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SPOTLIGHT

CENP-C in two acts: Dynamic roles in male meiotic centromere assembly

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In this issue, Keegan et al. (https://doi.org/10.1083/jcb.202512053) uncover molecular dynamics of biphasic centromere histone assembly in the *Drosophila* male germline: initially mitotic-like, then chaperone independent. These findings highlight germline-specific adaptations in centromere assembly that deviate from the somatic cell paradigm.

Accurate chromosome segregation is directed by the centromere, which assembles the kinetochore, a structure connecting chromosomes to spindle microtubules (1). Centromeres are epigenetically defined by the histone H3 variant, centromere protein-A (CENP-A, CID in *Drosophila*) (1). In mitosis, preexisting CENP-A is divided equally between sister chromatids during replication and replenished in the following G1 phase, a process tightly regulated by the cell cycle kinases CDK1/2, ensuring epigenetic propagation of the centromere mark (2). However, previous work has identified nascent CID assembly in prophase I (G2) during both male and female meiosis, a specialized, terminal division that produces haploid gametes, with an additional postmeiotic assembly phase in male meiosis (3). This departure from the tight G1 restriction of mitotic CENP-A assembly underscores deviations to accommodate germline-specific requirements during meiosis, but the dynamics and molecular requirements in each assembly phase remain unclear. In this study, the authors ask: How is biphasic CID assembly regulated in *Drosophila* male meiosis?

The authors began by characterizing CID abundance across spermatogenesis to precisely define when CID assembly occurs. They found that CID levels decrease between the spermatogonial 2-cell and 4-cell cyst stages, consistent with previous work reporting ~1.5-fold increased CID in germline stem cells compared with differentiating daughter cells, linking elevated CID abundance with stem cell identity (4). They identified a first assembly phase between S1 and S6 of spermatocyte prophase I, with a CID increase of 2.5-fold, and a second phase between T1 and T5 in postmeiotic spermatids, with a 1.8-fold increase, consistent with previous evidence of biphasic assembly (3).

At the molecular level, a previous study found three key centromeric proteins to be interdependent for centromere propagation in *Drosophila* mitosis: CID; CAL1, a CID-specific chaperone; and CENP-C, a constitutive CENP-A–binding partner (5). These proteins interact with one another and are essential for centromere specification and chromosome segregation. In this study, Keegan et al. ask whether these same principles of interdependency hold true in meiosis (6). To assess this, they used RNAi lines expressed with two stage-specific germline drivers to selectively knock down CID, CAL1, and CENP-C: *bam*-GAL4, which is active from the 8-cell spermatogonial stage through late prophase I, and the later *Rbp4*-GAL4 driver, which begins expression at the S1 stage in spermatocytes (Fig. 1). They found that at early prophase I (S1 stage), CAL1 is required for the stability of chromatin-bound CID and, in turn, requires newly synthesized CID for localization to the centromere. Analysis at prometaphase I revealed that while CID abundance was reduced in all three RNAi lines driven by *bam*-GAL4, CENP-C localization was not strictly dependent on CAL1 or on CID levels, highlighting a key difference from mitosis. This reveals that, unlike mitosis, early prophase I contains a pool of CENP-C that is not strictly coupled to CID or CAL1 abundance (Fig. 1).

Next, the authors investigated the requirement of these proteins in forming a functional centromere and kinetochore. Using the same RNAi lines, they found that recruitment of the outer kinetochore protein, Spc105, was strongly reduced only in CENP-C RNAi. This indicates that newly assembled CENP-C in prophase I is specifically required for outer kinetochore recruitment, a phenotype that could be rescued by supplying an RNAi-resistant form of CENP-C. Imaging of tagged microtubules in CENP-C RNAi revealed >30% abnormal meiotic spindles, compared with almost none in the control, resulting in a substantial increase in autosomal and sex chromosome mis-segregation in both meiosis I and II, far exceeding the milder elevations observed with CID or CAL1 RNAi. Despite this, individualized mature sperm were morphologically normal and present at levels comparable with control, with males producing normal numbers of offspring in all conditions. This is striking, given that only around 10% of postmeiotic spermatids had a normal karyotype following CENP-C

