Meiotic HORMA domain proteins prevent untimely centriole disengagement during Caenorhabditis elegans spermatocyte meiosis

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AUTHOR SUMMARY

Sexual reproduction relies on the production of complementary gametes that, together, contribute all the components necessary for normal embryonic development. In addition to each gamete contributing a single haploid set of chromosomes, the sperm and the egg (or oocyte) in many animal species also provide the zygote, or newly fertilized single-cell embryo, with complementary components of the centrosome. Centrosomes are organelles that nucleate and help organize arrays of microtubules within the cell. Each centrosome contains either one or two cylindrical microtubule structures called centrioles, which recruit additional proteins termed pericentriolar material (PCM) to form a functional centrosome. A commonly used strategy for ensuring that gametes provide complementary contributions of centrosome components to the zygote is to dispose of either the centrioles or the PCM selectively by the end of gametogenesis (1). In the nematode Caenorhabditis elegans, as in humans, oocytes dispose of the centrioles, whereas spermatocytes discard the PCM by the end of spermatogenesis. At fertilization, the oocyte contributes the PCM and the sperm contributes the centriole pair that, together, form the first centrosome of the embryo. Therefore, maintaining the correct organization of centrioles during male meiosis is key to ensuring formation of a normal bipolar mitotic spindle in the zygote.

During mitotic cell cycles, key events, such as DNA replication, chromosome segregation, centriole duplication, and cell division, are tightly coordinated and take place only once per cycle. Further, like sister chromatids, centrioles separate during or soon after anaphase, resulting in two daughter cells that each contain both a full genetic complement of chromosomes and a disengaged pair of centrioles. During meiosis, however, a single round of DNA replication is followed by two rounds of chromosome segregation: meiosis I, in which homologous chromosomes segregate away from each other but sisters stay together, and meiosis II, in which sister chromatids segregate. This meiotic pattern of chromosome segregation requires that sister chromatid cohesion be released in two steps during the meiotic program, and it represents a major difference between mitosis and meiosis. In previous work, we discovered that the HTP-1/2 protein, which is a member of a meiosis-specific protein family sharing a feature called the HORMA domain, is a crucial part of this strategy in C. elegans (2). HTP-1/2 localizes to the chromosome domains where cohesion is retained during meiosis I and prevents the untimely separation of sister chromatids by locally inhibiting the removal of cohesin complexes containing the meiosis-specific subunit REC-8 by a cytostatic protease called separase.

The fact that meiosis involves two rounds of cell division following a single round of DNA replication also necessitates a modification of the centriole duplication cycle: During male meiosis in C. elegans, as in mammals, centrioles normally undergo two rounds of duplication. In nematodes, this results in each haploid sperm containing a single orthogonally engaged centriole pair that will later disengage and duplicate in the zygote to form the centrosomes of the first mitotic division. Because disengagement can license centriole duplication, spermatocytes must somehow prevent centriole disengagement at meiosis II to ensure that sperm inherit a single engaged centriole pair. Indeed, in the current work, we show that inhibition of centriole disengagement during meiosis II is critical to guarantee the correct number of centrioles and centrosomes in the zygote (Fig. P1). Thus, prevention of centriole disengagement at meiosis II represents another important difference between mitosis and meiosis.

In this work, we investigate how proper centriole organization is maintained during C. elegans male meiosis. Our results reveal an unanticipated role for members of the HORMA domain protein family in regulation of centriole disengagement during meiosis. Specifically, we used high-resolution immunofluorescence...
cence imaging of WT and mutant *C. elegans* spermatocytes, spermatids, and zygotes to identify a previously unrecognized role for *C. elegans* HORMA domain proteins HTP-1/2 and HIM-3 in preventing centriole disengagement during the meiosis II division in spermatocytes. Our analysis revealed untimely centriole disengagement in *him-3* and *htp-1 htp-2* mutant spermatocytes, resulting in separated centrioles in sperm (Fig. P1). Further, an extra pair of centrosomes was detected in a subset of zygotes, suggesting that premature centriole disengagement may have enabled a single additional round of duplication (Fig. P1). We also provide evidence that these HORMA proteins likely function to maintain centriole engagement in meiosis II by inhibiting separase-dependent removal of meiosis-specific cohesion complexes containing REC-8. Thus, our data suggest that the same specialized meiotic mechanism that functions to prevent premature release of sister chromatid cohesion during meiosis I in *C. elegans* also functions to inhibit centriole separation at meiosis II, thereby ensuring that the zygote inherits the appropriate complement of both chromosomes and centrioles.

Our findings emphasize the fact that entire biological subroutines can be recruited to accomplish distinct tasks that require similar regulatory logic. In this case, the task is to maintain connections temporarily between structures that are ultimately destined for regulated separation. For mitotic cells, it was previously suggested that the use of cohesin and separase to regulate both sister chromatid cohesion and centriole engagement might have evolved as a means to couple the two types of separation events temporally during the cell cycle (3). However, HORMA-dependent mechanisms operate to maintain connections between sister chromatids during meiosis I (2) and to maintain connections between centrioles during meiosis II (our study). In both cases, these mechanisms prevent inappropriate separation events that could potentially impair the subsequent cell division. Thus, it is clear that parallel regulatory logic, rather than temporal coordination, is the relevant underlying commonality that drove the dual use of this HORMA-dependent cohesin maintenance strategy in regulating both chromosome and centriole separation during meiosis.