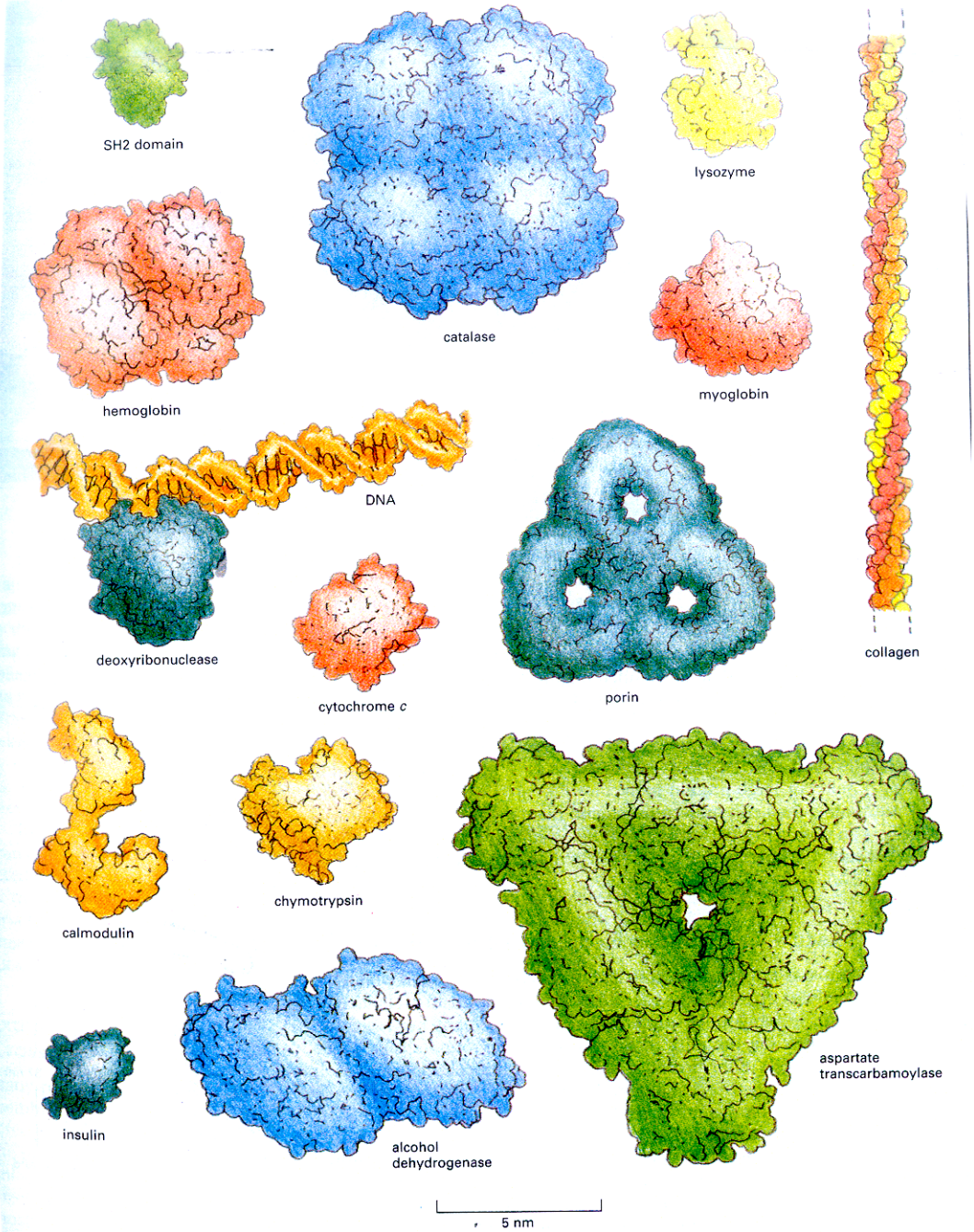
UNIQUE GENES IN A GENOME GIVE RISE TO UNIQUE PROTEINS



HOW DOES Gene # Relate to Protein #?

igure 3–24 A collection of protein molecules, shown at the same scale. For comparison, a DNA molecule bound to a protein is also ustrated. These space-filling models represent a range of sizes and shapes. Hemoglobin, catalase, porin, alcohol dehydrogenase, and aspartate anscarbamoylase are formed from multiple copies of subunits. The SH2 domain *(top left)* is presented in detail in Panel 3–2 (pp. 138–139). (After avid S. Goodsell, Our Molecular Nature. New York: Springer-Verlag, 1996.)

