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# BIOTECHNOLOGY AND OTHER SHENANIGANS

## CHAPTER 20: AN OVERVIEW

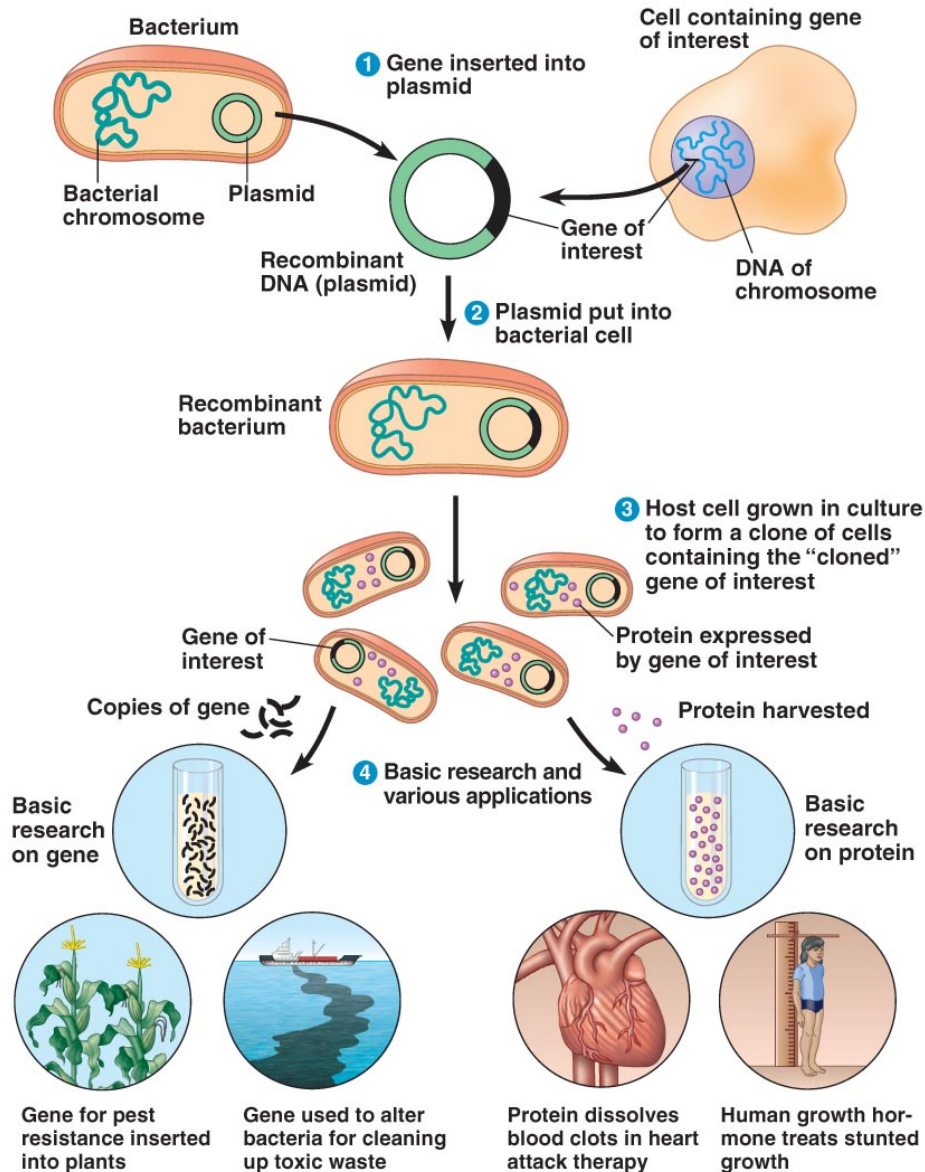
- **Recombinant DNA:** DNA molecules formed when segments of DNA from two different sources are combined *in vitro* (test tube)
- **Biotechnology:** manipulation of organisms or their components to make useful products. Biotech has a long history that includes early practices of selected breeding and using microorganisms to make wine and cheese
- **Genetic engineering:** direct manipulation of genes for practical purposes,

## 20.1 DNA CLONING YIELDS MULTIPLE COPIES OF A GENE OR OTHER DNA SEGMENT

- **DNA Cloning:** methods for preparing well-defined segments of DNA in multiple identical copies

### DNA CLONING AND ITS APPLICATIONS: A PREVIEW

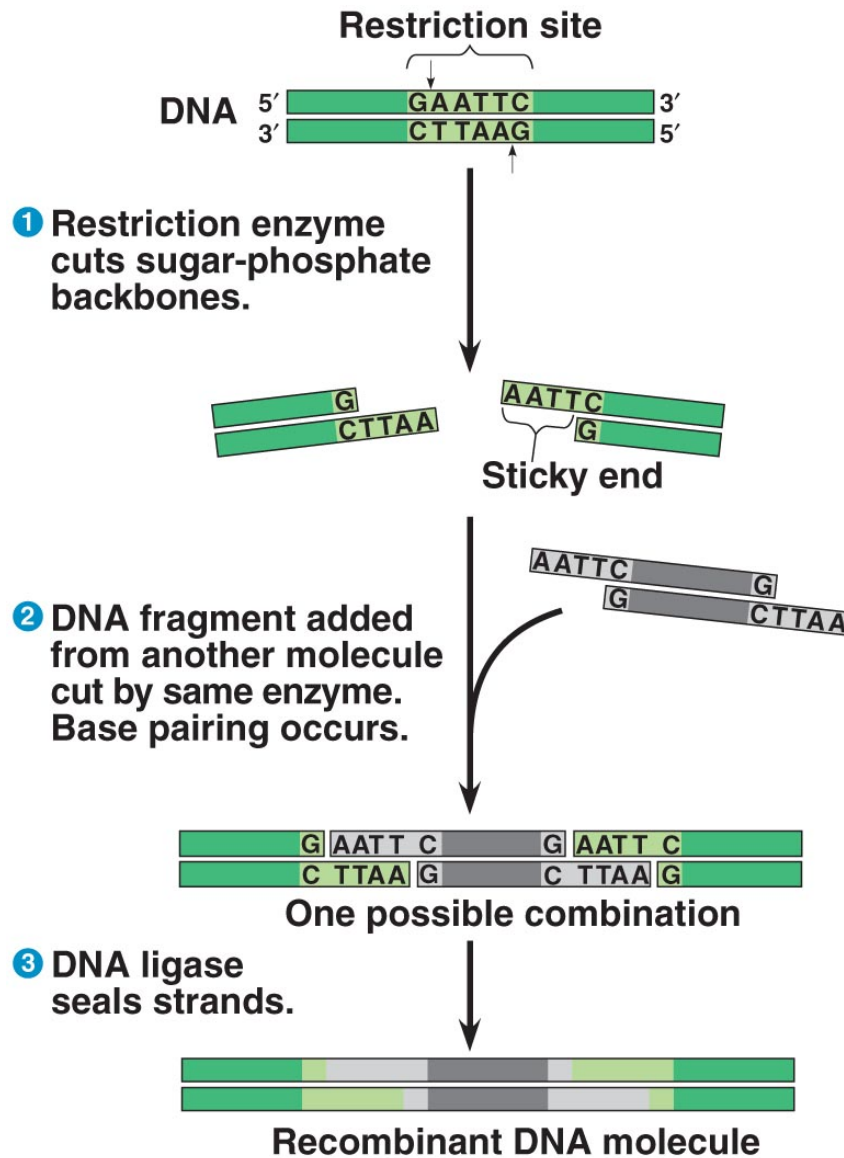
- Most use bacteria, most often *Escherichia coli*
- **Plasmids:** small circular DNA molecules that replicate separately from the bacterial chromosome, only has a small number of genes that may be useful when the bacterium is in a particular environment but may not be required for survival or reproduction under most conditions
- **Gene cloning:** production of multiple copies of a single gene
  - Many copies of a particular gene
  - Produce a protein product
  - Researchers can isolate copies of a cloned gene from bacteria for use in basic research or to endow an organism with a new metabolic capability
  - A protein with medical uses can be harvested in large quantities from cultures of bacteria carrying the cloned genes for the protein



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## USING RESTRICTION ENZYMES TO MAKE RECOMBINANT DNA

- **Restriction enzymes:** enzymes that cut DNA molecules at a limited number of specific locations, discovered in the late 1960s
- **Restriction site:** a short DNA sequence
- **Restriction fragments:** product of restriction enzymes when they make cuts in a DNA molecule
- **Sticky end:** the single-stranded end, it can form hydrogen-bonded base pairs with complementary sticky ends on any other DNA molecules cut with the same enzyme
- **DNA ligase:** enzyme that makes associations between sticky end fragments permanent



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## CLONING A EUKARYOTIC GENE IN A BACTERIAL PLASMID

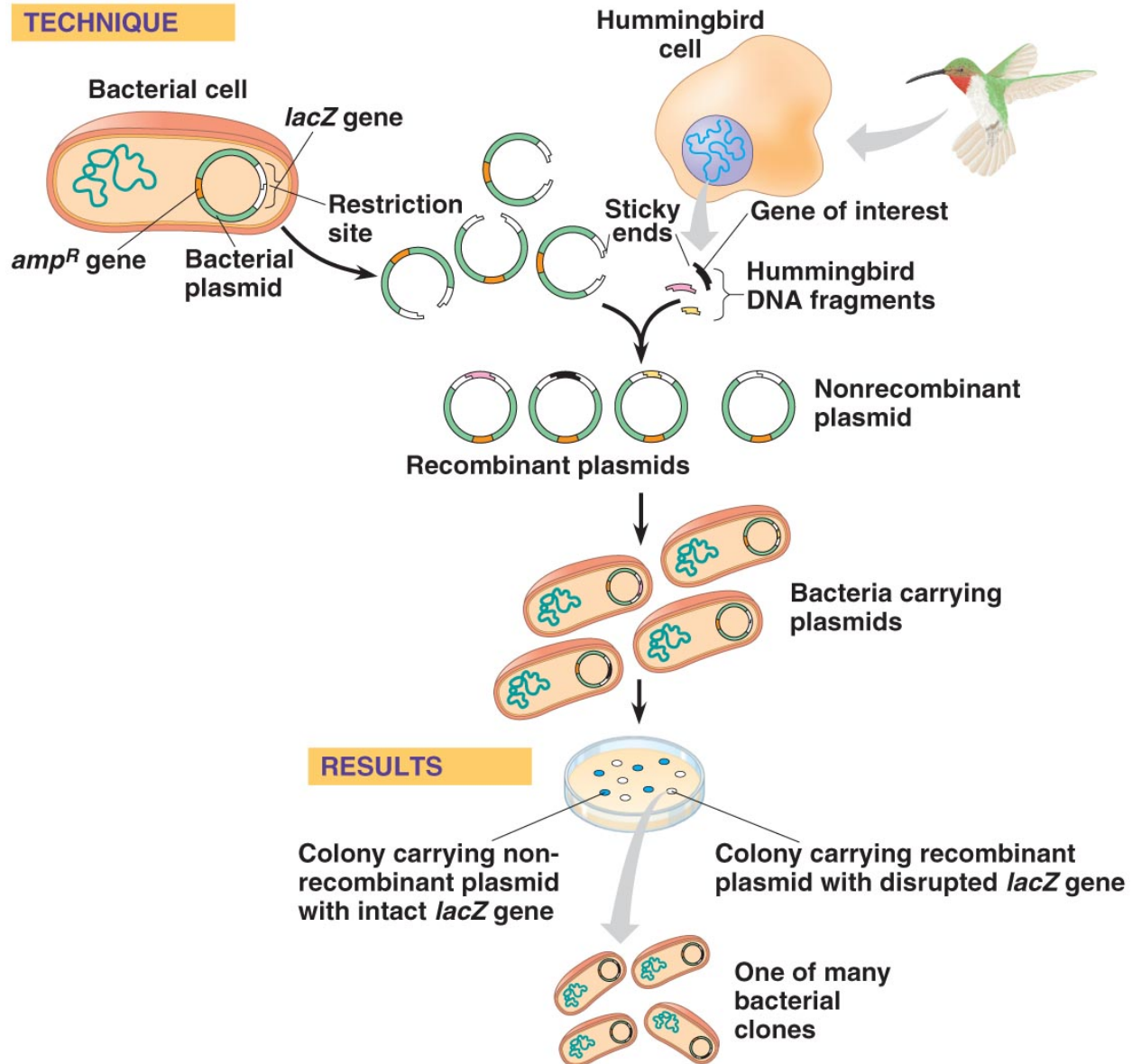
- **Cloning vector:** the original plasmid, a DNA molecule that can carry foreign DNA into a host cell and replicate
  - Usually bacterial plasmids
    - Easily isolated, manipulated and reintroduced into bacterial cells
    - They multiply easily

