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Causes of Mutation Rate Variability in Plant Genomes

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Abstract

Mutation is the source of all heritable diversity, the essential material of evolution and breeding. While mutation rates are often regarded as constant, variability in mutation rates has been observed at nearly every level—varying across mutation types, genome locations, gene functions, epigenomic contexts, environmental conditions, genotypes, and species. This mutation rate variation arises from differential rates of DNA damage, repair, and transposable element activation and insertion that together produce what is measured by DNA mutation rates. We review historical and recent investigations into the causes and consequences of mutation rate variability in plants by focusing on the mechanisms shaping this variation. Emerging mechanistic models point to the evolvability of mutation rate variation across genomes via mechanisms that target DNA repair, shaping the diversification of plants at phenotypic and genomic scales.

Contents

1. INTRODUCTION

Mutation occurs when DNA damage or error is unrepaired, generating the potential heritable variation that shapes evolution and provides fuel for breeding. The nature of mutation has intrigued generations of biologists, from Charles Darwin [who said, "I have hitherto sometimes spoken as if the variations...had been due to chance. This, of course, is a wholly incorrect expression, but it serves to acknowledge plainly our ignorance of the cause of each particular variation" (22, p. 131)] to Barbara McClintock. Research from the early twentieth century through today is replacing the black box of mutation with a mechanistic understanding of the forces and biological processes underlying mutation that "restructure the genome at various levels, from small changes involving a few nucleotides to gross modifications involving large segments of chromosomes, such as duplications, deficiencies, inversions, and other more complex reorganizations" (85, p. 800).

While the first genome-wide mutation rate in a plant species was measured in *Arabidopsis* in 2010 (97), a century of preceding research had already provided a preview of the remarkable variability in mutation rates now being uncovered in the age of genomics and molecular biology. In this review, we examine the progress made toward elucidating the proximate and ultimate causes of mutation rate variability in plants and consider the implications of emerging discoveries for understanding the origins and fate of plant diversity.

First, we review the various approaches that have been used to measure mutation rates in plants and how they provide the empirical basis for studying mutation rate variability. We then look more specifically at the variability that has been discovered—from differences in mutation rates between genome regions to the effect of environmental stress. Next, we take stock of the diverse sources of DNA damage and repair, yielding a conceptual model of mutation rate variability as a balance

Mutation: a change in the DNA sequence of an organism, which arises from DNA damage or an error in replication that is not accurately repaired

Mutation rate: the frequency of mutation, often expressed per generation, but can also describe the rate per cell division or per unit of time

between opposing biochemical forces that can be affected by targeting DNA repair machinery to specific regions of the genome. Finally, we consider the evolutionary relevance of mutation rate variability, reviewing how selection might act on DNA repair mechanisms, with potential consequences for plant adaptation. While we focus on plants, we draw from insights gained in other systems useful for understanding key processes and principles.

2. FROM TRAITS TO MOLECULES: A CENTURY OF MUTATION RATE RESEARCH

Mutation rate is a fundamental parameter in population, quantitative, and evolutionary genetics. Yet measuring mutation rates is notoriously difficult for several biological and technical reasons. First, rates of germinal mutation (i.e., a mutation with the potential to be passed to the next generation; see the end of Section 2 for a more thorough discussion of germinal versus somatic mutation in plants) are low—phenotypic mutation rates have been observed to be only as high as 10^{-4}, and the germinal per-base-pair mutation rate of single base-pair substitutions, for example, is between 10^{-11} and 10^{-8} for most species (**Supplemental Table 1**). Thus, large populations or numerous generations are required to enable researchers to observe these events. Second, mutations with a strong enough effect are often deleterious and would be rapidly removed because of reduced fitness, causing these mutations to be underrepresented or missed, meaning that it is difficult to measure mutation rates by analyzing population genetic diversity alone. Effective measurements of mutation rates require the absence of appreciable selection, motivating approaches that minimize the efficacy of selection influencing observed mutations. Here, we discuss various approaches that have been used to measure mutation rates, how they attempt to overcome these two challenges, and what they have revealed about the variability of mutation rates (**Figure 1**). In Section 3, we examine in greater detail the types of mutation rate variability that have already been discovered.

2.1. A Trait's-Eye View of Mutation Rate

The earliest studies of mutation rate measured the frequency of de novo phenotype generation at well-characterized loci controlling visible traits, a form of early high-throughput phenomics methodology. Geneticists pioneered this with loci such as the *R* gene in maize, which can mutate into the easily measured phenotypes of a colorless kernel aleurone (r^r) or colorless plant (R^g) alleles (122). Experiments based on measurements of phenotypic mutation rates in the early twentieth century led to the discovery of key principles of mutation rate variability that continue to be a focus of contemporary research:

1. Mutation rates increase as a consequence of radiation (120).
2. Mutation rates vary between environment and plant conditions (4, 15, 16).
3. Mutation rates vary between genes (111, 121).
4. Mutation rates of the same locus vary between genotypes (123).
5. Mutation rates can be affected by genetic modifiers (124, 125).
6. Mutation rates are affected by mobile elements (84).

These early studies of phenotypic mutation rates revealed that genes can vary in mutation rates by many orders of magnitude: from as high as 10^{-4} mutations per gamete per generation to 10^{-6} and lower (possibly approaching the lower bound, which is measurable by manually counting de novo phenotypes in single experiments). This variability led to the idea of stable and unstable genes having low and high mutation rates, respectively. These early phenotypically measured observations of variation in genic mutation rates anticipated modern studies of mutation at the molecular level, which can also vary considerably between loci.
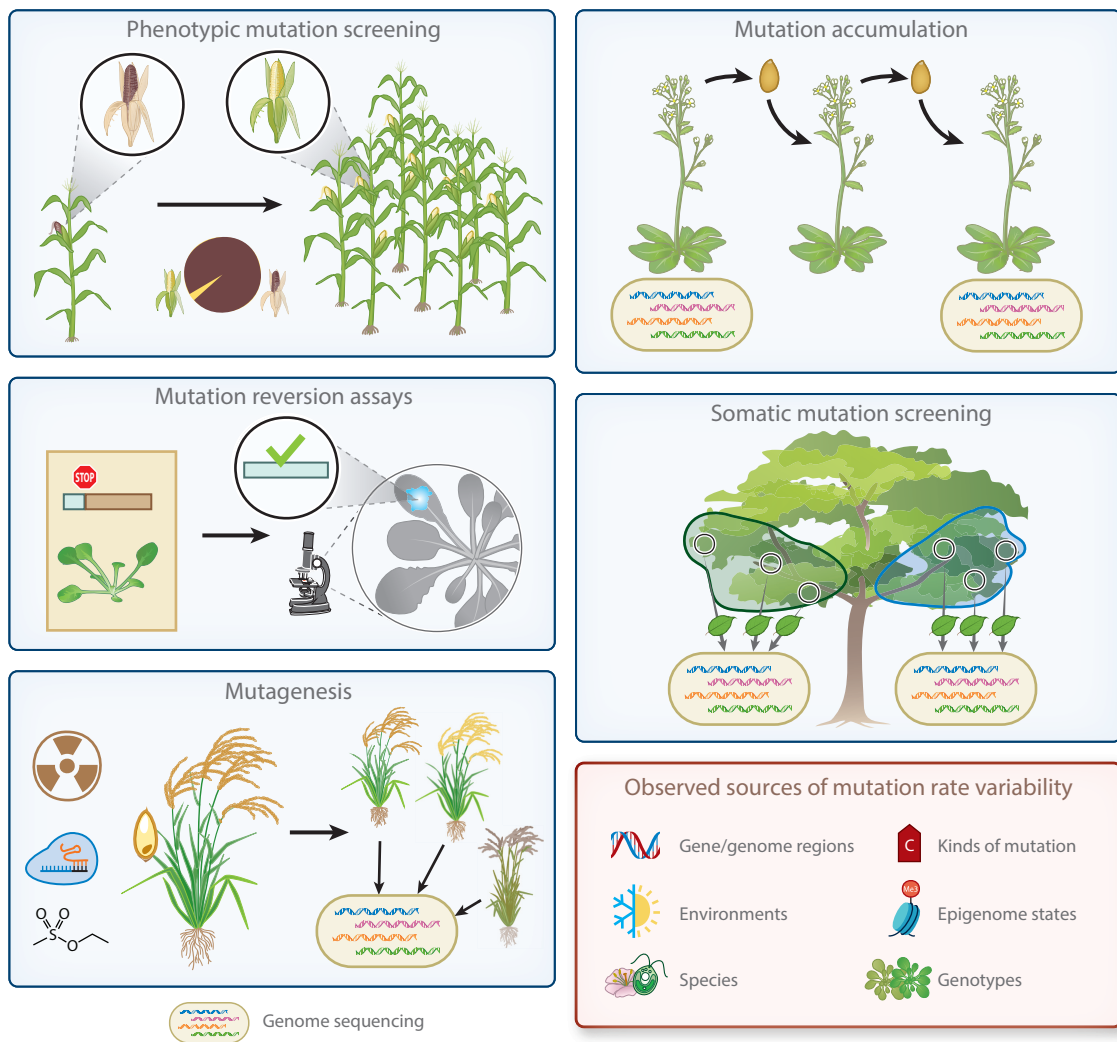
DNA repair: the process by which cells detect and correct errors or damage in the DNA sequence

