TOOLS FOR SAFETY ASSESSMENT

The Release of Transgenic Plants. Horizontal Gene Transfer

Kirsten Schlüter

Institute of Plantscience, Swiss Federal Institute of Technology,
Universitätsstrasse 2, 8092 Zurich

Agency for Biosafety Research and Assessment of Technology Impacts of the
Swiss Priority Programme Biotechnology

February 1995

Table of Contents

1 Introduction

Plant genetic engineering is rapidly proceeding leading to numerous genetically modified cultivars. Several field releases have taken place and the first product is on the market now.
One of the main safety concerns in terms of the release of transgenic plants is the transfer of a transgene to other organisms. Such gene transfer can be caused by pollen flow resulting in a hybridization with related species (BATS report 1-94), or it may happen by non sexual processes.
The present report focuses on non-sexual exchanges of genetic information between genomes, the so called ***horizontal gene transfer***. A general overview on horizontal gene transfer events is given in chapter 2, while the potential recipients of plant derived DNA, the respective transfer mechanisms and the different detection methods are presented in greater detail in the following chapters (see Fig. 1).

Fig. 1: Gene transfer from plants to other organisms. Classes of potential recipients of plant derived DNA and the transfer mechanisms.

Requirements

To cause a durable alteration of the recipient by a transferred gene, three requirements have to be met:

- the transfer of the transgene
- the expression of the transgene
- the inheritance of the transgene of the transgene.

The newly acquired gene must code for features having a selective advantage for the recipient, since otherwise this gene will get lost again or stays as a silent sequence in the recipient's genome.

1. Gene transfer

- **The plant DNA has to be accessible to the recipient.**
  The recipient has to interact closely with the gene donor like symbionts or pathogens do, or the plant DNA has to be released from the plant tissue and to remain stable in the environment until it is taken up by the recipient.
  DNA can be released from rotting plant material in soil or water and will remain stable to some degree by binding to soil or sediment particles (chapter 7.4), or plant-DNA can be released from animals after food uptake and may be not completely degraded (chapter 7.5).
- **The recipient should be naturally competent for DNA uptake.**
  An enhanced competence for DNA-uptake is proved for some bacteria during special phases of their growth cycle (chapter 7.2.1). It can be caused by the lack of one main nutrient or by other environmental stress factors like a heat shock or the presence of organic solvents (ethanol), acids, bases and detergents (chapter 7.2.1). There are also evidences that the conditions in the intestine of insects might increase bacterial competence (see 7.5.1).

2. Gene expression

- **The transferred gene should have a complete coding region.**
  A transferred gene with a complete coding region will be expressed when it inserts just behind a promoter in the recipient's genome.
  If the insertion occurs somewhere else, the transgene must bring along a promoter which can be recognized by the RNA polymerase of the recipient.
  Introns should only be present in the transgene, when the recipient has an appropriate splicing mechanism to remove eukaryotic introns from the precursor mRNA.

3. Gene inheritance

For the stable inheritance of the transferred DNA in the recipient, two alternatives are conceivable:

- **Integration into chromosomal or extrachromosomal DNA-sequences of the recipient.**
  The transferred DNA can be either a non-mobile or mobile genetic element.
  The stable integration of **non-mobile** DNA fragments will be facilitated by the presence of homologous sequences, but also non-homologous recombination is possible.

Mobile genetic elements include transposons and retrotransposons. While the transposons move in form of DNA, the retrotransposons are mobile through an RNA intermediate. Transposable elements (TEs) integrate as dsDNA fragments at a new locus due to the action of a TE encoded integrase, which cuts the host genome while having bound a TE copy. Most of the integrases do not have special sequence preferences for cutting DNA (chapter 6).

- **Persistence of the transgene as an extrachromosomal element by itself,** e.g. as a bacterial or fungal plasmid or as a viral satellite. For a bacterial plasmid the minimal requirement is the presence of an origin of replication next to the transgene, which is important for plasmid multiplication and plasmid transfer to future generations.

Detection

Indirect and direct approaches have been used for the detectionof possible HGT.

Indirect evidence is based on **sequence comparison** and assembly of gene specific phylogenetic trees (chapter 3). Usually, all genes of an organism co-evolve and will be placed at similar positions in a phylogenetic tree. Genes acquired by HGT will appear at unexpected positions in such a tree, because they were added to the genome at a later time. Appearance of such out-of-place genes can therefore be taken as indication for a gene transfer event. This assumption will be strengthened by a life-history indicating close contact of both partners.
For direct evidence HGT is investigated **experimentally**. HGT is expected to be an extremely rare event because otherwise separate species could not have persisted during evolution. In the experiments done so far artifical gene transfer promoting systems were used in which the donor-DNA was easily accessable to the recipient and transgene expression was assured.

2 Horizontal Gene Transfer - General

Horizontal gene transfer is best known for bacteria, where it is caused by conjugation, transduction and transformation. In some cases horizontal gene transfer crosses the borders of different kingdoms and therefore is called **interkingdom gene transfer**. A well known example is *Agrobacterium* inserting its T(transfer)-DNA into the genome of its plant hosts. An overview on the possible transfer mechanisms and the different organism classes involved in the process of horizontal gene transfer is given on the leaflet at the end of this report.

The scheme refers to the following literature:

- **Gene transfer by bacterial conjugation** (Mazodier and Davis, 1991; Tri-Cuot and Poyart, 1994)
- **Gene transfer by phage transduction** (Schmieger, 1994)
- **Gene transfer by viral recombination** (Falk and Bruening, 1994; Lai, 1992)
- **Gene transfer by transposable elements** (Kidwell, 1993)
- **Potential gene transfer events deduced from sequence homology studies** (Mazodier and Davis, 1991; Smith *et al.*, 1992; Syvanen, 1994)

Kidwell (1993), Mazodier and Davis (1991) and Syvanen (1994) present a complex survey on HGT. Kidwell describes the gene transfer from or to eukaryotes and distinguishes between non-mobile genomic sequences and mobile elements. Mazodier and Davis concentrate on gene exchange between bacteria, including higher organisms as gene donors or recipients as well.

C.F. Amabile-Cuevas and M.E. Chicurel
Horizontal gene transfer.
American Scientist 81 (1993) p. 332-341
horizontal gene transfer, review

G. Bell
The sexual nature of the eukaryote genome.
Journal of Heredity 84 (1992) p. 351-359
horizontal gene transfer, review

I. Broer and A. Pühler
Stabilität von HR-Genen in transgenen Pflanzen und ihr spontaner horizontaler Gentransfer auf andere Organismen.
In: W. van den Daele, A. Pühler, H. Sukopp (eds). Verfahren zur Technikfolgenabschätzung des Anbaus von Kulturpflanzen mit gentechnisch erzeugter Herbizidresistenz. Heft 3. FS II 94-303. Wissenschaftszentrum Berlin für Sozialforschung (WZB). (1994)
horizontal gene transfer, review

B.W. Falk and G. Bruening
Will transgenic crops generate new viruses and new diseases?
Science 263 (1994) p. 1395-1396
horizontal gene transfer, transgenic plant, virus, viral recombination, review

M.G. Kidwell
Lateral transfer in natural populations of eukaryotes.
Annual Review of Genetics 27 (1993) p. 235-356
horizontal gene transfer, review, mobile elements, transposon, retrotransposon

K. Kubitzki, P. v. Sengbusch and H.-H. Poppendieck
Parallelism, its evolutionary origin and systematic significance.
ALISO 13 (119) p. 191-206
horizontal gene transfer, review

M.M.C. Lai
RNA recombination in animal and plant viruses.
Microbiological reviews 56 (1992) p. 61-79
horizontal gene transfer, virus, viral recombination, review

P. Mazodier and J. Davis
Gene transfer between distantly related bacteria.
Annual Review of Genetics 25 (1991) p. 147-171
horizontal gene transfer, review

O. Schieder
Gentransfer und Sicherheitsforschung: Können Erbinformationen über Artschranken in der Umwelt weitergegeben werden?
In: D. Bartsch, H. Sukopp (ed): Ermittlung und Bewertung des ökologischen Risikos beim Umgang mit gentechnisch veränderten Organismen. Dokumentation eines Fachgesprächs. 14.-15. Oktober, 1991. Umweltbundesamt, Forschungsbericht 10802086/01, UBA-FB 93-037 (1993) p. 61-67
horizontal gene transfer, review

H. Schmieger
Transduction and its relevance for intra- and interspecific gene transfer.
In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld (1994)
horizontal gene transfer, phage, transduction, review

M.W. Smith, D-F. Feng and R.F. Doolittle
Evolution by acquisition: the case for horizontal gene transfers.
TIBS 17 (1992) p. 489-493
horizontal gene transfer, plant, bacteria, glyceraldehyde-3-phosphate dehydrogenase, glutamine synthetase II, glucose-6-phosphate isomerase, sequence homology

M. Syvanen
Horizontal gene transfer: evidence and possible consequences.
Annual Review of Genetics 28 (1994) p. 237-261
horizontal gene transfer, sequence homology, review

P. Trieu-Cuot and C. Poyart
Conjugation between gram-negative and gram-positive microorganisms.
In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld (1994)
horizontal gene transfer, bacteria, conjugation, transposon, review

C.J. Thompson
Horizontal gene transfer and evolution.
In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld (1994)
horizontal gene transfer, review

3 Horizontal Gene Transfer Plant ⇒ Bacteria

3.1 Sequence homology

Unexpectedly close sequence homologies between plant and bacterial genomes have been detected in different cases:

- **glyceraldehyde-3-phosphate dehydrogenase (GAPDH)**
- **glucose-6-phosphate isomerase (GPI)**
- **glutamine synthetase II (GSII)**
- **hemoglobin and**
- **T-DNA.**

Smith *et al.* (1992) discuss the degree of sequence homology and the probability of a potential HGT for the first three examples. While the transfer of genes coding for the GAPDH and the GPI is seen to be likely, it seems to be unlikely for the GSII gene.

M.W. Smith, D-F. Feng and R.F. Doolittle
Evolution by acquisition: the case for horizontal gene transfers.
TIBS 17 (1992) p. 489-493
horizontal gene transfer, plant, bacteria, glyceraldehyde-3-phosphate dehydrogenase, glutamine synthetase II, glucose-6-phosphate isomerase

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

The bacterium *Escherichia coli* and also other enteric bacteria have two GAPDHs. GAPDH A is similar to the eukaryotic form of this enzyme while GAPDH B is typical for bacteria. The phylogenetic trees indicate that transfer of the GAPDH A gene occurred before the divergence of animals and plants (Smith *et al.* 1992).

R.F. Doolittle, D.F. Feng, K.L. Anderson and M.R. Alberro
A naturally occuring horizontal gene transfer from a eukaryote to a prokaryote.
Journal of Molecular Evolution 31 (1990) p. 383-388
horizontal gene transfer, plant, bacteria, glyceraldehyde-3-phosphate dehydrogenase, sequence homology

Glucose-6-phosphate isomerase (GPI)

The GPI of *Escherichia coli* is highly homologous to the GPI from the chloroplasts of the wild flower *Clarkia sp.* In the gut of its animal host this enteric bacterial species might have taken up the plant DNA.

