Genetic Manipulation
Part 1

Wendell Wiggins
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Genetic Manipulation
The Basis of Genetics

- Genetics: the science of heredity, dealing with resemblances and differences of related organisms

- We deal primarily with the molecular mechanisms underlying genetics and methods for manipulating them

Genetic Manipulation

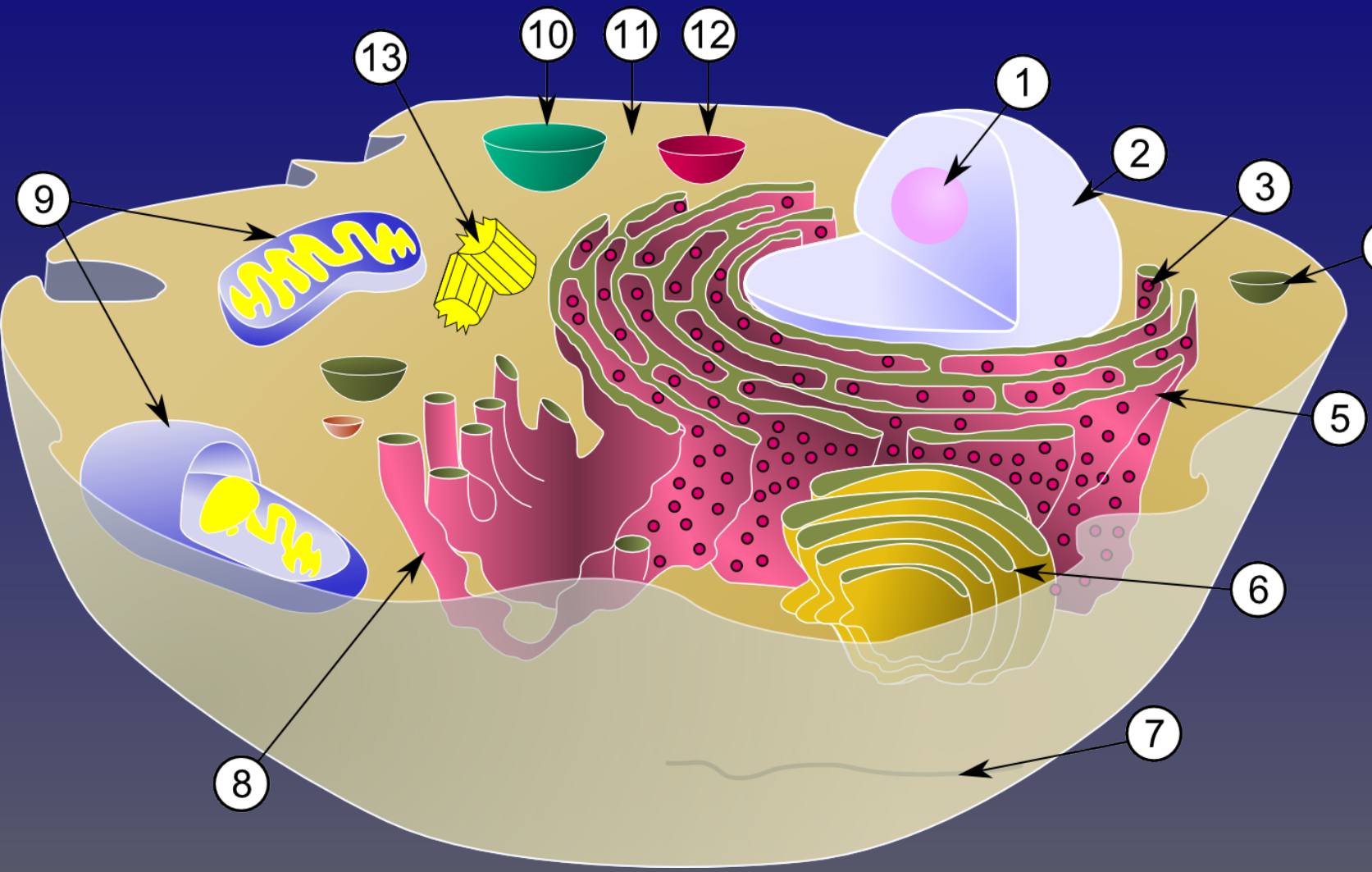
The Basis of Genetics

Why is a cow a cow?

Why does a single-cell cow
embryo make a whole cow?

Genetic Manipulation

The Basis of Genetics



1. Nucleolus
2. Nucleus
3. Ribosome
4. Vesicle
5. Rough endoplasmic reticulum
6. Golgi apparatus
7. Cytoskeleton
8. Smooth endoplasmic reticulum
9. Mitochondria
10. Vacuole
11. Cytosol
12. Lysosome
13. Centriole

Genetic Manipulation

The Basis of Genetics

Why is a cow a cow?

If the nucleus (DNA) is removed and replaced by a banteng nucleus, a single cell cow embryo produces a banteng

Advanced Cell Technologies, Inc. 2003

Genetic Manipulation

The Basis of Genetics

Why is a human a human?

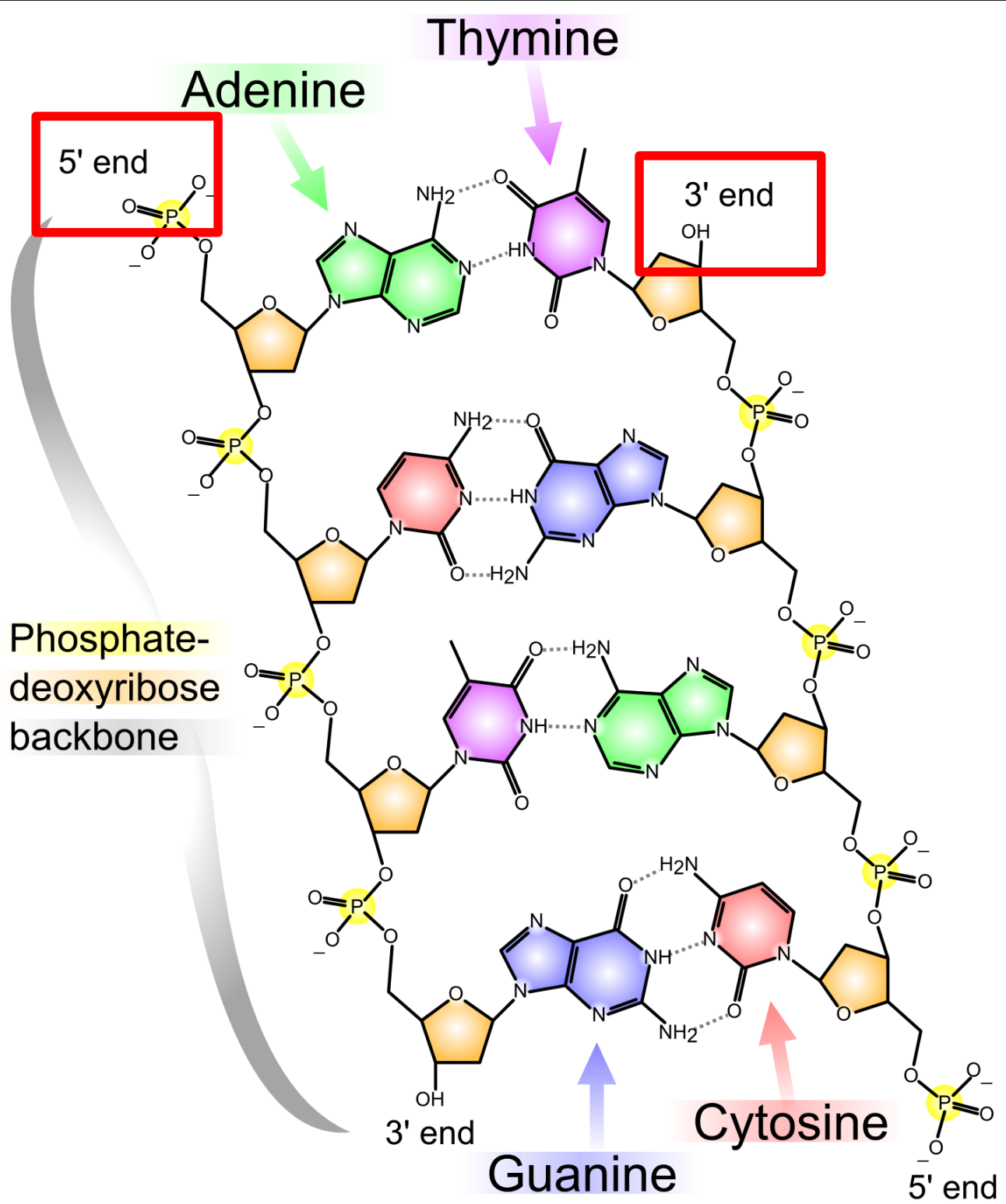
Would a single cell cow embryo produce a human if the nucleus were replaced?

