on genomes that are very different from mouse.

Massively parallel sequencing technology has already revolutionized the way we study genomes, and the capacity and quality of sequencing data continue to improve at a rapid pace. Trapnell *et al.*[4] and Guttman *et al.*[5] have demonstrated the power of RNA-Seq combined with novel transcript discovery to greatly improve the annotation of an already well-studied genome and to add substantially to our understanding of transcriptional and post-transcriptional regulation. By making their software available, they provide powerful tools that will facilitate future RNA-Seq studies.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Haploidy with histones

Gregory P Copenhaver & Daphne Preuss

An engineered centromere-specific histone could enable homozygous diploid lines to be generated at high frequency, simplifying crop breeding.

Sexually reproducing plants carrying a set of chromosomes from each parent are the rule in nature, but, for crop breeders, haploid plants represent a more useful resource. Arising either spontaneously at very low frequencies or generated by protracted cross-breeding or tissue-culture methods, haploid plants allow fully homozygous lines to be screened for desirable traits in one generation. A recent study in *Nature* reports that haploid plants can now be rapidly produced through the introduction of a single genetic alteration. Ravi and Chan[1] show that perturbing a centromeric histone in the model plant *Arabidopsis thaliana* makes it possible to reliably create haploid plants and 'doubled haploid' progeny from those plants. If this approach can be translated to crop species, it would find immediate application in agricultural biotechnology, shortening crop breeding programs by years.

In most eukaryotic organisms, the movement of a chromosome during cell division is regulated by its centromere, which is bound by the centromeric histone H3 (CENH3), a variant of the more ubiquitous histone H3. After DNA replication, CENH3 is loaded onto the newly formed daughter strands, targeting epigenetic marks in the centromere region[2,3]. In a zygote, the centromeres of the maternal and paternal chromosomes are bound by CENH3 proteins from the maternal and paternal germ cells, respectively. Normally, these two sets of CENH3 help to move the maternal and paternal chromosomes with equal efficiency in the first few mitotic divisions that form the developing embryo. Ravi and Chan[1] show that altering CENH3 from one parent can induce targeted elimination of the chromosomes inherited from that parent (**Fig. 1**).

The authors modify CENH3 in two ways. In the first, green fluorescent protein (GFP) is fused to the N terminus of CENH3. In the second, the N-terminal tail of CENH3 is replaced with the corresponding domain from histone H3, and GFP is fused to the new tail (**Fig. 1a**). Both the H3 and CENH3 N-terminal tails are targets for multiple post-translational modifications and are thought to regulate chromatin structure. The modified CENH3s do retain some function, but their recognition of the chromosome segregation machinery is diminished. As a result, the only chromosomes in the zygote that are moved properly are those that harbor CENH3 from the wild-type parent (**Fig. 1b**).

As new histone synthesis takes place within a developing embryo, one would expect that DNA strands are loaded with a mixture of CENH3 proteins encoded by the maternal and paternal alleles. Consistent with this view, Ravi and Chan[1] find that the distinction between chromosomes having maternal or paternal CENH3 is lost after the first few divisions and the remaining divisions are able to proceed normally throughout development, resulting in a haploid plant. These haploids can produce diploid (doubled-haploid) progeny, presumably either through somatic chromosome doubling or rare non-reductional divisions during meiosis.

For nearly a century, crop breeders have recognized that haploid plants can be used to accelerate the development of new inbred lines[4]. In a typical program, genetically diverse parents are crossed to create hybrids (F_1), and populations of their offspring (F_2, F_3, F_4 and so on) are surveyed to identify desirable traits and to select individual plants for further propagation. After several generations, the traits under selection become fixed, and the inbred line is typically homozygous for chromosomal regions of interest. Incorporating doubled haploids into a breeding program has the advantage of saving considerable time by achieving homozygosity more quickly; however, this strategy requires that more lines be planted and screened in a single generation, allowing a sufficiently complete survey of genetic combinations.

Although haploids occur spontaneously in many crop species, they are extremely rare, often forming prezygotically from gametophyte cells that develop into a mature plant. Haploids can be formed at a higher (albeit still extremely low) frequency from 'inducer' lines, from gametophytes cultured *in vitro*, or from intra- or interspecies hybrids that undergo post-zygotic chromosome elimination. What is most exciting about the breeding approach described by Ravi and Chan[1] is the high frequency at which they recover haploid plants from a diploid parent (~1–10% of a normal seed set in *A. thaliana*). In addition, they show that the same scheme can be used to create diploid plants from tetraploids, which may be useful for breeding crops with complex ploidy, such as hexaploid wheat.

