July 2006

The Nature of Genetic Engineering and the Uses and Potential Abuses of Biotechnology

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The Nature of Genetic Engineering and the Uses and Potential Abuses of Modern Biotechnology

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Abstract: Over the past decade the topic of genetic engineering has been has been readily debated in the media, but often these debates consist of political rhetoric and fail to offer objective information on the methods and the potential benefits to human health and their environment. In truth, humans have been manipulating the genomes of organisms for thousands of years, and it has been an evolution of scientific knowledge that has led to the more precise methods of genetic engineering. This paper discusses how scientists utilize natural processes to alter the genetic constituents of both prokaryotic and eukaryotic organisms, benefits to human health and the environment, as well as potential misuses of biotechnology such as bioterrorism.

I) Introduction

Some have argued that genetic engineering is unnatural, that humans are forcing the hand of God by tinkering with genes and making products that would have never been produced if nature had been left alone. However, humans have been using organisms to produce certain products and selectively steering evolution since the beginning of civilization. In fact, one can argue that human intervention into biological processes has allowed civilization to form in the first place. Yogurt and beer have been made for centuries using the metabolic pathways of organisms. Yet nobody has criticized Yoplait or Budweiser of playing God. Similarly, animals have been selectively bred to produce desired characteristics, such as breeding wild wolves into dogs and wild oxen into passive dairy cows, yet people still drink milk and there are no rallies against puppy dogs. Genetic engineering is merely an extension of a process that began at the advent of the agrarian society as a means of survival. Legitimate concerns can be voices against widespread use of recombinant genetic technology. Unfortunately, too often the loudest protests are merely knee-jerk diatribes of what they think (or fear) genetic engineering to be, which is certainly not a fair or credible discussion, and completely fails to acknowledge the amazing potential that this burgeoning technology can bring to mankind. This paper is an honest discussion of just how natural genetic engineering is, the technology used, the potential problems of wide scale application of the technology, as well as the solutions that scientists have devised to answer these problems.

II) Genetic Engineering: Using Nature's Tools

Although a discussion of what "natural" means falls outside the realm of molecular biology, it seems to be a necessary discussion since the supposed unnaturalness of genetic engineering is a sticking point in most people's minds. Bad Hollywood movies and large public relations campaigns have blurred what exactly modern science is trying to do. Nevertheless, a definition of "natural" is more elusive than it may seem. If a number of people on the street were asked whether natural was good, chances are all of them would say yes. Yet if they are pushed for a definition of what exactly "natural" means, they would probably struggle at first, and then after several attempts probably come up with something along the lines "as God intended," or "as nature created things." Natural is universally thought of as good, no matter how difficult its definition is to pin down. On the other hand, topics such as genetic engineering (or perhaps any other modern technology) has always been met with unease. There has always been a hasty reaction to resist scientific progress. It conjures up the archetypal image of Adam eating from the tree of knowledge, that something sinister lurks behind the thrust of progress. Perhaps the concepts of natural vs. unnatural can be more accurately distilled as rational vs. irrational. The concept of "natural" seems to be a refuge people take from the rational; an oasis to people who feel an impulse to interpret the causes of events as mysterious, divine, and unknowable.

It is extremely easy to disprove the argument that natural is good, and when humans tinker with nature, it's bad. This is a naïve judgment. In fact, it's a complete misinterpretation of how life perpetuates itself through evolution. Evolution doesn't particularly care specifically about human life, or for that matter, any form of life over

another. Organisms try to survive, and if by chance they are allowed to survive and reproduce more efficiently than other organisms, then they have a decided advantage, no matter if they are bacteria, insects, or humans.

It is one of the most cited fears of genetic engineering that an organism will acquire a resistance to an insecticide, herbicide, or even an antibiotic marker gene used to test for transformation, and this will lead to a "superbug" that is resistant to all known methods of suppression. Bacteria obtaining resistance to all prescribed forms of antibiotics and causing widespread human illness and death is a worry in many people's minds. This, of course, is possible, but antibiotic resistance is hardly a phenomenon introduced by genetic engineering. There are probably thousands of these "superbugs" in most people's back yards. A January 2006 article in Nature [1] describes an experiment where a team of microbiologists collected random samples of dirt from towns and forests around Canada, and isolated 460 different strains of *Steptomyces*, a common soil bacterium On average the strains were unaffected by seven of eight antibiotics (both natural and synthetic varieties) commonly prescribed for bacterial infections, with two strains being resistant to 15 drugs! [2]. There are superbugs all over the place!

Most of these microorganisms were found far from farms and hospitals, so there is no possibility that they evolved antibiotic resistance due to human activity. Rather, these microorganisms may simply lack transport mechanisms to take up the antibiotic, which renders them impervious to these molecules. Of course, *Streptomyces* species are known to secrete antibiotics naturally, perhaps to ward off any other bacterial species that may try to invade their area and resources, so it is not surprising that there are numerous species found that won't be affected by antibiotics. If they were affected, they wouldn't

have been permitted to survive in the first place.

The central problem with the "natural is always better" viewpoint is that it's an egocentric viewpoint, as if nature exists to serve man. Unfortunately for us, all organisms try to survive, and sometimes they survive at the expense of human life. *Yersinia pseudotuberculosis* is a bacterium which causes mild intestinal disorders to animals and humans who are infected by it. Centuries ago, long before the advent of genetic engineering, the genes encoding adhesion proteins which once allowed *Yersinia pseudotuberculosis* to adhere to the intestinal walls of the infected animal were altered [3]. This alteration, although very slight, gave this organism a decided advantage over non-mutant varieties. Of course, this minor mutation occurred merely by chance, perhaps due to UV damage affecting its genome. Now these altered bacteria not only had the ability to infect the intestines, but also move to other organ systems and cause more severe complications, causing death to 1 in 4 of every human being infected. The altered bacterium is called *Yersinia pestis*, which is the culprit that caused the plague in Europe during the Middle Ages. This is an instance of nature producing something that wasn't so good for human beings.

It may seem like getting a plant or other organisms to produce a non-native protein is a radical feat, as if humans are playing God with nature, but in fact scientists are only using the tools that organisms gave them. Humans didn't invent restriction enzymes to cut a specific position along an organism's genome. Bacteria naturally evolved restriction enzymes to cut foreign DNA from invading viruses. Of course, this ability to cut DNA would be useless without the ability to paste the genes in the desired location. This is the job for ligase, which again is naturally produced by all cells. Each

living cell contains this ligase enzyme to reconnect the sugar backbone of our DNA in the event that our DNA polymerase made a mistake during replication and had to remove a base. Once again, the tools for genetic engineering weren't invented by scientists. Scientists merely use the processes that the natural world provided them.

In fact, the natural abilities of the organisms perform all the work in genetic engineering, and the scientists merely tweak the processes. A good example is *Agrobacterium tumefaciens*, which is a soil bacterium that infects dicotyledonous plants such as grapes, peaches, and roses, eventually causing crown gall tumors [6]. It does so through the naturally occurring Ti plasmids, that allows a portion of its DNA (T-DNA) to insert into a plant's genome through illegitimate recombination. Among the essential sequences on this plasmid are left and right borders that specify where the plasmid is to be cut to yield the single stranded piece of T-DNA, as well as numerous virulence genes which encode the complex machinery that performs the conjugation process. There are also genes on the T-DNA segment that code for the synthesis of opines. These genes show how brilliant evolution is in furnishing organisms unique niches for survival. Opines are a carbon source only for *A. tumefaciens*, and can't be used by plant cells, or even other soil bacteria living in the environment. Essentially *A. tumefaciens* infects a plant with its extrachromosomal plasmid, and uses the plant as a factory to make nutrients so it can survive. This is an ingenious mechanism that is also harnessed by scientists. If bacteria can use plants for biosynthetic factories, humans should be able to as well. And in fact they do.

Perhaps the most commonly used product of genetic engineering, the Bt insecticide, is introduced into plant cells using a modification of the mechanism used by

A. tumefaciens. A variety of genes can be inserted in the T-DNA region of a Ti plasmid. This DNA can then be inserted into the plant genome, and use the plant's biosynthetic machinery to make the proteins encoded by these genes. This system came about long before genetic engineering. Slight modifications can be made by the scientist to cut out unwanted genes (such as the auxin, cytokinin, and opine genes) as well as other manipulations to get the Ti plasmid system to work in a wider range of plants than just dicots (the methodology is covered in more depth later in the paper).

However, the broader message is that exchange of genetic material is a common feature of many organisms in nature. Without it life wouldn't have evolved to what it is today. In fact, humans wouldn't have evolved at all if it weren't for some genetic cutting and pasting. Opponents of genetic engineering frequently complain that strange and unnatural genes are being inserted into our foods—the genetic purity of our natural environment is being disturbed. This is a naïve belief. Genetic purity simply doesn't exist. The notion of genetic purity is actually antithetical to how the complexities of life came about. Human beings are a prime example. DNA sequences that are viral in origin make up nearly half of our genome [7]. Viruses are the best genetic engineers that nature has, especially retroviruses, which on occasion can infect a germ cell and be passed on from generation to generation like any other gene. Although most people associate viruses with such nuisances as the common cold, or fatal diseases such as HIV and Ebola, viruses have provided a major driving force in the evolution of all life, including many beneficial traits. Gene expression, polyadenylation of mRNA, genomic recombination, and pregnancy are all influenced by these viral fragments in our genes [8]. These sequences continue to be a potent force for genetic change in human evolution. Since

retrotransposons are essentially retroviruses in our own genomes, an RNA copy can be made after the initial integration of the viral sequence, and using the reverse transcriptase encoded in the sequence, can make a complementary DNA sequence that can integrate into another place in the genome. It is estimated that there are at least 1 retrotransposition event in every 50 sperm [11], which potentially can create and introduce new regulatory regions or genes into the human gene pool.

Most of these HERV (human endogenous retrovirus) sequences are non-coding due to accumulation of mutations or deletions. However, it has been found that 18 fully coding envelope genes of retroviral origin are produced in healthy tissues, including two recent finds that some of these genes assist in cell to cell fusion during the synciotrophoblast formation during pregnancy [8]. In fact, three separate sequences that were once retroviruses supply vital components of human pregnancy and fetal development [9]. Virally derived genes control the immunosuppressant characteristics of the placenta, so the mother's immune system doesn't attack the developing fetus, as well as cell fusions so that the placenta can implant on the mother's uterine walls [8].

The LTR (long terminal repeat) of one of these segments, named ERV3, also contains several sex-hormone *cis* response elements such as a progesterone control element [8]. Since progesterone controls trophoblast proliferation, the presence of viral elements plays an essential role in early development. This is an example of a viral remnant that doesn't necessarily exist in a coding region, so it isn't a gene, but nonetheless provides a regulatory region that influences the expression of a gene. There exists a vast potential for these sequences to control gene expression and perform regulatory functions in the nearly 98.5% of the human genome that doesn't code for

proteins.

