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# An extracellular matrix protein prevents cytokinesis failure and aneuploidy in the *C. elegans* germline

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**Interactions between extracellular matrix (ECM) proteins and their transmembrane receptors mediate cytoskeletal reorganization and corresponding changes in cell shape during cell migration, adhesion, differentiation and polarization. Cytokinesis is the final step in cell division as cells employ a contractile ring composed of actin and myosin to partition one cell into two. Cells undergo dramatic changes in cell shape during the division process, creating new membrane and forming an extracellular invagination called the cleavage furrow. However, existing models of cytokinesis include no role for the ECM. In a recent paper, we demonstrate that depletion of a large secreted protein, hemicentin, results in membrane destabilization, cleavage furrow retraction and cytokinesis failure in *C. elegans* germ cells and in preimplantation mouse embryos.**

**Here, we demonstrate that cytokinesis failure produces tetraploid intermediate cells with multipolar spindles, providing a potential explanation for the large number of aneuploid progeny observed among *C. elegans* hemicentin mutant hermaphrodites.**

**Key words:** aneuploidy, cytokinesis, extracellular matrix, *C. elegans*, cleavage furrow, hemicentin, tetraploid intermediate

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The karyotype of *C. elegans* has five autosomes and one or two X chromosomes in males and hermaphrodites, respectively. The majority of self-progeny produced by wild-type hermaphrodites are hermaphrodites (~99.8%), while rare meiotic nondisjunction of the X chromosome produces nullo-X gametes and 0.2% males. Mutations in over 30 genes result in a 10–150-fold increase in the frequency of males among hermaphrodite self-progeny, due to increases in defects in X chromosome segregation.[1] The majority of these '*him*' (*high incidence of males*) loci are genes that encode proteins associated with the intracellular machinery of meiotic chromosome segregation.[2,3] Unique among *him* genes, the *him-4* locus encodes hemicentin, a large, highly conserved component of the extracellular matrix (ECM).[4] In addition to defects in germline chromosome segregation, *him-4* mutants have pleiotropic defects in somatic cell adhesion and migration.[1,4] The extracellular distribution of hemicentin at cell junctions that are defective in *him-4* mutants dovetails with current models of cell adhesion and migration.[5] However, it leaves unexplained several questions about how a secreted ECM component promotes correct chromosome segregation in the *C. elegans* germline.

*C. elegans* hermaphrodite gonads are composed of two U-shaped tubes, and gametogenesis proceeds sequentially from the distal to the proximal end of each tube. Germ cells in *C. elegans* have incomplete cleavage furrows that connect them to a central cytoplasmic core, allowing distal cells to act as "nurses" while allowing more mature proximal oocytes to fill with bulk cytoplasm.[6-8] Several genetic and cytogenetic observations suggest a mitotic rather than a meiotic origin for germline chromosome segregation defects observed in the absence of hemicentin.[4] For example, jackpots of male progeny from individual