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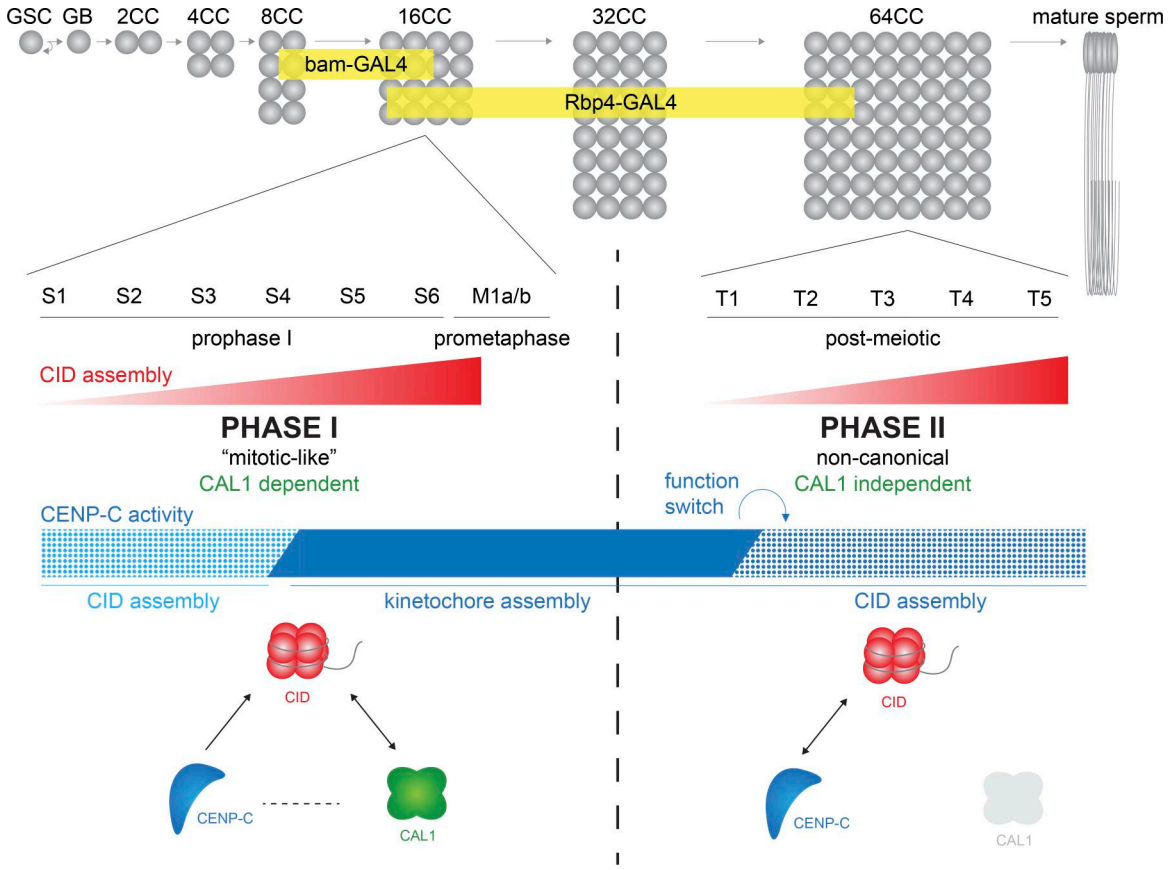

Figure 1. **Meiotic CENP-C supports two mechanistically distinct phases of centromere assembly.** The first phase occurs during prophase I and resembles the mitotic pathway, in which CENP-C acts together with CAL1: an early pool of CENP-C sustains CID assembly, then a later pool recruits the kinetochore as CAL1 is lost from centromeres. A second, noncanonical phase occurs after meiosis II in early spermatids, in which CID is assembled independently of CAL1 but still requires the late pool of CENP-C. These two phases were functionally dissected using two temporally distinct GAL4 RNAi drivers (*bam* and *Rbp4*, yellow bars). Black arrows indicate interdependencies of CID loading, while dotted line indicates partial dependence. Blue curved arrow indicates a switch in CENP-C function, supported by a pool independent of CAL1.

