Over the last few years biotech laboratories and industry have developed two new techniques – artificial minichromosomes and transformed organelles – which, the industry claims, will allow it to overcome the problems it has faced until now with GMOs, especially their low efficiency and genetic contamination. But basic biology and maths indicate that, contrary to what the industry claims, the new technology will not prevent genetic contamination in plants. In fact, as the two technologies converge, the frightening possibility arises that contamination will reach a new level of toxicity, and occur not only within organisms of the same species but also between species as different from each other as plants and bacteria, or plants and fungi.

The new weapons of genetic engineering

GRAIN

From its very beginning, genetic engineering has faced two tremendous barriers. First, there is the undeniable fact that the theory that each gene is responsible for a single characteristic (one gene–one trait), if it is true at all, holds true for only some genes. The more that is learnt about the functioning of cells and organisms, the more flexible and multiple the links between gene and function are found to be.[1] Second, there is the complex and powerful self-regulating capacity of chromosomes and genomes, which leads them to expel, delete or "silence" genetic material which is not part of their normal make-up. Mutations occur very often in nature, and most of the time the genetic material itself triggers mechanisms that "correct" or delete these mutations. The result is an amazing and stubborn stability of form and function.[2]

Three major practical effects derive from this: multiple and unexpected side-effects from genetic engineering; a very low rate of successful, stable expression of the engineered traits; and an overwhelming difficulty in genetically engineering traits that involve several genes. The biotech industry has addressed the first problem by not releasing engineered organisms with obviously harmful side-effects and by denying side-effects when they have occurred in the field or lab, or in animals and human beings. Industry has also been very careful to avoid acknowledging that fewer than one per cent of their attempts at genetic engineering are successful in any way. They are also reluctant to admit that none of the attractive initial promises of biotechnology – that it would make all plants capable of fixing nitrogen and acquiring phosphorus, that it would produce plants tolerant of drought, salt and heavy metals, and that it would manufacture new vaccines – has been delivered. A key factor in explaining this is that all these characteristics or products involve gene complexes; by contrast, almost all current biotech products are based upon single genes (plants that are tolerant of herbicide and plants that contain Bt toxin are two good examples).

1 See, for example: "Now: The Rest of the Genome", *New York Times*, 11 November, 2008.

2 Rachel Shulman, "New gene-silencing pathway found in plants", American Association for the Advancement of Science: Eurekalert, 17 November 2008. http://tinyurl.com/6q3fqv

As well as harming their public image, these failures have serious practical consequences for the companies, as they reduce their efficiency and limit their potential profits. Not surprisingly, the industry has long sought new approaches to overcome these limitations. Biotechnologists and the biotech industry are now saying that a major breakthrough has taken place: they are now able to build small artificial chromosomes that carry multiple genes and become fully functional once inserted into a cell. Due to their small size, these artificial chromosomes are called "minichromosomes". It is claimed that they will make the engineering of complex traits possible and that they will dramatically reduce side-effects, as they will not disrupt the native genetic material of the engineered organisms.[3]

A second important development has also taken place, with much less media coverage: the genetic engineering of cell organelles, such as chloroplasts and mitochondria. Because there may be multiple organelles (up to hundreds) per cell, this technique would allow a much stronger expression of the engineered traits. As GE organelles are not transferred through pollen, the industry also claims that genetic contamination of plants would be prevented.

There is still much that is unknown. New research is uncovering a remarkable level of complexity in the web of interactions between genetic material, whole organisms and the environment, which raises questions about how efficient the new technologies will be. Looked at from a commercial point of view, however, it is certainly true that, even if it works only partially, the technology will open up for the industry a whole new world of biotech products and patents. This is because it extends the range of patentable "inventions" beyond genes and traits to chromosomes and complete physiological processes.[4]

What are artificial minichromosomes?

