Cracking the Code

- A codon in messenger RNA: Witten in 5’ to 3’ direction
  - Is either translated into an amino acid or serves as a translational stop signal

The genetic instructions for a polypeptide chain are written in DNA as a series of three-nucleotide words.

The task of matching each codon to its amino acid counterpart began in the early 1960s.

UCU CUCUCU CUC

S L S L

The Nobel Prize: Physiology or Medicine 1968: Robert W. Holley, Har Gobind Khorana and Marshall W. Nirenberg: *"for their interpretation of the genetic code and its function in protein synthesis"*.

Second mRNA base

First mRNA base (5’ end)	U		C		A		G		Third mRNA base (3’ end)
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop	A
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A
	AUG	Met or start	ACG	Thr	AAG	Lys	AGG	Arg	G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly	G

Properties of Genetic Code

- The genetic code is *redundant* but not *ambiguous*.
  - There are typically several different codons that would indicate a specific amino acid.
  - Codons synonymous for the same amino acid often differ only in the third codon position.
  - GAA and GAG : Glu

    If you have a specific codon, you can be sure of the corresponding (only one) amino acid, but if you know only the amino acid, there may be several possible codons.

- To extract the message from the genetic code requires specifying the correct starting point. This establishes the **Open Reading Frame** and subsequent codons are read in groups of three nucleotides.

Our ability to extract the intended message from a written language depends on reading the symbols in the correct groupings- that is, in the correct reading frame.

In molecular genetics, an **open reading frame** (**ORF**) is the part of a **reading frame** that has the potential to code for a protein or peptide.

Every region of DNA has six possible **reading frames**, three in each direction. The reading frame that is used determines which amino acids will be encoded by a gene. Typically only one reading frame is used in translating a gene (in eukaryotes), and this is often the longest open reading frame. Once the open reading frame is known the DNA sequence can be translated into its corresponding amino acid sequence. **An open reading frame starts with an atg (Met) in most species and ends with a stop codon (taa, tag or tga).**

Genetic Code: Insulin gene (Matured)

```
  1 F  V  N  Q  H  L  C  G  S  H  L  V  E  A  L
  1 TTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCTC
 16 Y  L  V  C  G  E  R  G  F  F  Y  T  P  K  T
 46 TACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACC
 31 G  I  V  E  Q  C  C  T  S  I  C  S  L  Y  Q
 91 GGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCTCTACCAG
 46 L  E  N  Y  C  N  *
136 CTGGAGAACTACTGCAACTAG
```

To extract the message from the genetic code requires specifying the correct starting point. Group the letters incorrectly by starting at the wrong point, and the result will be meaningless (gibberish)

She had bun

Redundant

<u>UGG</u> <u>GGU</u> <u>GGC</u> : Codes particular amino acid sequence

:Code for different amino acid sequences

Non-overlapping

Although a genetic message is written with no spaces between the codons, the cell's protein synthesizing machinery reads the message as a series of non-overlapping three-letter codons. Base/letter of a one word/codon cannot be part of other word/codon. Machinery cannot skip the base (letter)

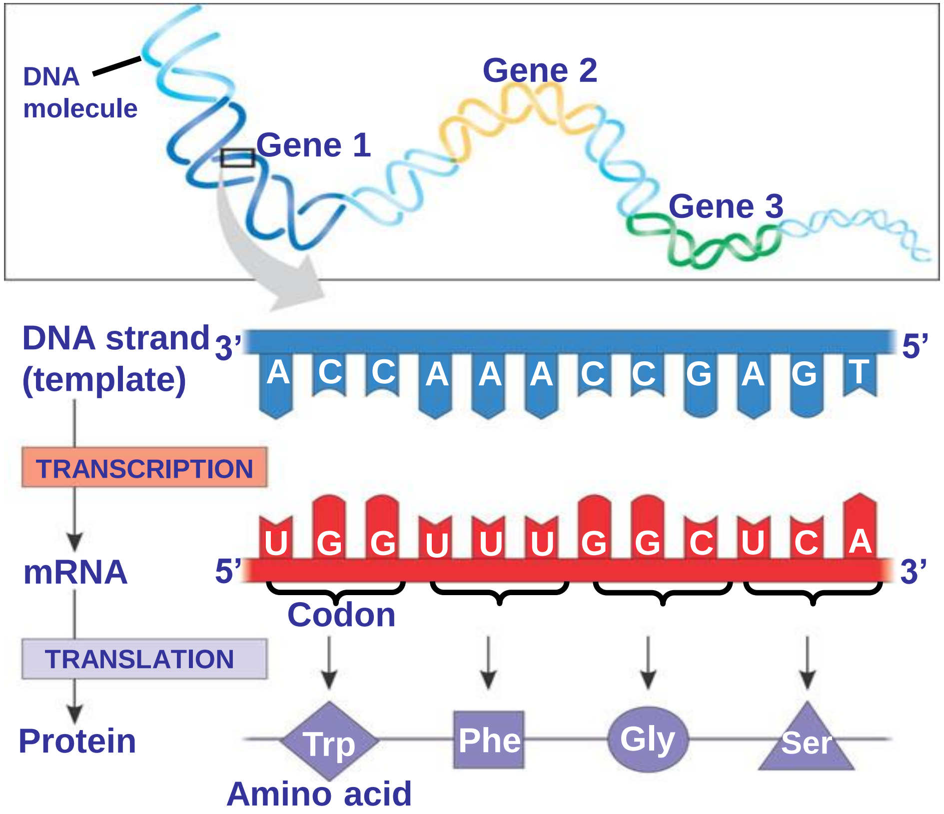
Frame shift mutation?

Multiple of 3 insertion or deletion?

F LC

TTTGTG AAC

Start with first nucleotide: FVN
Start with second nucleotide: L stop
Start with third nucleotide: CE



Non overlapping codons

The genetic code must have evolved very early in the history of life

- The genetic code is **nearly universal**, shared by organisms from the simplest bacteria to the most complex plants and animals.
- Applications in recombinant DNA Technology

Because of diverse forms of life share a common genetic code, one species can be programmed to produce proteins characteristic of a second species by introducing DNA from the second species into the first.

BT cotton, golden rice and Insulin

-Ex: mRNA codon CCG, is translated as the amino acid Proline in all organisms whose genetic code has been examined.

Tobacco with firefly gene

Molecular Components of Translation

Replication and Transcription: Transfer (decoding) of chemical (genetic) information from DNA to DNA or DNA to RNA through ………

Difficult (complex) problems, very simple solutions!!!!!!!

Translation: ???????

