Biosafety Briefings

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FAQs on Genetic Engineering

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1. What is a GMO?

A GMO is short for genetically modified organism, also known as genetically engineered organism, or transgenic organism. It carries genetic material that has been made in the laboratory and transferred into it by genetic engineering.

2. What is the genetic material, and where is it found?

The genetic material is DNA (deoxyribonucleic acid). It is usually found in every cell, from microorganisms that have only one cell, to plants and animals that have many cells, where the cells make up tissues and organs. The cell and its constituents can be seen only with the help of increasingly powerful microscopes (see Fig. 1).

Inside a cell from a plant or animal, the genetic material is enclosed in a spherical compartment, the nucleus.

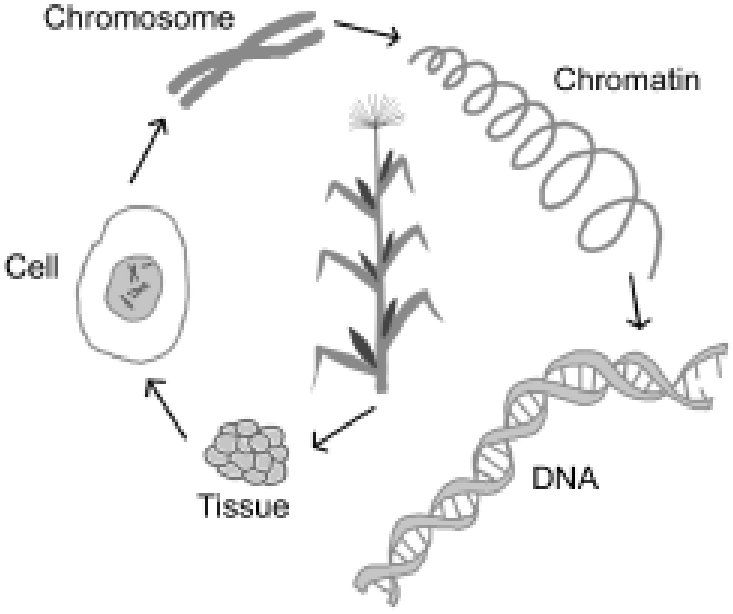

Figure 1. From plant to DNA

It is packaged into long compact structures called chromosomes. The totality of all the genetic material packaged into chromsomes is the genome. Each species has a different genome. For example, there are 23 pairs of chromosomes in the human genome, one of each pair from each parent. Bacteria have chromosomes which are not enclosed in a nucleus. The *E.coli* bacterium, which lives in the gut of mammals and human beings, has only

one chromosome in its genome.

Each chromosome is a very long molecule of DNA wound up and coiled around special proteins to form *chromatin*. (In animals and plants, each chromosome is duplicated but remains joined up at one point.) The DNA molecule itself, when stripped of all the bound proteins, consists of two strands wound around each other in a double helix. Each thread is made up of a long string of units joined end to end.

There are four different units in DNA, labelled with the letters A, T, G, and C, which stand for the identifying bases for each unit, adenine, thymine, guanine and cytosine, linked to a sugar (deoxyribose) that's linked in turn to a phosphate group (Pi). This unit is called a *nucleotide* (Fig. 2).

Pi — Sugar — Base

Figure 2. Structure of the monomer unit, the nucleotide, that makes up the DNA polymer

The bases of the two DNA strands pair up — A in one strand with T on the other, and G with C — with the bases sticking out at right angles from the backbone of each strand, which consists of alternating phosphate and sugar (Figure 3).

Note that the two strands of the DNA are anti-parallel, each going in opposite directions. As a result, the double helix looks like a spiral ladder, with the paired bases forming the rungs of the ladder.

