Asam Nukleat, Sintesis Protein dan Bioteknologi Pangan

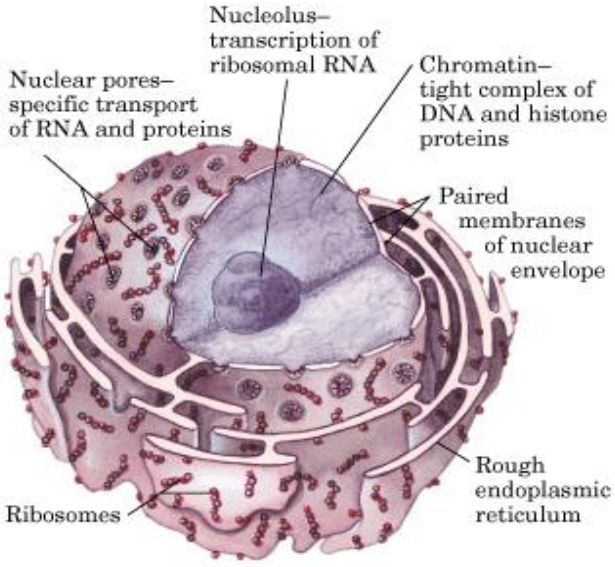
TIK :

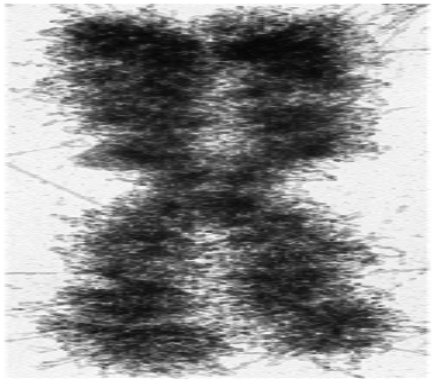
Mahasiswa mampu menguraikan pengertian asam nukleat dan building blocknya, menjelaskan proses sintesis protein dan proses-proses untuk menghasilkan pangan hasil rekayasa genetika

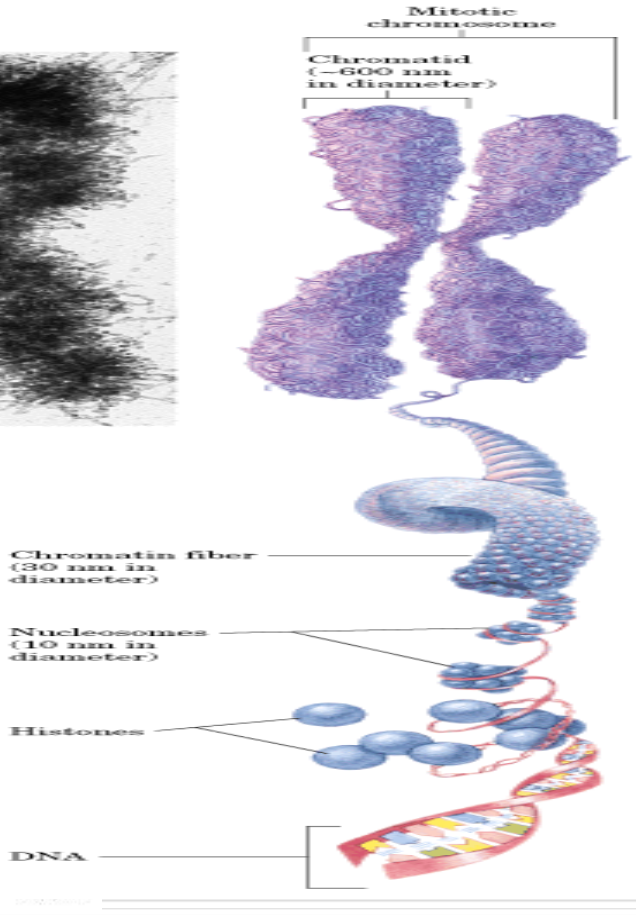
Pokok Bahasan

- Asam Nukleat dan building blocknya
- Sintesis Protein
  - Replikasi, transkripsi, translasi dan kejadian sesudahnya
- Teknologi Gen
- Pangan Transgenik
  - Produksi
  - Deteksi
  - Aturan dan kontroversi

