HC 70A

Winter 2003

Professor Bob Goldberg

Learning Unit #4

Turning Cells and Organisms into Factories

THEMES/CONCEPTS

1-1.5 hr lecture

2/12/03

1. TRANSgenic Bacteria
2. TRANSgenic Fungi
3. TRANSgenic Animals & Concerns
4. TRANSgenic Plants & Concerns

Genetic Engineering & Recombinant DNA ARE USED IN A VARIETY OF Applications

Similar CLASSES of Applications CAN be Engineered in Several organisms

1 TRANSGENIC Animals

4 DNA, RNA, Oligonucleotides

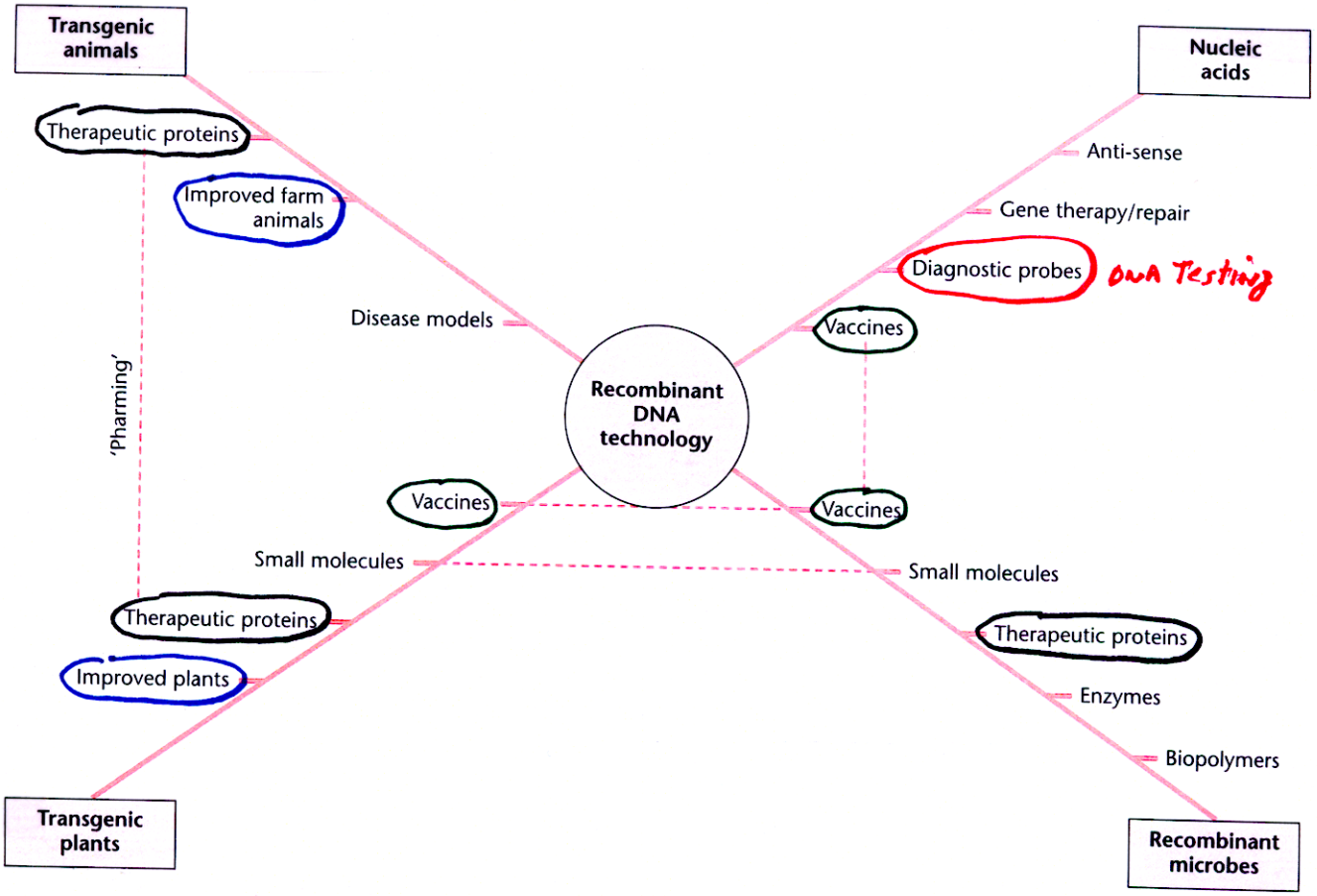


Fig. 14.1 The different ways that recombinant DNA technology has been exploited.

TRANSGENIC PLANTS

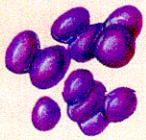
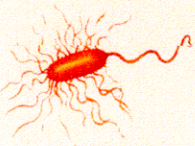
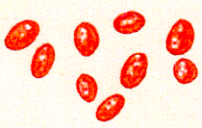
2

TRANSGENIC Microbes – Bacteria & Fungi

3

Using Bacteria as Factories

Table 34.1 Bacteria

Major Group	Typical Examples		Key Characteristics
			ARCHAEBACTERIA
Archaebacteria	Methanogens, thermophiles, halophiles		Bacteria that are not members of the kingdom Eubacteria. Mostly anaerobic with unusual cell walls. Some produce methane. Others reduce sulfur.
			EUBACTERIA
Actinomycetes	*Streptomyces*, *Actinomyces*		Gram-positive bacteria. Form branching filaments and produce spores; often mistaken for fungi. Produce many commonly used antibiotics, including streptomycin and tetracycline. One of the most common types of soil bacteria; also common in dental plaque.
Chemoautotrophs	Sulfur bacteria, *Nitrobacter*, *Nitrosomonas*		Bacteria able to obtain their energy from inorganic chemicals. Most extract chemical energy from reduced gases such as H_2S (hydrogen sulfide), NH_3 (ammonia), and CH_4 (methane). Play a key role in the nitrogen cycle.
Cyanobacteria	*Anabaena*, *Nostoc*		A form of photosynthetic bacteria common in both marine and freshwater environments. Deeply pigmented; often responsible for "blooms" in polluted waters.
Enterobacteria	*Escherichia coli*, *Salmonella*, *Vibrio*		Gram-negative, rod-shaped bacteria. Do not form spores; usually aerobic heterotrophs; cause many important diseases, including bubonic plague and cholera.
Gliding and budding bacteria	Myxobacteria, *Chondromyces*		Gram-negative bacteria. Exhibit gliding motility by secreting slimy polysaccharides over which masses of cells glide; some groups form upright multicellular structures carrying spores called fruiting bodies.
Pseudomonads	*Pseudomonas*		Gram-negative heterotrophic rods with polar flagella. Very common form of soil bacteria; also contain many important plant pathogens.
Rickettsias and Chlamydias	*Rickettsia*, *Chlamydia*		Small, gram-negative intracellular parasites. *Rickettsia* life cycle involves both mammals and arthropods such as fleas and ticks; *Rickettsia* are responsible for many fatal human diseases, including typhus (*Rickettsia prowazekii*) and Rocky Mountain spotted fever. Chlamydial infections are one of the most common sexually transmitted diseases.
Spirochaetes	*Treponema*		Long, coil-shaped cells. Common in aquatic environments; a parasitic form is responsible for the disease syphilis.

BACTERIAL FACTORIES FOR DRUGS

INSULIN

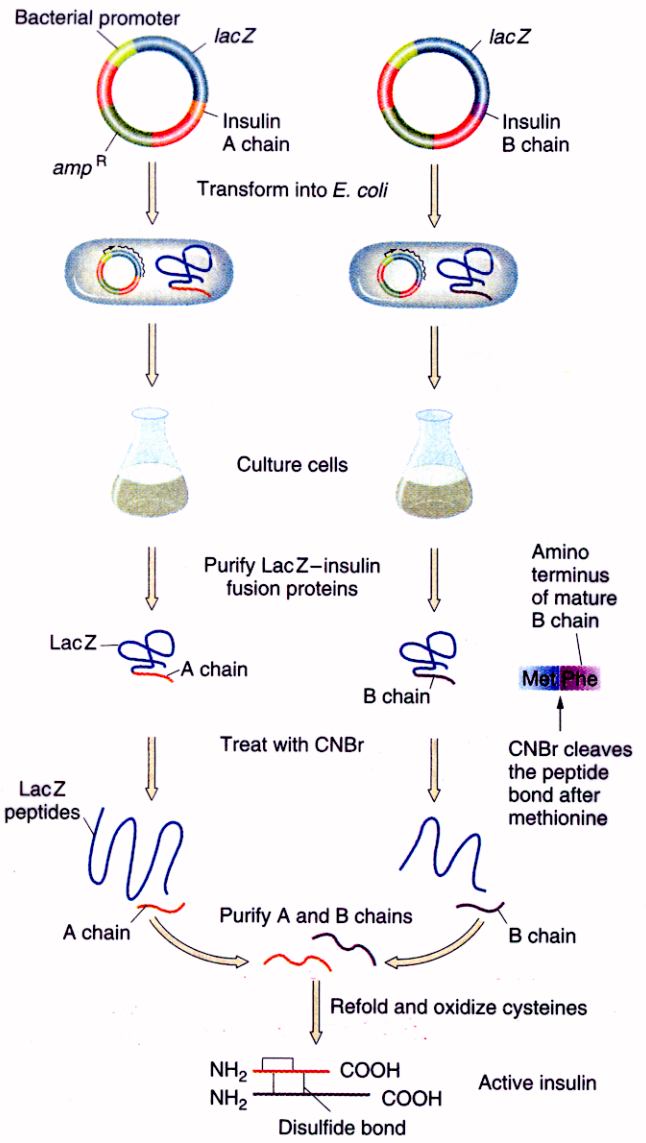


Figure 13-6 Expression of human insulin in *E. coli*. The two chains of insulin are made separately as fusion proteins with β-galactosidase. They are processed chemically and then mixed, and active insulin forms. (Copyright © 1992 by J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright © Scientific American Books.)

(+) MANY other Protein CLASSES — ENZYMES for Food Processing, etc.

GROWTH HORMONE

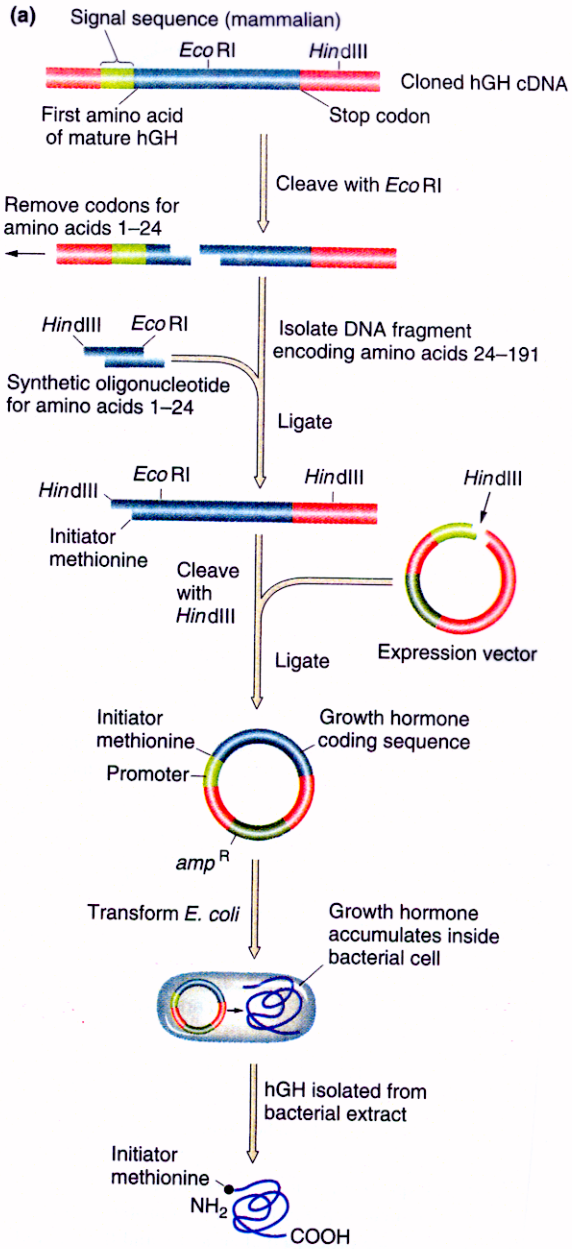


Figure 13-7 Expression of human growth hormone (hGH) in *E. coli*. (a) The human signal sequence is removed, enabling the protein to be produced in bacterial cells. The product contains an extra bacterial methionine. (b) A bacterial signal sequence that targets the protein for secretion to the outside can be added. In this method, the product has no extra methionine. (Copyright © 1992 by J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright © Scientific American Books.)

