ANAEROBIC FERMENTATION BY yeasts

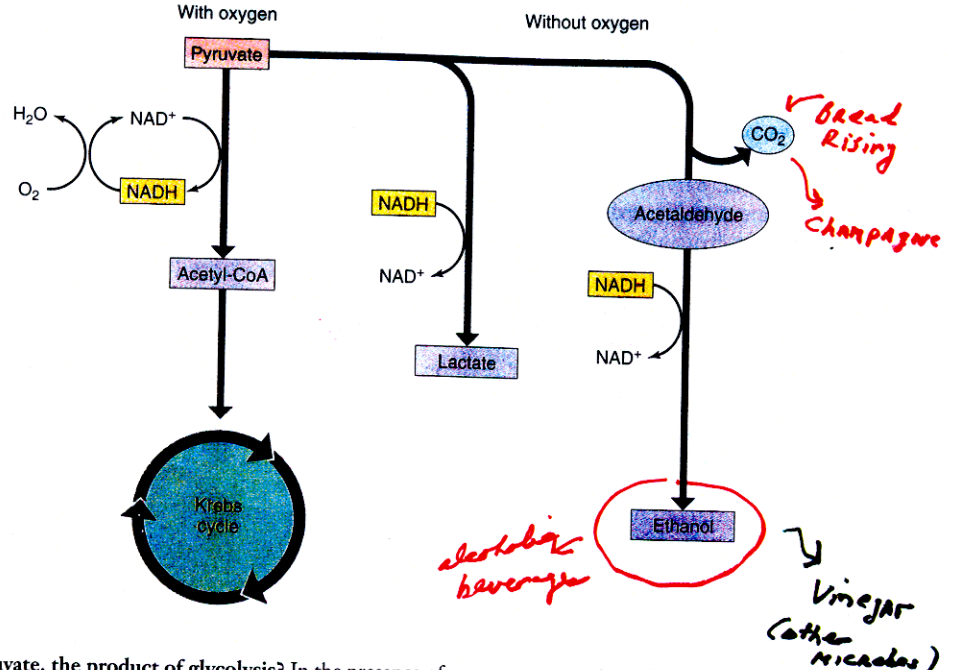


FIGURE 9.9

What happens to pyruvate, the product of glycolysis? In the presence of oxygen, pyruvate is oxidized to acetyl-CoA, which enters the Krebs cycle. In the absence of oxygen, pyruvate is instead reduced, accepting the electrons extracted during glycolysis and carried by NADH. When pyruvate is reduced directly, as in muscle cells, the product is lactate. When CO_2 is first removed from pyruvate and the product, acetaldehyde, is then reduced, as in yeast cells, the product is ethanol.

Yeasts could be Genetically Engineered to Enhance Alcohol Production

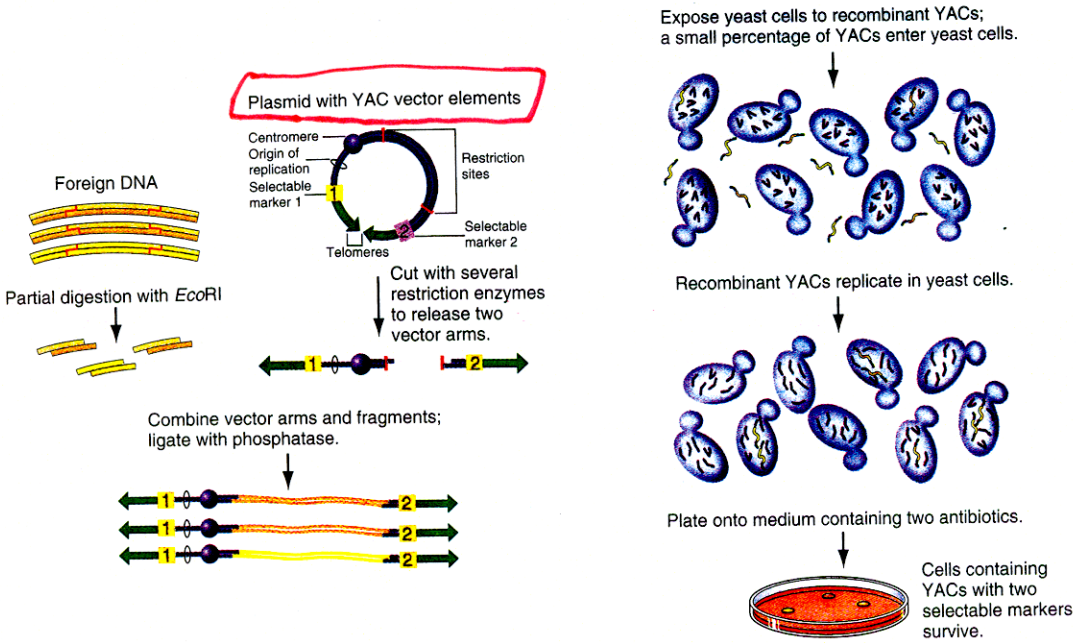


Figure 8.7 YAC vectors take advantage of DNA elements used for normal chromosome segregation within yeast cells. Two distinct arms make up each YAC vector. At the end of one arm is a telomere followed by a selectable marker, then a centromere, and finally a restriction site. The second arm lacks a centromere but has a telomere at one end, a restriction site at the other, and a second selectable marker in the middle. One of the two arms must also contain a yeast origin of replication. To make YAC-insert recombinants, you cut the two YAC arms and large foreign genomic fragments with the same restriction enzyme, mix the YAC arms with the foreign restriction fragments, and treat the mixture with phosphatase. As with bacteria exposed to plasmids, a small percentage of yeast cells exposed to YAC-insert recombinants will take up the recombinant molecules. And like bacteria that harbor plasmid vectors, yeast cells transformed by properly constructed recombinant YACs containing two selectable markers will survive and propagate in a medium infused with two antibiotics. Yeast cells with one or no marker will not. The properly constructed YAC recombinants will replicate and be transmitted along with other chromosomes inside the surviving yeast cells. Such proper YACs must meet three requirements: (1) They must contain an insert; (2) they must carry one—and only one—centromere, since those with more than one centromere will not segregate properly during mitosis; and (3) they must have a telomere at both ends. Tips without a telomere will fuse with another chromosome or decay. Since only those recombinants composed of two different arms flanking an insert will satisfy these requirements, the ability to segregate properly after replication ensures the reproduction of mostly single vector–single insert recombinants.

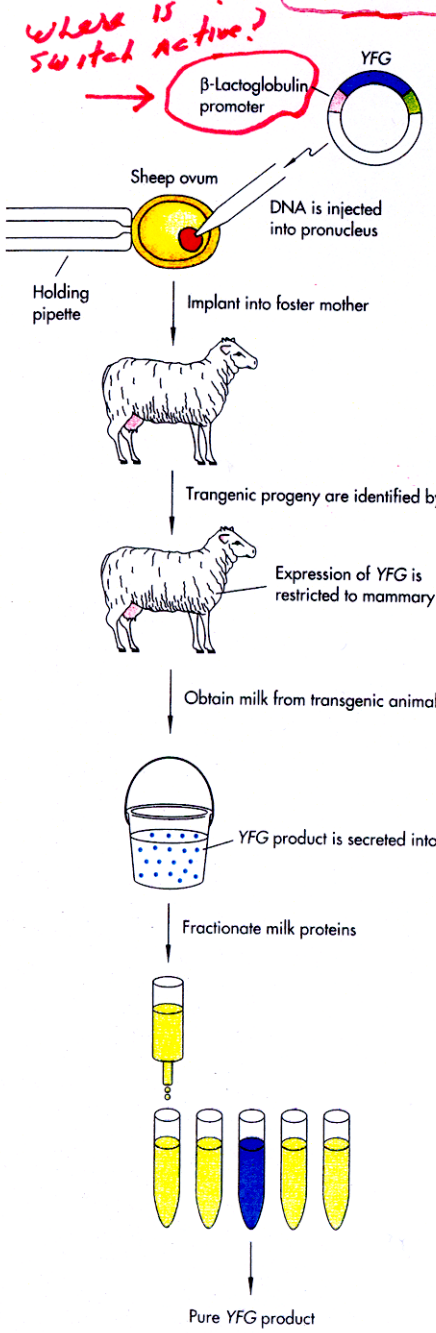
What is a YAC?

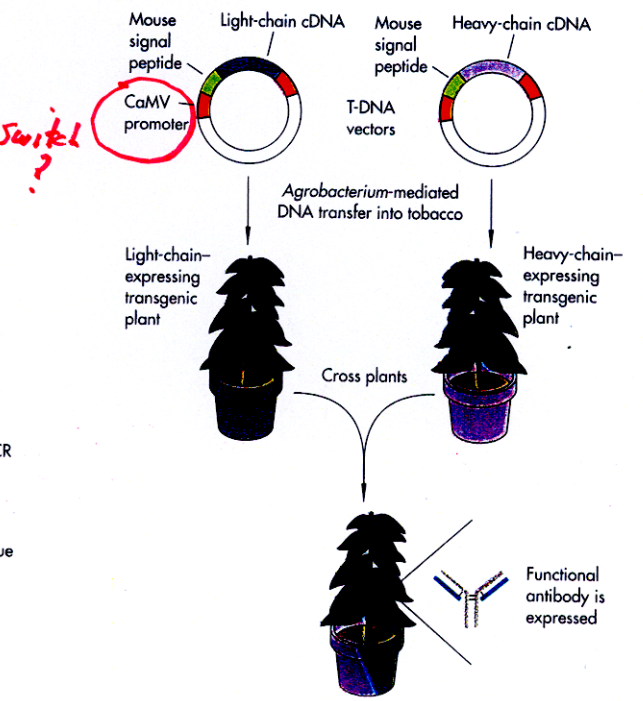
Haven't yet Why?

? Function ?

