JAGIELLONIAN UNIVERSITY
IN KRAKOW

Medical biotechnology
introduction

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Rules

15 hours course – 2 ECTS

Final exam: multiple choice test

In the begining was…

What is biotechnology

Biotechnology:

bio - the use of biological processes;

technology - to solve problems or make useful products.

Pre-History:

10,000 years ago - humans domesticate crops and livestock.

6,000 years ago - Biotechnology first used to leaven bread and ferment beer, using yeast (Egypt).

6,000 years ago - Production of cheese and fermentation of wine (Sumeria, China and Egypt).

2,500 years ago - First antibiotic: moldy soybean curds used to treat boils (China).

Wall paintings from the Tomb of Kenamun

Some traditional products of biotechnology

Wheat

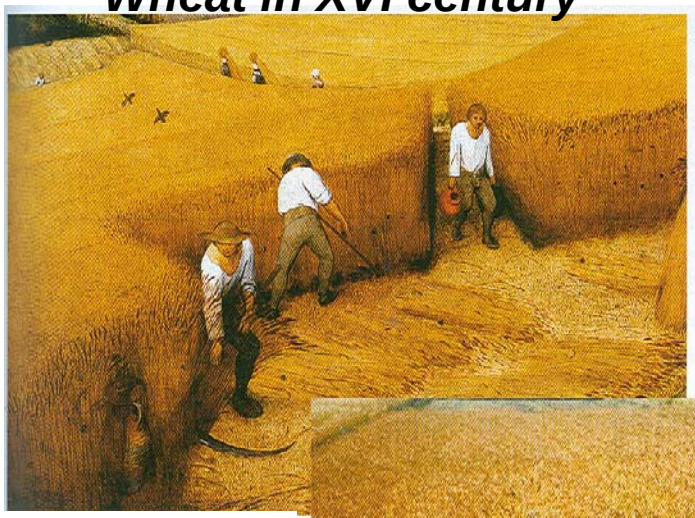
Maize

Plant modification is made by humans since centuries

Wild wheat

Cultivated wheat

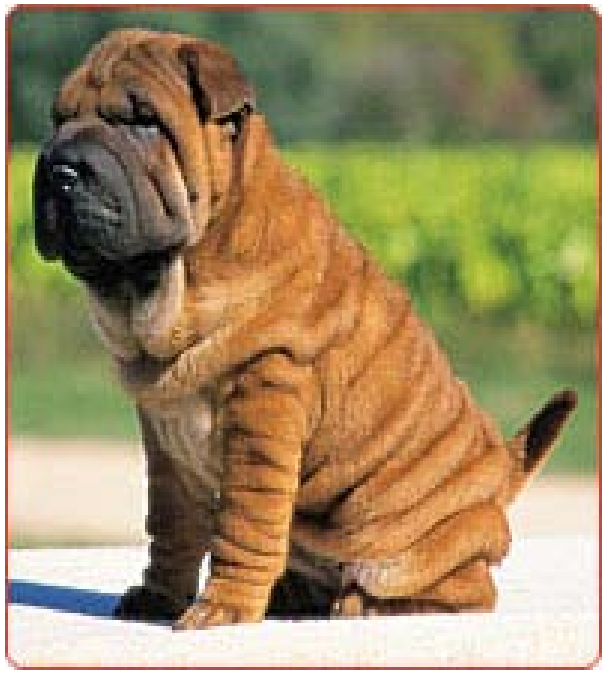
Wheat in XVI century



Wheat today

natural dog

selected dog



History

1797 - Jenner inoculates a child with a viral vaccine to protect him from smallpox.

1919 - First use of the word *biotechnology* in print.

1928 - Penicillin discovered as an antibiotic: Alexander Fleming.

1938 - The term *molecular biology* is coined.

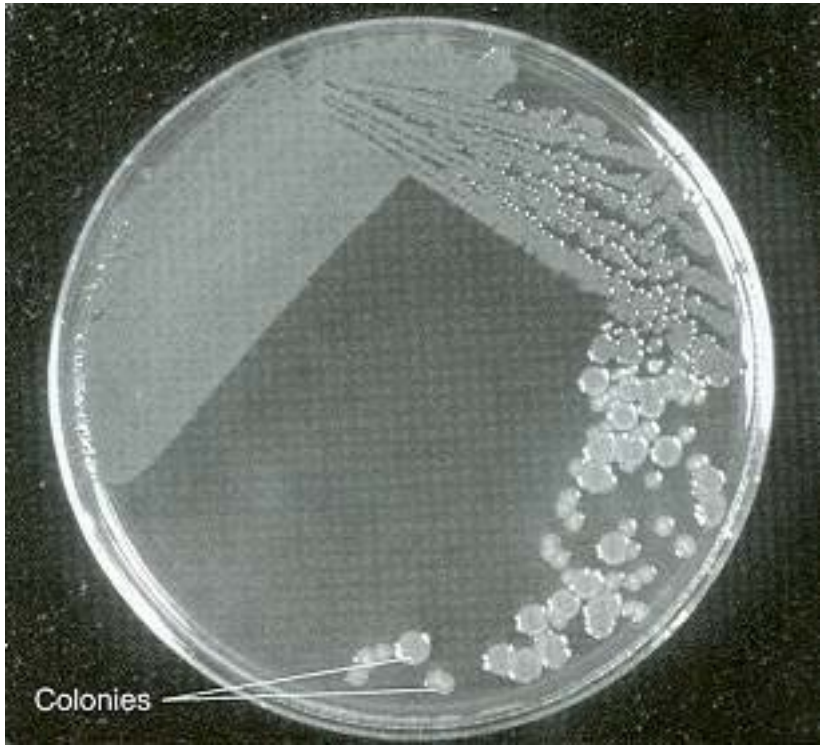
1941 - The term *genetic engineering* is first used, by Danish microbiologist A. Jost in a lecture on reproduction in yeast at the technical institute in Lwow, Poland.

1942 - Penicillin mass-produced in microbes.

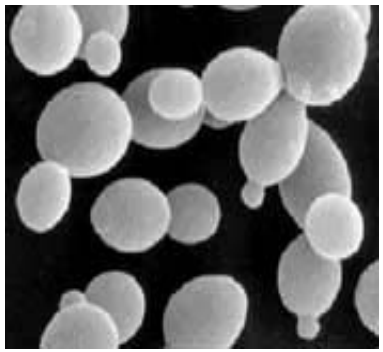
1944 - Waksman isolates streptomycin, an effective antibiotic for tuberculosis.

Edward Jenner's first vaccination

Bioreactors enable large-scale cultivation of microorganisms



bacteria



yeast

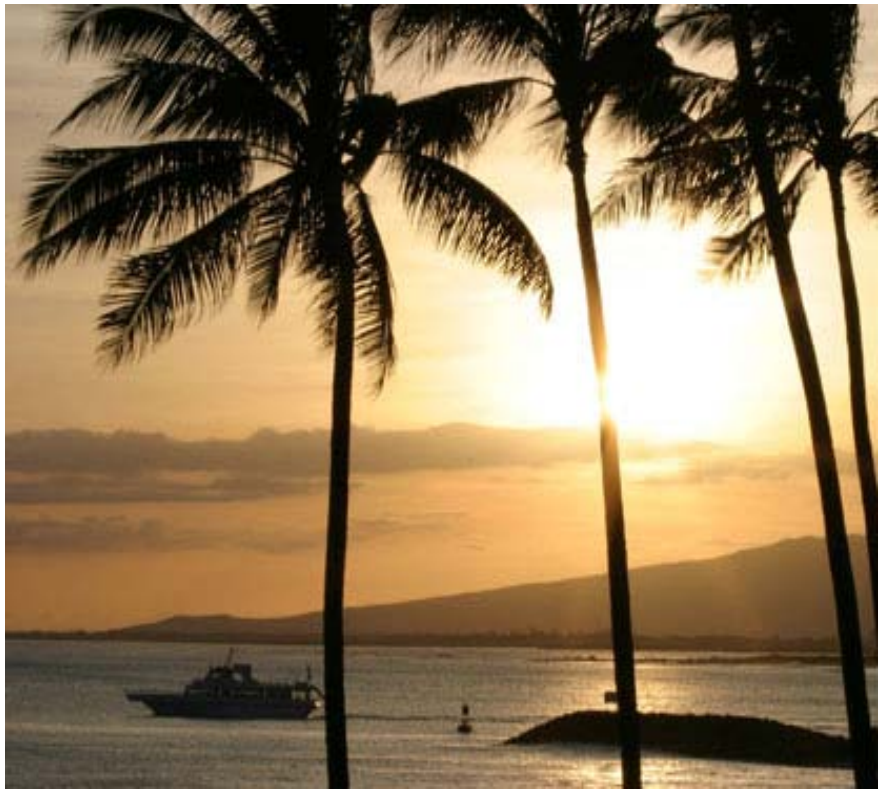
bioreactor

What is biotechnology

During the 1960s and ’70s understanding of biology reached a point where use the biological molecules - in addition to using whole organisms could begin.

A more appropriate definition in the new sense of the word is this:

“New” Biotechnology - the use of cellular and biomolecular processes to solve problems or make useful products.



Here the biotech industry was born

What is biotechnology

The first steps of biotechnology industry:

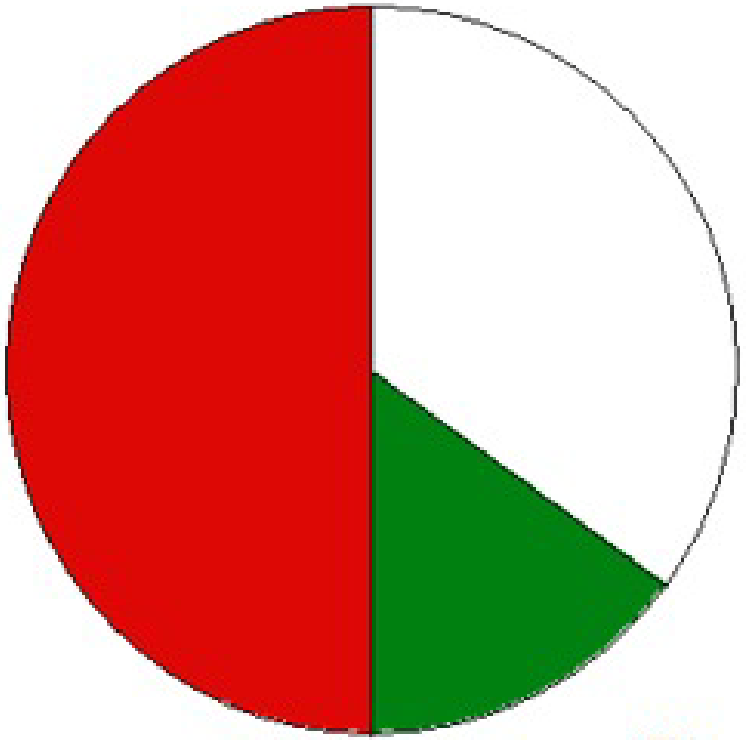
- The biotechnology industry originated in the 1970s, based largely on a new recombinant DNA technique whose details were published in 1973 by Stanley Cohen from Stanford University and Herbert Boyer from the University of California, San Francisco. Herbert Boyer went on to co-found Genentech, which today is biotechnology's largest company by market capitalization.

