CURRENT PERSPECTIVE ESSAY

Rapid Changes in Plant Genomes

Beginning with Mendel, plant research has been the source of revolutionary discoveries that have changed our views of heredity. Therefore, baffling new observations in plant genetics that challenge current concepts continue a long tradition. One of these challenges comes from the study of heritable phenotypic and genetic alterations that are consistently produced after a flax cultivar has undergone an environmental change. Another challenge comes from the startling discovery that mutations in the *Arabidopsis HOTHEAD* (*HTH*) gene can revert at an extraordinarily high frequency with genome-wide effects. This study has received attention in the popular press because it is thought to reveal the existence of extragenomic inheritance in the form of an RNA cache of correction templates. The observations in flax have received relatively little attention, even though they are remarkable by any standard, in that environmental induction consistently leads to the sudden appearance of a large element in a genome that previously lacked it.

Both studies reveal the appearance of sequence information from an unidentified source in plants, and it is attractive to think that they occur by related mechanisms. Here, I compare the two phenomena and attempt to weigh the relative merits of extragenomic versus intragenomic explanations.

ENVIRONMENTAL INDUCTION OF GENOMIC CHANGES

For more than three decades, Cullis and colleagues have studied heritable changes in flax that are triggered by environmental stress (Cullis, 1973, 2005). Remarkably, changes in nutrients consistently lead to immediate phenotypic alterations that are accompanied by gross genomic modifications during vegetative growth (Figure 1). Among these are consistent reductions in ribosomal gene copy number and widespread insertion events that appear to be distributed among all 15 flax chromosomes. In their latest report, Cullis' group focused on a single insertion site, identifying a novel 5.8-kb element called Linum Insertion Sequence 1 (LIS-1) that appeared at precisely the same position in all five independent lines when subjected to the same environmental regimen (Chen et al., 2005). Repeated precise insertion into a single site is of course very interesting, but what makes this observation astonishing is the apparent absence of intact LIS-1 from the original line. In other words, LIS-1 materializes reproducibly at a unique site in a single generation after an environmental stress from a source that cannot be identified as an intact donor element. Intact LIS-1 elements are readily detected in some other flax and linseed varieties, suggesting that it has an ancient origin.

The sequence of LIS-1 provides possible clues as to its source. A 3-bp duplication is found at the target site, suggesting a transposition event of some kind, but there are no other features that identify it as belonging to any known class of transposon. Rather, a complex set of short repeats in both orientations comprise the bulk of LIS-1. PCR amplification using primers from various repeat units indicated that the repeat units themselves can be detected in the genome, including sequences that immediately flank LIS-1, but these are peppered with single-base differences. Thus, it appears that LIS-1 was assembled in a complex series of programmed DNA rearrangement and editing events at a single site. Given that the LIS-1 insertion is only one of many programmed genomic changes that occur under inducing conditions (Figure 1), it seems likely that it is not an isolated example. Such events are not without precedence because programmed genome-wide rearrangements and insertions have been well documented in various ciliates (Prescott, 1999), and programmed rearrangements and point mutations in the vertebrate adaptive immunity system (Honjo et al., 2004) are among the best studied of all biological processes.

INSTABILITY OF MUTATIONS IN THE *HTH* GENE

Rapid genome-wide changes are also seen in *Arabidopsis hth* mutants (Lolle et al.,
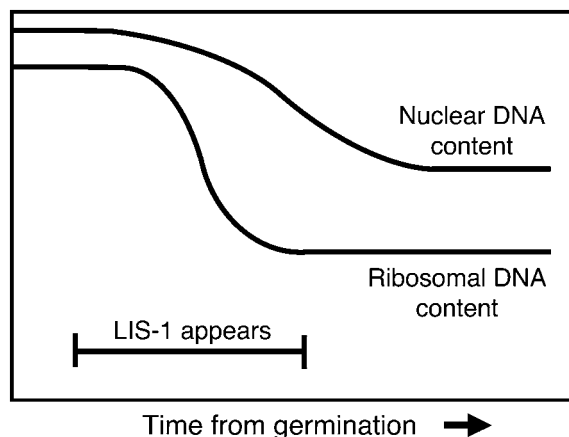
Figure 1. Rapid Appearance of LIS-1.

LIS-1 appears during vegetative growth of flax under inducing conditions at the same time that nuclear DNA and rDNA decreases are seen. Data are taken from Cullis (2005).

