# Hazards of transgenic plants containing the cauliflower mosaic viral promoter

## *Authors' reply to critiques of "The Cauliflower Mosaic Viral Promoter—a Recipe for Disaster?"*

Mae-Wan Ho[1], Angela Ryan[1] and Joe Cummins[2]

From the [1]Institute of Science in Society and Biology Department, Open University, Walton Hall, Milton Keynes, MK7 6AA, UK. [2]Department of Plant Sciences, University of Western Ontario, Ontario, Canada

Correspondence to: Mae-Wan Ho Biology Department, Open University, Walton Hall, Milton Kenyes MK7 6AA, UK

Microbial Ecology in Health and Disease 2000; 12: 6–11

(We are ignoring the comments of P. Christou, as they bear little relationship to the actual article that we submitted, and was published in your Journal. Our remarks are directed to the critiques from Hull, R., Covey, S. N. and Dale, P. of The John Innes Centre, and from Oliver Rautenberg of Biolinx.)

### WE REVIEWED AND SYNTHESIZED EXISTING FINDINGS TO PREDICT POTENTIAL HAZARDS

As Rautenberg (1) rightly points out, our paper (2) was not drawn from research work that we have done ourselves, rather it was written to review and synthesize the scientific literature on and around the CaMV 35S promoter. This is a legitimate and important part of scientific activity, as science does not consist of isolated facts which bear no relationship to one another. It is precisely the web of mutual interrelationships of the findings that constitute science. More importantly, this maps out the universe of possibilities both for further research and for predicting potential hazards in risk assessment. Our critics disagree with the implications we draw from the scientific findings, and especially with our conclusions and recommendation.

To recapitulate, we pointed out that the CaMV 35S promoter is promiscuous in function, and works efficiently in all plants, as well as green algae, yeast and *E. 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, and is 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. Finally, recombination between viral transgenes and infecting viruses has been demonstrated in the laboratory.

Transgenic constructs are already well-known to be unstable, and the existence of a recombination hotspot will exacerbate the problem. Consequently, transgenic constructs containing the CaMV promoter may be more prone to horizontal gene transfer and recombination than non-transgenic DNA. The potential hazards include genome rearrangement, insertion mutagenesis, insertion carcinogenesis, the reactivation of dormant viruses and generation of new viruses (reviewed in refs. 3 and 4). These considerations are especially relevant in the light of recent findings that certain transgenic potatoes-containing the CaMV 35S promoter and transformed with *Agrobacterium* T-DNA-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). Consequently, we called for all transgenic crops and products containing the CaMV promoter to be withdrawn and banned, which is in accordance with the precautionary principle as well as sound science.

### OBJECTIONS RAISED BY OUR CRITICS

Our critics do not disagree with our description of the findings but with the implications we draw, and with our conclusion. They raised a number of objections, which are listed as follows.

1. Cauliflower mosaic virus infects a wide range of crucifers eaten by human beings, and no ill effects have been found either through recombination to cause over-ex-

 ISSN 0891-060X

pression of normal genes or by producing new viruses (1, 6).

2. CaMV and other pararetroviruses, as well as the numerous related retrotransposons present in plant genomes have never been found to generate new viruses by recombination "in spite of intensive research on these virus groups" (6).
3. CaMV promoter is not unique. Many plant promoters are expected to share similar features. Recombination is a normal feature of conventional plant breeding and of all populations. Therefore, no new viruses could be generated by CaMV promoter in transgenic plants.
4. The recombination events described by Kohli et al (7) which we refer to, gives evidence for recombination events *before* the transgene is integrated and is therefore not relevant to its stability in the transgenic plants and may be due to their particular construct consisting of the CaMV 35S promoter being associated in all three linked gene-expression cassettes (6).
5. Relationship between CaMV and hepadnaviruses such as the human hepatitis B virus is not sufficient for the CaMV promoter to recombine with it. They have significantly different replication cycles and there is no sequence similarity between the hotspot of the CaMV 35S promoter and the hepadnavirus sequences important in replication (6).
6. For CaMV promoter to recombine with human or other animal or plant viruses, or to cause over-expression of host genes, the entire promoter would have to be excised and reinserted precisely at the new site or its 3' end linked precisely with the host gene or the viral gene. This is not likely, as there is only one recombination hotspot associated with the CaMV 35S promoter (6). Furthermore, the intact promoter will not survive digestion in the animal's gut.
7. Recombination involving the CaMV 35S promoter, if it occurs, is a rare event, as transgenic sequences are present in very low concentrations, i.e., one or at most several copies per cell. Furthermore, rare recombinants will be selected out when seeds are bulked up at an early stage (6).
8. Potentially carcinogenic compounds already exist in abundance in natural food plants (6). Therefore it is unnecessary to be concerned about generating new toxins or carcinogens in transgenic plants.

