**diaphanous** is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the *limb deformed* gene

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**SUMMARY**

We show that the *Drosophila* gene *diaphanous* is required for cytokinesis. Males homozygous for the *dia*mutation are sterile due to a defect in cytokinesis in the germline. Females trans-heterozygous for *dia* and a deficiency are sterile and lay eggs with defective eggshells; failure of cytokinesis is observed in the follicle cell layer. Null alleles are lethal. Death occurs at the onset of pupation due to the absence of imaginal discs. Mitotic figures in larval neuroblasts were found to be polyploid, apparently due to a defect in cytokinesis. The predicted 123×10^3 M_r protein contains two domains shared by the formin proteins, encoded by the *limb deformed* gene in the mouse. These formin homology domains, which we have termed FH1 and FH2, are also found in Bni1p, the product of a *Saccharomyces cerevisiae* gene required for normal cytokinesis in diploid yeast cells.

Key words: cytokinesis, mitosis, spermatogenesis, oogenesis, P element, *Drosophila*

**INTRODUCTION**

To complete mitosis successfully, a cell must carry out two separate processes: karyokinesis, or chromosome segregation, and cytokinesis, or the division of the entire cell. In animal cells, cytokinesis is believed to be mediated by the contractile ring, a transient cytoskeletal structure located midway between the spindle poles at the cell cortex. Actin and myosin, which are concentrated at the cleavage furrow in a variety of cells, are believed to generate the force leading to contraction of the ring and, ultimately, the separation into two daughter cells (Satterwhite and Pollard, 1992).

Despite increasing knowledge about mitosis and the cell cycle, many aspects of cytokinesis remain poorly understood. For example, it is not clear how actin and myosin are recruited to the contractile ring, nor how the contractile ring is attached to the cell membrane. The position of the contractile ring is believed to be determined by a signal originating at the mitotic spindle (Rappaport, 1986), but the nature of this signal is not known. Lastly, few of the structural and regulatory proteins involved in cytokinesis have been identified.

The isolation of mutations that disrupt cytokinesis in organisms such as yeast or *Drosophila* promises to be a useful approach for identifying genes required for cytokinesis. Indeed, mutations identified through genetic screens in *Drosophila* (Karess et al., 1991), or through a ‘reverse genetic’ approach in *S. cerevisiae* (Watts et al., 1987) and *Dictyostelium* (DeLozanne and Spudich, 1987), have demonstrated that myosin function is required for cytokinesis. More recently, the *peanut* locus has been shown to be required for cytokinesis in *Drosophila* and to encode a homolog of Cdc12 and related proteins required for cytokinesis in yeast (Neufeld and Rubin, 1994).

The *diaphanous* (*dia*) locus was identified in a P element screen for recessive male-sterile mutations (Castrillon et al., 1993). Here we present evidence that *dia* is generally required for cytokinesis. A failure in cytokinesis during spermatogenesis results in multinucleate spermatids. A defect in oogenesis in females is associated with a failure of cytokinesis in the somatically derived follicle cells, which surround each developing egg chamber. We further show that null *dia* alleles result in early pupal lethality and that the lethal phenotype is consistent with a defect in cytokinesis. Lastly, we have defined two evolutionarily conserved domains that are common to the protein products of *diaphanous*, the *limb deformed* locus of mouse and chicken, and *BNI1*, a yeast gene identified on the basis of its interaction with *CDC12*.

**MATERIALS AND METHODS**

*Drosophila* markers and manipulations

All crosses were performed at 25°C on yeastae cornmeal molasses agar. Genetic markers and balancers are described and referenced by Lindsley and Zimm (1992). Lethal *dia* mutations were balanced with *In(2LR)Gla*, *Bc*. RNA from germlineless flies was prepared from the progeny of females homozygous for the *tud* mutation.

Remobilization of P element to generate new alleles

The *dia* chromosome was brought together with a transposase source by crossing *dia/Cyo* flies to flies carrying the P[ry+, Δ2-3] transposase source on the third chromosome (Robertson et al., 1987). In the next generation, the P[ry+, Δ2-3] chromosome was crossed out, and *ry* derivatives of the *dia* chromosome were selected to establish lines.

