PLANT GENETICS

Hothead healer and extragenomic information

Arising from: S. J. Lolle, J. L. Victor, J. M. Young & R. E. Pruitt *Nature* **434,** 505–509 (2005)

Lolle *et al.* suggest that non-mendelian inheritance in *Arabidopsis thaliana* might be attributable to an ancestral RNA-sequence cache[1], whereby the RNA genome of previous generations causes a high rate of reversion of the plant's mutant *hothead* (*hth*) and *erecta* (*er*) genes. Here I describe a 'distributed genome' model that also explains their results, in which mutant *hth* DNA is restored by homologous sequences present in the genome itself. This model has implications for the generation of diversity without mating.

DNA-homology searches of the *Arabidopsis* genome based on the 21 nucleotides surrounding *hth-4*, *hth-8*, *hth-10* and *er* reveal the presence of short, perfectly homologous DNA stretches (known as 'reverting sequences') that include nucleotides needed to correct these mutations (Fig. 1). There are also many examples of short homology in genes tested for polymorphism (including *GL1*, *UFO* and *GAPC*).

These sequences might be transcribed into short RNA molecules directed against other chromosomal loci by the cellular machinery, perhaps with the involvement of *DRD1* (ref. 2), producing short RNA–DNA hybrids with potential mismatches that can be corrected by mismatch repair[3]. Consistent with this model, the *hth-4* allele — with 6 reverting sequences of 13–15 nucleotides each — has a lower reversion frequency than *hth-10*, which has 24 reverting sequences of 13–18 nucleotides. As a result of such differences in number, as well as differences in the production of short RNA, some sequences might be changed more than others.

These short sequences should also produce forward mutations, so it is important to measure forward-mutation frequency for several loci. Also, of the several short sequences available for reversion, some might express more short RNA in the male gamete, explaining the preferential transmission of reverted alleles through pollen. The messenger RNA of the corresponding gene may competitively hybridize with a small length of RNA and prevent its interaction with DNA but, owing to the shortness of the base-paired sequence, the mRNA would not be totally inactivated and so would not produce a mutant phenotype. As both nonsense and missense alleles can cause a reduction in transcription compared with the wild-type allele[4], there would be more opportunity for reversion to wild type than for forward mutation, which would account for the shielding of the genome against forward mutations.

Conversion to neutral alleles could also occur by this mechanism, but again some sequence stretches might be more effective than others. Lolle *et al.* did not find any neutral mutation in nine reverted *HTH* genes[1]. Even if the activity of all sequence stretches were comparable, the active reversion at any site should be independent of events at other sites: therefore, the frequency of a neutral mutation among revertants is expected to be around 1%, lower than the level of detection in Lolle *et al.*[1].

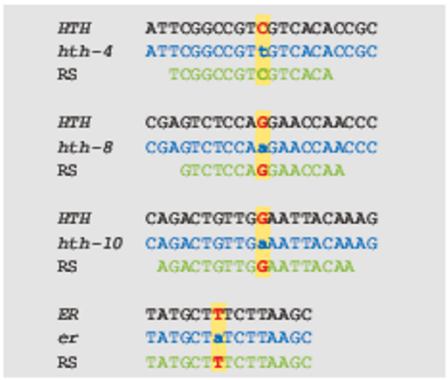

Figure 1 | DNA nucleotide sequences of the *hth-4*, *hth-8*, *hth-10* and *er* mutants in the region of the mutation, compared with wild type. Sequences of the mutants are shown in blue, with the mutated nucleotide in lower-case; the corresponding wild-type sequences are in black and the nucleotide at the site of mutation is highlighted in red. Homologous sequences that might cause the mutations to revert (RS sequences), obtained by BLAST-searching the *Landsberg erecta* database from www.arabidopsis.org, are shown in green, with the wild-type nucleotide in red. Reversion frequency is lower for the *hth-4* allele (with 6 RS sequences of 13–15 nucleotides), than for *hth-8* (with 20 RS of 13–15 nucleotides) and *hth-10* (with 24 RS and 13–18 nucleotides).

The proposed sequence-mediated reversion frequency could be boosted by another mechanism not described by Lolle *et al.*[1]. The *hth* mutant cuticles have increased cellular permeability compared with the wild type[5]. I suggest that the *hth* embryo sac is also more porous than the *HTH* embryo sac, causing DNA in the *HTH*/*hth* heterozygote to enter the *hth* embryo sac from the two degraded *HTH* spores and become 'archived', as in the P22 phage[6,7]. Although the increased cellular permeability would allow the *hth* gametophyte to obtain *HTH* molecules in the F_1 generation, in subsequent generations these DNAs need not replicate; the endogenous reverting sequence might provide a basal level of reversion.

Lolle *et al.* propose that metabolic stress in *hth* increases its reversion frequency[1], which might increase information transfer between selected short sequences to alter DNA and create genetic diversity.

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RNA cache or genome trash?

Arising from: S. J. Lolle, J. L. Victor, J. M. Young & R. E. Pruitt *Nature* **434,** 505–509 (2005)

According to classical mendelian genetics, individuals homozygous for an allele always breed true. Lolle *et al.*[1] report a pattern of non-mendelian inheritance in the *hothead* (*hth*) mutant of *Arabidopsis thaliana*, in which a plant homozygous at a particular locus upon self-crossing produces progeny that are 10% heterozygous; they claim that this is the result of the emerging allele having been reintroduced into the chromosome from a cache of RNA inherited from a previous generation. Here I suggest that these results are equally compatible with a gene conversion that occurred through the use as a template of DNA fragments that were inherited from a previous generation and propagated in archival form in the meristem cells that generate the plant germ lines. This alternative model is compatible with several important observations by Lolle *et al.*[1].

