Cauliflower Mosaic Virus Promoter: Potential Risks

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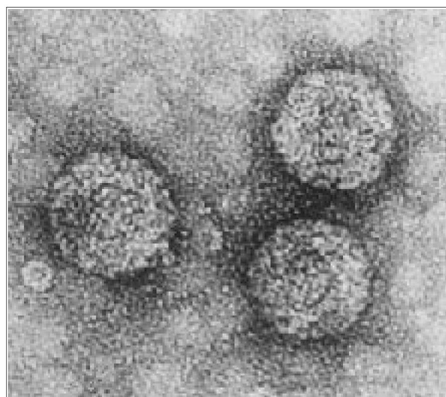

Issue

Potential risks associated with the application of the 35S promoter from the plant cauliflower mosaic virus in genetically engineered plants have recently been the focus of much attention. The risks have been described as including the free movement of these sequences from transgenic plants to other organisms and the reactivation of dormant viruses (Ho *et al.* 1999).

Background

Since the production of the first transgenic plant in 1983, the 35S promoter from cauliflower mosaic virus (CaMV) has seen widespread application in regulating the constitutive expression of foreign genes in genetically engineered plants. As proof of its versatility, this promoter has been used in at least 10 crop species, including canola, carnation, corn, cotton, potato, rice, soybean, squash, sugar beet and tomato.

CaMV is a spherical, DNA-containing plant virus that is naturally transmitted by aphids to crops such as broccoli, cabbage and turnips. The virus is worldwide in its distribution and is found wherever susceptible crops are grown and is present in the food products derived from these crops.

Much has been made of the fact that CaMV belongs to a class of viruses termed pararetroviruses, and in comparing it to true retroviruses such as human immunodeficiency virus (HIV). These two classes of viruses are similar in that the genome of each encodes a reverse transcriptase enzyme that is used during virus replication. In other respects these types of viruses are quite different. Retroviruses encapsidate an RNA genome and replicate through a DNA intermediate that is integrated into host chromosomes. Pararetroviruses, such as CaMV, encapsidate a DNA genome and replicate through an RNA intermediate. Their genomes remain as episomes in the nuclei and are never integrated into host chromosomes. It is also important to note that plant viruses such as CaMV do not replicate in animal cells and that animal retroviruses do not replicate in plant cells.

What then are the legitimate risks associated with taking this small piece of DNA from a virus which already occurs in the food we eat, and inserting it into the genome of a plant?

The only experimentally demonstrable risk has been the potential for genetic recombination between the genome of an infecting plant virus and the plant transgene, or more precisely, its messenger RNA (mRNA) transcript. The potential risk is the creation of a new, possibly more virulent strain of the infecting virus.

The acquisition of viral genes from transgenic plants has recently been demonstrated for at least three different plant viruses (Lommel & Xiong, 1991; Greene & Allison, 1994), including CaMV (Gal *et al.* 1992; Schoelz & Wintermantel, 1993; Wintermantel & Schoelz, 1996). A feature common to the experimental design of all of these studies was the introduction of a medium or strong selection pressure in order to maximize the likelihood of observing recombination events. An example of strong selective pressure would be the inability of the virus to systemically infect a host unless a recombination event occurs. In three of the studies, deletion mutants that were defective for systemic infection were inoculated into transgenic plants expressing the complementary or restorative function. The only viruses capable of systemic infection were those formed by recombination between the mutant virus and the transgene mRNA. In each of these cases, recombination was independent of the promoter sequences present and was dependent on the existence of sequence homology between the infecting virus RNA and the mRNA of the engineered gene.

These studies suggest that recombinant viruses would likely not become established in transgenic plants unless the transgene conferred a significant selective advantage over the wild type virus (Falk & Bruening, 1994). In the case of a herbicide tolerance or insecticidal trait under control of the CaMV 35S promoter, it is hard to envision an RNA-based recombination mechanism similar to that described above, as there is no sequence homology between the genes for these traits and any viral genes. And if it were to occur, it is equally hard to imagine how a gene encoding for herbicide tolerance or insecticidal activity would confer any selective advantage to a plant virus.

At the level of the plant genome, genetic recombination during meiosis is a naturally occurring phenomenon that is responsible for creating new arrangements of genes on chromosomes, thus adding to genetic diversity. In the absence of meiotic recombination, all genes residing on the same chromosome would segregate as a unit and thus display absolute genetic linkage. It has long been known that this type of recombination does not occur randomly across the lengths of chromosomes, but rather at so called recombination hotspots. The characterization of such hotspots and where they occur in the genome is an area of active research.

The recent work by Kohli *et al.* (1999) in examining the genomic DNA – transgene junctions following plant transformation may have implications with respect to transgene stability and segregation within the plant genome. In analyzing 12 different transgenic rice lines, produced via injection with plasmid DNA, they found a range of genetic rearrangements which were the result of recombination and they also demonstrated the presence of a recombination hotspot within the sequence of the CaMV 35S promoter.

These findings do not, however, imply an increased risk of horizontal gene transfer due to the incorporation of the CaMV 35S promoter into the plant genome. Any mechanism postulating a potential risk of transfer of the 35S promoter from transgenic plant DNA to other organisms must also apply in the reverse. Over the millions of years during which CaMV and related viruses have been infecting and replicating in plants there has been ample opportunity for recombination and the possible transfer of the 35S promoter to the plant genome. This event has yet to be recorded.

To date, the only observed horizontal gene transfers involving CaMV have been RNA mediated recombination events involving homologous defective, or closely related, plant viruses in experimental systems that were purposefully biased to favour the observation of a recombination event. Mixed infections of different viruses within the same host allow for a similar type of RNA recombination that has been experimentally verified in a number of cases (Rao & Hull, 1993; Cascone *et al.* 1993; Allison *et al.*1990).

Position

The available scientific information does not support the contention that the application of the CaMV 35S promoter in the genetic engineering of plants would pose an increased risk of horizontal gene transfer or any additional risk to human health.

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