Genetic Manipulation

DNA

Deoxyribose Nucleic Acid

- Sugar-phosphate chain
- Four base types
- Complementary strands
- Strong backbones, weak base pairing

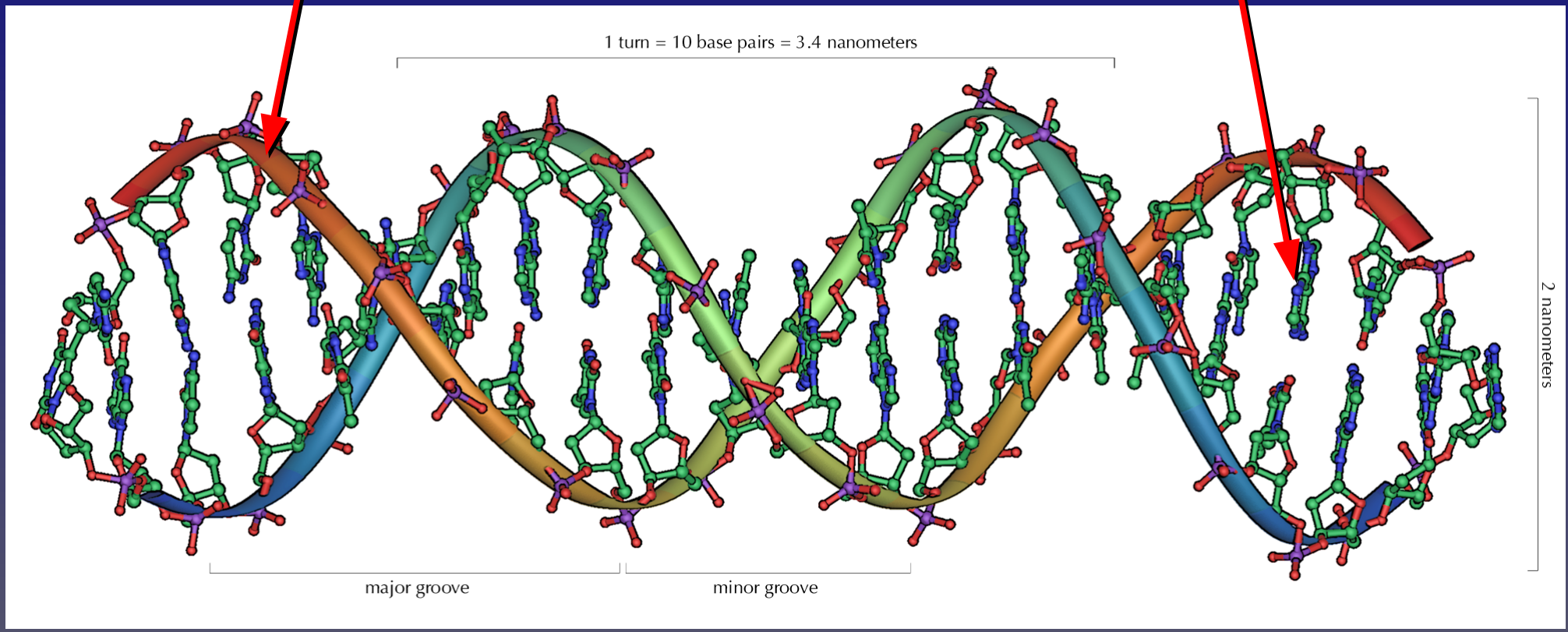


Genetic Manipulation

The Basis of Genetics

Backbone: Deoxyribose sugar plus phosphate

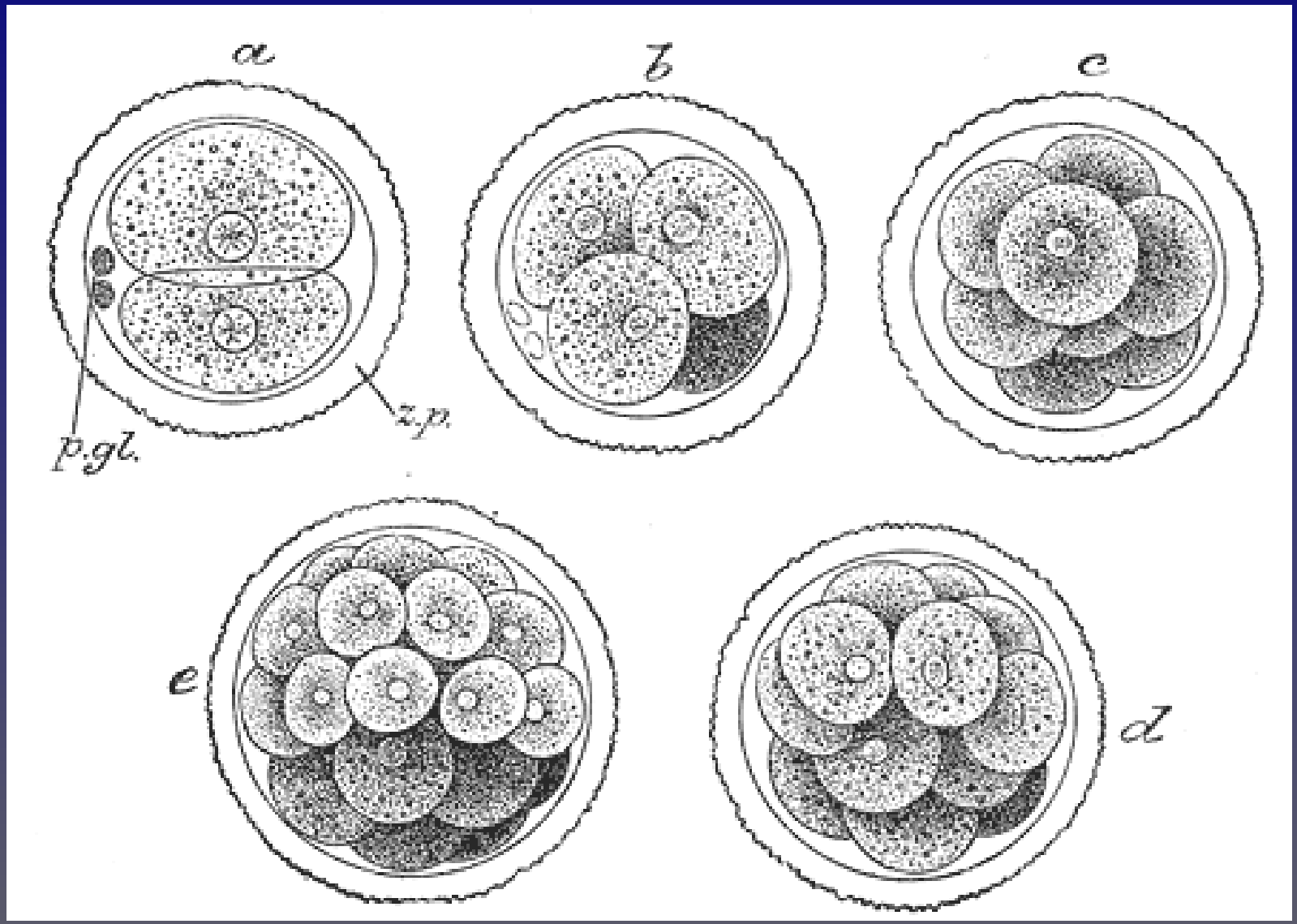
Nucleotides (bases)



Deoxyribonucleic Acid DNA

Genetic Manipulation

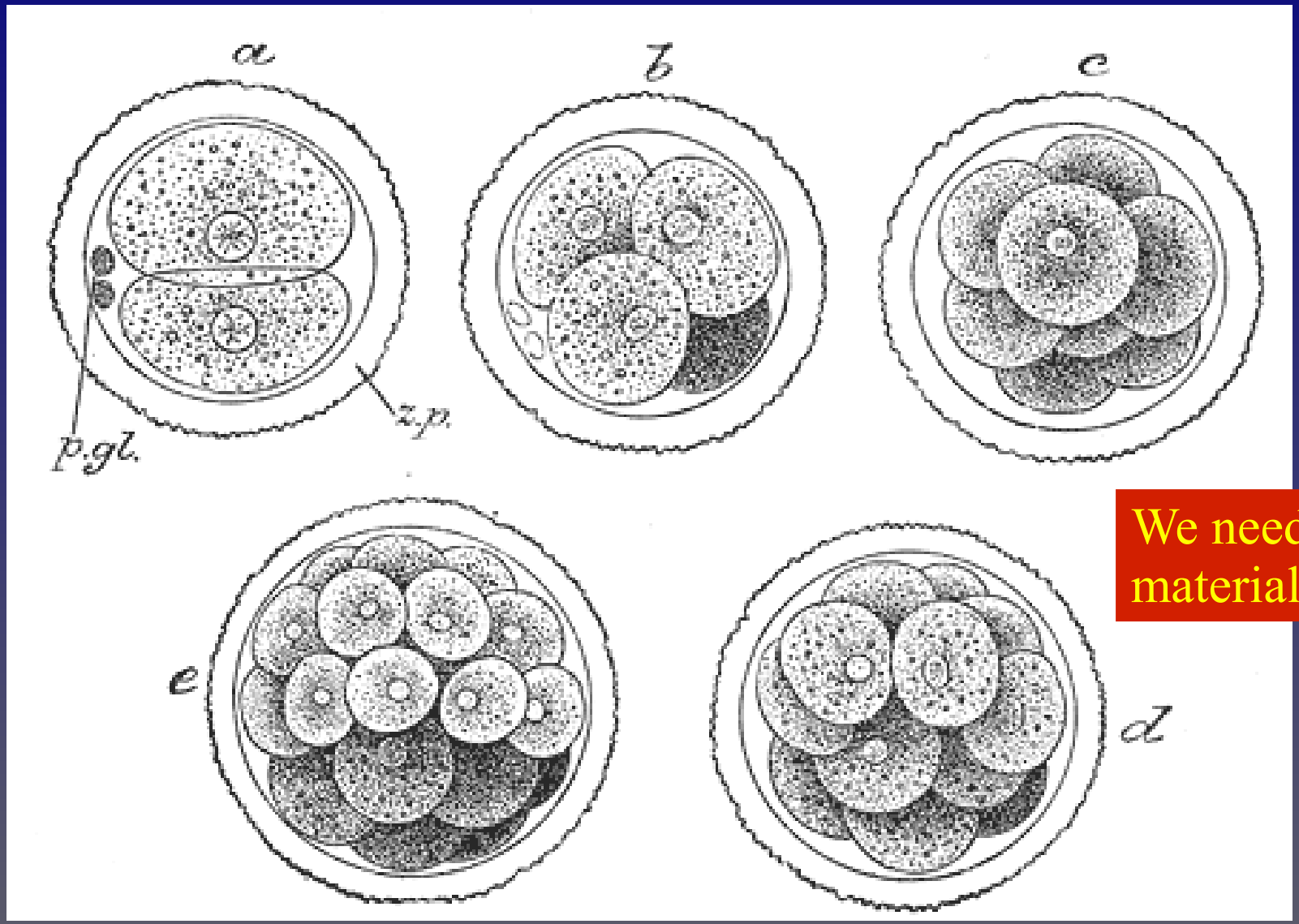
Growth and Cell Division



From
Gray's Anatomy, 1918

Genetic Manipulation

Growth and Cell Division



We need building materials!