A Protein is Synthesized by TRANSLATING THE GENETIC CODE of the Gene/mRNA into the Amino Acids of a specific Protein

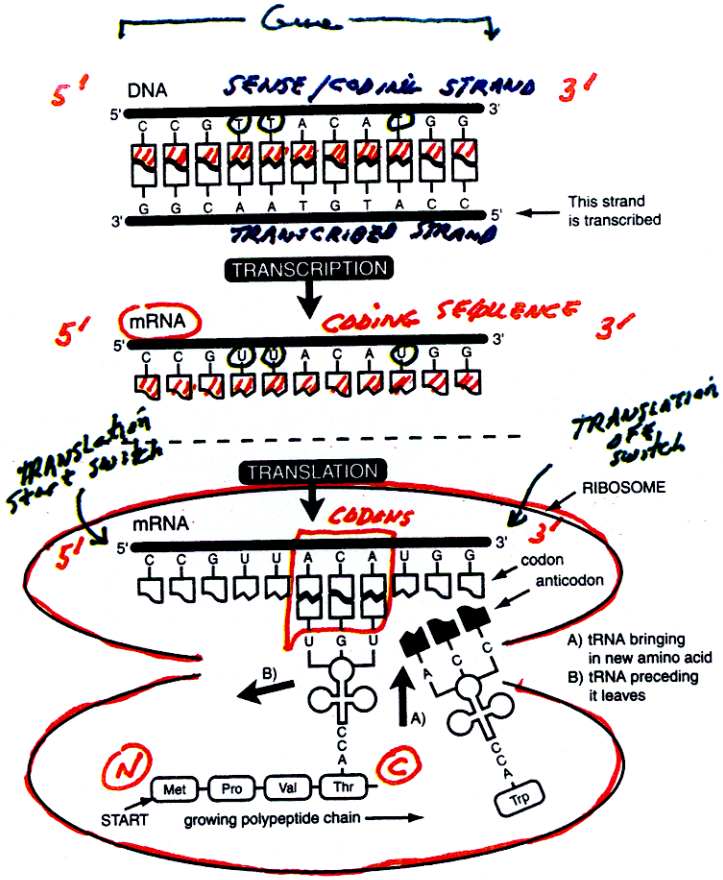


FIGURE 3.7 The process of translation.

Note:
1. Gene has unique 5'→3' sequence
2. mRNA has unique 5'→3' sequence
3. protein has unique aa sequence
4. gene sequence "translated" into protein sequence!
5. mRNA has signals or sequences that tell ribosome ① to bind or ② fall off & where start of translation is!