Of the 226 *rosy* lines generated, 17 were homozygous lethal. The results of several genetic tests argue that these events are lethal alleles...
of the dia locus: (1) the cytologically visible deficiency Df(2L)TW84, which deletes the entire dia region, fails to complement any of these lethal mutations; (2) complementation tests indicate that these 17 lethal events are due to mutations in the same locus; (3) the lethal events fail to complement the original dia allele and (4) a genomic fragment containing a single intact transcription unit can fully rescue the male-sterile and lethal phenotypes (discussed in more detail below).

**Nucleic acid manipulations and analysis**

Standard protocols were performed as described (Sambrook et al., 1989). For plasmid rescue, genomic DNA prepared from adults homozygous for dia+ was digested to completion with XbaI and Ncol, self-ligated and transformed into competent E. coli cells following a standard protocol (Ashburner, 1989) with selection for kanamycin resistance. The 140 bp fragment from one of the plasmid rescue clones was gel-purified, labelled and used to screen a Drosophila genomic library (EMBL3 vector) provided by J. Tamkun. The 3.6 kb dia cDNA was obtained by screening a 12-24 hour embryonic library (Brown and Kafatos, 1988). fod cDNAs were cloned from an adult testis library provided by T. Hazelsey.

RNA amplification (RT-PCR) was performed as described in Kawasaki (1990). Synthetic oligonucleotides were synthesized to the library provided by T. Hazelrigg.

RESULTS

**Spermatogenesis phenotype**

The male-sterile dia allele was identified in a P element screen for mutations affecting spermatogenesis. Although both spermatocytes and spermatids are initially present in dia+ testes, these cells degenerate and are not replenished. By 5 days after eclosion, most mutant testes are devoid of germinal contents. Female fertility is not significantly affected, and the viability of both males and females is normal. The locus maps to polytene interval 38E (Castrillon et al., 1993). During spermatogenesis, a spermatagonium (the product of a stem cell division) undergoes four rounds of mitotic division to give rise to a cyst of 16 spermatocytes; meiosis then produces a cyst of 64 haploid spermatids. Wild-type chromosomal segregation and cytokinesis result in spermatids that each contain two major cytological structures of identical size and shape (Fig. 1A): a pale round nucleus (arrowhead), and an adjacent dark nebenkern (arrow). The nebenkern results from the fusion of all the mitochondria in a spermatid (see Fuller, 1993); its size thus serves as a marker for the amount of cytoplasm inherited by a spermatid.

The contents of testes from 50 newly eclosed dia male flies were examined. dia+ testes contain far fewer spermatids than normal, due to the reduced germinal content. The majority of spermatids were large and multinucleate (Fig. 1B). These abnormal spermatids contained either 2, 4 or 8 nuclei, with the size of the nebenkern proportional to the number of nuclei. Of 159 unelongated spermatids identified, 51 (32%) contained one nucleus (phenotypically normal), 26 (16%) contained two nuclei, 81 (51%) contained four nuclei, and 1 (1%) contained 8 nuclei.

The presence of spermatids containing 2 or 4 nuclei within a common cytoplasm can be explained by a failure in cytokinesis in one or both of the meiotic divisions. Likewise, the rare spermatid containing 8 nuclei can be explained by a failure of cytokinesis in three consecutive cell divisions, the first being the mitotic division preceding meiosis. The nuclei in defective spermatids are almost always of wild-type size, indicating that described in this paper are marked with cn, which allowed us to confirm that the rescued flies were mutant for dia.

**Double-labelling of follicle cells**

All steps were carried out at room temperature. Ovaries from 5-day-old females were dissected out and ovarioles were teased apart in PBS, fixed in PBT (PBT is PBS, 0.1% Tween-20) + 3.7% formaldehyde for 30 minutes, and washed 3 × 5 minutes in PBT. To minimize non-specific propidium iodide staining, the ovaries were incubated overnight in PBS + 10 µg/ml RNAse (shorter incubations may be sufficient), and washed 3 × 5 minutes in PBT. The ovaries were then stained in PBT + 25 µg/ml BODIPY-Concanavalin A (Molecular Probes) + 0.5 µg/ml propidium iodide (Sigma). Following three 10 minute washes, the ovaries were viewed immediately. Concanavalin A strongly binds to the epithelial sheath of the ovariole, which is closely apposed to the follicle cell layer; the follicle cells could be visualized by focusing just below this layer.

