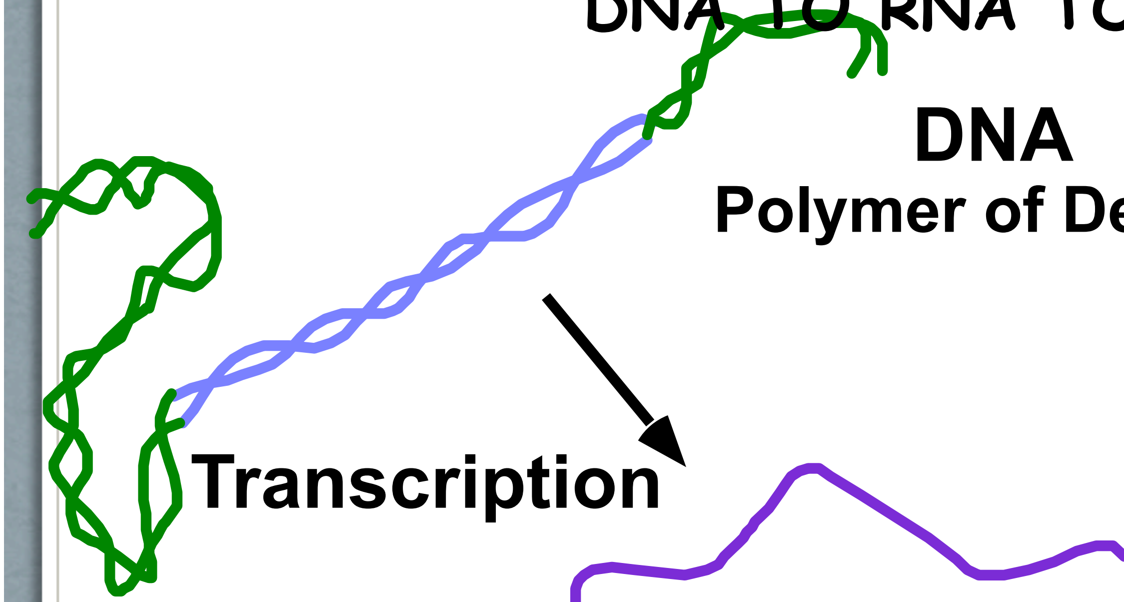
Lecture 6

RNA Structure

April 14, 2016
Pyle

BIOLOGICAL INFORMATION FLOWS FROM DNA TO RNA TO PROTEIN



DNA

Polymer of Deoxy-Nucleotides

Transcription

RNA

Polymer of Ribonucleotides

Translation

PROTEIN

Polymer of Amino Acids

Nucleotide sequence of gene determines protein sequence.

Protein sequence determines folded structure and hence function.

Location of Players involved in the Flow of Cellular Information

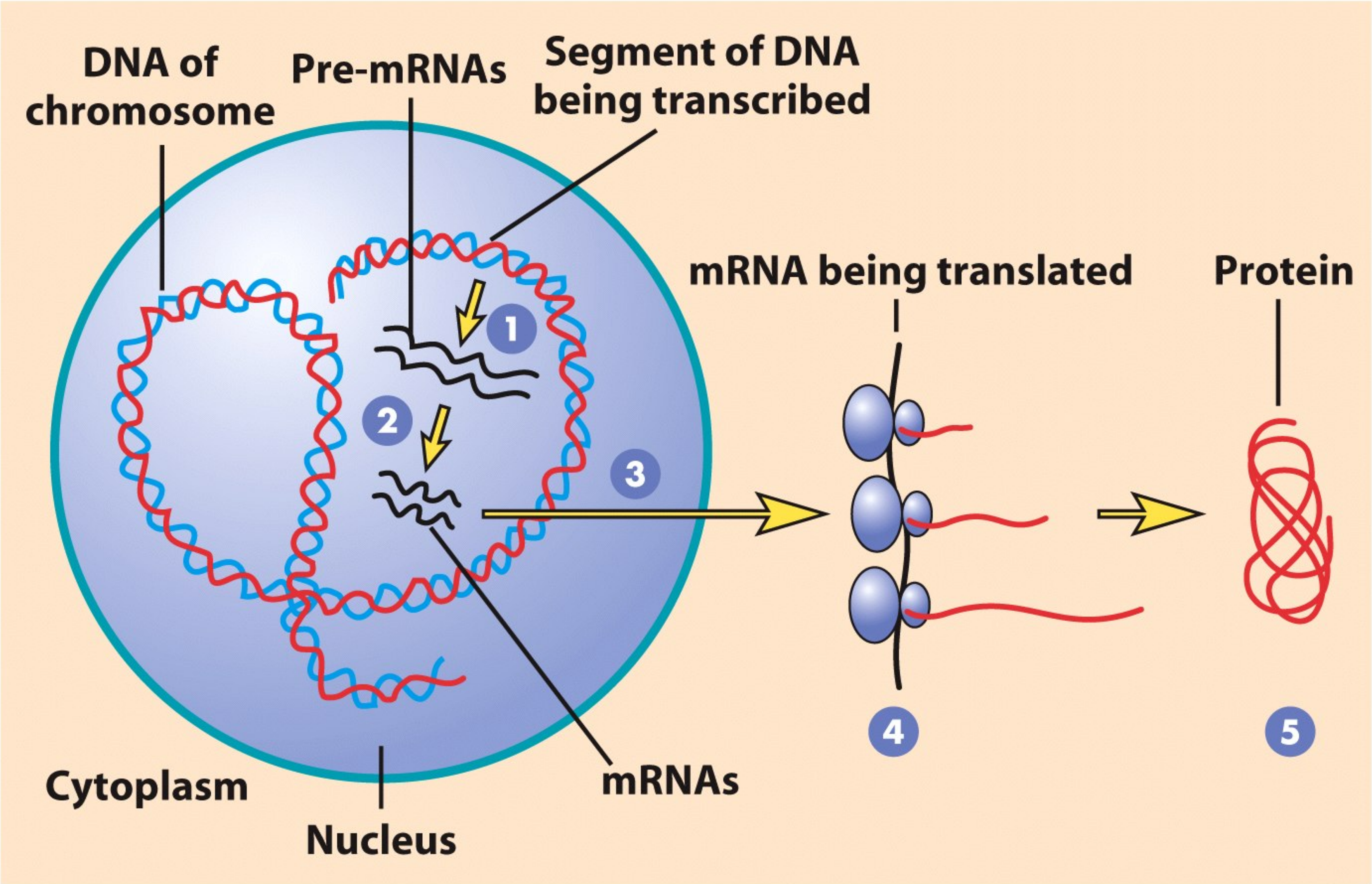
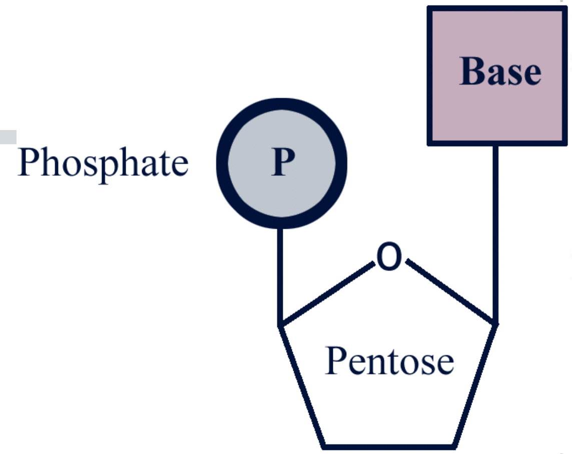


Figure 11-2 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Building blocks of RNA and DNA



Nucleoside

pentose + base

Pyrimidine type (one-ring):

C (cytidine), T (thymidine in DNA),

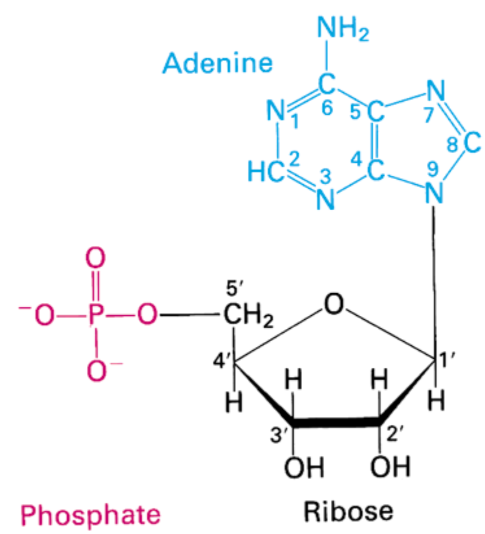
U (uridine, in RNA)

Purine type (two-ring):

A (adenosine), G (guanosine),

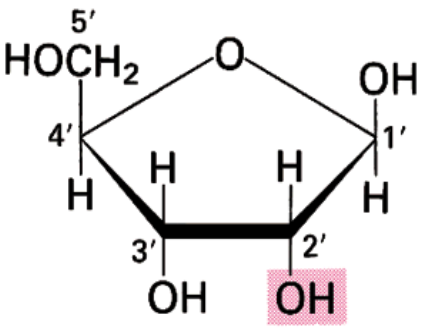
Nucleotide:

nucleoside + phosphate



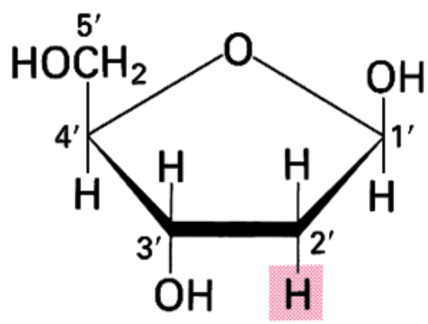
Adenosine 5′-monophosphate (AMP)

Pentose



Ribose

(in RNA)

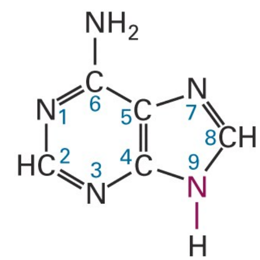


2-Deoxyribose

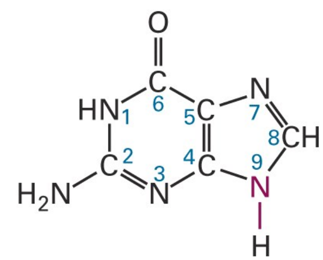
(in DNA)

Bases

PURINES

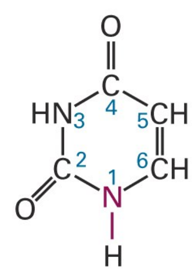


Adenine (A)



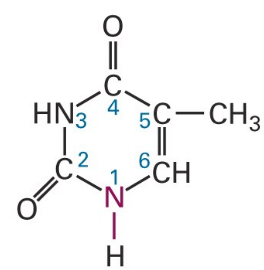
Guanine (G)

PYRIMIDINES



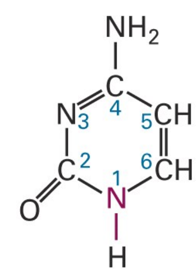
Uracil (U)

RNA



Thymine (T)