Such archival forms of DNA have been described previously[2]. The template DNA could have originated in the fragmented genomes of three of the four haploid female meiotic products in the germline of a heterozygous plant. Within the ovule of the original heterozygous plant, the surviving haploid female germline cell containing the mutant *hth*

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allele acquires from the degenerating sister nuclei a collection of chromatin fragments that are presumably heterochromatinized and silenced; they might also be covalently modified and therefore hard to detect by Southern blotting or by amplification in the polymerase chain reaction.

I propose that these supernumerary chromatin fragments propagate within the meristem cells of succeeding generations, and so are present in a very few cells of the plant; nevertheless, they are often present in the germ lines that are themselves derived from the meristem. Within a second-generation descendant that is homozygous for a chromosomal allele, such supernumerary chromatin fragments might harbour another allele in a cryptic state. These supernumerary fragments might pair with normal chromosomes in the male germ line preferentially, as indicated by Lolle *et al.*[1], where they direct gene conversion of the chromosomal alleles. The gene-converted allele, therefore, would reappear as a mendelian factor in the third or a subsequent generation.

This proposal dispenses with the hypothetical RNA cache[1], for which little evidence exists. It is consistent with the incidence of supernumerary chromosome fragments in plants, particularly with their known effects on pollen function[3], and explains the lack of somatic convertant sector in any generation[1]; it also invokes standard molecular processes of DNA-directed gene conversion over relatively long regions of homology. The differences in the frequency of conversion of the three tested alleles[1] may reflect co-conversion polarity[4].

My proposal can also explain the curiously high frequency of allele reappearance in dissected embryos, which is roughly twice that found in mature plants (it increased from 10% to 20%). Assume that gene conversion produces a heteroduplex DNA that escapes mismatch repair. If conversion occurs in the haploid generative nucleus of the pollen, the two sperm cells will be non-identical at the converted allele. After double fertilization, the embryo and the endosperm will contain two different alleles. An embryo dissected from such a seed will inevitably be associated with endosperm cells, and will yield a signal in the polymerase chain reaction that is indistinguishable from a heterozygous embryo, although the embryo itself is homozygous for the allele. As this will occur as often as a conversion, the frequency of the total converted allele in dissected embryos and endosperms together should be about 20%, an estimate close to the experimentally observed rate.

The model proposed here, but not one in which gene conversion is directed by RNA[5] or by very short, dispersed, repeated DNA sequences[6], is easily reconciled with the notion of co-conversion polarity[4]. It should be possible to test whether co-conversion polarity is a factor in the phenomenon revealed by Lolle *et al.*[1] by producing double- and triple- mutant alleles through exploitation of this effect. Further investigations into the possibility and nature of DNA archiving in plants and of plant germ lines should prove interesting.

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Lolle et al. *reply*

Reply to: A. Chaudhury (doi:10.1038/nature04062) and A. Ray (doi:10.1038/nature04063)

Chaudhury[1] and Ray[2] propose alternative models to account for our observed pattern of non-mendelian inheritance in the *hothead (hth)* mutant of *Arabidopsis*[3].

Chaudhury suggests that the information required to restore correct genetic sequences in *hth* mutant plants could be stored in short stretches of nucleotide sequence within the genome[1]. Although the sequences required for restoration are indeed present in the genome, the length of similarity seen in the 'reverting sequences' identified by Chaudhury is barely greater than would be expected from random chance. An appropriate control for his *in silico* experiment would be to establish how many similar sequences (13–18 nucleotides in length, with a single nucleotide mismatch relative to the sequence in the parent plant) are present in the genome that could likewise introduce silent nucleotide substitutions into the *hth* gene under the same conditions.

Should there be a significant number of these sequences (and given that no such silent mutations occur in the corrected alleles[3]), then an explanation is needed for why some of them are used for correction whereas the majority are not. Even if silent mutation events occur independently of the reversion of the *hth* mutation, we should still detect them in our small sample of sequenced reverted alleles owing to the relatively large number of possible silent mutations.

Chaudhury also suggests that the increased permeability of mutant *hth* female gametophytes could allow DNA or RNA from the degenerating non-functional megaspores to enter the functional *hth* megaspore and then be archived and carried forward to allow gene conversion in the next generation. This may be a possibility, but our previous work examined only changes in the permeability of the extracellular cuticle covering the outside of the epidermal cell layer[4]. We have no data concerning increased cellular permeability in *hth* mutants. The fact that we see no obvious drop in the rate of reversion over several generations[3] is inconsistent with Chaudhury's suggestion that this could be a second mechanism to bolster the rate of reversion for only a single generation.

Ray[2] claims that our results could be explained by the stable inheritance of supernumerary chromosomal fragments that are archived in a way that makes them inaccessible to DNA hybridization and the polymerase chain reaction. These fragments might also be restricted to meristematic cells and therefore be present in such low concentrations that they are undetectable in a conventional experiment. This is an interesting possibility that is consistent with our observations, but it postulates a novel system of segregation to restrict the chromosome fragments to what would constitute a hitherto undetected germ line in plants. Considering also Ray's explanation for the doubled rate of conversion in embryos, we note that it would be necessary for all conversion events to take place in the generative cell and to fail to be corrected by mismatch repair.

In summary, we agree with Ray that there is little direct evidence to support any given molecular identity for the cryptic templates that allow genetic restoration in *hth* mutant plants. We proposed that the templates might be a replicating form of RNA, but the data are also consistent with a form of DNA that is segregated into a limited number of cells in the plant or that is not readily detectable by conventional molecular techniques. This sequence archive (whether DNA or RNA) would therefore require the same basic properties as those we proposed[3]: it would need to be replicated, transmitted with high fidelity over several generations, and retain the ability to restore nuclear DNA sequences.

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