**Cytological examination of dividing neuroblasts**

Aceto-orcein squashes of the larval CNS were performed as described by Karess and Glover (1989).
chromosome segregation is normal in spite of the failure of cytokinesis. In contrast, in mutants that cause nondisjunction during meiosis, nuclei are of variable size (Gonzalez et al., 1989; Karess and Glover, 1989).

The finding that the initial failure of cytokinesis can occur at distinct points along the spermatogenesis pathway suggests why the germline in dia1 testes is eventually depleted. Since the 5-9 stem cells present in each testis continually divide to give rise to spermatogonia (Hardy et al., 1979), failure of cytokinesis during stem cell divisions should result in the permanent inactivation of these cells.

**Oogenesis phenotype**

In trans to a chromosomal deficiency or a null allele such as dia2, the dia1 allele exhibits an oogenesis phenotype. Although the viability of such trans-heterozygous adults is normal and the male germline phenotype is similar to that of dia1 males, female fertility is dramatically decreased. The ovaries of dia1/dia2 females are smaller than wild type. Egg chambers of all stages are present, the great majority of which contain 15 nurse cells and one oocyte. However, the eggs laid by dia1/dia2 females are shorter than wild-type and have short, fused, or extra dorsal appendages (Fig. 2). Only 10% of the eggs hatch.

This eggshell phenotype suggested a defect in the somatically derived follicle cells, which surround each developing egg chamber and secrete the eggshell. Indeed, follicle cells in mutant ovaries have an abnormal appearance when viewed by differential interference contrast (DIC) microscopy (Fig. 3B). Their nuclei vary considerably in size (arrowheads) relative to wild-type controls (Fig. 3A). Some of the cells appear to contain two nuclei (arrows).

To visualize the follicle cell layer better, ovaries were double labelled with a fluorescent Concanavalin A derivative (to stain plasma membranes) and with propidium iodide (to stain nuclei) and examined by confocal microscopy. Individual follicle cells containing two nuclei were frequently observed in egg chambers from mutant mothers (Fig. 3D, arrows); such cells were not found in wild-type egg chambers (Fig. 3C). Therefore, it appears that cytokinesis fails to occur in some follicle cells. The abnormally large nuclei seen in Fig. 3B are likely due to nuclear fusion following the failure of cytokinesis.

**Null mutations result in early pupal death and absence of imaginal discs**

Lethal dia mutations, including null mutations, were generated by imprecise excision of the P element in the dia1 allele. Null alleles such as dia2 result in early pupal lethality and absence of imaginal discs. This phenotype is consistent with dia being an essential mitotic gene. Due to the presence of maternal gene products, an embryo with a null mutation in such a gene can develop into a larva (Gatti and Baker, 1989). However, since imaginal disc cells divide during the larval stages, by which time zygotic gene expression is required,
such larvae will have defective or absent discs and will die at the onset of pupation.

Homozygotes for less severe alleles such as dia3 also die as early pupae, but third instar larvae contain imaginal discs, albeit somewhat smaller than normal. Homozygotes for the weakest lethal allele, dia9, have imaginal discs of normal appearance but die as late pupae or pharate adults. Very few (less than 1%) of dia9 flies eclose. These flies are sickly and have a weak ‘rough eye’ phenotype (not shown), consistent with a mitotic defect that affects a small fraction of cells. Testes from these surviving adults are almost completely devoid of germinal content.

Polyploidy in dividing neuroblasts from the larval CNS

The larval central nervous system (brain and ventral ganglion) is a rich source of dividing cells (neuroblasts) and is the most suitable tissue for examining mitosis in larvae. Chromosome morphology and segregation can be examined in aceto-orcein-stained preparations of the larval CNS (Gatti et al., 1974). In wild type, a mitotic figure consists of 3 pairs of major chromosomes (Fig. 4A, arrowheads).

