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The CaMV 35S Promoter

Government and Corporate Scientific Incompetence: Failure to assess the safety of GM crops

By Dr. Ricarda A. Steinbrecher – December 2002

Bacteria and fungi are able to take up DNA from their surroundings, whether this is water, soil or gut. They can use this DNA as a food source or as genetic information, integrating it into their own DNA. This form of *horizontal gene transfer* is part of an ongoing evolutionary process. Bacteria are capable of exchanging genetic information between each other, such as genes for antibiotic or herbicide resistance - a very common form of horizontal gene transfer.

The transfer of GM genes from GM crops to soil or gut bacteria is hence a distinct possibility and has already been observed in different laboratory settings. The question of whether a transferred GM gene could be utilised or activated by bacteria is of crucial importance for a sound risk assessment.

The risks and danger associated with the transfer of antibiotic resistance marker genes from GM crops to human and animal bacterial pathogens has been recognised by bodies like the British Medical Association (BMA) and in the Cartagena Protocol on Biosafety; their use as markers is now being phased out.

Less attention has been given to horizontal gene transfer of the other genes used in genetic engineering, like genes for herbicide tolerance or insect resistance. Often risk assessments carried out for the approval of a GM crop do not consider any risks related to horizontal gene transfer or define them without evidence as "negligible to zero". So far no tests have been carried out as to whether or how bacteria or yeast can make use of these novel genes, whether having a gene to counteract a toxic herbicide might give a selective advantage and shift the balance of micro-organisms in gut or soil.

Assessments are based on assumed lack of risk and lack of evidence. In the case of the CaMV 35S promoter any question of risk was immediately discounted as this promoter was regarded to be plant specific and not active in other organisms such as bacteria, fungi or human cells. This assumption is wrong (see below), putting the whole current procedure of risk assessment and accuracy of company safety data in question.

Horizontal Gene Transfer

Horizontal gene transfer can be defined as the movement of genetic information (DNA) between cells and organisms by means other than sexual reproduction. This process enables the transfer of genetic information between sexually incompatible organisms and works across the boundaries of species, genera and even kingdoms. Such transfer of a gene from e.g. plant to bacteria is called horizontal gene transfer.

Promoter

For an organism to be able to activate or turn off its own genes, each gene has its own molecular switch, called a promoter. A promoter is made of DNA and located at the front of a gene. A promoter is usually gene specific, and reacts to signals given by the organism, thus allowing the fine-tuning of the gene product (e.g. enzymes or hormones) according to need and developmental stage.

CaMV - Cauliflower Mosaic Virus

The Cauliflower Mosaic Virus (CaMV) is a double-stranded DNA virus which infects a wide range of crucifers, especially brassicas such as cabbages, cauliflowers, oilseed rape or mustard. In order to get itself and its DNA replicated (multiplied) within a plant cell, the virus must trick the plant's own molecular 'machinery' to do this task. For this purpose the virus has two promoters (35S and 19S) in front of its genes, which the plant cell believes to be its own. Furthermore, these promoters override the plant's own regulatory system, as they are *constitutive*, i.e. they are constantly switched on and can't be regulated or switched off by the plant.

Examples of artificial/novel genes:

Promoter | Sequence coding for protein | Regulatory element

- *pat* Gene - herbicide resistance (Glufosinate)
- *RR* Gene - herbicide resistance (Glyphosate)
- *Bt* Gene - insect resistance (toxin from the bacteria *Bacillus thuringiensis*)

Modified genes used in plant genetic engineering

Genes used to transform plants are commonly an artificial mosaic of different DNA components of various origins. A simple version consists of a promoter known to work in the target plant, followed by the information for a particular protein and ended by another regulatory sequence.

Genetic engineering commonly makes use of the viral CaMV 35S promoter, as this promoter is a strong as well as a constitutive promoter. It leads to high expression of the gene in almost any type of cell and tissue of the plant at any developmental stage.