## PRODUCING CLONES OF CELLS CARRYING RECOMBINANT PLASMIDS

Scientists want to see the B-globin gene in a particular hummingbird to see if it's different from another less metabolically active species

1. Isolate the hummingbird genomic DNA from the hummingbird cells, isolate the vector from *E. coli*.
2. Cut both DNA samples with the same restriction enzyme

3. Mix cut plasmids and DNA fragments, some join by base pairing, add DNA ligase to seal them together
  - a. Products are recombinant plasmids and many non-recombinant plasmids
4. Mix DNA with bacterial cells that have a mutation in their own *lacZ* gene, some cells take up a recombinant plasmid or other DNA molecule by transformation
5. Plate bacteria on agar containing ampicillin and X-gal

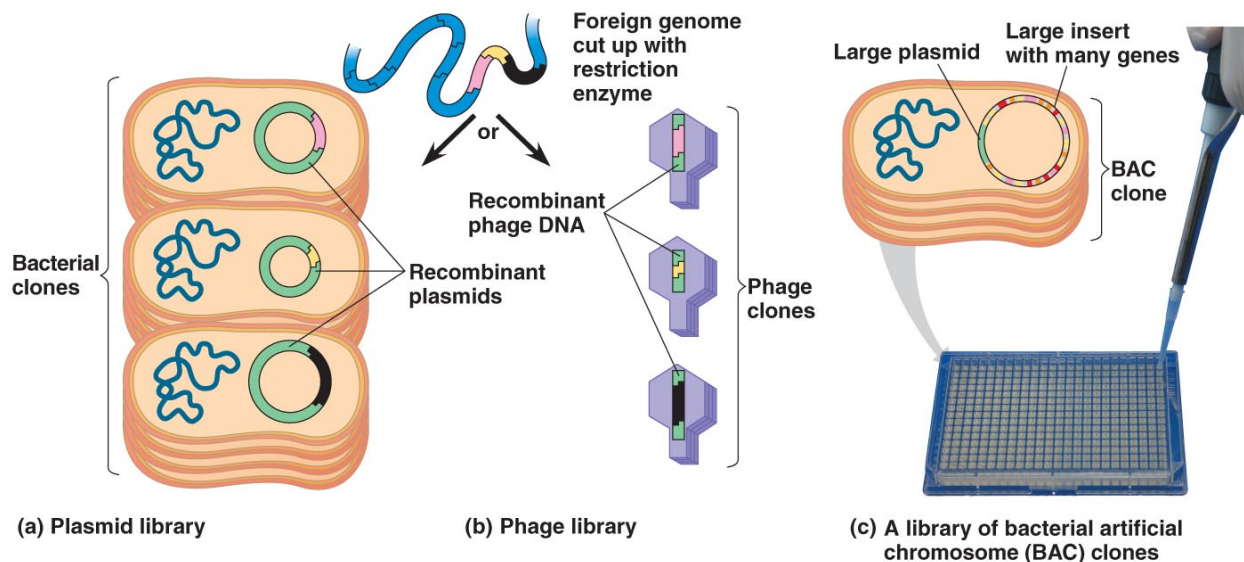


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## STORING CLONED GENES IN DNA LIBRARIES

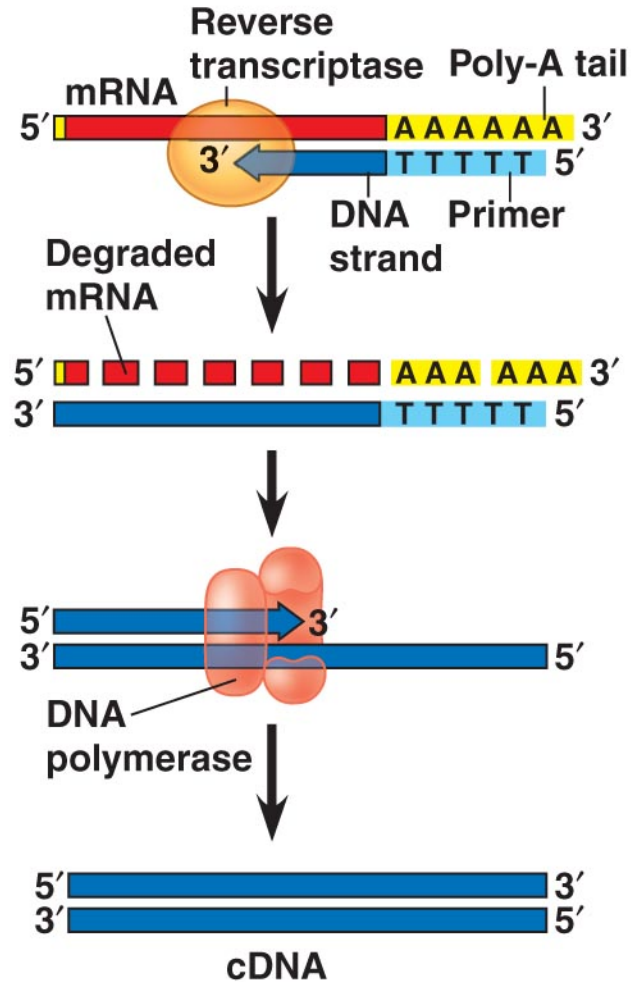
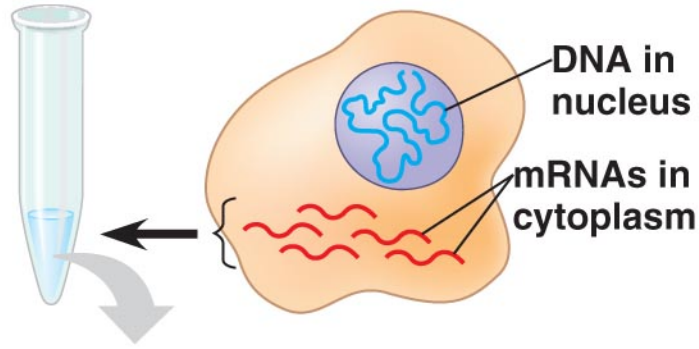
- **Genomic library:** complete set of plasmid-containing cell clones, each carrying copies of a particular segment from the initial genome
  - Certain bacteriophages have also been used as cloning vectors for making genome libraries
  - Genomic library made using phages is stored as a collection of phages clones

- Fragments of foreign DNA can be spliced into a trimmed-down version of a phage genome by restriction enzyme and DNA ligase
- **Bacterial artificial chromosome (BAC):** a type of vector used in library construction
  - Simply large plasmids, trimmed down so they contain just the genes necessary to ensure replication and capable of carrying inserts of 100-300 kb
  - Usually stored in multi-welled plastic plates
- **Complementary DNA (cDNA):** resulting double-stranded DNA from the synthesis of a second DNA strand by DNA polymerase
- **cDNA Library:** cDNAs that are cloned are part of this collection of genes, represents only part of the genome
- Genomic and cDNA libraries each have advantages
  - If you want to clone a gene, but don't know what cell type expresses it or cannot obtain enough appropriate cells, a genomic library is almost certain to contain the gene
  - If you are interested in regulatory sequences or introns associated with a gene, a genomic library is required because these sequences are absent from the fully processed mRNAs used in making a cDNA library
  - If you are interested in only the coding sequence of a gene, you can obtain a stripped-down version of the gene from a cDNA library



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#### SCREENING A LIBRARY FOR CLONES CARRYING A GENE OF INTEREST

- **Nucleic acid hybridization:** we use this to detect a gene's DNA by its ability to base-pair with a complementary sequence on another nucleic acid molecule
- **Nucleic acid probe:** the complementary molecule, short, single-stranded nucleic acid that can be either RNA or DNA
  - We can synthesize a probe complementary to any of a gene's nucleotides
  - 5' (GGCTAACTTAGC) 3' to...
  - 3' (CCGATTGAATCG) 5'

- We can use the cloned gene as a probe to identify similar/identical genes in DNA from other sources

## EXPRESSING CLONED EUKARYOTIC CELLS

- Once a particular gene has been cloned in host cells, its protein product can be produced in large amounts for research purposes or valuable practical applications

## BACTERIAL EXPRESSION SYSTEMS

- **Expression vector:** a cloning vector that contains a highly active bacterial promoter just upstream of a restriction site where the eukaryotic gene can be inserted in a correct reading frame
- Bacterial host cell will recognize the promoter and proceed to express foreign gene now linked to that promoter
- Such expression vectors allow the synthesis of many eukaryotic proteins in bacterial cells
- Another problem is the presence of noncoding regions in most eukaryotic genes, the prevent correct expression of the gene by bacterial cells, which don't have RNA-splicing machinery,
  - Problem can be solved by using a cDNA form of the gene, which includes only the exons

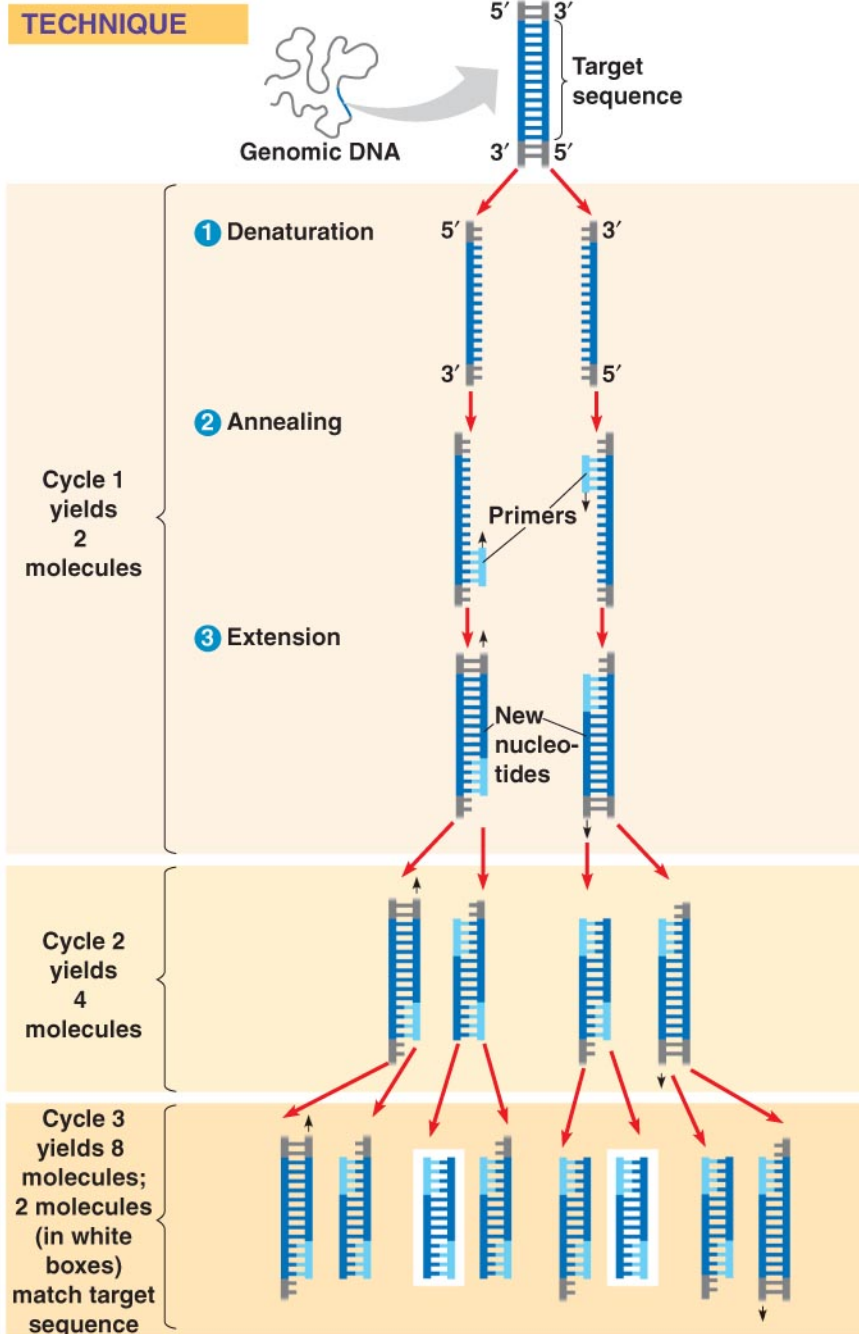
## EUKARYOTIC CLONING AND EXPRESSION SYSTEMS

