



# Unintended Horizontal Transfer of Recombinant DNA

by **Kaare M Nielsen & Daniele Daffonchio**

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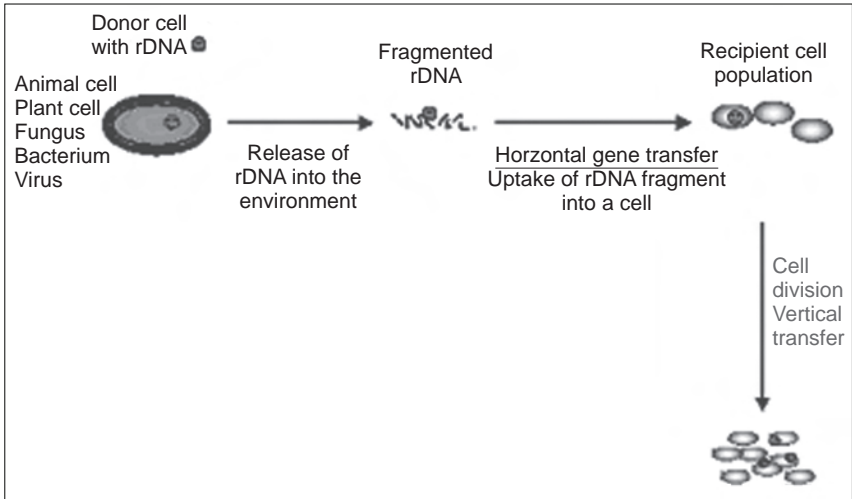
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# Chapter 1

## Introduction

DNA is usually transferred over generations following the normal reproduction pathway of the organism involved (e.g. sexual reproduction/inheritance by descent). This process is called *vertical gene transfer* and an example is pollen flow between the same or related plant species.<sup>1</sup> Thus, vertical gene transfer is the normal mode in which DNA is shared among individuals and passed on to the following generations. DNA can, however, also more infrequently spread to unrelated species through a process called *horizontal gene transfer* (HGT). HGT, sometimes also called lateral gene transfer, occurs independently of normal sexual reproduction and is more common among single-celled organisms such as bacteria. HGT is a one-way transfer of a limited amount of DNA from a donor cell/organism into single recipient cells (Figure 1). Examples of HGT are the spread of antibiotic resistance among bacterial species, gene therapy in humans, and *Agrobacterium*-infection in plants. HGT of recombinant DNA from genetically modified organisms (GMOs) to bacteria is a potential biosafety concern (Nielsen et al. 2005b). In this paper we introduce the main biosafety aspects of unintended<sup>2</sup> HGT processes as they relate to the use of recombinant DNA.



**Figure 1.** A schematic representation of horizontal gene transfer (HGT). A donor cell (of any origin) can release DNA (the presence of a particular gene is indicated with a black dot in the figure) that can persist in the environment. The subsequent uptake of DNA fragments by exposed recipient cells is called HGT. Such HGT can occur deliberately, e.g. by gene therapy in humans or genetic engineering of plants. Bacteria have several processes that can facilitate HGT, including transformation, conjugation and transduction.

## Chapter 2

# Introduction to Some Biosafety Aspects of Recombinant DNA

GMOs often contain recombined genes (transgenes) collected from different species to enable the expression of new traits. Most commercialised GMOs harbour < 5 protein-encoding transgenes assembled into unique genetic combinations and regulatory contexts that provide new functions to the host organism. The intended horizontal transfer and recombination of genetic material across species barriers is thought to be of little concern by many scientists active in genetic engineering, as genes are considered to be mechanistic entities or modules that can function equally well in many organisms, regardless of their historical and evolutionary context. This reductionistic understanding of genes as functional modules acting more or less independently of their organismal background and genetic networks underlies also the way risks of potential subsequent horizontal transfer of recombinant DNA to unintended recipients are presented and addressed in the biosafety assessment of GMOs.

The prevailing gene-centric perspective on GMO production is also shaping the approaches to, and understanding of, biological-mechanistic consequences of unintended HGT events.<sup>3</sup> The health and environmental impacts of potential unintended HGT from GMOs is a debated concern and risk scenario (Nielsen et al. 1998; 2001; 2005b; van den Eede et al. 2004). For instance, whereas *vertical* spread of recombinant DNA from GMOs (e.g.



