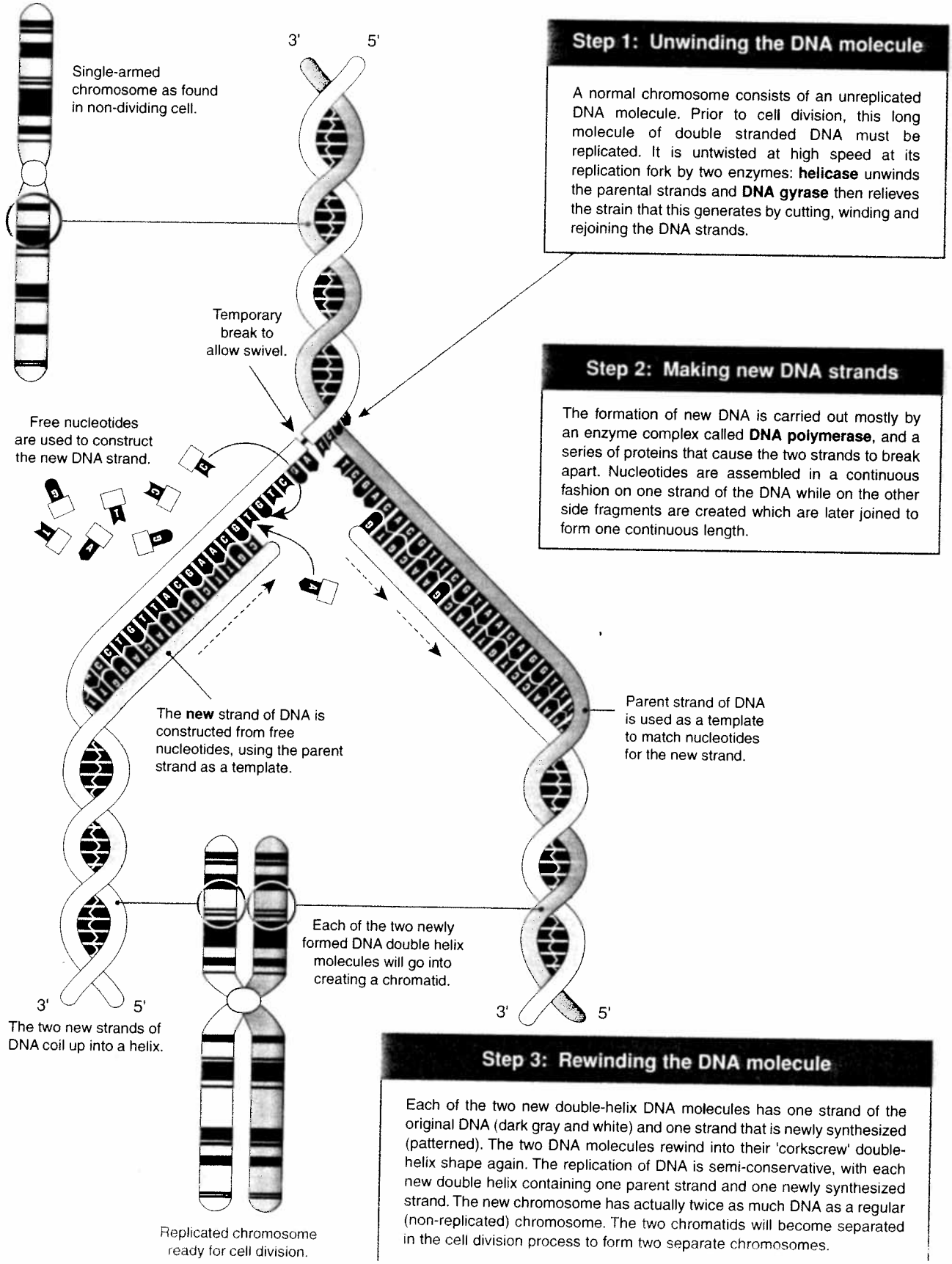
Protein Synthesis: Translating the Code

DNA Replication

Cells carry out the process of **DNA replicatio**n (DNA duplication) prior to cell division (mitosis and meiosis). This process ensures that each resulting cell is able to receive a complete set of genes from the original cell. After the DNA has replicated, each chromosome is made up of two chromatids, which are joined at the centromere. Each chromatid contains half original (parent) DNA and half new (daughter) DNA. The two chromatids will become separated during cell division to form two separate chromosomes. During DNA replication, new nucleotides become added at a region called the **replication fork**. The position of the replication fork moves along the chromosome as the replication progresses. This whole process occurs simultaneously for each chromosome of a cell and the entire process is tightly controlled by enzymes.



Step 1: Unwinding the DNA molecule

A normal chromosome consists of an unreplicated DNA molecule. Prior to cell division, this long molecule of double stranded DNA must be replicated. It is untwisted at high speed at its replication fork by two enzymes: **helicase** unwinds the parental strands and **DNA gyrase** then relieves the strain that this generates by cutting, winding and rejoining the DNA strands.

Step 2: Making new DNA strands

The formation of new DNA is carried out mostly by an enzyme complex called **DNA polymerase**, and a series of proteins that cause the two strands to break apart. Nucleotides are assembled in a continuous fashion on one strand of the DNA while on the other side fragments are created which are later joined to form one continuous length.

Step 3: Rewinding the DNA molecule

Each of the two new double-helix DNA molecules has one strand of the original DNA (dark gray and white) and one strand that is newly synthesized (patterned). The two DNA molecules rewind into their 'corkscrew' double-helix shape again. The replication of DNA is semi-conservative, with each new double helix containing one parent strand and one newly synthesized strand. The new chromosome has actually twice as much DNA as a regular (non-replicated) chromosome. The two chromatids will become separated in the cell division process to form two separate chromosomes.