In larvae homozygous for a weak lethal allele, dia9, only a small fraction of neuroblasts are polyploid and the number of mitotic figures is not affected (Table 1). Homozygotes for a stronger allele, dia3, exhibit ploidy ranging from 2n, 4n and 8n to extreme hyperploidy (Fig. 4B,C). In addition, significantly fewer mitotic figures are present in dia3 homozygotes than in wild type (Table 1). However, the fraction of mitotic figures in anaphase is the same as in wild type (see Table 1), whereas mutations that disrupt spindle function result in a decrease in the number of anaphases (Gatti and Baker, 1989). In addition, chromosome morphology is generally normal, although a small number of mitotic figures contain highly condensed chromosomes. Homozygotes for a null allele, dia2, have very few mitotic figures (Table 1), and all are...
The morphology of anaphase figures provides direct evidence that cytokinesis is defective in dia3 cells. Cleavage furrows are sometimes evident in wild-type anaphase figures, especially in well-isolated cells (Fig. 4D). Cleavage furrows have not been observed, however, in any dia3 anaphases and the cells appear completely round even when the chromosomes have finished migrating to opposite poles (Fig. 4E).

Despite the cytokinesis defect, chromosome segregation appears to be relatively normal in polyploid cells. In bipolar dia3 anaphases, the spindles are well organized and the chromosomes are equally segregated, with no lagging chromosomes or other abnormalities. This is true even in anaphases that are clearly hyperploid (Fig. 4F). In more extremely hyperploid cells, anaphases are typically multipolar. Such multipolar spindles have also been observed in other mutants that produce hyperploid cells (Gatti and Baker, 1989; Karess et al., 1991).

Taken together, the male-sterile, female-sterile and lethal phenotypes associated with dia mutations demonstrate that dia is required for cytokinesis in both the soma and germline and in mitosis as well as meiosis.

Cloning and characterization of dia genomic region
A 140 bp fragment of DNA extending from the 5′ end of the
P element to a genomic NheI site was cloned by plasmid rescue (Fig. 5A, B). Overlapping genomic clones of the diaphanous region (Fig. 5C) were obtained by screening a genomic library with the 140 bp fragment.

Northern analysis revealed two transcription units near the P insertion site; both were found to be expressed throughout development (data not shown). cDNAs for both genes were obtained and mapped onto the genomic clones. As discussed below, we showed by germline transformation with a genomic fragment that one transcription unit represents dia; the other we have named friend of diaphanous (fod) (Fig. 5D). The P insertion disrupts the 5′ untranslated end of the dia gene, since both the 140 bp fragment and fragments to the right of the insertion site detect the dia but not the fod transcripts (Fig. 5).

**Mapping of dia P element excision events**

The extent of the genomic deletion associated with each lethal allele was determined by Southern analysis using DNA prepared from homozygous third instar larvae; the results for three representative alleles are shown in Fig. 5E. The 3.4 kb NheI-XbaI fragment is deleted in the dia2 allele. Moreover, the polymorphic band detected when dia2 DNA was probed with the 2.4 kb XbaI-BamHI fragment was very faint, indicating that almost all of this genomic DNA is deleted. Thus, at least 5 kb of DNA from the dia locus are deleted by the dia2 mutation.

The dia2 allele, either when homozygous or in trans to Df(2L)TW84, results in early pupal lethality, complete absence of imaginal discs and extremely hyperploid neuroblasts. Although several alleles among the 17 lethal dia mutations recovered have a similar phenotype, none is more severe. Furthermore, the female-sterile phenotype of dia1 in trans to dia2 is as strong as that observed in dia1/Df(2L)TW84 females. On the basis of both the genetic and molecular data, we conclude that dia2 represents a null allele.

