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

Kathryn A. Swan[1], Aaron F. Severson[2], J. Clayton Carter[1], Paula R. Martin[1,], Heinke Schnabel**

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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 *Xba*I- or *Nde*I-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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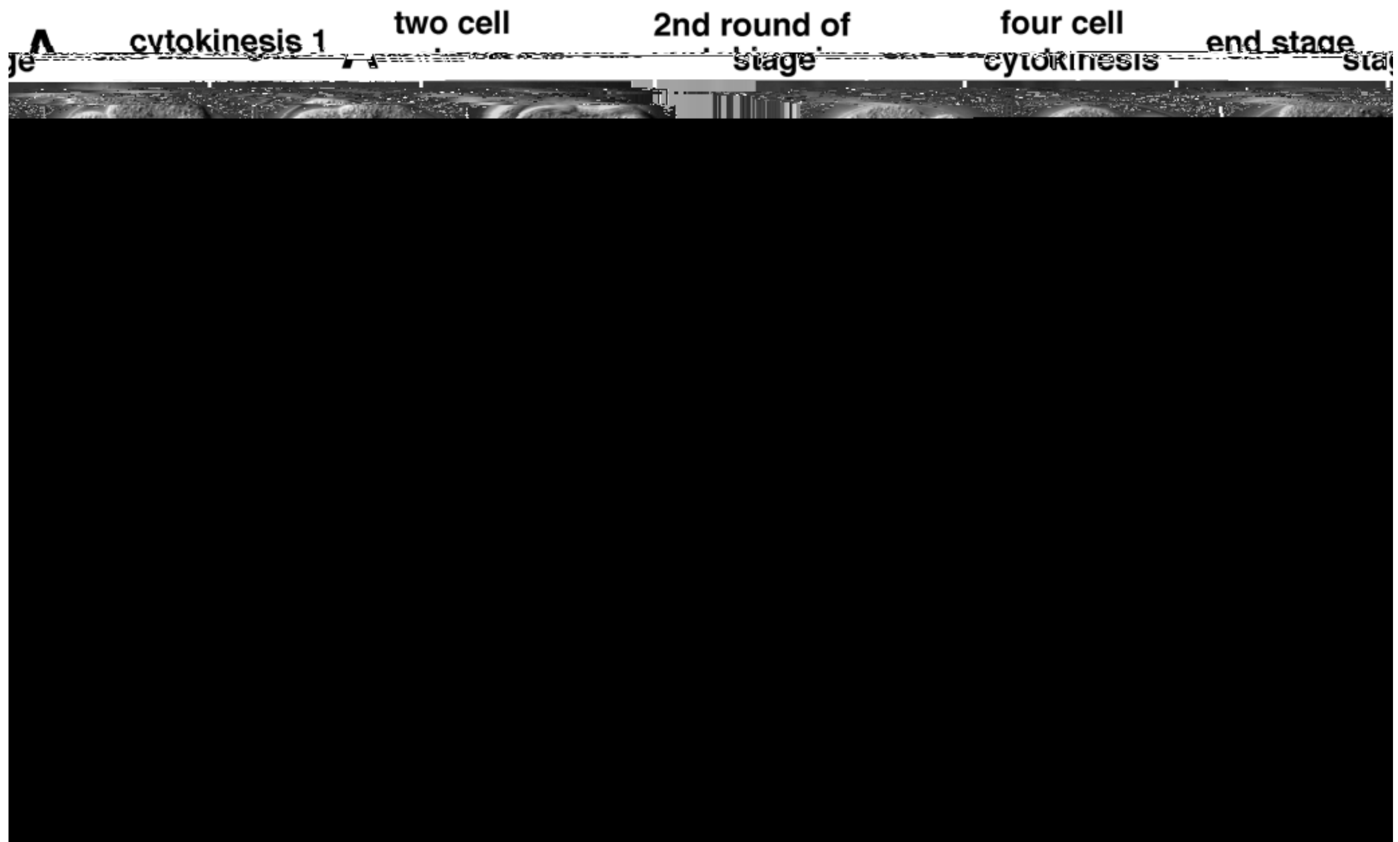

Fig. 1. *cyk-1* embryos are unable to complete cytokinesis. (A) Nomarski micrographs showing cytokinesis occurring at the 1- and 2-cell stages in wild type and at the equivalent stages in *cyk-1* mutant embryos. Note that the cleavage furrows in *cyk-1* mutant embryos persist even as nuclei reform. A tetrapolar spindle forms (observable in Nomarski images as an area cleared of yolk granules) at the second cleavage in *cyk-1* embryos, with bisecting tetrapolar cleavage furrows. A late stage wild-type embryo is shown just prior to hatching; a late stage *cyk-1* mutant embryo is shown with hypodermal-like nuclei visible in a multinucleated single cell. We also have observed octapolar spindles during the third round of embryonic mitosis, with pronounced cleavage furrows bisecting each spindle arm. In most embryos after the first two rounds of mitosis, though, large numbers of interconnected spindles appear to trigger widespread contractions of the plasma membrane without extensive furrow formation (K.A.S. and B.B., data not shown). In this and subsequent figures, anterior is to the left and ventral at the bottom; bar, approximately 10 μm. *C. elegans* embryos are about 50 μm in length. (B) Cell cycle timing during the first and second cell cycles in *cyk-1* mutant embryos compared to wild-type embryos (±1 s.d.).

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 *nop-1* mutant embryos do not undergo pseudocleavage but otherwise develop normally (Rose et al., 1995), it seems unlikely that the near absence of pseudocleavage furrows in *cyk-1* embryos significantly affects subsequent steps in development.

Most cytoskeletal functions appear normal in *cyk-1* mutant embryos

Many events in the early embryo that require a functional cytoskeleton occur normally in *cyk-1* mutant embryos. These events include pronuclear migration, spindle assembly and positioning, and anaphase chromosome segregation (Figs 1A, 2A,B and data not shown). Thus, mutational inactivation of *cyk-1* appears not to affect most cytoskeletal functions. We also examined P-granule segregation, a process that, like cytokinesis, requires microfilaments (Hird et al., 1996). P-granules are cytoplasmic structures initially present throughout the early 1-cell stage zygote that are segregated to the posterior cortex before the first embryonic mitosis. In contrast to the cytokinesis defect, P-granule localization appears normal in *cyk-1* mutant embryos (Fig. 3A), consistent with other functions of the actin cytoskeleton being unaffected.

The cleavage defect in *cyk-1* mutant embryos occurs late in cytokinesis

Observation of dividing embryonic cells suggests that cytokinesis initiates normally in *cyk-1* mutant embryos and that extensive ingression of the cleavage furrow occurs (Fig. 1A). To more accurately assess cleavage furrow ingression, we used phalloidin staining of fixed *cyk-1* mutant embryos to visualize filamentous actin associated with the cytoplasmic cortex (Strome, 1986; Aroian et al., 1997). The cleavage furrows that form during the second attempt at cytokinesis in *cyk-1* mutant embryos extend far into the cell and often meet in the center (Fig. 3B). Although in living *cyk-1* embryos, the first attempt at cytokinesis results in extensive migration of the cleavage

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 development, [illegible]

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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