1. State the purpose of DNA replication: _____

2. Summarize the three main steps involved in DNA replication:

   (a) _____

   (b) _____

   (c) _____

3. For a cell with 22 chromosomes, state how many chromatids would exist following DNA replication: _____

4. Discuss the importance of enzymes in DNA replication: _____

   _____

   _____

   _____

   _____

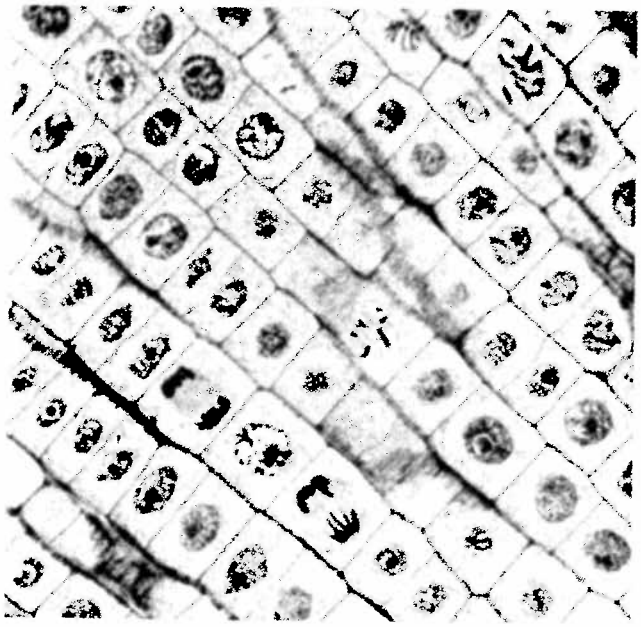
   _____

5. DNA replication occurs during the S (synthesis) phase of the **cell cycle**. This is part of a larger phase called interphase. It is the phase in which the cell is not dividing (in mitosis).

   The light micrograph (right) shows a section of cells in an onion root tip. These cells have a cell cycle of approximately 24 hours. The cells can be seen to be in various stages of the cell cycle. By counting the number of cells in the various stages it is possible to calculate how long the cell spends in each stage of the cycle.

   Count and record the number of cells in the image which are undergoing mitosis and those that are in interphase. Estimate the amount of time a cell spends in each phase.

Onion Root Tip Cells



Stage	No. of cells	% of total cells	Estimated time in stage
Interphase			
Mitosis			
Total		100	

6. Match the statements in the table below to form complete sentences, then put the sentences in order to make a coherent paragraph about DNA replication and its role:

The enzymes also proofread the DNA during replication...	...is required before mitosis or meiosis can occur.
DNA replication is the process by which the DNA molecule...	...by enzymes.
Replication is tightly controlled...	...to correct any mistakes.
After replication, the chromosome...	...and half new DNA.
DNA replication...	...during mitosis.
The chromatids separate...	...is copied to produce two identical DNA strands.
A chromatid contains half original ...	...is made up of two chromatids.