**Northern analysis of dia**

In wild-type adult males and females, two dia transcripts of 4.4 and 4.8 kb are observed in northern analysis (Fig. 6, lanes 1 and 2). In adults homozygous for dia1, two dia transcripts are also observed, but they are larger than the corresponding wild-type transcripts (Fig. 6, lane 3). Reversion to the wild-type pattern is observed in a phenotypic revertant line in which remobilization of the P element resulted in its precise excision (Fig. 6, lane 4). Both dia transcripts are present in flies that lack a germline (Fig. 6, lane 5); therefore, the expression of neither dia transcript is limited to the germline. Of the two fod transcripts, the 3.5 kb species, which is specific to the male germline (Fig. 6, compare lanes 1, 2 and 5) is absent in dia1 flies (Fig. 6, lane 3); this is expected, however, since mature males of this genotype have no germline (Castrillon et al., 1993).

**Germline transformation and phenotypic rescue**

P element-mediated germline transformation allowed us to confirm that the disrupted gene is dia. An 11 kb fragment of genomic DNA was subcloned into the Drosophila germline transformation vector pCaSpeR 4 (Pirrotta, 1988) to yield the recombinant plasmid pDC4 (Fig. 5F). The 11 kb fragment encodes the entire dia transcription unit. A portion of the fod transcription unit also lies within the transgene, but is not

![Fig. 5. Schematic diagram of the diaphanous region.](image)
An RNA loading control (the same blot reprobed with an
transcript sizes, in kilobases, are indicated to the right of the fi gure.

Homozygous for the lethal 
errors. Four sequence polymorphisms were identifi ed;

The consensus PCR sequence contains a long ORF contiguous
to the 5′ end of the cDNA. Therefore, this cDNA appeared to

An RNA amplification strategy (RT-PCR) was devised to
detectable levels (Fig. 6B, lane 7, compare to
to generate transgenic flies. One transformant line was identifi ed that carried a copy of pDC4 (P[w+, pDC4]) on the third chromosome. As shown in Fig. 6, lanes 6-8, this construct provided wild-type levels of the 4.8 kb dia transcript and somewhat lower levels of the 4.4 kb RNA. This transgene fully rescued the mutant phenotypes of all the alleles described in this paper: dia1 males and dia1/dia2 females are rescued to wild-type levels of fertility, and dia2, dia3 and dia5 flies are rescued to wild-type levels of viability and fertility.

Cloning of the 5′ end of the dia protein-coding sequence

The longest dia cDNA that we have identified (from an embryonic library) is 3.6 kb in length, signifi cantly shorter than the dia transcripts detected by northern analysis. DNA sequencing revealed a single long open reading frame (ORF) extending to the 5′ end of the cDNA. Therefore, this cDNA appeared to be missing some protein-coding sequence at its 5′ end.

An RNA amplifi cation strategy (RT-PCR) was devised to clone the missing 5′ cDNA sequence (see Experimental Procedures). RT-PCR reactions using the primers shown in Fig. 7 consistently resulted in a single product of 0.9 kb (data not shown). Two PCR products from each of two separate reactions were cloned and sequenced to resolve any Taq polymerase errors. Four sequence polymorphisms were identifi ed; for each the correct base could be unambiguously determined, since the other sequences matched at these sites.

Analysis of dia protein sequence

The consensus PCR sequence contains a long ORF contiguous with the ORF from the 3.6 kb cDNA. The PCR and cDNA sequences were linked and the complete predicted amino acid sequence of the diaphanous protein was generated (Fig. 7). The nucleotides fl anking the fi rst start codon are a close match to the Drosophila translational start consensus sequence (Cavener, 1987). The next methionine in the ORF occurs 77 amino acids into the ORF.