2005). Reversion of *hth* point mutations appear spontaneously at an extraordinarily high frequency. Control experiments ruled out artifactual explanations for revertants, such as contamination. The fact that spontaneous mutations were discovered at unlinked loci at high frequency in *hth/hth* plants suggested that the genomic plasticity observed at *HTH* occurs throughout the *Arabidopsis* genome.

To explain the high reversion frequencies observed for *hth* alleles, the authors and others proposed that *Arabidopsis* maintains a cache of correction templates that is independent of the chromosomal genome (Lolle et al., 2005; Ray, 2005; Siddiqi, 2005; Weigel and Jurgens, 2005). For each gene, ancestral wild-type copies would be available for correction of mutations under conditions of stress. An attractive paradigm for template-directed correction is small interfering RNA-directed DNA methylation, a well-established and efficient phenomenon in plants (Aufsatz et al., 2002). If RNA templates can direct sequence-specific methylation in the genome, then a similar templating process might revert a mutation, for example, by recruiting a DNA repair enzyme rather than a DNA methyltransferase.

How would a cache of correction templates be inherited? *hth/hth* plants retain their ability to revert over multiple generations in the homozygous state (Lolle et al., 2005). Thus, *HTH* correction templates seem to be indefinitely replicated in the cache. This is the basis for the extraordinary proposal that templates were cached under selection in the past for restoration during stressful conditions (Lolle et al., 2005).

It is worth noting that evidence for an ancestral cache of correction templates applies only to *HTH* itself, not genome-wide. When Lolle and coworkers crossed *hth/hth* in one genetic background (Landsberg *erecta* [L*er*]) to *HTH/HTH* in a different genetic background (Columbia [Col]), they recovered frequent unselected changes among F3 plants at a variety of loci, either from L*er* to Col or from Col to L*er* (Lolle et al., 2005). With no bias reported in either direction, this observation is consistent with the reconstitution of the cache from parental copies every generation, as diagrammed in Figure 2. However, by back-crossing an F3 *hth/hth* with a homozygous L*er* marker to the parental *hth/hth* (L*er*) line, one can ask whether the Col-to-L*er* reversion frequency drops as expected from the fact that both Col and L*er* entered the hypothesized cache and must have diluted one another in the parental cross. If no drop is observed, this would contradict the implied dilution of the cache in the parental cross, thus providing a critical genetic test of the model.

THE TOXIC MUTATOR AND SELECTION HYPOTHESIS

Comai and Cartwright (2005) propose an alternative to the cache model. They argue that the high rate of unselected mutation in *hth/hth* plants can be explained if mutations in the *hth* protein have mutagenic effects. Because mutagenicity is likely to be toxic, *HTH* revertants should be better fit than *hth* parents. Selection for *HTH* will be especially strong in pollen, where *HTH* pollen would have the opportunity to out-compete *hth* pollen. Note that toxicity and strong selection could also occur in somatic tissues, and a flower from an *HTH/hth* sector would explain the occasional appearance of *HTH/HTH* double revertants in F1 selfed progeny.

How can *hth* mutations produce a toxic mutagen? *HTH* is a member of a plant-specific gene family that includes mandelonitrile lyase (MDL), which releases hydrogen cyanide from the simple aromatic compound, mandelonitrile, causing cyanogenicity. MDL has been purified from several cyanogenic plant sources (Evans, 1996; Comai and Cartwright, 2005). In *Prunus* tissues, its natural substrates are the *O*-linked monoglucoside, prunasin, and the diglucoside, amygdalin, of mandelonitrile, which are also found in other plants. When Pruitt's group first reported that HTH is related to MDL, they proposed that accumulation of the substrate of HTH is responsible for the floral fusion phenotype of *hth* plants that initially attracted their attention (Krolikowski et al., 2003). However, the natural substrate for HTH is unknown, and *Arabidopsis* has six other MDL family members. Although key catalytic residues are conserved in members of the MDL family and *hth* mutations are consistent with loss of enzymatic activity, HTH belongs to a different subfamily from MDL. Nevertheless, it seems likely that the natural substrate for HTH is similar to prunasin and amygdalin and that diversity of MDLs reflects the multiplicity of substituted forms of mandelonitrile or related aromatic hydroxynitriles (Evans, 1996).
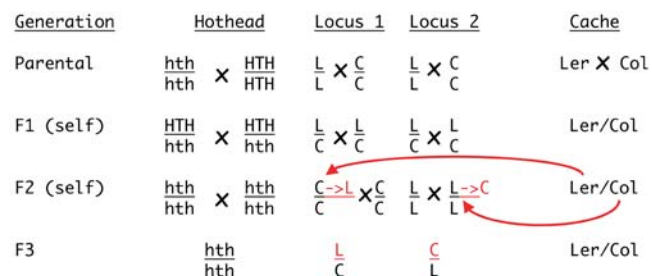
Figure 2. Crosses That Uncovered Unselected Col-to-L*er* (Locus 1) and L*er*-to-Col (Locus 2) Changes in *hth/hth* Mutants.

