The Flightless I homolog, \textit{fli-1}, regulates anterior-posterior polarity, asymmetric cell division and ovulation during \textit{C. elegans} development

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Abstract

Flightless I (Fli I) is an evolutionarily conserved member of the gelsolin family, containing actin binding and severing activity in vitro. The physiological function of Fli I during animal development remains largely undefined. In this study, we reveal a key role of the C. elegans Fli I homolog, fli-1, in specifying asymmetric cell division and establishing anterior-posterior polarity in the zygote. The fli-1 gene also regulates the cytokinesis of somatic cells, the development of germline, and interacts with the phosphoinositol signaling pathway in the regulation of ovulation. The fli-1 reporter gene shows that the localization of FLI-1 coincides with actin-rich regions, and the actin cytoskeleton is impaired in many tissues in the fli-1 mutants. Furthermore, the function of fli-1 in C. elegans can be functionally substituted by the Drosophila Fli I. Our studies demonstrate that fli-1 plays an important role in regulating the actin-dependent events during C. elegans development.
Introduction

The actin microfilament cytoskeleton regulates multiple cellular processes, including cytokinesis, cell morphology, establishment of cell polarity and cell motility. Actin-binding proteins, such as the members of the gelsolin family, have been shown to modulate the actin filament network by regulating the polymerization and depolymerization of actin (for review, see Sun et al., 1999). The actin filament severing and capping activity of gelsolin is regulated by Ca$^{2+}$ and phosphoinositol 4, 5-bisphosphate (PIP2) (for review, see Kwiatkowski, 1999; Silacci et al., 1999).

Despite their crucial role in regulating actin dynamics in tissue cultures, knockouts of gelsolin and several other related genes are viable in mice, indicating the existence of compensatory mechanisms in the regulation of actin cytoskeleton turnover and reorganization (Witke et al., 1995). Consequently, this functional redundancy of the actin-binding proteins complicates study of their physiological roles during animal development.

Flightless I (Fli I) is a unique member of the gelsolin family of proteins (Campbell et al., 1993). In addition to the gelsolin domain, Fli I contains 16 tandem leucine rich repeats (LRR) at its N-terminus. The LRR motif is known to be involved in protein-protein or protein-lipid interactions, suggesting a role for Fli I in linking the actin cytoskeleton with the signal transduction pathways (Campbell et al., 1997; Claudianos et al., 1995). Consistent with having the gelsolin domain, the human and C. elegans FLI-1 proteins bind to both actin monomers (G-actin) and actin filaments (F-actin) in vitro and possess F-actin serving activity (Goshima et al., 1999; Liu and...
Unlike other gelsolin family proteins, the actin-binding and severing activity of Fli I appears to be calcium independent (Goshima et al., 1999). Consistently, the residues essential for calcium binding in the gelsolin region of the other gelsolin family proteins are not conserved in Fli I (Goshima et al., 1999).

Analysis of the viable and lethal mutants in *Drosophila* suggests that Fli I may be involved in regulating the actin cytoskeleton reorganization (de Couet et al., 1995; Straub et al., 1996). Depletion of Fli I causes a defect in the cellularization of the syncytial blastoderm during early embryogenesis, a process with similarities to cytokinesis (Straub et al., 1996). This defect is associated with a disorganized cortical actin cytoskeleton in the embryo (Straub et al., 1996). However, flies lacking Fli I do not have defects in cytokinesis at other developmental stages, nor is Fli I needed for cell division of the germline (Straub et al., 1996). In mouse, the Fli I knockout is embryonic lethal (Campbell et al., 2002). In human, the Fli I locus is mapped to a region deleted in Smith-Magenis syndrome, a disorder that exhibits many developmental and behavioral abnormalities (Chen et al., 1995). Thus, elucidating the physiological function of Fli I during animal development will help us to understand the causes of the defects associated with Fli I loss-of-function in mammals.

Studies in *C. elegans* have revealed an essential role of the actin cytoskeleton in the establishment of cell polarity, asymmetric distribution of cell fate determinants and in morphogenesis (Strome and Wood, 1983; Strome et al., 1986). The establishment of cell polarity has been extensively characterized in the division of the one-cell stage embryo (Albertson et al., 1984; Gonczy et al., 1999). A polarized
cytoplasmic flow, which involves the simultaneous movement of cortical cytoplasm away from and the interior cytoplasm toward the sperm pronucleus, occurs during the pronuclear stage (for review, see Nance 2005; Cowan and Hyman; 2004). Correlated with the polarized cytoplasmic flow, some cytoplasmic components become asymmetrically distributed. For example, the germline specific P granules are segregated exclusively in the posterior end of the P1 blastomere after first cell division (Hird et al., 1996; Kemphues and Strome, 1997). Depolymerization of the actin microfilaments by cytochalasin D blocks polarized cytoplasmic flow, prevents P-granule segregation and causes other losses in anterior/posterior asymmetry (Hill and Strome; 1988). Coordination of the dynamics of the actin cytoskeleton within different tissues also regulates other more complex biological processes during C. elegans development. For example, successful ovulation requires the orchestrated actions of the gonad sheath cell contracting and the spermatheca dilating (McCarter et al., 1997; Clandinin et al., 1998). Therefore, C. elegans offers a model by which to study comprehensively the physiological role of actin-binding proteins in the regulation of actin turnover and reorganization.

Here, we show that mutations in the C. elegans Fli I homolog, fli-1, cause defects in actin-based events, including cytokinesis, the establishment of the cell polarity, asymmetric cell division and ovulation. fli-1 is expressed in actin-rich regions. Abnormalities in the organization of actin filaments exist in fli-1 mutants. The function of fli-1 can be functionally substituted by the fly Fli I, suggesting a conserved role of Fli I in regulating the dynamics of actin cytoskeleton during animal
development.

Materials and Methods

Strains and alleles

The following mutant alleles were used in this study. LG I: *lfe-2*(sy326). LG III: *fli-1*(bp130), *sma-3*(e491), *unc-32*(e189), *ruIs32*(pie-1::GFP::H2B). LG IV: *jcls1*(ajm-1::gfp). LGV: *pgl-1::gfp*, *zuIs45*(nmy-2::gfp), *wIs51*(scm::gfp), *him-5*(1490), *bxIs14*(pkd-2::gfp). LG X: *ipp-5*(sy605). The location for *qIs56*(lim-7::GFP) was not determined.

Identification, genetic mapping and molecular cloning of *fli-1*

*fli-1*(bp130) was identified in a screen to isolate mutants with altered number of seam cells at young adult animals. In brief, strains carrying the seam cell specific marker, *scm::gfp*, were mutagenized by EMS and mutants with increased or reduced number of seam cells were cloned. From 6,000 haploid genomes screened, 31 mutants were obtained. *fli-1* mutants have reduced number of seam cells and other defects, including reduced brood size and defects in the development of germline and tail morphogenesis.

Three-factor mapping placed *fli-1* between *sma-3*(-0.93) and *unc-32*(0.0) on linkage group III, at an approximate chromosomal position of -0.1. From the +*fli-1* +/*sma-3* +*unc-32* cross, nine out of 10 Sma non-Unc recombinants and one out of 11 Unc non-Sma recombinant carried the *fli-1* mutation.