Herbert Boyer *Stanley Cohen*

- In 1982, recombinant human insulin became the first biotech therapy to earn FDA approval. The product was developed by Genentech and Eli Lilly and Co.

Divisions of biotechnology

50%
biotechnologia czerwona
(ochrona zdrowia, diagnostyka)



35%
biotechnologia biała (m.in. fermentacja, synteza farmaceutyków)

15%
biotechnologia zielona
(m.in. produkty dla rolnictwa)

T. Twardowski, S. Bielecki, European Biotechnology 2005

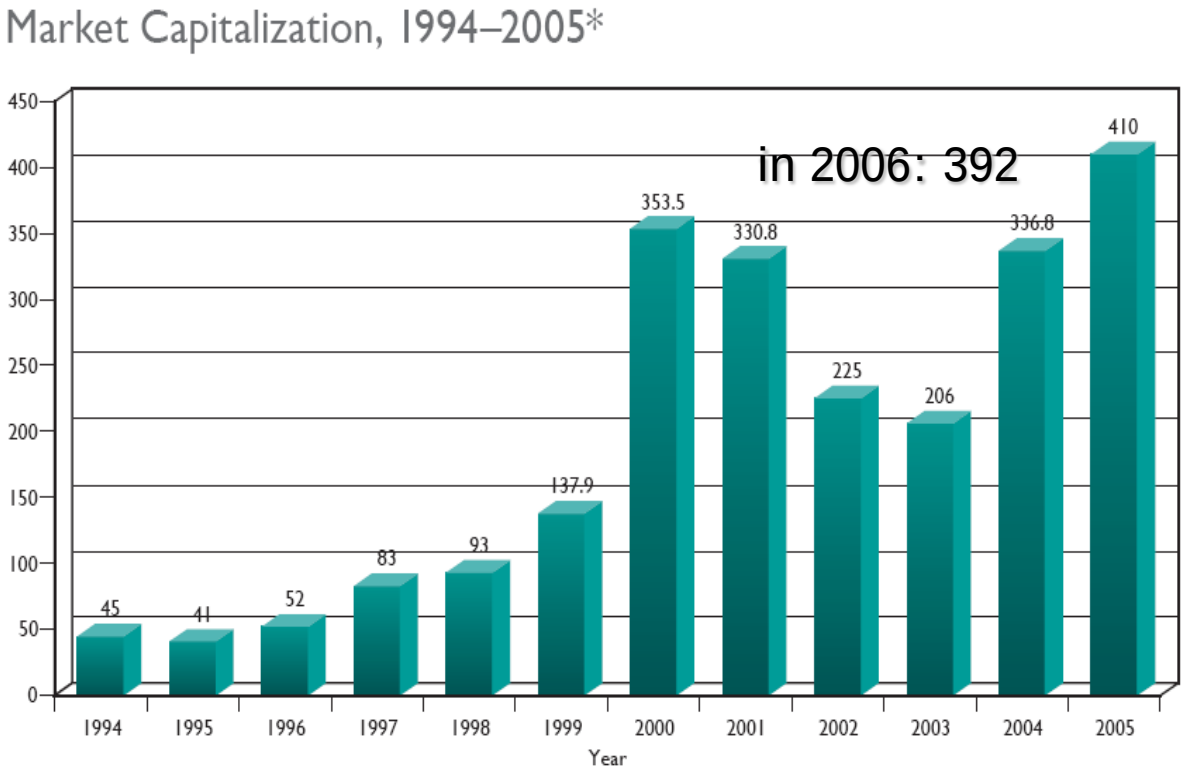
Biotechnology: one of the most research-intensive industries in the world

* The U.S. biotech industry spent **$19.8 billion** on research and development in 2005 (academic bioscience: **$29.0 billion** in 2006). The top five biotech companies invested an average of **$170,000** per employee in R&D in 2006.

* The biotechnology industry has mushroomed since 1992, with U.S. health-care biotech revenues increasing from $8 billion in 1992 to $58.8 billion in 2006.

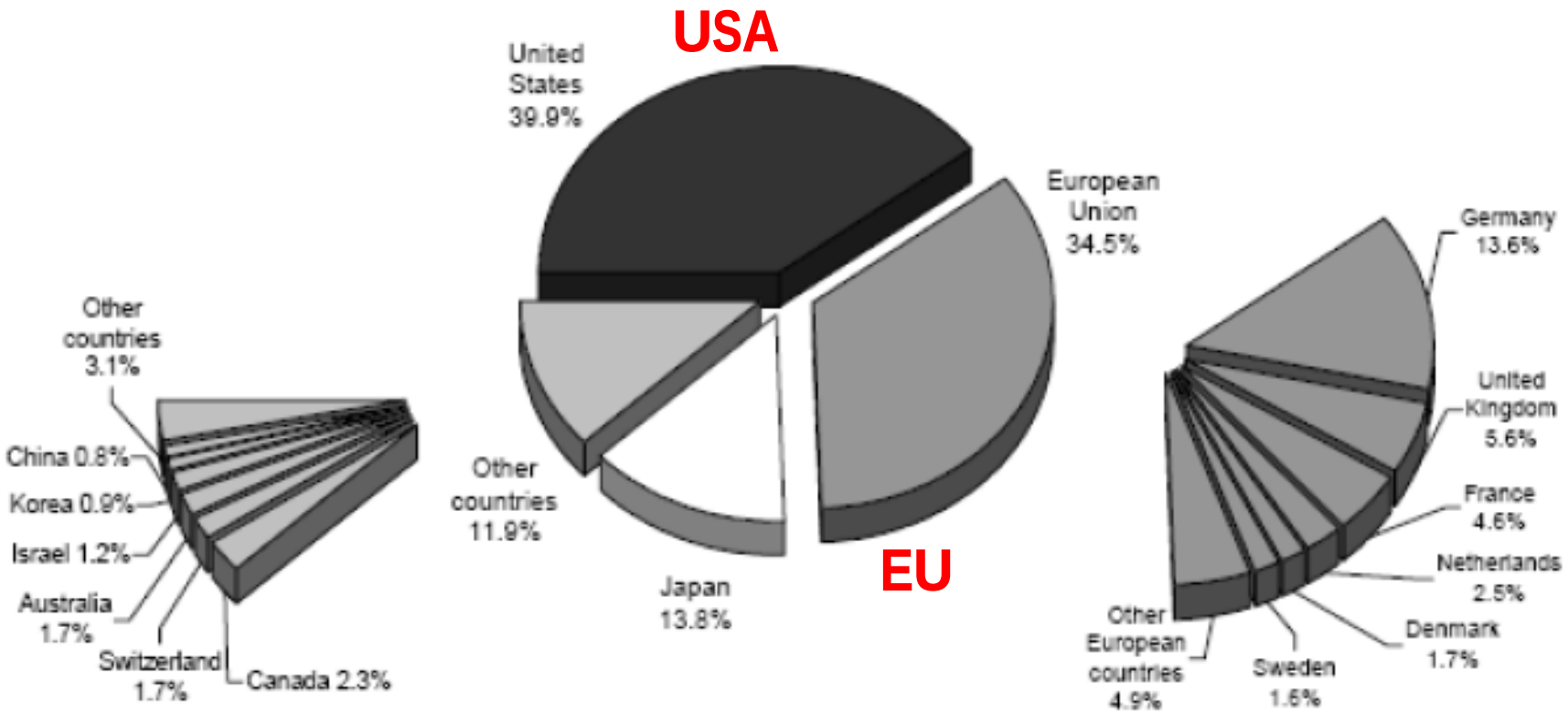
* The biosciences - including all life sciences activities - **employed 1.3 million people** in the United States in 2006 and generated an additional **7.5 million related jobs**.

* The average annual **wage** of U.S. bioscience workers was **$71,000** in 2006, more than 29,000 greater than the average private sector annual wage.