Animals & Plants can also be used as factories to produce large amounts of Human Proteins

MOLECULAR PHARMING





Reasons

Advantages

1. Proteins need to be modified after translation to be active — only eukaryotic cells can do this
2. Bacteria need big fermenters & elaborate protein purification schemes -- farm animals & plants can be used for this purpose w/o special processing / machinery
3. Proteins in plants (e.g., seeds) is finitely stable - can be stored cheaply (e.g., grain cheaply) for long periods of time!

TRANSgenic Animals Have Many Pharmaceutical Uses

TABLE 3.1 Potential uses of transgenic animals for pharmaceutical production.

Species	Theoretical Yield (g/yr of Raw Protein)	Examples of Products Under Development
Chicken	250	Monoclonal antibodies Lysozyme Growth hormone Insulin Human serum albumin
Rabbit	20	Calcitonin Superoxide dismutase Erythropoietin Growth hormone IL-2 α-glucosidase
Goat	4,000	Antithrombin III Tissue plasminogen activator Monoclonal antibodies α-1-Antitrypsin Growth hormone
Sheep	2,500	α-1-Antitrypsin Factor VIII Factor IX Fibrinogen
Cow	80,000	Human serum albumin Lactoferrin α-Lactalbumin

Source: Modified from Dove, 2000.

cow highest amount of protein!

And other uses —

enhanced milk

larger animals

MAKING RECOMBINANT HUMAN PROTEINS IN ANIMALS

Table 19.3 Some exogenous proteins that have been expressed in the mammary glands of transgenic animals

Antithrombin III
Calcitonin
Erythropoietin
Factor IX
Factor VIII
Fibrinogen
Glucagon-like peptide
Granulocyte colony-stimulating factor
Growth hormone
Hemoglobin
Human serum albumin
Insulin
Insulin-like growth factor 1
Interleukin 2
Lactoferrin
Lysozyme
Monclonal antibodies
Nerve growth factor β
Protein C
Superoxide dismutase
Tissue plasminogen activator
α1-Antitrypsin
α-Glucosidase
α-Lactalbumin

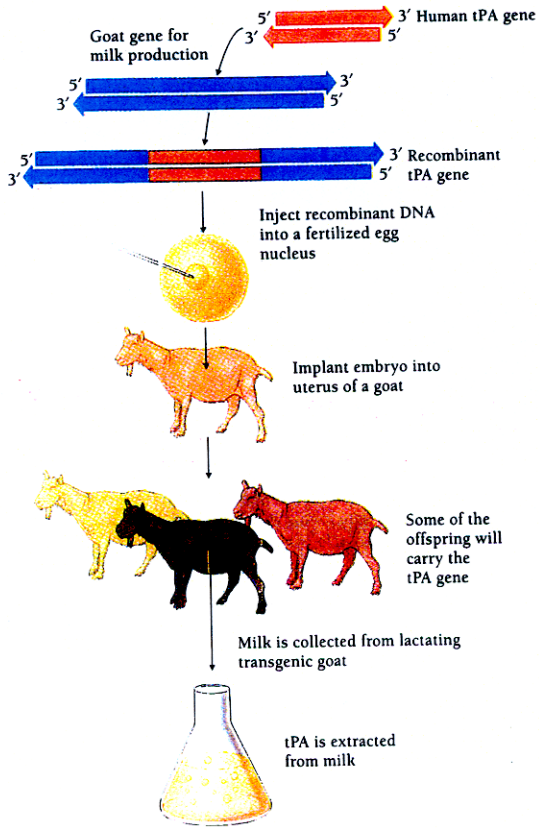
Advantages over Bacteria?

Table 19.2 Milk production and estimated recombinant protein yields from organisms used for the expression of transgenes in mammary glands

Organism	Annual milk yield (liters)	Estimated recombinant protein per female (kg/yr)
Rabbit	5	0.02
Pig	300	1.5
Sheep	500	2.5
Goat	900	4
Cow	10,000	60 !!!

Advantages?

Producing TPA in a Goat



Also!
Sheep
Pigs
Cattle

Advantages!

1. Cost → no special equipment needed
2. Mammalian Gene active in Mammalian Cell
   ↳ use goat switch for controls
3. By-Product of other uses of Goats
4. Eukaryotic Protein Modification Processes

But → Generation time long to establish transgenic farm animals & only few offspring :: scale-up hard.... but..... →

Designer milk from transgenic clones

Biotechnology gets a step closer in the pre-harvest production of "new milks" by generating cows that overexpress casein proteins in their milk.

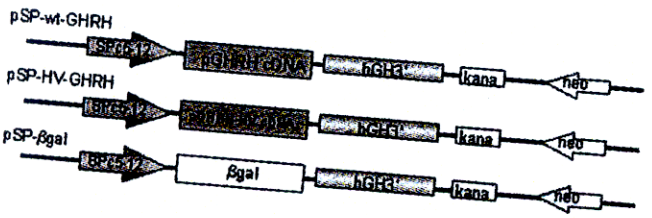
Table 1. Potential modifications of milk composition by gene addition, with expected functional outcome (modified from ref. 2).

Modification	Functional consequence
Introduction of casein genes	
Increase ratio of κ-casein to β-casein or concomitant increase of all caseins by transferring casein locus	Increase in protein and calcium content. Reduction in micelle size, enhancement of heat stability
Modification of casein genes	
Add phosphorylation sites	Increase in calcium content, micelle size, and stability of milk. Enhanced amphiphilicity of β-casein increases its emulsifying and foaming properties
Introduction of protease (chymosin) cleavage sites	Increase in rate of cheese-ripening
Deletion of protease (plasmin) site from β-casein	Increase in emulsifying properties. Elimination of bitter flavor in cheese
Introduction of other functional proteins	
Add lysozyme, lactoferrin, or lysostaphin	Milk with antimicrobial activity
Add reversibly inactive lactase that is activated in gastrointestinal tract upon ingestion of milk	Elimination of sweet taste of lactose-hydrolyzed milk and alleviation of lactose intolerance symptoms

Table 19.1 Protein composition (grams/liter) of milk from cattle and sheep

Proteins	Cattle	Sheep
Casein		
α_{s1}-Casein	10.0	12.0
α_{s2}-Casein	3.4	3.8
κ-Casein	3.9	4.6
β-Casein	10.0	16.0
Major whey proteins		
α-Lactalbumin	1.0	0.8
β-Lactalbumin	3.0	2.8
Other proteins		
Serum albumin	0.4	Unknown
Lysozyme	Trace	Unknown
Lactoferrin	0.1	Unknown
Immunoglobulins	0.7	Unknown

Using Gene Therapy to "Engineer" Farm Animals



NATURE BIOTECHNOLOGY VOL 17 DECEMBER 1999 *http://biotech.nature.com*

Myogenic expression of an injectable protease-resistant growth hormone–releasing hormone augments long-term growth in pigs

Ruxandra Draghia-Akli[1,4*], Marta L. Fiorotto[2], Leigh Anne Hill[1,4], P. Brandon Malone[1,4], Daniel R. Deaver[3], and Robert J. Schwartz[1,4,5*]

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PRODUCTION OF TRANSGENIC ANIMALS By Injecting Eggs with Genes is NOT Efficient

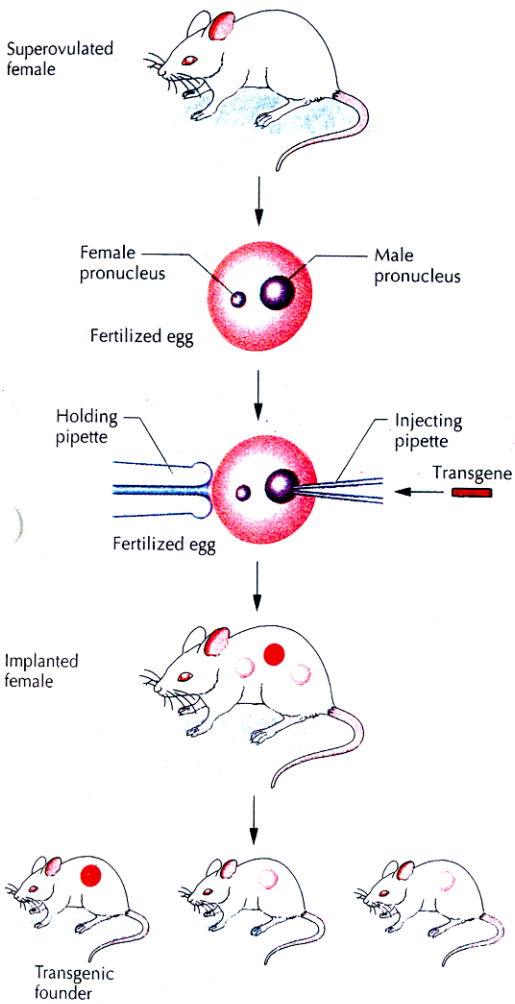
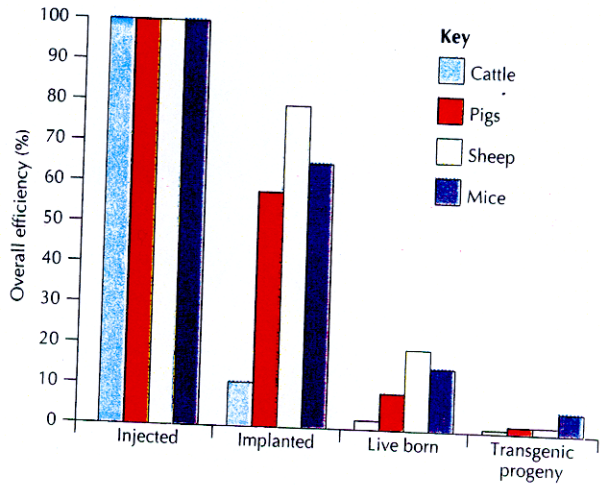


