

Chapter 10C

Applications of Genetic Engineering and Biotechnology

Sections to study

1 Production of biological and pharmaceutical products in genetically engineered organisms

- Insulin, growth hormones, vaccines

2 Genetic engineering in plants has revolutionized agriculture

- Herbicide-resistant crops
- Pest-resistant crops
- Nutrition-enriched crops

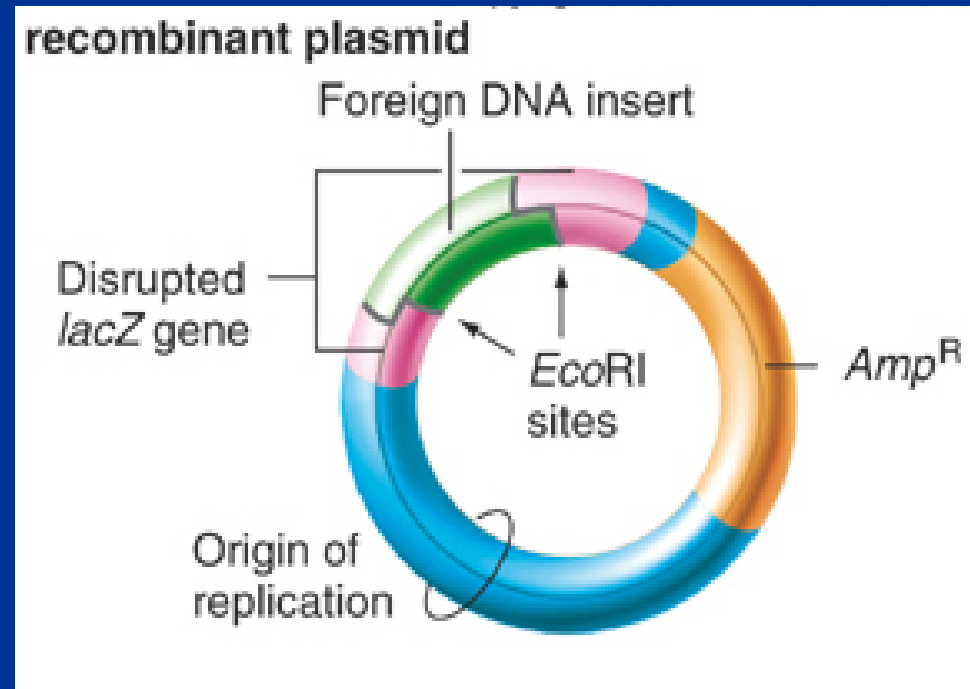
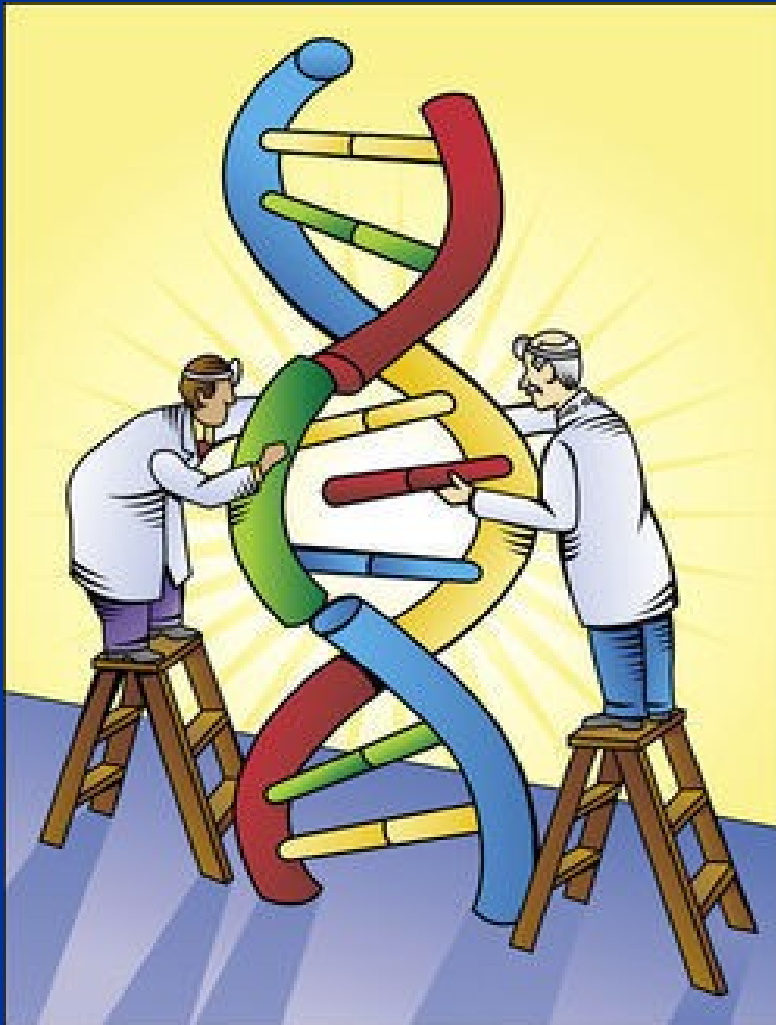
3 Genetic engineering in animals

- Transgenic fishes, cows, pigs

4 Gene therapy in humans

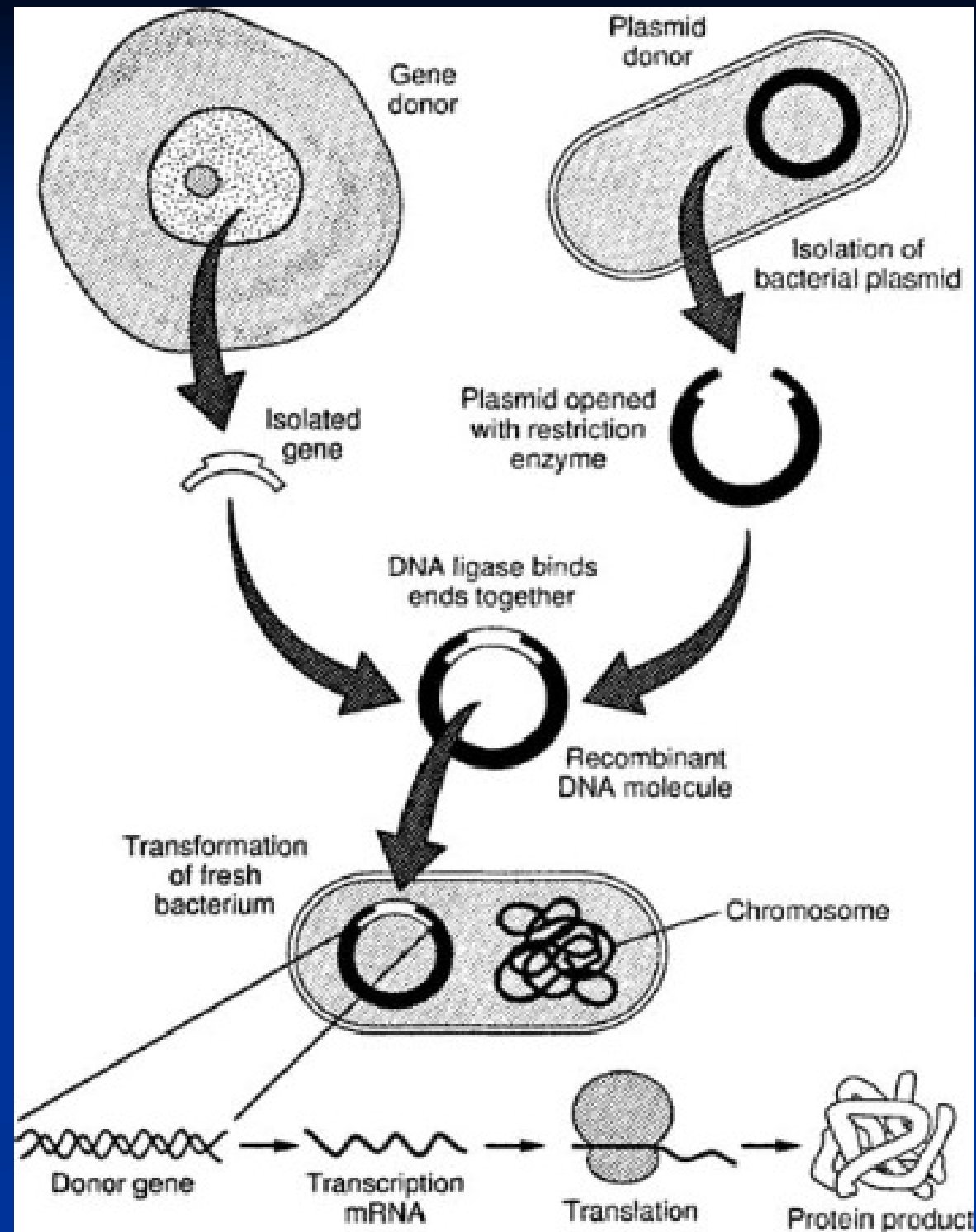
Recombinant DNA technology

- Restriction enzymes
- Plasmid vectors

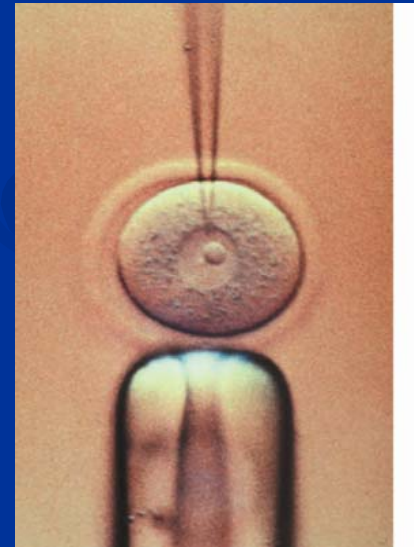


- **Biotechnology:** The use of living organisms to create a product or a process that helps improve the quality of life for humans or other organisms.
 - People in ancient civilizations used microbes to make important products, such as wine, beer, vinegar, breads, and cheeses.

- Modern biotechnology relies heavily on **recombinant DNA technology, genetic engineering, and genomics applications.**



- **Genetic engineering:** The alteration of an organism's genome.
 - **Genetically Modified Organisms (GMO):** New varieties of plants, animals, and other organisms with specific traits.
 - Required technologies
 - The ability to manipulate DNA in vitro
 - The ability to introduce genes into living cells



Applications of biotechnology and genetic engineering

- 1. Production of biological and pharmaceutical products in genetically engineered organisms**
 - Insulin, growth hormones, vaccines
- 2. Genetic engineering in plants has revolutionized agriculture**
 - Herbicide-resistant, pest-resistant, nutrition-enriched crops
- 3. Genetic engineering in animals**
 - Transgenic fishes, cows, pigs
- 4. Human gene therapy**

