

MOLECULAR BASIS OF INHERITANCE

NOTE to STUDENTS and FACILITATORS: One of the main purposes of the Workshops is to allow free exchange of information by **having each member of a Learning Community in turn answer one part** of a discussion question. As each student explains a term or gives a definition in their own words, it should allow for free verbal EXCHANGE and promote learning by interaction. **Try to insure that everyone in your Learning Community does a question or two and the purpose of the exercise is that they must EXPLAIN THEIR ANSWERS to the rest of the community.**

Framework:

The goal of today's exercise is for you to look at DNA, its structure, and its replication. Deoxyribose-nucleic acid, DNA, is the genetic material, the substance of genes, and the basis of heredity. Nucleic acids unique ability to direct their own replication allows for precise copying and transmission of DNA into all the cells of an organism from one generation to the next. Have one member, in turn, of your Workshop answer one part of each of the questions or problems, then let the next member go on to the next part...

Part 1. Proof of DNA as the Genetic Material

1. Hershey and Chase (1952) devised an experiment using radioactive isotopes to determine whether a bacteriophage's DNA or its proteins were transferred during viral replication.

- What and/or how did that label the phage protein?
- How did they label the phage DNA?

Separate experimental samples of E. coli were infected with the differently (protein & DNA) labeled T4 virus particles, then agitated in a blender to stop infection and centrifuged to isolate the bacterial cells separate from the virus particles.

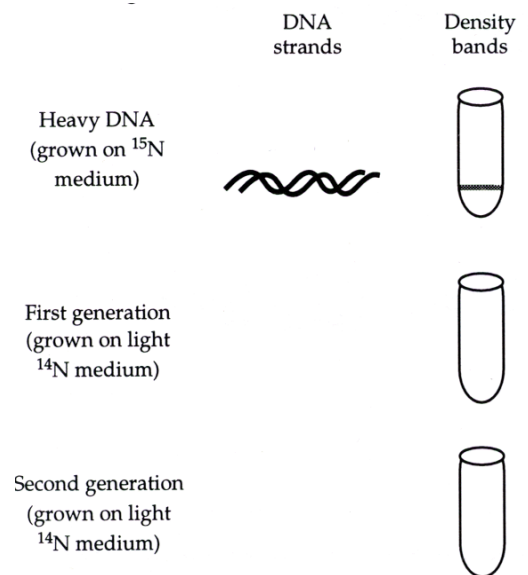
- Where was the radioactivity found in the samples with labeled phage protein and why?
- Where was the radioactivity found in the samples with labeled phage DNA and why?
- What were Hershey & Chase's conclusions from their experiment?

Part 2. Patterns of DNA Replication

A. Have a member of your group use a large piece of paper or go to a backboard and using different colors or different patterns for the **light** and **heavy** strands of DNA in a Mesleson /Stahl-like experiment, sketch the results of the semi-conservative replication cycles of heavy DNA when the E. coli cells are moved to an ^{14}N medium for two successive generations. Show the resulting light, hybrid, heavy density bands in the centrifuge tubes to the right.