Artificial minichromosomes are small chromosomes built by incorporating genes into a DNA molecule that initially contains only the units that regulate the replication of chromosomes (called telomeres); those that initiate the replication, and those that ensure the right distribution of chromosomes in new cells (called crentromeres).[5] Multiple genes can be added to these two basic units and, to render them functional, there is no need to include the regulating DNA that makes up more than 90 per cent of most natural chromosomes. The biggest artificial minichromosomes built so far carry between a dozen and 20 genes but, in theory, there is no limit to the number of genes that can be included in one single artificial chromosome. Artificial minichromosomes can be built and inserted into all kind of species, from yeast and bacteria, to higher plants, insects, mammals and humans. In fact, in the early years bigger advances were made in developing artificial chromosome technology for animals and humans than for other species, but more recently the technology for plants, yeasts and bacteria has been catching up.[6]

There are natural minichromosomes too, and they are encountered widely among different species and kingdoms. They may be present in the nucleus, as well as in the cell "organelles" that are responsible for photosynthesis, energy processing and other fundamental processes of life. They characteristically lack regulating DNA and may exist in highly variable numbers of copies in the same cell. The role and functioning of natural minichromosomes is little understood, but they may be important in the process of adjusting to very different or changing habitats and conditions.

One characteristic of natural and artificial minichromosomes that has attracted the attention of biotechnologists is that they seem to be more "independent" from the rest of the genetic material than larger nucleus chromosomes. That is, their expression seems not to be determined by – and seems to have little influence on – the behaviour of other chromosomes. When foreign genes are inserted, the genetic material of the artificial minichromosomes is not "silenced" or "deleted", as often happens with genes inserted into existing chromosomes. Once inserted into the cell, artificial minichromosomes also remain physically independent from other chromosomes and genetic material; they are not incorporated into the native DNA and therefore do not cause mutations in the native DNA. Industry and labs developing and using the technology thus claim that minichromosomes will avoid the side-effects of genetic engineering because there will be no disruption of genetic material.[7]

What are transformed organelles?

Organelles – also called plastids – are tiny structures that exist within animal and plant cells. They are the sites where fundamental processes take place, such as photosynthesis and cell respiration. They include chloroplasts, ribosomes and mitochondria. There are multiple copies per cell, each with their own DNA. If a foreign gene or an artificial chromosome is inserted into an organelle, the cell will multiply it, producing new cells with multiple copies of the inserted gene. Under certain conditions that can

3 University of Missouri College of Arts and Sciences press release, 17 December 2007: http://tinyurl.com/a32fpp; entry in Yenra online encyclopaedia, 24 September 2003: http://tinyurl.com/ay2r9v

4 Weichang Yu and James A. Birchler, "Minichromosomes: the next generation technology for plant genetic engineering", University of Missouri, Division of Biological Sciences, August 2007. http://tinyurl.com/7k26mn

5 See, for example patent WO 2007137114 20071129 at http://tinyurl.com/8bxone

6 Arnaud Ronceret, Christopher G. Bozza and Wojciech P. Pawlowski, "Naughty Behavior of Maize Minichromosomes in Meiosis", *The Plant Cell*, American Society of Plant Biologists, 2007. http://tinyurl.com/9vhxup

7 "Transplastomics: a convergence of biotechnology and evolution", WordPress.com blog, posted 16 November 2008. http://tinyurl.com/82rs2d

The main corporate players

The development of artificial minichromosomes and transformed organelles has followed the same pattern as earlier biotech developments: from publicly funded basic research to fully private application and use, with growing concentration in the hands of a few corporations. Two labs have led the way in research into artificial minichromosomes: one headed by Dr Daphne Preuss at the University of Chicago, the other headed by Dr James Birchler at the University of Missouri.

