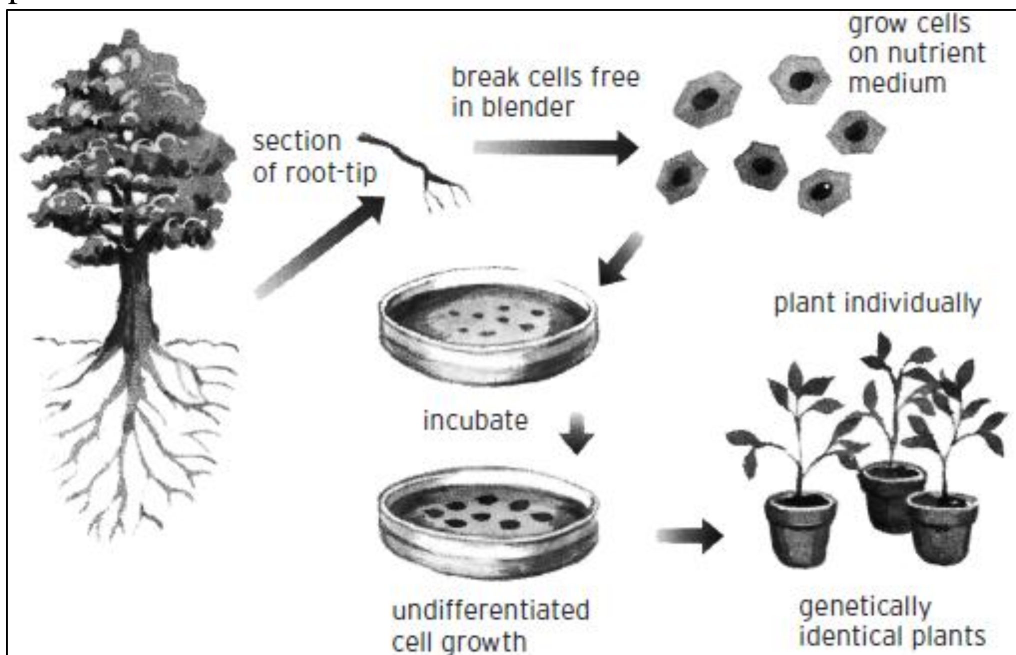
Cloning plants, animals, and cells

Take a cutting from a plant, put it in a pot of soil, and you have cloned an organism. The plant that grows from the cutting will be genetically identical to the one from which you took the cutting. Its development is made possible because each cell at the cut edge has the genetic potential to develop into any type of plant tissue needed to form a whole new plant.

Cloning does not necessarily involve genetic engineering. In plant regeneration from individual cells, young cells from a root tip can each be encouraged to form a new plant. The process shown here can be used along with recombinant DNA techniques to develop new strains with particular properties.