No N-terminal signal peptide or obvious transmembrane domain is present in the 123×10^3 Mr protein. The protein does contain an unusual proline-rich domain near its middle. This proline-rich domain contains six repeats of 5 to 8 consecutive prolines separated by short stretches rich in the amino acids glycine, methionine, alanine or arginine, with a few interspersed prolines (Fig. 8C). The diaphanous protein sequence was compared to protein sequences in available databases with the BLASTP program (Altschul et al., 1990). When the ‘fi lter’ directive was used to mask off segments of low compositional complexity, such as the proline-rich region, a region of about 130 amino acids was identifi ed that was conserved in the yeast protein Bni1p, and in both mouse and chicken forms (Fig. 8B). Mutations in BNII (bud neck involved) affect bud site selection and cytokinesis in diploid strains, and have a synthetic lethal interaction with CDC12, a gene required for cytokinesis in S. cerevisiae (Hartwell, 1971; H. Fares and J. Pringle, personal communication). In mice, the formin proteins are produced by the limb deformity gene; mutations in this gene result in limb malformations (Kleinebrecht et al., 1982; Woychick et al., 1985, 1990; Jackson-Grusby et al., 1992). In searching a six-frame translation of the DNA databases we found a fourth example of this conserved domain: a peptide encoded by a partial cDNA sequence from a rice expressed sequence tag (EST) (Fig. 8B).

The formins and Bni1p, like diaphanous, contain a large proline-rich domain located near the middle of the proteins (Fig. 8A,C). Although a number of proteins contain proline-rich domains (e.g. Mermod et al., 1989), those of the formins and Bni1p are the most similar to diaphanous in that they contain multiple stretches of consecutive prolines (Fig. 8C); proline-rich regions in other proteins usually consist of interspersed prolines or long stretches of polyproline. We have given the name FH1 (formin homology domain 1) to the proline-rich domains found in diaphanous, formin and Bni1p, and the name FH2 to the conserved 130 amino acid region in these proteins (Fig. 8). It is striking that not only the sequence composition of the FH1 and FH2 domains, but also the spacing between these domains, is conserved among diaphanous, Bni1p, and the formins (Fig. 8A).

Secondary structure analysis, using the algorithm devised by Stock and colleagues, revealed two regions of the diaphanous protein that are very likely to form coiled-coil domains (defi ned as a mean score >1.0; see Materials and Methods and Lupas et al., 1991). One of these, spanning amino acids 441 to 500 (mean score 2.1), ends just at the beginning of the FH1 domain. The second, spanning amino acids 863 to 1053 (mean score 1.3), begins 17 amino acids before the end of the FH2 domain, just after the region of highest sequence similarity. Thus it appears that coiled-coil domains flank the FH1 and FH2 domains of diaphanous. Moreover, sequence analysis also predicts the existence of coiled-coil domains at equivalent positions in mouse formin IV and Bni1p (H. Fares, personal communication).

DISCUSSION

Interpretation of mutant phenotypes

In this paper, we present the genetic and molecular characterization of the diaphanous locus. Mutations in dia affect a range

Fig. 6. Effect of dia mutations on fod and dia transcripts. Autoradiograph of northern blot probed with dia and fod cDNAs. Each lane was loaded with 5 μg of poly(A)+ RNA. The dia and fod transcript sizes, in kilobases, are indicated to the right of the fi gure. No N-terminal signal peptide or obvious transmembrane domain is present in the 123×10^3 Mr protein. The protein does contain an unusual proline-rich domain near its middle. This proline-rich domain contains six repeats of 5 to 8 consecutive prolines separated by short stretches rich in the amino acids glycine, methionine, alanine or arginine, with a few interspersed prolines (Fig. 8C). The diaphanous protein sequence was compared to protein sequences in available databases with the BLASTP program (Altschul et al., 1990). When the ‘filter’ directive was used to mask off segments of low compositional complexity, such as the proline-rich region, a region of about 130 amino acids was identified that was conserved in the yeast protein Bni1p, and in both mouse and chicken forms (Fig. 8B). Mutations in BNII (bud neck involved) affect bud site selection and cytokinesis in diploid strains, and have a synthetic lethal interaction with CDC12, a gene required for cytokinesis in S. cerevisiae (Hartwell, 1971; H. Fares and J. Pringle, personal communication). In mice, the formin proteins are produced by the limb deformity gene; mutations in this gene result in limb malformations (Kleinebrecht et al., 1982; Woychick et al., 1985, 1990; Jackson-Grusby et al., 1992). In searching a six-frame translation of the DNA databases we found a fourth example of this conserved domain: a peptide encoded by a partial cDNA sequence from a rice expressed sequence tag (EST) (Fig. 8B).