- **Yeast artificial chromosomes: (YACs)** combine the essentials of a eukaryotic chromosome, an origin for DNA replication, a centromere, and two centromeres, with foreign DNA
  - They behave like normal chromosomes during mitosis, cloning the foreign DNA as the yeast cell divides
  - It can carry much longer DNA segments than a plasmid vector, a cloned fragment is more likely to contain an entire gene
- **Electroporation:** a brief electrical pulse applied to a solution containing cells that creates temporary holes in their plasma membranes through which DNA can enter

## AMPLIFYING DNA *IN VITRO*: THE POLYMERASE CHAIN REACTOR (PCR)

- **Polymerase chain reaction (PCR):** is quicker and more selective when it comes to preparing large quantities of a particular gene/DNA sequence
  - Any specific target segment within one or many DNA molecules can be quickly amplified in a test tube
  - PCR can make billions of copies of a target segment of DNA in a few hours
  - Being used increasingly to make enough of a specific DNA fragment to insert it directly into a library
  - Photocopying one page rather than checking out all the books in a library
  - Three-step cycle brings about a chain reaction that produces an exponentially growing population of identical DNA molecules
  - During each cycle, the reaction mixture is heated to denature the DNA strands then cooled to allow annealing of short, single stranded primers complementary to the sequences on the opposite strands at each end of the target sequence. Then a DNA polymerase extends the primers in the 5'→3' direction
  - Also extremely specific
    - The primers only bond to sequences at opposite ends of the target segment
  - Although it is fast and specific, it can't substitute for gene cloning in cells when large amounts of a gene are desired

- Occasional errors impose limits on the number of good copies that can be made
- It has been used to amplify DNA from a wide variety of sources



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- Heat briefly to separate DNA strands
- Cool to allow primers to form h-bonds with ends of target sequence
- DNA polymerase adds nucleotides to the 3' of each primer

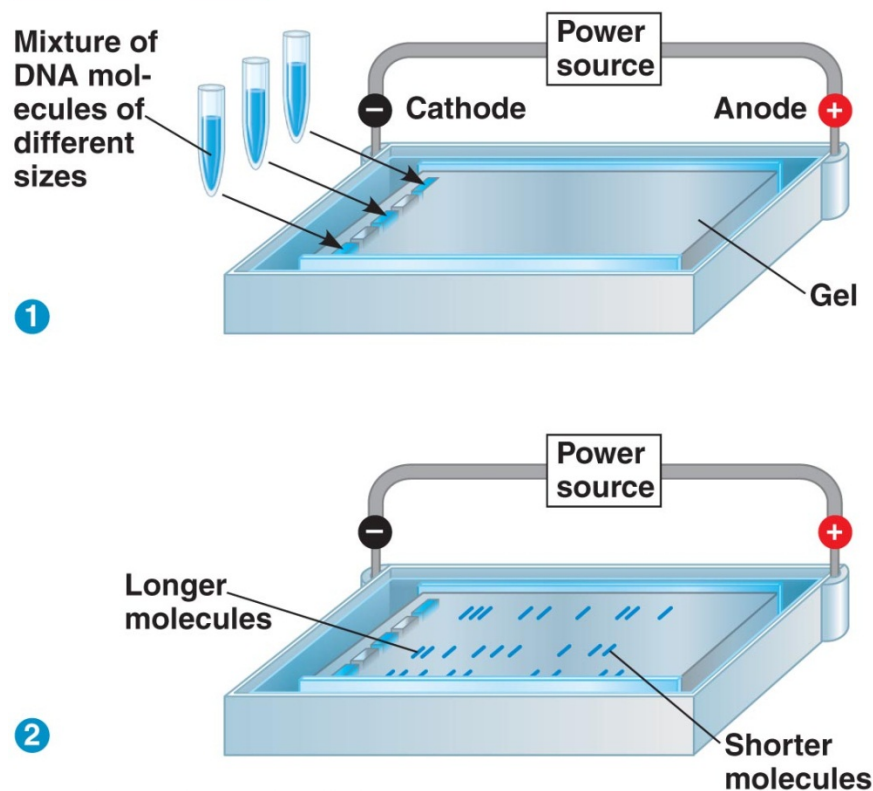


## 20.2: DNA TECHNOLOGY ALLOWS US TO STUDY THE SEQUENCE, EXPRESSION, AND FUNCTION OF A GENE

### GEL ELECTROPHORESIS AND SOUTHERN BLOTTING

- **Gel electrophoresis:** uses gel made of a polymer, which acts as a molecular sieve to separate nucleic acids or proteins on the basis of size, electrical charge and other physical properties
  - Because nucleic acid molecules carry negative charges on their phosphate groups, they travel toward the positive pole in the an electric field
  - Shorter ones move faster

#### TECHNIQUE

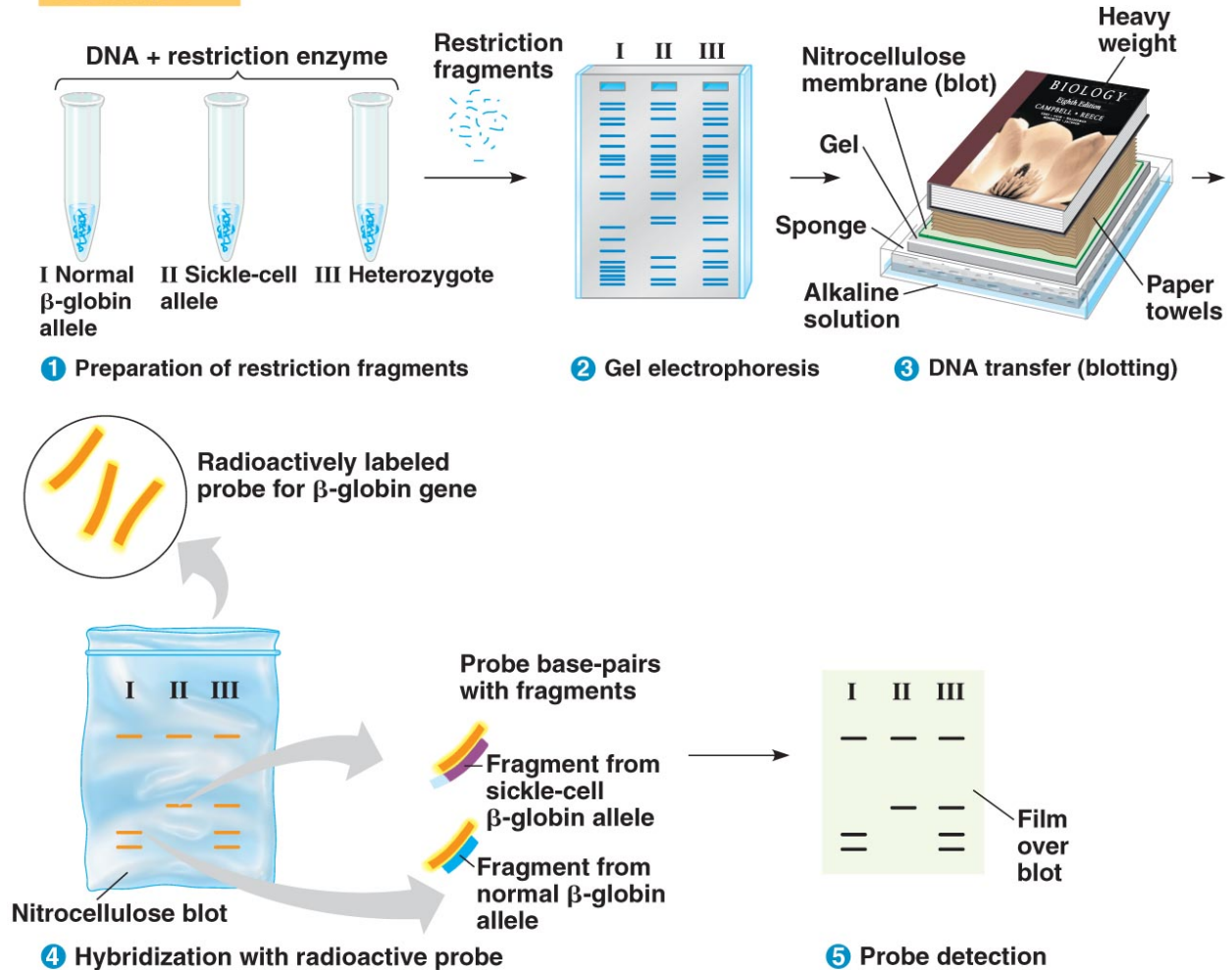


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1. Each sample, a mixture of DNA molecules, is placed in a separate well near one end of the thin slab of gel. The gel is set into a small plastic support and immersed in an aqueous solution in a tray w/ electrodes at each end
  2. When the current is turned on, the negatively charged DNA moves towards the anode, with shorter molecules moving faster than longer ones
- **Restriction fragment analysis:** rapidly provide useful info about DNA sequences
    - Fragments produced by restriction enzyme digestion of a DNA molecule are sorted by gel electrophoresis

- Useful for comparing two different DNA molecules
- Distinguish normal and unhealthy cells
- **Southern blotting:** combines gel electrophoresis and nucleic acid hybridization allows us to detect just those bands that includes parts of the B-globin gene
  - Probe is usually a radioactive single-stranded DNA molecule that is complementary to the gene of interest
  - Identification of carriers of mutant alleles associated with genetic diseases is one of the ways Southern Blotting can be used