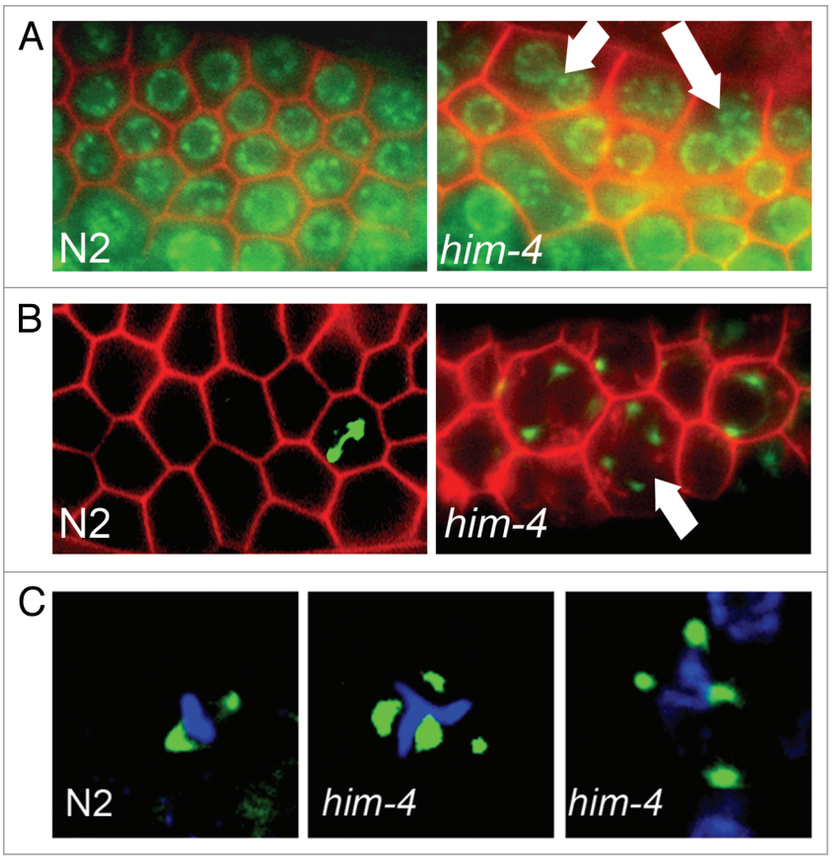


**Figure 1.** Multinucleate germ cells and multipolar germ cells observed in the mitotic zone of him-4 mutant hermaphrodites. (A) PH::RFP and histone::GFP in the mitotic region of wild-type (left) and *him-4 (rh319)* hermaphrodite gonads. Large numbers of multinucleate cells are observed among mitotic germ cells in mutant gonads (arrows). (B) PH::RFP and tubulin::GFP in the mitotic region of wild-type (left) and *him-4 (rh319)* hermaphrodite gonads. A significant fraction (**Table 1**) of mitotic germ cells in *him-4 (rh319)* hermaphrodite gonads have multipolar spindles (arrow). (C) Tubulin:GFP and DAPI-stained germ cells in wild-type and *him-4* (rh319) distal gonads. In wild-type animals (left), single bipolar spindles segregate chromosomes aligned on the metaphase axis. In contrast, multipolar spindles cause chaotic mitoses in *him-4* mutant animals. The GFP and RFP constructs are described in reference 14–16.

**Table 1.** Severity and types of defective germ cells in him-4 gonads

| Defect | Wild type | *him-4 (rh319)* |
|---|---|---|
| Mitotic germ cells with multiple nuclei | 3/107 (3%) | 28/105 (27%) |
| Mitotic germ cells with multipolar spindles | 0/108 (0%) | 16/115 (14%) |
| Aneuploid pachytene germ cells | 6/524 (1%) | 257/741 (35%) |
| Aneuploid diakinesis germ cells | 0/58 (0%) | 18/57 (32%) |

hermaphrodites and nullisomy of primary meiocytes in *him-4* mutants suggest a defect in a mitotic germline stem cell rather than in a post-mitotic process. Our recent work describing hemicentin localization at the cleavage furrows of dividing cells in the early mouse embryo and *C. elegans* germline, in addition to membrane destabilization, cleavage furrow retraction and cytokinesis failure in the absence of hemicentin, suggests that hemicentin has an evolutionarily conserved role in stabilizing and preventing retraction of nascent cleavage furrows.[9]

Aneuploid cells are frequently observed in, and may be associated with the generation of, human tumor cells. Recent work from several laboratories suggests that cytokinesis failure is one of several mechanisms whereby tumor cells generate tetraploid intermediates that result in the production of aneuploid daughter cells in subsequent cell divisions. One proposed mechanism for the generation of aneuploid daughter cells from a tetraploid intermediate is thought to involve multipolar mitotic spindles that result in asymmetric mitoses.[10-13]

To determine whether a similar mechanism might be responsible for the aneuploidy observed in the absence of hemicentin, *him-4 (rh319)* animals were examined for multipolar mitotic spindles. A significant fraction (14%) of mitotic germ cells have multipolar spindles that are not observed in a wild-type background (**Fig. 1** and **Table 1**). In contrast to wild-type mitoses with bipolar spindles, which produce a symmetric segregation of chromosomes aligned on the metaphase plate, the multipolar spindles found in *him-4* mutant animals cause asymmetric mitoses (**Fig. 1**).