DNA



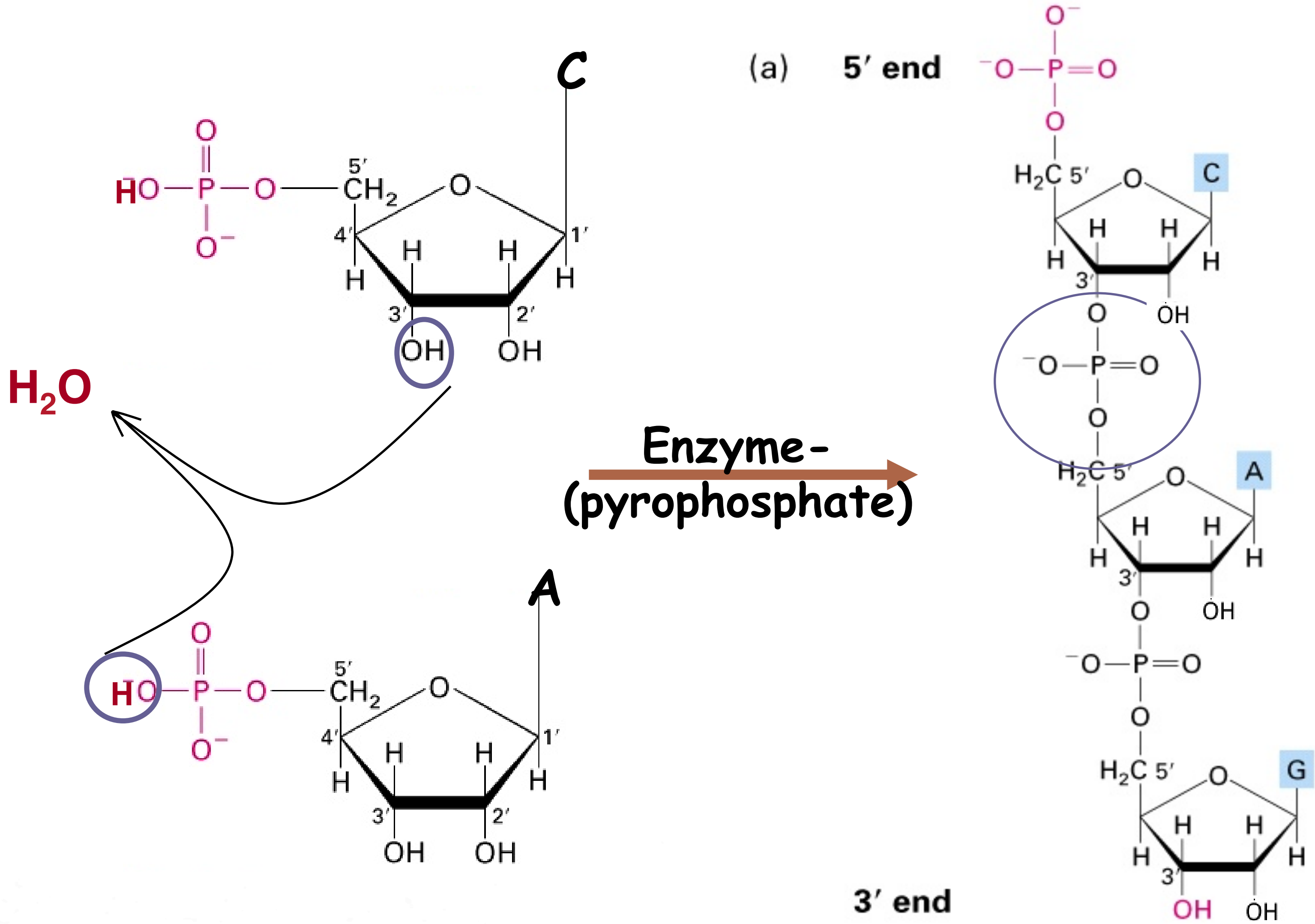
Cytosine (C)

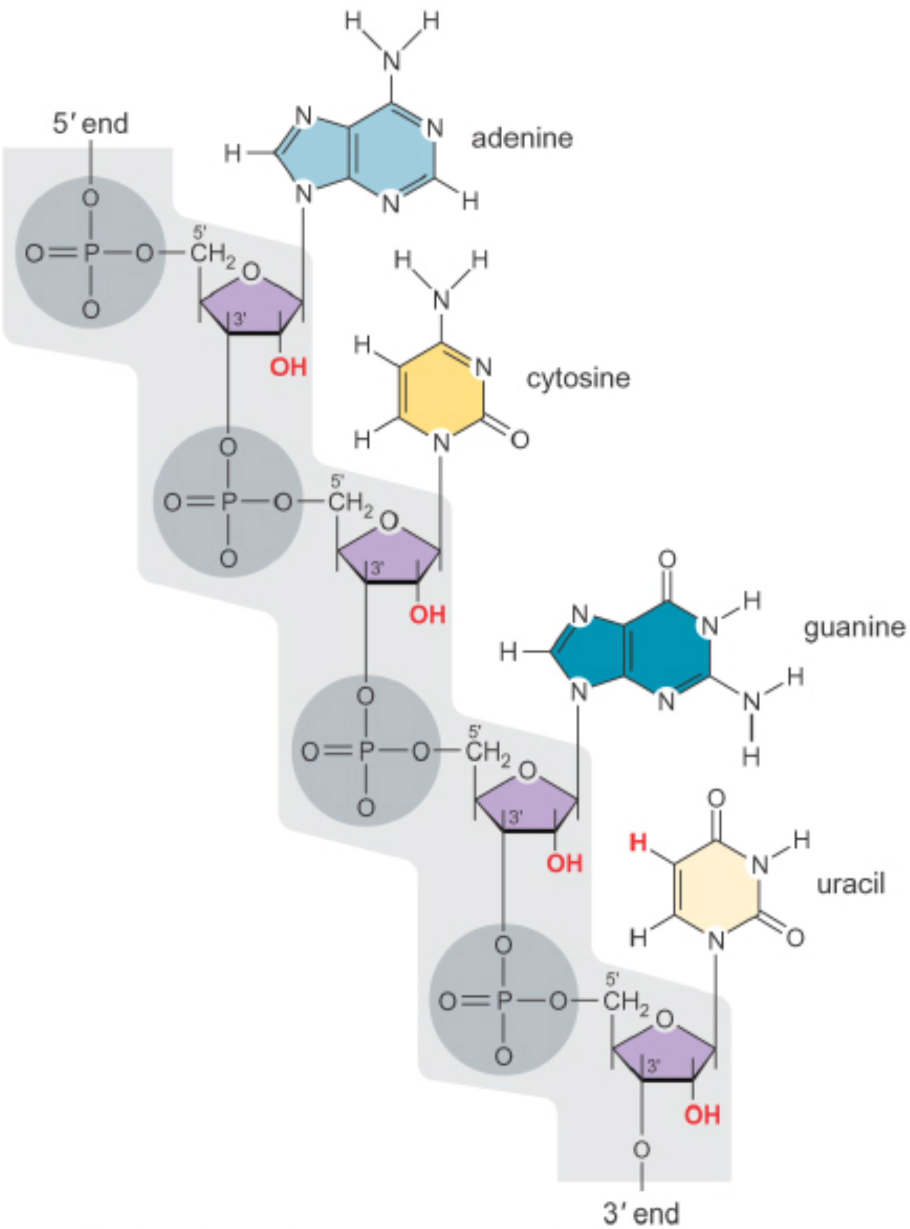
Nomenclature of DNA and RNA

TABLE 2-2 Terminology of Nucleosides and Nucleotides

		Bases			
		Purines		Pyrimidines	
		Adenine (A)	**Guanine** (G)	**Cytosine** (C)	**Uracil** (U) **Thymine** [T]
Nucleosides	in RNA	Adenosine	Guanosine	Cytidine	Uridine
	in DNA	Deoxyadenosine	Deoxyguanosine	Deoxycytidine	Deoxythymidine
Nucleotides	in RNA	Adenylate	Guanylate	Cytidylate	Uridylate
	in DNA	Deoxyadenylate	Deoxyguanylate	Deoxycytidylate	Deoxythymidylate
Nucleoside monophosphates		AMP	GMP	CMP	UMP
Nucleoside diphosphates		ADP	GDP	CDP	UDP
Nucleoside triphosphates		ATP	GTP	CTP	UTP
Deoxynucleoside mono-, di-, and triphosphates		dAMP, etc.			

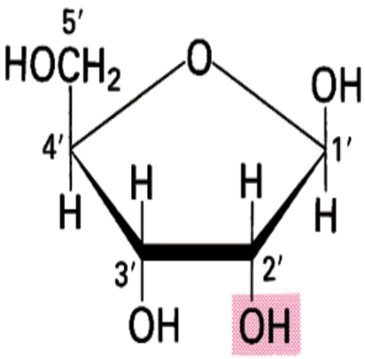
Formation of the phosphodiester bond by a condensation reaction, in which one H_2O is removed



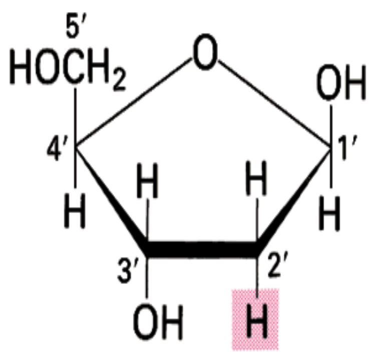


RNA is a single strand polynucleotide composed of 4 ribonucleotides: A, C, G, U.

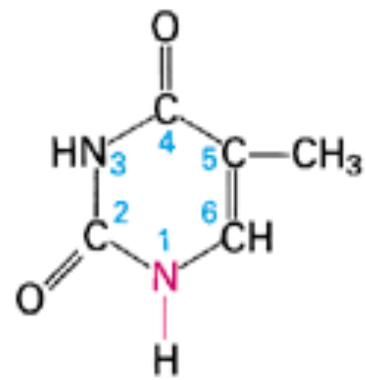
RNA and DNA



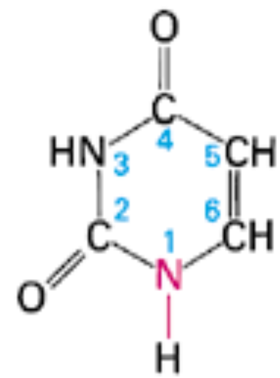
Ribose



2-Deoxyribose



Thymine (T)



Uracil (U)

RNA **DNA** **DNA** **RNA**

ssDNA is stable in alkaline solution, but RNA is not. Why?