Human Therapeutic Proteins Made in Bacteria

Protein	Application	Current status
Human growth hormone (somatotropin)	Pituitary dwarfism	Approved for sale
Human insulin	Diabetes	Approved for sale
Interferon-α2b	Hairy cell leukaemia, genital warts and other applications	Approved for sale
Interferon-α2a	Hairy cell leukaemia, Kaposi's sarcoma and other applications	Approved for sale
Erythropoietin (EPO)	Anaemia associated with kidney dialysis and AZT treatment of AIDS	Approved for sale
Tissue plasminogen activator	Myocardial infarction	Approved for sale
Hepatitis B coat protein	Vaccination	Approved for sale
Granulocyte colony stimulating factor	Neutropenia arising from cancer chemotherapy	Approved for sale
Interleukin-2	Cancer therapy	Approved for sale
Consensus interferon	Cancer therapy	Late clinical trials
Interferon-γ	Rheumatoid arthritis and cancer therapy	Late clinical trials
Interferon-β	AIDS therapy	Late clinical trials
Superoxide dismutase	Free radical damage of reperfusion, renal transplants	Late clinical trials
Factor VIII	Haemophilia	Late clinical trials
Lung surfactant protein	Respiratory distress syndrome	Late clinical trials
Tumour necrosis factor	Cancer therapy	Clinical trials
Epidermal growth factor	Healing of ulcers	Clinical trials
Fibroblast growth factor	Healing of ulcers	Clinical trials
Relaxin	Facilitation of childbirth	Early clinical trials

1978 List

Proteins Used to Treat Human Disorders Made By Recombinant DNA

Table 10.1 Some human proteins that have been produced by recombinant DNA technology for treating various disorders

Protein	Disorder(s)
α_1-Antitrypsin	Emphysema
Adrenocorticotropic hormone	Rheumatic diseases
B-cell growth factors	Immune disorders
Bactericidal/permeability-increasing protein	Infections
Brain-derived neurotrophic factor	Amyotrophic lateral sclerosis (Lou Gehrig's disease)
Calcitonin	Osteomalacia
Colony-stimulating factors	Cancer
Chorionic gonadatropin	Female infertility
Endorphins and enkephalins	Pain
Epidermal growth factor	Burns
Erythropoietin	Anemia, kidney disorders
Factor VIII	Hemophilia
Factor IX	Hemophilia
Growth hormone	Growth defects
Growth hormone-releasing factor	Growth defects
Hemoglobin	Anemia
Insulin	Diabetes
Insulin-like growth factor	Diabetes, renal failure
Interferons (α, β, γ)	Viral diseases, cancer, multiple sclerosis
Interleukins	Cancer, immune disorders
Interleukin-1 receptor	Asthma, rheumatoid arthritis
Lymphotoxin	Cancer
Macrophage-activating factor	Cancer
Nerve growth factor	Nerve damage
Platelet-derived growth factor	Atherosclerosis
Relaxin	Birthing
Serum albumin	Insufficient plasma proteins
Somatomedin C	Growth defects
Thyroid-stimulating hormone	Thyroid cancer
Tissue plasminogen activator	Blood clots
Tumor necrosis factor	Cancer
Urogastrone	Ulcers
Urokinase	Blood clots

Table 10.2 Some recombinant proteins that have been approved for human use by the U.S. Food and Drug Administration

Compound	Company	Disorder
Antihemophilic factor	Miles, Baxter Healthcare, Genetics Institute	Hemophilia A
DNase I	Genentech	Cystic fibrosis
Erythropoietin	Amgen and Ortho Biotech	Anemia, kidney disease
Glucocerebrosidase	Genzyme	Gaucher disease
Growth hormone	Genentech	Growth hormone deficiency in children
Insulin	Eli Lilly	Diabetes
IFN-α_{2a}	Hoffmann-La Roche	Hairy cell leukemia, Kaposi sarcoma
IFN-α_{2b}	Schering-Plough	Hairy cell leukemia, genital warts, Kaposi sarcoma, hepatitis B and C
IFN-α_{n3}	Interferon Sciences	Genital warts
IFN-β_{1b}	Berlex Laboratories and Chiron	Relapsing multiple sclerosis
IFN-γ_{1b}	Genentech	Chronic granulomatous disease
Interleukin-2	Chiron	Renal cell carcinoma
Somatotropin	Eli Lilly	Growth hormone deficiency
Tissue plasminogen activator	Genentech	Acute myocardial infarction, acute massive pulmonary embolism

Other Uses of Proteins Synthesized in Engineered Bacteria

Table 5.2 Examples of Microbial Enzymes and Their Uses

Enzyme	Uses
Lipase	Enhances flavor in cheese making
Lactase	Breaks down lactose to glucose and galactose; lactose-free milk products
Protease	Detergent additive; hydrolyzes suspended protease proteins in beer that form during brewing for a less cloudy chilled beer
α-amylase	Used in production of high fructose corn syrup
Pectinase	Degrades pectin to soluble components, reduces cloudiness in chilled wine, fruit juice
Tissue plasminogen activator (TPA)	Dissolves blood clots

LARGE BIOREACTORS & FERMENTORS ARE NEEDED TO GROW RECOMBINANT BACTERIA FOR LARGE SCALE Protein PRODUCTION

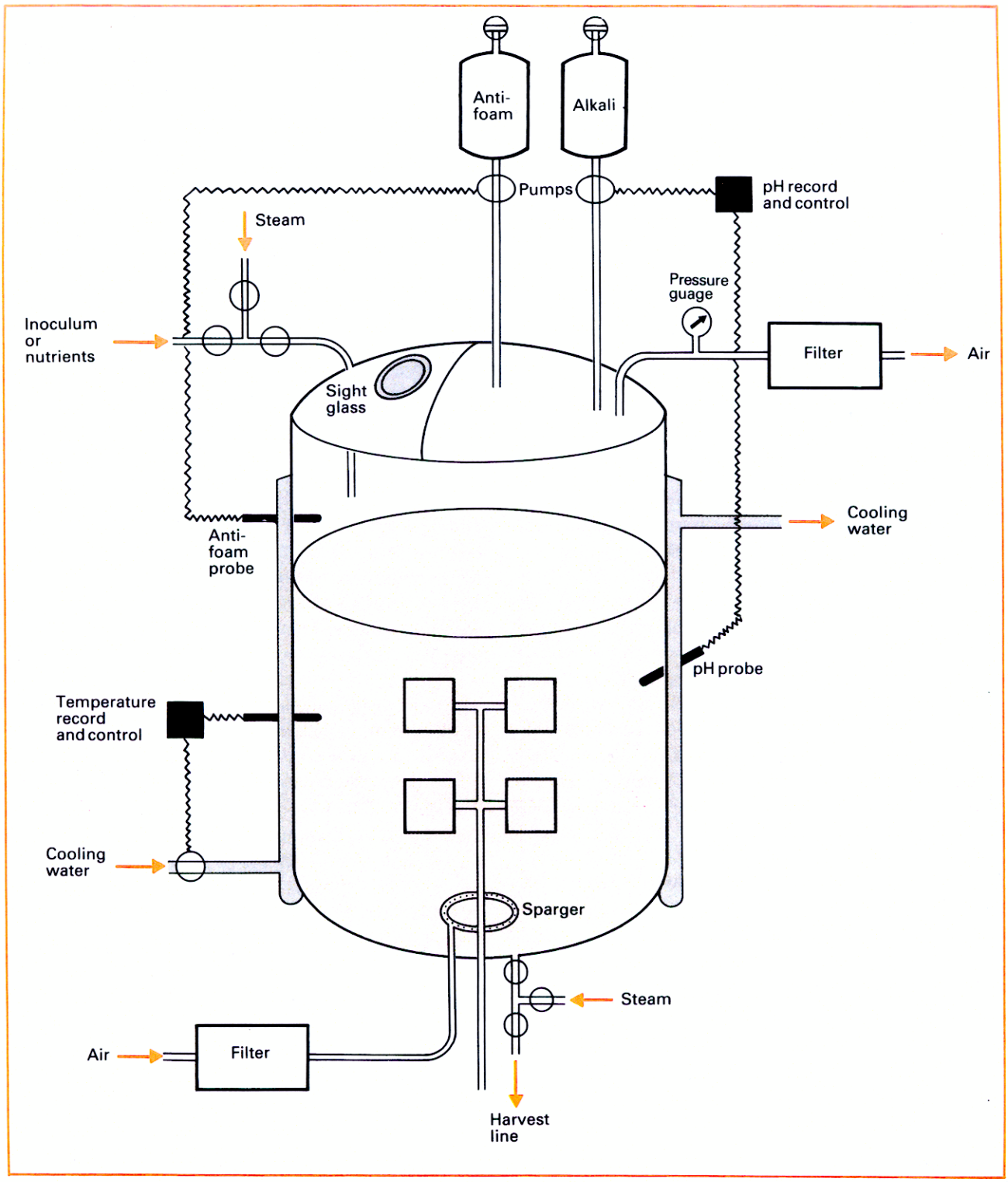


Fig. 5.4 Schematic representation of a stirred tank reactor. For clarity no seal is shown between the agitator shaft and the fermenter body and baffles have been omitted.

INDUSTRIAL-SCALE PROCESSES HAVE BEEN DEVELOPED TO COLLECT BACTERIAL CELLS + ISOLATE HUMAN PROTEINS

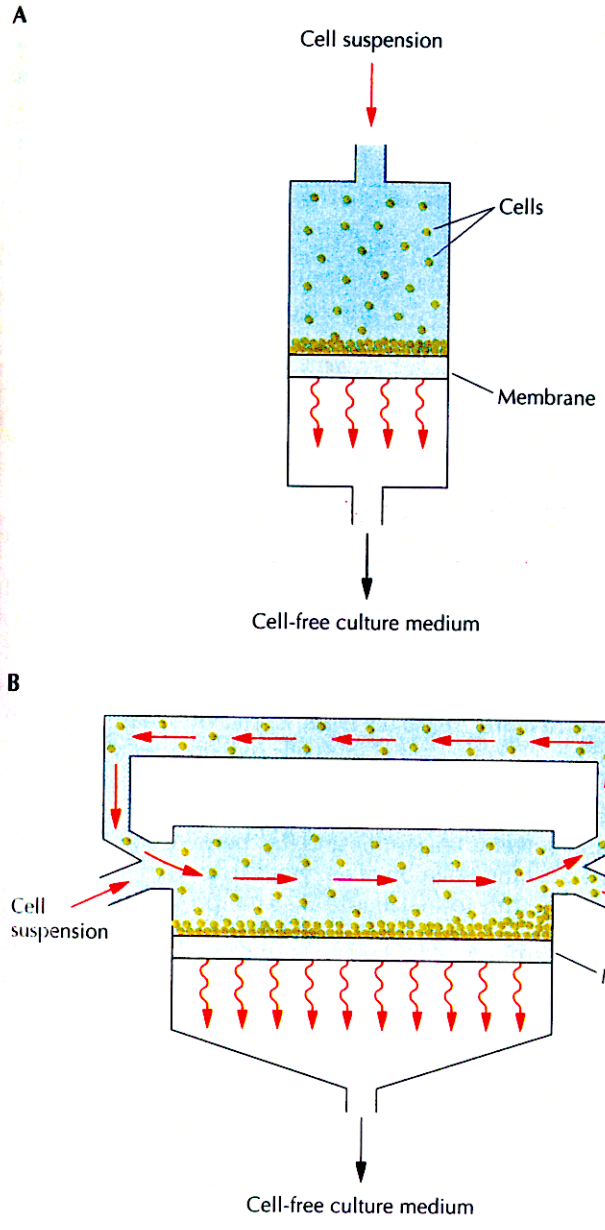


Figure 16.7 Membrane filtration systems for concentrating microbial cells. **A.** Dead-end filtration. **B.** Cross-flow filtration. Arrows within each unit show the direction of the liquid flow.

Specific Human Protein Purification e.g. insulin

→ MUST BE 100% Pure for Drug Use + FDA Approval!!

Microbes - Including Bacteria - HAVE MANY Useful Metabolites

Table 6.2 Some applications of microbial cells.

Organism	Application
Bacillus thuringiensis and related organisms	Microbial insecticide
Lactobacillus sp., *Streptococcus cremoris* and related species	Starter cultures for the manufacture of dairy products, e.g. yoghurt, cheese
Penicillium roquefortii and related species	Inocula for the production of blue-veined cheeses
Rhizobium sp.	Inoculants for adding to legume seeds to promote nodulation and nitrogen fixation
Pseudomonas syringae	Creation of artificial snow. Ice-nucleation-defective mutants for the prevention of frost damage to crops
Many different organisms	Single-cell protein production

Table 6.8 Sources and applications of some microbial enzymes.

Enzyme	Source	Applications
α-amylase	*Aspergillus oryzae*	Preparation of glucose syrups
	Bacillus amyloliquefaciens	Removal of starch sizes
	Bacillus licheniformis	Liquefaction of brewing adjuncts
β-glucanase	*Aspergillus niger*	Liquefaction of brewing adjuncts
	Bacillus amyloliquefaciens	Improvement of malt for brewing
Glucoamylase	*Aspergillus niger*	Starch hydrolysis
	Rhizopus sp.	
Glucose isomerase	*Arthrobacter* sp.	High-fructose corn syrup
	Bacillus sp.	
Lactase	*Kluyveromyces* sp.	Removal of lactose from whey
Lipase	*Candida lipolytica*	Flavour development in cheese
Pectinase	*Aspergillus* sp.	Clarification of wines and fruit juices
Penicillin acylase	*Escherichia coli*	Preparation of 6-aminopenicillanic acid
Protease, acid	*Aspergillus* sp.	Calf rennet substitute
Protease, alkaline	*Aspergillus oryzae*	Detergent additive
	Bacillus sp.	Dehairing of hides
Protease, neutral	*Bacillus amyloliquefaciens*	Liquefaction of brewing adjuncts
	Bacillus thermoproteolyticus	
Pullulanase	*Klebsiella aerogenes*	Starch hydrolysis

Table 6.7 Commercially available microbial polysaccharides and their uses.

Polysaccharide	Producing organism	Uses
Xanthan gum	*Xanthomonas campestris*	**1** Food additive for stabilizing liquid suspensions and gelling soft foods, e.g. ice cream, cheese spreads **2** Lubrication in, for example, toothpaste preparations **3** Enhanced oil recovery
Gellan	*Pseudomonas* sp.	**1** Solidification of food products
Emulsan	*Acinetobacter calcoaceticus*	**1** Cleaning oil spills
	Arthrobacter	**2** Enhanced oil recovery
Pullulan	*Aureobasidium pullulans*	**1** Biodegradable material for food coating and packaging
Dextrans	*Leuconostoc mesenteroides*	**1** Blood expander **2** Adsorbents for pharmaceutical preparations

BACTERIAL METABOLIC PATHWAYS CAN BE ENGINEERED TO OPTIMIZE PRODUCTION OF NOVEL INDUSTRIAL PRODUCTS

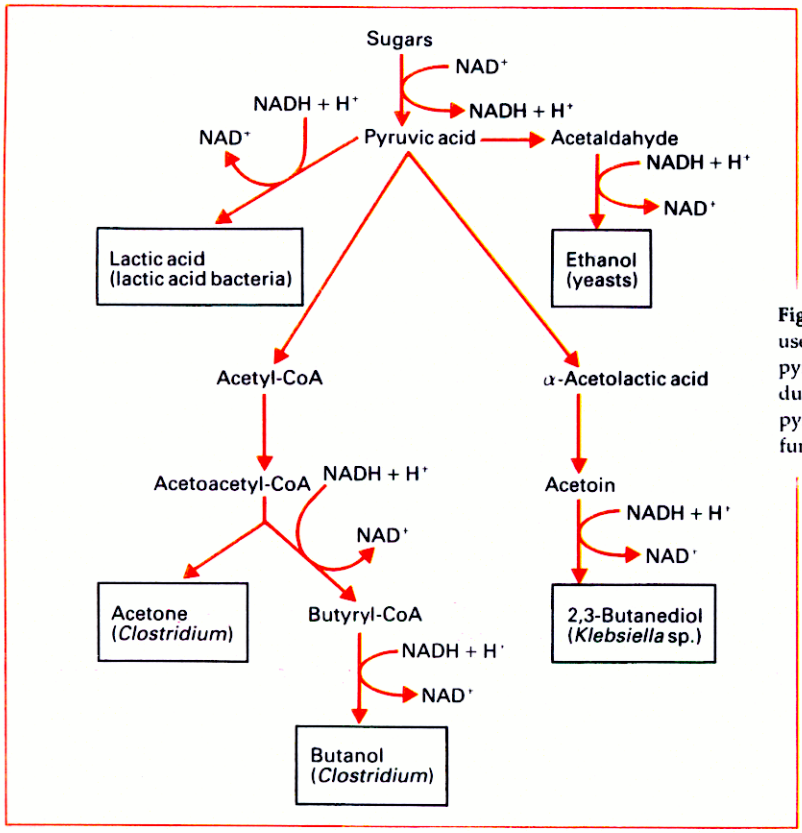


Fig. 6.5 The formation of commercially useful metabolic end-products. Note that pyridine nucleotide cofactors are reduced during the conversion of sugars to pyruvate and subsequently oxidized by further metabolism of pyruvate.