Germinal mutation: also referred to as a germline mutation; a mutation that has the potential to be passed to the next generation

Somatic mutation: mutation arising in nonreproductive tissue

Supplemental Material >

Figure 1

Measuring mutation. Diverse methods have been explored in pursuit of measuring mutation rates. A century of research deploying these various approaches has led to fundamental discoveries of variability in mutation rates across different levels (e.g., genome regions and environments).

However, in addition to simple differences in molecular mutation rates between loci, another nonmutually exclusive explanation exists for genic variability in mutation rates between distinct traits. Some phenotypes may arise at a higher frequency due to more mutational paths (at the molecular level) that give rise to that particular phenotype. A classic example is phenotypes caused by loss-of-function versus gain-of-function alleles (86). If a phenotype is caused by loss of function, many different mutations could be responsible (e.g., any potential insertion or deletion in the coding region causing a frameshift). By contrast, when considering a phenotype at another locus, there may be only a few potential mutations (e.g., a specific single base-pair substitution causing a specific amino acid change). Synthesizing variability discovered using classic phenotype-based studies of mutation rate with modern molecular genomics reveals that to predict the rate of new

mutations at phenotypic scales, considering both loci-specific mutation rates and the functional molecular genetics of specific phenotypes is important.

While mutation rate research has shifted from measuring phenotypic mutations to measuring the sequence changes directly, the relevance of phenotypic mutation rates has not diminished. This is especially true when it comes to measuring the mutation rate of fitness as a trait to estimate the distribution of fitness effects of new mutations (28), which is of major importance for understanding the evolutionary relevance of de novo mutation.

2.2. Mutation Reversion Assays: Mutations in Broken Genes

A hybrid approach that blends the simplicity of phenotype-based mutation measurement with insights into mutation at precise molecular scales was obtained using mutation reversion assays. Here, a defective reporter gene is constructed, and specific mutations can rescue this defect, thus allowing the detection of specific de novo mutations. For example, by screening for blue patches in leaves of transgenic *Arabidopsis* lines containing a β-glucuronidase gene with a premature stop codon, one can infer that a mutation reverting to the original amino acid occurred at that stop site, restoring the function of β-glucuronidase and leading to blue fluorescence in cells with this mutation (56, 154). This approach has the benefit of using a neutral reporter gene that should have little to no selective consequence on the host but suffers by having a very narrow mutational target site, in this case, a single codon.

These experiments provided key insights that continue to have relevance to ongoing discoveries: Genes with lower rates of expression have higher mutation rates, and mutation rates vary between different single base-pair substitutions. They also revealed that somatic mutation rates can increase in developmentally mature tissue and under stressful environments (10, 34, 154). Mutation reversion assays suggest the potential for *Arabidopsis* to have an exceptionally high somatic mutation rate in differentiated tissues compared to other species (56). This is especially interesting in light of recent discoveries of the relationship between life span and somatic mutation rate, which suggest that short-lived plants such as *Arabidopsis*, especially in transient leaves and petals, may have particularly high mutation rates in differentiated cells (13, 96, 141).

2.3. Increasing Mutation Rate with Mutagenesis

Another way to address the limitations of mutation research is to utilize treatments to dramatically increase the mutation rate. For example, the use of radiation (e.g., X-rays) to increase mutation rates has origins in the early 1900s (120). Today, mutagenesis by radiation, chemicals [e.g., ethyl methanesulfonate (EMS)], and biological agents (e.g., CRISPR-Cas) continues to be widely used to introduce new genetic variation into breeding pools (94); to generate resources for functional genomics (70); and to scale up the study of DNA damage, repair, and other processes underlying mutation rate variability across plant genomes (109, 145, 151). The spectra (e.g., transition versus transversion ratio and trinucleotide context) of mutations generated by chemical and radiation mutagenesis can differ from spontaneous mutations. Still, the patterns of mutability observed across genomes can reveal variation in the activity of DNA repair and mutation rate that is similar to patterns observed in mutations arising in the absence of exogenous mutagens (67, 151). Studies of mutagenesis rates have found that mutation rates are predicted by epigenomic features such as lower CRISPR efficiency at loci marked by the histone modification H3K4me1 and elevated mutation rates at methylated cytosine, findings that are expanded upon in subsequent sections (109, 145, 151). Elucidating and leveraging the mechanisms responsible for variability in the rate of mutagenesis in plants could prove valuable for next-generation efforts to introduce novel adaptive genetic variation for accelerated crop improvement.

Histone modification: a chemical modification, such as methylation or acetylation, added to the histone proteins that package and organize DNA

2.4. Mutation Rates in the Molecular Age

The availability of rapid, affordable whole-genome DNA sequencing has ushered in an exciting era of mutation rate research. Today, germinal mutation rates are measured by whole-genome sequencing of individuals in mutation accumulation experiments, in which lineages accumulate mutations in experiments designed to minimize the effects of selection. A single seed is randomly propagated at each generation, inducing total genetic bottlenecks that reduce the effective population size of a lineage to one. This eliminates all but the most extreme selection against fully lethal mutations. De novo mutations can be identified by comparing the genome sequence at the end of one or many generations to the initial sequence (5, 6, 48, 77, 88, 97, 146). The total number of mutations measured by these experiments reflects the number of lineages multiplied by the number of generations and mutation rate. This approach has been used extensively in *Arabidopsis thaliana*, providing some of the first per-base-pair mutation estimates in plants, and continues to shed light on mutation rate variability.

Mutation accumulation experiments have also been widely used to study single-celled algae such as *Chlamydomonas reinhardtii*. While bottlenecks to an effective population size of one are not tenable at each generation (i.e., each cell division) in experiments involving single-celled organisms, imposing bottlenecks at high frequency, such as every 20 generations, appears to adequately remove the effect of selection, allowing even strongly deleterious mutations to accumulate. The ability to reduce selection proves to be important when interpreting findings such as the lower mutation rates in gene bodies observed in several alga species (59, 76), which is similar to what has been observed in *Arabidopsis* (**Supplemental Table 2**). From these studies we have learned, for example, that the frequency of single base-pair substitutions is tightly linked to local nucleotide contexts, small deletions occur much more frequently than small insertions, and germinal mutation rates are sensitive to environmental conditions.