Translation is the RNA-directed synthesis of a polypeptide

- A cell translates an mRNA message into protein
  - With the help of **transfer RNA (tRNA)**
    - tRNA's job is to transfer amino acids from the cytoplasmic pool of amino acids to the ribosome
    - The ribosome adds each amino acid carried by tRNA to the growing end of the polypeptide chain.
    - How tRNA does? How it knows that which codon on mRNA is for what amino acid?

The Structure and Function of Transfer RNA

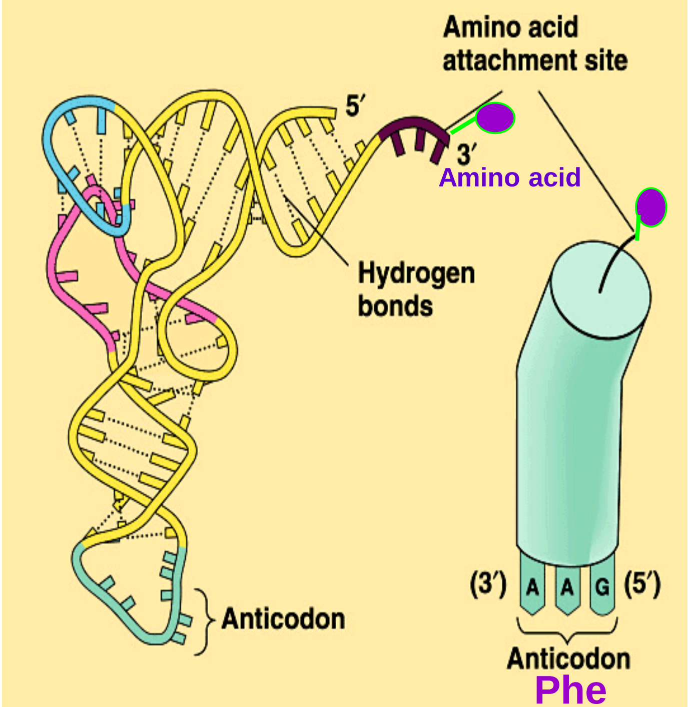
- A tRNA molecule
  - Each carries a specific amino acid on one end
  - Each has an anticodon on the other end, which decodes the codon of the mRNA

Structure determines the function…….

Robert William Hollev

Cloverleaf

Phe-tRNA^{Phe}



(b) Three-dimensional structure (c) Symbol

Two-dimensional structure: The four base-paired regions and three loops are characteristic of all tRNAs. 3 base sequence of the **amino acid attachment site at the 3' end**. The **anticodon triplet** is unique to **each tRNA type**. (The asterisks mark bases that have been chemically modified, a characteristic of tRNA.)

Each amino acid is joined to the correct tRNA by aminoacyl-tRNA synthetase.

During translation, each type of tRNA links a mRNA codon with the appropriate amino acid

If anti-codon is 5' GAA 3' then codon is 5'…….3' and what's the amino acid it codes for?

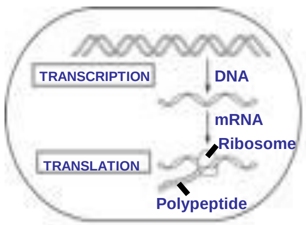
The ribosomal subunits

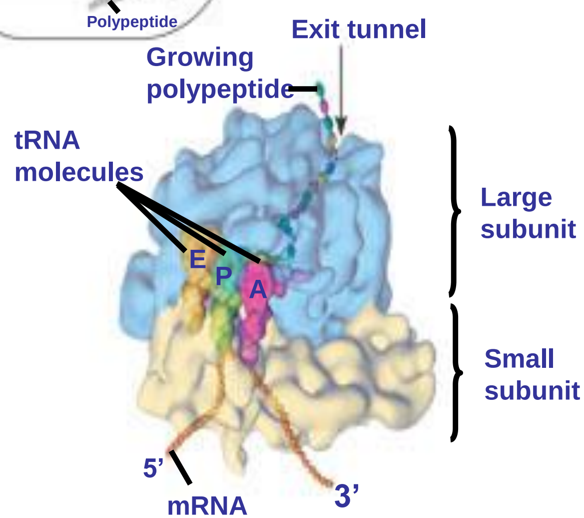
Are constructed of proteins and RNA molecules named ribosomal RNA or rRNA

Facilitate the specific coupling of tRNA anticodons with mRNA codons during protein synthesis

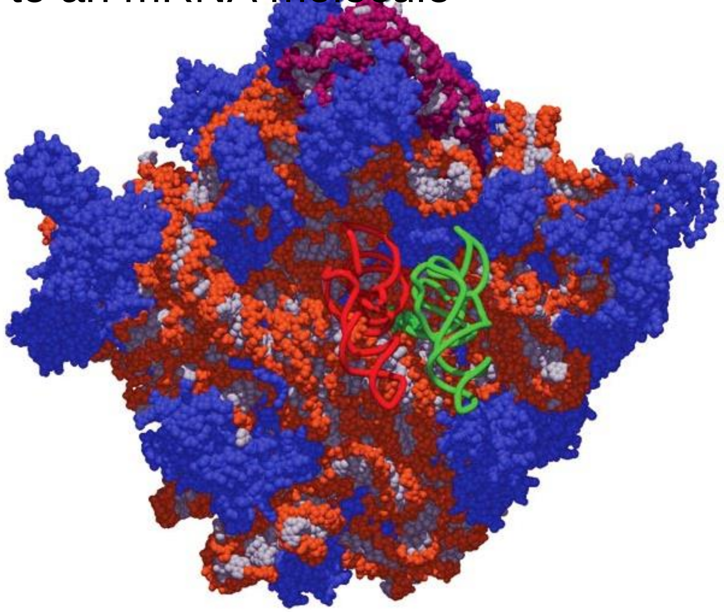
Codon by codon, tRNAs deposit amino acids in the prescribed order and the ribosome joins them into a polypeptide chain.

Made of large and small subunits that join together in the cytosol when they attach to an mRNA molecule





(a) Computer model of functioning ribosome. This is a model of a bacterial ribosome, showing its overall shape. The eukaryotic ribosome is roughly similar. A ribosomal subunit is an aggregate of ribosomal RNA molecules and proteins.



- The ribosome has three binding sites for tRNA
  - The A site
  - The P site
  - The E site

We can divide translation into three stages also
-Initiation
-Elongation
-Termination

Why cell cannot use DNA (gene) directly for translation?