These results raise several questions about chromosome dynamics during cell division. What is the nature of the competition between centromeres bound to different CENH3s? The authors suggest that the modified CENH3s

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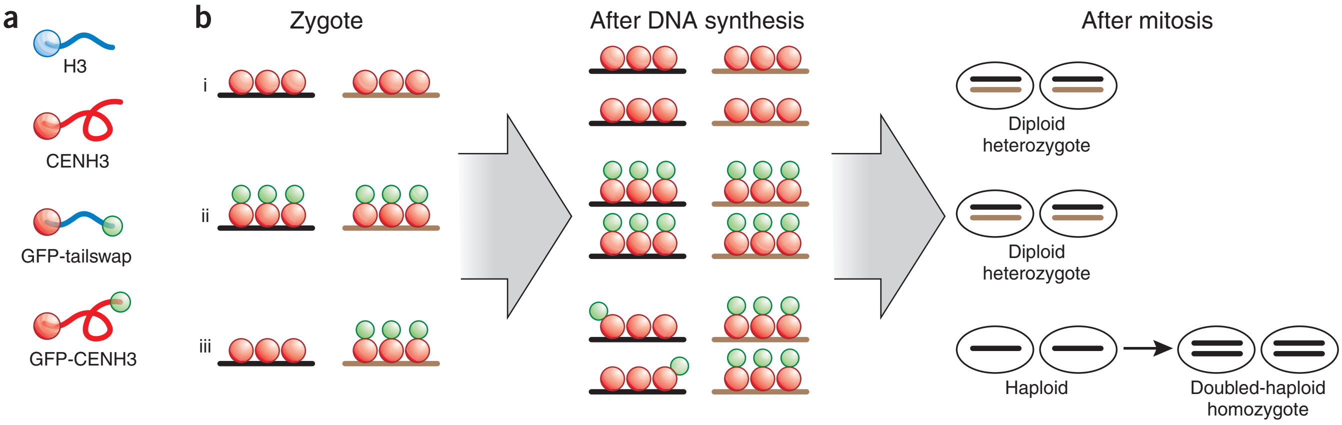

Figure 1 Manipulation of CENH3 structure perturbs chromosome segregation in plants. (**a**) Centromere-specific histone variants. Centromere-specific CENH3 differs from the ubiquitous histone H3 at its N-terminal tail. Ravi and Chan[1] modify CENH3 by fusing GFP to the N termini of CENH3 (GFP-CENH3) or of a CENH3 variant whose tail has been replaced by the tail of H3 (GFP-tailswap). (**b**) Inheritance patterns of chromosomes bearing normal and modified CENH3 in their centromeres. Self-pollination of plants bearing normal (i) or GFP-tagged CENH3 (ii) generates zygotes that replicate and transmit chromosomes normally. A cross between a plant with normal CENH3 and a plant with GFP-tagged CENH3 (iii) generates chromosome strands that remain primarily decorated with their respective parental CENH3 variants. Wild-type CENH3 has an advantage in promoting chromosome segregation to daughter cells, resulting in haploid plants that can be selfed to form doubled-haploid, homozygous progeny.

may slow the kinetics of interaction with cellular machinery, leading to the loss of chromosomes bound mostly by modified CENH3s. Other possible explanations include differences in the interactions with other centromere-binding proteins (as many as 19 have been identified) or in the physico-mechanical properties of histone-bound centromeres[5,6]. Is CENH3 the only component of the centromere whose variants can compete in this manner? For example, another centromere component, CENP-C, is functionally distinct from CENH3 but shares the quality of significant diversity at the amino acid level in phylogenetic analyses, suggesting that variants of CENP-C might also have different competitive efficiencies[7].

Ravi and Chan[1] have shown that a single genetic change can alter the efficiency of haploid induction in plants. Translating this technology to crops will require overcoming a few hurdles. First, appropriate CENH3 alleles must be identified—a null mutation in CENH3 will be required, and a stable line encoding a suitably altered form of CENH3 will have to be generated. Second, because most crop plants have more chromosomes (and often fewer seeds) than does *A. thaliana*, it is not clear how efficiently the set of chromosomes contributed by the CENH3 mutant parent will be eliminated. Despite these questions, the potential benefits for crop breeding coupled with the broad conservation of CENH3 across plant families clearly justify commercial investment in this approach.

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High-content imaging

Arnold Hayer & Tobias Meyer

Multiparametric imaging of siRNA screening data sheds light on endocytosis.

Gaining a systems-level understanding of complex cellular processes will require new analytic approaches that account for the effects of perturbations on a large number of functional parameters with high resolution and high throughput. A recent study by Collinet *et al.*[1] in *Nature* provides an instructive example of how this might be achieved. Focusing on endocytosis, the authors combine multiparametric imaging with a genome-wide RNA interference (RNAi) screen in HeLa cells to analyze many parameters of the endocytic system in unprecedented detail.

Endocytosis allows eukaryotic cells to remove signaling receptors from their surfaces and to take up extracellular molecules. Internalized cargo are shuttled through a maze of intracellular sorting and transport stations until they reach their destinations. Primary endocytic vesicles fuse with early endosomes, from where cargo is either recycled back to the plasma membrane or sorted into the endo-lysosomal pathway for degradation. Clathrin-mediated endocytosis is a major endocytic route used by transferrin, growth-factor receptors and pathogenic viruses during infectious entry. Although clathrin-dependent uptake is the best-studied endocytic pathway, a ystems-level understanding of the dynamic

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