Retrotransposon-Induced Mutations in Grape Skin Color

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The color of grape skins is determined by the accumulation of red plant pigments called anthocyanins. White cultivars of grape are thought to have arisen from different red cultivars by independent mutations (*1*), but the molecular bases of these color mutations are unknown. *Myb*-related genes (such as *VlmybA1-1*, *VlmybA1-2*, and *VlmybA2*) regulate anthocyanin biosynthesis in Kyoho, a black-skinned cultivar of *Vitis labruscana* (*2*). We show that a retrotransposon-induced mutation in *VvmybA1*, a homolog of *VlmybA1-1*, is associated with the loss of pigmentation in white cultivars of *V. vinifera*.

Two red-skinned cultivars of *V. vinifera*, Ruby Okuyama (Ru) and Flame Muscat (Fl), are derived by bud mutation from the white-skinned cultivars Italia (It) and Muscat of Alexandria (Al), respectively. Using VlmybA1-1 from Kyoho as a probe, we detected two transcripts in white-skinned cultivars and three in the red-skinned sports. Sequencing identified transcripts VvmybA2 (DNA Data Bank of Japan accession no. AB097924) and VvmybA3 (AB097925) in all four cultivars and VvmybA1 (AB097923) in the red cultivars (Fig. 1A). In the other cultivars examined, *VvmybA1* transcript was also detected only in the colored ones (Fig.1B). VvmybA1 cDNA induced red pigmentation when introduced into the skin tissues of white grapes (*3*).

Genomic clones for VvmybA1 indicated that *VvmybA1* is homozygous (*VvmybA1a*, AB111100) in It, but heterozygous in Ru (Fig. 1C). The heterozygous alleles, *VvmybA1a* and *VvmybA1b* (AB111101), differed in their 5′-flanking region but were identical in their coding sequences. *VvmybA1a* contained a retrotransposon, designated *Gret1* (grapevine retrotransposon 1), upstream of the *VvmybA1*-coding sequences. *Gret1* was 10,422 base pairs (bp) long: 824 bp of a 5′–long terminal repeat (LTR), 8774 bp of an internal region, and 824 bp of a 3′-LTR. The sequences of the two LTRs differed at only four nucleotides, suggesting a relatively recent insertion event. The internal region of *Gret1* showed similarities to the *gag-pol* region of the Ty3-*gypsy*–type retrotransposons *RetroSor1* (AF098806), *RIRE2* (AB030283), and *Cinful-1* (AF049110). Mutations caused by retrotransposon insertions in or near genes can alter gene expression or the structure of the encoded proteins (*4*). Since no *VvmybA1* transcript was detected in It, the *Gret1* insertion in *VvmybA1a* must block expression of the gene.

Southern blot analyses indicated that *Gret1*-related retrotransposons are present in multiple copies in grapevine genomes (*3*). The *VvmybA1a* allele is widely distributed among cultivars of *V. vinifera* and *V. labruscana* (fig. S1). Cultivation of the grapevine likely began during the Neolithic era (6000 to 5000 B.C.) along the eastern shores of the Black Sea (*5*). We hypothesize that *Gret1* originally inserted upstream of one of the *VvmybA1*-coding sequences of a black-skinned ancestor and that, subsequently, a white-skinned grape was produced by spontaneous crossing.

In the *VvmybA1b* allele, *Gret1* was missing, leaving behind its 3′-LTR flanked by 5 bp of a duplicated target site (Fig. 1C). This structure is reminiscent of a reversion event previously documented in yeast that was attributed to recombination between LTRs (*6*). Polymerase chain reaction (PCR) fragments amplified from *VvmybA1b* of Fl and Ru had identical sequences (fig. S1), suggesting that these red cultivars are derived from their white-skinned progenitors by the same mechanism. Smaller-sized fragments, representing *VvmybA1* alleles without *Gret1* or a solo LTR, were produced in the other colored cultivars tested (fig. S1). Our data indicate that a retrotransposon insertion in *VvmybA1* is the molecular basis of white coloration in It and Al and that the same mutant allele has spread among most, if not all, white grape cultivars in the world.

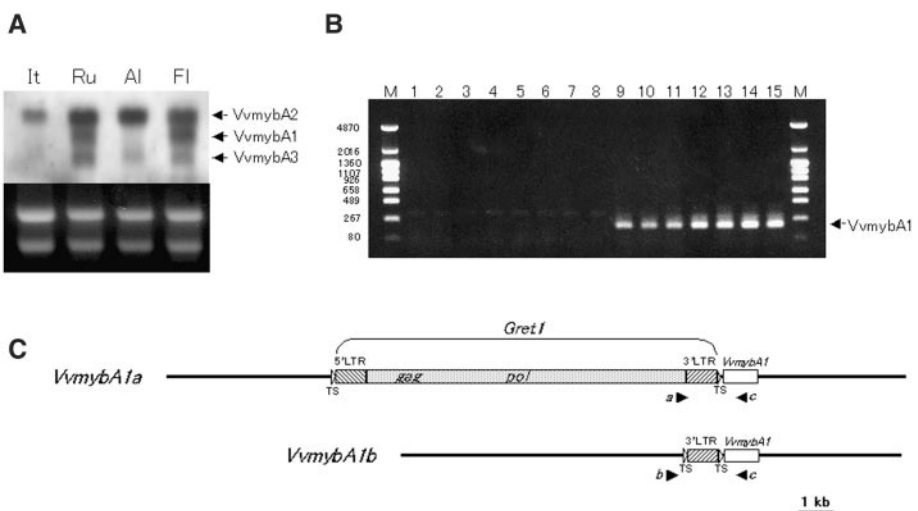

Fig. 1. (**A**) Top: Northern blot analysis. Bottom: Ethidium bromide–stained gel. (**B**) Reverse transcription–PCR detection of *VvmybA1* transcript in other cultivars of *V. vinifera*. Lanes 1 to 8, white cultivars; lanes 9 to 15, colored cultivars (*7*). (**C**) *VvmybA1a* clones contained *Gret1*, but *Gret1* was missing in *VvmybA1b* clones. *a*, *b*, and *c* represent the positions of primers used for PCR analysis (fig. S1); TS represents a duplicated target site.

References and Notes

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7. Materials and methods are available as supporting material on *Science* Online.

Supporting Online Material
www.sciencemag.org/cgi/content/full/304/5673/982/DC1
Materials and Methods
Fig. S1
References and Notes

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