Write the complete paragraph here: _____

_____

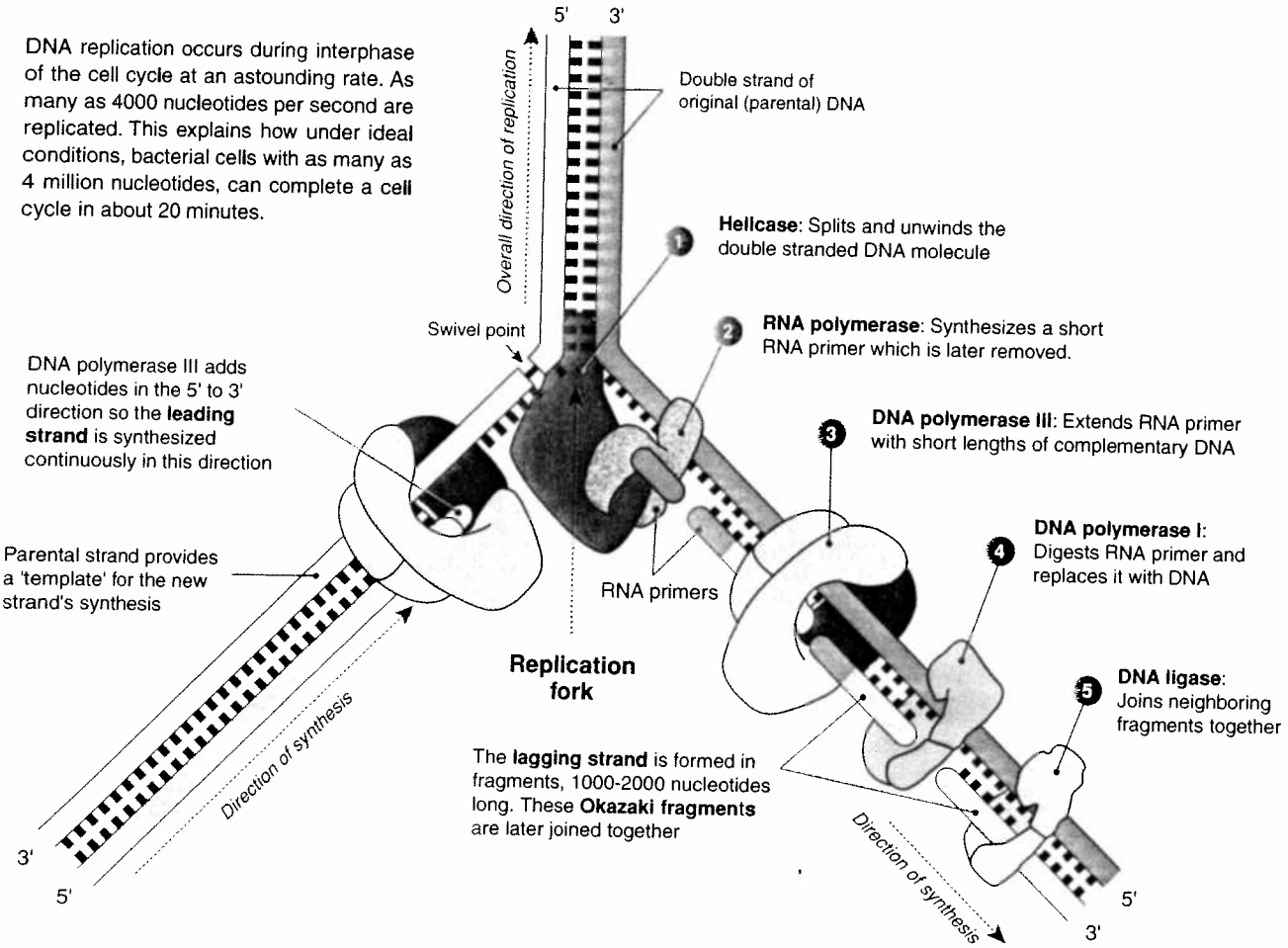
_____

_____

_____

Enzyme Control of DNA Replication

The sequence of enzyme controlled events in DNA replication is shown below (1-5). Although shown as separate, many of the enzymes are found clustered together as enzyme complexes. These enzymes are also able to 'proof-read' the new DNA strand as it is made and correct mistakes. The polymerase enzyme can only work in one direction, so that one new strand is constructed as a continuous length (the leading strand) while the other new strand is made in short segments to be later joined together (the lagging strand). **NOTE** that the nucleotides are present as deoxynucleoside triphosphates. When hydrolyzed, these provide the energy for incorporating the nucleotide into the strand.



1. What is the purpose of DNA replication? _____

_____

2. Summarize the steps involved in DNA replication (on the previous page):

   (a) Step 1: _____

   _____

   (b) Step 2: _____

   _____

   (c) Step 3: _____

   _____

3. Explain the role of the following enzymes in DNA replication:

   (a) Helicase: _____

   (b) DNA polymerase I: _____

   (c) DNA polymerase III: _____

   (d) Ligase: _____

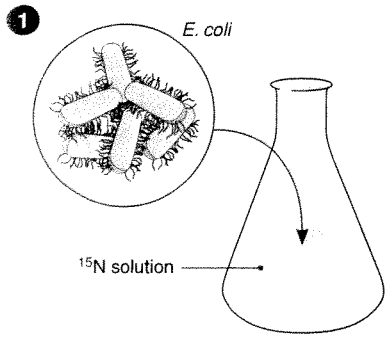
4. Determine the time it would take for a

Meselson and Stahl's Experiment

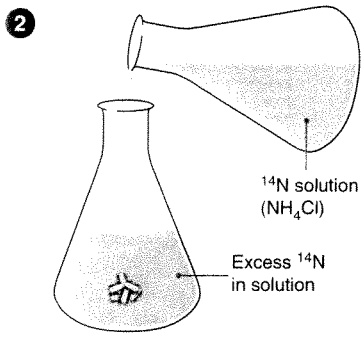
When Watson and Crick identified the structure of DNA in 1953 it became apparent that its structure could help to explain how DNA was replicated. Three models were proposed. Watson and Crick proposed the **semi-conservative model** in which each DNA strand served as a template, forming a new DNA molecule that was half old and half new DNA. The **conservative model** proposed that the original DNA served as a complete template so that the resulting DNA comprised two completely new strands. The **dispersive model** proposed that the two new DNA molecules had part new and part old DNA interspersed (mixed) throughout them. **Meselson and Stahl** devised a simple experiment using *E. coli* grown in differing isotopes of nitrogen, to determine which theory was correct.

Meselson and Stahl's Experiment

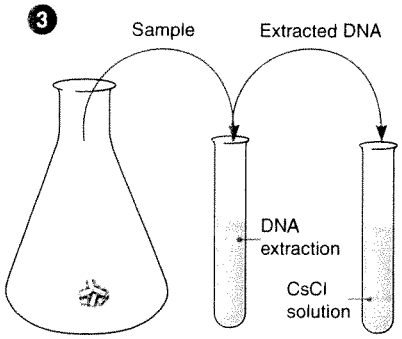
E. coli were grown for several generations in a medium containing a **heavy nitrogen isotope** (^{15}N). Once all the bacterial DNA contained ^{15}N, they were transferred to a medium containing a **light nitrogen isotope** (^{14}N). After the transfer, newly synthesized DNA would contain ^{14}N and old DNA would contain ^{15}N.



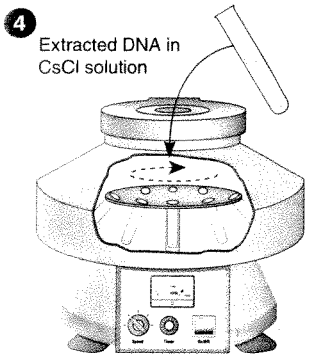
E. coli were grown in a nutrient solution containing ^{15}N. After 14 generations, all the bacterial DNA contained ^{15}N. A sample is removed. This is **generation 0**.