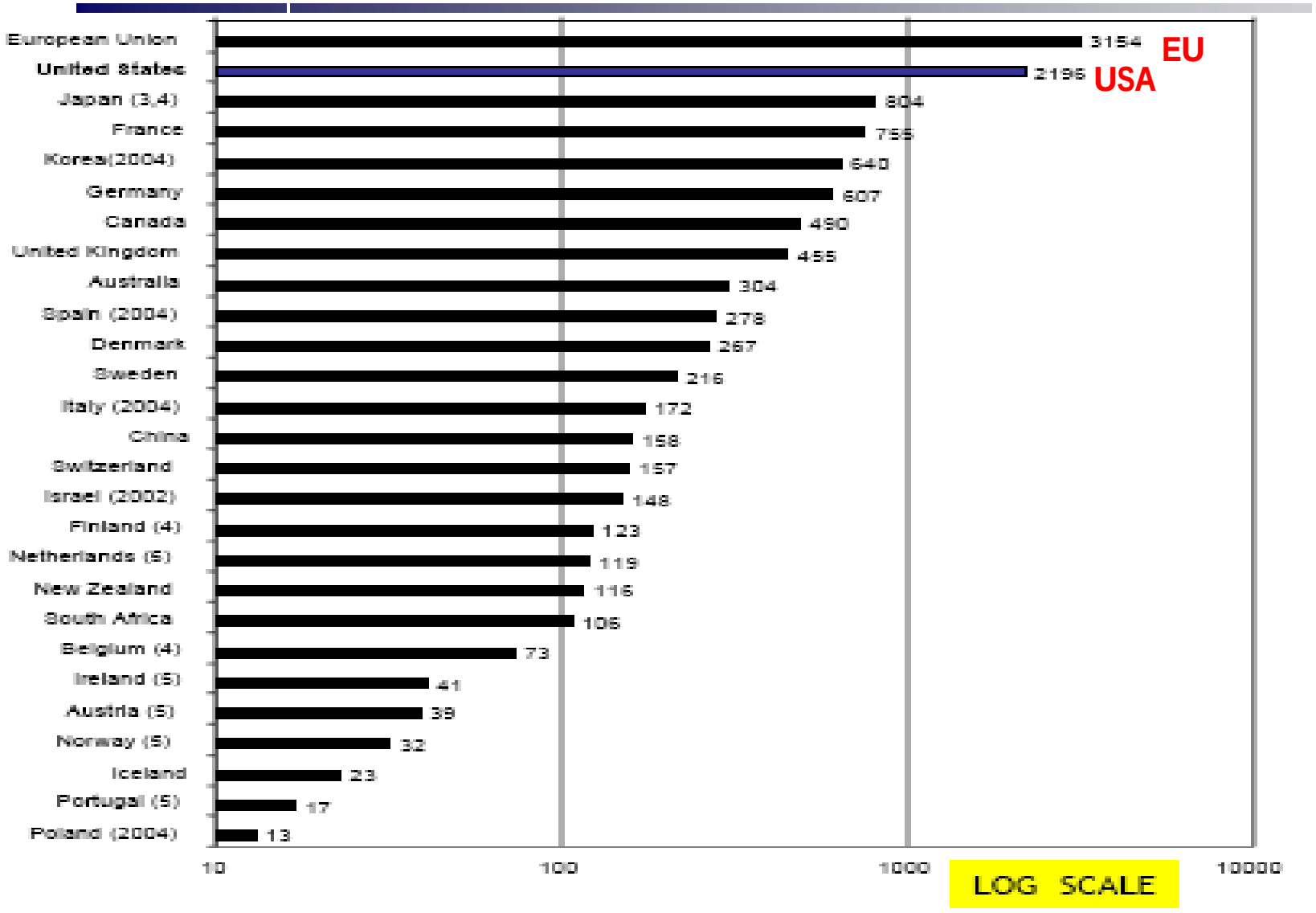
OECD Biotechnology Statistics, 2006

Biotechnology - number of patents



OECD Biotechnology Statistics, 2006

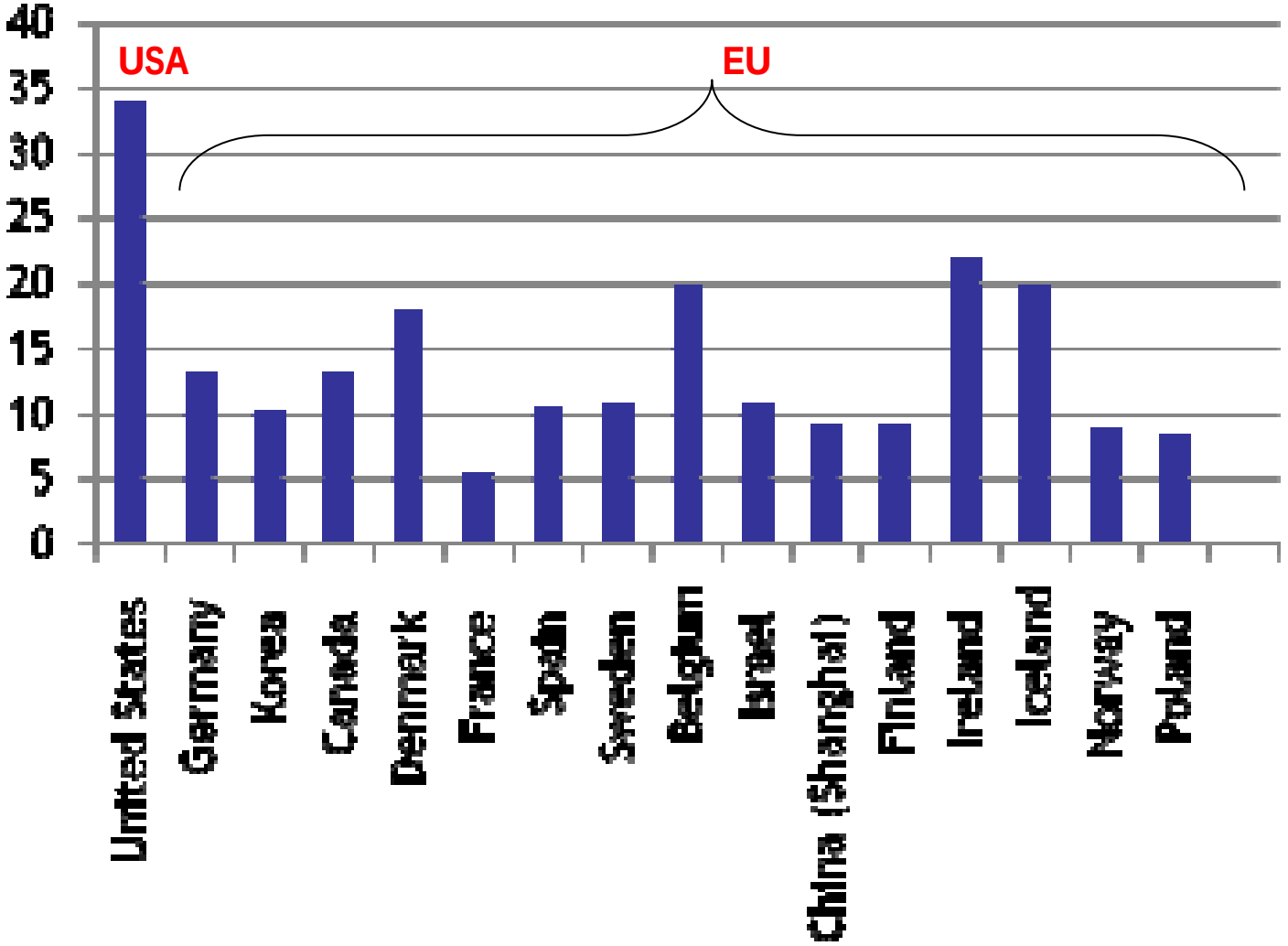
Biotechnology - number of firms



OECD Biotechnology Statistics, 2006

Biotechnology - number of employees

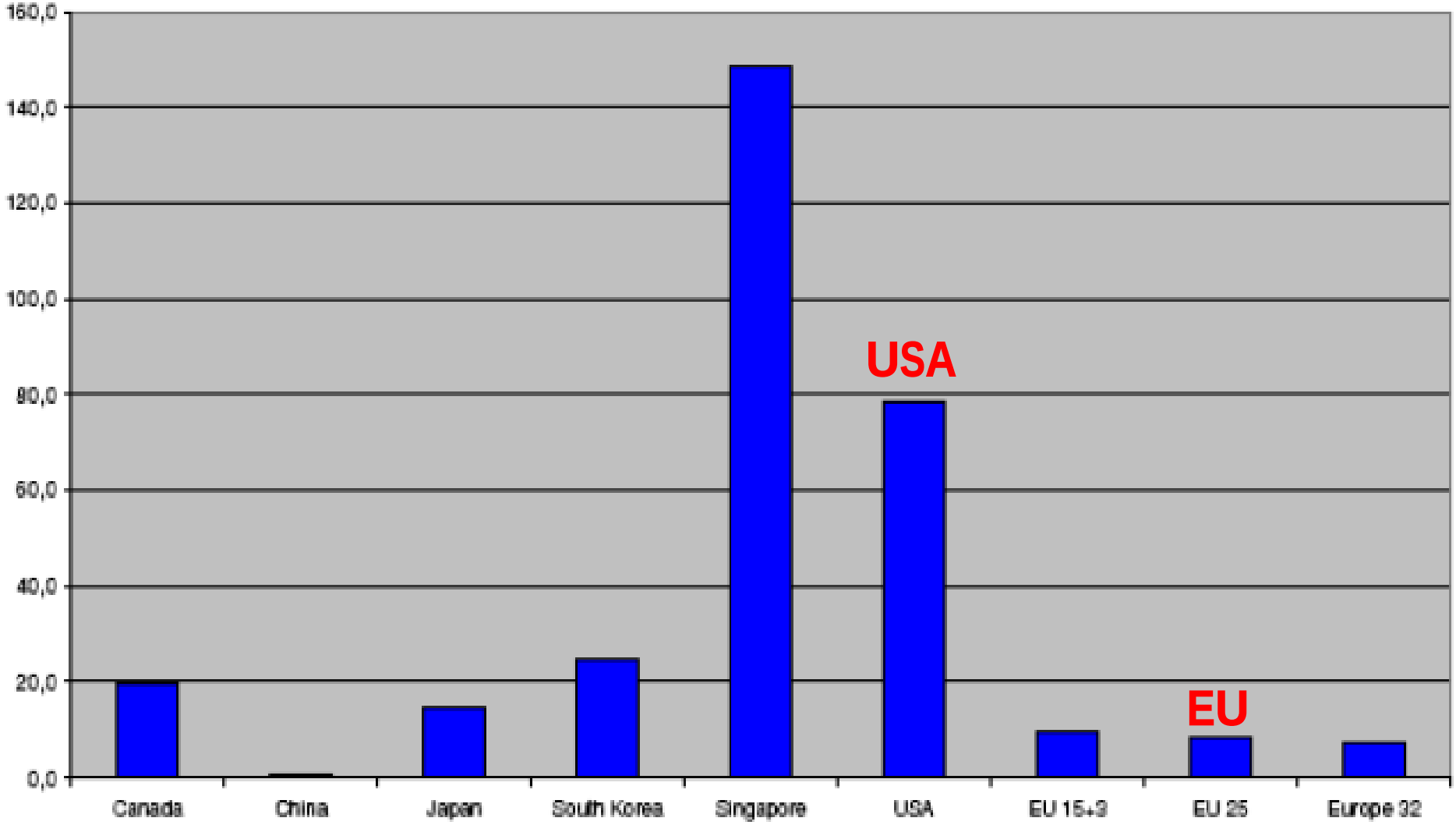
However, while the average US Biotech has 33 employees/company, the EU has on average 10 employees/company