We shall try to deal with all of these objections, in the spirit of "enhancing the debate" (b).

## THE INTACT VIRUS DIFFERS SIGNIFICANTLY FROM THE NAKED VIRAL GENOMES

The intact, encapsidated CaMV, consisting of the CaMV genome wrapped in its protein coat, is not infectious for human beings nor for other non-susceptible animals and plants, as is well-known; for it is the coat that determines host susceptibility in the first instance. So eating the intact virus (objection 1 above) is of little consequence. However, the naked or free viral genomes may be more infectious and have a wider host-range than the intact virus. Human T-cell leukemia viral genomes formed complete viruses when injected into the bloodstream of rabbits (8). Similarly, the genomes of the human polyomavirus BK (BKV) gave a full-blown infection when injected into rabbits, despite the fact that the intact BKV is not infectious (9). Recent developments in gene therapy and nucleic acid vaccines leave little doubt that naked or free nucleic acids can readily gain access to all cells of model animals and human beings (4). The fate of such nucleic acids, once internalized, depends on the particular constructs. For example, it is recently found that integrated viral sequences in genomes of dead cells are much more readily transferred horizontally to the genomes of live cells that have taken up the fragmented DNA (10). It is also found that non-integrated viral sequences replicated as episomes are rarely, if ever, transferred.

There is a world of difference between viral genomes containing the CaMV 35S promoter as an integral part of the virus-adapted to the virus and to the host over millions of years of evolution-and the CaMV 35S promoter taken out of context, joined up with a strange gene and inserted into a strange genome. So, eating naked viral genomes (objection 1) may have little effect but that says nothing about the transgenic DNA containing the CaMV 35S promoter.

## THE CaMV 35S PROMOTER IN THE VIRAL GENOME DIFFERS SIGNIFICANTLY FROM THE CaMV 35S PROMOTER IN TRANSGENIC DNA

As Hull et al (6) emphasize, there are many constraints on natural recombination, and natural recombination between viruses is very rare (objection 7), possibly because each viral genome has a natural integrity and stability resulting from millions of years of evolution. The 35S promoter in the virus does not transfer into genomes because pararetroviruses like CaMV, do not need to integrate into host genomes to complete their lifecycle; and the virus replicates in the cytoplasm away from the host genome. Nevertheless, some pararetroviral sequences have been found integrated into plant genomes (11).

It is also clear that recombination between viral transgenes and infecting viruses *can* occur. A number of studies have demonstrated that plant viruses can acquire a variety of viral genes from transgenic plants. It indicates that the viral transgene, isolated from the virus and integrated into the host genome, cannot be equated with the same gene in the virus itself. This is relevant to objections 2, 3, 4 5 and 7 raised by our critics.

Defective red clover necrotic mosaic virus lacking the cell-to-cell movement gene, and hence not infectious, recombined with a copy of that gene in transgenic *Nicotiana*

*benthamiana* plants to regenerate infectious viruses (12). Transgenic *Brassica napus* containing CaMV gene VI, a translational activator, recombined with the complementary part of the virus missing that gene (13), and gave infectious virus in 100% of the transgenic plants. The same experiment carried out in *Nicotiana bigelovii* (14) gave infectious recombinants that expanded the host range of the virus. *N. benthamiana* plants expressing a segment of the cowpea chlorotic mottle virus (CCMV) coat-protein gene recombined with defective virus missing that gene (15). A later report stated that recombination between transgenes and infecting virus in CCMV was much more frequent than recombination between co-infecting viruses (16), despite the fact that transgenic sequences occur at very low concentrations compared with co-infecting viruses (objection 7). The same plants transformed with three different constructs containing the coat protein coding sequence of African cassava mosaic virus (ACMV) recombined with a deletion mutant of the coat protein to regenerate the wild-type virus that produced severe symptoms of infection in the transgenic plants (17).