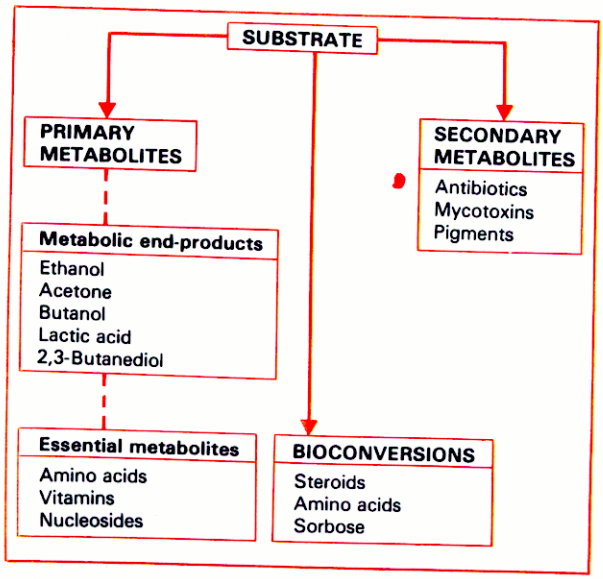


Fig. 6.4 The different classes of low-molecular-weight compounds synthesized by microorganisms.

These pathways can be optimized +/or changed by adding genes on plasmids that encode novel enzymes

e.g. Maxygen ®
gene shuffling
protein evolution

Useful Bacterial Metabolites that can be Engineered

Table 5.1 Examples of Primary and Secondary Metabolites Produced by Fermentation

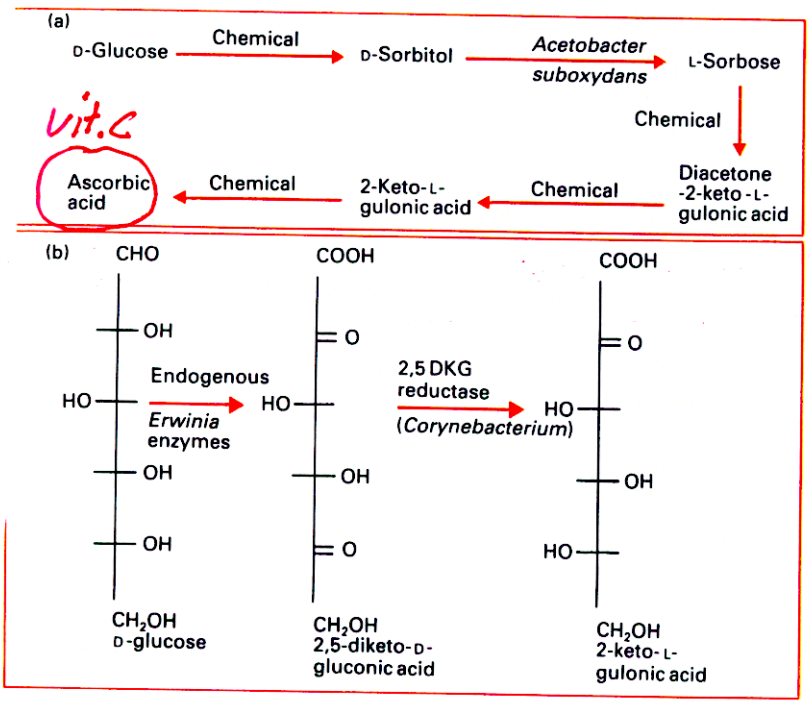
Primary Metabolites	Secondary Metabolites
Amino acids	Antibiotics
Vitamins	Pigments
Nucleotides	Toxins
Polysaccharides	Alkaloids
Ethanol	Many active pharmacological compounds (e.g., the immunosuppressor cyclosporin, hypotensive compound dopastin)
Acetone	
Butanol	
Lactic acid	

Organic Chemical	Microbial Sources	Selected Uses
Acetic acid	*Acetobacter*	Industrial solvent and intermediate for many organic chemicals, food acidulant
Acetone	*Clostridium*	Industrial solvent and intermediate for many organic chemicals
Acrylic acid	*Bacillus*	Industrial intermediate for plastics
Butanol	*Clostridium*	Industrial solvent and intermediate for many organic chemicals
2,3-Butanediol	*Aerobacter, Bacillus*	Intermediate for synthetic rubber manufacture, plastics and antifreeze
Ethanol	*Saccharomyces*	Industrial solvent, intermediate for vinegar, esters and ethers, beverages
Formic acid	*Aspergillus*	Textile dyeing, leather treatment, electroplating, rubber manufacture
Fumaric acid	*Rhizopus*	Intermediate for synthetic resins, dyeing, acidulant, antioxidant
Glycerol	*Saccharomyces*	Solvent, plasticizer, sweetener, explosives manufacture, printing, cosmetics, soaps, antifreeze
Glycolic acid	*Aspergillus*	Textile processing, pH control, adhesives, cleaners
Isopropanol	*Clostridium*	Industrial solvent, cosmetic preparations, antifreeze, inks
Lactic acid	*Lactobacillus, Streptococcus*	Food acidulant, dyeing, intermediate for lactates, leather treatment
Methylethyl ketone	*Chlamydomonas*	Industrial solvent, intermediate for explosives and synthetic resins
Oxalic acid	*Aspergillus*	Printing and dyeing, bleaching agent, cleaner, reducing agent
Propylene glycol	*Bacillus*	Antifreeze, solvent, synthetic resin manufacture, mold inhibitor
Succinic acid	*Rhizopus*	Manufacture of lacquers, dyes and esters for perfumes

optimize using genetic Engineering

MICROBES CAN BE ENGINEERED TO PRODUCE IMPORTANT MOLECULES that were Made Previous By Chemical Reactions

e.g. → VITAMIN C synthesis



Chemical

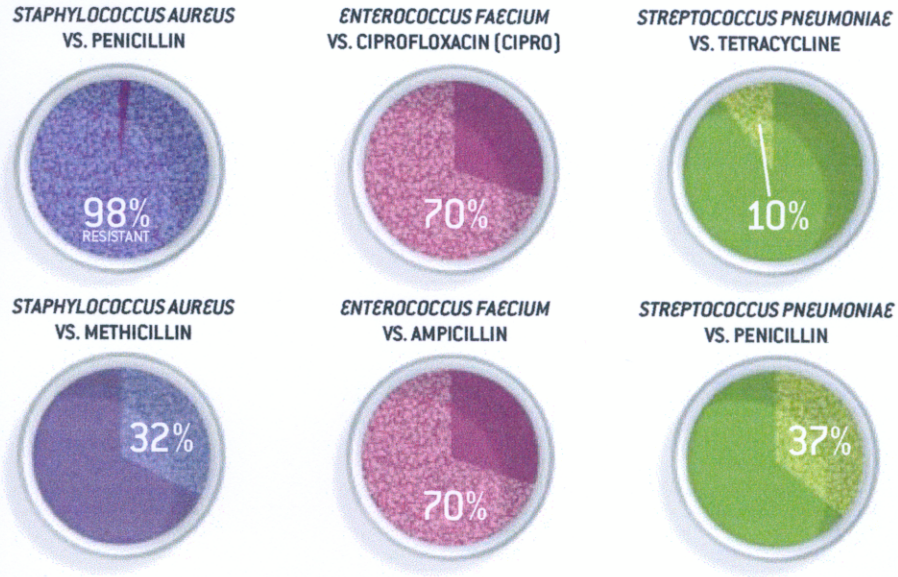
Biology-based

Fig. 6.12 Simplified route to vitamin C (ascorbic acid) developed by cloning in *Erwinia* the *Corynebacterium* gene for 2,5-diketogluconic acid reductase. (a) Classical route to vitamin C. (b) The simplified route to 2-ketogulonic acid, the immediate precursor of vitamin C.

Antibiotic Resistance is a major problem

RISING RESISTANCE

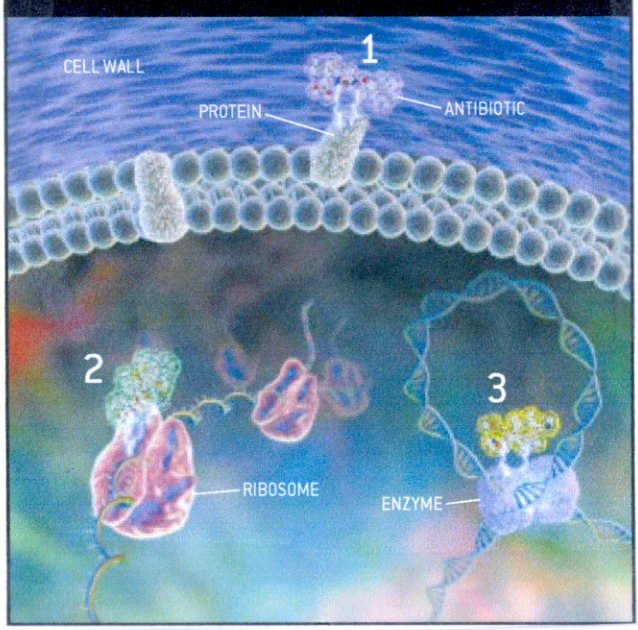
MANY ANTIBIOTICS are no longer effective against certain strains of bacteria, as these examples—collected from different hospitals in the late 1990s—show. One strain of *Staphylococcus aureus* found in Korea, for instance, is 98 percent resistant to penicillin (*top left*); another, found in the U.S., is 32 percent resistant to methicillin (*bottom left*). All these strains are not resistant to vancomycin, for now.



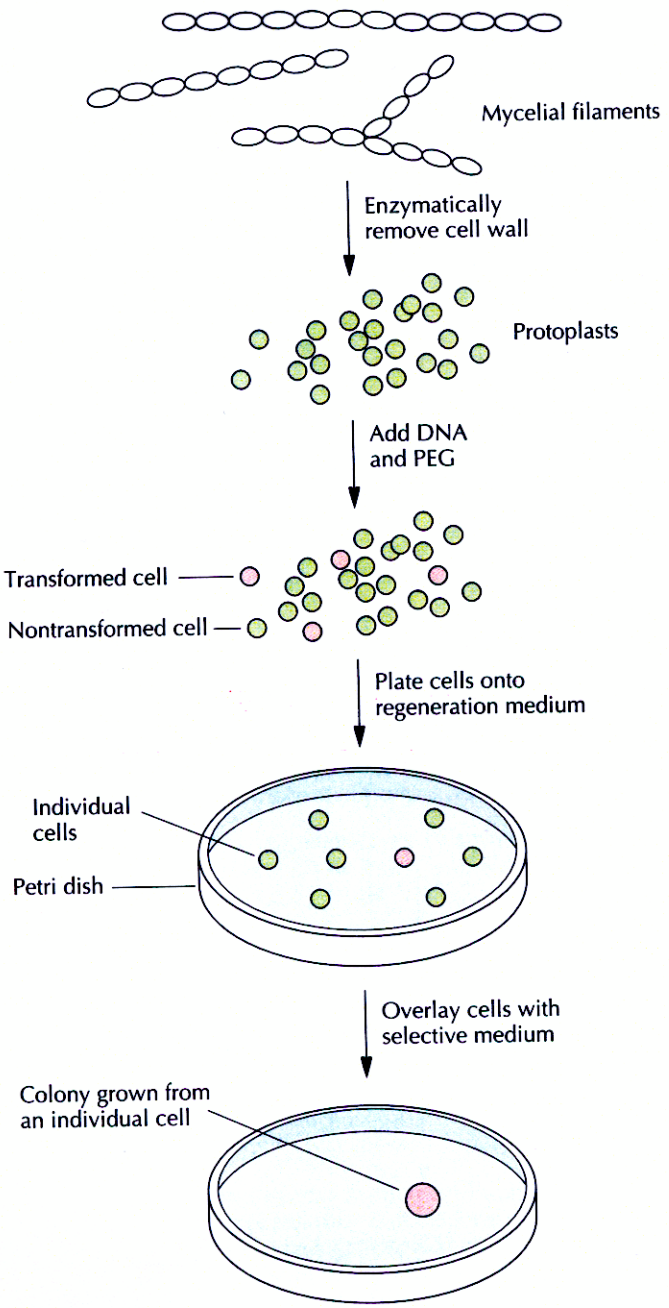
How Antibiotics Work

ANTIBIOTICS AT WORK

EXISTING ANTIBIOTICS fight infections by preventing bacteria from making essential substances. Vancomycin and ß-lactam antibiotics interfere with synthesis of the cell wall (*1*). Erythromycin and tetracycline disrupt ribosomes that make proteins (*2*). Quinolone antibiotics inhibit enzymes involved in replicating DNA (*3*), and sulfonamide antibiotics also interfere with DNA synthesis (*not shown*).



Novel Antibiotics CAN BE Engineered in Bacteria



Streptomyces

↓

Streptomycin!

Figure 12.9 Schematic representation of DNA transformation and selection of transformants of *Streptomyces* strains. The pink circles represent transformed cells, and the green circles represent nontransformed cells. PEG, polyethylene glycol.