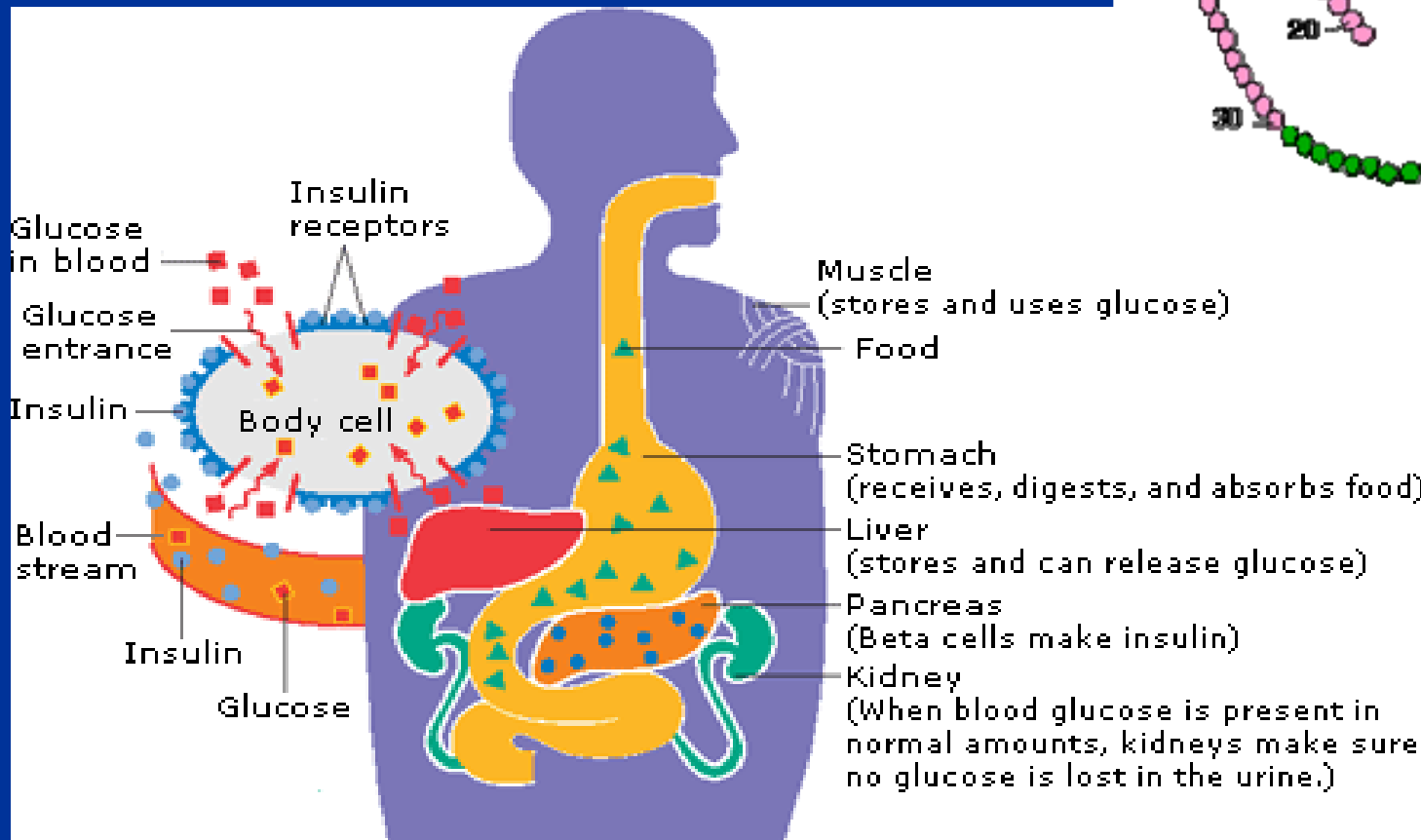
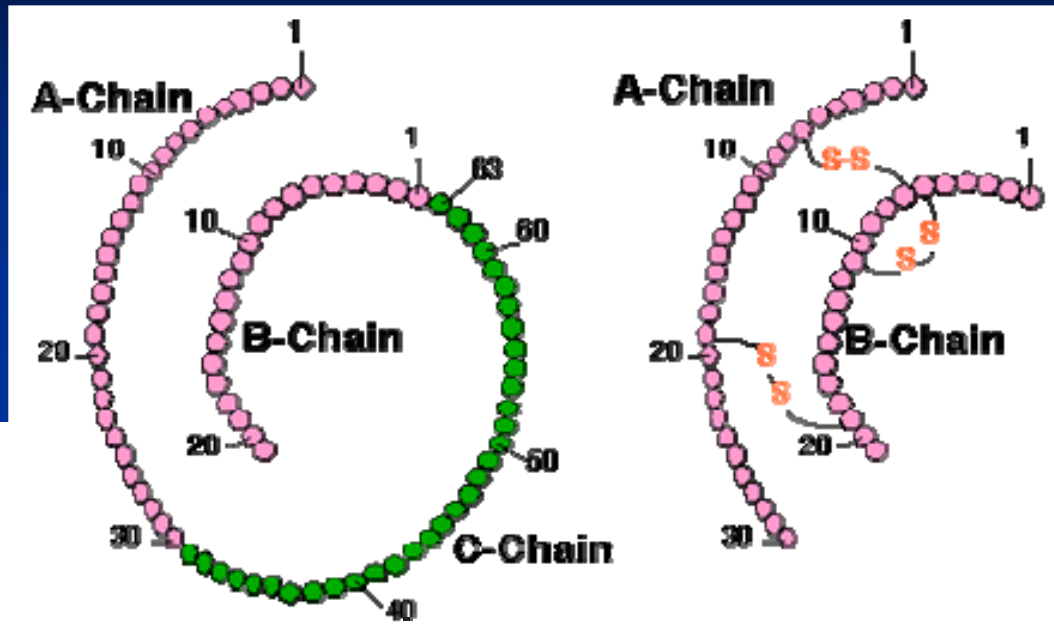
1 Production of biological and pharmaceutical products in genetically engineered organisms

- **Biopharmaceutical products**
 - Therapeutic proteins: insulin, growth hormones, clotting factors were traditionally purified from tissues.
- **Biopharming:** A commonly used term to describe the production of valuable proteins in genetically modified animals and plants.



Insulin

- A growth hormone produced by the pancrea.
- Used to be extracted from the pancreas of cows and pigs.



■ **Humulin:** The first human gene product manufactured by recombinant DNA technology.

- Developed by Genentech, a biotech company founded in 1976
- Licensed to therapeutic use in 1982 by FDA

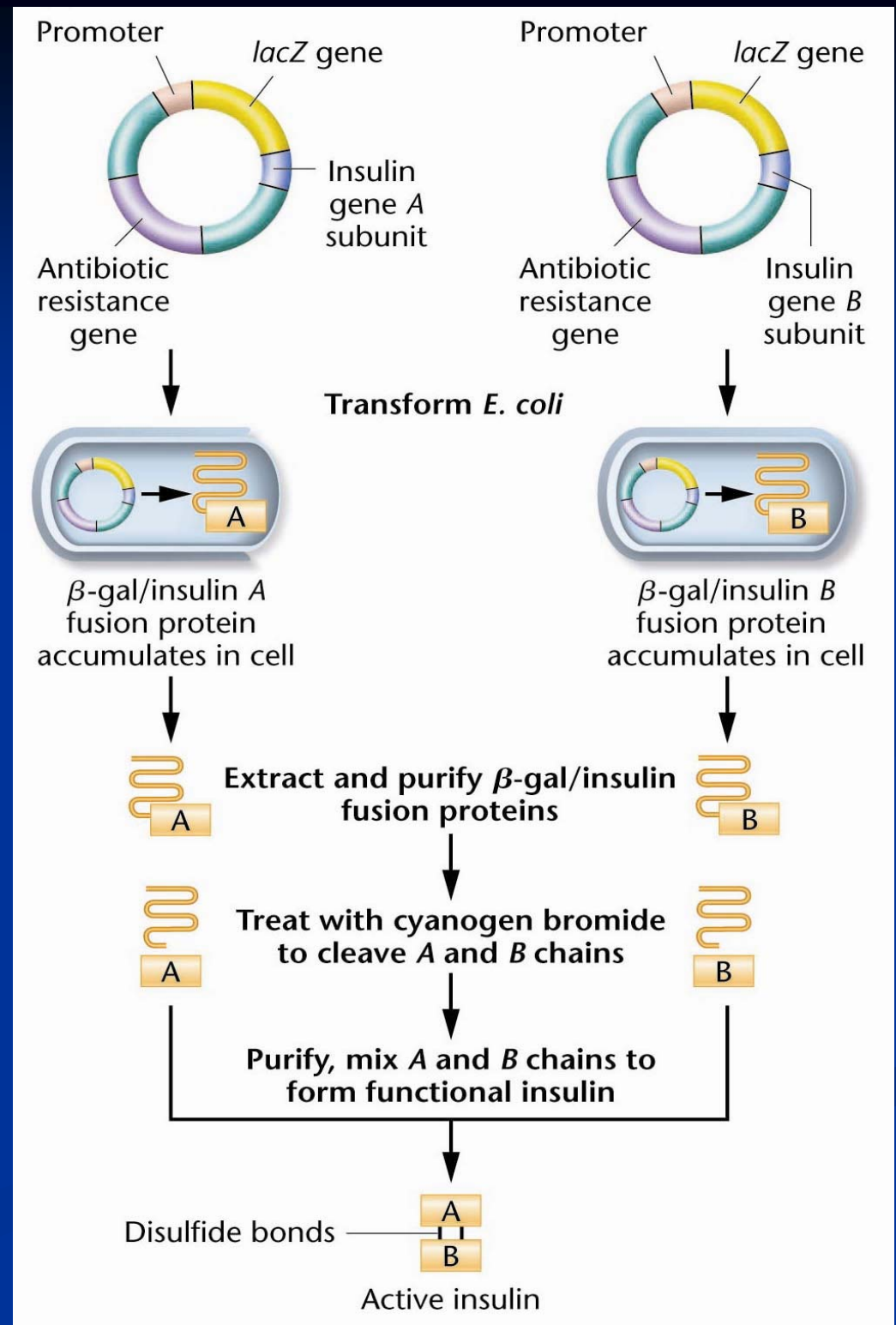
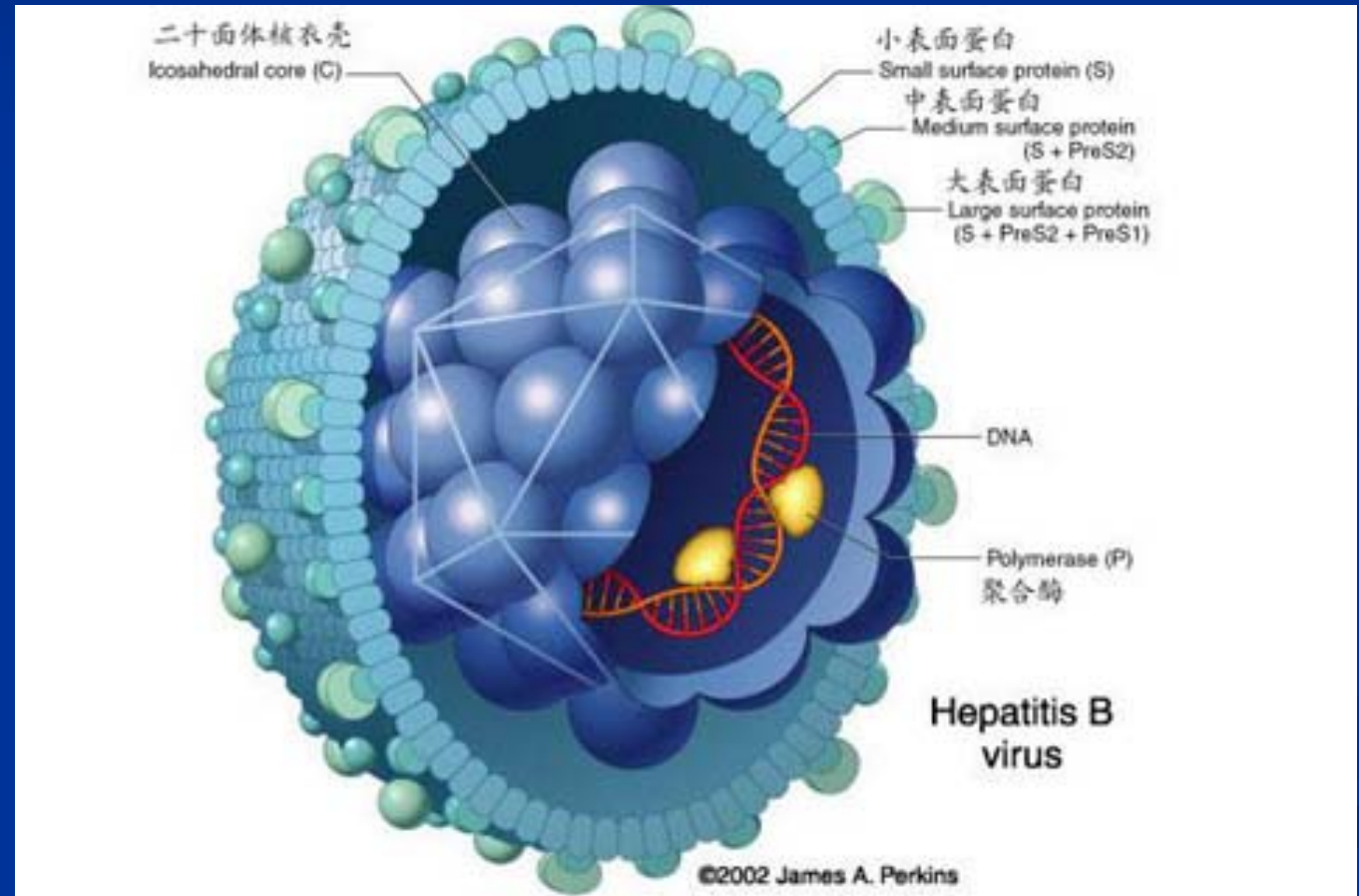
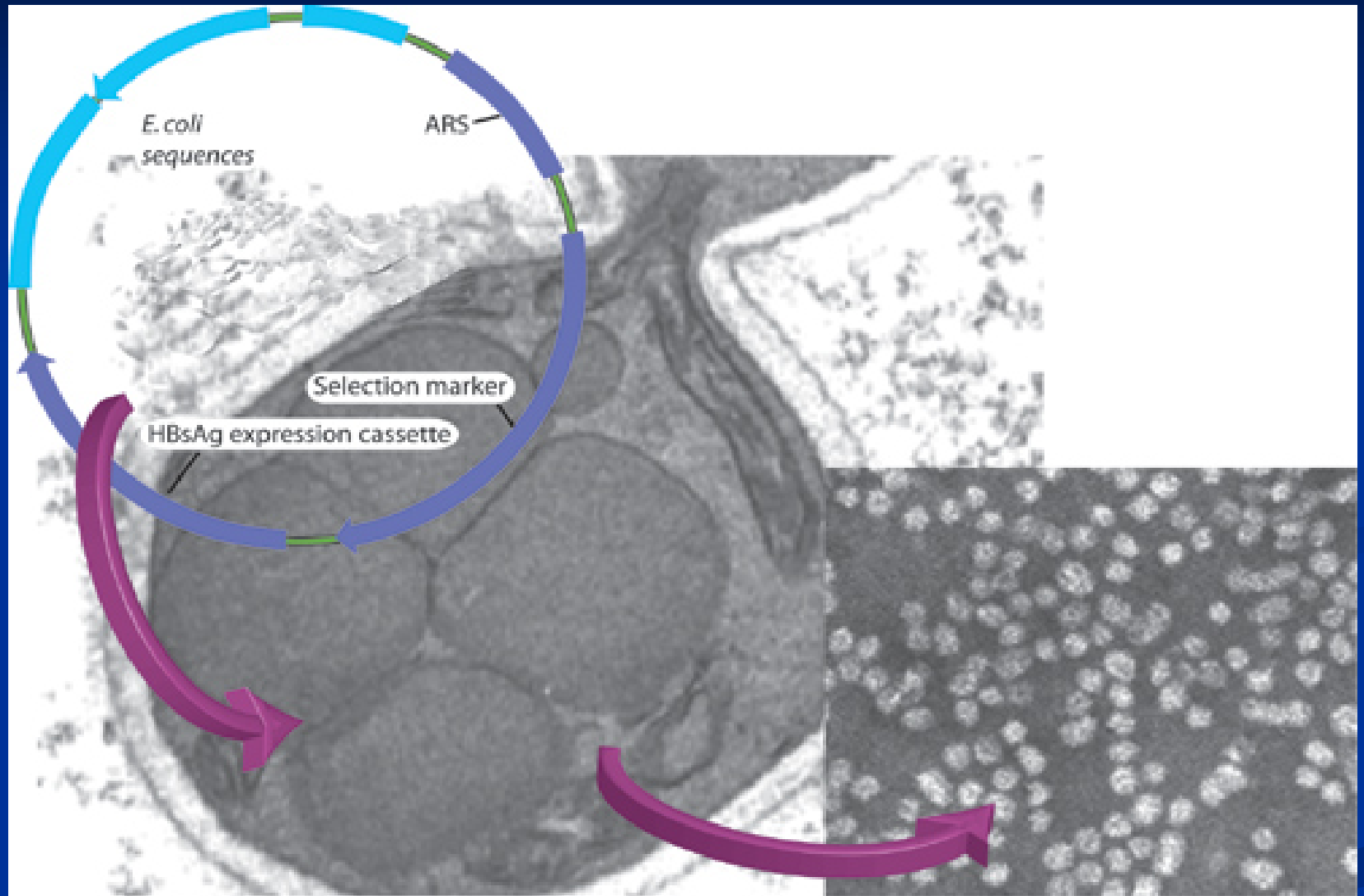


