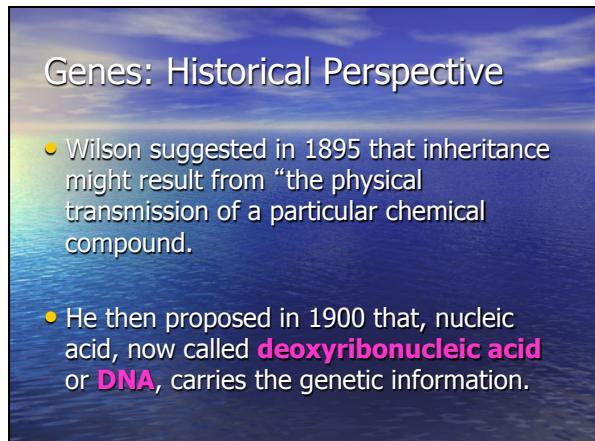


## Genes: Historical Perspective

- A Danish geneticist, Wilhelm Johannson in 1911, first used the word “gene” as a convenient term for the elementary unit of Mendelian genetics.
- An American cell biologist, Edmund B. Wilson, was the first to note that chromosomes are composed of **nuclein** (i.e. nucleic acid and protein).



## Genes: Historical Perspective

- It must be noted that, until the 1950's, virtually all geneticists believed that genes were constructed or made up of proteins.
- This was because the development of nucleic acid chemistry lagged behind that of protein chemistry.

## Genes: Historical Perspective

- Two types of nucleic acid had been identified.
  - (i) **deoxyribonucleic acid (DNA)**
  - (ii) **ribonucleic acid (RNA)**

## Empirical Tests of the Chemical Nature of Genes

- From the late 1930's through the 1950's, experiment provided direct insight into the chemical nature of genes.
- These experiments correlated known properties of genes with physical or chemical properties of nucleic acid and proteins.

## Chemical Nature of Genes

- One of the earliest involved the induction of mutations.
- Using high energy radiation such as X' ray or gamma rays and by ultra-violet (UV) light, Stadier postulated that for UV light to act as a mutagen (*i.e. all physical and chemical agents that increase the rate of mutation significantly above normal*), the energy of the light might be absorbed by genetic molecules and produce some change in the structure of the molecules.

## Chemical Nature of Genes

- Both nucleic acids and proteins absorb UV light.
- The maximum absorption of UV by proteins is at a wavelength of 280nm while that of nucleic acid is at 260nm

## Chemical Nature of Genes

- If UV light at 260nm induced mutations more efficiently than UV light at 280nm, then, it might suggest that genes are made up of nucleic acid and NOT protein.

## Griffith's Transformation Experiment

- Frederick Griffith, a British bacteriologist, in 1928 described a phenomenon known as **transformation** when he was investigating the reasons for the presence of different strains of *Streptococcus pneumoniae* (Pneumococcus) infected mice.

## Transformation of Bacteria

- Commonly practiced technique today
- Bacteria incorporate foreign DNA
- Developing vaccine for *Streptococcus pneumoniae* (pneumonia)
- Two bacterial strains
  - R strain – appeared rough
  - S strain – appeared smooth (had capsule)

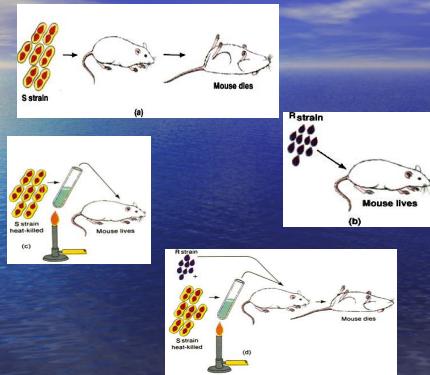
## Griffith's Transformation Experiment

- When he injected live virulent type **III S** pneumococci, many mice died.
- If he first killed the virulent bacteria by heat, the mice lived.
- When live, non-virulent type **IIR** bacteria was injected, the mice survived.

### Griffith's Transformation Experiment

- But when live non-virulent type **II R** bacteria was injected simultaneously with a large quantity of heat-killed, virulent type **II IS** bacteria, many mice died.
- Bacteria recovered from the dead mice proved, when grown on nutrient agar plates to be virulent type **II IS** pneumococci.

