



“[Lolle *et al.*] bred each plant so that it was homozygous for the mutant gene, yet 1-10% of these progeny contradicted Mendel’s laws and showed wild-type genes.”

Our Body’s Own Historian

a look into the phenomenon of HOTHEAD revertants

By Ashish Agrawal

Every cell in the human body contains about three billion base pairs of genetic information that must be duplicated each time the cell divides (1). Although DNA replication is orders of magnitude more reliable than any man-made invention, mutations nevertheless arise because of occasional mismatched base pairs or externalities such as radioactivity or UV light. Mutations are important for the survival of humans as a species, since they allow for greater genetic diversity and for the expansion of the gene pool. Yet despite their evolutionary importance, mutations in the human genome are much more likely to be harmful than helpful.

In light of these ideas, the scientific community immediately understood the significance of data published by Lolle *et al.*, which showed that *Arabidopsis thaliana*, a small flowering plant commonly used in laboratory experiments, can revert to a wild-type genotype from a mutant genotype even if the wild-type genotype did not exist in either parent (2, 3). In other words, imagine a situation where an organism can revert to a “backup” copy of its genome if the organism is hurt by mutations in its genetic code.

Mendelian Genetics

Gregor Mendel is often called the father of modern genetics because of his landmark 1865 paper, “Experiments on Plant Hybrids.” Over a period of eight years, Mendel tracked seven different characteristics in over 33,500 pea plants that were grown behind the monastery in which he lived. He kept meticulous records on each trait, and used these to develop his foundational theories of genetics (4).

Using his data, Mendel realized that some parent plants could produce progeny that did not resemble either parent. For example, two plants that produced round seeds could produce a small proportion, about 25%, of wrinkled seed progeny. Mendel predicted that the wrinkled trait was recessive, or “hidden”, behind the dominant round trait. He also realized that in order for his data to make sense, each parent had to have two copies of each trait, one of which was passed down to the offspring. This idea, known today as the Principle of Independent Segregation, states that each allele, or copy of a trait, has an equal likelihood of being passed on to the progeny (4). Thus, if both parents have only domi-

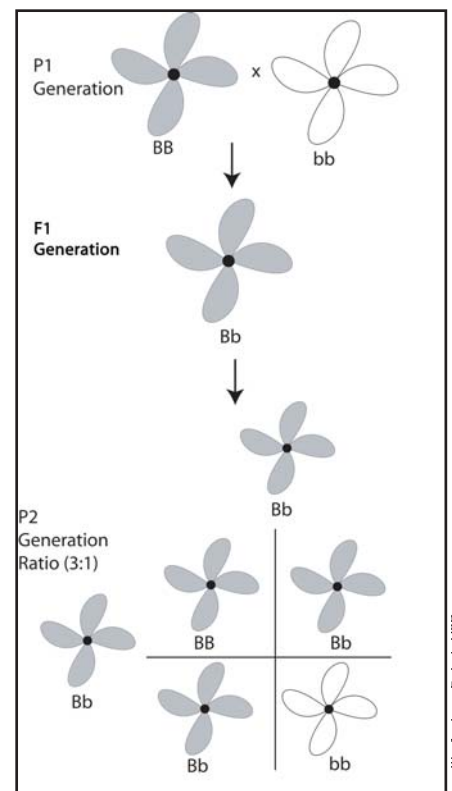


Figure 1. Mendel’s laws predict that two parents who are homozygous (pure breeding) for different alleles produce progeny that each have one copy of these alleles. The same principle applies to heterozygous (mixed breeding) parents; the progeny have an equal likelihood of obtaining either of their two alleles from each parent, resulting in 3:1 ratio of dominant to recessive types. Note that Mendel’s laws would not explain any dominant progeny from recessive parents.

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nant genes, the progeny will all be dominant. If both parents have only recessive genes, the progeny will all be recessive. If both parents have both types of genes, then the progeny will have varying combinations of these genes in different proportions (Figure 1).

Mendel's work is often seen as an example of science at its best. Mendel spent years collecting observations and testing his theories on thousands of plants, and emerged with a brilliantly simple theory that is now taught to high school students as a fundamental tenet of genetics.

The HOTHEAD Discovery

Unfortunately, nature is rarely as simple as we think, and Mendel's theories have required subtle reshaping over the years to allow for such unforeseen phenomena as co-dominance, gene linkage, and incomplete dominance. However, the principles underlying Mendel's experiments have remained the same: traits are passed down from parents to offspring in a predictable manner and as discrete entities.