B.E. Froman, R.C. Tait and L.D. Gottlieb
Isolation and characterization of the phosphoglucose isomerase gene from *Eschericha coli*.
Molecular & General Genetics 217 (1989) p. 126-131
horizontal gene transfer, plant, bacteria, Clarkia xanthiana, Escherichia coli, glucose-6-phosphate isomerase, sequence homology

M.W. Smith and R.F. Doolittle
Anomalous phylogeny involving the enzyme glucose-6-phosphate isomerase.
Journal of Molecular Evolution 34 (1992) p. 544-545
horizontal gene transfer, plant, bacteria, Clarkia ungulata, Escherichia coli, glucose-6-phosphate isomerase, sequence homology

Glutamine synthetase II (GSII)

Of the two glutamine synthetases, GSI is characteristic for prokaryotes, while GSII is present in eukaryotes. The discovery of GSII in plant symbiotic bacteria of the *Rhizobiaceae* family was originally seen as an example for HGT (Carlson and Chelm, 1986). But the detection of both GS types also in non-symbiotic bacteria belonging to *Streptomytaceae* argues against HGT and the arrangement of both GS genes in tandem position in the plant symbiotic bacterium *Frankia* indicates that these two genes could be derived from gene duplication and subsequent divergent development during evolution (Kumada *et al.* 1993).

T.A. Carlson and B.K. Chelm
Apparent eukaryotic origin of glutamine synthetase II from the bacterium *Bradyrhizobium japonicum*.
Nature 322 (1986) p. 568-570
horizontal gene transfer, plant, bacteria, Bradyrhizobium japonicum, glutamine synthetase II, sequence homology

Y. Kumada, D.R. Benson, D. Hillemann, T.J. Hosted, D.A. Rochefort, C.J. Thompson, W. Wohlleben and Y. Tateno
Evolution of the glutamine synthetase gene, one of the oldest existing and functioning genes.
Proceedings of the National Academy of Sciences of the United States of America 90 (1993) p. 3009-3013
horizontal gene transfer, plant, bacteria, glutamine synthetase II, sequence homology

G. Pesole, M.P. Bozzetti, C. Lanave, G. Preparata and C. Saccone
Glutamin synthetase gene evolution: A good molecular clock.
Proceedings of the National Academy of Sciences of the United States of America 88 (1991) p. 522-526
horizontal gene transfer, plant, bacteria, glutamine synthetase II, sequence homology

R.G. Shatters and M.L. Kahn
Glutamine synthetase II in *Rhizobium*: reexamination of the proposed horizontal transfer of DNA from eukaryotes to prokaryotes.
Journal of Molecular Evolution 29 (1989) p. 422-428
horizontal gene transfer, plant, bacteria, Rhizobium meliloti, glutamine synthetase II, sequence homology

Hemoglobin

HGT has been assumed for the hemoglobin of *Vitreoscilla* which shows a maximum sequence homology with the lupine leghemoglobin (Wakabayashi *et al.*, 1986). Sequence homology is observed also for the untranslated 3'ends of this bacterial gene and several plant leghemoglobin genes (Khosla and Baily, 1988). As *Vitreoscilla* is found in oxygen-poor environments such as decaying vegetable matter, it might have taken up the hemoglobin gene from this rotting plant material.

C. Khosla and J.E. Bailey
The Vitreoscilla hemoglobin gene: molecular cloning, nucleotide sequence and genetic expression in *Escherichia coli*.
Molecular & General Genetics 214 (1988) p. 158-161
horizontal gene transfer, plant, bacteria, Vitreoscilla, hemoglobin, sequence homology

S. Wakabayashi, H. Matsubara and D.A. Webster
Primary sequence of a dimeric bacterial haemoglobin from *Vitreoscilla*.
Nature 322 (1986) p. 481-483
horizontal gene transfer, plant, bacteria, Vitreoscilla, hemoglobin, sequence homology

T-DNA

Sequence homology has been detected between the T(transfer)-DNA of the Ri (root inducing) plasmid of *Agrobacterium rhizogenes* and the genome of *Nicotiana glauca*. White *et al.* (1983) suggested two theories for explaining this homology:
(1) The bacterium might have captured plant genes which became linked to the bacterial chromosome and now are reinserted as a part of the T-DNA into plant genomes during infection by *Agrobacterium*.
(2) The plant may have acquired bacterial genes during past *Agrobacterium* infections and retained these genes.
Sequencing data from other members of the genus *Nicotiana* and the family *Solanaceae* supported the second theory. Since homology of T-DNA was limited to species of the subgenera *N. rustica* and *N. tabaccum*, infection of a common ancestor of both subgenera is assumed or, alternatively, the infection of one subgenera followed by lateral spread of the bacterial sequence through plant interspecific hybridization (Furner *et al.*, 1986).

I.J. Furner, G.A. Huffman, R.M. Amasino, D.J. Garfinkel, M.P. Gordon and E.W. Nester
An *Agrobacterium* transformation in the evolution of the genus *Nicotiana*.
Nature 319 (1986) p. 422-427
horizontal gene transfer, plant, bacteria, tobacco, Nicotiana glauca, Agrobacterium rhizogenes, T-DNA, sequence homology

F.F. White, D.J. Garfinkel, G.A. Huffman, M.P. Gordon and E.W. Nester
Sequences homologous to *Agrobacterium rhizogenes* T-DNA in the genome of uninfected plants.
Nature 301 (1983) p. 348-350
horizontal gene transfer, plant, bacteria, tobacco, Nicotiana glauca, Agrobacterium rhizogenes, T-DNA, sequence homology

3.2 Experimental approach

Different types of experiments have been performed dealing with gene transfer from plants to bacteria.
Reports on the transfer of released DNA present in soil, water and in the intestine of animals are listed in the chapter "DNA-persistence and DNA-uptake" (chapter 7), while this chapter refers to experiments with intact transgenic plant tissue serving as a gene source and with bacterial recipients living in close contact with the transgenic plant.

Broer *et al.* (1994) chose plant tumors caused by *Agrobacterium* infections as a system for analyzing gene transfer. Transgenic tobacco plants carrying marker genes which can be expressed in bacteria, were infected with an *Agrobacterium* wildtype strain. After tumor formation the bacteria were reisolated and analyzed for the presence of these marker genes. With an experimental detection limit of one transformant per 6×10^{12} bacteria, no gene transfer event could be detected.
In preexperiments the natural competence of the *Agrobacterium* strain for taking up DNA has been proven. One transformant resulted out of 2×10^{7} bacteria when using plasmid DNA but no transformant was found when adding linearized transgenic plant DNA. Additionally it has been shown that the induction of the bacterial virulence genes by applying acetosyringon and the presence of plant material did not improve transformation capacity. The advantage of this model system is the close interaction between plant and bacterium. But as the bacterium is thought to stay in the intercellular space of the plant tissue, it is uncertain whether the plant DNA in the nucleus is directly accessible to the bacterium.

This problem might be overcome by using a model system of plant-pathogen interaction from which it is known that the pathogen lyses plant tissue. In the project of Schlüter (1994), transgenic potatoes containing the prokaryotically expressed β-lactamase gene are inoculated with the bacterial pathogen *Erwinia chrysanthemi*, which produces cell wall dissolving enzymes, especially pectate lyases, and causes stem rot and wet rot of the tubers. Experimental prerequisites for gene transfer are the natural competence of the bacterial recipient for DNA uptake and the *in planta* conservation of the transgene in an unmodified, active form so that gene transfer to the bacterium could be followed by gene expression. Calculations based on experimental results gave a transformation frequency lower than 10^{-17} (unpublished results).

Possible gene transfer events have been also investigated by Lebrun *et al.* (1993, 1992) for soil microorganisms and leaf surface bacteria which are in contact with transgenic tobacco plants containing a copy of a prokaryotically functional antibiotic- or herbicide resistance gene. Data have not been published yet.

I. Broer, W. Dröge-Laser, M. Gerke, I-M. Pretorius-Güth and A. Pühler
Horizontal gene transfer from transgenic plants to associated soil bacteria.
In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld (1994)
horizontal gene transfer, transgenic plant, bacteria, Agrobacterium tumefaciens, antibiotic resistance, neomycin, kanamycin, gentamycin, luciferase, experiment

M. Lebrun, A. Clapot and K.E. Pallett
Putative gene transfer between transgenic plants and soil microorganisms: Design of an experimental system based on the use of the bxn gene in transgenic tobacco plants.
In: Commission of the European Communities (ed). BRIDGE/BIOTECH. Final sectorial meeting on biosafety and first sectorial meeting on microbial ecology. October 24-27,1993. Granada (1993) p. 26
horizontal gene transfer, transgenic plant, bacteria, tobacco, herbizide resistance, bromoxynil, antibiotic resistance, gentamycin, experiment

K. Schlüter
Risk assessment concerning the deliberate release of transgenic plants.
In: Schweizerischer Nationalfond (ed) Prisma 94. Schwerpunktprogramme (1994) p. 46
research, National Foundation, SchwerPunktProgramm-Biotechnology, Switzerland, horizontal gene transfer, transgenic plant, bacteria, potato, Erwinia chrysanthemii, antibiotic resistance, ampicillin, experiment

4 Horizontal Gene Transfer Plant ⇒ Fungi

The results of three experiments dealing with HGT from plants to fungi have been published. In one case an unmodified plant acted as gene donor while in the other two cases GMPs were used.

Bryngelson *et al.* (1988) worked on the host-pathogen system *Brassica napus* - *Plasmodiophora brassicae*. In the host derived fungus repetitive DNA sequences have been detected. After infecting a susceptible variety of *Sinapsis alba* with *P. brassicae*, the new resting spores lacked the *B. napus* sequences but instead contained a diverged version specific for *S. alba*. It was concluded that *P. brassicae* takes up host specific sequences during each infection cycle.

Oliver and Curtis (1993) have attempted to detect HGT between transgenic tomato and the fungal pathogen *Cladosporum fulvum*. The genetic elements of interest are retrotransposons (Tnt-1 of plants, CfT-1 of fungi) which have been marked by integrating an antibiotic resistance gene under control of a fungal or plant viral promoter. Results of these experiments have no been published yet.

Hoffmann *et al.* (1994), cocultivated transgenic *Brassicaceae* resistant to hygromycin with the naturally transformable fungus *Aspergillus niger* in microcosms under sterile conditions. An increased number of resistant fungi was obtained after cocultivation with GMPs compared to cocultivation with unmodified plants. Only in 5% from over 200 resistant *Aspergillus* colonies the transgene could be detected by Southern blot hybridization. Nearly all of these supposed transformants lost their transgenic character after further cultivation. Only in one case the fungus maintained its acquired resistance. DNA reisolation and analysis revealed a sequence corresponding to the original plant transformation vector, but did not contain the hygromycin resistance gene. The authors concluded that spontaneous gene transfer from *Brassica sp*. to *Aspergillus niger* is possible in soil microcosms but the extent and the mechanism of this HGT are still not known.