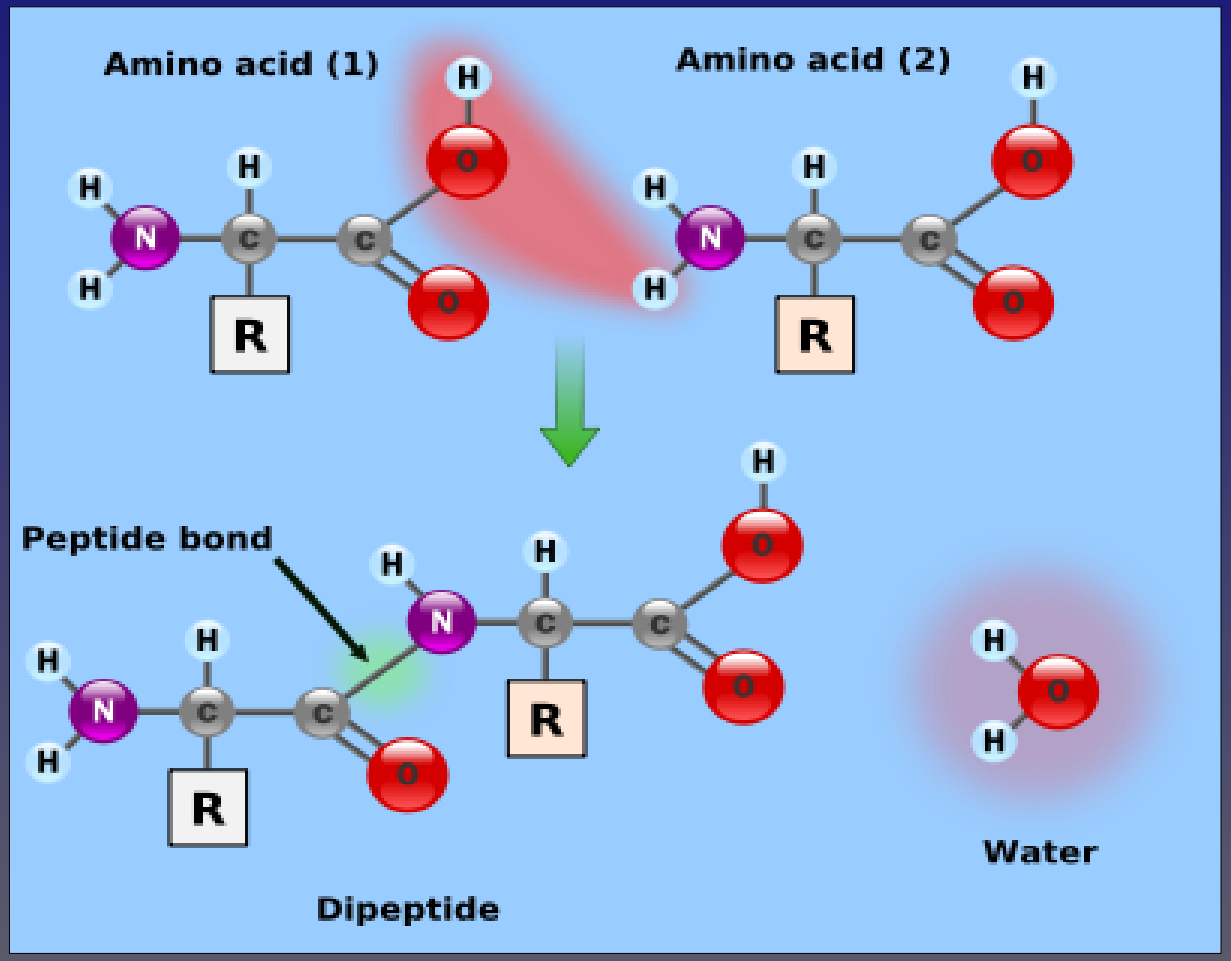
From Gray's Anatomy, 1918

Genetic Manipulation

Growth and Cell Division: Protein

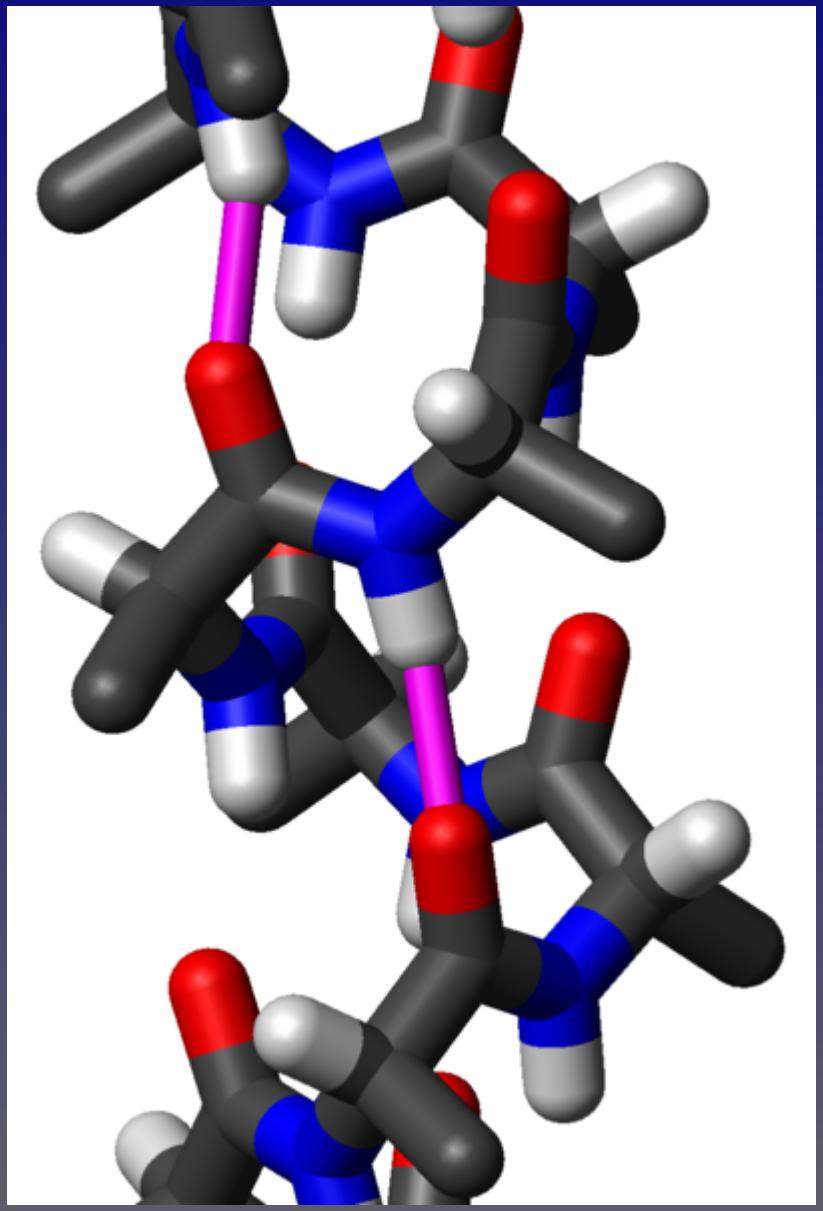
- Proteins are large organic compounds made of amino acids arranged in a linear chain.
- Twenty different kinds of standard AAs, depending on the residue

Common Amino Acids

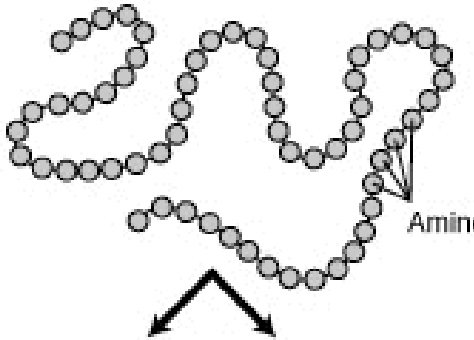


Genetic Manipulation

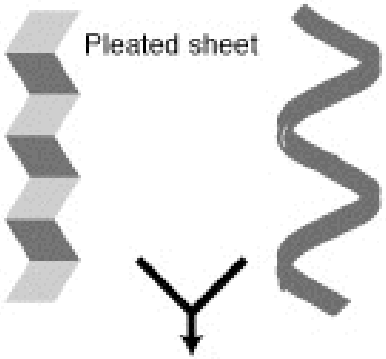
Growth and Cell Division: Protein



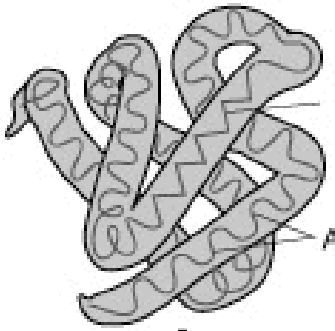
Polyalanine helix



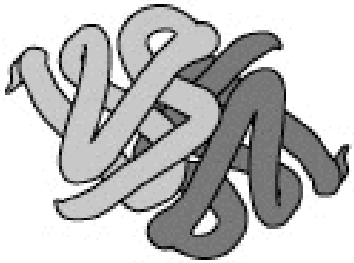
Primary protein structure
is sequence of a chain of amino acids



Secondary protein structure
occurs when the sequence of amino acids are linked by hydrogen bonds



Tertiary protein structure
occurs when certain attractions are present between alpha helices and pleated sheets.



Quaternary protein structure
is a protein consisting of more than one amino acid chain.