### Griffith's Experiment



### Griffith's Transformation Experiment

His interpretation to this, was that, the live, non-virulent bacteria cells had been genetically transformed into virulent ones by something from the dead cells. This "something" was called the Transforming Factor.

### Griffith's Transformation Experiment

In retrospect, the simplest interpretation of his result is that **molecules carrying hereditary information (i.e. genes)** had been transformed from the dead virulent cells into the chromosomes of the live, non-virulent cells and had made the live cells virulent.

## Avery, Macleod and McCarty

- Sixteen years after Griffith's publication (1944), these scientists proposed that the transforming factor is DNA.
- Their conclusion was based on following experiments.
  - (i) they demonstrated that the chemical composition and absorption spectrum of the purified transforming factor was not different from those of DNA (was 99.98% pure of DNA).

## Avery, Macleod and McCarty

(ii) they studied the effects of different degradative enzymes especially, **trypsin** and **chymotrypsin**, which are proteolytic enzymes that break proteins down into smaller peptides. They found that these enzymes had no effect on the transforming activity of the purified material.

## Avery, Macleod and McCarty

Because the molecules that cause transformation are not sensitive to these enzymes, they presumably are not proteins.

## Avery, Macleod and McCarty

(iii) only DNA from type **IIIS** bacteria transforms **IIR** bacteria into type **IIIS** (not vice versa).  
Thus, transformation of **IIR** cells into **IIIS** requires an attribute that is only found in the DNA of **IIIS** cells.

## Avery, Macleod and McCarty

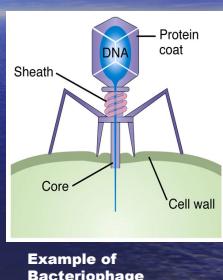
(iv) they determined the molecular weight of the purified DNA and found it to be about 500,000 D (Dalton).

## Alfred D. Hershey and Martha Chase

- They conducted a series of experiments in which replication of genes was used as an assay (*analysis to determine the presence or absence of one or more components in a compound, solution or a mixture*) for determining the nature of genetic molecules.

## Their Experiment

- They prepared phages (*i.e. viruses that attack bacteria*) that had either their DNA or protein labeled with a radioactive compound.
- The DNA was labeled with  $^{32}\text{P}$  and the protein was labeled with  $^{35}\text{S}$ .
- Proteins generally have no phosphorus and nucleic acid have no sulphur.



## Hershey and Chase Experiment

- The radioactive phages were allowed to infect or attack *E.coli* cells, and the phage heads, empty of their chromosomes were separated from the bacteria by first placing the phage-bacterial mixture in a blender to dislodge the phage ghost from the surface of the bacteria.
- The mixture was then gently centrifuged, forcing large bacterial cells to the bottom of a centrifuge tube while the small phage ghosts remained in suspension.

### Hershey and Chase Results

- After separation, the phage ghosts were seen to contain about 85% of the original protein of the parent phages with only 15% of the original nucleic acid as revealed by the amount of  $^{35}\text{S}$  and  $^{32}\text{P}$ .
- Thus, most of the protein never entered the bacterial cells.

### Hershey and Chase Experiment

- Later, following lysis (break up) of the bacterial cells, they recovered the next generation of newly produced phages and determined the amount of radioactive parental DNA and protein present.
- They found that, less than 1% of the original protein was present in the progeny phages, but about 30% of the parental DNA was present.

### Hershey and Chase Experiment

- There was substantial conservation of DNA from generation to generation, but almost no conservation of protein.**
- Hence, it appeared that the genetic information was embodied in the DNA and not in the protein.**

### The Structure of DNA

- All the experiments described so far point to nucleic acid, DNA as the carrier of genetic information.
- The determination of the structure of DNA then became a matter of great importance.

## The Structure of DNA

- In the early 1950's, through effective investigations, scientists like Rosalind Franklin and Maurice Wilkins (X-ray crystallographers), Francis Crick, Watson, Chargaff and others were able to elucidate the structure of DNA.

## DNA is a Double Helix

- Levine provided an important information about the chemical composition of DNA and concluded that it is a polymer of **purine** and **pyrimidine** nucleotides.