T. Bryngelsson, M. Gustafsson, B. Gréen and C. Lind
Uptake of host DNA by the parasitic fungus *Plasmodiophora brassicae*.
Physiological and Molecular Plant Pathology 33 (1988) p. 163-171
horizontal gene transfer, plant, fungi, oilseed rape, Brassica napus, Sinapsis alba, Plasmodiophora brassicae, sequence homology

T. Hoffmann, C. Golz and O. Schieder
Foreign DNA sequences are received by a wild-type strain of *Aspergillus niger* after co-culture with transgenic higher plants.
Current Genetics 27 (1994) p. 70-76
horizontal gene transfer, transgenic plant, fungi, Brassica, Aspergillus niger, antibiotic resistance, hygromycin, experiment

C. Moreau, C. Audeon, E. Huntner and M.A. Grandbastien
Expression of the Tnt1 tobacco retrotransposon in tomato (*Lycopersicon esculentum*).
In: Commission of the European Commmunities (ed). BRIDGE/BIOTECH. Final sectorial meeting on biosafety and first sectorial meeting on microbial ecology. October 24-27, 1993. Granada (1993) p. 82-83
horizontal gene transfer, transgenic plant, fungi, tomato, Lycopersicon esculentum, tobacco, Nicotiana tabaccum, Cladosporium fulvum, retrotransposon, ß-glucuronidase, experiment

R.P. Oliver and M.D. Curtis
An experimental approach to investigate horizontal gene transfer between organisms.
In: Commission of the European Communities (ed). BRIDGE/BIOTECH. Final sectorial meeting on biosafety and first sectorial meeting on microbial ecology. October 24-27, 1993. Granada (1993) p. 84
horizontal gene transfer, transgenic plant, fungi, tomato, Lycopersicon esculentum, Cladosporium fulvum, retrotransposon, experiment

5 Horizontal Gene Transfer Plant ⇒ Viruses

5.1 General

The mechanism of HGT from plants to viruses is recombination. As the genome of most plant viruses consists of RNA, recombination has to take place between plant mRNA and the virus genomic RNA. The ability of RNA viruses to undergo genetic recombination in general has been demonstrated for a few viruses. Unlike DNA recombination which usually involves double stranded DNA, only single stranded RNA has been shown to undergo RNA recombination. Since recombination occurs only during RNA synthesis a copy choice mechanism is assumed which involves a RNA polymerase jumping from one template to the other. In the overview by Lai (1992) the three different types of RNA recombinations are listed:

- **Homologous recombination**
  involves two homologous RNA molecules with the crossover occurring at the same site of each molecule.
- **Aberrant homologous recombination**
  involves two RNAs with homologous or similar sequences, but the crossovers occur at nearby sites of the molecules resulting in duplication or deletion of sequence areas.
- **Non-homologous or illegitimate recombination**
  occurs between two RNA sequences which do not have any sequence homology.

In experiments with viral deletion mutants homologous and aberrant homologous recombination between the different RNA segments of one virus have been demonstrated for members of the bromovirus family. Illegitimate recombination was assumed for one satellite RNA of a member of the carmovirus family. In this case three recombination events took place between two different satellite RNAs and the helper virus genome. RNA recombination has also been shown between different virus strains or even between distinct members of a virus family (e.g. tobraviruses) during coinfection of a plant (Lai, 1992).
Another overview on recombination events involving viral RNA was given in a paper by Falk and Bruening (1994). Investigations on gene transfer from non-transgenic and transgenic plants to infecting viruses were listed in this review (see 5.3).

B.W. Falk and G. Bruening
Will transgenic crops generate new viruses and new diseases?
Science 263 (1994) p. 1395-1396
horizontal gene transfer, transgenic plant, virus, viral recombination, review

M.M.C. Lai
RNA recombination in animal and plant viruses.
Microbiological reviews 56 (1992) p. 61-79
horizontal gene transfer, virus, viral recombination, review

M. Tepfer
Viral Genes and Transgenic Plants.
Bio - Technology 11 (1993) p. 1125-1132
horizontal gene transfer, transgenic plant, virus, viral recombination, review

5.2 Sequence Homology

Sequence homologies between viral RNA or viral satellite RNA and chloroplast DNA suggesting HGT by recombination are described in two papers:

C. Masuta, S. Kuwata, T. Matzuzaki, Y. Takanami and A. Koiwai
A plant virus satellite RNA exhibits a significant sequence complementarity to a chloroplast tRNA.
Nucleic Acids Research 20 (1992) p. 2885
horizontal gene transfer, plant, virus, cucumber mosaic virus, viral recombination, sequence homology

M.A. Mayo and C.A. Jolly
The 5'-terminal sequence of potato leafroll virus RNA: evidence of recombination between virus and host RNA.
Journal of General Virology 72 (1991) p. 2591-2595
horizontal gene transfer, plant, virus, tobacco, potato leafroll virus, viral recombination, sequence homology

5.3 Experimental Approach

Experiments have been done on gene transfer from transgenic plants to

- **RNA viruses**
- **DNA viruses**
- **viroids**.

Gene transfer from transgenic plants to RNA viruses

Lommel and Xiong (1991) created transgenic tobacco plants which carried the sequence for the movement protein of red clover necrotic mosaic virus. Inoculation with infectious transcripts lacking the movement protein sequence resulted in systemic infection. Reisolated viruses contained a complete RNA genome and sequence analysis indicated that recombination had taken place.

In the experiment of Greene and Allison (1994), transformed plants expressing 2/3 of the coat protein (CP) of the cowpea chlorotic mottle virus were inoculated with a partial CP deletion mutant of this RNA virus. Systemic infection could only occur if recombination restored the capsid gene. Even under this strong selection pressure, a recombination occurred only in 3% of the tested plants. By the introduction of marker mutations in the CP sequence present in the GMPs it could be ascertained that the horizontally transferred sequence came actually from the GMP and not from another source, e.g. a coinfecting virus.

There are two ongoing projects on viral recombination involving GMPs and either luteoviruses (Bruening and Falk 1994) or potyviruses (Malnoe, 1994). The frequency of recombination and factors affecting recombination will be determined in these projects.

Gene transfer from transgenic plants to DNA viruses

A possible transfer mechanism of DNA from GMPs to DNA viruses might be intermolecular recombination by RNA template switching of the reverse transcriptase during virus replication. First the enzyme starts reverse transcription at the viral RNA intermediate, then it switches the template at a highly homologous region and proceeds with DNA synthesis on the transgenic mRNA of the GMP until it switches back to its original template, again at a region of sequence homology (Schoelz and Wintermantel, 1993).

Experimental proof for the transfer of viral sequences stably integrated into a plant genome to a dsDNA virus has been given in two cases. Gal *et al.* (1991) constructed transgenic *Brassica napus* containing one of the six genes of the cauliflower mosaic virus (CaMV) in its genome. GMPs were inoculated with *Agrobacterium* containing the other five genes of CaMV. To allow recombination the two viral sequence constructs contained regions of homology. After some weeks virus particles could be reisolated from the plants. These viruses were able to infect nontransgenic turnip plants. By this, gene transfer from chromosomally located viral genes to extrachromosomal viral genes has been evidenced.

Schoelz and Wintermantel (1993) used host range differences of closely related CaMV strains to assay for recombination between viral genomes and transgenes: A

host-range determining gene from a strain able to multiply in tobacco was stably integrated into tobacco plants. These transgenic plants now supported viral growth even of strains that normally are not able to infect tobacco. By sequence analysis it was confirmed that these viruses had incorporated the transgene by recombination and therefore showed an altered host-range corresponding to the transgene.

Gene transfer from transgenic plants to viroids.

In the project of Owens *et al.* (1994) mutant or wildtype **viroids** RNA are tested for their ability to recombine with modified potato spindel tuber viroid cDNAs present in transgenic tobacco.

B. Falk and G. Bruening
Risk assessment: recombination between virus genomes and genome segments in plants.
US Environmental Protection Agency, US Department of Agriculture (eds) Risk Assessment Methodologies. An International Conference for Regulatory Agencies, Academy and Industry. June 22-25, 1994. Maryland (1994)
horizontal gene transfer, transgenic plant, virus, beet western yellow luteovirus, viral recombination, experiment

S. Gal, B. Pisan, T. Hohn, N. Grimsley and B. Hohn
Agroinfection of transgenic plants leads to viable cauliflower mosaic virus by intermolecular recombination.
Virology 187 (1992) p. 525-533
horizontal gene transfer, transgenic plant, virus, oilseed rape, Brassica napus, cauliflower mosaic virus, Agrobacterium tumefaciens, experiment

A.E. Greene and R.F. Allison
Recombination between viral RNA and transgenic plant transcripts.
Science 263 (1994) p. 1423-1425
horizontal gene transfer, transgenic plant, virus, tobacco, Nicotiana benthamiana, cowpea chlorotic mottle bromovirus, viral recombination, experiment

S. Lommel and Z. Xiong
Reconstitution of a functional red clover mosaic virus by recombinational rescue of the cell-to-cell movement gene expressed in a transgenic plant.
Journal of Cellular Biochemistry 15A (1991) p. 151
horizontal gene transfer, transgenic plant, virus, Nicotiana benthamiana, red clover necrotic mosaic virus, movement protein, viral recombination, experiment

P. Malnoë
Viral resitance and RNA recombination in transgenic plants expressing viral sequences.
In: Schweizerischer Nationalfond (ed) Prisma 94. Schwerpunktprogramme (1994) p. 43
research, National Foundation, SchwerPunktProgramm-Biotechnology, Switzerland, horizontal gene transfer, transgenic plant, virus, potato virus, viral recombination, experiment

R.A. Owens, R.W. Hammond and T.O. Diener
Stability of disabled viroid sequences in transgenic plants: Generation of wild-type viroids via sequence reversion and recombination.
US Environmental Protection Agency, US Department of Agriculture (eds) Risk Assessment Methodologies. An International Conference for Regulatory Agencies, Academy and Industry. June 22-25, 1994. Maryland (1994)
research, United States, Department of Agriculture, horizontal gene transfer, transgenic plant, viroid, tobacco, Nicotiana benthamiana, potato spindle tuber viroid, viral recombination, experiment

J.E. Schoelz and W.M. Wintermantel
Expansion of viral host range through complementation and recombination in transgenic plants.
Plant Cell 5 (1993) p. 1669-1679
horizontal gene transfer, transgenic plant, virus, tobacco, Nicotiana bigelovii, cauliflower mosaic virus, viral recombination, experiment

6 Horizontal Gene Transfer - Mobile Elements

HGT is also assumed for **mobile elements** since these sequences occur quite well conserved in non-related or only distantly-related organisms while being absent in close relative ones. Sequence homologies and restricted distribution in the different organism classes (plants, animals, yeast) serve here as an indication for gene transfer. Starting from the gene donor, the mobile element most probably will not insert directly into the genome of the recipient, but will involve a vector. Therefore two gene transfer events are required:

- transfer from the gene-donor to a vector
- transfer from the vector to a gene-acceptor.

The most obvious candidates for such vectors are viruses (Smith, 1993). Both classes of transposable elements (TEs), transposons and retrotransposons, have been found in plants. Kidwell (1993) summarizes reports providing evidence for possible HGT of TEs by listing the name of the mobile element, its host and the strength of evidence for HGT. The strength of evidence is based on studies of sequence homologies between the TE encoded proteins, which function in propagation of the respective element, namely transposases in case of transposons and reverse transcriptases in case of retrotransposons.
The discovery that several TEs which inserted into transcribed sequences will be spliced out from the precursor-mRNA, strengthens the idea that nuclear introns may have their origin in a transposable ancestor (Kidwell, 1993, Purugganan, 1993).

A description of plant **retrotransposons** and their distribution in divergent hosts is given in the review article of Smith (1993). For members of the *copia*-like retroelement family, Voytas *et al.* (1992) concluded HGT, since *copia*-like TEs were detected in 64 plant species from 9 of 10 plant divisions and since the phylogenetic tree based on the sequence homology of the reverse transcriptase showed little congruence with the phylogenic relationship of the analyzed species.

A special case of gene transfer in which cellular DNA had been transduced by a retrotransposon within a cell, is reported by Bureau *et al.* (1994). The retroelement was isolated as a recent insertion into a maize gene. Sequence analysis revealed that the transfer function must have been supplied in trans since the retroelement lacked the gene which is required for autonomous retrotransposition. It had been replaced by a sequence with significant similarity to a plant ATPase. Therefore, this altered retrotransposon provides evidence for transfer not only of incomplete TEs but also of cellular genes being integrated in the TE sequence. Since retrotransposons are assumed to move between species, the same can be supposed for modified retrotransposons carrying a cellular gene.

An example for HGT in a **transposon** family containing also plant TEs was given by Brian *et al.* (1991). The transposases of the Ac (activator) element of maize, the Tam3 element of snapdragon and the hobo element of *Drosophila* show in colinear order three regions of strong similarities. From these observations a common evolutionary origin was proposed for the three TEs.