### TECHNIQUE



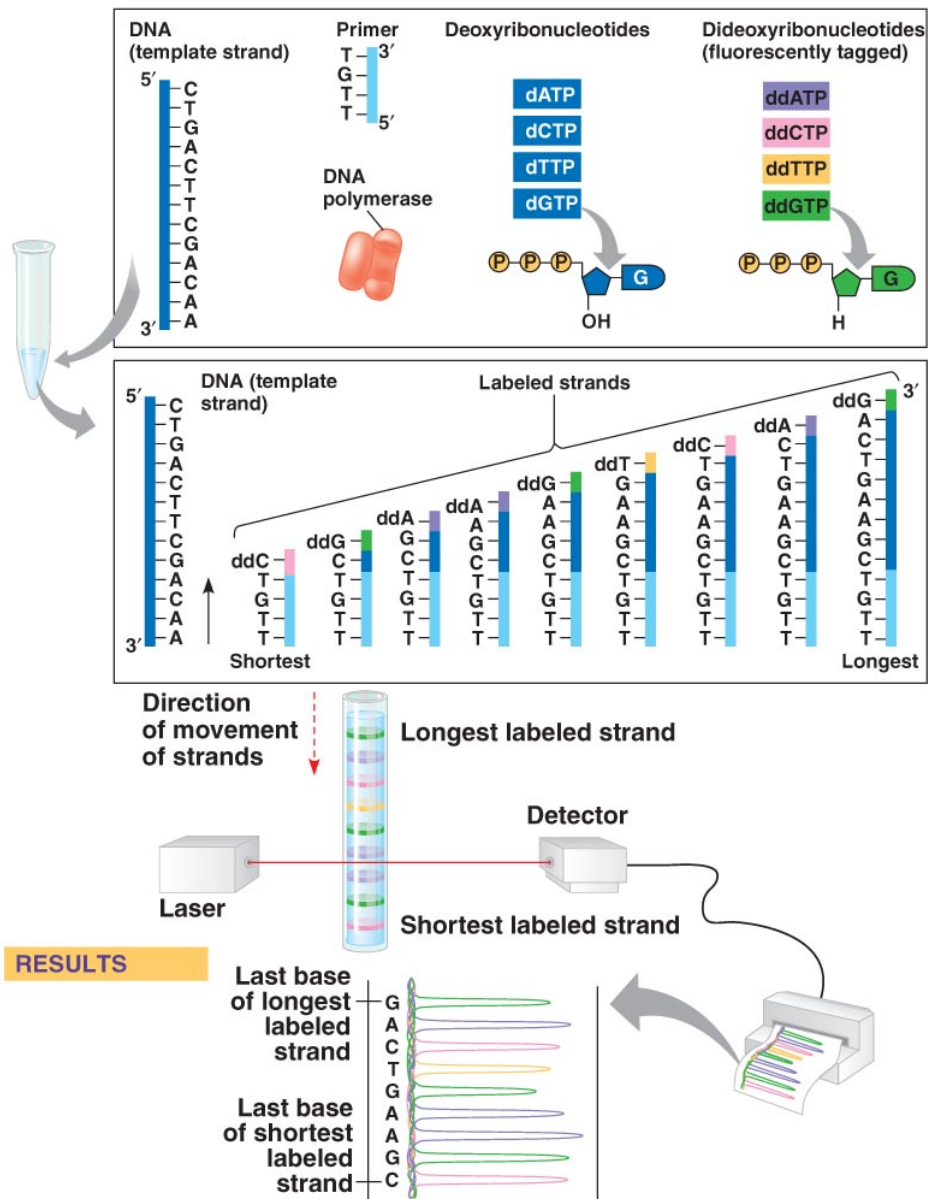
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1. Each DNA sample is mixed with the same restriction enzyme, digestion yields a mixture of thousands of restriction fragments
2. Fragments are separated by electrophoresis
3. Capillary action pulls alkaline solution up through the gel, transferring DNA to a microcellulose membrane, producing a blot (DNA is denatured)
4. Exposed to a solution containing a radioactively labeled probe
5. Sheet of photographic film is laid of the blot, forms an image corresponding to those bands containing DNA that base-paired with the probe

# DNA SEQUENCING

- Once a gene is cloned, its complete nucleotide sequence can be determined
- Carried out by sequencing machines
- Based on a technique called **dideoxyribonucleotide chain termination method**
  - Developed by Frederick Sanger (Nobel Prize)
  - Knowing the sequence of the gene allows researchers to compare it directly with genes in other species
  - If two genes from different species are quite similar, it is reasonable to suppose their gene products perform similar functions

## TECHNIQUE



## RESULTS

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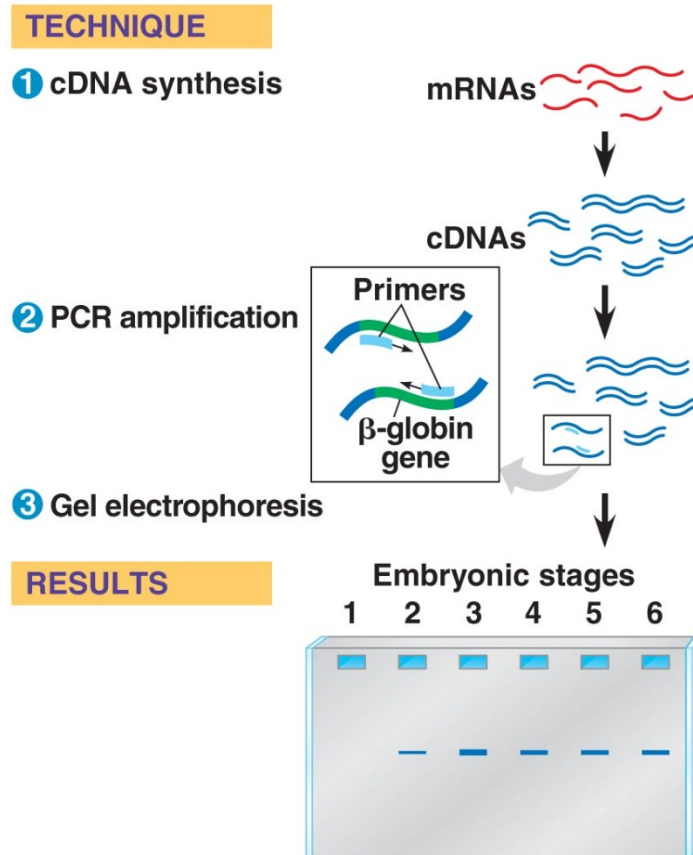
1. Fragment is denatured into single strands and incubated in a test tube with a primer, DNA polymerase and four deoxyribonucleotides and for dideoxyribonucleotides
2. Synthesis of each new strand starts at the 3' end of the primer and continues until a dideoxyribonucleotide is inserted instead of the normal deoxyribonucleotide, which prevents further elongation of the strand
3. Labeled strands in the mixture are separated by electrophoresis, the gel is formed in a capillary tube for DNA sequencing

## ANALYZING GENE EXPRESSION

- Probes can provide info about when/where in an organism the gene is transcribed

### STUDYING THE EXPRESSION OF SINGLE GENES

- **Northern blotting:** carry out gel electrophoresis of samples of mRNA
  - If we expose a film to the membrane, resulting image will look similar to the Southern blot, with one band of a given size showing up in each sample
  - If the mRNA band is seen at a particular stage, we can hypothesize that the protein functions during events taking place during that stage
- **Reverse transcriptase—polymerase chain reaction (RT-PCR):** quicker and more sensitive than Northern Blotting
  - Shows all the embryonic stages, can be carried out with mRNAs collected from different tissues at one time to discover which tissue is producing a specific mRNA



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1. cDNA synthesis: carried out by incubating the mRNAs with reverse transcriptase and other necessary components
  2. PCR amplification: of the sample is performed using primers specific to the gene
  3. Gel electrophoresis: will reveal amplified DNA products only in samples that contained mRNA transcribed from the gene
- **In situ hybridization:** carried out with probes labeled by attachment of fluorescent dyes
    - Different probes can be labeled with different dyes
    - Can determine which tissues/cells are expressing certain genes to track down the location of specific mRNAs

### STUDYING THE EXPRESSION OF INTERACTING GROUPS OF GENES

- Basic strategy: isolate mRNAs made in particular cells, use these molecules as templates for making corresponding cDNAs by reverse transcription and employ nucleic acid hybridization to compare this set of cDNAs with a collection of DNA fragments representing all of the genome
- **DNA microarray assays:** consists of tiny amounts of a large number of single-stranded DNA fragments representing different genes fixed to a glass slide in a tightly spaced array (grid)
  - Fragments represent all the genes of an organism
  - May contribute to better understanding of diseases and suggest new diagnostic techniques/therapies
  - Should provide a grander view of how ensembles of genes interact to form an organism and maintain its vital systems

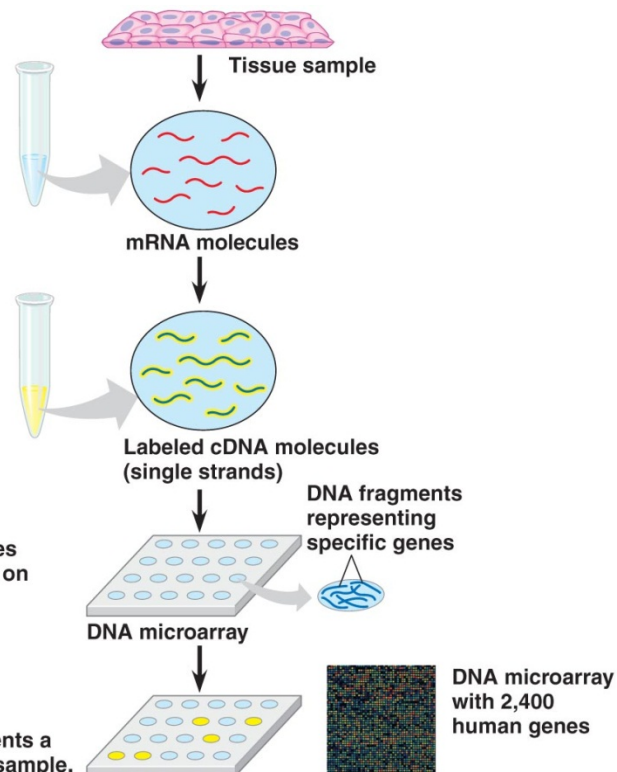
#### TECHNIQUE

##### 1 Isolate mRNA.

##### 2 Make cDNA by reverse transcription, using fluorescently labeled nucleotides.

##### 3 Apply the cDNA mixture to a microarray, a different gene in each spot. The cDNA hybridizes with any complementary DNA on the microarray.

##### 4 Rinse off excess cDNA; scan microarray for fluorescence. Each fluorescent spot represents a gene expressed in the tissue sample.