Colinearity

DNA Sequence → mRNA Sequence → Protein Sequence → trait

RIBOSOME FACTORY for protein PRODUCTION

CODONS ON the MRNA Specify Specific Amino Acids

Protein is made on Ribosomes using the Genetic Code on mRNA for Instructions

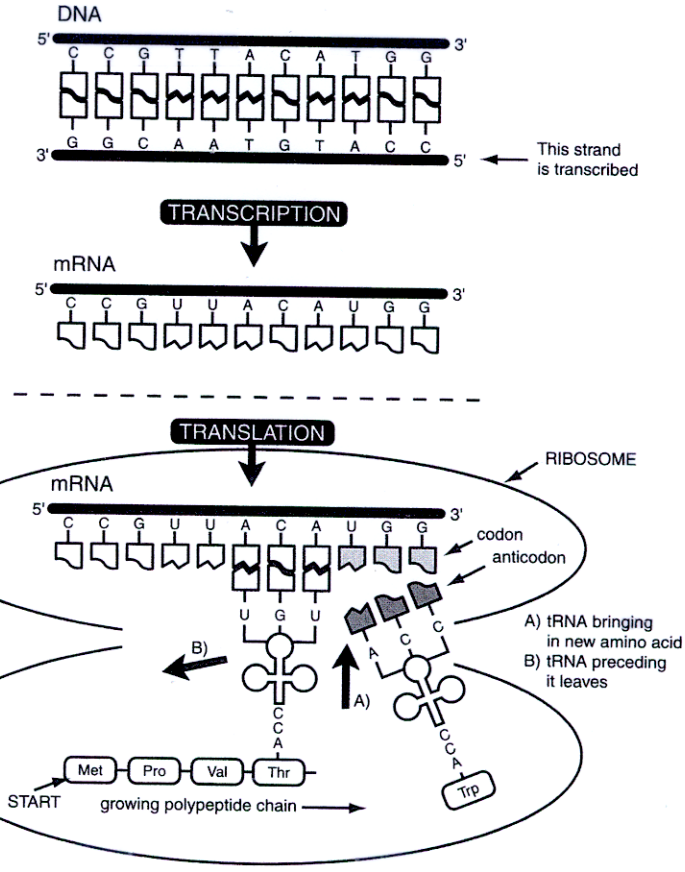


FIGURE 3.7 The process of translation.

Codons on mRNA specify order & kinds of amino acids in proteins

Ribosomes TRANSLATE the Genetic Code

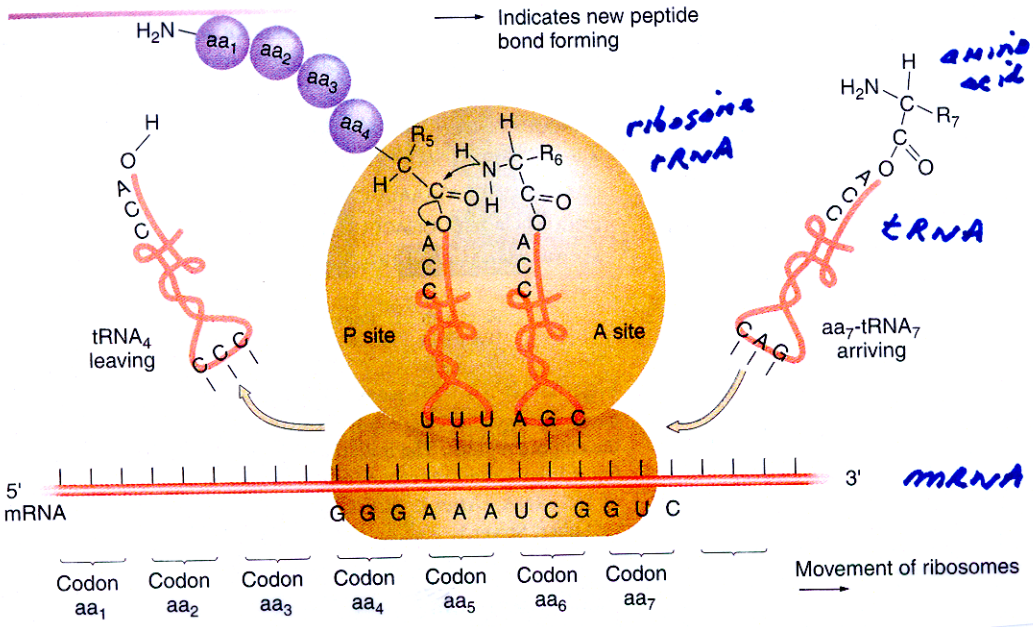
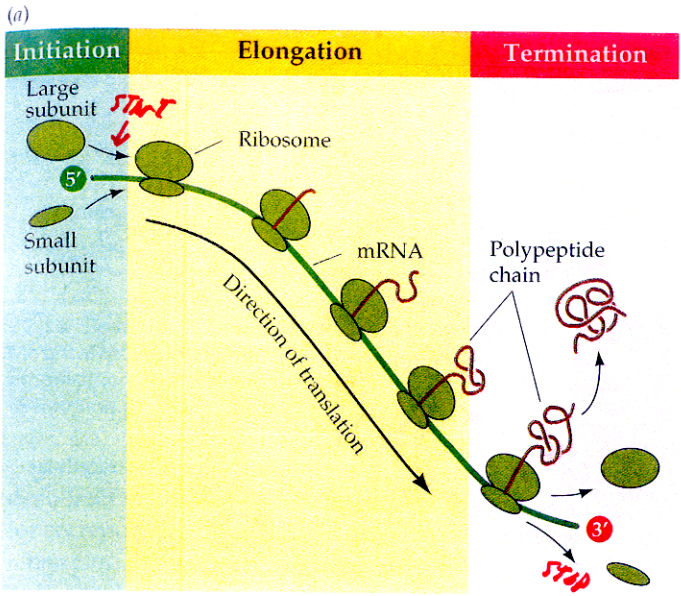


