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REVIEWS

Review

Haploid Induction and Genome Instability

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The advent of affordable, large-scale DNA sequencing methods, coupled with advanced computing power, is empowering a detailed analysis of the structure and function of chromosomes. Genomic instability, involving chromosome number and structure changes, has been documented in multiple systems. In plants, haploid induction through genome elimination has recently been connected mechanistically to the formation of complex chromosome reorganizations, known collectively as chromoanagenesis. These abnormalities can be triggered by altering the specialized centromeric histone 3, the epigenetic determinant of centromeres, which leads to loss of centromere function and chromosome missegregation. Other historical and recent instances of genomic instability, at the same time, suggest multiple causes. Their study provides a unique opportunity for a synthesis encompassing genome evolution, its response to stress, as well as the possibility of recruiting the connected mechanisms for genome engineering-based plant breeding.

Highlights

Advances in DNA sequencing and genome analysis enable both reinterpretation of historical data as well as discovery of plant genome instability in the field and in experimental systems.

Genome instability, which in animals is associated with cancer, can be triggered in plants by multiple causes, including crosses between parents with incompatible genomes.

Mechanisms leading to instability are common across plant and animal kingdoms, involving failure in chromosome partitioning between dividing cells, DNA breaks, and faulty repair.

Haploid induction, an important tool in plant breeding, can result from alteration of a chromatin protein that determines centromeres and promotes genome instability.

Plant tolerance to genomic imbalance and to aneuploidy may provide increased opportunity for evolutionary success of karyotypic novelty generated by genome instability.

The Fluid Genome

The plastic and potentially unstable nature of the genome was obvious to the Boveris, over 120 years ago [1]. Sequence analysis of the genomes of different species and of different individuals within the same species, is now revealing how chromosomes evolve and how genic and nongenic regions can be reorganized. Our understanding of processes that lead to genomic remodeling, however, is limited by the difficulty in filling large time gaps. To address this deficiency, we will need to identify the mechanisms that produce genomic changes, as well as the selective environment that drives successful rearrangements.

Discoveries in the past decade have provided mechanistic insights concerning genome instability. Large-scale and rapid episodes of genomic instability within a single chromosome can profoundly alter the phenotype, for example, leading to cancer in animals [2,3]. We now focus on analogous phenomena in plants and evaluate their impact on plant breeding and evolution. We address genome instability in the context of **haploid** (see Glossary) induction, also known as uniparental genome elimination [4], and compare this with other sources of instability of the nuclear genome. We consider evidence for genome elimination as a form of postzygotic incompatibility between parental genomes, we discuss the molecular mechanisms that underlie this incompatibility, and finally speculate on its evolutionary implications. Several reviews have covered the chromosome remodeling phenomena collectively known as **chromoanagenesis (CAG)** in animals [5–8]; we focus here on related phenomena in plants and connect historical findings to contemporary data.

We conclude that **missegregation** of chromosomes can lead to similar genome instability in animal and plants. In plants, however, specific features such as flexible ploidy types and meristematic development may confer different evolutionary potential to genome variants. Biotechnological exploitation could also be possible.

Genome Stress

Genomes are subjected to various types of stress, although a common and most severe result of stress is a double-stranded DNA (dsDNA) break [9]. The consequence of a broken chromosome end was a focus of McClintock's research [10–12]; highlighted in her Nobel address: she described how such an 'unexpected' event triggered activation of previously silent mobile elements, leading to local and large-scale disruption of the genome [13]. She interpreted this disruption as being both a 'programmed' and an 'accidental' or 'not so precisely programmed' response: faced with cataclysm, the organism improvises using available tools for genome remodeling. McClintock thought that hybridization between species was also a stressful event and provided examples of genome remodeling events in plant hybrids. Thirty-five years later, this insightful evaluation can be revised in light of our understanding of DNA repair, the redundancy of its mechanisms, and the largely

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disordered nature of eukaryotic genomes [14,15]. Our contemporary understanding of genomic and **epigenetic** diversity has led to models of genomic dysfunction syndromes that may be general among eukaryotes and could have medical implications.

dsDNA breaks can be induced directly, by physical and chemical mutagens, or indirectly, by problems in DNA replication, chromosome segregation, or by epigenetic remodeling that mobilizes transposons. Both failure of centromeric determination and unexpected recombination events can lead to missegregation of chromosomes. A persistent dsDNA break would be deleterious because it can generate an acentric fragment that cannot be transmitted efficiently to daughter cells. Furthermore, dsDNA breaks trigger DNA repair responses with potentially damaging outcomes [9]. Incomplete or low fidelity repair might be sufficient in a somatic, differentiated cell. However, in order to enable mitotic cell divisions, all chromosomes must be compatible with mitosis and proceed through mitotic checkpoints. Karyotypic novelty in this situation could be deleterious as even simple translocations could compromise meiosis and result in unbalanced meiotic products. In vertebrates, some unbalanced rearrangements can lead to cancer. Plants are more resilient to the deleterious effects of restructured chromosomes: clonal propagation is possible in many species and allows meiotically inviable rearrangements to survive. Polyploidy provides buffering such as shielding deficiencies or copy number changes in dosage-sensitive genes [16,17]. When restructured chromosomes are transmitted through the germline, a successful evolutionary event is possible, but unlikely. It may occur through chance fixation, selfish behavior [18], or, less likely, because the new chromosome represents an advantageous innovation [8]. Importantly, in genetics and evolution, rare does not mean unimportant.