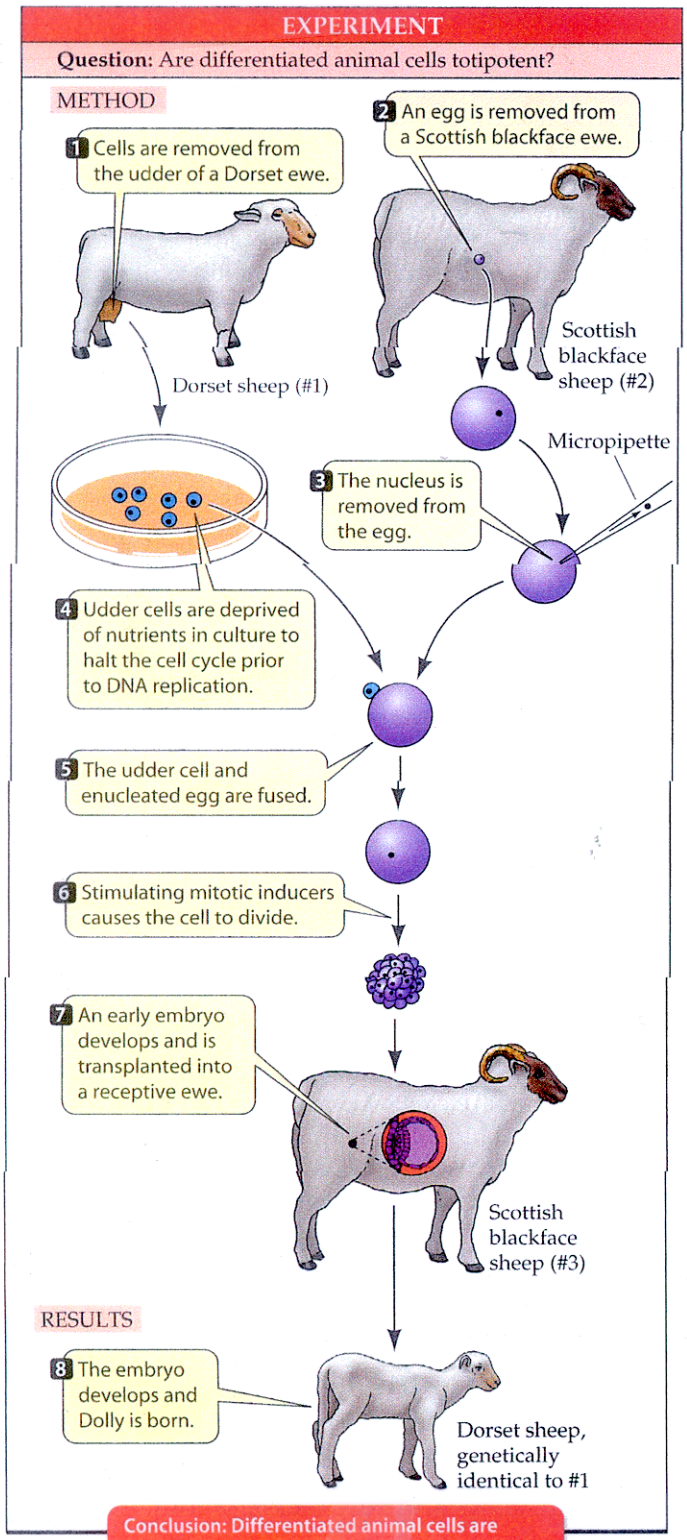
Table 11.3 Efficiency of production of transgenic animals by microinjection of a growth hormone gene. (Adapted from Hammer *et al.* 1985.)

Animal species	No. of ova injected	No. of offspring	No. of transgenic offspring
Rabbit	1907	218	28
Sheep	1032	73	1
Pig	2035	192	20



Limits use of Molecular Pharming for Pharmaceutical Production

CLONING CAN BE USED TO GENERATE TRANSGENIC PHARM ANIMALS

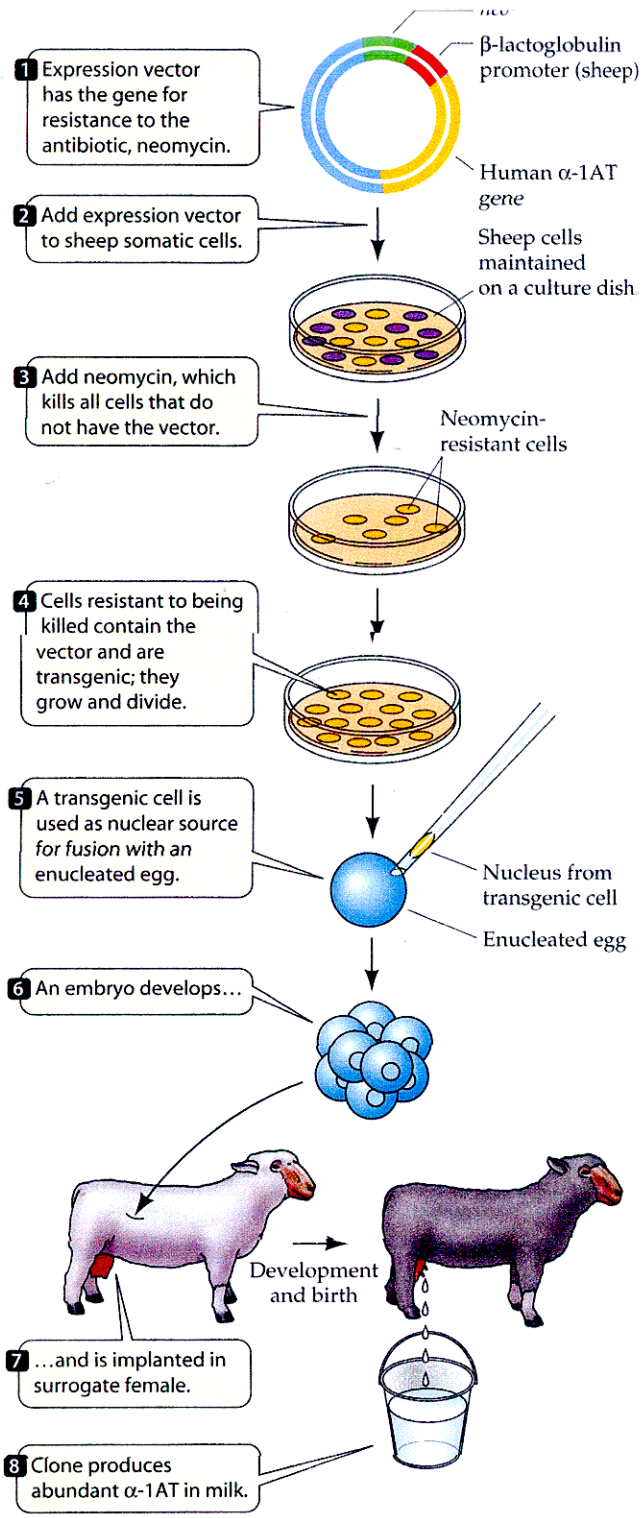


16.4 A Clone and Her Offspring
Although Dolly herself (right) is a clone with only one parent, she has mated and given birth to "normal" offspring (the lamb on the left), proving the genetic viability of cloned mammals.

TRANSGENIC LINES

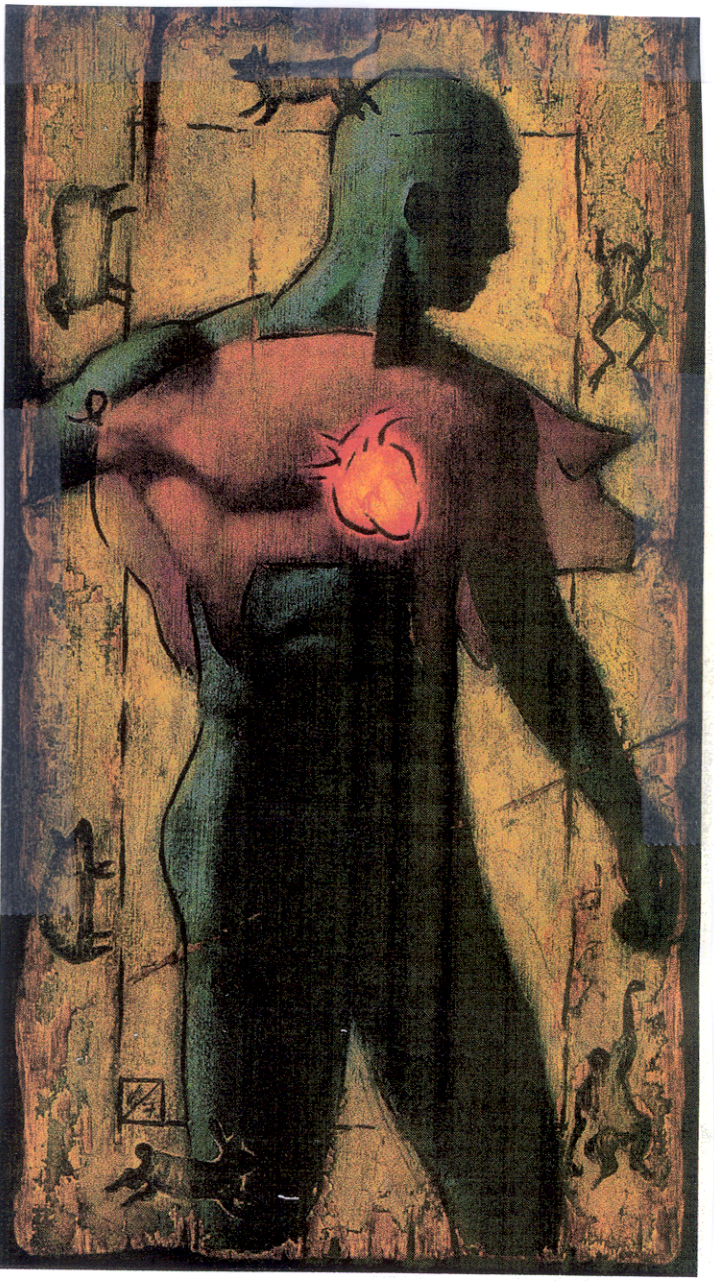
Most Mammals Have Now been Cloned
Mouse → Monkeys

USING CLONING & RECOMBINANT DNA TO MAKE TRANSGENIC PHARM ANIMALS



17.15 Production of Transgenic Clones for "Pharming"
The production of transgenic animals involves a combination of DNA technology and reproductive technology.