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## DETERMINING GENE FUNCTION

- ***In vitro* mutagenesis:** specific mutations are introduced into a cloned gene, and then the mutated gene is returned to a cell in such a way that it disables the normal cellular copies of the same gene
- **RNA interference (RNAi):** uses synthetic double-stranded RNA molecules matching the sequence of a particular gene to trigger breakdown of the gene's messenger RNA/block its translation
  - Has been used to reduce the expression of specific genes in mammalian cells for the treatment of human disorders



## 20.3: CLONING ORGANISMS MAY LEAD TO PRODUCTION OF STEM CELLS FOR RESEARCH AND OTHER APPLICATION

### CLONING PLANTS: SINGLE-CELL CULTURES

- **Totipotent:** a cell that is able to give rise to all specialized cell types of an organism
  - FC Steward successfully found differentiated cells taken from a carrot could be grown into normal plants
- Plant cloning is used extensively in agriculture
  - Sometimes cloning is the only practical mean of reproducing plants
  - Used to reproduce plants with valuable characteristics (immunity)

### CLONING ANIMALS: NUCLEAR TRANSPLANTATION

- Differentiated cells from animals don't divide in culture and they aren't totipotent
- **Nuclear transplantation:** removal of the nucleus of an egg and replace it with the nucleus of a differentiated cell
  - Robert Briggs and Thomas Kling, John Gurdon
    - Transplanted a nucleus from an embryonic tadpole cell into an enucleated egg of the same species
    - Direct normal development was inversely related to the age of the donor
    - Gurdon: The nucleus does change as animal cells differentiate
    - Nuclear potential tends to be restricted more as embryonic development progresses

### REPRODUCTIVE CLONING OF MAMMALS

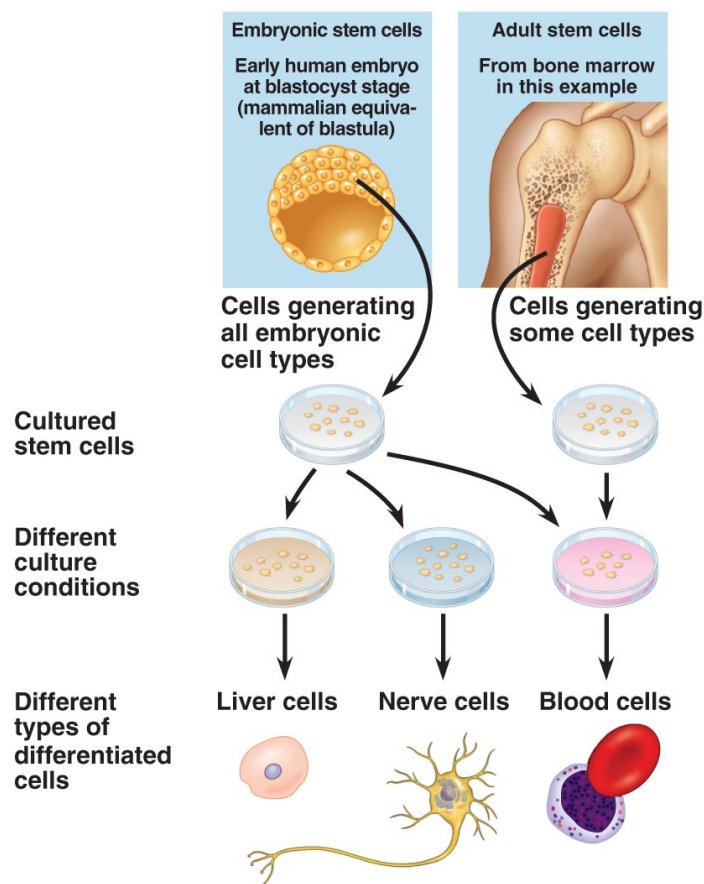
- Researchers have been able to clone mammals using nuclei/cells from a variety of early embryos
  - Dolly: a lamb cloned from an adult sheep by nuclear transplantation (1997)
    - Died at age 6 from a lung disease
    - Her cells were not as healthy as a normal sheep, reflecting the incomplete reprogramming of the original transplanted nucleus
  - **Reproductive cloning:** production of new individuals
    - Cloned animals don't always look the same/behave identically
    - Environmental influences and random phenomena can play a significant role during development
- Cloning humans?
  - Massachusetts and SK
  - Ethnical issues?

### PROBLEMS ASSOCIATED WITH ANIMAL CLONING

- Small percentage of cloned embryos develop normally to birth
- Cloned animals are likely to have subtle defects
- Reprogramming of donor nuclei requires chromatin restructuring, which occurs incompletely during cloning procedures
- Success of cloning attempt may depend on whether or not the chromatin structure in the donor nucleus can be restored to that of a newly fertilized egg

## STEM CELLS OF ANIMALS

- **Stem cell:** relatively unspecialized cell that can both reproduce itself indefinitely and differentiate into specialized cells of one or more types
  - Are able to replenish their own population and generate cells that travel down specific differentiation pathways
  - **Embryonic Stem (ES) cells:** reproduce indefinitely and can be made to differentiate into a variety of specialized cells
    - Can be isolated from early embryos at the blastula stage/blastocyst stage
  - **Adult stem cells:** serve to replace nonreproducing specialized cells as needed
    - Ex: bone marrow cells generate all kinds of blood cells
    - With the right culture conditions, cultured stem cells from adult animals have been made to differentiate into multiple types of specialized cells



- Research with stem cells is a source of valuable data about differentiation and has enormous potential for medical applications
  - Ultimate aim is to supply cells to repair damaged/diseased organs
- **Pluripotent:** capable of differentiating into many different cell types
  - The only way to obtain ES cells is to harvest them from human embryos (yikes!)
- **Therapeutic cloning:** produce ES cells to treat disease

- **Induced pluripotent stem cells (iPS cells):** transformed cells that are reduced by introducing retroviruses to create master regulatory genes

## 20.4: THE PRACTICAL APPLICATIONS OF DNA TECHNOLOGY THAT AFFECT OUR LIVES IN MANY WAYS

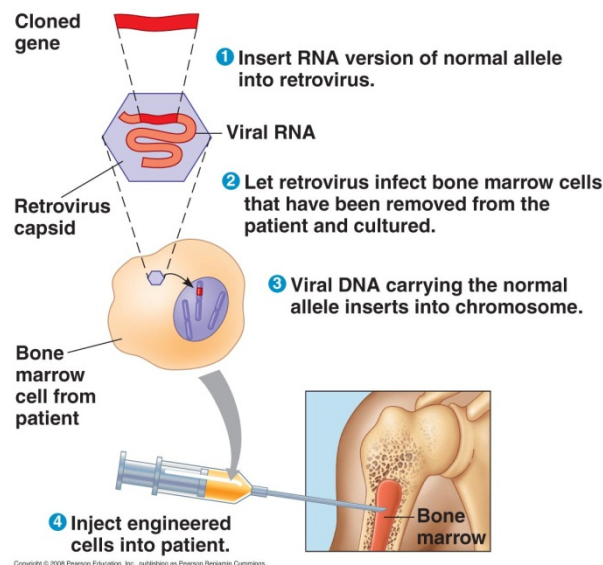
### MEDICAL APPLICATIONS

#### DIAGNOSIS OF DISEASES

- Doctors can...
  - Use PCR to track down pathogens
  - Diagnose hundreds of human disorders with primers that target the genes associated with these disorders
  - Use the amplified DNA by sequencing it to reveal the presence/absence of a disease-causing mutation
    - Hemophilia, Huntington's disease, sickle-cell, cystic fibrosis, etc
  - Test for **genetic markers** that are close to a normal, disease-causing allele
- **Polymorphism:** variation in a DNA sequence
- **Single nucleotide polymorphism (SNP):** single base-pair site where variation is found
  - Occurs about once in 100-300 base pairs and are found in both introns/exons
  - **Restriction fragment length polymorphism (RFLP):** SNPs that alter the sequence recognized by a restriction enzyme
    - Use Southern blotting to find these
- The presence of an abnormal allele can be diagnosed with accuracy if a closely linked SNP marker has been found

#### HUMAN GENE THERAPY

- **Gene therapy:** introducing genes into an afflicted individual for therapeutic purposes, capable of treating disorders traceable to a single defective gene



- SCID: 9/10 young kids with SCID showed improvement over two years
  - 3/10 developed leukemia
  - 1/10 died
  - A recent experiment in mice showed a high incidence of lymphoma
- Raises ethical and technical questions...
  - Specificity?
  - Practicality?
  - Eugenics?

## PHARMACEUTICAL PRODUCTS

### SYNTHESIS OF SMALL MOLECULES FOR USE AS DRUGS

- Recent synthesis of small molecules that are tailored to combat certain cancers by blocking the function of a protein crucial to the tumor's survival
  - Imatinib (gleevec) is a small molecule that inhibits a receptor
  - Success with a few types of lung and breast cancers
  - Can only be used with cancers whose molecular basis are well understood

### PROTEIN PRODUCTION IN CELL CULTURES

- Human insulin and human growth hormone
- Cells in culture can be used to produce vaccines, etc

### PROTEIN PRODUCTION BY PHARM ANIMALS AND PLANTS

- Scientists can use whole animals to produce protein products
- **Transgenic** animals: animals that have a gene from a different animal introduced in it
- **Transgene:** the foreign DNA into host genomes, and some transgenic animals are able to express the foreign gene
  - Transgenic animals can act as pharmaceutical factories
  - Human proteins produced in farm animals may differ in some ways from naturally produced human proteins
  - “pharm” animals and “pharm” plants: use plants and animals to make human proteins

## FORENSIC EVIDENCE AND GENETIC PROFILES

- **Genetic profile:** an individual's unique set of genetic markers
- **Short tandem repeats (STRs):** the variations in length of genetic markers
  - # of repeats present is highly variable from person to person
  - PCR is used to amplify particular STRs, sets of primers that are labeled with different-colored fluorescent tags
  - The length of the region is determined by electrophoresis
- Used for parental tests, identifying victims of mass casualties
- How reliable is it?
  - The greater the # of markers examined, the more likely that the profile is unique to one individual
  - More ethical questions

## ENVIRONMENTAL CLEANUP

- Microbes can be used to extract waste from the environment
- Biofuels: corn, soybeans and cassava can be used to produce biodiesel

## AGRICULTURAL APPLICATIONS

### ANIMAL HUBANDRY

- Goals of creating a transgenic are to make animals better for human consumption
- Problems with fertility and increased susceptibility

### GENETIC ENGINEERING IN PLANTS

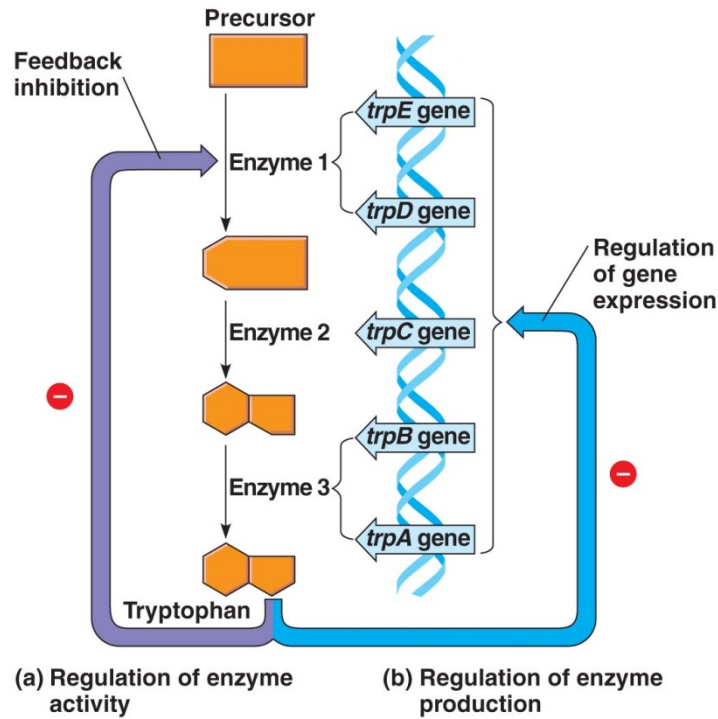
- Endowing crops with genes for desirable traits: delayed ripening and resistance to spoilage/disease
  - **Ti plasmid:** from *Agrobacterium tumefaciens* which integrates a segment of its own DNA into the chromosomal DNA of its host plants, most commonly used vector
- Genetic engineering is rapidly replacing traditional plant-breeding programs
  - Crops can be engineered to be resistant to herbicides, salinity-resistant, more nutritious, etc

## SAFETY AND ETHNICAL QUESTIONS RAISED BY DNA TECHNOLOGY

- **Genetically modified (GM) organism:** an organism that has acquired 1+ gene from another species by artificial means
  - GM Crops are widespread in US, Argentina and Brazil
    - GM products are not required to be labeled in the US
    - Strong controversy in Europe
    - Fear that transgenic plants may pass their new genes to close relatives in nearby wild areas
    - Some fear protein products of transgenes might lead to allergic reactions