Haploid Induction via Genome Elimination

Uniparental Genome Elimination Consistent with Interspecific Postzygotic Incompatibility

Plant crosses that result in uniparental haploids have been studied for many years because of the utility of haploids [19]. Two general mechanisms can produce haploids: parthenogenesis (a gamete develops without fertilization) and parent-specific genome elimination. The latter is relevant to genome instability. Missegregation of one parent's chromosomes in interspecific crosses was first observed within the genus *Nicotiana* in the 1920s [20]. Barbara McClintock was well aware of these findings: author Ar-Rushdi acknowledges her for 'valuable criticism'. The phenomenon was subsequently connected with uniparental chromosomal instability [21]. Uniparental genome inheritance has since been observed in embryos produced by other interspecific crosses, such as between barley spp. [22], wheat × maize, maize × oat, and others (reviewed by [4]). The syndrome entails postzygotic incompatibility: parents that reproduce normally when crossed to individuals of the same species display 'weak' genomes when crossed to more distant partners. The 'weak' parent chromosomes are missegregated, leaving behind haploid progeny that carry only chromosomes from the other parent. In some cases, the weak genome appears affected by centromeric deficiency, such as in barley interspecific crosses where the centromeric histone H3 variant, the principal determinant of centromeric chromatin, can be seen to vacate the presumed weak chromosomes [23]. Common forms of postzygotic incompatibility are consistent with the interaction of two factors following genetic rules and resulting in death or impaired growth [24]. In this case, however, the deleterious outcome affects a single parental genome, which must be either marked by its sequence or epigenetically, such as in centromeric chromatin. A recent hypothesis proposes that individuals with different **centromere** sizes could be incompatible [25]. Consistent with this, the size of centromeres varies and is determined for any given species [26]. For example, oat centromeres are larger than those of maize. Hybrids of oat and maize eliminate maize chromosomes. Occasionally, chromosomes of maize persist and in these cases their centromere is enlarged, conforming to those of oat [27]. Genome elimination, however, can also be observed between individuals of the same species, suggesting the involvement of additional mechanisms.

Genome Elimination Resulting from Defective Sperms

Haploid induction in maize does not appear to result from incompatibility of mating partners, but it may still be connected to genome instability. A recessive mutant of maize, stock 6, was identified that resulted in haploids when either selfed or crossed as a male [28]. Although it is clear that accessory loci

Glossary

Aneuploid: an organism or cell with an abnormal chromosome number that involves an incomplete set.
B chromosome: a supernumerary, often highly heterochromatic accessory chromosome encoding few or no traits, found occasionally in outcrossing plants and animals.
Centromere: a chromosomal region that becomes attached to spindle fibers during mitosis and meiosis, facilitating the movement of chromosomes to the metaphase plate and subsequently to the poles of the spindle apparatus.
Centromeric Histone 3: (CENH3 in plants, CENP-A in vertebrates, CID in fruit fly). A replacement histone 3 that epigenetically determines centromeric chromatin and promotes formation of the kinetochore.
Chromoanagenesis (CAG): encompasses the various mechanisms that result in highly rearranged chromosomes. We use the general chromoanagenesis term (remodeled chromosomes are thus 'chromoanagenic') to define restructuring processes affecting chromosomes, of which there are four classes: chromothripsis, kataegis, chromoplexy, and chromoanansynthesis [5,116,117].
Chromoanasynthesis (CS): replication-fork failure followed by unguided repair with amplifications in the form of duplications, triplications, or quadruplications of certain regions.
Chromoplexy: series of rearrangements involving multiple chromosomes.
Chromothripsis (CT): fragmented chromosomes joined up in random fashion.
Disomy: two copies of a given chromosome (normal diploid state).
Epigenetic: a genetic trait or property heritable through mitosis or meiosis and determined by DNA or chromatin modification without DNA sequence changes.
Haploid: an organism or cell containing a single set the chromosomes constituting the nuclear genome.

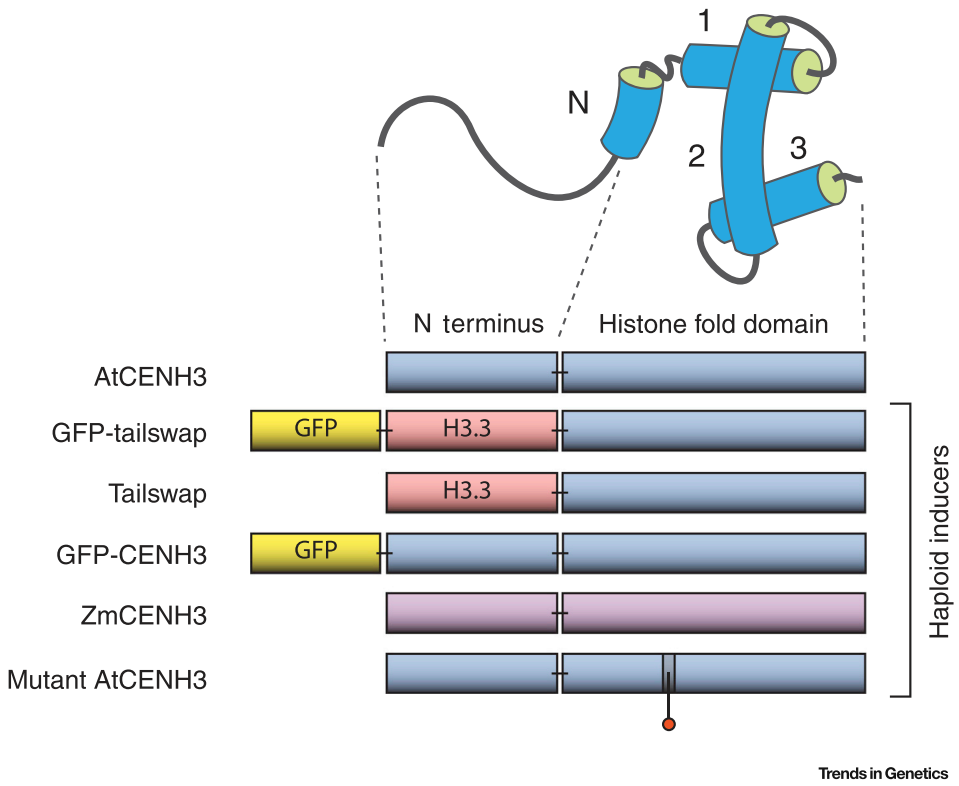

Figure 1. Structure of CENH3 and Its Derivatives.