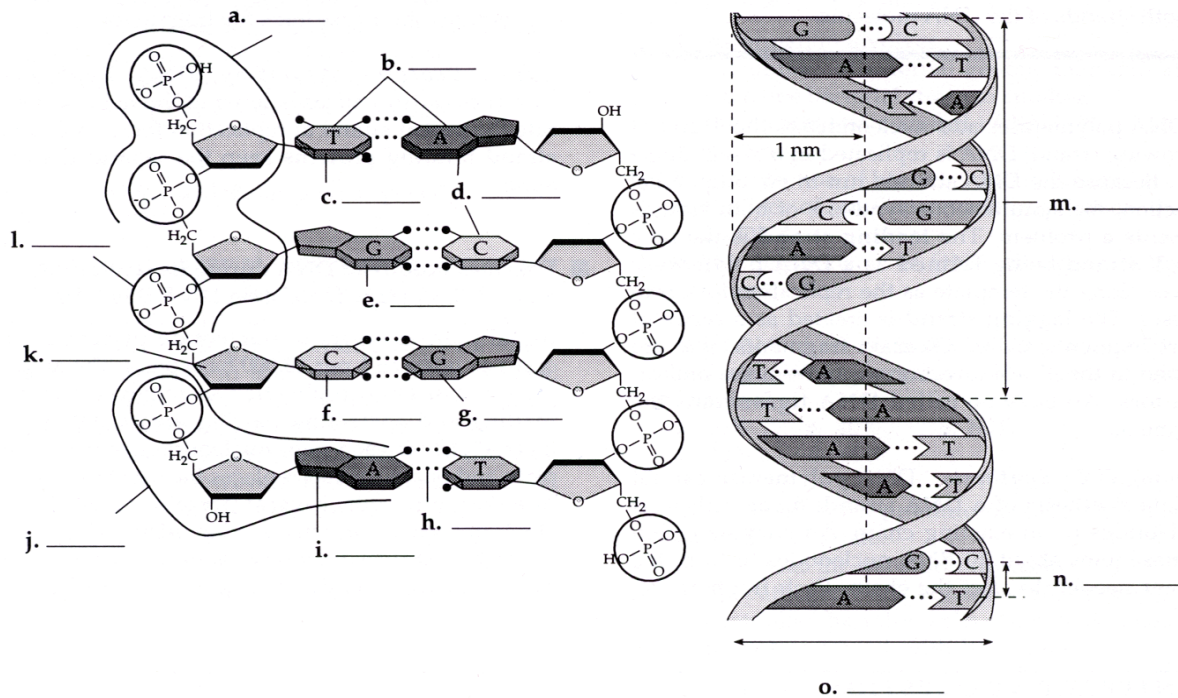
B. Now do the same for a conservative pattern of replication