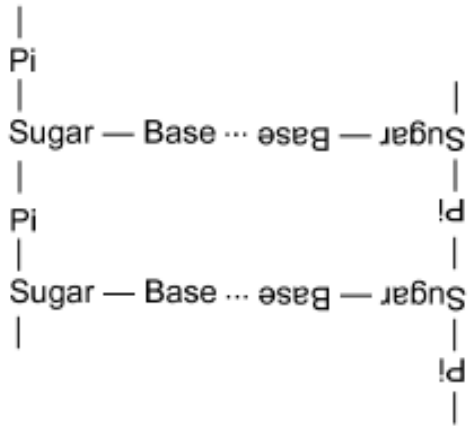

Figure 3. Structure of DNA showing anti-parallel strands engaged in complementary base pairing

On account of the specific base-pairing, the sequence of the bases on one strand is complementary to that on the other. In other words, each strand is a template for making the other strand, and this provides the basis for exact replication, which is one of the functions of the genetic material.

DNA is the genetic material in all organisms. Many viruses — genetic parasites that depend on the cell to multiply copies of themselves — make use of RNA as genetic material. RNA is similar to DNA except that the sugar is ribose instead of deoxyribose, and in place of the base thymine (T), it has uracil (U). Furthermore, RNA usually does not exist in double-stranded forms. RNA is involved in transcribing the base sequence of DNA in the first step of protein synthesis (see later).

3. What does the genetic material do?

The genetic material is replicated and passed on from one generation to the next in reproduction, and accounts for some of the resemblance between parents and offspring, although the way the

genetic material works is highly complex and strongly dependent on the environment.

One of the earliest discoveries on what DNA does, besides providing for its own replication, is that certain stretches, called *genes*, specify the structure of proteins that are made, through a 'genetic code'. Three successive bases, a 'triplet', codes for one of twenty different amino acids that are strung together to make proteins. There are 4^3 (4 x 4 x 4) or 64 possible triplets from 4 bases, so more than one triplet often codes for one amino acid, and there are triplets for 'start' and 'stop'.

Proteins perform all the vital functions in the body, and the amino acid sequence of each protein and its folded three-dimensional structure are especially suited to carry out a specific function. Other stretches of the DNA are *regulatory elements*, and enable the proteins to interact with one another and with the environment, to regulate when, where, by how much and for how long each gene is expressed, i.e., when the protein specified by the gene is made in the cell.

It is a mistake to think, as most biologists did at least up to the mid-1970s, that there is a one-way flow of 'genetic information', from DNA to protein. Feedback from the environment is crucial, and results in a lot of chopping and changing in between genes and proteins, often altering the DNA itself (see *Genetic Engineering Dream or Nightmare?* by Mae-Wan Ho, especially the chapter on 'The Fluid and Adaptable Genome').

4. What is genetic engineering?

Genetic engineering is a set of laboratory techniques for isolating genetic material from organisms, cutting and rejoining it to make new combinations, multiplying copies of the recombined genetic material (also called recombinant DNA) and transferring it into organisms, bypassing the process of reproduction. Genes can be exchanged between species that would never interbreed in nature. Thus, spider genes end up in the goat, human genes in plants, mice, and bacteria, and bacterial genes in plants.

5. How is a GMO made?

To make a GMO, the new combination of genetic material must first be constructed by using enzymes (proteins that catalyze reactions in organisms) to cut and join DNA from different sources into one stretch. To make a GM plant, say, that's tolerant to herbicide, a gene coding for a protein that inactivates the herbicide is linked with regulatory elements to enable it to be read by the cell to make the protein.

One important regulatory element is the start signal or *promoter*, and often, a stop signal, *terminator* is also provided. This trio of promoter-gene-terminator makes an 'expression cassette', a unit construct (Figure 4).

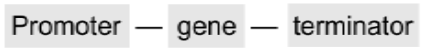

Figure 4. An expression cassette

Often, more than one expression cassettes are linked (or stacked) together, and the whole

construct is spliced into a *plasmid*, a parasitic piece of DNA in bacteria that multiplies independently of the chromosome, so that the construct can be copied millions or tens of millions of times. The copies are then introduced into the cells or embryos or an organism, such as maize or mouse, so that the construct can be inserted into the cell's genome. Geneticists use either mechanical means to force the foreign constructs into the cells, or else they splice the genes into a vector which smuggles them into the cells (see Fig.5).