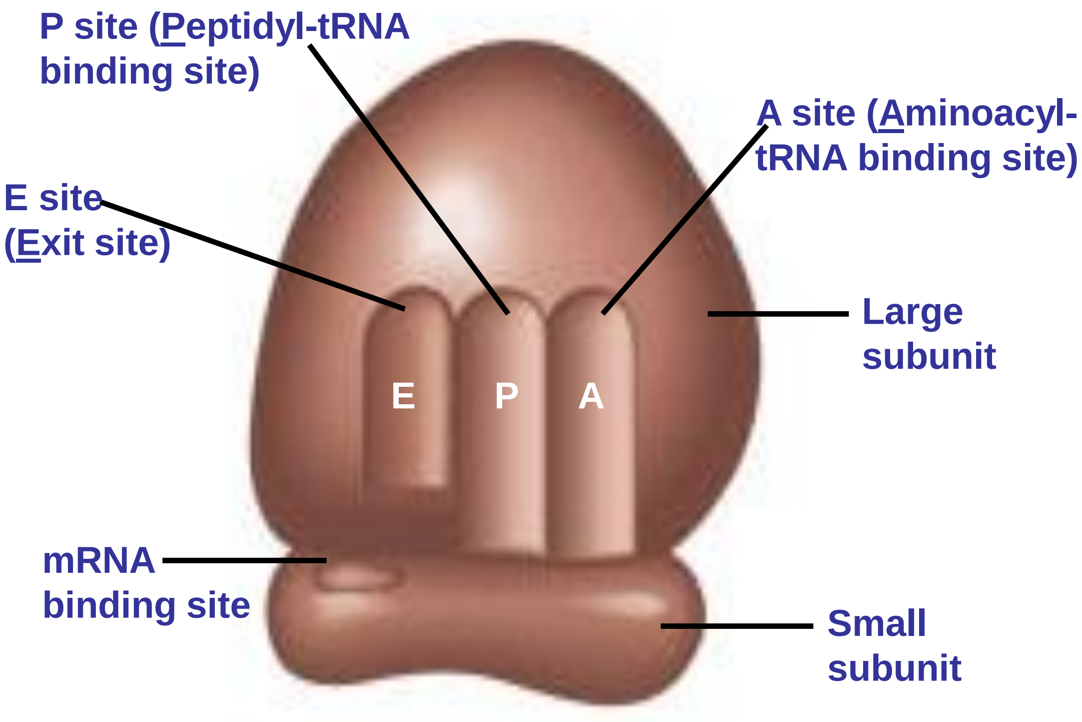
Gene (DNA)

↓

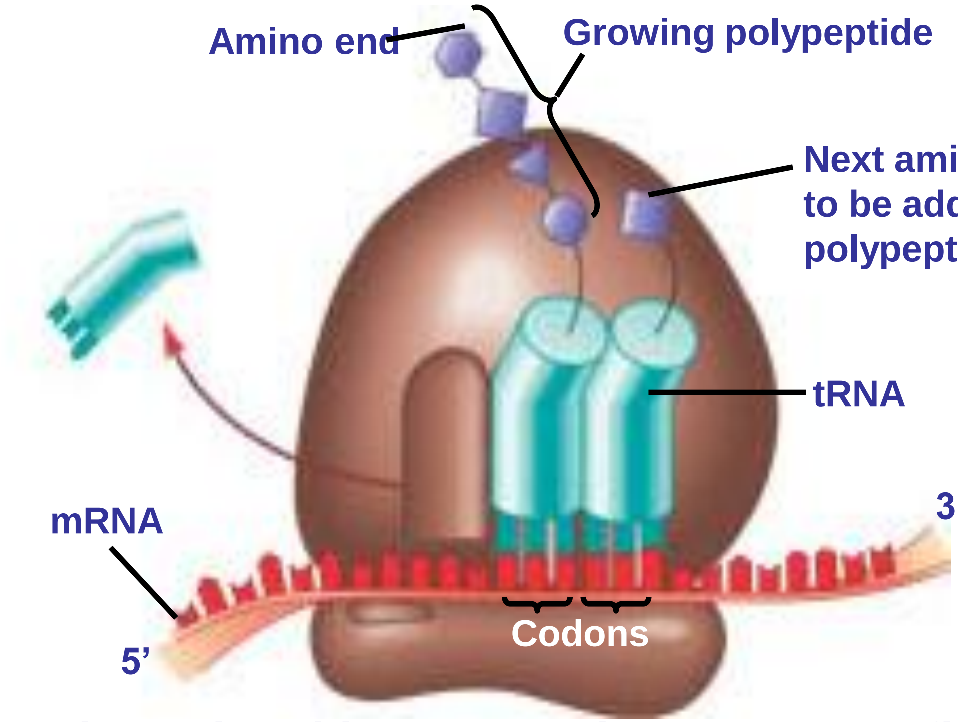
~10^6 mRNA

↓

~Each mRNA used for Synthesis of ~10^3 copy of protein



(b) Schematic model showing binding sites. A ribosome has an mRNA binding site and three tRNA binding sites, known as the A, P, and E sites. This schematic ribosome will appear in later diagrams.



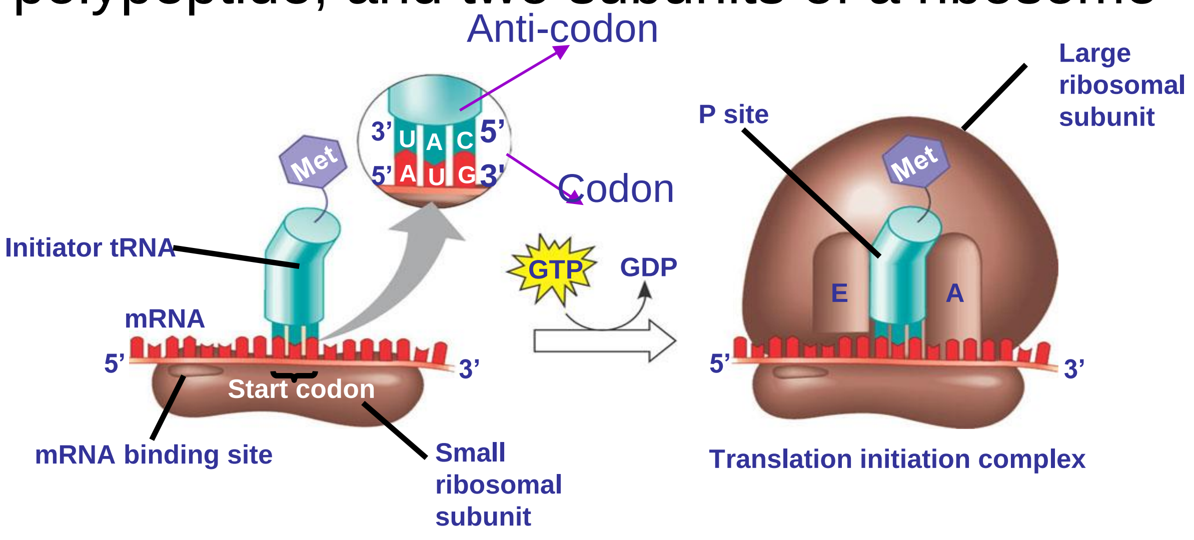
(c) Schematic model with mRNA and tRNA. A tRNA fits into a binding site when its anticodon base-pairs with an mRNA codon. The P site holds the tRNA attached to the growing polypeptide. The A site holds the tRNA carrying the next amino acid to be added to the polypeptide chain. Discharged tRNA leaves via the E site.