A detailed discussion of the influence of retroviruses on human evolution would take thousands of pages. The bottom line is that nature cuts, pastes, mutates, inverts, and makes numerous alterations to the genomes of every organism, and it is these fortuitous alterations that provide the driving force of evolution. What humans do in genetic engineering is mild compared to the power that microorganisms possess. Once again, humans are only using some of the vast number of tricks that organisms already possess to alter genetic information. In this respect, genetic engineering is a poor term to describe the work being done on genetic material, since it suggests that humans can alter organisms in whatever ways they desire, as if they could (or desire to) make strange and exotic creatures at will. It would surprise some opponents of genetic engineering just how limited scientists are in their actions. The introduction of genes into the correct position in another organism's genome is not a simple or precise activity, and even if the genes are integrated, gene regulation is far more complex than once expected. A gene can't simply be introduced into another organism and be expected to produce the correct product in the right quantities for a particular cellular function. Metabolic pathways are also complex, and there are specific characteristics of the host organism, such as its endogenous pool of tRNA's, that may limit the amount of a gene product that is produced.

Whatever complexities may arise in the manipulation of organisms, it is clear that the desire to alter organisms to perform a particular function in not a new one. It is borne out of the instinct for survival. This same instinct drove humans to domesticate wild animals, selectively breed wild weeds to produce nutritious crops, and to continue to

pursue scientific knowledge. We do all these things so we are less vulnerable to the seemingly random shifts in our environment that are caused by a near infinite amount of factors that are far beyond the control of humans.

In fact, in many instances humans are at a severe disadvantage in the fight for survival. Bacteria can replicate at a much faster rate. “Humans would require a millennium to produce the same number of generations that a bacterium can in a single day” [7]. This leads to much fewer humans, as well as fewer opportunities for genetic mutations that can lead to beneficial traits. Also, humans can’t transfer genes from individual to individual like bacteria can. Three known processes exist in which one bacterium can horizontally transfer its genetic information to other bacteria: transduction, conjugation, and transformation. Transduction is the movement of genetic information via bacteriophage, which infect one bacterial cell, replicate their genome, then somewhat imprecisely excise themselves, sometimes taking a chunk of the host cell’s genome with them. Then following the bursting of the host cell, the bacteriophage can infect another bacterial cell, now carrying a portion of the previous cell’s genome with it. The second method is conjugation, which involves cell to cell contact and the transfer of a plasmid among two bacterial cells. The third process is transformation. This involves the uptake and incorporation of naked DNA between bacterial cells.

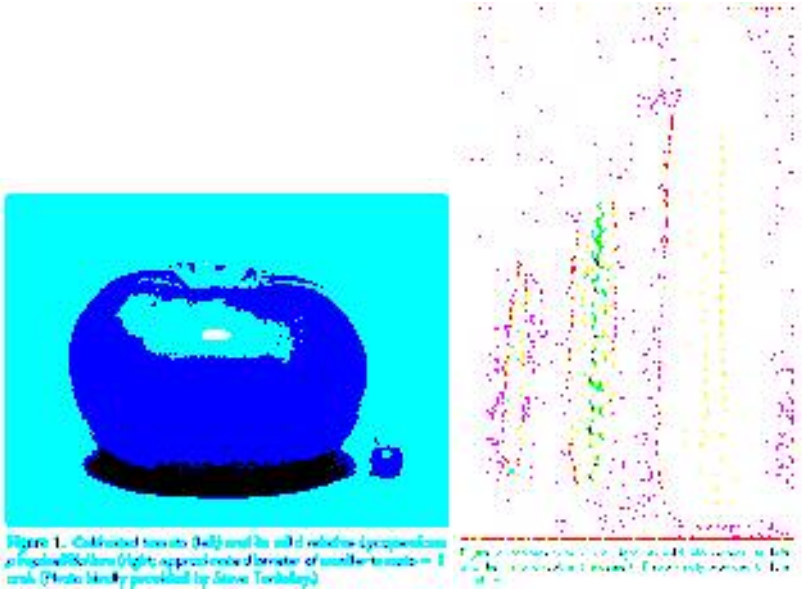
Obviously, humans can’t freely swap genes from one person to another. This, added to the fact that our generation time is much longer than the generation time of microorganisms, makes us decidedly less fit for survival. However, perhaps the most important quality that gives humans an advantage over all other organisms is our intellect. In this respect, intelligence is an advantage that evolution has afforded us, so

any activity of that intellect can be seen as an extension of evolution. And since genetic engineering is a product of our intellect, and intellect is a means of survival for us just like swapping DNA is a means of survival for bacteria, it doesn't make sense to forfeit our selective advantage and not use our intelligence to alter the biological world in our favor to give ourselves any advantage we can get. Again, because humans are at a disadvantage in so many aspects of life, not doing everything we can to survive is antithetical to evolution. Pathogenic bacteria aren't going to forfeit the ability to infect us and our food sources, so we shouldn't forfeit the ability to increase crop yields, protect our food sources, make cheap and reliable vaccines, and the numerous other benefits that genetic engineering can provide us.

Besides, the manipulation of the genetic material of organisms is not a new activity for man. Ever since the start of primitive societies, humans began to tinker with the DNA of different organisms, all toward the accomplishment of a particular aim. The cows grazing in the pastures of today's farms were once wild oxen, but through selective breeding were "engineered" to be large, lazy and perfect creatures for the production of milk and meat. Wild boars were engineered into pigs, wolves were engineered into dogs, and all the animals that serve some function in modern societies were once wild animals that were selectively bred to serve our needs. Now the definition of genetic engineering—the tinkering with an organism's genes to produce a desired outcome—seems to have been practiced for some 10,000 years. The desire was the same then as it is now. The only difference is that our tools for executing that desire have improved tremendously.

This is also a point where the argument that genetic engineering is "unnatural" breaks down. One cannot simply say that natural is the way the world would be if man

hadn't tinkered with it, or look back to previous generations and claim those societies lived more naturally, since the scaffolding of even the most ancient societies was based on the manipulation of the genes of the organisms around them. All the major crops around the world were once weeds, incapable of feeding more than a small group of people at a time. "Most people who are concerned about modern biotechnology have little or no knowledge of the processes that were used to transform crops in the past. Nor are they likely aware that crops have been continually altered over time or that, without human care, they would cease to exist" [4]. If plants weren't crossed, recrossed with wild, related species and selectively bred over a period of thousands of years, potatoes and apples would be smaller than the size of a marble! "Today's wheat, even before the advent of genetic engineering, differs from its ancestors in so many ways that it is classified as a different species" [3].



Figures from Prakash, Channapatna. (2001) "The Genetically Modified Crop Debate in the Context of Agricultural Evolution. Plant Physiology. 126, 8-15.

Figures 1 and 2 shows a native corn and tomato before and after humans began to

selectively breed these species. These phenotypic traits are the result of humans purposefully altering the genotypes of these plants. Although humans in ancient societies had no idea that DNA was the source of the phenotypic changes they sought, they deliberately guided evolution to produce crops that could sustain large groups of people in a stable residence, thus making them less vulnerable to the myriad of factors that could compromise their survival. It was crude genetic engineering. But without it we would still be primitive creatures, wandering from area to area wherever naturally occurring foods are plentiful, leaving when the source has been diminished, and perpetually vulnerable to every frost, flood, drought, infestation—every sudden change in environment that's an inevitable part of a large, complex ecosystem. Without man's manipulation of the genetic world around him, a historical consciousness wouldn't have even developed to a point of questioning what is natural and what is not.

Hundreds of pages can be written to show how natural modern genetic engineering really is. However, the undisputable difference between the processes that occur in the laboratory and those that occur in the wild can be succinctly listed on a single page. First of all, evolution in the wild takes a much longer time to alter the genes of a species. This is something of an understatement. Evolution may take hundreds of thousands to millions of years to alter a species, whereas in a laboratory this can take only weeks. Modern genetic engineering can be described as speedy, controlled evolution. In fact, it's more accurate, controlled evolution compared to traditional breeding. If a farmer tries to get a particular trait expressed in one of his plants, he must cross two of the plants with the desired traits, wait, cross again, wait, and repeat this procedure many times until the desired trait is expressed in all of the generations, if the

traits can be expressed at all. This traditional breeding method is not specific, introducing perhaps thousands of genes at a time. A farmer using the conventional methods may achieve resistance to a disease, for instance, but also introduce a variety of unwanted genes that may be potential allergens or toxins. On the other hand, genetic engineering allows a specificity that can't be matched by traditional methods. A single known gene can be inserted into another organism's genome and be controlled by a promoter that allows the gene to be activated when desired. Genetic engineering is as natural as traditional breeding methods, but more efficient, more accurate, and safer. Modern scientists use nature in a more intelligent way than past generations.

Secondly, for the most part there is a species barrier in the movement of genetic information in the wild, with the rare exception of gene movement through the infection by viruses. However, in the laboratory the species barrier has been knocked down. Now animal genes can be expressed in bacteria and plants (and vice versa), and a gene from one species of animal can be expressed in another species of animal. Of course, this manipulation of genes in the laboratory requires an *in vitro* step, followed by the target organism carrying out the brunt of the work *in vivo*, whereas evolution carries out its business completely outside of the test tube.

II) Human Health and Genetic Engineering

A) Addressing Fears about Biotechnology and Human Health

Another common protest against genetically altering crops is that it will produce synthetic, unsafe products that will cause damage to human health in the long term. People have even called genetically modified food crops "Frankenfood," as if scientists are huddled away in dank basements concocting strange substances that will have horrible and unforeseen consequences for humankind.

Once again, those who protest against genetic engineering generally don't understand the principles by which transgenic foods are made. First of all, just because something is produced through natural breeding methods doesn't mean it is necessarily healthy. Secondly, nature has produced a huge variety of plants that just so happen to be hazardous to human health. "Our daily food naturally contains thousands of chemicals, and many of them are shown to be carcinogens or hazardous in lab animal studies with huge doses. We consume roughly 5,000 to 10,000 natural toxins daily, as plants have evolved to produce an array of chemicals to protect themselves against pests, disease, and herbivores" [4].

Genetic engineering has an enormous potential to enhance the nutritional contents of crops and other organisms. By altering one or a few select genes, an organism that once provided little or no nutrients can be stimulated to produce a valuable product that can serve to fulfill nutritional needs for humans. Of course this is not a new activity. Thirty years ago, traditional breeding was used to select rapeseed plants which didn't produce the nutritionally undesirable erucic acid, but instead produced canola oil, which has much greater health benefits to humans [5]. Now canola oil is so commonly used that

it supplies a large proportion of fatty acids consumed in most developed nations. Flax seeds were similarly bred to produce edible high linolenic acids similar to those produced in corn oil, instead of high linolenic oils used for industrial purposes [5].