Fig 22-1

Recombinant vaccines

- **Engerix B, hepatitis B** vaccine produced in yeast, developed by GlaxoSmithKline.

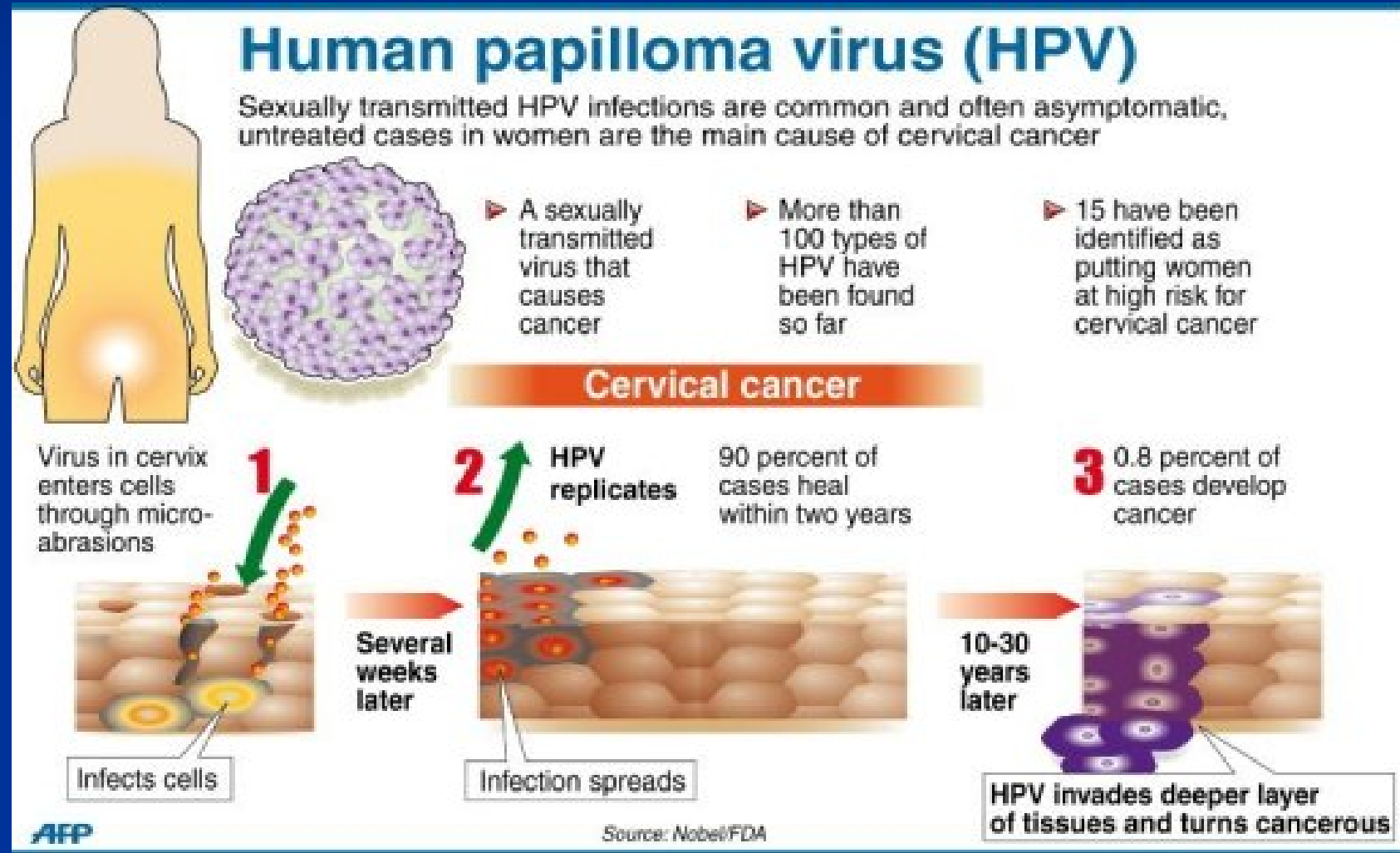




Dane particles
(ϕ 42 nm)

■ Vaccines against cancer

- **Gardasil**, a subunit vaccine against **human papillomavirus (HPV, 人乳头瘤病毒)** that cause ~ 70% of cervical cancers. Developed by Merck and was approved by FDA in 2005.



Human papillomavirus (HPV, 人乳头瘤病毒)



Disease	HPV type
Common warts	2, 7, 22
Plantar warts	1, 2, 4, 63
Flat warts	3, 10, 8
Anogenital warts	6, 11, 42, 44 and others ^[13]
Anal dysplasia (lesions)	6, 16, 18, 31, 53, 58 ^[14]
Genital cancers	<ul style="list-style-type: none"> • Highest risk:^[13] 16, 18, 31, 45 • Other high-risk:^{[13][15]} 33, 35, 39, 51, 52, 56, 58, 59 • Probably high-risk:^[15] 26, 53, 66, 68, 73, 82
Epidermodysplasia verruciformis	more than 15 types
Focal epithelial hyperplasia (oral)	13, 32
Oral papillomas	6, 7, 11, 16, 32
Oropharyngeal cancer	16
Verrucous cyst	60
Laryngeal papillomatosis	6, 11

■ Edible vaccines

- Vaccines are produced in plants

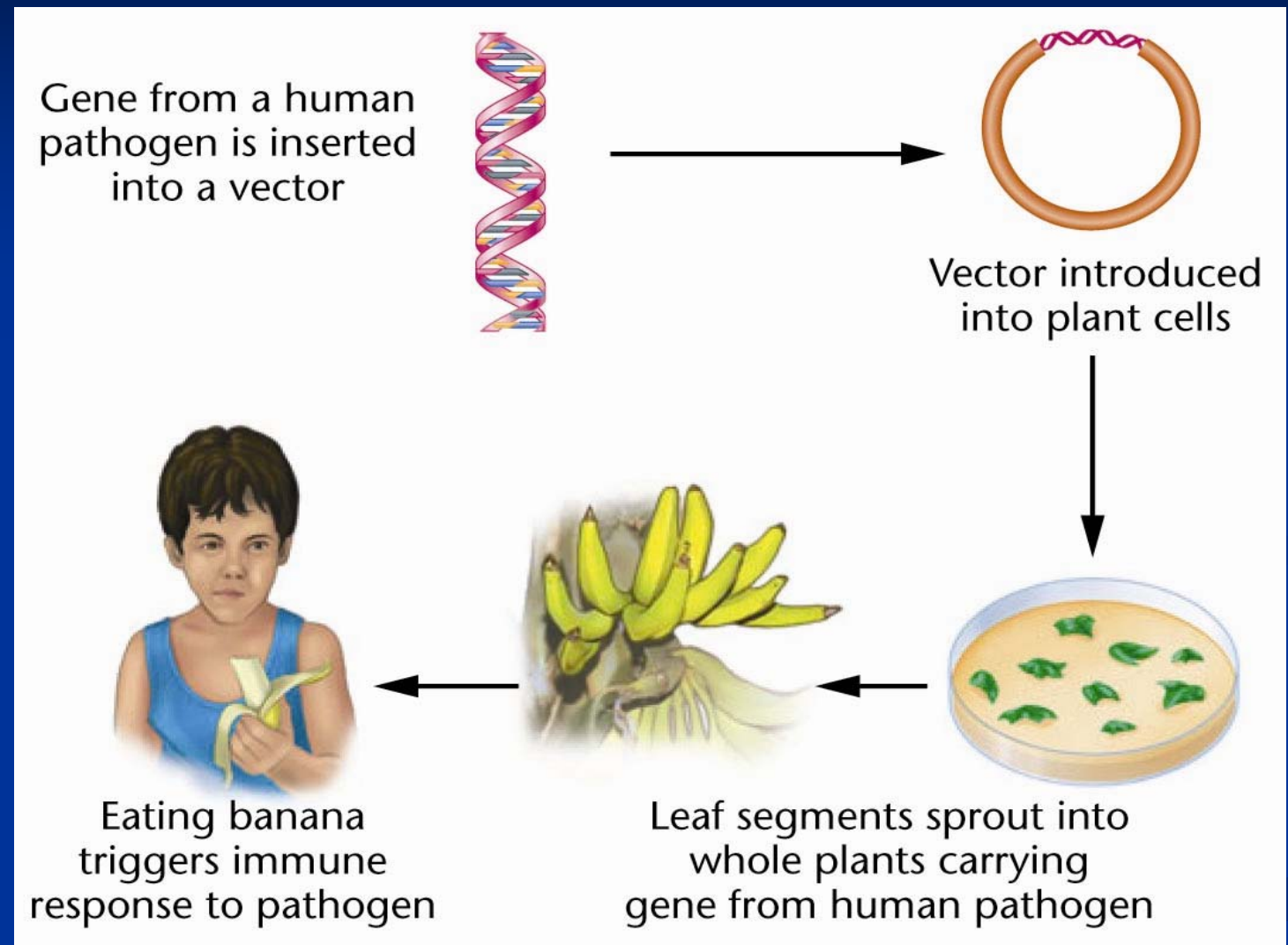


Fig 22-2

- **Transgenic sheep as a biofactory**

- Valuable products are produced in the milk.

