GREENPEACE Briefing

Genetic Engineering Campaign

April 2003

50 years since the double helix: Genetic Engineering is crude and old-fashioned

50 years ago the structure of DNA was determined[1] and hailed as the "secret of life". The determination of the structure of DNA made it seem as if the complete understanding of living organisms was possible, even though fundamental questions regarding DNA function were unanswered, and remain so today. Later, in the 1970s and 1980s, the technology to insert genes at random into the genomes of organisms were developed and termed genetic engineering (GE). Genetic engineering was hailed as a "life" science, as a technology to shape and design living organisms as required. Some GE crops have now been commercialised by the GE industry and deliberately released to the environment. Unexpected effects occurring in GE organisms, including commercial GE crops, are regarded as technical problems to be overcome by more research or adapted technologies. However, these unexpected effects may be due to more a more fundamental reason – that the basis of GE is invalid. In the 50 years since the discovery of the double helix, science has shown that gene expression is not nearly as simple as the GE industry would like to believe.

The fundamental basis of GE is the Central Dogma of molecular biology, as stated in the 1950s. Today, however, this Central Dogma is considered a highly over-simplistic model for gene expression. It is now known that, in higher organisms (such as plants), which genes are expressed and when is the result of many reactions and interactions between elements other than DNA (e.g. proteins, RNA). These reactions and interactions were not originally part of the Central Dogma. The original Central Dogma has given way to the concept of complex regulatory networks that controls gene expression in a manner that is far from being fully understood. Therefore, the fundamental basis of GE is an over-simplistic and old-fashioned dogma. GE can never produce an organism that is acceptable to be released into the environment and food chain: it cannot incorporate the complex regulatory networks now known to exist in organisms.

This article details how changes in the understanding of the nature of DNA and the Central Dogma of molecular biology have invalidated the GE paradigm.

Definitions

The Central Dogma – A dogma is a set of principles or beliefs. The central dogma of molecular was first proposed in 1957[2] and states that DNA (genetic information) generates RNA (an intermediary), RNA then generates protein (which performs a function) as shown in Fig. 1. The RNA intermediary between DNA and protein is a "messenger" type of RNA, or mRNA. The Central Dogma stipulates that no genetic information is transferred from protein to protein, protein to RNA or protein to DNA. However, as detailed below, it is now known that many types of regulatory information are transmitted to DNA by, e.g. proteins.

The genetic code - The genetic code is the "alphabet" contained in DNA. It was deciphered in the 1960's[3]. The sequence of bases in DNA defines the type of protein building blocks (amino acids) and their sequence in the eventual protein. The bases in DNA/RNA are "read" 3 bases at a time (a triplet), with each triplet coding for one amino acid.

How DNA was viewed in the 1950s

Ever since its discovery, the simplicity of the double helix has stunned scientists and non-scientists alike. This structure is not only elegant and stable, but also easily replicated, by splitting into two stands and assembling two new strands to complement the existing ones. The discovery of the structure of DNA occurred at a time when physicists and chemists were discovering that many materials were made of simple regular, repeating patterns of atoms. Biological scientists were looking for clear simple building blocks of life, DNA and the genes it encoded seemed the perfect solution.

