A sword in the rosetta stone

According to Mendel's rules, genes are passed on from one generation to the next in a stable manner and there is a continuity to the process: each allele of a gene carried by an individual is identical to a copy that is present in the genome of its parent(s). There are exceptions to this rule: one is that genes can become mutated by the time they pass on to the next generation, but these are rare events (typically 10^{-7} to 10^{-8} per base pair). A truly remarkable finding has come recently from the laboratory of Robert Pruitt (Lolle *et al* 2005); it pertains to observations on a mutant called *hothead* (or *hth*) in the plant *Arabidopsis thaliana*. The *hothead* mutant shows fusion of floral organs (see figure 1). In brief, it appears that the genome of *hth* mutant plants can acquire DNA sequences that are absent in the genome of the immediate parents but can be traced back to older ancestors: the DNA appears to 'jump' across two or more generations.

Pruitt and co-workers noticed something unusual in plants grown from seeds derived from an *hth* parent in which both the *hth* gene copies were functionally defective due to a single base change in the DNA sequence: contrary to expectation up to 10% of such plants showed the normal wild type phenotype. It turned out that this was due to the presence of a functional *hth* copy with a different DNA sequence from that of the mutant. By examining several independent revertants they found that in every case the sequence was identical to a wild type copy that was present, not in the immediate parents but in the grandparents or an earlier ancestor in the lineage. This phenomenon also occurred

Figure 1. Inflorescence of the *hothead* mutant showing joining together of individual flowers. The flowers remain partially closed due to fusion of floral organs (ref. http://news.uns.purdue.edu/images/+2005/pruitt-mutant.jpg).

for genes other than *hth*: in fact mutations in all of five other genes that they examined showed comparable reversion rates to wild type. In other words, a mutant, defective copy of the gene, had been changed to a functional one. A 10% reversion rate for a mutation is very high, a million-fold higher than would be expected for a point mutation. Furthermore the high rate was observed for three different mutant alleles of the *hth* gene.

How did this functional copy arise? A mutational change in a DNA sequence can result in the replacement of one base by any of the other three bases. Since more than one sequence change could restore gene function to a mutant copy, it would be expected that different functional copies representing distinct reversion events would often have different sequences. This was not the case: each time the changed sequence was the same, and identical to the wild type sequence, namely to a stretch of DNA that should not have been present in the genome of the plant that displayed the reversion. This was a complete surprise. The investigators were also able to rule out the possibility that the information was coming from an additional cryptic copy of the *hth* gene located somewhere else in the genome, or that functional sequence information from a related gene was being used to replace the mutant copy. Based on this the authors suggest a template-dependent mechanism which could change the mutant information specifically to wild type. However, the template would appear to be something outside the canonical DNA genome, since they could not detect it by methods that should have revealed its presence if it was there in the DNA. They speculate that the most likely candidate is an RNA template, and propose the existence of a stable double-stranded RNA molecule capable of being replicated and passed on over successive generations. This could then be converted to DNA when required.

It should be reiterated that Lolle *et al* (2005) observed a high rate of sequence change not just in the *hth* locus but wherever they looked for in the genome including introns, exons, and untranslated regions (they do not say what happens in intergenic regions). In other words, whatever is going on is not confined to the *hth* gene but appears to operate on DNA sequences throughout the genome; but it does require the plant to be mutant for *hth*. Wild type plants and other mutants do not behave in this away or are not known to (if they did one presumes the phenomenon would have been discovered long ago). The authors hypothesise that a similar "inheritance of non-genomic templates" might take place in the wild type too, but might be more frequent in *hth* mutants, perhaps as a result of stress resulting from the consequences of the *hth* mutation. Thus something about *hth* seems to result in a very high rate of genetic change to wild type. The *hth* gene encodes an enzyme responsible for a step in epicuticular wax biosynthesis. Indirect consequences of the mutation may be monitored by a mechanism that surveys the cell's physiology for indications of stress; and the sensing of stress could activate the mutagenic transfer of 'cache' information into the genome. Perhaps the existence of such a mechanism would have some evolutionary advantage by providing a means for clearing out those genetic changes which lead to deleterious physiological consequences, and reverting to a backup copy of information.

Like all major discoveries this one raises more questions than it answers. The most obvious relate to the mechanism of the phenomenon: the nature of the postulated template, and its mode of propagation. DNA and RNA are the only two types of molecules in the cell that are known to act as self-templates. The evidence leans against DNA, so RNA appears to be the most likely candidate. But what type of RNA molecule and how is it made and stored? It must be very low in abundance relative to mRNA since standard RT-PCR experiments do not amplify intronic regions.

How widespread is the phenomenon and does it exist in other species? If the phenomenon is genuine and the explanation offered – that of an RNA template existing outside the DNA genome – correct, the findings will have a major impact in all areas of biology. This is apparent from the responses of members of the scientific community. Reactions to the findings and the heretical model proposed range from excited recognition of the implication of these findings among molecular biologists to a more cautious "let us see if other creatures also do this" among evolutionary biologists. One consequence of such a phenomenon would be to allow for a mutation to be road tested, as it were, before it becomes fixed in the lineage. The authors point out that although the model is heretical, the individual component steps that go into it are not new. Double-stranded RNA induced gene silencing is known to persist for several generations in the worm *Caenorhabditis elegans* (Fire *et al* 1998), and

site-directed mutagenesis can be carried out by using hybrid RNA-DNA oligonucleotides (Cole-Strauss *et al* 1996). Therefore the plausible pieces do exist.

References

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