Building a Polypeptide

- We can divide translation into three stages also
- Initiation
  Elongation
  Termination

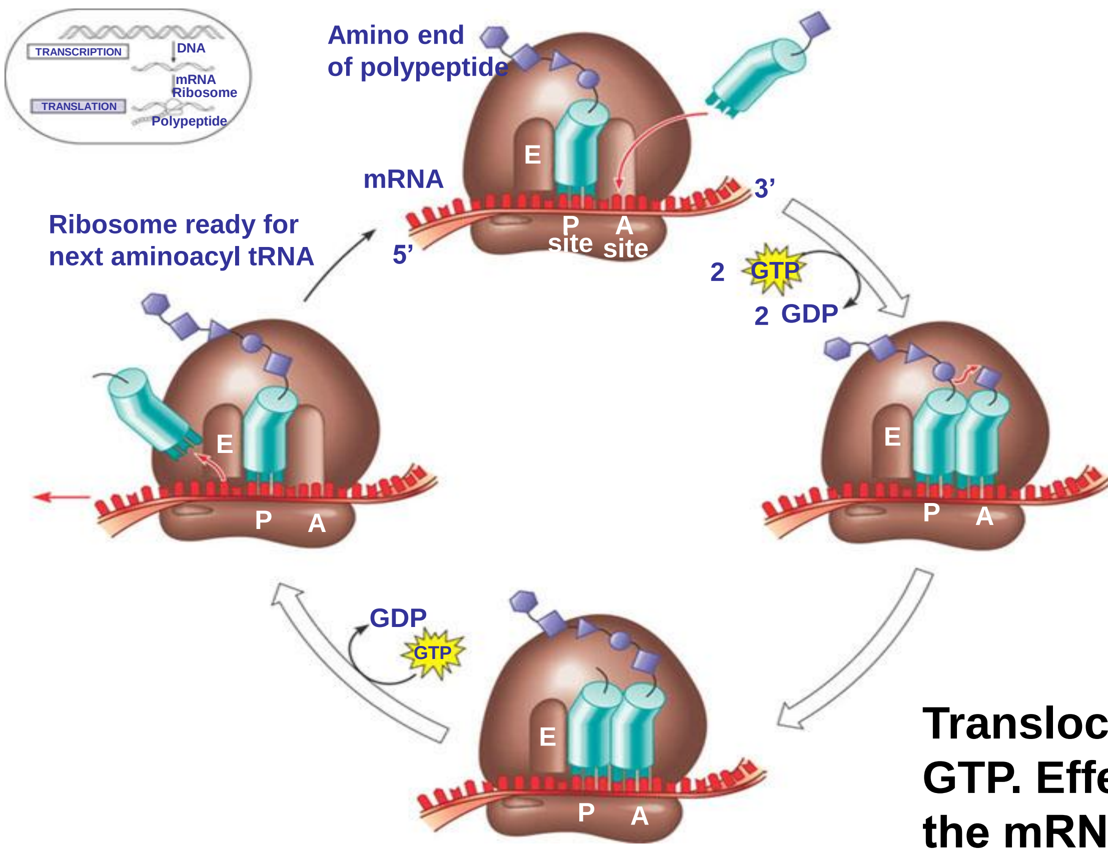
Ribosome Association and Initiation of Translation

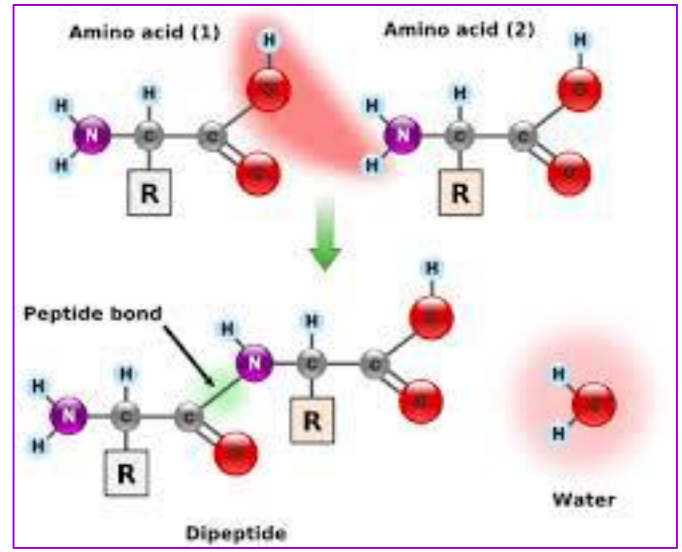
- The initiation stage of translation
  - Brings together mRNA, tRNA bearing the first amino acid of the polypeptide, and two subunits of a ribosome



Elongation of the Polypeptide Chain

- In the elongation stage of translation
  - Amino acids are added one by one to the preceding amino acid