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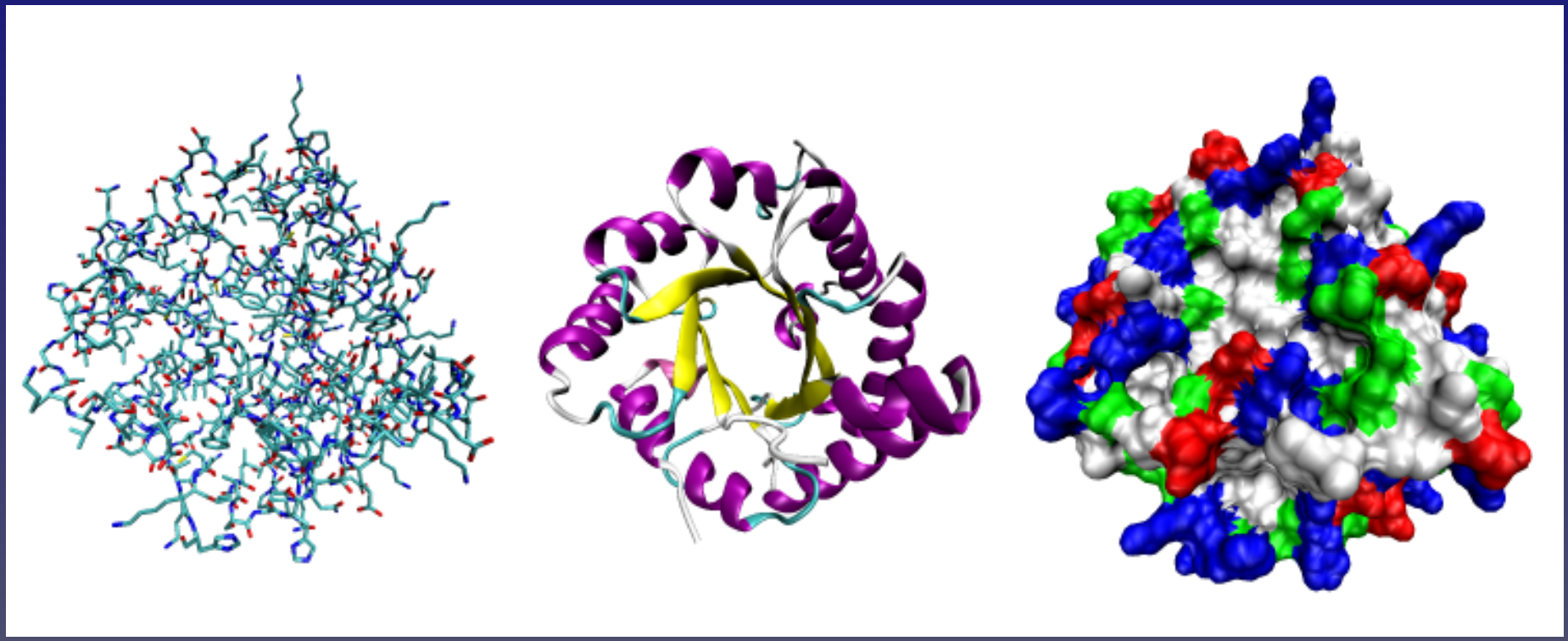
Growth and Cell Division: Protein

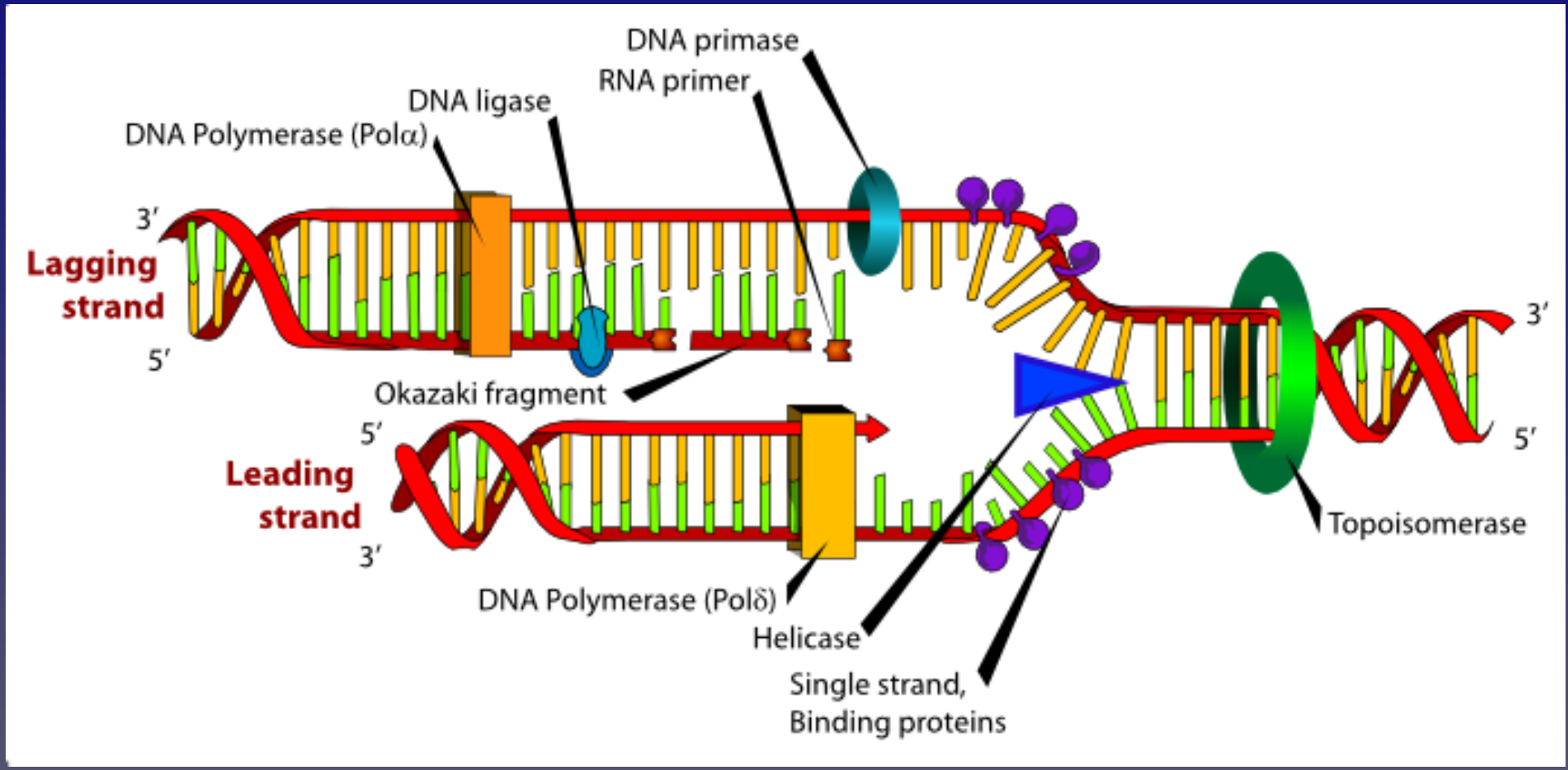


Protein Structure

Genetic Manipulation
Growth and Cell Division: Enzyme

- Enzymes: Biomolecules that catalyze (dramatically increase the rates of) specific chemical reactions.
  Billion years → Milliseconds
- Almost all enzymes are proteins
- A name ending in "ase" indicates an enzyme

Genetic Manipulation
DNA Replication



DNA Replication

Genetic Manipulation
DNA to Proteins

Gene

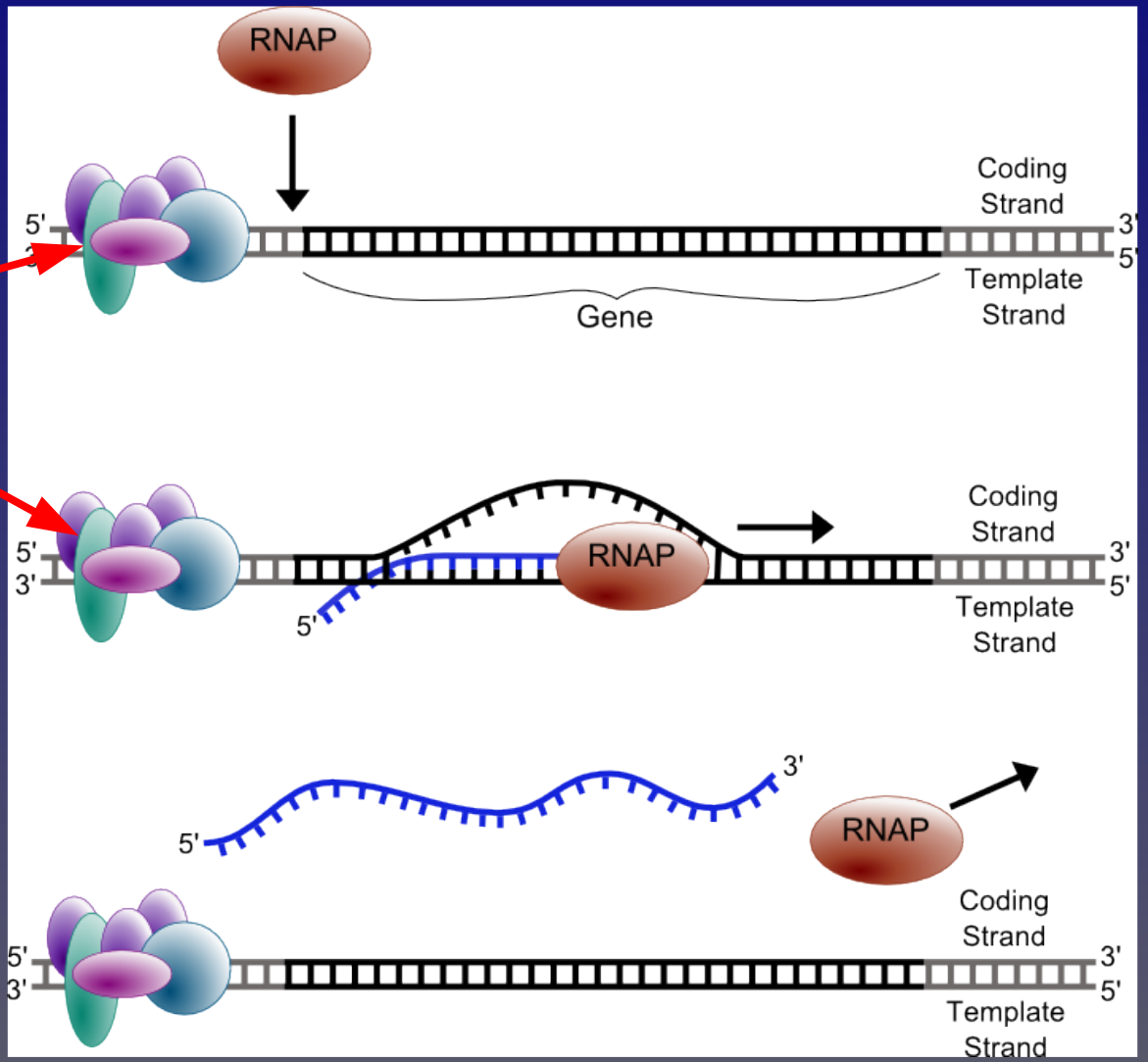
The basic physical unit of heredity; a linear sequence of nucleotides along a segment of DNA that provides the coded instructions for synthesis of RNA, which, when translated into protein, leads to the expression of hereditary character.