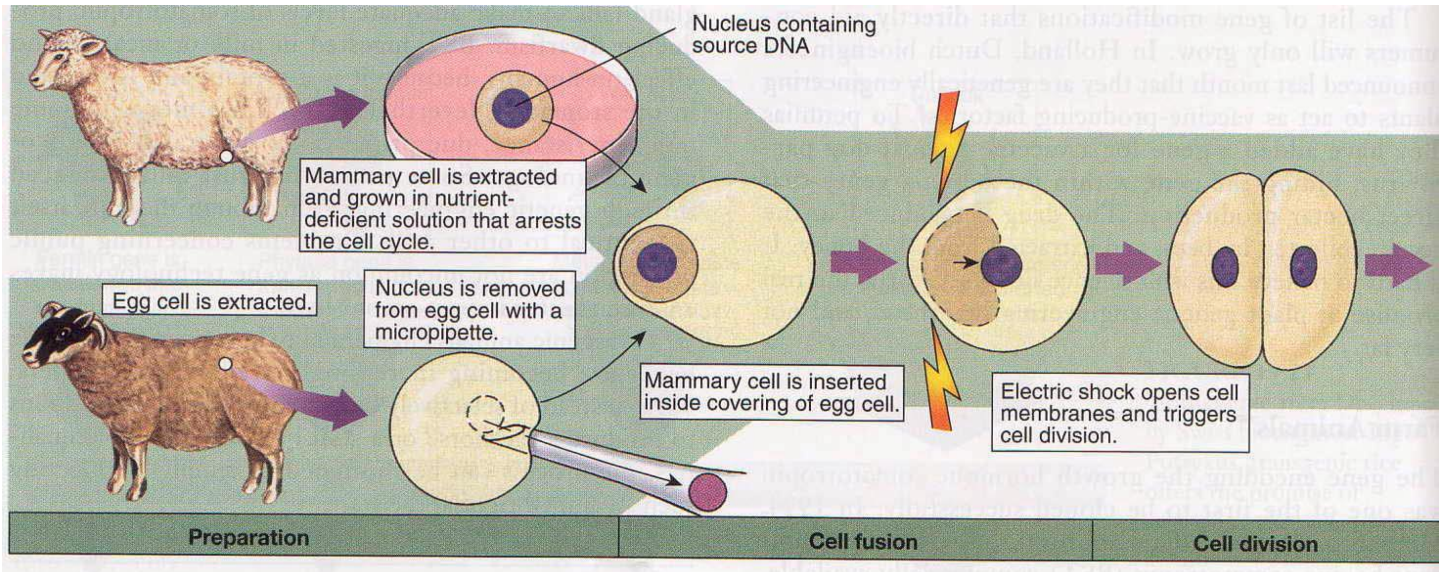
Cloning from plant cells

While whole plants have been regenerated from cuttings for centuries, biologists in the late 1950s discovered that whole plants can be regenerated from individual cells (see figure above). Plant cells seem to retain the potential to express any of their genes and thus repeat the developmental process from a single cell to a whole plant. Biotechnologists have taken great advantage of this characteristic of plant cells.

The number of cells you can take from a plant is obviously much greater than the number of cuttings. This offers the possibility of rapidly developing new strains of crops or trees from a single plant that has desirable traits.

The extraordinary ability of any body cell to give rise to a whole new identical organism has been demonstrated in several species of animals by a technique called nuclear transplanting. The procedure involves destroying the nucleus of an egg cell, then replacing it with the nucleus taken from any cell — say, a skin cell — of another individual. In the rare cases where success

is achieved, the egg with the transplanted nucleus goes on to develop into a complete new organism identical to the one that supplied the skin cell nucleus. Nuclear transplanting was first successfully used to clone frogs, but animal cloning suddenly became a global news item following the birth of the celebrated sheep named Dolly in July 1996.