## 18.1: BACTERIAL OFTEN RESPOND TO ENVIRONMENTAL CHANGE BY REGULATING TRANSPORTATION

- Metabolic control occurs on two levels
  - 1: cells can adjust the activity of enzymes already present
    - A fast response, relies on the sensitivity of many enzymes to chemical cues that increase/decrease their catalytic activity
  - 2: cells can adjust the production level of certain enzymes- regulate the expression of the genes encoding enzymes
    - Many genes of the bacterial genome are switched on/off by changes in the metabolic status of the cell
    - **Operon model:** basic mechanism for the above control of gene expression, discovered by Francois Jacob and Jacques Monod

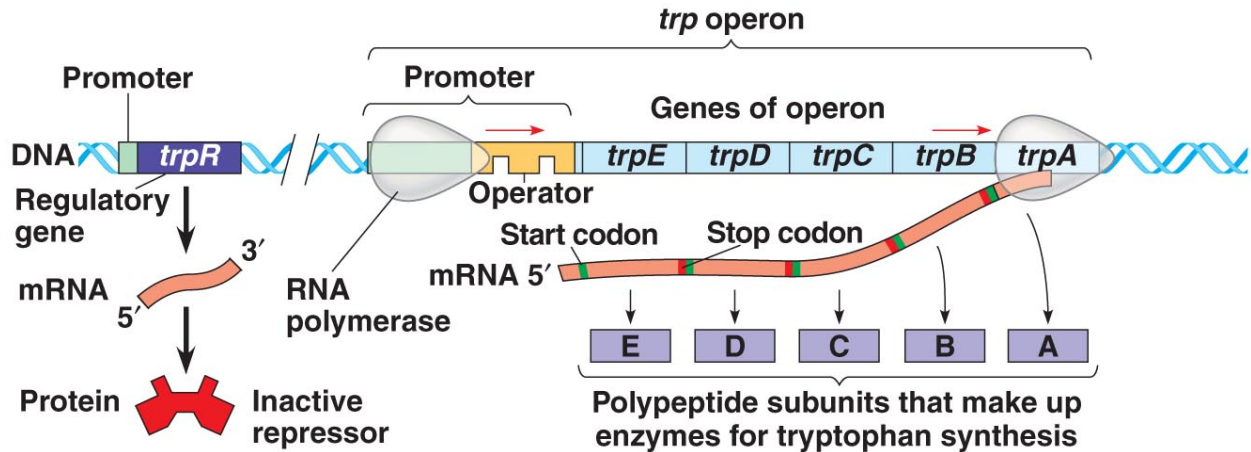


## OPERONS: THE BASIC CONCEPT

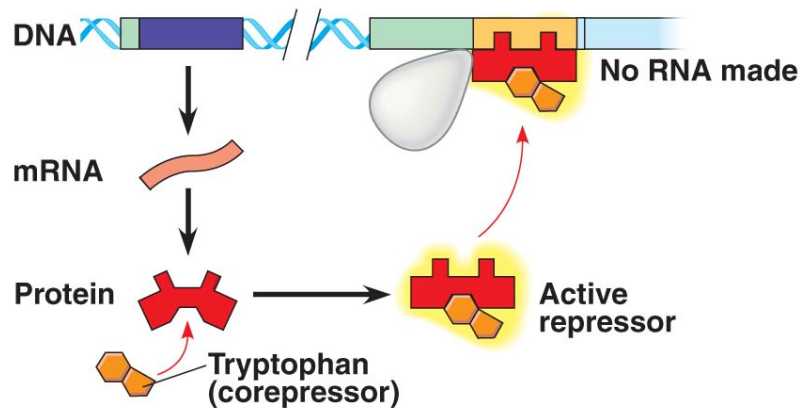
- Ex: *E. coli*:
  - Synthesizes the amino acid tryptophan from a precursor molecule
  - Each reaction in the pathway is catalyzed by a specific enzyme
  - Five genes that code for the subunits of these enzymes are clustered together on the bacterial chromosome
  - A single protein serves all five genes
  - Transcription gives rise to one long mRNA that codes for 5 polypeptides
- Advantage of grouping genes of related function into one transcription unit is that the single off-on switch can control the whole cluster of functionally related genes: **coordinate control**
- **Operator:** the switch for a segment of DNA
  - The operator, promoter and the genes they control constitute the **operon**
  - *Tpr* operon in *E. coli*
- By itself, an operon is turned on
  - Operons can be turned off by a **repressor**, which binds to the operator and blocks attachment of RNA polymerase to the promoter, preventing the transcription of genes
    - This protein is specific for the operator of a particular operon
    - *Tpr* repressor
    - Repressors are products of **regulatory genes**, located some distance from the operon and promoter
      - *trpR* in *E. coli*
      - the binding of repressors is reversible: operator vacillate between two states: one w/o the repressor and one with the repressor



- *trp* repressor is an allosteric protein with two alternative shapes, active and inactive
  - only if tryptophan binds to *trp* repressor allosteric site, does the protein change to the active form that can attach to the operator
- **corepressor:** small molecule that cooperates with the repressor protein to switch an operon off



(a) Tryptophan absent, repressor inactive, operon on



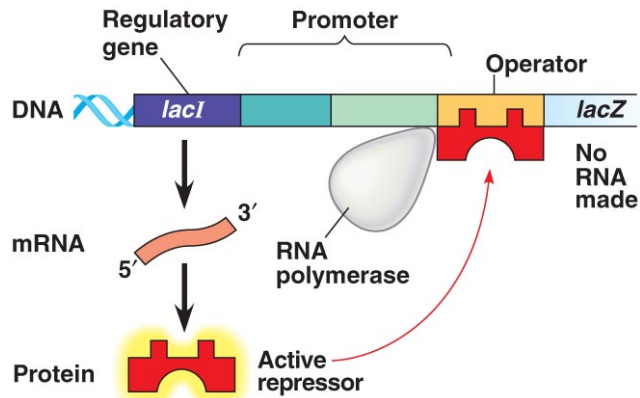
(b) Tryptophan present, repressor active, operon off

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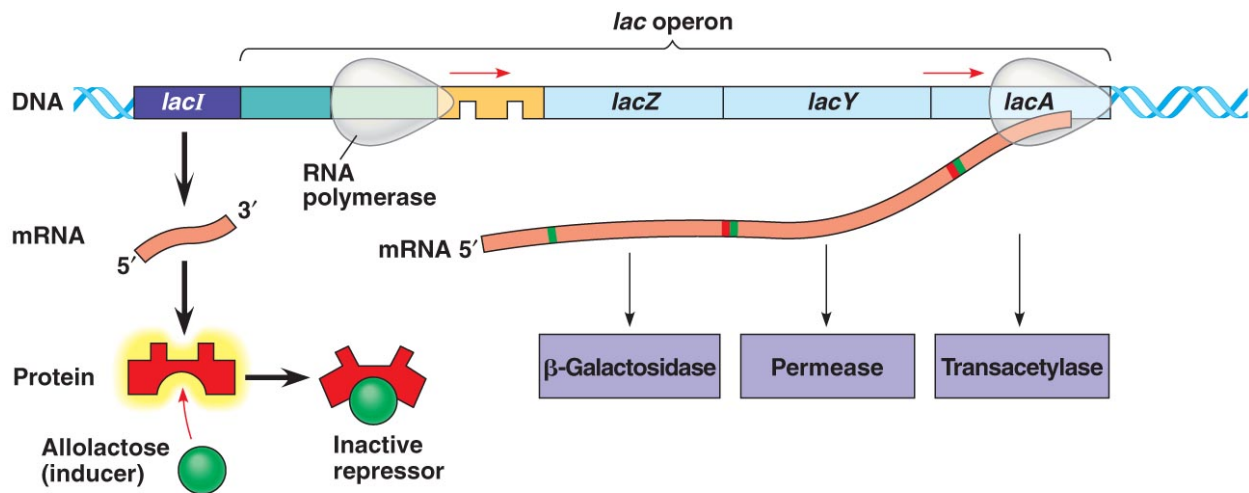
## REPRESSIBLE AND INDUCIBLE OPERONS

- **Repressible operon:** transcription is usually on, but can be repressed when a specific small molecule binds allosterically to a regulatory protein
- **Inducible operon:** usually off but can be stimulated when a specific small molecule interacts with a regulatory protein
- **Inducer:** a small molecule that inactivates the repressor
- **Inducible enzymes:** enzymes of the lactose pathway because their synthesis is induced by a chemical signal
- **Repressible enzyme:** function in anabolic pathways, which synthesize essential end products from raw materials

- By producing the appropriate enzymes only when the nutrient is available, the cell avoids wasting energy and precursors making proteins aren't needed



(a) Lactose absent, repressor active, operon off

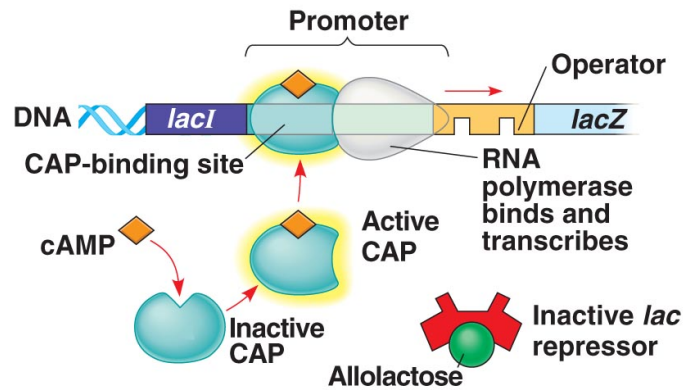


(b) Lactose present, repressor inactive, operon on

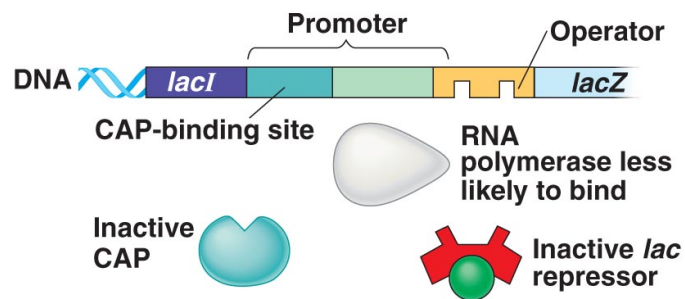
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## POSITIVE GENE REGULATION

- Glucose is usually used, enzymes for glucose breakdown in glycolysis are continually present
- **Cyclic AMP (cAMP):** small organic molecule that accumulates when glucose is scarce and lets the bacterium know that they can use lactose
  - **Activator:** *catabolite activator protein (CAP)*, a protein that binds to DNA and stimulates transcription of a gene
  - When cAMP binds to a regulatory protein made by CAP, CAP assumes its active shape and can attach to a specific site at the upstream end of a promoter
    - Increases the affinity of RNA polymerase for the promoter, which is actually low even when no repressor is bound to the operator
    - The attachment of CAP to the promoter stimulates gene expression
  - If the amount of glucose in the cell increases, cAMP concentration falls and CAP detaches from the operon



