In a battle between parental chromosomes, a failure to reload

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What can go wrong after an egg is fertilized by sperm from a different species? One of the most intriguing outcomes to the chromosome biologist is uniparental genome elimination. In this phenomenon, one of the parental chromosome sets is completely lost during embryonic cell divisions, creating sterile haploid offspring (1). Uniparental genome elimination occurs in diverse taxa, including several distantly related plant species and even in fish (1, 2). The mechanism by which chromosomes from one parent are selectively discarded has been studied cytologically in crosses between different grass species. However, these experiments have not revealed what is defective about the eliminated genome in a hybrid. In PNAS, Sanei et al. (3) make a major breakthrough by showing that missegregated chromosomes in a classic barley interspecies cross (*Hordeum vulgare* × *Hordeum bulbosum*) fail to assemble kinetochores, the microtubule attachment sites that mediate chromosome inheritance.

The chromosomal location of the kinetochore, termed the centromere, is marked by incorporation of a histone H3 variant named CENH3. CENH3-containing nucleosomes are found at centromeres in all eukaryotes and are essential for recruiting other kinetochore proteins. Sanei et al. (3) used an antibody raised against rice CENH3 to stain embryos created by pollinating cultivated barley (*H. vulgare*) with its wild relative *H. bulbosum*—the antibody recognizes CENH3 from both parents. A fascinating observation about this cross is that elimination of the paternal *H. bulbosum* chromosomes is temperature-dependent. At low temperature, CENH3 was found at the predicted foci on all chromosomes, which segregated accurately. In embryos created by fertilization at higher temperature, chromosomes that lagged behind during mitosis and were eventually discarded showed a lack of CENH3 antibody staining. Fluorescence in situ hybridization with labeled genomic DNA (combined with a wealth of previous genetic data) indicates that lagging chromosomes without functional kinetochores were derived from the *H. bulbosum* parent.

Loss of CENH3 from *H. bulbosum* chromosomes neatly explains their failure to segregate faithfully during embryonic mitosis but also raises important questions about how centromere identity and consequently kinetochore location are preserved when chromosomes are copied. Elimination of the *H. bulbosum* genome in unstable hybrids is gradual, taking place over several days after pollination (4). When chromosomes are replicated during S phase of the cell cycle, histones including CENH3 are distributed between the two sister chromatids (5). These observations suggest that *H. bulbosum* chromosomes enter the zygote with a normal complement of CENH3, which is gradually depleted by several rounds of DNA replication until the kinetochore is no longer able to function. Therefore, reloading of CENH3 after DNA replication is specifically defective in *H. bulbosum*-derived chromosomes, although it is normal in chromosomes from the *H. vulgare* parent. What could cause CENH3 loading to fail in one of the two parental genomes? A possible clue is that *H. bulbosum*-derived chromosomes in hybrids appear less condensed than their *H. vulgare* counterparts. This might indicate that replication of *H. bulbosum* chromosomes is poorly synchronized with the hybrid cell cycle, a factor that could explain why CENH3 reloading does not occur. If asynchronous replication of parental chromosome sets is responsible for a CENH3 loading defect, it would indicate that the time window for replenishment is quite narrow (Fig. 1). Increasing the ploidy of the *H. bulbosum* parent can prevent genome elimination, further suggesting that subtle differences in cell cycle timing may affect whether CENH3 reloading is effective in hybrids (6). Cell cycle asynchrony between *H. vulgare* and *H. bulbosum* chromosome replication may be testable through kinetic analysis with cytological markers in the barley interspecies cross. A wild card in the interpretation of these results is the possibility that histones are reprogrammed on a large scale during reproductive development. A substantial fraction of *Arabidopsis thaliana* CENH3 is unloaded after fertilization and apparently resynthesized from the zygotic genome (7). Whether such a process takes place in the grasses is unknown, but zygote-specific CENH3 dynamics could have a major influence on the segregation of parental genomes in interspecies hybrids.

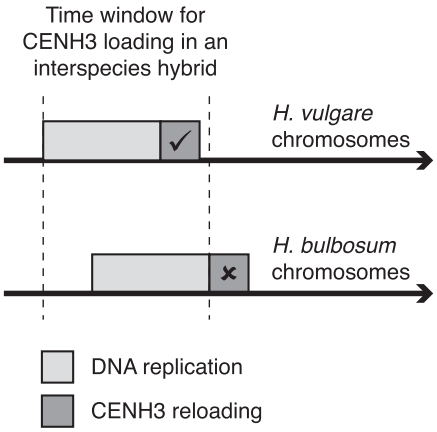

Fig 1. Cell cycle asynchrony could explain why *H. bulbosum* chromosomes fail to reload the centromere-specific histone CENH3 in an interspecies hybrid. In this model, DNA replication is a chromosome-intrinsic property, so *H. bulbosum* chromosomes proceed through S phase more slowly than *H. vulgare* chromosomes. This delay means that *H. bulbosum* chromosomes miss a narrow time window for CENH3 replenishment and show less condensation. The cell cycle might proceed more slowly at low temperature, possibly explaining why *H. vulgare* × *H. bulbosum* hybrids are stable when cultivated below 18 °C.