MAKING TRANSGENIC PIGS FOR HUMAN ORGAN TRANSPLANTS



STEVE JOHNSON AND LOU FANCHER

TRANSPLANTS OF TISSUES from animals to humans (xenotransplants) have been attempted experimentally using a variety of donor animals, from frogs to baboons and pigs. Most efforts quickly failed. But doctors may soon perfect ways to transplant organs, such as the heart, from specially bred pigs.

Knocking out xenograft rejection

Two reports on the knockout of one allele of the α1,3-galactosyltransferase gene in pigs bring us one step closer to the transplantation of pig organs into people.

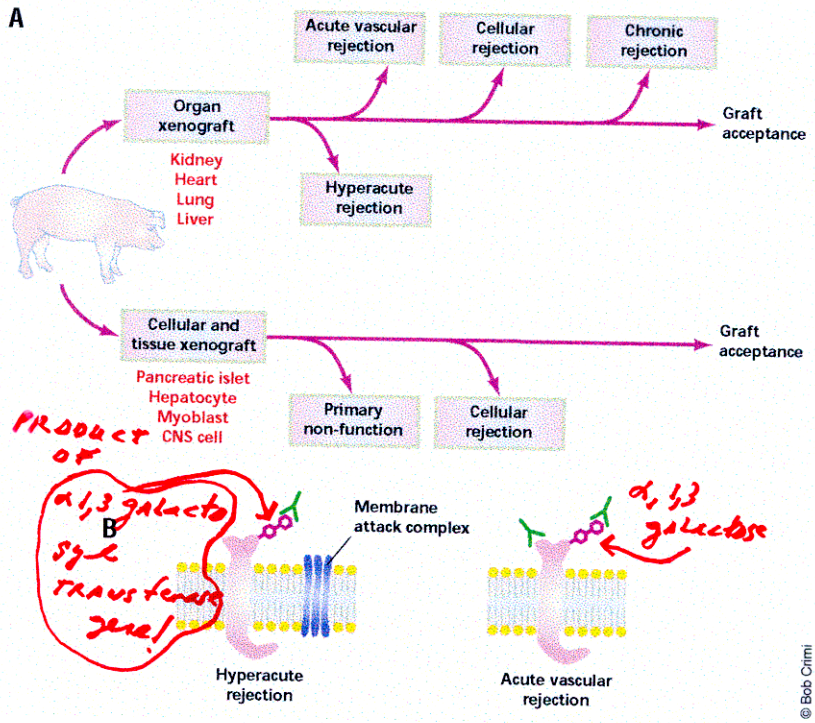


Figure 1. α1,3Gal in the fate of xenografts and the mechanisms of tissue injury. (A) Fate of xenografts. The impact of immunity on xenografts depends on the type of graft. Organ xenografts are subject to vascular rejection of various types thought to be induced by anti-donor antibodies and cellular rejection caused by T cells. Cell and tissue xenografts are subject to primary non-function, thought to be caused by macrophages and cellular rejection. Expression of α1,3Gal and the action of anti-Gal antibodies is expected to have a far more profound impact on the fate of organ grafts than on the fate of cell or tissue grafts[3]. (B) The role of α1,3Gal in hyperacute and acute vascular rejection. Hyperacute rejection is caused by binding of large amounts of antibody, consisting predominantly of anti-α1,3Gal, to graft blood vessels, activating large amounts of complement. It is prevented by anything that inhibits antibodies or complement. Acute vascular rejection is caused by binding of antibodies to the graft with or without complement. The antibodies causing acute vascular rejection may be directed against α1,3Gal[14] or against other xenogeneic proteins[12]. Acute vascular rejection is not prevented by complement inhibitors, but may be inhibited by depleting antibodies or by modifying antigenic targets, as might be seen in the α1,3GT-knockout pig.

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Targeted disruption of the α1,3-galactosyltransferase gene in cloned pigs

Yifan Dai[1]*, Todd D. Vaught[1], Jeremy Boone[1], Shu-Hung Chen[1], Carol J. Phelps[1], Suyapa Ball[1], Jeff A. Monahan[1], Peter M. Jobst[1], Kenneth J. McCreath[2], Ashley E. Lamborn[1], Jamie L. Cowell-Lucero[1], Kevin D. Wells[1], Alan Colman[2], Irina A. Polejaeva[1], and David L. Ayares[1]

Galactose-α1,3-galactose (α1,3Gal) is the major xenoantigen causing hyperacute rejection in pig-to-human xenotransplantation. Disruption of the gene encoding pig α1,3-galactosyltransferase (α1,3GT) by homologous recombination is a means to completely remove the α1,3Gal epitopes from xenografts. Here we report the disruption of one allele of the pig α1,3GT gene in both male and female porcine primary fetal fibroblasts. Targeting was confirmed in 17 colonies by Southern blot analysis, and 7 of them were used for nuclear transfer. Using cells from one colony, we produced six cloned female piglets, of which five were of normal weight and apparently healthy. Southern blot analysis confirmed that these five piglets contain one disrupted pig α1,3GT allele.

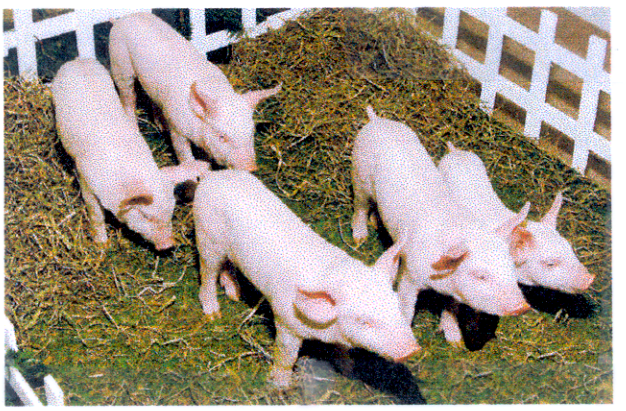

Figure 3. Five α1,3GT gene knockout piglets at 2 weeks of age.

This gene not present in humans!

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MAMMalian Clones Often Have Serious Problems

Table 1. Literature survey of developmental problems in cloned animals

Species	Percentage healthy animals (healthy/total born)	Problems (% of reported problem cases) after birth	Follow-up period	Reference [a] Unpublished data
Cattle	100 (10/10)	None	4 weeks	1
	100 (2/2)	None	2 months	2
	100 (1/1)	None	7 months	3
	100 (1/1)	Diabetes (100). This animal survived into adulthood	8 months	4
	100 (5/5)	None	8–15 months	5
	80 (24/30)	Pulmonary hypertension, dilated cardiomyopathy (17)	1–4 years	6
	75 (3/4)	Internal hemorrhage umbilical artery (100)	NA	7
	66 (4/6)	Viral infection (50), dystocia (50)	10–12 months	8
	54 (13/24)	Dystocia (15), bacterial infection (8), kidney problems (42)	2–12 months	9
	50 (1/1)	Oversized, leg malformation (100)	NA	10
	50 (4/8)	Pneumonia (25), drawing in amniotic fluid (50), dystocia (25)	2–4 months	11
	44 (11/25)	Heart defects (57), liver fibrosis (29), pneumonia (7), osteoporosis (21), joint defects (14), anemia (42)	4 weeks	12
	40 (4/10)	None described	1 year	13
	25 (1/4)	Viral infection (66)	1 month	14
	0 (0/1)	Thymic atrophy, lymphoid hypoplasia (100)	NA	15
Sheep	100 (1/1)	None	6 years	16, 17 (K. Campbell)[a]
	100 (1/1)	None	3 weeks	18
	83 (5/8)	None described	3 years	19 (K. Campbell)[a]
	21 (3/14)	Kidney, liver, and brain defects	6 months	20
	0 (0/1)	Kidney and liver defects	NA	21
Goats	100 (3/3)	None	3 years	22 (E. Behboodi)[a]
	100 (5/5)	None	1 year	23
	50 (3/6)	Bacterial infection in the lungs (100)	1 year	24
Pigs	100 (1/1)	None	7 weeks	25
	100 (4/4)	None	1 week	26
	100 (2/2)	None	2 months	27
	100 (5/5)	None	9 months	28 (I. Colman)[a]
Mice	100 (8/8)	None	>3 months	29
	100 (4/4)	Obesity (100). This was not a lethal disorder	6 months	30
	100 (5/5)	Enlarged placenta (20)	6 months	31
	100 (6/6)	None	>2 months	32
	100 (3/3)	None	2 months	33
	99 (79/80)	None described	>3 months	34
	93 (15/16)	Umbilical hernia (100)	>3 months	35
	86 (19/22)	None described	>1 year	36
	40 (2/5)	Respiratory failure/umbilical hernia (40), failure to foster (20)	>3 months	37
	33 (1/3)	Respiratory failure (100)	>3 months	38
Total	**77** (259/335)			

Nature - January, 2002

IMPRINTING - Male/Female - Specific Gene Modifications!

Other TRANSGENIC ANIMALS Have Been created

TABLE 2.1 State of the art of transgenic technology for selected organisms.

Organism	Transfection	Viral vectors	Transposon	ES cells	Nuclear transfer
Mouse	4[a]	2	1	4[a]	2
Cow	3	1	0	0	2
Sheep	3	0	0	0	2
Goat	3	0	0	0	2
Pig	3	0	0	0	2
Rabbit	3	0	0	1	0
Chicken	1	2	1	0	0
Altlantic salmon	3	0	0	0	0
Channel catfish	2	0	0	0	0
Tilapia	3	0	0	0	0
Zebrafish	1	0	0	1	1
Crustaceans	1	1	0	0	0
Mollusks	1	1	0	0	0
Drosophila	2	2	2	2	0
Mosquito	1	0	2	0	0

NOTE: 0: No significant progress.
1: Has been accomplished experimentally (proof of concept).
2: Routine experimental use.
3: Commercialization sought.
4: Widespread production.
[a] For experimental uses.
See (Dove, 2000)

TRANSGENIC SALMON

control

superfish

Figure 8.11 Comparison of 1-month-old coho salmon siblings; nonengineered fish are at left, transgenic fish are at right. The largest fish (top right) is 41.8 cm in length.