As impressive as this was, genetic engineering can make even greater advances in nutritional value than traditional breeding. It does not require a large overhaul of the organism's genome to get it to produce a desired product. Specific organisms are selected that naturally synthesizes an intermediate or possess certain biosynthetic pathways, so only slight modifications are needed to produce a new product. It should console the opponents of genetic engineering to know that the alterations scientists make to organisms are slight, and it is the natural ability of the organism that provides most of the work. Transgenic plants aren't Frankensteinish creations, but ordinary plants that differ by as little as a single gene. And while these alterations pose virtually no risk to human health, the potential benefits are amazing.

Take for example "Golden Rice." Rice is a staple in the diets of billions of people around the world, especially those in developing countries. Unfortunately the oil-rich aleurone layer of rice is removed to allow it to be stored for longer periods of time without going rancid, leaving behind the starchy endosperm containing few essential vitamins [12]. By adding two genes coding for enzymes in the β-carotene biosynthetic pathway, an essential nutrient for humans can be produced by the rice endosperm that would have never been able to be achieved through traditional breeding.

While vitamin deficiencies have been eradicated decades ago in America and other first-world countries, there continues to be millions of people around the world that can't obtain nutrients in order to achieve basic health. For instance, inadequate supply of

vitamin A is a serious public health concern in at least 26 countries, including highly populated countries in Asia, Africa, and Latin America [12]. More than 250,000 children go blind every year in Southeast Asia due to retinol deficiency [3]. This is an instance where poor countries not only lack the luxury of modern medicines and technologies, but lack the essential factors to survive. More than 125 million people in the world experience vitamin A deficiency [3]. Yet it only takes the addition of two genes to potentially protect millions of people from the crippling effects of vitamin A deficiency.

Rice is a perfect target for this engineering for a couple of reasons. First of all, rice is plentiful and relatively cheap to grow in these poor countries. Secondly, the rice endosperm synthesizes an early intermediate in the beta-carotene biosynthetic pathway, called geranyl diphosphate, that can be acted on by the enzyme phytoene synthase to produce carotene phytoene, which is the next substrate in this pathway [12]. The product is a provitamin form of vitamin A, which is cleaved in the body to produce retinal, the usable form of vitamin A.

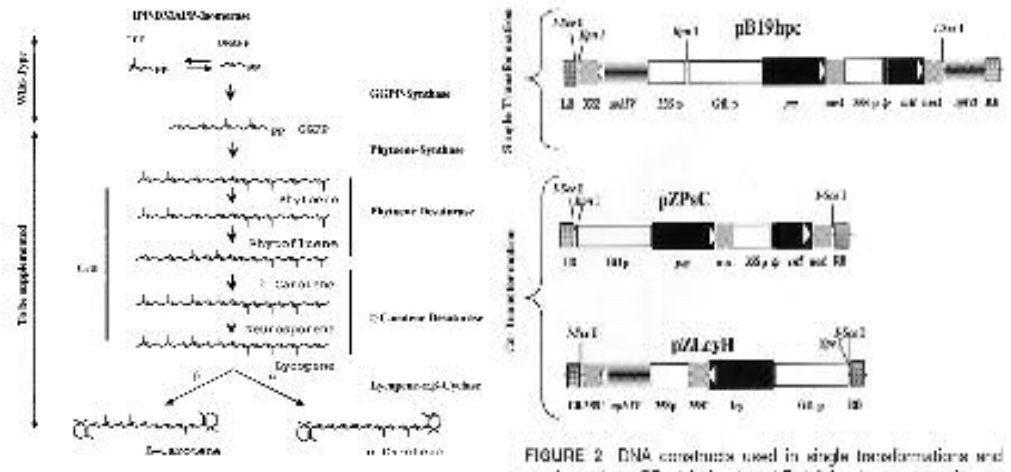


FIGURE 1 Provitamin A biosynthetic pathway. The names of enzymes are given. Crt1 denotes a bacterial carotene desaturase capable in performing all necessary desaturation reactions for which two enzymes are required in plants. Arrows indicate the prenyllipid biosynthetic capacity of wild-type rice endosperm and the necessary reaction sequence to be completed to yield provitamin A.

FIGURE 2 DNA constructs used in single transformations and cotransformations. RB, right borders; LB, left borders; tp, transit peptide from pea ribulose bisphosphate carboxylase; t, termination; p, promoter; gt, gutelin; psy, phytoene synthase; crtI, bacterial carotene desaturase; lcy, lycopene ß-cyclase. DNA sequence coding for carotenoid biosynthetic enzymes are given in black.

From Beyer, Peter et al. “Golden Rice: Introduing the β-Carotene Biosynthesis Pathway into Rice Endosperm by Genetic Engineering to Defeat Vitamin A Deficiency.” J. Nutr. 132:506S-510S (2002).

It's an extremely simple answer to a major problem. However, the "Golden Rice" experiment came under harsh criticism because it yielded lower amounts of β-carotene than expected. Some critics even went as far as to claim that the scientists performing the experiment committed fraud. It's unfortunate that people tend to cast off an experiment as a failure simply because a single attempt failed to produce miraculous results. The demand for immediate and ideal results is hardly ever satisfied in science, since any kind of groundbreaking work is a process, one which takes the cooperative efforts of groups of scientists and constantly evolving protocols to achieve the desired results. The "Golden Rice" experiment was a success, although it may not have produced the amounts of β-carotene that some people expected. However, just a few years later the yield of β-carotene was greatly increased by a simple adjustment.

It was discovered a few years later that the step limiting the accumulation of β-carotene in the rice endosperm was the phytoene synthase originating from *Narcissus pseudonarcissus* [13]. Instead, the phytoene synthase from maize was found to be more effective. When added to the carotene desaturase from the original golden rice experiment, β-carotene accumulation increased up to 23 fold! A maximum yield of 1.6 mg/g of carotenoids was achieved in the original experiment, compared to 37 mg/g by using the maize phytoene synthase [13]. Based on the current RDA, 50% of a child's daily requirements for vitamin A could be satisfied by the β-carotene in 72 grams of the golden rice made in this experiment [13].

It's clear that genetic engineering is far superior to traditional breeding in enhancing and adding nutrient content to foods. Genetic engineering can even make foods that were once unhealthy a little better for us. Researchers have actually found a

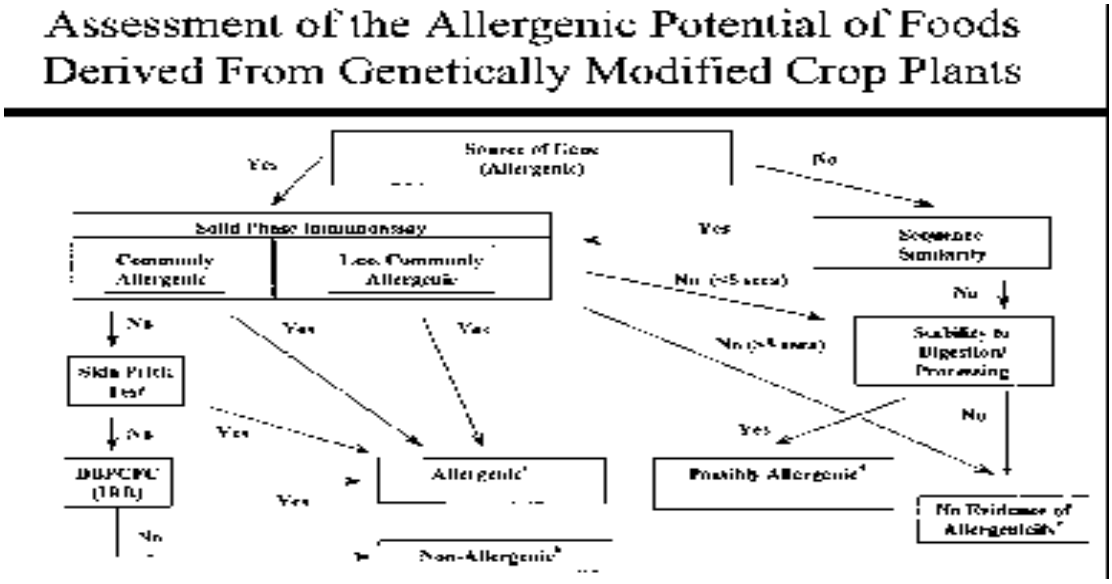
way of making bacon healthy [14]. Nuclear transfer cloning was used to insert the fat-1 gene into pig primary fetal fibroblasts, allowing pigs to convert the abundant omega-6 fatty acids into omega-3 fatty acids, which are the unsaturated fatty acids that have been shown to improve cardiovascular health. This breakthrough will allow scientists to further study the health benefits of omega-3 fatty acids, as well as giving people an alternative source for these healthy fatty acids besides fish (which contains mercury as well). Once again this engineering was only a slight alteration, adding a single gene—the fatty acid desaturase gene—which pigs naturally lack.

If genetic engineering can help prevent blindness and even make eating bacon healthier, who in their right mind would protest against it? If people objectively examine the facts they would come to the conclusion that the potential benefits far outweigh the potential risks. Unfortunately, every time a new technology or a new paradigm about doing things is introduced, there is always the initial apprehension, even sometimes outright revolt. However, if the changes don't lead to harm, or if the changes are so badly needed that it becomes a necessity, then acceptance follows and the changes become the norm. People in the United States have been eating genetically modified foods for several years now, yet there hasn't been a mass outbreak of allergic reactions or other health problems. If you've consumed breakfast cereals, junk foods, bread, etc in America in the past five years, then chances are that you've consumed genetically modified food. Genetically modified ingredients are present in 60 to 70% of supermarket products in the United States [15]. In 2003, 81% of the soybean crops in America were genetically modified, and many soybean based ingredients such as oil, flour, lecithin, and protein extracts are added to most processed foods [15].

While only 40% of corn crops in America were genetically modified in 2003, genetically modified and non-genetically modified corn are not separated by growers and processors, so if someone has consumed a corn-based product, then they have undoubtedly consumed a genetically modified product [15]. Yet there hasn't been a mass rush of people to emergency rooms with allergies against new protein in foods. In fact, it can be said that a massive practical experiment has been performed on the American people over the past several years. Millions of people have consumed the thousands of products on supermarket shelves which contain at least some genetically modified ingredients in them, and there have been no evident health effects. Most people didn't even realize they were consuming foods that were genetically modified.