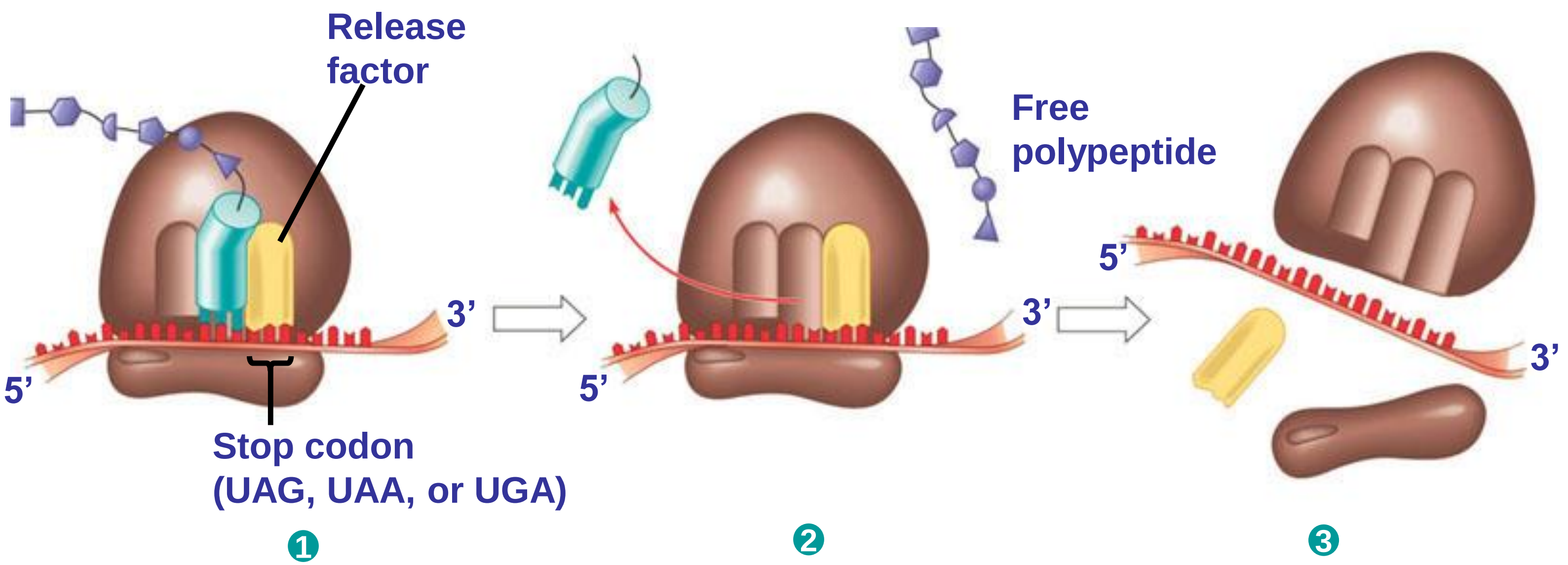


Direction
N-terminus to C-terminus

Translocation is fueled by the hydrolysis of GTP. Effectively, translocation ensures that the mRNA is “read” 5’ -> 3’ codon by codon.

Termination of Translation

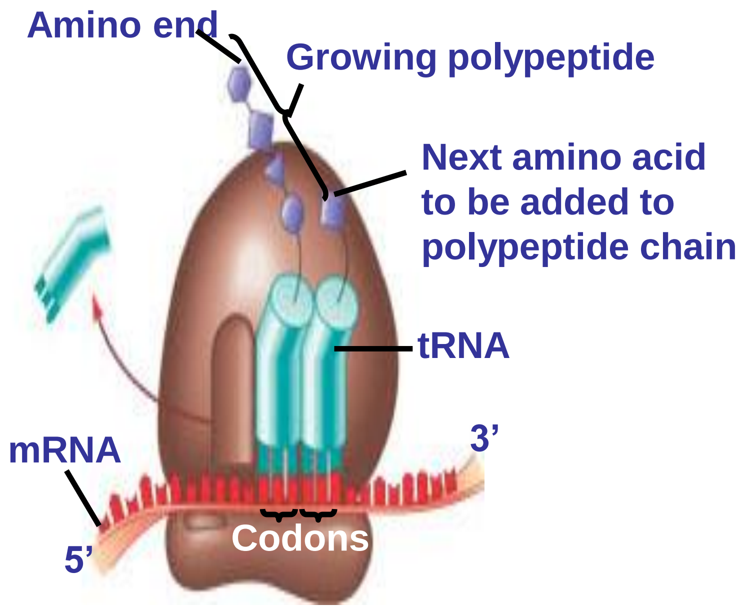
- The final stage of translation is termination
  - When the ribosome reaches a stop codon in the mRNA

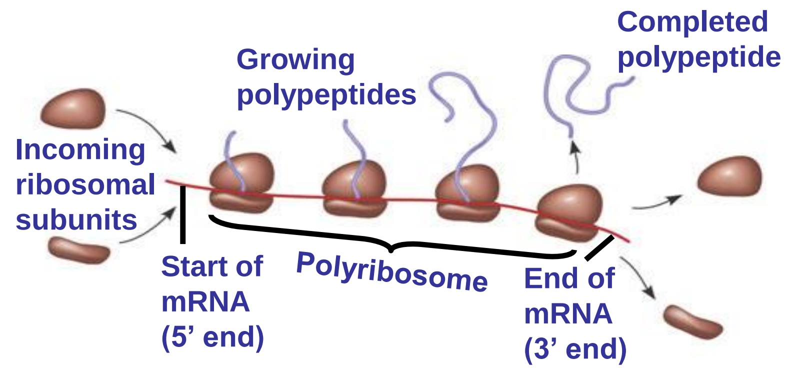


Translation-1 video?

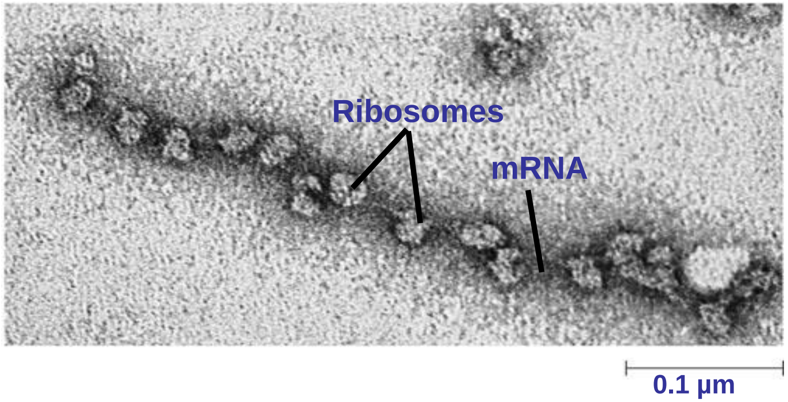
Polyribosomes

- A number of ribosomes can translate a single mRNA molecule simultaneously
  - Forming a polyribosome