GM plants) to conventional crops, landraces and to some wild relatives has been documented in several studies, no studies have conclusively proven *horizontal* spread of recombinant DNA from GMOs into naturally occurring host tissues or bacteria. The reason for the absence of observations of horizontal transfer of DNA from GMOs is currently debated and can be due to:

- Lack of receptive host cells or bacteria, conducive environments, or limited availability or insufficient amounts of recombinant DNA in a given environment (e.g. the gastrointestinal tract, agricultural fields).
- Lack of a selective advantage of the horizontally transferred recombinant DNA so that rare host cells or bacterial transformants never surface in investigations working with limited sample sizes.
- Lack of funding, and hence, conducted and published studies that have examined the process with a reasonable effort and detection limit.
- Lack of motivation among scientists to investigate such HGT processes due to the many levels of conflicts of interest and highly vocal opinion leaders in the field.<sup>4</sup>
- Lack of methods preventing an investigation of HGT processes with a sensitivity that is relevant to somatic cell dynamics or bacterial evolutionary processes.

As outlined in Nielsen (2003a), some commonly occurring characteristics of recombinant DNA in GMOs can make their transgenes more likely to be taken up and expressed in unintended host or bacterial cell recipients than the majority of the genes present in naturally occurring higher organisms (Table 1). Given the many specific characteristics of transgenes

exemplified in Table 1, it is clear that the argument that ‘native plant genes are not observed in bacterial genomes, therefore plant transgenes will have the same constraints and, hence hypothesized occurrence of HGT processes from GM plants should be dismissed’ is not relevant.

Here, we briefly present the state of knowledge concerning horizontal transfer of recombinant DNA from GM plants into human cells or into bacteria present in the gastrointestinal tract or in agricultural fields. We discuss knowledge gaps and describe various types of uncertainty embedded in the prevailing biological paradigms underlying the evaluation of HGT processes in biological risk assessments.

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**Table 1. Characteristics of recombinant DNA that may alter the likelihood of horizontal transfer, expression and stabilisation in unintended hosts**

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<b>Modification</b>	<b>Recombinant DNA has an altered likelihood of mediating:</b>
Use of bacterial gene constructs and vector sequences	– Homologous recombination with prokaryotic genomes because the recombinant DNA have regions with high sequence similarity to commonly occurring bacterial genomes. <sup>a</sup> Homologous recombination frequently facilitates integration of dissimilar DNA (through additive or substitutive integration mechanisms), based on linkage to DNA regions with high DNA similarity.
Functional assembly into a single genetic unit (linked)	– Transfer of novel multi-gene encoded traits because only a single transfer event is necessary for a recipient to acquire a genetically linked and functionally optimised genetic trait complex. The trait may have previously been distributed across the donor genome (with a lower likelihood for simultaneous multi-gene transfer), or the trait was absent from the evolving species/lineages.

- Introduced changes in gene expression and protein composition – Expression of the modified traits in novel hosts, if horizontally acquired, because broad host range promoters (often derived from microbial pathogens) are used to drive the expression of the engineered trait. Codon and promoter modifications may also change the expression levels and protein characteristics (e.g. mRNA processing and editing, post-translational modifications) affecting protein composition, function, stability, and cellular location in unintended recipients.
- Insertion of a transgene construct into an unrelated genome – Host-specific differences in the gene expression and regulation systems between the transgene's original host and the modified recipient host, can lead to unpredictable changes in the global gene regulation in the new host and in the transgene's transcription level and mRNA modifications, the translation process and composition of the translation product, altered post-translational modifications, and hence protein stability, activity and degradation.
- Removal of introns from cDNA cloned genes – Expression of the modified traits in a broader set of species and domains because intron processing (specific to eukaryotes) is regarded as a main barrier for functional assembly and expression of eukaryotic genes in bacteria.
- Insertion of transgenes into organelles – Increased exposure rates (relative to nuclear-inserted genes) to unintended recipients due to high transgene copy number in organelles, recombination (homology-based) and functional expression of the modified traits in unintended bacterial recipients because organellar genomes resemble bacteria in overall genome organization and regulation.

Large-scale release of modified gene constructs – The large-scale and continual cultivation, processing and consumption of GMOs may result in a very low-frequency horizontal gene transfer event becoming statistically likely. Empirically derived HGT frequencies obtained in laboratory-scale models are therefore of little use to understand the occurrence and impact of HGT at field scales.<sup>b</sup>

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<sup>a</sup> De Vries et al. 2001; Bensasson et al. 2004

<sup>b</sup> Heinemann & Traavik 2004; Nielsen & Townsend 2004; Pettersen et al. 2005

## Chapter 3

# Recombinant DNA Introduction and Potential Impact in Various Environments

The large-scale approval, cultivation and consumption of GM commodity crops will necessarily lead to the release and, to some extent, persistence of recombinant DNA in the environment. DNA is continually released from living organisms (e.g. crop plants) shedding tissues or cells or from their decaying debris. The release of DNA is therefore not specific to GMOs and the effect thereof should be seen in the context of DNA released from other organisms present in the same natural system (e.g. by conventional agriculture).