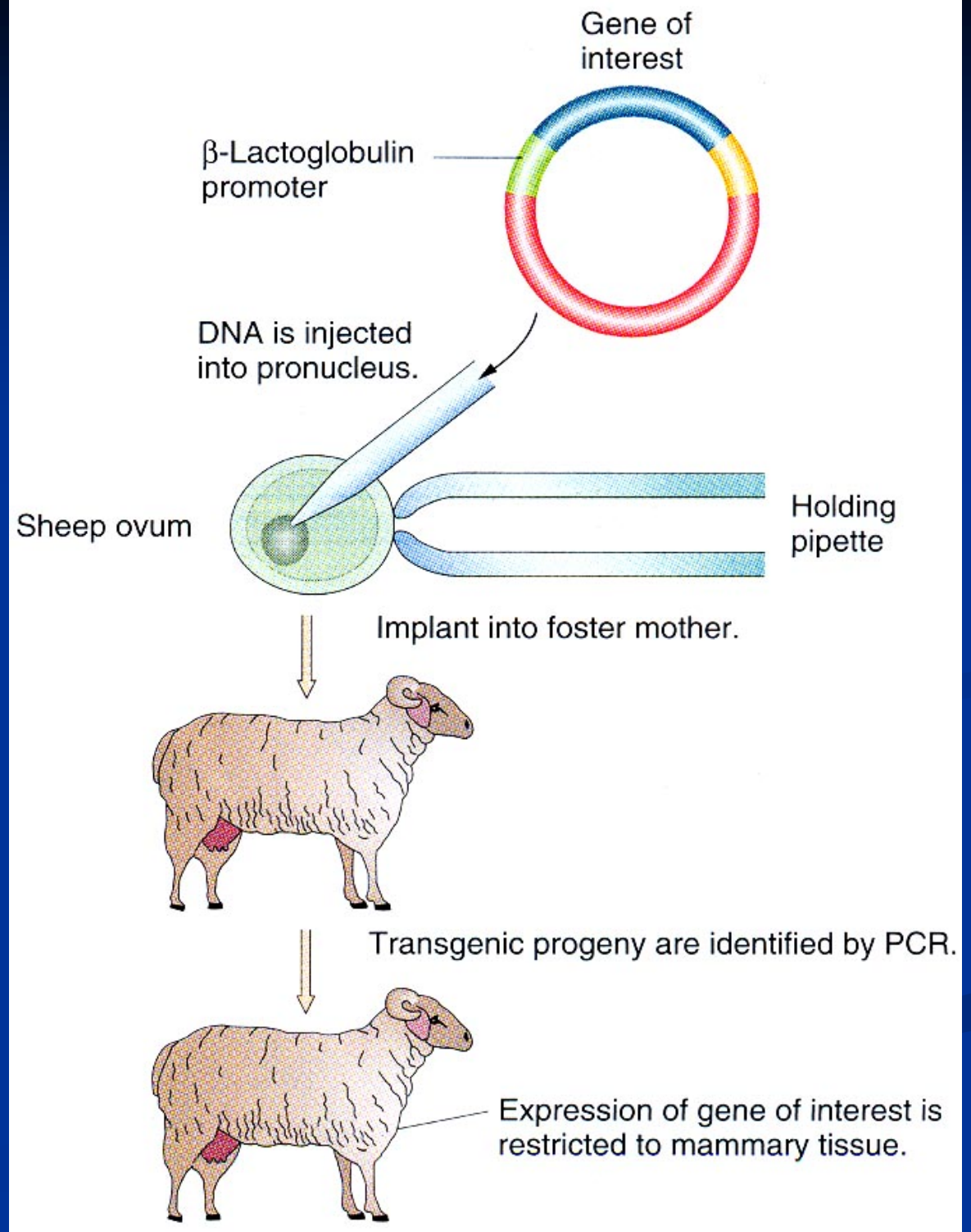
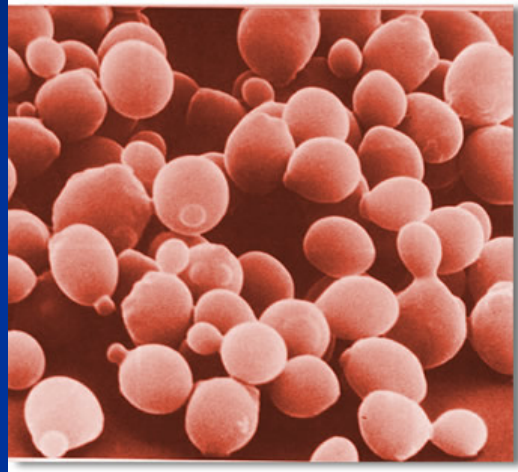


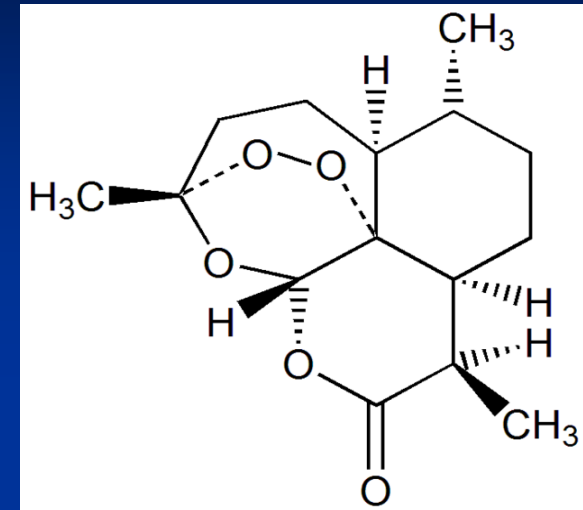
TABLE 22.1
Examples of Genetically Engineered Biopharmaceutical Products Available or under Development

Gene Product	Condition Treated	Host Type
Erythropoitin	Anemia	<i>E. coli</i> ; cultured mammalian cells
Interferons	Multiple sclerosis, cancer	<i>E. coli</i> ; cultured mammalian cells
Tissue plasminogen activator tPA	Heart attack, stroke	Cultured mammalian cells
Human growth hormone	Dwarfism	Cultured mammalian cells
Monoclonal antibodies against vascular endothelial growth factor (VEGF)	Cancers	Cultured mammalian cells
Human clotting factor VIII	Hemophilia A	Transgenic sheep, pigs
C1 inhibitor	Hereditary angioedema	Transgenic rabbits
Recombinant human antithrombin	Hereditary antithrombin deficiency	Transgenic goats
Hepatitis B surface protein vaccine	Hepatitis B infections	Cultured yeast cells, bananas
Immunoglobulin IgG1 to HSV-2	Herpesvirus infections	Transgenic soybeans glycoprotein B
Recombinant monoclonal antibodies	Passive immunization against rabies (also used in diagnosing rabies), cancer, rheumatoid arthritis	Transgenic tobacco, soybeans, cultured mammalian cells
Norwalk virus capsid protein	Norwalk virus infections	Potato (edible vaccine)
<i>E. coli</i> heat-labile enterotoxin	<i>E. coli</i> infections	Potato (edible vaccine)

Synthetic biology provides new possibilities



Artemisinin (青蒿素) fights malaria and cancer

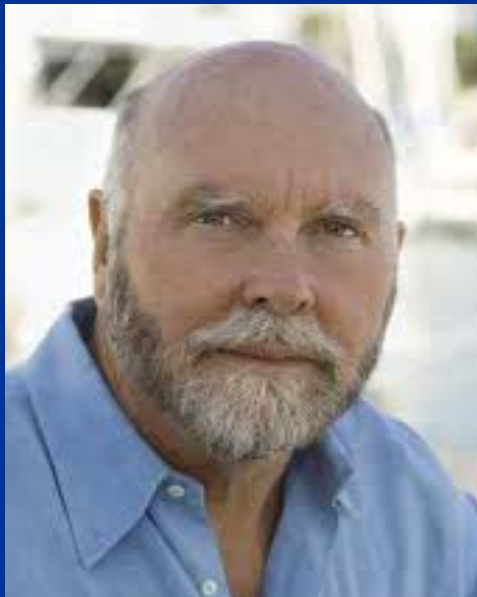


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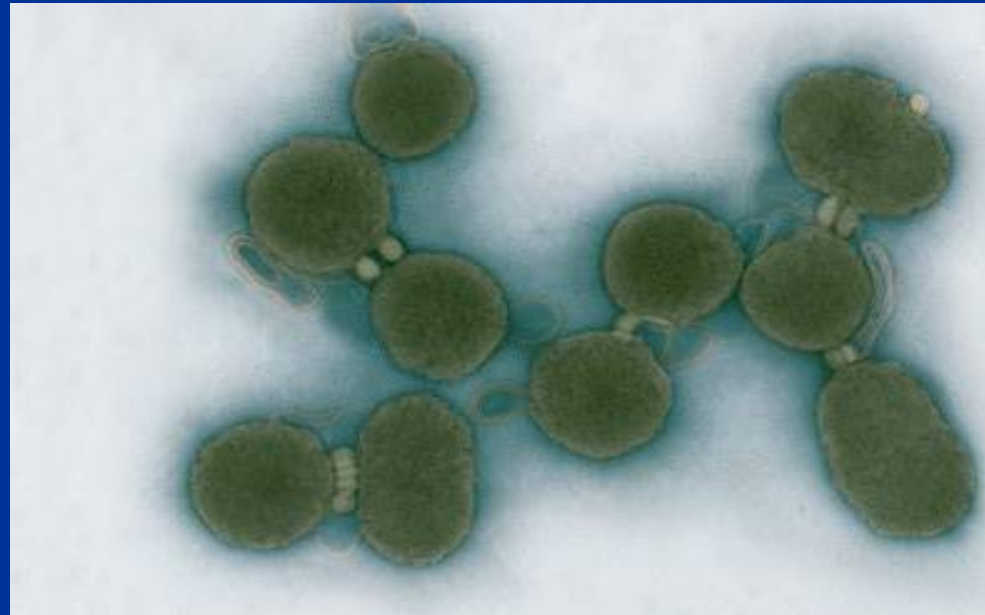
Artemisia annua (黄花蒿)

Artificial design and synthesis of a genome

- *Mycoplasma mycoides* JCVI-syn1.0: The first single-celled organism with a completely artificially synthesized genome.
- 2010, J. Craig Venter Institute

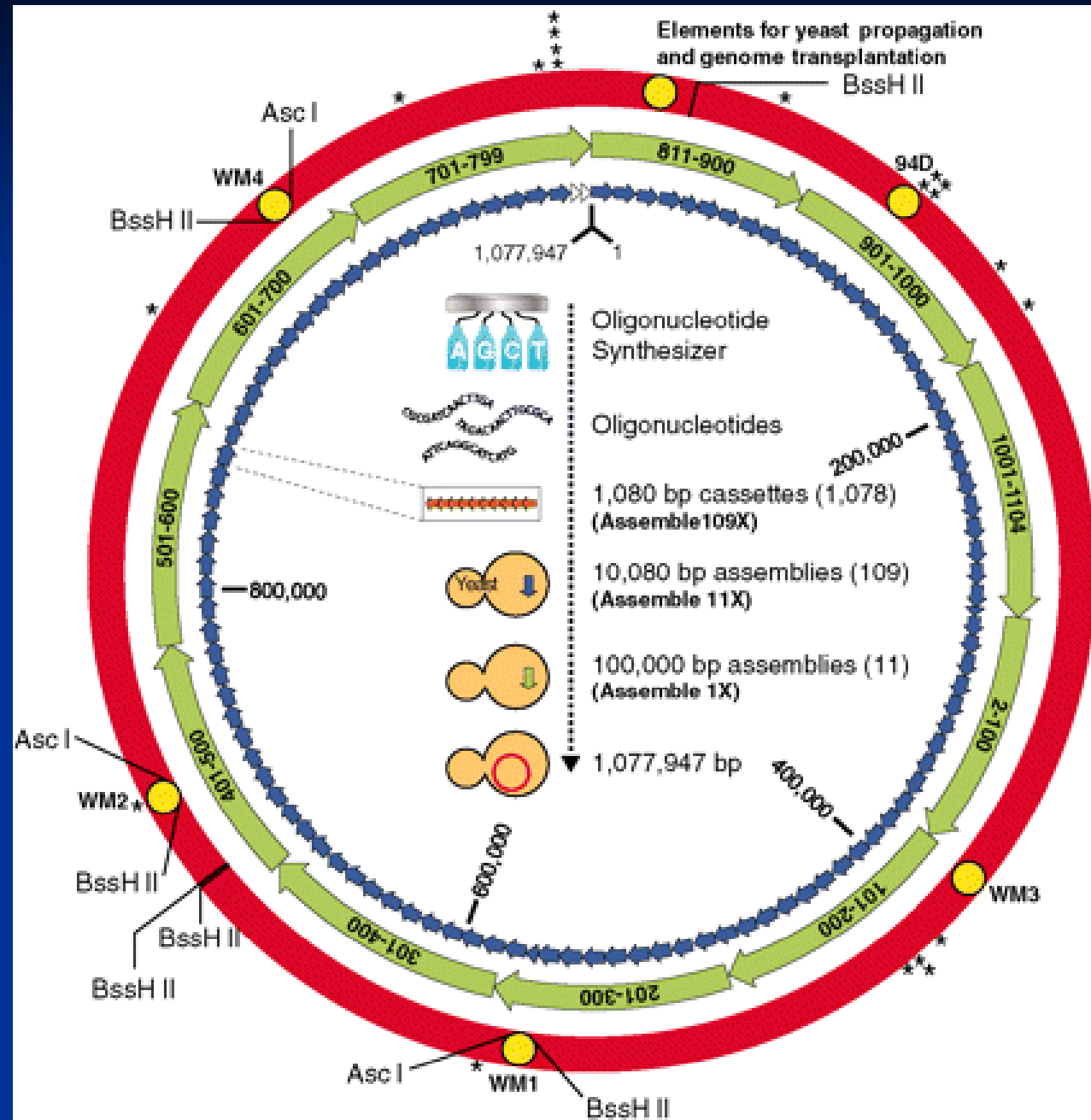


J. Craig Venter



The negatively stained transmission electron micrographs of aggregated *M. mycoides*.

- Artificially synthesized *Mycoplasma mycoides* genome with modifications. Deleted 4000 bp (2 genes), replaced 10 genes with four “watermark” sequences, each over 1000 bp.
- 1.08 Mb genome, assembled *in vitro* and in yeast cells.
- Recipient: *Mycoplasma capricolum*.



