

# An Alternate Hypothesis to Explain the High Frequency of “Revertants” in *Hothead* Mutants in *Arabidopsis*

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**Abstract:** Lolle et al. reported a high frequency of genomic changes in *Arabidopsis* plants carrying the *hothead* mutation and proposed that the changes observed were the result of a gene correction system mediated by a hypothetical RNA cache. Here, we propose a very different hypothesis to explain the data reported by Lolle et al. Our hypothesis is based on a relatively straightforward developmental aberration in which maternal cells (“Legacy cells”) fuse with the developing embryo, resulting in a chimera, which could then give rise to the aberrant genetic segregations reported by Lolle et al.

**Key words:** Hothead, legacy cell, non-Mendelian.

Lolle et al. (2005) reported non-Mendelian inheritance in *Arabidopsis* plants from the *hth/hth* (*hothead*) genotype. When *hth/hth* plants were self-pollinated, they observed 4.0 to 8.2% reversion of *hth* to *HTH*, the progeny inheriting a genotype found in the grandparent but not detected in the parent. The reversion to ancestral sequence was not limited to the *HTH* locus but also occurred at other molecular marker sequences tested.

Lolle et al. proposed an RNA template-mediated editing mechanism. According to this model, a yet undetected cache of RNA templates is passed on from generation to generation, and is used as template for “correcting” changes to the genome sequence. This mechanism may occur normally at a very low frequency in non-mutant plants, and at elevated frequencies in the *hth/hth* mutant background.

Since the original publication by Lolle et al., at least three alternative explanations have been advanced. Chaudhury (2005) proposed a mechanism in which the *hth* DNA sequence is restored by a gene conversion mechanism using homologous genomic sequences as templates. In addition, it was suggested that *hth* mutant cuticles may have increased permeability and so the embryo sac could be more porous to DNA fragments arising from the degraded spores, facilitating the homology-

based sequence editing. Similarly, a model proposed by Ray (2005) involves gene conversion based on chromatin fragments originating from the degenerating non-functional megaspores which could be taken up by the egg cell and archived in a way that is inaccessible to detection by PCR or DNA hybridization. The supernumerary chromatin fragments could propagate within the meristem and serve as template for conversion of the altered genome sequences. Finally, Comai and Cartwright (2005) proposed a biochemical mechanism of mutagenesis and selection to explain the *hth* reversion phenomena. According to this model, *hth* mutant plants may accumulate a mutagenic and toxic metabolite; the mutagenicity could enhance the rate of reversion, while the toxicity may lead to selection for the *Hth* revertant genotype.

The four hypotheses proposed previously to explain the observations of Lolle et al. are not mechanistically related to the *hothead* mutant phenotype and the known function of the *Hothead* gene. Here, we propose an alternative mechanism that is based on the phenotypic alteration in *hothead* mutant plants. *HOTHEAD* is involved in the formation of extracellular matrix and the biosynthesis of long-chain alpha-omega-dicarboxylic fatty acids (Kurdyukov et al., 2006). These fatty acids are required for the cross-linking that ensures the integrity of the outer epidermal cell wall. *Hothead* mutants are defective in epidermal cell interaction, leading to fusion of the floral organs (Krolikowski et al., 2003). We propose that in both *hth/hth* and *hth/HTH* plants, some of the maternal diploid cells may fuse with the developing embryo and become incorporated into the meristematic zone. The number of these “*Latent Legacy Cells*” that become incorporated into the developing meristem may be very few, and thus would escape detection by DNA hybridization or PCR of the progeny somatic tissue. However, when the floral meristem forms gametes, the latent legacy cells may pass on their allele to the next generation. Depending on the proportion of latent legacy cells in the floral meristem, the numbers of progeny exhibiting the grandparental genotype may range from 0 to 10%.

A key prediction of our model is that progeny plants that exhibit “genomic instability” of *hth* or other markers should contain the entire genome derived from the ancestral legacy cell; whereas, models involving stochastic molecular changes at individual loci would be expected to generate plants in which the genotype of different loci are not necessarily correlated. Pertinent to this point, Lolle et al. reported that the progeny of

a cross of *hth/hth* (Ler.) X *HTH/HTH* (Col.), exhibited “genomic instability” at six different molecular markers, including AG, GAPC, GL1, HTH, RGA, and UFO (Table 3 in Lolle et al.), although it was not stated whether changes at different loci were correlated.

Our model is based on the observation that *hth* mutants are defective in epidermal cell interaction and exhibit inappropriate cell fusions. The origin of the hypothetical legacy cells that undergo fusion with the developing embryo in *hothead* plants is unclear. It is tempting to speculate that these cells could be derived from the integuments, which are epidermal in origin. Another possible candidate is a megaspore mother cell (MMC); Grossniklaus and Schneitz (1998) have observed that about 5% of wild-type *Arabidopsis* ovules contain two megaspore mother cells (MMCs), of which only one matures.

The major disadvantage of our model is that it proposes two biological phenomena with little or no precedence. First, the fusion of a “legacy cell” of unknown origin with the developing embryo. Second, the persistence of the “legacy cell” in a latent state within the meristem from which it can contribute to the gametes giving rise to the following generation. On the other hand, our model is mechanistically less complicated than the alternative hypotheses, and it makes straightforward, testable predictions regarding the genotypes of *hothead* progeny plants that exhibit “reversion” to grandparental genotypes.

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