Genetic Manipulation

DNA → Proteins (Transcription)

Transcription Factors

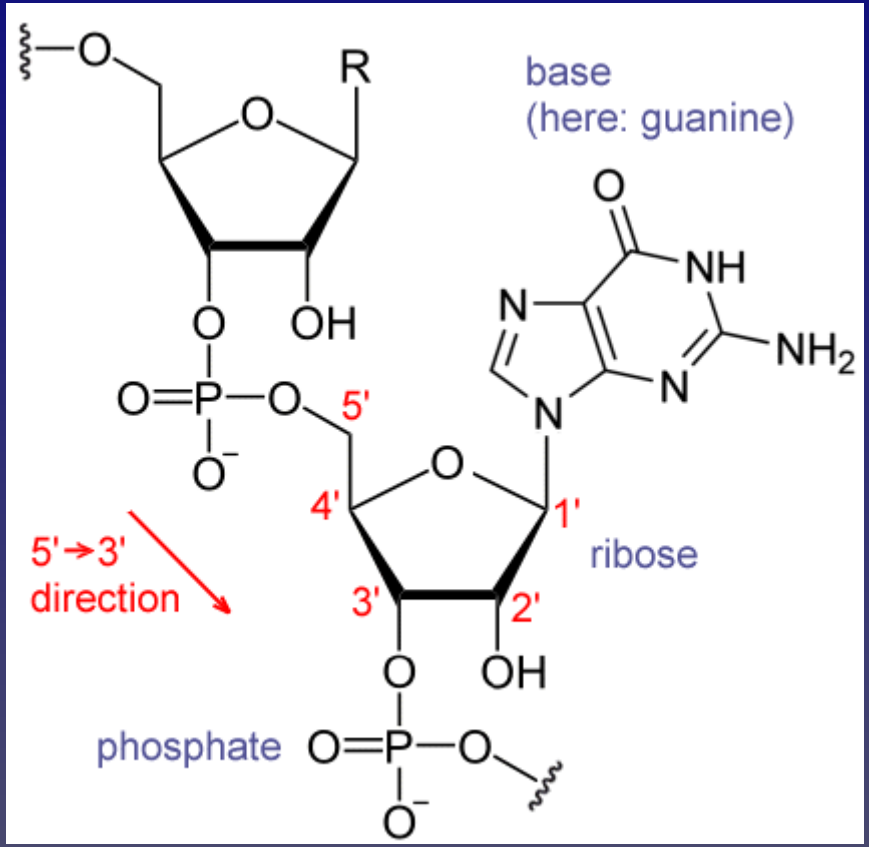
RNAP = RNA polymerase



Genetic Manipulation

DNA → Proteins (RNA)

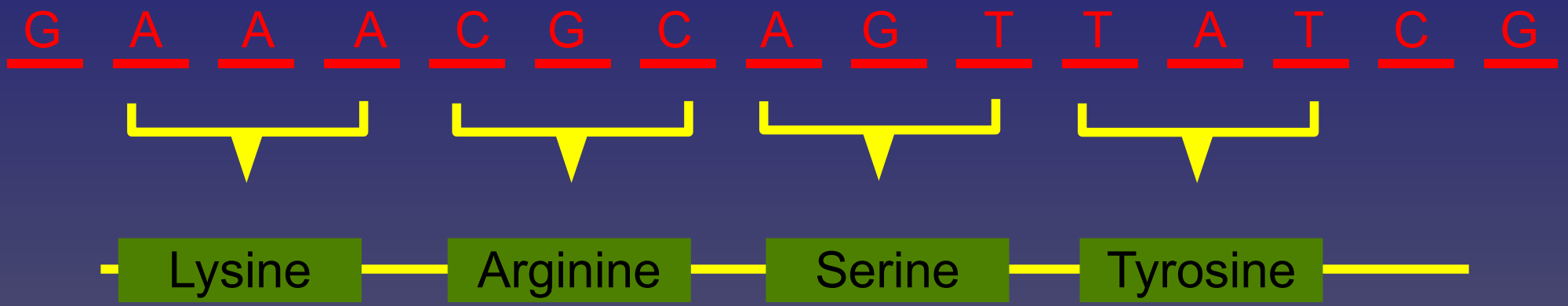
- First step: make messenger RNA (mRNA) from one strand of DNA
- RNA is just like DNA except for the sugar (added OH)
- Thymine is replaced by uracil
- Pairing occurs between GC, AU
- No simple double helix



Genetic Manipulation

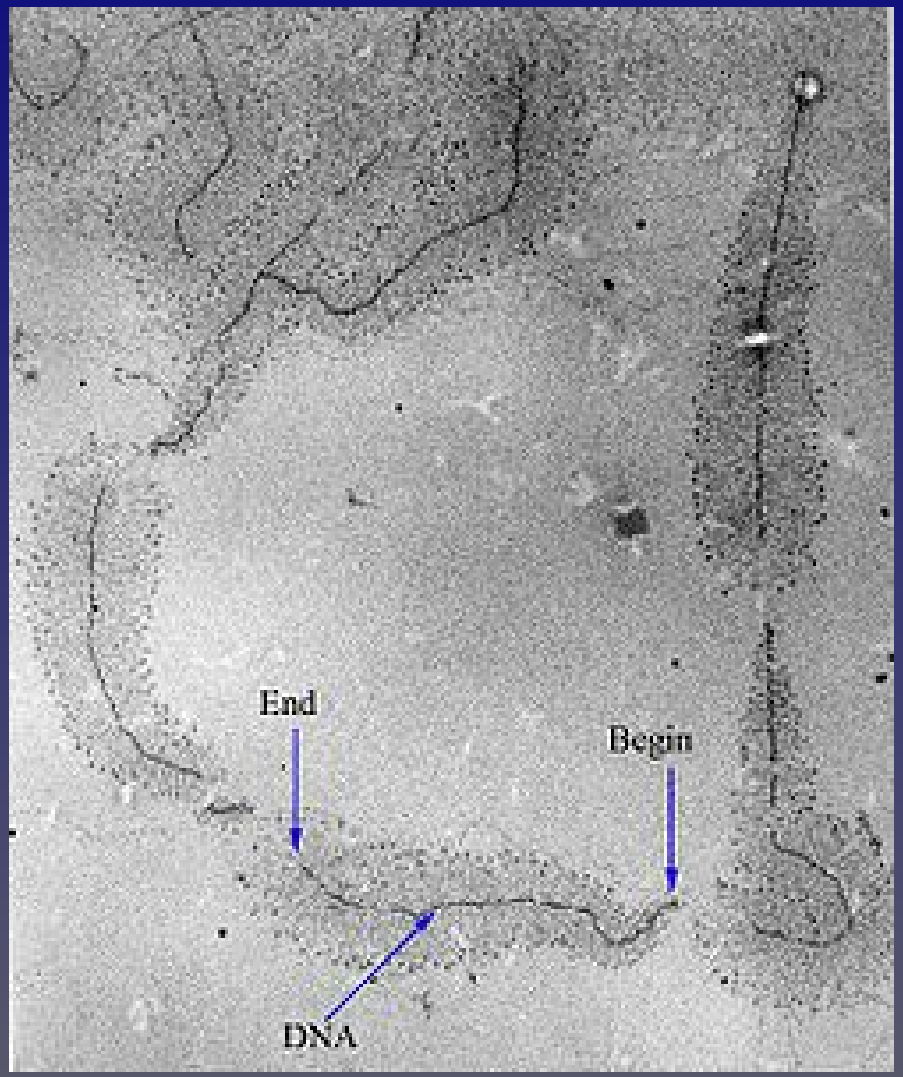
DNA → Proteins

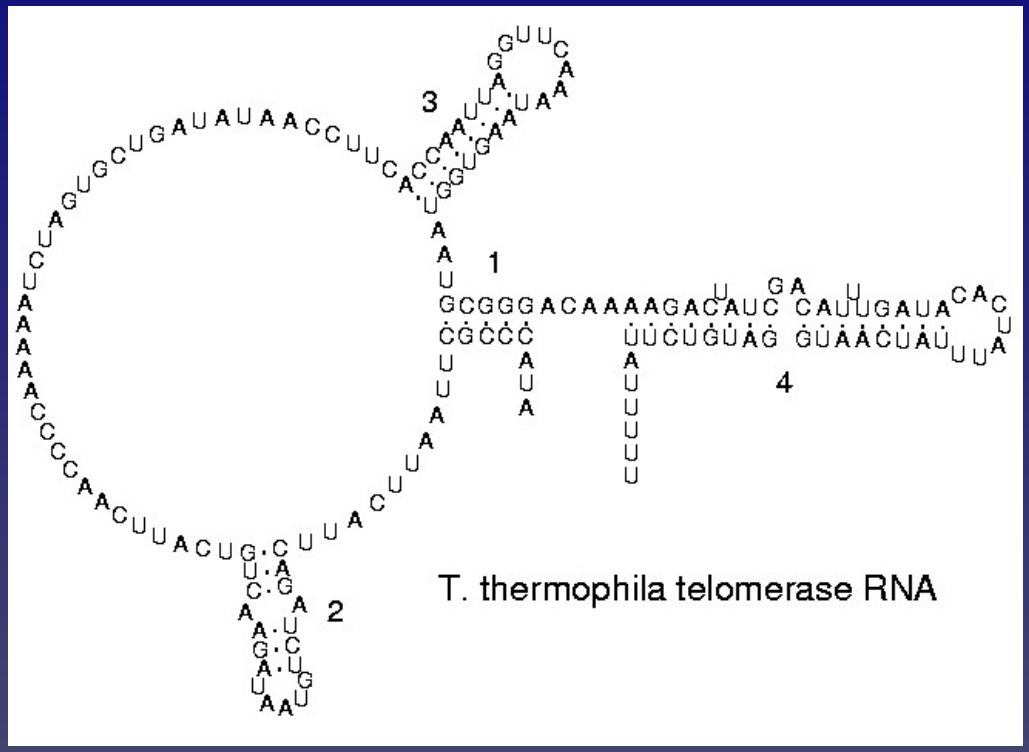
- A part of the DNA (a gene) is copied into messenger RNA
- The messenger RNA is read by a ribosome and each three nucleotides specifies an amino acid