## The Structure of DNA

- Erwin Chargaff examined base content of DNA – (1940s)
- Four (4) different bases
- Two (2) **purine** (A) and (G) bases
  - Have double ring
- Two (2) **pyrimidine** (T) and (C) bases
  - Have single ring

(a) Key features of DNA structure: A 3D model of a DNA double helix with dimensions 1 nm width, 3.4 nm pitch, and 0.34 nm rise per base pair. Labels include G-C, C-G, A-T, T-A base pairs, and hydrogen bonds between them.

(b) Partial chemical structure: A detailed chemical diagram of the four DNA nucleotides: Adenine (A), Thymine (T), Guanine (G), and Cytosine (C). Each shows the purine or pyrimidine ring system with attached nitrogenous bases and deoxyribose sugar rings.

## The Structure of DNA

Pyrimidines	
	Cytosine C
	Thymine T

Purines	
	Guanine G
	Adenine A

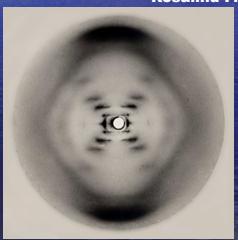
## Chargaff's rules

- 1. Amount of A, T, G, and C in DNA varies from species to species
- 2. In each species, the amount of A = T and the amount of G = C
- And each species has unique nucleotide content of DNA
  - Variability
  - Constancy
- Now known average chromosome contains 140 million base pairs

Species	A	T	G	C
<i>Homo sapiens</i> (human)	31.0	31.5	19.1	18.4
<i>Drosophila melanogaster</i> (fruit fly)	27.3	27.6	22.5	22.5
<i>Zea mays</i> (corn)	25.6	25.3	24.5	24.6
<i>Neurospora crassa</i> (fungus)	23.0	23.3	27.1	26.6
<i>Escherichia coli</i> (bacterium)	24.6	24.3	25.5	25.6
<i>Bacillus subtilis</i> (bacterium)	28.4	29.0	21.0	21.6

## X-Ray Diffraction Data

- Rosalind Franklin studied structure of DNA using X-rays.
- Concentrated solution can be separated into fibers and form a crystal arrangement
- X-ray diffraction clearly showed DNA in double helix arrangement

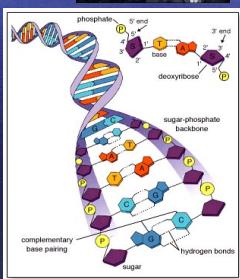



Rosalind Franklin

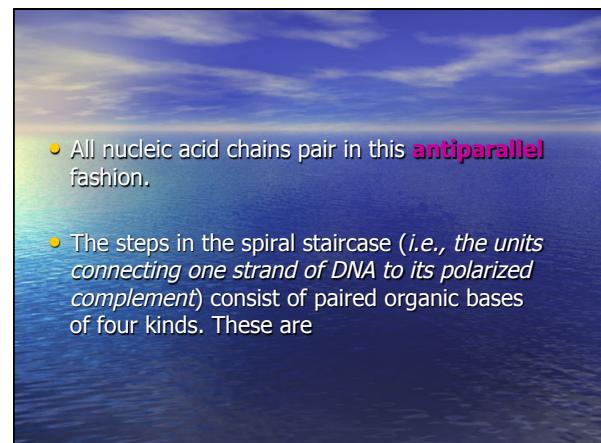
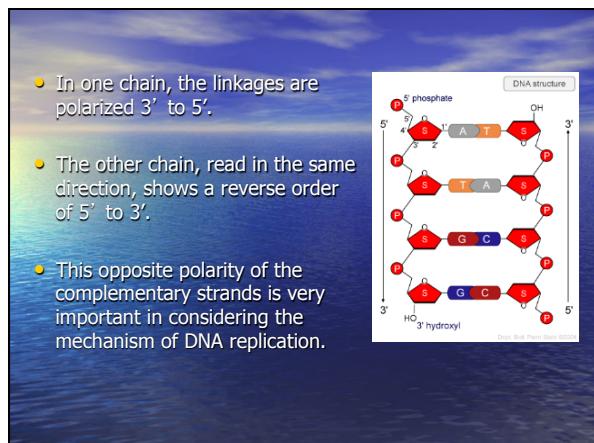
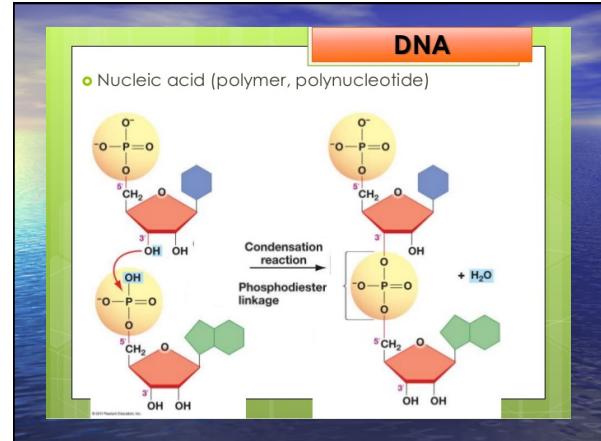
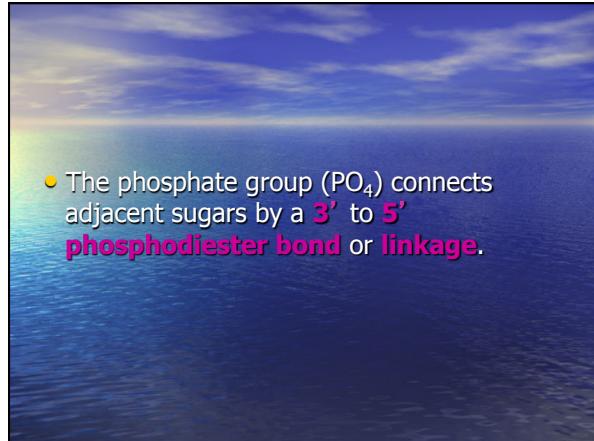
X - ray diffraction results

