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HORIZONTAL GENE TRANSFER — The Hidden Hazards of Genetic Engineering

by Mae-Wan Ho

TWN
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Contents

Abstract

Genetic engineering involves designing artificial constructs to cross species barriers and to invade genomes. In other words, it enhances horizontal gene transfer - the direct transfer of genetic material to unrelated species. The artificial construct (transgenic DNA) typically contains genetic material from bacteria, viruses and other genetic parasites that cause diseases as well as antibiotic resistance genes that make infectious diseases untreatable. Horizontal transfer of transgenic DNA has the potential to create new viruses and bacteria that cause diseases, spread antibiotic resistance genes to pathogenic bacteria and trigger cancer in mammalian cells. There is an urgent need to establish effective regulatory oversight to prevent the escape and release of these dangerous constructs into the environment, and to consider whether some of the most dangerous experiments should be allowed to continue at all.

(Key words: Antibiotic resistance genes; dormant viruses; CaMV promoter; cancer; naked DNA; transgenic DNA)

Chapter 1

Transgenic pollen and baby bees

Prof. Hans-Hinrich Kaatz from the University of Jena is reported to have new evidence, as yet unpublished, that genes engineered into transgenic plants have transferred via pollen to bacteria and yeasts living in the gut of bee larvae.[1]

If Prof. Kaatz's claim can be substantiated, it indicates that the new genes and gene-constructs introduced into transgenic crops (and other transgenic organisms) can spread, not just by ordinary cross-pollination to closely-related species, but also by the genes and gene-constructs invading the genomes (the totality of the organisms' own genetic material) of completely unrelated species, including the microorganisms living in the gut of animals eating transgenic material.

This finding is not unexpected. Some scientists have been drawing attention to this possibility recently,[2] but the warnings actually date

[1] Thanks to Dr. Beatrix Tappeser, Institute for Applied Ecology, Postfach 6226, D-79038, Freiburg, for this information. See also Barnett, A. (2000). GM genes 'jump species barrier'. *The Observer*, May 28, 2000.

[2] See Stephenson, J.R., and Warnes, A. (1996). Release of genetically-modified miroorganisms into the environment. *J. Chem. Tech. Biotech*. 65, 5-16; Harding, K. (1996). The potential for horizontal gene transfer within the environment. *Agro-Food-Industry Hi-Tech* July/August, 31-35; Ho, M.W. (1996). Are current transgenic technologies safe? In Virgin, I. and Frederick R.J., eds. *Biosafety Capacity Building*, pp. 75-80, Stockholm Environment Institute, Stockholm; Traavik, T. (1999). *Too Early May be Too Late*, Report for the Directorate for Nature Research, Trondheim, Norway.

back to the mid-1970s when genetic engineering began. Hundreds of scientists around the world are now demanding a moratorium on all environmental releases of transgenic organisms on grounds of safety,[3] and horizontal gene transfer is one of the major considerations.

Some of us have argued that the hazards of 'horizontal' gene transfer to unrelated species are inherent to genetic engineering.[4] The gene-constructs created in genetic engineering have never existed in billions of years of evolution. They consist of new combinations of genetic material originating from dangerous bacteria, viruses and other genetic parasites, including genes coding for antibiotic (see Box 1). The artificial constructs are designed to cross all species barriers and to invade genomes, in the course of which, new viruses and bacteria that cause diseases may be created, and antibiotic resistance genes spread to bacterial pathogens, making infectious diseases untreatable.

Box 1: **What are antibiotic resistance marker genes?**

Antibiotics are chemicals that kill bacteria and cells. There are many kinds of antibiotics. An antibiotic resistance gene codes for a protein that makes the bacteria or cells resistant to, that is, survive treatment with, specific kinds of antibiotics. An antibiotic resistance marker gene is one that accompanies foreign genes to be transferred into cells. It is placed next to the foreign genes in the construct, so that those cells that have taken up the construct will also be resistant to the antibiotic. This gives a convenient way to select for transformed cells, simply by using the antibiotic(s) to kill off the rest.

[3] See World Scientists' Open Letter to All Governments Concerning GMOs <www.i-sis.org>.

[4] See Ho, M.W. (1998, 1999). *Genetic Engineering Dream or Nightmare? The Brave New World of Bad Science and Big Business.* Gateway, Gill & Macmillan, Dublin; Ho, M.W., Traavik, T., Olsvik, R., Tappeser, B., Howard, V., von Weizsacker, C. and McGavin, G. (1998). Gene Technology and Gene Ecology of Infectious Diseases. *Microbial Ecology in Health and Disease* 10, 33-59.