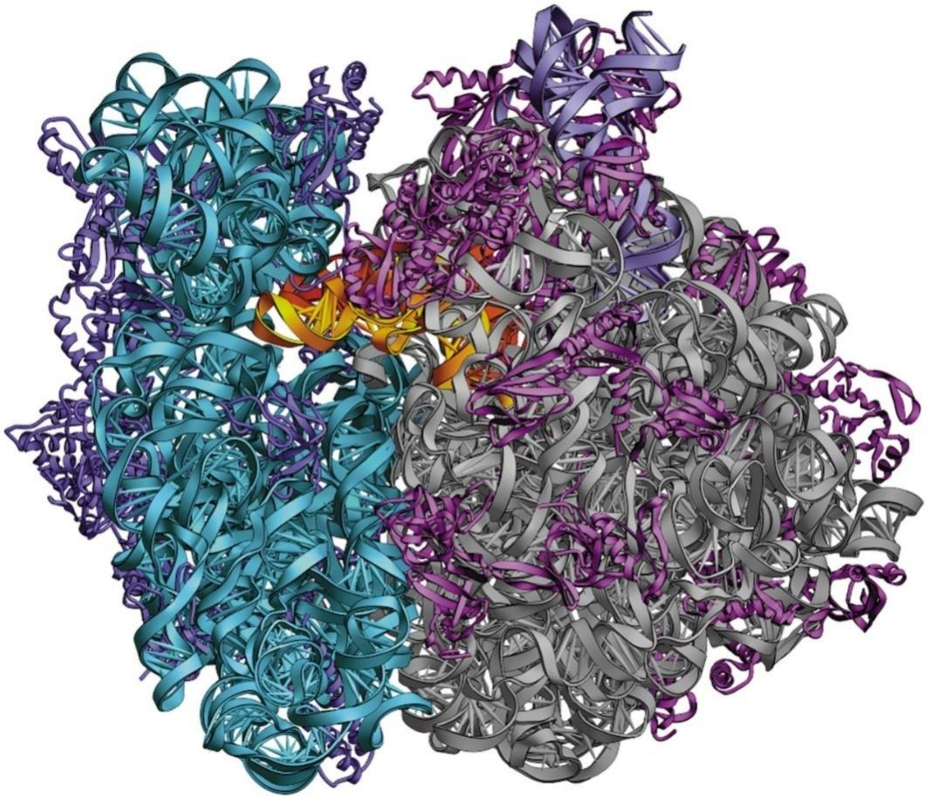
(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

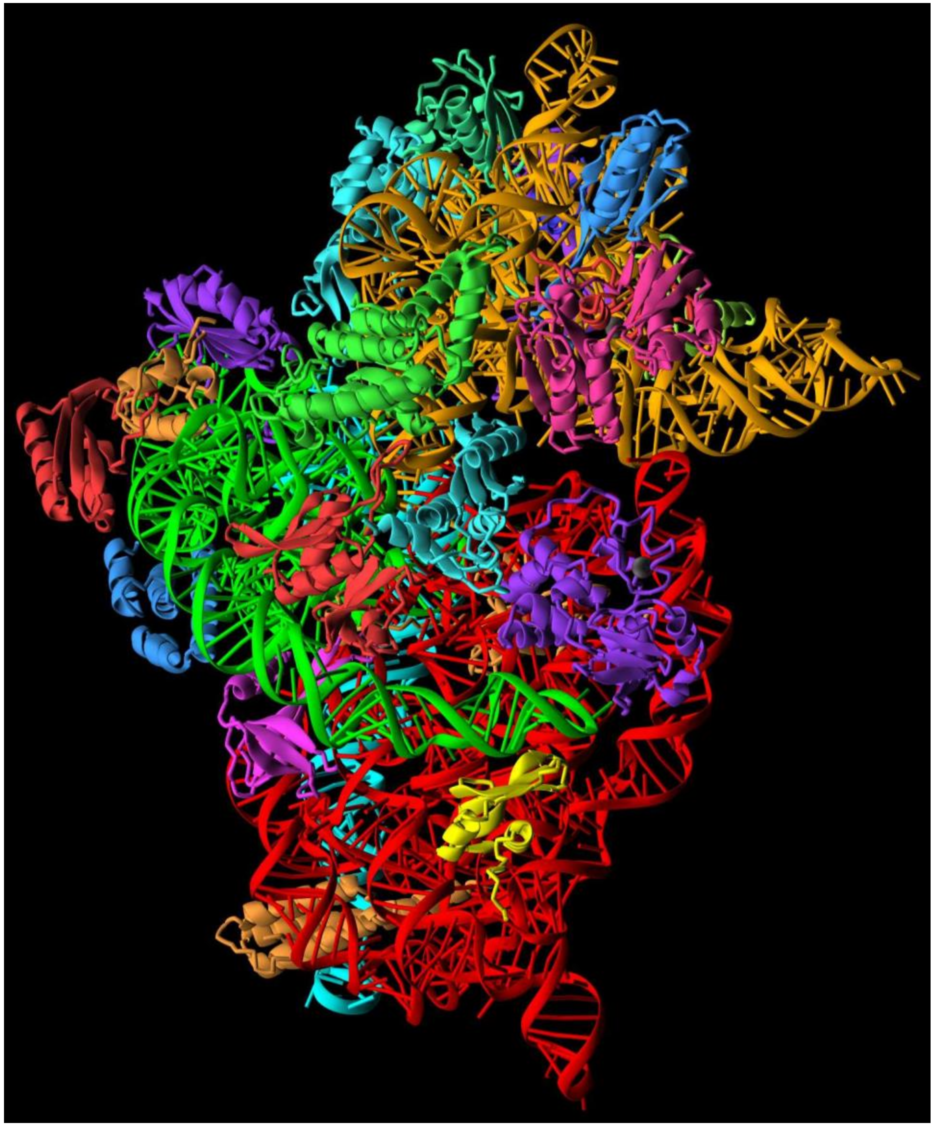
A ribosome requires less than a minute to translate an average-sized mRNA into a polypeptide.

Ribosome and Antibiotics



Small + Big Subunit

The Nobel Prize in Chemistry 2009 : Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath *"for studies of the structure and function of the ribosome"*.



Small Subunit

-Antibiotics-Ribosome Video ?