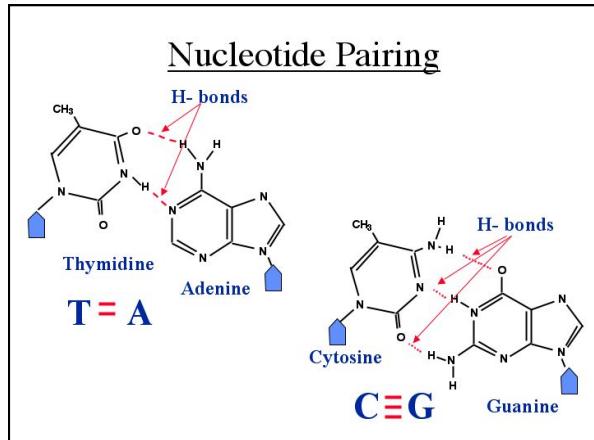
## Watson and Crick Model

- James Watson and Francis H. C. Crick
- Used data provided from X-ray diffraction and other sources
- Deduced that DNA is a **double helix**
  - Model agreed with Chargaff's rules
  - Purine always bonded to a pyrimidine
    - Right size
    - Purine too wide
    - Pyrimidine too narrow

- The backbone of the helix is composed of two chains with alternating sugar (S)-phosphate (P) units.
- The sugar is a pentose (five-carbon molecule) called **deoxyribose**.





- Adenine pairs with thymine by **two hydrogen** bonds; guanine and cytosine pair by **three hydrogen** bond.
- Hydrogen bonds between the base-pairs accounts in part, for the high degree of stability of DNA double helices.
- Hydrophobic bonding** between the stacked base pairs also account in part for the high degree of stability.

- A base-sugar complex is called a **nucleoside**;
- A nucleoside plus a phosphate is called a **nucleotide**.

- ### Complementarity
- Once the sequence of bases in one strand of a DNA double helix is known, the sequence of bases in the other strand is also known because of the specific base-pairing.
  - Thus, the two strands of DNA double helix is said to be **complementary (not identical)**.

- X-ray diffraction data have shown that DNA has the form of a regular helix, with a distance of  $34\text{\AA}$  (3.4nm) at every complete turn.
- It has a diameter of about  $20\text{\AA}$  (20 Angstroms or 2nm).
- Since the distance between adjacent nucleotides (or stacked nucleotides) is  $3.4\text{\AA}$  (0.34nm), then, there are 10 nucleotides per turn.

## RIBONUCLEIC ACID (RNA)

- Another class of nucleic acids is ribonucleic acid.
- RNA is slightly different from DNA in the following respects:
  - Cellular RNA is **single-stranded** while DNA is **double-stranded**.
  - RNA contains **ribose sugars** instead of the **deoxyribose** sugars in DNA.
  - RNA contains the pyrimidine **uracil** (U) instead of **thymine** (T), and **U** pairs with **A**.
  - RNA molecules are much **shorter** than DNA molecules.