To date, sequencing to study mutation rate has predominantly used short-read technologies. These generate sequence data for DNA molecules on the order of 100 to 150 base pairs and are well suited to identify single base-pair substitutions and short insertions and deletions but perform poorly at calling de novo structural variation such as large deletions or transpositions. However, with adequate sequencing depth, some large structural variants can be confidently called, as has been demonstrated in analyses of fast-neutron mutagenesis lines in rice (70). They have also been used to study transposition in *Arabidopsis*, which has revealed that transposable elements exhibit highly biased insertion sites (108, 113). Still, the mutation rates of structural variants, especially complex ones such as inversions, remain difficult to measure. The emergence of long-read sequencing technologies, such as circular consensus sequencing of single molecules up to tens of kilobases, may shed new light on mutation rates of complex structural variants and transposable element dynamics (66, 75).

2.5. Is It Heritable? Somatic Versus Germinal Mutation Rates

In animals, somatic mutations (e.g., in skin cells) are clearly distinct from mutations of the germinal cells (e.g., in sperm). Because they contribute to aging, cancer, and other diseases, variability in somatic mutation rates in mammals has been closely studied. However, the molecular rate of somatic versus germinal mutation in plants is less clear (**Figure 2**). In part, it can be difficult to define somatic mutation in plants, given the less discrete nature of plant germline segregation (63, 144). A mutation that arises in a meristem and is shared by an entire branch is often referred to as somatic, yet it can contribute to the germline because offspring can inherit that mutation from gametes derived from reproductive organs on that branch. And in plants that propagate clonally, mutations arising late in development in leaves, roots, and other differentiated tissue could
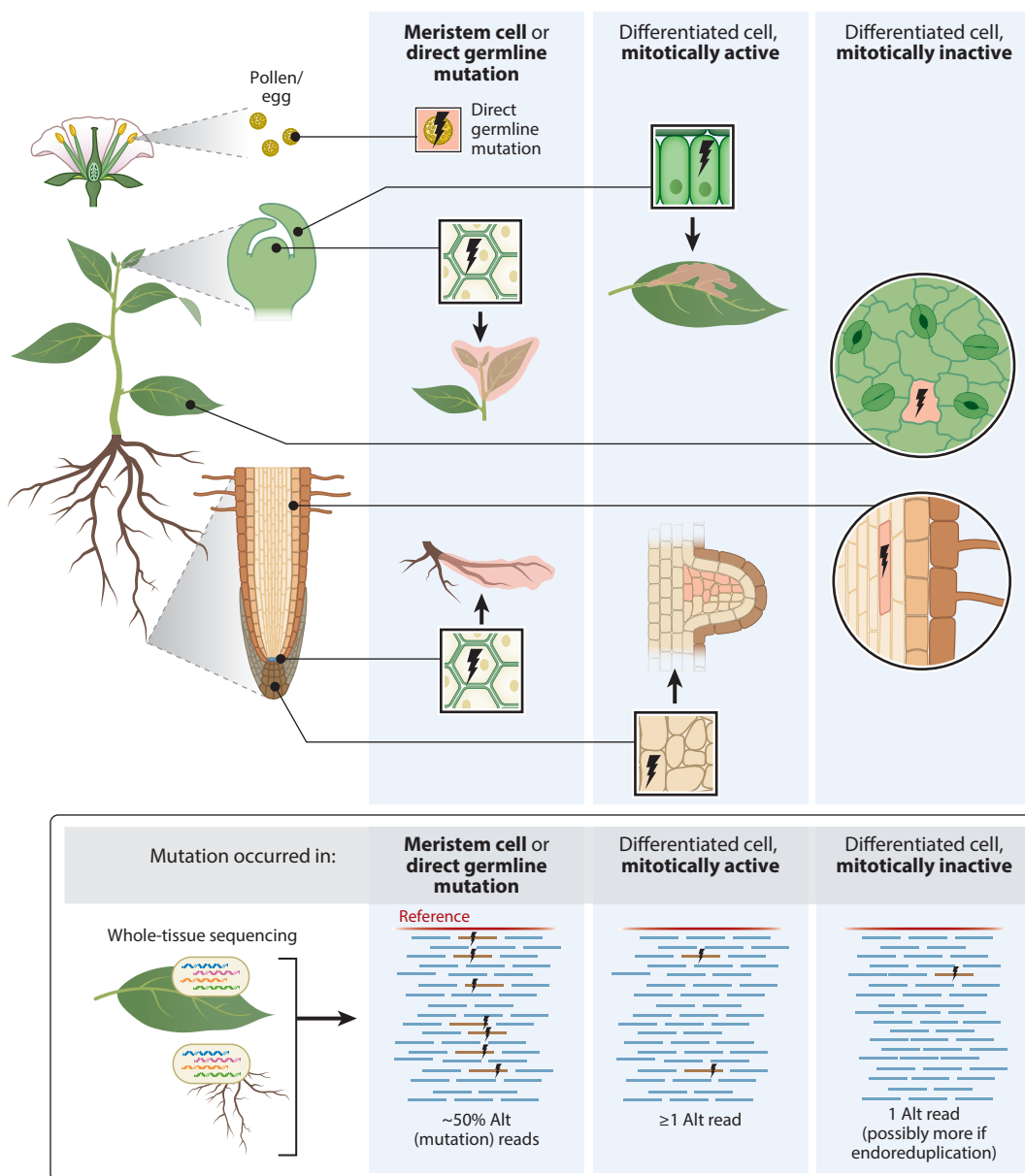
Figure 2

Mutation rate from the perspective of plant development. Mutations (shown as *black lightning bolts in individual cells* or *pink regions in tissues and organs*) can arise in cells during different stages of growth and development. Where and when a mutation occurs will determine how many cells inherit that mutation and whether it can be passed to the next generation. When detecting mutations within a single plant by sequencing, researchers expect that most mutations would be found in a single cell and thus supported by a single sequencing read, whereas mutations that arise earlier in development can be found in many cells with multiple reads supporting that new mutation.

contribute to future generations in some species (e.g., *Kalanchoe* and African violets, from which sexually reproducing plants can be propagated directly from leaves) (141).

Mutations of meristematic origin, shared by an entire branch, are said to be fixed. Measuring the rate of such mutations (e.g., by sequencing multiple tissues across a branch) in long-lived plants has proven valuable for calculating mutation rates in trees where multigenerational mutation accumulation experiments are currently untenable (38, 45, 96, 102, 117). These measures are useful because they yield estimated mutation rates relevant for plant evolution or breeding, as these fixed mutations are more likely to be transmitted to subsequent generations. They are also relatively easy to measure since they are observed at a high frequency (∼50% of reads) in sequencing data as a mutation that arose in the common ancestor of all leaf cells; all will have that mutation and be heterozygous. Such studies have led to surprising findings, most notably that the fixed somatic mutation rate is lower than anticipated. Estimated on a mutations-per-site-per-year basis, the annual rate of fixed single base-pair substitutions is on the order of 1e-10 mutations per site in trees (102, 117), which is more than an order of magnitude less than the per-year germinal mutation rate of *Arabidopsis*. However, when considering the total life span of many trees, the final mutation rate on a per-generation scale is higher (117). The low fixed somatic mutation rate in trees supports the argument that while plants may lack a completely segregated germline, some plants may maintain a population of cells in meristems that experience exceptionally infrequent cell divisions, have less DNA damage, have elevated DNA repair, or a combination of these (63). The case for elevated DNA repair in meristems is supported by evidence that DNA repair genes are highly expressed in meristems and that plant age is not strongly correlated to germinal mutation rate (21, 55, 115, 136, 138, 144).