C. and the same for a dispersive pattern of DNA replication



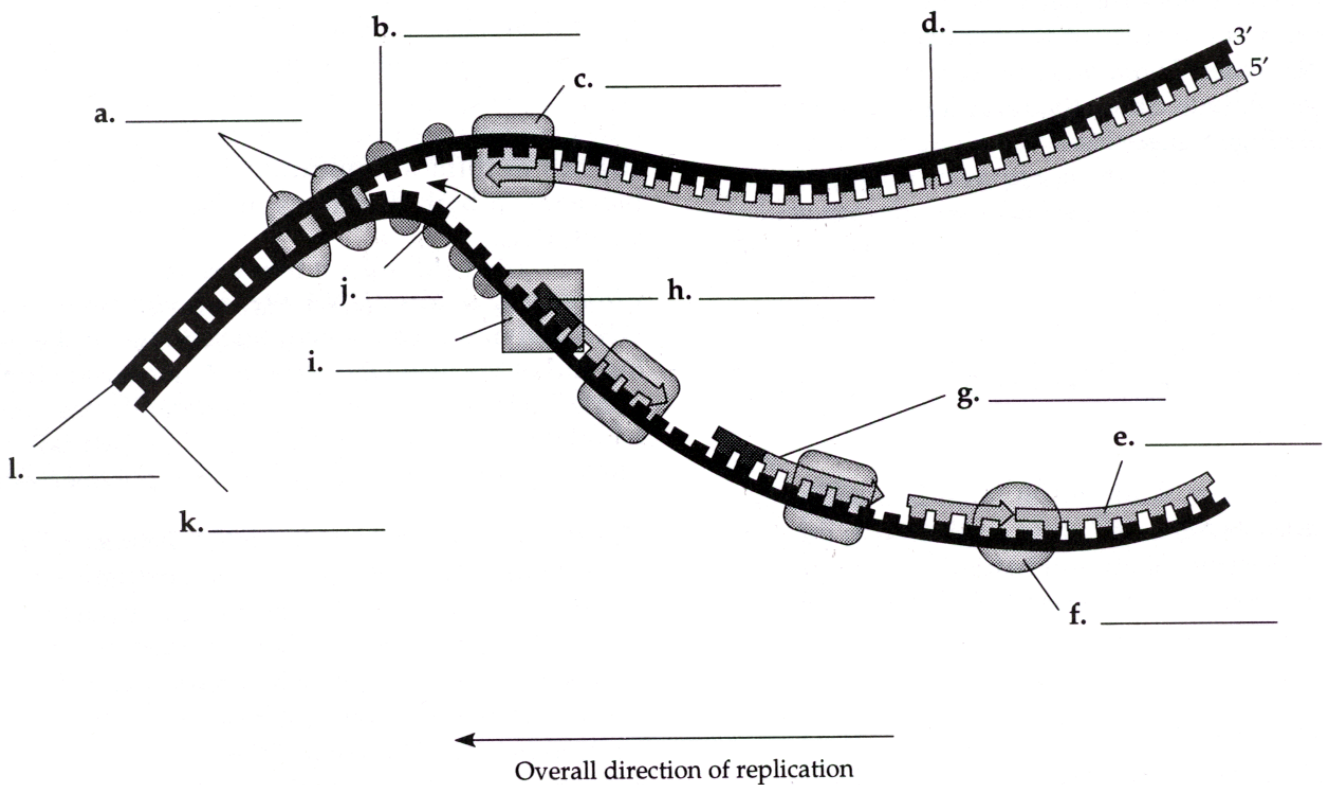
Part 3. Structure of DNA

2. Review the Structure of DNA by having each member of your Learning Community, in turn, label the following diagram (note the important dimensions 2nm, 0.34 nm, 3.4nm and 10 np's).



Part 4. Enzymatic Replication of DNA

In the diagram below each should in turn identify the label for the following items: leading strand, lagging strand, Okazaki fragment, DNA polymerase, DNA ligase, helicase, primase (RNA polymerase), binding proteins, RNA primer molecule, replication fork, and the 5' and 3' ends of the parental DNA, and newly made DNA.



Part 5. Matching Functions with the enzymes of DNA Replication

| Enzyme | Function |
|-----------------------|---|
| A. Topoisomerase | 1. _____ reads DNA template and synthesizes new DNA complimentary strand |
| B. Helicase | 2. _____ binds and stabilizes open DNA fork for template replication |
| C. SSBP | 3. _____ makes RNA primer at 5' end of DNA strands |
| D. Primase | 4. _____ joins Okazaki fragments on lagging strand |
| E. DNA Polymerase I | 5. _____ removes the RNA bases in 5' to 3' and replace with DNA nucleotides |
| F. DNA Polymerase III | 6. _____ relieves the overwinding of DNA ahead of replication fork |
| G. DNA Ligase | 7. _____ unwinds DNA helix at the replication fork |

Part 6.

The goal of this part is for you to look at RNA, its structure, its transcription, and its function in making proteins. Triplet codon instructions from DNA are transcribed into a sequence of codons in mRNA. In eukaryotes mRNA is processed before it leaves the nucleus to produce a mature functional cytoplasmic mRNA. Complexed with ribosomes, mRNA is translated into a linear sequence of amino acids in a polypeptide as tRNAs match their anticodons to the codons of the mRNA. Have one member, in turn, of your Learning Community answer one part of each of the questions or problems, then let the next member go on to the next part in the materials below.

a. Transcription of DNA & Genetic Code

At the end of this worksheet is a copy of the genetic code. We'll practice using the dictionary of the genetic code by determining the proper amino acid sequence for the polypeptide coded by the following DNA. Have someone first make the mRNA, then someone else make the correct polypeptide. Remember the initiator & proper polarity.

5'- ATGCCTGACTTTAAGTGA -3'
3'- TACGGACTGAAATTC ACT -5'

the mRNA is...

the polypeptide is...

b. Using the codons and amino acids you identified in part 1a. above have one member of your group fill in the following table.

| DNA Triplet 3'→5' | mRNA codon 5'→3' | Anticodon 5'→3' | Amino acid |
|----------------------|---------------------|--------------------|------------|
| | | | methionine |
| | | GCA | |
| TTC | | | |
| | UAG | | |

c. How does a mature cytoplasmic, eukaryotic mRNA differ physically from its primary transcript?