The 2’-OH group makes RNA unstable in alkaline conditions, because it can attack the phosphodiester bonds of the RNA chain and break RNA into small pieces or nucleotide monomers

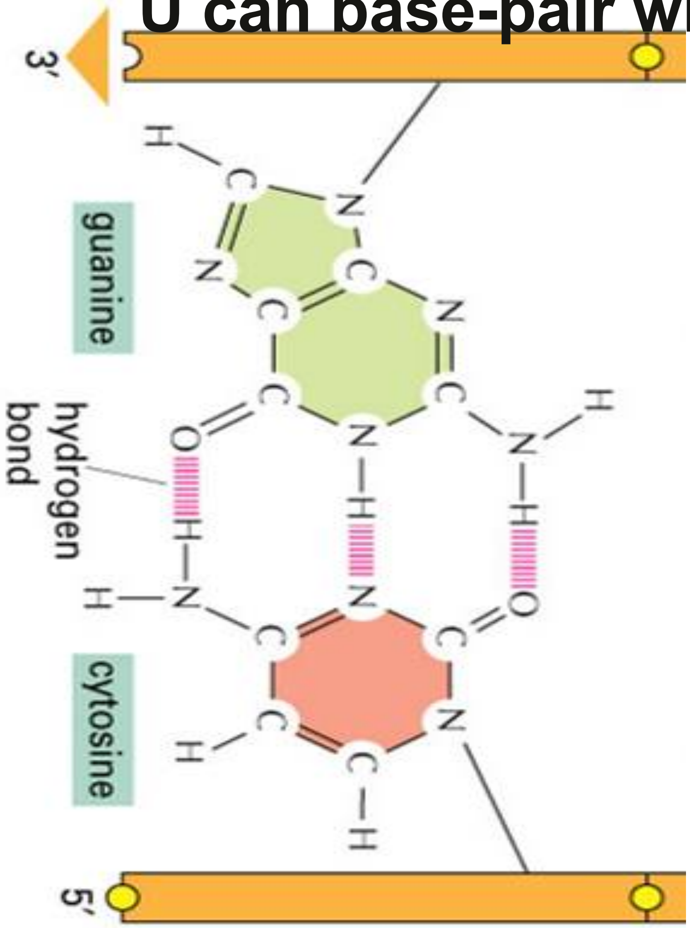
Comparison between RNA and DNA

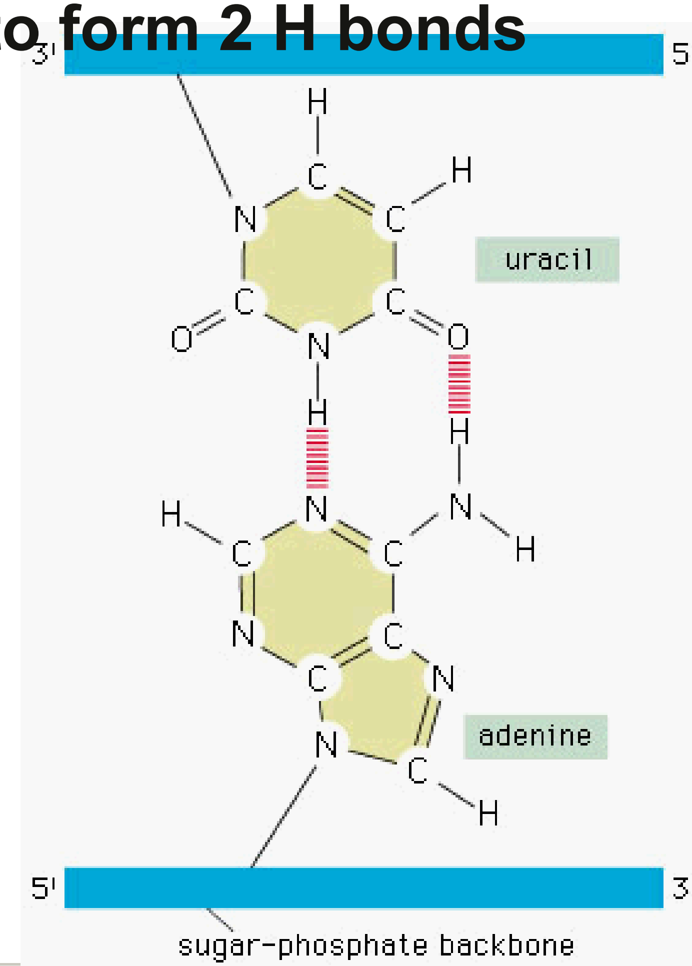
	DNA	RNA
Sugar	Deoxyribose (**2' -H**)	Ribose (**2' –OH**)
base	A, C, G, **T**	A, C, G, **U**
structure	double strand	single strand
Size	large (kb-Mb)	Small (bp-kb)
type	DNA	mRNA, tRNA, rRNA, snRNA
Stability in alkaline	Stable-dsDNA denatured at high pH but it is not hydrolyzed	Unstable-Hydrolyzed to nucleotides

A single strand RNA molecule can form short stretches of double strands when there are enough intra-molecule complementary base-pairing.

G can base-pair with C to form 3 H bonds

U can base-pair with A to form 2 H bonds





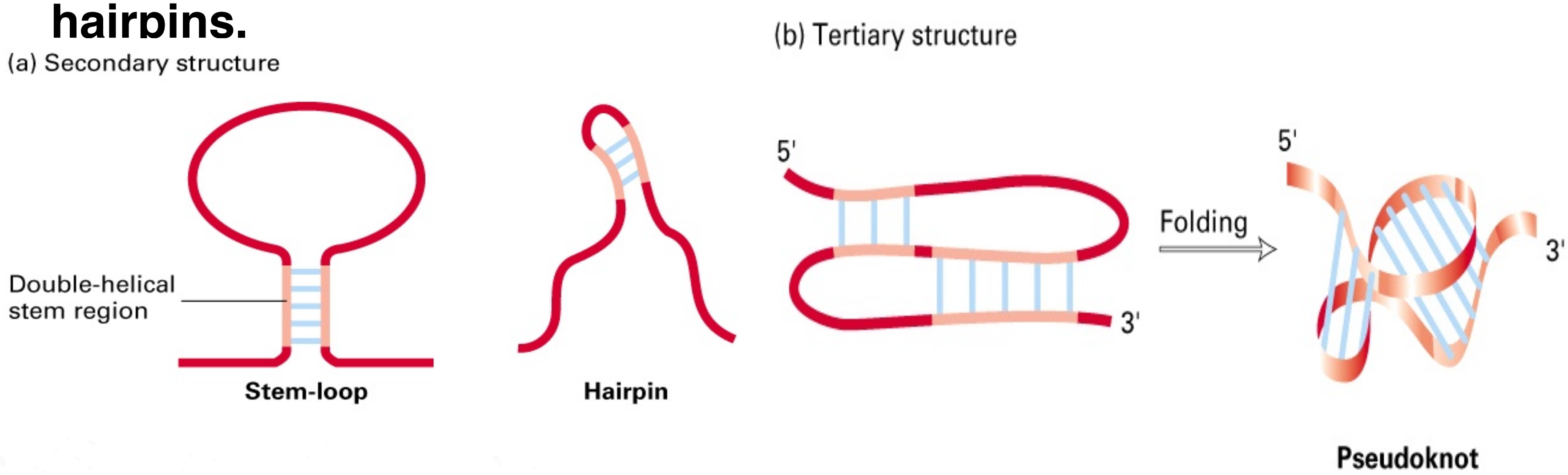
RNA can have 3-D structures

1. Secondary structure

The secondary structures are stem-loops and hairpins, which are formed through intra-molecule complementary base-pairing (A::U, C:::G).

2. Tertiary structures

The tertiary structures are 3-D structures such as pseudoknots found in tRNA and rRNA, formed by folding of stem-loops and hairpins.



Major types of RNA in the cell

1. **mRNA**:

   message RNA—used as template for protein translation, usually a linear structure

2. **tRNA**

   transfer RNA—used to bring amino acids for translation reaction. tRNA has an unique clover-leaf like structure

3. **rRNA**

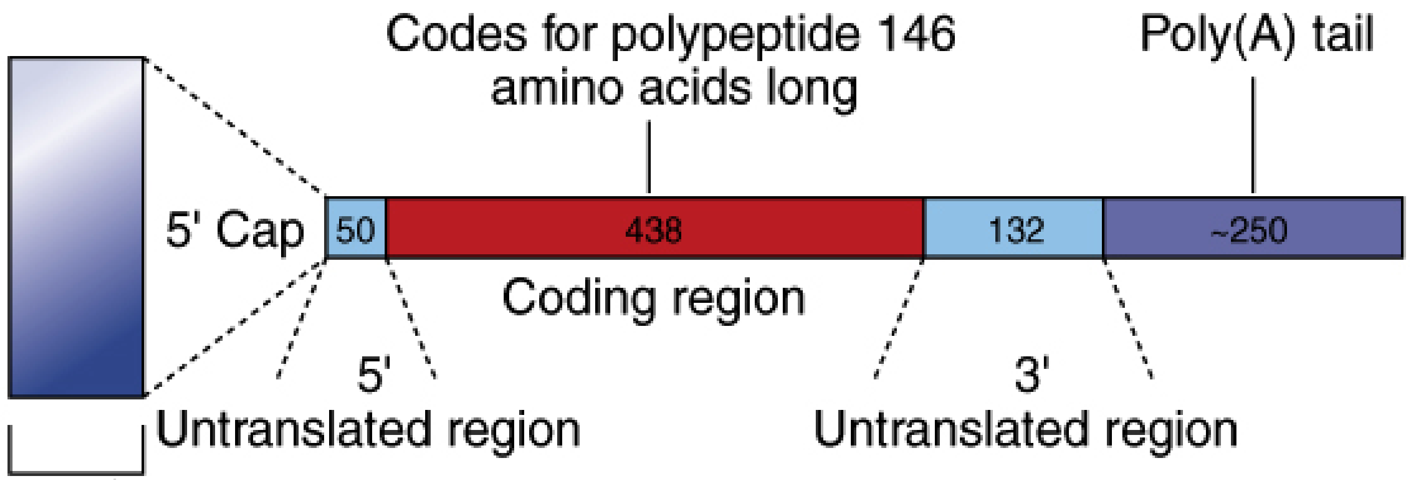
   ribosomal RNA—rRNA always associate with proteins to form ribosomes. Ribosomes are the protein synthesis apparatus.