The mutation rate of somatic tissues arising in cells that have already transitioned from the meristem toward differentiated tissues may be much higher but also considerably more difficult to measure because only a subset of sampled cells (as few as one) will contain each mutation (110, 118). The trade-off between sensitivity and precision challenges somatic mutation calling at this scale: Mutation rates may increase late in tissue development [given elevated DNA repair observed in meristems (21, 55, 115, 136, 138)], yet true positives may be more difficult to distinguish since late-occurring mutations will be shared by fewer cells and thus supported by fewer sequencing reads. Practical progress in studying somatic mutation at these spatiotemporal scales in plant development may arrive from single-cell or sequencing approaches using unique molecular identifiers to detect true positive singleton mutations, as have been effective in other systems (50).

3. MUTATION RATE VARIABILITY AT DIFFERENT SCALES

The previously described diverse approaches used to study plant mutation rates have given a view of the nonuniformity and variability of mutation rates. In this section, we examine mutation variability rate and how it differs across genomic scales and environments (**Figure 3**).

3.1. Variability Among Different Kinds of Mutations

Mutation rate varies between different types of single base-pair substitutions (97). Most notably, GC to AT are particularly common, resulting in a characteristic enrichment in transitions relative to transversions. This mutational bias is believed to play a key role in the evolution of GC content between species and within genomes (74) and potentially contributes to selection for codon usage biases (52, 100).

Just as in mutation spectra of other organisms (13, 89), mutation rates also vary with the trinucleotide context (i.e., the nucleotide of the mutated site along with the nucleotides of neighboring base pairs) of each site in plants (92, 95, 151). Studies in cancer and human somatic mutation have

established that the rate of mutations in relation to trinucleotide contexts reflects the combined activity of specific DNA damage and repair pathways (132). Applications of methods such as deconvolution of trinucleotide mutation spectra (155) in plants may prove valuable, especially in understanding how a plant's unique ecology can affect mutational processes and some forms of mutagenesis can leave distinct mutational signatures (7, 69, 150). For example, plants must

a Base substitutions, Indels, Structural variants

b SBS transitions, transversions, spectra and trinucleotide context

c SBS versus indel

d Interspecific variability

e Intragenomic

f Environment
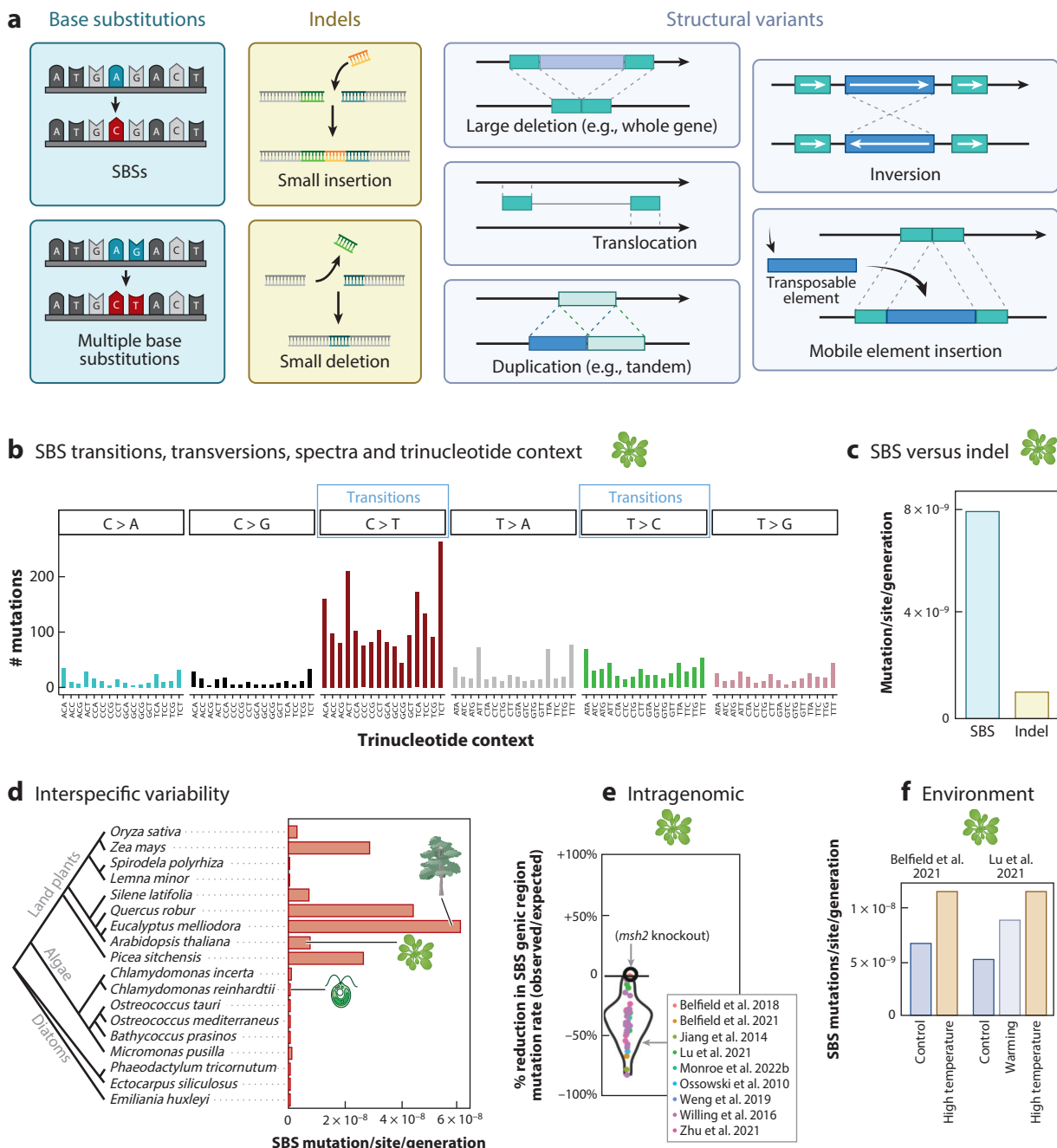
(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Examples of mutation rate variability. (*a*) Mutations constitute a diverse array of potential changes to DNA sequences, from SBSs to insertions of transposable elements. Not all mutations are shown here. For example, chromosome duplications (including the whole genome) can also be regarded as mutations. (*b*) The rate of single base-pair substitutions depends on the trinucleotide context at each site. Data are germinal mutations in *Arabidopsis* from References 88 and 146. (*c*) Single base-pair substitutions occur more frequently than small insertions and deletions (and deletions occur more frequently than insertions). Data compiled from *Arabidopsis* mutation accumulation experiments in **Supplemental Table 1**. (*d*) The mutation rate differs between species. Data in panels *c* and *d* from References 5, 6, 20, 38, 45, 48, 58–62, 75–77, 91, 92, 96, 97, 102, 114, 117, 146, 148, 152, 153, and 158 (see also **Supplemental Table 1**). (*e*) Mutation rates are systematically lower in *Arabidopsis* genic (transcribed) genomic regions. A notable exception—mismatch repair-deficient *msh2* knockout lines (6)—is highlighted. Data from References 5, 6, 48, 77, 88, 97, 146, 148, and 158 (see also **Supplemental Table 2**). (*f*) Mutation rates are higher under elevated temperatures in *Arabidopsis*. Data from References 5 and 77. Panels marked with leaf rosettes show data from *Arabidopsis thaliana* only, while panel *d* compares numerous species. Abbreviations: indel, insertion–deletion mutation; SBS, single base substitution.

constantly cope with ultraviolet (UV) radiation, which causes a characteristic spectra of mutations with particular trinucleotide sites being particularly prone to UV-induced damage (41, 148).