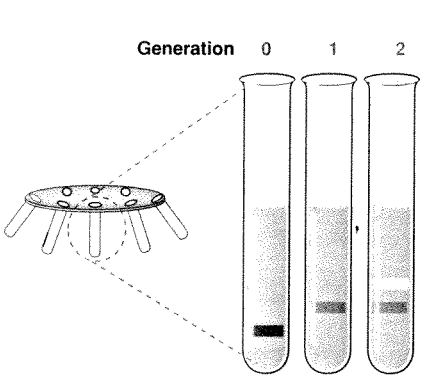
Generation 0 is added to a solution containing excess ^{14}N (as NH_4Cl). During replication, new DNA will incorporate ^{14}N and be 'lighter' than the original DNA (which contains only ^{15}N).



Every generation (~ 20 minutes), a sample is taken and treated to release the DNA. The DNA is placed in a CsCl solution which provides a density gradient for separation of the DNA.

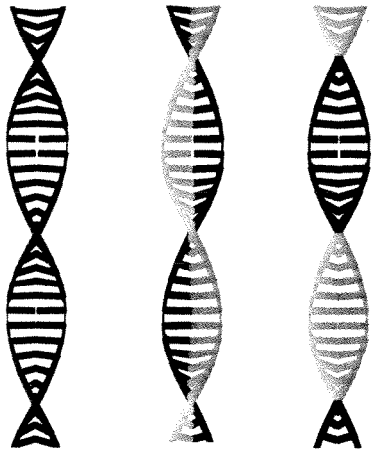


Samples are spun in a high speed ultracentrifuge at 140,000 *g* for 20 hours. Heavier ^{15}N DNA moves closer to the bottom of the test tube than light ^{14}N DNA or intermediate $^{14}N/^{15}N$ DNA.



All the DNA in the generation 0 sample moved to the bottom of the test tube. All the DNA in the generation 1 sample moved to an intermediate position. At generation 2 half the DNA was at the intermediate position and half was near the top of the test tube. In subsequent generations, more DNA was near the top and less was in the intermediate position.

Models for DNA Replication



Conservative | Semi-conservative | Dispersive

1. Describe each of the DNA replication models:

   (a) Conservative: _____

   _____

   (b) Semi-converative: _____

   _____

   (c) Dispersive: _____

   _____

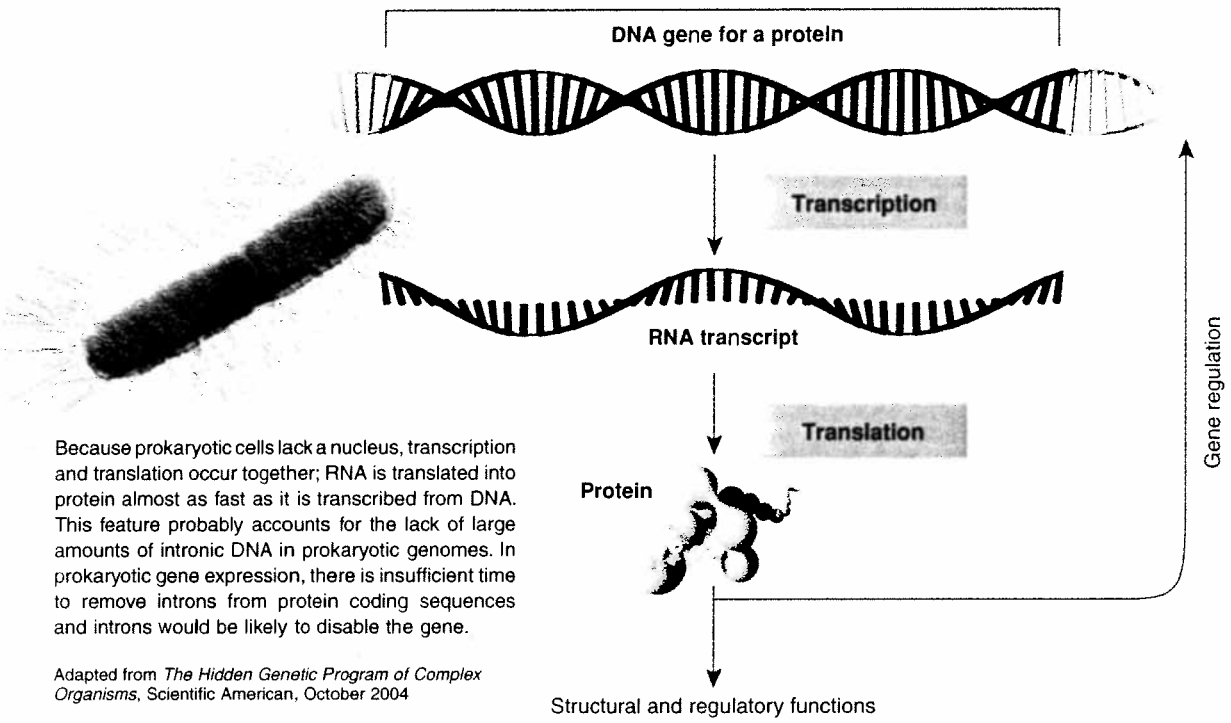
2. Explain why the *E. coli* were grown in an ^{15}N solution before being transferred to an ^{14}N solution: _____

   _____

Gene Expression: Prokaryotes vs Eukaryotes

The process of transferring the information encoded in a gene to its functional gene product is called **gene expression**. The central dogma of molecular biology for the past 50 years or so has stated that genetic information, encoded in DNA, is transcribed as molecules of RNA, which are then translated into the amino acid sequences that make up proteins. The established opinion was often stated as "one gene-one protein" and proteins were assumed to be the main regulatory agents for the cell (including its gene expression). The one gene-one protein model is supported by studies of prokaryotic genomes, where the DNA consists almost entirely of protein-coding genes and their regulatory sequences.