All living cells harbour long DNA molecules. In higher organisms, some of the DNA is broken down (fragmented) within the host during controlled cell death (apoptosis). In contrast, in single-celled organisms such as bacteria, DNA breakdown is mainly facilitated by nearby organisms with specific enzymes (called nucleases or DNases) that facilitate the degradation process. Thus, released DNA is routinely and continually degraded and recycled into nutrients in all ecosystems. Yet, evidence obtained both from DNA sequencing of whole organismal genomes and laboratory studies of DNA exchange between organisms demonstrates that some, often minor, fragments of DNA, can be integrated into the genome of the exposed recipient organism (Ochman et al. 2000; Rosewich & Kistler 2000; Nakamura et al. 2004; Thomas & Nielsen 2005).

The uptake process of DNA molecules into the cytoplasm of a cell is considered to be random and independent of the DNA's subsequent biological utility. Most foreign DNA taken up and integrated into the genome of an organism will have a deleterious effect due to its interference with the host cell biology and genome structure (Elena et al. 1998; Doerfler 2000). HGT processes thus resemble mutational processes, that is, they may occur by chance and repeatedly over time, but a very low proportion of the HGT events will confer a benefit, and be retained in the host over time (Heinemann & Bungard 2005). For multi-cellular organisms, HGT events occurring in somatic (i.e. not germ-line) cells will be lost when the organism dies. In contrast, HGT events occurring into germ-line cells or single-celled organisms such as bacteria will be passed on to the following generations. Predicting the long-term survival and competitive ability (fitness) of the transformed host organism is therefore essential to understanding whether the transformant cells will expand in numbers or eventually die out (Pettersen et al. 2005).

The potential impact of unintended HGT of recombinant DNA from GMOs to exposed organisms must be seen within the broader picture of naturally occurring processes, including: (i) the continual large-scale release of genetically diverse DNA molecules from a broad range of naturally occurring or introduced species in a given environment, (ii) the infrequent and random HGT events occurring naturally in the same environment that the GMO will be released into, and (iii) the low likelihood that any DNA taken up will improve the fitness of the exposed host organism. Within the aforementioned naturally occurring HGT context one can ask biosafety-relevant questions such as:

- Will recombined DNA released from GMOs have an altered and increased capacity to be transferred to, and change the fitness of, exposed host cells and bacteria?

- Can the likelihood of this HGT process and the subsequent population genetic trajectories of the transformed cell be accurately predicted?
- Do the currently available scientific literature and empirically-founded knowledge base on HGT processes allow a scientifically-robust impact assessment to be made?

Some scientists would argue that a hypothesised low-frequency HGT event is irrelevant from a GMO risk perspective; others may argue that the HGT issues are case- and transgene-specific, requiring a more detailed understanding of the natural selection context of each GMO case. Common to all biosafety viewpoints is that they are founded on expert opinion, familiarity with the gene donor and inference, rather than conclusive empirical evidence. The latter is unachievable given the limited understanding of the complexity of host cells and microbial communities exposed to GMOs.

Familiarity with the gene donor as a starting point for safety assessment is important. For instance, a GMO-specific and credible risk hypothesis can be difficult to design and test if the protein-coding regions of the recombined DNA ('the transgene') are already present naturally in the same environment as the GMO is being introduced to. If the recombined DNA sequences (present in the transgene) are also present naturally, then the HGT risk aspect would be narrowed to the potential biological effects caused by the recombinant DNA's altered genome location, context and regulation. Identifying and understanding the effects of the novel genetic compositions in GMOs are thus key elements in HGT risk assessment. Risk assessments based on absence of effects due to a predicted low frequency of HGT events are invalid, given the minor (non-linear) relationship between gene transfer frequencies and environmental impact (Pettersen et al. 2005).

We encourage a shift in the focus of the further development of GMOs to the use of intragenic and genomic modifications; that is, to limit the genetic modification to within the genome of an organism without the introduction of recombinant DNA from several unrelated species. Doing so may alleviate many of the current HGT concerns (Nielsen 2003b). The interest in developing an intragenic approach is currently limited by a prevailing gene-centric approach to genetic engineering (that assumes a gene's biological performance is independent of genome context) and a lack of in-depth understanding of the regulation and traits in the genomes of organisms that are of commercial interest.