T.E. Bureau, S.E. White and S.R. Wessler
Transduction of a cellular gene by a plant retroelement.
Cell 77 (1994) p. 479-480
horizontal gene transfer, mobile elements, retrotransposon, maize, sequence homology

B.R. Calvi, T.J. Hong, S.D. Findley and W.M. Gelbart
Evidence for a common evolutionary origin of inverted repeat transposons in drosophila and plants: hobo, Activator, and Tam3.
Cell 66 (1991) p. 465-471
horizontal gene transfer, mobile elements, transposon, maize, Zea mays, snapdragon, Antirrhinum majus, Drosophila melanogaster, Ac, Tam3, hobo, sequence homology

M.G. Kidwell
Lateral transfer in natural populations of eukayotes.
Annual Review of Genetics 27 (1993) p. 235-356
horizontal gene transfer, review, mobile elements, transposon, retrotransposon

M.D. Purugganan
Transposable elements as introns: evolutionary connections.
TREE 8 (1993) p. 239-243
horizontal gene transfer, mobile elements, introns, review

D.R. Smith
Plant retrotransposons.
In: DPS Verma (ed). Control of plant gene expression. CRC Press (1993) p. 1-15
horizontal gene transfer, mobile elements, retrotransposon, review

D.F. Voytas, M.P. Cummings, A. Konieczny, F.M. Ausubel and S.R. Rodermel
***Copia*-like retrotransposons are ubiquitous among plants.**
Proceedings of the National Academy of Sciences of the United States of America 89 (1992) p. 7124-7128
horizontal gene transfer, mobile elements, retrotransposon, copia, sequence homology

G.J. Stewart
The mechanism of natural transformation.
In: S. B. Levy and R. V. Miller (eds). Gene transfer in teh environment. McGraw-Hill Publishing Company. New York.1989 (1989), p. 139-164
horizontal gene transfer, DNA persistence, DNA uptake, bacteria, review

7 DNA-Persistence and DNA-Uptake

7.1 General

From rotting plant material, DNA can be released into the environment and may become available to bacteria. The requirements for a successful gene transfer are

- **natural competence of bacteria for DNA uptake**
- **persistence of DNA in the environment**
- **transformability of mineral-bound DNA**.

Reviews dealing with this different aspects are:

M.G. Lorenz, W. Wackernagel
Bacterial gene transfer by natural genetic transformation in the environment.
Microbiological Reviews 58 (1994), p. 563-602
horizontal gene transfer, DNA persistence, DNA uptake, bacteria, review

E. Paget, P. Simonet
On the track of natural transformation in soil.
FEMS Microbiology Ecology 15 (1994) p. 109-118
horizontal gene transfer, DNA persistence, DNA uptake, bacteria, review

G.J. Stewart
The mechanism of natural transformation.
In: S. B. Levy and R. V. Miller (eds). Gene transfer in the environment. McGraw-Hill Publishing Company. New York.1989 (1989) p. 139-164
horizontal gene transfer, DNA persistence, DNA uptake, bacteria, review

7.2 Soil and Sediment

7.2.1 Natural bacterial competence for DNA-uptake

Natural competence is defined as the ability of bacteria to take up free DNA from the surrounding medium. This property has been demonstrated for different bacterial taxomomic groups. The bacterial species and their respective transformation frequencies for chromosomal marker genes ranging from 10^{-2} to 10^{-7}, are listed in the review of Lorenz and Wackernagel (1994). Competence is found to be most often related to the **physiological state** of the bacteria (e.g. to the log phase or the stationary phase) and is seldom constitutive. The concentration of **nutrients** seems to effect bacterial competence. *Pseudomonas stutzeri* which did not grow in soil extract media, was shown to be transformable in fully supplemented ones (with carbon, nitrogen, and phosphorus). It can be assumed that *P. stutzeri* is competent for DNA uptake in soil sites supplied with nutrients, e.g. in the rhizosphere or following natural and artificial fertilization (Lorenz and Wackernagel, 1992). The transformation frequency of Pseudomonas stutzeri could be still increased up to 290 fold in partly supplemented soil extract media leaving one nutrient, nitrogen or phosphorus, limited (Lorenz and Wackernagel, 1992)

An enhanced competence for DNA uptake is caused also by other **environmental stress factors**. Heat treatment, organic solvents (ethanol), acids, bases and detergents (SDS) were shown to increase significantly interspecific gene transfer most probably due to a stress-dependent inactivation of the restriction system in the bacterial recipient (Schäfer, 1994; Schäfer *et al.*, 1993). Although these results refer to gene transfer by conjugation and transduction, similar effects are conceivable for natural transformation.

The frequency of natural transformation depends not only from the state of competence but also on the presence of **homologous sequences** in the bacterial recipient. It was shown by Becker *et al.* (1994) that for naturally competent *Acinetobacter calcoaceticus* homologous recombination resulted in an increase in the number of transformants.

J. Becker, H. Siegert, J. Logemann and J. Schell
Begleitende Sicherheitsforschung zur Freisetzung gentechnisch veränderter Petunien.
In: Bundesministerium für Forschung und Technik (ed). Biologische Sicherheit. Forschung Biotechnologie. Band 3 (1994) p. 563-578
horizontal gene transfer, transgenic plant, bacteria, petunia, tobacco, Bacillus, Pseudomonas, Acinetobacter calcoaceticus, antibiotic resistance, neomycin, kanamycin, hygromycin, homologous recombination, DNA degradation, experiment

M.G. Lorenz, W. Wackernagel
Bacterial gene transfer by natural genetic transformation in the environment.
Microbiological Reviews 58 (1994), p. 563-602
horizontal gene transfer, DNA persistence, DNA uptake, bacteria, review

M.G. Lorenz and W. Wackernagel
Stimulation of natural genetic transformation of *Pseudomonas stutzeri* in extracts of various soils by nitrogen or phophorus limitation and influence of temperature and pH.
Microbial releases 1 (1992) p. 173-176
horizontal gene transfer, DNA persistence, DNA uptake, soil, bacteria, Pseudomonas stutzeri, experiment

M.G. Lorenz and W. Wackernagel

High frequency of natural genetic transformation of *Pseudomonas stutzeri* in soil extract supplemented with a carbon/energy and phosphorus source.
Applied and Environmental Microbiology 57 (1991) p. 1246-1251
horizontal gene transfer, DNA uptake, soil, bacteria, Pseudomonas stutzeri, experiment

A. Schäfer, J. Kalinowski, A. Pühler
Increased fertility of *Corynebacterium glutamicum* recipients in intergeneric matings with *Escherichia coli* after stress induction.
Applied and Environmental Microbiology 60 (1994) p. 756-759
horizontal gene transfer, bacteria, conjugation, Escherichia coli, Corynebacterium glutamicum, phage, transduction, stress enhanced transformation efficiency, experiment

A. Schäfer, J. Kalinowski, A. Pühler
Analysis of the major restriction system of *Corynebacterium glutamicum* by conjugation with *E. coli*.
In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld (1994)
horizontal gene transfer, bacteria, conjugation, Escherichia coli, Corynebacterium glutamicum, stress enhanced transformation efficiency, experiment

7.2.2 DNA-Persistence

DNA-Adsorption

The persistence of DNA in soil is probably caused by its adhesion to soil compounds. The extent of adsorption is affected by the pH, the concentration and the valency of the cations, the kind of soil minerals (Khanna and Stotzky, 1993), and the DNA conformation.
The adsorption of DNA to montmorillonite (Paget *et al.*, 1992; Khanna and Stotzky, 1992) and sand (Romanowsky *et al.*, 1991, Lorenz and Wackernagel, 1987) increases at a lower **pH** and at a higher concentration of **bivalent cations**. It is assumed that bivalent cations act as bridges in electrostatic interactions by neutralizing the negative charges of mineral surfaces and therefore favouring the adsorption of greater amounts of DNA (Romanowsky *et al.,* 1991). Supercoiled plasmid **DNA** molecules adsorbed slightly less than linearized or open circular ones (Romanowsky *et al.*, 1991).

M. Khanna and G. Stotzky
Transformation of *Bacillus subtilis* by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA.
Applied and Environmental Microbiology 58 (1992) p. 1930-1939
horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, Bacillus subtilis, experiment

M. Khanna, M. Roy and G. Stotzky
Adsorption of DNA from *Bacillus subtilis* on clay minerals and transformation in soil.
93rd general meeting of the American Society for Microbiology, May 16-20, 1993. Atlanta, Georgia, United States (1993) p. 377 (Q-172)
horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, Bacillus subtilis, experiment

M.G. Lorenz and W. Wackernagel
Adsorption of DNA to sand and variable degradation rates of adsorbed DNA.
Applied and Environmental Microbiology 53 (1987) p. 2948-2952
horizontal gene transfer, DNA degradation, soil, experiment

E. Paget, L.J. Monrozier and P. Simonet
Adsorption of DNA on clay minerals: protection against Dnase I and influence on gene transfer.
FEMS Microbiology Letters 97 (1992) p. 31-40
horizontal gene transfer, DNA persistence, experiment

G. Romanowski, M.G. Lorenz and W. Wackernagel
Adsorption of plasmid DNA to mineral surfaces and protection against DNase I.
Applied and Environmental Microbiology 57 (1991) p. 1057-1061
horizontal gene transfer, DNA persistence, experiment

DNA-Stability

It has been demonstrated that DNA bound to soil components is more resistant against nucleases. While free DNA in solution starts to be degraded at **DNase I** concentrations of 50 ng/ml, sand adsorbed plasmid DNA requires nearly a 100 fold higher concentration of DNase I for acid-soluble product formation (Romanowsky *et al.*, 1991).

However DNA protection is only partial, since DNase I treatment of plasmids results in a decrease of the covalently closed circular form and in an increase of the open and linear forms (Paget *et al.*, 1992). DNase I treated genomic DNA shows a loss of fragments longer than 15 kb (Paget *et al.*, 1992).

Since DNase I comes from the bovine pancreas and is of no environmental relevance, Wackernagel and Lorenz (1994) started experiments with the DNase of the ubiquitously spread bacterium *Serratia marcescens*. First results demonstrate that also against this nuclease bound DNA is more resitant than free DNA.

The intactness of DNA was demonstrated even in **unsterile soil** during a longer term. After 60 days 0,01% - 0,2% of the initially added plasmid DNA were still detectable, the varying DNA amounts depended on the soil specific DNA degradation kinetics (Romanowsky *et al.*, 1993). This DNA reextracted from soil was found to be still usable for artificial transformation of *Escherichia coli* cells. The transformation frequency was in maximum 0,01% of the initial transforming activity after 1 h of soil incubation (Romanowsky *et al.*, 1993).