To identify the *fli-1* gene in this region, cosmid DNA (provided by Alan
Coulson at Sanger Center, UK) was coinjected with the rol-6(su1006) dominant marker into fli-1/qC1; scm::gfp animals. Heterozygous F1 Rol transformants were picked and stable transgenic lines were obtained. Individual F2 Rol animal was cloned and its progenies that did not segregate Dpy worms (qC1 animals are Dpy) were further analyzed for the number of seam cells and Emo phenotypes. We found that cosmid B0523 rescued the fli-1 mutant defects. The five candidate genes located in B0523 were PCR amplified for delimiting the rescuing activity and we found that B0523.5 gave rescue activity.

The fli-1(bp130) mutation was determined by sequencing the PCR products from the corresponding genomic sequence. To determine whether the bp130 mutation affects the fli-1 splicing, RT-PCR was performed using primers located in the exon 3 (5' atgcctccaccacagatcg 3') and exon 5(5' ctgtcaattgatattgtc 3') of fli-1 and the PCR products were sequenced.

**Time-lapse Recordings**

Time-lapse Nomarski images were performed as described previously (McCarter et al., 1997). In brief, worms were anesthetized in 0.1% tricaine, 0.01% tetramisole in M9 for 30 min, mounted on 2% agarose pads, and then were observed under a Zeiss axivert microscope with a 100x Fluor objective (numerical aperture 1.3). Images were captured by CCD (AxioCam) and recorded every 10 seconds for 60 to 100 minutes (software, Axiovision: Re14.2).

**RNA interference**

Single-stranded RNA (ssRNA) was transcribed from the T7 and SP6-flanked
PCR templates. The primers used for amplifying the *fli-1* templates for synthesizing RNA are: 5' cactagatttagtgacactatagaacaggtgcctgatgagctg 3' and 5' cactagtaatagactcactatagccagccagctgcctgcac 3'. The dsRNA was then injected into animals carrying *scm::gfp* reporter. Eggs laid by the injected animals between 4 to 48 hours were collected for further analysis. The average number of seam cells was 13.4 (ranging from 11 to 15, n=20) in *fli-1(RNAi)* animals. Furthermore, two out of 16 *fli-1(RNAi)* animals showed defects in tail morphogenesis and six out of 16 *fli-1(RNAi)* animals showed the EMO phenotype.

**Construction of *fli-1::gfp* reporter and *fli-1::Fli I cDNA**

The *fli-1::gfp* reporter was constructed by PCR fusion based approach. The fused PCR products were derived from two overlapping PCR DNA fragments. One contained the DNA derived from fosmid WRM0621aG11 (nt 20547 to 30147), which includes a 2kb promoter region and the entire ORF of *fli-1*. Another one contained *gfp* and the *unc-54 3' UTR* from pPD95.67 (The NLS was not included in our reporters). The reporter DNA was coinjected with pRF4 (rol-6). *fli-1::gfp* rescued the *fli-1* mutant defects (Table 2).

The chimeric gene *fli-1(promoter)::Fli I cDNA* was constructed by following: the full length of fly-1 Fli I cDNA was subcloned into pPD95.67 backbone that contains the *fli-1* promoter (WRM0621aG11, nt 28146 to 30147), *gfp* and *unc-54 3'UTR*. The construct was injected into *fli-1/qC1* animals (Table 2).

**Immunostaining**

A rapid one-step fixation/permeabilization/staining procedure was used for
R-ph visualization of F-actin as previously described by Strome, 1986. In brief, the dissected animal parts, such as gonad arms, or the cut gravid adult hermaphrodites (for body muscle staining) were placed in 5ul M9 on a polylysine-treated microscope slide. Samples were fixed (1.5% paraformaldehyde, 0.1% glutaraldehyde) and stained with R-phalloidin (0.33 uM in M9) for 20-30 minutes at room temperature, washed by PBS, and then were observed. As for staining of F-actin in embryos, the eggs were collected from the bleached gravid adult hermaphrodites. Embryos were washed twice in M9 buffer, then were fixed and stained.

**Dye-filling assays of the phasmid**

The worms were stained with 25 ug/ml DiO solution at room temperature for two hours and then destained for one hour. The stained animals were visualized under fluorescence microscope using a Rhodamine filter.

**Results**

The *fli-1* mutants display defects in the asymmetric cell division at post-embryonic stage

**A. Defects in the post-embryonic development of seam cells**

In wild type animals, seam cells divide at each of the four larval stages, with one daughter cell fusing with the hypodermal syncytium, hyp7, and the other daughter cell maintaining seam cell fate for further division. At the L2 larval stage, seam cells H1, V1-V4 and V6 undergo an extra round of symmetric cell division with both daughter cells adopting seam cell fate, resulting in an increase in the number of seam
cells from 10 at the hatching stage to 16 at the later L2 larval stage and onwards (Fig. 1A,B) (Sulston and Horvitz, 1977). In order to determine how the stage specific seam cell division is specified, we performed genetic screens to identify mutants with altered number of seam cells at adult stage. The bp130 mutation was identified that caused reduced number of seam cells (Fig. 1C). In the bp130 mutant adults, the average number of seam cells was 11.2 (n=83, ranging from 6 to 14), compared to an average number of 16.3 (n=32, ranging from 15 to 17) in wild type animals.

Subsequent genetic and molecular analysis indicated that bp130 encodes the C. elegans Fli I homolog, fli-1. fli-1(bp130) showed maternal effect (Table 1) and only the fli-1(bp130) mutants derived from fli-1(bp130) homozygous were analyzed in the subsequent studies unless otherwise noted.

We further analyzed seam cell division in the fli-1 mutants and found that the asymmetric cell division of seam cells was defective, resulting in both daughter cells either fusing with hyp 7 or retaining seam cell fates (Fig. 1D). 25% and 67% of fli-1 mutant animal sides (n=21, seam cells on each side of animal develop independently, representing separate developmental process) have one or more seam cells with both daughter cells retaining the seam cell fate or fusing with hyp 7, respectively. Other abnormalities in the seam cell development in fli-1 mutants included the transformation of the seam cell fate to other hypodermal cell type and failure to divide (Fig. 1E,F). In 15% of fli-1 mutant animal sides (n=32), some daughter seam cells expressed the epidermal cell marker ajm-1::gfp, but failed to express the seam cell specific marker, scm::gfp (Fig. 1E), indicating the change of the seam cell fate to
other hypodermal cell fate. Moreover, in 9% of mutant animal sides (n=32), two nuclei were present in one cell, indicating a defect in the cytokinesis of seam cells (Fig. 1F). Thus, the development of seam cells shows multiple defects in fli-1 mutants.

We further characterized the development of seam cell T, which undergoes asymmetric cell division with the anterior daughter cell maintaining seam cell fate and the posterior daughter cell acquiring a neuronal fate, giving rise to a group of neuronal cells, called phasmid (Fig. 1A). The phasmid can be detected by its ability to take up Dye (Fig. 1G) (Herman and Horvitz, 1994). One or both of the two socket cells in the phasmid failed to take up dye in the dye filling assay in 46% and 10% of the fli-1 mutant animal sides (n=100), respectively (Fig. 1H). Consistent with this, we found that both daughters of T, T.a and T.p, expressed the seam hypodermal fate in 33% of animal sides (n=12) (Fig. 1I-L). In summary, asymmetric cell division of seam cells is defective in the fli-1 mutants.