L and C are polymorphisms at unlinked loci that differ between L*er* and Col accessions. Parental reconstitution of a hypothetical cache is shown at right, where correction sequences in the cache are either L*er* or Col. Note that it is unnecessary to postulate the existence of an ancestral cache to account for the frequent appearance of changes in either direction. However, by successively backcrossing an F3 nonrevertant *hth/hth*;L/L line to the parental *hth/hth*;L/L line, it should be possible to test whether the Col templates that entered the cache are still able to efficiently cause L*er*-to-Col changes as predicted by the ancestral cache model or are diluted as expected for parental reconstitution.

CURRENT PERSPECTIVE ESSAY

INHIBITION OF BASE EXCISION REPAIR MIGHT ACCOUNT FOR *hth* REVERSIONS

Is there a plausible mechanism for mutagenicity of a mandelonitrile-based metabolite? An intriguing possibility is suggested from a study searching for inhibitors of mammalian DNA polymerase β in plant extracts (Mizushina et al., 1999). Polymerase β is the primary DNA polymerase involved in base excision repair (BER), which is the major pathway for repair of endogenous DNA damage (Figure 3). Purification of a DNA polymerase β inhibitor from two different plant species yielded prunasin in both cases. Prunasin is competitive with the deoxyTTP substrate, similar to inhibition by dideoxyTTP. Inhibition by prunasin appears to be highly specific: amygdalin does not inhibit, perhaps because, like deoxyTTP, prunasin consists of a single cyclic sugar moiety attached to a single aromatic ring derivative. Inhibition by prunasin was confirmed in vivo by showing that it inhibits BER in tumor cells with an effect similar to the effect of a DNA polymerase β knockout (Tomicic et al., 2001). The failure to complete BER through inhibition of β-like DNA polymerases causes misincorporations to be converted to DNA breaks because of failure to remove the damaged 3′ base after base excision (Figure 3) (Dianov et al., 2003). Thus, prunasin is a potential recombinogenic and mutagenic agent in that its presence

is expected to inhibit BER, leading to unrepaired DNA lesions.

Plants and animals use somewhat different DNA polymerases for BER, which requires a polymerase with 5′-deoxyribose-5-phosphate lyase activity (Braithwaite et al., 2005). Animals have several such enzymes, including DNA polymerase λ, which appears to play only a backup role in BER. Plants lack DNA polymerase β, but instead have λ as their only known polymerase with 5′-deoxyribose-5-phosphate lyase activity (Uchiyama et al., 2004). β and λ DNA polymerases appear to have diverged prior to the plant animal split, with β being lost from the plant lineage. Thus, an excellent candidate for a toxic mutator is a glycosylated mandelonitrile inhibitor of DNA polymerase λ. Moreover, plant DNA polymerase λ activity is specific for meristematic and meiotic tissues (Uchiyama et al., 2004), so that its inhibition might lead to defective gamete production, a key prediction of the toxic mutator hypothesis (Comai and Cartwright, 2005).

Accumulation of an inhibitor of BER is also consistent with gene conversion underlying the high rate of *hth* reversion, given that DNA breaks are repaired by strand invasion from a homolog. Chaudhury (2005) has speculated that short patches of homology only 13 to 18 bp in length might serve as templates for ectopic conversion. However, sequence identities that would revert *hth* alleles are no better than expected by chance in the *Arabidopsis* ge-

nome (data not shown). This is a serious shortcoming because if gene conversion were that promiscuous, then the genome would be in constant flux, as sequences would convert one another almost indefinitely. That is, Chaudhury's hypothesis is implausible unless *hth/hth* somehow facilitates the gene conversion process, and creation of DNA breaks by inhibition of BER would do exactly that. An overwhelming frequency of strand breaks would presumably allow some of them to be repaired from ectopic templates with only limited homology.