Chapter 2

Horizontal gene transfer may spread transgenes to the entire biosphere

Horizontal gene transfer is the transfer of genetic material between cells or genomes belonging to unrelated species, by processes other than usual reproduction. In the usual process of reproduction, genes are transferred *vertically* from parent to offspring; and such a process can occur only within a species or between closely related species.

Bacteria are known to exchange genes across species barriers in nature. There are three ways in which this is accomplished. In *conjugation*, genetic material is passed between cells in contact; in *transduction*, genetic material is carried from one cell to another by infectious viruses; and in *transformation*, the genetic material is taken up directly by the cell from its environment. For horizontal gene transfer to be successful, the foreign genetic material must become integrated into the cell's genome, or become maintained in the recipient cell in some other form. In most cases, foreign genetic material that enters a cell by accident, especially if it is from another species, will be broken down before it can incorporate into the genome. Under certain ecological conditions which are still poorly understood, foreign genetic material escapes being broken down and becomes incorporated in the genome. For example, heat shock and pollutants such as heavy metals can enhance horizontal gene transfer; and the presence of antibiotics can increase the frequency of horizontal gene transfer 10 to 10 000 fold.[5]

[5] See Ho *et al.*, 1998 (note 4) and references therein.

While horizontal gene transfer is well known among bacteria, it is only within the past 10 years that its occurrence has become recognized among higher plants and animals.[6] The scope for horizontal gene transfer is essentially the entire biosphere, with bacteria and viruses serving both as intermediaries for gene trafficking and as reservoirs for gene multiplication and recombination (the process of making new combinations of genetic material).[7]

There are many potential routes for horizontal gene transfer to plants and animals. Transduction is expected to be a main route as there are many viruses that infect plants and animals. Recent research in gene therapy indicates that transformation is potentially very important for cells of mammals including human beings. A great variety of 'naked' genetic material are readily taken up by all kinds of cells, simply as the result of being applied in solution to the eye, or rubbed into the skin, injected, inhaled or swallowed. In many cases, the foreign gene-constructs become incorporated into the genome.[8]

Direct transformation may not be as important for plant cells, which generally have a protective cell wall. But soil bacteria belonging to the genus *Agrobacterium* are able to transfer the *T* (tumour) segment of its Tumour-inducing (*Ti*) plasmid (see below) into plant cells in a process resembling conjugation. This *T*-DNA is widely exploited as a gene transfer vehicle in plant genetic engineering (see below). Foreign genetic material can also be introduced into plant and animal cells by insects and arthropods with sharp mouthparts. In addition, bacterial pathogens that enter plant and animal cells may take up foreign genetic material and carry it into

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[6] See Lorenz, M.G. and Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev.* 58, 563-602.

[7] See Ho,1998, 1999; Ho, *et al.*, 1998 (note 4).

[8] See Ho, M.W., Ryan, A., Cummins, J. and Traavik, T. (2000a). *Unregulated Hazards: 'Naked' and 'Free' Nucleic Acids*, ISIS & TWN Report, London and Penang <www.i-sis.org>.

the cells, thus serving as vectors for horizontal gene transfer.[9] There are almost no barriers preventing the entry of foreign genetic material into the cells of probably any species on earth. The most important barriers to horizontal gene transfer operate after the foreign genetic material has entered the cell.[10]

Most foreign genetic material, such as those present in ordinary food, will be broken down to generate energy and building blocks for growth and repair. There are many enzymes (proteins that act as catalysts for chemical reactions in living organisms) that break down foreign genetic material; and in the event that the foreign genetic material is incorporated into the genome, chemical modification can still put it out of action and eliminate it.

However, viruses and other genetic parasites, such as plasmids and transposons, have special genetic signals and probably overall structure to escape being broken down. A virus consists of genetic material generally wrapped in a protein coat. It sheds its overcoat on entering a cell and can either hijack the cell to make many more copies of itself, or it can jump directly into the cell's genome. Plasmids are pieces of 'free', usually circular, genetic material that can be indefinitely maintained in the cell separately from the cell's genome. Transposons, or 'jumping genes', are blocks of genetic material which have the ability to jump in and out of genomes, with or without multiplying themselves in the process. They can also land in plasmids and be propagated there. Genes hitch-hiking in viruses, plasmids and transposons, therefore, have a greater probability of being successfully transferred into cells and genomes. They are *vectors* for horizontal gene transfer.