E. Paget, L.J. Monrozier and P. Simonet
Adsorption of DNA on clay minerals: protection against DNase I and influence on gene transfer.
FEMS Microbiology Letters 97 (1992) p. 31-40
horizontal gene transfer, DNA persistence, experiment

G. Romanowski, M.G. Lorenz and W. Wackernagel
Use of Polymerase chain reaction and electroporation of *Escherichia coli* to monitor the persistence of extracellular plasmid DNA intoduced into natural soils.
Applied and Environmental Microbiology 59 (1993) p. 3438-3446
horizontal gene transfer, DNA persistence, soil, experiment

G. Romanowski, M.G. Lorenz and W. Wackernagel
Adsorption of plasmid DNA to mineral surfaces and protection against DNase I.
Applied and Environmental Microbiology 57 (1991) p. 1057-1061
horizontal gene transfer, DNA persistence, experiment

G. Romanowski, M.G. Lorenz, G. Sayler and W. Wackernagel
Persistence of free plasmid DNA in soil monitored by various methods, including a transformation assay.
Applied and Environmental Microbiology 58 (1992) p. 3012-3019
horizontal gene transfer, DNA persistence, soil, experiment

W. Wackernagel and M.G. Lorenz
DNA-Entlassung aus Bakterien, DNA-Übertragung und genetische Transformation im natürlichen Lebensraum.
In: Bundesministerium für Forschung und Technik (ed). Biologische Sicherheit. Forschung Biotechnologie. Band 3 (1994) p. 9-33
horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, Pseudomonas stutzeri, Bacillus subtilis, Acinetobacter calcoaceticus, experiment

7.2.3 Transformability of mineral-bound DNA

DNA has been shown to be still active in transformation not only after reextraction from soil (chapter 7.2.2) but also when bound to soil minerals. The transformation frequency was effected by environmental parameters as the pH, the temperature, the kind of minerals to which the DNA adsorbs and by the DNA type.

In the case of ***Bacillus subtilis***, the binding of DNA to the clay mineral montmorillonite reduced the transformation frequency by one order of magnitude to about 2 x 10^{-5} (Gallori *et al.,* 1994). When clay-DNA complexes were incubated together with non-sterile soil for 15 days the transformation frequency decreased again one order of magnitude to about 2 x 10^{-6} (Gallori *et al.*,1994).

Clay-DNA complexes built up at a low **pH**, resulted in a decrease of the transformation frequency which was below the detection limit at a pH of 1, probably because of a partial denaturation of the DNA (Khanna and Stotzky, 1992). Although the transformation frequency is also affected by the **temperature**, transformation still took place at 0°C and 45°C (Khanna and Stotzky, 1992).

The transformation efficiency depended also on the **minerals** to which the DNA could adsorb. It was reduced by DNA binding to montmorillonite (Gallori *et al.*, 1994) and to natural groundwater aquifer material (Romanowski *et al.*, 1993) while it was increased by DNA adsorption to sand grains (Lorenz and Wackernagel, 1988, Lorenz *et al.*, 1988).

In microcosms of ground water aquifer material the transformation frequency of sediment adsorbed **plasmid DNA** was 1000 fold reduced and that of **chromosomal DNA** only 10 fold reduced in comparison to a standard liquid assay (Romanowsky *et al.*, 1993). From this it might be concluded that in natural environments chromosomal DNA is more active in transformation than plasmid DNA.

E. Gallori, M. Bazzicalupo, L.D. Canto, R. Fani, P. Nannipieri, C. Vettori, G. Stotzky
Transformation of *Bacillus subtilis* by DNA bound on clay in non-steril soil.
FEMS Microbiology Ecology 15 (1994) p. 119-126
horizontal gene transfer, DNA persistence, DNA uptake, soil, bacteria, Bacillus subtilis, experiment

M. Khanna and G. Stotzky
Transformation of *Bacillus subtilis* by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA.
Applied and Environmental Microbiology 58 (1992) p. 1930-1939
horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, Bacillus subtilis, experiment

M.G. Lorenz and W. Wackernagel
Impact of mineral surfaces on gene transfer by transformation in natural bacterial environments.
In: Klingmüller (ed) Risk Assessment for Deliberate Releases. Springer-Verlag. Berlin, Heidelberg. 1988 (1988) p. 110-119
horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, Bacillus subtilis, experiment

M.G. Lorenz, B.W. Aardema and W. Wackernagel
Highly efficient genetic transformation of *Bacillus subtilis* attached to sand grains.
Journal of General Microbiology 134 (1988) p. 107-112
horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, Bacillus subtilis, tryptophan deficiency, experiment

G. Romanowski, M.G. Lorenz and W. Wackernagel
Plasmid DNA in a groundwater aquifer microcosm - adsorption, DNAase resistance and natural genetic transformation of *Bacillus subtilis*.

Molecular Ecology 2 (1993) p. 171-181
horizontal gene transfer, DNA persistence, DNA uptake, sediment, bacteria, Bacillus subtilis, experiment

For the soil bacterium ***Pseudomonas stutzeri*** uptake of sand-adsorbed DNA (Lorenz and Wackernagel, 1990) as well as natural transformation in soil extract microcosms has been demonstrated (Paget and Simonet, 1993).

Acinetobacter calcoaceticus can take up free high molecular weight as well as plasmid DNA in unsterile soil extracts and groundwater (Lorenz *et al.*, 1992). In microcosms of sterile groundwater aquifer material and groundwater, transformation by plasmid DNA was lower than transformation by chromosomal DNA, but seems to be promoted by the presence of chromosomal DNA on mineral surfaces (Chamier *et al.*, 1993).

M.G. Lorenz and W. Wackernagel
Natural genetic transformation of *Pseudomonas stutzeri* by sand-adsorbed DNA.
Archives of Microbiology 154 (1990) p. 380-385
horizontal gene transfer, DNA uptake, soil, bacteria, Pseudomonas stutzeri, experiment

E. Paget and P. Simonet
Evidence of gene transfer in soil via transformation.
In: Commission of the European Communities (ed). BRIDGE/BIOTECH. Final sectorial meeting on biosafety and first sectorial meeting on microbial ecology. October 24-27,1993. Granada (1993) p. 32
horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, Escherichia coli, Acinetobacter calcoaceticus, Bacillus subtilis, Pseudomonas stutzeri, experiment

M.G. Lorenz, K. Reipschläger and W. Wackernagel
Plasmid transformation of naturally competent *Acinetobacter calcoaceticus* in non-sterile soil extract and goundwater.
Archives of Microbiology 157 (1992) p. 355-360
horizontal gene transfer, DNA uptake, soil, water, bacteria, Acinetobacter calcoaceticus, experiment

B. Chamier, M.G. Lorenz, W. Wackernagel
Natural transformation of *Acinetobacter calcoaceticus* by plasmid DNA adsorbed on sand and groundwater aquifer material.
Applied and Environmental Microbiology 59 (1993) p. 1662-1667
horizontal gene transfer, DNA uptake, sediment, bacteria, Acinetobacter calcoaceticus, experiment

7.3 Marine Environments

The possibility for natural transformation was also investigated in **marine environments**. The estuarine bacterium ***Vibrio*** sp. was shown to be naturally transformable in sterile seawater and sediment microcosms with a transformation frequency between 10^{-6} and 10^{-10} (Paul *et al.*, 1991). In unsteril seawater the presence of the ambient microbial community maximally reduced the transformation efficiency by one order of magnitude while in unsteril sediment microcosms transformation could not be detected any longer (Paul *et al.*, 1991).

The marine bacterial species ***Pseudomonas stutzeri* ZoBell** is able to take up chromosomal DNA in non-sterile sediments. With the size of the DNA fragment, the number of transformants increased. DNA fragments bigger than 3,0 kb did not result in a further increase in transformation frequency (Stewart *et al.*, 1991).

More than 10% of the indigenous marine bacterial isolates were naturally transformable by plasmid DNA and 14% by chromosomal DNA. Several transformed bacteria belonged to the genera *Vibrio* and *Pseudomonas*. The acquisition of the plasmid was accompanied by a modified restriction enzyme digestion pattern. A quantitative estimate resulted in a transformation rate ranging from 0,0005 to 1,5 transfomants per l per day. From extrapolation to ecosystem scales it was concluded that natural transformation may be an important mechanism for DNA exchange in bacterial communities (Frischer *et al.*, 1994).

W.H. Jeffrey, J.H. Paul and G.J. Stewart
Natural transformation of a marine *Vibrio* species by plasmid DNA.
Microbial Ecology 19 (1990) p. 259-268
horizontal gene transfer, DNA uptake, sediment, bacteria, Vibrio, experiment

M.E. Frischer, G.J. Stewart and J.H. Paul
Plasmid transfer of indigenous marine bacterial populations by natural transformation.
FEMS Microbiology Ecology 15 (1994) p. 127-136
horizontal gene transfer, DNA uptake, bacteria, Pseudomonas stutzeri, Vibrio, experiment, risk assessment

M.E. Frischer, J.M. Thurmond and J.H. Paul
Natural plasmid transformation in a high-frequency-of-transformation marine *Vibrio* strain.
Applied and Environmental Microbiology 56 (1990) p. 3439-3444
horizontal gene transfer, DNA uptake, bacteria, Vibrio, experiment

J.H. Paul, M.E. Frischer and J.M. Thurmond
Gene transfer in marine water column and sediment microcosms by natural plasmid transformation.
Applied and Environmental Microbiology 57 (1991) p. 1509-1515
horizontal gene transfer, DNA uptake, sediment, water, bacteria, Vibrio, experiment

G.J. Stewart and C.D. Sinigalliano
Detection of horizontal gene transfer by natural transformation in native and introduced species of bacteria in marine and synthetic sediments.
Applied and Environmental Microbiology 56 (1990) p. 1818-1824
horizontal gene transfer, DNA persistence, DNA uptake, sediment, bacteria, Pseudomonas stutzeri, experiment

G.J. Stewart, C.D. Sinigalliano and K.A. Garko
Binding of exogenous DNA to marine sediments and the effect of DNA /sediment binding on natural transformation of *Pseudomonas stutzeri* ZoBell in sediment columns.
FEMS Microbiology Ecology 85 (1991) p. 1-8

horizontal gene transfer, DNA persistence, DNA uptake, sediment, bacteria, Pseudomonas stutzeri, experiment

7.4 Transgenic plant material

In the project of Widmer and Seidler (1994) an assay for the transformability of released, plant derived transgenes (marker genes) shall be developed. The transformability of **GMP derived DNA** has been already tested by Becker *et al.* (1994). Two different approaches were used:
(1) Untreated or digested DNA extracts of modified tobacco harboring two prokaryotically expressed antibiotic resistance genes, were incubated with soil isolates of *Bacillus* and *Pseudomonas*.
(2) Mortared tobacco tissue was mixed with unsterile, microbiologically active soil.
Until now HGT events have not been detected.

Over a time period of more than two years Becker *et al.* (1994) have taken soil samples before, during and after a release experiment with transgenic petunia harboring the kanamycin/neomycin resistance gene. From 400 samples tested by PCR amplification, 4 gave a positive signal: one signal came from a sample taken during the experiment and the other three signals derived from samples taken two month after underploughing of the plants. It could not be excluded that the PCR amplification products might have been arisen from transgenes in still unrotted plant tissues.

Paget *et al.* (1993, 1994) conducted a field test with transgenic tobacco carrying a marker gene for antibiotic resistance. One year after harvest of the plants, the marker gene could still be detected by PCR in total DNA extracts from soil samples by PCR, but not in DNA extracts from soil bacteria. Even when DNA persisted in soil either in surviving or dead root cells or as free DNA, gene transfer to the indigenous soil microflora has not been detected up to now.

Analyses of soil and soil microflora have also been conducted for German field tests with transgenic potatoes and sugar beets. The results were similar: transgene persistence could be detected, but no gene transfer (Smalla, 1995).

In a Swiss release experiment with transgenic potatoes marker gene sequences have not been detected in soil samples up to now. (Zeyer, 1994 unpublished results).