As all the experiments involved recombination between defective virus and transgene, it was thought that under natural conditions, when viruses are not defective, no recombinant viruses would be generated (18). However, recombination between wild-type CaMV and transgene VI was demonstrated in *N. bigelovii* (19). At least one of the recombinant viruses was more virulent than the wild type.

Green and Allison (20) found that trimming the 3′ end of the CCW transgene containing the untranslated region (UTR) reduced recombination to zero, as compared with 3% in the controls. As ribonucleotide sequences within the 3′ UTR are involved in initiating viral replication, the presence of this sequence may encourage the participation of the transgene in RNA recombination. This suggests that most, if not all of the recombinations may involve template-switching between homologous sequences during viral replication. Recent findings also indicate that the viral RNA-dependent RNA polymerase of several potyviruses and tomato aspermy virus have the ability to recognize heterologous 3′ UTR (from lettuce mosaic virus and cucumber mosaic virus) included in transgene mRNAs, and to use them as transcription promoters (21). These findings have important implications for the safety of viral resistant transgenic plants in general.

It has been noted in experiments involving CaMV (19), that the frequency of recombination is much higher than for other viruses. While recombinant CCMV was recovered from 3% of transgenic *N. benthamiana* containing CCMV sequences, recombinant CaMV was recovered from 36% of transgenic *N. bigelovii*. It was suspected that double-stranded DNA breaks may be involved in the case of CaMV recombination. This may be due to the fact that the transgenic DNA included the CaMV 35S promoter.

## STRUCTURAL INSTABILITY OF TRANSGENIC DNA VERSUS NATURAL HOST DNA

Our critics believe that the recombination hotspot of CaMV 35S promoter in transgenic DNA is not unique, as all promoter contain recombination hotspots and recombination in genomes is a normal event (objection 3).

It is well-known, however, that pieces of DNA taken out of context and recombined in novel configurations are likely to be unstable, so much so that structural instability of artificial vectors for genetic engineering-made by joining pieces of DNA from different viruses, plasmids, transposons and other sources-is a topic discussed in a text-book on genetic engineering (22). This instability also renders the artificial vectors prone to recombination and rearrangement.

The CaMV 35S promoter in the transgenic plant is part of the transgenic DNA introduced, which is a highly mosaic construct. The CaMV promoter is joined to a gene it has never been linked to before, to form an 'expression cassette'. Several expression-cassettes are often stacked in series, and spliced in turn into an artificial vector, most often T DNA, which is also known to be flanked by recombination hotspots (see ref; 7). Such a structure typical of transgenic DNA is recognized to be unstable and to have a propensity for rearrangements and for horizontal gene transfer (23, 24). This is stated explicitly in a recent scientific report commissioned by the UK Health and Safety Executive (25),

> "The location of released genes on mobile genetic elements or in close association within IS sequences, transposons, gene cassettes or hot-spots for recombination ... can all increase the probability of horizontal gene transfer." p. 70.

## CaMV PROMOTER IN TRANSGENIC DNA DIFFERS SIGNIFICANTLY FROM THE PLANT'S OWN PROMOTERS, INTEGRATED VIRUSES AND OTHER POSSIBLE RECOMBINATION HOTSPOTS

The CaMV promoter in transgenic DNA is also quite different from the promoters of the plant's own genes (objection 3). Structurally, the plant's own promoters will be expected to be much more stable than the CaMV promoter in transgenic DNA for the following reasons. First, the plant's genes and their promoters exist in an organized genome, where recombination is predominantly between homologous alleles, so each promoter will remain associated with alleles of the same gene after recombination. Second, each host gene and its promoter have been adapted to each other, structurally and functionally, in the context of the whole genome, for hundreds of millions of years, and therefore expected to be much more stable than the transgenic DNA containing CaMV promoter. Integrated, inactive viruses and retrotransposons, similarly, have been 'tamed' by the plant, probably for millions of years and hence, again, more likely to be stable than the

new intruder (objection 3). As Hull et al (6) state, most, if not all of the retrotransposons are no longer mobile. Third, the mere integration of transgenic DNA into a host chromosome creates regions of non-homology, which will be expected to further disrupt chromosomal structure due to unequal cross-over in mitotic and meiotic recombination.