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[9] Grillot-Courvalin, C., Goussand, S., Huetz, F., Ojcius, D.M. and Courvalin, P. (1998). Functional gene transfer from intracellular bacteria to mammalian cells. *Nature Biotechnology* 16, 862-866.

[10] See Nielsen, K.M., Bones, A.M., Smalla, K. and van Elsas, J.D. (1998). Horizontal transfer from transgenic plants to terrestrial bacteria – a rare event? *FEMS Microbiology Reviews* 22, 79-103.

Natural genetic parasites are restricted by species barriers, so for example, pig viruses will infect pigs, but not human beings, and cauliflower viruses will not attack tomatoes. It is the protein coat of the virus that determines host specificity, which is why naked viral genomes (the genetic material stripped of the coat) are generally found to have a wider host range than the intact virus.[11] Similarly, the signals for propagating different plasmids and transposons are usually specific to a limited range of host species, although there are exceptions.

As more and more genomes have been sequenced, it is becoming apparent that gene trafficking or horizontal gene transfer has played an important role in the evolution of all species.[12] However, it is also clear that horizontal gene trafficking is regulated by internal constraints in the organisms in response to ecological conditions.[13] Overall, gene exchange across species barrier has been limited to a minimum.

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[11] See Ho *et al*., 2000a (note 8).

[12] See Doolittle, W.F. (1999). Lateral genomics. *Trends Cell Biol.* 9, 5-8.

[13] See Jain, R., Rivera, M.C. and Lake, J.A. (1999). Horizontal gene transfer among genomes: The complexity hypothesis. *Proc. Natl. Acad. Sci. USA* 96, 3801-3806; Shapiro, J. (1997). Genome organization, natural genetic engineering and adaptive mutation. *TIG* 13, 98-104; Ho, 1998, 1999 (note 4).

Chapter 3

Genetic engineering is unregulated horizontal gene transfer

Genetic engineering is a collection of laboratory techniques used to isolate and combine the genetic material of any species, and to multiply the constructs in convenient cultures of bacteria and viruses in the laboratory. Most of all, the techniques allow genetic material to be transferred between species that would never interbreed in nature. That is how human genes can be transferred into pig, sheep, fish and bacteria; and spider silk genes end up in goats. Completely new, exotic genes are also being introduced into crops, livestock and fish for a variety of purposes from the production of food and textiles to pharmaceuticals and industrial chemicals.

In order to overcome natural species barriers restricting gene transfer and maintenance, genetic engineers have made a huge variety of artificial vectors (carriers of genes) by combining parts of the most infectious natural vectors – viruses, plasmids and transposons — from different sources. These artificial vectors generally have their disease-causing functions removed or disabled, but are designed to cross wide species barriers, so the same vector may now transfer, say, human genes spliced into the vector to the genomes of all other mammals, or of plants. Artificial vectors greatly enhance horizontal gene transfer (see Box 2). [14]

Although different classes of vectors are distinguishable on the basis of the main frame genetic material, practically every one of them

[14] See Ho *et al.*, 1998 (note 4) for references.

Box 2: **Artificial vectors enhance horizontal gene transfer**

- They are derived from natural genetic parasites that mediate horizontal gene transfer most effectively.
- Their highly chimaeric nature means that they have sequence homologies (similarities) to DNA from viral pathogens, plasmids and transposons of multiple species across Kingdoms. This will facilitate widespread horizontal gene transfer and recombination.
- They routinely contain antibiotic resistance marker genes that will enhance horizontal transfer in the presence of antibiotics, either intentionally applied, or present as pollutant in the environment. Antibiotics are known to enhance horizontal gene transfer between 10- and 10 000-fold.
- They often have 'origins of replication' and 'transfer sequences', signals that facilitate horizontal gene transfer and maintenance in cells to which they are transferred.
- Chimaeric vectors are well known to be structurally unstable, that is, they have a tendency to break and rejoin incorrectly or join up with other DNA, and this will increase the propensity for horizontal gene transfer and recombination.
- They are designed to invade genomes and to overcome mechanisms that break down or disable foreign DNA, thus increasing the probability of horizontal transfer.

is chimaeric, being composed of genetic material originating from the genetic parasites of many different species of bacteria, animals and plants. Important chimaeric 'shuttle' vectors enable genes to be multiplied in the bacterium *E. coli* and transferred into species in every other Kingdom of plants and animals. Simply by creating such a vast variety of promiscuous gene transfer vectors, genetic engineering biotechnology has effectively opened up highways for horizontal gene transfer and recombination, where previously the process was tightly regulated, with restricted access through narrow, tortuous footpaths. These gene transfer highways connect

species in every Domain and Kingdom with the microbial populations via the universal vessel used in genetic engineering, *E. coli*.