BACTERIA CAN BE Engineered TO HAVE NOVEL Degradative PATHWAYS FOR BIOREMEDIATION

PLASMIDS

Table 13.1 *Pseudomonas* plasmids, their degradative pathways, and their sizes

Name of plasmid	Compound(s) degraded	Plasmid size (kb)
SAL	Salicylate	60
SAL	Salicylate	72
SAL	Salicylate	83
TOL	Xylene and toluene	113
pJP1	2,4-D	87
pJP2	2,4-D herbicide	54
pJP3	2,4-D	78
CAM	Camphor	225
XYL	Xylene	15
pAC31	3,5-Dichlorobenzoate	108
pAC25	3-Chlorobenzoate	102
pWWO	Xylene and toluene	176
NAH	Naphthalene	69
XYL-K	Xylene and Toluene	135

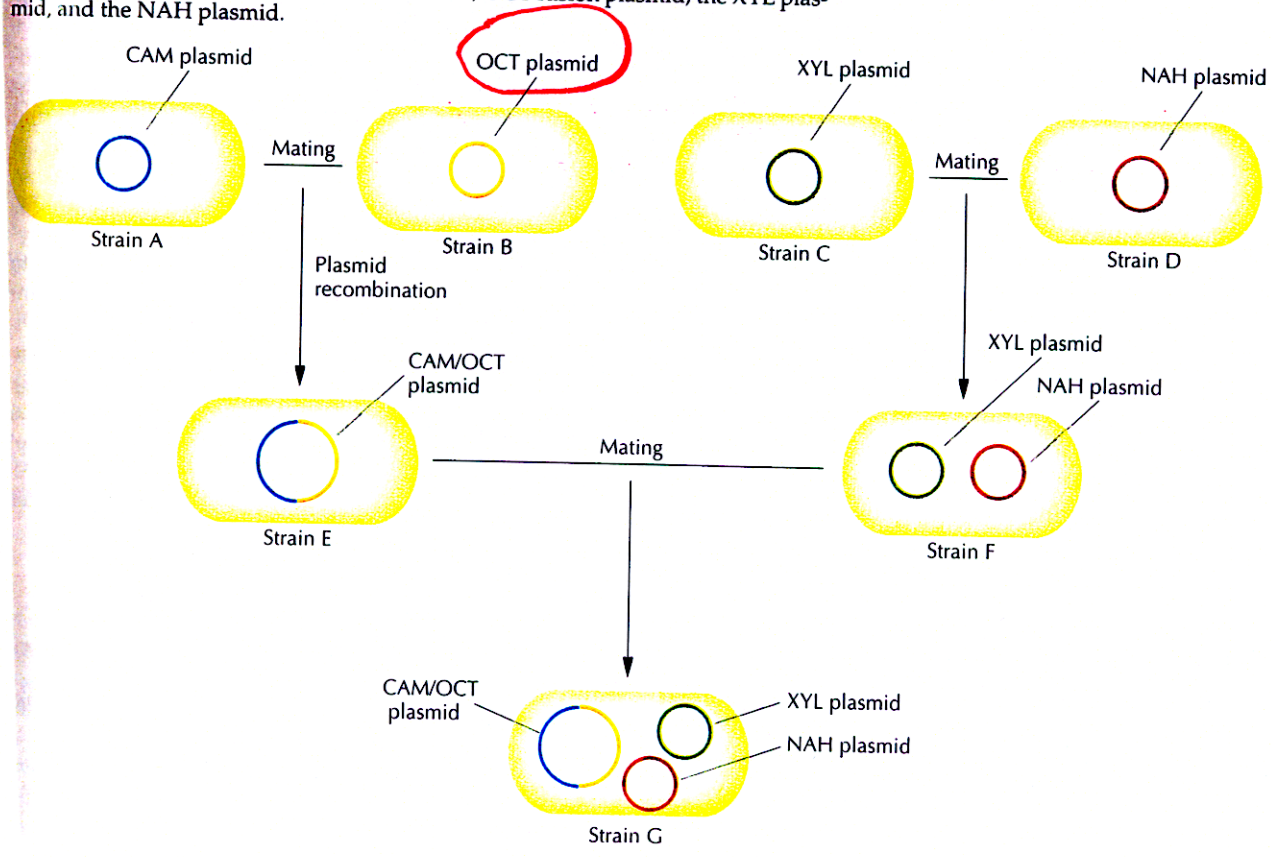
Adapted from Cork and Krueger, *Adv. Appl. Microbiol.* **36**:1–66, 1991.

Plasmids with the same name encode a similar degradative pathway even though they have different sizes and were described in different laboratories. 2,4-D, 2,4-dichlorophenoxyacetic acid.

BACTERIA CAN BE Engineered to degrade Several Different "toxic" Compounds

PSEUDOMONAS

Figure 13.5 Schematic representation of the development of a bacterial strain that can degrade camphor, octane, xylene, and naphthalene. Strain A, which contains a CAM (camphor-degrading) plasmid, is mated with strain B, which carries an OCT (octane-degrading) plasmid. Following plasmid transfer and homologous recombination between the two plasmids, strain E carries a CAM and OCT biodegradative fusion plasmid. Strain C, which contains a XYL (xylene-degrading) plasmid, is mated with strain D, which contains a NAH (naphthalene-degrading) plasmid, to form strain F, which carries both of these plasmids. Finally, strain E and strain F are mated to yield strain G, which carries the CAM/OCT fusion plasmid, the XYL plasmid, and the NAH plasmid.



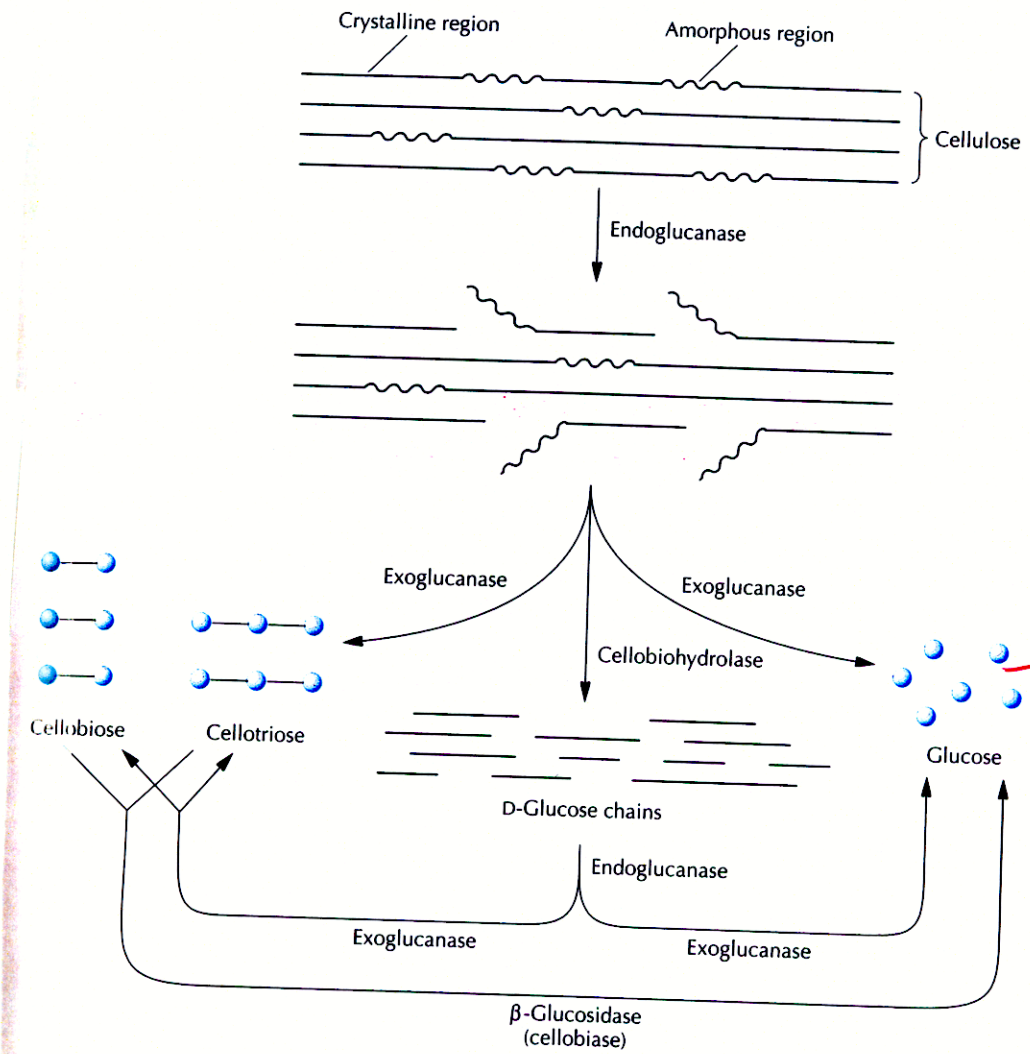
LANDMARK DECISION

CHAKRABARTY US PATENT 4,259,444 1981
genetically engineered MICROORGANISMS ARE "inventions"

BIOTEC INDUSTRY

BACTERIA CAN BE Engineered To degrade BIOMASS WASTE Products

WASTE containing cellulose



Energy for Bacteria

Figure 13.15 Enzymatic biodegradation of cellulose. Cellulose hydrolysis begins with the cleavage of β-1,4-linkages within the accessible amorphous regions of the cellulose chains by endoglucanase(s). This reaction is followed by the removal of oligosaccharides from the reducing ends of the partially cleaved cellulose chains by exoglucanase(s) and cellobiohydrolase(s). The degradation of cellulose is completed when the cellobiose and cellotriose are converted to glucose by β-glucosidase.

Agriculture, Timber Processing, Human Activities:
e.g.) plants left after harvests, animal manure with grasses, municipal waste paper, cotton left-overs, hay, etc

Bacteria

Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments

Hassan Brim[1], Sara C. McFarlan[2], James K. Fredrickson[3], Kenneth W. Minton[1], Min Zhai[1], Lawrence P. Wackett[2], and Michael J. Daly[1]*

RESEARCH ARTICLES

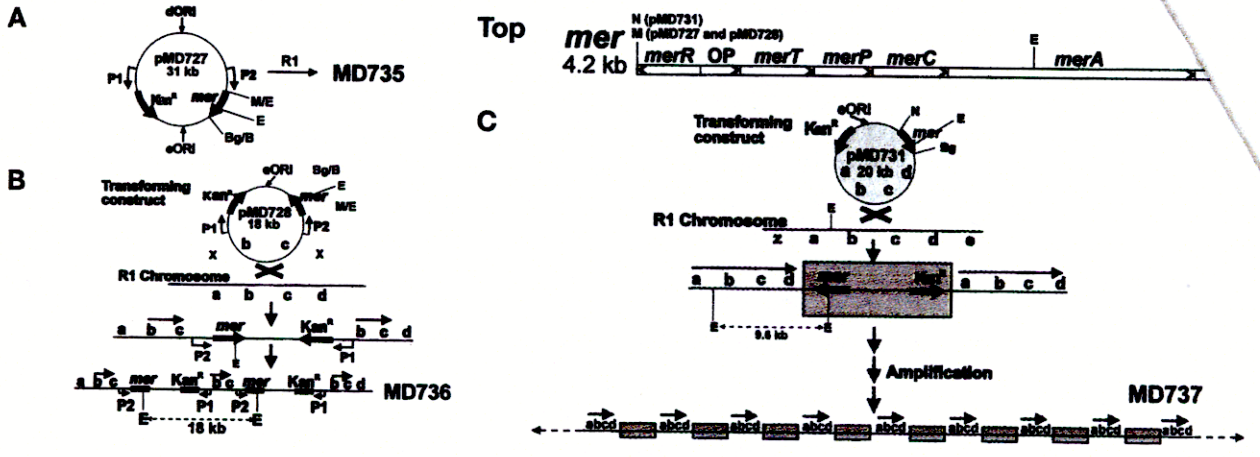


Figure 1. Plasmid and chromosomal maps. (A) 4.2-kb *mer* operon of pBD7[24] encodes six proteins: MerR, activation/repression of the *mer* operon; MerT, mercuric ion transport protein; MerP, periplasmic mercuric ion binding protein; MerC, transmembrane protein; MerA, mercuric reductase; and MerD, putative secondary regulatory protein. OP, operator/ promoter sequence; M, *MfeI*; N, *NcoI*; E, *EcoRI*; Bg, *BglII*. (A) pMD727 was transformed into *D. radiodurans* strain R1 by selection with kanamycin (Kan), giving MD735. dORI, deinococcal origin of replication[18]; eORI, *E. coli* origin of replication[18]. P1 and P2 are two different constitutive deinococcal promoters[21,25]. Kan^R, kanamycin resistance gene *aphA*; *mer*, mercury operon. Bg/B, *BglII*/*BamHI* fusion; M/E, *MfeI*/*EcoRI* fusion. (B) pMD728 was transformed into strain R1 with *Km* selection, giving MD736. Two rounds of recombinative duplication are illustrated, yielding two vector copies on a chromosome. bc, duplicated chromosomal target sequence; X, *Xba*1; all other abbreviations and symbols, as in A. (C) pMD731 was transformed into strain R1 with *Km* selection, giving MD737. Several rounds of recombinative duplication are illustrated, yielding many insertions per chromosome. abcd, duplicated chromosomal target sequence; all other abbreviations and symbols, as in A and B above.