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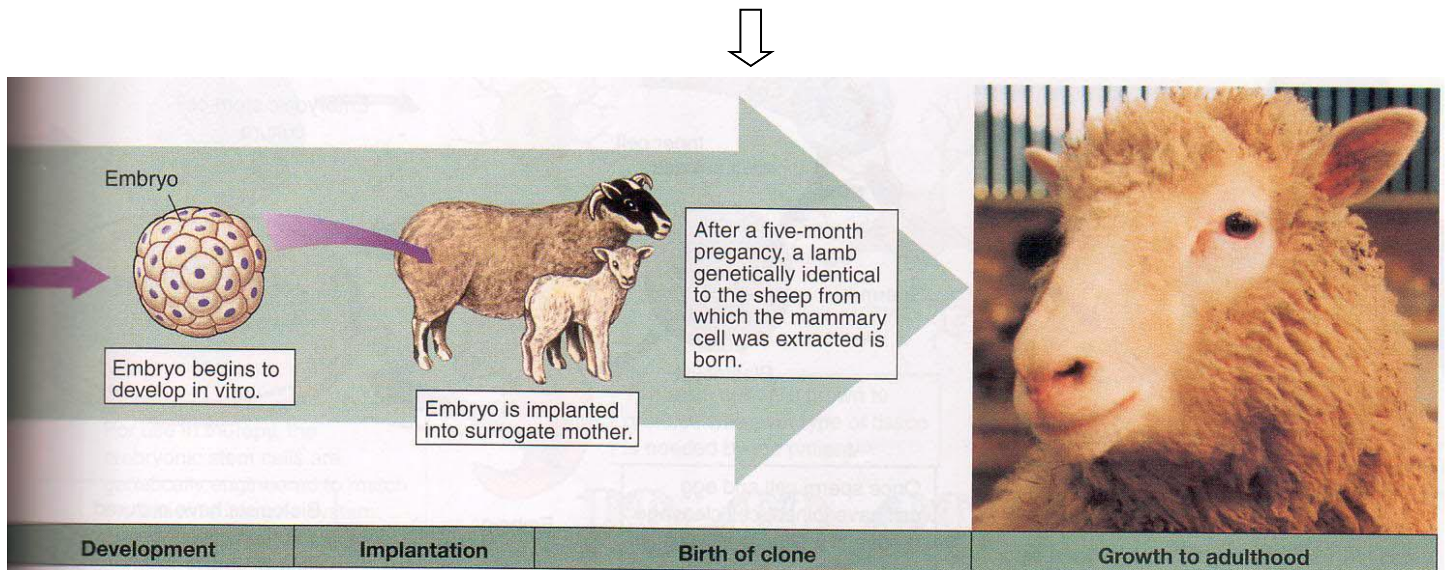


Dolly, first successful clone generated from a differentiated cell

The first mammal to be cloned from an adult cell, Dolly was identical to her mother — and did not have a father. To produce Dolly, scientists took the nucleus of an udder cell from a six-year-old Finn Dorset white sheep and injected it into an unfertilized egg cell from a Scottish Blackface ewe after removing the egg cell's nucleus. They treated the reconstructed egg with chemicals to stimulate cell division, and then implanted the cloned embryo into the uterus of a third sheep — the surrogate mother that gave birth to Dolly.

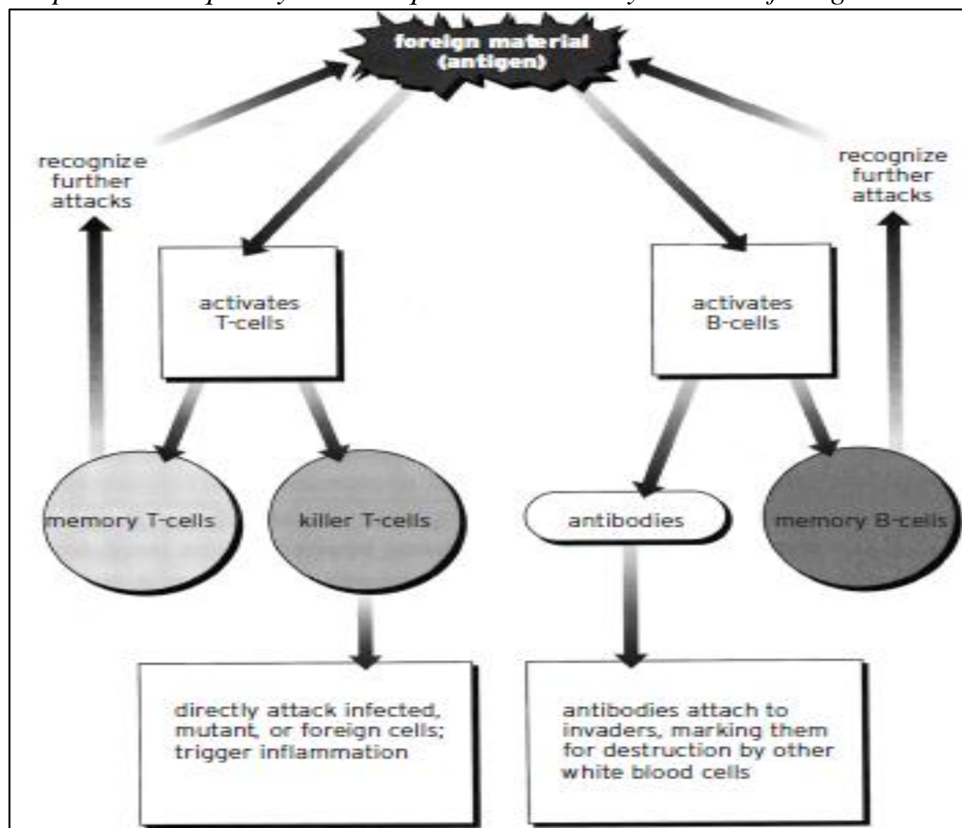
In the years since Dolly's birth, scientists have cloned other sheep as well as mice, pigs, goats, cattle, cats, and deer from adult cells. Although time and experience have made researchers more successful at cloning mammals, the technique is by no means straightforward. Dolly was the only lamb born from 277 attempts at cell fusion, and the success rate for producing live, healthy offspring from adult mammalian cells still stands at around only two percent.