Dr Preuss, who joined the University of Chicago in 1995, worked with her team in the development of methods to build artificial chromosomes. In 2000 she founded Chromatin Inc. as a way of marketing minichromosomes. In 2004 Unilever became the first major corporation to invest in the new firm. In 2007 Chromatin granted Monsanto a non-exclusive licence for the use of minichromosomes and, just four months later, did the same with Syngenta. Both agreements include funds for research, but the amounts involved and the terms of the agreements have been kept secret. All along, Chromatin has continued to receive public funding. Chromatin lists on its web page twelve patents as its own. Six of those patents, however, were actually granted to the University of Chicago[1] and four others are shared with the University. Neither party has disclosed whether the University of Chicago has transferred its rights to Chromatin Inc.

Dr Birchler has long been a professor and researcher at the University of Missouri. His work on artificial chromosomes has been funded by the National Science Foundation, the US Department of Agriculture, and Monsanto.[2] He recently strengthened his links with Monsanto by becoming scientific adviser to Evogene, a biotech company based in Israel that specialises in computer-assisted identification of commercially promising genes. Monsanto currently owns 13.6 per cent of Evogene and will have a 20 per cent stake within 3 years.[3] Evogene will grant Monsanto exclusive licences over identified genes. Monsanto will, in turn, use the technology developed by Birchler or Preuss to engineer those genes into plant varieties.

Transformed organelles have been developed by several University labs, and the privatisation processes have been similar. One of the leading labs, headed by Dr Pal Maliga of Rutgers University, is currently funded by public sources as well as by Monsanto. Another prominent laboratory is headed by Dr Henry Daniell at the University of Central Florida. Dr Daniell has raised record amounts of public money, and the work of his lab is "protected" by over 90 patents. In 2002 Dr Daniell set up a private firm, Chlorogen, to commercialise transformed chloroplasts.[4] In 2005 Chlorogen signed a major agreement with Dow AgroSciences to produce veterinary drugs in plant cells.[5] The company closed in September 2007, selling its technology to undisclosed parties.[6]

Monsanto and Bayer seem to be the corporations to have done most to develop fully commercial applications for both technologies. Monsanto has been very active: it has co-funded, invested, reached research agreements and licensed applications from a variety of university research groups and has also carried out in-house research. It has

be induced, plant cells also increase the number of copies of their organelles. This way GE organelles have the potential to secure multiple copies of the inserted DNA and hence a very high level of expression of the engineered genes, in theory much higher than the improved level that can be reached through minichromosomes.[8]

Although efforts to transform organelles – especially chloroplasts – have been going on for the last decade, they have succeeded in only a few plant species. It is still done "the old way", inserting foreign genes in the organelle DNA, and hence it still faces many of the serious limitations of that approach.[9]

What can be done with these technologies?

The biotech industry expects to solve some of its major hurdles by using minichromosomes. First, they will be able to insert several genes in a cell and thereby expect to make complex traits a feasible target for genetic engineering (although the actual feasibility is still to be seen: complex traits are exactly that and the presence of multiple genes does not guarantee the expression of a complex trait). Minichromosomes will also make "gene stacking" possible: several of the current single genes present in GM crops could be accumulated in one variety, providing a new opportunity to reap profits out of them. "Gene stacking" is currently possible, and is being done by companies such as Monsanto and Syngenta, but the time and work it requires make it far less profitable. Second, artificial minichromosomes should make genetic engineering more efficient by decreasing the type of side-effects that make so many engineered organisms unviable. Third, they will be by-passing many genetic control mechanisms so that the engineered genes will obtain higher and more stable levels of expression.

8 Melinda Mulesky, Karen K. Oishi, David Williams, "Chloroplasts: transforming biopharmaceutical manufacturing", *Biopharm international*, 1 September 2004. http://tinyurl.com/8em3je

9 See Patent Storm, US patent 7235711, 26 June 2007. http://tinyurl.com/9de8y3

been busy signing agreements and obtaining licences from biotech firms, including Chromatin, Evogen, Asgrow and BASF. It is already testing gene stacking through minichromosomes, and it expects to release commercially what it calls its SmartStax "platform" in 2010. On its web page for investors, Monsanto has highlighted the potential use of the technology to lower environmental requirements.[7]