Cell cycle differences are not the only reason why centromere assembly could differ between *H. vulgare* and *H. bulbosum* chromosomes. However, any explanation must account for the fact that *H. bulbosum* chromosomes load CENH3 normally in selfed plants yet are defective when mixed with *H. vulgare* chromosomes (even this depends on the environmental factor of high temperature). An alternative hypothesis is that *H. vulgare* CENH3 cannot be loaded into *H. bulbosum* centromeres in unstable hybrids and has a dominant-negative effect on *H. bulbosum* CENH3 loading in this situation. The lack of evidence for obligate interactions between CENH3 and centromere DNA argues against this model; like conventional nucleosomes, CENH3 nucleosomes seem to package DNA with relatively little sequence specificity. First, Sanei et al. show that barley expresses two CENH3 variants, both of which can coexist at either *H. vulgare* centromeres or at *H. bulbosum*

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centromeres in stable low-temperature hybrids (3). Second, CENH3 from Chinese cabbage (*Brassica rapa*) or maize can be loaded into *A. thaliana* chromosomes in the presence of the wild-type protein, even though these three species have dissimilar centromere DNA sequences (8). Third, there are many examples of CENH3 loading into chromosome locations completely lacking the normal centromere DNA sequence (9, 10). Centromere sequence differences may still play a role in some genome elimination phenomena. Fusing somatic cells of mouse and tammar wallaby yielded a hybrid cell line that had lost almost all of the marsupial genome (11). Surviving fragments of tammar wallaby chromosomes were fused to mouse chromosomes and had replaced their centromere DNA with corresponding sequences from the mouse genome, possibly indicating that wallaby centromeres were deleterious to sustained inheritance when the two parental genomes were mixed.

The involvement of centromere maintenance defects in genome elimination should stimulate a search for histone chaperones and other factors required to replenish CENH3 in plants. This is complicated by the fact that the CENH3 loading machinery changes rapidly during evolution: the CAL1 chaperone of *Drosophila melanogaster*, KNL-2 of *Caenorhabditis elegans*, and the HJURP histone chaperone identified in human cells are not conserved between all sequenced animal genomes, let alone in plants (12–15). Improvements in barley genomic resources should allow fine genetic mapping of loci that control genome elimination in *H. vulgare* × *H. bulbosum* crosses. Preliminary mapping experiments already suggest that the *CENH3* genes themselves are not responsible for chromosome loss in hybrids (16). The efficiency of uniparental genome elimination in barley depends on the *H. bulbosum* cultivar, providing another source of potentially informative genetic variation.

The barley chromosome elimination mechanism may also be relevant to a provocative hypothesis for postzygotic speciation.

A broader question is whether the mechanism discovered in barley interspecies hybrids is responsible for other natural cases of uniparental genome elimination. *A. thaliana* experiments using artificial CENH3 variants have shown that parental centromere differences can cause specific loss of either the maternal or paternal genome (17). An important difference between these observations and those made in barley hybrids is that parental chromosomes in *Arabidopsis* experiments were genetically identical, differing only in the proteins incorporated into centromeres. It is likely that genome elimination in this case must be faster than the gradual process seen in barley, because loading of both versions of CENH3 into maternal and paternal chromosomes after DNA replication would rapidly abolish functional differences between the two sets. Therefore, there are two ways for centromeres to differ when divergent parental genomes are mixed in the fertilized zygote. Either CENH3 reloading into one genome can be defective (as shown by Sanei et al.), or functional differences between CENH3 alleles brought into the zygote could cause immediate missegregation during the first few mitotic divisions. The barley chromosome elimination mechanism may also be relevant to a provocative hypothesis for postzygotic speciation. Centromere DNAs and CENH3 protein sequences evolve very rapidly, and parental centromere differences have been proposed to cause chromosome segregation errors in mitosis or meiosis (18). This infidelity would reduce fitness or fertility when two different species are crossed. Uniparental genome elimination may therefore represent an extreme case of how centromere differences can affect genetic inheritance in interspecies hybrids.

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