OECD Biotechnology Statistics, 2006

Biotechnology - public funds

spending per million capita



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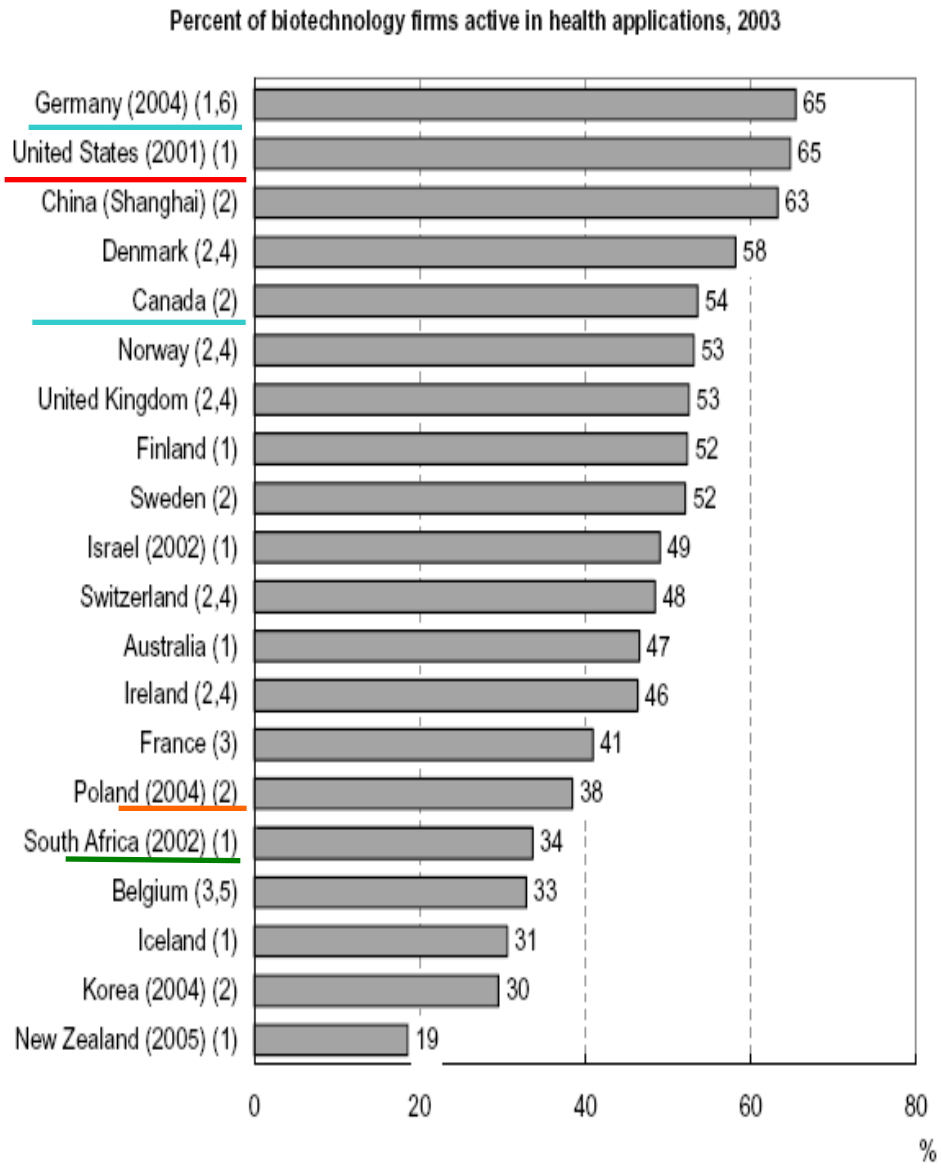
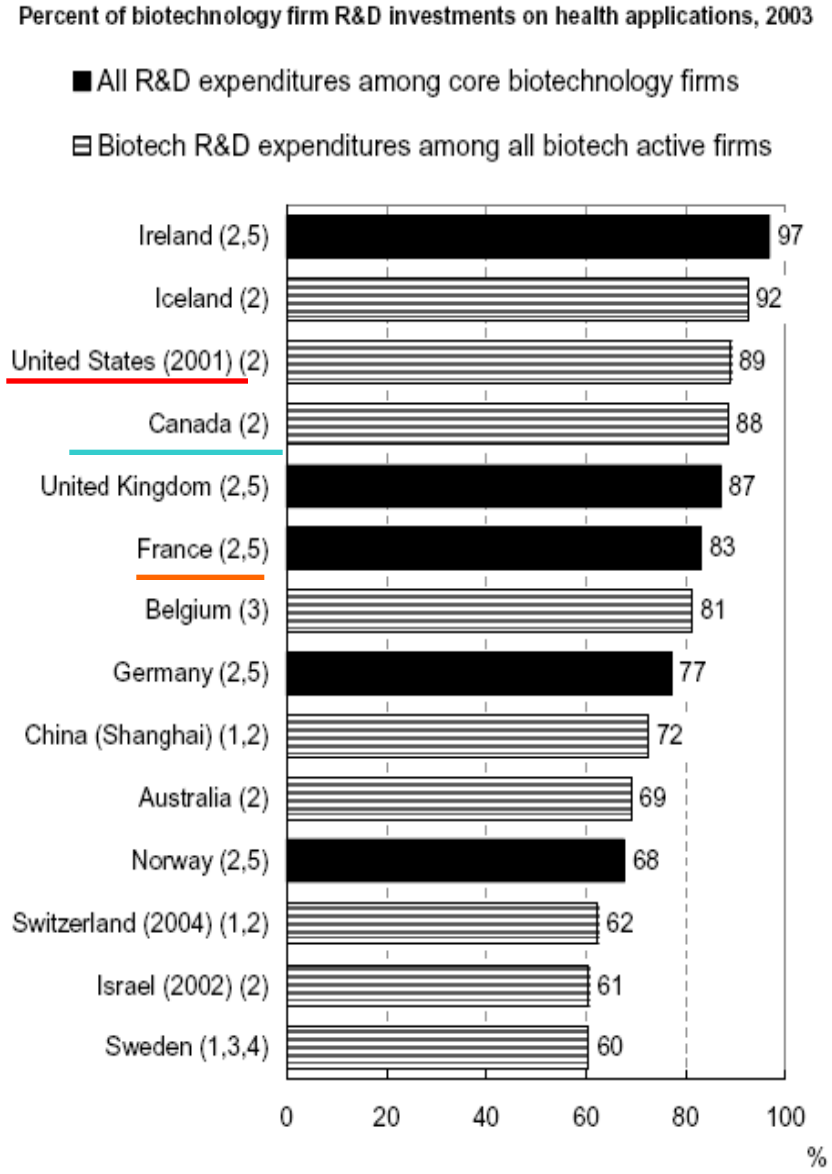




Biotechnology - achievements

Biotechnology therapeutics approved by the U.S. Food and Drug Administration (FDA) to date are used to treat many diseases, including leukemia and other cancers, anemia, cystic fibrosis, growth deficiency, rheumatoid arthritis, hemophilia, hepatitis, genital warts, and transplant rejection.

Biotechnology has created:

* more than 200 new therapies and vaccines, including products to treat cancer, diabetes, AIDS and autoimmune disorders.

* more than 400 drug products and vaccines currently in clinical trials targeting more than 200 diseases, including various cancers, Alzheimer's disease, heart disease, diabetes, multiple sclerosis, AIDS and arthritis.

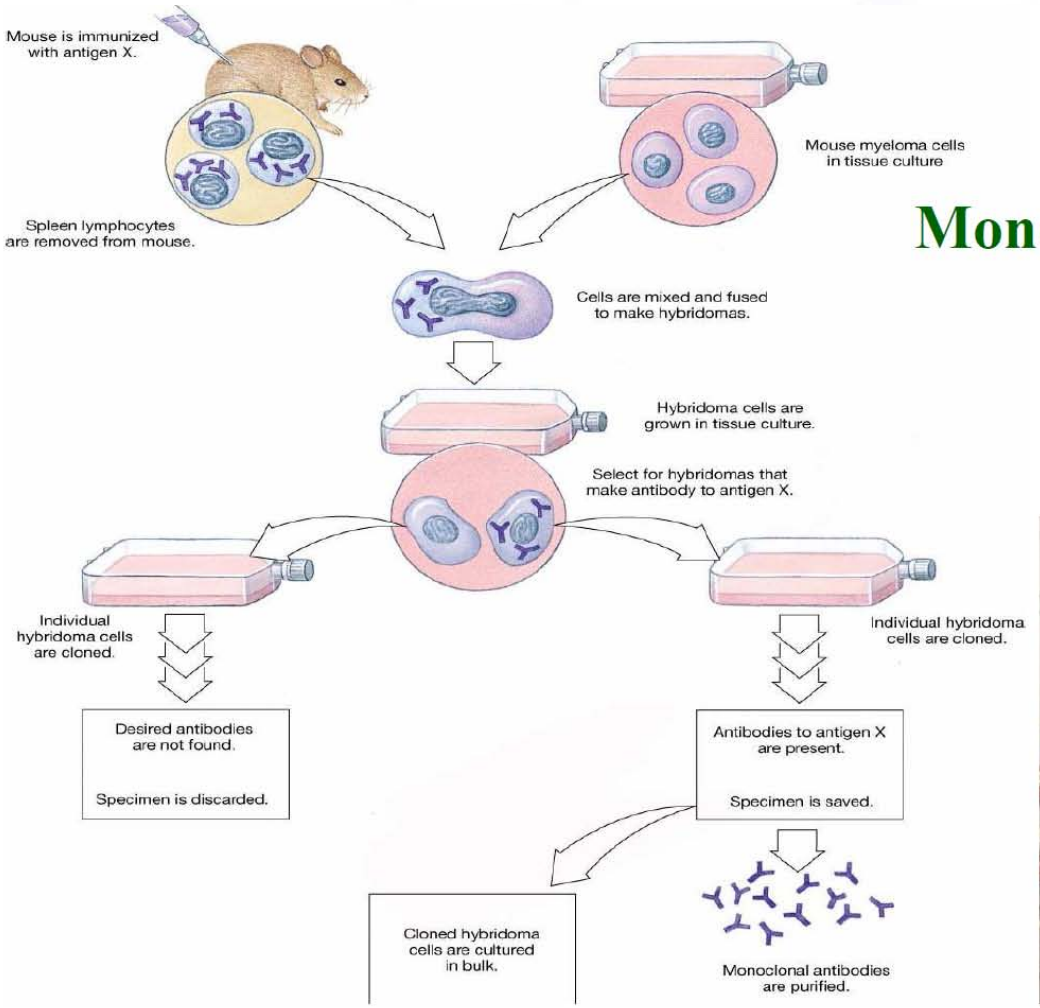
* hundreds of medical diagnostic tests for early detection of diseases, for keeping the blood supply safe, or for detection of pregnancy at home.

* DNA fingerprinting, which has dramatically improved criminal investigation and forensic medicine.

Tools and products of medical biotechnology

an overview

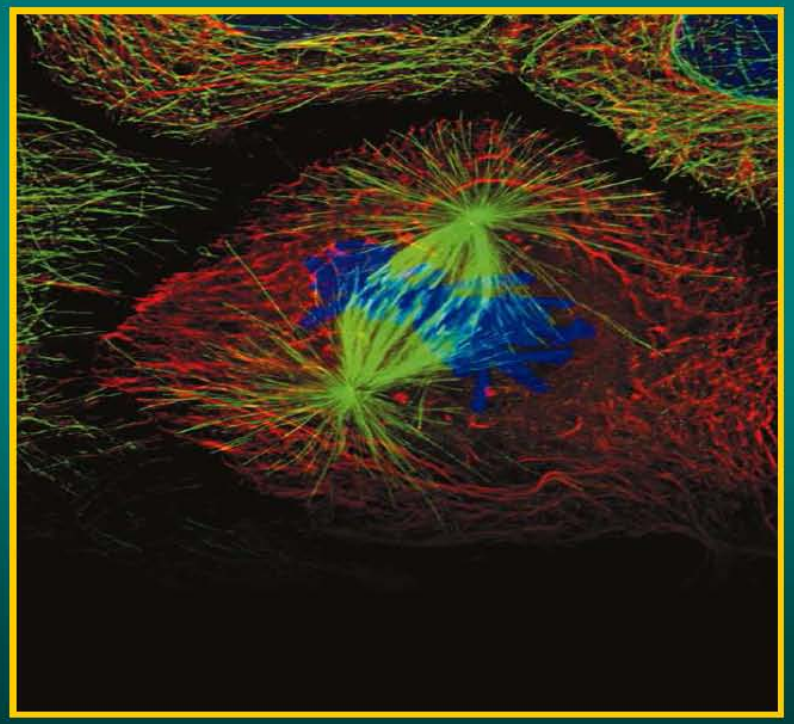
Monoclonal antibodies (MAbs)



Monoclonal antibodies (MAbs)

- The specificity of antibodies makes them powerful *diagnostic tools*. They can locate and measure substances that occur in minuscule amounts. For example, they can be used to:

* locate environmental pollutants
* detect harmful miroorganisms in food.
* detect specific antigens and to localize cells or specific proteins.
* diagnose infectious diseases very quickly and accurately.



- MAbs can be *therapeutic compounds*. Joined to a toxin they can selectively deliver chemotherapy to a cancer cells. They also are developing to treat organ-transplant rejection and autoimmune diseases.

Therapeutic monoclonal antibodies

* Avastin - inhibits angiogenesis by blocking the vascular endothelial growth factor (VEGF). It has been approved for the treatment of colorectal cancer.

* Bexxar - a conjugate of a MAb against CD20 and the radioactive isotope iodine-131. It has been approved to treat lymphoma.

* Campath - binds to CD52, a molecule found on white blood cells; produced complete remission of chronic lymphocytic leukemia (for at least18 months).

* Erbitux - blocks HER1, epidermal growth factor (EGF) receptor, and has been approved to treat colorectal cancer.

* Herceptin - binds to HER2, a receptor for EGF found on some breast cancers and lymphomas.

* Mylotarg - a conjugate of a MAb that binds to CD33, a cell surface molecule expressed by the cancerous cells in acute myelogenous leukemia, and calicheamicin, a complex oligosaccharide that makes double-stranded breaks in DNA. The drug is the first immunotoxin that shows promise in the fight against cancer.