Figure 10-31 The addition of a single amino acid to the growing polypeptide chain in the course of translation of mRNA.

CODON by CODON!

ONE mRNA is TRANSLATED MANY TIMES



12.13 A Polysome
(*a*) A polysome consists of ribosomes and their growing polypeptide chains moving in single file along an mRNA molecule. (*b*) An electron microscopic view of a polysome.

IMPLICATIONS FOR BIOLOGY

Gene Activity can be visualized!

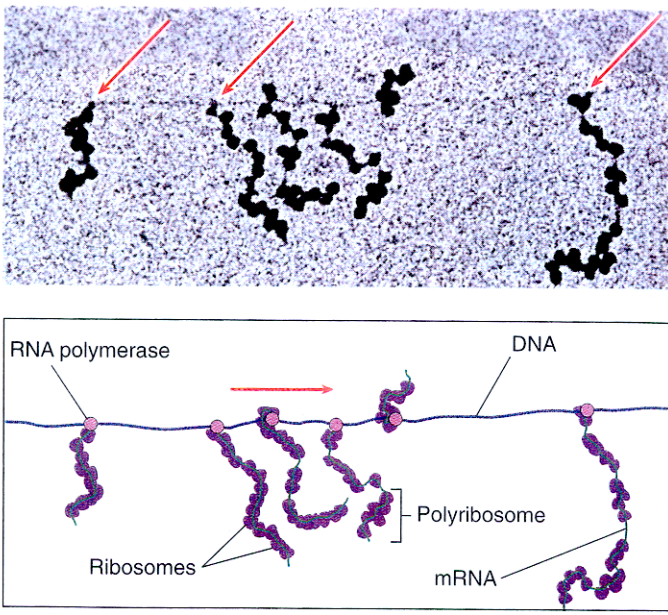


FIGURE 15.10
Translation in action. Bacteria have no nucleus and hence no membrane barrier between the DNA and the cytoplasm. In this electron micrograph of genes being transcribed in the bacterium *Escherichia coli*, you can see every stage of the process. The arrows point to RNA polymerase enzymes. From each mRNA molecule dangling from the DNA, a series of ribosomes is assembling polypeptides. These clumps of ribosomes are sometimes called "polyribosomes."

Transcription / translation are coupled in Bacteria

EUKARYOTIC & PROKARYOTIC Gene Expression Processes differ Slightly

Genes Differ
Switches/RNA Polymerases Differ } Because Cells & Life Cycles Differ
Genetic Code the SAME
General Processes the SAME

but.....