4. **Other types of RNAs**

   hnRNA — heterogenous nuclear RNA-stability, processing
   snRNA — small nuclear RNA-processing
   miRNA — micro RNA-regulation
   siRNA — small interfering RNA -regulation

mRNA

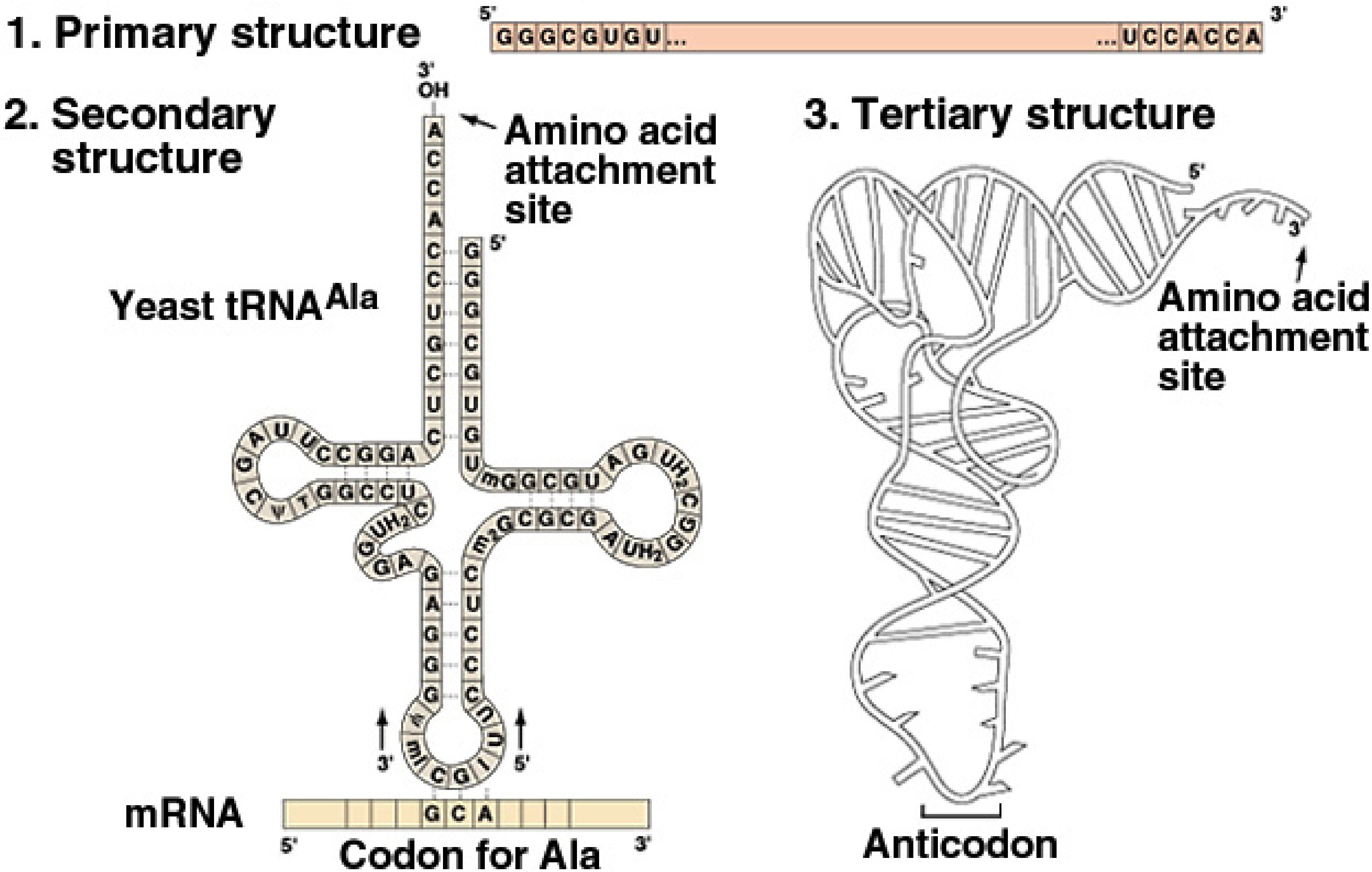
1. An eukaryotic mRNA contains sequences encoding a specific polypeptide (ORF=open reading frame).
2. A mRNA also contains noncoding regions at 3’ and 5’ ends, which help translation.
3. mRNAs are mainly in cytosol, where translation occurs.
4. An eukaryotic mRNA has special modifications that are 5’ cap and 3’ polyA, neither modifications are found in prokaryotic mRNA or other types of eukaryotic RNAs.



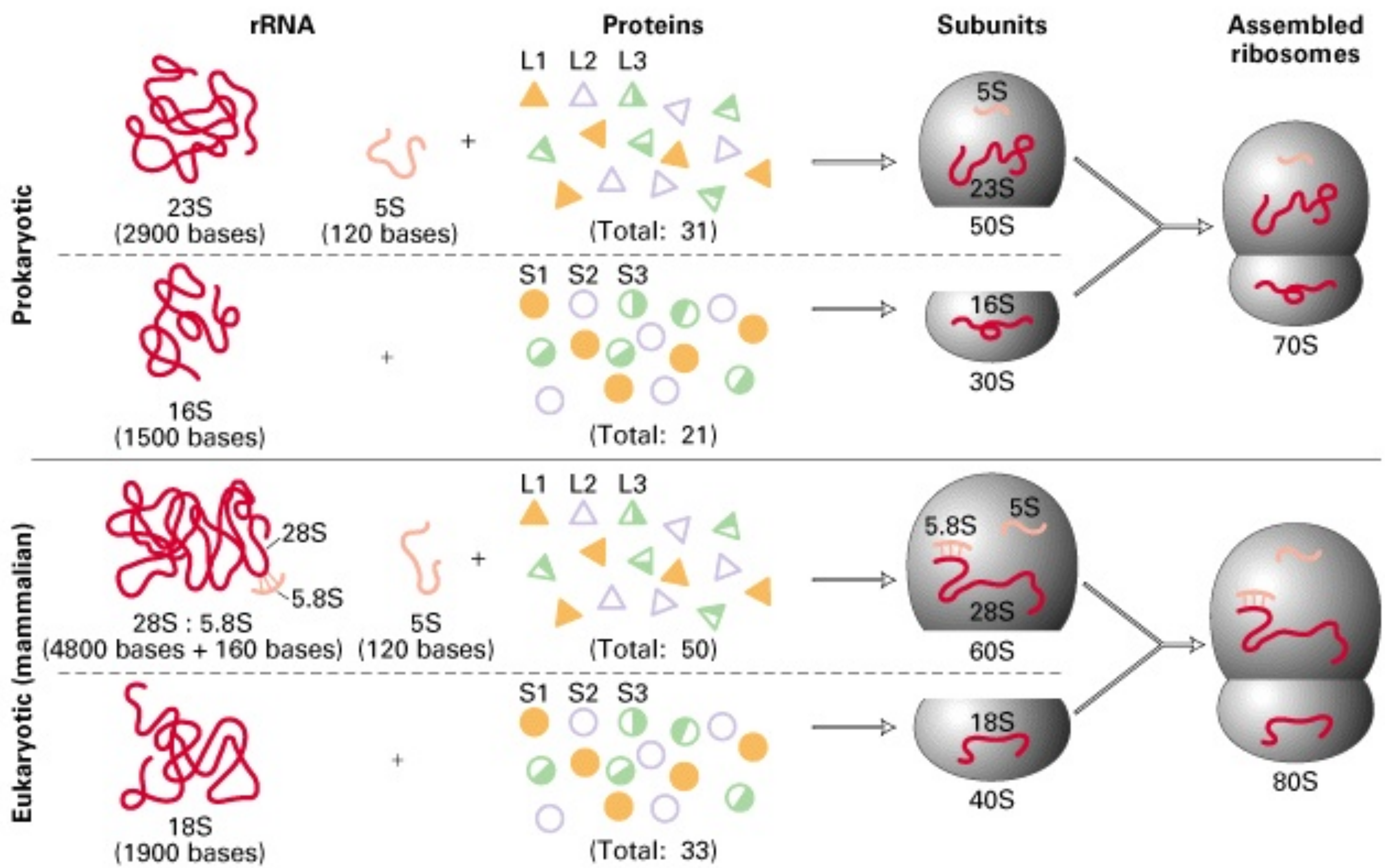
An example of a mature mRNA

tRNA structure

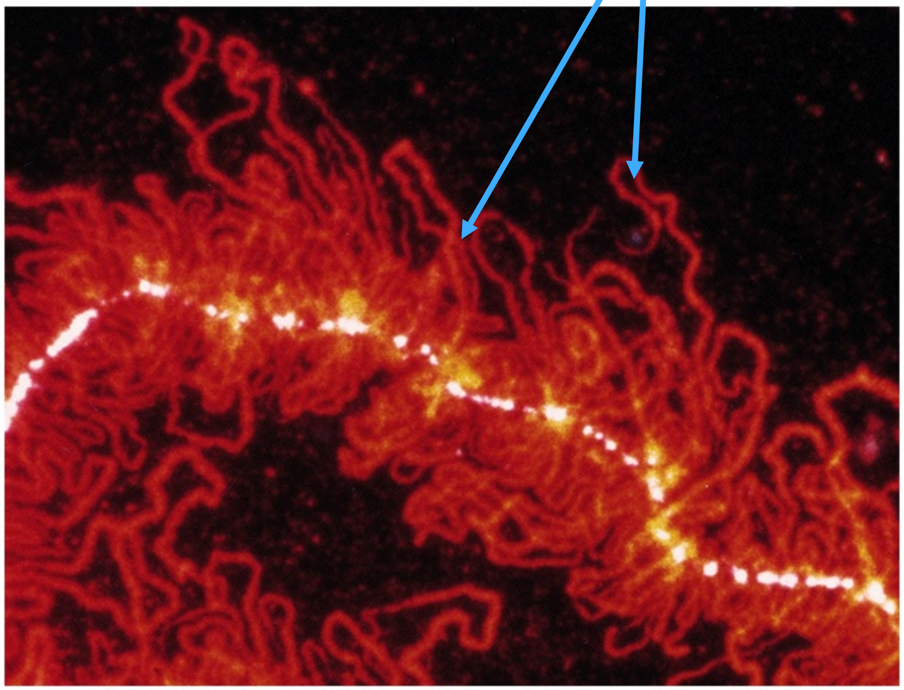
(b)Each tRNA has a primary, secondary, and tertiary structure



rRNAs as part of ribosomes



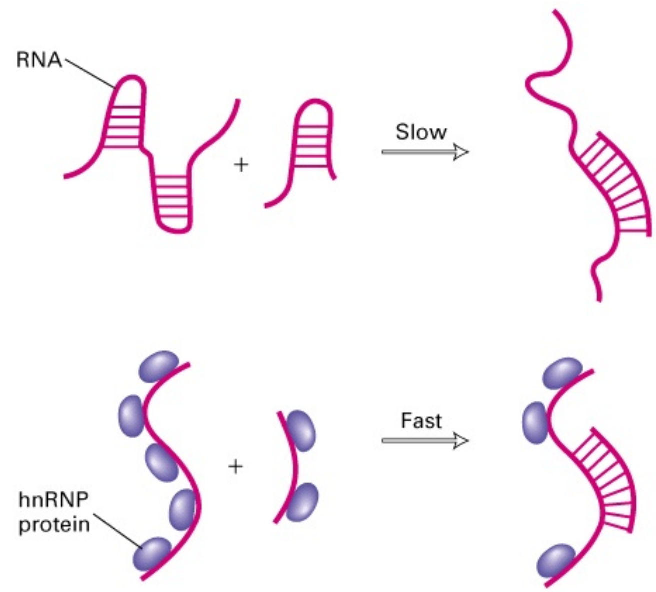
Newly transcribed RNA bound by hnRNP



hnRNA and hnRNP

hnRNA:
heterogenous nuclear RNA (pre-mRNA, snRNAs, etc)

hnRNP: heterogenous ribonucleoprotein particle (hnRNAs+proteins)



As soon as a RNA is made, it is bound by hnRNP that is a complex of different proteins (34-120 kD) and RNAs. Functions of hnRNPs include stabilization of ssRNA, RNA processing, and RNA transportation.