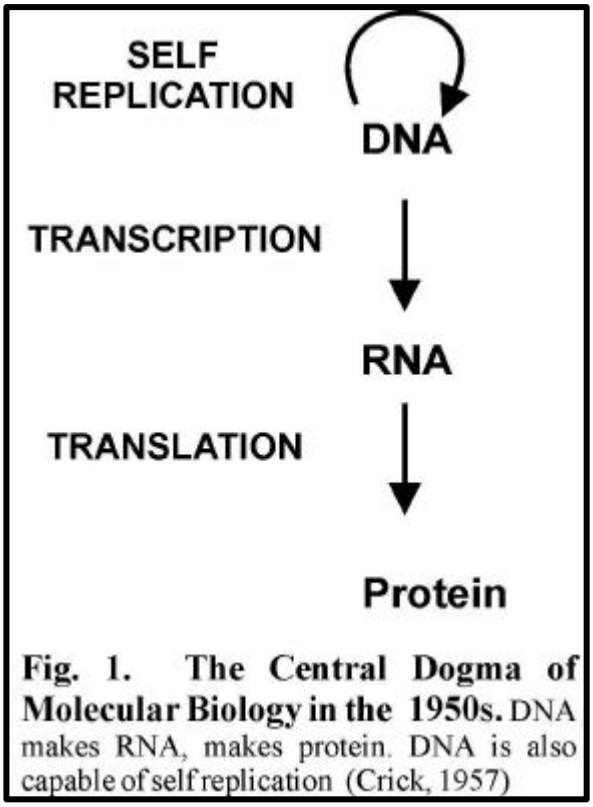


Fig. 1. The Central Dogma of Molecular Biology in the 1950s. DNA makes RNA, makes protein. DNA is also capable of self replication (Crick, 1957)

The discovery of the genetic code allowed DNA to be thought of as the master in the cell, as the superior entity that defines life. The discovery of the genetic code occurred at the same time as rapid developments in information technologies. Sequences of DNA, with all information they stored, were called the "book of life". The DNA was described as the carrier of information in addition to being the builder who uses this information to "build" the cell. Descriptions in the scientific and non-scientific literature on the topic considered DNA as "active". The active nature of DNA is shown in the colloquial paraphrase of the Central Dogma[4]: "DNA makes RNA, RNA makes proteins". The common understanding was that DNA was the initiator, with genes causing protein production. This perception of DNA as the master molecule forms the conceptual foundation of GE.

The basic structure of DNA and the genetic code have not been disputed, but their initial interpretation has proven to be too simplistic. Unfortunately, this over-simplistic understanding has hampered progress towards a fuller understanding of the function of DNA and has led instead to the commercial development of GE organisms with hardly any understanding, and certainly no appreciation of, the complex regulation of gene expression. This perceived simplicity resulted in the significance of some discoveries being overlooked because they did not fit into the general picture. For example, transposons are mobile genetic elements present in some organisms. They occasionally move (or are moved) or copied from one position in the genome to another. Transposons were described by Barbara McClintock in maize in the 1950s. However, the significance of this discovery, that genetic elements in certain organisms could move, was not realised at the time. Then, it was commonly thought that, as DNA had a fixed structure, genes would be fixed in their positions within the genome in all organisms at all times, and it was only in 1983 McClintock was finally awarded a Nobel Prize for this discovery.

There are now many such discoveries regarding the nature of DNA and gene expression. The collective evidence gives a picture of a much more complex Central Dogma than first proposed in the 1950s. This completely undermines the whole philosophy of GE, which is based on the 1950s Central Dogma.

Discoveries in the last 50 years

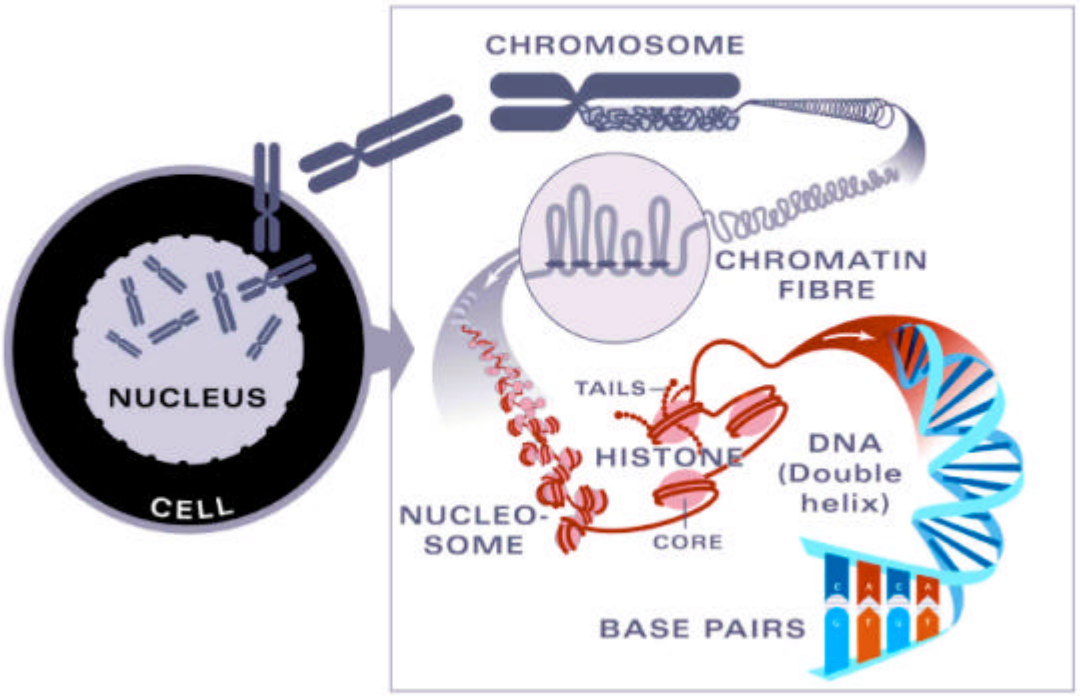
Over the last 50 years, many discoveries, both small and large have challenged the original Central Dogma and the fixed, deterministic view of DNA. These studies now form the cornerstones of modern molecular biology. They reveal that, whilst the essential elements of the Central Dogma still holds, it is an over-simplistic model. Gene expression is subject to a regulatory network of a complexity that it only just being realised[5].