B: Defects in the development and division of distal tip cells

The development of distal tip cells (DTCs), located at the tip of each gonad arm (Fig. 2A,B), was also analyzed in the fli-1 mutants. The DTC plays an essential role in the migration and extension of the gonad arm. In 12% of the fli-1 mutant animals (n=43), two DTCs (labeled by a lag-2::gfp reporter) were present in one gonad arm (Fig. 2C). Consistently, two gonad branches were formed in those gonad arms containing two DTCs (Fig. 2D). In 5% of the fli-1 mutant branches, two nuclei were found in one DTC, indicating a defect of the cytokinesis (data not shown). The
morphology of DTC was also abnormal in the \textit{fli-1} mutants. In wild type young adult animals, the processes of DTC extend and branch down the side of germline (Fig. 2E) (Finger et al., 2003). In 72\% of the \textit{fli-1} mutant young adults (\(n=45\)), the processes of the DTCs were much longer and disorganized (Fig. 2F).

The migration of the gonad was also defective in the \textit{fli-1} mutants. Normally, in wild type animals, the gonad migrates away from the mid-body region and then makes a turn from the ventral to the dorsal site. Finally, it reorients and migrates back towards the mid-body (Fig. 2G) (Finger et al., 2003). However, in the \textit{fli-1} mutants, 67\% of the gonad branches (\(n=102\)) migrated for only a short distance along the ventral body wall muscles. 45\% of the mutant gonad branches (\(n=102\)) showed defects in the migration from the ventral to dorsal site (Fig. 2H), while 54\% (\(n=102\)) showed defects in the migration back to the mid-body (note that single gonad arm could display multiple migration defects). Taken together, wild type \textit{fli-1} is required for the DTC and gonadal development.

\textbf{Reduction of the function of \textit{fli-1} causes defects in the establishment of the A/P polarity and cytokinesis in the first mitotic cell cycle}

To further determine the role of \textit{fli-1} in asymmetric cell division, we analyzed the first mitotic cell division in living \textit{fli-1} mutant embryos by time-lapse recording. In wild type zygotes, the polarized cytoplasmic flow, shown by the movement of the cortical yolk droplets, occurs rapidly in the posterior region of the embryo (see Movie 1 in supporting online materials) (Hird and White 1993; Hird et al., 1996). Correlated with the polarized flow, the maternal pronucleus migrates to meet the paternal
pronucleus in the posterior hemisphere of the embryo, approximately 70% of the length of the embryo (Fig. 3A) (for review, see Cowan and Hyman, 2004). After the pronuclear meeting, the first mitotic spindle forms and becomes displaced posterior, resulting in the production of a smaller P1 cell and a larger AB cell (Fig. 3E and see Movie 1). We found that reducing the function of fli-1 caused multiple defects in the division of the zygotes. The movement of the yolk droplets was greatly reduced in the fli-1 mutants (see Movie 2). The polarized localization of P granules in the posterior end of the embryo and the asymmetric meeting of the pronuclei were also severely impaired in the fli-1 mutant zygotes (Fig. 3 and Fig. 4) (Hird et al., 1996; Kawasaki et al., 1998). In 30% of the fli-1 mutant embryos (n=42), pgd-1::gfp labeled P granules were distributed in the whole P1 cell instead of being confined to the posterior end of the P1 cell (Fig. 4A to D). In ~5% of these mutant embryos (n=42), the P granules were distributed in both the AB and P cells (Fig. 4E, F). We analyzed the first mitotic cell division in 12 normal looking fli-1 mutant zygotes in detail. The maternal and paternal pronuclei were initially properly positioned and the pseudocleavage furrow was formed in most of the fli-1 mutants. The migration of the pronucleus, however, was abnormal in four of the 12 fli-1 zygotes (Fig. 3B-D). The paternal pronucleus migrated only small distance and the pronuclei met near at the about 80 to 90% of the embryo in the posterior region in two mutant embryos (Fig. 3B), while both pronuclei migrated and met in the center of the embryo in other two (Fig. 3C). Three of these four embryos with abnormal pronuclear meeting proceeded through the mitotic cycle without division and underwent endomitotic division. The rest of the eight fli-1
mutant embryos successfully progressed through the first division. However, five mutant embryos took one hour or more to complete division, compared to ~20 minutes in wild type embryos (Fig. 3F).

Mutations in *fli-1* cause other defects in the first mitotic cell division. For example, segregation of chromosomes, visualized by the histone 2B (H2B::GFP) marker, showed abnormality at anaphase in 9% of *fli-1* mutant embryos (n=23) (Fig. 3G-J). In addition to the defects in the first mitotic cell division, mutations in *fli-1* also affected subsequent cell divisions. For example, in wild type embryos, the AB cell completes division prior to the initiation of the cleavage of the P1 cell (Fig. 3K, L). In the *fli-1* mutants, however, the temporal order of the division of the AB and P cells could be disrupted, such that P1 cell division occurred before that of the AB cell in 13% of mutant embryos (n=23) (Fig. 3M,N).

To determine whether the defects in early cell division in *fli-1* mutants were the indirect consequence of defects in eggshell production, we examined the DAPI absorption of the embryos (Kawasaki et al., 2004). *fli-1(bp130)* and wild type embryos were released from the uterus of mothers into water containing DAPI (final concentration 10ug/ml). We found that all seven *fli-1(bp130)* one-cell-stage embryos excluded DAPI. Thus, *fli-1* mutant embryos produced functional eggshells.

In summary, mutations in *fli-1* cause multiple defects in the first mitotic cell division, including defects in the polarized cytoplasmic flow, in the establishment of the A/P asymmetry of P granules, in the migration of the pronuclei, in the formation of the cleavage furrow and in the chromosomal segregation.
The **fli-1** mutants display defects in other developmental processes

The **fli-1** mutants also showed defects in morphogenesis, cell migration and axon guidance. Four out of 20 **fli-1** mutant embryos displayed bulges at dorsal surfaces, a phenotype that is similar to that of **hmp-1** and other mutants causing the humpback phenotype (Fig. 5A,B) (Costa et al., 1998). Also instead of having a gradually tapered tail spike, the **fli-1** mutant tail was malformed and contained a bulge (100%, n=13) (Fig. 5C, D). The development of male specific structures was also severely deformed in the **fli-1** mutants. For example, the cuticular fan structure was small and the male specific sensory rays were missing in all the male sides examined (n=6) (Fig. 5E, F). Thus, **fli-1** plays an important role in morphogenesis and/or in cell fate determination.

The migration of Q cell descendants and axon guidance was also defective in the **fli-1** mutants. In wild type animals, **mec-7::gfp** labeled touch neuron AVM, a descendant of QR neuroblast, migrates to the anterior and positions anterior to the touch neurons ALMs (Fig. 5G) (Ch'ng et al., 2003). In 8% of the **fli-1** mutant sides (n=52), the migratory distance of AVM was shorter and positioned posterior to ALMs (Fig. 5H). In 11% of the **fli-1** mutant sides (n=23), AVM was missing, indicating a defect in the development of QR. The axon guidance of various neuronal types, including DD and VD motor neurons, the PVM touch neuron and the male ray neurons, was defective in **fli-1** mutants. For example, in 33% of the **fli-1** mutants (n=16), the axon trajectory followed by ray 1 B-type neuron (R1B), labeled by **pkd-2::gfp**, failed to make the turn around the body, but continued to migrate to the
anterior (Fig. 5I,J) (Jia and Emmons, 2006). Therefore, *fl-i-1* plays essential roles in controlling the migration of cells and neuronal axons.