Advanced Information on The Nobel Prize in Physiology or Medicin 2006

Nobelförsamlingen
The Nobel Assembly at Karolinska Institutet

Karolinska Institutet

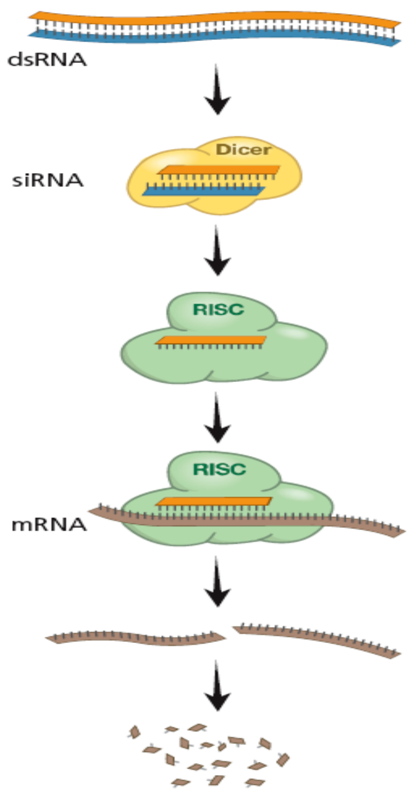
RNA INTERFERENCE

This year's Nobel Prize in Physiology or Medicine is shared by Professor Andrew Z. Fire at Stanford University, California, USA, and Professor Craig C. Mello at the University of Massachusetts Medical School in Worcester, USA. They receive the prize for their discovery that double-stranded RNA triggers suppression of gene activity in a homology-dependent manner, a process named RNA interference (RNAi). Their discovery revealed a new mechanism for gene regulation, and the biochemical machinery involved plays a key role in many essential cellular processes. Double-stranded RNA synthesized within the cell can reduce or abolish gene activity by RNAi-like mechanisms. This control system for gene expression has proven to be important for both the development of an organism and the physiological functions of cells and tissues. Furthermore, RNAi protects against RNA virus infections, especially in plants and invertebrate animals, and secures genome stability by keeping mobile elements silent. Today, double-stranded RNA is used as a powerful tool to experimentally elucidate the function of essentially any gene in a cell. The discovery of RNAi has already had an immense impact on biomedical research and will most likely lead to novel medical applications in the future.

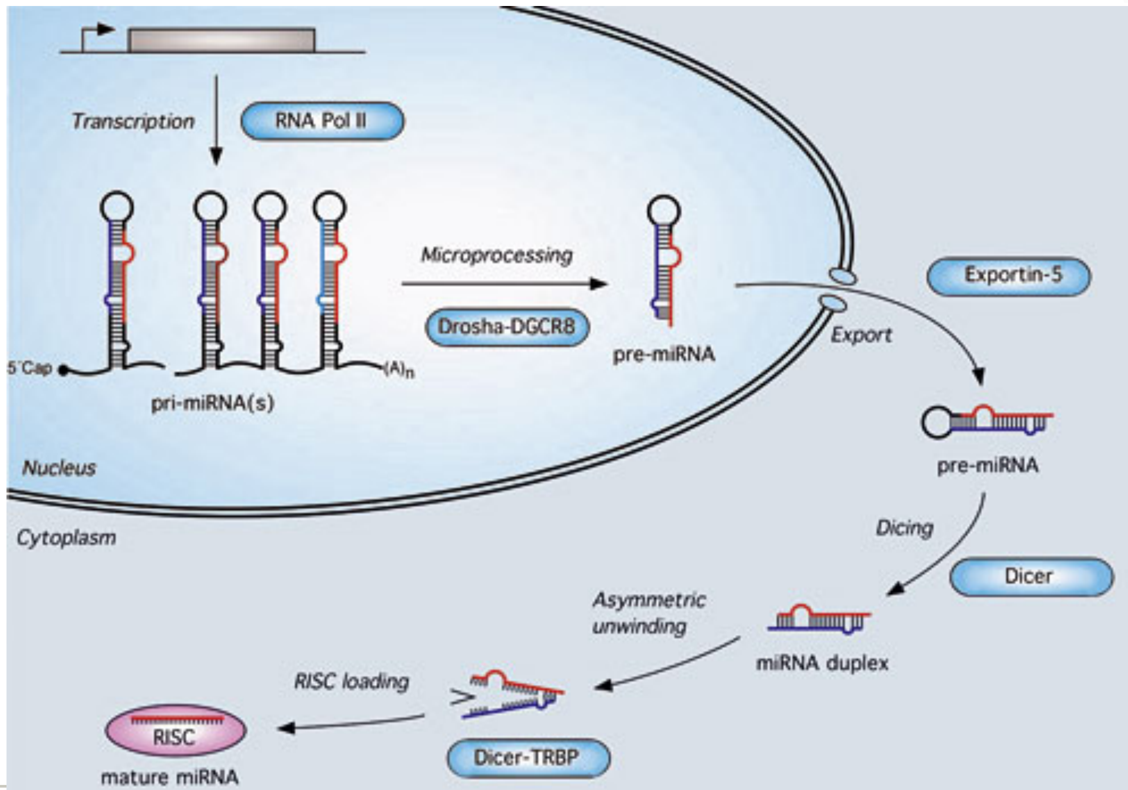
miRNA and siRNA

RNA interference (RNAi) is triggered by double-stranded RNA helices that have been introduced exogenously into cells as small interfering (si)RNAs or that have been produced endogenously from small non-coding RNAs known as microRNAs (miRNAs). miRNAs are regulators of endogenous genes, and siRNAs are defenders of genome integrity in response to foreign or invasive nucleic acids such as viruses, transposons, and transgenes.