GROWTH HORMONE GENE

What ARE THE ISSUES WITH THESE FISH?

TRANSGENIC FISH - A PROBLEM?

Transgenic Fish: A Boon or Threat?

ERIK STOKSTAD'S ARTICLE "ENGINEERED FISH: friend or foe of the environment?" (News Focus, 13 Sept., p. 1797) entertains the premise that the culture of transgenic fish, which grow two to six times faster than conventional fish, "might alleviate pressure on wild stocks." Two key points not addressed by Stokstad challenge this premise.

First, the culture of carnivorous species, such as salmon and trout, already represents a net drain on wild fish populations. Over 2 kg of wild fish are required to produce 1 kg of aquacultured conventional carnivorous fish (*1*). In North America and Europe, fish are usually reared in high densities and therefore rely completely on manufactured feeds for sustenance. Manufactured feeds for carnivorous species are typically composed of 35 to 50% fish meal and up to 20% fish oil (*1*). The accelerated growth rate of transgenic fish will necessitate an enormous increase in the ...age of feeds and their constituent marine feedstuffs. Fish meal and fish oil are typically made from menhaden and anchoveta harvested from the wild. As these species are already being exploited near their maximum sustainable levels (*2*), using more of them to create even more feed for transgenic fish can hardly be considered an easing of pressure.

Second, on the basis of the Law of Conservation of Matter, increased feed inputs will result in more outputs of waste in aquaculture effluents [e.g., (*3*)]. Reclamation of aquaculture waste is already problematic. In net-pen culture, for example, untreated wastes are expelled directly into the surrounding waters and commonly cause local eutrophication, buildup in sediments of feed-borne antibiotics, and benthic anoxia (*4*). Although the degree of these impacts depends on husbandry practices and the hydrodynamics of the site, the potential for serious environ...ntal damage will increase with the increased feed usage required by transgenic fish culture. Add the potential effects of interbreeding between transgenic escapees and wild fish discussed by Stokstad, and transgenic fish culture appears more threat than boon to the wild fishery.

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References
1. R. L. Naylor *et al.*, *Nature* **405**, 1917 (2000).
2. Food and Agricultural Organization (FAO), *The State of World Fisheries and Aquaculture 2000* (FAO, Rome, 2000).
3. H. Ackefors, M. Enell, *Ambio* **19**, 28 (1990).
4. British Columbia Environmental Assessment Office, *Salmon Aquaculture Review*, vol. 3 (British Columbia Environmental Assessment Office, Victoria, Canada, 1997).

Dealing with the Risks of Transgenic Fish

ERIK STOKSTAD'S ARTICLE "ENGINEERED FISH: friend or foe of the environment?" (News Focus, 13 Sept., p. 1797) correctly points out the risk to the environment associated with potential releases of genetically modified aquatic animals. This risk is a function of the specific genes, specific species and strain, and environment, and is independent of whether the genes came from genetic engineering, conventional breeding, or inadvertent selection.

The scientific research community must remain attentive to the details of how these very complex problems are being addressed. Researchers can become "collateral damage" to groups with agendas ranging from real environmental concern, to antitechnology, anti–genetically modified organism activists, to crass commercial interests.

In California, State Senator Byron Sher introduced legislation (*1*) SB 1525 that would have made it "unlawful to import, transport, possess... any live transgenic fish." When it was clear that this legislation would shut down many zebra fish researchers in California, it was amended to allow researchers to get a permit for noncommercial purposes only. This could still affect researchers by impacting zebra fish suppliers like Scientific Hatcheries and Exelixis, along with the added burden of another layer of permits. This bill with its amended variations and reincarnations posed a real risk to scientific research in California, before it was finally stopped for this year.

The proponents of a ban on transgenic fish (*2*) submitted a petition to the California Fish and Game Commission to adopt a moratorium on "transgenic" fish and stated that the moratorium would "specifically apply... [to] ornamental aquatic species, such as transgenic zebra fish." Senator Sher's letter of support (*3*) specified plans for "mass producing a transgenic form of these zebra fish" as "wrong." When the zebra fish research community heard about these plans and showed up at the Fish and Game Commission meeting on 29 August 2002, the proposal was defeated. Efforts are under way to find a solution to the real problem of unwanted gene movement in the environment, without impacting scientific research and other insignificant environmental risk situations.

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References and Notes
1. See info.sen.ca.gov/pub/bill/sen/sb_1501-1550/sb_1525_bill_20020220_introduced.html.
2. Letter to R. Treanor, California Fish and Game Commission by the Natural Resources Defense Council (NRDC), Institute for Fisheries Resources, Pacific Coast Federation of Fishermen's Associations (PCFFA) and The Ocean Conservancy, 23 July 2002.
3. Letter to M. Flores, California Fish and Game Commission, by State Senator Byron Sher, 30 July 2002.

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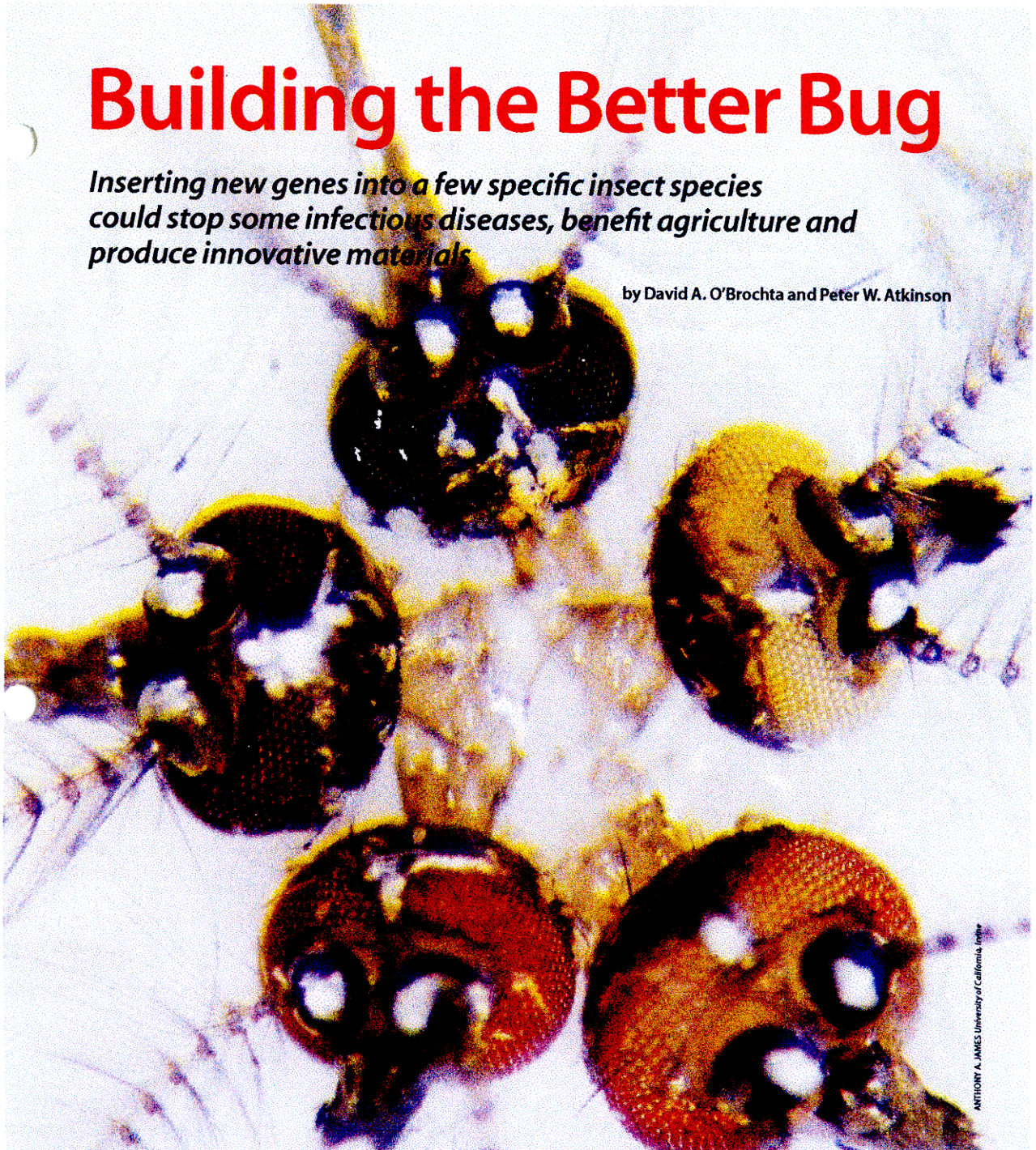
Building the Better Bug

Inserting new genes into a few specific insect species could stop some infectious diseases, benefit agriculture and produce innovative materials

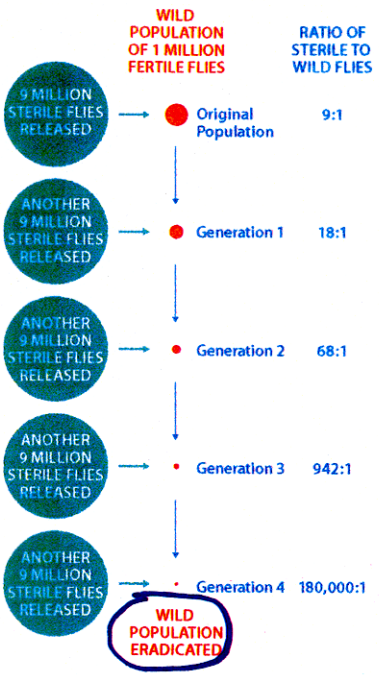
by David A. O'Brochta and Peter W. Atkinson