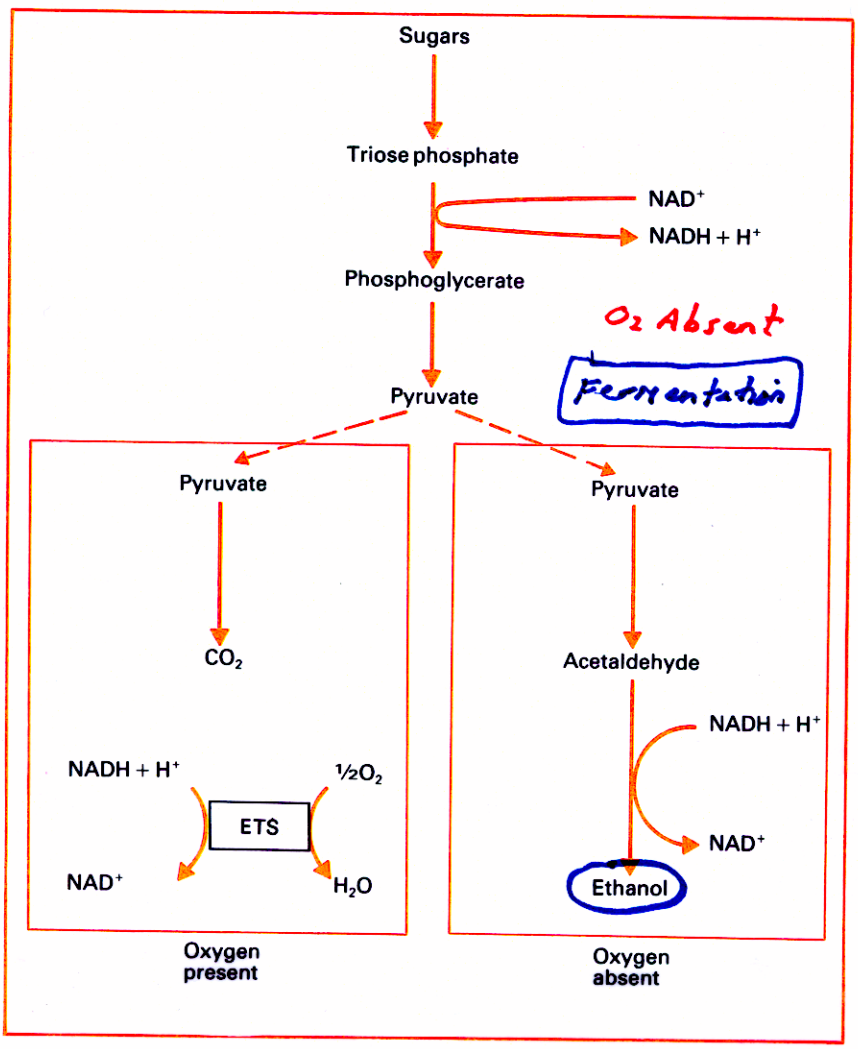
Hg(II) → Hg(0) → Vapor

Metal Chelate in Soil

USING YEAST AS FACTORIES AND "CATALYSTS"

Table 36.1 Fungi

Phylum	Typical Examples		Key Characteristics	Approximate Number of Living Species
Ascomycota	Yeasts, truffles, morels		Develop by sexual means; ascospores are formed inside a sac called an ascus; asexual reproduction is also common	32,000
Imperfect fungi	*Aspergillus*, *Penicillium*		Sexual reproduction has not been observed; most are thought to be ascomycetes that have lost the ability to reproduce sexually	17,000
Basidiomycota	Mushrooms, toadstools, rusts		Develop by sexual means; basidiospores are borne on club-shaped structures called basidia; the terminal hyphal cell that produces spores is called a basidium; asexual reproduction occurs occasionally	22,000
Zygomycota	*Rhizopus* (black bread mold)		Develop sexually and asexually; multinucleate hyphae lack septa, except for reproductive structures; fusion of hyphae leads directly to formation of a zygote, in which meiosis occurs just before it germinates	1050



USING YEAST TO MAKE ALCOHOLIC BEVERAGES

Table 6.5 The origins of the different kinds of alcoholic beverages.

Alcoholic beverage	Origin
Non-distilled	
Beer	On germination, starch in barley grains is converted to sugar, which is extracted by boiling in water to produce wort and this is fermented
Cider	Fermentation of apple juice
Wine	Fermentation of grape juice
Sake	Starch in steamed rice is hydrolysed with *Aspergillus oryzae* and the sugars released are fermented with yeast
Distilled	
Whisky (Scotch)	Distillation of alcohol produced from barley
Whiskey—Irish	Pot still whiskey produced from alcohol derived from a mixture of barley, wheat and rye. Grain whiskey produced from alcohol derived from maize
—Rye	Produced from alcohol derived from rye
—Bourbon	Produced from alcohol derived from maize
Rum	Distillation of fermented molasses, a by-product of sugar cane refining
Vodka	Distillation of alcohol produced from any non-grain carbohydrate source, e.g. potatoes
Gin	Distillation of alcohol derived from maize or rye and redistillation in presence of herbs and juniper berries
Tequila	Distillation of fermented extracts of Mexican cactus

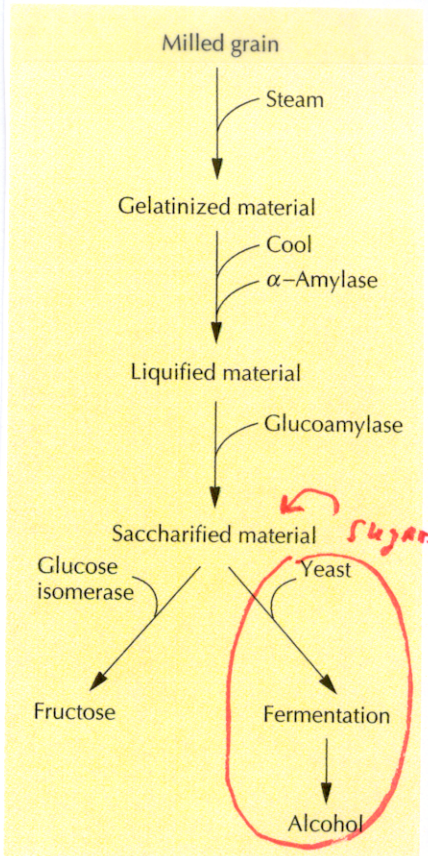


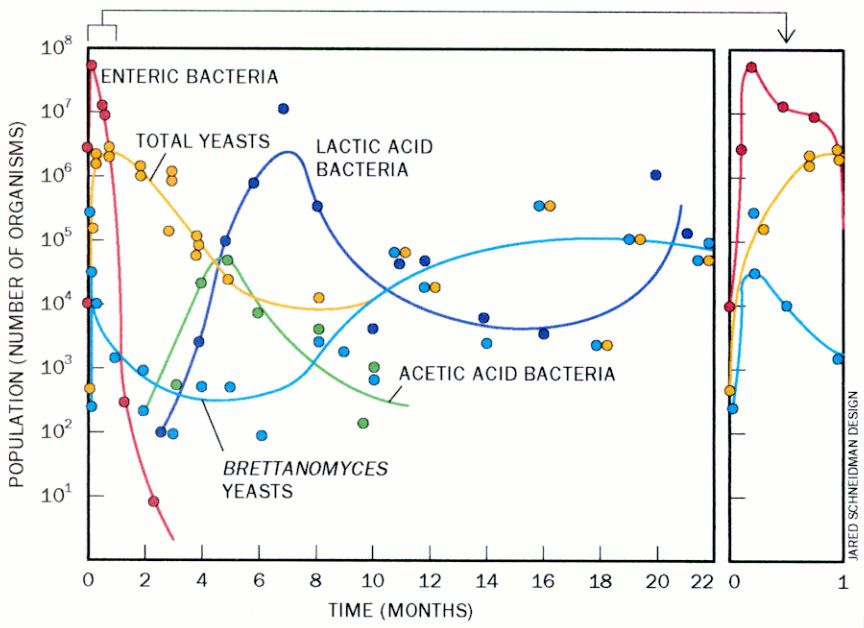
Figure 13.10 Industrial production of fructose and alcohol from starch.

FIGURE 9.10
How wine is made. The conversion of pyruvate to ethanol takes place naturally in grapes left to ferment on vines, as well as in fermentation vats of crushed grapes. Yeasts carry out the process, but when their conversion increases the ethanol concentration to about 12%, the toxic effects of the alcohol kill the yeast cells. What is left is wine.

YEASTS ARE USED IN BEER MAKING

taneously fermented, like lambic, with wild yeasts only. A brew called *sikaru,* for instance, was produced 5,000 years ago by Sumerians in Mesopotamia. Instead of hops, of which they had no

HIGHLY AROMATIC LAMBICS are always served, in their native Belgium, in glasses designed to convey their aromas. Fruit lambics, such as cherry, peach, raspberry and plum, are usually poured into snifters or flutes. More traditional gueuze and faro are often served in tumblers.



LAMBIC FERMENTATION encompasses the rise and fall of many different populations of yeast and bacteria in four basic stages. In the first, enteric bacteria and wild yeasts predominate and break down glucose into ethanol, carbon dioxide and acids. Then various yeasts create additional ethanol. In stage three, lactic and acetic bacteria make more of these acids. Finally, *Brettanomyces,* a yeast genus, creates the many esters that make the beer uniquely aromatic.

ANAEROBIC FERMENTATION BY yeasts

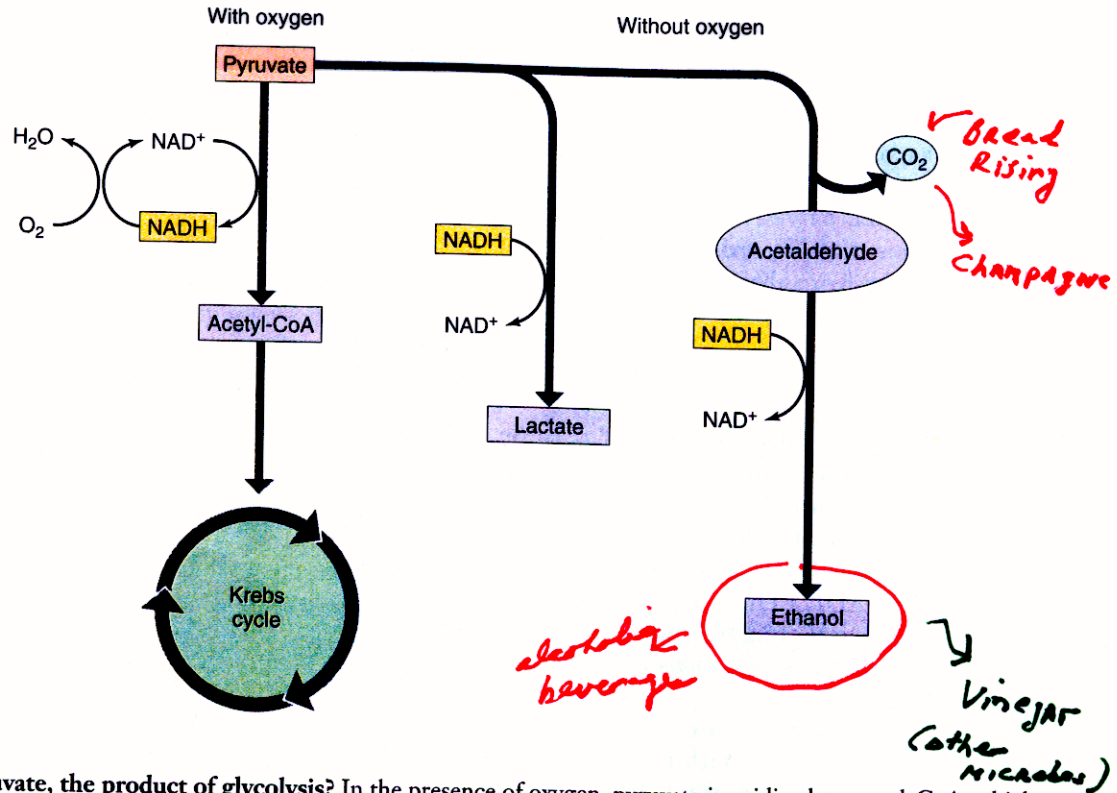


FIGURE 9.9
What happens to pyruvate, the product of glycolysis? In the presence of oxygen, pyruvate is oxidized to acetyl-CoA, which enters the Krebs cycle. In the absence of oxygen, pyruvate is instead reduced, accepting the electrons extracted during glycolysis and carried by NADH. When pyruvate is 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

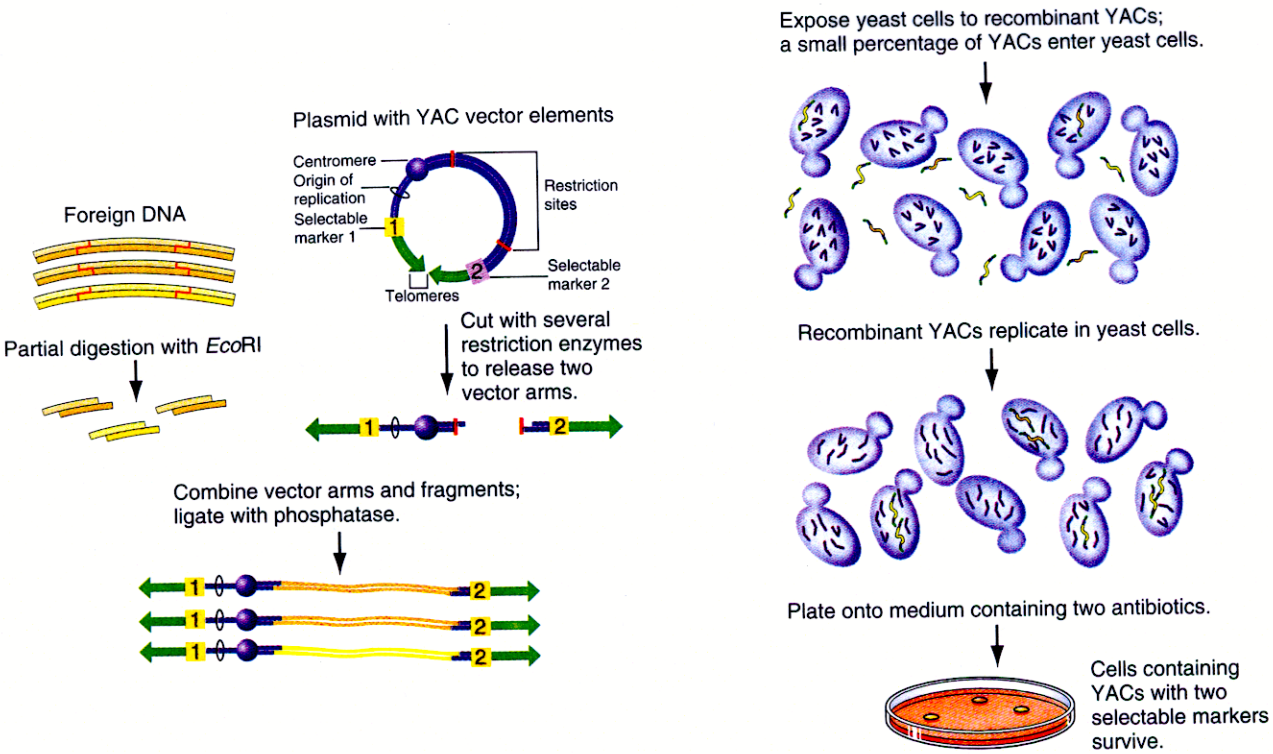
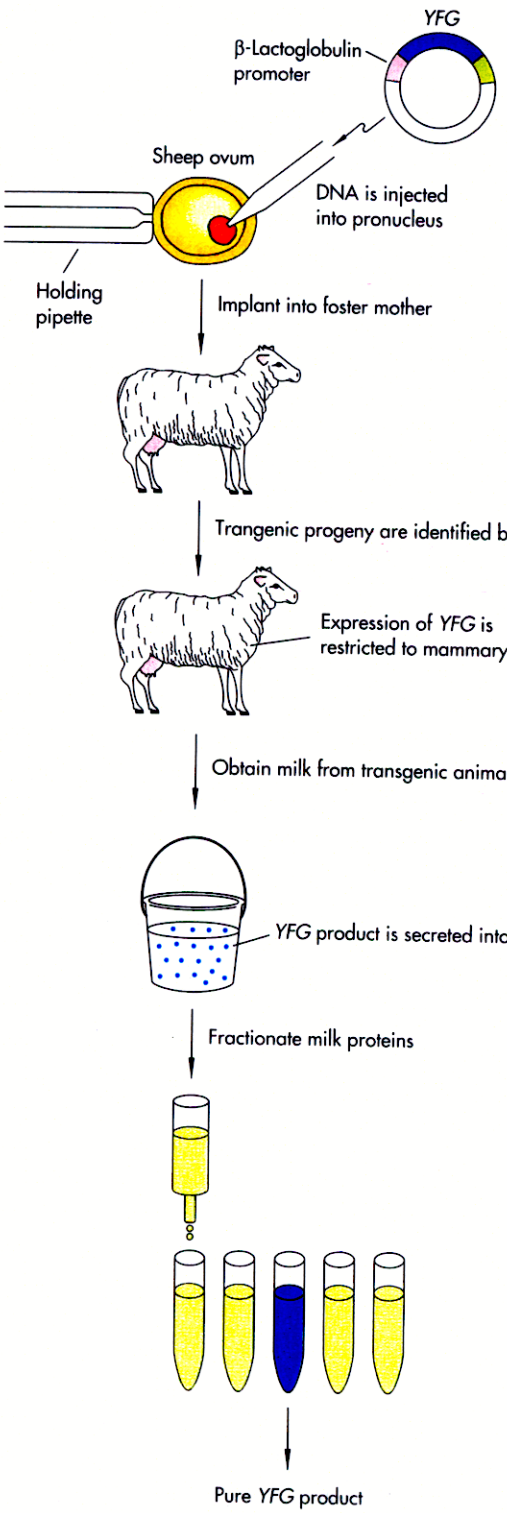