Genes and Gene Expression in Prokaryotes



Because prokaryotic cells lack a nucleus, transcription and translation occur together; RNA is translated into protein almost as fast as it is transcribed from DNA. This feature probably accounts for the lack of large amounts of intronic DNA in prokaryotic genomes. In prokaryotic gene expression, there is insufficient time to remove introns from protein coding sequences and introns would be likely to disable the gene.

Adapted from *The Hidden Genetic Program of Complex Organisms*, Scientific American, October 2004

1. Describe the important features of gene expression in prokaryotes: _____

2. The traditional (old) view of gene expression in eukaryotes (table right) was based on a modification of the one gene-one protein model. This model does not adequately explain gene expression in eukaryotes, but it is probably still appropriate for prokaryotes. Suggest why:

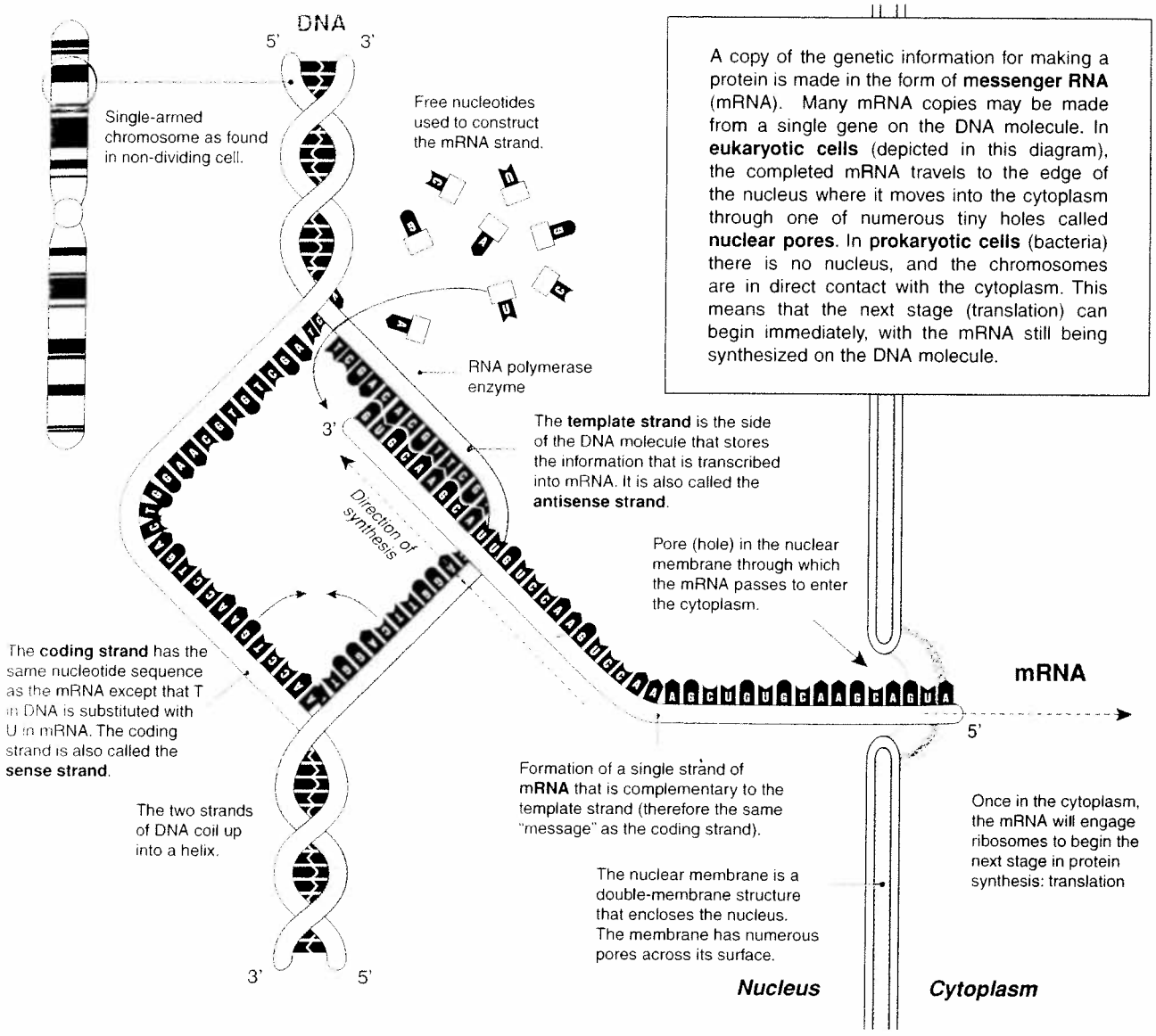
Gene Expression in Eukaryotes

The Old View	The New View
Introns are spliced out of a primary RNA transcript	Introns are spliced out of a primary RNA transcript
All the exon RNA is translated into proteins.	Not all exon RNA is translated into proteins. Non-protein-coding exonic DNA may have its own function or may contribute to microRNAs
Introns are junk DNA with no function; they are degraded and recycled.	Introns are processed into microRNAs which are involved in regulating development.