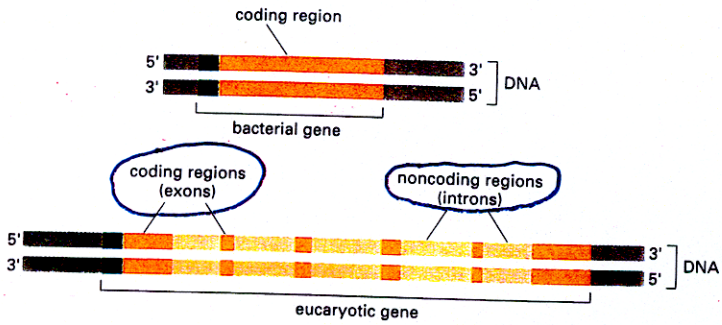


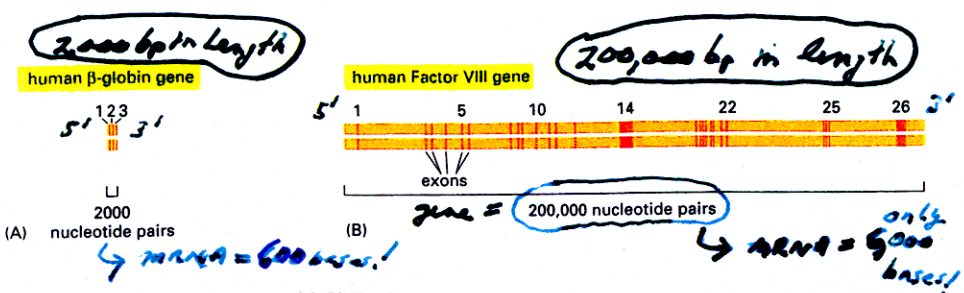
Figure 7–13 Comparison of a bacterial gene with a eucaryotic gene. The bacterial gene consists of a single stretch of uninterrupted nucleotide sequence that encodes the amino acid sequence of a protein. In contrast, the coding sequences of most eucaryotic genes (*exons*) are interrupted by noncoding sequences (*introns*). Promoters for transcription are indicated in *green*.

SWITCHES UNIQUE TO BACTERIA & TO PLANTS/HUMANS

Eukaryotic Genes (CAN) have NON-coding regions "Stuck" in coding regions

Prokaryotic Genes only have coding regions!

Thus: Eukaryotic cells must remove non-coding regions in mRNA BEFORE genetic code can be translated CONTINUOUSLY!



Note: Human Genes can be Mostly Intron Sequences!

NOTE: gene CAN be Huge!

EUKARYOTIC GENES HAVE INTRONS OR NON-CODING DNA INTERSPERSED IN CODING SEQUENCES OR EXONS

INTRONS ARE TRANSCRIBED BUT MUST BE SPLICED OUT IN NUCLEUS TO MAKE MRNA WITH CONTINUOUS GENETIC CODE!!

GENE → RNA → SPLICING → MRNA

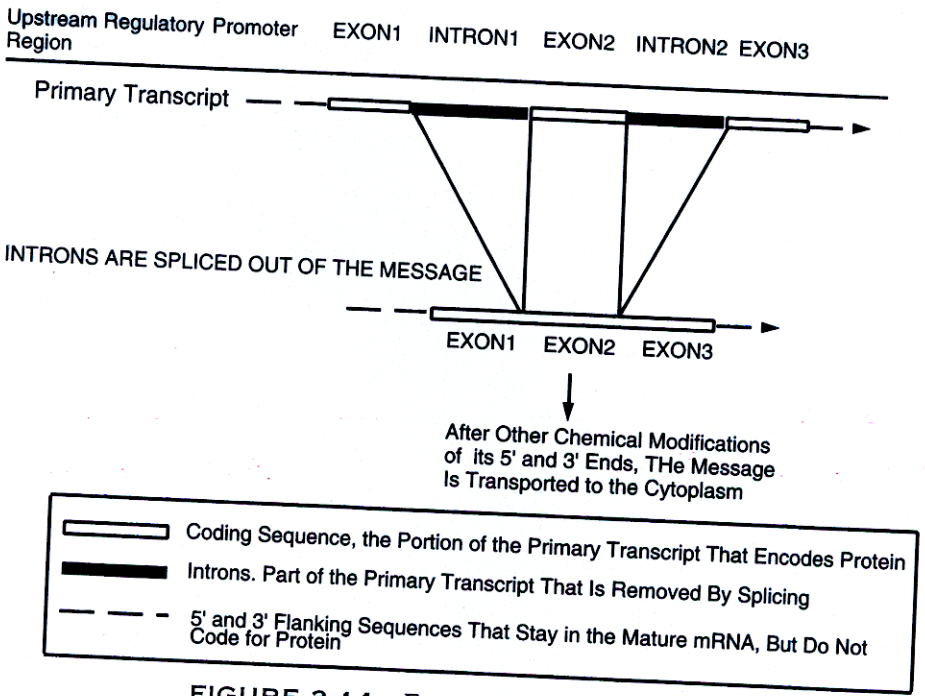
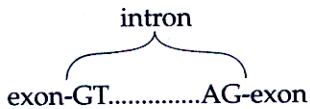


FIGURE 3.14 Exons, introns, and splicing.