Top. Structural analysis has revealed that the very rapidly evolving amino terminal region (N terminus) is disorganized. In the histone fold domain (HFD), four alpha-helices (green cylinders) form a globular domain, which is rapidly evolving but still displays conservation of many residues. Haploid inducers result from three types of CENH3 gene manipulations, all forming recessive alleles. The first type is fusion to another protein such as GFP or substitution of the N terminus (GFP-tailswap, Tailswap, and GFP-CENH3). The second type is replacement of the native CENH3 with a CENH3 from a different species (such as ZmCENH3). The third type is a missense mutation in conserved HFD residues (mutant AtCENH3).

can modify this trait, a mutation at a major locus was demonstrated. It affects a gene encoding a phospholipase, called MATRILINEAL (MTL) [28–32]. MTL is expressed in pollen, the gametophytic haploid tissue formed by two divisions of male meiotic products. Sperm nuclei in *mtl* pollen undergo genomic instability, as indicated by DNA fragmentation detected by single sperm sequencing [33]. These observations suggest that MTL is required for genome stability. In its absence, sperm genome is degraded and, although delivered to the zygote, it is subsequently eliminated, resembling the outcome of using irradiated sperms for fertilization [19,34]. Residual paternal chromosomes and DNA segments found in **aneuploids** [35,36] as well as paternally mediated CRISPR editing of resulting haploids [37], indicate that *mtl* pollen is competent for karyogamy, but delivers an unstable paternal genome. In mouse–human hybrid cells, elimination of human chromosome is facilitated by the persistence of DNA damage on human DNA [38]. The mechanistic basis of the *mtl* mutation awaits further clarification.

Genome Elimination from Defects in CENH3

Genetic studies of the centromere-specific histone H3 variant (CENH3) in *Arabidopsis* revealed that its alteration or substitution can lead to genome elimination when plants expressing them are crossed to the wild type. In the original study, the *cenh3-1* mutant, a splice-site point mutation that is presumed null because homozygous *cenh3-1/cenh3-1* mutant state is lethal, was complemented with a fusion product called GFP-Tailswap (Figures 1 and Figures 2) [39]. Further studies indicated that complementation of the null mutation with CENH3 from divergent species or with missense

Haploid inducer: a plant type that in specific crosses results in uniparental progeny lacking the haploid inducer genome.
Karyotype: a visual representation or image of the chromosomes in a cell or organism.
Kataegis: clusters of hypermutation within fragmented regions.
Meristem: small clusters of stem cells that form the plant body. The two embryonic meristem types, one in the shoot, the other in the root, are formed very early in embryo development and produce continued growth after germination.
Micronuclei: cellular bodies that have a defective nuclear membrane and that form around chromatin separated from the proper nucleus as a result of missegration and DNA damage.
Minichromosome: an abnormal, supernumerary chromosome that can be linear or circular. It is smaller than any in the original set and is formed by deletion of one or multiple segments in a regular chromosome.
Missegregation: failure of proper delivery of a chromosome to the designated spindle pole.
Neocentromere: newly formed centromere appearing at a locus where no centromere was visible in previous cell divisions.
Nonhomologous end joining (NHEJ): a DNA repair pathway that joins broken DNA ends regardless of their sequence. Two types are known: 'canonical' and 'noncanonical'.
Restitution: reunion of previously separated chromosomes by nuclear fusion. Also, reattachment of a broken fragment to the chromosome.
Trisomy: three copies of a given chromosome.
Truncation: loss of a terminal chromosome segment.

mutations affecting conserved residues of the histone fold domain also yielded **haploid inducers** [40–42]. The use of genotyping and genetic markers indicates the preferential elimination of chromosomes associated with hypomorphic centromeres in the early embryo [43,44]. Haploid production via CENH3 manipulation has been demonstrated in *Zea mays* [45], suggesting that this is a general phenomenon. The discovery of genome elimination in *Arabidopsis* provides a mechanistic model for centromere-mediated genome elimination in plants.

The efficacy of CENH3 manipulation in inducing missegregation in the embryo was also observed in *Drosophila*, where CID depletion (the CENH3 ortholog) in the male lineage leads to maternally haploid embryos due to failure of spindle fibers attachment to the sperm-contributed chromosomes [46]. In human cell lines, selective depletion of Y-chromosome CENP-A (the CENH3 ortholog) leads to Y-specific centromere inactivation and chromosome elimination [47]. Taken together, these results indicate that preferential missegration takes place as a form of incompatibility between mating partners, when one contributes chromosomes in which centromere specification is defective. Remarkably, CENH3 variants that function as haploid inducer in *Arabidopsis* exist in nature [40,42]. Together with the observation that *Arabidopsis* with altered or replaced CENH3 can self efficiently (or cross to individuals with the same genotype), this suggests that CENH3-based hypomorphic centromeres may exist in nature and may lead to incompatibility in outcrosses [40,41].

Role of CENH3 and Centromeric DNA Sequences in Chromosomal Stability

The monocentric, regional centromere was originally noted as the primary constriction of metaphase chromosomes [48]. Its mechanistic properties [10,49,50] emerged in the 1930s: the centromere is the DNA foundation of the kinetochore, which connects DNA to spindle fibers, resulting in faithful segregation of chromosomes and sister chromatids into the daughter cells produced by meiosis and mitosis. At the same time, centromeres have distinct genetic properties as they suppress recombination [51]. Following discovery of specialized kinetochore proteins (reviewed in [52,53]), it was determined that centromeres are defined by the deposition of nucleosomes containing the centromere-specific H3 variant, CENH3, a.k.a CENP-A [52,54]. The relationship between the bound DNA sequences and CENH3 appear paradoxical as both are rapidly evolving, contrary to the expectation for conserved mechanism [55]. Rapid evolution could be a response to centromere drive (i.e., selection for changes that suppress emerging selfish centromeres through alteration of kinetochore proteins) [56], or other unknown centromere functions.