3. How is gene expression in prokaryotes fundamentally different from gene expression in eukaryotes?

Transcription in Eukaryotes

Transcription is the process by which the code contained in the DNA molecule is transcribed (rewritten) into a **mRNA** molecule. Transcription is under the control of the cell's metabolic processes which must activate a gene before this process can begin. The enzyme that directly controls the process is RNA polymerase, which makes a strand of mRNA using the single strand of DNA (the **template strand**) as a template (hence the term). The enzyme transcribes only a gene length of DNA at a time and therefore recognizes start and stop signals (codes) at the beginning and end of the gene. Only RNA polymerase is involved in mRNA synthesis; it causes the unwinding of the DNA as well. It is common to find several RNA polymerase enzyme molecules on the same gene at any one time, allowing a high rate of mRNA synthesis to occur.



A copy of the genetic information for making a protein is made in the form of **messenger RNA** (mRNA). Many mRNA copies may be made from a single gene on the DNA molecule. In **eukaryotic cells** (depicted in this diagram), the completed mRNA travels to the edge of the nucleus where it moves into the cytoplasm through one of numerous tiny holes called **nuclear pores**. In **prokaryotic cells** (bacteria) there is no nucleus, and the chromosomes are in direct contact with the cytoplasm. This means that the next stage (translation) can begin immediately, with the mRNA still being synthesized on the DNA molecule.

1. Explain the role of messenger RNA (mRNA) in protein synthesis: _____

_____

_____

2. Explain the difference between the template and coding strands: _____

_____

3. For the following triplets on the DNA, determine the codon sequence for the mRNA that would be synthesized:

(a) Triplets on the DNA: T A C T A G C C G C G A T T T

Codons on the mRNA: _____

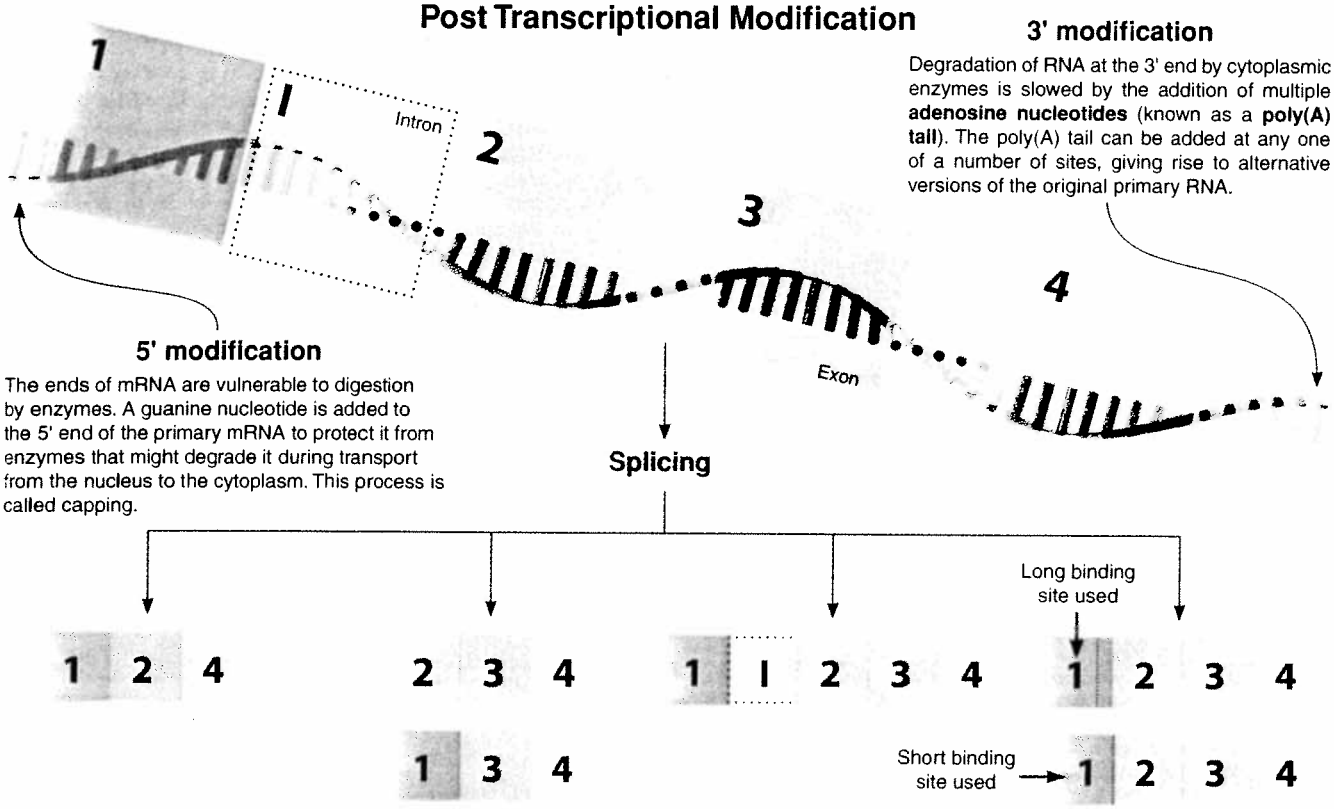
(b) Triplets on the DNA: T A C A A G C C T A T A A A A

Codons on the mRNA: _____

Post Transcriptional Modification

Human DNA contains only 25,000 genes, but produces 90,000 different proteins. Each gene must therefore produce more than one protein. This is achieved by both **post transcriptional** and **post translational modification**. Primary mRNA molecules contain exons and introns. Usually **introns** are removed after transcription and the **exons** are spliced together, this is post transcriptional modification. However, the number of exons joined together and the way they are spliced together is not always the same. This creates variations of the polypeptide chain that results. These mechanisms allow for the production of the diverse range of proteins.