2 Genetic engineering in plants has revolutionized agriculture

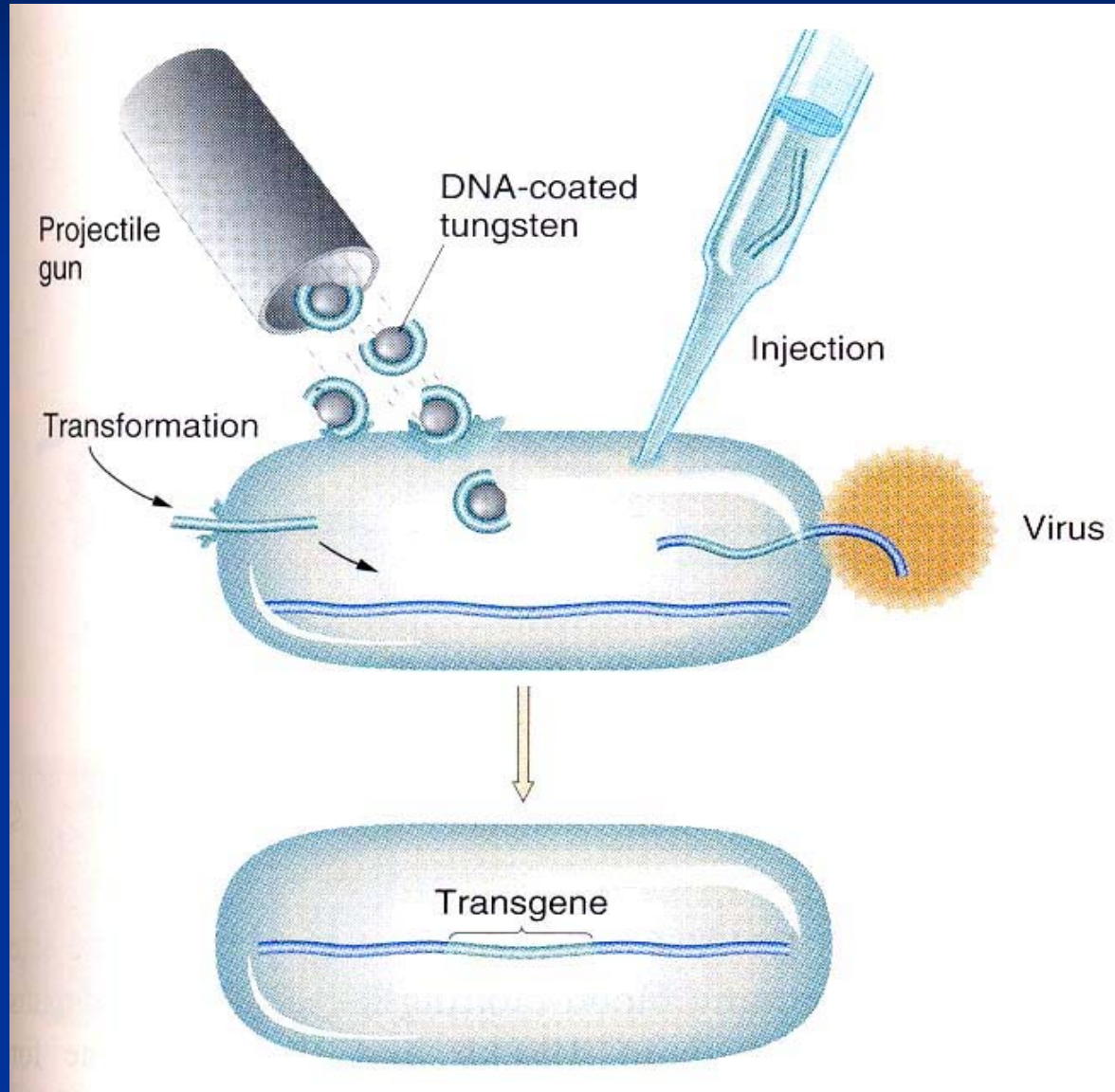
- **Genetically modified (GM) plants as bioreactors producing biological and pharmaceutical products**
 - Hormones, enzymes, vaccines
- **Genetically modified crops: GM foods.**
 - In 2000, 26% corns, 68% soybeans, and 69% cottons planted in the U.S. were GM crops.



- **Herbicide resistance**
 - Corn, soybean, rice, cotton, sugarbeet, canola
- **Insect resistance**
 - Corn, cotton, potato
- **Virus resistance**
 - Potato, squash, papaya
- **Delayed ripening**
 - Tomato
- **Nutritional enhancement**
 - Golden rice

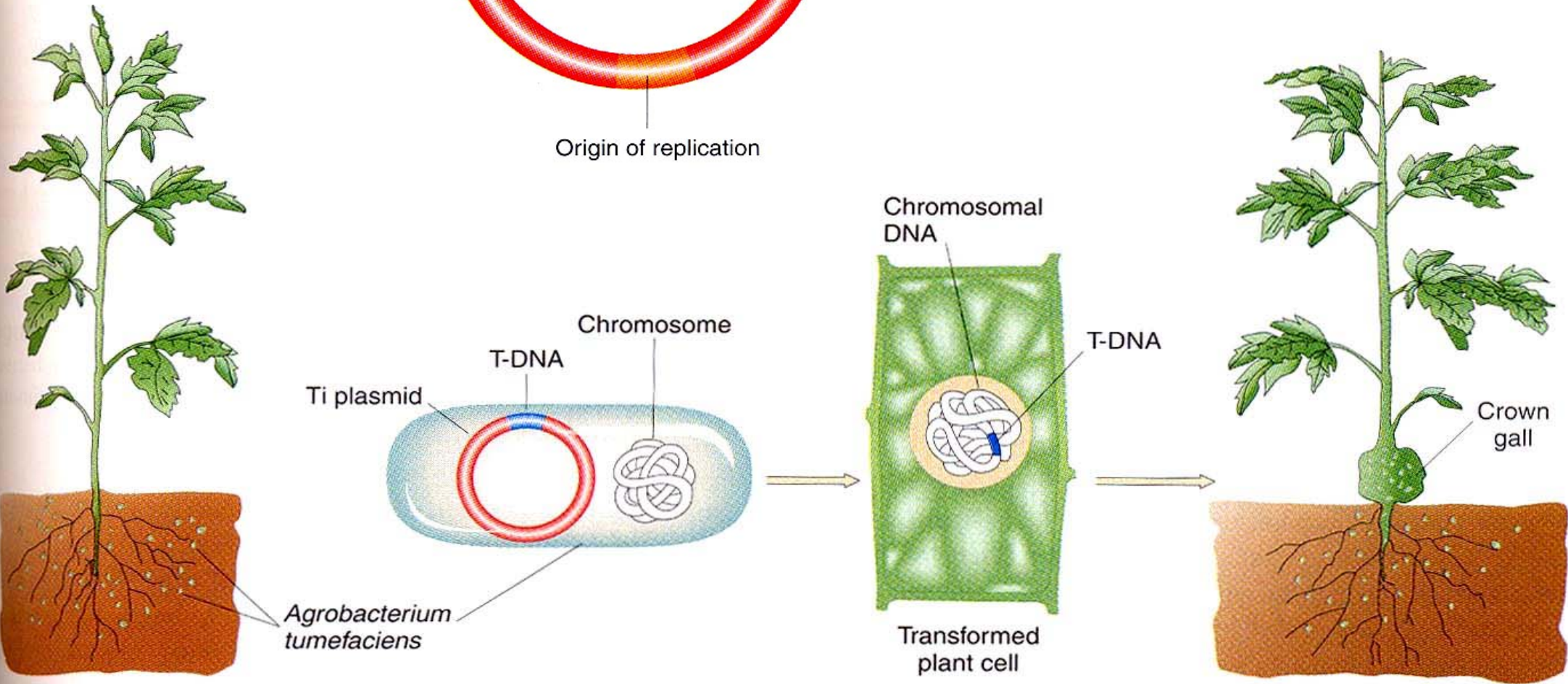
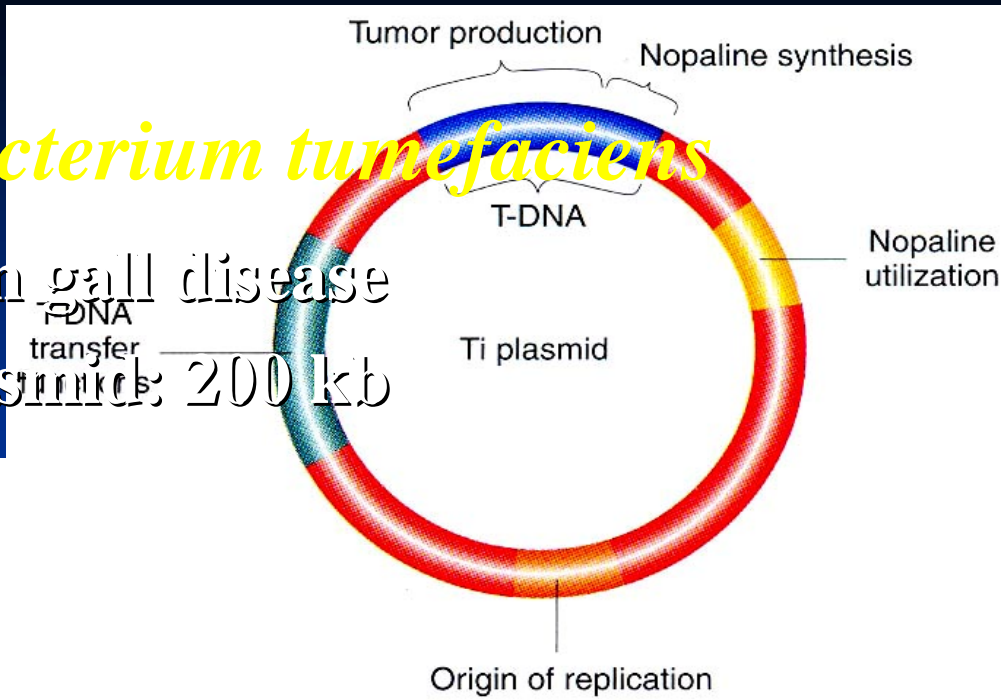


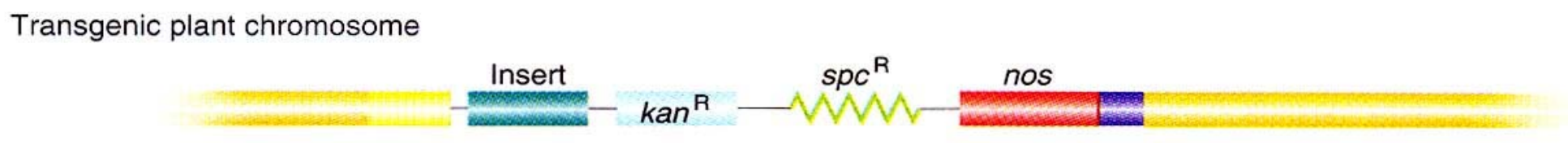
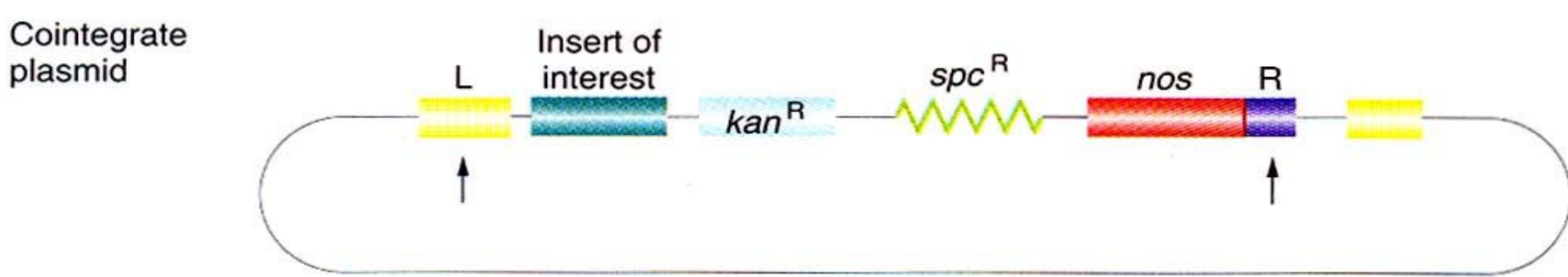
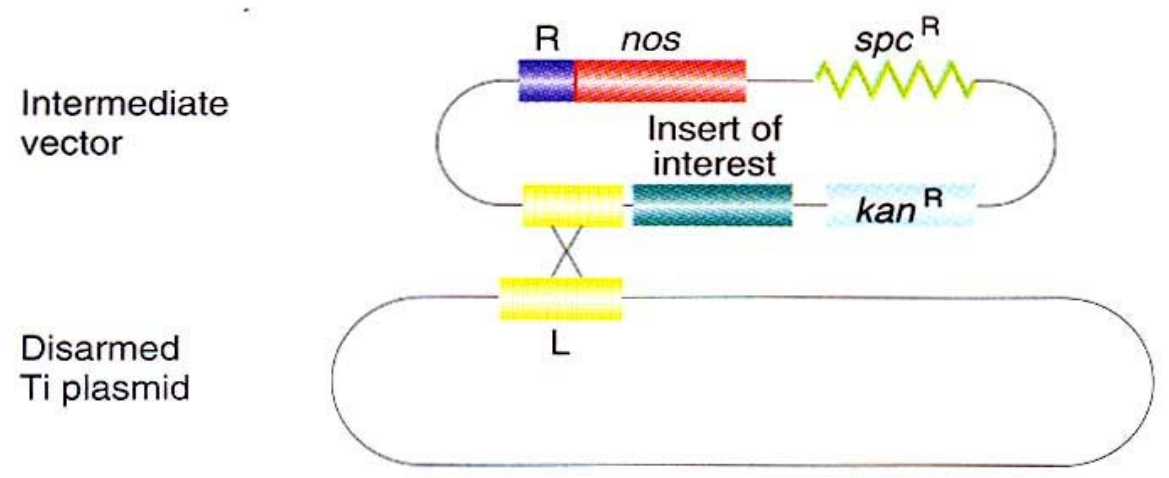
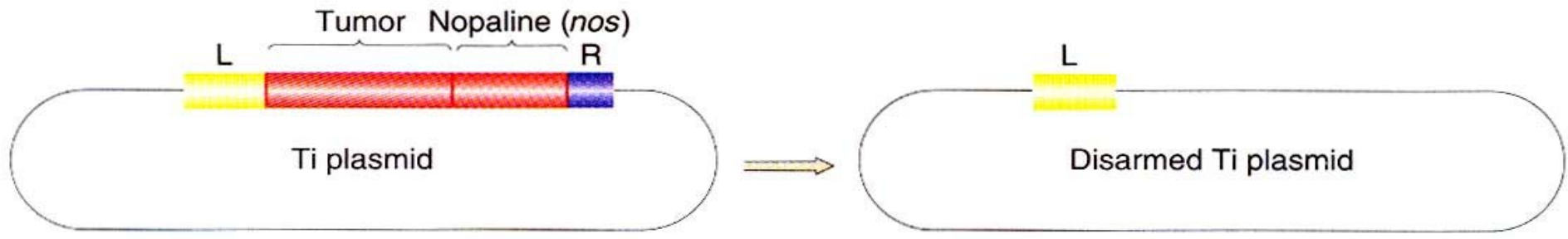
Methods of introducing foreign DNA into a cell