What is the role of centromeric sequences in centromere function? Flexible formation of **neocentromeres** and other evidence ([53], reviewed in [57]) supports epigenetic determination rather than DNA sequence-dependence for centromeric identity. Nevertheless, the association of centromeric meiotic drive in mouse and monkeyflower with changes in centromeric repeats [58,59] seems at odd with the notion of DNA as a passive partner of CENH3 in determining centromere function. This conundrum awaits characterization of molecular mechanisms involved in each case. In a third case, in which meiotic drive is associated with maize knobs [60], interaction of the knob repeats with spindle fibers is based on a mechanism independent of normal centromeric function and requires a specialized class of kinesin motor proteins [61]. Genome elimination, however, is consistent with epigenetic determination of centromeres. First, in CENH3-based elimination, centromeres with identical sequences appear to be differentiated in the resulting embryos during elimination [39–42]. Second, the highly diverged maize CENH3 is capable of complementing the native *Arabidopsis* CENH3, even occupying the same centromeric repeat class [62], but it promotes genome elimination in outcrosses [41]. This clearly indicates that centromeres marked by different CENH3 can assume different epigenetic states with varying strengths. It remains to be determined if the apparent epigenetic weakness of CENH3 haploid inducers results from decreased size of the centromere [25] or from another property of the centromeric chromatin.

Plant CAG

Genome Instability in CENH3-Based Haploid Inducers

CENH3-based haploid-induction crosses produce multiple progeny types, the genetic origin of which can be determined with accuracy if the parents are polymorphic [63,64]. They consist of

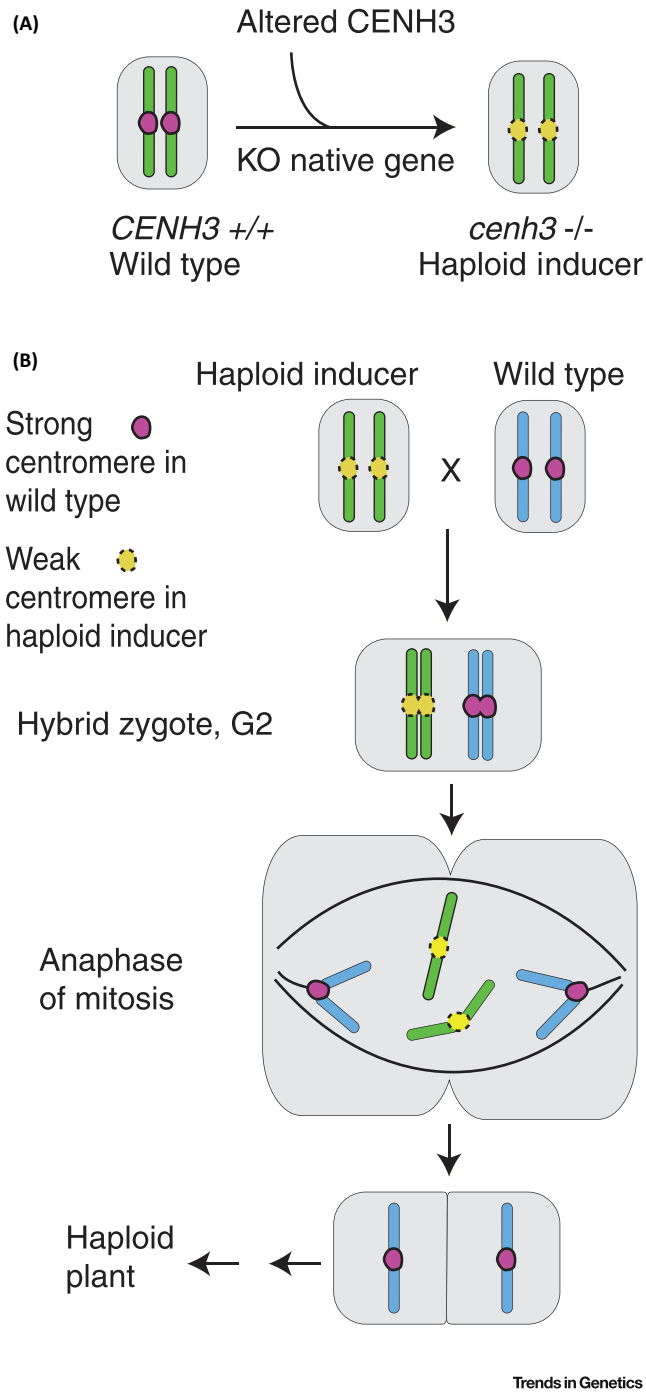

Figure 2. Engineering and Use of a Haploid Inducer through CENH3 Manipulation.
(A) A CENH3 allele determining haploid induction acts recessively. Engineering a plant to express altered CENH3 can be done in at least two ways. First, a CENH3 knockout (KO) is complemented with an altered CENH3. Second, a single mutation, such as one induced by TILLING alters CENH3, as demonstrated by Kuppu *et al.* [40]. (B) Haploid inducers can be selfed without generating instability or production of haploid progeny [39,44]. To produce haploids, they are crossed to a wild type.

individuals that died during embryonic development, biparental diploids, uniparental haploids, and aneuploids (Figure 3). Dead seeds are probably the result of extreme genomic unbalance, either in the embryo or in the nutritive endosperm tissue. Because of parental imprinting, both paternal and maternal contributions are needed for successful endosperm development [65]. Biparental diploids and uniparental haploids are the result of retention or loss, respectively, of the haploid inducer genome.