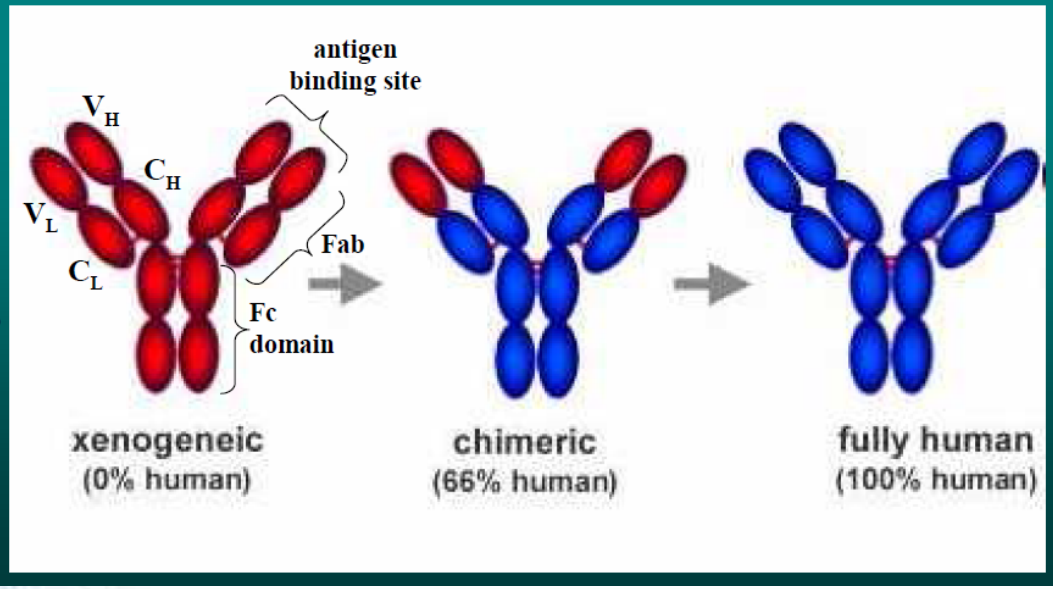
Chimeric monoclonal antibodies

MAbs can be created in mice, but mouse antibodies are recognized by the human immune system, which not only eliminates the therapeutic MAb administered, but also causes damage to the kidneys. Therefore, the chimeric or humanized antibodies are used.

To form a chimeric antibody, one must combine the antigen binding parts (variable regions) of the mouse antibody with the effector parts (constant regions) of a human antibody.

Examples:

* Remicade - rheumatoid arthritis
* Rituxan - B-cell lymphomas
* ReoPro - blood clot complications

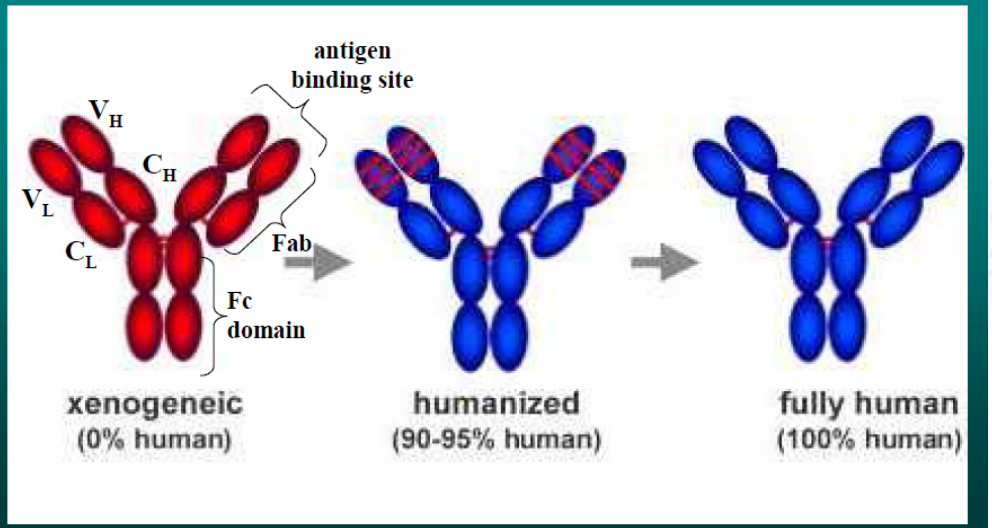


Humanized monoclonal antibodies

To create humanized antibodies, one combines only the amino acids responsible for making the antigen binding site (the hypervariable regions) of a mouse antibody and the rest of a human antibody molecule, thus replacing its own hypervariable regions.

Examples:

* Zenapax - rejection of transplanted kidneys, T-cell lymphomas
* Vitaxin - inhibition of angiogenesis in solid tumors
* Mylotarg - acute myeloid leukemia
* Herceptin - breast cancer and lymphomas
* Xolair - asthma



Newest history of biotechnology

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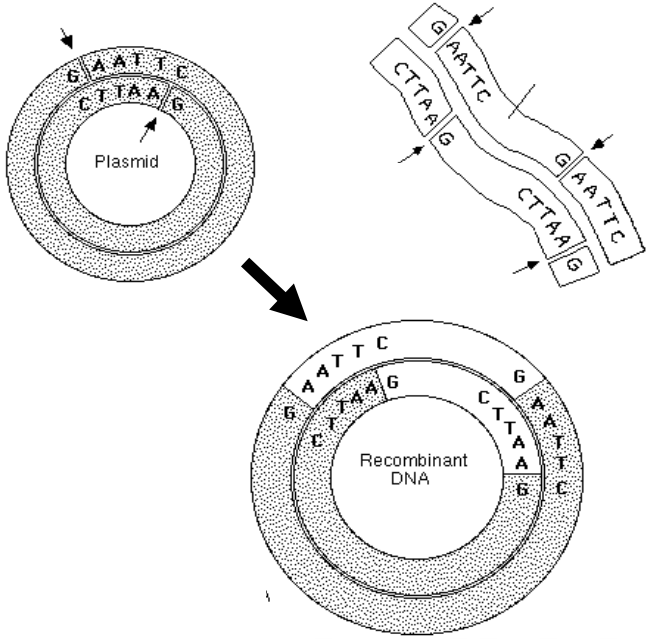
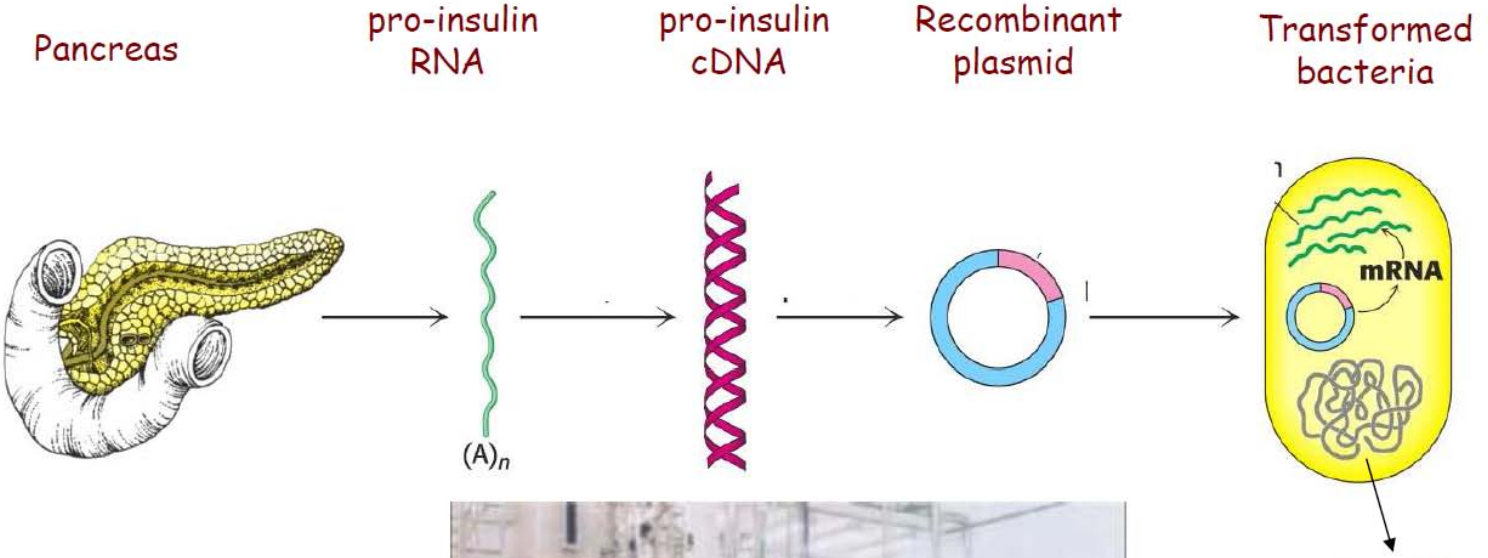
Genetic engineering

Modification of genetic material of different organisms in a designed and controlled way, by means of techniques of DNA recombination



Genetically modified bacteria produce insulin



The manufacturing of insulin

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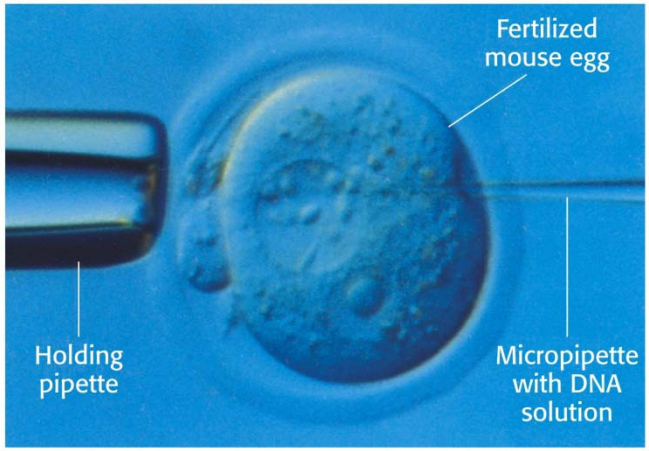
First transgenic mice



Mysz-"giganta" uzyskano poprzez wprowadzenie do zapłodnionej komórki jajowej plazmidu z genem hormonu wzrostu

Palmiter et al., Nature 1982: 300: 611-615

Transgenic animals

- Transgenic animal-made antibodies can be produced from animals that have had human antibody genes transferred to them. These animals can then be vaccinated against human diseases and antibodies can be collected from their blood and used for treating diseases in humans.

- Animals are often used as models for research as many of the technologies developed for animals can be transferred to human.

Pigs expressing GFP

Transgenic animals

Researchers are developing transgenic animals, including cows, goats and sheep, that produce milk containing therapeutic proteins. Some interesting ongoing projects include:

* **Atryn** - The first drug product for humans produced by a transgenic animal was in July 2006 approved by the European Commission. This protein is human anti-thrombin, a naturally occurring plasma protein that has both anti-coagulant and anti-inflammatory properties. The protein is produced by transgenic goats whose milk contains human anti-thrombin.

* Growth hormon - In 2005 in Argentina, cows were improved with biotechnology to produce human growth hormone. Scientists estimate that just 15 of these Jersey cows could produce enough human growth hormone to meet the current world demand.