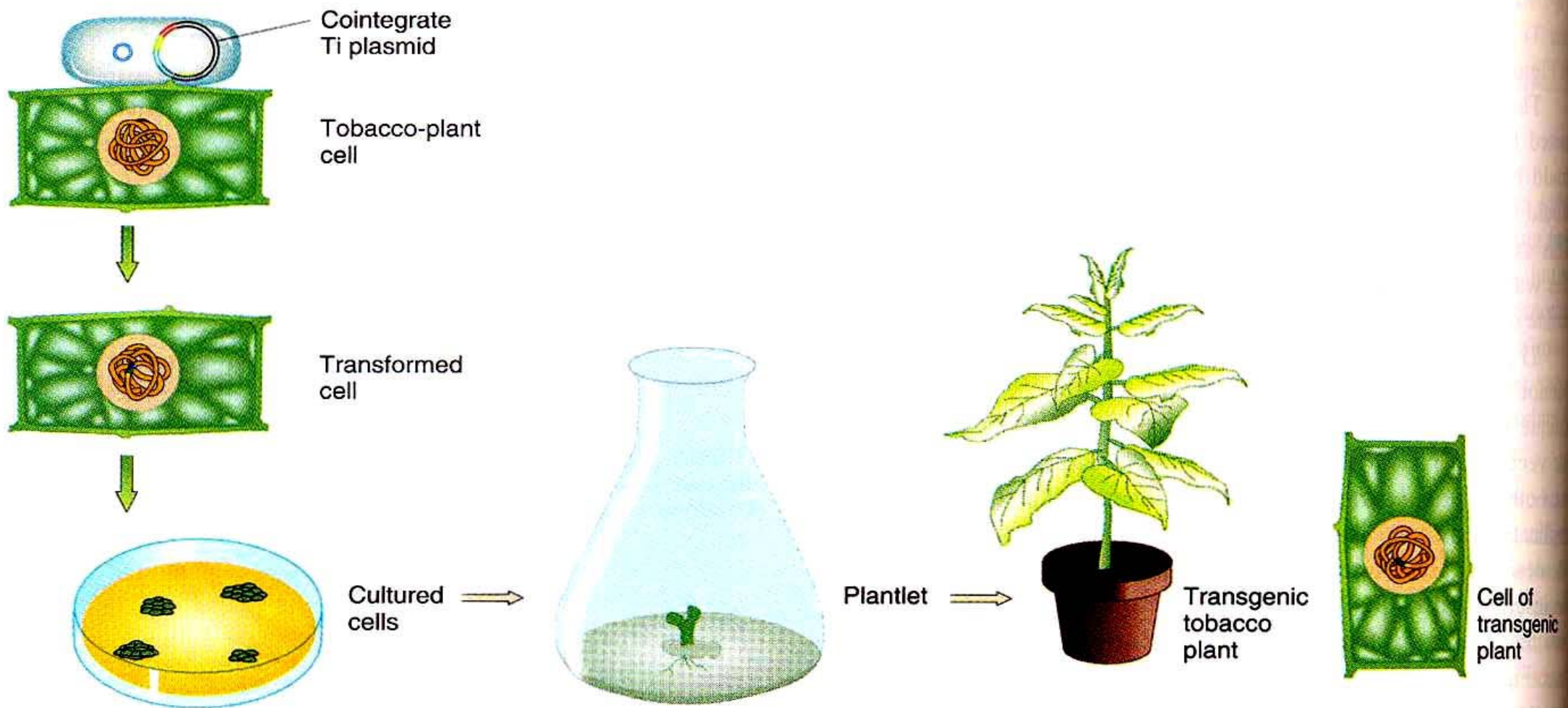


Agrobacterium tumefaciens

- Crown gall disease
- Ti plasmid: 200 kb







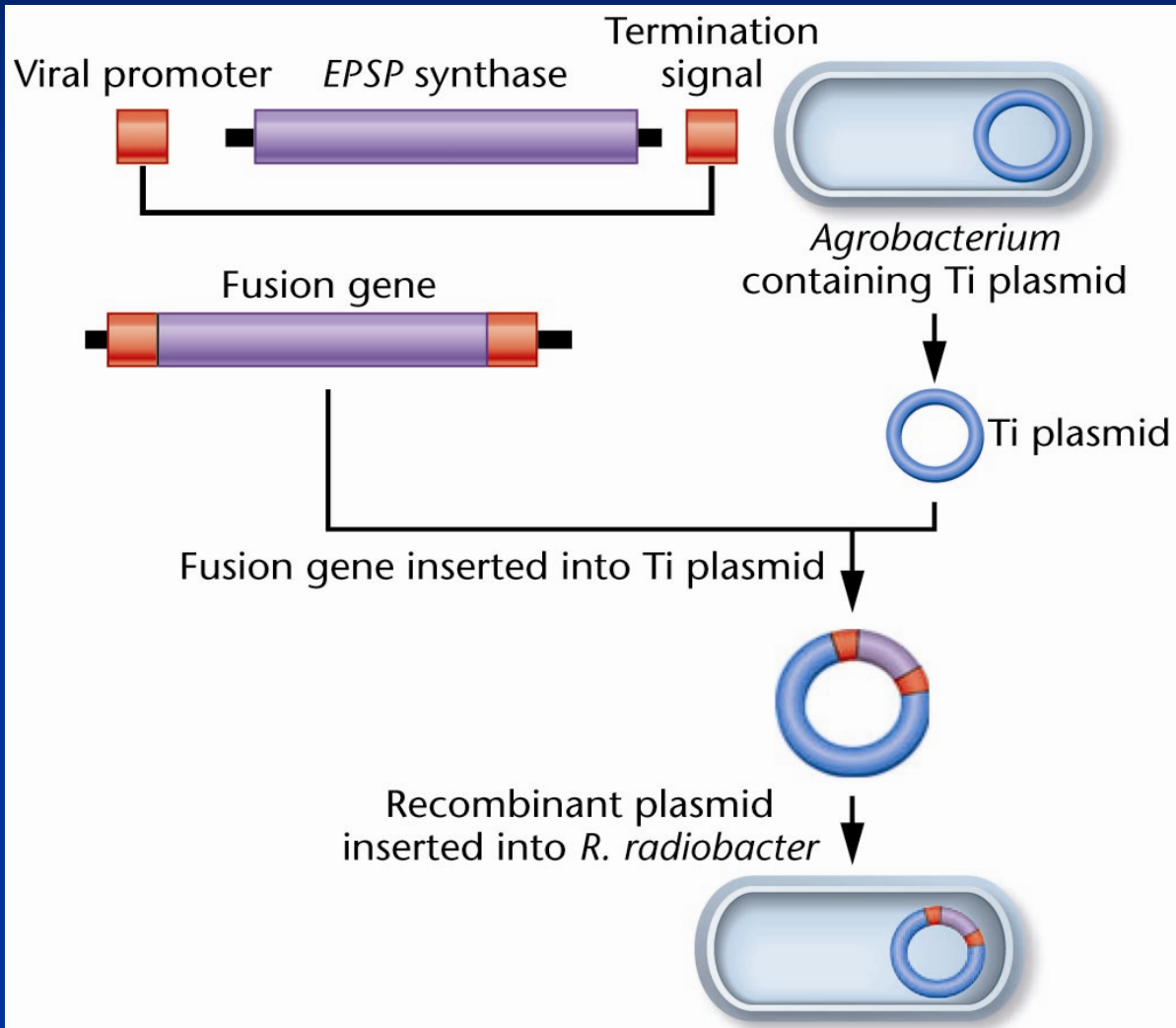
Herbicide-resistant crops

- Damage from weed infestation destroys ~ 10% of crops worldwide
- Herbicides kill weeds
 - **Glyphosate (草甘膦)**, a commercial weed killer, marketed by the name Roundup
 - By 2012, over 75% of soybeans and cottons in the U.S. are resistant to glyphosate.



Fig 22-5

Creation of glyphosate-resistant transgenic plants



R. radiobacter transfers plasmid carrying **EPSP fusion gene** into plant chromosome, resulting in high levels of **EPSP synthase**, making cell resistant to glyphosate

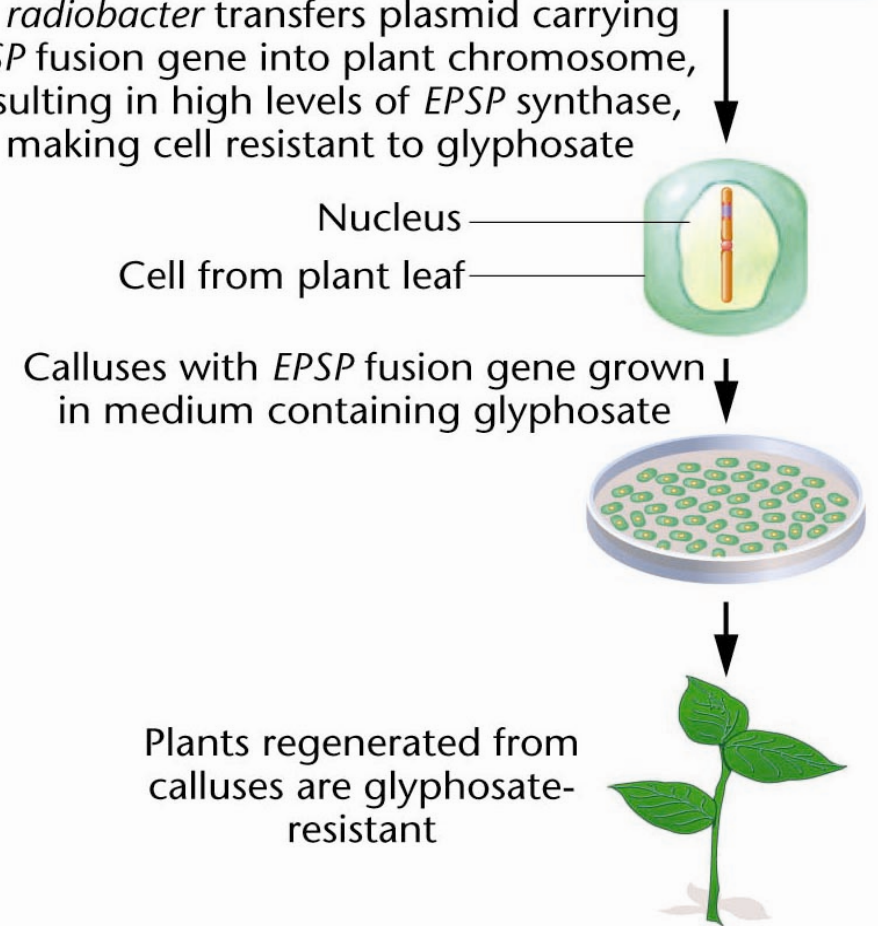


Fig 22-6

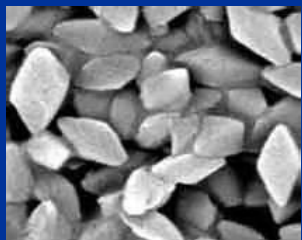
Pest-resistant crops

- **Corn-borer (玉米螟) larvae** are responsible for millions of dollars of crop damage worldwide