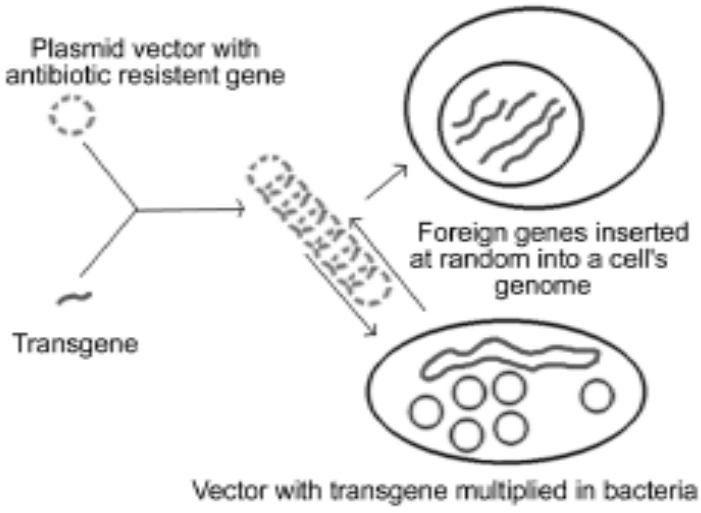

Figure 5. How to make a GMO

Mechanical means include injection with a fine glass pipette in the case of mouse embryos, or particle bombardment, in which fine particles of gold or tungsten are coated with the DNA construct and fired into the cells with a 'gene gun'. Or else strong electric fields could be used to create pores in the cell membrane letting in the foreign DNA. These usually cause a lot of damage to the cells.

Vectors or gene carriers are made from viruses or bacteria that are adept at getting into cells. The construct is spliced into the vector and taken into the cell with the vector. Within the cell, the vector carrying the construct, or the construct itself, becomes inserted into the genome.

There are key features of the transgenesis process that makes it unpredictable and unreliable, regardless of whether it is carried out by mechanical means or mediated by vector. Insertion of the transgenic construct is uncontrollable and entirely random. The genetic engineer cannot yet target the insert to a specific site in the genome, or preserve the intended structure of the insert itself. This results in many unpredictable and unintended effects. Depending on where in the genome and in what form the foreign genetic material is inserted, the resultant transgenic organism will have distinctly different properties.

The insert could jump into a gene of the host and disrupt its function, or the strong promoter in the construct, often from a pathogenic virus, could lead to inappropriate over-expression of host genes. In addition, the transgenic construct could be rearranged, duplicated or deleted in part or in whole.

In order to select and identify those cells that have taken up the insert, genetic engineers use *antibiotic resistance marker genes* that are stitched next to the genes to be inserted, so by using antibiotics, only the cells that have taken up the foreign insert will survive.

In the case of an embryo that has the foreign construct inserted into the genome of some of its cells, it will grow into an organism carrying the foreign genes in some of its cells. And by subsequent breeding, a GMO can be obtained which, theoretically at least, carries the same

foreign genes in every one of its cells. In the case of plant cells that have taken up the foreign insert, each cell can be stimulated to grow into a transgenic plant, from which a transgenic line is produced.

On account of the vagaries of the transgenic process, each transgenic line is different from all the rest, and it is important to have molecular data that uniquely identify each line.

Unfortunately, the artificial constructs contain a lot of weak links and have proven to be unstable (see below). Further changes may take place during growth or regeneration to obtain the transgenic plant and during reproduction of subsequent generations. In order to make sure that the transgenic line obtained is stable, 'event-specific' molecular data must be collected to demonstrate that both the structure of the transgenic insert and its location in the host genome have remained unchanged in successive generations. **There are, up to now, no data supporting the genetic stability of any transgenic line of plants that has been approved for commercial use.**

For more on transgenic instability, read "The best kept secret of GM crops", ISIS Report, February 2002, also many other reports in *Transgenic Instability*, ISIS Reprints, ISIS Members website.