Despite the now-copious evidence for the potential of horizontal gene transfer, *there is still no legislation in any country to prevent the escape and release of artificial vectors and other artificial constructs into the environment*.[15]

[15] See Ho *et al.*, 2000 (note 8).

Chapter 4

What are the hazards of horizontal gene transfer?

Most artificial vectors are either derived from viruses or have viral genes in them, and are designed to cross species barriers and to invade genomes. They have the potential to recombine with the genetic material of other viruses to generate new infectious viruses that cross species barriers. Such viruses have been appearing at alarming frequencies recently, although good epidemiological data are still wanting. The antibiotic resistance genes carried by artificial vectors can spread to bacterial pathogens, and there appears to have been an accelerated rate at which bacteria become resistant to new antibiotics in recent years.

Has the growth of commercial-scale genetic engineering biotechnology contributed to the resurgence of drug and antibiotic infectious diseases within the past 25 years? [16] There is already overwhelming evidence that horizontal gene transfer and recombination have been responsible for creating new viral and bacterial pathogens and for spreading drug and antibiotic resistance among the pathogens. One way that new viral pathogens may be created is through recombination with dormant, inactive or inactivated viral genetic material that are in all genomes, plants and animals without exception. Recombination between external and resident, dormant viruses have been implicated in many animal cancers.[17]

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[16] Reviewed in Ho *et al.*, 1998 (note 4).

[17] Reviewed in Ho, 1998, 1999 (note 4) Chapter on 'The mutable gene and the human condition'.

As stated earlier, the cells of all species including our own can take up foreign genetic material. Artificial constructs that are designed to invade genomes may well invade our own. These insertions may lead to inappropriate inactivation or activation of genes (insertion mutagenesis), some of which may lead to cancer (insertion carcinogenesis).[18] The hazards of horizontal gene transfer are summarized in Box 3.

Box 3: **Potential hazards of horizontal gene transfer from genetic engineering**

- Generation of new cross-species viruses that cause disease
- Generation of new bacteria that cause diseases
- Spreading drug and antibiotic resistance genes among the viral and bacterial pathogens, making infections untreatable
- Random insertion into genomes of cells resulting in harmful effects including cancer
- Reactivation of dormant viruses, present in all cells and genomes, which may cause diseases
- Spreading new genes and gene-constructs that have never existed
- Multiplication of ecological impacts due to all of the above

[18] See Ho *et al*., 2000 (note 8) and references therein.

Chapter 5

Transgenic DNA may be more likely to transfer horizontally than non-transgenic DNA

Both the artificial vectors used in genetic engineering and the genes transferred to make trangenic organisms are predominantly from viruses and bacteria associated with diseases, and these are being brought together in combinations that have never existed in billions of years of evolution.

Genes are never transferred alone. They are transferred in unit-constructs, known as an 'expression cassettes'. Each gene has to be accompanied by a special piece of genetic material, the *promoter*, which signals the cell to turn the gene on, that is, to transcribe the DNA gene sequence into RNA. At the end of the gene there has to be another signal, a *terminator*, to end the transcription and to mark the RNA, so it can be further processed and translated into protein. The simplest expression cassette looks like this:

Typically, each bit of the construct — promoter, gene and terminator — is from a different source. The gene itself may also be a composite of bits from different sources. Several expression cassettes are usually linked in series, or 'stacked' in the final construct. At least one of the expression cassettes will be that of an antibiotic resistance marker gene to enable cells that have taken up the foreign construct to be selected with antibiotics. The antibiotic resistance gene cassette will often remain in the transgenic organism.