We observed that most haploid seeds display a variegated biparental endosperm, suggesting that loss of the haploid inducer genome in the endosperm is lethal [43] and potentially explaining seed death during genome elimination crosses. Aneuploids fit three karyotypic categories (Figure 3), based either on hybrid genomes or on haploid genomes. Aneuploids in the first category display whole chromosome changes (numerical), such as **trisomy** in a diploid background or **disomy** of chromosome 4 (disomy of other chromosomes was not observed) in a haploid background. Aneuploids in the second category have one or more chromosomes displaying a **truncation** or a simple deletion. Plants in the third category usually display a single chromothriptic (reshuffled) chromosome. In all cases, aneuploidy-determining chromosomes are derived from the haploid inducer genome.

The outcomes with reshuffled chromosomes fit well in our current understanding of **chromothripsis (CT)** observed in cancer cells, where missegregated chromosomes captured in **micronuclei** undergo catastrophic pulverization and reassembly [66]. At some frequency, the remodeled chromosome is returned to the nucleus proper [3]. The cytological events leading to the observed aneuploids are exemplified in Figure 4 and are postulated to resemble events in mammalian cells leading to CT [3].

Models involving missegregations of haploid inducer chromosomes and their **restitution** to form aneuploids suggest that multiple **karyotypes** are formed in the embryo, resulting in frequent chimerism (Figure 4). Their developmental separation raises the question whether the shoot versus root apical **meristems** will undergo differential rates and fates of genome elimination. Based on the genome instability model (Figure 4), one should expect chimerism in the embryo, particularly in root versus shoot.

Chimerism of meristem might also induce somatic competition, since the development potential of different cell types is likely to vary. Hybrid cells, for example, should be more competitive and proliferate faster than haploids because of heterosis. An (x+1) aneuploid would be more severely unbalanced than a (2x+1) aneuploid [67]. In haploid production studies, seedlings formed from few selected embryonic cells in the shoot meristem are sampled. The profile of plant types emerging from these crosses is thus likely to involve cell competition dynamics during early embryonic growth.

The majority of restructured chromosomes observed in the primary progeny of a haploid induction cross confer a more severe phenotype than regular trisomics and are not transmitted to sexual progeny [44]. The reason for this is unclear, since all primary trisomics of *Arabidopsis* transmit aneuploidy at considerable rates [68,69]. In some cases, the altered chromosomes carry triplicated regions, presumably causing higher imbalance. Cytological characterization of one such chromosome indicated that it does not pair during meiosis and perhaps this compromises its segregation. Last, CT junctions are common in and around genes, potentially creating gene fusions that may be deleterious [44].

Formation of Minichromosomes

Comparison of *Arabidopsis* haploids revealed that some (about 1%), while phenotypically not distinguishable from simple haploids, had an unexpected feature. In addition to a haploid genome, these haploids carried a duplication corresponding to the centromeric and some pericentromeric regions of a single chromosome [Figure 3(V)]. In all cases studied, the duplicated region originated from the haploid inducer genome [40,44] (Seymour *et al.*, unpublished results), suggesting that a fragment containing the centromere was retained, forming a **minichromosome**. Such a genetic element would carry fewer genes and would have minimal, if any, deleterious dosage effect. In summary, while most remodeled chromosomes could not be transmitted due to their deleterious effects, some could, suggesting that evolutionary competent karyotypic novelty could emerge. For example, the first step in B

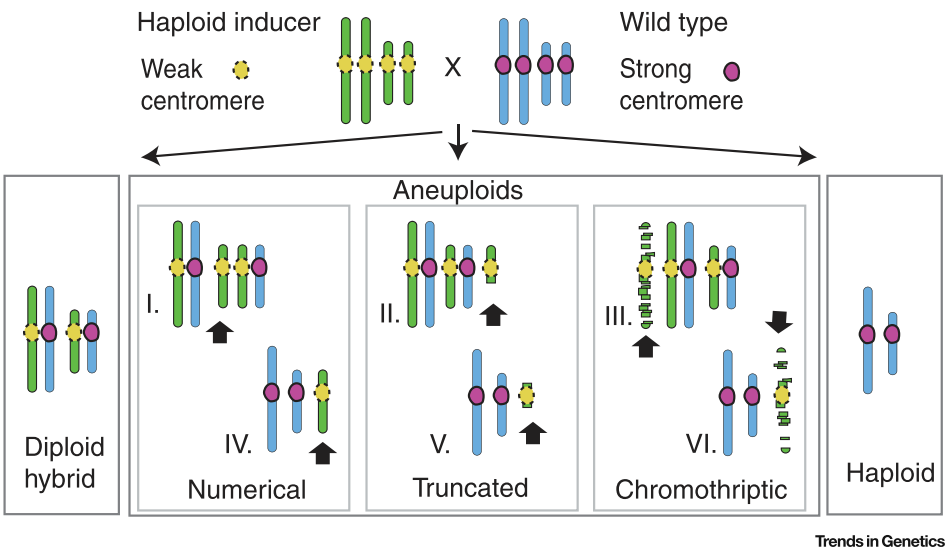

Figure 3. Types of Live Progeny Produced in a CENH3-Haploid Inducer Cross in *Arabidopsis*.
The chromosomal colors represent the genome of polymorphic parents. For simplicity, only two chromosomes pairs are used to represent the parental genome. Thick black arrows point to chromosomal irregularities in the aneuploids. Among aneuploids, I, II, and III are based on a hybrid genome (2x+1). IV, V, and VI are based on a haploid genome (x+1). Not all possible patterns are illustrated and different chromosomal numbers are possible, such as (2x+2) or (2x-1). Pattern V illustrates the presence of a putative minichromosome. In a set of characterized aneuploid seedlings, type III frequency was about $^{1}/_{3}$ [44].

chromosome formation could entail remodeling of a single chromosome during uniparental genome elimination [4].