### **3.1 Human Exposure to Foreign DNA**

Humans are continually exposed to DNA in inhaled organisms (e.g. bacteria, viruses, pollen etc.), from a broad variety of food sources including the microorganisms present in food, via microorganisms normally present in and on humans, and infectious agents entering the body. Thus, the human body has mechanisms to protect host cells, and utilise and degrade or remove foreign DNA molecules.

For instance, free bacterial DNA in the blood triggers immune system reactions (Stacey et al. 1996; Cohen 2002). It is estimated that humans ingest 0.1 g to 1 g of DNA per day (Doerfler 2000). Moreover, DNA is also released continually in the gastrointestinal tract from dead microorganisms and shed intestinal cells. The quantity of any recombinant DNA ingested will be a minor fraction of the total DNA consumed per human per day. Transgenes are considered chemically equivalent to any other gene present in food (Jonas et al. 2001) (with the possible exception of transgene-induced epigenetic modifications and protein interactions). Therefore, risk hypotheses of an unintended impact of recombinant DNA are mainly focused on the novel genetic composition of the recombinant DNA and not the overall chemical structure.



In the following sections, the presence of DNA in food, and its subsequent degradation in the intestine are briefly discussed. We then consider potential uptake of food-derived DNA into host intestinal cells or tissues, or into exposed bacterial cells present in the gut or in agricultural settings.

### 3.1.1. *DNA in food*

DNA molecules of broad size ranges are present in large numbers in all raw and unprocessed food sources. Depending on the extent of processing, various fractions of DNA molecules of a reduced size may be present in the consumed product. The proven persistence of DNA molecules in raw or many types of processed food is crucial for the identification of GMO ingredients. The broad application of sensitive PCR (polymerase chain reaction) technology has thus exemplified the widespread occurrence and persistence of DNA molecules in various food sources, including processed food such as corn chips and chocolate (Rizzi et al. 2001; 2003; 2004). However, the PCR protocols applied for GMO detection routinely target small DNA fragments, typically 100–400 nucleotides long. This size range is less than the length of a single transgene with a complete protein coding sequence. Thus, the overall concentration and distribution of DNA of a size that enables entire protein coding genes to be horizontally acquired from various food sources by host cells or bacteria remains largely undetermined. Many studies have demonstrated the persistence of DNA in food, for instance in canned food, whole seeds, cracked seeds and meal of canola, wet sugar beet pulp, cereal grains, and silage (Bauer et al. 1999; Chiter et al. 2000; Einspanier et al. 2001; Duggan et al. 2003). Processing often decreases the size of DNA, and such molecules can be undetectable in extensively processed food (Pauli et al. 2000; Kharazmi et al. 2003). See Nielsen et al. (2007) for a more extensive review of DNA in various environments. Table 2 lists several major knowledge gaps related to the general state of knowledge of the fate of DNA in food and during digestion.

### 3.1.2. *DNA stability in the digestive tract*

Most free DNA molecules entering the digestive system undergo substantial degradation by enzymes attacking DNA (nucleases, DNases), released from the pancreas and by bacteria present in the intestine (Wilcks et al. 2004). In addition, the low pH of the stomach may chemically modify the DNA molecules. Remaining DNA fragments are excreted in the faeces with variation in the degradation efficiency between mammals. For instance, Chowdhury et al. (2003a; 2003b) reported that maize DNA could be detected in pig faeces. Few studies have been conducted on the digestion of food-derived DNA within the 6–8 m long digestive tract of adult humans. One study by Netherwood et al. (2004) reported that whereas some DNA fragments survived passage through the small bowel, transgenes could not be detected in the faeces of human volunteers fed GM soy products.

In general, studies of the degradation of DNA in the gastrointestinal tract face many methodological challenges. Ingested food contains DNA present within tissues and cells or as complex biochemical mixtures in heat- or mechanically-damaged cells. Therefore, each food source, preparation conditions, and host physiology will determine the DNA degradation efficiencies in the digestive tract. Most studies on DNA stability in the digestive systems of mammals have used purified DNA and may therefore not capture the impact of various food components, treatments and locations on DNA degradation and stability (Martín-Orúe et al. 2002). Whereas it is generally acknowledged that DNA molecules in food are substantially degraded upon digestion in animals, there are many knowledge gaps related to the specific circumstances leading to survival of smaller DNA fragments during digestion (Table 2).