* Dutch researchers are working with biotech rabbits that secrete a potential drug for Pompe's disease (acid maltase deficiency) in their milk. Pompe's disease is an extremely rare genetic disorder that can result in crippled muscles, breathing problems and sometimes death.

* Biotech cows can now produce the human milk protein lactoferrin, which is an antibacterial protein that can be used to treat immunosupressed patients or incorporated into infant formula.

Gene knockouts

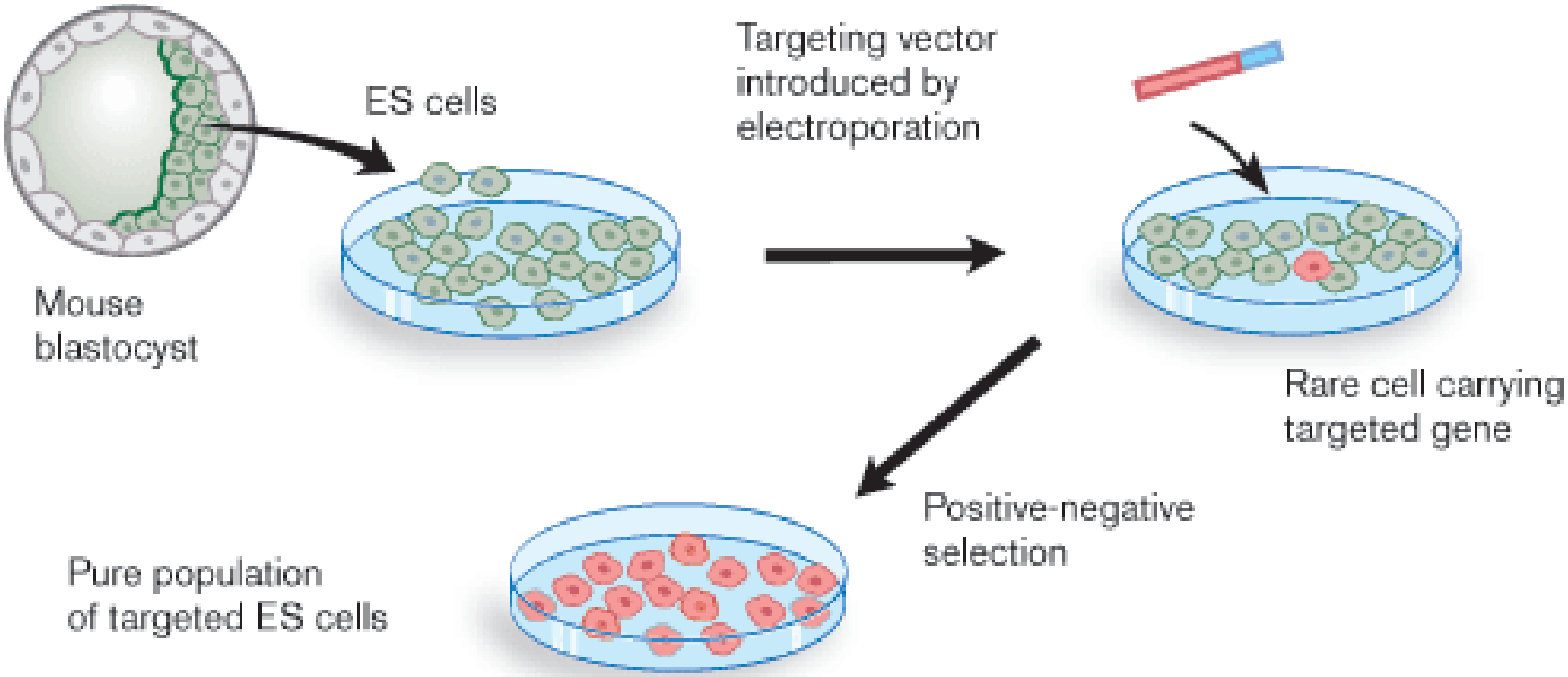
- Targeted mutations or gene knockouts are one of biotech's most powerful research tools for elucidating gene function. By deleting or disrupting specific genes in cells, we gain valuable information about the role a given gene plays in the expression of a certain protein.

- When gene-knockout technology is combined with ability to derive genetically identical animals from cultured cells, we can determine how the absence of this protein affects the whole organism.

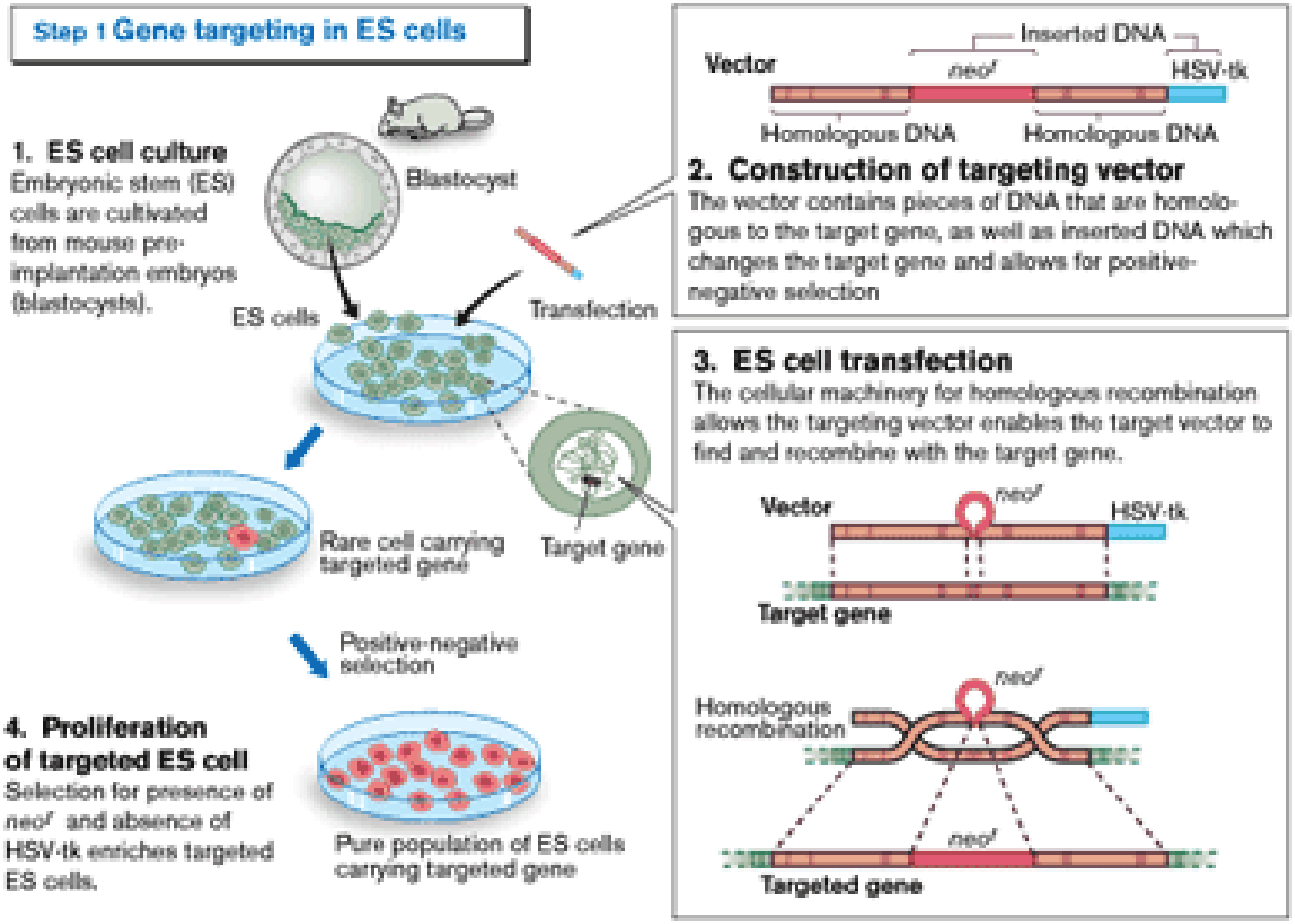
- There is a wide variety of genetically identical colonies of mice with very specific genes knocked out to study the processes of gene regulation, DNA repair and disease development.

Gene knockout technology

A. Gene targeting of embryonic stem cells



Gene knockout technology

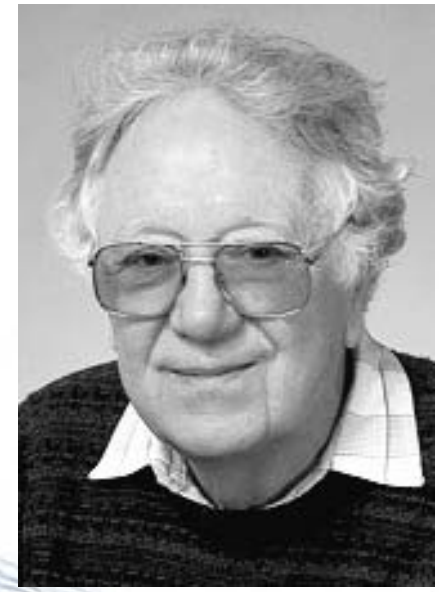


The Nobel Prize in Physiology or Medicine, 2007

Mario R. Capecchi, **Martin J. Evans** and **Oliver Smithies**
for their discoveries of **"principles for introducing specific gene modifications in mice by the use of embryonic stem cells"**

M. Capecchi
Univ. of Utah

Sir M. Evans
Cardiff Univ., UK



O. Smithies
UNC Chapel Hill

First KO mice using ES cell technology were produced in 1989

Biotechnology vaccine production

- Conventional vaccines use weakened or killed forms of a virus or bacteria to stimulate the immune system to create the antibodies that will provide resistance to the disease. Usually only one or a few proteins on the surface of the bacteria or virus, called antigens, trigger the production of antibodies.

- Most of the new vaccines consist only of the antigen, not the actual microbe. The vaccine is made by inserting the gene that encodes the antigen into a manufacturing cell, such as bacteria, yeast or insect cells. The antigen is later purified from the cell culture.

- By producing and isolating antigens, it is possible to make vaccines that cannot transmit the virus or bacterium itself. This method does not require animal using and allows for a large-scale vaccine production.

- Using these techniques, the antigen-only vaccines against life-threatening diseases such as hepatitis B and meningitis have been developed.

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Gene therapy

Therapeutic gene (transgene)

Vector

Patient

Expression of therapeutic gene

Gene therapy is succesful in treatment of diseases

Some Gene Therapy Successes

Disorder	Disease type	Patients benefiting	First publication
X-SCID	Immunodeficiency	17/20	2000
ADA-SCID	Immunodeficiency	26/37	2002
Adrenoleukodystrophy	Neurologic	2/4*	2009
Leber's congenital amaurosis	Blindness	28/30	2008
Wiskott-Aldrich syndrome	Immunodeficiency	8/10	2010
β-thalassemia	Hemoglobinopathy	1/1	2010
Hemophilia	Coagulation	6/6	2011?

*Includes a patient treated too recently to see benefit

Science, 7th October 2011

Newest history of biotechnology

1990 - First experimental gene therapy treatment is performed successfully on a 4-year-old girl suffering from an immune disorder.

1992 - Technique for testing embryos in vitro for genetic abnormalities such as cystic fibrosis and hemophilia.

1997 - First animal cloned from an adult cell: a sheep named Dolly in Scotland.