Tetracycline and Streptomycin

Bacterial Ribosome

Biotechnology
DNA Technology
Genetic Engineering

Overview

- Sequencing of the human genome was completed by 2007
- DNA sequencing has depended on advances in technology, starting with making recombinant DNA
- In **recombinant DNA**, nucleotide sequences from two different sources, often two species, are combined *in vitro* into the same DNA molecule

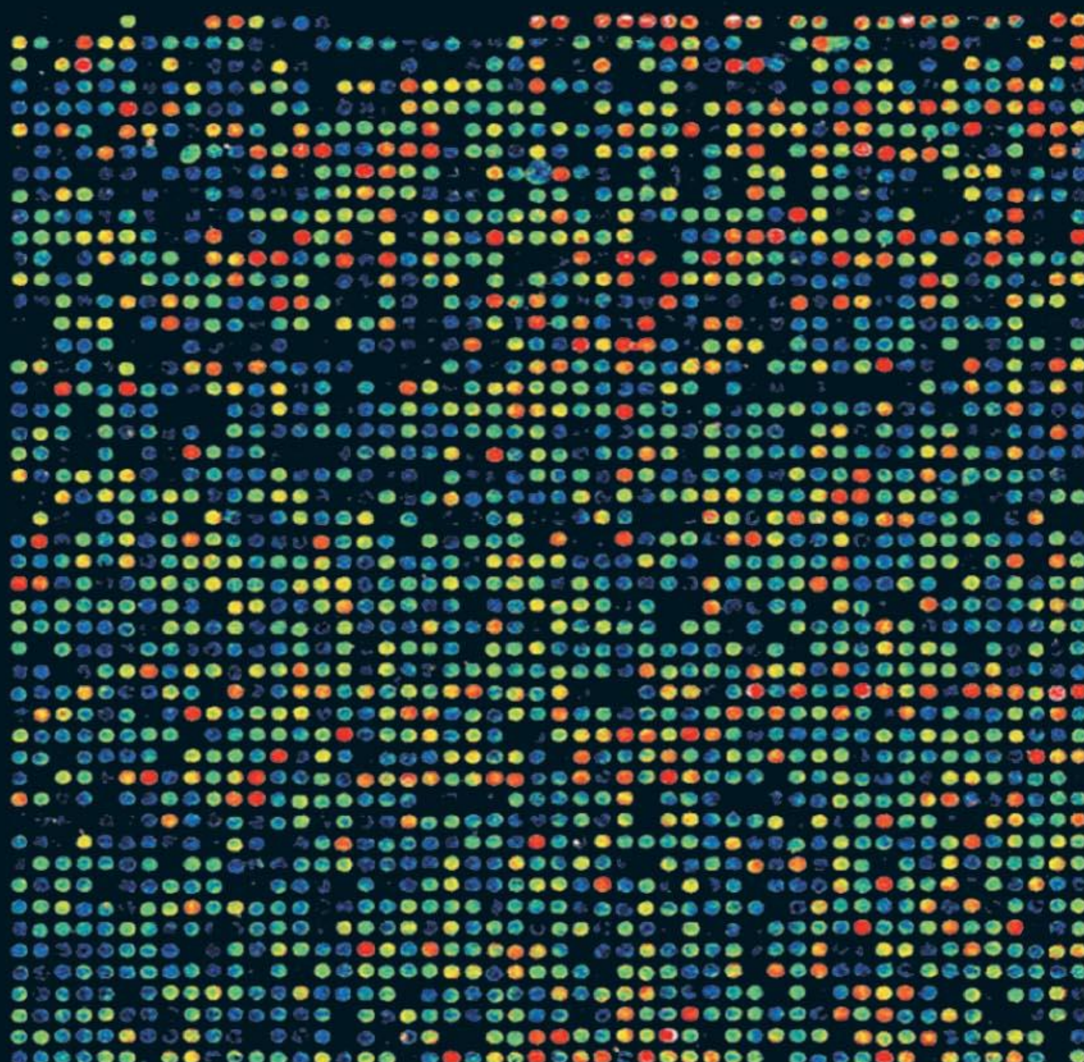
How scientists prepare recombinant DNA and use DNA technology to answer fundamental biological questions are one focus of this chapter. Another focus of the chapter is how our lives are affected by biotechnology, the manipulation of organisms or their components to make useful products.

DNA toolbox

- Methods for making recombinant DNA are central to **genetic engineering**, the direct manipulation of genes for practical purposes
- DNA technology has revolutionized biotechnology, the manipulation of organisms or their genetic components to make useful products
- An example of DNA technology is the microarray, a measurement of gene expression of thousands of different genes

How can this array of spots be used to compare normal and cancerous tissues?

The colored spots represent the relative level of expression of 2,400 human genes



DNA cloning yields multiple copies of a gene or other DNA segment

Molecular biologists studying the particular gene faces a challenge. Naturally occurring DNA molecule are very long, and, a single molecule usually carries many genes.
Moreover, genes occupy only a small portion of the chromosomal DNA (1/100000).

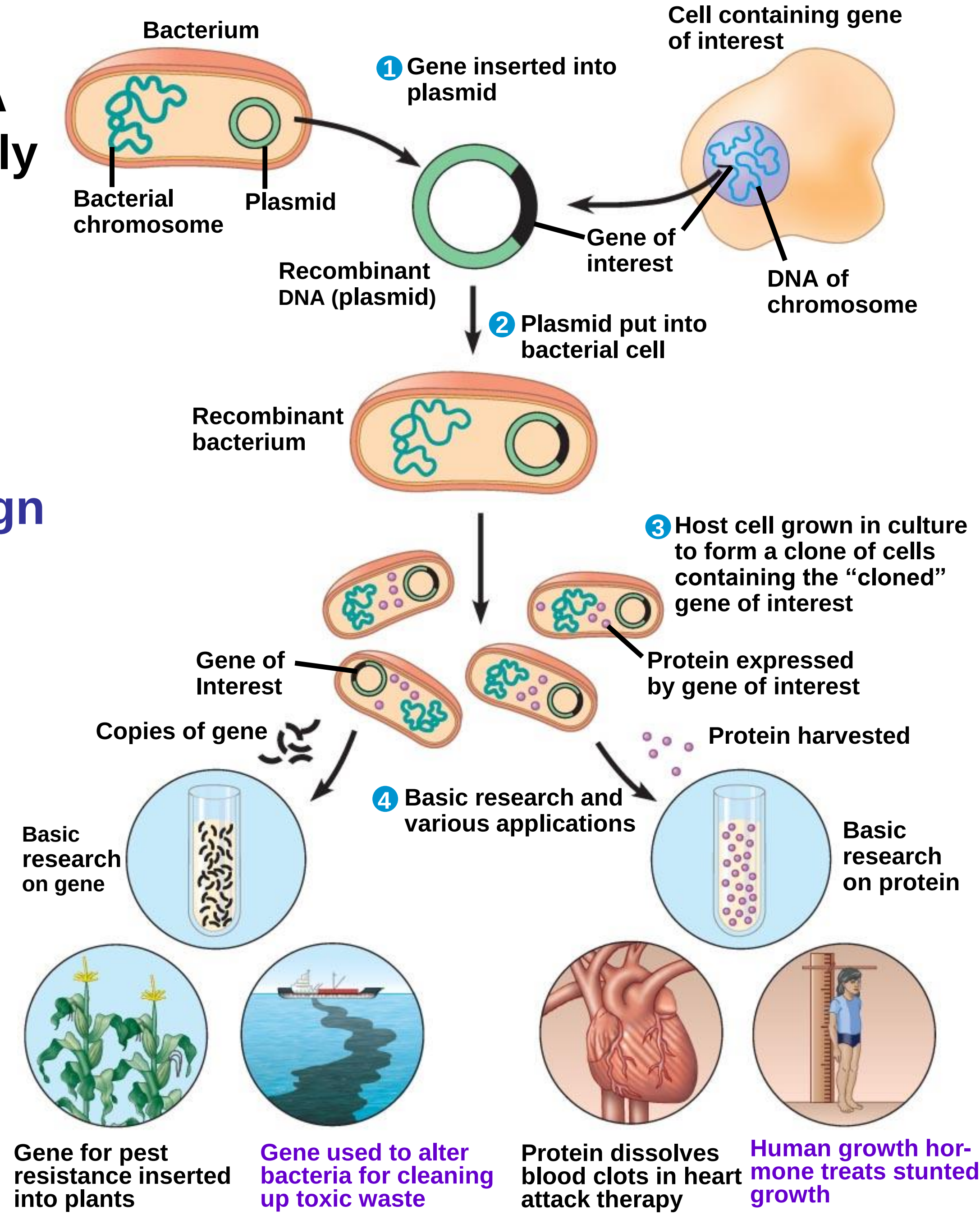
- To work directly with specific genes, scientists prepare gene-sized pieces of DNA in identical copies, a process called *DNA cloning*
- Cloned genes are useful for making **copies of a particular gene and producing a protein product**