**Table 2. Knowledge gaps in the understanding of the fate of (recombinant) DNA in food and the GIT**

<b>Location/ process</b>	<b>Lack of detailed biological understanding of:</b>
DNA in food	<ul style="list-style-type: none"> <li>– The amount, size distribution, stability and degradation dynamics in various types of raw food sources.</li> <li>– The effects of various types of processing and subsequent storage.</li> <li>– The protective or degradative role of cellular/nuclear proteins, the cytoplasmic content and cell membranes/walls.</li> <li>– The combined effects of the above in complex food sources.</li> </ul>
Food-derived DNA in the GIT	<ul style="list-style-type: none"> <li>– The amount, size distribution, stability, and degradation dynamics in various compartments of the GIT as a function of food source, food mixtures and prior processing.</li> <li>– The specific degradation mechanisms active and their relative role.</li> <li>– The relationship between degradation mechanisms, degradation rate and DNA availability to epithelial or bacterial cells.</li> <li>– Quantitative DNA exposure rates to epithelial or bacterial cells.</li> <li>– Intra- and interspecies host variation in the above parameters.</li> </ul>
HGT of DNA in the GIT to host cells	<ul style="list-style-type: none"> <li>– The DNA uptake mechanisms, transport pathways and degradation mechanisms in host tissues and cells.</li> <li>– The quantitative aspects of DNA uptake from the GIT into the bloodstream of mammals.</li> <li>– The cellular locations of DNA after uptake, the potential transcription, and the elimination mechanisms active.</li> <li>– The overall uptake process such that sensitive methods and models can be developed to adequately address the fate and possible biological effects of DNA taken up into host cells from the GIT.</li> </ul>

HGT of DNA  
in the GIT to  
intestinal  
bacteria

- The proportion, size distribution, location and nature of DNA complexes exposed to bacteria in various parts of the GIT.
- The diversity, function, variability, and population dynamics of the microbiota in the GIT of mammals.
- The species distribution of, and tempo-spatial variability in natural transformation of bacteria present in the GIT.
- The host, microbial and food factors influencing uptake of feed-DNA into bacteria.
- The overall uptake process such that sensitive methods and models can be developed to adequately address the occurrence of, the relevant recipient bacterial species, and the possible biological effects of bacterial DNA uptake in the GIT.

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*Revised from Nielsen et al. 2005b. HGT: horizontal gene transfer, GIT: gastrointestinal tract.*

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## Chapter 4

# HGT of Recombinant DNA to Eukaryotic Cells (e.g. Human Cells)

The uptake of food-derived DNA into host intestinal cells or tissues has been raised as a potential concern related to the introduction of GMO-based food sources. As discussed, such exposure must be seen in relation to the broad variety of DNA naturally present in food, and hence, whether specific qualitative or quantitative genetic changes are present in the GMO that would create a higher risk/impact of DNA exposure from this source.

Experimental data are readily available that support the notion that intestinal cells of the host will be exposed to DNA molecules present in food (see the following). The potential transfer of transgenes from GM food into epithelial cells of the gastrointestinal tract can thus be hypothesised to take place but experimental studies have not yet shown such transfer to occur. The lack of such observation is likely due to the fact that the total surface area of the small intestine (microvillus) alone is more than 40 m<sup>2</sup>, with approximately 100,000,000,000 mucosal cells. Rare gene transfer events into a few of these cells are practically impossible to detect with currently available methods. In risk assessment, such hypothesised HGT events are considered to have little effect on the host because intestinal cells are continually shed from the lumen wall. The life span of mucosal cells of the small intestine is 1–2 days, and less than 10 days for most epithelial cells in the human gastrointestinal tract.

Humans eat natural food products that when combined contain > 1 million genes, some that would likely cause adverse effects if inadvertently inserted and expressed in human cells. The high general genetic diversity of DNA that enters and undergoes degradation in the intestinal system is astonishing. For instance, a simple meal consisting of chicken and two vegetables will contain a genetic diversity of more than 1 million different unique (non-overlapping) DNA fragments of 1000 bp and more than 10 million unique (non-overlapping) DNA fragments of 100 bp. Assuming a normal diet will consist of at least 50 different food sources over a limited time period, the routine exposure to DNA fragments with different compositions is between 50 and 500 million. This rough calculation does not take into account the highly diverse DNA leaking from microorganisms (eaten or present in the intestine). Thus, it can be concluded that humans are continually and naturally exposed to a genetic diversity ranging from 50 million to 5 billion different and unique DNA compositions in the size range of 100–1000 bp. Given the high variety of DNA compositions already present in conventional food sources, few, if any, specific and testable hypotheses have been put forward that suggest commercially-used transgenes would elicit more adverse effects if horizontally acquired by intestinal cells than their conventional counterparts.<sup>5</sup>