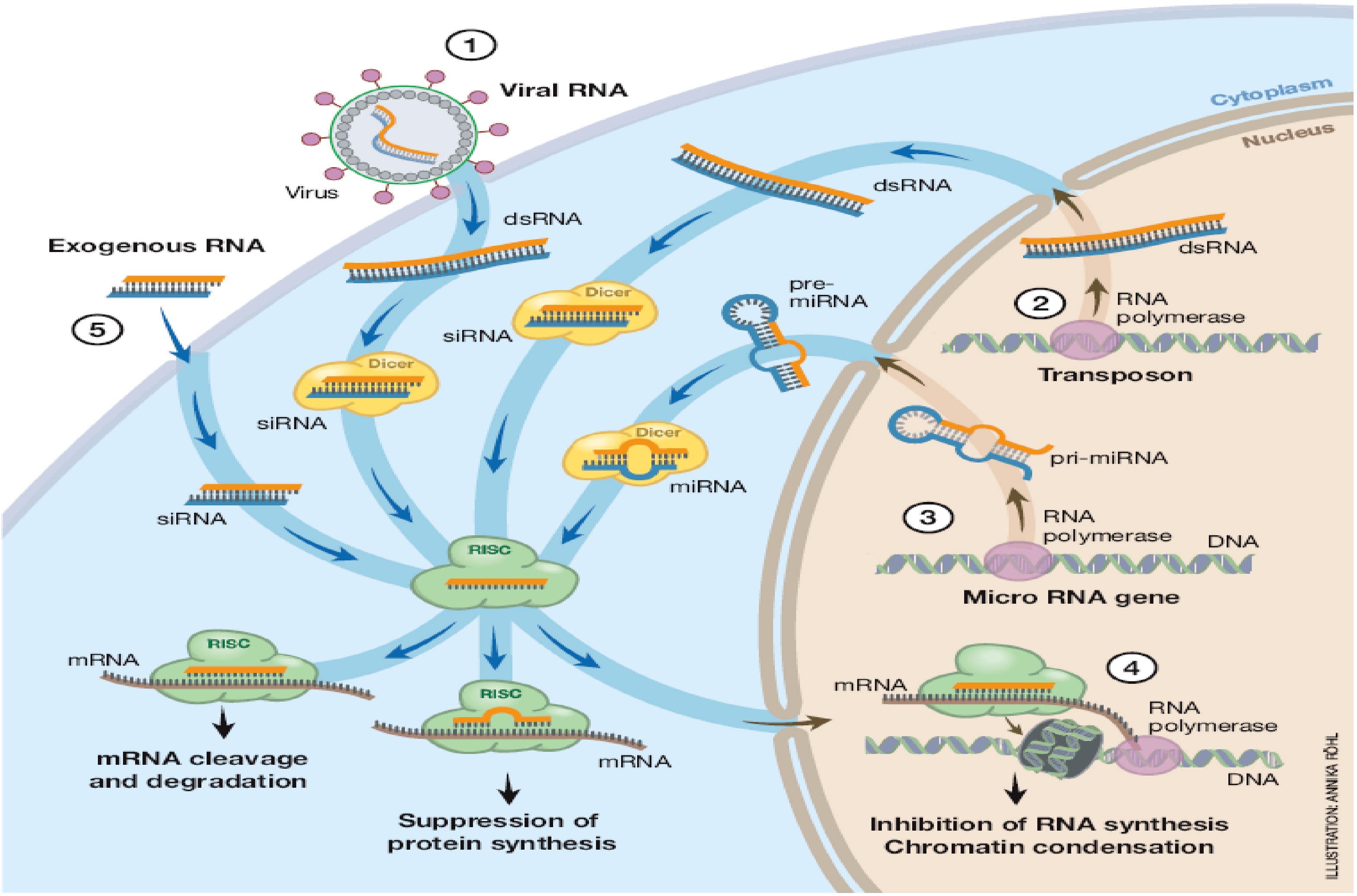
siRNA



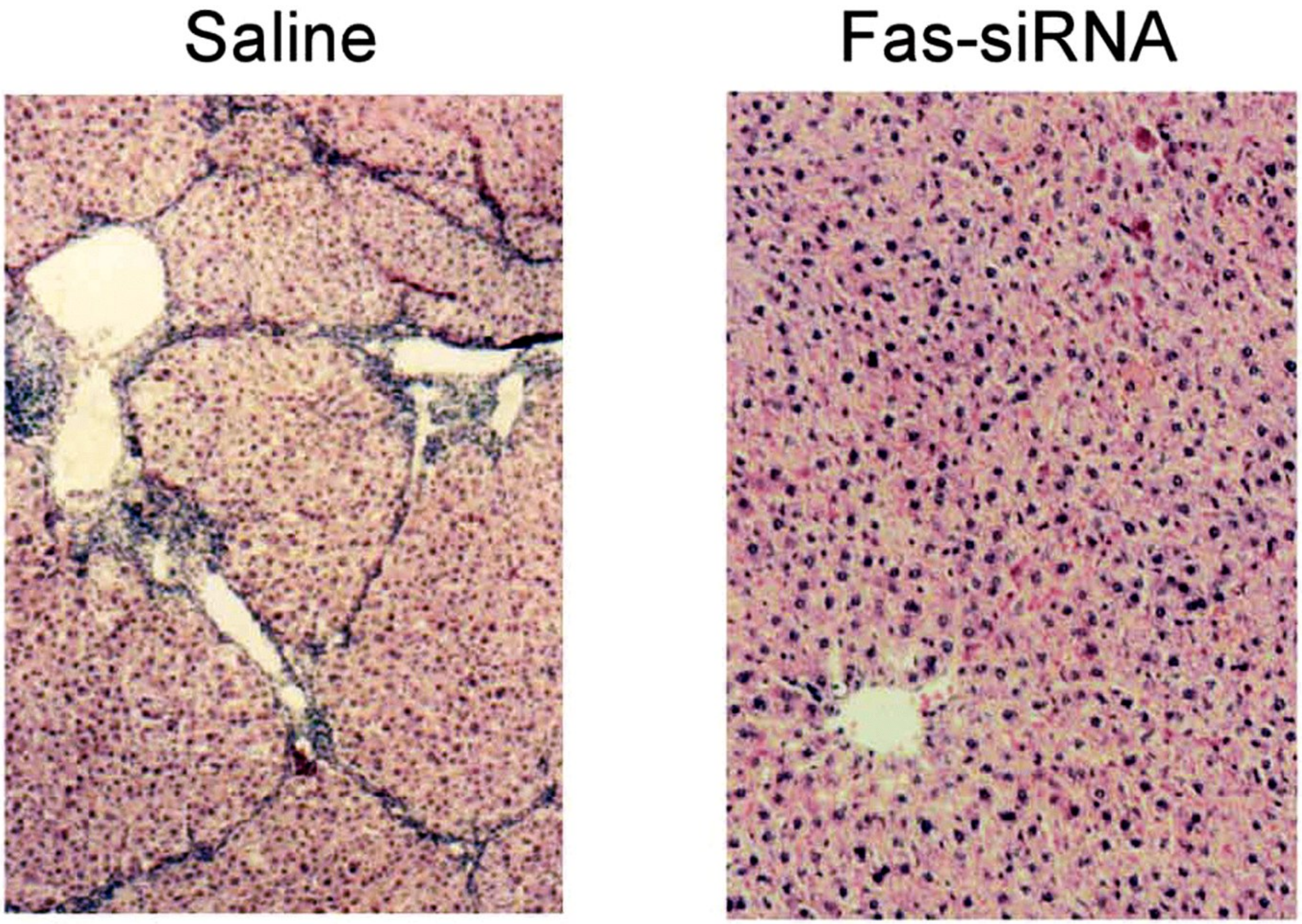
miRNA



RNAi has many different functions including regulation of gene expression and defense of viral infection. RNAi has also become a standard experimental tool and its therapeutic potential is being aggressively harnessed.



RNAi can be used as a Therapeutic Strategy



HP Figure 11-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

In this example, the liver is scarred with fibrosis (saline only). However treatment with siRNA that targets a cell death gene called *Fas* leads to normal histology (Fas-siRNA).

MicroRNAs are synthesized in tissue specific manner during development

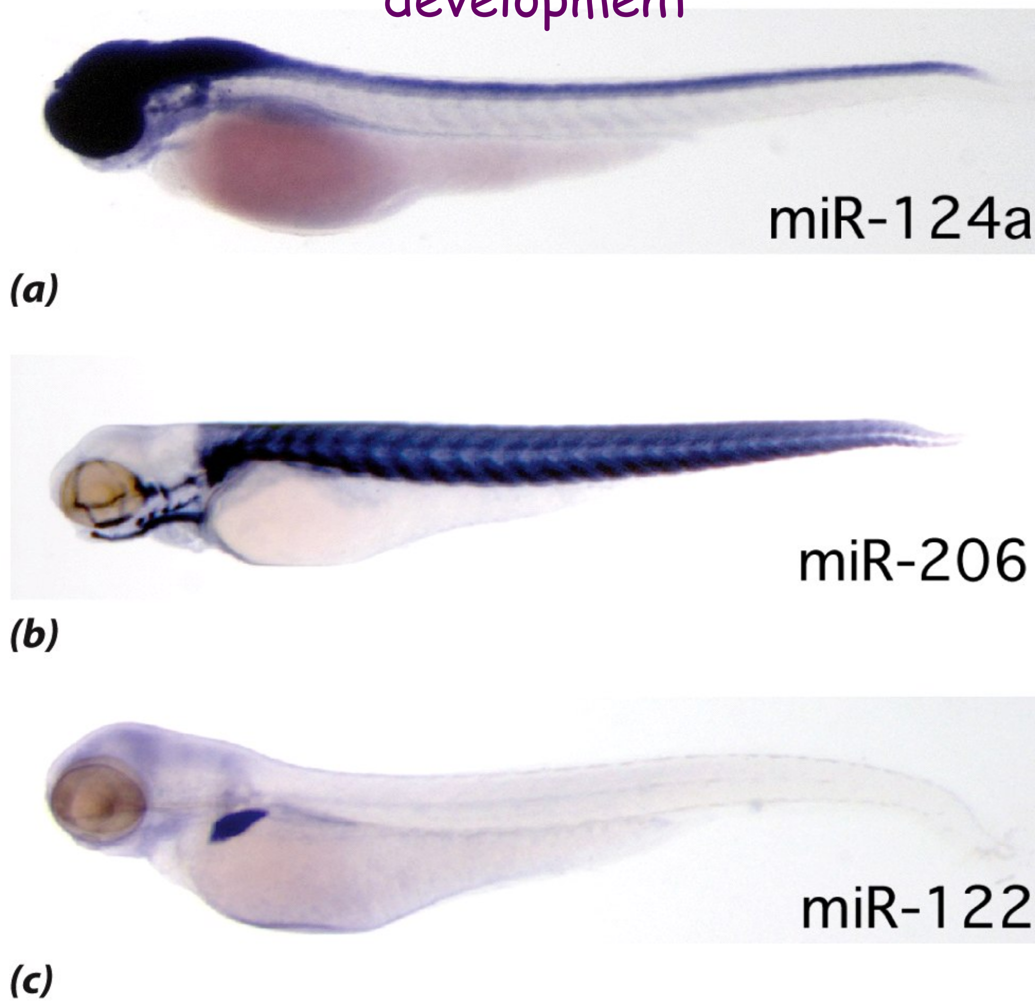
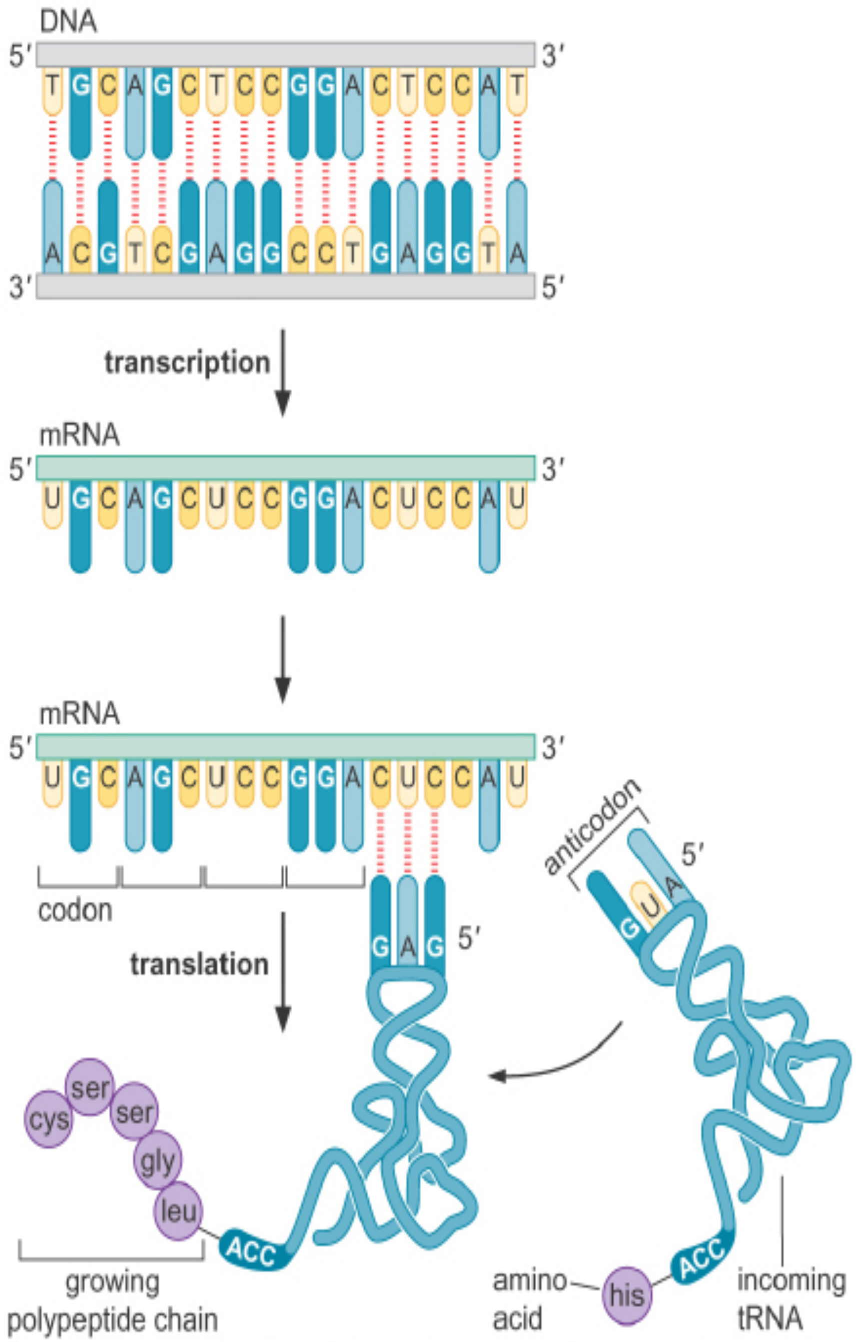


Figure 11-39 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

In this example, zebrafish embryos have specific expression of three different microRNAs during development: a. Nervous system b. Muscle c. Liver