6. How does a GMO differ from one that is derived from conventional breeding?

In conventional breeding by reproduction, only individuals from the same species or related species can be mated to produce offspring. The offspring will have genes from both parents, but the genes are just different variants of the same genes coding for the same functions. A GMO, however, bypasses reproduction altogether, so completely new genes with new functions, as well as new combinations of genes can be introduced, which will interact with the organism's own genes in unpredictable ways.

Conventional breeding involves crossing many individuals of one variety or species with another. The result is a population that preserves much of the initial genetic diversity of the parental lines, and selection occurs in successive generations until the desired results are achieved. It is therefore more controllable and predictable.

A transgenic line, in contrast, results from gene insertion events in a single original cell, out of which the entire line is produced. It is genetically very impoverished.

Furthermore, the genetic engineering process for making the GMO is uncontrollable and error-prone (see above), it causes random disturbances to the system, making the result highly unpredictable as well as unstable. Genetic instability of GMOs is now a well-known problem. GM crops are failing and GM animals have had little success.

7. Is GM food safe to eat and safe for the environment?

There are reasons to be very cautious about the safety of GM food.

New genes and gene products, mostly from bacteria, viruses and other non-food species are being introduced that we have never eaten before, at least not in such large quantities. They may be toxic or may cause allergic reactions.

These new genes and gene products may also harm other organisms that interact with the

GM crops.

The vast majority of GM crops are engineered to be tolerant to broad-spectrum herbicides that not only kill all other plants, but are also known to be toxic for wild animals and human beings.

The transgenes and antibiotic resistance marker genes may spread out of control, not just through crossing with unrelated species, but through the transgenic DNA being taken up by unrelated species, including domestic animals and human beings eating the food. This is referred to as horizontal gene transfer (see below).

For more on this topic, read "Special safety concerns of GMOs" in *Hazards of GM Crops*, ISIS Reprints, ISIS Members website.

8. What is horizontal gene transfer, and why is it dangerous?

A cell can pick up pieces of genetic material directly from its environment, and instead of digesting it as food, ends up inserting the genetic material into its own genome. The genetic material picked up could belong to the same species or to unrelated species. This 'illicit' gene trafficking is called *horizontal* gene transfer, to distinguish it from the vertical transfer that takes place in reproduction.

Horizontal gene transfer across species barriers is a rare event in nature, especially in multi-cellular organisms. Foreign genetic material is largely broken down or otherwise put out of action. And even after it has become inserted into the genome, it can still be thrown out.

Genetic engineering consists to a large extent, of *artificial* horizontal gene transfer. New combinations of genetic material from different species are made (recombined) in the laboratory. The artificial constructs are designed to cross all species barriers and to jump into genomes. They are also structurally unstable, consisting of many weak links, and tend to break and rejoin incorrectly, or to join up with genetic material from other genomes. In other words, the process of genetic engineering has greatly enhanced the potential for uncontrolled horizontal gene transfer.

Horizontal transfer of transgenic DNA could create new disease-causing viruses and bacteria, spread antibiotic resistance genes to the pathogens to make the diseases untreatable. Insertion of foreign DNA into animal cells could also trigger cancer.

For more details on this topic, read, "What is horizontal gene transfer?" and "Techniques and dangers of genetic engineering in Horizontal Gene Transfer", ISIS Reprints, ISIS Members website.

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Third World Network is a network of groups and individuals involved in bringing about a greater articulation of the needs, aspirations and rights of the people in the Third World and in promoting a fair distribution of world resources and forms of development which are humane and are in harmony with nature.

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Instability and Horizontal Gene Transfer

There is a wealth of literature on gene silencing, in which the transgenes remain in the genome, but are not expressed. But more seriously, from the safety point of view, is structural instability, that is, the tendency for the transgenic DNA to come loose, to rearrange or become lost in part or in whole in successive generations. This could change the transgenic line in unpredictable ways in terms of health and environmental risks. And it will increase the chance of transgenic DNA being taken up by unrelated species to make new combinations with their genetic material. That's referred to as horizontal gene transfer and recombination.