1) DNA packing affects gene expression

DNA is generally shown as a double helix spiralling about a straight central axis. In reality, this axis is not straight but coiled again and again to form a complex 3-D structure. It is now known that this higher order coiled structure has an important influence on gene expression, not originally envisaged in the Central Dogma.

DNA consists of six different "building blocks": sugar, phosphate and four types of bases. They bind to together to form a string-like molecule and, together with a complementary DNA strand (with the four different bases matching in pairs); they form the double helix. DNA is pictured as this double helix revolving around a straight axis and DNA sequences are shown as linear strings of bases. However, in reality, the axis is coiled and coiled again to form a supercoiled or higher order coiled structure, known as chromatin, shown in Fig. 2. The fundamental subunit of chromatin is the nucleosome, which consists of DNA wrapped around a core of proteins (histones). Each nucleosome is connected to its neighbours by a short segment of link DNA and this "beaded" string is coiled into a compact fibre, chromatin, resulting roughly in 50-fold compaction of the DNA. The chromatin is then arranged into chromosomes and all the chromosomes together in the nucleus of the cell make up the genome of the organism.

Figure 2 The organisation of DNA into a chromosome. DNA is not arranged as a simple double helix (as often shown in articles) but wound around histone cores, coiled into chromatin fibre and then tightly packed into a chromosome. Recent studies have shown that the position of a gene on the chromosome can control gene expression and that the packing arrangement changes with time, affecting gene expression.



Grafik: Nicole Krohn, 3/03

GREENPEACE

The coiled structure of DNA is dynamic. The extent of the coiling, or rather, the structure of the chromatin is constantly changing, not randomly but in an organised manner that exerts a control over gene expression[6]. *"Regulatory signals entering the nucleus encounter chromatin, not DNA, and the rate-limiting biochemical response that leads to activation of gene expression in most cases involves alternation in chromatin structure."*[7]

Some regions of the chromatin are more condensed than others. There is open chromatin and more densely packed, closed chromatin, which seems to be impenetrable to regulatory proteins and RNAs. Within chromatin, genes that are frequently transcribed appear to be right at the edges of the chromatin. Transcription takes place at the outer edge of the

chromosomes, it is speculated although not yet known, because of the easier accessibility of the DNA to transcription enzymes[8]. It is unclear whether the location of the genes determines the activity or the other way around. Presence within, or close proximity to, densely packed, closed chromatin can permanently silence genes and this might even be inherited as a trait that does not involve any alteration of the DNA sequence (an epigenetic trait)[9].