RNAi. The authors suggest that the large number of sperm generated during spermatogenesis may allow the remaining chromosomally normal sperm to sustain fertility. It also remains possible that reduced paternal CID is compensated for after fertilization, or a quality-control step removes aneuploid spermatids prior to individualization.

To investigate the second CID assembly phase, the authors quantified CID in late spermatids after early knockdown using the *bam*-GAL4 driver. CAL1 RNAi does not reduce CID beyond prometaphase I levels, which surprisingly suggests that CAL1 is not needed for this phase. Using the more temporally restricted late-stage *Rbp4*-GAL4 driver (Fig. 1), they found no change in CID at prometaphase I in any of the three RNAi treatments. This indicates that the endogenous CID deposited during prophase I is normally synthesized earlier, during the 8-cell mitotic stage. However, the authors note that differences in GAL4 expression strength cannot be excluded, and *Rbp4*-driven CID overexpression showed that CID synthesized during prophase I is nevertheless capable of assembling at centromeres. By the late spermatid stage, however, CENP-C and CID RNAi both reduced CID, while CAL1 RNAi still had no effect. Together, these results show that this second, postmeiotic wave of CID assembly uses protein synthesized during prophase I and requires CENP-C, but not CAL1, unlike mitotic assembly, where CAL1 is essential (5). This is notable because CAL1 performs functions analogous to those of HJURP and the Mis18 complex, which mediate CENP-A assembly in most other systems.

Altogether, Keegan and colleagues showed that CENP-C has stage-specific meiotic functions: an early pool supports CID assembly, whereas a later pool recruits the outer kinetochore independently of CID levels and promotes postmeiotic CID assembly. This functional switching is a major conceptual advance and parallels findings in female meiosis (7). The identification of a CAL1-independent CID assembly phase is highly novel, representing the first known example of chaperone (CAL1)-independent CID deposition in flies, or indeed in any species. This may mean that meiosis-specific factors act to assemble or maintain CID in postmeiotic haploid cells. However, the findings do not yet establish that assembly is entirely chaperone-independent, as CENP-C may instead recruit an unidentified CID chaperone or a more general histone assembly factor.

Strikingly, this study reveals a flexible relationship between cell cycle timing and centromere assembly in meiosis compared with mitosis, especially the uncoupling of CID synthesis and deposition. How the soluble CID pool is stabilized and carried through meiosis I and II remains unclear, particularly because CAL1 is no longer detectable in later stages. In mammals, CENP-A

assembly is normally confined to a narrow G1 window, since CDK activity inhibits assembly (8). Meiosis breaks this rule: CENP-A assembles in prophase I in mammalian spermatogenesis (9), when CDK2 activity is hypothesized to be elevated, as is seen in mice (10, *Preprint*), raising the possibility that germ cells broadly decouple centromeric assembly from CDK-based restriction. This may reflect a germline-specific need to overload CID, which must survive histone-to-protamine exchange in sperm and then support paternal chromosomes in the embryo until *de novo* assembly resumes. Consistent with this idea, haploid T5 spermatids overload CID, perhaps to contribute to this preparation, although the functional significance of the excess CID remains speculative.

Many interesting questions remain regarding the requirements for the second assembly phase: What facilitates CID assembly, if not CAL1? Does CENP-C switch completely from CID assembly to kinetochore recruitment swedin late prophase I, or does it perform both functions concurrently? What regulates the functional transition: posttranslational modifications, association with meiosis-specific chaperones, or other complexes? Further, CID is one of the few histones retained in mature sperm, and how it is maintained there in the absence of both CAL1 and CENP-C remains an open area of investigation.

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