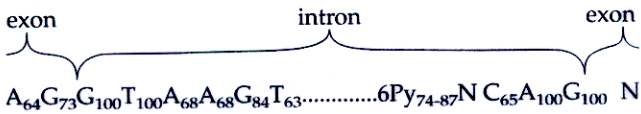
Implication for Engineering Eukaryotic genes in Bacteria?

BACTERIAL GENES DO NOT HAVE INTRONS & DO NOT PROCESS EUKARYOTIC RNAS!

Yo! It's in the Sequences!



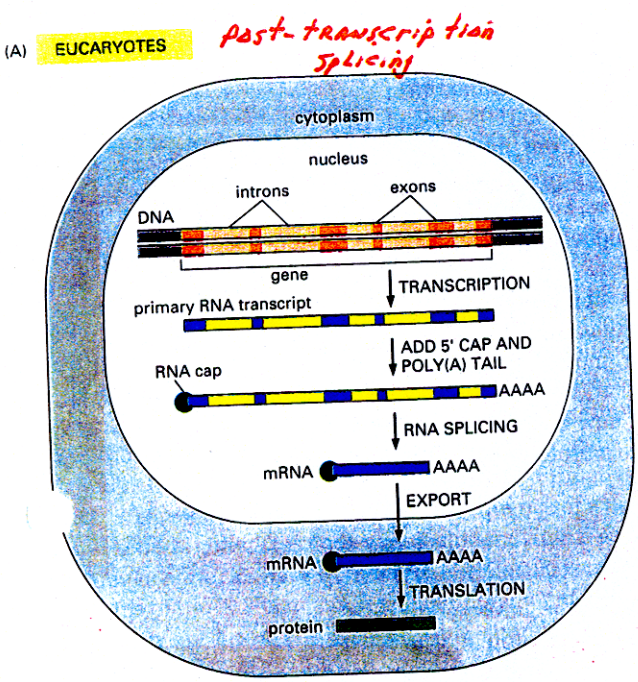
The sequences shown here are for the DNA nontemplate strand (equivalent to the RNA transcript, but with T rather than U). In addition, there are short consensus sequences at the exon–intron junctions. For nuclear genes, the consensus junctions are



Specific Sequences required for RNA Splicing!

What happens if these Sequences are Mutated in a Gene?

Eukaryotic Gene TRANSCRIPTS Are PROCESSED By Splicing in the Nucleus to FORM mRNAS



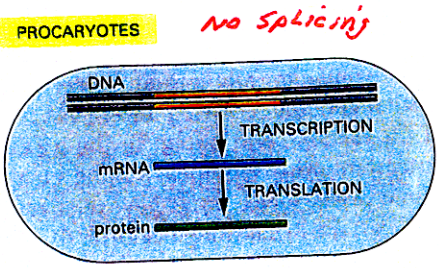


Figure 7–19 Summary of the steps leading from gene to protein. The final level of a protein in the cell depends on the efficiency of each step and on the rates of degradation of the RNA and protein molecules. (A) In eucaryotic cells, the initial RNA molecule produced by transcription (the primary transcript) contains both intron and exon sequences. Its two ends are modified, and the introns are removed by an enzymatically catalyzed RNA splicing reaction. The resulting mRNA is then transported from the nucleus to the cytoplasm, where it is translated into protein. Although these steps are depicted as occurring one at a time, in a sequence, in reality they often occur simultaneously. For example, the RNA cap is typically added and splicing typically begins before the primary transcript has been completed. (B) In procaryotes, the production of mRNA molecules is simpler. The 5′ end of an mRNA molecule is produced by the initiation of transcription by RNA polymerase, and the 3′ end is produced by the termination of transcription. Since procaryotic cells lack a nucleus, transcription and translation take place in a common compartment. In fact, translation of a bacterial mRNA often begins before its synthesis has been completed.