Clearly these foods are safe. But this is not a coincidence. Genetically modified foods are the most stringently tested foods on the market. The figure below is an example of all the tests performed on a new food before it enters the marketplace.

Fig 3



From World Health Organization. "Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology." (2000)

Any transgenic crop engineered with pesticidal traits is closely examined by three different government agencies: the Environmental Protection Agency (EPA), the US Department of Agriculture (USDA) and the Food and Drug Administration (FDA) [17]. All three organizations closely scrutinize new products to make sure that they are safe for the environment and human health.

The FDA requires that each company proposing a new transgenic product for public consumption do their own stringent independent testing to determine any potential allergic reactions before these products are even considered for sale. Normally *in vitro* digestion assays are performed (to see if the product will remain in the digestive system), amino acid sequence comparisons to known allergens are made, and acute oral toxicity tests are done with large doses of the purified transgenic protein in question [17]. The last test involves the ingestion of the purified protein in mice at 3,280 to 5,000 mg per kg of body weight [17]. It should be noted that only the protein that is added by the new gene is administered in these trials, and not the complete product, in which only a small fraction of the total protein is transgenic. Of course, these tests administer far more of the added protein than could even be consumed in the normal diet. Government agencies like the FDA err on the side of caution, so that anything short of falling into a vat of purified protein and having to eat your way out will be safe.

There have been examples where the FDA stopped a product from being produced because it was suspected of containing a known allergen. The structure of any novel protein that may appear in food is compared to the structure of common allergens, and anything showing amino acid sequence similarities to known allergens is extensively

scrutinized. When a genetically modified soybean was suspected of containing an allergen, it was never allowed to see the grocery store shelf. Soybeans offer many nutritional benefits, but their nutritional value is somewhat compromised by the fact that they lack substantial levels of methionine.

Traditional breeding methods can be used to increase the total amount of protein in the soybean, and site directed mutagenesis in conjunction with traditional breeding may be able to increase the production of sulfur-containing amino acids, but the most realistic and efficient way to get a soybean to produce methionine is to genetically modify it [18]. This is exactly what was done. The methionine-rich 2S albumin gene from the Brazil-nut was introduced into the genome of soybean [19]. When subjects with Brazil-nut allergies became allergic to the transgenic soybean but not the regular soybean, it was a clear indication that the major allergen in the Brazil-nut was this particular 2S albumin. Consequently, this transgenic soybean was never sold to the general public, even though the modified soybean would have been harmless to the majority of people who don't experience Brazil-nut allergies.

The Starlink corn incident is another example of the government's willingness to protect people from any potential problem related to transgenic organisms. Starlink corn was genetically modified with the Bt toxin Cry9C, which is produced by a different subspecies of *Bacillus thuringiensis* that produces the more popularly used insecticide Cry1Ac. The bacterial protein Cry1Ac and its closely related derivative had been used as a topical insecticide for over 30 years for both traditional and organic crops, and in the past decade the gene coding for it has been inserted in a growing number of crops such as corn and potatoes. However, with such widespread use, it was feared that insects would

acquire a resistance to the toxin through a mutation of the toxin binding site in the insect midgut. This was when Cry9C was introduced into corn, which binds to a different binding spot than Cry1Ac [20], thus providing an additional line of defense in case of insect resistance.

Initially the corn carrying the Cry9C protein— Starlink corn—was approved by the USDA only for animal feed. Since Cry9c broke down more slowly than Cry1Ac, it was feared that it would remain in the digestive tract of humans too long, therefore increasing its risk of allergenicity. However, somehow this corn entered the general corn grain supply of the United States. An activist group— the Genetic Engineering Food Alert— did independent research and found trace amounts of the Cry9C protein in several of the products it tested [21]. Although this protein had never been shown to cause harm to humans, a large-scale response was launched that sampled over 110,000 different sources of grain [22]. A year later it was determined that consuming the corn containing Cry9C had a low chance of affecting consumers, due partly to the fact that it breaks down readily during processing [21]. However, it should be comforting to some opponents of genetic engineering that there are agencies that are willing to take swift and decisive action at the threat of harm.

Even if people are skeptical about the government's efficacy about regulative transgenic products, the fact still remains that traditional breeding methods introduce allergens into food as well. If a farmer tries to selectively breed a new trait into any crop, even if he is successful in adding that particular gene, undoubtedly a number of unintended genes come along with it. Breeding two organisms together can never simply transfer a single gene. Therefore, the chance that a new fruit or vegetable from a

nontransgenic crop will carry a protein that elicits an allergic reaction is much higher than a genetically engineered product. With genetic engineering a single gene can be added. And the product of that gene is probably well known before it is ever used to transform a plant, so necessary and specific testing can be done to determine possible allergenicity.

Of course, the shelves of American grocery stores are lined with products that elicit an allergic response in small segments of the population. A normal diet will introduce many thousands of proteins, and only a few of these have been shown to sensitize a person to an allergy [24]. If 2% of the people in America happen to have a peanut allergy, does that mean peanut butter, mixed nuts, peanut butter cups, and the remainder of products that contain peanuts should be pulled off the shelves? Of course not. Transgenic foods are so stringently tested that there is virtually no chance that a product will be introduced into the market that causes nearly the rate of allergies as do peanuts.

Another common fear about genetically modified foods concerns the markers used to test for transformation. When the public hears about the use of antibiotic resistance genes, there is instantly a panic that somehow our last line of defense against bacterial infections will be destroyed if these markers were ever to be picked up by pathogenic bacteria. The mucosal surfaces of our bodies are homes to billions of bacteria, most of which provide valuable services such as consuming nutrients so pathogenic bacteria can’t grow. However, some of this normal microflora can become pathogenic if for some reason its population suddenly increases. There are also pathogenic bacteria mixed in this normal flora that will cause infections in instances such as a compromised immune system. The fear is that if an antibiotic resistance gene is left in the final product

being consumed, that it could spread to pathogenic bacteria living along our digestive tracts and lead to serious infections that are difficult to treat.

This isn't a very realistic concern. First of all, antibiotic resistance markers can be removed from transgenic plants, so the marker may never reach the human digestive tract. Removal of markers will be discussed later in this paper. Also, commonly used markers, such as for antibiotic resistance, will not pose a health risk if ingested by people. DNA is genetic material of all organisms more complex than viruses, and any plant or animal meat we eat will contain DNA. We can consume up to 1.0 grams of RNA and DNA per day, which is a huge amount of genetic information [5]. However, if the marker remains in the final product, the overall percentage of DNA from the novel genes is infinitesimally small. Marker DNA will account for less than 1/250,000 of the total DNA consumed [5]. Besides, the genes would have to survive the nucleases of the digestive tract, and then bacterial cells in the digestive tract would have to be competent for DNA uptake.

Even if the antibiotic resistance marker remained in the eaten product, if the DNases fail to break it down, and if conditions happen to favor uptake of exogenous DNA (these are a lot of ifs), markers that are most commonly used pose no threats to human health. One of the most commonly used markers to test for transformation is npt II, which confers resistance to the antibiotics kanamycin and neomycin [25]. This gene is commonly present in many bacteria in the gut, so humans already have a high exposure to the product of this gene. In fact, nptII was initially taken from microbes in the human gut [25]. For this reason, kanamycin and neomycin are not prescribed as antibiotics. Commonly prescribed antibiotics would never be used to test for transformation in plants

that were targeted for human consumption.

It's clear that genetically modified organisms pose little threat to human health in America, since there exists an elaborate infrastructure for testing any product to be approved for human consumption. Transgenic products are even more stringently tested than conventional foods, so they are not merely as safe to eat, but even safer in many respects than unmodified foods. Also, the antibiotic resistance markers used to test for transformation can be removed before the product reaches the grocery store, but even if left in, poses an insignificant threat to human health. Beside, there are other markers besides antibiotic resistance genes that can be used to test for transformation.

B) Improvements to Human Health: Genetic Engineering and Medicine

The potential benefits of genetic engineering are immense. Not only can it serve to raise the quality of life in wealthy industrial countries, it can also provide nutrients and medicines cheaply so that life may be possible in poorer countries that can't afford the rising costs of western medicine. The biosynthetic capabilities of plants and animals can be harnessed to produce medicines and vaccines that are normally produced in pharmaceutical laboratories. Using recombinant DNA technology to make medical products is also cheaper in many respects, since larges amounts of proteins can be synthesized at a relatively low cost, and storage expenses such as refrigeration can be dramatically decreased. This is a particular advantage in poor countries in hot, humid climates.

Plants can be used as factories to produce complex molecules in the correct conformation for pharmaceutical activity. Since plants are eukaryotic cells, they contain the endoplasmic reticulum and Golgi apparatus necessary for posttranslational

modifications. Also, some plants have chaperones that are homologous to those in mammalian cells, which control the efficiency of protein assembly and the extent of protein degradation [26]. Plants are even able to make molecules with several subunits such as antibodies. Bacteria are somewhat limited in their ability to make complex proteins for human use, since they lack the ability to perform many posttranslational modifications necessary for the proteins to obtain their correct conformation.

Fig. 4

Table 1 | Plant-derived pharmaceutical proteins that are closest to commercialization for the treatment of human diseases

Product	Class	Indication	Company/Organization	Crop	Status
Various single-chain Fv antibody fragments	Antibody	Non-Hodgkin's lymphoma	Large Scale Biology Corp	Viral vectors in tobacco	Phase I
CaroRx	Antibody	Dental caries	Planet Biotechnology Inc.	Transgenic tobacco	Phase II
E. coli heat-labile toxin	Vaccine	Diarrhoea	Prodigene Inc.	Transgenic maize	Phase I
			Arouzen group (Tacket *et al*, 1998)	Transgenic potato	Phase I
Gastric lipase	Therapeutic enzyme	Cystic fibrosis, pancreatitis	Meristem Therapeutics	Transgenic maize	Phase II
Hepatitis B virus surface antigen	Vaccine	Hepatitis B	Arouzen group (Richter *et al*, 2000)	Transgenic potato	Phase I
			Thomas Jefferson University/ Polish Academy of Sciences	Transgenic lettuce	Phase I
Human intrinsic factor	Dietary	Vitamin B12 deficiency	Cobento Biotech AS	Transgenic *Arabidopsis*	Phase II
Lactoferrin	Dietary	Gastrointestinal infections	Meristem Therapeutics	Transgenic maize	Phase I
Norwalk virus capsid protein	Vaccine	Norwalk virus infection	Arouzen group (Tacket *et al*, 2000)	Transgenic potato	Phase I
Rabies glycoprotein	Vaccine	Rabies	Yusibov *et al* (2002)	Viral vectors in spinach	Phase I

From Ma, Julian K.C et al. "Molecular farming for new drugs and vaccines: Current perspectives on the production of pharmaceuticals in transgenic plants." European Molecular Biology Organization. 6:7. 593-599. (2005).