The most commonly used promoters are from viruses associated with serious diseases. The reason is that such viral promoters give continuous over-expression of genes placed under their control. The same basic construct is used in all applications of genetic engineering, whether in agriculture or in medicine, and the same hazards are involved. There are reasons to believe that transgenic DNA is much more likely to spread horizontally than the organisms' own DNA (see Box 4).[19]

Box 4: **Transgenic DNA may be much more likely to spread horizontally**

- Artificial constructs and vectors are designed to invade genomes and to overcome species barriers.
- All artificial constructs are structurally unstable,[20] and hence prone to recombine and transfer horizontally.
- The mechanisms that enable foreign gene-constructs to jump into the genome may enable them to jump out again, to re-insert at another site, or in another genome. For example, the enzyme, *integrase*, which catalyzes the insertion of viral DNA into the host genome, also functions as a *disintegrase* catalyzing the reverse reaction. These integrases belong to a superfamily of similar enzymes present in all genomes from viruses and bacteria to higher plants and animals.[21]
- The integration sites of most commonly used artificial vectors for transferring genes are 'recombination hotspots', and may therefore have

[19] See Ho, M.W. (1999). Special Safety Concerns of Transgenic Agriculture and Related Issues. Briefing Paper for Minister of State for the Environment, The Rt. Hon. Michael Meacher <www.i-sis.org>.

[20] See Old, R.W. and Primrose, S.B. (1994). *Principles of Gene Manipulation.* 5th ed. Blackwell Science, Oxford; Kumpatla, S.P., Chandrasekharan, M.B., Iuer, L.M., Li, G. and Hall, T.C. (1998). Genome intruder scanning and modulation systems and transgene silencing. *Trends in Plant Sciences* 3, 96-104.

[21] Asante-Appiah E. and Skalka, A.M. (1997). Molecular mechanisms in retrovirus DNA integration. *Antiviral Research* 36, 139-56.

an increased propensity to transfer horizontally.

- Viral promoters, such as that from the cauliflower mosaic virus, widely used to make transgenes over-express, contain recombination hotspots, and may further enhance horizontal gene transfer.[22]
- The metabolic stress on the host organism due to the continuous over-expression of the foreign genes linked to aggressive viral promoters may also contribute to the instability of the transgenic DNA.[23]
- The transgenic DNA is typically a mosaic of DNA sequences from many different species and their genetic parasites; that means they have sequence homologies with the genetic material of many species and their genetic parasites, thus facilitating wide-ranging horizontal gene transfer and recombination.[24]

[22] See Kohli A., Griffiths S., Palacios N., Twyman R.M., Vain P., Laurie D.A., 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, and references therein.

[23] Finnegan, J. and McElroy, D. (1994). Transgene inactivation: plants fight back! *Bio/Technology* 12, 883-888.

[24] See Ho *et al.*, 1998 (note 4) and references therein.

Chapter 6

Additional hazards from viral promoters

We have recently drawn attention to additional hazards associated with a promoter of the cauliflower mosaic virus (CaMV) most widely used in agriculture.[25] It is in practically all transgenic plants already commercialized or undergoing field trials, as well as a high proportion of transgenic plants under development, including the much acclaimed 'golden rice'.[26]

CaMV is closely related to human hepatitis B virus, and less so, to retroviruses such as the AIDS virus.[27] Related viruses exchange genes much more readily than unrelated ones. Although the intact virus itself is infectious only for cruciferae plants, its promoter is promiscuous in function across Kingdoms and Domains. It is ac-

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[25] Ho, M.W., Ryan, A. and Cummins, J. (1999). The cauliflower mosaic viral promoter – a recipe for disaster? *Microbial Ecology in Health and Disease* 11, 194-197; Ho, M.W., Ryan, A. and Cummins, J. (2000). Hazards of transgenic plants containing the cauliflower mosaic viral promoter. *Microbial Ecology in Health and Disease* 12, 6-11; Ho, M.W., Ryan, A. and Cummins, J. (2000). CaMV 35S promoter fragmentation hotspot confirmed, and it is active in animals. *Microbial Ecology in Health and Disease* (in press).

[26] Ye, X., Al-Babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P. and Potrykus, I. (2000). Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287, 303-305; see also Ho, M.W. (2000). *The Golden Rice – An Exercise in How Not to Do Science.* ISIS Sustainable Science Audit #1 <www.i-sis.org>.

[27] Xiong, Y. and Eikbush, T. (1990). Origin and evolution of retroelements based upon the reverse transriptase sequences. *The Embo Journal* 9, 3363-72.

tive in all higher plants, in algae, yeast, and *E. coli*,[28] *as well as frog and human cell systems*.[29]

All promoters of viruses and cellular genes have a modular structure, with common, interchangeable parts. The CaMV 35S promoter has been joined artificially to the cDNAs of a wide range of viral genomes, and infectious viruses were produced. Recombination between viral transgenes and infecting viruses has already been demonstrated many times in the laboratory. In some cases, the recombinant viruses are more infectious than the original.

The CaMV 35S promoter 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 requires little or no DNA sequence homologies.