What are Consequences For Expressing a Human Gene with Introns in a Bacterial Cell???

Engineer mRNA not gene!!

Modular Organization of Sequences

1. DNA Replication
   - ORI
2. TRANSCRIPTION
   - Switch / Regulator
   - Terminator
3. Processing of RNA (Eukaryotes)
   - Splicing Sites
4. Translation
   - Start
   - Stop
   - Genetic Code / Codons
5. Coding Sequence
   - Genetic Code

Modules → anything you want to do genetically!

Engineering Genes Requires:

1. The Gene & its Sequence
2. A Roadmap of where Coding Sequence & ALL switches located (What's the roadmap?)
3. TRANSCRIPTION Start & Stop Switches
4. Coding Part of Gene / Genetic Code Part
5. TRANSLATION Start & Stop Switches
6. Kingdom-Specific Switches/Signals

Note! The general process of gene → protein is the same in all organisms but the Specific switches & enzymes (e.g., RNA polymerase) differ in Kingdoms !!

PLANT TRANSCRIPTION ON SWITCH + HUMAN CODING Sequence + Plant TRANSCRIPTION off switch

T

It's that easy Because living cells use SAME overall genetic processes!

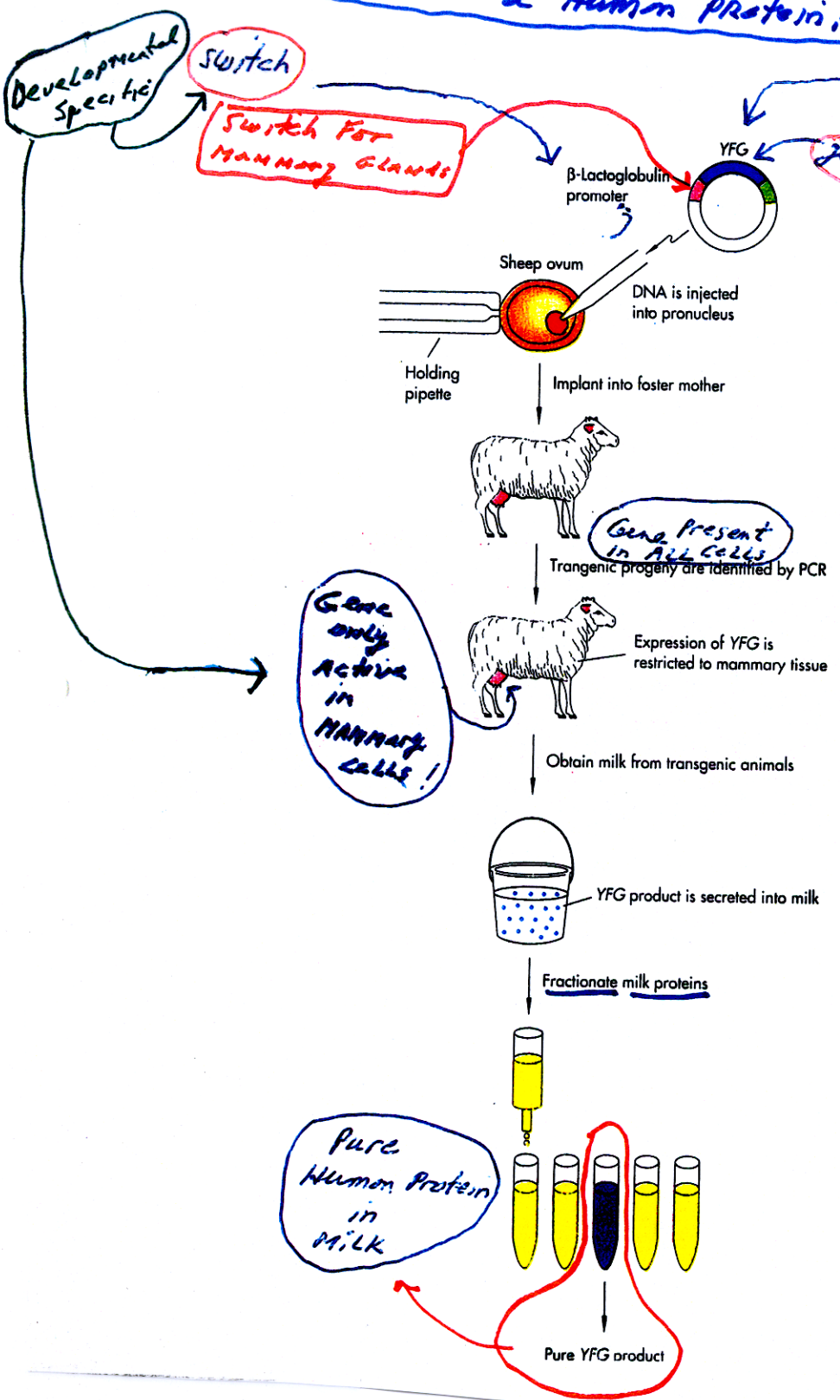
→ human protein in plant

D

The Limit?

There are NO LIMITS!

How to Engineer Goat's Milk to contain a Human protein!



Questions?

1. How identify presence of gene in sheep?
2. How Engineer to be Active only in Mammary Cells?
3. How can human gene work in sheep?