**Mutations in *fl-i-1* cause defects in germ cell development**

Having established the important role of *fl-i-1* in the development of somatic cells, we next investigated the role of *fl-i-1* in the development of the germline. During germline development, germ cells undergo mitotic cell division at the distal tip of the gonad and enter meiosis as they move proximally (for review, see Crittenden et al., 2003). In the wild type gonad, the meiotic germ cells are nearly round in shape and are evenly distributed at the germ cell plasma membrane, forming a single layer of nuclei around the nucleus-free center (the rachis) (Fig. 6A,C). The number of germ cells was greatly reduced from an average of 320 in wild type animals (n=5) to an average number of 78 in the *fl-i-1* mutants (n=12). Also, in the *fl-i-1* mutants the shapes of the germ cell were irregular and the size of the germ cell varied (Fig. 6B). The alignment of the germ cells in the gonad was also disorganized and the rachis was misshaped in 12 out of 19 *fl-i-1* mutant gonad arms examined (Fig. 6B,D). Thus, *fl-i-1* is required for the development of germ cells.

**Mutations in *fl-i-1* cause ovulation defects**

The *fl-i-1* mutants displayed a defect in ovulation. In wild type hermaphrodites, oocytes are aligned along the proximal-distal axis (Fig. 7A). During ovulation, the proximal oocytes are engulfed by the spermatheca and pulled into the oviduct (Fig. 7C) (McCarter et al., 1997; McCarter et al., 1999). We analyzed 11 single ovulation processes in the *fl-i-1* mutants. In five of them, the proximal oocyte was not engulfed
by the spermatheca and the trapped oocyte in the distal side underwent multi-rounds of DNA replication, causing an endomitotic oocyte (EMO) phenotype (Fig. 7B) (Iwasaki et al., 1996). In three of the ovulation processes analyzed in the *fli-1* mutants, oocytes were spliced during the dilation of the spermatheca. These went on to be fertilized, developing into small embryos in two of the cases and undergoing endomitosis in the third case (Fig. 7D). The egg-laying apparatus also appeared to be defective in the *fli-1* mutants, as the fertilized embryos were often trapped in the uterus.

Ovulation requires the coordinated action of the contraction of the gonad sheath cells and the dilation of the spermatheca (McCarter et al., 1997). In wild type animals, the contraction of the sheath cells and spermatheca is vigorous and increases in rate and intensity during ovulation (see Movie 3). However, in the *fli-1* mutants, we found that the contraction of the sheath cells and the spermatheca was weak and infrequent (see Movie 4). The number and organization of the sheath and spermathecal cells appeared to be normal in the *fli-1* mutants (data not shown), suggesting that other defects, such as the interaction between the oocyte and the spermatheca, may cause the failure of ovulation in the *fli-1* mutant animals.

**The ovulation defect in the *fli-1* mutants can be partially rescued by mutations in *lfe-2* and *ipp-5* in the phosphoinositol signaling pathway**

The LIN-3 (expressed in the oocyte)/LET-23 (expressed in the spermatheca and sheath cells) EGF signaling pathway has been shown to play an important role in controlling the contraction of sheath cells and the dilation of spermatheca through the
regulation of the cellular level of inositol 1, 4, 5-trisphosphate (IP3) (Clandinin et al., 1998; Yin et al., 2004). Loss-of-function of Ife-2 (encoding the IP3 kinase) or ipp-5 (encoding type I inositol polyphosphate 5-phosphatase) bypasses the defect in the interaction between the oocyte and the spermatheca (Clandinin et al., 1998; Bui and Sternberg, 2002). We found that loss of function of ipp-5 and Ife-2 partially rescued the defects in the contraction of the sheath cells and the dilation of the spermatheca in the fli-1 mutants. Consistently, the brood size of fli-1 mutants was dramatically increased (p<0.05) in fli-1; ipp-5 and Ife-2; fli-1 double mutants (Fig. 7E). Other mutant phenotypes in the fli-1 mutants, including defects in seam cell division and abnormal tail morphology, however, were not rescued by mutations in Ife-2 and ipp-5 (Fig. 7E and data not shown). Thus, the defect in the ovulation in the fli-1 mutants is probably in part due to the defective signaling between the oocyte and the spermatheca.

fli-1 encodes the C. elegans Flightless I homolog

Genetic mapping placed bp130 between sma-3 and unc-32 on chromosome III. Cosmids from this region were used for transformation rescue experiments. We found that cosmid B0523 rescued the mutant phenotypes in the fli-1 mutants in all four stable transgenic lines analyzed. Further analysis demonstrated that the PCR product containing the single predicted gene, B0523.5, had rescuing activity (Fig. 8A). Furthermore, B0523.5 (RNAi) exhibited phenotypes similar to those observed in the fli-1(bp130) mutants (details see Materials and Methods and Fig. 8E), indicating that the defects are in fact due to loss of function.
B0523.5 encodes the *C. elegans* Fli I homolog (Campbell et al., 1993). The N-terminus of FLI-1 consists of 16 tandem repeats of a 23-amino acid (aa) leucine-rich motif and the C-terminus consists of contains six copies of a 125 aa residues gelsolin related repeating unit (Fig. 8B).

By sequencing, we found that an invariant residue located at the 3' splicing site of the third intron is mutated from G to A in the *fli-1(bp130)* mutants (Fig. 8C). RT-PCR was performed using primers located in the exon 3 and exon 5 of *fli-1*. We found that intron 3 was present in *fli-1(bp130)* mRNA (Fig. 8D), which resulted in a frameshift at amino acid 194.

*fli-1(bp130)* does not appear to be null, as the mutant defects in the *fli-1(bp130)/nDf17* animals were more severe than the ones in the *fli-1(bp130)* mutant homozygous (Fig. 8F). The non-null nature of *fli-1(130)* could be due to that the truncated FLI-1(bp130), which contains the C-terminal eight copies of LRR repeats, retains some function or could be due to the presence of other alternative spliced forms of *fli-1(bp130)* that were not detected in our analysis.

*fli-1* functions cell autonomously in determining the seam cell division

To further examine the role of *fli-1* in controlling seam cell division, we expressed *fli-1* using a seam cell specific promoter. The defects of seam cells in *fli-1(bp130)*, including the reduced number of seam cells, were rescued in the animals carrying a *scm::fli-1* transgene (Table 2). This indicates that *fli-1* acts cell autonomously in regulating the development of seam cells. This also suggests that the pleiotropic defects observed in *fli-1* mutants cannot be solely attributed to the defects
in the development of germline or early embryos.

**fli-1 can be functionally substituted by the fly Fli I**

*C. elegans* FLI-1 displays 49% identity to *Drosophila* Fli I at protein sequence level. We determined whether *Drosophila* Fli I can substitute the function of *fli-1* in *C. elegans*. The fly Fli I cDNA was expressed under the control of the *fli-1* promoter. This transgene was functional in rescuing the defects in the *fli-1(bp130)* mutants, including the number of seam cells, EMO, and reduced brood size (Table 2), indicating that the function of Fli I is evolutionarily conserved.