Although some genetic defects in the mitotic machinery produce a consistent syndrome of chromosome loss or gain, multipolar mitoses are predicted to result in a broad spectrum of chromosome sorting defects.[13] To determine the types of chromosome sorting defects found in the absence of hemicentin, fluorescent in situ hybridization (FISH) was performed with a probe specific for the X chromosome and an autosomal probe specific for chromosome 5. Analysis of FISH experiments reveal a variety of abnormal karyotypes in germ cells throughout the gonad in *him-4(rh319)* mutant animals (**Fig. 2**). *him-4* pachytene nuclei are generally larger than those found in wild-type animals and chromosome numbers are frequently elevated. Examination of oocytes in diakinesis indicates that the aneuploidy observed in *him-4* mutant animals can affect all five autosomes in addition to the X chromosome and may include massive aneuploidy and more subtle "near-diploid" defects in chromosome number (either missing or supernumerary chromosomes) and complement (correct total number of chromosomes produced by absence of one chromosome and duplication of another chromosome, **Fig. 2**).

Our recent work showing that absence of hemicentin in the cleavage furrow can lead to cytokinesis failure in *C. elegans*

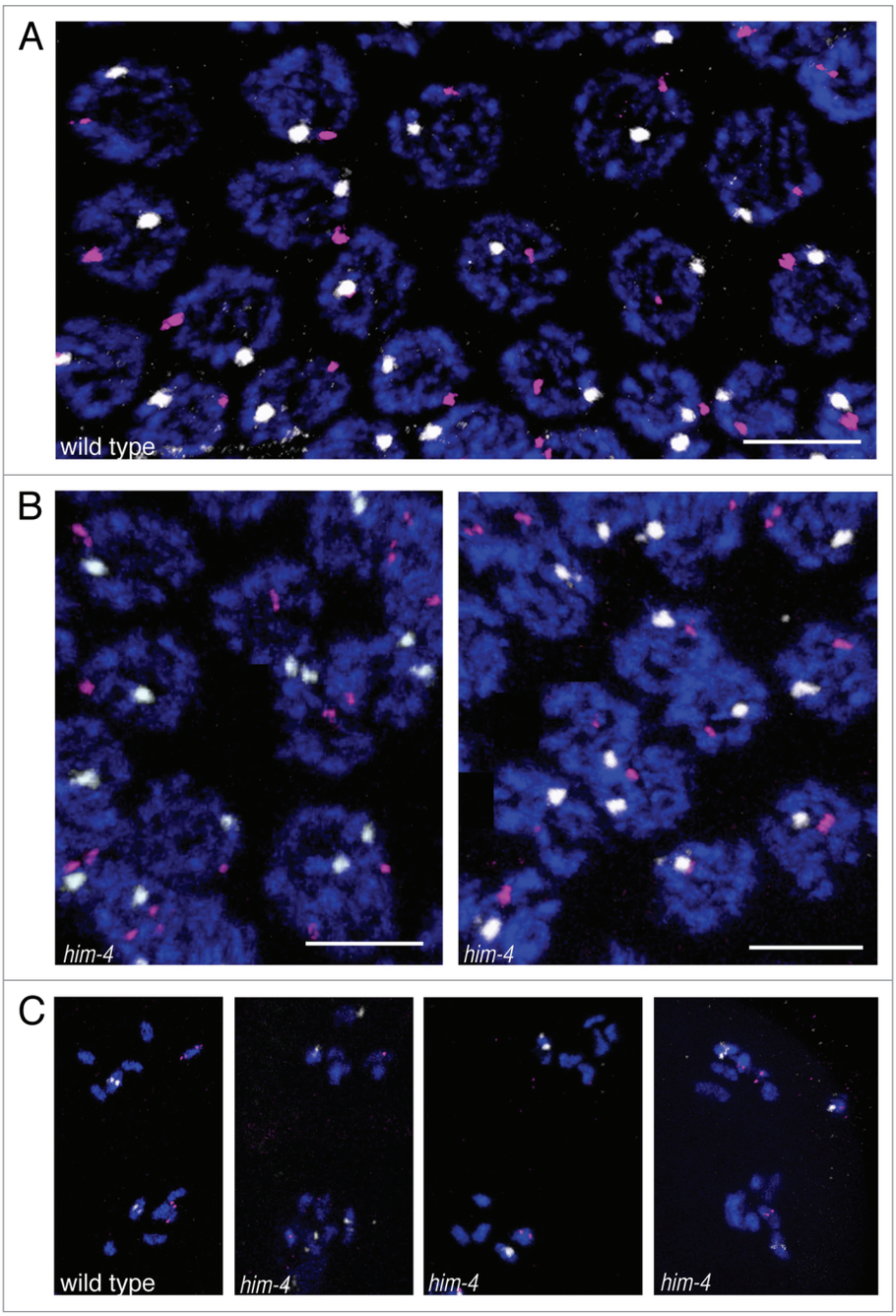