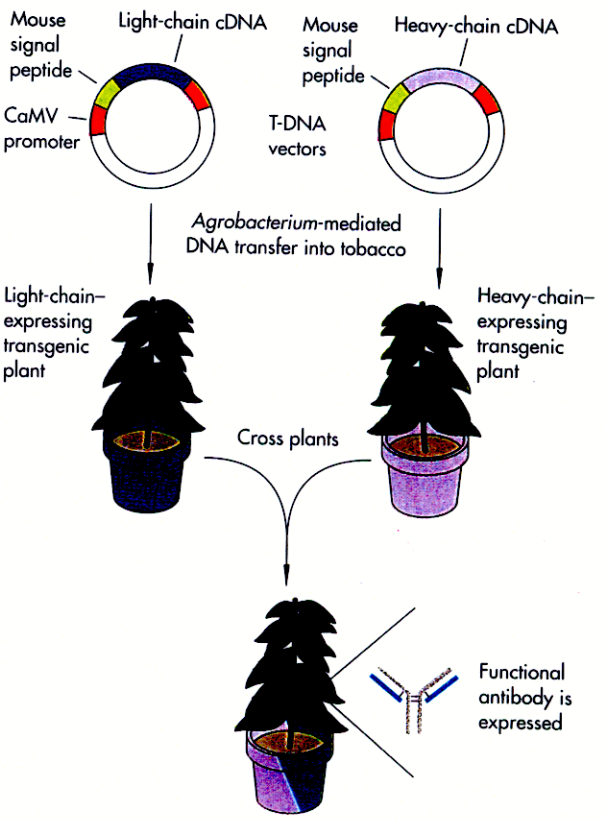
Figure 8.7 YAC vectors take advantage of DNA elements used for normal chromosome segregation within yeast cells. Two distinct arms make up each YAC vector. At the end of one arm is a telomere followed by a selectable marker, then a centromere, and finally a restriction site. The second arm lacks a centromere 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.

Haven't yet Why?

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 fermentors & elaborate protein purification schemes -- from animals & plants can be used for this purpose w/o special processing / machinery
3. Proteins in plants (e.g., seeds) are finitely stable - can be stored cheaply (& grown 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.

And other uses —

enhanced milk

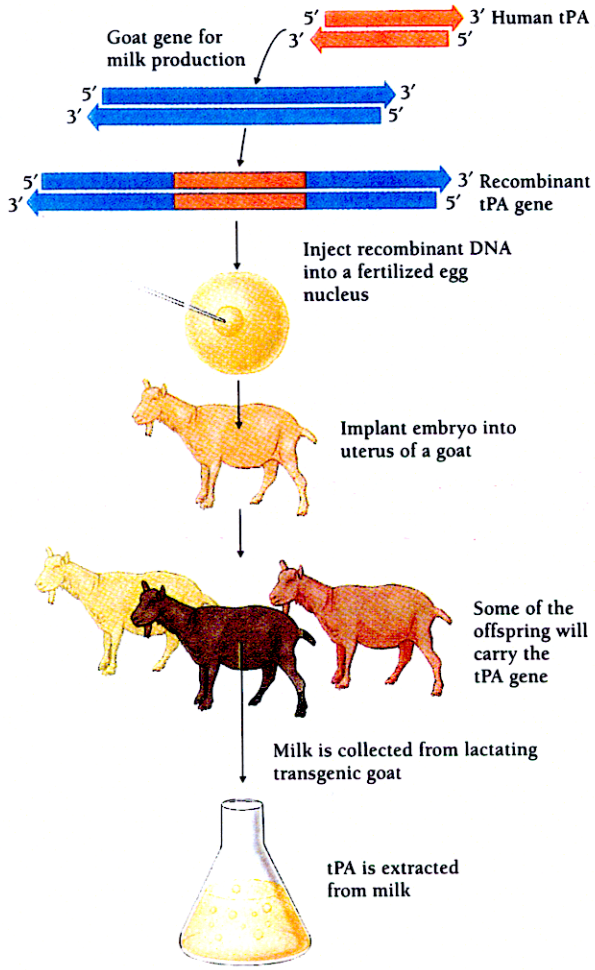
larger animals

Recombinant Proteins Produced in Animals

Table 14.4 Some recombinant proteins produced in the secretions of animal bioreactors.

System	Species	Product	Reference
Milk	Mouse	Sheep β-lactoglobulin	Simons *et al.* 1987
		Human tissue-plasminogen activator	Gordon *et al.* 1987
		Human urokinase	Meade *et al.* 1990
		Human growth hormone	Devinoy *et al.* 1994
		Human fibrinogen	Prunkard *et al.* 1996
		Human nerve growth factor	Coulibaly *et al.* 1999
		Spider silk	Karatzas *et al.* 1999
	Rabbit	Human erythropoietin	Massoud *et al.* 1996
	Sheep	Human α_1-antitrypsin	Wright *et al.* 1991
	Goat	Human tissue-plasminogen activator	Ebert *et al.* 1991
Blood serum	Rabbit	Human α_1-antitrypsin	Massoud *et al.* 1991
	Pig	Recombinant antibodies	Lo *et al.* 1991, Weidle *et al.* 1991
Urine	Mouse	Human growth hormone	Kerr *et al.* 1998
Semen	Mouse	Human growth hormone	Dyck *et al.* 1999

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..... →

Human Proteins Synthesized in Pharm Animal Milk

TABLE 8.2 Some human proteins that have been expressed in the milk of transgenic "pharm" animals

Human gene product	Pharmaceutical use	Mammary gland-specific promoter	Transgenic animal
Factor IX	Blood clotting protein, treatment of hemophilia B	Sheep β-lactoglobin	Sheep
α-1-Antitrypsin	Protease inhibitor, treatment of emphysema and cystic fibrosis	Sheep β-lactoglobin	Sheep
Antithrombin III	Blood clotting protein, treatment of ATIII deficiency disease and use in open heart surgery	Cow casein	Goat
Tissue plasminogen activator	Dissolves blood clots, used as an acute treatment of heart attacks	Mouse whey acidic protein	Goat
Lactoferrin	Iron transport protein, infant formula additive	Cow α-S-casein	Cow
Protein C	Anticoagulant, treatment of hemophilia and used for surgery	Mouse whey acid protein	Pig

CFTR	Cystic Fibrosis	β-casein	Mouse
Interleukin-2	Renal Cell Carcinoma	β-casein	rabbit

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.

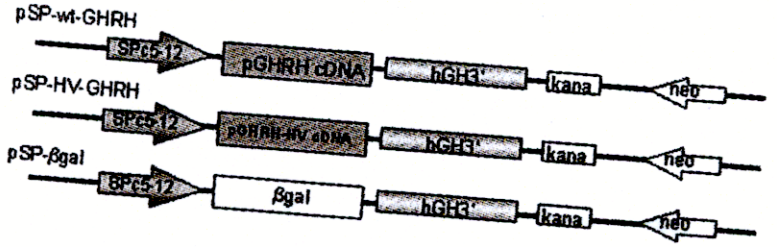
Table 1. Potential modifications of milk composition by gene addition, with expected functional outcome (modified from ref. 2).

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]*

31

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

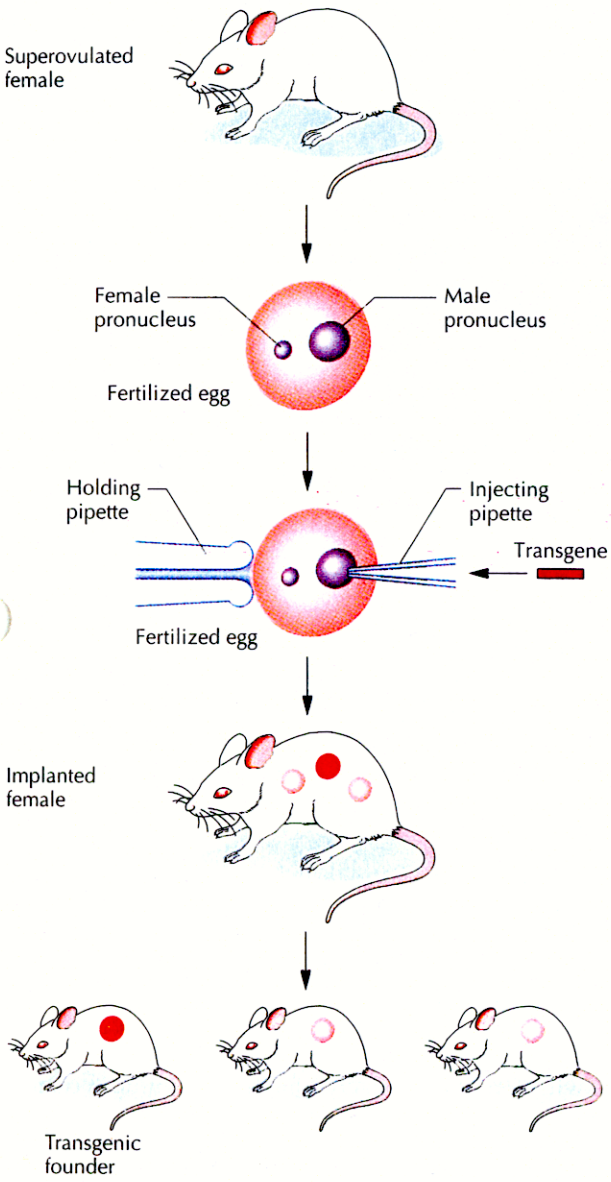
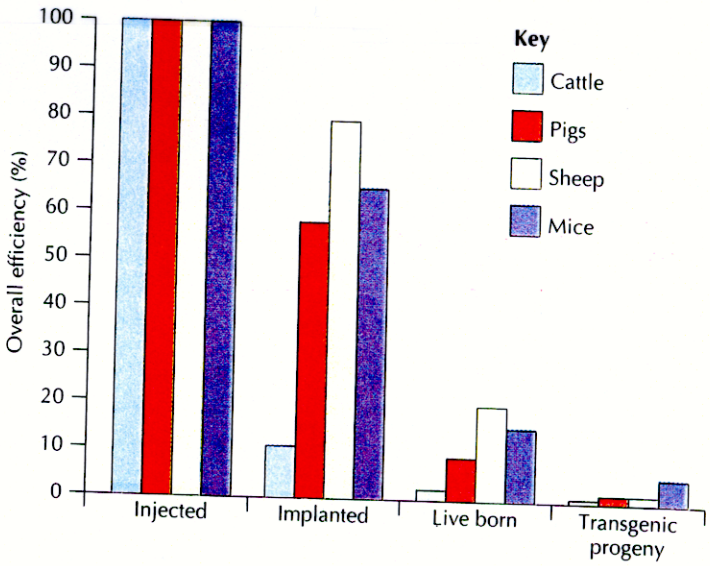