4. How does this experiment show DNA is genetic material?

CAN ANIMAL GENES BE ENGINEERED TO WORK IN PLANTS?

which kingdom switch?

Note)

plant switch

developmental specific

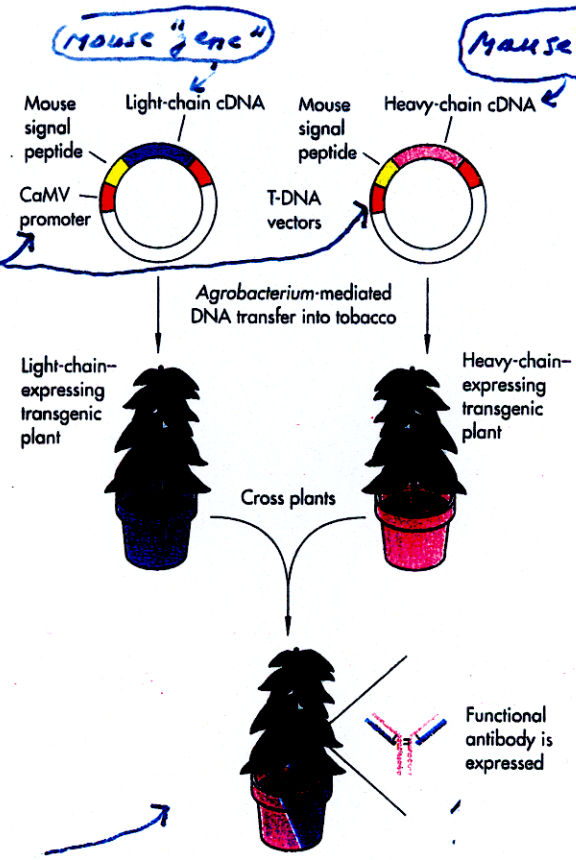


FIGURE 24-5
Plants as bioreactors to produce antibodies. Cloned cDNAs encoding the light and heavy chains from a mouse monoclonal antibody were ligated into separate T-DNA vectors and placed under control of a constitutive CaMV promoter. The plasmids were transferred separately into tobacco plants by *Agrobacterium* infection. Transgenic plants containing the light- and heavy-chain genes were sexually crossed to produce progeny plants that contained both genes. Examination of protein extracted from leaves demonstrated the expression of functional antibody molecules in these progeny plants. Other experiments showed that the presence of a signal sequence was necessary for high-level expression. These results suggest that the plant secretion machinery can recognize the mouse signal peptide.

Mouse protein in Leaves of PLANT!

QUESTIONS?

① How identify gene in plant?

② How Engineer to be active in Leaves of plant?

③ What does this experiment tell us about genetic processes + gene + protein synthesis?

IMPORTANT CONCEPT!

Yo! It's ALL in the Sequences!!

DNA, Gene, Switch, ori, mRNA, Protein!

NO HOCUS POCUS!

What does this IMPLY for BIOLOGY & Genetic Engineering!