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They should be addressed to:
Correspondence
Nature Biotechnology
345 Park Avenue South
New York, NY 10010-1707, USA
or sent by e-mail to biotech@natureny.com
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Hazardous CaMV promoter?

To the editor:

In your account (January 2000) of our prepublication manuscript[1], you quote the criticisms but ignore completely our full rebuttal, which was posted on the web last November[2].

Our manuscript[3] reviews and synthesizes the scientific literature on the 35S promoter of the cauliflower mosaic virus (CaMV), used to give constitutive overexpression of transgenes in practically all GM crops already commercialized or undergoing field trials. The promoter functions efficiently in all plants, as well as green algae, yeast, and *Escherichia coli*. It has a modular structure, with parts common to, and interchangeable with promoters of other plant and animal viruses. It also has a recombination hotspot, flanked by multiple motifs involved in recombination, similar to other recombination hotspots including the borders of the *Agrobacterium* T DNA vector most frequently used in making transgenic plants. The suspected mechanism of recombination—double-stranded DNA break repair—requires little or no DNA sequence homologies, and recombination between viral transgenes and infecting viruses has been demonstrated in the laboratory[4].

The findings suggest that transgenic constructs with the CaMV 35S promoter may be structurally unstable and prone to horizontal gene transfer and recombination. The potential hazards are mutagenesis, carcinogenesis, reactivation of dormant viruses, and generation of new viruses. These considerations are especially relevant in the light of recent findings that certain transgenic potatoes—containing the CaMV 35S promoter—may be unsafe for young rats, and that a significant part of the effects may be due to "the construct or the genetic transformation (or both)"[5].

Our critics believe the CaMV 35S promoter is not harmful because people have been eating the virus in infected cabbages and cauliflower for many years. What we have been consuming is predominantly intact virus and not naked viral genomes. Naked viral genomes have been found to give full-blown infections in nonhost species that are not susceptible to the intact virus[6,7]. Moreover, the 35S promoter in the CaMV is a stable, integral part of the virus, and cannot be compared to the 35S promoter in artificial transgenic constructs. Artificial constructs are well known to be structurally unstable[8]. We know that the 35S promoter in the virus does not transfer into genomes because pararetroviruses, such as CaMV, do not integrate into host genomes to complete their life cycle; and viral replication takes place in the cytoplasm[9]. But that says nothing about the 35S promoter in transgenic constructs that are integrated into host genomes.

Proviral sequences are present in all genomes, and have at least one module—the TATA box—in common, if not more; it is not inconceivable that the 35S promoter in transgenic constructs can reactivate dormant viruses or generate new viruses by recombination. The CaMV 35S promoter has been joined artificially to the cDNAs of a wide range of viral genomes, and infectious viruses produced in the laboratory[10]. There is also evidence that proviral sequence in the genome can be reactivated[11].

The fact that plants are "loaded" with potentially mobile elements can only make things worse. Most, if not all, of the elements will have been "tamed" in the course of evolution and hence no longer mobile. But integration of transgenic constructs containing the 35S promoter may mobilize the elements. The elements may in turn provide helper functions to destabilize the transgenic DNA, and may also serve as substrates for recombination to generate more exotic invasive elements. In signing on to the International Biosafety Protocol in January, more than 150 governments agreed to implement the precautionary principle. The available evidence clearly indicates that there are serious potential hazards associated with the use of the CaMV promoter. All GM crops and products containing the CaMV promoter should therefore be withdrawn both from commercial use and from field trials unless and until they can be shown to be safe.

1. Hodgson, J. *Nat. Biotechnol*. **18**, 13 (2000).
2. Institute of Science in Society (www.i-sis.org).
3. Ho, M-W., Ryan, A. & Cummins, J. *Microbial ecology in health and disease*, **in press** (2000), and available in electronic form (www.scup.no/mehd/ho).
4. Wintermantel, W. & Schoelz, J. *Virology* **223**, 156–164 (1996).
5. Ewen, S.W.B. & Pusztai, A. *Lancet* **354**, 1353–1354 (1999).
6. See for example, Rekvig, O.P. et al. *Sc and. J. Immunol.* **36**, 487–495 (1992).
7. Structural instability of artificial vectors is a textbook topic. See Old, R.W. & Primrose, S.B. *Principles of gene manipulation,* Edn. 5. (Blackwell, Oxford; 1994).
8. Covey, S. et al. *Proc. Natl. Acad. Sci. USA* **87**, 1633–1637 (1990).
9. Maiss, E. et al. *J. Gen. Virol*. **73**, 709–713 (1992).
10. Meyer, M & Dessens, J. *J. Gen. Virol*. **78**, 147–151 (1997).
11. Nowora, T. et al. *Virology* **255**, 214–220 (1999).

Joe Cummins
Dept. Plant Sciences, University of Western Ontario, Ontario, Canada
Mae-Wan Ho and Angela Ryan
Department of Biological Sciences
Open University, Walton Hall
Milton Keynes, UK
MK7 6AA

John Hodgson replies:

An apology is clearly due to Cummings et al. for not having drawn attention in my article to their rebuttal of the critics I cited. In my defense I can only say that my nearly 20 years' experience of science publishing had not prepared me to expect a rebuttal to unpublished comments on an unpublished manuscript.

The criticism of my article and their critics is largely misdirected and their choice of supporting data (other people's) questionable.

They point, for instance, to a range of "potential hazards" of the structural instability of constructs containing CaMV 35S promoter sequences. Their choice of key published data in support of this proposition is the work of Stanley Ewen and Arpad Pusztai on the intestinal effects of potato diets on rats. Whatever its scientific merits (and those have been widely discussed), that paper does not claim that "a significant part of the effects may be due to" the construct or the transformation but merely that "other parts of the GM construct, or the transformation, could have contributed to the overall effects." In any case, identifying potential hazards is of little use unless the relative magnitude of either the potential or of the hazard is described. Exposure to sunlight after all, even in Milton Keynes or Ontario, will elicit many if not all of the same "potential hazards." A more proper comparison, perhaps, would be with the risks from the ingestion of plants produced by "conventional breeding" (i.e., through random mutagenesis, crossing, and selection).

The other key point made—that naked transgenic DNA containing CaMV 35S sequence might be harmful to humans—is also not well supported by their choice of reference. The work of Rekvig et al. that they cite concerns the inoculation of rabbits with naked BK virus, a human polyomavirus. How does this evidence of an experimental infection of one mammalian species by an integrating virus from another mammalian species support a thesis that, in essence, seems to be that laboratory-created constructs including promoter regions from a plant virus that does not integrate naturally into host genomes will (1) infect humans (2) integrate into human genomes, and (3) cause "potential hazards."

The final paragraph of Cummings and Ho's letter linking their arguments to the Biosafety Protocol could be considered revealing. A cynic might posit that the reason their review paper was publicized in December 1999 and January 2000—even though it was not actually published then—was to try to influence decision makers involved in finalizing global rules on the transborder shipment of living modified organisms. However, such a supposition, unsupported as it is by any real data, would require such a convoluted and contorted train of logic as to be utterly unbelievable.