**fli-1 is wildly expressed and localized in actin-rich regions**

In order to determine the expression pattern of *fli-1*, a *fli-1* green fluorescence reporter was constructed, including the 2kb promoter region and the full-length genomic DNA of *fli-1*. This reporter was functional in rescuing the defects in the *fli-1(bp130)* mutants (Table 2). The reporter was widely expressed and the expression level was dynamic, varying among tissues and developmental stages. The GFP was mainly localized in the cytoplasm. The expression of *fli-1::gfp* was first detected during embryogenesis before the apparent morphogenesis occurred (Fig. 9A,B). In the comma stage embryo, expression of *fli-1::gfp* was detected in muscle cells (Fig. 9C,D). The expression of *fli-1::gfp* was not observed before 32-cell stage embryos (*n>40* embryos examined). The early developmental defects in *fli-1* mutants suggest that maternally produced FLI-1 is present in the early embryo. Such maternal expression cannot be recapitulated by the reporter construct due to the germline silencing effect.
At post-embryonic stages, expression of \textit{fli-1::gfp} was detected in many tissues, including the pharyngeal muscle, rectum muscle, vulva muscle, proctodeum muscle in males and somatic gonad tissues (including spermatheca and distal tip cell) (Fig. 9E-I and data not shown). The \textit{fli-1} expression in the spermatheca was unevenly distributed with strong expression in the distal and proximal part of spermatheca (Fig. 9G). \textit{fli-1} was also expressed within neuronal migratory structures such as axons (Fig. 9J). In the body wall muscle, \textit{fli-1::gfp} was localized in a distinct striated pattern, with accumulation of FLI-1::GFP at dense bodies, which is similar to the Z-disc in the muscle cells in other organisms (Fig. 9K). The expression pattern of \textit{fli-1} in body wall muscle cells is reminiscent of the distribution of the actin filaments. We stained F-actin in body wall muscle cells with Rodamine-labeled phalloidin and found that it colocalized with FLI-1::GFP (Fig. 9K-M). In summary, the expression pattern of \textit{fli-1} appears to be coincident with actin-based structures.

\textbf{Organization of F-actin microfilaments is impaired in the \textit{fli-1} mutants}

We next examined the actin cytoskeleton in the \textit{fli-1} mutant animals at various stages and tissues. In the wild type pronuclear one-cell embryo, F-actin is uniformly distributed in the cortex with the dispersed localization of actin foci (Strome, 1986; Hill and Strome, 1988). The actin filaments are enriched in the cortical surfaces where the cleavage furrow forms in multi-cell embryos (Fig. 10A-D) (Strome, 1986). In three out of five \textit{fli-1} mutant zygotes analyzed, the actin foci were reduced in number and increased in size. In two out of 12 \textit{fli-1} mutant multi-cell embryos, F-actin was unevenly distributed along the membrane and it diffused in the peripheral cytoplasm.
membranes (Fig. 10E-H).

In gonad tissues, the actin filaments form cortical meshwork structures in the sheath cells and run roughly circumferentially in spermathecal cells, with strong accumulation at the valve from the spermatheca to the uterus (Fig. 10I,K) (Strome, 1986; McCarter et al., 1997). In the \textit{fli-1} mutants, normal arrays of the actin filaments appeared to be formed in the sheath and spermathecal cells. However, the organization of the actin filaments was irregular and disorganized (Fig. 10J,L). In body wall muscle cells, the \textit{fli-1} mutants had a variably disorganized myofilament lattice; the actin filaments were disorganized and unevenly distributed with more actin-rich dense body appearance structures (Fig. 10M, N).

During the wild type germline development, the actin filaments are distributed along the membranes that surround germ nuclei, forming a honeycomb arrangement (Fig. 10O) (Strome, 1986). In the \textit{fli-1(bp130)} mutants, the actin filaments were unevenly distributed along the membrane and appeared to diffuse in the periphery of the membrane (Fig. 10P). Furthermore, actin foci were observed surrounding the germ nuclei. \textit{nmy-2}, encoding the nonmuscle myosin II, displays the same expression pattern as actin filaments in the wild type germline (Guo and Kemphues, 1996; Piekny et al., 2003). Consistently, NMY-2::GFP showed abnormal accumulation, as actin filaments, in the \textit{fli-1} mutant germ line (Fig. 10Q,R). Taken together, wild type \textit{fli-1} plays an essential role in organization of the actin filaments during \textit{C. elegans} development.
Discussion

Defects in the establishment of A/P polarity and asymmetric cell division in the *fli-1* mutants

We show here that *fli-1* regulates the establishment of cell polarity in the one-cell stage embryo, including polarized cytoplasmic flow, asymmetric meeting of pronuclei and asymmetric segregation of P granules, while at the post-embryonic stage, *fli-1* regulates asymmetric cell division.

How is *fli-1* involved in specifying the distinct cell fate of daughters?

Mutations in *fli-1* can lead to disruption of the asymmetric distribution of the key cell fate determinants, such as P granules. The defects in the spindle position (data not shown) and chromosome segregation observed in the *fli-1* mutant embryos indicate that *fli-1* may also play a role in regulating the spindle alignment and function, which is crucial for asymmetric segregation of the cell fate determinants. Alternatively, cells in *fli-1* mutants could be defective in responding to extrinsic cues that are involved in specifying the distinct cell fate.

The failure of cytokinesis in the one-cell stage embryo and the presence of two nuclei in seam cells and DTCs also indicate that *fli-1* is essential for cell division. In fly, the cellularization of the syncytial blastoderm is defective (Straub et al., 1996). The cytoplasmic contraction waves and nuclear migration in the syncytium, however, are normal in *Fli I* mutants. Fly Fli I is also not needed for the cytokinesis at late developmental stage and is not required for the development of the oocyte (Straub et al., 1996). The less severe defects in fly could be due to the redundancy of the Fli I
with other proteins, such as with other gelsolin family members, in controlling the dynamics of actin network. The development of the muscle structure is defective in both *C. elegans* and the fly. In *fli-1* mutants, the muscle fibers are irregular and malformed. Viable Fli I mutant flies show abnormal formation of the indirect flight muscle fibers, which display severely disrupted Z-discs (Miklos and de Couet, 1990), suggesting a conserved role of Fli I in regulation of the development of muscle cells.

**The role of FLI-1 in controlling the dynamics of the actin network**

The *fli-1* mutant phenotypes argue that *fli-1* plays an important role in controlling the dynamic arrangement of the actin filaments or in generation of the force of actomyosin structure. First, the defects in *fli-1* mutant embryos resemble some of the effects caused by cytochalasin D-induced actin depolymerization (Strome and Wood, 1983; Hill and Strome, 1988). Mutations in two actin-associated proteins, NMY-2, the nonmuscle myosin II, and MLC-4, the nonmuscle myosin II regulatory light chain, also result in similar defects in the polarized cytoplasm flow, establishment of the A/P polarity and cytokinesis in the first mitotic cell division (Guo and Kemphues, 1996; Shelton et al., 1999). Mutations in several genes that are involved in regulating actomyosin have also been reported to influence the timing of cytokinesis. For example, in *mel-11* (which encodes myosin phosphatase) mutants, the furrow ingression completed approximately twice as fast as in wild type, while mutations in *let-502*, which encodes Rho-binding kinase, had slower cytokinesis (Piekny and Mains, 2002). Multinucleate single embryos were observed in *mlc-4* and *let-502* mutants due to cytokinesis failures (Shelton et al., 1999; Piekny and Mains,
Second, FLI-1 is localized at the sites where dynamic actin rearrangement appears to occur, such as in the distal tip cells, spermatheca and vulval muscle cells. Moreover, FLI-1 is colocalized with actin bundles in the body wall muscles. Third, the distribution of the actin filaments is impaired in the fli-1 mutants. In general, the actin filaments appear to be disorganized and also accumulate in some parts in the fli-1 mutants. Fourth, FLI-1 can directly interact with the G and F-actin and contains the actin severing activity (Goshima et al., 1999). The abnormality of chromosome segregation in fli-1 mutants suggests that fli-1 may also regulate some microtubule-dependent events, such as in regulating the spindle alignment and function. In mammalian cells, Fli I is also localized to centrosomes and accumulated at the midbody (Davy et al., 2001). Alternatively, the defects in chromosome segregation in fli-1 mutants could be an indirect effect, such as due to the severe germline defects.