Single base-pair mutations occur more frequently than small insertions and deletions, with deletions being more common than insertions (see references in **Supplemental Table 1**). This may be relevant for understanding the evolutionary dynamics of genome size in the context of plants' cyclical evolutionary histories of whole-genome duplications followed by rediploidization (36, 107).

Pan-genome studies reveal that structural variation, including whole-gene presence-absence, constitutes a major proportion of functional genomic variation (42, 71, 157). These variations, and transposable elements, possibly play an outsized role in driving phenotypic evolution, given their potential to have large effects on gene function (3, 14, 18, 39, 85, 133). However, quantifying the rate of structural mutations, such as large deletions, insertions, and inversions, including the movement of transposable elements, is a notable frontier for research. New bioinformatics approaches and sequencing technologies are beginning to shed light on the rate and spectra of structural mutations. Transposition rates in *Arabidopsis* have been estimated to be as high as a third of that measured for single base-pair substitutions, and the rate of transposition varies between transposable element families (108, 113). Estimates of structural variant mutation rates in two genotypes of *C. reinhardtii* find between 5% and 20% of mutations are classified as structural mutations (75).

3.2. Lower Mutation Rates in Genes

Beyond variation at the individual base level, the mutation rate is also nonuniform at regional (i.e., kilobase to megabase) scales across plant genomes. The earliest studies of mutation rate in *Arabidopsis* mutation accumulation lines revealed that mutation rates are highest in pericentromeric regions and lower in gene bodies (i.e., transcribed regions) (97). These observations have been confirmed and refined in multiple subsequent investigations, with whole-genome mutation rate studies in *Arabidopsis* finding that mutation rates are systematically lower in genic (transcribed) regions (**Figure 3e**). This pattern has also been observed in some species of algae (59, 76), but not *C. reinhardtii*, suggesting interspecific variation in gene body hypomutation (75, 91, 92). To begin understanding potential mechanisms, mutation rate was studied in knockout lines of the mismatch repair (MMR) gene *MSH2*. These showed that *msh2* mutants do not have reduced gene body single base-pair mutation rates in *Arabidopsis* (6), suggesting that DNA repair genes can preferentially target and protect gene bodies. This phenomenon is consistent with known mechanisms of targeted repair in animals, though, as we discuss in Section 4, it may be mechanistically distinct and of independent evolutionary origin in plants (46, 51, 68, 130).

3.3. In Relation to Epigenome Features

The epigenome refers to nonpermanent chemical changes to DNA and chromatin, including DNA methylation, histone variants, and histone posttranslational modifications. Different epigenome states correspond to alternative levels of genome activity and transcriptional activation (72). As such, epigenome features are distributed nonrandomly across genomes, and surveys of mutation rate variation across plant genomes find that mutation rates and epigenomes covary (88, 97, 109, 145, 146, 151, 158). One of the most well-studied examples of epigenome-associated mutation rate variability is the observation that methylated cytosines are nearly twice as likely to mutate as nonmethylated cytosines, likely due to cytosine deamination, in which methylated cytosines mutate into uracil and ultimately thiamine (146). Because transposable elements tend to be enriched for methylated cytosines, this contributes to their elevated mutation rate (146).

Mutation rates also vary in relation to histone variants and modifications. A survey of CRISPR mutagenesis efficiency revealed that regions marked by H3K4me1, H3.3, and H3.1 experience significantly lower mutation rates (145). This is consistent with surveys of mutation rates in mutation accumulation in *Arabidopsis* and fast-neutron lines of rice, which have found that mutation rates are significantly lower in regions marked by H3K4me1. This histone modification marks gene bodies of active genes in plants (88, 98, 109). Similarly, in EMS mutagenesis lines of rice, mutation rates are significantly lower in genic regions marked by activating histone modifications (151).

Transposon insertion is also tightly correlated to epigenomic features. On one hand, ATCOPIA78/ONSEN retrotransposons have been found to preferentially insert in exons of genes enriched with H3K27me3 and H2A.Z, which mark transcriptionally silenced genes (108, 113). VANDAL21 transposable elements, on the other hand, preferentially target chromatin states associated with active promoters (108). Such discoveries are transforming our understanding of the role of mutation by transposition in plant evolution.

3.4. Between Environments

In addition to internal sources of variation, mutation rate can also be influenced by external stimuli from the environment. The effect of the environment on mutation has been observed since the earliest studies of mutation in plants (4, 15, 16). While not a steadfast rule, an emerging trend is that mutation rates increase under stressful conditions. For example, germinal mutation rates in mutation accumulation lines increase in heat-stressed *Arabidopsis* plants but not under colder conditions (5, 77). This is consistent with observations of elevated somatic mutation and microsatellite instability in heat-stressed mutation reversion assays (154). Similarly, *Arabidopsis* plants exposed to salt stress can experience an increase in mutation rate (48, 158). In addition to changes in the overall mutation rate, the environment can affect intragenomic variability in the mutation rate. In particular, mutation rates can increase in gene bodies under heat and salt stress (77, 158). Further, specific transposable element families can be activated by particular environmental conditions. For example, *ATCOPIA78/ONSEN* transposition is significantly increased under heat stress (101, 108, 134), and in tomatoes, *Rider* transposons are activated in response to drought stress (8). These recent findings complement a history of discovering environmentally responsive transposable elements, including those activated by biotic stress (35), cold (12), and viruses (49).

4. INFERRING THE MOLECULAR BASIS OF MUTATION RATE VARIABILITY

Where mutation is the raw material of evolution, DNA damage is the raw material of mutation. DNA repair, then, is the initial sieve that works to filter out this damage to prevent mutation. DNA repair is a massive undertaking, as a single cell might experience up to 100,000 instances
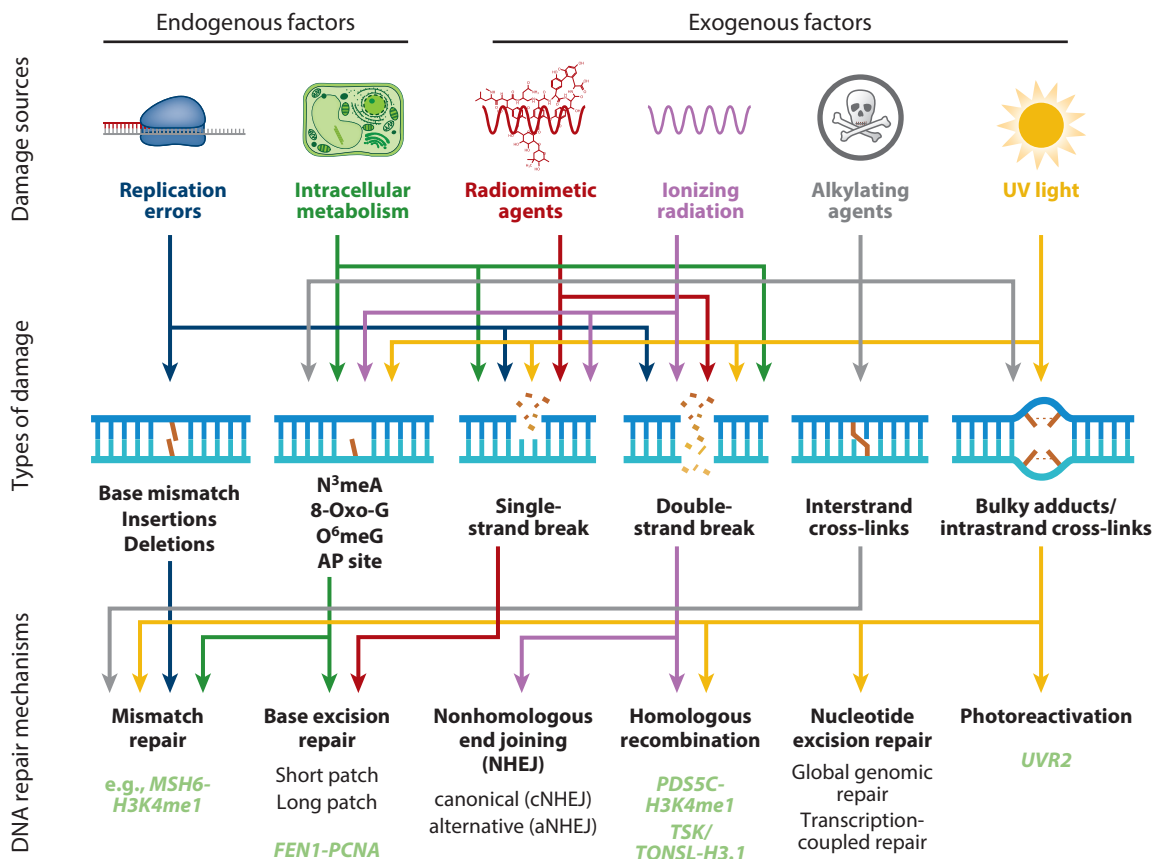
Figure 4