Instability of transgene expression as the result of 'gene-silencing' is now well-recognized and actively researched (see ref. 26 for the latest mechanism discovered). Structural instability involving secondary mobility or rearrangement of integrated transgenes is also a common cause of break-down of transgenic lines (discussed in ref. 27).

Structural instability of transgenes is generally underestimated, as cells which lose transgenes are automatically eliminated during the selection process necessary for producing transgenic lines. However, instability may arise even in later generations of plant propagation (objection 7). We are aware of no published data documenting the structural stability of transgenic lines in successive generations, even though phenotypic instability has been documented, for example, in transgenic bt-cotton commercially grown in Southern United States in 1996 (28), and Roundup cotton in 1997 (29). Physiological stress due to extremes of temperature, or drought, which can mobilize transposons, may increase transgene instability. The constitutive overexpression of transgenes linked to the CaMV 35S promoter, similarly is a metabolic stress-factor that may increase transgene instability (30).

Hull et al (6) suppose that as there is only one recombination hotspot in the CaMV 35 promoter, it is not likely to undergo horizontal gene transfer (objection 6). Of course, even one double-stranded break can give rise to genetic rearrangement, and result in the CaMV 35S promoter being associated with host gene or proviral sequences. But more to the point, the transgenic construct typically contains multiple recombination hotspots. For example, most transgenic plants created with the *Agrobacterium* T-DNA vector will have transgenic DNA flanked by the left and right borders, both recombination hotspots. In addition, gene expression cassettes include terminators that are also recombination hotspots (as discussed in ref. 7). So all or part of the transgenic DNA may be prone to horizontal transfer.

It is quite likely that stacking CaMV promoters in three successive expression cassettes, as Kohli et al. (7) have done, will increase structural instability further (objection 4). The recombinations and rearrangements they have observed in the different transgenic lines, however, may have occurred both before and after the primary transformation events, during propagation and selection in cell and plant culture. This is something that must be addressed by empirical investigations.

## FUNCTIONAL CONSEQUENCES OF THE MODULAR CONSTRUCTION OF ALL PROMOTERS, INCLUDING THE CaMV 35S PROMOTER

The modular construction of promoters has two important consequences, the first of which is related to the function of promoters. Different modules or elements in the promoter respond to signals from a battery of other genes coding for transcription factors, which determine the tissue specificity, timing and amplitude of gene expression. Thus, promoters allow genes to talk to one another, forming complex intercommunication networks that enable the tens of thousands of genes in an organism to function as a coherent whole, and as appropriate to the environment. Some of the elements in the promoter also enable it to respond to recombinases and other enzymes that result in recombination, rearrangement, mobility and mutation. This kind of 'natural genetic engineering' (31) is quite precise, as it is regulated by the organism as a whole, in ways that we still do not understand (32). The structure of chromatin itself is now known to contribute to this regulation. Histones are modified in accordance to an as yet unknown 'histone code' which modulates gene function through chromatin structure in all eukaryotic genomes (33).

One can see why random insertion of foreign DNA into this incredibly complex, mutually entangled and subtle regulatory system will give a range of unexpected, unintended effects, especially when the foreign DNA includes the strong constitutive CaMV 35S promoter. The integration of transgenic DNA into genomes is known to have many unexpected effects, including mutations, cancers (in the case of mammalian cells) and changes in DNA methylation, a chemical modification of DNA which can affect activities of host genes. The effects are known to extend far away from the site of insertion (34). Hull et al (6) are mistaken to suppose that the CaMV promoter has to be placed exactly next to a gene in order to make it over-express (objection 6). In a recent experiment in insertion mutagenesis using a synthetic mini-transposon, researchers discovered an event resulting in the over-expression of a host gene which is 164 basepairs away from the site of insertion (35).

Thus, the possibility of new toxins and allergens arising cannot be easily dismissed on account of both position effects due to random insertion of transgenic DNA and pleiotropic effects due to functional interactions with host genes. The suggestion that potentially carcinogenic compounds occur in abundance in natural plants (objection 8) is irrelevant. These foods have been eaten for tens of thousands of years, and compounds which are carcinogens in isolation may have very different effects when eaten in combination with all the other constituents of the food itself.

The important point is that transgenic constructs do contain new genes and new combinations of genes that have never existed in nature, not in billions of years of evolution. That, at least, is one reason on which patent claims are made. Furthermore, over-expression of host genes that become associated with the CaMV 35S promoter as the result of horizontal gene transfer or genomic rearrangement may also increase the concentration of otherwise safe metabolites to toxic or carcinogenic levels.