How does FLI-1 modulate the actin network? FLI-1 could function as a structural component, holding the actin filaments together. FLI-1 might also be involved in the delivery of actin to or in the stabilization of the actin network. Furthermore, FLI-1 could have a regulatory role in establishing the actin cytoskeleton. The LRR repeats of FLI-1 have been shown to directly bind to Ras in vitro (Goshima et al., 1999), raising the possibility that FLI-1 integrates the Ras signaling pathway with the actin filaments.

**Ovulation defect in the fli-1 mutants**

The fli-1 mutants show a great reduction in the frequency and intensity of the
contraction of the sheath cells and the dilation of the spermatheca during ovulation. The contraction of the sheath cells and dilation of the spermatheca is triggered by LIN-3/LET-23 EGF signaling, which most likely stimulates hydrolysis of PIP2 into inositol 1, 4, 5, trisphosphate (IP3) (Bui and Sternberg, 2002; Yin et al., 2004). In other systems, IP3 has been shown to excite intracellular calcium release channels and cause a transient increase in intracellular calcium (Berridge 1993). Mutations in ipp-5 and lfe-2 suppress the fli-1 ovulation defect, strongly arguing in favor of a defect in signaling between the oocyte and the spermatheca in fli-1 mutants. However, it is unlikely that the increase in intracellular calcium concentration directly regulates the activity of FLI-1, as the actin-binding activity of FLI-1 is calcium independent (Goshima et al., 1999). In mammalian cells, the EGF receptor signaling pathway drives cytoskeleton rearrangement and cell protrusion by regulating the activity of gelsolin. The F-actin severing activity of gelsolin is regulated by PIP2 (Janmey and Stossel, 1987; Janmey et al., 1992; Yu et al., 1992). EGF signaling activates phospholipase C-γ (PLCγ), which hydrolysis PIP2, and subsequently leads to the dissociation of gelsolin from the plasma membrane (Chen et al., 1996; Chou et al., 2002). Thus, it is possible that the activity of FLI-1 in modulating the actin cytoskeleton in the spermatheca is regulated by PIP2, which could be dissociated from FLI-1 in lfe-2 and ipp-5 mutants. Alternatively, the defects in ovulation could be due to the oocyte, which secretes EGF, being defective in fli-1 mutants. Further studies could help us to elucidate how the EGF signaling pathway regulates the activity of fli-1 in controlling the dynamics of the actin network during animal
development.

In summary, our studies reveal a key role of fli-1 in establishing anterior-posterior polarity, specifying asymmetric cell division, interacting with the phosphoinositol signaling pathway in the regulation of ovulation and other actin-dependent events. These findings have significant implications for our understanding of the causes of the defects associated with Fli I loss-of-function in mammals and also provide insight in the physiological functions of gelsolin family members during animal development, especially in early embryogenesis and muscle development.

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We thank Dr. Xiaochen Wang, Ms. Andrea Christoforou and members in our laboratory for their helpful comments on the manuscript. We thank Dr. Bob Goldstein for pgl-1::gfp strain. Some strains used in this work were received from the Caenorhabditis Genetics Center, which is supported by a grant from the NIH. This work was supported by the National High Technology Projects 863 (2005AA210910).

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Figure legends:

Figure 1. Defects in the post-embryonic development of seam cells in the fli-1 mutants

A. The post-embryonic division pattern of a subset of seam cells. The proliferative S2 seam cell division is highlighted in red box. Seam cell T undergoes a distinct division pattern at the L1 larval stage, with one daughter cell generating neuronal structures (highlighted in red circle) and the other maintaining seam cell fate. The post-embryonic stages are indicated along the vertical axis, separated by larval stage.

B. Sixteen seam cells, visualized by the seam cell specific marker scm::gfp, are evenly distributed along the anterior/posterior axis in a wild type young adult.
C. In the \textit{fli-1} mutant animal shown, there were 11 seam cells, which were also unevenly distributed.

D. Defects in the asymmetric seam cell division in the \textit{fli-1} mutants. Both V1 daughter cells adopted seam cell fate, while both V4 daughter cells failed to adopt the seam cell fate (The V4 daughters remained unfused with hyp 7 at the stage shown and thus retained weak \textit{gfp} signal).

E. Two cells labeled by \textit{ajm-1::gfp} (arrows) did not express \textit{scm::gfp} in a \textit{fli-1} mutant animal.

F. Presence of two nuclei in one seam cell (arrows) in a \textit{fli-1} mutant animal, indicating a defect in the cytokinesis.

G. The phasmid structure, which takes up the dye in the Dye-filling assay, is generated on each side of a wild type animal (two socket neurons in each phasmid can be stained by the dye).

H. Failure of staining with dye in a \textit{fli-1} mutant side.

I-J. Asymmetric cell division of seam cell T in a wild type L2 larva. The anterior daughter cell (arrow in I) maintaining the hypodermal seam cell fate (expressing \textit{scm::gfp}) and the posterior daughter adopting a neuronal fate, which has distinct nuclear morphology.

K-L. In a \textit{fli-1} mutant larva, both daughter cells of T had hypodermal cell appearance and expressed \textit{scm::gfp} (arrows in K and L). I, K: Nomarski micrograph. J, L: expression of \textit{scm::gfp} in the same animal shown in I and K, respectively.
Figure 2. Defects in the development of somatic gonad in the fli-1 mutants

A-B. One DTC, labeled by lag-2::gfp, is present on each gonad arm in a wild type hermaphrodite. The tip of the gonad, expressing lag-2::gfp, is indicated by arrow (B).

C-D. In a fli-1 mutant animal, two DTCs were present in one side. Consistently, two gonad branches were present (arrows in D).

E. The processes of DTC (arrow) extend down the side of the germline and contain two longer branches at the outer edges in a wild type hermaphrodite gonad.

F. The processes of DTC (arrow) were much longer and less organized in a fli-1 mutant.

G. Migration of the gonad arm in a wild type hermaphrodite. The gonad grows out of the vulva position, then makes a turn from ventral to dorsal site, and then reorients and migrates back to the mid-body. The anterior gonad is shown in G and H.

H. Defects in the migration of the gonad in a fli-1 mutant hermaphrodite. The turning from ventral to dorsal site was defective in the mutant gonad arm shown (arrow).

Figure 3. Defects in the first mitotic cell division in the fli-1 mutants

A: The pronucleus meet at about 70% egg length in the posterior in a wild type zygote. Anterior is to the left in all panels.

B-C: Defects in the polarized meeting of the pronucleus in the fli-1 zygotes. The
pronucleus met at about 80% (B) or 50% (C) of the zygotes in the posterior.

D: Schematic summary of the position where pronuclei met in eight fli-1 mutant zygotes analyzed.

E. Time-lapse DIC images of the first cleavage of a wild type zygote. The pronuclear, pronuclear meeting, fusion and centration, formation of the spindle, formation of cleavage furrow and the two-cell stages were shown. The time when the event occurred was shown in each panel in minutes and seconds. The first mitotic division took about 20 minutes to complete. Bar, 10 μm.