Diverse sources of DNA damage and the pathways responsible for repair. Genes shown in green text exhibit some evidence of targeting to specific genomic regions. Abbreviations: AP, abasic; FEN1, flap endonuclease 1; PCNA, proliferating cell nuclear antigen; UV, ultraviolet.

of DNA lesions daily due to endogenous and exogenous processes that damage DNA (73, 81, 156). These changes will become mutations if left unrepaired. But plants, like all organisms, have evolved an array of complex repair pathways to counterbalance damage and maintain the integrity of their genome sequence (140). Here, we address the internal and external mutagenic agents, their consequent lesions on the DNA, varying DNA repair mechanisms, and how chromatin states affect the propensity for DNA damage and the activity of DNA repair (**Figure 4**).

4.1. Types of DNA Damage and Their Sources

Mutagenic factors are sometimes distinguished as endogenous or exogenous factors, where endogenous refers to processes that arise directly from the outcome of biological processes occurring in cells. An important endogenous source of damage is DNA-associated processes like replication, which can lead to insertion, deletion, or mismatches of the DNA. These processes can also lead to single-strand breaks (SSBs) and double-strand breaks (DSBs) in plants (26). The error rate of DNA polymerase is not uniform across the genome. For example, polymerase slippage can occur in the presence of homopolymers, often leading to the expansion of homopolymeric sequences (139, 159). Other cellular processes such as metabolism can increase free radical concentrations,

damaging DNA. Damage from free radicals is particularly high at alkylated, deaminated, and oxidized bases, contributing to damage in methylated cytosines. And because cytosine deamination is limited by the in vivo rate of DNA denaturation, it is sensitive to GC content, with regions of high GC content being less likely to experience damage (29, 30).

The most common exogenous factor affecting plant mutation rate is UV radiation (11). UV-induced lesions include cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts (PPs). The relative proportion of CPDs and (6-4) PPs has a nonrandom distribution in the genome, depending on the sequence composition and chromatin structure (65, 135), potentially contributing to the relationship between trinucleotide context and mutation rate. UV radiation may also lead to oxidative DNA damage in plants (143).

Alkylating agents, such as methyl methanesulfonate (MMS) and EMS, are widely used as mutagenic chemical agents in plants (e.g., for mutagenesis breeding). These agents cause methylation of the DNA bases, intrastrand and interstrand cross-links, or bulky adducts (24, 119). EMS-induced single-nucleotide polymorphisms (SNPs) are negatively correlated with gene expression and active epigenetic markers, suggesting that chromatin structure might reflect the sensitivity of DNA EMS-induced damage, but this may also reflect differential repair (151). Ionizing radiation (IR) is another agent with mutagenic potential in plants. Common ionizing agents used for this purpose are X-rays, gamma rays, and ion beams (137). Similarly, fast neutron mutagenesis is also a mutagenic agent that has been shown to cause a higher number of large deletions and has therefore been used as a tool for functional genomics in rice and other crops (70, 150). IR produces various lesions, directly ionizing DNA molecules and generating DSBs and possibly DNA fragmentation (116). Indirectly, it can cause oxidative damage and SSBs by water radiolysis and the production of highly reactive species (1, 32, 54). Evidence suggests that chromatin organization influences the distribution of IR-induced DSBs, where DSBs would be enhanced by chromatin relaxation, and that large-scale domains surrounding active genes and replicating DNA are more sensitive to DSBs induced by IR (64).

Lastly, radiomimetic agents are exogenous mutagenic chemicals that mimic radiation and can induce various lesions. Radiomimetic agents like bleomycins, a family of antibiotic glycopeptides used as mutagens in plants, can cause DSBs, SSBs, and oxidative damage as IR (31). Linker DNA of nucleosomes shows higher breakage rates in bleomycin-induced mutagenesis in barley (80).

We focus here on DNA damage in terms of replication error, physical damage, and chemical changes. Transposable elements are a special form of damage—a transposable element that jumps or replicates and inserts into a genome region. See Section 3 for a review of how the epigenome governs transposable elements' activity and insertion rate, with different families of transposons interacting with different histone states and preferentially inserting near transcription start sites or silenced genes (108, 113).

4.2. DNA Repair Mechanisms and Their Distributions Across the Genome

Several DNA repair pathways have evolved to correct errors and DNA damage. These pathways have been widely characterized in plants, and there is accumulating evidence that these mechanisms are more efficient in some regions of the genome. Here, we review central repair mechanisms: photoreactivation, base excision repair (BER), and nucleotide excision repair (NER) for damaged base repair; MMR to correct misincorporated nucleotides; and nonhomologous end joining (NHEJ) and homologous recombination (HR) for DSB repair, and we focus on the observed variability in their activity across plant genomes.

4.2.1. Photoreactivation.
Photoreactivation is an error-free DNA repair mechanism acting on UV-induced lesions. Damage from CPDs and (6-4) PPs can be repaired by class II CPD

photolyase and (6-4) photolyase, respectively. The interaction between the pyrimidine dimers and the photolyase binding pocket allows the lesions to be flipped out from the DNA. The environment plays an important role in the modulation of the photoreactivation DNA repair pathway because of its light-dependent nature, and spontaneous variants of CPD photolyases can be associated with UV-tolerance phenotypes in plants (43).

Besides environmental and genetic regulation of photoreactivation, chromatin states directly impact photolyase repair, with some photolyases preferentially targeting open chromatin, promoters, and the nontranscribed strand of transcriptionally active genes. As a result, at least in yeast, the nontranscribed strand in active genes is repaired faster by photolyases, while the transcribed strand is preferentially repaired by the NER pathway (131). A relation between the epigenome and photoreactivation has also been demonstrated in maize. Histones 3 and 4 posttranslational modifications are involved in UV-B tolerance, with chromatin remodeling being a key process in acclimatization to UV-B (17). In *Arabidopsis*, knockout lines of the photolyase UVR2 exhibit an increase in the relative mutation rate of coding regions, in the presence and absence of high UV-B stress, with further work needed to determine the potential role of epigenomic features in this pattern (148).

4.2.2. Base excision repair. The BER pathway repairs oxidation, alkylation, and deamination on the DNA nitrogenous bases (112). The first step of this pathway consists of recognizing and cleaving the *N*-glycosidic bond by glycosylases, resulting in an abasic (AP) site. AP sites can also be generated spontaneously. After the AP site is generated, there are two possible paths for BER: the short patch, where AP lyase treats the AP site, integrating one nucleotide to the end, and the long patch, where AP endonuclease treats the AP site, integrating multiple nucleotides at the end and requiring the processing of flap endonuclease 1 (FEN1) before ligation and the δ/ε–proliferating cell nuclear antigen (PCNA)-FEN1 complex is formed. Both patches leave blocked ends that are further processed to facilitate DNA polymerase and DNA ligase activity. Expression levels of FEN1 and polymerase λ (Pol λ) are lower in mature leaves in rice, resulting in different mutation rates through development and between tissues (55, 136). PCNA interacts with FEN1 in plants (57) and is a known central coordinator of several functions in eukaryotes. FEN1 further recruits additional BER proteins to replication forks (25), potentially contributing to variation in BER across the genome.