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required for cytokinesis of cell types and result in diverse phenotypes, including male sterility, female sterility and lethality. Examination of mutant tissues suggests that a single underlying cellular defect, a failure of cytokinesis, is responsible for all of these phenotypes. Furthermore, analysis of null mutations indicates that \(\text{dia}^+\) is likely to be required for cytokinesis in all cells.

Sterile phenotypes
Failure of cytokinesis during meiosis is readily apparent in \(\text{dia}^+\) spermatids. The presence of rare spermatids containing 8 nuclei indicates that cytokinesis can also fail in the mitotic divisions preceding meiosis. Finally, the fact that testes become devoid of germinal contents suggests that cytokinesis can also fail during stem cell divisions. Because the 5-9 germline stem cells present in each testis continually divide (Hardy et al., 1979), failure of cytokinesis in stem cells should block the initiation of spermatogenesis.

\(\text{dia}^+\)/\(\text{dia}^2\) females lay short eggs with defective dorsal appendages. These defects are presumably secondary to the failure of cytokinesis observed in the follicle cell layer. Eggshell synthesis is a complex process requiring the precise migration and positioning of specialized follicle cells to lay down the various layers and structures of the eggshell (Spradling, 1993). The failure of \(\text{dia}^+\) follicle cells to undergo at least two more rounds of mitotic divisions than the germline cells leads to the development of abnormal follicle cells. These cells then fail to undergo mitosis and the accompanying additional opportunities for the lack of \(\text{dia}^+\) function to have phenotypic consequences, is the cause of the apparent selectivity of the cytokinesis defect for follicle cells.

The failure of cytokinesis that we have observed in follicle cells has also been observed in \(\text{Drosophila}\) females lacking \(\text{peanut}\) function (Neufeld and Rubin, 1994). In addition, other female-sterile mutants that produce short eggs and short dorsal appendages have been described (Spradling, 1993). Since many of these lines are relatively uncharacterized, it is conceivable that a similar underlying defect is responsible for the phenotypes of some of these mutants.

Lethal phenotype
The lethal \(\text{dia}^+\) phenotypes are likely the result of a gradual dilution of maternal \(\text{dia}^+\) gene product over many cell cycles. Presumably, when the amount of protein in a cell falls below a certain threshold, the cell ceases to divide and eventually dies.
below a certain critical point, cytokinesis fails in subsequent cell cycles. In flies homozygous for the null allele dia2, the defect is manifested early, perhaps in the first few larval cell divisions, such that by the end of larval development, very few neuroblasts are present and all are highly hyperploid. In contrast, in partial-loss-of-function dia3 mutants, the cytokinesis defect apparently is manifested after a variable number of normal cell cycles. The number of cells and the degree of polyploidy is therefore intermediate between dia2 and wild type.

The survival of embryos homozygous for null dia mutations is likely due to a maternal contribution of dia mRNA or protein. Consistent with this hypothesis, dia transcripts are detectable in 0-3 hour embryos. Maternal dia gene product may be required for cytokinesis in postblastoderm mitoses and perhaps for cellularization of the syncytial blastoderm, a process similar in some respects to cytokinesis.

Role of diaphanous

We believe that dia is specifically required for cytokinesis; as such, the protein might be a component of the contractile ring or regulate its function. Other aspects of the cell cycle, including DNA replication, spindle assembly and function, and nuclear reformation appear to proceed normally in dia mutants. The chromosomes of salivary glands from larvae homozygous for a null allele reach wild-type levels of polytenization (unpublished data), demonstrating that dia is not required for DNA replication. Chromosome segregation occurs in mutant spermatids, follicle cells and neuroblasts.

In dia mutant cells that have failed to undergo cytokinesis, nuclear reformation appears to occur normally, although fusion of nuclei occurs in neuroblasts and possibly in follicle cells. In Drosophila and other species, fusion of nuclei is common in cells that become multinucleate due to a failure of cytokinesis. This phenomenon has been observed in the Drosophila cytokinesis mutant spaghetti-squash1 (sqh1) (Karess et al., 1991; see also Gatti and Baker, 1989) and in a hamster cell line mutant defective for cytokinesis (Hatzfeld and Buttin, 1975). In dia1 spermatids, fusion of nuclei does not take place. This may be due to the fact that the spermatogenesis differentiation program, which includes the condensation of nuclei for packaging into sperheaders, proceeds to some extent in these spermatids.