The use of genetic engineering to make products to promote human health and prevent disease is more widespread than people recognize. There are over 2500 biotech companies worldwide, with over 1300 in the US alone [6]. The result of this intensive research and the billions of dollars invested is a myriad of products that people take every day to prevent disease and serve to improve health. For instance, monoclonal antibodies can be produced by genetically modified organisms that can aid in the prevention of transplant rejection as well as serve as research tools. Recombinant clotting factors are produced in mass quantities to prevent hemophilia. The list goes on and on.

In most cases the product of the transgenic organism, whether it be a plant, yeast, or animal, produces a pharmaceutical product that is far superior to and causes far fewer complications than the product obtained before modern genetic engineering. Bovine insulin was once given to people with type II diabetes mellitus. However, the bovine insulin caused allergic reactions in people due to the fact that bovine insulin differs from human insulin by three amino acids [3]. Thanks to genetic engineering, scientists were able to take pig insulin (which differs from human insulin by only one amino acid: it has an alanine at position 30 instead of threonine [3]) and engineer it so that it is identical to human insulin. Now potentially deadly allergic reactions are not a concern, and everyone with type II diabetes can be treated with this recombinant product. Another example is growth hormone, which used to be extracted from human corpses! It once took the pituitary glands of 650 corpses to produce merely two to three grams of growth hormone [3]. However, thanks to genetic engineering, microorganisms produce all the growth hormone needed.

Perhaps the most intriguing possibility for genetic engineering is the use of a cheap, stable, edible vaccine against some of the common diseases of our day. Plants are suitable biosynthetic factories for the production of complex proteins in high amounts. And plants are biosynthetic factories that can be eaten! As pointed out in ref. [27], many "experiments demonstrated that plants can express, fold, assemble, and process foreign antigens and can provide both a simple vaccine-manufacturing process as well as a matrix for suitable oral immunization."

Using plants as for the production of medicine is advantageous in affluent countries like America, which could significantly decrease the costs of production. This in turn can be passed along to the consumer as cheaper prices for drugs. However, the countries that stand to benefit the most from having plants produce vaccines are the poorer countries. Diseases that were eradicated decades ago in America, like polio, continue to plague poor countries for a variety of reasons (including political and social instabilities). One of the reasons is that only major pharmaceutical companies in affluent countries possess the resources to mass-produce medications and vaccines. However, if something as simple as a gene inserted into a common plant could produce the same bioactive compounds created in American labs, then genetic engineering may potentially be a relatively inexpensive way of vaccinating billions who otherwise wouldn't have the opportunity.

One particular disease that can successfully be vaccinated against with an edible subunit vaccine is hepatitis B. As of 1996 the hepatitis B virus had infected 115 million people worldwide, particularly in poor countries that can't afford the current licenses for

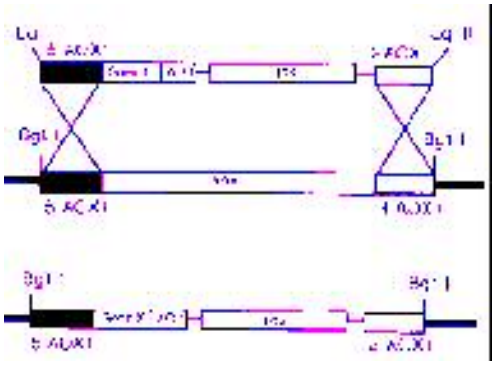
the vaccines, or means of mass production and storage. In these countries hepatitis B is endemic [27].

A means of rapidly and cheaply vaccinating large numbers of people is to get a common edible plant, such as a potato, to express the surface antigen of the pathogen. This way the immune system will recognize the foreign proteins of the subunit vaccine, make antibodies and memory B lymphocytes in response, and leave the person with a lasting immunity against a future infection of the actual pathogen. The most advantageous aspect to having plants produce subunit vaccines is that no injections are needed. The fruit or vegetable is consumed, and the antigens are processed like any other protein eaten, ultimately getting absorbed through the mucosal layers of the small intestine [27]. Amazingly these antigens survive the journey through the acidic environment of the stomach and retain their necessary conformation. In 1996 121 million Indian children were vaccinated against polio in just two days using oral vaccines [27].

Plants are not the only organisms that can produce complex, correctly folded proteins for human use. Yeast cells also have amazing biosynthetic abilities. They also possess the advantage of proliferating faster than plant cells, so large amounts of protein can be produced relatively quickly. The disadvantage is that if the protein isn't secreted from the yeast cell, then it will have to be purified from the rest of the constituents of the cell. The yeast *Pichia pastoris* can be used to make vaccines. *P. pastoris* is an excellent expression system since it has an alcohol oxidase gene that is tightly controlled by its promoter [28]. In the presence of methanol, the alcohol oxidase promoter is activated and the gene downstream of it is transcribed.

Mutant strains of *P. pastoris* that can't grow on minimal media can be used to test for transformation. The initial transfer vector requires the transgene of interest, the gene that codes for the biosynthetic enzyme lacking in the mutant strain (such as a histidinol dehydrogenase gene if the strain is unable to synthesize histidine), and regions that share sequence similarity with the region of insertion of the *P. pastoris* genome. A single recombination event takes place where this cassette gets inserted, and the gene coding for alcohol oxidase is removed. Only those *P. pastoris* cells that have been transformed will grow on minimal media, and the product of the inserted gene, such as a subunit vaccine for hepatitis B, will be produced with the addition of methanol.

Fig. 5: Production of Vaccine in Yeast



From Dominguez, Angel et al. "Non-conventional yeasts as hosts for heterologous protein production." International Microbiology. 1:131-142. (1998).

Bacteria, yeasts, and plant cells can be used to make a variety of products that have nutritional and therapeutic compounds for humans. Even animals can be used to make proteins that are correctly folded and modified for human use. One example is the product for the gene alpha-1-antitrypsin, which is lacking in people who have a heritable form of emphysema. To make this protein a vector is constructed which contains the alpha-1-antitrypsin gene next to the gene that controls milk production [29]. This vector

is then microinjected into fertilized sheep eggs, and then the eggs were placed back in the mother sheep to develop like any other sheep [29]. In fact, the sheep will look and act like any other sheep, only that it produces alpha-1-antitrypsin in its milk. These sheep are absolutely normal, and are not harmed in any way by making this protein.

Allowing genetically modified organisms to produce pharmaceutical products is an efficient and safe way to keep the ever increasing human population healthy. Many therapeutic compounds are produced in this way already. In a world where non-renewable resources such as fossil fuels are being consumed rapidly, it is nice to see a technology that takes advantage of renewable sources to benefit the population. Once again this production of pharmaceutical proteins is completely natural by nearly every definition of the word, as we are merely using the biosynthetic capabilities of the organisms around us to make a desired product.

IV) Genetic Engineering and the Environment

A) Environmental Benefits of Genetic Engineering

Genetic engineering can be used not only to enhance and sustain the health of humans, but also enhance and sustain the health of our environment. By lowering the amounts of herbicides and pesticides, by making crops less vulnerable to environmental stresses, and by increasing the yields that a typical crop will produce, genetic engineering has the potential to help every imaginable facet of agriculture. Since the advent of the agrarian society, farmers have been seeking ways to protect their crops from pests, droughts, floods—anything that could compromise their crops. Maintaining crops wasn't a luxury; it was necessary to survive. And society hasn't changed all that much. Agriculture is still the largest industry in most countries. With populations increasing at an exponential rate, and with it the demand for food, it is necessary for agriculture to be as efficient, productive, and environmentally friendly as possible.

Farmer have been fighting the battle against microorganisms and other pests since the beginning of agriculture. The first recorded instance of pest control was 4000 years ago, when Chinese farmers used ants to destroy leaf-eating insects [3]. Since then pesticides have increased in their ability to kill insects, but also in their toxicities. A good example is DDT, which targeted insects that were reducing annual crop yields. And it did kill many species of insect pests. Unfortunately, it also killed many non-target insects, even those that ate common pests of the crops, along with many species of birds like the bald eagle which was almost driven to extinction by widespread use to DDT. Of course, DDT is now illegal to use, but there are still toxic chemicals that are being used by the ton that are seeping into water supplies, contaminating the soil, and having detrimental

health effects on any living creature in the areas where they are being used. Clearly the indiscriminate dumping of harsh chemicals by methods such as crop-dusting is not the most efficient or safe way to protect crops.

The environmental toll of using pesticides is only the beginning of the problem. The human toll is also immense. “Malignancies linked to pesticides in case reports or case-control studies include leukemia, neuroblastoma, Wilms' tumor, soft-tissue sarcoma, Ewing's sarcoma, non-Hodgkin's lymphoma, and cancers of the brain, colorectal, and testes” [30]. Organophosphates, which are a type of chemical insecticide currently in use, were initially developed as chemical warfare agents that inhibit the enzyme acetylcholinesterase and disrupt the function of cholinergic neurons [6]. This should alarm some people, since our neuromuscular junctions are controlled by acetylcholine.

On the next page is a list of 46 chemicals typically used as pesticides which have been shown to have carcinogenic effects by the International Agency for Research on Cancer. Eight pesticides with sufficient evidence of carcinogenicity are still in use today, with 15 more being strongly suspected as carcinogens [30]. It should be noted that other countries may not adhere to American agricultural laws and may use compounds outlawed here long ago. More alarmingly, the United States imports a large amount of food from other countries, and we may be consuming large amounts of toxins that continue to be used in other countries.

Table 1. Pesticides with limited or sufficient evidence of carcinogenicity in animals [International Agency for Research on Cancer(*2*)].