4.2.3. Nucleotide excision repair. The NER mechanism removes various structural lesions in DNA, mainly UV-induced photoproducts and other bulky DNA adducts. NER constitutes two main pathways: global genomic repair (GGR), which is a whole-genome repair pathway, and transcription-coupled repair (TCR), which specifically accelerates the removal of transcription-blocking lesions from the template DNA strand of expressed genes (37). While the TCR pathway provides a clear connection between elevated repair and active genes (99), the GGR pathway can also preferentially target active genes, with evidence that the underlying mechanism is mediated by epigenome scanning in NER proteins (2). MED17 is reported to contribute to the recruitment of multiple NER proteins (27, 53) and, in *Arabidopsis*, interacts with PDS5C, a protein that specifically binds active gene bodies via H3K4me1 targeted by its Tudor domain (27, 33, 93, 104). Also in *Arabidopsis*, MED14 was shown to be required for heterochromatin transcription during heat stress, together with UVH6 forming part of the IIH complex that participates in TCR and NER (9).

4.2.4. Mismatch repair. MMR corrects mismatches or nucleotide insertions and deletions occurring during DNA replication. Besides that, MMR has been shown to participate in interstrand cross-links, oxidative DNA damage, and UV photoproducts. MMR proteins form part of the MSH

and MLH gene families. In plants, 7 MSH proteins have been described, functioning as dimeric complexes such as MSH2*MSH6 (MutSα). In plants, MSH1 is responsible for maintaining low mutation rates in mitochondrial and plastid genomes (149). And MSH2 preferentially repairs gene bodies in *Arabidopsis*. Knockout lines of *msh2* do not show the reduced gene body mutation observed in all other mutation accumulation experiments (6). MSH6 from rice and *Arabidopsis* shows evidence of targeting the H3K4me1 mark, which is enriched in active gene bodies, through a Tudor reader domain that has been observed in multiple DNA repair genes (93, 109). H3K4me1 recognition would explain how MSH2 (as part of MutSα) could be anchored to active gene bodies (6). This finding in plants is functionally analogous to the well-studied MSH6 targeting in humans, which also leads to genic hypomutation, but rather than H3K4me1 being targeted via the Tudor domain as appears to be the case in plants, in animals, gene body–targeted MMR instead involves H3K36me3 targeting via a PWWP domain of vertebrate MSH6 (68).

4.2.5. Repair of double-strand breaks.
DSBs arguably represent the most harmful class of DNA damage: Unrepaired DSBs often result in cell death. Several repair pathways have evolved to repair DSBs. The two general classes of DSB repair mechanisms are homology-directed repair, which involves HR, and NHEJ.

4.2.5.1. Nonhomologous end joining.
NHEJ is regarded as an error-prone mechanism of DSB repair and may be more error-prone in plants than in other organisms. Species-specific NHEJ has been reported, with an inverse correlation between the size of deletion and genome size, suggesting that NHEJ dynamics could contribute to the evolution of genome size (105).

In plants, there are two main NHEJ pathways, the canonical (cNHEJ) and the alternative (aNHEJ). After a DSB is produced, in cNHEJ, the two double-stranded ends are protected from degradation by binding a Ku heterodimer. After this, phospho and hydroxyl groups at 5′ and 3′ DNA ends are corrected by several proteins, and, finally, ligase 4 is targeted to the break site and religates the ends with various proteins mediating these processes (XRCC1, XLF, and PARP3). aNHEJ usually leads to deletions at the junction sites and is therefore highly mutagenic (106). PARP1 and XRCC1 proteins participating in the cNHEJ final ligation step are conserved in this process (47). Given the mutagenic nature of NHEJ, it often occurs only in the absence of homologous DNA, with homology-directed repair being considerably less error prone.

4.2.5.2. Homology-directed repair.
The most common mechanism of homology-directed repair is HR, which uses the sister chromosome as a template to repair a DSB. It is considered the most effective DSB repair, being essentially error free (129). However, a variant on homologous DNA can be integrated into the sequence being repaired, a possibility being explored for use with the CRISPR-Cas system to induce specific target mutations (142).

Several mechanisms involved in HR have been shown to interact with the epigenome, demonstrating the potential for targeting of HR proteins to active and essential genes. For example, PDS5 proteins are cohesion cofactors that contribute to HR (104), with cohesion being central in HR across eukaryotes as it contributes to sister chromosome interactions (90, 103). In *Arabidopsis*, each of five PDS5 (PDS5A–PDS5E) copies contains a Tudor domain, a histone reader that recognizes methylated lysine on histone tails. The Tudor domain of PDS5C was experimentally demonstrated to specifically bind H3K4me1, and analyses of PDS5C by chromatin immunoprecipitation sequencing (ChIP-seq) confirm that it preferentially binds H3K4me1-enriched regions: active gene bodies and essential genes (93, 109). These findings may explain why CRISPR-induced mutations are inefficient in H3K4me1-enriched sequences (145) and other observations of lower mutation rates in H3K4me1-marked genome regions (87, 88, 109). However, other HR-related proteins may also contribute, as well as other repair pathways.

Histone variants (H3.1 versus H3.3) may also recruit HR machinery. And while H3K4me1 may contribute to recruiting HR proteins, other histone modifications may prevent binding of HR complexes. For example, H3.1 is bound, via its tetratricopeptide repeat domain, by TONSOKU, which initiates HR in the HDR pathway when replication is interrupted by DSBs. However, methylation of H3.1K27, which is a signature of gene silencing (147), blocks the capacity for TONSOKU to bind H3.1 (23).

5. EVOLUTION OF INTRAGENOMIC MUTATION RATE VARIABILITY

The adaptive relevance of mutation rate variability is of longstanding interest in evolutionary biology (126, 128). With a deeper understanding of the molecular processes affecting mutation rates, we can explore classic questions examining the evolvable, biological processes (127) whose underlying mechanisms (i.e., targeting of DNA repair genes) can be subjected to evolutionary forces, including selection. Here, we focus on mechanisms that target DNA repair genes to different regions of plant genomes, primarily through interactions with transcription and epigenomic marks.

5.1. Potential for Adaptive Benefits of Mutation Rate Variability

Multiple mechanisms of DNA repair proteins targeted to transcriptionally active genes have been discovered. Examples described in more detail in the preceding section include targeting of PDS5C and MutSα to active gene bodies via Tudor domains that bind H3K4me1. These mechanisms are consistent with the target regions (gene bodies) exhibiting reduced mutation rates observed experimentally (**Figure 3**; **Supplemental Table 2**). The result is a potential connection between regions of the genome that experience preferential DNA repair (and therefore lower mutation rates) and regions that experience greater proportions of deleterious mutational effects. Coding regions, for example, in *Arabidopsis*, are under considerably stronger purifying selection than intergenic regions (i.e., they have a more negative distribution of fitness effects of new mutations) (28, 40). The preferential reduction of the mutation rate in active gene bodies by DNA repair may provide an evolutionary benefit of reducing mutation load. However, the resulting mutation rate variability in genes could also be maladaptive in some cases. For example, there are genes under balancing and diversifying selection that may provide likely fitness gains (e.g., NLR genes involved in innate immunity in plants) from novel mutations, and many nongenic regions are under strong purifying selection. Indeed, gene body hypomutation is not universal: The alga *C. reinhardtii* appears to lack the reduced genic mutation rate that has been observed in *Arabidopsis*, rice, and several other algae (59, 76, 88, 97, 109, 146).