Micronuclei Formation and Role in CAG

Observations of micronuclei in tomato, maize, *Fritillaria*, and lily date to nearly a century ago [50,70–72]. Plant geneticists recognized immediately that they formed around lagging chromosomes appearing during meiosis of triploids [70–72] and of polyploids [73]. They are also formed by treatments that damage the spindle [74] or DNA [75,76], or when genome elimination is induced by wide crosses in plants [23,77,78]. Micronuclei may represent an attempt to salvage missegregated or damaged chromosomes. Nevertheless, chromatin captured in micronuclei is often unstable: the structural, enzymatic, and metabolic insufficiency of micronuclei produced after irradiation of onion or faba bean roots was evidenced by their inability to replicate nucleic acids unless they included ribosomal RNA genes [79]. The defective state of micronuclei was confirmed by detailed studies in mammalian cells [80,81]. When micronuclei are formed during genome elimination in wheat × pearl millet crosses, they contain pearl millet chromatin, which is eliminated in the resulting hybrid embryos [78]. Consistent with the connection of micronuclei to damaged DNA, chromatin fragmentation was also evident. Crosses of cultivated barley to *Hordeum bulbosum* results in genome elimination. The resulting micronuclei, formed during elimination of *H. bulbosum* chromosomes, contain only chromatin of this species and are depleted of CENH3 and RNA polymerase II [23]. Micronuclei also appear in embryos formed by crosses between a wild type *Arabidopsis* and a plant with altered CENH3. The genome marked by the defective CENH3 undergoes elimination in the developing embryos [44]. It is thus likely that instability of missegregated chromatin in plants is also explained by failure of nuclear processes.

CAG in *Arabidopsis*

Sequencing aneuploids that were derived from centromere-mediated genome elimination crosses in *Arabidopsis* led to the observation of restructured chromosomes in ~10% of the aneuploid

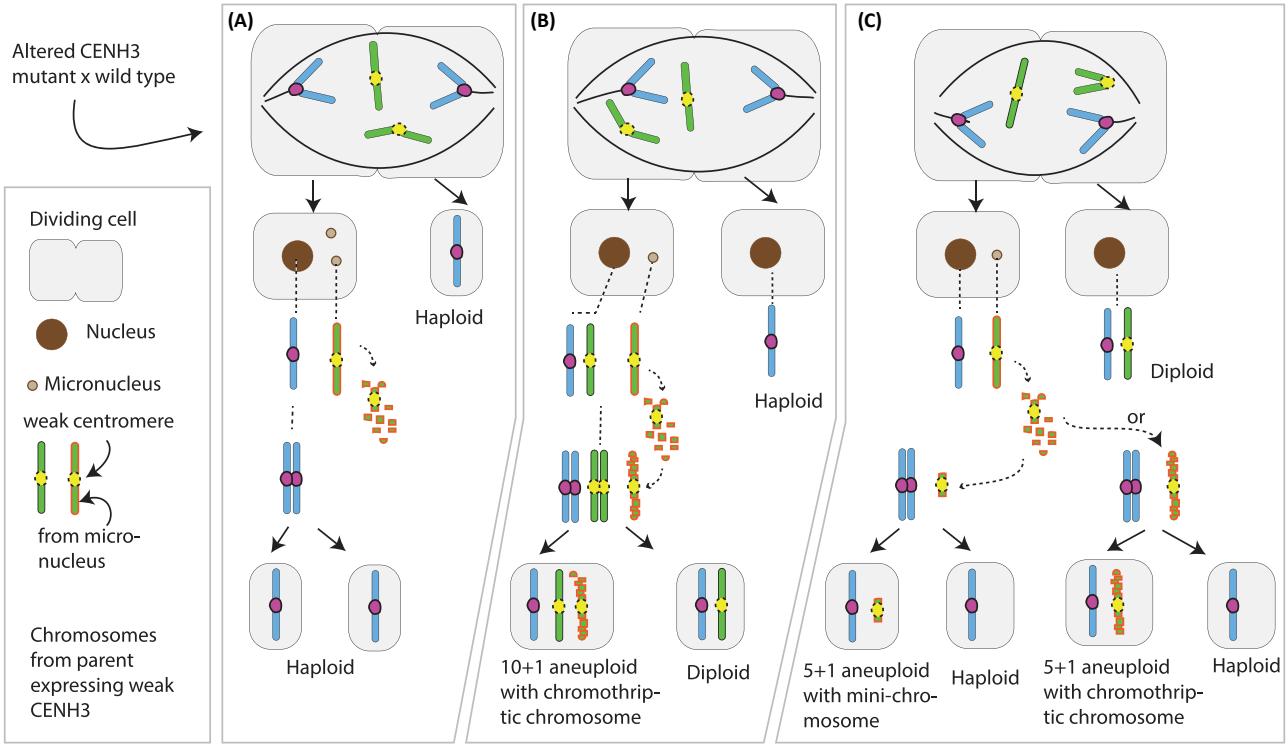

Figure 4. Formation of Different Karyotypes during Genome Elimination.
Following the cross on the top left (female parent listed first) genome instability results. A single chromosome pair is shown in an embryonic anaphase cell, with color signifying parent of origin. Instability of missegregated chromosomes (illustrated as shattered chromosomes within micronuclei) eventually leads to complete loss of chromosome fragments, or through chromoanagenesis and restitution, to aneuploids with rearranged chromosomes. (A) Haploid cell production. Chromosomes with weak centromeres are missegregated, bounded within micronuclei, and degraded. A uniparental complement is retained forming haploids. (B) Production of (2x+1) aneuploid cell. One missegregated chromosome incorporated into a micronucleus is degraded, reassembled, and restituted to a normal diploid nucleus. (C) Formation of (x+1) aneuploid cell. Same as B, but restitution takes place into haploid nucleus. Further, formation of a minichromosome from a centromeric fragment is shown. Not shown, is the formation of whole-chromosome numerical aneuploids. These may originate without involvement of micronuclei by accidental inclusion of missegregated chromosomes into the normal nucleus or to restitution of a micronucleus that has not undergone failure. Modified from [3].

population [41,44]. Interestingly, cytological evidence for related events has been documented in grass hybrids [82]. Typically, a single chromoanagenic chromosome was present. Rarely, severe aneuploids harbored two shattered chromosomes. Because the parents were polymorphic, it could be determined that all the aneuploid chromosomes, including the shattered, chromoanagenic ones, were derived from the haploid inducer genome [44]. Therefore, genomic instability leading to genome elimination is a uniparental phenomenon acting exclusively on the haploid inducer chromosomes, while the wild type chromosomes segregate faithfully (Figure 4).