Pesticide	Animal evidence of carcinogenicity	Currently registered in the United States[a]
Herbicides		
Amitrole	Sufficient	Y
Atrazine	Limited	Y
Diallate	Limited	Y
Monuron	Limited	Y
Nitrofen	Sufficient	N
Picloram	Limited	Y
Sulfallate	Sufficient	N
Trifluralin	Limited	Y
Insecticides		
Aldrin	Limited	N
Aramite	Sufficient	N
Arsenic and arsenical compounds	Limited	N[b]
Chlordane/heptachlor	Sufficient	N
Chlordecone	Sufficient	N
Chlorobenzilate	Limited	N
DDT	Sufficient	N
Dichlorvos	Sufficient	Y
Dicofol	Limited	Y
Dieldrin	Limited	N
HCH, α-HCH	Sufficient	N
β-HCH, γ-HCH (lindane)	Limited	Y
Methyl parathion	Limited	Y
Mirex	Sufficient	N
Tetrachlorvinphos	Limited	Y
Toxaphene	Sufficient	N
Nonarsenical insecticides	Limited	Y
Fungicides		
Captafol	Sufficient	N
Captan	Limited	Y
Chlorothalonil	Limited	Y
Ethylene thiourea	Sufficient	Y[c]
Formaldehyde	Sufficient	Y
Hexachlorobenzene	Sufficient	N
Pentachloronitrobenzene	Limited	Y
Pentachlorophenol	Sufficient	Y
ortho-phenyl phenol	Sufficient	N
Sodium *ortho*-phenyl phenate	Sufficient	N
2,4,6-Trichlorophenol	Sufficient	Y
Ziram	Limited	Y
Other		
Creosote	Sufficient	Y
1,2-Dibromo-3-chloropropane	Sufficient	N
1,3-Dichloropropene	Sufficient	Y[b]
Dimethylcarbamoyl chloride	Sufficient	–
1,1-Dimethyl hydrazine	Sufficient	–
Ethylene dibromide	Sufficient	N
Methyl bromide	Limited	Y
Methylmercury chloride	Sufficient	N

Abbreviations: –, could not be determined. HCH, hexachlorocyclohexane; N, no; Y, yes. [a]Data from IARC (*2,3*) and the U.S. EPA (*4,5*). [b]Severely restricted. [c]Contaminant or metabolite of a registered product.

From Zahm, Shelia Hoar and Ward, Mary H. "Pesticides and Childhood Cancer." Environmental Health Perspective 106(Suppl 3):893-908 (1998).

Fortunately, there are natural insecticides produced by bacteria that are extremely effective in killing common pests in agriculture. "The delta-endotoxin proteins of *B. thuringiensis* have been intensively studied, and no indications of mammalian toxicity have been reported. Furthermore, approximately 176 different *B. thuringiensis* products have been registered since 1961, and the regulatory agency [EPA] has not received any reports of dietary toxicity attributable to their use" [31]. The gram-negative soil bacterium *Bacillus thuringiensis* produces these insecticidal crystalline proteins (encoded by Cry genes) during sporulation, and the spores can remain in a dormant state for long

periods of time when nutrients are scarce or environmental conditions are unfavorable for survival. Presumably the *Bacillus thuringiensis* endospores will be consumed by the insect, and the insect will subsequently die, leaving behind fresh tissue which the bacteria can use as nutrient.

The Bt toxin is an ideal insecticide for agricultural use for a number of reasons. First of all, there are a large number of subspecies of *Bacillus thuringiensis*, many of which produce unique versions of the toxin which affect different types of insects. Secondly, the toxin only stays active in the environment for a short time. Sunlight degrades over 50% of the tryptophan residues of the parasporal crystal within 24 hours, so the active toxin may exists in the environment for less than a day [6]. It may seem strange to say that a substance's fragility can actually be an attribute, but in this case it is. The lack of persistence in the environment will drastically reduce the amount of toxin affecting non-target organisms, while also decreasing the chance that a target insect will acquire a resistance against the toxin. To go along with this point, it should be noted that the Bt toxin is remarkably specific to its target insect, and has little or no effect on those insects that aren't pests to crops. This is a claim that can't be made about synthetic insecticides. At one time a hypothesis was made that various Bt toxins would wipe out the monarch butterfly population, since the larvae consume milkweed which is found predominantly around cornfields, and deposition of transgenic corn pollen can be found on the surface of those milkweeds. However, this assertion was ultimately found to be false, as purified Bt toxin was fed to butterflies and it had little detrimental effects on them [32].

Butterflies aren't the only organisms unaffected by the Bt toxins. Humans, cattle,

and wild animals living around sprayed crops would experience no direct harm by widespread application. The toxin is ingested by insects and binds to specific receptors in the gut only under alkaline conditions. The acidic environment and lack of specific receptors in animals' stomachs prevents the toxin from binding, and therefore it is perfectly harmless.

This insecticide was so beneficial that approximately 90% of the microbiological products designed to control insects are based on topically applied Bt toxin [33]. In fact, since the toxins are naturally produced by bacteria, organic farmers have been using the Bt toxin as an insecticide for over 35 years. As effective as these toxins were, however, there was one major problem: topically applied toxin could not affect those species of insect that burrow into the tissue of the plant. Insects like the Southwestern and European cornborers were impervious to the Bt toxins, no matter how much was applied.

The solution to this problem is genetic engineering. By using the *A. tumefaciens* Ti vector system, the gene or multiple genes coding for Bt toxins can be placed under the control of a strong promoter (such as the 35S promoter from the cauliflower mosaic virus), terminator and polyadenylation sequences (typically from the nopaline synthase gene), and inserted directly into the genome of the crop to be transformed. This way the Bt toxin will be produced in the tissue of the plant as well as on the surface, so all ranges of insects susceptible to the toxin will be killed. Fusion proteins can also be made by putting two or more segments of different Cry genes together under the same promoter to make a novel protein with an increased range of effectiveness.

The amazing flexibility to manipulate genes through genetic engineering provides ways to increase the amount and type of toxin produced, as well as a means to prevent

insects from acquiring resistance to the toxin. Large amounts of the toxins are used, both by transgenic crops and by topical application, and this widespread use puts tremendous selective pressure on the susceptible insects to evolve resistance. Nature certainly isn't static. Any act to suppress a certain species will lower the population initially, but those mutant members that, by chance, are not susceptible to the treatment will survive, and it is only a matter of time before they build up the population with strictly resistant organisms. In the case of the Bt toxins, tolerance has been acquired by insects through alterations in the insect's midgut receptors that prevents the toxin from binding and causing its physiological effect [34]. It only took 1-2 years of widespread use of the Bt toxin for mosquitoes to evolve a resistance in some tropical countries [35].

It is this resistance argument that is most commonly used to argue against genetic engineering. However, it often seems like people making this argument assume that scientists are powerless in the face of nature, that these problems that science runs into can't be addressed by science. Evolution is not idly sitting by and letting scientist do what they want with the biological world, so it is unfair to expect scientist to sit by passively and not act to fix any problems that modern technology may come across. There will always be a push and pull, a move and a countermove when dealing with nature. An action is taken, and life molds itself around that action. There will never be a technology that perfectly and permanently fixes a problem. However, science can always address problems that may arise. If the insect's midgut receptors change, then scientists can always alter the toxin to bind once again.

Scientists have created a novel protein that causes the same pharmacological effects as the regular Bt toxin, but also has an additional, non-native polypeptide at its

carboxy-terminus that binds with a greater affinity to toxin receptor in the insect's midgut [33]. It is necessary for the toxin to bind to its specific receptor to have its toxological effect, which is creating pores in the insect's gut. It has been discovered that N-acetyl galactosamine residues on this receptor are an important component for binding of the toxin to the receptor [33]. Ricin-B is a galactose and N-acetyl galactosamine specific lectin that binds to these specific residues with a high efficiency, so adding the gene coding for Ricin-B to the Cry1Ac gene will create a protein with a very high affinity for the receptor. This new protein not only killed a greater percentage of insects that are normally susceptible to the Bt toxins (due to the increased binding affinity), but also affected a wider range of insects than those that are normally susceptible to the toxin [33]. Now it would most likely take a mutation of two different genes simultaneously to fully prevent toxin binding, which is unlikely, and therefore resistance is prevented.

The important aspect of this experiment is not that Ricin-B binds with a high affinity to the insect receptor, since there are many galactose-specific lectins that can be used besides Ricin-B (and probably more appealing than Ricin-B). Rather, this experiment shows that measures can be taken to combat possible resistances to transgenic crops. If by chance the toxin's receptor undergoes a mutation which allows the insect to tolerate the Bt toxin, then scientists can study the new structure, and make adjustments by merely changing the binding characteristics of the protein produced. It is possible that in the near future other insect receptors can be targeted, perhaps with a toxin other than Bt. The possibilities are endless.

Besides, there are already strategies to combat insect resistance, such as refugia, which requires that a certain proportion of the total acreage of land be set aside for non-

transgenic crops. This way the non-susceptible insects will be able to mate with those susceptible to the toxin, and the genes that make insects vulnerable to the toxin will remain in the gene pool.

The increasingly widespread use of transgenic Bt crops against several prominent pests has had positive effects for the environment. A four-year agriculture research study sponsored by the USDA measured pesticide runoff into the Mississippi river, which flows through a 7000 square mile cotton producing area. “The fewer pyrethroid applications needed on Bt cotton sites reduced the amount of pesticides released into the environment. And while runoff from non-Bt cotton sites contained very slight amounts of pyrethroid insecticides, runoff from Bt cotton sites had almost none at all” [36].

As the population increases genetic engineering will become a necessary facet of agriculture. More crops will be needed, and this means more pesticides and herbicides will be required to protect the crops, which means that an increasing amounts of chemicals toxic to humans and animals will enter the environment. A smarter way of producing food for the world is needed, and genetic engineering can do this in a number of ways. Genetic engineering is already a part of all of our lives. As of 1999, 40% of corn, 45% of soybeans, and 50% of cotton produced in the United States came from genetically modified crops [35]. Now genetically modified plants cover 131 million acres of American soil [35]. Clearly these transgenic products are safe, and the advantages to agricultural production and sustainability that genetic engineering provides will only increase in the future.

B) Addrssing Fears about Biotechnology and the Environment

Fears about possible allergic reactions to new proteins added into food have already been addressed in previous chapters. Also, the concern about antibiotic resistance markers affecting soil bacteria has been touched on. However, since the potential creation of "superbugs" is a common fear that persists in many people's minds, it is necessary to explain why the creation of antibiotic resistant soil bacteria does not warrant too much concern.

The United States Food and Drug Administration stated in 1995 that horizontal gene transfer does not occur between plants and bacteria [37]. However, this might be something of an overstatement. Nucleotide sequences in databases have shown that some genes in soil bacteria come from plants [38]. Therefore, there must be some mechanism for horizontal gene transfer between plants and bacteria, even if it occurs infrequently under very specific conditions.

While there are well known cases of gene transfer from a bacterium such as *A. tumefaciens* to a plant and from viruses to plants, it appears that the transfer of a gene between a eukaryotic plant and a prokaryotic bacterium faces many barriers if it can happen at all. One likely way that this would occur is through transformation: the loss of naked DNA from the plant and the uptake of that DNA by a bacterium. Several conditions must be met in order for this to occur. "These conditions include release of DNA very close to those few bacteria that have the relevant molecular mechanisms and which actually develop the physiological state of competence. The naked DNA must also escape any rapid chemical and enzymatic degradation and avoid being irreversibly adsorbed onto soil components" [39]. The DNA sequence in question also must be

integrated into the bacterial genome. For this to happen, there needs to be sequence similarity between the plant gene and a portion of the soil bacterium genome. There also needs to be a promoter that comes along with the plant gene that works effectively in a prokaryotic cell.