CURRENT SITUATION: The CaMV 35S promoter is being used in almost all GM crops currently grown or tested, especially GM maize. It is the promoter of choice for plant genetic engineering, as it is a strong and constitutive promoter. Failure to recognise or to ignore its capacity to be universally active in almost any organism is irresponsible and careless and shows a serious lack of scientific rigour and commitment to safety.
Any safety assessment can be expected to be flawed that does not resort to actual laboratory test of the capacity of bacteria and fungi to utilise the particular genes and their promoters.

DENIAL OF PROMOTER ACTIVITY:

EU Scientific Committee on Plants, 10 February 1998, regarding genetically modified T25 maize:

> *"pat gene - The gene is under the control of a plant promoter which is not functional in bacteria. Consequently, in the unlikely event of gene transfer from the transgenic maize to intestinal bacteria, expression of the pat gene would not occur. ..... "*

UK Department of the Environment, Food and Rural Affairs & Advisory Committee on Releases into the Environment (ACRE) February 2002:

> *"In the unlikely event that the T25 maize pat gene is transferred to a soil bacterium then it would not be expressed. This is because it is linked to the cauliflower mosaic virus promoter that expresses genes in plants - not bacteria."*

AVENTIS CropScience:- written submission to ACRE T25 Maize Hearing, 20 February 2002

> *"The cauliflower mosaic promoter, associated with the pat gene is only active in plants, not in bacteria, thus even if horizontal gene transfer did take place, the PAT protein would not be expressed in the soil bacteria without the presence of a suitable promoter."*

PROOF OF CaMV 35S ACTIVITY:

Already in 1990 it had been established that the CaMV 35S promoter is not only active in plants but also in the gut bacterium *E.coli,* in yeast and in extracts of human cancer cell lines. It has more recently been shown that the promoter is active in gut pathogens (i.e. *Y. entorocolitica*) and soil bacteria (*A. rhizogenes*) - see table. It is vital to include such knowledge and findings if a risk assessment is to be dependable, trustworthy and scientific.

Publications on CaMV 35S promoter activity

Bacteria:

• Escherichia coli	- Assaad and Signer 1990
• Escherichia coli	- Lewin et al. 1998
• Yersinia enterocolitica	- Lewin et al. 1998
• Agrobacterium rhizogenes	- Lewin et al. 1998

Fungi/yeast:

• Schizosaccharomyces pombe	- Pobjecky et al. 1990

Human:

• Cell extract of HeLa cells: (human cancer cell line)	- Guilley et al. 1982 - Cooke and Penon 1990 - Burke et al. 1990

References

Assaad FF and Signer ER (1990). Cauliflower mosaic-virus p35S promoter activity in *Escherichia-coli*. *Molecular and General Genetics* 223(3): 517-520;

Burke C, Yu X-B, Marchitelli L, Davis EA and Ackerman S (1990). Transcription Factor IIA of wheat and human function similarly with plant and animal viral promoters. *Nucleic Acid Research* 18(12):3611-3620

Cooke R and Penon P (1990). In vitro transcription from cauliflower mosaic virus promoters by a cell-free extract from tobacco cells. *Plant Molecular Biology* 14:391-405

Guilley H, Dudley RK, Jonard G, Balazs E and Richards KE (1982). Transcription of Cauliflower Mosaic Virus DNA: Detection of promoter sequences, and characterization of transcripts. *Cell* 30:763-773

Lewin A, Jacob D, Freytag B, Appel B (1998). Gene expression in bacteria directed by plant-specific regulatory sequences. *Transgenic Research* 7:403-411

Pobjecky N, Rosenberg GH, Dintergottlieb G, Kaufer NF (1990). Expression of the beta-glucuronidase gene under the control of the CaMV-35S promoter in *Schizosaccharomyces-pombe*. *Molecular & General Genetics* 220 (2): 314-316.