J. Becker, H. Siegert, J. Logemann and J. Schell
Begleitende Sicherheitsforschung zur Freisetzung gentechnisch veränderter Petunien.
In: Bundesministerium für Forschung und Technik (ed). Biologische Sicherheit. Forschung Biotechnologie. Band 3 (1994) p. 563-578
horizontal gene transfer, transgenic plant, bacteria, petunia, tobacco, Bacillus, Pseudomonas, Acinetobacter calcoaceticus, antibiotic resistance, neomycin, kanamycin, hygromycin, homologous recombination, DNA degradation, experiment

E. Paget and P. Simonet
On the track of natural transformation in soil.
FEMS Microbiology Ecology 15 (1994) p. 109-118
horizontal gene transfer, DNA persistence, DNA uptake, bacteria, review

E. Paget, D. Jouan, M. Lebrun and P. Simonet
Persistence of plant DNA in soil.
In: Commission of the European Communities (ed). BRIDGE/BIOTECH. Final sectorial meeting on biosafety and first sectorial meeting on microbial ecology. October 24-27, 1993. Granada (1993) p. 33
horizontal gene transfer, DNA degradation, DNA uptake, soil, transgenic plant, bacteria, tobacco, antibiotic resistance, gentamicin, experiment

H. Siegert, J. Schell and J. Logemann
Analysis of natural gene transfer mechanisms between transgenic plants and microorganisms under field test conditions.
In: R. Casper, J. Landsmann (eds) 2nd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms. May 11-14, 1992. Goslar, Germany (1992) p. 273
horizontal gene transfer, transgenic plant, bacteria, petunia, antibiotic resistance, neomycin, kanamycin, DNA degradation, experiment

K. Smalla
Microbial communities influeced by transgenic plants.
In: D. D. Jones (ed). Third International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms. November 13-16 1994. Monterey. In press (1995)
horizontal gene transfer, DNA persistence, DNA uptake, soil, bacteria, transgenic plant, potato, sugar beet, experiment

F. Widmer
Microcosm experiments on the fate of DNA derived from decomposing transgenic plants in soil and its biological activity on soil organisms.
In: Schweizerischer Nationalfond (ed) Prisma 94. Schwerpunktprogramme (1994) p. 46
research, National Foundation, SPP-Biotechnology, Switzerland, horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, antibiotic resistance, neomycin, kanamycin, proteinase inhibitor, transgenic plant, experiment

F. Widmer and R.J. Seidler
Fate and microbial transformation of plant-derived transgenes in non-sterile agricultural soil.
US Environmental Protection Agency, US Department of Agriculture (ed) Risk Assessment Methodologies. An International Conference for Regulatory Agencies, Academy and Industry. June 22-25, 1994. Maryland (1994)
horizontal gene transfer, DNA degradation, DNA uptake, soil, bacteria, antibiotic resistance, neomycin, kanamycin, proteinase inhibitor, transgenic plant, experiment

J. Zeyer
Detection of nucleic acids in heterogenous environmental systems.
In: Schweizerischer Nationalfond (ed) Prisma 94. Schwerpunktprogramme
research, National Foundation, SPP-Biotechnology, Switzerland, horizontal gene transfer, DNA persistence, soil, transgenic plant, potato, experiment

7.5 Intestine

7.5.1 Experimental Approach

Favourable conditions for gene transfer may be also provided within the gut of higher organisms. A model system has been developed by Adamo *et al.* (1993). Following a release of GMOs, soil nematodes might take up modified microbes as well as transgenic plant tissue. Preliminary investigations with a nematode species of the genus *Rhabditis* demonstrated that it is possible to recover internalized bacteria carrying a marker gene from faceal depositions.

More advanced are the experiments of Tebbe *et al.* (1994). The soil insect *Folsomia candida* was investigated for its ability to induce gene transfer in its gut. The insect had to feed on agar cubes inoculated with bacterial or yeast donor strains containing selectable plasmids, and just afterwards they had to feed on cubes inoculated with streptomycin resistant bacterial recipients. From faecal depositions recipient strains containing the donor plasmids could be reisolated with a rate of one natural transformant per 585 recipient cells. Since the transformation rate was similar for dead as well as viable donor cells, the mechanism of gene transfer is most likely transformation. The low transformation rates (one transformant of at least 3 x 10^7 recipients) for plasmid addition to faecal depositions implied that it must have been the gut of the soil insect which stimulated gene transfer.

J.A. Adamo, C.M. Stadnick and M.A. Gealt
A nematode model system for the study of gene transfer in soil organisms.
93rd General Meeting of the American Society for Microbiology. May 16-20, 1993. Atlanta, Georgia, United States (1993) p. 164 (Q-214)
horizontal gene transfer, DNA degradation, DNA uptake, intestine, transgenic bacteria, Escherichia coli, nematode, Rhabditis, experiment

C.C. Tebbe, W. Vahjen and H. Borkott
Interspecies gene transfer by "artificial" transformation of recombinant, broad host range plasmids in the gut of the soil insect *Folsomia candida*.
In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld (1994)
horizontal gene transfer, DNA uptake, intestine, transgenic bacteria, Saccharomyces cerevisiae, Corynebacterium glutamicum, Agrobacterium radiobacter, Arthrobacter citreus, Pseudomonas putida, Escherichia coli, insect, Folsomia candida, experiment

7.5.2 Theoretical models

The frequency of indirect gene transfer by DNA uptake through bacterial or eukaryotic cells in the intestine has been calculated in two projects. Redenbaugh *et al.* (1993) calculated the transformation probability for the kanamycin resistance gene (nptII) of the transgenic FLAVR SAVR tomatoes based on the following assumptions:

1. 10^{12} bacterial cells are present in a person's non-anaerobic gut microflora, with each bacterium being
2. equally competent and
3. transformable.
4. From transformation experiments with competent *Streptococcus pneumoniae* under optimal laboratory conditions, the frequency for natural transformation of gut bacteria was estimated to be 6.7×10^{-5} for a mean consumption rate of fresh fruits and vegetables.
5. The real transformation frequency will be lower because only a fraction of total DNA consumed contains the marker gene and because the marker gene will be partly degraded by enzymatic processes in the digestive tract. Only DNA fragments being as long as the marker gene are relevant for transformation. Based on *in vitro* experiments with simulated gastro-intestinal fluid, gastric and intestinal DNA survival was assumed to be 0.1%.
6. The integration of the transgene into the recipient's genome is determined by the relative frequency of illegitimate recombination. Data on this subject are already present in literature. Since nothing is known on
7. gene disruption by recombination and on the
8. probability of gene expression, these factors are considered as not influencing negatively the transformation rate.

Taken all listed values into account, the actual transformation rate should be 1×10^{-3} transformed bacteria / person for a mean daily intake of 30g transgenic tomatoes. For a single person eating 30 g tomatoes per day, only one single transformant will arise in two and a half years time.

A similar analysis done in the Netherlands (Bergmans, 1993) resulted in 10 potential transformants per year for the gut microflora of a single person. However kanamycin resistant bacteria are already present in the normal gut microflora at much higher frequencies. The resorption of DNA by gut epithelial cells was described to be a common phenomenon which never has been seen as a risk (Bergmans, 1993).

H. Bergmans
Acceptability of the use of antibiotic resistance genes as marker genes in transgenic plants.
In: OECD (ed.). Report on the scientific approaches for the assessment of research trials with genetically modified plants. April 6-7, 1992. Jouy-en-Josas, France (1993) p. 106-108
horizontal gene transfer, DNA degradation, DNA uptake, intestine, transgenic plant, bacteria, eukaryotic cells, antibiotic resistance, neomycin, kanamycin, risk assessment

K. Redenbaugh, T. Berner, D. Emlay, B. Frankos, W. Hiatt, C. Houck, M. Kramer, L. Malyj, B. Martineau, N. Rachman, L. Rudenko, R. Sanders, R. Sheehy and R. Wixtrom
Regulatory issues for commercialization of tomatoes with an antisense polygalacturonase gene.
In Vitro Cellular & Developmental Biology 29P (1993) p. 17-26
horizontal gene transfer, DNA degradation, DNA uptake, transgenic plant, bacteria, tomato, Lycopersicon esculentum, Flavr savr, antibiotic resistance, neomycin, kanamycin, risk assessment

8 Additional aspects

Unwanted transfer of a transgene can be caused by the transformation technique itself.

Unwanted transformants by *Agrobacterium* mediated gene transfer.

In the case of *Agrobacterium* mediated gene transfer, removal of the bacteria by antibiotic application might be incomplete. E.g., Cefotaxim as selection agent inhibits bacterial growth but does not completely kill all bacteria. By tissue-print immunoblotting it could be demonstrated that agrobacteria are still present in plants six till nine month after transformation (Matzk and Schiemann, 1994). It is not clear whether such persisting agrobacteria might be transmitted to progeny plants and by this will be released during field tests.

It has been demonstrated so far that **engineered agrobacteria** are able to survive in soil and to **infect neighboring plants** (Nir *et al.*, 1993). It could be hypothesized that such *Agrobacterium* infections might result in a production of new transgenic plants. Infections by wildtype *A. rhizogenes* can result in transgenic plant roots having an unusual ability to grow in culture and in some cases spontaneously regenerate to transformed shoots (Pellegrineschi *et al.*, 1994). It is assumed that similar gene transfer events with subsequent regeneration might have taken place also in nature since fragments of the Ri T-DNA have been detected in tobacco (chapter 3.1).

Kyriakides *et al.* (1993) investigated whether **transgenic agrobacteria** are able to **transfer their transgenes to other gram-negative bacteria**. Gene transfer from modified *Agrobacterium tumefaciens* to natural soil isolates of *Rhizobium leguminosarum* could not be verified until now. Similar experiments have been done by Hwang and Farrrand (1994) using *Agrobacterium tumefaciens* that carries a broad host range recombinant plasmid with the origin of replication from the Ti plasmid. Although the plasmid itself is non conjugal, it can be mobilized by the Ti plasmid system present in *A. tumefaciens*. Plasmid transfer could be detected from *Agrobacterium tumefaciens* to *Pseudomonas fluorescens* at a frequency of 9×10^{-4} and to *Escherichia coli* at a frequency of 7×10^{-4}.

Agrobacterium mediated transformation might also result in unwanted **transgenic endophytes**. Langridge *et al.* (1992) suggested this possibility for three reasons when trying to transform cereals by floret inoculation with *Agrobacterium*:
(1) Plants showed positive hybridization signals for the marker gene only in the first generation, but not in the second.
(2) All potentially transformed plants showed the same banding pattern in a Southern blot probed with the marker gene. This would indicate that the foreign DNA inserted into a similar site of each plant which is extremely unlikely.
(3) The undigested DNA from potentially transformed plants showed a hybridization signal for a band of 8 kb demonstrating that the T-DNA did not insert into the plant genome. Therefore, it is hypothesized that the T-DNA might have integrated into a plasmid of an endophytic bacterium or into the genome of some chloroplasts which were not passed to future generations. Attempts to isolate bacteria containing the marker gene from the plants were not successful.

Unwanted transformants by protoplast transformation and particle bombardment

Chen *et al.* (1994) assumed that PEG induced protoplast transformation and particle bombardment might result in **transgenic plant endophytes**, when they transformed wheat cells. The presence of the transgene was demonstrated by Southern blot analysis of DNA samples taken 10 months after transformation. Several common bands between independently transformed cell lines were detected. Since most of these common bands had disappeared 30 months after transformation, the transgene was probably not stably integrated into the wheat genome. This assumption was strengthened by an analysis of the methylation pattern. While the transgene persisted as N^6 adenine methylated DNA (typically for bacteria), such methylation was not present in the organellar and nuclear genomes of the investigated wheat cell lines. Analysis of chromosome preparations revealed a high A&T content of the DNA which is unusual for wheat chromosomal DNA, but applies to mycoplasma like organisms, which might have been present in the cell culture and therefore were cotransformed. Since these organisms are difficult to cultivate on artificial media, this might explain why no possible bacterial contaminants could be isolated from the cell cultures.

Schiemann and Gunson (1994) demonstrated for an bacterial endophyte of yam that it is transformable and that a plant transformation vector can be stably maintained and expressed. Next they want to investigate whether this endophyte can be also transformed in planta by direct gene transfer.