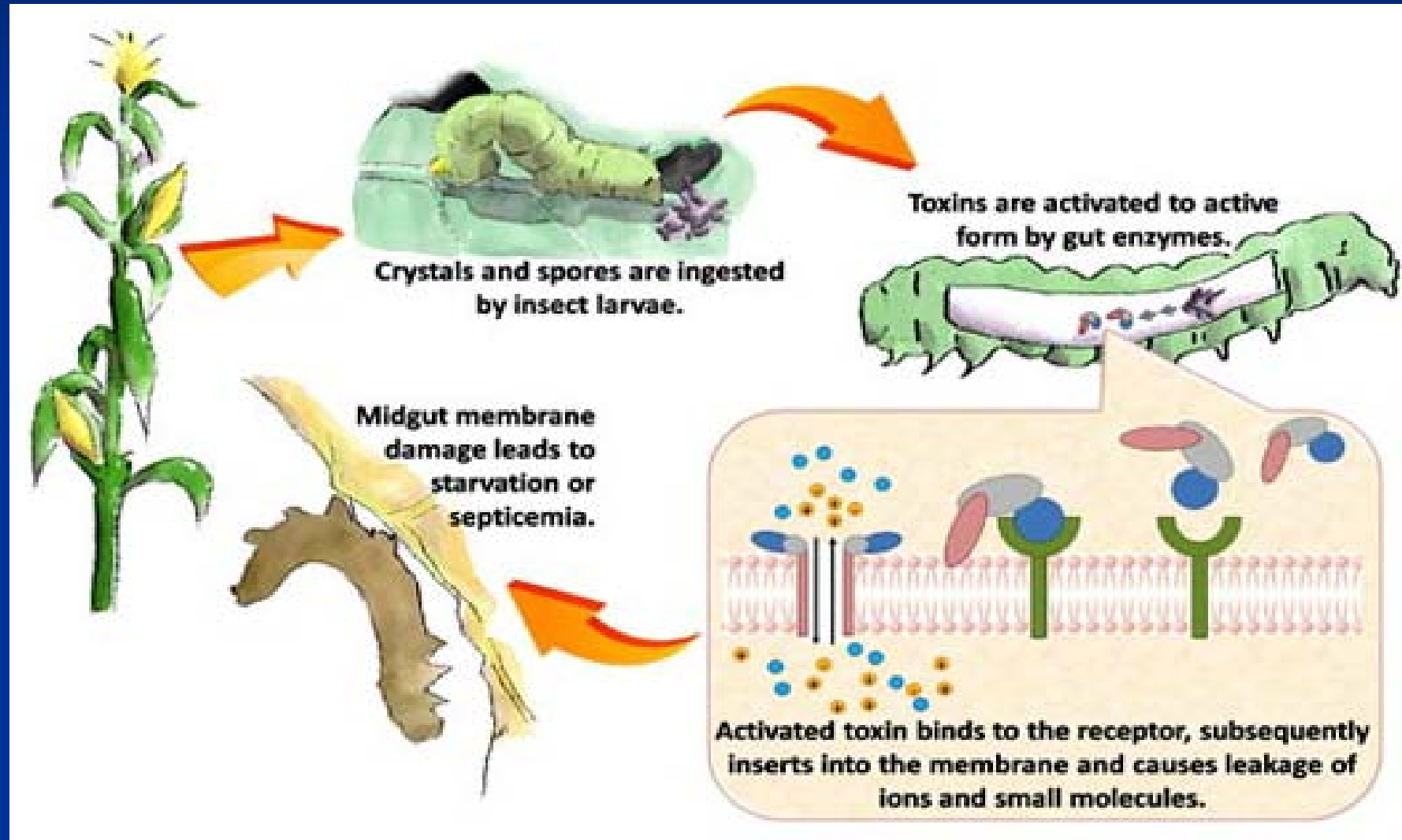


European corn borer

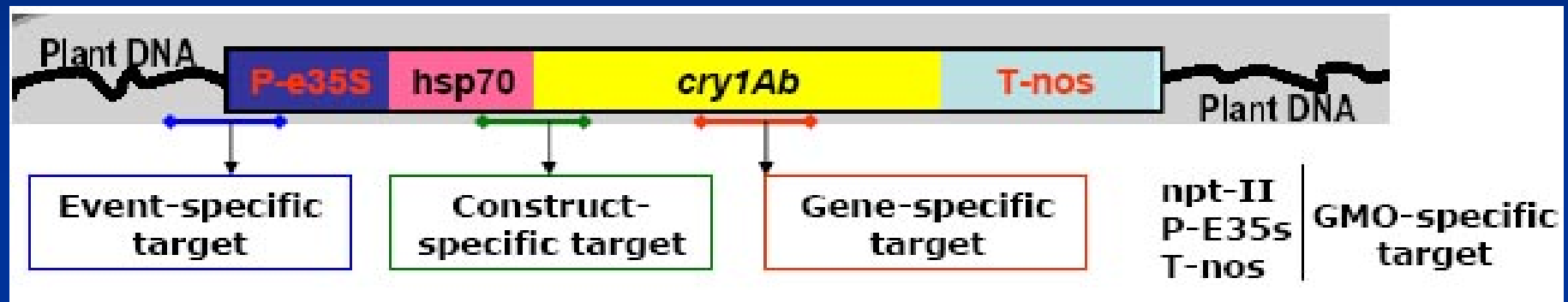
■ **Bt crystalline protein** produced by *Bacillus thuringiensis* kill insects



Bacillus Thuringiensis Crystals
Source: WikiMedia



- **Bt crops:** Transgenic crops producing Bt crystalline protein.



■ **Bt cotton** (抗虫棉)

- Transgenic cotton that are engineered to be insect resistant.
- In China, >30% of cotton planted in 2000 were Bt cotton.



Bt cotton

Regular cotton

Nutrition-enriched crops

- **Golden rice:** Transgenic rice that produces **β -carotene**, a precursor to **vitamin A**.
 - **Vitamin A deficiency** is prevalent in many areas of Asia and Africa.
- **Decaffeinated teas and coffees**



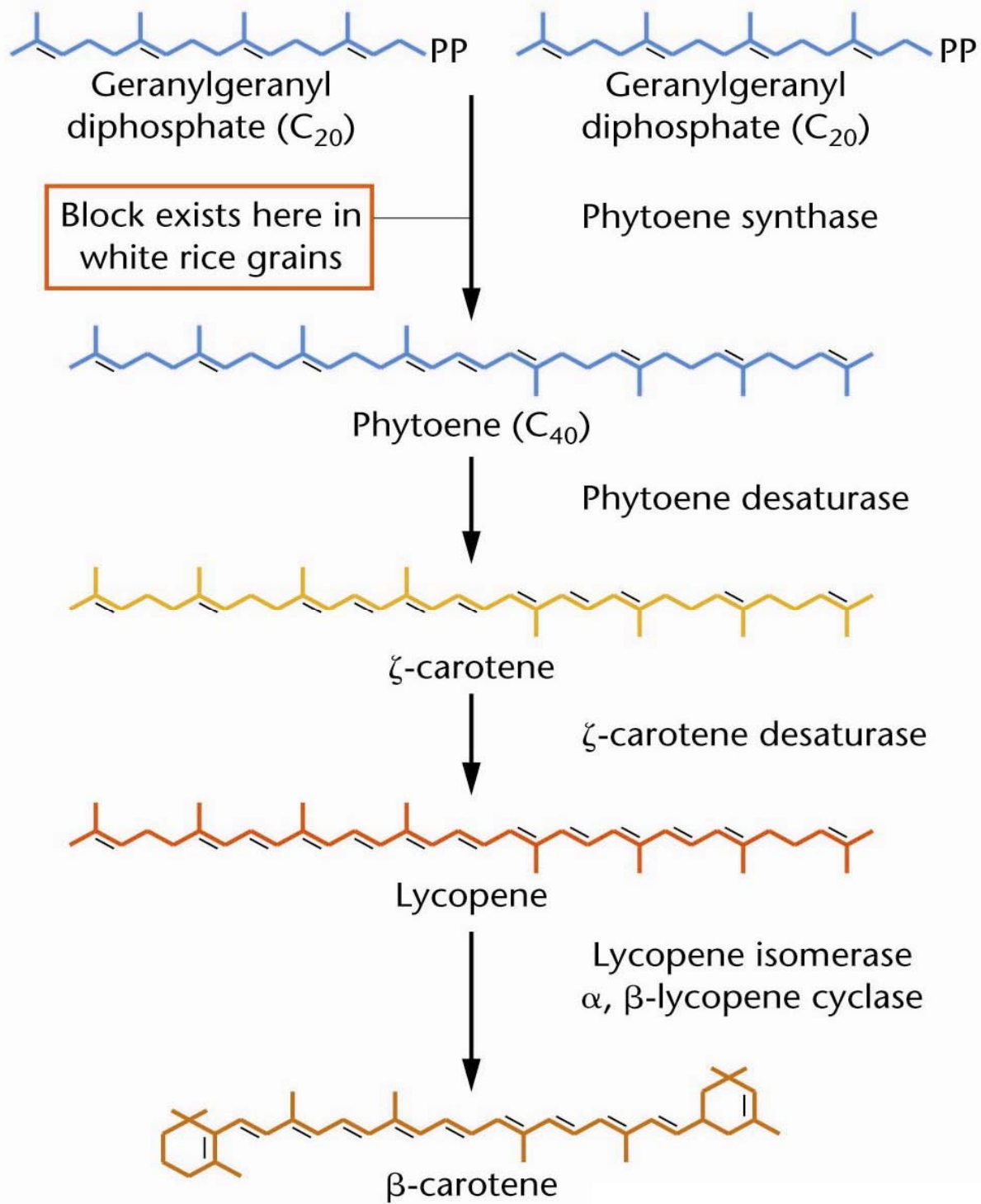
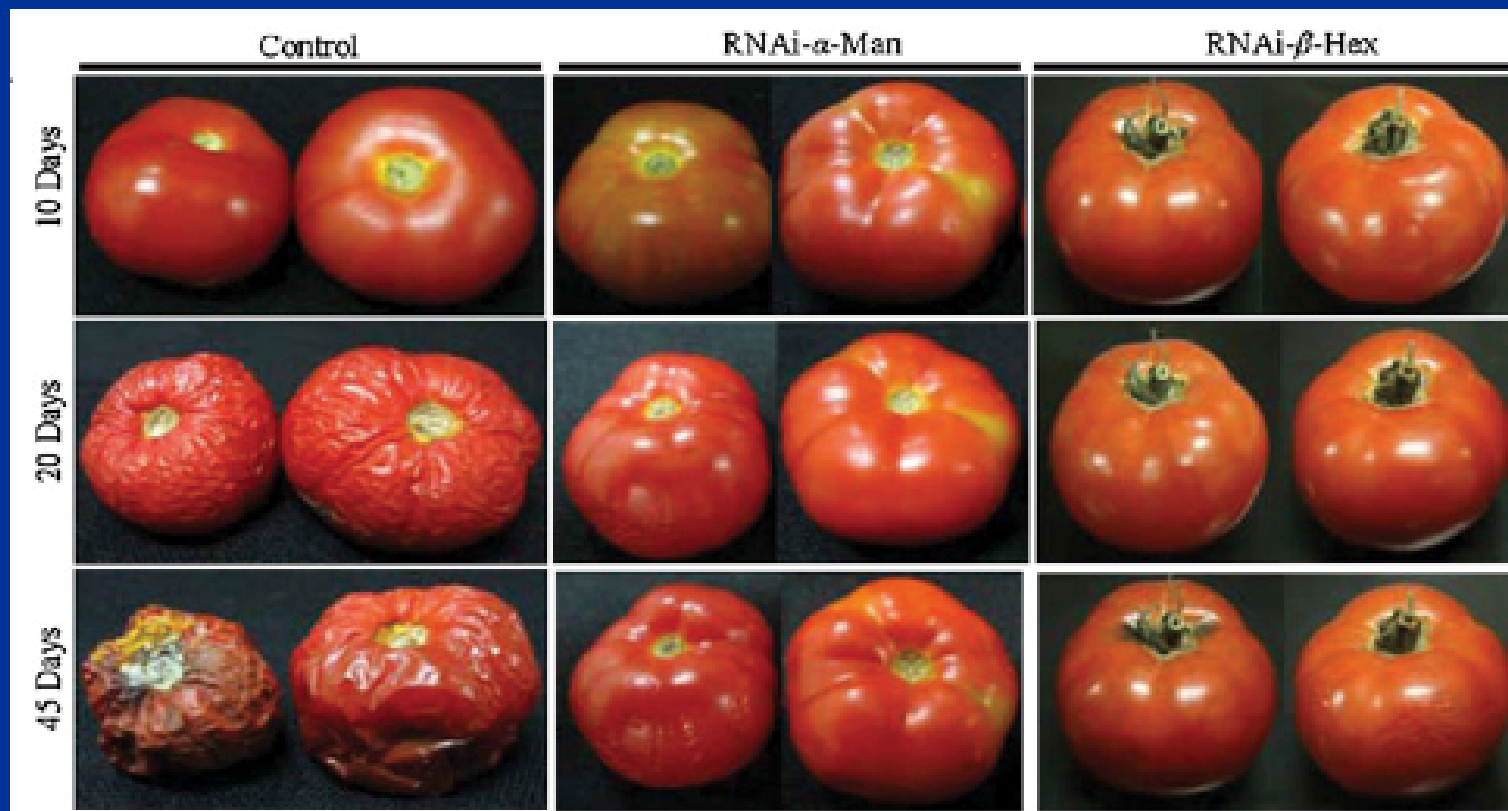