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

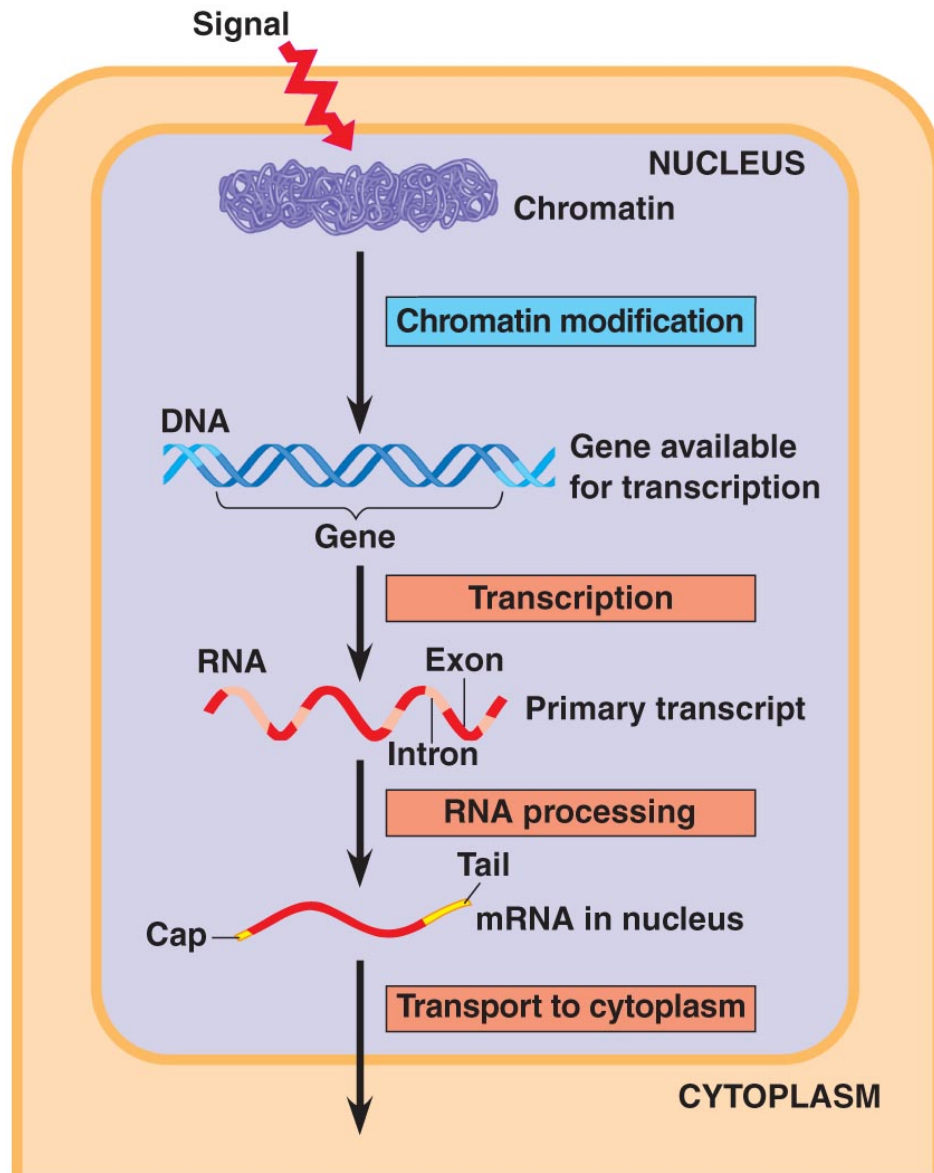
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- CAP helps regulate other operons that encode enzymes used in catabolic pathways
- Compounds present in the cell at the moment determine which operons are switched on—the result of simple interactions of activator and repressor proteins with the promoters of the genes in question

## 18.2: EURKARYOTIC GENE EXPRESSION CAN BE REGULATED AT ANY STAGE

### DIFFERENTIAL GENE EXPRESSION

- Human cells don't express a lot of its genes at any given time (usually <20%)
- **Differential gene expression:** expression of different genes by cells with the same genome
  - Genomes of eukaryotes may contain tens of thousands of genes, but only a small amount of the DNA codes for proteins
    - The rest codes for RNA products (tRNAs) or isn't transcribed at all



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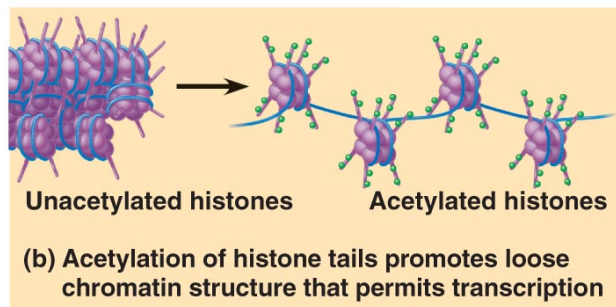
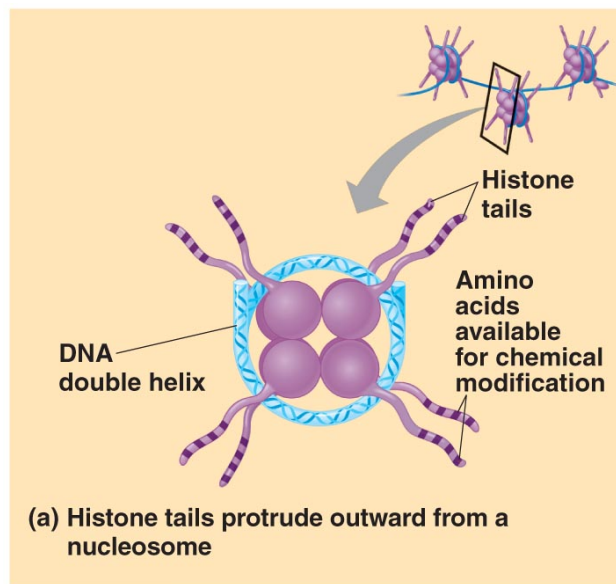
## REGULATION OF CHROMATIN STRUCTURE

- Structural organization of chromatin packs a cell's DNA into a compact form that fits inside the nucleus and helps regulate gene expression
  - Location of a gene's promoter relative to nucleosomes and to the sites where the DNA attaches to the chromosome scaffold can affect whether the gene is transcribed
  - Genes within heterochromatin aren't usually expressed
  - Certain chemical modifications to histones and DNA of a chromatin can influence chromatin structure and gene expression

## HISTONE MODS

- Chemical mods to **histones**, proteins around which DNA is wrapped in nucleosomes, play a direct role in the regulation of gene transcription

- **Histone acetylation:** acetyl groups ( $-COCH_3$ ) are attached to lysines in histone tails
  - Deacetylation is the removal of acetyl groups
  - When lysines are acetylated, their positive charges are neutralized and histone tails no longer bind to neighboring nucleosomes
  - Histone acetylation enzymes may promote the initiation of transcription not only by remodeling chromatin structure, but also by binding to and thus “recruiting” components of transcription machinery
  - Other chemical groups can be reversibly attached to amino acids in histone tails
    - Methyl groups, phosphate groups, etc
    - **Histone code hypothesis:** proposes that specific combinations of mods help determine chromatin configuration



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## DNA METHYLATION

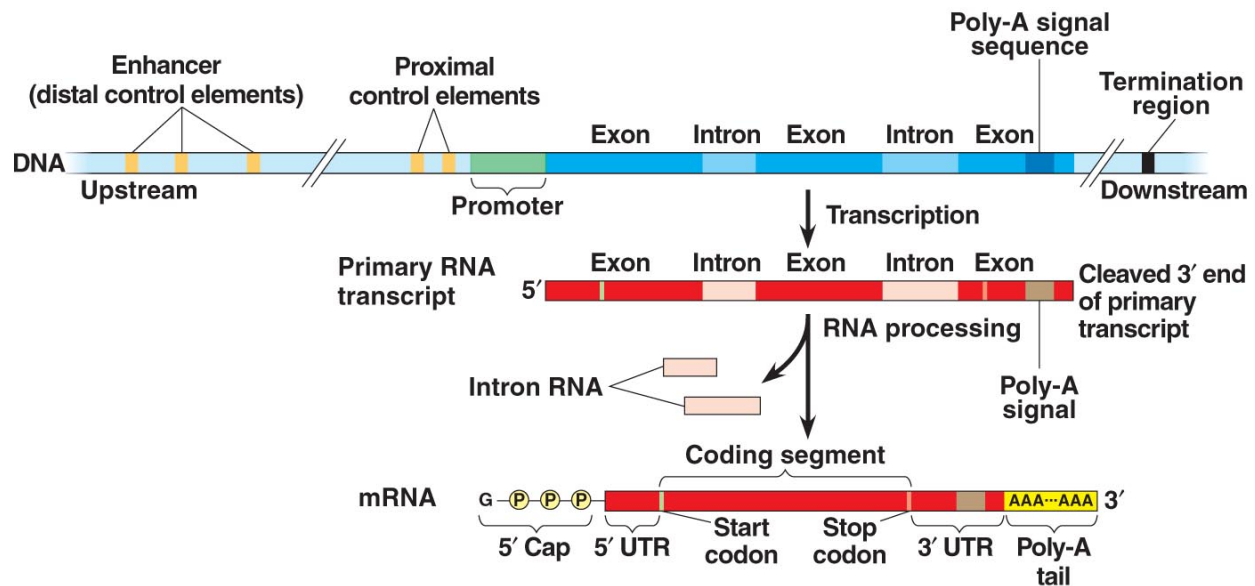
- Seems to be essential for the long-term inactivation of genes that occurs during normal cell differentiation in the embryo
- Deficient DNA methylation due to lack of methylating enzymes leads to abnormal embryonic development
- Genes usually stay methylated and patterns are passed on, with cells forming specialized tissues to keep chemical record of what occurred during embryonic development

- **Genomic imprinting:** methylation permanently regulates expression of either maternal/paternal allele of particular genes at the start of development

## EPIGENETIC INHERITANCE

- **Epigenetic inheritance:** inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence
  - Mods to the chromatin can be reversed
  - Epigenetic variations might help explain why one identical twin acquires a genetically based disease while the other doesn't
  - Alterations in normal patterns of DNA methylation are seen in some cancers
  - Enzymes that modify chromatin structure are integral parts of the eukaryotic cell's machinery for regulating transcription

## REGULATION OF TRANSCRIPTION INITIATION



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## ORGANIZATION OF A TYPICAL EUKARYOTIC GENE

- **Control elements:** segments of noncoding DNA that help regulate transcription by binding certain proteins
- RNA polymerase II transcribes the gene, synthesizes pre-mRNA and provides the 5' cap and poly-A tail