However, Lolle *et al.* provide data that seems to contradict the basis of our understanding of genetics. The HOTHEAD mutants used in Lolle *et al.*'s experiments cause the fusing of the flowers of the *Arabidopsis* plant. Lolle *et al.* isolated 11 unique point, or single base-pair, mutations in the recessive HOTHEAD gene that cause this fusion, and bred each plant so that it was homozygous for the mutant gene. Mendel's theories would predict that since each parent has only the mutant copy of the gene, all progeny should inherit those mutant copies. However, one to ten percent of these progeny were wild-type plants (Figure 2). Although we might expect a very small proportion to mutate back to the wild-type genome, the normal mutation rate alone could not account for such a high reversion rate (3).

After obtaining these results, Lolle *et al.* wanted to rule out any possible

sources of error, particularly those arising from external contamination. By analyzing the DNA through gel electrophoresis and other techniques, they were able to determine conclusively that all of the heterozygotes had inherited the specific copy of the mutant HOTHEAD allele from one of their parents, making external contamination unlikely. In addition, a small proportion of homozygotes was detected, meaning that not only the paternal, but also the maternal allele must have reverted. Moreover, dissections of seeds showed that a fraction of the unfertilized seeds also consisted of revertants. Finally, examination of pollen derived from a homozygous mutant plant showed transmission of a wild-type gene in some fraction of the cases (3). These exhaustive follow-up experiments showed that it unlikely that external contamination was the cause of these results.

Also, the lab ruled out the possibility that other genetic mechanisms may have given rise to the revertants. There is no other large sequence on the genome (or

on HOTHEAD-like genes) that could take part in recombination, or the exchange of long stretches of DNA from different parts of the genome. Moreover, the revertants possessed exactly the same sequence as the original wild-type gene, without any nearby base changes. Therefore, the idea of recombination with another section of the genome is unlikely (3). It is worth noting that Lolle *et al.* found a strong bias for genetic changes to occur in the male reproductive system, a fact that may be important to remember for future experiments.

Follow-Ups and Connections

The discovery of HOTHEAD revertants holds a great deal of promise for the future of science and medicine, but how do we know that it does not represent an isolated phenomenon unique to this particular gene in this particular laboratory plant?

Lolle *et al.* have already shown that mutant HOTHEAD alleles can destabilize other mutant alleles in the *Arabidopsis* genome. For example, the

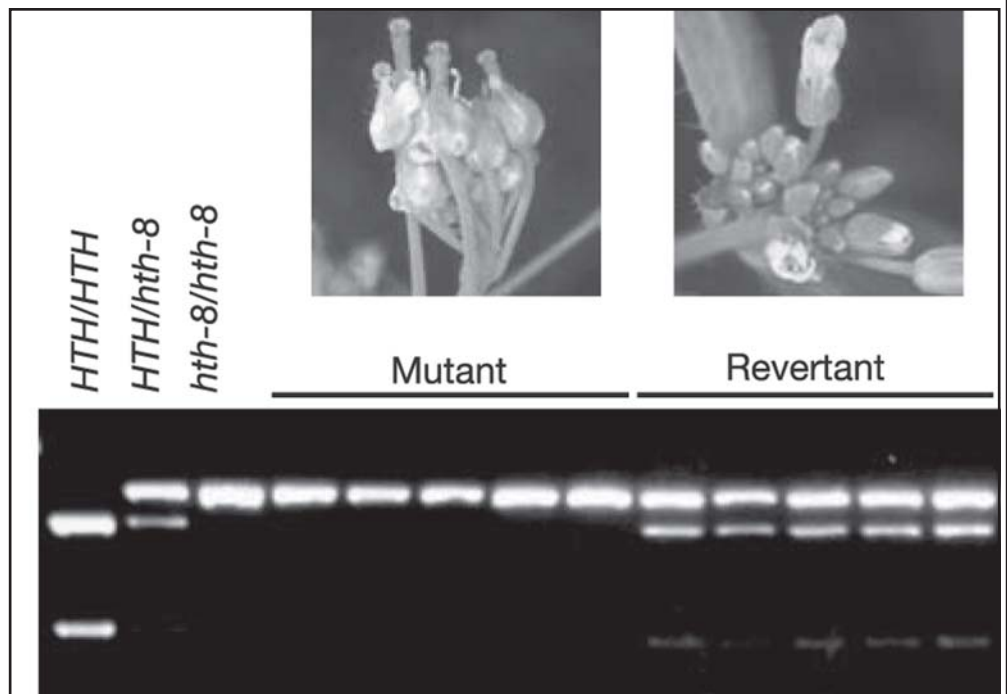


Figure 2. The *Arabidopsis* HOTHEAD mutant possesses organs that are fused (left). After plants homozygous for the mutant gene were bred, one to ten percent of progeny reverted to the normal *Arabidopsis* wild-type phenotype (right). Gel electrophoresis of the alleles in the plant show that revertant actually possess the wild-type allele (bottom).