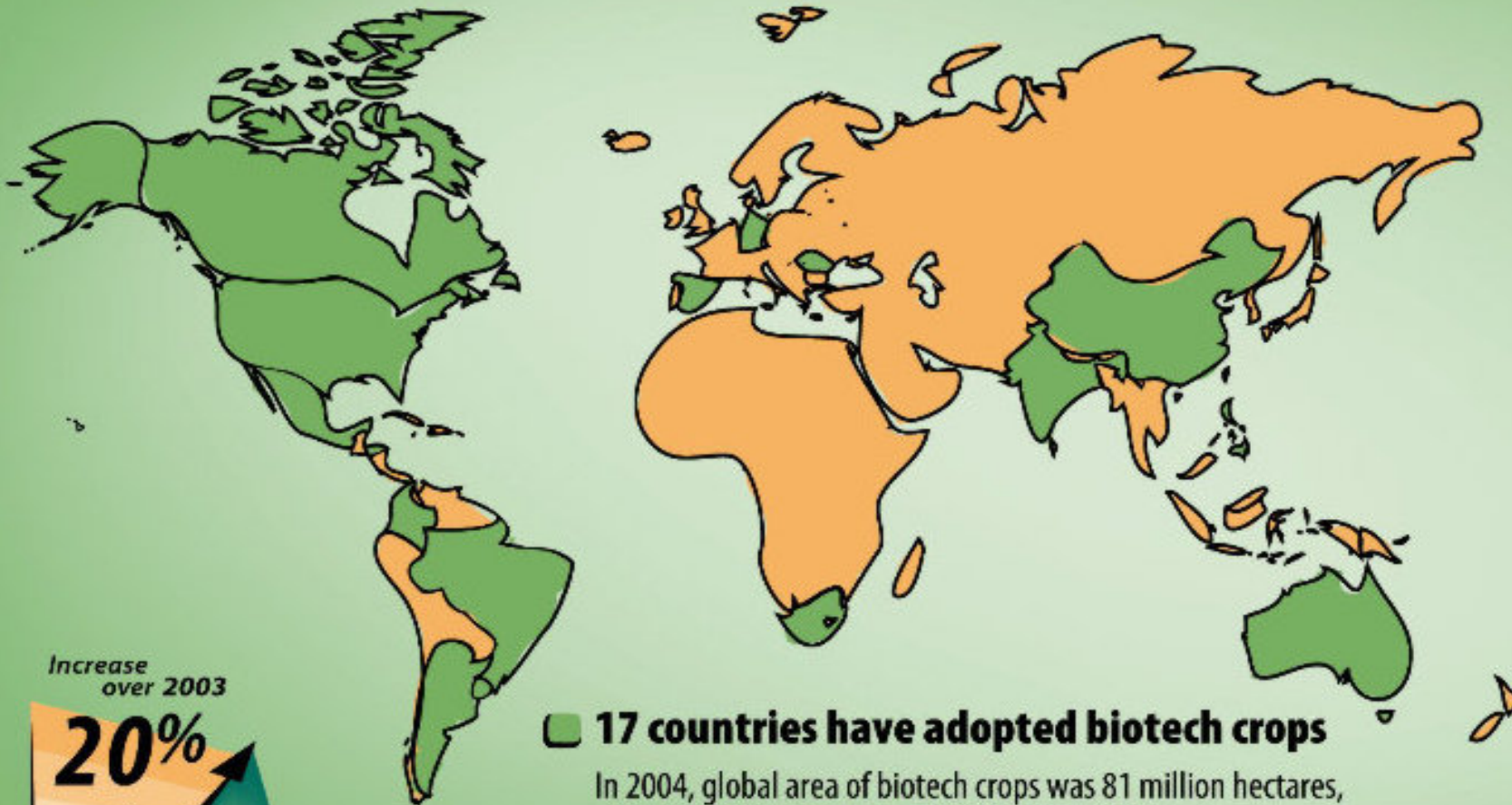
Fig 22-7b

Longer-lasting tomato

- **Flavr Savr tomato:** Transgenic tomato that has longer shelf life (several weeks). Developed by Calgene.
 - Antisense RNA technology to inhibit the production of polygalacturonase, an enzyme that digests pectin in the cell wall of tomatoes.



Global Status of Biotech Crops in 2004



Increase over 2003

20%

17 countries have adopted biotech crops

In 2004, global area of biotech crops was 81 million hectares, representing an increase of 20% over 2003, equivalent to 13.3 million hectares.

Source: Clive James, 2004 ISAAA Briefs 32

BIOTECH MEGA-COUNTRIES

50,000 hectares or more

USA:	47.6 million
Argentina:	16.2 million
Canada:	5.4 million
Brazil:	5.0 million
China:	3.7 million
Paraguay:	1.2 million
India:	0.5 million
South Africa:	0.5 million
Uruguay:	0.3 million
Australia:	0.2 million
Romania:	0.1 million
Mexico:	0.1 million
Spain:	0.1 million
Philippines:	0.1 million

50,000 hectares or less

Colombia	Honduras
	Germany

Concerns about GMOs and GM foods

- **Consumer safety**
 - Are GM plants safe to eat?
- **Environmental consequences**
 - Possible gene transfer by cross breeding with wild plants
 - Toxicity to animals
 - Loss of biodiversity due to the invasiveness of the modified plant

3 Genetic engineering in animals

- Genetically engineered animals as bioreactors producing biological and pharmaceutical products
 - Hormones, enzymes, vaccines
- Genetically engineered animals as products
 - Transgenic fish

Creation of transgenic animals



Fig 22-8

The first GM pet in the U.S.

■ GloFish

- Transgenic **zebrafish** containing red fluorescent protein gene from sea anemones.



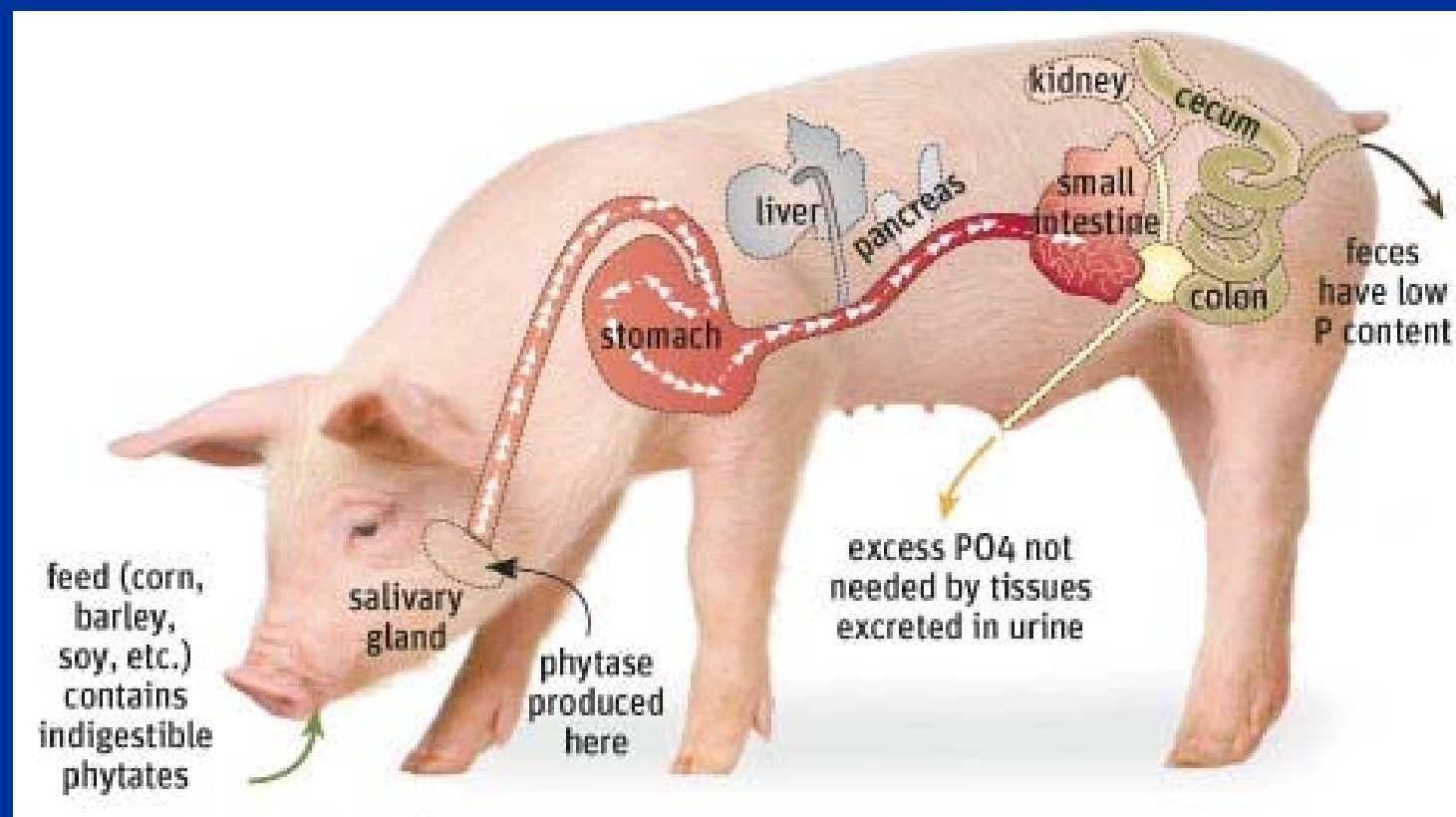
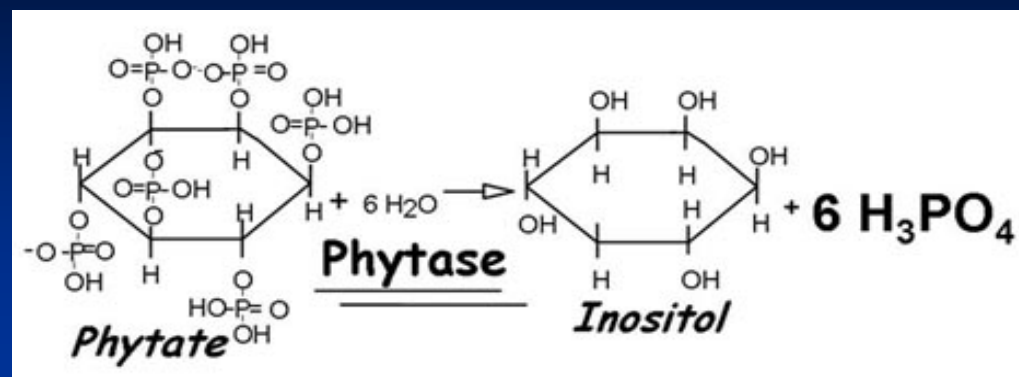
Fig 22-11

Environment friendly GM pigs



■ EnviroPig

- Expressing *E. coli* phytase (植酸酶) gene in the salivary gland.
- Reducing phosphorus excretion.



Creation of oversize farm animals by overexpressing growth hormone

- **Most have failed!**
 - Growth is a complex, multigene trait?
- **Transgenic Atlantis salmon**
 - Expressing Chinook salmon GH gene
 - Weighs 10 times more than non-transgenic strains

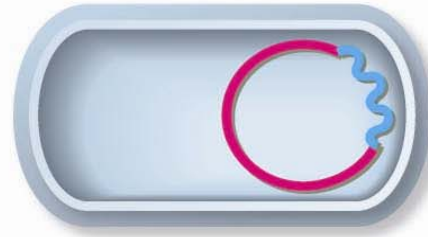


4 Human gene therapy

- Began in 1990 with the treatment of a young girl with **severe combined immunodeficiency (SCID)**
 - Caused by a mutation in the adenosine deaminase (ADA) enzyme
- **Delivery systems**
 - Adeno-associated virus (AAV)
 - Retroviral vectors
- **RNAi therapy**
 - Several RNAi clinical trials are underway in the U.S.
 - Treatment of a form of blindness, due to a mutation in *VEGF* gene (promotes blood vessel growth).



Bacterium carrying plasmid with cloned normal human ADA gene



Genetically disabled retrovirus



Cloned ADA gene is incorporated into virus



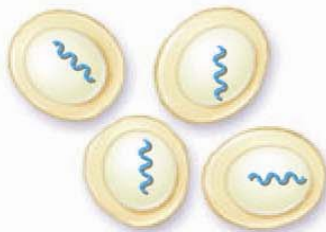
T cells isolated from SCID patient



Retrovirus infects blood cells, transfers ADA gene to cells



Genetically altered cells are reimplanted, produce ADA



Cells are grown in culture to ensure ADA gene is active

Fig 22-27

- **From 1990 to 1999, more than 4000 people underwent gene therapy for a variety of genetic disorders. Many of them have failed.**
 - **In 1999-09, Jesse Gelsinger died while treating a liver disease in the U.S.**
 - **In 2003, 2 of the 10 children treated for X-linked SCID developed leukemia in France. Due to the activation of *LMO2* gene.**

Ethical concerns about gene therapy

- **Somatic gene therapy**
 - All gene therapy trials are restricted to using somatic cells.
- **Germ-line therapy**
 - Germ cells or mature gametes are used as targets for gene transfer.
 - Not approved.