F. Time-lapse DIC images of the first cleavage of a fli-1 mutant embryo. The pronucleus met in the center of the embryo. The mutant embryo shown took much longer to complete division.

G-H: Equal amount of DNA, labeled by H2B::GFP, is segregated into the AB and P cells in a wild type embryo.

I-J: Unequal distribution of DNA into two daughters of a fli-1 mutant embryo. More H2B::GFP containing DNA content was segregated into the posterior P1 cell.

K-L. The second mitotic division in a wild type embryo. The division of the AB cell completes prior to the initiation of the cleavage of the P1 cell.

M-N. Both AB and P1 cells appeared to be at the same cell division stage in a fli-1 mutant embryo. The arrangement of the AB and P1 cells was also abnormal.

**Figure 4. Defects in the asymmetric segregation of P granules in the fli-1 mutants**

A-B: Distribution of P granules, labeled with PGL-1::GFP, in a wild type embryo after
the first mitotic cell division. P granules are confined in the posterior end of the P1 cell. A, Nomarski micrograph. B. Fluorescence micrograph of the same embryo.

C-F: Abnormal segregation of P granules in the fli-1(bp130) mutant embryos. P granules were localized in the whole P1 cell (C) or were localized in both AB and P1 cells (E). The localization of P granules in the P1 cell in (E) was marked with arrow, which was not at the same focus plane as the ones in the anterior AB cell. C, E, Nomarski micrograph. D, F. Fluorescence micrograph of the same embryos shown in C and E, respectively.

**Figure 5. Mutations in fli-1 cause other developmental defects**

A-B. Compared to a wild type embryo (A), a fli-1 mutant embryo exhibited a bulge at body surface (arrow).

C. The wild type hermaphrodite has a tapered tail spike (arrow).

D. The tail morphology was malformed in a fli-1 mutant animal (arrow).

E. Nine pairs of rays, embedded in the culticule fan-like structure, are present in a wild type male tail.

F. The fli-1 mutant male tail is grossly abnormal. In the mutant animal shown, most of the rays were missing. Other male specific structures, including spicule (arrow), were also abnormal.

G. AVM (arrow), labeled by mec-7::gfp, positions anterior to the ALMs (arrowhead) in a wild type animal.
H. In a $fli-1$ mutants, AVM positioned posterior to ALMs.

I. PKD-2::GFP marks the axons of B-type neurons of all the rays. R1B has distinct pathfinding route (arrow).

J. Defects in the axon pathfinding of R1B in a $fli-1$ mutant male. The R1B axons failed to make a turn to the ventral side and continued to migrate toward the anterior body region (arrow).

**Figure 6. Defects in the germline development in the $fli-1$ mutants**

A. In the meiotic region of a wild type hermaphrodite, the germ cells are round in shape (arrow) and evenly aligned at the germ cell plasma membrane. The upper surface of the gonad was shown in A and B.

B. The morphology of the germ cell was irregular, the size of the germ cell varied, and the germ cells were disorganized in a $fli-1$ mutant (arrows).

C. In a wild type hermaphrodite, the syncytial germ cells are arranged around a central cytoplasm core, termed the rachis (arrow). The gonad was stained with R-phalloidin and the mid-focal plane of the gonad was shown in C and D.

D. The rachis in $fli-1$ mutants was smaller (arrow) and the alignment of the germ cells in the rachis was disorganized in a $fli-1$ mutant animal (arrowheads).

**Figure 7. Mutations in $fli-1$ cause defects in ovulation**

A. DAPI staining in the proximal gonad in a wild type hermaphrodite. Chromosomes of the oocytes in the proximal gonad are condensed in diakinesis (arrow). The
spermatheca contains characteristic small, compact nuclei (arrowhead).

Anterior gonad was shown in A and B.

B. DAPI staining showed endomitotic oocyte nuclei in a fli-1 mutant animal (arrows).

C. The oocyte (arrow) is engulfed by the spermatheca and located in the uterus in a wild type hermaphrodite.

D. In the fli-1 mutant shown, the oocyte was spliced into two pieces (arrows) during ovulation. One part was engulfed in the spermatheca and the small part was left behind.

E. Suppression of the ovulation defect in the fli-1 mutants by mutations in ipp-5 and lfe-2. Allele used: fli-1(bp130), ipp-5(sy605), lfe-2(sy326). The number of animals analyzed are: fli-1: 8 and 83 (for brood size and number of seam cells, respectively); ipp-5: 15 and 12; fli-1; ipp-5: 7 and 18; lfe-2: 18 and 17; lfe-2; fli-1: 12 and 12.

**Figure 8. fli-1 encodes the C. elegans Flightless I homolog**

A. Cosmid B0523 and PCR product containing the single predicted gene, B0523.5, had the rescuing activity.

B. fli-1 encodes the fly Flightless I homolog, it contains 16 repeats of leucine rich motif and six folds of the gelsolin domain.

C. fli-1(bp130) contains a G to A mutation at the 3' splicing site of the third intron of fli-1. The reading frame was underlined in red. Failure of splicing of intron 3 caused a frameshift at amino acid 194.
D. The RT-PCR products covering the exon 3 to exon 5 of fli-1 in wild type and 

\textit{fli-1}(bp130) mutants. Failure of removing intron 3 led to the increase size of 

the product from 355 bp in wild type animals to 398 bp in \textit{fli-1}(bp130) 

mutants.

E. \textit{fli-1}(RNAi) also showed reduced number of seam cells. In the animal side shown, 

only nine seam cells were present.

F. \textit{fli-1}(bp130)/nDf17 caused more severe defects in lethality and sterility than those 

in \textit{fli-1}(bp130) homozygotes, indicating that \textit{fli-1}(bp130) is a partial loss of 

function allele. \textit{nDf17}: deletion in chromosome III from -1.50 to 2.11. 25% of 

lethal progenies derived from \textit{fli-1}(bp130)/nDf17 should be \textit{nDf17} 

homozygotes, which are embryonic lethal. The progenies derived from six 

\textit{fli-1}(bp130) homozygotes (n=194) and 11 \textit{fli-1}(bp130)/nDf17 (n=80) were 

analyzed. Both \textit{fli-1}(bp130) and \textit{fli-1}(bp130)/nDf17 were derived from m/+ 

hermaphrodites.

\textbf{Figure 9. fli-1 is wildly expressed}

A-B: The onset of the expression of \textit{fli-1::gfp} was detected at the embryonic stage 

before obvious morphogenesis.

C-D: Expression of \textit{fli-1::gfp} in a comma stage embryo. Strong expression was 

evident in muscle cells.

E-J. Expression of \textit{fli-1::gfp} in pharyngeal muscles (E), vulva muscles (F), 

spermatheca (G), male proctodeum muscles (I), distal tip cell (arrow, H), and
axons (arrow, J). In the spermatheca, strong expression was seen in the valve
connected to the uterus (arrows).

J-L: Colocalization of fli-1::gfp and actin filaments in body wall muscles. Both

\textit{fli-1::gfp} and phalloidin labeled actin filaments were enriched at the dense
bodies (arrow). FLI-1::GFP (K), actin filaments (L), merged picture (M).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure10.png}
\caption{The actin filaments are disorganized in the fli-1 mutants}
\end{figure}

A-C: The distribution of F-actin in a wild type pronuclear stage embryo. Actin is
uniformly distributed and distinct actin foci are dispersed through the cortex.
R-ph staining seen at mid-focal plane (B) and on the top surface (C).