D.F. Chen, P.J. Dale, J.S. Heslop-Harrison, J.W. Snape, W. Harwood, S. Bean and P.M. Mullineaux
Stability of transgenes and presence of N^6 methyladenine DNA in transformed wheat cell.
The Plant Journal 5 (1994) p. 429-436
horizontal gene transfer, additional aspects, endophytes, myxoplasma like organisms, methylation, wheat, experiment

I. Hwang and S.K. Farrand
Assessing heterologous horizontal gene transfer between a soil organism and genetically modified microorganisms.
US Environmental Protection Agency, US Department of Agriculture (ed) Risk Assessment Methodologies. An International Conference for Regulatory Agencies, Academy and Industry. June 22-25, 1994. Maryland (1994)
horizontal gene transfer, additional aspects, bacteria, Agrobacterium tumefaciens, Pseudomonas fluorescens, Escherichia coli, opine catabolism, agropine catabolism, experiment

X. Kyriakides, D. Gennimata, A. Tsaftaris and AS. Tsiftsoglou
Evaluation of gentamycin acetyltransferase (aacC1) gene transfer from genetically modified *Agrobacterium tumefaciens* into *Rhizobium leguminosarum* natural isolates and other bacteria.
In: Commission of the European Communities (ed). BRIDGE/BIOTECH. Final sectorial meeting on biosafety and first sectorial meeting on microbial ecology. October 24-27, 1993. Granada (1993) p. 29-30
horizontal gene transfer, additional aspects, bacteria, Agrobacterium tumefaciens, Rhizobium leguminosarum, Escherichia coli, experiment

A. Matzk and J. Schiemann
Persistence of *Agrobacterium tumefaciens* in transgenic plants.
In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld (1994)
horizontal gene transfer, additional aspects, bacterial persistance, bacteria, Agrobacterium tumefaciens, transgenic plant, experiment

P. Langridge, R. Brettschneider, P. Lazzeri and H. Lörz
Transformation of cereals via *Agrobacterium* and the pollen pathway: a critical assessment.
The Plant Journal 2 (1992) p. 631-638
horizontal gene transfer, additional aspects, endophytes, wheat, Triticum aestivum, barley, Hordeum vulgare, maize, Zea mays, experiment

M. Nir, Z. Dan, G. Ron, M. Bar-Joseph
The persistance of engineered *Agrobacterium tumefaciens* in agroinfected plants.
In: 9th International Congress of Virology. August 8-9, 1993. Glasgow, Scotland
horizontal gene transfer, additional aspects, bacterial persistance, bacteria, Agrobacterium tumefaciens, plant, tomato, Gynura aurantium, avocado, grapefruit, experiment

A. Pellegrineschi, J-P. Damon, N. Valtorta, N. Paillard and D. Tepfer
Improvement of ornamental characters and fragrance production in lemon-scented geranium through genetic transformation by *Agrobacterium rhizogenes*.
Bio/Technology 12 (1994) p. 64-68
horizontal gene transfer, additional aspects, bacteria, plant, Agrobacterium tumefaciens, geranium, Pelargonium, spontaneous regeneration, experiment

J. Schiemann and H. Gunson
Gene transfer in bacterial endophytes.
In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld (1994)
horizontal gene transfer, additional aspects, endophytes, bacteria, Sphingomonas paucimobilis, Pseudomonas paucimobilis, yam, Dioscorea, experiment

J. Schiemann, J. Landsmann, C.v. der Hoeven, A. Riedel-Preuss, R. Zweigerdt, A. Matzk, R.S. Conlau and H. Gunson
Extrachromosomale Fremd-DNA in transgenen Pflanzen - Untersuchungen zur Persistenz von Agrobakterien und zum Gentransfer in Endophyten.
In: Bundesminesterium für Forschung und Technik (ed) Biologische Sicherheit . Forschung Biotechnologie. Band 3 (1994) p. 223-241
horizontal gene transfer, additional aspects, bacterial persistance, bacteria, Agrobacterium tumefaciens, tobacco, endophytes, Sphingomonas paucimobilis, Pseudomonas paucimobilis, yam, Dioscorea, luciferase, transgenic plant, experiment

9 Summary

In experiments in natural systems (field tests)

- HGT from plants to microorganisms (bacteria and fungi) and from plants to viruses has not been detected hitherto.

In experiments in artifical systems

- HGT from plants to fungi has been described.
- HGT from plants to viruses has been detected under selective pressure.
- Uptake of free DNA by microorganisms has been detected.

By sequence homology analyses

- HGT from plants to bacteria has been implied.
- HGT from plants to viruses has been implied.

gene donor	gene acceptor	gene transfer has been implied by		
		field tests	laboratory experiments	sequence homology analyses
free DNA	bacteria		+	
free DNA	fungi		+	
viral RNA present in the cytoplasm of a plant	viruses		+	
plant	bacteria			+
plant	fungi		+	
plant	viruses		+	+
plant (mobile elements)	plants			+

⇒⇒

The experimental and sequence homology data indicate that horizontal transfer of plant endogenous genes as well as chromosomally integrated transgenes might occur under specific conditions. However, with the current methods it has not been possible to detect HGT from plants to microorganisms or viruses in field tests. It can be assumed that HGT is an extremely rare event in nature which seldom results in a selective advantage for the recipient and therefore will be only of importance during evolutionary time periods.

Explanations for figure and table which present an OVERVIEW ON THE POSSIBLE HORIZONTAL GENE TRANSFER EVENTS including the involved organism classes and genes, the direction and mechanism of transfer and the way of detection

Explanations for colors, symbols and abbreviations:

Prokaryotes.
Eukaryotes.
Viruses and bacteriophages.

Gene transfer events between the different organism classes.
Transfer of mobile elements between eukaryotes.

[ ] = HGT has been discussed controversially.
ε = HGT has been demonstrated in **experiments**.
σ = HGT has been implied by **sequence homology** analyses.
hp = helper plasmid

Abbreviations for genes

ANKs	=	ankyrin-like proteins
CM 1	=	muscle specific chicken calmoduline
Cu-Zn SOD	=	Cu-Zn superoxid dismutase
Cyt c	=	cytochrome c
Cyt P-450	=	nitrate / nitrite inducible cytochrome
Fe-SOD	=	Fe superoxid dismutase
Fn 3	=	fibronectin type III
GAPDH	=	glyceraldehyde-3-phosphate dehydrogenase
GPI	=	glucose-6-phosphate isomerase
GS II	=	glutamine synthetase II
T-DNA	=	transfer-DNA of the Ti/Ri plasmid of *Agrobacterium tumefaciens* / *rhizogenes*

CONJUGATION

"Mating" between two bacterial cells with (part of) the chromosome being transferred.
Conjugative plasmid transfer depends generally upon the host range of the transfer system and the replication system.

The transfer system
Gene transfer starting from gram negative bacteria depends on

- *tra* (= transfer) genes, encoding components of cell surface interactions.
- *mob* (= mobilization) genes, encoding proteins which nick the plasmid DNA at *oriT*.
- *oriT* = origin of transfer of DNA.

Plasmids containing all three components are selftransmissable. Plamids lacking the *tra*- or *mob* genes can still be mobilized, when these genes are present on a helper plasmid (**hp**).

The replication system
The replication of a plasmid in a bacterium depends on the respective

- oriV = origin of vegetative replication.
  - oriV* = oriV active in the donor bacterium.
  - oriV** = oriV active in the recipient bacterium.
  - oriV*** = oriV acitve in the donor as well as in the recipient bacterium.

In yeast, plasmid replication is possible in the presence of

- *2μ* replicative sequence.
- *ARS* = autonomously relicating sequence.

Natural plasmids with a broad conjugational host range of gram negative bacteria:

- PR4 (= RK2 = RP1 = R68) of the incompatibility group Pα (IncPα) [*tra*:+, *mob*:+, *oriT*:+].
- R751 of IncPβ [*tra*:+, *mob*:+, *oriT*:+].
- RSF1010 (= R300B = R1162) of IncQ [*tra*:-, *mob*:+, *oriT*:+].

of gram positive bacteria:

- pAMβ1

Natural plasmids with a limited conjugational host range of gram negative bacteria:

- ColE1 [*tra*:-, *mob*:+, *oriT*:+]
- F factor [*tra*:+, *mob*:+, *oriT*:+]

Artificial, mobilizable plasmids

- **for transfer from gram positive to gram negative bacteria**

Such plasmids contain the transfer function of the broad host range gram positive plasmid pAMβ1 next to two oriVs, one is active in the donor-, the other is active in the recipient bacterium.

- **for transfer from gram negative to gram positive bacteria / yeasts / cyanobacteria**

Such plasmids contain the oriT of a broad host range plasmid and one or rather two oriVs active in the donor bacterium and the recipient. *Tra*- and *Mob* genes, with the latter corresponding to the *oriT*, may be present on the mobilized plasmid itself or on a co-resident helper plasmid (**hp**). The helper plasmid is usually of the IncP group.
For plasmid transfer to yeast also the oriT of ColE1 or of the F factor can be used. For plasmid replication in yeast the *2μ* or the *ARS* element has to be present.

- **for transfer from a gram negative bacterium to a plant cell**

In the case of the non-selftransmissable plasmid RSF1010 tra-functions can be provided by the agrobacterial *vir* genes. Replication of the transferred sequences is only possible after integration into the plant genome.

Tn*916* from *Enterococcus faecalis*

Tn916 is a conjugative transposon integrated in the bacterial chromosome. It has transfer-, excision- and integration functions for conjugal transfer, since it is excised from the host genome, circularized to a non-replicative intermediate, and finally integrated into the recipient's genome.

List of natural plasmids which have been involved in the construction of the artificial plasmids mentioned in the table

plasmid	source
ColE1	*E.coli*
F (=Fertility factor)	*E. coli*
p15A	*E. coli*
pAL5000	*Mycobacterium fortuitum*
pAMβ1	*Enterococcus faecalis*
pCV35	*Corynebacterium glutamicum*
pIJ101	*Streptomyces lividans*
pMB1	*E. coli*
PR4	*Pseudomonas aeruginosa*
R751	*Klebsiella aerogenes*
RSF1010	*E. coli*

TRANSDUCTION

Transfer of bacterial genes from one bacterium to another by a phage or transfer of eukaryotic cellular sequences by retroviruses.

Bacteriophages can cause

- **Specialized transduction**
  = Aberrant prophage excision caused by illegitimate recombination between the prophage's genome and adjacent segments of the bacterial chromosome.

- **Generalized transduction**
  = Packaging mistakes by encapsulation of bacterial DNA caused by sequences of the bacterial genome which are similar to the specific sequence signal marking the beginning of prophage DNA.

MOBILE ELEMENTS

HGT of mobile elements has been assumed only by sequence homology analyses.

RT (LTR) Retrotransposons with long terminal repeats.
(1) ***copia***-like family
(2) ***gypsy***-like family.

RT (non-LTR) Retrotransposons lacking long terminal repeats.
(1) ***jockey***-elements
(2) ***R1*/*R2*** elements
(3) ***LINE*s** (long interspersed nuclear elements).