Post Transcriptional Modification



3' modification

Degradation of RNA at the 3' end by cytoplasmic enzymes is slowed by the addition of multiple **adenosine nucleotides** (known as a **poly(A) tail**). The poly(A) tail can be added at any one of a number of sites, giving rise to alternative versions of the original primary RNA.

5' modification

The ends of mRNA are vulnerable to digestion by enzymes. A guanine nucleotide is added to the 5' end of the primary mRNA to protect it from enzymes that might degrade it during transport from the nucleus to the cytoplasm. This process is called capping.

Splicing

Exon Skipping
During splicing, an exon may be skipped. This is a relatively common way to produce protein variants in mammals.

Mutually Exclusive Exons
In some cases, only one of two exons (but never both) will be incorporated into the mature mRNA.

Intron Retention
Introns are not always removed during the splicing process. In some rare cases the intron is retained in the mature mRNA.

Alternative Binding Sites
Exons may contain more than one site for binding to other exons. If the shorter version is used, the remaining code is discarded, and results in a shorter mRNA sequence.

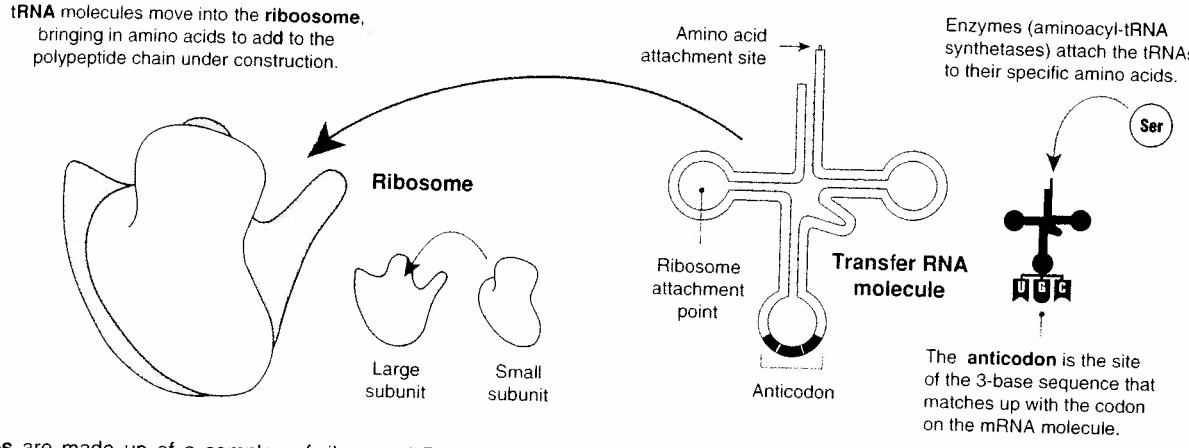
1. Explain why it is difficult to identify the true number of genes that are in the human genome: _____

2. Describe the ways in which mRNA can be modified to code for different proteins: _____

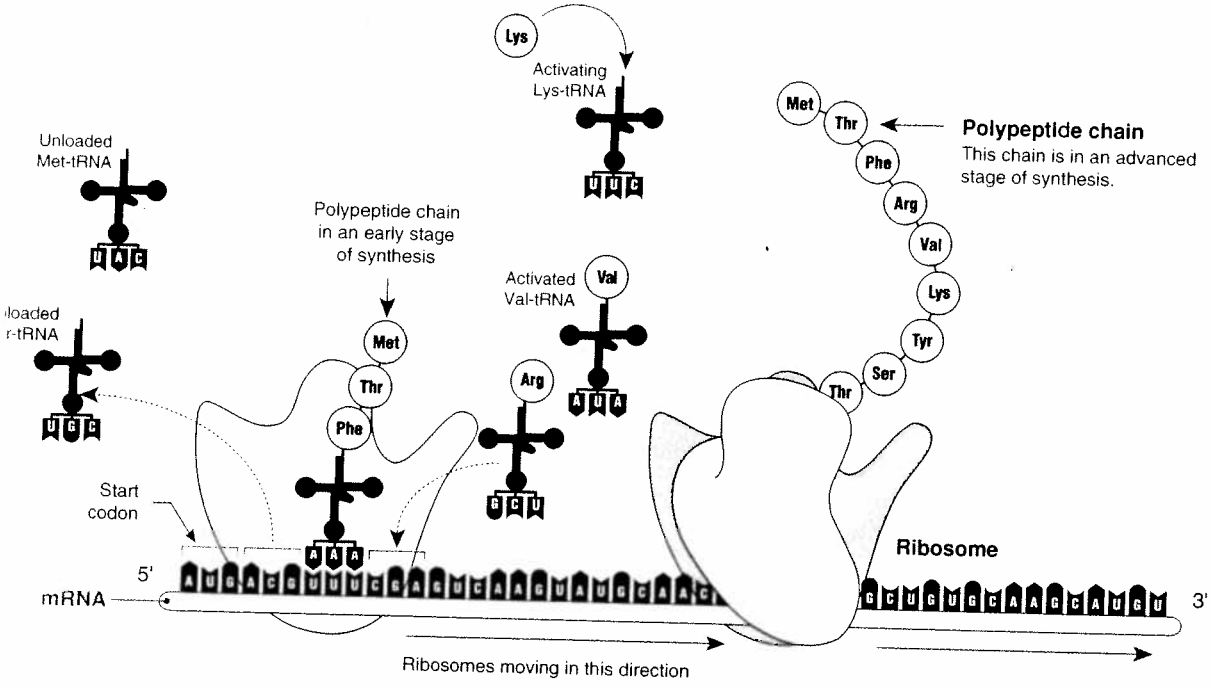
3. Explain the advantage of being able to modify the mRNA to produce different proteins: _____