Dolly eventually died in February 2003 at the age of six, about one-half the normal life span for her breed. During her life, she had conceived and given birth to six lambs in the normal way, but her premature death raised questions about the risks of cloning. Dolly was suffering from arthritis and a lung infection — diseases more typical of older animals. Although a post-mortem showed no abnormalities, it was speculated that when an adult cell is used as the source of a clone, the clone is effectively born with the cellular age of its parent. The result may be premature aging. (That is, premature for the "newborn," but not necessarily premature for the older source.).

Monoclonal antibodies

How the immune system works

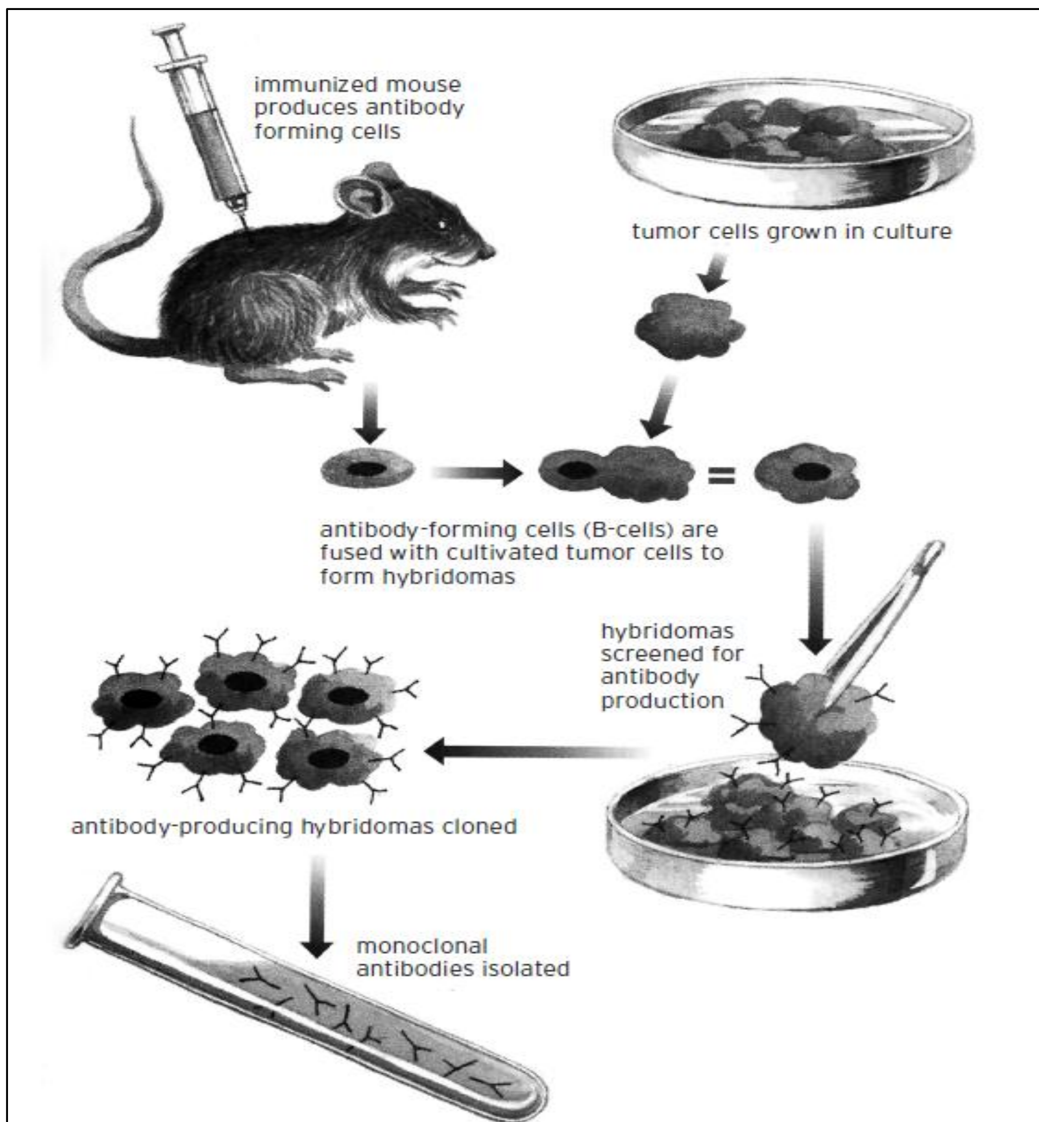
The immune system has two branches, using two main types of white blood cells. T-cells respond to foreign materials by becoming killer cells and memory cells. B-cells produce antibodies and memory cells. Memory cells help the body respond more quickly to subsequent invasions by the same foreign material.