## ROLE OF TRANSCRIPTION FACTORS

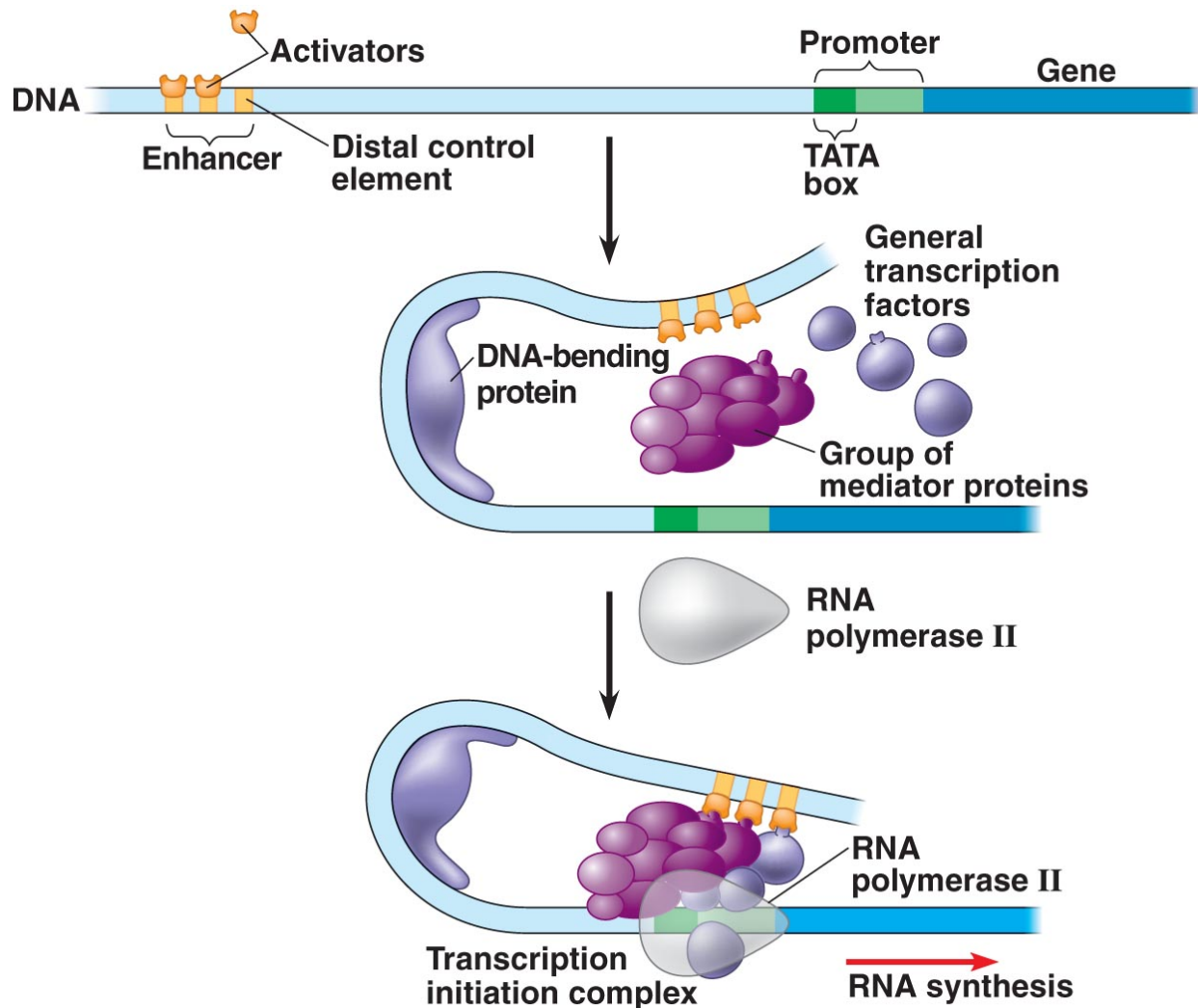
- RNA polymerase requires the assistance of proteins called transcription factors
  - Essential for the transcription of all protein-coding gene- *general transcription factors*
  - Interaction of general transcription factors and RNA polymerase with a promoter usually leads to a low rate of initiation and production of few RNA transcripts
  - High levels of transcription of particular genes at the appropriate time and place depend on the interaction of control elements with other set of proteins- *specific transcription factors*

## ENHANCERS AND SPECIFIC TRANSCRIPTION FACTORS

- *Proximal control elements*- located close to the promoter



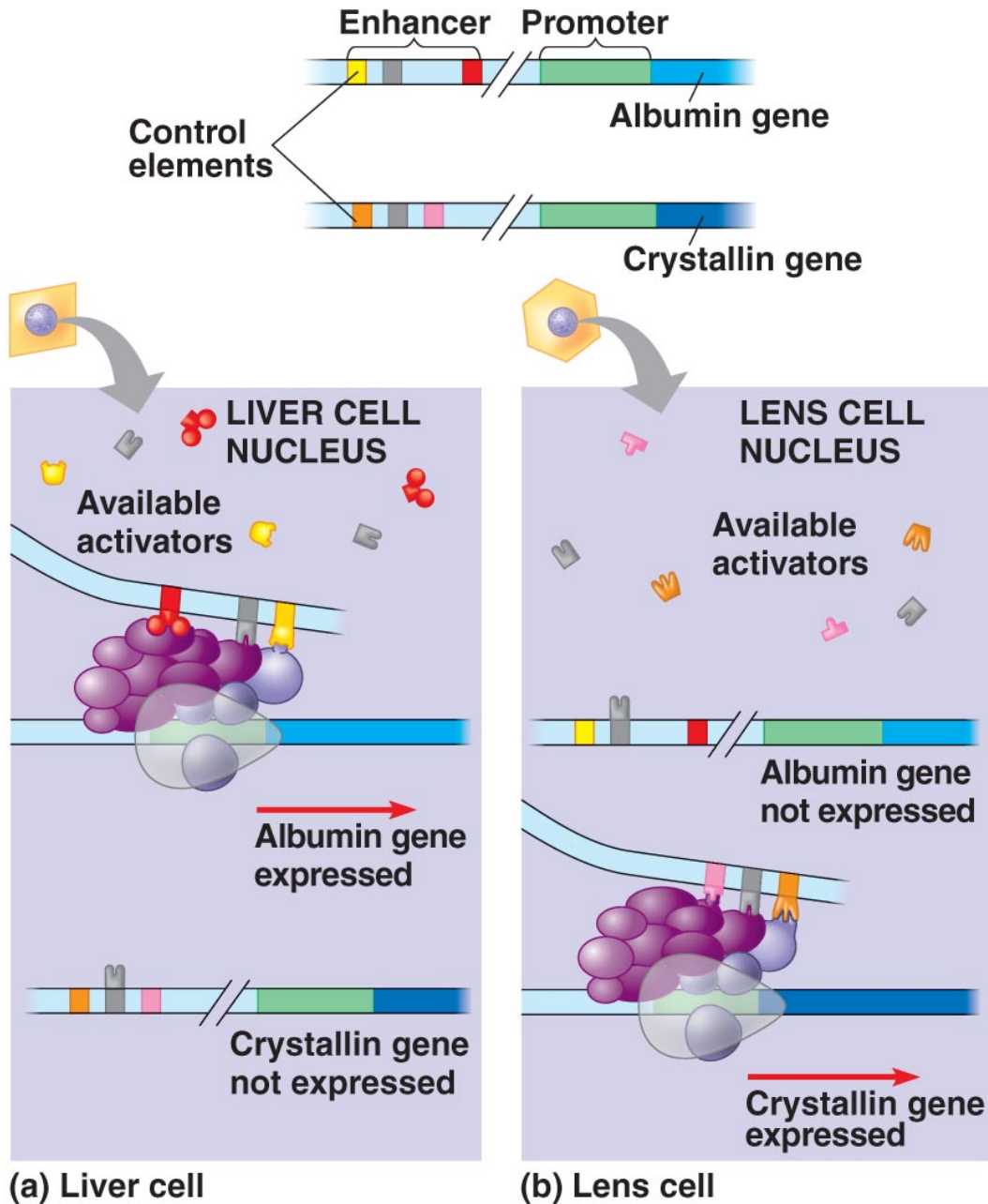
- *Distal control elements (enhancers = groupings of these)*: may be thousands of nucleotides upstream/downstream of a gene
  - A given gene may have multiple enhancers, each active at a different time or in a different cell type
- In eukaryotes: Rate of gene expression can be strongly increased/decreased by the binding of proteins to control elements of enhancers
- *Mediator proteins*: interact with proteins at the promoter – assemble and position the initiation complex on the promoter
- Specific transcription factors that function as repressors can inhibit gene expression in several different ways
  - Some bind directly to control element DNA, blocking activator binding or turning off transcriptions
  - Block the binding of activators to proteins that allow the activators to bind DNA
  - Act indirectly by affecting chromatin structure



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## COMBINATION CONTROL OF GENE ACTIVATION

- Precise control of transcription depends largely on the binding of activators to DNA control elements
- The number of completely different nucleotide sequences found in control elements is surprisingly small
- A particular combination of control elements in an enhancer associated with a gene turns out to be more important than the presence of a single unique control element in regulating transcription of a gene
- A very large number of combinations is possible



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## COORDINATELY CONTROLLED GENES IN EUKARYOTES

- Co-expressed genes are clustered near one another on the same chromosome
  - Each gene in a cluster has its own promoter and is individually transcribed
  - Coordinate regulation of these clustered genes is thought to involve changes of chromatin structure that make the entire group of genes either available or unavailable for transcription
- Co-expressed eukaryotic genes are found scattered over different chromosomes
  - Coordinate gene expression depends on the association of a specific combination of control elements with every gene of a dispersed group
  - Occurs in response to chemical signals from outside the cell
  - Every gene whose transcription is stimulated by a particular steroid hormone has a control element recognized by that hormone-receptor complex
  - Many signal molecules bind to receptors on a cell's surface and never actually enter the cell

## MECHANISMS OF POST-TRANSCRIPTIONAL REGULATION

### RNA PROCESSING

- **Alternative RNA splicing:** regulation at an RNA-processing level, different mRNA molecules are produced from the same primary transcript, depending on which RNA segments are treated as exons and introns
  - Regulatory proteins specific to a cell type control intron-exon choices by binding to regulatory sequences within the primary transcript

### MRNA DEGRADATION

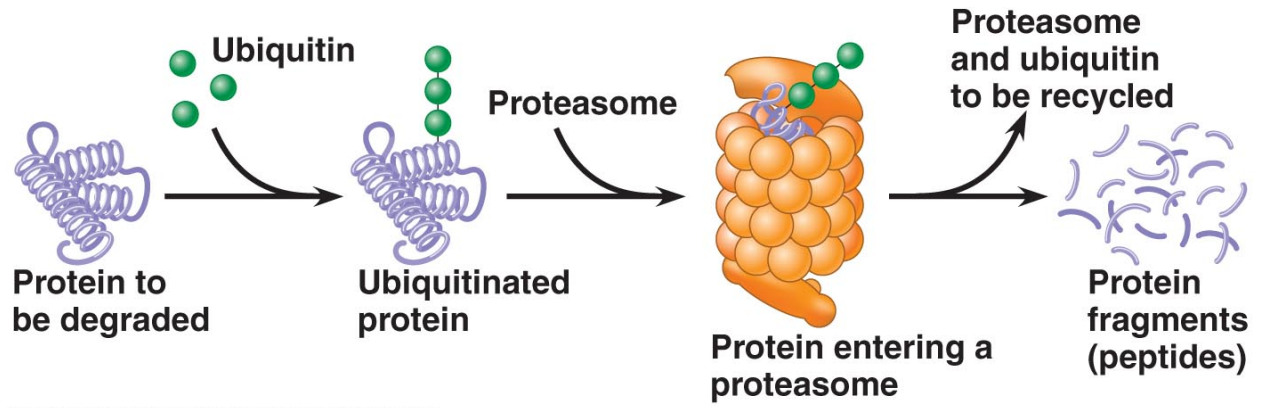
- Short life span of mRNA- bacteria change their patterns of protein synthesis quickly in response to environmental changes
  - mRNAs in eukaryotes survive for hours/days/weeks+
  - mRNA breakdown begins with the shortening of the poly-A tail, then the 5' cap is removed
- nucleotide sequences affect how long an mRNA remains intact is founded in the untranslated region at the 3' of the molecule

### INITIATION OF TRANSLATION

- Translation presents opportunity for regulating gene expression
- Initiation of translation of some mRNAs can be blocked by regulatory proteins that bind to specific sequences
- Translation of *all* mRNAs in a cell may be regulated simultaneously, plays a role in starting translation of mRNA that are stored in eggs, etc

### PROTEIN PROCESSING AND DEGRADATION

- Many proteins undergo chemical modifications that make them function
- Regulatory proteins are commonly activated/inactivated by the reversible addition of phosphate groups
- Length of time each protein functions in the cell is regulated by selective degradation
  - **Proteasomes:** giant protein complexes that recognize the ubiquitin-tagged proteins and degrade them



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