## STRUCTURAL CONSEQUENCES OF THE MODULAR STRUCTURE OF PROMOTERS

Hull et al (6) may also be mistaken to think that the entire CaMV 35S promoter has to be transferred before it can either lead to over-expressing of host genes or to reactivating or generating new viruses (objection 6). On account of the modular construction of all promoters, it is already clear that many elements are common to many promoters, so much so that gene therapists are now making synthetic super-promoters by random recombination of different elements (36). As reviewed in our earlier paper (2), the CaMV 35S promoter is promiscuous in function, and is active in combination in whole or in part with other promoters. Therefore, the transfer of parts of the CaMV 35S containing enhancer or other elements into genomes may be sufficient to cause over-expression of genes or to reactivate dormant viruses or generate new viruses.

As pointed out by Hull et al (6), proviral sequences (37) and related retrotransposons are now found to be present in all genomes, including those of higher plants (38). The fact that the CaMV promoter is different in sequence from other promoters does not prevent it from substituting for a range of viruses. The CaMV 35S promoter has been joined artificially to the cDNAs of a wide range of viral sequences, and infectious viruses produced in the laboratory (39, 40). There is also evidence that proviral sequence in the banana genome *can* be reactivated, especially in tissue culture, as demonstrated by a group of researchers that include one of our critics, Roger Hull (41). He had earlier warned that viral coat proteins in transgenic plants not only can offer disguise to related viruses to move around the plant and infect it, but also that the protein may wrap up retrotransposons in plants and allow them to be transmitted horizontally to other species (42).

The fact that plants are "loaded" with potentially mobile elements, such as retrotransposons, can only make things worse. Most, if not all, of the elements are 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. It is already known, from experiments in gene therapy, that retroviral and other sequences can integrate into mammalian genomes in the absence of viral integrase (43). Although CaMV 35S promoter and promoters of animal viruses do not have the same base sequence, they have at least one element (the TATA-box) in common, if not more. It is therefore possible therefore, for host protooncogenes and proviral sequences to become activated and reactivated (objections 5 and 6). Also, completely new cross-species viruses may arise from recombination between elements and motifs of the CaMV 35S promoter and those of animal viruses, dormant or otherwise (objection 5).

New research in our critics' own research institute are revealing how plants naturally resist viral infections by making small antisense RNA of 25 nucleotides against viral genes. Exactly the same mechanism is directed against transgenes to silence them (26). The authors remark that the gene-silencing "may represent a natural antiviral defence mechanism and transgenes might be targeted because they, or their RNA are perceived as viruses." So much for the claim that genetic engineering is just like conventional plant breeding.

In signing on to the International Biosafety Protocol in Montreal in January, more than 130 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 35S promoter. The hallmark of science is that it is always provisional and uncertain. Molecular genetics is a new discipline and our ignorance regarding gene regulation and the ecological impacts of horizontal gene transfer is profound. The social responsibility of science and the proper use of scientific evidence are therefore to set precaution. We appeal to our critics as fellow scientists to join us in calling for the withdrawal of all GM crops and products containing the CaMV 35S promoter, both from commercial use and from field trials, unless and until they can be shown to be safe. Meanwhile, much more good quality basic research—such at that carried out in the John Innes Institute—should continue under strictly contained conditions.

## ACKNOWLEDGEMENTS

We thank Mark Griffiths for drawing our attention to some important papers.

## REFERENCES

1. Rautenberg O. Opinion in the guise of science. Microbial Ecology in Health and Disease (in press): will be published in next number of Microbial Ecology in Health and Disease; 12/2.
2. Ho MW, Ryan A, Cummins J. Cauliflower Mosaic Viral Promoter—A Recipe for Disaster? Microbial Ecology in Health and Disease 1999; 11: 194–7.
3. Ho MW, Traavik T, Olsvik R, Tappeser B, Howard V, von Weizsacker C, McGavin G. Gene Technology and Gene Ecology of Infectious Diseases. Microbial Ecology in Health and Disease 1998; 10: 33–59.
4. Ho MW, Ryan A, Cummins J, Traavik T. Unregulated Hazards: 'Naked' and 'Free' Nucleic Acids. ISIS Report, Jan. 2000, prepared for TWN and circulated at the Biosafety Protocol Conference in Montreal, Jan. 24–28, 2000, available