**Figure 2.** Meiotic defects in *him-4* mutant germlines. FISH probes were used to mark the right end of the X chromosome (white) and the 5S locus on chromosome V (pink). DNA was stained with DAPI (blue). (A) Pachytene nuclei in wild type are evenly spaced and show one focus or 2 very closed foci for each chromosome, indicating that all chromosomes are properly paired and synapsed. (B) In *him-4(rh319)* mutants, pachytene nuclei are often larger than those found in wild-type animals and frequently have elevated chromosome numbers and the wrong complement of chromosomes. (C) Diakinesis nuclei in wild type show six spots corresponding to each of the six pairs of chromosomes, held together as chiasmata. In *him-4*, diakinesis defects reveal an array of chromosomal abnormalities: nuclei may have the correct number but the wrong complement of chromosomes (left), missing chromosomes (center, bottom) or supernumerary chromosomes (right). FISH XR, XL and 5S probes were synthesized and labeled as previously described in reference 17. FISH gonad preparation, fixation and hybridization of were performed according to published protocols.[18] Scale bars = 5 μm.

germ cells and mouse embryonic cells undergoing cytokinesis,[9] coupled with the observation of mitotic germ cells with multi-polar spindles (**Fig. 1** and **Table 1**) and the large variety of chromosome sorting defects that result (**Fig. 2**) suggest that the aneuploidy observed in *him-4* mutant animals may arise by a mechanism similar to that described for the generation of aneuploid tumor cells.[10-13]

We suggest a model where cytokinesis failure in the absence of hemicentin generates a tetraploid intermediate cell similar to those found in tumor cell precursors. These cells may have several distinct fates that