The adaptive value of genic hypomutation by DNA repair targeting is not certain, but some evidence suggests that it may have evolved in response to selection. Mechanisms of targeted repair could represent a classic example of convergent evolution. In animals, gene body hypomutation is also mediated by DNA repair proteins via similar mechanisms, yet possibly independent from those in plants (46, 68, 130). Specifically, in animals, H3K36me3-modified histones mark gene bodies and are targeted by PWWP domains in several DNA repair genes such as MSH6. In plants, H3K4me1 marks gene bodies and is targeted by Tudor domains in DNA repair genes, including MSH6. Animals and plants may have therefore evolved different histone modifications that mark gene bodies and binding domains that specifically recognize those marks in DNA repair systems. It is also notable that targeted repair may be highly conserved in land plants: Both rice and *Arabidopsis* contain Tudor domains in MSH6 and PDS5C, whose binding specificity to H3K4me1 appears to be conserved across over 100 million years of divergence (109). The examination of other DNA repair pathways and a deeper investigation into the evolutionary history of

convergence, conservation, and diversity across diverse taxa may yield insight into the role that selection has played on mechanisms of repair that affect intragenomic mutation rate variability.

5.2. Possible Evolutionary Histories of Targeted DNA Repair

The possibility of mutation rate variability providing an evolutionary benefit raises questions about how the mechanism responsible for it has evolved. Although empirical evidence is not yet entirely available or conclusive, we can look to established theory on the evolution of mutation modifiers to gain insight into potential evolutionary histories where selection played a role. When we consider the idea of mutation rate variability across genomes being fine-tuned via selection, a problem is quickly encountered that has been central to contemporary research of mutation rate evolution (19, 44, 78, 79, 82, 83, 88). Mutation rates cannot evolve on a gene-by-gene basis: Because of genetic drift, adaptive genic hypomutation "is theoretically untenable, especially in the lack of mechanisms that simultaneously tune the mutabilities of multiple genes with similar fractions of deleterious mutations" (19, p. 1559). As such, it is unlikely that the targeted DNA repair observed in extant plants today is the product of evolution by individual modifiers unique to each gene that contribute to locally reduced mutation rates. Instead, selection can only act on mutation modifiers that operate on genome-wide scales, affecting the mutation rates of thousands of genes simultaneously. As others have articulated, this is indeed possible if mutation rates are linked to the epigenome (82).

When considering the evolutionary origins of targeted DNA repair, we therefore face a chicken-and-egg question (**Figure 5**): Which came first, the concentration of epigenomic features to functionally distinct genome regions (e.g., H3K4me1 to active gene bodies) or the mechanisms that link DNA repair to these features (e.g., the Tudor domain of PDS5C)? It also may be the case that these systems evolved in tandem. Addressing this question may yield further insight into the reciprocal nature of the relationship between genome biology and genome evolution.
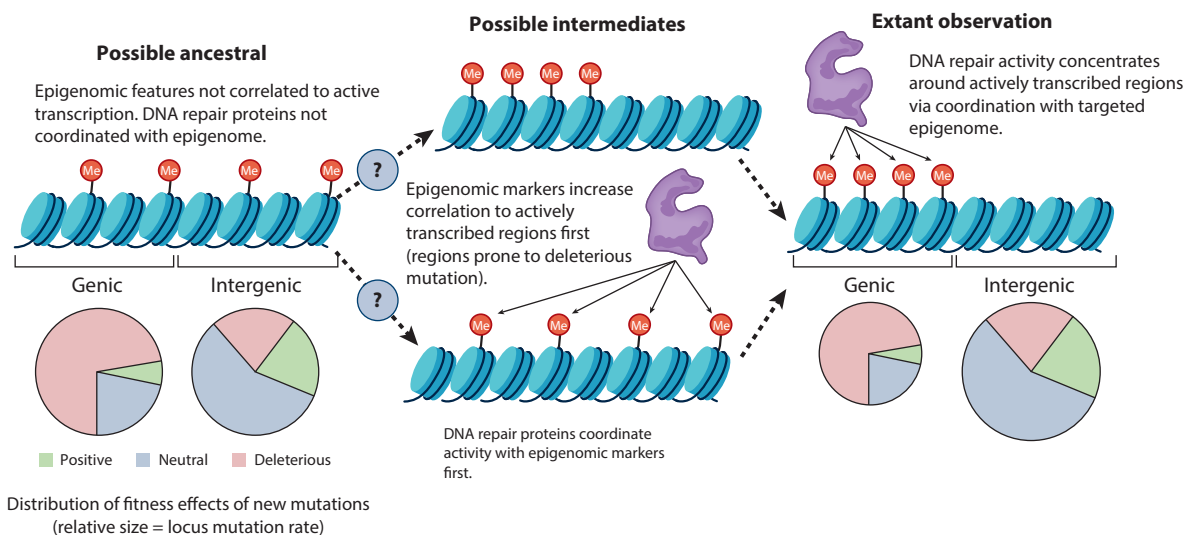
Figure 5

Conceptual model of potential evolutionary histories of DNA repair–related proteins (e.g., PDS5C) that evolved specific targeting of histone modifications (e.g., H3K4me1) that are enriched in active gene bodies in plants. The result is that regions that tend to experience a more deleterious distribution of fitness effects are more likely to have lower mutation rates due to the effects of elevated repair.

6. CONCLUSIONS

Wondering at the incredible advances in knowledge of genome biology in her 1984 Nobel Lecture, Barbara McClintock noted that our increased molecular understanding of processes such as mutation was comparable in magnitude to the synthesis of Mendelian genetics with Darwinian evolution in the early twentieth century: "I believe we are again experiencing such a revolution. It is altering our concepts of the genome" (85, p. 793).

Our knowledge of the causes and consequences of mutation rate variability has increased dramatically with new experimental approaches, especially in recent decades, supported by a century of research on the nature of mutation. Recent discoveries provide a higher-resolution understanding of mutation rate variability that has intrigued generations of biologists. Mutation rates vary considerably among different kinds of mutation and genomic contexts. Mutation rate responses to environmental conditions have potentially radical implications for our understanding of plant evolutionary responses to rapidly changing environments. Observations of reduced gene body mutation in plants raise questions about the adaptive relevance of targeted DNA repair. Exploring and exploiting DNA repair mechanisms may be valuable in accelerating breeding via targeted mutagenesis in plants, helping achieve critical goals in crop improvement. Deeper investigations into the mechanisms governing mutation rate variability between species and across genomes and environments will lead to a more unified understanding of the relationship between plant genome biology and the origins of plant diversity.

SUMMARY POINTS

1. Mutation rates reflect the balance between DNA damage/error and DNA repair.

2. Mutation rates differ across genomes, in relation to the environment, among different classes of mutation, and between species and genotypes.

3. In plants, the distinction between germline and somatic mutation is complex, especially in plants that can reproduce from vegetative tissues.

4. Chromatin dynamics play a central role in affecting mutation rate variation.

5. Plants have evolved mechanisms that link DNA repair to chromatin features such as histone modifications.

FUTURE ISSUES

1. Sequencing with long-read technologies will help determine the mutation rate of structural variants, an important source of genetic variation whose mutation rate remains largely unknown.

2. A better understanding of the relationship between meristem biology (stem cell and chromatin) and mutation rate in plants is needed, especially in those that propagate vegetatively.

3. Dissecting the evolutionary history of mechanisms that link DNA repair and chromatin (e.g., histone modifications) will shed light on the origins and long-term evolutionary consequences of targeted DNA repair.

4. Experimental and theoretical work is needed to understand how mutation rate variation will affect plant adaptation to changing climates.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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