PLANT GENETICS

Hothead healer and extragenomic information

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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

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).

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].

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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1. Lolle, S. J., Victor, J. L., Young, J. M. & Pruitt, R. E. *Nature* **434,** 505–509 (2005).
2. Matzke, M. A. & Birchler, J. A. *Nature* **455,** 24–34 (2005).
3. Watson, J. D., Losick, R., Bell, S. P., Gann, A. & Levine, M. *Molecular Biology of the Gene* (Cummings, San Francisco, 2003).
4. Mustajoki, S., Kauppinen, R., Mustajoki, P., Suomalainen, A. & Peltonen, L. *Genome Res.* **7,** 1054–1060 (1997).
5. Lolle, S. J., Hsu, W. & Pruitt, R. E. *Genetics* **149,** 607–619 (1998).
6. Downs, D. M. & Roth, J. R. *Genetics* **117,** 367–380 (1987).
7. Ray, A. *Nature* doi:10.1038/nature04063 (2005).

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RNA cache or genome trash?

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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*