Chromosome restructuring In *Arabidopsis* can fit the signature of CT or that of **chromoanasynthesis (CS)**. Consistent with the latter, triplicated regions were nested randomly within the duplicated copy of aneuploid chromosomes (Figure 5). These triplicated blocks were substantially smaller in size compared with the duplicated regions and are interspersed throughout the length of the chromosome. How were these additional copies derived? Breakpoints surrounding triplicated regions appeared to be more often associated with replication origins [83] than predicted by chance alone [44]. However, breakpoints surrounding duplicated regions appeared to be under-represented for replication origins than by chance alone. Thus, a replicative mechanism consistent with CS was the likely source of triplicated segments. During CT, when chromosomes are trapped in micronuclei,

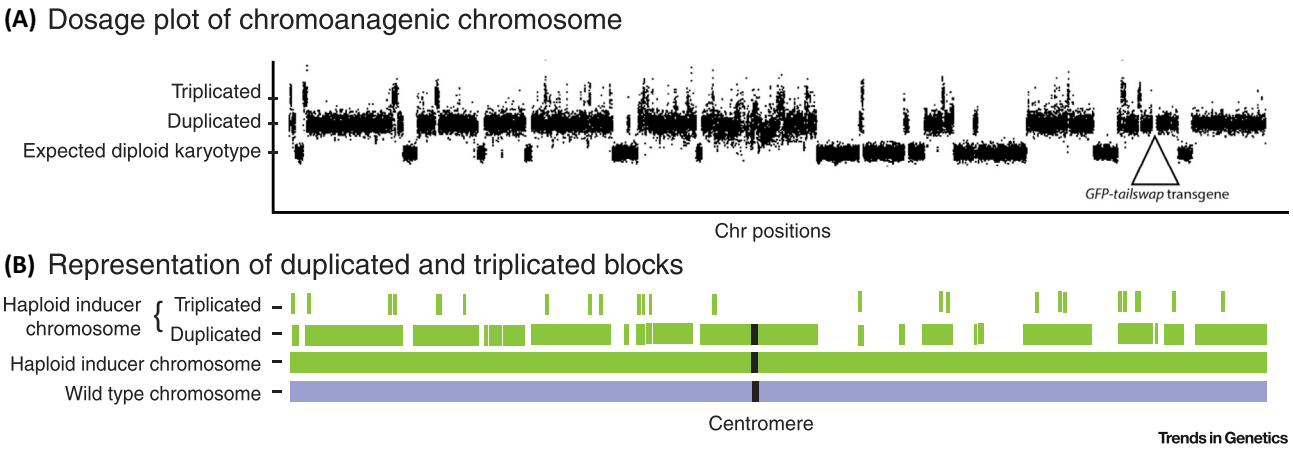

Figure 5. Genomic Characterization of an *Arabidopsis* Chromosome Showing Signatures of Chromoanagenesis.
(A) A high resolution dosage plot reveals triplication and duplication of certain chromosomal segments. The GFP-tailswap transgene insertion site in the haploid inducer located on the same chromoanagenic chromosome, is also depicted. (B) Representation of genomic content from the triplicated regions and duplicated regions derived from the haploid inducer chromosome based on bioinformatics analysis. The wild type chromosome remains intact. Duplicated and triplicated regions are not derived from the wild type chromosome.

easily accessible and open chromatin sites near genes should be much more likely to be damaged. Consistent with this, breakpoints surrounding duplicated regions were substantially enriched for gene-containing DNA sequences, while triplicated breakpoints were not [44].

The association of triplicated breakpoints with replication origins is consistent with abortive replication [84]. In animal cells, DNA replication timing malfunctions within micronuclei and is out of sync with the proper nucleus [66]. Rupture of defective nuclear envelope is a common feature for micronuclei [80,85]. Therefore, if a late-replicating micronucleus ruptures, replication fork collapse would cause DNA breaks that need to be repaired. In reconstructed breakpoints, microhomology-mediated repair sites were consistent with repair of exposed single-stranded DNA [44]. Coincident with triplicated regions being associated with replication origins and with microhomology-mediated repair, CS is likely to play a major role during the restitution phase of micronuclei [7,86]. In the scenario when a single haploid inducer chromosome is captured within a micronucleus, dsDNA breaks may initiate CT. This in turn could be followed by ectopic abortive replication, leading to fork failure and CS. Restitution of such a micronucleus could then lead to a newly reconstructed chromosome with hallmarks of both CT and CS. Although we have not detected signatures of **kataegis** or **chromoplexy**, the observation of multiple distinct signatures in genomic reconstruction of chromoanasynthetic chromosomes in *Arabidopsis* suggest that all the associated CAG processes could be at work as cells recover from catastrophic genome instability.

The majority of dsDNA breaks from reconstructed *Arabidopsis* chromosomes were repaired by the canonical **nonhomologous end joining (cNHEJ)** pathway [44], involving blunt end fusion, microhomology-mediated fusion, and fusion involving unrecognizable DNA insertions [87]. Impairment of *DNA Ligase IV* (*LIG4*), a conserved DNA ligase in the cNHEJ pathway, increased haploid induction frequency, consistent with a crucial role for cNHEJ [44]. Studies in animal cells also suggest a similar role for LIG4 that promote survivability of cells during periods of genomic instability [88].