Bayer is focusing its action in the field through Icon Genetics Inc. Founded by two University professors in 1999, Icon Genetics focuses on producing pharmaceuticals through plants. Throughout its life, it has managed to obtain important public grants and has displayed a highly diversified portfolio of agreements with pharmaceutical companies. It was bought by Bayer in 2006. Its products are mostly based on chloroplast engineering, but the company is also working on the engineering of other organelles. It holds at least one patent over a method to produce minichromosomes. It recently opened a new factory in Germany to produce biotech drugs in tobacco plants.[8]

Syngenta has licensed minichromosome technology from Chromatin Inc., and it has already stacked tolerance to glyphosate, rootworm resistance and European corn borer resistance in maize.[9] It holds at least one patent over a method to engineer organelles. Biofuels is one of its main areas of interest. Novartis, Calgene (owned by Monsanto), Pioneer Hi-Bred, and Assgrow are also using the new technologies.

1 They are US Patents 6156953, 6900012, 6972197, 7015372, 7119250, 7132240.

2 University of Missouri College of Arts and Sciences press release, 29 September 2005. http://rcp.missouri.edu/articles/birchler_chromosome.html

3 Evogene–Investor Conference, September 2008. http://www.evogene.com/investors_presentations.asp

4 "About Dr. Henry Daniell", Daniell Lab for Molecular Biotechnology Research, University of Central Florida College of Medicine, 2008. http://daniell.ucf.edu/people/daniell

5 "Dow AgroSciences, Chlorogen to co-develop chloroplast transformation technology for plant cell culture and crop improvements", Dow AgroSciences press release, 16 September 2005. http://www.dowagro.com/newsroom/corporatenews/2005/20050916a.htm

6 "Biotech Startup Chlorogen Shuts Down, Starts Selling Off Its Technology", BioSpace, 12 September 2007. http://www.biospace.com/news_story.aspx?NewsEntityId=69496

7 See http://www.monsanto.com/pdf/investors/2008/12-09-08.pdf

8 "Pilot plant for future-oriented technology opens in Halle", Icon Genetics press release, 16 June 2008. http://www.icongenetics.com/html/5948.htm

9 See Syngenta's Research & Development front page on its website. http://www.syngenta.com/en/about_syngenta/researchanddevelopment.html

If the industry is to be believed, artificial minichromosomes will make the engineering of complex traits possible, which means that it will possible to produce almost any substance through genetic modification. What does this mean for the future of genetic engineering? The industry puts forward two versions. When it is being careful about its public image, it presents this new technique as an effective and safe technology for – yet again – saving the world from hunger and environmental problems. Daphne Preuss, a leading scientist from the University of Chicago, who is now the president of Chromatin Inc., has made presentations for the Gates Foundation and the United Nations on how this technology could herald a breakthrough for African agriculture.[10] However, when discussing the possible applications of the new technology in patent applications, the biotech industry deals with the genetic engineering of crops for food production as only a secondary target, the main goal being pharming (the production of drugs and chemicals through engineered crops). Companies want to create GE plants that will produce drugs, human and animal proteins, and biofuels, as well as specific industrial raw materials, including toxins. Other possible uses include "the production of nutraceuticals, food additives, carbohydrates, RNAs, lipids, fuels, dyes, pigments, vitamins, scents, flavours, vaccines, antibodies, hormones, and the like."[11]

The idea of using crops to produce drugs is an interesting one for industry for two reasons: crops can be employed more efficiently in this process than animals or bacteria, with a larger output achieved with fewer resources; and it is easier for the drugs produced to be delivered orally to people and animals.[12] Other types of organisms have not been discarded, however. Bacteria remain an important target, because they are easier to engineer and they can be more easily used to produce high-value molecules in small quantities; they may, however,

10 See http://tinyurl.com/7hafo7

11 WIPO Patent N°.2007/030510. http://tinyurl.com/a9crbb

12 Melinda Mulesky, Karen K. Oishi, David Williams, "Chloroplasts: transforming biopharmaceutical manufacturing", *Biopharm international*, 1 September 2004. http://tinyurl.com/8em3je

face important regulatory problems. Other species being transformed and tested as possible drug factories are insect larvae and moss.