The influence of location goes beyond the 3-D structure of the chromatin. At least in certain cell types, each chromosome tends to keep to its own territory within the nucleus, returning to its original spot after each cell division[10]. Chromosomes with a lot of activated genes are found more in the centre of the nucleus, while other chromosomes with fewer active genes are in the outskirts of the nucleus[11,12]. All this leads to the question in the DNA anniversary issue of *Nature* of *"How many celebrations of the double helix will admit that, 50 years on, we don't really know what DNA at large in the cell looks like?"* [13]

The nucleosomal histones (the proteins around which the double helix is wound) also play an important role in regulating gene expression. The histones can be modified chemically in various ways in regions known as their "tails" (see Fig. 2), which is now seen as a central regulatory control mechanism for gene activation or silencing. It has even been suggested that the patterns of modifications of the histone tails may constitute a histone code, which determines gene activity in an organism, in addition to a genetic DNA code[14].

In summary, the structure of DNA within a chromosome affects gene expression. Both the histones that DNA is wound round and the higher order coiled structure of chromatin, exert important regulatory controls on gene expression.

2) Gene expression – the "on" and "off" switches

DNA contains genetic information. However, it is now clear that this genetic information must be accessed and the process of protein production initiated. This initiation requires transcription factors, which are proteins that bind to specific sequences of DNA, such as promoters and enhancers, which activate gene expression. The chromatin must be in such a way that the transcription factors can interact with it (i.e. open rather than closed). Hence, whilst the Central Dogma states that genetic information may not be transferred from protein to DNA, it is clear that vital regulatory information, that required to initiate gene expression, is transferred from protein to DNA in the form of transcription factors.

Genes can be switched off, or silenced. Small hydrocarbon molecules or methyl groups can be added to the DNA in the genome. This blocks transcription and has the effect of "shutting down" gene expression. A clear picture of how methylation is caused and what activates silencing at the molecular level has yet to emerge, but there are suggestions that histone modification may be involved[15].

3) From RNA to DNA – "reverse transcription"

The Central Dogma considers that the transfer of genetic information in reverse, from RNA to DNA, is "rare or special"[16]. However, viruses bring genetic material into the cell and include it into the DNA of the host cell. Certain RNA viruses are able to use this process to make a DNA copy of their RNA, which is then inserted in the host DNA[17]. This is reverse transcription.

4) The role of RNA – viewed with increasing importance

The Central Dogma (Fig. 1) sees RNA[18] only as the messenger from the DNA in the nucleus to create the proteins in the cell, hence its name, messenger RNA or mRNA. However, it is now well accepted that there are different types of RNA that do not code for protein called non-coding RNA or ncRNA[19]. Several other forms of ncRNA have also been discovered, mostly very recently. Most of these forms of RNA went undetected for so long because they did not code for proteins and hence did not fit easily with the Central Dogma: "*…Almost all means of gene identification assume that genes encode protein, so even in the era of complete genome sequences, ncRNA has been effectively invisible.*"[20] and "*Could it be possible that a large class of genes has gone relatively undetected because they do not make protein?"* [21]

One of the newest discoveries of ncRNA is RNA interference (RNAi), reported as a scientific highlight of the year 2002 in the leading scientific journal, *Nature*[22]. These are small pieces of RNA, which can bind to complementary mRNA to form double stranded RNA, which in turn are destroyed by specific enzymes. Thus, RNAi has the ability to interfere with mRNA, i.e. to stop the production of proteins from genes by acting on the intermediary, RNA. It is thought that this might act as a defence mechanism against RNA viruses, as an intracellular immune system, but it can also silence genes[23]. Other classes of ncRNA seem to function as enzymes. Genes that do not code for proteins but for ncRNA can be located in non-coding stretches of DNA. Such stretches of non-coding DNA have previously been dismissed in genome sequencing programmes as "junk DNA"[24]. However, it is becoming clear that this so-called "junk DNA" is not junk but probably a vital part of the gene regulatory network.

Many questions regarding these ncRNAs remain and will be the subject of ongoing research into the controls of gene expression: *"How many RNA genes are there? How important are they? What function does a cell delegate to RNA instead of to protein, and why?*" [25]

5) The DNA Sequence of the Human Genome

The publication of the human genome sequence[26,27] in 2001 has been heralded as starting a new era in genomics. However, the most startling fact about the human genome is its size. There are only 30,000 to 40,000 genes in the human genome, much less than the expected 100,000 genes. This finding has severely challenged the current understanding of DNA regulation and control. The simplistic Central Dogma can no longer explain gene expression. How can such an apparently complex and highly evolved species such as *Homo sapiens* have so few "extra" genes, compared to such simple creatures as the roundworm or nematode, *Caenorhabditis elegans* (approximately 19,000 genes); the fruit fly, *Drosophila malogaster* (approximately 14,000) or the simple plant *Arabidopsis thaliana* (approximately 25,500)? There are simply too few genes to account for the diversity of proteins that are manufactured by the human genome on a one gene, one protein basis.