B. Actin filaments are enriched at the cleavage furrow structures in a wild type
four-cell stage embryo.

E-G: The distribution of F-actin in a fli-1 mutant pronuclear embryo. The actin foci
were reduced in number and increased in size (arrow in G). F: mid-focal plane.
G: top surface.

H. The actin filaments in the cortical surface of embryo were not evenly distributed
and reached varying depth in peripheral cytoplasm (arrow) in a fli-1 mutant
multi-cell embryo (H).

I, K: In a wild type animal, the actin filaments form a meshwork in the sheath cells (I)
and are densely packed and aligned circumferentially around the spermathecal
cell (K).

J, L: The distribution of F-actin was irregular and disorganized in the sheath cells (J)
and in the spermathecal cells (L) in a *fli-1* mutant.

M: Distribution of F-actin in body wall muscles. The actin filaments form organized myofibril lattice and are enriched in dense bodies (arrow).

N: The muscle thin fibers were abnormal in appearance in *fli-1* mutants. The actin filaments were disorganized and appeared to concentrate in many places along the fibers (arrow).

O: A honeycomb arrangement of the actin network in the wild type germline. The F-actin evenly surrounds the germ cell nuclei.

P: Distribution of F-actin in the *fli-1* mutant germ cells. F-actin appeared to accumulate around the nuclei in some parts (arrow).

Q-R. NMY-2::GFP displayed the same expression pattern as actin filaments in both wild type germline (Q) and *fli-1* mutant germline (R).
Table 1. *fli-1(bp130)* mutants display maternal effect

<table>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>embryo</td>
<td>larvae</td>
</tr>
<tr>
<td><em>fli-1(bp130)^1</em></td>
<td>32.3±8.4 (n=6)</td>
<td>124.2±32.2 (n=9)</td>
<td>19.5%</td>
<td>11.7%</td>
<td>9.9%</td>
</tr>
<tr>
<td><em>fli-1(bp130)^2</em></td>
<td>13.0±8.6 (n=8)</td>
<td>78.4±20.4 (n=12)</td>
<td>24.5%</td>
<td>32.3%</td>
<td>21.5%</td>
</tr>
<tr>
<td><em>wild type[^3]</em></td>
<td>189.0±32.2 (n=5)</td>
<td>320.4±42.5 (n=5)</td>
<td>4.0%</td>
<td>1.5%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

1. Six *fli-1* mutant animals derived from *m/+* hermaphrodites were analyzed for brood size, sterility and lethality.
2. Eight *fli-1* mutant animals derived from *m/m* mother were analyzed for brood size, sterility and lethality.
3. Five animals carrying the *wls51(scm::gfp)* transgene were analyzed.
4. Brood size referred to the eggs laid by the mother.
5. The number of germ cells were counted in each gonad arm by DAPI staining.
6. The number of progenies analyzed.

[^4]: brood size referred to the eggs laid by the mother.
[^5]: The number of progenies analyzed.
[^6]: The number of germ cells were counted in each gonad arm by DAPI staining.
Table 2. The phenotypes of *fli-1(bp130)* mutant animals carrying various transgenes

<table>
<thead>
<tr>
<th>genotype</th>
<th>brood size</th>
<th>number of germ cells</th>
<th>EMO</th>
<th>Number of seam cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>189.0±32.2 (n=5)</td>
<td>320.4±42.5 (n=5)</td>
<td>0 (n=5)</td>
<td>16.3±0.4 (n=32)</td>
</tr>
<tr>
<td><em>fli-1(bp130)</em></td>
<td>13.0±8.6 (n=8)</td>
<td>78.4±34.2 (n=12)</td>
<td>6 (n=8)^5</td>
<td>11.2±0.5 (n=83)</td>
</tr>
<tr>
<td>line 1</td>
<td>32.0±8.4 (n=2)</td>
<td>189.3±10.1 (n=3)</td>
<td>1 (n=10)</td>
<td>14.5±1.3 (n=4)</td>
</tr>
<tr>
<td>line 2</td>
<td>38.5±4.9 (n=4)</td>
<td>192.5±30.1 (n=4)</td>
<td>1 (n=9)</td>
<td>15.3±0.75 (n=6)</td>
</tr>
<tr>
<td>line 3</td>
<td>68.3±7.4 (n=3)</td>
<td>210.0±62.3 (n=4)</td>
<td>0 (n=12)</td>
<td>15.7±1.0 (n=6)</td>
</tr>
<tr>
<td>line 4</td>
<td>58.7±6.3 (n=3)</td>
<td>86.5±32.4 (n=2)</td>
<td>2 (n=11)</td>
<td>15.6±1.3 (n=8)</td>
</tr>
<tr>
<td><em>fli-1(bp130); fli-1::gfp</em></td>
<td>line 1</td>
<td>18.2±5.8 (n=5)</td>
<td>76.3±8.9 (n=3)</td>
<td>8 (n=8)</td>
</tr>
<tr>
<td></td>
<td>line 2</td>
<td>11.6±6.0 (n=3)</td>
<td>89.2±12.6 (n=5)</td>
<td>8 (n=9)</td>
</tr>
<tr>
<td></td>
<td>line 3</td>
<td>12.6±2.3 (n=3)</td>
<td>92.5±10.6 (n=2)</td>
<td>7 (n=7)</td>
</tr>
<tr>
<td><em>fli-1(bp130); scm::fli-1::gfp</em></td>
<td>line 1</td>
<td>64.3±10.6 (n=3)</td>
<td>168.3±34.2 (n=3)</td>
<td>1 (n=8)</td>
</tr>
<tr>
<td></td>
<td>line 2</td>
<td>74.5±13.8 (n=4)</td>
<td>154.5±20.2 (n=4)</td>
<td>0 (n=9)</td>
</tr>
<tr>
<td></td>
<td>line 3</td>
<td>56.0±5.7 (n=2)</td>
<td>192.0±27.7 (n=4)</td>
<td>1 (n=12)</td>
</tr>
</tbody>
</table>

1. The number of germ cells were counted in each gonad arm by DAPI staining.
2. The number animals displayed the EMO phenotype.
3. The other two mutants were sterile. The total number of animals examined were shown in parenthesis.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
**E**

<table>
<thead>
<tr>
<th>genotype</th>
<th>fli-1</th>
<th>ipp-5</th>
<th>fli-1; ipp-5</th>
<th>Ife-2</th>
<th>Ife-2; fli-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>brood size</td>
<td>13.0±8.6</td>
<td>132.0±12.4</td>
<td>62.4±8.2</td>
<td>150.3±21.5</td>
<td>34.4±1.5</td>
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<tr>
<td>number of seam cell</td>
<td>11.2±0.5</td>
<td>16.4±0.8</td>
<td>12.2±0.5</td>
<td>16.2±0.3</td>
<td>11.5±0.8</td>
</tr>
</tbody>
</table>

*Figure 7*
Figure 8

<table>
<thead>
<tr>
<th>genotype</th>
<th>brood size</th>
<th>lethal (%)</th>
<th>sterile (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fli-1(bp130)/fli-1(bp130)</td>
<td>32.3±8.4</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>fli-1(bp130)/nDf17</td>
<td>7.3±5.2</td>
<td>73</td>
<td>27</td>
</tr>
</tbody>
</table>
Figure 10