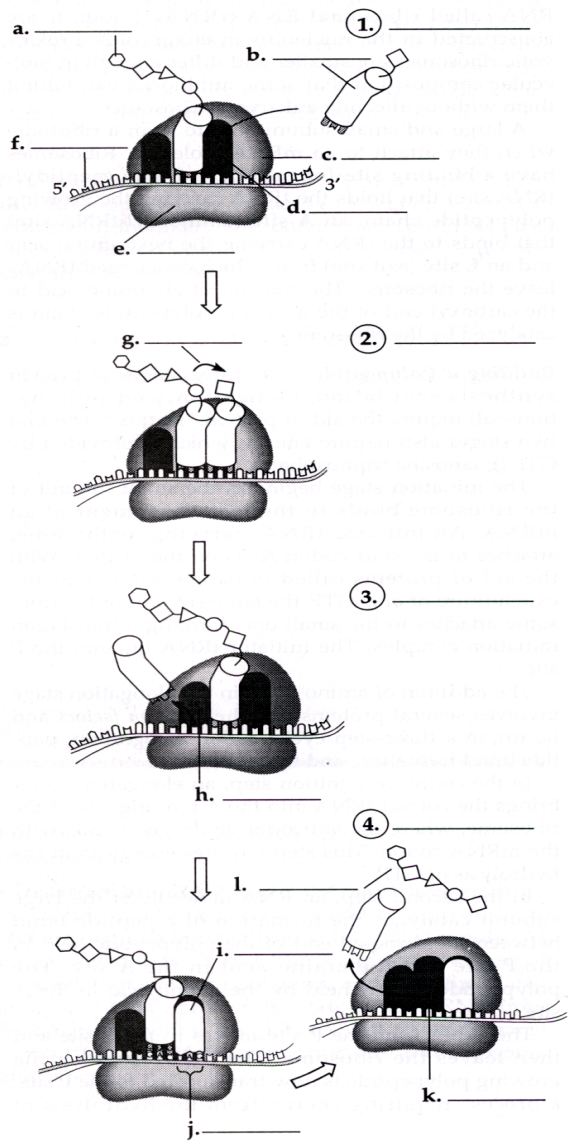
d. Have one member of your group, in turn, define the function of each of the following types of RNAs.

1. mRNA
2. tRNA
3. rRNA
4. snRNA
5. SRP-RNA

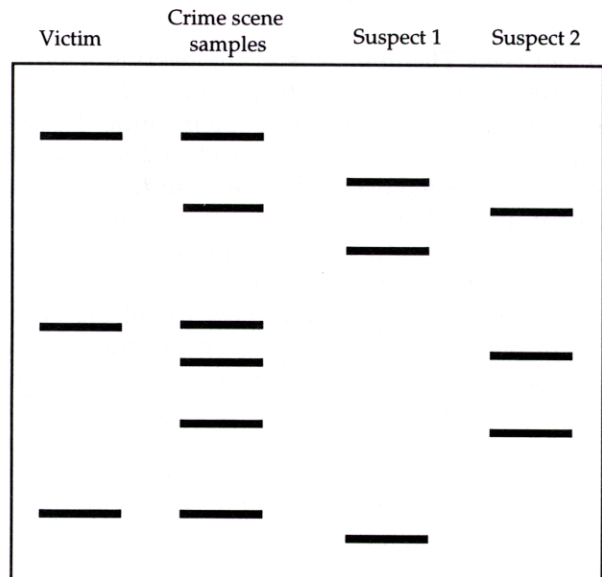
e. Define the differences between a redundant, non-sense and mis-sense mutation.

Part 7. In the figure to the right of the details involved in protein translation:

- Name the stages of translation (1-4)
- Briefly describe what happens at each stage
- Identify the components (a. thru l.)



Part 8. A bloody crime scene has occurred in Miami. The CSI New Orleans groups heads to the SoBe and collects blood samples from a victim, two different suspects, and from a possible murder scene. From the DNA electropherograms below, tell us which suspect you would charge with the crime and why. Also describe what techniques or procedures were used?



Part 9.

Fill in this table on the basic tools of gene manipulations used in DNA biotechnology.

| Technique or tool | Brief description | Some uses in DNA technology |
|---------------------|-------------------|-----------------------------|
| Restriction enzymes | | |
| Gel electrophoresis | | |
| cDNA | | |
| Labeled probes | | |
| Southern blots | | |
| DNA sequencing | | |
| PCR | | |
| RFLP analysis | | |

| | U | C | A | G | |
|----------|----------|----------|----------|----------|----------|
| U | Phe | Ser | Tyr | Cys | U |
| | Phe | Ser | Tyr | Cys | C |
| | Leu | Ser | STOP | STOP | A |
| | Leu | Ser | STOP | Trp | G |
| C | Leu | Pro | His | Arg | U |
| | Leu | Pro | His | Arg | C |
| | Leu | Pro | Gln | Arg | A |
| | Leu | Pro | Gln | Arg | G |
| A | Ile | Thr | Asn | Ser | U |
| | Ile | Thr | Asn | Ser | C |
| | Ile | Thr | Lys | Arg | A |
| | Met | Thr | Lys | Arg | G |
| G | Val | Ala | Asp | Gly | U |
| | Val | Ala | Asp | Gly | C |
| | Val | Ala | Glu | Gly | A |
| | Val | Ala | Glu | Gly | G |