1998 - Cloning three generations of mice from nuclei of adult ovarian cumulus cells.

1998 - Human embryonic stem cell lines are established.

1998 - Cloning eight identical calves using cells taken from a single adult cow.

2002 - Successful results for a vaccine against cervical cancer, the first demonstration of a preventative vaccine for a type of cancer.

2002 - An endangered species (the banteng) is cloned for the first time.

2003 - Cloning mules, horses and deer.

2003 - Dolly, is euthanized after developing progressive lung disease.

Cloning

- Cloning technology allows to generate a population of genetically identical molecules, cells, plants or animals. Any legislative or regulatory action must take great care in defining the term.

Artificial embryo twinning (AET):

- It is the old way to clone. AET mimics the natural process of creating identical twins. Researchers manually separate a very early embryo into individual cells and then allow each cell to divide and develop on its own.

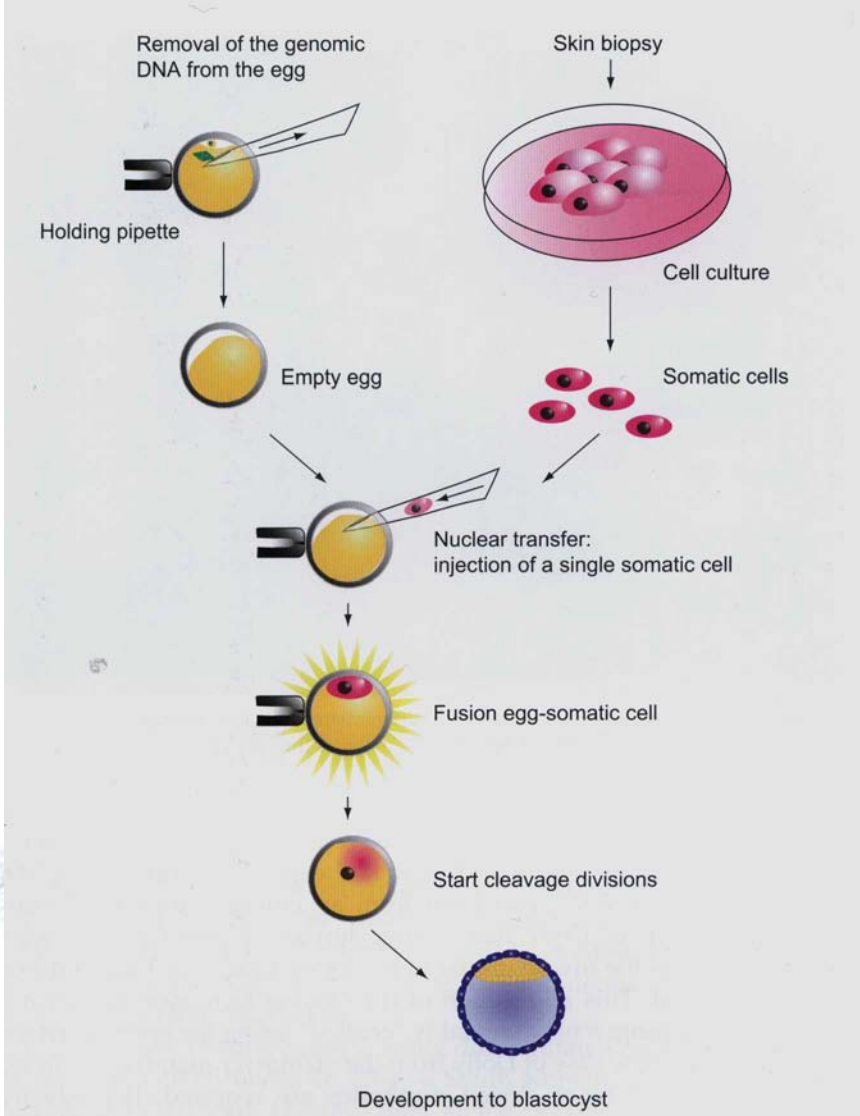
Somatic cell nuclear transfer (SCNT):

- Involves the isolation of a somatic cell and transfer of its nucleus to an egg cell from which the nucleus had been removed. After some chemical manipulation, the egg cell, with the new nucleus, behaves like a freshly fertilized zygote.

Not Dolly only...

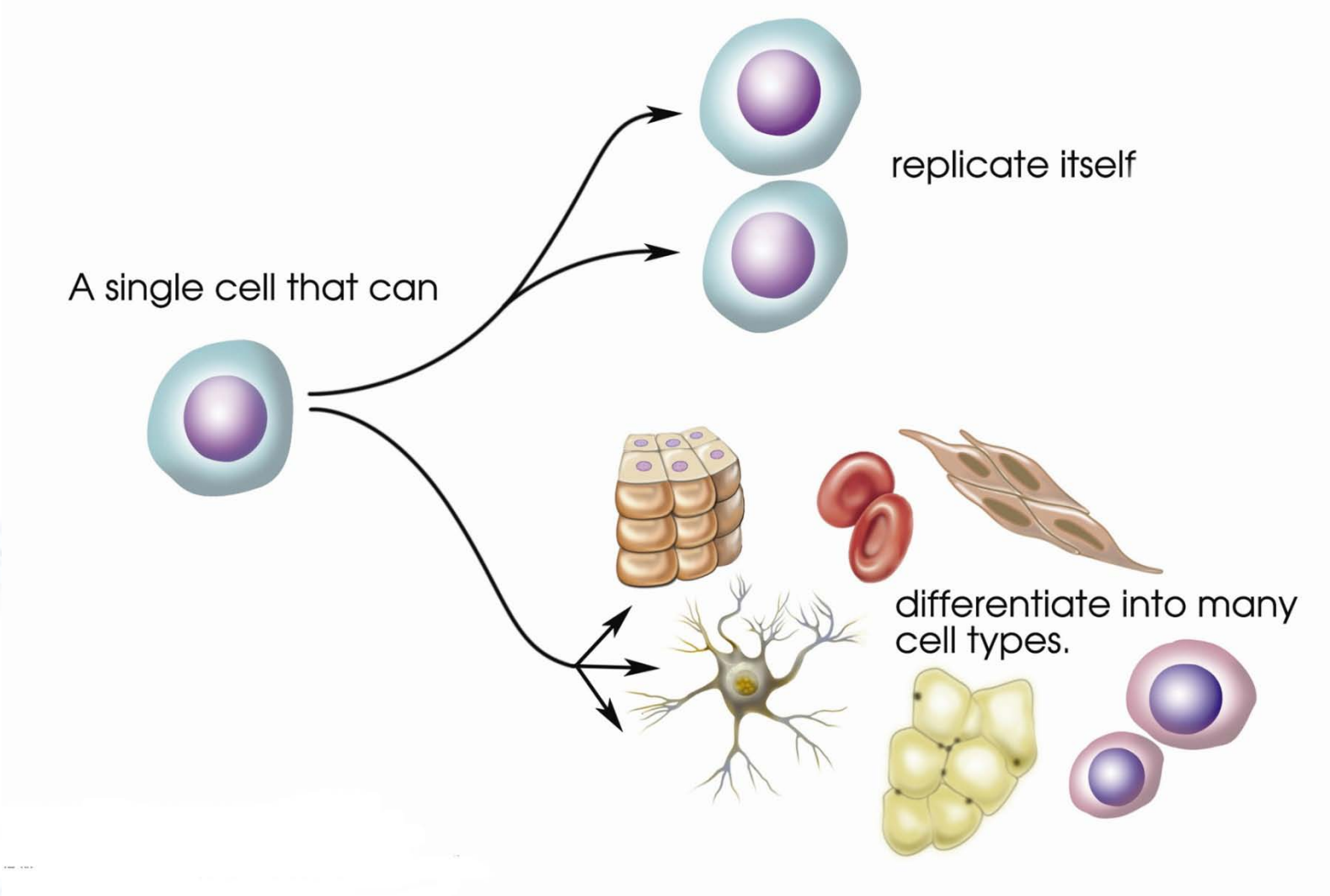

Cloned banteng

Somatic cell nuclear transfer – cloning



Stem cells: Scientific facts and fiction; Academic Press 2011

What is a stem cell?



Classification of SCs

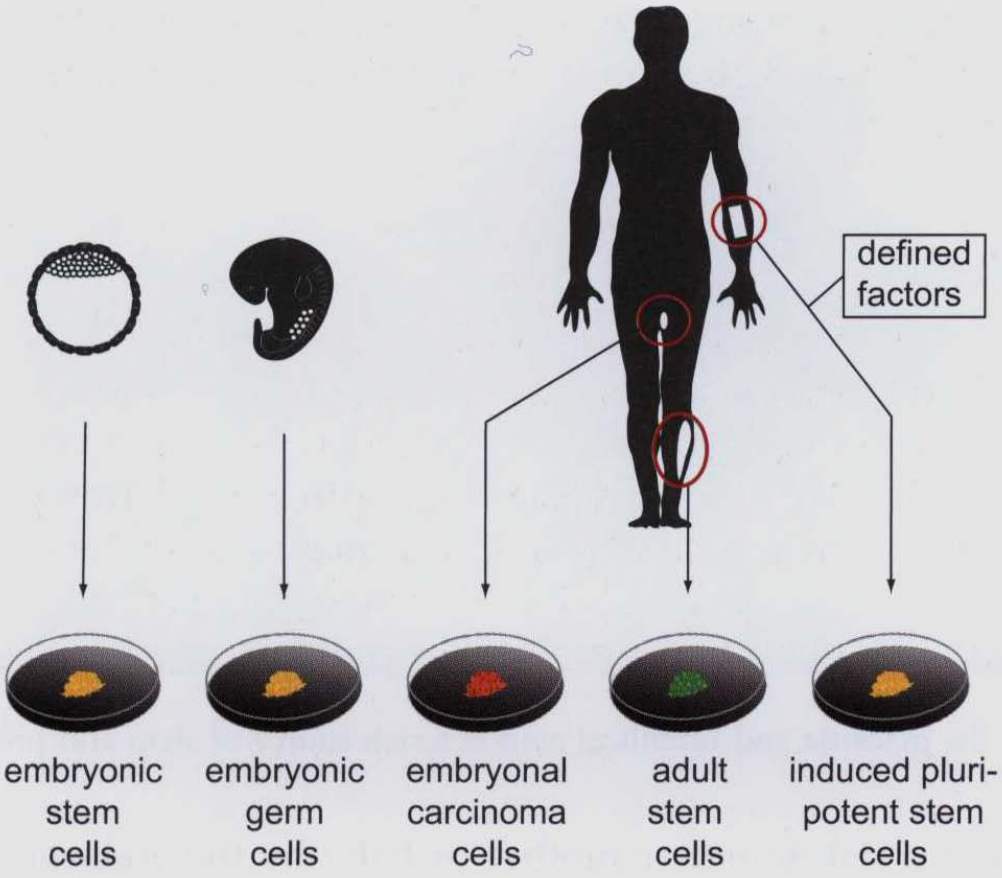
1. Based on the tissue commitment/ differentation capacity

- Totipotent SCs - zygote
- Pluripotent SCs
- Multipotent SCs
- Unipotent SCs (Tissue progenitors)

2. Based on their origin

- Embryonic SCs (ESCs)
- Fetal SC („adult" SC)
- Umbilical cord blood stem cells („adult")
- Postnatal - Adult SCs
- Reprogrammed SCs (Inducible Pluripotent SCs = iPS cells)

Different origin of stem cells



Stem cells of different origins can be cultured. Pluripotent stem cells are depicted in orange, multipotent cells in green. Embryonal carcinoma cells (in red) can also be pluripotent but their behavior can change rapidly due to their malignant (cancer) characteristics which are associated with genomic instability and abnormal chromosomes.