Many experiments have tried unsuccessfully to simulate gene transfer between a plant and a bacterium. Neilson et al. tried to accomplish gene transfer in non-sterile soil, claiming that if it happens at all, it occurs at a frequency of once in every 10^{10} to 10^{11} bacterial cells, which is below the level of detection [40]. Clearly this occurrence under field conditions is a rare event. One obvious reason for this is sheer probability. The native plant genome is vast compared to the antibiotic resistance gene and any other transgenes that were added, so transfer of these select genes will be infrequent. It was hypothesized that as the complexity of the donor DNA increases, the frequency of transformation for a given gene decreases [39].

The bottom line is that the number of physiological events that need to occur in order to allow transformation of a soil bacterium by plant DNA makes this phenomenon rare. If one is to take action to prevent the creation of superbugs that are resistant to all commonly prescribed forms of antibiotic, then genetic engineering is a poor place to focus one's energies. Rather, the haphazard use of antibiotics in the beef and milk industries, as well as physicians' propensity to prescribe antibiotics far too often are more logical places to start. Furthermore, bacteria are naturally resistant to many of the antibiotics that are used by humans [1], which has absolutely nothing to do with modern genetic engineering at all.

There are several options if one desires to remove the antibiotic resistance marker from an edible plant. One way is to co-transform the desired plant with two separate plasmids: one carrying the marker gene, and the other one carrying the gene of interest into the plant. These genes will integrate into different portions of the host's genome, and then traditional selective breeding can produce offspring which contain the gene of interest, but not the marker gene.

Another way to remove the marker is through site-specific recombination through the Cre/loxP system. The marker gene is flanked by specific DNA sequences called the loxP recombination sites, and Cre is the enzyme that cuts out anything between these two sites after it has been inserted into chromosomal DNA. The gene coding for the Cre enzyme is located on its own plasmid and is controlled by a specifically induced promoter. In one experiment, the XVE system was used, which is induced by the addition of estradiol [45]. When estradiol is added, the antibiotic resistance marker was removed, activating a GFP gene that will be the evidence that the marker has been removed [45]. This way the antibiotic resistance marker can be removed, and the desired gene remains to have the designed effect.

Analogous to the fear of antibiotic resistance markers spreading to soil bacteria is the fear that herbicide resistance will spread to weeds, creating a weed that grows out of control and destroys crops. Pollen from transgenic crop can travel over long distances, even be carried across international boarders and germinate in countries that never intended to harvest transgenic crops. A perfect example are the mountains of Oaxaca, Mexico, where the world's greatest genetic variety of maize is maintained in open-pollinated fields [41]. These fields are a great source of historic and cultural pride for the

Mexican people, and it was never intended for transgenes to make their way into these fields. In fact, Mexico outlawed the planting of genetically engineered maize within its borders in 1998 [42].

However, a 2001 article in *Nature* claims that transgenes were found in some species of corn in Oaxaca [42]. A 35S promoter sequence (from the cauliflower mosaic virus) was discovered in kernel samples and in grain sold in local stores [42]. The 35S promoter is a strong and constitutive promoter that is commonly used to drive the expression of an inserted transgene, so it is a logical target to test for transgenic contamination in species. Also, a nopaline synthase terminator sequence and a Bt Cry1AB endotoxin sequence from *Bacillus thuringiensis* was detected in some samples of maize [42]. Of course, these genes could have never appeared in wild species of maize unless the Oaxaca fields were specifically contaminated with genes from transgenic plants. It is possible that Mexican farmers may have unknowingly planted transgenic kernels within their borders.

However, a more recent publication in Nature claims that the Oaxaca maize was uncontaminated. The seeds of 870 plants from 125 different fields in Oaxaca were screened for the CaMV 35S promoter and the nopaline synthase terminator. Neither one of these genes were found in the maize [41]. Certainly, more research needs to be conducted on the maize in these remote Mexican fields.

What is clear is that pollen can potentially carry transgenes into lands where they are not welcome, just as pollen can carry any gene. In fact, it's difficult to conceive how the spread of transgenes can be stopped, even to remote mountainous lands like Oaxaca, Mexico. With trade occurring freely between America —which is the world's leader in

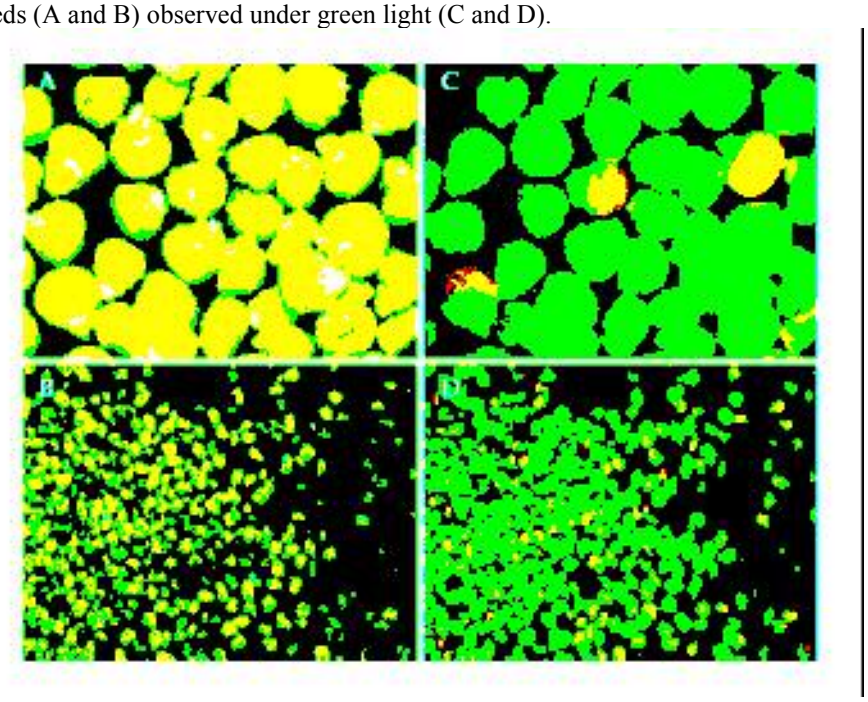
producing genetically modified organism— and the rest of the world, it is impossible to prevent these transgenes from crossing borders. The United States exports several million tons of maize to Mexico alone every year (6.5 million tons in 2001 alone), some transgenic and some conventional [43]. Mexican farmers could easily plant these in their own fields, unknowing whether they are planting transgenic or conventional kernels—perhaps not even knowing that such a thing as a transgenic plant exists.

It is unfortunate that the genes for the Bt toxin contaminated Mexican maize. However, the spread of transgenes can have more dramatic consequences than merely offending cultural pride. A much more harmful prospect is that a gene for herbicide tolerance will end up in the wild population, perhaps in a weed, creating a superweed that will be difficult to control. This is not as far fetched as it seems. "Of the 60 crop plants under widespread cultivation, at least 49 have wild and often weedy relatives to whom they have the potential to cross" [35]. The same mechanisms that allows plants to spread desirable traits considerable distances is the same mechanism that will allow transgenes to spread rapidly if it creates some sort of selective advantage. This is how plants evolved. The risk of crop gene flow to weedy relatives has always existed, and such 'gene flow' occurs where possible. The risk of gene transfer to weeds is similar with both conventional and GM crops and is not contingent on how we introduce these genes into plant" [4]. And pollen can spread genes over long distances. Separating different maize varieties by two-hundred meters—the length of two football fields—will result in contamination due to cross pollination of pure plants of about 0.1% [44].

With the number of different crops in widespread cultivation, and the sheer volume of international trade, the unintended spread of transgenes is inevitable. However,

once again scientists aren't helpless against this undesired consequence of genetic engineering. Due to our ever-increasing knowledge of the molecular world, there are ways to track and even prevent the spread of transgenes. Scientists aren't powerless to prevent unwanted gene flow, however. One intriguing way to track the spread of transgenes is to use a marker, such as green fluorescent protein. This is used extensively in research laboratories, and can easily be used to mark only those plants that possess the transgene in them. This way the farmers of the mountains of Oaxaca, Mexico could tell which maize crops were of native origin and which have been contaminated with the transgene.

Fig 7: Detection of transgenic seeds using red fluorescent protein as a visible marker. Maize and tobacco seeds (A and B) observed under green light (C and D).



From: Ma, Julian K.C et al. (2005) "Molecular farming for new drugs and vaccines: Current perspectives on the production of pharmaceuticals in transgenic plants." European Molecular Biology Organization. 6:7. 593-599.

If the prospect of eating a glowing tomato is a little too strange to bear, there are precautions that can be taken to severely limit the chance that a transgene will spread to a related plant. One interesting solution is to insert the transgene into a plastid, such as the chloroplasts in green photosynthetic plants. Chloroplasts contain their own distinct DNA that is separate from the chromosomal DNA, since chloroplasts, like the mitochondria in virtually all eukaryotes, were once free living organisms that evolved to have a symbiotic relationship in eukaryotic cells. The advantage of inserting the gene of interest into the chloroplast genome is that its DNA is inherited in a non-Mendelian fashion. In most angiosperms, the plastids are maintained solely in the egg cell during reproduction, so it is transmitted only by the female [46]. This means that the pollen will not contain any genetic information concerning the plastids, and therefore any transgenes contained in the plastid cannot be spread to distant, related plants.

Another major benefit of using plastids as a carrier of transgenes is that they are present in such high numbers in a plant. “A chloroplast gene will usually be present thousands of times per cell with the result that chloroplast genes produce the most abundant proteins on the planet” [47]. With many chloroplasts per cell, and many copies of chloroplast DNA per chloroplast, any gene inserted into the chloroplast genome will be expressed at high levels, thus producing large amounts of protein.