ANTHONY A. JAMES University of California, Irvine

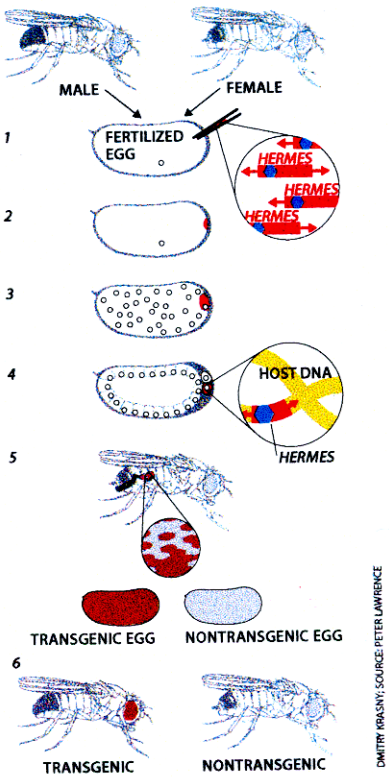
TRANSGENIC INSECTS can be given new characteristics, as illustrated by these five *Aedes aegypti* mosquitoes. Normal individuals have what appear to be black eyes, the result of large amounts of red pigment. A mutant version of *Ae. aegypti* has white eyes because of the lack of an enzyme, kynurenine hydroxylase, required to synthesize the red pigment. This white-eyed condition can be altered via the insertion of the gene for the enzyme. The resultant mosquitoes produce enough pigment to have visibly pink eyes. Such eye-color changes merely point out the potential of transgenic technology for producing a strain incapable of transmitting yellow fever or dengue.

TRANSGENIC INSECTS



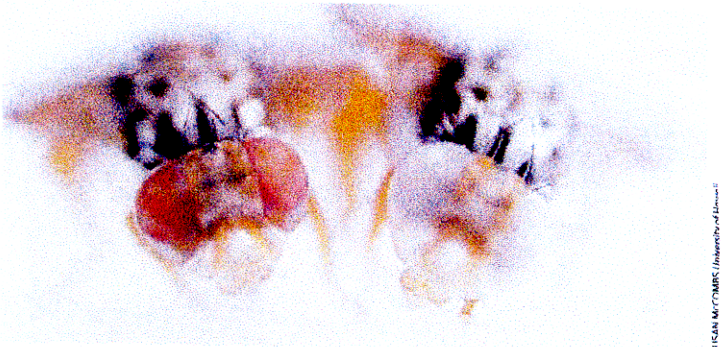
STERILE INSECT TECHNIQUE (SIT) can be an effective weapon against pests. Wave after wave of sterile insects, mostly males when possible, far outnumber the fertile members of the same species, and cause most matings to be fruitless. Within a few generations, the pest population is decimated. Traditional breeding programs have made for successful SIT interventions, but transgenic technology has the potential to streamline these procedures.

Has been done with non-transgenic flies!



DMITRY KRASNY; SOURCE: PETER LAWRENCE

MAKING TRANSGENIC INSECTS requires the insertion of a gene (*blue*), carried by a transposable element such as *Hermes* (*red*), into a fertilized egg (*1*). The new genetic material is strategically placed at the polar plasm (*2*), that section of the egg destined to become the still nascent insect's own egg cells when it reaches maturity. After numerous divisions of the egg's nuclear material (*3*), most of it segregates to the periphery, where it will become the nuclei of the cells of the insect's body; two nuclei, however, will migrate to the pole to become the insect's egg cells (*4*) when it reaches maturity (*5*). Should those cells have incorporated the transgene, progeny will be transgenic (*6*).



TRANSGENIC MEDFLY has its natural eye color restored. White-eyed mutants produce red pigment but cannot transport the pigment to the eyes. The red-eyed Medfly on the left is a transgenic that has been given the transposable element *piggyBac*, which is carrying a normal copy of the gene enabling pigment transport to the eye.

TRANSGENIC INSECTS

Sterile Mosquitos

Mosquitos that cannot harbor malaria protozoan

ISSUES?

Potential Risks of TRANSgenic Animals?

TABLE 5.1 Factors contributing to level of concern for species transformed.

Animal	Factor Contributing to Concern: Number of Citations[1]	Ability to Become Feral[2]	Likelihood of Escape Captivity[3]	Mobility[4]	Community Disruptions Reported[5]	Level of Concern[6]
Insects[8]	1804	High	High	High	Many	High
Fish[7]	186	High	High	High	Many	
Mice/ Rats	53	High	High	High	Many	
Cat	160	High	High	Moderate	Many	
Pig	155	High	Moderate	Low	Many	
Goat	88	High	Moderate	Moderate	Some	
Horse	93	High	Moderate	High	Few	
Rabbit	8	High	Moderate	Moderate	Few	
Mink	16	High	High	Moderate	None	
Dog	11	Moderate	Moderate	Moderate	Few	
Chicken	11	Low	Moderate	Moderate	None	
Sheep	27	Low	Low	Low	Few	Low
Cattle	16	Low	Low	Low	None	

[1] Number of scientific papers dealing with feral animals of this species.
[2] Based on number of feral populations reported.
[3] Based on ability of organism to evade confinement measures by flying, digging, swimming, or jumping ability for any of the life stages.
[4] Relative dispersal distance by walking, running, flying, swimming, or hitchhiking in trucks, trains, boats, etc.
[5] Based on worldwide citations reporting community damage and extent of damage.
[6] A ranking based on the four contributing factors.
[7] Did not include shellfish, some of which (such as zebra mussel and asiatic clam) have proven highly invasive.
[8] Limited to gypsy moth and Africanized honeybee.

could be Food issues as well - Pharma products

TRANSGENIC PLANTS/CROPS HAVE MUCH PROMISE FOR AGRICULTURE & MEDICINE

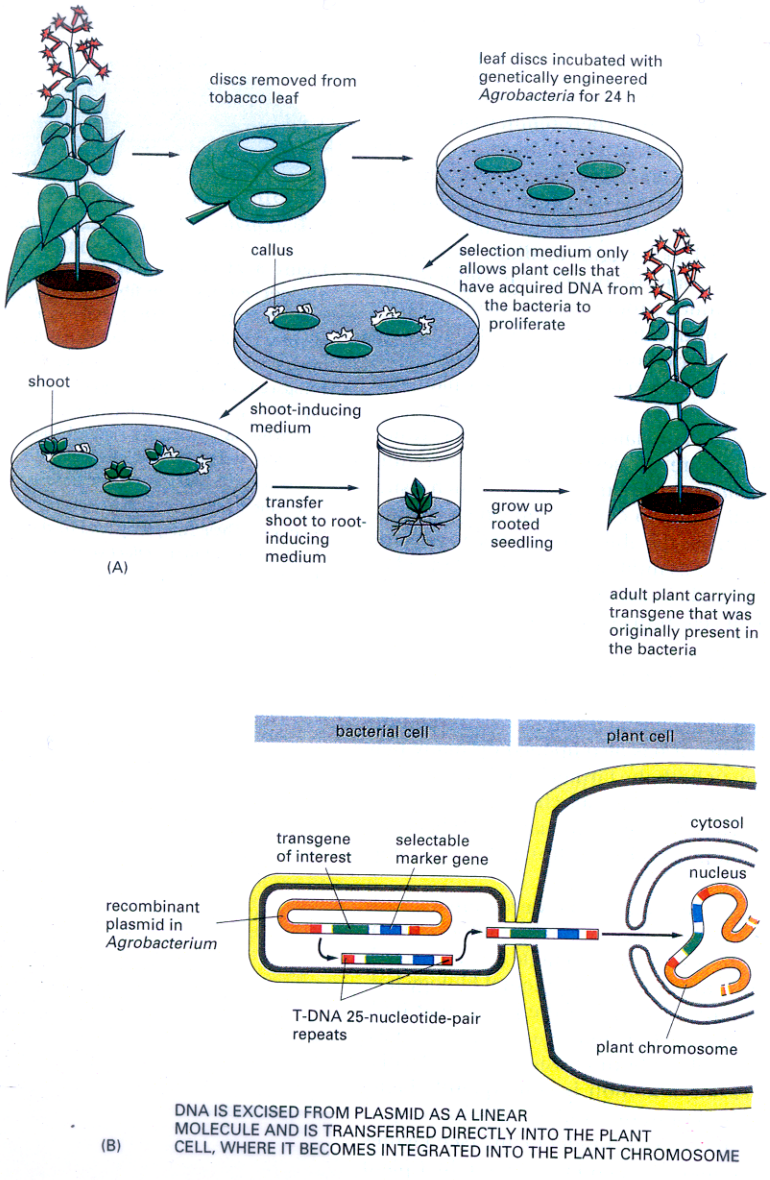


Figure 8–72 A procedure used to make a transgenic plant. (A) Outline of the process. A disc is cut out of a leaf and incubated in culture with *Agrobacteria* that carry a recombinant plasmid with both a selectable marker and a desired transgene. The wounded cells at the edge of the disc release substances that attract the *Agrobacteria* and cause them to inject DNA into these cells. Only those plant cells that take up the appropriate DNA and express the selectable marker gene survive to proliferate and form a callus. The manipulation of growth factors supplied to the callus induces it to form shoots that subsequently root and grow into adult plants carrying the transgene. (B) The preparation of the recombinant plasmid and its transfer to plant cells. An *Agrobacterium* plasmid that normally carries the T-DNA sequence is modified by substituting a selectable marker (such as the kanamycin-resistance gene) and a desired transgene between the 25-nucleotide-pair T-DNA repeats. When the *Agrobacterium* recognizes a plant cell, it efficiently passes a DNA strand that carries these sequences into the plant cell, using the special machinery that normally transfers the plasmid's T-DNA sequence.

Pharming in Plants

NICOTIANA BENTHAMIANA, a tobacco plant, serves as a biofactory for producing antibodies against cancer.

Advantages
1. Cost
2. Simplicity of method
3. Stability of proteins etc.

Table 14.5 A selection of pharmaceutical recombinant human proteins expressed in plant systems.