Proviral sequences – generally inactive copies of viral genomes — are present in all plant and animal genomes. As all viral promoters are modular, 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. There is evidence that proviral sequence in the genome *can* be reactivated.[30] In fact, this is one of

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[28] Assad, F.F. and Signer, E.R. (1990). Cauliflower mosaic virus P35S promoter activity in E. coli. *Mol. Gen. Genet*. 223, 517-20.

[29] Ballas, N., Broido, S., Soreq, H. and Loyter, A. (1989). Efficient functioning of plant promoters and poly(A) sites in Xenopus oocytes. *Nucl. Acids Res*. 7891-903; Burke, C., Yu, X.B., Marchitelli, L., Davis, E.A., Ackerman, S. (1990). Transcription factor IIA of wheat and human function similarly with plant and animal viral promoters. *Nucl. Acids Res*. 18, 3611-20.

[30] Ndowora, T., Dahal, G., LaFleur, D., Harper, G., Hull, R., Olszerski, N.E. and Lockhart, B. (1999). Evidence that badnavirus infection in Musa can originate from integrated pararetroviral sequences. *Virology* 255, 214-20.

the main problems encountered in human gene therapy. Viral vectors have to be 'packaged' in human cell lines, and replicating viruses are often generated by the viral vector recombining with proviral sequences in the cells.[31]

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 to 'the construct or the genetic transformation (or both)'.[32] The authors also report an increase in lymphocytes in the intestinal wall, which is a non-specific sign of viral infection.[33]

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[31] Martinez I. and Dornburg R.(1996) Partial reconstitution of a replication-competent retrovirus in helper cells with partial overlaps between vector and helper cell genomes. *Hum. Gene Ther.* 7, 705-12.

[32] Ewen S., Pusztai A. Effect of Diets Containing Genetically Modified Potatoes Expressing *Galanthus nivalis* Lectin on Rat Small Intestine. *The Lancet* 1999, 354: 1353-1354.

[33] Arpad Pusztai, personal communication.

Chapter 7

Evidence for horizontal transfer of transgenic DNA

It is often argued that transgenic DNA, once incorporated into the transgenic organism, will be just as stable as the organism's own DNA. But there is both direct and indirect evidence against this supposition. Transgenic DNA is more likely to spread, and has been found to spread by horizontal gene transfer.

Transgenic lines are notoriously unstable and often do not breed true.[34] There is a paucity of molecular data documenting the structural stability of the transgenic DNA, both in terms of its site of insertion in the genome and the arrangement of genes, in successive generations. Instead, transgenes may be silenced in subsequent generations or lost altogether.[35]

A herbicide-tolerance gene, introduced into *Arabidopsis* by means of a vector, was found to be up to 30 times more likely to escape and spread than the same gene obtained by mutagenesis.[36] One

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[34] Reviewed by Pawlowski, W.P. and Somers, D.A. (1996). Transgene inheritance in plants genetically engineered by microprojectile bombardent. *Molecular Biotechnology* 6, 17-30; See also Ho, 1998, 1999 (note 4) Chapter 'Perils amid promises of genetically modified food'.

[35] See Pawlowski and Somers, 1996 (note 34), also Srivastava V., Anderson O.D., Ow D.W. Single-copy Transgenic Wheat Generated through the Resolution of Complex Integration Patterns. *Proc. Nat. Acad. Sci. USA* 1999, 96: 11117-11121.

[36] Bergelson, J., Purrington, C.B. and Wichmann, G. (1998). Promiscuity in transgenic plants. *Nature* 395, 25.

way this may have happened is by means of horizontal gene transfer via insects visiting the plants for pollen and nectar.[37] The reported finding that pollen can transfer transgenic DNA to bacteria in the gut of bee larvae is relevant here.

Secondary horizontal transfer of transgenes and antibiotic resistance marker genes from genetically engineered crop-plants into soil bacteria and fungi has been documented in the laboratory. Transfer to fungi was achieved simply by co-cultivation,[38] while transfer to bacteria has been achieved by both re-isolated transgenic DNA or total transgenic plant DNA.[39] Successful transfers of a kanamycin resistance marker gene to the soil bacterium *Acinetobacter* were obtained using total DNA extracted from homogenized plant leaf from a range of transgenic plants: *Solanum tuberosum* (potato), *Nicotiana tabacum* (tobacco), *Beta vulgaris* (sugar beet), *Brassica napus* (oil-seed rape) and *Lycopersicon esculentum* (tomato).[40] It is estimated that about 2500 copies of the kanamycin resistance genes (from the same number of plant cells) are sufficient to successfully transform one bacterium, *despite the fact that there is six million-fold excess of plant DNA present*. A single plant with, say, 2.5 trillion cells would be sufficient to transform one billion bacteria.

