Hill, D. P. and Strome, S. (1990). Brief cytochalasin-induced disruption of microfilaments during a critical interval in 1-cell *C. elegans* embryos alters the partitioning of developmental instructions to the 2-cell embryo. *Development* **108**, 159-172.
- Hird, S. N. and White, J. W. (1993). Cortical and cytoplasmic flow polarity in early embryonic cells of *Caenorhabditis elegans*. *J. Cell Biol.* **121**, 1343-1355.
- Hird S. N., Paulsen, J. E. and Strome, S. (1996). Segregation of germ granules in living *Caenorhabditis elegans* embryos: cell-type-specific mechanisms for cytoplasmic localization. *Development* **122**, 1303-1312.

- Imamura, H., Tanaka, K., Hihara, T., Umikawa, M., Kamei, T., Takahashi, K., Sasaki, T. and Takai, Y.** (1997). Bni1p and Bnr1p: downstream targets of the Rho family small G-proteins which interact with profilin and regulate actin cytoskeleton in *Saccharomyces cerevisiae*. *EMBO J.* **16**, 2745-2755.
- Kemphues, K. J., Priess, J. R., Morton, D. and Cheng, N.** (1988). Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* **52**, 311-320.
- Lupas, A., Van Dyke, M. and Stock, J.** (1991). Predicting coiled coils from protein sequences. *Science* **252**, 1162-1164.
- Lynch, E. D., Lee, M. K., Morrow, J. E., Welsh, P. L., Leon, P. E. and King, M. C.** (1997). Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the *Drosophila* gene *diaphanous*. *Science* **278**, 1315-1318.
- Manseau, L., Calley, J. and Phan, H.** (1996). Profilin is required for posterior patterning of the *Drosophila* oocyte. *Development* **122**, 2109-2116.
- Mello, C. C., Kramer, J. M., Stinchcomb, K. and Ambros, V.** (1991). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**, 3959-3970.
- Petersen, J., Weilguny, D., Egel, R. and Nielsen, O.** (1995). Characterization of *fus1* of *Schizosaccharomyces pombe*: a developmentally controlled function needed for conjugation. *Mol. Cell. Biol.* **15**, 3697-3707.
- Rose, L. S., Lame, M. L., Hird, S. N. and Kemphues, K. J.** (1995). Pseudocleavage is dispensable for polarity and development in *C. elegans* embryos. *Dev. Biol.* **168**, 479-489.
- Seydoux, G. and Fire, A.** (1994). Soma-germline asymmetry in the distributions of embryonic RNAs in *Caenorhabditis elegans*. *Development* **120**, 2823-2834.
- Strome, S.** (1986). Fluorescence visualization of the distribution of microfilaments in gonads and early embryos of the nematode *Caenorhabditis elegans*. *J. Cell. Biol.* **103**, 2241-2252.
- Strome, S. and Wood, W. B.** (1983). Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos. *Cell* **35**, 15-25.
- Sulston, J. E., Schierenberg, E., White, J. G. and Thomson, J. N.** (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64-119.
- Theriot, J. A. and Mitchison, T. J.** (1983). The three faces of profilin. *Cell* **75**, 835-838.
- Wasserman, S.** (1998). FH proteins as cytoskeletal organizers. *Trends Cell Biol.* **8** (in press).
- Watanabe, N. P., Madaule, P., Reid, T., Ishizaki, T. and Watanabe, G.** (1997). p140mDia, a mammalian homolog of *Drosophila diaphanous*, is a target protein for a Rho small GTPase and is a ligand for profilin. *EMBO J.* **16**, 3044-3056.
- Wheatley, S. P. and Wang, Y-L.** (1996). Midzone microtubule bundles are continuously required for cytokinesis in cultured epithelial cells. *J. Cell Biol.* **135**, 981-989.
- Woychik, R. P., Maas, R. L., Zeller, R., Vogt, T. F. and Leder, P.** (1990). 'Formins': proteins deduced from the alternative transcripts of the limb deformity gene. *Nature* **346**, 850-853.