Whereas potential events of uptake and integration of food-derived DNA into exposed lumen (epithelial) cells remain unidentified, many studies have shown that food-ingested DNA can pass luminal cells in the gastrointestinal tract, and be detected in the bloodstream and tissues of mammals. Specific examples are feed-derived DNA taken up from the gastrointestinal tract and detection in leucocytes, spleen, liver, and kidneys in mice (M13 DNA), in the brain, eyes, liver, and heart of the offspring of mice (plasmid DNA), detection in the liver and spleen of mice following feeding with soybean leaves (Schubbert et al. 1994; 1997; 1998; Hohlweg & Doerfler 2001), and detection of fragments of plant DNA

in muscle, liver, spleen, and kidneys in chicken and cattle (Ein-spanier et al. 2001). It has been estimated that approximately 0.1% to 1% of dietary DNA is absorbed from the gastrointestinal tract (Nielsen et al. 2005a; 2006). A precise measurement of this process is complicated because absorption from the gastrointestinal tract takes place over several hours and absorbed DNA undergoes continuous transport, degradation and elimination. Nevertheless it is clear that DNA in food may reach the bloodstream and be exposed to and localised to various host cells and tissues. Some infrequent horizontal transfer events into somatic cells can thus be hypothesised to take place. Thus, the genetic composition of transgenes must be assessed in the 'worst-case-scenario' of being inadvertently taken up into the body from the gastrointestinal system.

This gene-centric assessment may still be ignorant of, yet to be identified, effects of higher order genome structures and chromosome modifications of importance for the HGT potential and subsequent inheritance. It can be concluded from Table 2 that the many gaps in the general biological understanding of food DNA limit the scientific basis and quality of the current risk assessment of HGT processes in this environment. The final risk assessment may therefore often be founded on expert opinion, experience and inference, rather than an in-depth understanding of the biological fate of food DNA in the gastrointestinal tract.

## Chapter 5

# HGT of Recombinant DNA to Prokaryotic Cells (e.g. Bacterial Cells)

HGT of transgenes into pathogenic, beneficial or environmental microorganisms, resulting in potential unanticipated (absolute and relative) fitness effects, has been voiced as a potential biosafety issue. As discussed so far in this paper, a broad range of DNA compositions is continually released from decaying organic matter. Microorganisms are responsible for the majority of organic matter decomposition and therefore also DNA degradation. Thus, microorganisms present in the human gastrointestinal tract and in agricultural environments experience continual exposure to DNA released from themselves and the organisms in their immediate surroundings.

DNA fragments exposed to bacteria will most often be utilised as a nutrient source (Nielsen et al. 2007). However, in rare circumstances, foreign DNA may also be integrated into the bacterial genome (Dröge et al. 1998; Davison 1999, Ray et al., 2009). Many experimental observations show that bacteria can integrate DNA molecules from their environment at measurable frequencies in the laboratory. The mosaic genetic composition of bacterial genomes also strongly suggests that horizontal transfer of chromosomal DNA has shaped their composition over evolutionary timescales (Ochman et al. 2000; Feil & Spratt 2001). However, the comparative analysis of bacterial genomes identifies HGT events that are evolutionarily stable and have occurred over a time span of millions of years. Comparative



DNA analysis does not provide information on the gene transfer frequency itself or provide a historical account of the diversity of prior DNA exposure into the bacterium in question (Pettersen et al. 2005). Thus, it remains unclear to what extent chromosomal DNA from unrelated higher organisms is exposed to and taken up into bacterial cells under natural conditions over the time course of modern agriculture.<sup>6</sup>

Experimental studies do not suggest bacteria integrate foreign unrelated chromosomal DNA at measurable frequencies over the limited time span (hours to days) and population size examined in laboratories (De Vries et al. 2001; Nielsen et al. 1998; 2005b). A high uptake frequency is also unlikely because bacteria are continually exposed to a high diversity of DNA compositions in their environments, and unchecked uptake of DNA would quickly reduce the fitness of the bacterium and soon become lethal (Elena et al. 1998). Thus, an advantage of carrying the horizontally transferred DNA is assumed necessary to cause a biologically significant amplification and impact of the transfer event (see Figure 2). It is therefore suggested that biosafety risk assessments question, determine, and identify qualitative changes in the transgenes of GMOs that would make them likely to:

- Transfer horizontally, establish, and be expressed in exposed bacterial recipients.
- Increase the fitness of transformed bacteria more extensively than any other transforming DNA source present in the same environment, so that altered bacterial population size or habitat utilisation can be expected.