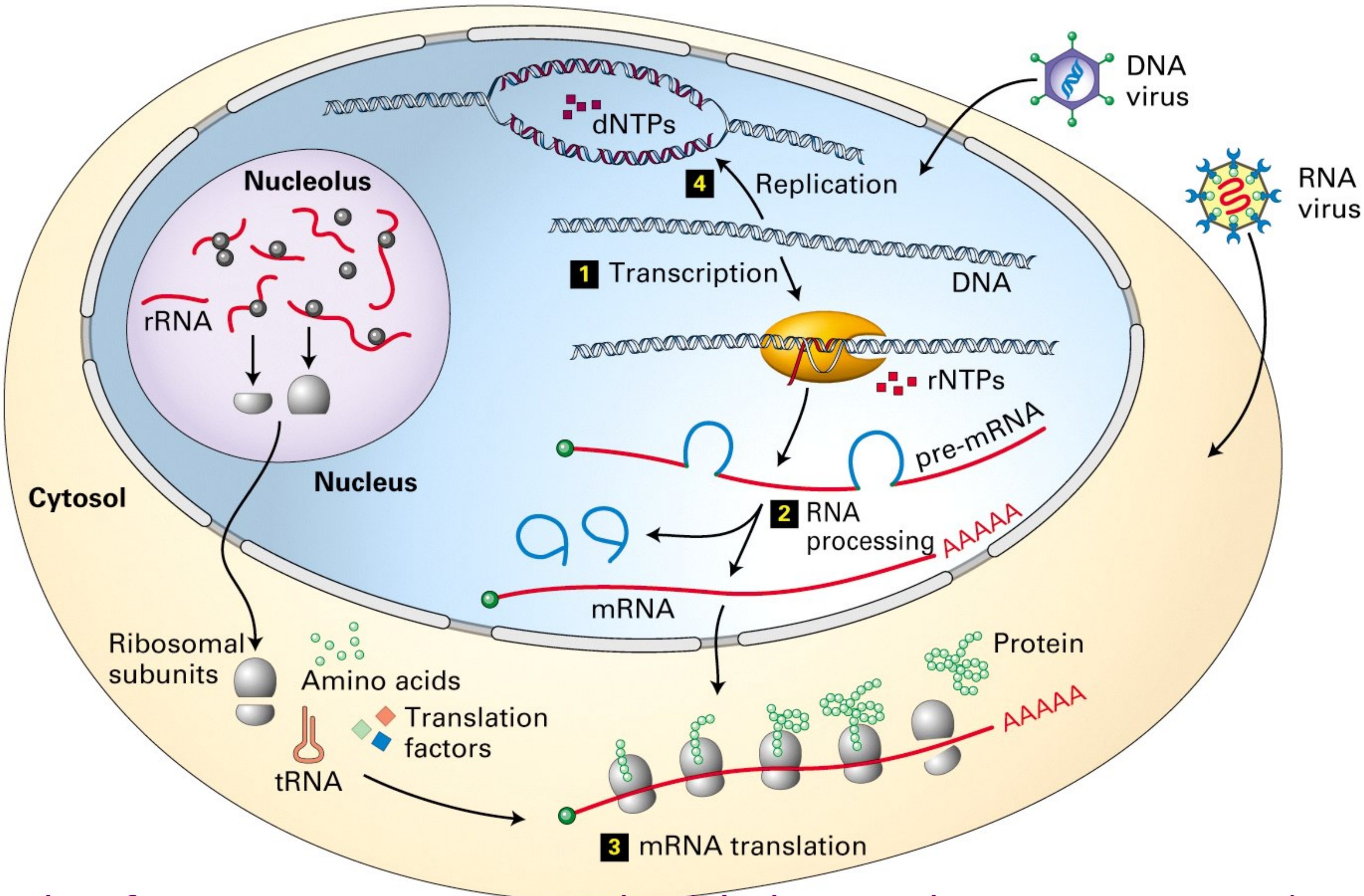
All cellular RNAs are made by transcription.

Transcription is a DNA-dependent RNA synthesis process, it is catalyzed by the RNA polymerase

Only one of the two strands of DNA are copied into RNA for a given gene.

The sequence of nucleotides in the DNA determines the sequence of nucleotides in the RNA, which determines the sequence of amino acids in the protein

How is genetic information contained in the DNA processed into usable information?



The first experiments to shed light on this process used a technique commonly referred to as a Pulse-Chase Experiment.

New technologies arising from improved understanding or RNA!

https://www.youtube.com/watch?v=FL-3qIme-Hw&feature=youtu.be

Radioisotopes

Isotopes:
Atoms that contain the same number of protons but different number of neutrons

Radioisotopes:
Isotopes that can decay and emit electromagnetic radiation

two isotopes of hydrogen:

radioactive

^{1}H ^{3}H

Non radioactive

Radioisotopes often used in molecule biology:

$^{31}P/^{32}P$, $^{32}S/^{35}S$, $^{12}C/^{14}C$,

Radioactive isotopes (radioisotope) are widely used in molecular biology studies

- because they are physically distinguishable but chemically identical from each other.

- Presence of various particles emitted during decay of a radioisotope can be detected by:

  - Geiger counter (monitor)
  - exposure to X-ray film (autoradiography)

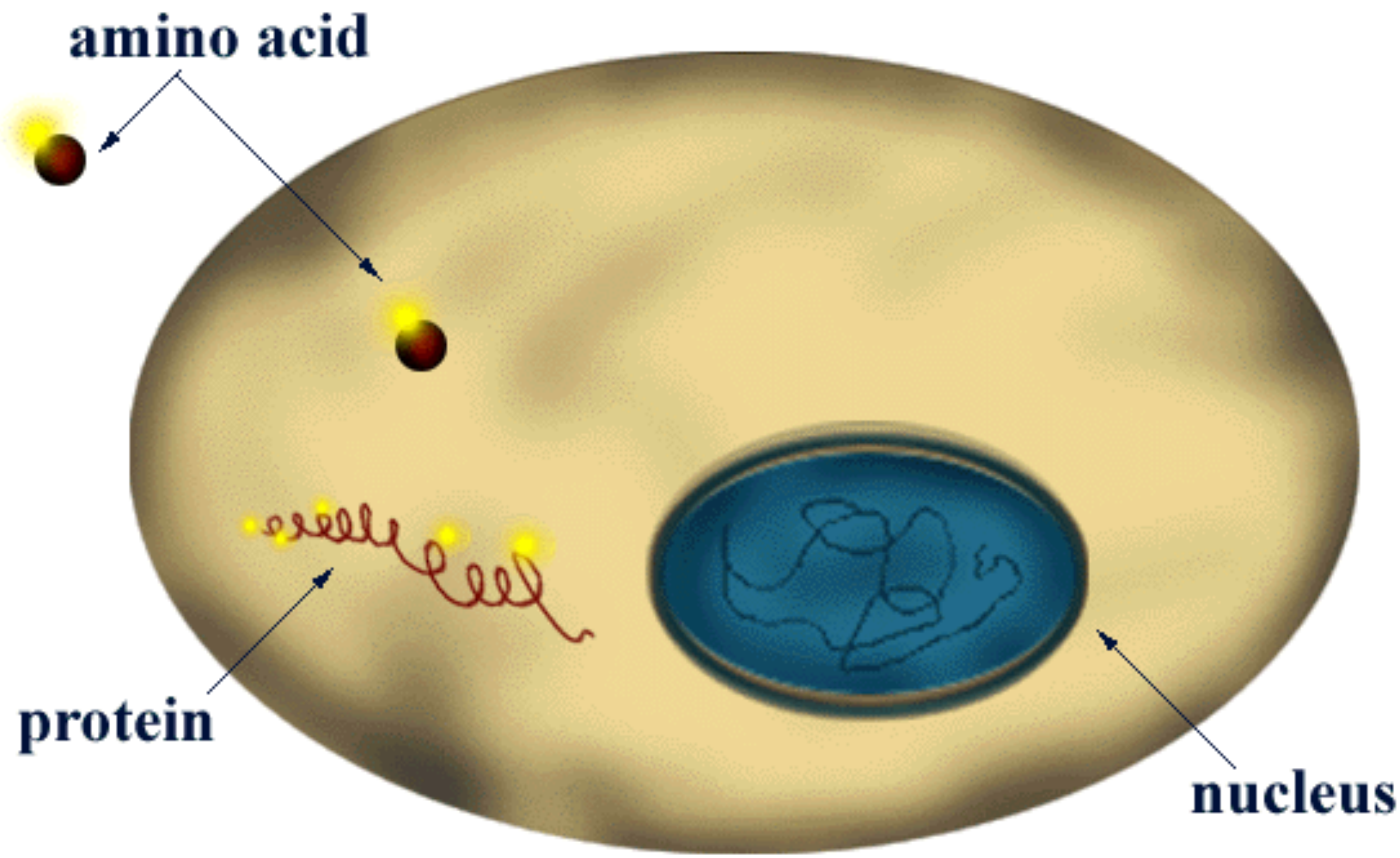
Pulse-Chase Experiments

- Pulse - short exposure to labeled precursors

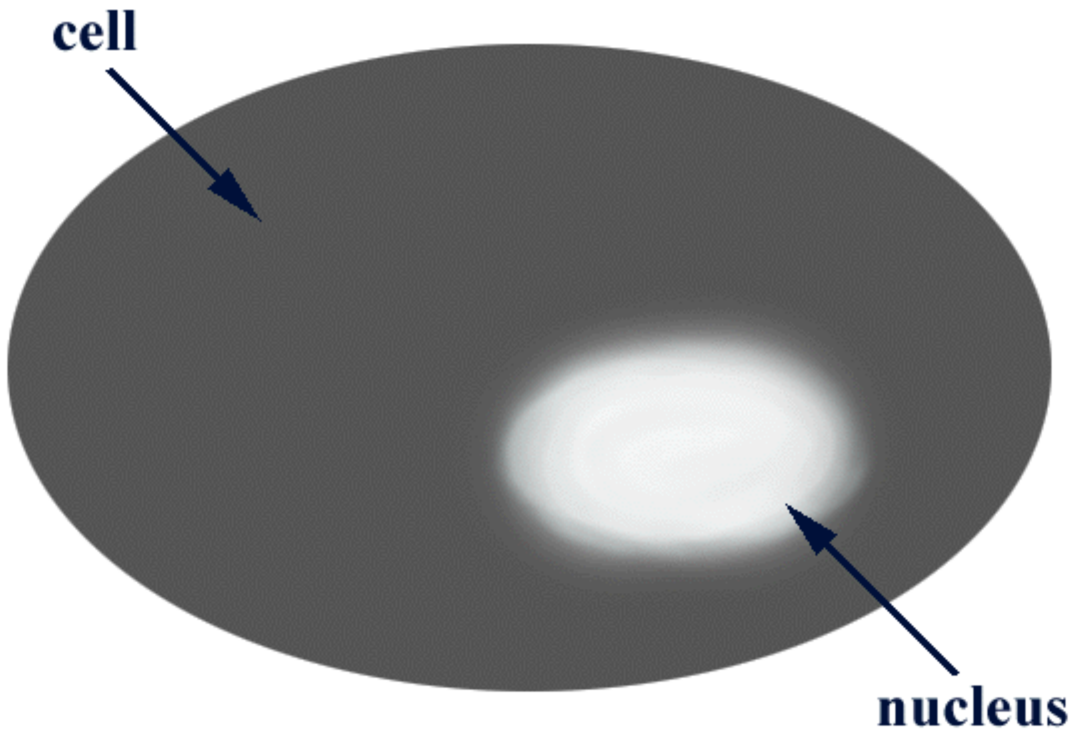
•Synthesis of DNA, RNA or protein involves the polymerization of precursor molecules (nucleotides or amino acids).

•If these precursors are radioactively labeled and supplied to cells in a culture media, then the cells will transport them into the cell and use them to synthesize the macromolecule.

•If cells are examined immediately for the location of the radioactivity, the site of synthesis can be determined.