Translation

The diagram at the bottom of the page shows the translation phase of protein synthesis. The scene shows how a single mRNA molecule can be 'serviced' by many ribosomes at the same time. The ribosome on the right is in a more advanced stage of constructing a polypeptide chain because it has 'translated' more of the mRNA than the ribosome on the left. The anticodon at the base of each tRNA must make a perfect complementary match with the codon on the mRNA before the amino acid is released. Once released, the amino acid is added to the growing polypeptide chain by enzymes.



Ribosomes are made up of a complex of ribosomal RNA (rRNA) and proteins. They exist as two separate sub-units (above) until they are attracted to a binding site on the mRNA molecule, when they join together. Ribosomes have binding sites that attract transfer RNA (**tRNA**) molecules loaded with amino acids. The tRNA molecules are about 80 nucleotides in length and are made under the direction of genes in the chromosomes. There is a different tRNA molecule for each of the different possible anticodons (see the diagram below) and, because of the degeneracy of the genetic code, there may be up to six different tRNAs carrying the same amino acid.



1. For the following codons on the mRNA, determine the **anticodons** for each tRNA that would deliver the amino acids:

   Codons on the mRNA: U A C U A G C C G C G A U U U

   Anticodons on the tRNAs: _____

2. There are many different types of tRNA molecules, each with a different anticodon (HINT: see the mRNA table).

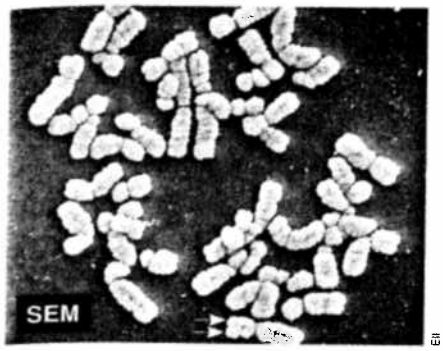
   (a) How many different tRNA types are there, each with a unique anticodon? _____

   (b) Explain your answer: _____

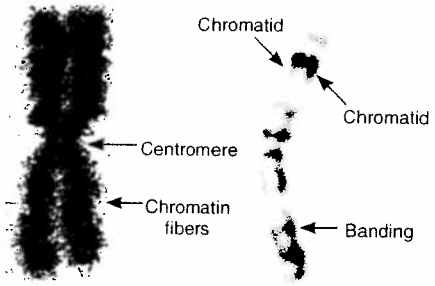
   _____

Eukaryotic Chromosome Structure

The chromosomes of eukaryote cells (such as those from plants and animals) are complex in their structure compared to those of prokaryotes. The illustration below shows a chromosome during the early stage of meiosis. Here it exists as a chromosome consisting of two chromatids. A non-dividing cell would have chromosomes with the 'equivalent' of a single chromatid only. The chromosome consists of a protein coated DNA strand which coils in three ways during the time when the cell prepares to divide (below).

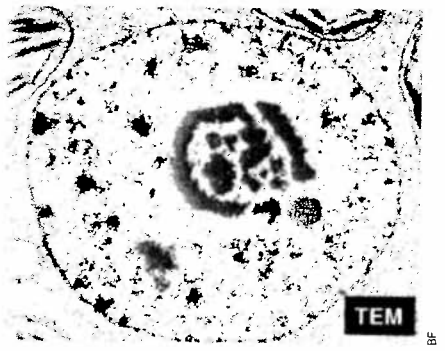


A cluster of human chromosomes seen during metaphase of cell division. Individual chromatids (arrowed) are difficult to discern on these double chromatid chromosomes.

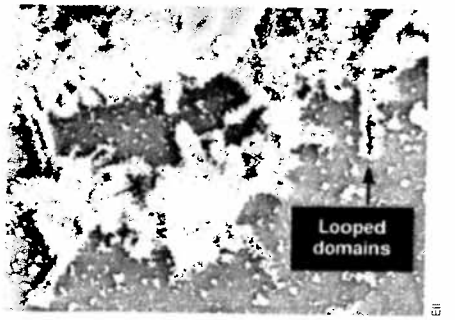


Chromosome TEM Human chromosome 3

A human chromosome from a dividing white blood cell (above left). Note the compact organization of the chromatin in the two chromatids. The LM photograph (above right) shows the banding visible on human chromosome 3.



In non-dividing cells, chromosomes exist as single-armed structures. They are not visible as coiled structures, but are 'unwound' to make the genes accessible for transcription (above).



The evidence for the existence of looped domains comes from the study of giant lampbrush chromosomes in amphibian oocytes (above). Under electron microscopy, the lateral loops of the DNA-protein complex appear brushlike.

The Packaging of Chromatin

Chromatin is the combination of DNA and proteins that make up the contents of the cell nucleus. Chromatin structure is based on successive levels of DNA packing. **Histone proteins** are responsible for packing the DNA into a compact form. Without them, the DNA could not fit into the nucleus. Five types of histone proteins form a complex with DNA, in a way that resembles "beads on a string". These beads, or **nucleosomes**, form the basic unit of DNA packing.