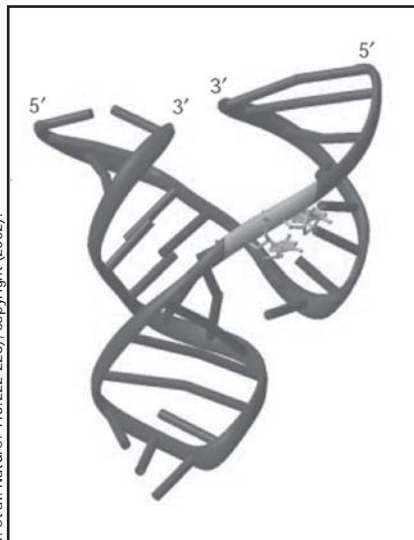


Figure 3. Scientists have only recently discovered many previously unknown functions for RNA. One category of RNA molecules, called ribozymes, has catalytic properties previously associated only with proteins. Ribozymes have now been found to be involved in many basic cellular functions, including protein synthesis.

erecta mutant, which causes smaller growth and helps minimize seed contamination (5), shows no revertants when it is the only mutant in an otherwise wild-type plant. When the plant contains both the *erecta* and HOTHHEAD mutants, both genes show revertants at about the same rate (3). The destabilization by HOTHHEAD mutants of other mutants is therefore an important effect to explore.

In addition, prior research on adaptive mutations has shown that increased environmental stress can increase the rate of adaptive mutations and revertants (6). Increased stress can be caused by a decrease in the fitness of the plant, which is often the result of deleterious mutations. Thus, when an organism experiences a genetic change that is disadvantageous for its survival, environmental stress increases and there is a greater likelihood that the change may be reversed in future generations.

Perhaps most interestingly, there has been evidence that some of these patterns may also apply to humans (7). For example, recent studies of type I tyrosinemia, an autosomal recessive disease that results in the production of a

dysfunctional liver protein, have examined the role of mosaics as revertants (8). Kvittingen *et al.* have found many patients who qualified as mosaics, meaning that some of their liver cells could create the functional protein while others created only dysfunctional protein. Kvittingen confirmed that though the individuals were homozygous recessive, some liver cells had reverted to the wild-type genotype by an unknown mechanism (9). Additional experiments conducted by different laboratories have shown that three homozygous individuals possessed reverted liver cells with a heterozygous genotype (10, 11).

Plausible Mechanisms and Future Directions

Though the discovery of HOTHHEAD revertants is still new and on the forefront of our scientific knowledge, there are already theories that attempt to explain how this process of reverting to a wild-type genotype might occur. Lolle *et al.* have suggested that there might be a stable cache of RNA that is transmitted over multiple generations (3, 12, 13). In the event that an undesirable mutation occurs, the RNA can reverse transcribe itself onto a template DNA strand. An article published in 1998 by Fire *et al.* supports this theory by showing that double stranded RNA is transmitted over multiple generations in *Caenorhabditis elegans*, a common laboratory worm (14).

In addition, recent research has discovered unusual functions for RNA in cells (Figure 3). RNA interference, the binding of short, double-stranded pieces of RNA to longer RNA strands, has been implicated in gene silencing, a previously unknown function. It is also widely believed that RNA was the original genetic material of life. The infrastructure that exists with RNA for its survival and replication would make it an obvious choice as a backup copy of DNA in the cell.

Regardless, much more experimentation needs to be done in order to find

the limits of this new phenomenon and the methods by which it can be harnessed for research or medical use. Future experiments should use different model organisms as well as different genes. Will this backup copy work with larger mutations or deletions? For how many generations does the revertant phenomenon last? The discovery of HOTHHEAD revertants has the potential to spark a revolution in our understanding of evolutionary biology, molecular biology, and the practice of medicine. **H**

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