## Functions of RNA

- RNA functions primarily in protein synthesis; acting in one capacity as a messenger carrying information from the instructions coded into the DNA to the ribosomal sites of protein synthesis in the cell. This form of RNA is called **messenger RNA (mRNA)**.
- Ribosomes contain a special class of RNA called **ribosomal RNA (rRNA)**. During polypeptide or protein synthesis, rRNA molecules provide the site on the ribosome where the polypeptide is assembled.

## Functions of RNA

- A third kind of RNA, called **transfer RNA (tRNA)**, attaches to amino acids and during protein synthesis brings them into proper positioning with other amino acids using the mRNA-ribosome complex as a template.

## Functions of RNA

- Some RNA molecules, called **ribozymes**, have enzymatic capabilities.
- It must be noted that all cellular RNA molecules are made from a DNA template.
- A single-stranded RNA may fold back upon itself and form localized “double-stranded” sections by complementary base pairing.

## Genetic Information Flow

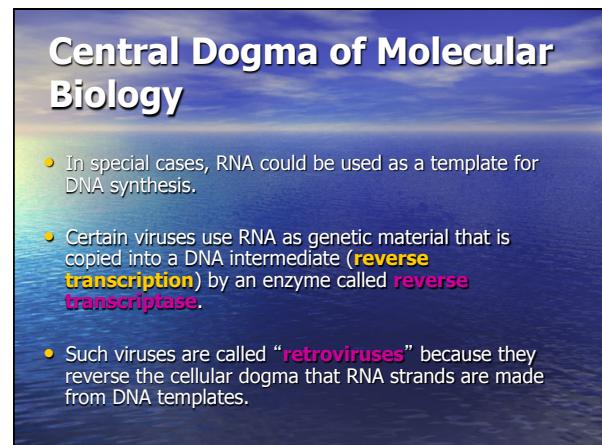
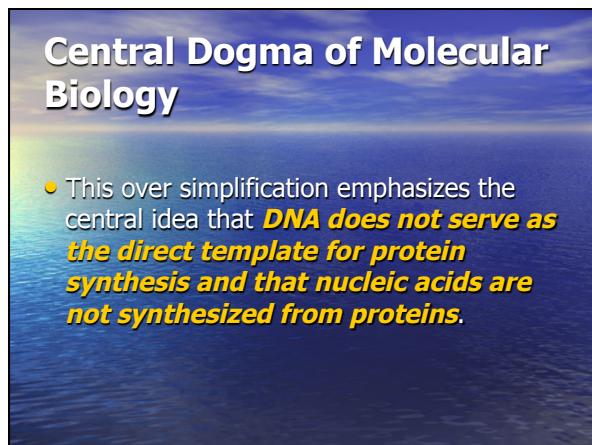
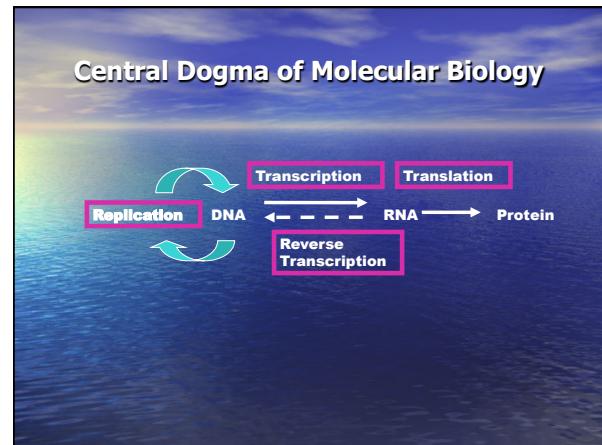
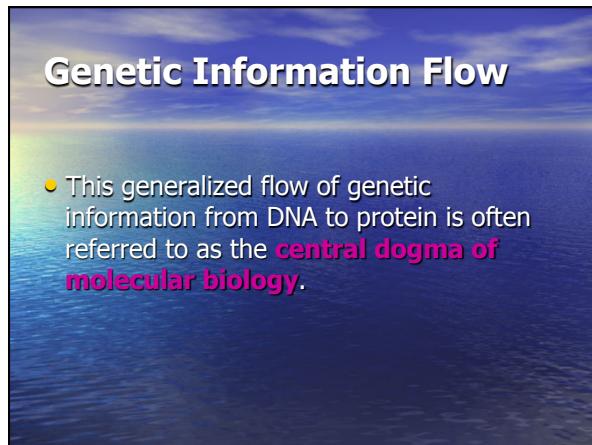
- DNA serves as the main repository of genetic information within a cell.
- Each strand of the DNA double helix serves as a **template** for its own **replication**.