Species	Recombinant human product	Reference
Tobacco, sunflower (plants)	Growth hormone	Barta *et al.* 1986
Tobacco, potato (plants)	Serum albumin	Sijmons *et al.* 1990
Tobacco (plants)	Epidermal growth factor	Higo *et al.* 1993
Rice (plants)	α-Interferon	Zhu *et al.* 1994
Tobacco (cell culture)	Erythropoietin	Matsumoto *et al.* 1995
Tobacco (plants)	Haemoglobin	Dieryck *et al.* 1997
Tobacco (cell culture)	Interleukins-2 and 4	Magnuson *et al.* 1998
Tobacco (root culture)	Placental alkaline phosphatase	Borisjuk *et al.* 1999
Rice (cell culture)	α_1-Antitrypsin	Terashima *et al.* 1999
Tobacco (seeds)	Growth hormone	Leite *et al.* 2000
Tobacco (chloroplasts)	Growth hormone	Staub *et al.* 2000

Antigen	Host-plant system	Reference
Herpes virus B surface antigen	Tobacco	Mason *et al.* 1992
Rabies glycoprotein	Tomato	McGarvey *et al.* 1995
Norwark virus coat protein	Tobacco, potato	Mason *et al.* 1996
Foot-and-mouth virus VP1	*Arabidopsis*	Carrillo *et al.* 1998
Cholera toxin B subunit	Potato	Arakawa *et al.* 1998
Human cytomegalovirus glycoprotein B	Tobacco	Tackaberry *et al.* 1999

Table 14.7 A selection of recombinant vaccines against animal viruses produced in plants.

RE-ENGINEERING PLANTS AS DRUG FACTORIES

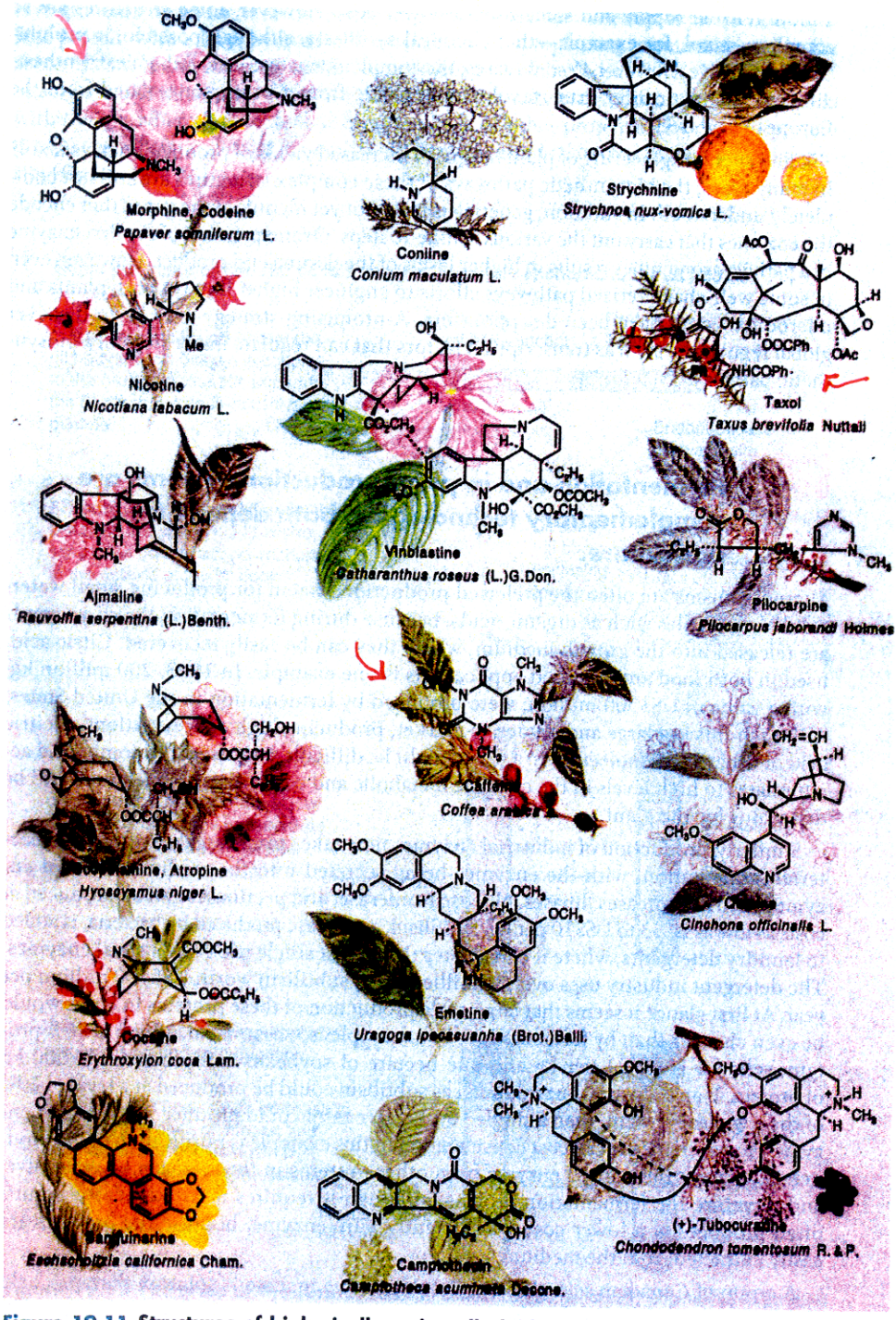


Figure 19.11 Structures of biologically active alkaloids and the plants that produce them. *Source:* Kutchan, T. M. 1995. Alkaloid biosynthesis—The basis for metabolic engineering of medicinal plants. *Plant Cell* 7:1059–1070.

RE-ENGINEERING PLANTS AS SOURCES OF SPECIALTY OILS

Table 19.4 Some specialty uses of plant fatty acids and oils

Lipid Type	Example	Major and Alternative Sources	Major Uses	Approx. U.S. Market Size (10^3 t)	10^6 US Dollars
Medium chain (C8–C14)	Lauric acid	Palm kernel, coconut, Cuphea	Detergents	640	320
Long chain (C22)	Erucic acid	Rapeseed, Crambe	Lubricants, nylon, plasticizers	30	80
Epoxy	Vernolic acid	Epoxidized soybean oil, Vernonia	Plasticizers	64	64
Hydroxy	Ricinoleic acid	Castor bean, Lesquerella	Lubricants, coatings	45	40
Trienoic	Linolenic acid	Flax	Coatings, drying agents	30	45
Low melting solid	Cocoa butter	Cocoa bean, illipe (*Shovea stenoptera*)	Chocolate, cosmetics	100	500
Wax ester	Jojoba oil	Jojoba	Lubricants, cosmetics	0.35	

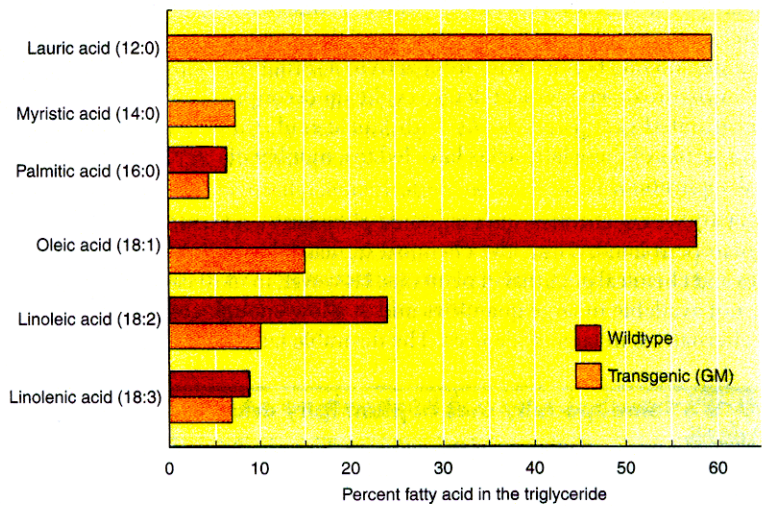


Figure 19.9 Genetic engineering of canola oil that is high in lauric acid, a fatty acid with 12 carbon atoms. By introducing a single gene from the California bay tree, the canola oil was changed from containing 60% oleic acid to 60% lauric acid. This new canola oil resembles the oil found in coconut and oil palm. *Source:* Courtesy of T. Voelker, Calgene/Monsanto.

PLANTS AS FACTORIES

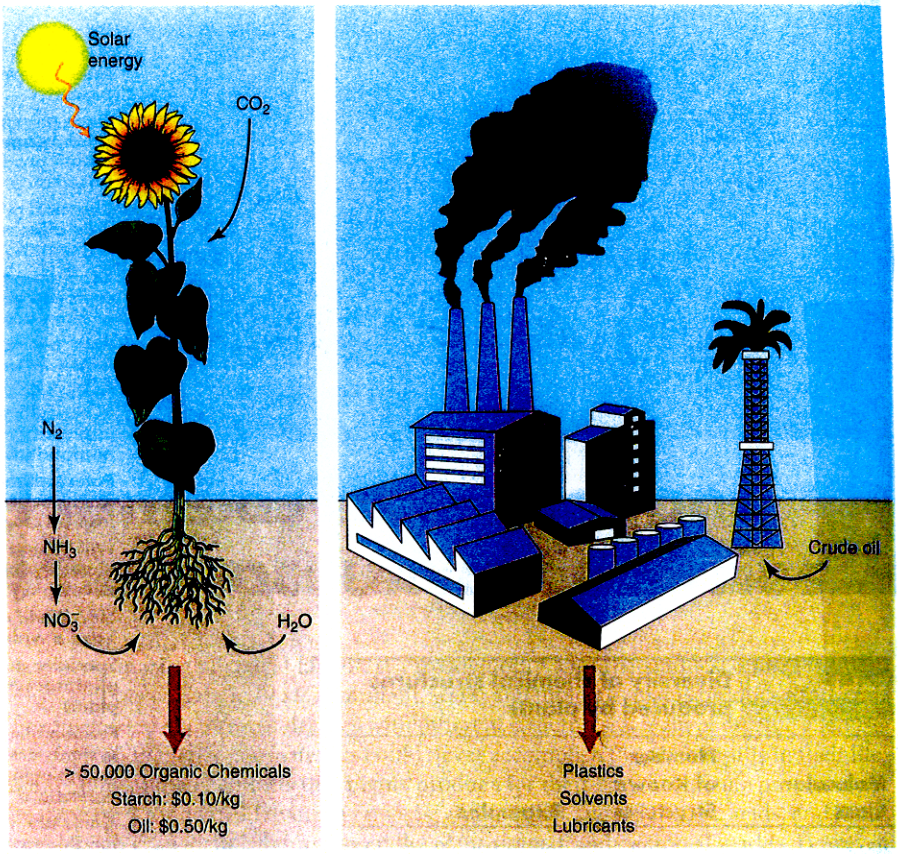


Figure 19.1 Can plants replace plants? In green plants the inputs are carbon dioxide and solar energy, in chemical plants the input is petroleum.