For example, many of the commercially introduced first-generation of plant transgenes are derived from soil microorganisms. Thus, microbial communities are in some cases already exposed to naturally occurring counterparts to these protein-encoding genes (Nielsen 2003a; EFSA 2004; Nielsen et al. 2005b) although the

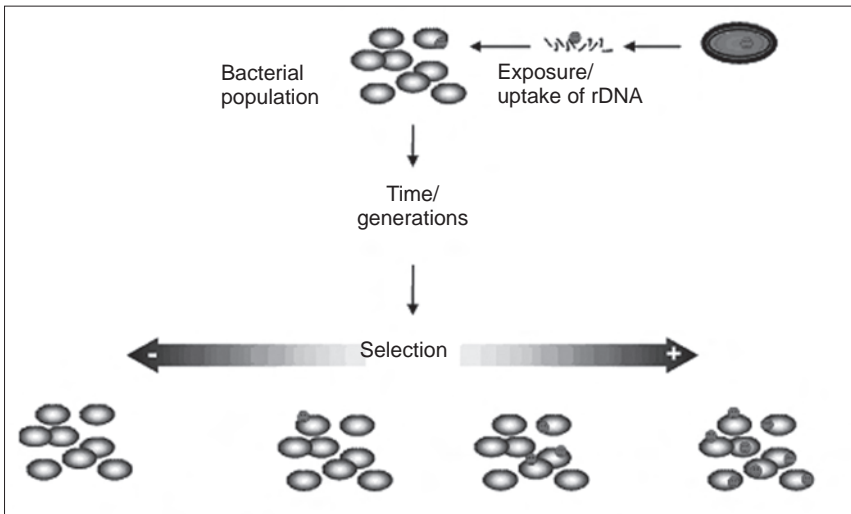


Figure 2. Schematic illustration of the fate of a horizontally acquired gene (black dot) over time. As shown, depending on directional selection, loss maintenance or amplification of the transformant population will occur. If the acquired gene has little effect on fitness of the transformed bacterium, random processes will determine the survival and distribution of the transformant population (this process is called genetic drift). Because most bacterial populations consist of high numbers of individuals, rare transformants will in most cases disappear from the bacterial population by chance, unless they confer a clear fitness gain to their host. Such disappearance is explained by the fact that only some members of a bacterial population will contribute to the next generation with daughter cells.

combinations of associated regulatory elements are unique. The introduction of similar protein-coding genes from recombinant sources to soil is therefore often inferred in biological risk assessments to cause little additional environmental impact, if an HGT event occurred (Nielsen 2003a; EFSA 2004). The HGT risk of some of the commercialised GM commodity crops currently cultivated may thus be confined to the altered genetic locations, context and regulation, and overall gene copy number concentrations. See Nielsen et al. (2005b) for a further discussion

on some risk considerations related to the use of antibiotic marker genes in GM plants.<sup>7</sup>

The novelty of the transgenes inserted into GMOs is likely to increase in the future due to development of novel gene constructs (synthetic and artificial bifunctional and multifunctional proteins) obtained through gene fusions, reshuffling and de novo construction of novel protein-encoding domains (Nielsen, 2003b). For instance, GM plants producing novel pharmaceuticals or chemicals are in development and have already been tested in field trials. Specific, reasonable and testable hypotheses can be put forward that some of these novel plant varieties may release recombinant genes that will cause a selective advantage if taken up by exposed bacteria. Thus, HGT of recombinant DNA into bacteria will become a bigger biosafety issue in the future if the current directions in GMO production are continued. The current genetic modification approaches have little focus on the gene sources and the cellular context of the recombinations made.

## Chapter 6

# Concluding Remarks

There are a number of knowledge gaps relating to the fate of DNA in the environment and if, when, and how exposed cells and bacteria will take up and incorporate such DNA. Knowledge gaps are themselves not indicative of harm, but are the driving motivation for new hypothesis formation and data collection. Discrepancy between the regulatory agencies' need for exact information on HGT processes and the iterative, dynamic process of knowledge formation creates a situation with no clear scientific answers or regulatory or consumer consensus.

Assumption-based reasoning and a variety of information sources of variable quality have been used to aid in the assessment of potential HGT of recombinant DNA. The basis for the current risk assumptions consists of:

- Laboratory test results submitted by the GMO developers.
- Experimentally collected laboratory data available in the peer-reviewed literature.
- Published and/or communicated historical and comparative experiences and observations of HGT processes in similar biological systems.
- Submitted or conducted expert evaluations of the outcomes of conceived worst-case scenarios.

Public trust in, and scientific consensus, confidence and support of HGT risk assessment conducted by regulatory bodies depends on the quality of the data used and how uncertainty has been addressed, acknowledged and communicated. Public trust also depends on the value sets underlying scientific expert opinion formation and to what extent the consumer adheres to the same values. The current lack of standards in HGT research that can guide hypothesis construction, choice of models and methods, and data interpretation and presentation result in sometimes heavily contextualised and motivationally biased research communications. Thus, the regulatory agencies have a challenging job separating facts from opinions, keeping in mind that even the experimental study design may bias the study to lead to a certain outcome.

