

# ***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 *fus1* 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

control, *sma-3 unc-36(e251)/+* males were crossed into *nDf16/qC1* hermaphrodites. Small, Unc animals were identified (*nDf16* uncovers *unc-36*) and were compared with *sma-3 cyk-1/nDf16* animals. Oocyte production defects and the resulting sterility were not seen in the *sma-3 unc-36/nDf16* animals. Animals homozygous for the deficiency, produced by heterozygous mothers, die during embryogenesis and fail to hatch.

***cyk-1* mRNA and protein expression**

Synthesis of digoxigenin-labeled antisense RNA probes (Boehringer-Mannheim) and in situ hybridizations were done as described (Seydoux et al., 1994), except that no Proteinase K step was included. For SP6 (antisense) and T7 (sense) polymerase templates, we used *XbaI*- or *NdeI*-linearized p1.6A3, respectively. We compared antisense with sense staining as a negative control, and we used staining with *mex-3* antisense RNA made from the pJP621 plasmid as a positive control (Draper et al., 1996).

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N-erominalafrvagnmti(anmino acd ar11(gsisdus -35 Tfsed) o hGST  
 C-erominalafragnmti(anmino acd s 834-1159, with e as thae jfifv ag eno re a 54(amibg 20 h 5005) hqg) / JI\* C\* T\* C\* 0054 T\* C\* T\* C\* (at a n g a w p a t e r o n d s e W E) m b y o g s i n s

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, and we see *cyk-1* mRNA in the oocyte and in the early cleavage stages (Fig. 7A, 7B, 7C, 7D, 7E, 7F, 7G, 7H, 7I, 7J, 7K, 7L, 7M, 7N, 7O, 7P, 7Q, 7R, 7S, 7T, 7U, 7V, 7W, 7X, 7Y, 7Z).







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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