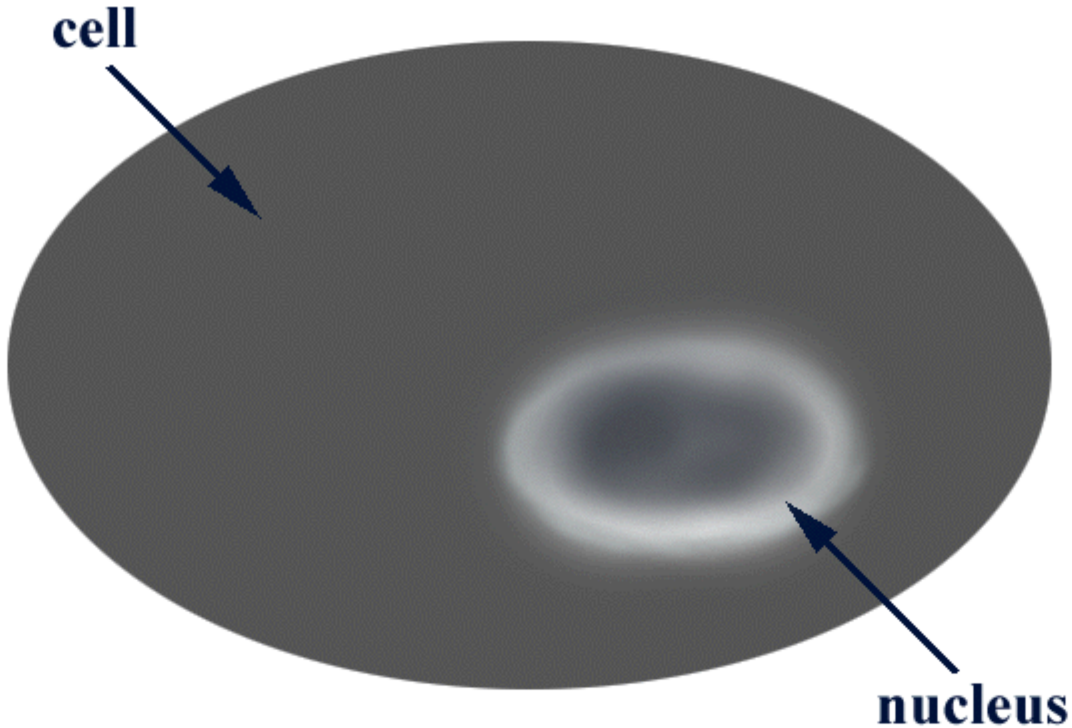
Position of macromolecules in cell are fixed and free *aa are washed away. Autoradiogram detects location of labeled protein.



X-ray film is dark where radioactive emissions are detected. In this experiment, protein synthesis is detected in the cytoplasm but not the nucleus.

The Chase Experiment

- Wash or dilute out label and allow the cell to continue growing for a period.
- During this period no new incorporation of radioactive precursor in macromolecule occurs; however, macromolecules may move around in the cell (determines final location of macromolecule).



Presence of radioactivity in nucleus suggests proteins move into nucleus after they are synthesized in the cytoplasm.

3 Pulse-Chase experiments done in eukaryotic cells

1: ^{35}S-methionine - protein
2: ^{3}H-uracil - RNA
3: ^{3}H -thymine -DNA

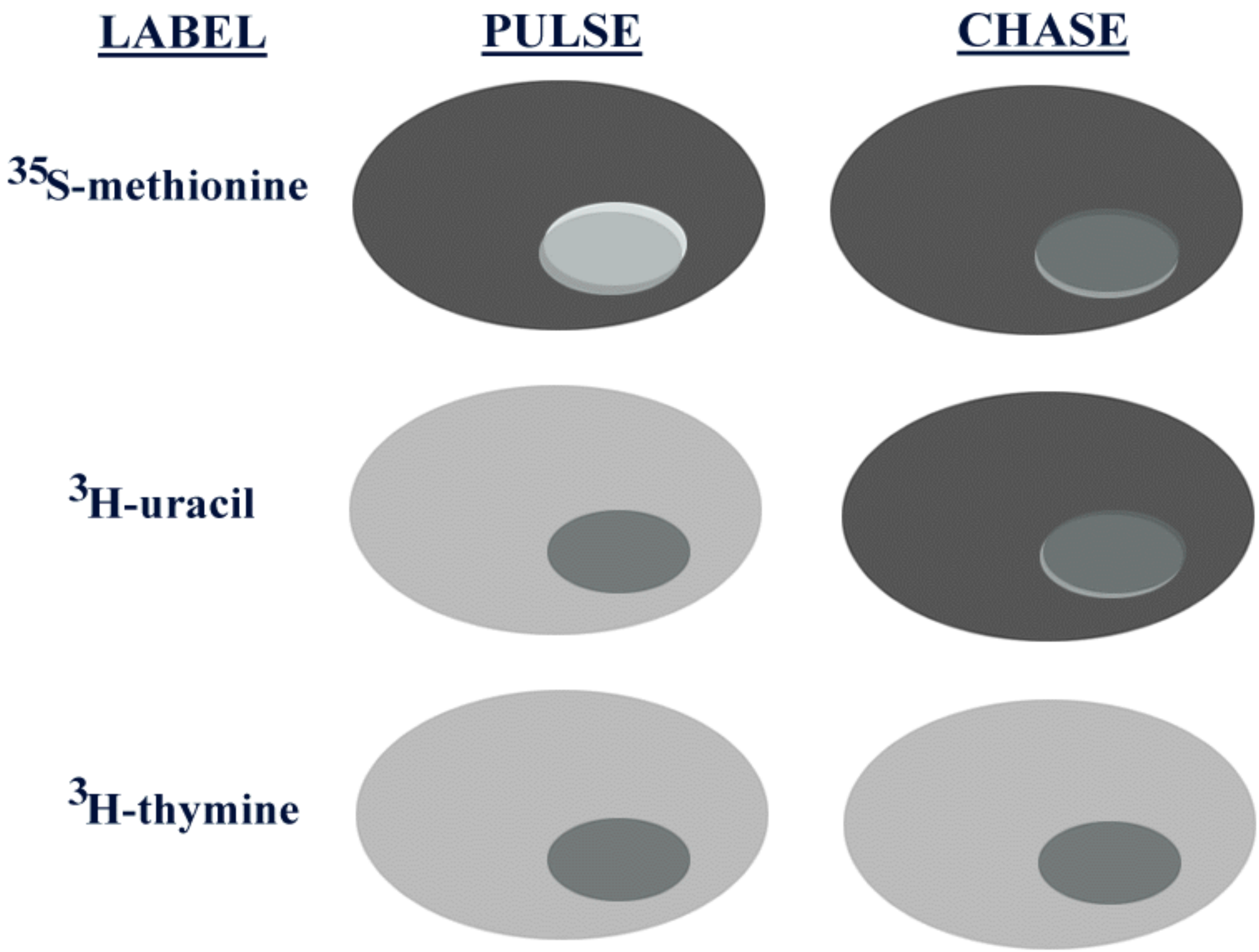
- Grow cells in presence of labeled precursor (this allows the labeled compound to become incorporated into the macromolecule during synthesis).

- Autoradiogram of pulsed cells

- Wash remaining cells, grow in presence of unlabeled precursor

- Autoradiogram of chased cells

Results

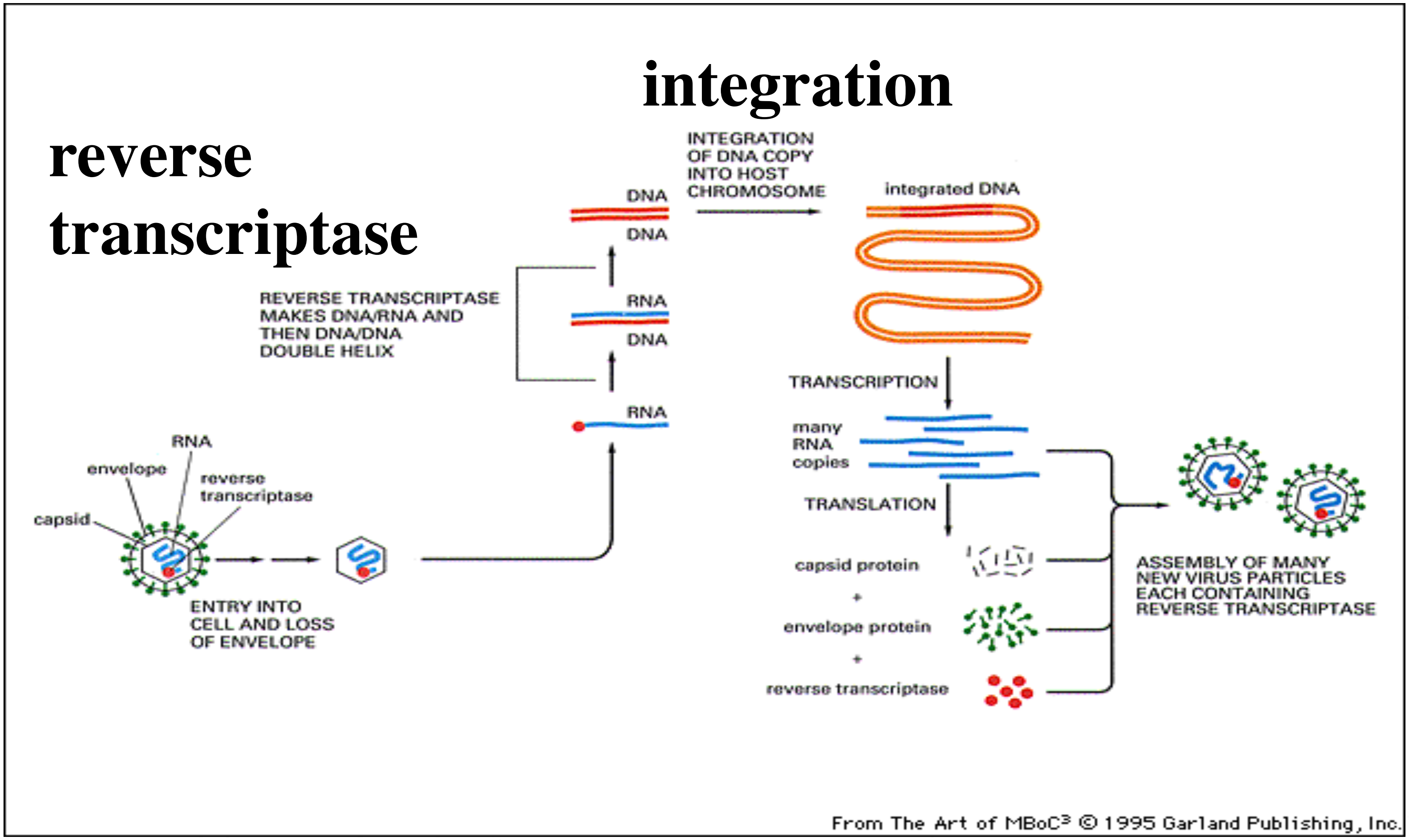


Conclusions

- **Protein is synthesized in the cytoplasm, and later may move to the nucleus**
- **DNA is synthesized in the nucleus and remains there**
- **RNA is synthesized in the nucleus and migrates to the cytoplasm**

Hypothesis:
RNA carries genetic information from the DNA to the site of protein synthesis

Retrovirus Life Cycle (HIV)



Central Dogma – Modified!!

$$\begin{tikzcd} \text{DNA} \arrow[r, "\text{transcription}"] & \text{RNA} \arrow[r, "\text{translation}"] & \text{Protein} \\ & \text{RNA} \arrow[ul, "\text{reverse transcription}"] \arrow[u, "\text{RNA-directed RNA synthesis}"'] & \end{tikzcd}$$

Implications and Critical Thinking-6

- Based on what you have learned in this lecture, what approaches could you take to target HIV?