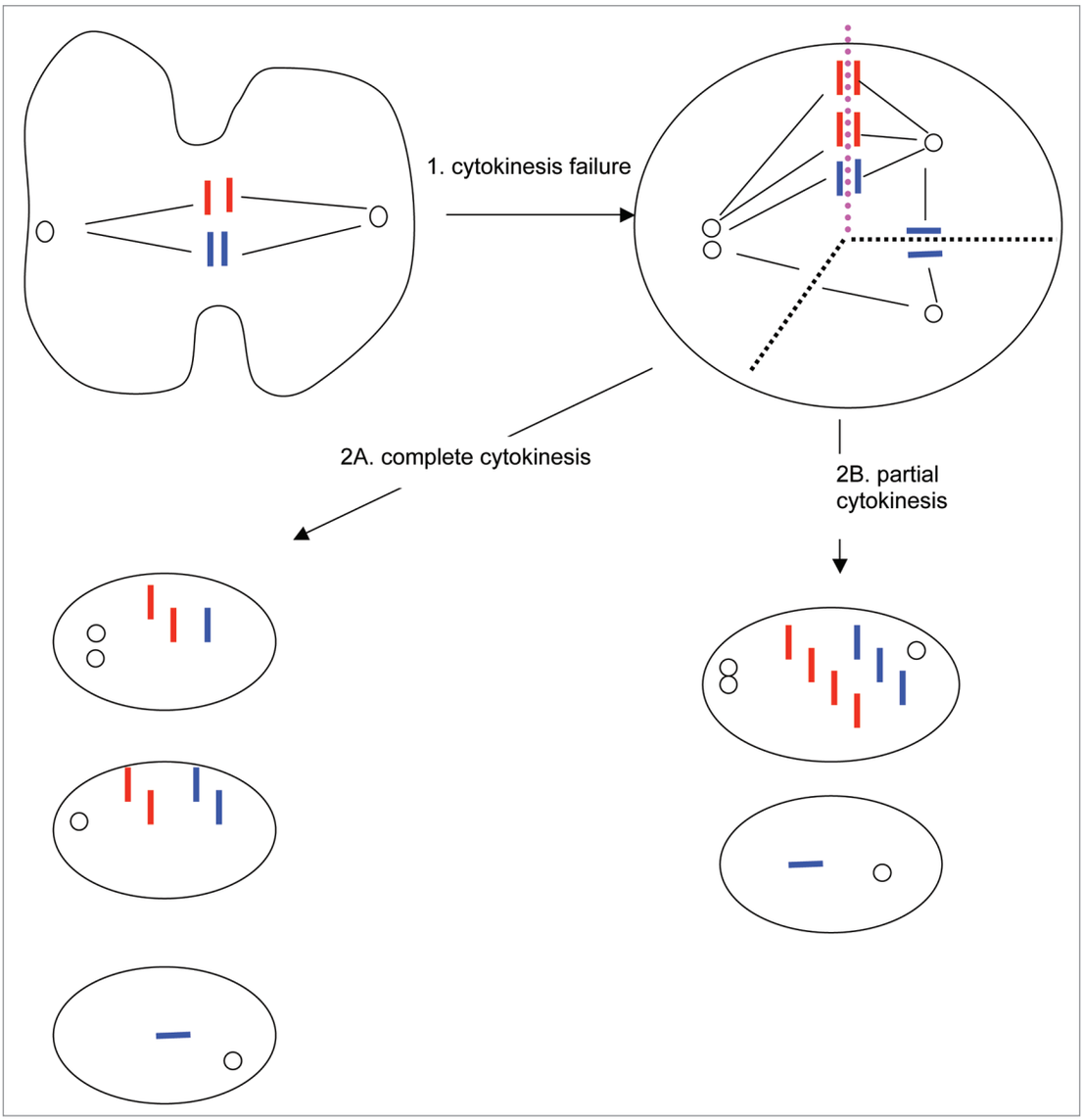


**Figure 3.** Model for the generation of monosomy in the germline of *him-4* mutant animals. Absence of hemicentin in the cleavage furrow can lead to cytokinesis failure (1) and generation of a tetraploid intermediate cell. Centrosome clustering may result in a tripolar nuclear division that will produce trisomies, tetrasomies and massive aneuploidy and monosomy for chromosomes (blue), where one homolog (red) is not aligned on the metaphase axis along which cytokinesis occurs. Cells with monosomic chromosomes will be generated if the second cytokinesis is complete (2A) or incomplete along one axis (dotted pink line, 2B). (See refs. 10–13 for discussions of centrosome clustering and generation of tumor cell aneuploidy).

include trisomies, tetrasomies and massive aneuploidy. Monosomy or nullisomy for chromosomes will occur when one or both homologs are not aligned on the metaphase axis, along which a subsequent cytokinesis occurs and will occur whether the subsequent cytokinesis is complete or incomplete along one axis (**Fig. 3**).[10-13] The observation of large numbers of primary meiocytes with seven or more bivalent chromosomes suggests that partial cytokinesis does indeed occur in *him-4* mutant animals.[4] The generation of primary meiocytes with monosomy or nullisomy for the X chromosome can account for the large number of males among the offspring produced by *him-4* mutant hermaphrodites, and autosomal aneuploidy is likely to account for the large numbers of inviable zygotes (~40%).[4]


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