The application of minichromosomes does not end there. As well as promising higher yields, nitrogen fixation and resistance to salt, drought, heavy metals, viruses, insects, diseases and changes in climate – or any combination thereof – companies are consistently claiming in their patent applications to have the ability to alter plant architecture and physiology, including the process of photosynthesis. In the words of WIPO patent 2007/030510, it may be possible to obtain "resistance or tolerance to drought, heat, chilling, freezing, excessive moisture, salt stress, mechanical stress, extreme acidity, alkalinity, toxins, UV light, ionising radiation or oxidative stress; increased yields, whether in quantity or quality; enhanced or altered nutrient acquisition and enhanced or altered metabolic efficiency; enhanced or altered nutritional content and makeup of plant tissues used for food, feed, fiber or processing; physical appearance; male sterility; drydown; standability; prolificacy; starch quantity and quality; oil quantity and quality; protein quality and quantity; amino acid composition; modified chemical production; altered pharmaceutical or nutraceutical properties; altered bioremediation properties; increased biomass; altered growth rate; altered fitness; altered biodegradability; altered CO_2 fixation; presence of bioindicator activity; altered digestibility by humans or animals; altered allergenicity; altered mating characteristics; altered pollen dispersal; improved environmental impact; altered nitrogen fixation capability".[13] There is, it would seem, a huge range of biologically possible alterations, and industry will establish its targets by seeing which GE modifications are most profitable.

The genetic engineering of organelles offers another set of rewards for the biotech industry, especially through the engineering of plant chloroplasts. The most important of these is much higher levels of productivity of whatever substance the engineered plant will make. If, for example, each cell holds tens of chloroplasts and each chloroplast holds over 200 copies of the foreign DNA, the potential production of the engineered substance will, in theory at least, be many times more than it is with the use of current techniques. And tests have, indeed, shown "hyperexpression" of the transgenes.

A second important promise for industry is the stable passing on to the next generation of the foreign DNA. Organelles are transferred through the so-called "maternal inheritance" as identical copies. A female animal will transfer identical copies to all its offspring and a plant to all the seeds it produces, without changes from one generation to the next. Industry claims that this will ensure the stability of the GE traits from generation to generation. They also claim that, as pollen grains and semen cells do not carry GM organelles, there is no possibility of them being accidentally transferred to other organisms. In other words, GM organelles will be a powerful biosafety tool for preventing genetic contamination, they say.[14]

An obvious powerful development would be to put these two techniques together. The different research groups that have been developing the new techniques do not seem to be talking much to each other, but some of the big biotech companies are working hard to combine the techniques and to use them together, mostly in plants. Bayer has been very active through Icon Genetics Inc. They already claim widespread success in engineering plastids, and have at least one patent related to minichromosomes. Monsanto, which was the first company to engineer chloroplasts, has funded research on minichromosomes at the University of Missouri and has signed a licence agreement with Chromatin Inc., one of the leading players in the new field, for the use of its minichromosome technology. Syngenta is also working with both technologies, although it seems less actively involved than Bayer and Monsanto.

What can be expected from all this?

Artificial minichromosomes and GE plastids are advancing fast, especially for plant species, and some of their field applications are already available. Their impact – independently or working together – may well be huge. The production of all types of molecules and chemicals is now within reach and economically promising, and for various biotech companies the opportunity is too attractive to let pass. It seems inevitable that in the not too distant future we will have multiple GE crops producing toxic substances. Due to their possible application in biofuels and industrial inputs, such toxic crops will eventually cover large areas. Because biotech companies claim that engineered organelles will contain genetic contamination, they will probably manage to introduce the new crops into the field without proper tests or regulation.

