CaMV 35S Promoter Fragmentation Hotspot Confirmed, and it is Active in Animals

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We would like to address the suggestion of Matzke et al. (1) and others that pararetroviral promoters such as the CaMV 35S are not exotic to plant genomes as they already contain many integrated pararetroviral sequences. The crucial question is whether the CaMV 35S promoter in transgenic constructs poses special risks. We would like to draw attention to several publications that are relevant to this issue.

Kumpatla and Hall (2) analyzed a transgenic rice locus and confirmed that fragmentation and recombination occur frequently within the CaMV 35S promoter, but not in the wheat plant ubiquitin promoter used in another transgenic cassette. This indicates that the CaMV promoter is not like any other promoter. Six out of seven recombination junctions in the CaMV promoter map near the 19 basepair palindrome identified as a recombination hotspot by Kohli et al. (3).

The conventional wisdom among plant molecular geneticists is that plant promoters, such as the CaMV 35S, are not active in animals (4). In fact, the CaMV 35S promoter was found to support high levels of reporter gene expression in mature *Xenopus* oocytes (5), and to give very efficient transcription in extracts of HeLa cell nuclei (6). The CaMV promoter worked at least as well as the SV40 promoter in *Xenopus* oocytes, and better than the major late promoter of the adenovirus-2 in HeLa cell extracts.

So, while the CaMV is specific for plants in the cruciferae family, its isolated promoter is promiscuous across domains and kingdoms of living organisms. It is the genetic (and evolutionary) context that makes all the difference. There is no justification for claiming that the promoter in transgenic constructs is as safe as the promoter in the intact viral genome, nor to consider it equivalent to the promoter of proviral sequences in the plant genome.

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