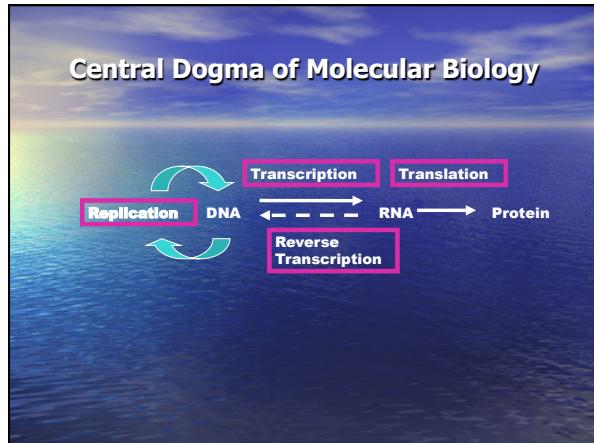
## Genetic Information Flow

- This activity precedes all cell division and this is how genetic information is transmitted, or “handed down” to new generations of cells.
- All cellular RNA molecules are synthesized from DNA templates in a process called **transcription**.

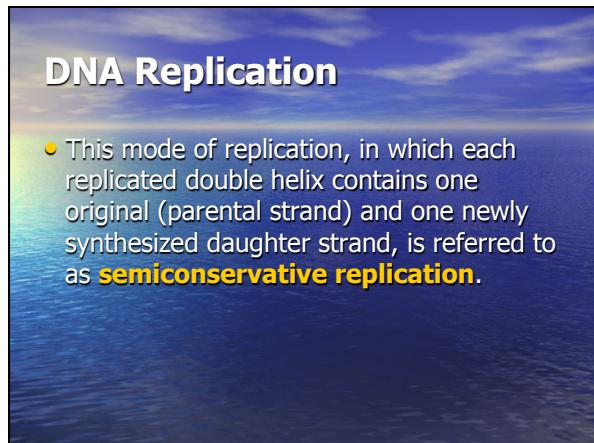
## Genetic Information Flow

- Genes are said to be active, or expressed, when they are being transcribed into RNA.
- Proteins are synthesized from mRNA templates by a process called **translation**.





- ### DNA Replication
- The hydrogen bonds linking bps together are relatively weak bonds.
  - During DNA replication, the two strands separate along this line of weakness in a zipper-like fashion.
  - Each strand of the DNA molecule can serve as a template against which a complementary strand is formed by the catalytic activity of an enzyme called **DNA polymerases**.



- ### DNA Replication
- All DNA pol enzymes add free nucleotides only to the **3' ends of existing chains so that the chains will grow from their 5' ends toward their 3' ends**.
  - All DNA pols can also degrade DNA in the **3' to the 5' direction**.

## DNA Replication

- Enzymes that degrade nucleic acids are called **nucleases**.
- If the enzyme cleaves nucleotides from the end of the chain, it is called an **exonuclease**; if it makes cuts in the interior of the molecule, it is termed an **endonuclease**.

## DNA Replication

- As long as deoxyribonucleotide precursors are present in even moderate amounts, the synthetic activity of a DNA polymerase is greatly favored over its degradation activity.
- During replication, incorrectly paired bases are removed by the exonuclease activity of the DNA pols before the next nucleotide is added. This "proofreading system" protects the DNA from errors (or mutations)*

## Sample Question

The percentage of nucleotide A in DNA isolated from human liver is observed to be 30.7%. What is the expected percentage of

- (a) T    (b) G    (c) C

## DNA REPLICATION

SYNTHESIZING IDENTICAL GENETIC MATERIAL



Cells, like these prokaryotic *E. coli* cells, replicate themselves quickly and efficiently. Part of the process of asexual