on Institute of Science in Society website www.i-sis.org; also submitted for publication elsewhere.
5. Ewen S, Pusztai A. Effect of Diets Containing Genetically Modified Potatoes Expressing *Galanthus nivalis* Lectin on Rat Small Intestine. The Lancet 1999; 354: 1353–4.
6. Hull R, Covey SN, Dale P. Genetically Modified Plants and the 35S Promoter: Assessing the Risks and Enchancing the Debate. Microbial Ecology in Health and Disease (in press).
7. Kohli A, Griffiths S, Palacios N, Twyman RM, Vain P, Laurie DA, Christou P. Molecular Characterization of Transforming Plasmid Rearrangements in Transgenic Rice Reveals a Recombination Hotspot in the CaMV 35S Promoter and Confirms the Predominance of Microhomology Mediated Recombination. The Plant journal 1999; 17: 591–601.
8. Zhaqo T, Robinson M, Bowers F, Kindt T. Infectivity of Chimeric Human T-cell Leukaemia Virus Type I Molecular Clones Assessed by Naked DNA Inoculation. Proc. Natnl. Acad. Sci. USA 1996; 93: 6653–8.
9. Rekvig OP, Fredriksen K, Brannsether B, Moens U, Sundsfjord A, Traavik T. Antibodies to Eucaryotic, Including Autologous, Native DNA are Produced during BK Virus Infection, but Not After Immunization with Non-infectious BK DNA. Scand. J. Immunol. 1992; 36: 487–95.
10. Holmgren L, Szeles A, Rajnavolgyi E, Foldman J, Klein G, Emberg I, Falk KI. Horizontal Transfer of DNA by the Uptake of Apoptotic Bodies. Blood 1999; 93: 3956–63.
11. Harper G, Osuju JO, Heslop-Harrison JS, Hull R. Integration of Banana Streak Badnavirus into the *Musa* Genome: Molecular and Cytological Evidence. Virology 1999; 255: 207–13.
12. Lommel SA, Xiong Z. Reconstitution of a functional red clover necrotic mosaic virus by recombinational rescue of the cell-to-cell movement gene expressed in a transgenic plant. J. Cell. Biochem. 1991; 15A: 151.
13. Gal S, Pisan B, Hohn T, Grimsley N, Hohn B. Aginfection of transgenic plants leads to viable cauliflower mosaic virus by intermolecular recombination. Virology 1992; 187: 525–33.
14. SchoeIz JE, Wintermantel WM. Expansion of viral host range through complementation and recombination in transgenic plants. Plant Cell 1993; 5: 1669–79.
15. Green AE, Allison RF. Recombination between viral RNA and transgenic plant transcripts. Science 1994; 263: 1423.
16. Allison, R. Update on Virus Recombination in Transgenic Crops. 22923mgr@msy.edu 1997.
17. Frischmuth T, Stanley J. Recombination between Viral DNA and the Transgenic Coat Protein Gene of African Cassava Mosaic Geminivirus. J. Gen. Virol. 1998; 70: 1265–71.
18. Falk BW, Buening G. Will Transgenic Crops Generate New Viruses and New Diseases? Science 1994; 263: 1395–6.
19. Wintermantel WM, Schoelz JE. Isolation of Recombinant Viruses between Cauliflower Mosaic Virus and A Viral Gene in Transgenic Plants under Conditions of Moderate Selection Pressure. Virology 1996; 223: 156–64.
20. Green AE, Allison RF. Deletions in the 3′Untranslated Region of Cowpea Chlorotic Mottle Virus Transgene Reduce Recovery of Recombinant Viruses in Transgenic Plants. Virology 1996; 225: 231–4.
21. Teycheney P-Y, Aaziz R, Dinant, Salanki K, Tourneur D, Balazs E, Jacquemond M, Tepfer M. Synthesis of (-)-Strand RNA from the 3′ Untranslated Region of Plant Viral Genomes Expressed in Transgenic Plants upon Infection with Related Viruses. J. Gen. Virol. 2000, 81: 1121–26.
22. Old RW, Primrose SB. Principles of Gene Manipulation 5th ed., Blackwell, Oxford, 1994.
23. Smith V. More T-DNA than meets the eye. Trends in Plant Science 1998; 3: 85.