Genes must logically be able to produce more than one protein through a complex DNA - RNA - protein route. They are thought to do this by splicing mechanims.

Genes and splicing mechanisms

What is a gene? The term "gene" still remains undefined. It was introduced in 1909 as a new term to describe, simply, a hereditary unit[28]. With the discovery of the DNA structure in 1953 and the genetic code in 1960s, a gene became a sequence of a DNA that codes for a protein, but the definition has since become blurred. To produce a protein from DNA, more information and regulatory controls are required than simply the DNA sequence for the amino acids that make up the protein (the genetic code). For example, "start" and "stop" instructions are required. In addition, one "gene" must be able to produce more than one protein.

In eukaryotes, the initial RNA copy of a gene within DNA undergoes numerous processing steps to produce mRNA before it is translated into proteins. A gene can be thought of as a succession of a number of smaller units, exons and introns. Exons carrying the information for part of the protein alternate with non-coding introns. A splicing process functions in eukaryotes, which accurately joins the protein coding exons to form the mature mRNA. Introns do not contain information for the formation of the protein, but may contain regulatory sequences in addition to splicing information. Different combinations of exons can produce different mRNA from DNA by the process known as "alternative splicing". Thus, mRNA can be spliced to give rise to the "right" template for a protein[29]. Both proteins and small ncRNAs are factors controlling the splicing[30]. It is thought that 40-60% of human genes have alternatively spliced forms[31]. *"Clearly, increasing protein diversity does not simply correlate with increasing gene number. It is dependent both on the number of genes in the genome and on the rate alternative splicing of those genes."*[32]

Cutting mechanisms

Different mechanisms can act on the proteins formed. Some proteins are formed in large molecular precursors to proteins, which are then cut to yield the final protein or proteins[33]. These are then cut down to a final protein or proteins by specialised enzymes (proteases).

The question "what a gene?" remains unanswered. In many cases, the DNA sequence, the "gene" can no longer be thought to code for one specific protein, but the final protein product is a result of the interaction of all the different RNAs, including ncRNA and regulatory proteins. Neither the basic structure of DNA, nor the genetic code offer a satisfactory explanation for the production of so many proteins from so few genes in the human genome.

As Stephen Jay Gould commented on the publication of the human genome sequences: *"First the key to complexity is not more genes, but more combination and interaction generated by fewer units of code – and many of these interactions (as emergent properties, to use the technical jargon) must be explained at the level of their appearance, for they cannot be predicted from the separate underlying parts alone."*[34]

How DNA is viewed today

The discoveries in the past 50 years have caused a paradigm shift from the concept of DNA as the master molecule that stores information, controls and produces protein, to DNA as an important store of information that is subject to a mutli-layered complex regulatory network.

The language of the Central Dogma is changing. DNA is not active anymore, it is activated. DNA is not self-replicating anymore, it is replicated by a system of interacting enzymes (proteins). DNA is not making DNA anymore, DNA is the template. In the new language, DNA is taking on a more passive role. The passive nature of DNA is consistent with its physico-chemical properties. DNA is very stable molecule despite its enormous size, suggesting an unreactive or passive nature.

The original Central Dogma of molecular biology was based on the understanding that DNA was active, controlling gene expression. Discoveries over the past 50 years (and old ones revisited) have changed this picture. Indeed, new discoveries continue to change the paradigm of gene expression. For example, almost every month during 2002, one of the two leading scientific journals, *Nature* and *Science,* published a commentary or review on some significant new aspect to our understanding of gene expression. The emerging picture is that of gene expression in a complex network of actors and regulatory processes in the cell.