Plasmids are small circular DNA molecules that replicate separately from the bacterial chromosome

Replication, transcription and translation machinery in bacterial cell responsible for production of protein of foreign gene

Almost everyday we do gene cloning

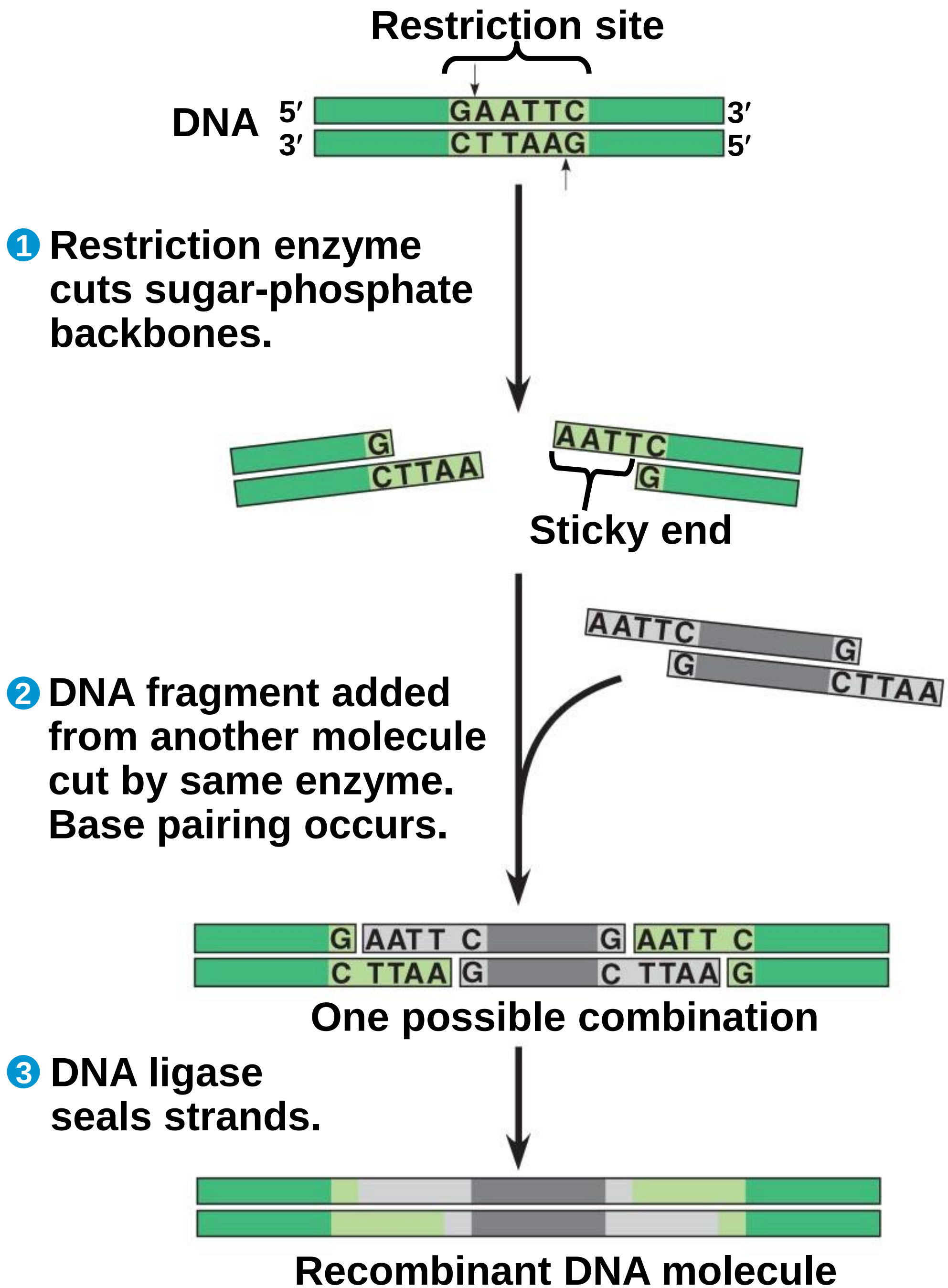
How to cut the plasmid DNA at precise position to insert the foreign gene?



Using Restriction Enzymes (molecular scissors) to Make Recombinant DNA

- Bacterial **restriction enzymes** cut DNA molecules at **specific DNA sequences** (precise position) called **restriction sites**
- A restriction enzyme usually makes many cuts, yielding **restriction fragments**
- Most useful are Producing fragments with **“sticky ends**” that can bond with complementary “sticky ends” of other fragments
- **DNA ligase** is an enzyme that seals the bonds between restriction fragments

Fig. 20-3-3



Restriction site
DNA
5′
3′
GAATTC
CTTAAG
3′
5′
1 Restriction enzyme cuts sugar-phosphate backbones.
G
CTTAA
AATTC
G
Sticky end
AATTC
G
G
CTTAA
2 DNA fragment added from another molecule cut by same enzyme. Base pairing occurs.
G AATT C G AATT C
C TTAA G C TTAA G
One possible combination
3 DNA ligase seals strands.
Recombinant DNA molecule