HGT processes occurring in nature are still not well understood and many years of further study and biological knowledge accumulation are required before precise predictions can be made on the effect or absence of effects of introduced, novel recombinant DNA. The acknowledgement of broad empirical knowledge gaps contrasts with some of the risk conclusions (the absence or presence of an HGT risk outcome) made by perhaps overly confident researchers drawing on poor data sets on HGT processes. A transparent communication of the current scientific understanding of HGT processes, the data basis applied for risk assessment, and the knowledge gaps addressed, are necessary to build public confidence in the regulatory process and to direct further HGT research on transgene ecology.

## Endnotes

- 1 Pollen transfer between related plant species is less frequent than within species, and is also called outcrossing or hybridisation. Note that hybridisation processes still follow the normal ways of plant reproduction and are therefore vertical gene transfer events. The participating plants contribute c.50% each to the DNA composition of the seeds, in contrast to HGT events where most often much less than 1% of the genome of one organism is transferred to another.
- 2 This paper focuses on the likelihood of unintentional HGT. Intentional HGT, i.e. the insertion of defined DNA fragments into the target organism, is the basis for all genetic engineering and production of GMOs.
- 3 We recognise that an implicit utilitarian value set frames the presentation of the biological aspects of unintended HGT of transgenes in this paper. Nevertheless, we acknowledge a non-consequentialist view on HGT processes: that any unintended HGT of a man-made, recombined gene construct with traits derived from many unrelated organisms represents an unacceptable violation of nature. This latter argument may be seen as an ethical objection. However, most gene constructs used in GMOs today could not have arisen by natural genetic processes or traditional breeding within the timescale of modern civilisation. Ethical concerns related to the novel origin, genome and biochemical composition of GMOs are, however, also founded in a comparative perspective taking into account the long-term complex processes underlying the evolution and composition of extant organisms.

- 4 A rapid transition from a scientific debate to personal attacks and attempts to discredit the researcher may soon follow if 'unwelcome' paradigm-challenging results are published. Hence, potential threats to a further scientific career development are to be considered prior to initiating risk-focused studies.
- 5 This argument assumes that there are no genome positional effects, epigenetic modifications or protein associations specific to the transgene that will affect its stability and likelihood of HGT.
- 6 The spread of antibiotic resistance genes in clinical bacterial communities demonstrates that strongly selected genes can spread between bacterial species and communities within a short time. Although most of these resistance genes are localised on mobile genetic elements, these events demonstrate that genes can spread rapidly between microbial species when they confer a strong selective advantage to the new host.
- 7 A precautionary-based decision to phase out antibiotic resistance plant marker genes has been made in the EU (EFSA 2004; Nielsen et al. 2005b). Such a decision also exemplifies the gaps in the knowledge of resistance development in bacteria. Some of the antibiotics to which the plant marker genes encode resistance are among the most widely used in the world. Thus, whereas resistance genes to these antibiotics are known to be distributed also in non-clinical environments, they are still not a part of the majority of the antibiotic-treated population of clinically troublesome bacteria. We have currently no predictive understanding to identify the specific environments, locations and conditions that will lead to the acquisition of resistance in previously sensitive bacterial populations. In the absence of such knowledge, it is impossible to accurately predict the contribution of, and long-term impact of, plant marker genes to overall resistance development in bacteria. It is also noteworthy that most emerging bacterial pathogens arise from positive selection

of single HGT events. Thus, most HGT events that have had an ecological impact are not a proportional result of a high DNA exposure or HGT rate. The lack of a direct relationship between exposure/bacterial uptake, and a subsequent biological population scale impact suggest that qualitative aspects and the selection present for a given HGT event are the most important contributor and predictors of risk, and that DNA exposure or HGT rates is of little informative value (Pettersen et al. 2005).



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Heritable material (DNA) is usually recombined and transferred to the progeny by sexual reproduction. This process is called *vertical gene transfer* and an example is pollen flow between the same or related plant species. DNA can, however, also more infrequently spread to unrelated species through a process called *horizontal gene transfer* (HGT). HGT occurs independently of normal sexual reproduction and is more common among single-celled organisms such as bacteria. Examples of HGT are the spread of antibiotic resistance among bacterial species, gene therapy in humans, and *Agrobacterium*-infection in plants.

HGT of recombinant DNA from genetically modified organisms (GMOs) to bacteria is a potential biosafety concern. In this paper, the authors introduce the main biosafety aspects of unintended HGT processes as they relate to the use of recombinant DNA.

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