Transgenic DNA can spread to every species that interact with the transgenic plant, in the soil, in the air, in the mouth and gut and the respiratory tracts of animals including human beings.

New viruses and bacteria that cause diseases could be generated, and antibiotic resistance marker genes could spread to the pathogens. Transgenic DNA may also get into human cells and insert into the human genome; and a large body of evidence from so-called gene therapy experiments have amply demonstrated this does occur.

The constructs used in gene therapy are very similar to those used in transgenic plants, and one main side-effect of transgenic DNA inserting into human genome during gene therapy is cancer.

The following are reasons why transgenic DNA is different from natural DNA, and is more likely to spread by horizontal gene transfer and recombination, both by design or otherwise:

l All artificial constructs tend to be unstable, so much so that this is a topic in a standard text-book on genetic engineering. Transgenic DNA is more likely to break and join up again, i.e., to recombine.

l Transgenic DNA typically contains DNA from widely different sources, mainly bacteria and viruses and other genetic parasites that cause diseases and spread antibiotic resistance, and hence, has the potential to recombine homologously with all those agents, i.e., due to similarities in DNA base-sequence. Homology enhances horizontal gene transfer 10 million to 100 million-fold.

l Transgenic DNA is designed to cross species barriers and to invade genomes. They are flanked by recombination sequences, such as the left and right borders of T-DNA or the terminal repeats of viral vectors, which enable them to jump into genomes. By the same token, they could jump out again. Enzymes catalysing jumping in also catalyse jumping out.

l Certain 'receptive hotspots' have now been identified in both the plant and the human genome. These may also be 'recombination hotspots', prone to breaking and rejoining. That would mean inserted transgenes are more likely to be lost, to recombine, or to invade other genomes.

l There are mechanisms in the cell that actively seek out, inactivate or eliminate foreign DNA from the genome.

l Cell and embryo culture methods are well-known to induce unpredictable, uncontrollable (*somaclonal*) variations that persist in the plants generated. There is now evidence that the transformation process for making transgenic plants induces further genetic instability leading to chromosomal rearrangements, genome scrambling, in other words.

l Monsanto's Roundup Ready soya, commercially grown for years, was finally analysed by molecular methods. Not only was the gene order of the insert found to be scrambled, the plant genome at the site of insertion was also scrambled, and there was a 534 bp fragment of unknown origin in there as well — all very different from the original data provided by Monsanto.

l Recombination hotspots within the transgenic DNA, such as that associated with the ubiquitous cauliflower mosaic virus (CaMV) 35S promoter, could enhance horizontal gene transfer and recombination. This was highlighted in 1999, and it was demanded that all transgenic crops with the promoter should be immediately withdrawn for safety reasons. Two years later, the researchers who discovered the promoter's recombination hotspot also recommended that it should no longer be used, not because of safety, but because its instability compromises agronomic performance.

l Recently, landraces of corn growing in remote regions of Mexico were found to be contaminated with transgenic corn DNA by probing with the CaMV 35S promoter. Molecular analysis showed that the sequences next to the promoter were very diverse, as consistent with horizontal gene transfer and recombination.

l CaMV 35S promoter is active in species across the entire living world, including frog eggs and human cells, as we uncovered in the literature more than ten years old that had apparently escaped the notice of plant geneticists. CaMV 35S promoter, if transferred to human or animal cells, could activate cancer-associated genes as well as dormant viruses that are in all genomes. Another side effect of gene-therapy is the generation of active viruses in cell lines used to package the gene-therapy vectors.

* Transgenic DNA from GM plants was found to transfer to soil bacteria. The possibility of transfer to bacteria in the mouth and gut of animals was suggested in laboratory investigations funded by the UK government. There is also evidence suggesting that transgenic DNA from crop plants has transferred to soil bacteria in the field.

Extracted from 'The Best Kept Secret of GM Crops', by Mae-Wan Ho; ISIS Report, February 2002.