Genetic Manipulation

DNA → Proteins (RNA)

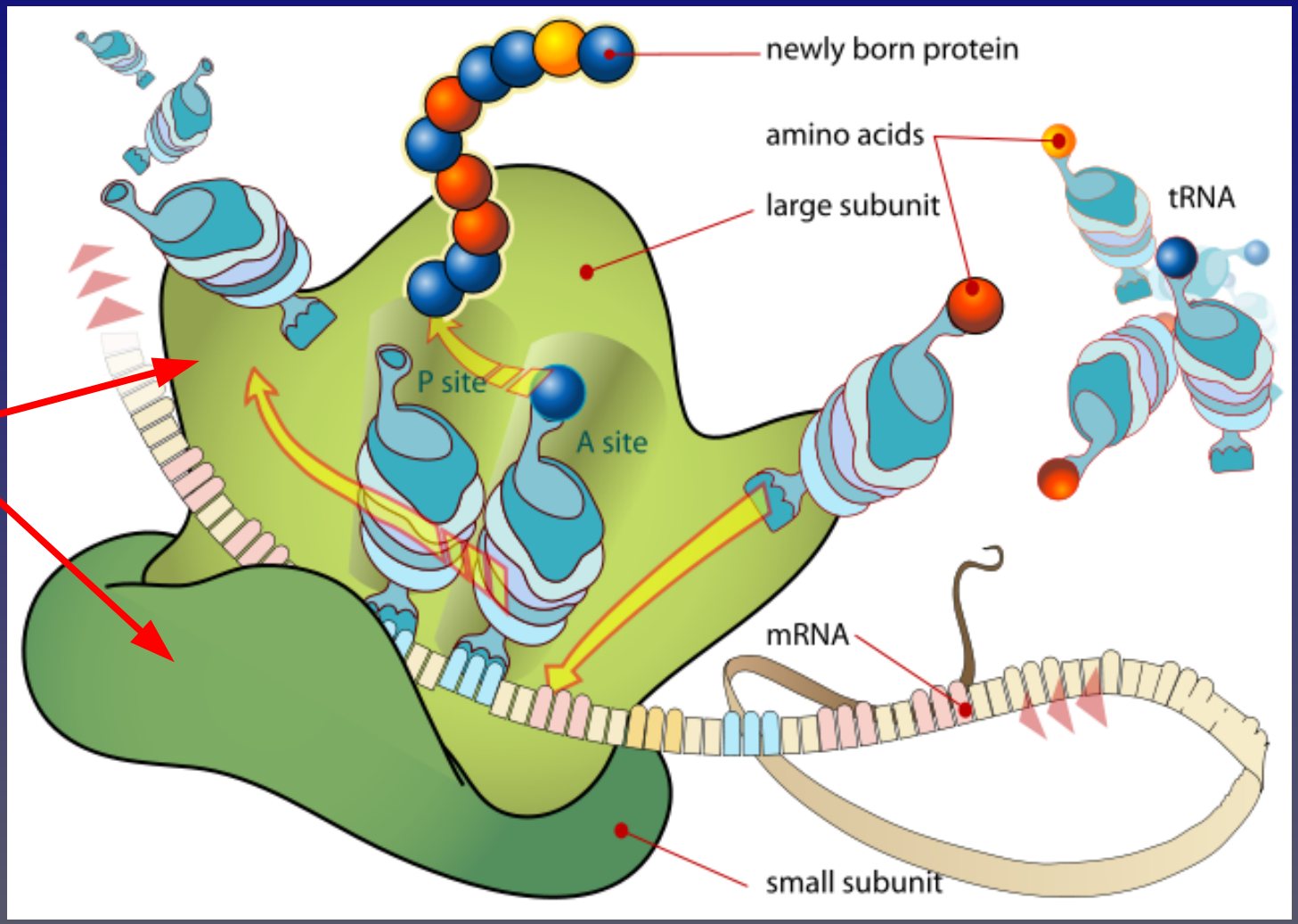




Genetic Manipulation

DNA → Proteins (Translation)

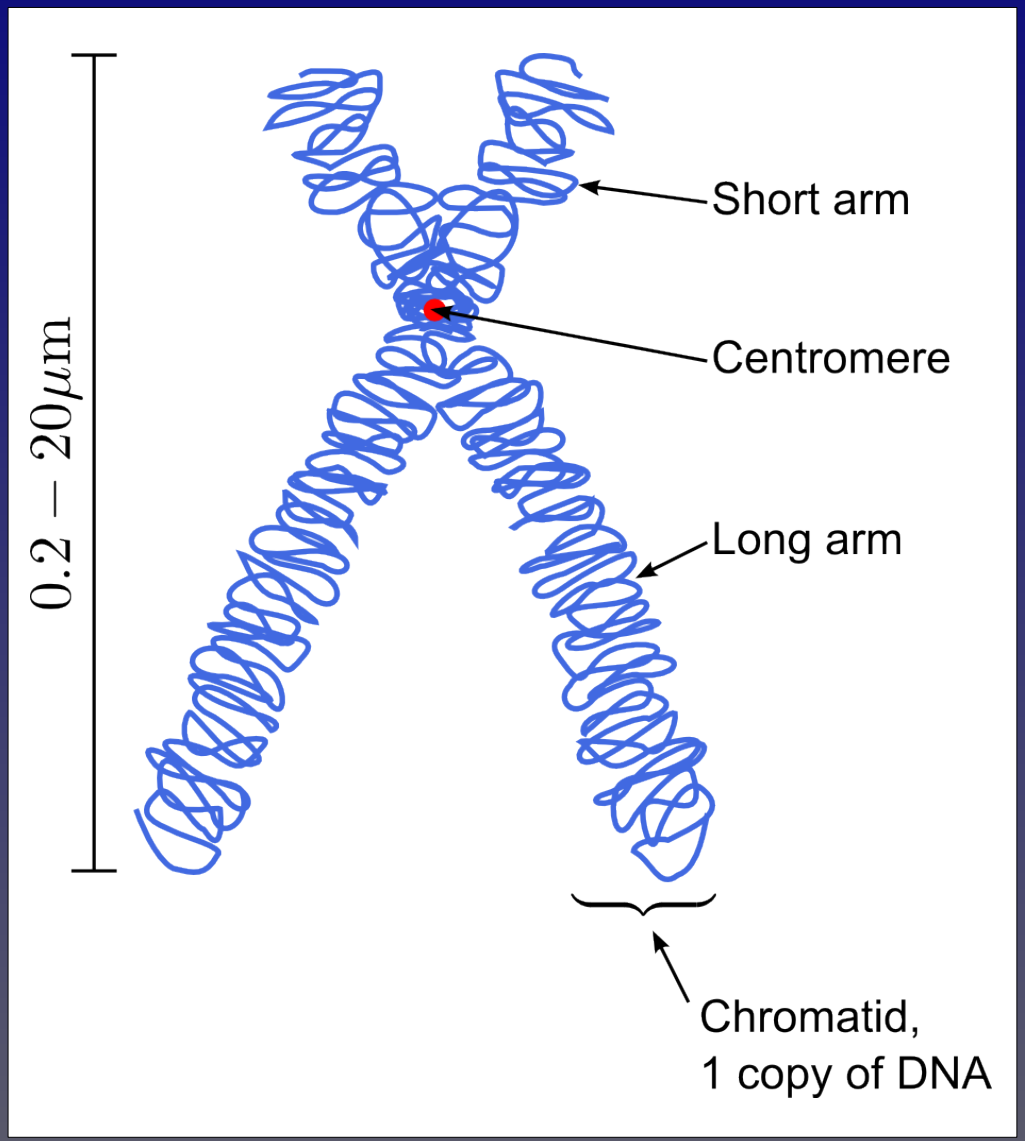
Ribosome
(two parts)



DNA to Protein

Genetic Manipulation

Chromosomes



Genetic Manipulation
DNA Replication: Cell Division

- Mitosis – Growth, creation of identical cells
- Meiosis – Creation of eggs or sperm (gametes)

Cell Division

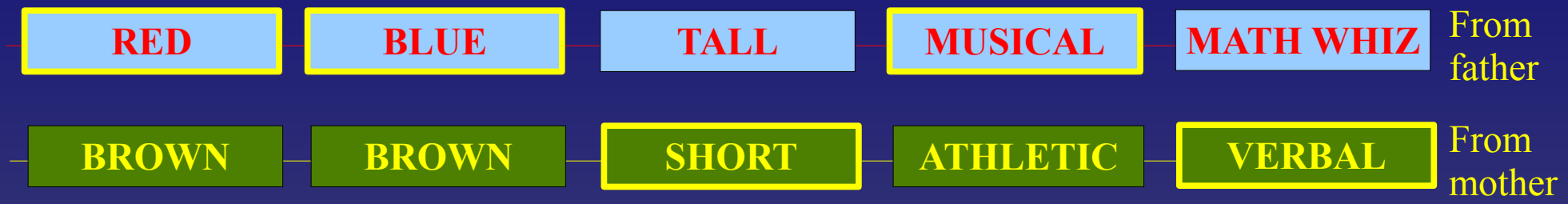
Genetic Manipulation
DNA Replication: DNA Silencing

- Each cell has two copies of each gene: one from each parent
- One of the copies may be passive while the other is active
- Both of the two copies can be active