The partial loss-of-function sqh1 allele results in a mutant phenotype similar to dia1: homozygotes die as early pupae, have small imaginal discs and exhibit a high frequency of polyploid neuroblasts in third instar larvae. In addition, cleavage furrows are missing in anaphase figures of sqh1 neuroblasts. Viable, sterile alleles of sqh have not been described. The sqh gene encodes the regulatory light chain of nonmuscle myosin, consistent with a requirement for myosin function during cytokinesis in Drosophila (Karess et al., 1991).

Significance of formin homology domains

Diaphanous, the formins and Bni1p contain two regions of sequence similarity, FH1 and FH2. Although no primary sequence similarity was detected in the N-terminal domains of these proteins, this portion of the mouse and chicken formins is much less conserved than the C-terminal domain (Trumpp et al., 1992; Jackson-Grusby et al., 1992). The fact that each of the proteins containing an FH2 domain also contains an FH1 domain, that the spacing between the two domains is also conserved and that the two domains in each protein are flanked by regions likely to form coiled-coil domains strongly suggests that the FH1 and FH2 domains function together.

Mutations in the mouse limb deformity gene, which encodes multiple formin isoforms, cause moderate to severe aplasia of both limbs and kidneys (Kleinebrecht et al., 1982; Woychick et al., 1985; 1990; Zeller et al., 1989). Within the limb bud, defects are observed in the apical epidermal ridge. However, complete loss-of-function mutations have not been identified, and little is known about the role of the formins at the cellular level. It remains possible that a defect in cytokinesis is responsible for the observed deformities.

A peptide representing a portion of the FH1 domains of the formins has been shown to bind in vitro to the SH3 domain of the Abl protein (Ren et al., 1993) and this sequence is a reasonable match with a recently defined consensus binding site for SH3 domains (Yu et al., 1994). However, the FH1 domains of diaphanous and Bni1p do not fit the defined consensus. Another possibility is that the FH1 domain serves as a hinge between two functionally separate domains. Hinge regions of proteins are also often rich in proline (e.g. Koenig and Kunkel, 1990), but tend to be smaller than the FH1 domain and have more evenly dispersed proline residues.

One intriguing possibility is that the FH1 domain binds to profilin, which is involved in the regulation of actin polymerization (Carlsson et al., 1977). Profilin binds with extremely high affinity to poly(L-proline), a feature that has been exploited to purify profilin from crude cell extracts in a single-step (Tanaka and Shibata, 1985). Profilin is therefore expected to interact in vivo with at least one as yet unidentified protein containing a proline-rich region. Dictyostelium discoideum cells mutated for both profilin genes form multinucleate cells due to a defect in cytokinesis (M. Schleicher, personal communication). Furthermore, male-sterile alleles of chickadee, which encodes the Drosophila homolog of profilin (Cooley et al., 1992) affect the proliferation of the germline (Castrillon et al., 1993). In this regard, it is notable that dia and the Drosophila profilin gene can both mutate to germlineless phenotypes.

The diaphanous protein might also interact directly with the product of the peanut gene (Neufeld and Rubin, 1994). Like diaphanous, peanut is required for cytokinesis in Drosophila. The peanut protein is similar throughout its length to the S. cerevisiae proteins Cdc3, Cdc10, Cdc11, and Cdc12, all of which are required for cytokinesis (Hartwell, 1971; Kim et al., 1991). Since CDC12 has a synthetic lethal interaction with BNI1 (H. Fares and J. Pringle, personal communication), there may be a direct interaction between the products of these two genes and, by analogy, between peanut and diaphanous. Whether or not this is the case, the observation that there are regions of similarity common to diaphanous, the formins and Bni1p indicates that these proteins are likely to share a common biochemical function.

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