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[37] This possibility was not considered by the authors Bergelson et al., 1998 (note 36), although when I put this possibility to the first author by e-mail, she replied that it could not be ruled out.

[38] Hoffman, T., Golz, C. & Schieder, O. (1994). Foreign DNA sequences are received by a wild-type strain of *Aspergillus niger* after co-culture with transgenic higher plants. *Current Genetics* 27: 70-76.

[39] Schluter, K., Futterer, J. & Potrykus, I. (1995). Horizontal gene-transfer from a transgenic potato line to a bacterial pathogen (*Erwinia-chrysanthem*) occurs, if at all, at an extremely low-frequency. *Bio/Techology* 13: 1094-1098; Gebhard, F. and Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Appl. Environ. Microbiol*. 64, 1550-4.

[40] De Vries, J. and Wackernagel, W. (1998). Detection of nptII (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue transformation. *Mol. Gen. Genet*. 257, 606-13; see also Gebhard and Smalla, 1998 (note 39).

Despite the misleading title in one of the publications,[41] a high gene transfer frequency of 5.8×10^{-2} per recipient bacterium was demonstrated under optimum conditions. But the authors then proceeded to calculate an extremely low gene transfer frequency of 2.0×10^{-17} under extrapolated 'natural conditions', *assuming that different factors acted independently*. The natural conditions, however, are largely unknown and unpredictable, and even by the authors' own admission, synergistic effects cannot be ruled out. Free transgenic DNA is bound to be readily available in the rhizosphere around the plant roots, which is also an 'environmental hotspot' for gene transfer.[42] Other workers have found evidence of horizontal transfer of kanamycin resistance from transgenic DNA to *Acinetobactor*, and positive results were obtained using just 100 ml of plant-leaf homogenate.[43]

Defenders of the biotech industry still insist that just because horizontal gene transfer occurs in the laboratory does not mean it can occur in nature. However, there is already evidence suggesting it can occur in nature.

First of all, genetic material released from dead and live cells is now found to persist in all environments; and not rapidly broken down as previously supposed. It sticks to clay, sand and humic acid particles and retains the ability to infect (transform) a range of microorganisms in the soil.[44] The transformation of bacteria in the

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[41] Schlutter *et al.*, 1995 (note 39).

[42] Timms-Wilson, T.M., Lilley, A.K. and Bailey, M.J. (1999). *A Review of Gene Transfer from Genetically Modified Micro-organisms*. Report to UK Health and Safety Executive.

[43] Gebhard and Smalla, 1998 (note 39).

[44] Reviewed by Lorenz, M.G. and Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev*. 58, 563-602.

soil by DNA adsorbed to clay sand and humic acid has been confirmed in microcosm experiments.[45]

Reseachers in Germany began a series of experiments in 1993 to monitor field releases of transgenic rizomania-resistant sugar beet (*Beta vulgaris*), containing the marker gene for kanamycin resistance. They looked for persistence of transgenic DNA and for horizontal gene transfer of transgenic DNA into soil bacteria.[46] It is the first such experiment to be carried out, after tens of thousands of field releases and tens of millions of hectares have been planted with transgenic crops. It will be useful to review their findings in detail.

Transgenic DNA was found to persist in the soil for up to two years after the transgenic crop was planted. Though they did not comment on it, the data showed that the proportion of kanamycin resistant bacteria in the soil increased significantly between 1.5 and 2 years. Could that be due to horizontal transfer of antibiotic resistance marker gene to soil bacteria?

A total of 4000 colonies of soil bacteria were isolated, a rather small number, and none was found to have taken up transgenic DNA by the probes available. However, two out of seven samples of *total* bacterial DNA yielded positive results after 18 months. This suggests that horizontal gene transfer may have taken place, but the specific bacteria that have taken up the transgenic DNA cannot be isolated as colonies. That is not surprising as less than 1% of all the bacteria in the soil can be cultured by current techniques. The au-

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[45] Paget, E. and Simonet, P. (1997). Development of engineered genomic DNA to monitor the natural transformation of *Pseudomonas stutzeri* in soil-like microcosms. *Can. J. Microbiol*. 43, 78-84.