"If all of this destroys the pretty illusion created by the iconic model of Watson and Crick, it surely also opens up a much richer panorama. The fundamental mechanism of information transfer in nucleic acids — complementary base pairing — is so elegant that it risks blinding us to the awesome sophistication of the total process. These molecules do not simply wander up to one another and start talking. They must first be designated for that task, and must then file applications at various higher levels before permission is granted, forming a complex regulatory network"[35]

Consequences for GE crops

Genetic engineering is based on the over-simplistic Central Dogma as shown in Fig. 1. GE assumes that genes act as isolated units within a system. This is simply not true. Gene position within the genome is crucial to the strict regulatory controls it is subjected to. Genes inserted at random into the genome means are outside of these regulatory controls - they are unregulated. GE goes against the current understanding of the complex nature of the genome. It does not take into account the regulatory actions of RNA, proteins, or any other cell components. The transgenic gene construct usually includes promoters as well, which initiate transcription of the DNA. However, these promoters are not part of the regulatory system of the cell, in most cases the cauliflower mosaic virus (CaMV35S) promoter is used, which keeps the inserted gene(s) switched on all the time, in every cell of the organism.

DNA is inserted at random into the genome. Insertion of DNA can cause deletions and rearrangements the original DNA at the insertion site[36]. In addition, DNA insertion is always forcibly introduced into the cell, e.g. by the "gene gun" method where particles coated with DNA are fired into cells. This method is becoming notorious for unintentionally introducing additional, randomly placed copies and fragments of the genetic insert, in addition to causing deletions and rearrangements of the host's DNA. The gene gun technique is commonly used by Monsanto to produce commercial GE crops and irregularities have been found in the genomes of several of their GE crops, e.g. Roundup Ready maize (GA21, NK603) and Roundup Ready soya.

The new paradigm of DNA being just one part, albeit an important part, of the network regulating living cells completely undermines the fundamental ideology on which GE is based. The complexity of the regulatory network makes the GE of higher organisms appear crude and old fashioned.

Problems with GE organisms have been acknowledged, e.g. events of gene silencing but are viewed by the developers simply as practical problems to be solved. The random, often forcible introduction of DNA into the genome must affect the 3-D structure of the higher order chromatin structure, the chromatin, but this has not being examined. Similarly, there are no studies on the effects of DNA insertion on the complex and tightly controlled regulatory network. However, it is likely that at least some of the untended and unexpected effects that occur in GE organisms can be attributed to disruption of the regulatory network.

During the development of GE plants, a high number of transformed plants is produced and most are rejected due to unintended effects. There have been several examples of unexpected effects of commercial GE plants, e.g. Monsanto's Roundup Ready soya gave rise to unexpected crop losses in hot, dry weather due to stem splitting caused, most probably, by increased lignin[37] and cotton bolls have inexplicably dropped from Roundup Ready cotton plants[38]. However, none of these have, as yet, been thoroughly investigated (or, at least, the results of any investigation have not been made publicly available) and satisfactorily explained. One of the most striking examples of unintended effects in Roundup Ready soya is the phenomenon of yield drag (or yield reduction). In side-by-side experiments, there was an average yield drag of 10% in Roundup Ready soya compared with sister non-GE lines, with 5% of the yield drag attributable either to the inserted gene or the GE process[39].

Proponents for releases of GE organisms would assert that any such interference in metabolic function would be self-eliminating during breeding due to substandard performance before release. However, not all effects may be easily detectable and effects may not be apparent for several generations[40] or until exposed to extreme field conditions such as drought or high temperatures.

GE regulation

Although the regulatory authorities (e.g. US and EU) recognise that unexpected effects will occur, risk assessments of GE organisms for both the environment and food/feed are still based upon the original, over-simplistic Central Dogma (Fig. 1); the linear concept of one gene, one function. Screening for interaction effects, which cannot be deduced from the (usually one) known function of an inserted gene, involves the difficult task of looking for the unexpected.

The risk assessments of GE crops are based on the DNA sequence of the insert and their expression. They do not take into account whether any genes interrupted upon insertion (whether they code for proteins or not), nor how the insert affects the overall structure of the genome. The risk assessments omit vital considerations so are unsound and irrelevant. The Precautionary Principle is more applicable to GE organisms than a risk assessment. It is intended for situations where there a potential for serious harm and requires action to be taken to avoid such threats even where definite proof of harm does not yet exist. Any release of GE organisms is irreversible and has potential to cause serious harm. Therefore, the Precautionary Principle must be applied and to prevent all releases of GE organisms to the environment.