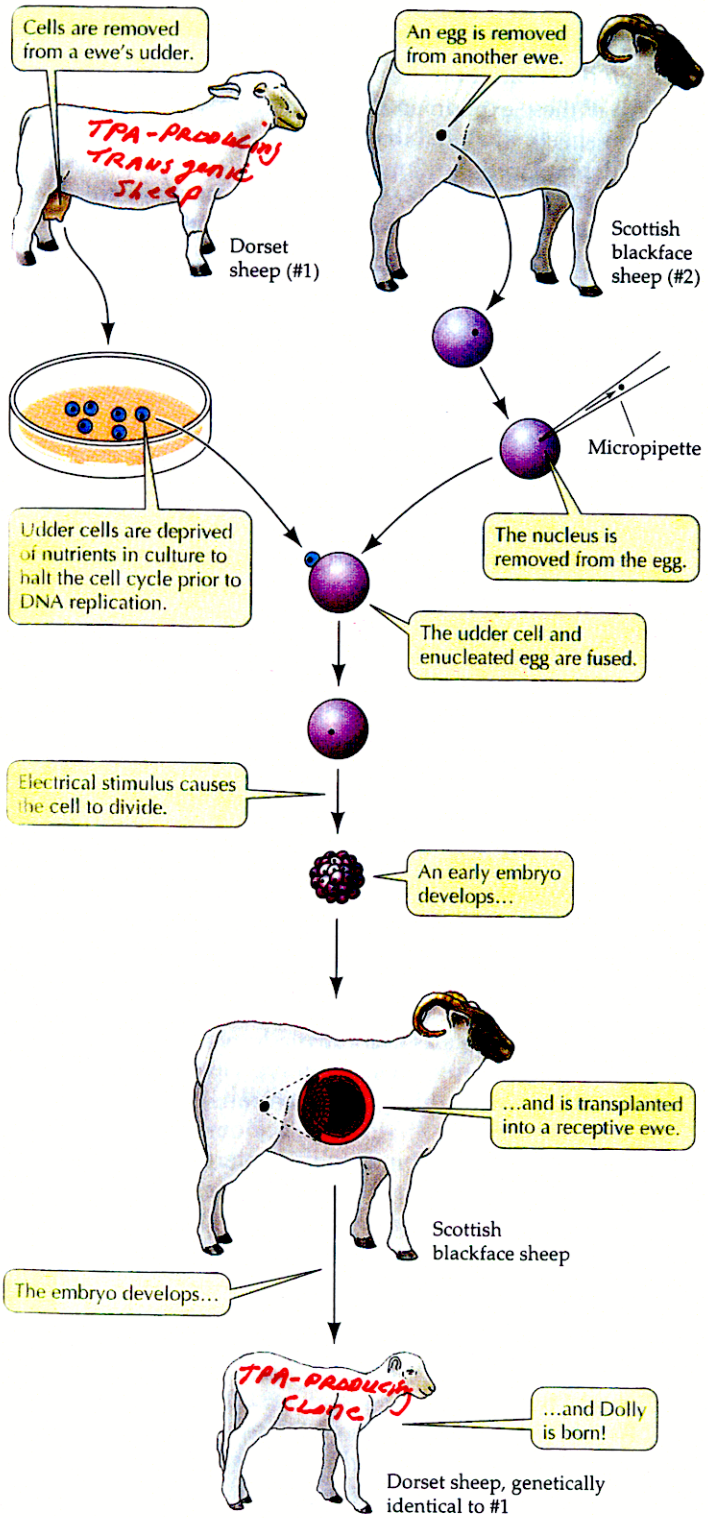
Table 11.3 Efficiency of production of transgenic animals by microinjection of a growth hormone gene. (Adapted from Hammer *et al.* 1985.)

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" AN ∞ NUMBER OF TRANSGENIC FARM ANIMALS



∴ CAN ESTABLISH LINES OF TRANSGENIC FARM ANIMALS THAT PRODUCE LARGE QUANTITIES OF MEDICALLY-IMPORTANT HUMAN PROTEINS!

15.4 Cloning a Mammal Dolly, a cloned sheep resulting from this experiment, has the same genes as the ewe that donated the udder cells.

FARM ANIMAL CELLS CAN BE Genetically Engineered BEFORE using nuclei for Cloning

Figure 8.12 Pharm animals can be generated by combining the molecular genetic techniques of DNA co-transfection and nuclear transfer. Flow scheme illustrating how the Roslin Institute researchers used lipofection-mediated gene transfer to transfect diploid fetal donor cells, isolated from a Poll Dorset sheep embryo, stably with a mammary gland-specific expression vector containing human Factor IX cDNA (pMIX1). The ovine β-lactoglobulin (BLG) promoter upstream of the Factor IX coding sequence on pMIX1 had previously been shown to direct high level expression of a heterologous gene in sheep mammary glands. In this co-transfection strategy, a second plasmid was included that encoded the *neo*^{r} gene expressed from the constitutive phosphoglycerate kinase promoter (pPGKneo), which provided a selectable marker for stable transfectants with G418. Molly and Polly represent the first two cloned transgenic pharm animals shown to contain a human gene of pharmaceutical importance.

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

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

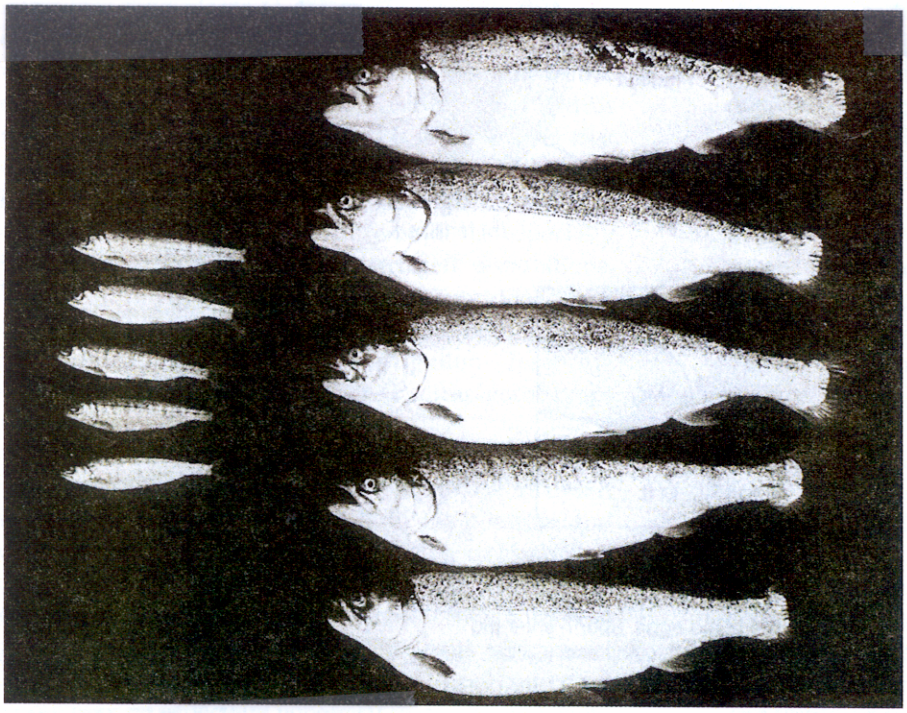


Figure 8.11 Comparison of 1-month-old coho salmon siblings; nonengineered fish are at left, transgenic fish are at right. The largest fish (top right) is 41.8 cm in length.

Building the Better Bug

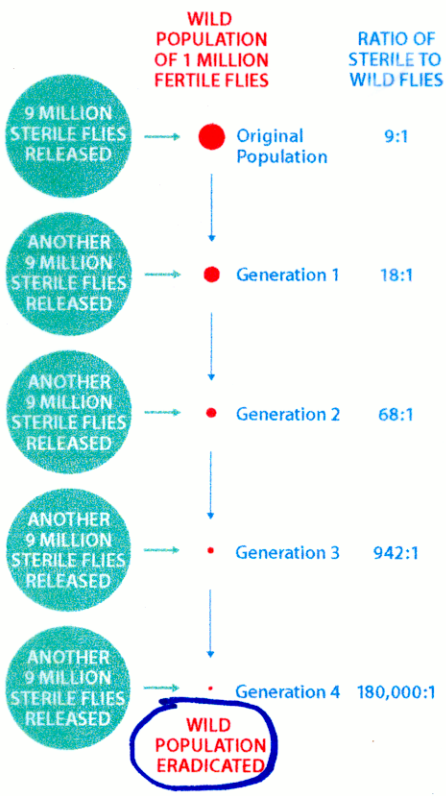
Inserting new genes into a few specific insect species could stop some infectious diseases, benefit agriculture and produce innovative materials

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

ANTHONY A. JAMES University of California, Irvine

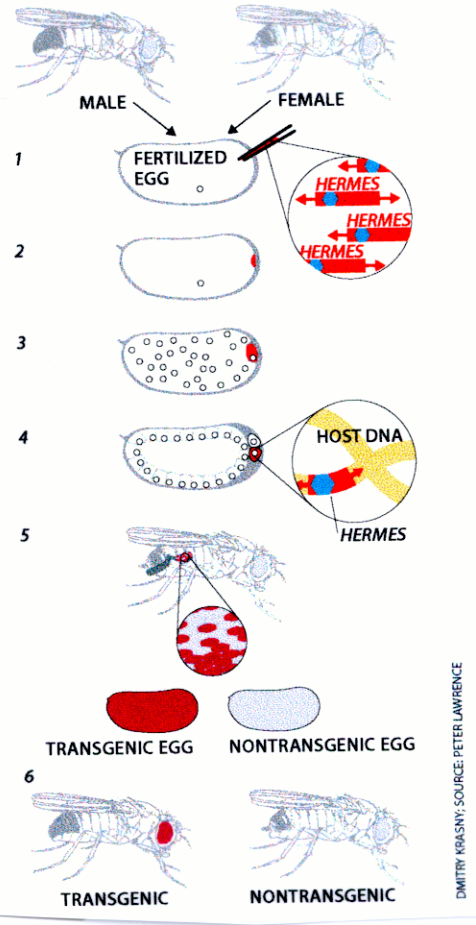
TRANSGENIC INSECTS can be given new characteristics, as illustrated by these five *Aedes aegypti* mosquitoes. Normal individuals have what appear to be black eyes, the result of large amounts of red pigment. A mutant version of *Ae. aegypti* has white eyes because of the lack of an enzyme, kynurenine 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



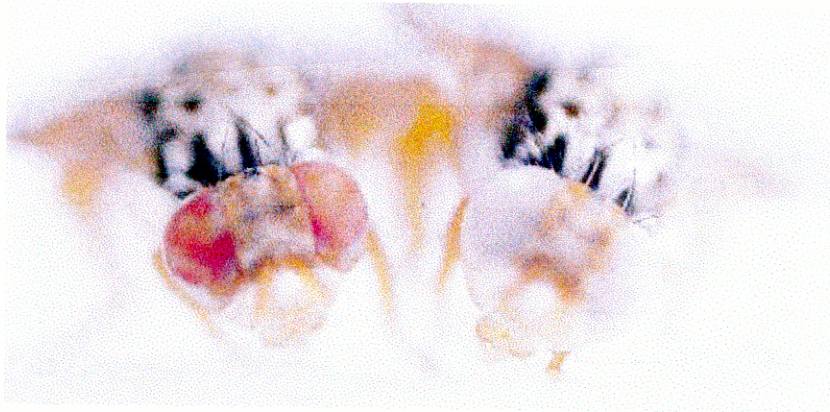
STERILE INSECT TECHNIQUE (SIT) can be an effective weapon against pests. Wave after wave of sterile insects, mostly males when possible, far outnumber the fertile members of the same species, and cause most matings to be fruitless. Within a few generations, the pest population is decimated. Traditional 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*).



SUSAN MCCOMBS, University of Hawaii

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.

Potential Risks of TRANSGENIC Animals?

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

Animal	Factor Contributing to Concern					Level of Concern[6]
	Number of Citations[1]	Ability to Become Feral[2]	Likelihood of Escape Captivity[3]	Mobility[4]	Community Disruptions Reported[5]	
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	
Cattle	16	Low	Low	Low	None	Low

[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.

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 usage 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 environmental 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.

DALLAS WEAVER

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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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Plants CAN BE CLONED & Genetically Engineered Too!

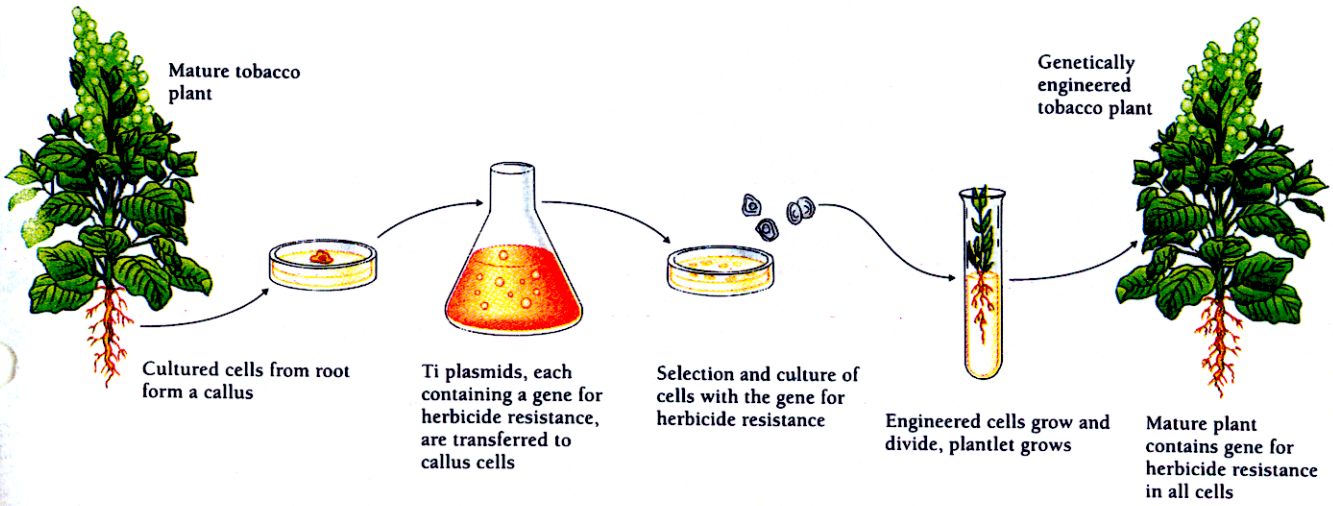

Figure 13-4 **Recombinant DNA technology in action.** Cells from a tobacco plant are grown in culture, then infected with a Ti plasmid carrying a gene for herbicide resistance and other genes. The growing cells are treated with herbicide and only the cells expressing the new genes survive. These herbicide-resistant cells can then be grown into mature plants, which will bear seeds carrying the new genes.