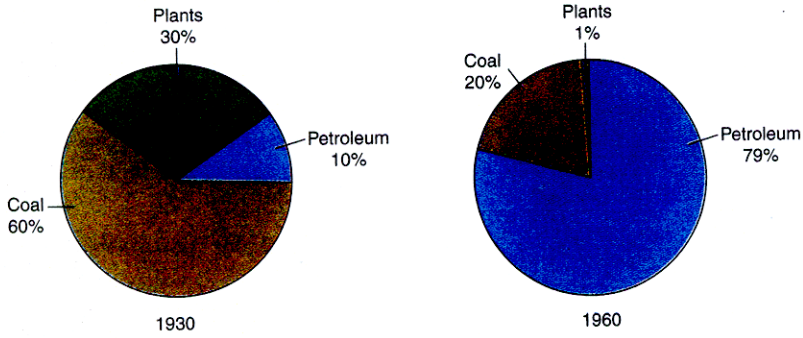


Figure 19.2 Change in the primary sources of industrial chemicals in the United States between 1930 and 1960. Note the rise of oil and the disappearance of plants and decreased importance of coal over this 30-year period. As of 2000, petroleum provides over 95% of organic chemicals used in the United States.

Using Plants for Environmental Detoxification

Feb 2000 Nature Biotechnology

Phytodetoxification of hazardous organomercurials by genetically engineered plants

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Methylmercury is a highly toxic, organic derivative found in mercury-polluted wetlands and coastal sediments worldwide. Though commonly present at low concentrations in the substrate, methylmercury can biomagnify to concentrations that poison predatory animals and humans. In the interest of developing an in situ detoxification strategy, a model plant system was transformed with bacterial genes (*merA* for mercuric reductase and *merB* for organomercurial lyase) for an organic mercury detoxification pathway. *Arabidopsis thaliana* plants expressing both genes grow on 50-fold higher methylmercury concentrations than wild-type plants and up to 10-fold higher concentrations than plants that express *merB* alone. An in vivo assay demonstrated that both transgenes are required for plants to detoxify organic mercury by converting it to volatile and much less toxic elemental mercury.

Bacteria isolated from organic mercury-contaminated environments possess two enzymes that convert methylmercury and other

Methylmercury

organomercurials to elemental mercury, [Hg(0)] (ref. 19). Elemental mercury is much less toxic than either Hg(II) or organic mercury and rapidly diffuses out of bacterial cells as a result of its volatility. The bacterial mercury-processing enzymes, organomercurial lyase (MerB) and mercuric reductase (MerA), catalyze the following reactions:

MerB — mercurial lyase

$$R\text{-}CH_2\text{-}Hg^+ + H^+ \rightarrow R\text{-}CH_3 + Hg(II)$$

MerA — mercuric reductase

$$Hg(II) + NADPH \rightarrow Hg(0) + NADP^+ + H^+$$

In theory, plants engineered with both genes should extract organomercurials from substrates and transpire Hg(0) into the atmosphere using the same mechanism as bacteria (Fig. 1). Because the atmospheric residence time of Hg(0) is about two years, it can be diluted to trace concentrations before redepositing into the terrestrial substrate[16]. Furthermore, the quantity of mercury released from polluted sites can be regulated and will, in all likelihood, be small in comparison with the atmospheric mercury load (~4 × 10^6 kg) (ref. 20).

Also Explosives!

WEEDS AND PATHOGENS REDUCE CROP YIELDS

Figure 17.7 Hand hoeing of weeds. Hand hoeing is backbreaking and time consuming but is still the primary means of weed control in developing countries. This couple in the Luang Prabang province of Laos is weeding upland rice. Note the numerous weeds among the young rice plants. If not removed at this stage the yield will be lost. *Source:* Courtesy of Eugene Hettel, International Rice Research Institute.

Table 16.1 Crop losses in farming from insect and mite pests worldwide

	% Crop Losses		
Crop	**1965**	**1988–1990**	**Change in Loss[a]**
Barley	3.9	8.8	+4.9
Maize	13.0	14.5	+1.5
Cotton	16.0	15.4	−0.6
Potatoes	5.9	16.1	+10.2
Rice	27.5	20.7	−6.8
Soybeans	4.4	10.4	+6.0
Wheat	5.1	9.3	+4.2
Average	10.8	13.6	+2.8

[a] Change in percentage losses (1988–1990 minus 1965). Includes losses due to viruses transmitted by insect vectors.

Source: Modified from N. Duck and S. Evola (1997), Use of transgenes to increase host plant resistance to insects: Opportunities and challenges, in N. Carozzi and M. Koziel, eds., *Advances in Insect Control: The Role of Transgenic Plants* (Bristol, PA: Taylor & Francis), p. 8.

PATHOGENIC MICROBES DESTROY CROPS!

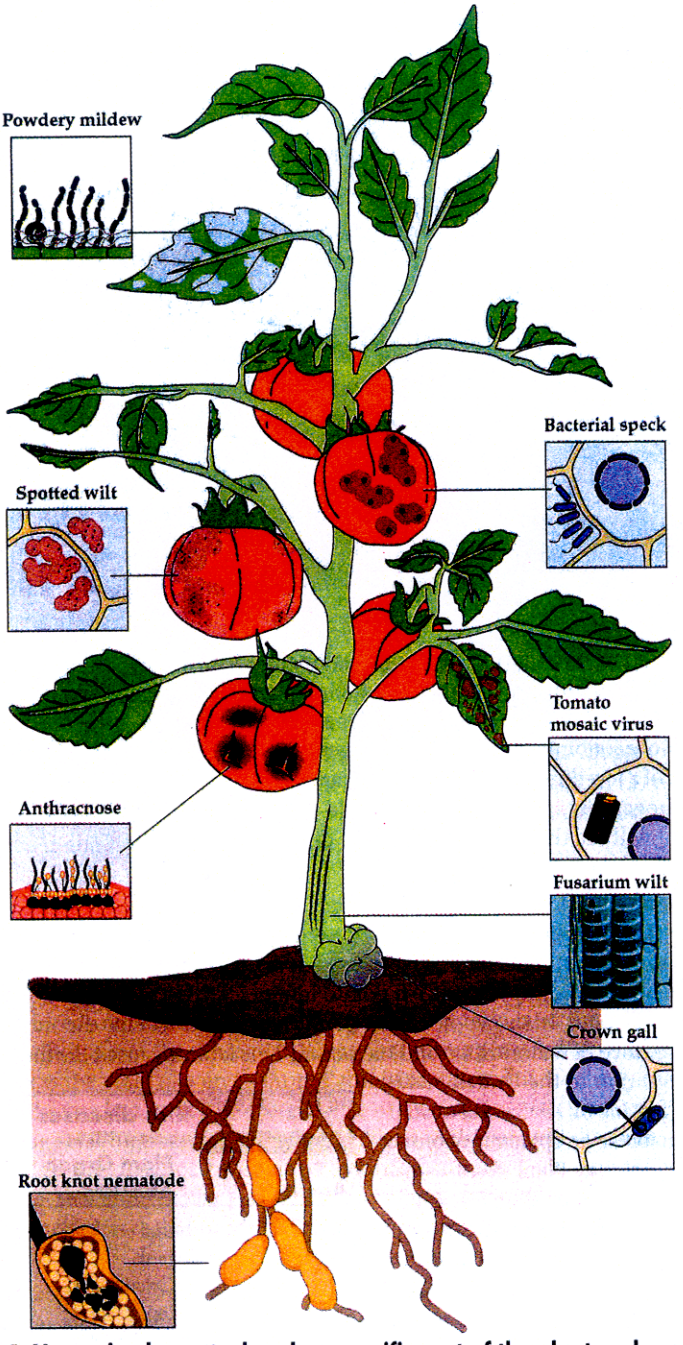

Figure 15.1 Most microbes attack only a specific part of the plant and produce characteristic disease symptoms. Tomato, shown here, can be attacked by more than 100 different pathogenic microorganisms. *Source:* B. B. Buchanan, W. Gruissem, and R. L. Jones, eds. (2000), *Plant Biochemistry and Molecular Biology* (Rockville, MD: American Society of Plant Physiologists), p. 1104.

OPPOSITION TO GENETICALLY MODIFIED PLANTS

1. Ideology - Don't CHANGE Nature (Politics)
2. Anti-Technology - Symbol for technology being central in western society - Anti-Science
3. Anti-Market - Globalization - Industry taking over food supply
4. Protectionism - American Agro companies out competing European Agro companies - First generation "Losers" -
5. Anti-Eugenics - Experience in WW II
6. Organic Growers
7. Ecology - Genetically Modified Crops / PLANTS out competing "natural" species
8. Do not Need in West - Personal Control / Liberty - Labeling
9. No Obvious Consumer Benefit
10. Easy Target for Anti-gene Technology
11. Lack of Confidence in Government - NO FDA, EPA, USDA - Symbol of all "disasters" - BSE, Bophal, etc.