[46] Gebhard, F. and Smalla, K. (1999). Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. *FEMS Microbiology Ecology* 28, 261-272.

thors were careful not to rule out transgenic DNA getting adsorbed to the surface of bacteria rather than being actually transferred into the bacteria.

The researchers also carried out microcosm experiments to which total transgenic sugar-beet DNA was added to non-sterile soil with its natural complement of microorganisms. The intensity of the signal for transgenic DNA decreased during the first days and subsequently increased. The most likely interpretation of this observation is that the transgenic DNA has been taken up by bacteria and become replicated as the bacteria multiply.

In parallel, soil samples were plated, and the total bacterial lawn allowed to grow for 4 days. After that, DNA was extracted and probed for transgenic DNA. Several positive signals were found, 'which might indicate uptake of transgenic DNA by competent bacteria'.

The authors were cautious not to claim conclusive results, simply because the specific bacteria carrying the transgenic DNA sequences were not isolated. The results do show, however, that horizontal gene transfer may have taken place both in the field and in the soil microcosm.

DNA is not broken down sufficiently rapidly in the gut either, which is why transfer of transgenic DNA to microorganisms in the gut of bee larvae would not be surprising. A genetically engineered plasmid was found to have a 6% to 25% survival after 60 minutes of exposure to human saliva. The partially degraded plasmid DNA was capable of transforming *Streptococcus gordonii*, one of the bacteria that normally live in the human mouth and pharynx. The frequency of transformation dropped exponentially with time of exposure to saliva, but it was still detectable after 10 minutes. Hu-

man saliva actually contains factors that promote competence of resident bacteria to become transformed by DNA.[47]

Viral DNA fed to mice is found to reach white blood cells, spleen and liver cells via the intestinal wall, to become incorporated into the mouse cell genome.[48] When fed to pregnant mice, the viral DNA ends up in cells of the foetuses and the new-born animals, indicating that it has gone through the placenta as well.[49] The authors remark that 'The consequences of foreign DNA uptake for mutagenesis [mutations] and oncogenesis [cancer] have not yet been investigated.'[50] As already mentioned, recent experiments in gene therapy leave little doubt that naked nucleic acid constructs can readily enter mammalian cells and in many cases become incorporated into the cell's genome.

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[47] Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A. and Flint, H.J. (1999) Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* 65, 6-10.

[48] Schubbert, R., Rentz, D., Schmitz, B. and Doerfler, W. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proc. Nat. Acad. Sci. USA* 94, 961-6.

[49] Doerfler, W. and Schubbert, R. (1998). Uptake of foreign DNA from the environment: the gastrointestinal tract and the placenta as portals of entry, *Wien Klin Wochenschr*. 110, 40-44.

[50] Doerfler and Schubbert, 1998, (note 49), p. 40.

Conclusion

Horizontal gene transfer is an established phenomenon. It has taken place in our evolutionary past and is continuing today. All the signs are that natural horizontal gene transfer is a regulated process, limited by species barriers and by mechanisms that break down and inactivate foreign genetic material. Unfortunately, genetic engineering has created a huge variety of artificial constructs designed to cross all species barriers and to invade essentially all genomes. Although the basic constructs are the same for all applications, some of the most dangerous may be coming from the waste disposal of contained users of transgenic organisms.[51] These will include constructs containing cancer genes from viruses and cells from laboratories researching and developing cancer and cancer drugs, virulence genes from bacteria and viruses in pathology labs. In short, the biosphere is being exposed to *all kinds of novel constructs and gene combinations that did not previously exist in nature, and may never have come into being but for genetic engineering*.

There is an urgent need to establish effective regulatory oversight, in the first instance, to prevent the escape and release of these dangerous constructs into the environment, and then to consider whether some of the most dangerous experiments should be allowed to continue at all.

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[51] See Ho, *et al.*, 1998 (note 4); Ho *et al.*, 2000 (note 8).

Genetic engineering involves designing artificial constructs to cross species barriers and to invade genomes. In other words, it enhances horizontal gene transfer – the direct transfer of genetic material to unrelated species. The artificial construct (transgenic DNA) typically contains genetic material from bacteria, viruses and other genetic parasites that cause diseases as well as antibiotic resistance genes that make infectious diseases untreatable. Horizontal transfer of transgenic DNA has the potential to create new viruses and bacteria that cause diseases, spread antibiotic resistance genes to pathogenic bacteria and trigger cancer in mammalian cells. There is an urgent need to establish effective regulatory oversight to prevent the escape and release of these dangerous constructs into the environment, and to consider whether some of the most dangerous experiments should be allowed to continue at all.

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