T Transposons.
(1) ***Ac*/*hobo***-like family
(2) ***Mariner***-like family
(3) ***P*** element family

gene donor	gene acceptor	plasmid or gene or gene product	natural gene or plasmid	artificial plasmid	transgene	sequence homology analysis	controversial discussion	experiment	literature
bacteria	**bacteria**								
gram positive	**gram negative**								
Enterococcus faecalis	E.coli	pAT191 [tra-pAMβ1, oriV*-MB1, oriV**-pAMβ1]		x				x	1
Enterococcus faecalis	Alcaligenes eutrophus, Citrobacter freundii, E.coli	Tn916	x					x	2
gram negative	**gram positive**								
E. coli	Bacillus thuringiensis, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus, Streptococcus lactis, Streptococcus agalactiae	pAT187 [oriT-RP4, oriV*-pMB1, oriV**-pAMβ1] + hp		x				x	3
E. coli	Bacillus anthracis	pAT187 + hp		x				x	4
E. coli	Clostridium acetobutylicum	pAT187, pCTC1 [oriT-RP4, oriV*-pMB1, oriV**-pAMβ1] + hp		x				x	5
E. coli	Corynebacterium	pECM1 [oriT-RP4, oriV*-p15A, oriV**-pCV35] + hp		x				x	6
E. coli	Mycobacterium smegmatis	pMY10 [oriT-RP4, oriV*-pMB1, oriV**-pAL500] + hp		x				x	7
E. coli	Streptomyces	pPM801 [oriT-RP4, oriV*-pMB1, oriV**-pIJ101] + hp		x				x	8
E. coli	Mycobacterium smegmatis, Streptomyces lividans	RSF1010 [oriT-RSF1010, mob-RSF1010, oriV***-RSF1010] + hp	x					x	9
E. coli	Bacillus subtilis, Clostridium acetobutylicum, Enterocoocus faecalis, Streptococcus lactis	Tn916	x					x	2
bacteria	**cyanobacteria**								
E. coli	Synetochocystis	pKT210 [oriT-RSF1010, mob-RSF1010, oriV***-RSF1010] + hp		x				x	10
bacteria	**protozoa**								
bacteria	Entamoeba histolytica	Fe superoxide dismutase	x			x			11
bacteria	**fungi**								
E. coli	Saccharomyces cerevisiae	pDPT51•LEU2 [tra-R751, oriT-R751, mob-R751, oriV*-R751, 2μ]		x				x	12
		pFLEU2 [tra-F, oriT-F, mob-F, oriV*-F, 2μ]		x				x	12
		YEp13 [oriT-ColE1, oriV*-pMB1, 2μ] + hp		x				x	12
		pAY205 [oriT-RSF1010, mob-RSF1010, oriV*-RSF1010, ars] + hp		x				x	13
E. coli	Schizosaccharomyces pombe	YEp13 [oriT-ColE1, oriV*-pMB1, 2μ] + hp		x				x	14
E.coli	yeast	aldolase type II	x			x			11
Flavobacterium, Streptomyces	Aspergillus, Penicillium, Cephalosporium	β-lactam antibiotic biosynthetic genes	x			x	x		15-17, 11
Streptomyces	Fusarium oxysporum	cytochrome p450	x			x	x		18-20
Rumoncoccus flavefaciens	Neocallimastix patriciarum (rumen fungus)	xylanase	x			x			21
bacteria	**plants**								
Agrobacterium tumefaciens	Nicotiana plumbaginifolia	pSUP104 [oriT-RSF1010, mob-RSF1010, oriV*-p15A] + hp		x				x	22
Agrobacterium tumefaciens/ rhizogenes	dicotyledonous plants	T-DNA	x					x	23
bacteria	**bacteriophages**								
Samonella typhimurium, E. coli, Bacillus subtilis	Samonella-phage P22, E. coli-phage P1, Bacillus-phage PBS1	transduction of nonspecific bacterial DNA							24, 25
E. coli	phage lamda	transduction of specific bacterial genes next to the prophage DNA							24, 25

gene donor	gene acceptor	gene or gene product	natural gene ↓	artificial plasmid ↓	transgene ↓	sequence homology analysis ↓	controversial discusion ↓	experiment ↓	literature
eukaryotes	**bacteria**								
eukaryote	E. coli	glyceraldehyde-3-phosphate dehydrogenase (GAPDH A)	x			x			26,11
eukaryote	Chromatium virosum, E. coli	ankyrin (ANK)-like proteins	x			x			27
protozoa	**animals**								
protozoan	chicken	CM1	x			x	x		28, 20
fungi	**bacteria**								
Kluyveromyces fragilis	Clostridium thermocellum	β-glucosidase B	x			x			29
fungi	**plants**								
Neurospora, yeasts	Arabidopsis thaliana	cytochrome c	x			x	x		30, 20
animals	**bacteria**								
mammalians & invertebrates	Streptomyces limosus	α-amylase	x			x			31
ponyfish	photobacterium leiognathi	Cu-Zn superoxide dismutase	x			x	x		32-35, 11
animals	bacteria	fibronectin type III	x			x			37
Echinodermata, mammalians	bacteria	sialidase	x			x			38
animals	**protozoa**								
human	Plasmodium falciparum	ankyrin (ANK)-like proteins	x			x			27
animals	**retroviruses**	*onc* genes	x			x			39, 40
plants	**bacteria**								
Clarkia ungulata	E.coli	glucose-6-phosphate isomerase (GPI)	x			x			41, 42, 11
plants	Rhizobiaceae (Bradyrhizobium japonicum), Frankiaceae, Streptomycetacea	glutamin synthetase II (GS II)	x			x	x		43-46, 11
plants (lupine, soybean)	Vitreoscillla	hemoglobin	x			x			47, 48
plants	**fungi**								
Brassica napus, Sinapsis alba	Plasmodiophora brassicae	host tandem repeats	x					x	49
Datura innoxia	Aspergillus niger	hygromycin resistance			x			x	50
plants	**plant viruses**								
plant chloroplasts	cucumber mosaic virus (CMV) satellite RNA (Y-sat RNA)	chloroplast tRNA	x			x			51
tobacco chloroplasts	potato leafroll virus (PLRV)	chloroplast DNA	x			x			52
Brassica napus	cauliflower mosaic virus (CaMV)	CaMV gene VI			x			x	53
Nicotiana benthamiana	cowpea chlorotic mottle virus (CCMV)	part of the CCMV-capsid			x			x	54
Nicotiana benthamiana	red clover necrotic mosaic virus (RCNMV)	RCNMV-movement protein			x			x	55
Nicotiana bigelovii	cauliflower mosaic virus (CaMV)	CaMV-strain D4 gene VI			x			x	56
bacteriophages	**bacteria**	integration of phage DNA into the host's genome							57
retroviruses	**animals**	integration of virus DNA into the host's genome							39
retroviruses	**bacteria**	RNaseH	x			x			58

horizontal transfer of mobile elements

Lit.: Kidwell (1993) Ann Rev Genet 27: 235-256 * = vertical and horizontal transfer are postulated

fungi	animals	plants	retrotransposons
yeast	Drosophila, hering*	64 plant species, Volvox	copia-like
yeast	Drosophila	lilly, pine	gypsy-like
	Drosophila		jockey
	Japanese beetle*, parasitic wasp*		R1/R2
	Drosophila, man, mouse, silk worm	lilly, maize	LINEs

fungi	animals	plants	transposons
	Drosophila	Antirrhinum, maize	Ac/hobo-like
	several insects and other athropods: Ceropid moth, Drosophila, Zapriosus		Mariner-like
	Drosophila, Lucilla, Scaptomyza		P element

References

1 Trieu-Cout et al. (1988) J Bacteriol 170: 4388-4391
2 Bertram et al. (1991) J Bacteriol 173: 443-448
3 Trieu-Cuot et al. (1987) FEMS Microbiol Lett 48: 289-294
4 Cataldi et al. (1990). Mol Microbiol 4: 1111-1117
5 Williams et al. (1990) J Gen Microbiol 136: 819-826
6 Schäfer et al. (1990) J Bacteriol 172: 1663-1666
7 Lazraq et al. (1990) FEMS Microbiol Lett 69: 135-138
8 Mazodier et al. (1989) J Bacteriol 171: 3583-3585
9 Gormley & Davies (1991) J Bacteriol 173: 6705-6708
10 Kreps et al. (1990) Mol Gen Genet (1990) 221: 129-133
11 Smith et al. (1992) TIBS 17: 489-493
12 Heinemann & Sprague (1989) Nature 340: 205-209
13 Nishikawa et al. (1992) Curr Genet 21: 101-108
14 Sikorski et al. (1990) Nature 345: 581-582
15 Landan et al. (1990) Mol Biol Evol 7: 399-406
16 Penalva et al. (1990) Proc R Soc Lond B 241: 164-169
17 Smith et al. (1990) EMBO J 9: 741-746
18 Degtyarenko & Archakov (1993) FEBS Lett 332: 1-8
19 Kizawa et al. (1991) Proc Natl Acad Sci USA 266: 10632-10637
20 Syvanen (1994) Annu Rev Genet 28: 237-261
21 Gilbert et al. (1992) Mol Microbiol 6: 2065-2072
22 Buchanan-Wollaston et al. (1987) Nature 328: 172-175
23 Binns & Thomashow (1988) Ann Rev Microbiol 42: 575-606
24 Schmieger (1994) In: Horizontal Gene Transfer - Mechanisms and Implications. Workshop. July 25-27, 1994. Bielefeld.
25 Schlegel (ed) Allgemeine Mikrobiologie. 6. Aufl. (1985) Thieme Stuttgart. 466-468
26 Doolittle et al. (1990) J Mol Evol 31: 383-388
27 Bork (1993) Proteins: Structure, Function, and Genetics 17: 363-374
28 Gruskin et al. (1987) Proc Natl Acad Sci 84: 1605-1608
29 Gräbnitz et al. (1989) Mol Gen Genet 217: 70-76
30 Kemmerer et al. (1991) J Mol Evol 32: 227-237
31 Long et al. (1987) J Bacteriol 169: 5745-5754
32 Bannister & Parker (1985) 82: 149-152
33 Cornish-Bowden (1985) Eur J Biochem 151: 333-335
36 Leunissen & Jong (1986) J Mol Evol 23: 250-258
34 Martin & Fridovich (1981) J Biol Chem 256: 6080-6089
35 Smith & Doolittle (1992) J Mol Evol 34: 175-184
37 Bork & Doolittle (1992) Proc Natl Acad Sci USA 89: 8990-8994
38 Roggentin et al. (1993) Mol Microbiol 9:915-921
39 Lewin (ed) Genes. 3rd edition (1987) Wiley & Sons New York. 614-616
40 Schlegel (ed) Allgemeine Mikrobiologie. 6. Aufl. (1985) Thieme Stuttgart. 149-150
41 Froman et al. (1989) Mol Gen Genet 217: 126-131
42 Smith & Doolittle (1992) J Mol Evol 34: 544-545
43 Carlson & Chelm (1986) Nature 322: 568-570
44 Kumada et al. (1993) Proc Natl Acad Sci USA 90: 3009-3013
45 Pesole et al. (1991) Proc Natl Acad Sci USA 88: 522-526
46 Shatters & Kahn (1989) J Mol Evol 29: 422-428
47 Khosla & Bailey (1988) Mol Gen Genet 214: 158-161
48 Wakabayashi et al. (1986) Nature 322: 481-483
49 Bryngelsson et al. (1988) Physiol Mol Plant Pathol 33:163-171
50 Hoffmann et al. (1994) Curr Genet 27: 70-76
51 Masuta et al. (1992) Nucleic Acids Res 20: 2885
52 Mayo & Jolly (1991) J Gen Virol 72: 2591-2595
53 Gal et al. (1992) Virology 187: 525-533
54 Greene & Allison (1994) Science 263: 1423-1425
55 Lommel & Xiong (1991) J Cell Biochem 15A: 151
56 Schoelz & Wintermantel (1993) Plant Cell 5: 1669-1679
57 Schlegel (ed) Allgemeine Mikrobiologie. 6. Aufl. (1985) Thieme Stuttgart. 142-147
58 Doolittle et al. (1989) Q Rev Biol 64:1-3