Immune system

Most cells divide a certain number of times and then die. The number of cell generations they produce is genetically determined. Cancer cells, however, are a special case. They continue to divide and copy themselves indefinitely. The origin of this condition seems to lie in a mutation that affects the regulatory genes. This mutation makes cancer cells all but immortal, their genes for promoting cell division stuck in the "on" position. Biotechnologists take advantage of cancer cells' property of unstoppable growth by joining cancer cells to cells that make desirable products. The hybrid cells that result from this marriage combine the cancer cells' proclivity for endless multiplication with their partner cells' production of enzymes, hormones, or whatever else is chosen. Cultures of such fused cells, called hybridomas, are used to mass-produce huge quantities of valuable proteins.

Probably the most important products now derived from hybridoma technology are monoclonal antibodies, whose development won George Koehler and Cesar Milstein a Nobel Prize in 1984.



Monoclonal antibodies are made by fusing antibody-forming cells with tumor cells.

Antibodies are proteins produced by certain white blood cells to fight infection. Each antibody is specific to a particular foreign particle invading the body, such as a bacteria or virus. As they attach themselves to the invader, antibodies deactivate the foreign particle.

It would obviously be of great value to medicine if antibodies could be produced in the lab in large amounts. That possibility was always limited, however, by the fact that white blood cells do not survive for very long outside the body. To overcome this problem, Koehler and Milstein "persuaded" some white blood cells to fuse with cancer cells taken from tumors. Although cells do not normally fuse with other bodies, fusion can be promoted by using chemicals or viruses, or by electroporation — placing the cells and DNA fragments together in a high-frequency electrical field. Hybridoma cells obtained in this way multiply and produce a continuous supply of antibodies called monoclonal because they are all descended from a single or clonal line of cells.