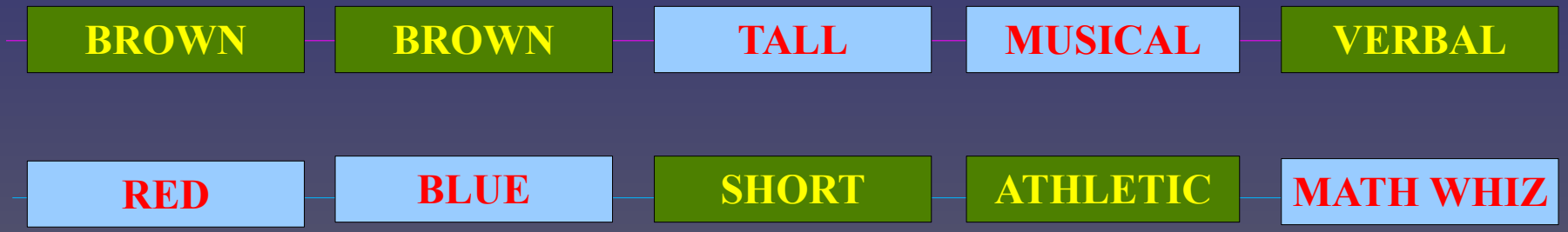
Genetic Manipulation

DNA Replication: Crossing Over

Mother's genes

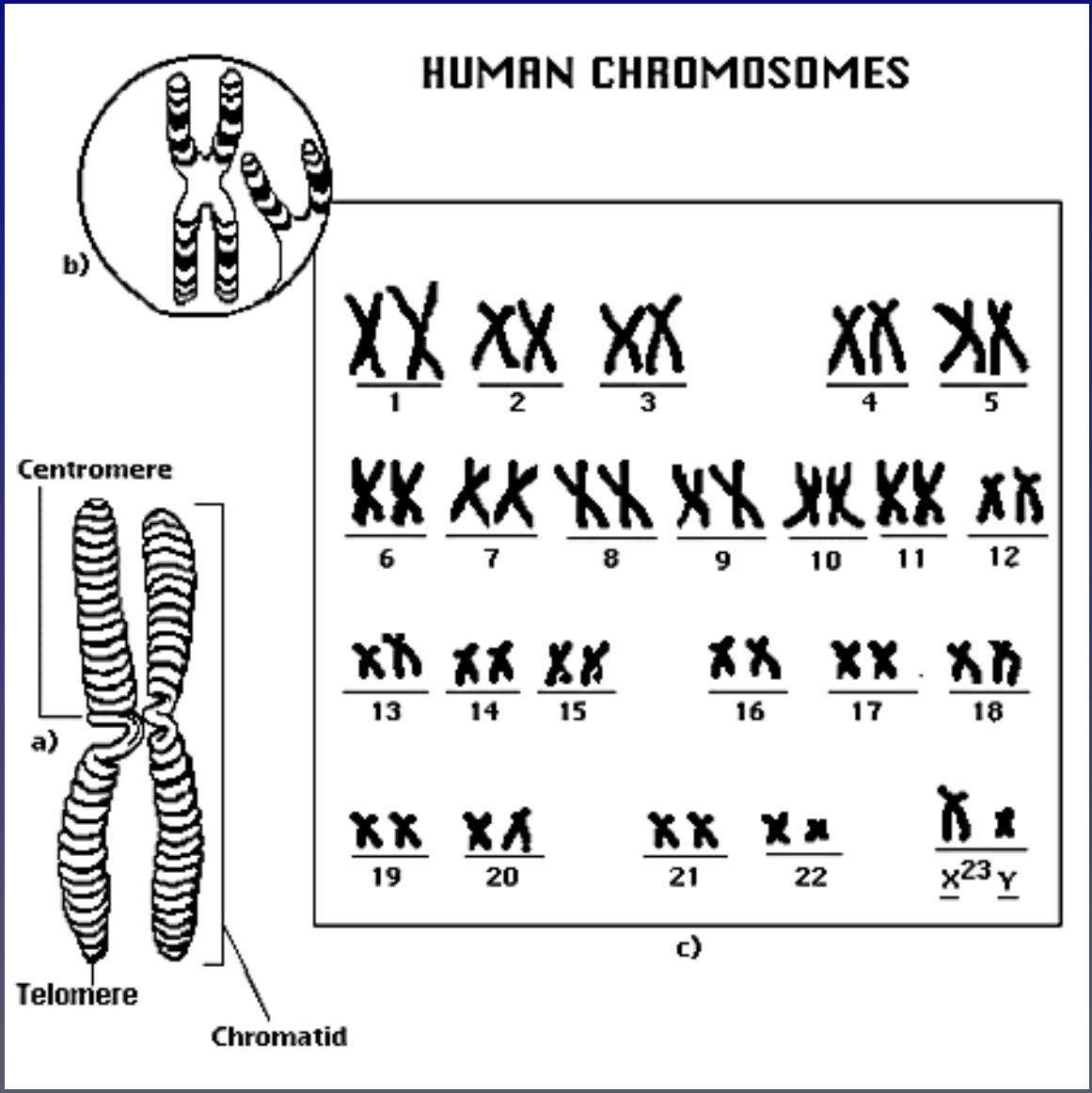


Her egg's genes

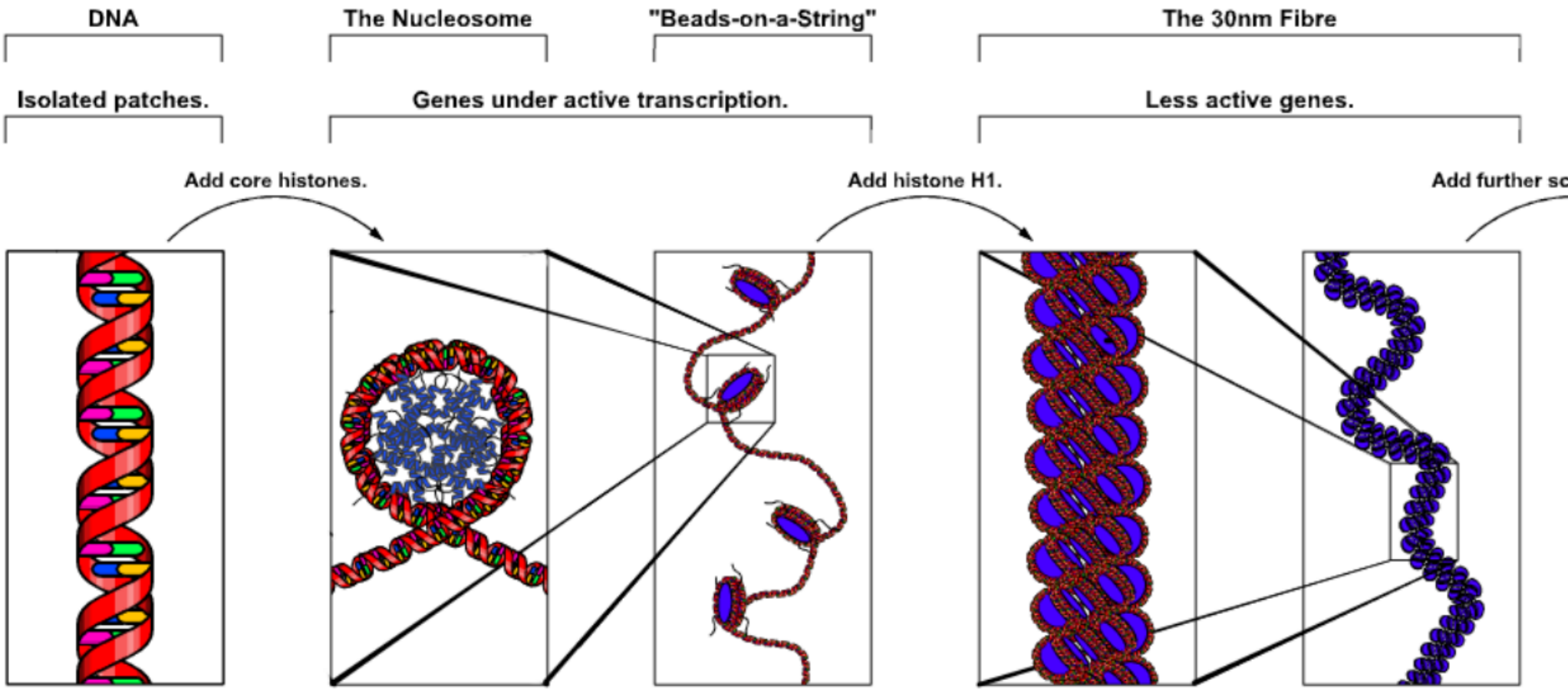


Genetic Manipulation

Chromosomes



Genetic Manipulation
Chromatin



Genetic Manipulation
Chromatin

- *Histone positions within the nucleosome using platinum labeling and the scanning transmission electron microscope*: C J Stoeckert, M Beer, J W Wiggins, J C Wierman, Journal of Molecular Biology, Aug 1984 (Vol. 177, Issue 3, Pages 483-505)

Genetic Manipulation
Mutations

- **Mutations**
  Changes to the nucleotide sequence of the genetic material of an organism

- **Point mutation**
  The replacement of a single base nucleotide with another nucleotide