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

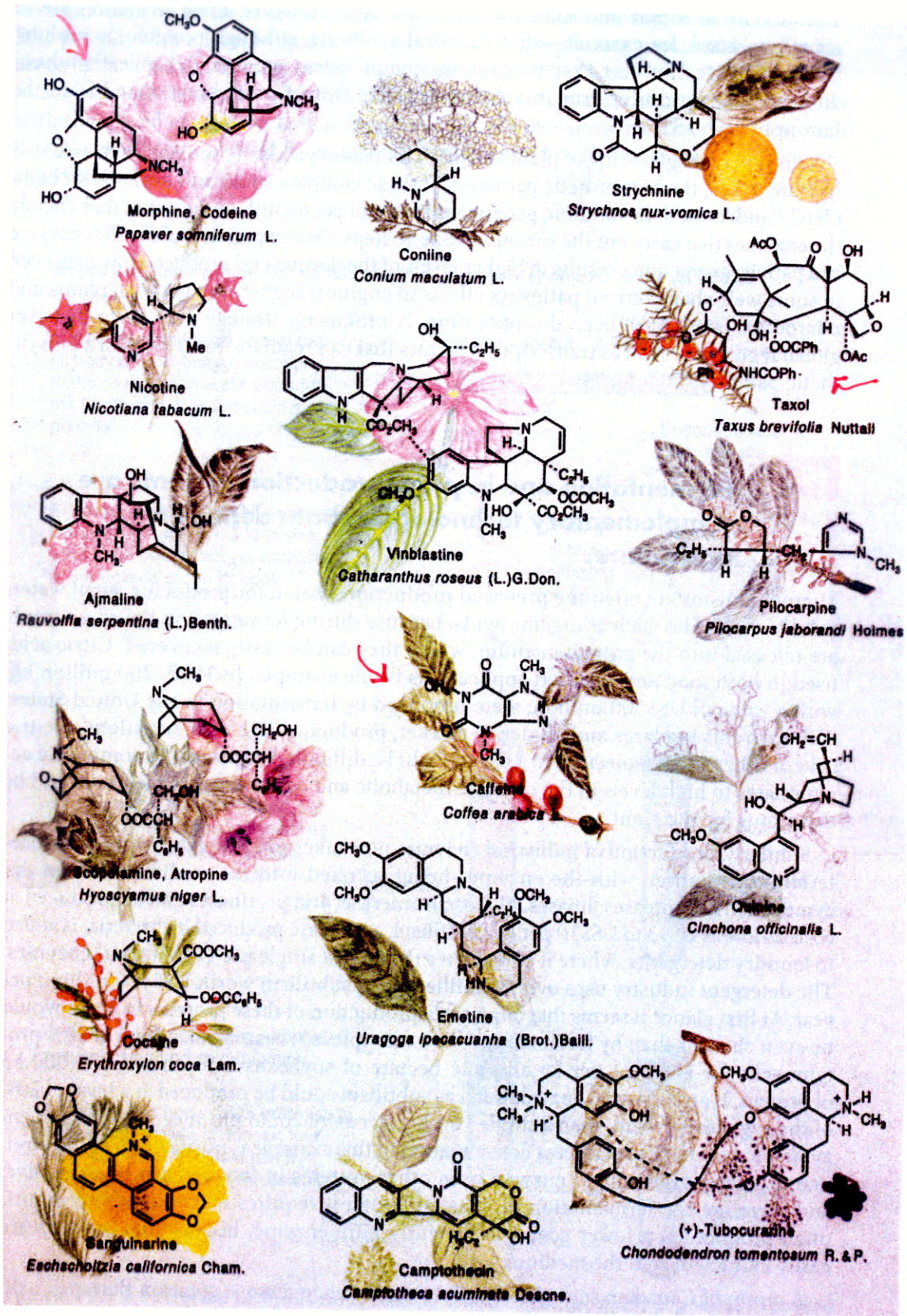

Figure 19.11 Structures of biologically active alkaloids and the plants that produce them. *Source:* Kutchan, T. M. 1995. Alkaloid biosynthesis—The basis for metabolic engineering of medicinal plants. *Plant Cell* 7:1059–1070.

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	

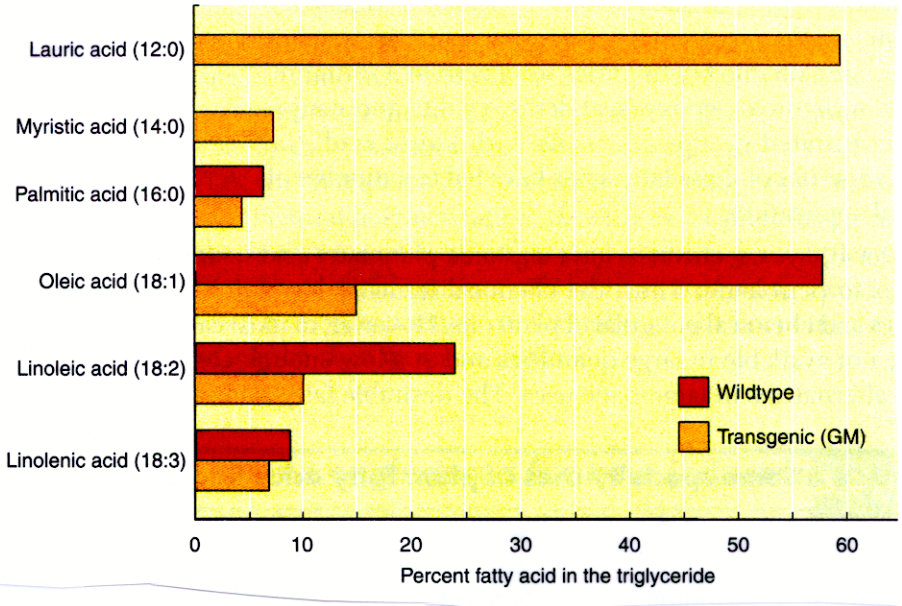

Figure 19.9 Genetic engineering of canola oil that is high in lauric acid, a fatty acid with 12 carbon atoms. By introducing a single gene from the California bay tree, the canola oil was changed from containing 60% oleic acid to 60% lauric acid. This new canola oil resembles the oil found in coconut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

PLANTS AS FACTORIES

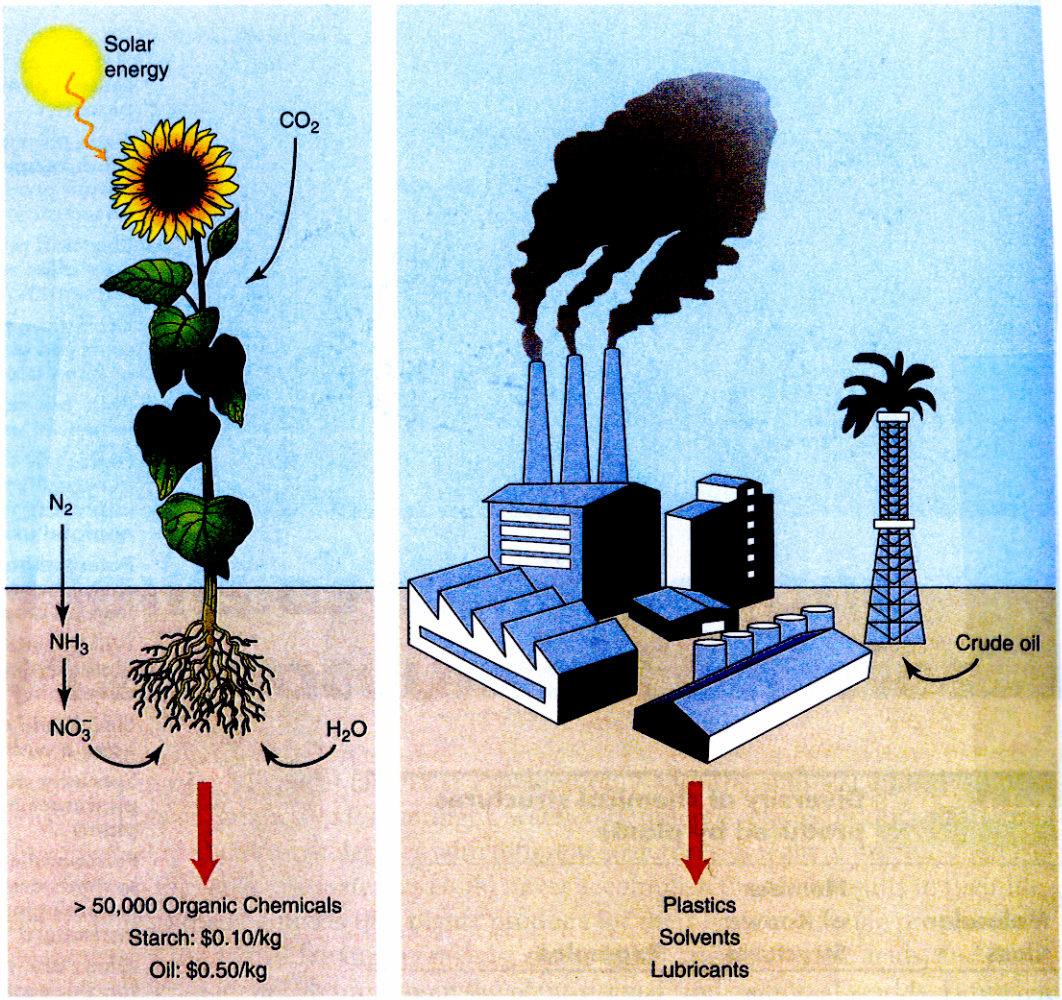

Figure 19.1 Can plants replace plants? In green plants the inputs are carbon dioxide and solar energy, in chemical plants the input is petroleum.

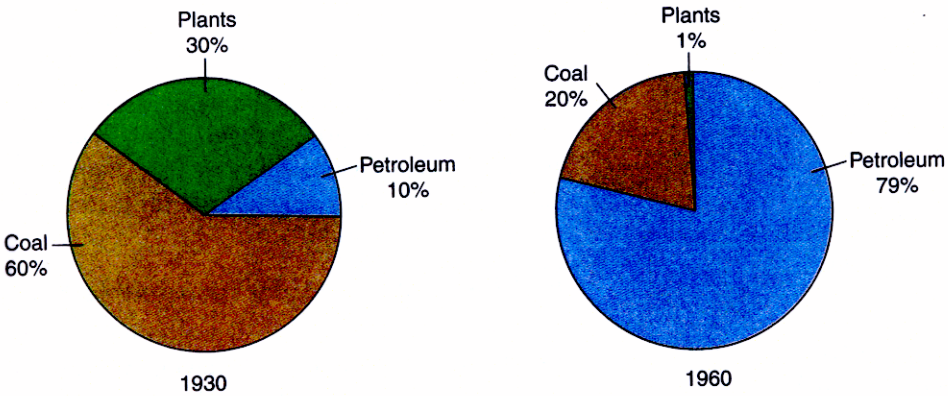

Figure 19.2 Change in the primary sources of industrial chemicals in the United States between 1930 and 1960. Note the rise of oil and the disappearance of plants and decreased importance of coal over this 30-year period. As of 2000, petroleum provides over 95% of organic chemicals used in the United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

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

MerB — mercurial lyase

$$Hg(II) + NADPH \xrightarrow{MerA} Hg(0) + NADP^+ + H^+$$

MerA — mercuric reductase

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!

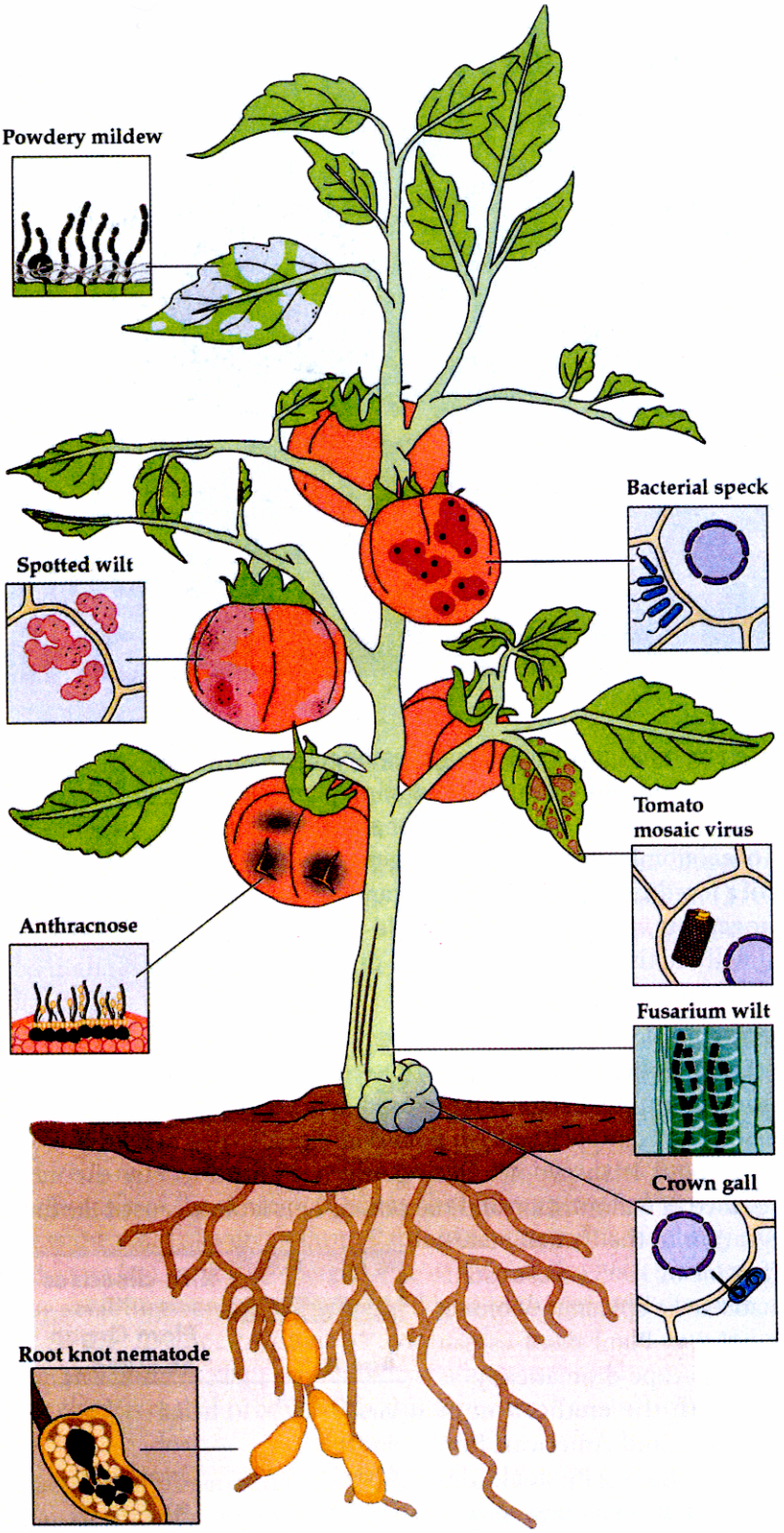

Figure 15.1 Most microbes attack only a specific part of the plant and produce characteristic disease symptoms. Tomato, shown here, can be attacked by more than 100 different pathogenic microorganisms. *Source:* B. B. Buchanan, W. Gruissem, and R. L. Jones, eds. (2000), *Plant Biochemistry and Molecular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