Evolutionary Impact of Genome Instability

Is there an evolutionary consequence for the type of genome instability described here? CAG can result in the concurrent establishment of multiple genic and chromosomal changes engendering large phenotypic effects, consistent with the formation of Goldschmidt's 'hopeful monsters' [118], the large-impact mutants underlying macroevolutionary theory [89]. A key requirement for the

success of such genomic innovations is generational transmission. *Arabidopsis* chromoanagenic chromosomes can indeed be transmitted meiotically into the next generation without apparent recombination [44]. In human, meiotic transmission of chromothriptic chromosomes is possible for at least three generations [90]. A second requirement is that the changes increase fitness. This is not an easy condition to satisfy: most chromoanagenic modifications observed in *Arabidopsis* had negative impacts on the plant phenotype and fecundity [44]. However, consistent with the hopeful monster concept, evolution is a blind and patient watchmaker.

Genome Instability and Gene Amplification

Spontaneous chromosome fragmentation and potential remodeling was originally observed cytologically in plant hybrids [21,91], aneuploids [92], and measles virus-infected human cells [93]. Formation of very small chromosomal circles was documented upon DHFR gene amplification in methotrexate-treated cell cultures [94]. Shattered and randomly reassembled chromosomes have been described in animals [8] and are likely to contribute to the pathology of cancer and developmental diseases [2]. A dramatic demonstration of the potential of genome instability has been provided by the rise of weeds resistant to the herbicide glyphosate via amplification of the gene encoding the target enzyme. This was documented in tissue cultured cells treated with increasing concentration of the wide-spectrum herbicide glyphosate, which inhibits the shikimate biosynthesis enzyme enolpyruvyl phosphoshikimate synthase (EPSPS) [95–97]. Instability was thought to be peculiar to tissue culture, which is known to cause frequent aneuploidy and polyploidy [13]. Recently, chromosomal rearrangements were documented by sequencing in cultured and regenerated potato, confirming the destabilizing effect of tissue culture on chromosomal stability [98]. Tissue culture, however, may just be one of the stresses that triggers genomic remodeling. Remarkably, gene amplification has been found in weeds growing in or around crop fields treated with the herbicide. Gene amplification is responsible for acquired resistance to glyphosate in these weeds [99,100], through both translocations and extrachromosomal circle formation of DNA encoding EPSPS genes [101]. Collectively, these findings demonstrate that naturally occurring chromosome instability can foster extreme karyotypic changes and useful and selectable variation through multiplication of a gene under selection.

Genome Instability Resulting from Wide Crosses

A requirement for evolutionary significance is the relatively common occurrence of conditions that induce genome instability. Observations consistent with genome instability have been made while studying the progeny of multiple wide crosses involving different plant species, such as potato [102], *Arabidopsis* [103–106], and tobacco [21]. Aneuploidy in the form of residual haploid inducer chromosomes have been described from maize crosses to oat [107]. These cases were described by cytogenetic and genotyping techniques, such as restriction fragment length polymorphism and amplified fragment length polymorphism, which reveal the presence of haploid inducer DNA, but do not report changes in DNA size or structure.

Epigenetic remodeling can also be evident [108–110]. In oat–maize addition lines, maize centromeric regions expand to match the size of the predominant genome without any obvious instability [27]. In related addition lines of oat and pearl millet, however, mitotic abnormalities consistent with persistent cohesion of sister chromatids affect pearl millet chromosomes, resulting in non-disjunction and chromosomal breakage [77].

Another phenomenon promoting instability occurs in newly formed allopolyploids where intergenomic pairing and meiotic recombination (homoeologous) lead to aneuploidy and chromosomal breaks [106,111,112]. In summary, it is likely that high-resolution genomic analysis of dysgenic progeny from wide crosses will reveal cases where pervasive changes are comparable with those seen in *Arabidopsis*.

Concluding Remarks and Future Perspectives

A long history of studies in plant genomic instability have been recently augmented by dramatic demonstrations of genomic restructuring under both natural and artificial conditions: rearrangements

potentially consistent with CAG have been documented in many plant species and systems, such as in irradiated poplar [64], in a grape color variant [113], after biolistic transformation of rice and maize [114], after *Agrobacterium*-mediated transformation of *Arabidopsis* [115], and of potato [98]. Although the precise signatures of different chromoanagenetic processes must still be characterized in some of these systems, we predict that genomic restructuring will be encountered with increasing frequency due to the increased use and affordability of powerful sequencing technologies.

These analyses suggest considerable similarities in the way that plant and animal cells react to genomic upheaval. Tolerance to genomic imbalance and various degrees of ploidy may endow plants with increased resilience to genomic restructuring and increased capacity to exploit these macromutations. The study of haploid induction and genome elimination in plants should provide tools for improving plant breeding. In addition, it will enable further interrogation of the fundamental aspects of genomic plasticity in times of genomic crisis (see Outstanding Questions).

Outstanding Questions

What factors and pathways address genome instability and outcomes? Plants provide a tractable system to test hypotheses in ways that may not be possible in human and animal models.

How often does centromere failure or competition between centromeres with different epigenetic characteristics produce genome instability? Interspecific matings are common in nature among plants and may result in epigenetic conflicts. How often do these result in karyotypic novelty?

What determines the severity of chromosome rearrangements? These can vary from simple translocations and inversions, to the reassembly of pulverized chromosomes.

Are certain genotypes found in nature more prone to rearrangements and gene amplification? Could these be responsible for the appearance of weeds resistant to a common herbicide?

What features and mechanisms of genome instability can be recruited to facilitate the breeding of sustainable crop varieties?

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