Stem cells: Scientific facts and fiction; Academic Press 2011

Zastosowanie komórek macierzystych w terapii

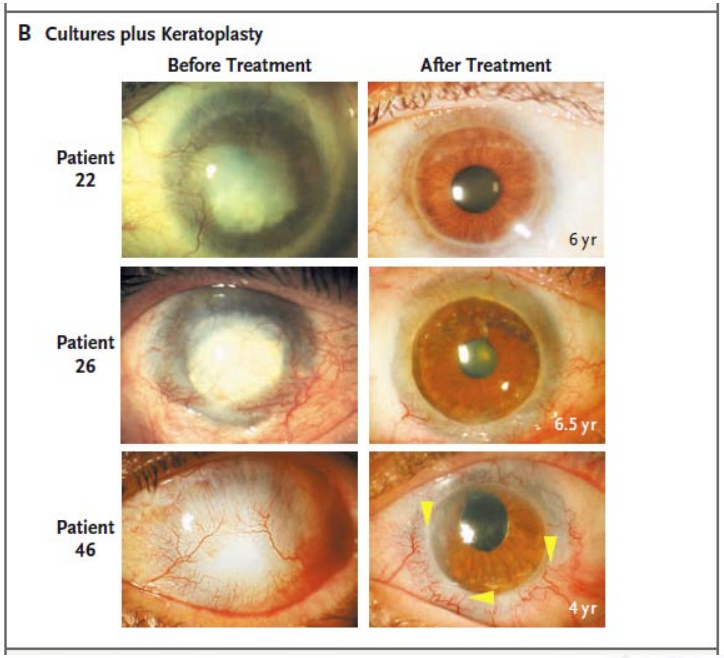
1. Krwiotwórcze komórki macierzyste:
   szpik, krew pępowinowa
   (białaczki, niedokrwistości,
   złożone niedobory odporności)

2. Rąbkowe komórki macierzyste –
   leczenie utraty wzroku wywołanej oparzeniem

3. Komórki macierzyste naskórka -
   leczenie oparzeń, owrzodzeń troficznych

4. Komórki macierzyste śróblonka i inne (CD34+)
   – terapia zawału serca;
   dusznicy bolesnej

5. Przeszczepy szpiku kostnego w terapii cukrzycy – próby kliniczne

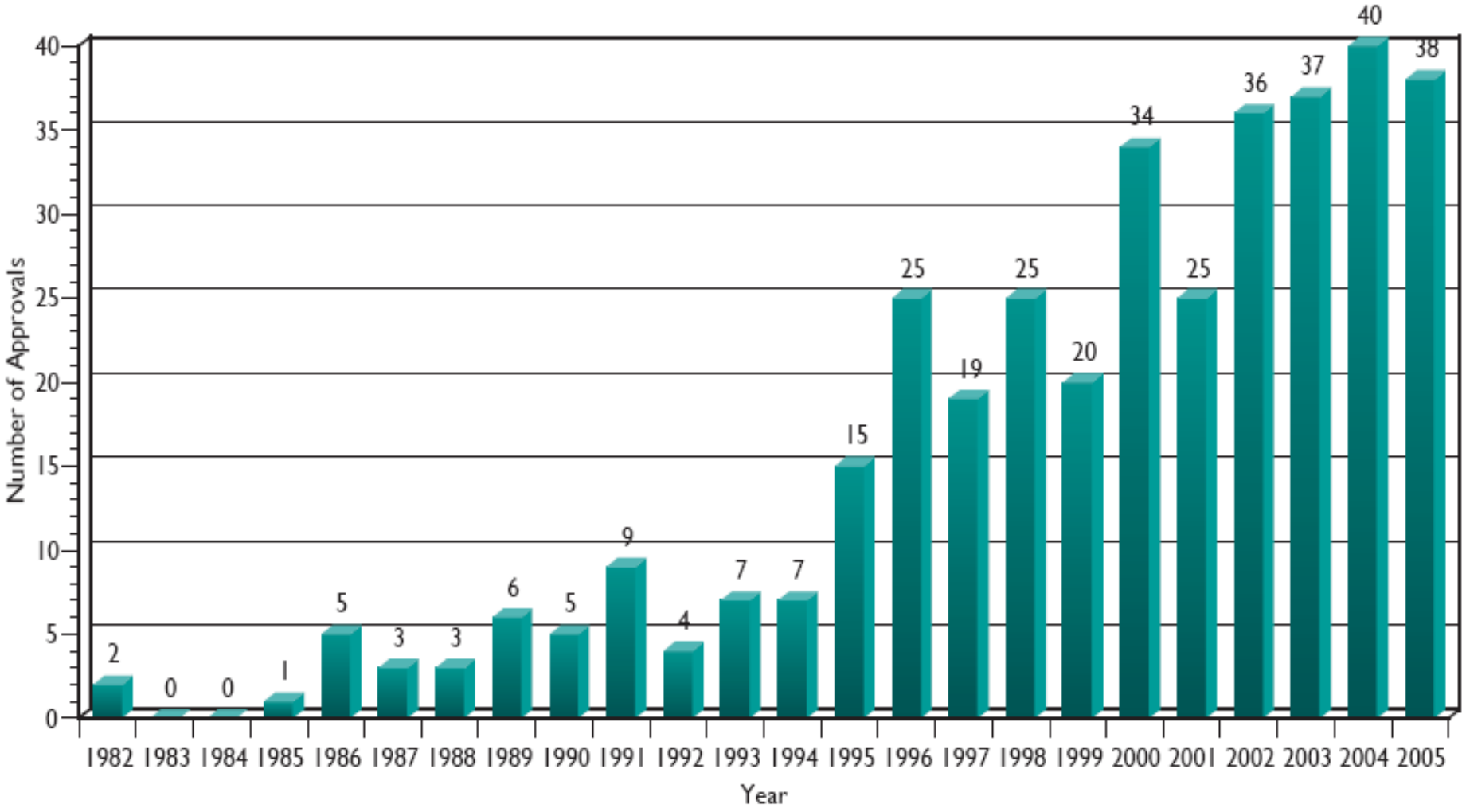


P. Rama (G. Pellegrini) et al., NEJM 2010

Pharmaceutical biotechnology

Biotechnology - achievements

New Biotech Drug and Vaccine Approvals/ New Indication Approvals by Year



Biotechnology - achievements

It typically takes 10 to 15 years and an average of more than $800 million (including the cost of failures) to develop a new therapy. The process is rigorous and conducted in multiple stages:

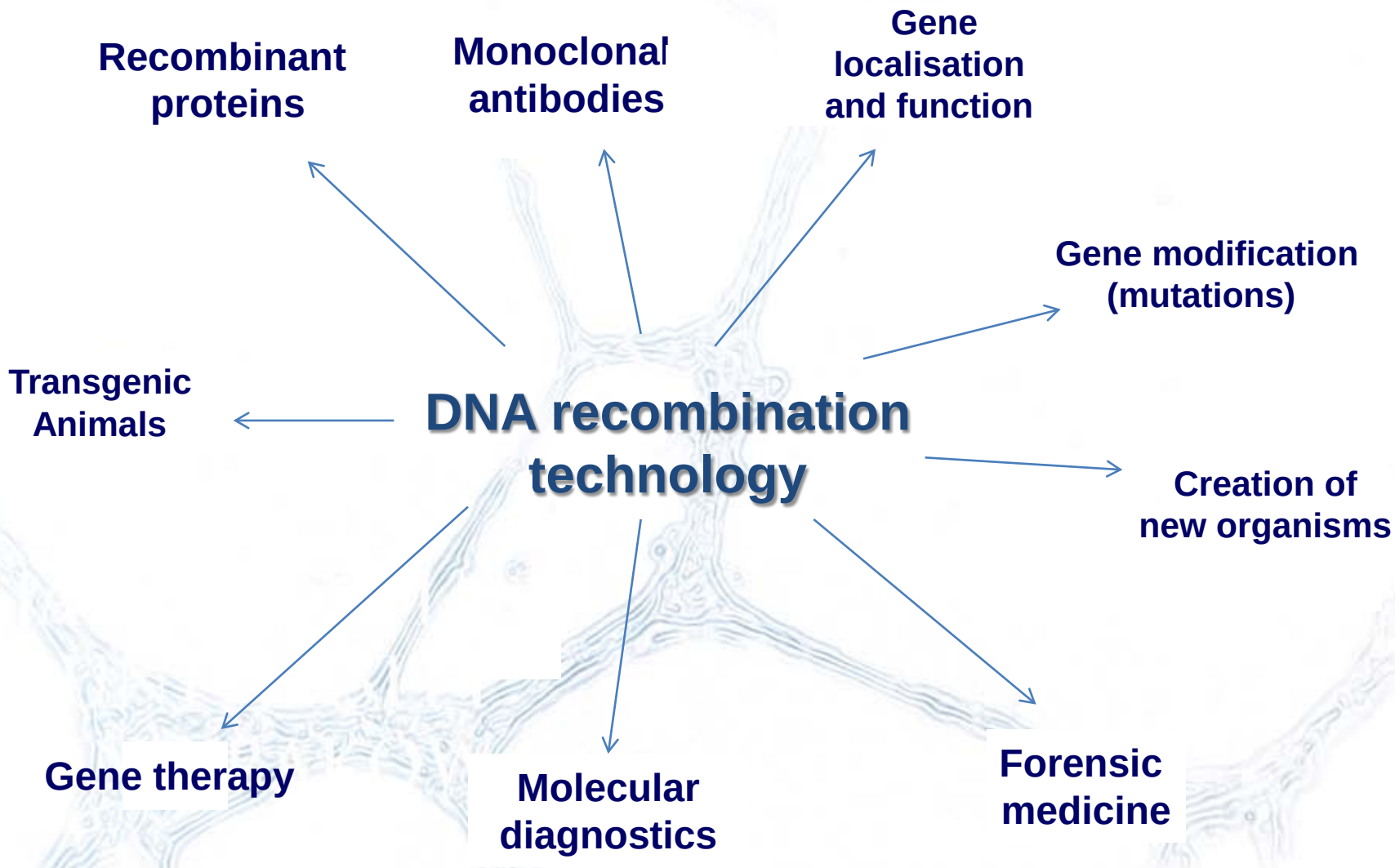
* Generating and validating a new hypothesis; by modulating the function of a chosen protein target, a related disease pathology could be symptomatically relieved, modified or prevented.

* Identifying chemical 'lead' compounds that modulate the function of the chosen target and have the general physico-chemical and toxicological properties needed for them to become drugs.

* Chemically optimising a chosen series of lead compounds to be selectively unique for the target and to have the appropriate pharmacokinetic, metabolic and safety properties.

At the completion of this stage a preferred compound is selected for pre-clinical safety testing using animal models.

Application of DNA recombination technology



Next lectures:

13 March

27 March

3 April

17 April

24 April

8 May

15 May

22 May (if necessary)

Thank you