24. Conner AJ. Case Study: Food Safety Evaluation of Transgenic Potato. In Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods or Food Components from Plants Derived by Modern Biotechnology, pp. 23–35, WHO/FNU/FOS/95.1, World Health Organization, Geneva, Switzerland, 1995.
25. Timms-Wilson TM, Lilley AK, Bailey MJ. A Review of Gene Transferfrom Genetically Modified Micro-organisms. Report to UK Health and Safety Executive, 1999.
26. Hamilton AJ, Baulcombe DC. A Species of Small Antisense RNA in Posttranscriptional Gene Silencing in Plants. Science 1999; 286: 950–2.
27. Srivastava V, Anderson OD, Ow DW. Single-copy Transgenic Wheat Generated through the Resolution of Complex Integration Patterns. Proc. Nat. Acad. Sci. USA 1999; 96: 11117–21.
28. Benson S, Arax M, Burstein R, Brokaw J. A Growing Concern. Mother Jones www.Motherjones.com/mother_jones/JF97/biotech_jump.html 1997.
29. Fox JL. Farmers Say Monsanto's Engineered Cotton Drops Bolls. Nature Biotechnology 1997, 15: 1233.
30. Finnegan J, McElroy D. Transgene Inactivation, Plants Fight Back!. Bio/Technology 1994; 12: 883–8.
31. Shapiro JA. Genome Organization, Natural Genetic Engineering, and Adaptive Mutation. Trends in Genetics 1997; 13: 98–104.
32. Ho MW. Genetic Engineering Dream or Nightmare? The Brave New World of Bad Science and Big Business. Gateway, Gill & Macmillan, 1998, 2nd ed. 1999.
33. Strahl BD, Allis CD. The Language of Covalent Histone Modifications. Nature 2000; 403: 41–5.
34. Doerfler W, Schubbert R, Heller H, Kammer C, Hfiger-Eversheim K, Knoblauch M, Remus R. Integration of Foreign DNA and Its Consequences in Mammalian Systems. Trends in Biotechnology 1997; 15: 297–301.
35. Rubin EJ, Akerley BJ, Novik VN, Lampe DJ, Husson RN, Mekalanos JJ. *In vivo* Transpostion of *mariner*-based Elements in Enteric Bacteria and Mycobacteria. Proc. Natl. Acad. Sci USA 1999; 96: 1645–50.
36. Li X, Eastman EM, Schwartz RJ, Draghia-Akli R. Synthetic Muscle Promoters: Activities Exceeding Naturally Occurring Regulatory Sequences. Nature Biotechnology 1999; 17: 241–6.
37. Jakowtisch J, Mette MF, van der Winden J, Matzke MA, Matzke AJM. Integrated Pararetroviral Sequences Define a Unique Class of Dispersed Repetitive DNA in Plants. PNAS 1999; 96: 13241–6.
38. Wright DA, Voytas DR. Potential Retroviruses in Plants: *Tat1* is Related to a Group of *Arabidopsis thaliana Ty3/gypsy* Retrotransposons that Encode Envelope-like Proteins. Genetics 1998; 149: 703–15.
39. Maiss E, Timpe U, Brisske-Rode A. Infectious *in vivo* transcripts of a plumpox potyvirus full lenth c-DNA clone containing the cauliflower mosaic virus 35-S RNA promoter. J. Gen. Virol. 1992; 73: 709–13.
40. Meyer M, Desserts J. 35S promoter driven cDNA of barley mild mosaic virus RNA-1 and RNA-2 are infectious in barley plants. J. Gen. Viol. 1997; 78: 147–51.
41. Ndowora T, Dahal G, LaFleur D, Harper G, Hull R, Olszerski NE, Lockhart B. Evidence that badnavirus infection in *Musa* can originate from integrated pararetroviral sequences. Virology 1999; 255: 214–20.
42. Hull, R. (1998). Detection of risks associated with coat protein transgenics. In Methods in Molecular Biology: Plant Virology Protocols: from Virus Isolation to Transgenic Resistance (Foster, G.D. and Taylor, S.C. eds.), Hurnana Press, New Jersey.
43. Zheng C, Baum BJ, Iadorola MJ, O'Connell BC. Genomic Integration and Gene Expression by a Modified Adenoviral Vector. Nature Biotechnology 2000; 18: 176–86.