Biotechnology doesn't mean only GE

After years of plunging head on into the development of GE organisms, there is now more emphasis on basic research, to examine how DNA actually functions. If this emphasis has been placed on basic research earlier, it may already have shown that life is too complex to be manipulated by GE. There is no one single building block in biology, anymore than there is in physics. We have to deal with a world of networks, of regulation, in a world where there are no easy solutions. So, if GE organisms are doomed to failure, how can genomic and genetic research be utilised to improve the quality of life?

Biotechnology is often thought of as meaning GE. But it does not. Biotechnology is simply a term given to biological technology, usually at the molecular level. There are many ways to utilise our knowledge of the genome of plants and animals and their genetic controls. Where genetic markers (specified DNA fragments) are known to correspond with specific traits, they can be used in marker-assisted selection. The selection and visualisation of the genetic marker requires knowledge of the concepts and techniques used for genetic and genomic studies. Marker-assisted selection (MAS) cannot be used to change the DNA of the plant, to genetically engineer it. However, in seed development and breeding programmes it can be used to select the plants for further breeding programmes because they contain the genetic marker. Unlike GE, MAS is a biotechnology that works in accordance with the new discoveries of gene expression. It acknowledges the complex regulatory network controlling gene expression. Marker-assisted selection has tremendous scope for creating new plant varieties because it allows complex traits (e.g. drought resistance), or even multiple complex traits, to be bred into plants. Marker-assisted selection is often viewed as accelerated conventional breeding. Hence, the release of crop developed by marker assisted selection to the environment does not give rise to the same concerns regarding unexpected and unpredictable effects as a GE crop.

Marker-assisted selection is becoming more widely utilised and this trend will undoubtedly increase as more markers become identified. For example, markers for drought resistance in rice, vitamin E in corn have recently been identified[41]. However, to develop such programmes further increased funding is necessary and, at present, much of the available funding is directed at developing GE organisms[42].

Conclusion

In the 50 years since the publication of the structure of DNA, there have been many discoveries regarding the nature of DNA that challenge the Central Dogma, especially in the last few years. Discoveries such as the influence of the coiling of DNA, gene silencing, reverse transcription and the role of various types of RNA have changed the picture of gene expression from a simple linear system (DNA makes RNA, makes protein) to one where gene expression is tightly regulated and controlled by many non DNA elements in a complex, multi-layered hierarchy.

GE technology is crude and old-fashioned. It is based on the over-simplistic 1950s Central Dogma and relies on a linear model of DNA. GE is bound to failure because it cannot incorporate the complex regulatory networks now known to exist in organisms. However, GE is not the only biotechnology. Other technologies such as marker-assisted selection, which utilise the knowledge and some of the tools of modern biotechnology are becoming more widely used and do not give rise to the same concerns as GE organisms.

DNA is important, but it is not the "secret of life".

References:

[1] Watson JD & Crick FHC (1953) Molecular structure of nucleic acids. Nature, 171, 737-738.
[2] Crick FHC (1957) On protein synthesis. Symp. Soc. Exp. Biol., 12, 138-163.
[3] Crick FHC, Barnett L, Brenner S, Watts-Tobin RJ (1961) General nature of the genetic code for proteins. Nature 192, 1227-1232.
[4] Crick FHC (1957) On protein synthesis. Symp. Soc. Exp. Biol., 12, 138-163.
Crick FHC (1970) Central Dogma of Molecular Biology. Nature, 227, 561-563.
[5] Lee T et al. (2002) Transcriptional Regulatory Networks in *Saccharomyces cervisiae*. Science, 298, 799-804.
[6] Felsenfeld G & Groudine M (2003) Controlling the double helix. Nature, 421, 448-453.
[7] Felsenfeld G & Groudine M (2003) Controlling the double helix. Nature, 421, 448-453.
[8] Pearson H (2003) Beyond the Double Helix. Nature, 421, 310-312.
Holmes B (2003) Location, location, location. New Scientist, 15 March 2003, 48-49.
[9] Holmes B (2003) Location, location, location. New Scientist, 15 March 2003, 48-49.
[10] Holmes B (2003) Location, location, location. New Scientist, 15 March 2003, 48-49.
[11] Pearson H (2003) Beyond the double helix. Nature, 421, 310-312.
[12] Tanabe H et al. (2002) Evolutionary conservation of chromosome territory arrangements in cell nuclei from higher primates Proceedings of the National Academy of Sciences USA, 99, 4424-4429.
[13] Ball P (2003) Portrait of a molecule. Nature, 421, 421-422.
[14] Felsenfeld G & Groudine M (2003) Controlling the double helix. Nature, 421, 448-453.
[15] Felsenfeld G & Groudine M (2003) Controlling the double helix. Nature, 421, 448-453.
[16] Crick FHC (1970) Central Dogma of Molecular Biology. Nature, 227, 561-563.
[17] Watson JD et al. (1992) Recombinant DNA. 2. edn. Scientific American Books, New York, USA.
[18] Messenger RNA (mRNA), transcribed as complimentary strand to a DNA sequence and translated into a protein, is considered coding RNA, while all other classes of RNA, especially transfer and ribosomal RNA are non-coding.
[19] Henikoff S (2002) Beyond the Central Dogma. Bioinformatics, 18, 223-225.
[20] Eddy SR (2001) Non-coding RNA and the modern RNA world. Nature Reviews Genetics, 2, 919-929.
[21] Eddy SR (2001) Non-coding RNA and the modern RNA world. Nature Reviews Genetics, 2, 919-929.
[22] Dennis C (2002) 2002 in context: the genome's guiding hand? Nature, 420, 732.
[23] Henikoff S (2002) Beyond the Central Dogma. Bioinformatics, 18, 223-225.
[24] Keller EF (2000) The century of the gene. 186p. Harvard University Press, USA.
[25] Eddy SR (2001) Non-coding RNA and the modern RNA world. Nature Reviews - Genetics, 2, 919-929.
[26] International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. Nature, 409, 860-921.
[27] Venter JC et al. (2001) The sequence of the humane genome. Science, 291, 1304.
[28] Keller EF (2000) The century of the gene. Harvard University Press, USA.
[29] Sorek R & Amitai M (2001) Piecing together the significance of splicing. Nature Biotechnology, 19, 196.
[30] Lewin B. (2000) Genes VII. Oxford University Press, Oxford.
[31] Modrek B & Lee C (2002) A genomic view of alternative splicing. Nature Genetics, 30, 13-19.
[32] Sorek R & Amitai M (2001) Piecing together the significance of splicing. Nature Biotechnology, 19, 196.
[33] see, e.g. Lewin B. (2000) Genes VII. Oxford University Press, Oxford.
[34] Gould SJ (2001) The New York Times, 19 February 2001.
[35] Ball P (2003) Portrait of a molecule. Nature, 421, 421-422.
[36] Svitashev, SK & Somers DA (2001) Genomic interspersions determine the size and complexity of transgene loci in transgenic plants produced by microprojectile bombardment. Genome, 44, 691–697.
[37] Coghlan A (1999) New Scientist, 20th November, p. 25.
[38] Fox JL (1997) Farmers say Monsanto's engineered cotton drops bolls. Nature Biotechnology, 15, 1233.
[39] Elmore RW, Roeth FW Nelson LA Shapiro CA Klein RN Knezevic SZ & Martin A (2001) Glyphosate-resistant soybean cultivar yields compared with sister lines. Agronomy Journal, 93, 408–412.
[40] Riha K McKnight TD Griffing LR & Shippen DE (2001) Living with instability: plant responses to telomere dysfunction. Science, 291, 797-1800.
[41] Price AH, Townend J Jones MP Audebert A & Courtois B (2002) Mapping QTLs associated with drought avoidance in upland rice grown in the Philippines and West Africa. Plant Molecular Biology, 48, 683–695.
Rocheford TR, Wong JC, Egesel CO & Lambert RJ (2002) Enhancement of Vitamin E Levels in Corn. Journal of the American College of Nutrition, 21, 191S–198S.
[42] Knight J (2003) A dying breed. Nature, 421, 568-569.