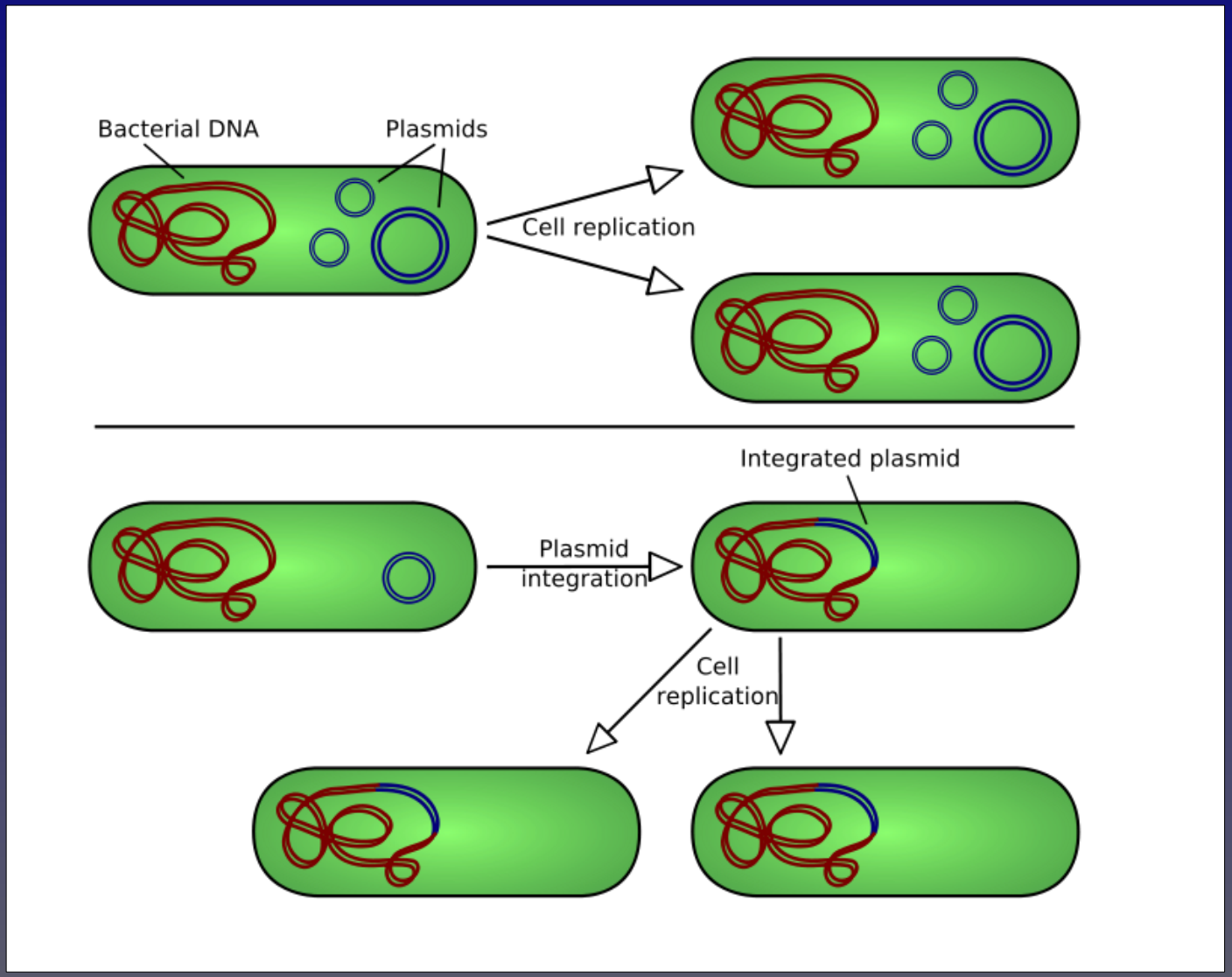
- **Nucleotide deletion**
  Deletion of a single nucleotide. Leads to a *frameshift* and incorrect translation to protein.

Genetic Manipulation
Mutations

Can we deliberately introduce mutations (changes) to an organism's DNA?

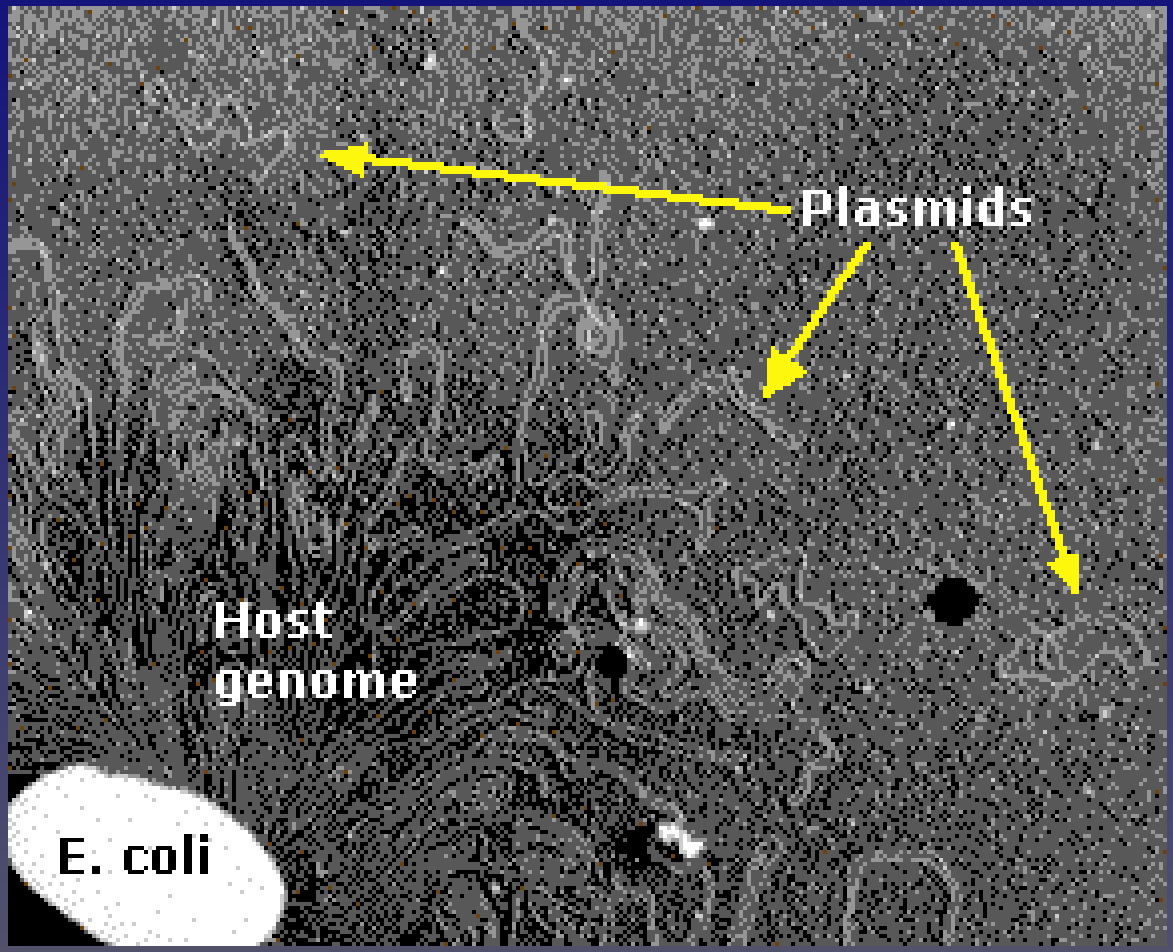
Genetic Manipulation

DNA Modification: Plasmids



Genetic Manipulation

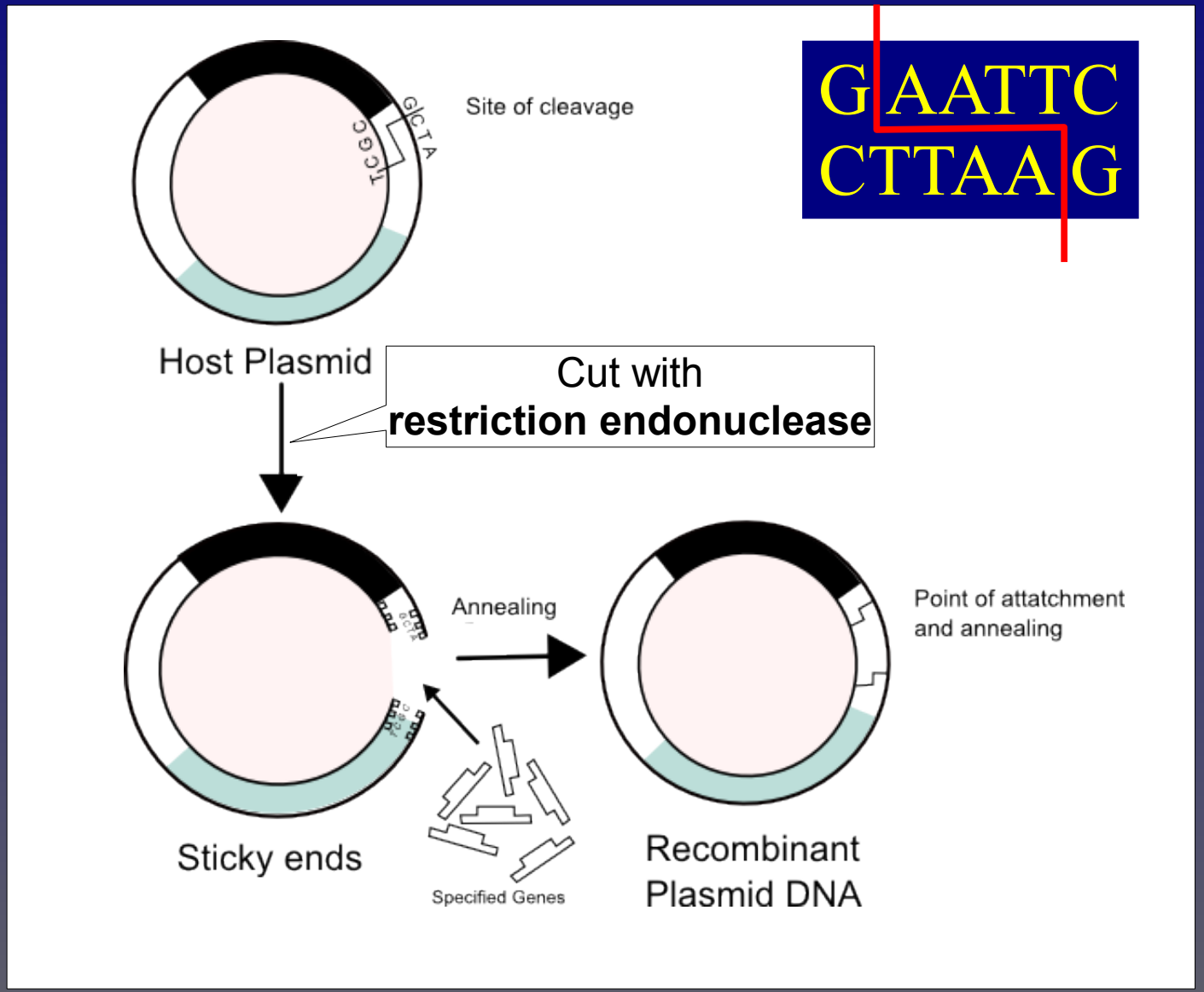
DNA Modification: Plasmids



Electron microscope image of plasmids

Genetic Manipulation

DNA Modification: Recombinant DNA



Genetic Manipulation
DNA Modification: Recombinant DNA

- Restriction endonucleases discovered by Werner Arber, Dan Nathans and Hamilton Smith, for which they received the Nobel prize in 1978
- Recombinant DNA technique in plasmids was engineered by Stanley Norman Cohen and Herbert Boyer in 1973

Genetic Manipulation
DNA Modification: Gene Insertion

- The gene for human insulin was inserted into E. Coli to produce insulin for diabetics. Also human growth hormone and oxytocin
- Over 3,000 restriction endonucleases are known