The new technologies are, however, far from safe. It may well be true that engineered plastids will not be transferred through pollen in 99 per cent of cases but, given the huge number of pollen grains

13 WIPO Patent N°.2007/030510. http://tinyurl.com/a9crbb

14 Bao-Rong Lu "Transgene escape from GM crops and potential biosafety consequences: an environmental perspective", International Centre for Genetic Engineering and Biotechnology, Collection of Biosafety Reviews, Vol. 4, 2008: 66–141. http://tinyurl.com/7nn3h7

that any plant can produce, one per cent transfer is enough to produce widespread contamination. Toxic genes will be disseminated at a lower speed than is the case with current transgenes, but they will still be disseminated.[15]

There is another route for genetic contamination by artificial chromosomes: widespread transfer through bacteria. Bacteria are readily able to acquire DNA from other bacteria[16] and to transfer it to other bacteria and micro-organisms, and to plants. The pathogen *Agrobacterium tumefaciens* is used in the genetic engineering of plants because it is particularly effective at doing this, but all bacteria have the potential to do the same. Artificial minichromosomes share important characteristics with bacterial DNA, and it is to be expected that bacteria will be able to incorporate some of their genes and transfer them to other bacteria, micro-organisms and plants. So artificial minichromosomes will create new forms of contamination, between species and, more alarmingly still, between kingdoms.

Industry acknowledges other dangers too. Icon Genetics, which is owned by Bayer, indicates in one of its patent applications that not only will the transgenes in chloroplasts lead to the production of different drugs and chemicals, but the hyperproduction of those substances can be highly toxic for the plants, to the point of endangering their development and survival. Instead of seeing this as a good reason for stopping the development of the technology, Icon Genetics is using this as a justification for developing different forms of Terminator-type technology. They are developing plants with genes that will control the expression of other genes at almost any point of development. The control can be switched on and off by externally applying substances as diverse as DNA, RNA, lactose, tetracycline, arabinose, ethanol, steroids, copper ions and so on.[17] Once this technology is accepted, nothing will stop industry from using it to produce Terminator seeds.

It must not be forgotten also that both new technologies will significantly broaden the scope of patentable "inventions". Gene patenting will be expanded to the patenting of chromosomes, organelles and entire physiological processes. Given the wide and diverse potential applications of minichromosomes and transformed plastids, patents and patent claims will multiply quickly and aggressively. The web pages for the laboratory of Dr H. Daniell at the University of Central Florida states that "Dr Daniell's chloroplast genetic engineering technology is protected by more than 90 US and international patents".[18] Industry is not lagging behind. In a list of patents published at MolecularFarming.com, two thirds of those related to pharming to have been filed or granted since 2001 are in the hands of major biotech companies.[19]

We urgently need to monitor these new developments closely and to strengthen social opposition to these and other forms of genetic engineering. Far from solving the many problems caused so far by genetic engineering, artificial chromosomes and transformed organelles create new dangers, exacerbate industrial concentration and corporate control, and open the way for serious and perhaps irreparable damage to all forms of life on our planet.

15 "Transplastomics: a convergence of biotechnology and evolution", WordPress.com blog, posted 16 November 2008. http://tinyurl.com/82rs2d; "Researchers attach genes to minichromosomes in maize", Biology News Net, 14 May 2007. http://tinyurl.com/92xlsk

16 Entry giving definition of "plasmid" at Answers.com. http://tinyurl.com/7yn9tb

17 Icon Genetics and Stefan Mühlbauer, WIPO patent application (WO/2005/054481) "Controlling gene expression in plastids", 16 June 2005. http://tinyurl.com/a5nzcc

18 "About Dr. Henry Daniell", Daniell Lab for Molecular Biotechnology Research, University of Central Florida College of Medicine, 2008. http://tinyurl.com/7mn99a

19 "Molecular farming and plant pharming/biopharming – Chloroplast transformation method and Chloroplast engineering patents", MolecularFarming.com. http://tinyurl.com/7fbobc