Myeloma cell *Neoplastic* ***plasma cell****. The proliferating plasma cells often replace all the others within the marrow, leading to immune deficiency, and frequently there is destruction of the bone cortex. Because they are monoclonal in origin they secrete a monoclonal immunoglobulin. Bence-Jones proteins are monoclonal immunoglobulin light chains overproduced by myeloma cells and excreted in the urine. Myeloma cell lines are used for producing* ***hybridomas*** *in raising monoclonal antibodies.*

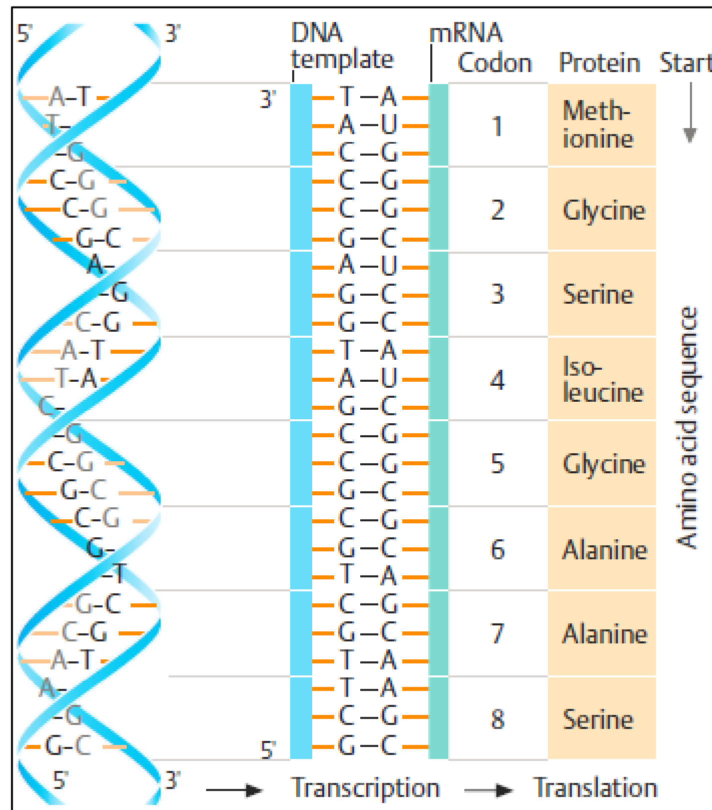
DNA probes

Suppose you want to find out where the human gene responsible for producing insulin is located on the chromosomes. You could break each chromosome into fragments, combine each fragment with a plasmid, insert the recombinant plasmids into bacteria, and check to see which bacteria make insulin. But the chance of any random fragment having the gene you want is remote, and to test all fragments in this way would consume too much time and money.

A much quicker way of doing this sort of research is now possible thanks to a machine that can assemble synthetic strands of DNA. Furthermore, if you know the sequence of amino acids in the protein, you can translate this into the sequence of bases in the gene. You can then use the machine to string together a short, distinctive strand of DNA that complements the sequence on the gene. This strip of DNA can be used as a probe, to find the gene you are looking for.

DNA from the organism that is being analyzed is cut into fragments with restriction enzymes and the fragments are separated according to their size. The DNA probes are made radioactive for later identification, and then added to the DNA fragments. After the DNA probes have paired with their corresponding genes on the fragments, the location of each probe is pinpointed using X-ray film. Each radioactive probe — and the gene it is bonded to — shows up on the X-ray film as a dark spot. Probes may also be made luminescent (light emitting) and their

locations found using light-sensitive film. DNA probes are used to map the position of genes on chromosomes, to locate the presence of recombinant DNA in bacterial cultures or intransgenic plants and animals, and to find oncogenes on a person's chromosomes, giving advance warning of cancer risk. They have recently been applied to detect microscopic killer plankton that produces toxins that kill fish. They are also part of the technology involved in the process of DNA "fingerprinting."



A synthetic probe can be designed on the basis of the genetic code and the known amino acid sequence of the protein encoded by the gene of interest.

Reference:
Biotechnology unzipped promises and realities (2006) by Eric S. Grace, 2nd edition