Perhaps the proposed BER mechanism is also responsible for triggering the genome-wide programmed rearrangements in flax. Environmental stress would inhibit DNA polymerase λ or another enzyme involved in repair of DNA breaks, leading to point mutations, rearrangements, and conversion from ectopic sites. How the damage would be targeted is unknown; however, targeted DNA deamination of immunoglobulin genes that overwhelms the mammalian BER system is believed to account for the co-occurrence of somatic hypermutation, gene conversion, and class-switch recombination in activated B-cells (Honjo et al., 2004). Rearrangements and point mutations that distinguish LIS-1 from its hypothesized set of templates in the flax genome could thus be accounted for by a single triggering mechanism. Presumably, the elaborate program of events that seems to occur
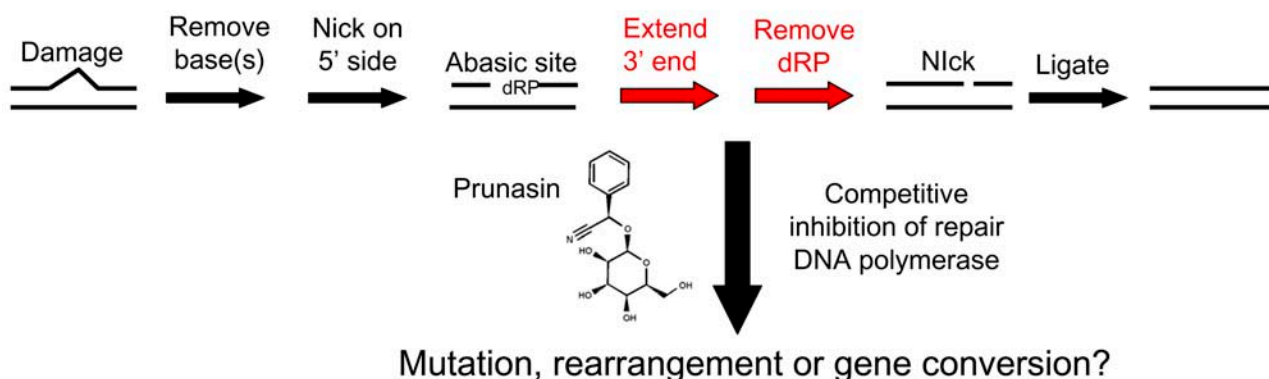
Figure 3. Hypothesized Effect of a Prunasin-Like Compound on the BER Pathway.

Reactions performed by repair DNA polymerases are indicated in red.

upon induction has evolved from a series of much simpler random events. In the same way that programmed rearrangements and point mutations of vertebrate adaptive immunity probably evolved from a simpler system of defense against environmental agents, so would the flax programmed events have originated from a more generic response to the environment. Starting with a toxic mutator, such as an inhibitor of BER, and adaptive selection for particular rearranged or mutated genomic regions, we might envision a stepwise evolutionary process leading to massive programmed rearrangements.

PERSPECTIVE

The proposal that environmental stress causes bacteria to direct genetic changes (Cairns et al., 1988) was followed by years of controversy and illuminating experiments that in the end rejected the concept. While the basis for the phenomenon remains controversial, none of the surviving hypotheses require a reexamination of the basic laws of heredity (Hastings et al., 2004). The more recent idea of an RNA cache that plants use in times of stress also has the potential of rewriting genetics textbooks, although in this case, it is hoped that a consensus will be achieved more quickly than in the case of directed mutation. To arrive at a consensus, it will be important to experimentally test the most plausible alternatives, such as the toxic mutator and selection hypothesis, and the possibility that *hth/hth* is defective in BER is a place to start. Only when all reasonable alternatives have been excluded and the mechanism behind the phenomenon elucidated will the RNA cache hypothesis gain acceptance.

Whereas the existence of an RNA cache that is induced during stress is tentative, the evidence for some kind of massive programmed rearrangement upon environmental induction in flax is unequivocal. Inheritance of acquired changes has been an anathema to evolutionary biologists ever since Darwin's time, but that is because

claims of Larmarckian inheritance were never accompanied by plausible mechanisms. However, in the case of flax at least, we may not be far from meeting this requirement.

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