However, a potential dilemma exists. If plastids were once free-living organisms that learned to rely on another cell to live, and therefore lost most of their genes to the larger nuclear genome, then it was obviously possible for genes to escape the plastid into the nuclear DNA. This seems to be the case, but at a very low level. Plastid inheritance was studied over a 3 year period of time between wild populations growing next to

Figure 8: List of potential solutions to the gene flow of transgene

Technique	Advantages	Disadvantages
Maternal inheritance	Prevents gene flow through out-crossing and volunteer seeds. Field tests indicate low incidence of sympantry and mixed stands extinct in three years. High levels of trans-gene expression and no evidence of gene silencing or position effects.	Techniques to export proteins are not yet available.
Male Sterility	Prevents outcrossing. Shelf-life of Flowers may also be extended. Several tapetup specific promoters available.	Crops need to be propagated By cross-pollination from non-GM crop or by artificial seed. Potential for volunteer seed dispersal
Seed Sterility	Controls both outcrossing and volunteer seed dispersal	If transgene is silenced, introgression will occur. All linked genes should segregate together
Cleistogamy	Pollination occurs before flower opens, theoretically preventing outcrossing	In practice, introgression occurs despite self-pollination
Apomixis	Seed is of vegetative origin and not from sexual cross. Controls both outcrossing and volunteer seed dispersal. Hybrid traits can be fixed	Only known in a few crops. Genes not yet available.
Incompatible genomes	Prevents recombination after pollination	May not be applicable to crops that exhibit homologous recombination. Crops will not produce seeds unless propagated with compatible plants
Temporal and tissue-specific control via inducible promoters	Gene either activates only when product is necessary or excised before flowering	May not be applicable to traits required throughout the plant's life. If chemical treatment fails to penetrate plant tissue, residual level of transgene may be present in pollen or seeds that could be outcrossed.

Modified from Daniell, Henry. "Molecular strategies for gene containment in transgenic crops." Nature Biotechnology. 20. 581-586. (2002).

oilseed rape, and it was discovered that gene flow was extremely rare between the transgenic and the weed species, even though some of the weeds were relatives of the oilseed rape [48]. This means that a transgene placed in the chloroplast of a plant will be safely retained in the chloroplast, preventing unwanted spread of the transgene.

Another potential solution to prevent the unwanted spread of genes is to make the gene only active under specific conditions, such as the application of a particular chemical. This way the transgene will be transcribed only when the farmer wants it to, and silent in those weeds and crops that the transgene wasn't intended for. One such system used ethanol vapor to successfully induce genes in tobacco, potatoes, and oilseed rape [49]. This system uses the alc expression system, which acts as a switch, turning on gene expression for any gene fused to the modified alc promoter [49]. This system allows the transcription of a gene under very low concentrations of ethanol vapor (as low as 0.4 μM of ethanol vapor). Again, this experiment is important in that it shows that transgenes can be selectively transcribed, so even if a Bt toxin gene finds its way into a distant corn field due to travel of pollen, that the gene won't be expressed in those fields unless the particular inducer is supplied. In the future other inducible systems can be devised that will be activated by a cheap compound that can be sprinkled onto crops, or added to water and taken up by the roots.

VI) Bioterrorism

So far this paper has dealt with unintended consequences that biotechnology may encounter due to natural mechanisms. However, there remains one major fear that many people are concerned about: the misuse of biotechnology to intentionally harm other people. Although it is not the fault of genetic engineering if someone uses it to harm others, just as it is not the gun's fault if a person shoots another person, it is a topic that has received a lot of attention in this post 9/11 era. It is possible to alter a microorganism to create a biological weapon, although it is not as simple as many people may believe, since it takes a certain level of expertise and resources that the average person simply doesn't possess. However, the mere possibility of bioterrorism warrants at least a mention in an honest discussion of genetic engineering.

The use of biological agents as weapons is not a novel notion. "Mongol invaders catapulted plague victims into besieged cities, probably causing the first plague epidemic in Europe, and British settlers distributed smallpox-infected blankets to Native Americans" [50]. This isn't an instance of engineered organisms through the use of recombinant DNA, but it shows how pathogenic microorganisms can cause far-reaching devastation (and it is yet another argument against the theory that natural is always good). Unfortunately, the desire to harm others with the use of pathogenic microorganisms persists in recent times. During the cold war the Soviet Union reportedly introduced alien genes into a strain of *Bacillus anthracis* to increase its pathogenicity [35]. In addition, the USSR's Biopreparat agency reported stockpiles of a wide range of potential deadly microorganisms, including hundreds of tons of anthrax and 20 tons of smallpox [35]. More recently, the Japanese Cult Aum Shinrikyo—the group that attempted a nerve gas

attack on a Tokyo subway—attempted to obtain *Ebola* virus during an outbreak in Zaire in the 1990's [39].

The constant advances in our understanding of the molecular world have given scientists powers that were once unthought-of just decades before. For instance, in 2002 the poliovirus was synthesized in a lab without using genetic material derived from a natural virus [51]. This was the first time that a virus fully capable of infection was created *de novo* in a laboratory from scratch. Since the genome of the poliovirus is available on-line, specific oligonucleotide sequences can be ligated end to end in a laboratory. The virus was then injected intracerebrally into mice and had the same effect as the wild poliovirus, with the only difference that the synthetic poliovirus required larger doses to cause flaccid paralysis or death [51]. Of course, most of the world has been vaccinated against poliovirus, so it isn't a likely weapon for bioterrorists. However, the fact that a viral genome can be assembled in a laboratory is enough to cause concern that a more virulent microorganism can soon be constructed.

The virus that has received most of the public's fear lately is smallpox. Fortunately, there are a couple of reasons why smallpox would be more difficult to construct in a lab from scratch. First of all, its genome is much larger, consisting of about 200,000 base pairs, as opposed to the 75,000 base pairs of the poliovirus [50]. Secondly, the smallpox genome is not published for the general scientific community to see. Perhaps most importantly, the World Health Organization prohibits DNA recombination studies between variola (virus that causes smallpox) and other members of the orthopoxvirus family [52].

Smallpox stirs such widespread fear since very few people in the population have actually been immunized against it. For this reason, there are supposedly only two stockpiles of the virus that remain in the world—one in the United States and one in the former Soviet Union [35]—each under tight security so a potential theft is unlikely. However, another route to reintroduce smallpox it to introduce the virulence factors of the variola virus into a more common organism. These virulence genes can be introduced into vaccinia, which is related to variola (the virus that causes smallpox) but harmless to humans. This is exactly what has been done at the University of Pennsylvania.

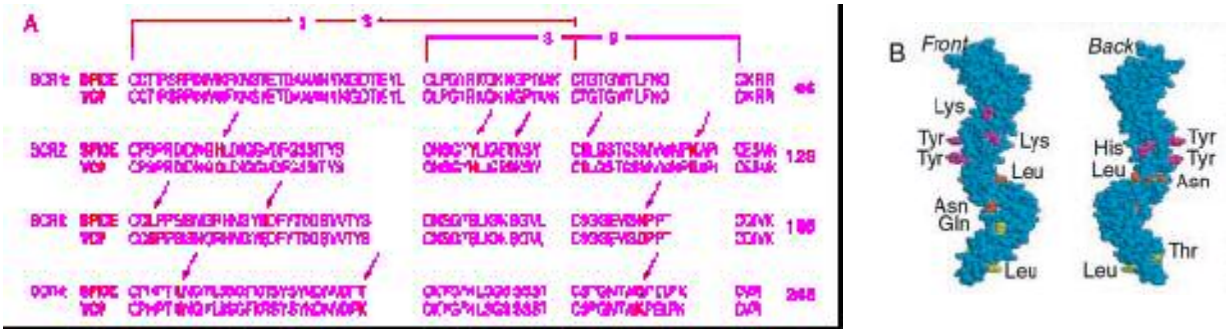
The complete variola genome may not be published to the general scientific community, but the genes, locations, and sequences that code for the virus' virulence factors has been published for anyone to see [52]. Both the vaccinia and the variola virus cause the cells they infect to make proteins that inhibit pivotal components of the immune complement system. However, the difference between the two is that variola causes infected cells to make smallpox inhibitor of complement enzyme (SPICE), and vaccinia causes infected cells to make a homologous protein called the vaccinia virus complement control protein [52]. Both viral gene products inhibit the immune complement system, but SPICE from variola is 100 times more efficient than the VCP of vaccinia, so variola is deadly to most humans, and vaccinia can only cause problems in immune-compromised individuals [52].

There is only an 11 amino acid difference between the VCP protein that is so harmless that it can be used as a live vaccine, and the deadly SPICE protein that causes a 30-40% fatality rate for those infected with smallpox [52]. Even more frightening, this research team from the University of Pennsylvania used site-directed mutagenesis on

only 13 VCP nucleotide base pairs and created the gene that encodes the deadly SPICE protein [52].

The ramification of this type of work are frightening. A relatively innate, easy to acquire vaccine such as vaccinia can be turned into a potential biological agent. This is perhaps the most frightening potential that genetic engineering has unlocked: that a harmless microorganism can be engineered to be highly pathogenic and resistant to modern medical care.

Figure 9: Comparison between SPICE from variola and VCP from vaccinia. In A, arrows point to the amino acids that are different between the two. B is a schematic view of SPICE mapped onto VCP.



From Rosengard, A., Liu, Y., Nie, Z., Jimenez, R. ‘‘Variola virus immune evasion design: Expression of a highly efficient inhibitor of human complement.” PNAS. 99:13, 8808-8813. 2002.

Most of the problems that may arise due to genetic engineering have relatively simple scientific solutions. The ease of fixing some of these potential issues, such as the unintended flow of genes, should be encouraging to those who are skeptical of widespread use of this burgeoning technology. The ingenuity of modern scientists should not be underestimated, and it can be trusted that if unforeseen problems do arise, that they can be fixed in one way or another. Other problems, such as the spread of markers to soil bacteria, are not serious and should not halt progress of genetic engineering. The only

problems that are outside the control of scientific intervention are the ill intentions of those people who want to do harm to others. This is not so much a scientific issue as it is a social and political issue. Perhaps the most assuring point to be made about this is that the potential to unleash smallpox on a population has existed for some time, and yet mass bioterrorism has yet to be actualized. It is probably more rational to go on with scientific advancement and not underestimate the humanity of others, even our enemies.

VI) Conclusion

Genetic engineering is the extension of methods and technologies that humans have been building on for thousands of years in order to survive. This world would be incompatible for civilized life if prehistoric humans hadn't initiated the process of guiding the world around them. The major crops we consume for nourishment everyday were once small, wild weeds that were incapable of sustaining more than a small group of people for an extended period of time. These weeds were plucked out of the forests, selectively bred to grow larger and faster in non-native environments, and gradually were directed into the plentiful crops we have today. The same process was repeated to domesticate wild animals. This was all accomplished through genetic engineering: the intentional manipulation of organisms on a genetic level to serve a particular function. The only difference between the genetic engineering of 5,000 years ago and today is that our techniques are more refined. However, the desire is the same. The need is the same. Societies can only be built around a stable environment, but the environment will only possess some semblance of stability if humans attempt to exert a measure of control over it. Quite simply there are many forces in this world that don't particularly care if humans survive or not. Genetic engineering can better protect and enhance our food sources, provide plentiful and easy to administer vaccines, and provide pharmaceutical products that are cheap to produce and store so that even impoverished countries can afford quality medical care. Forfeiting our most powerful technology to enhance and protect human life and provide a sustainable environment is sheer insanity.

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