DNA in Cell

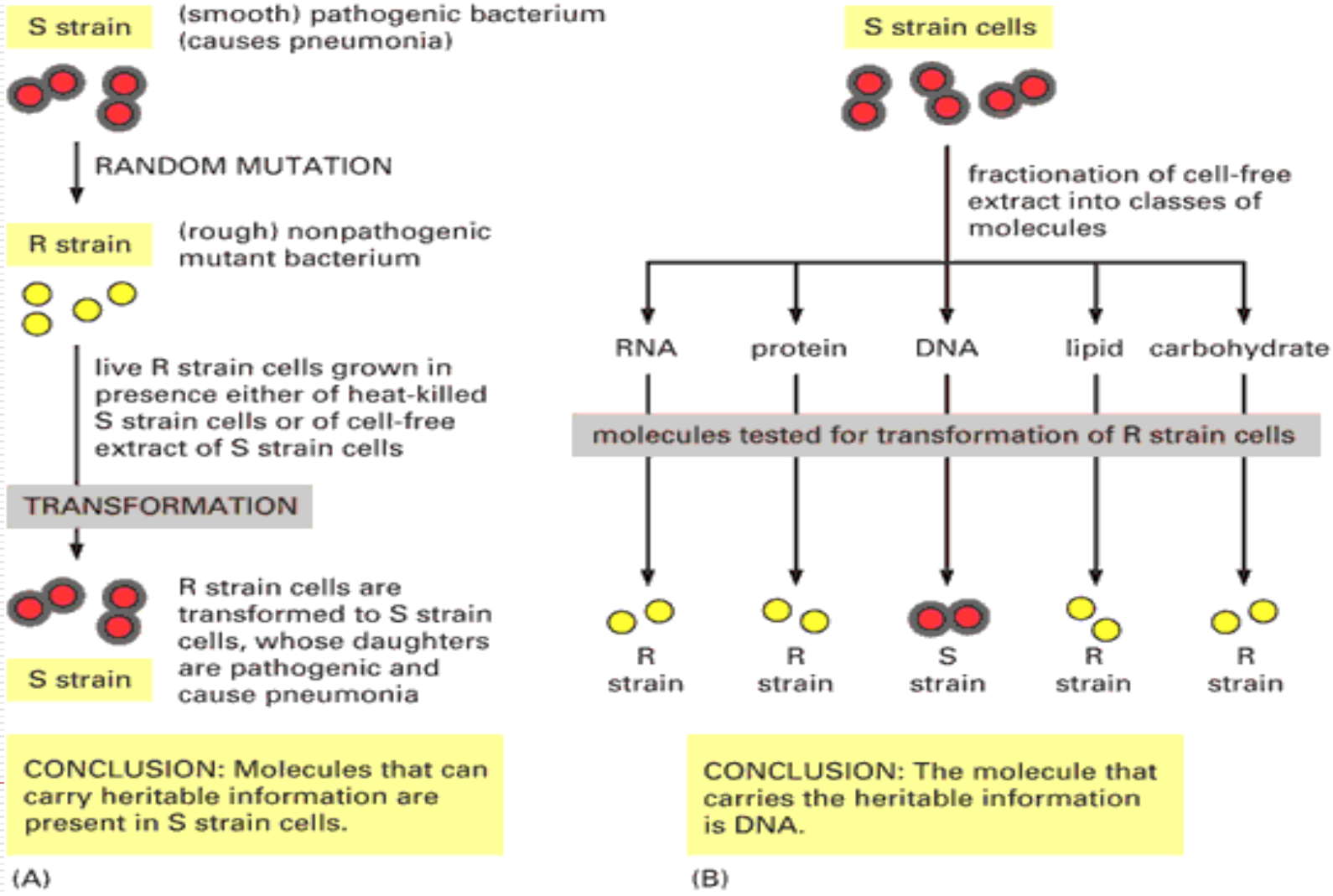






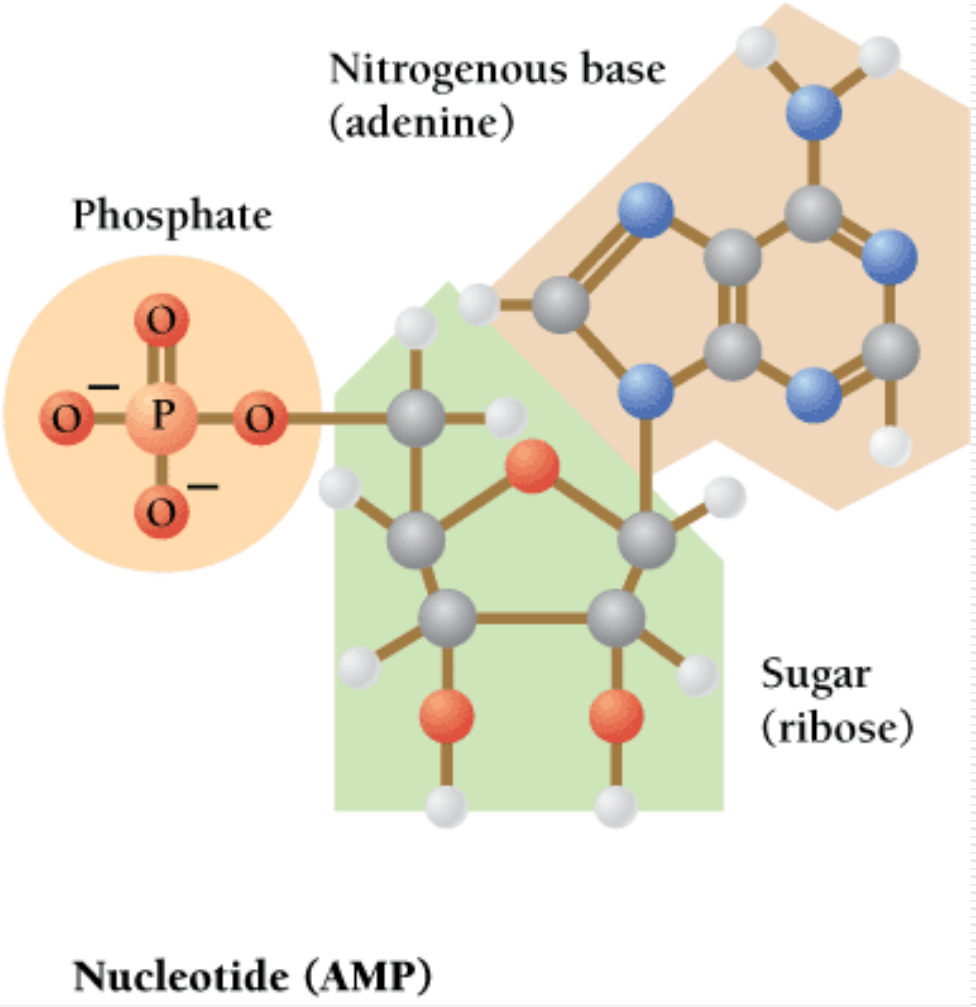
- Materi genetik
- Bersama-sama dengan protein (histone) menggulung menyusun nucleosomes, menyusun chromatin fiber dan menyusun kromosom

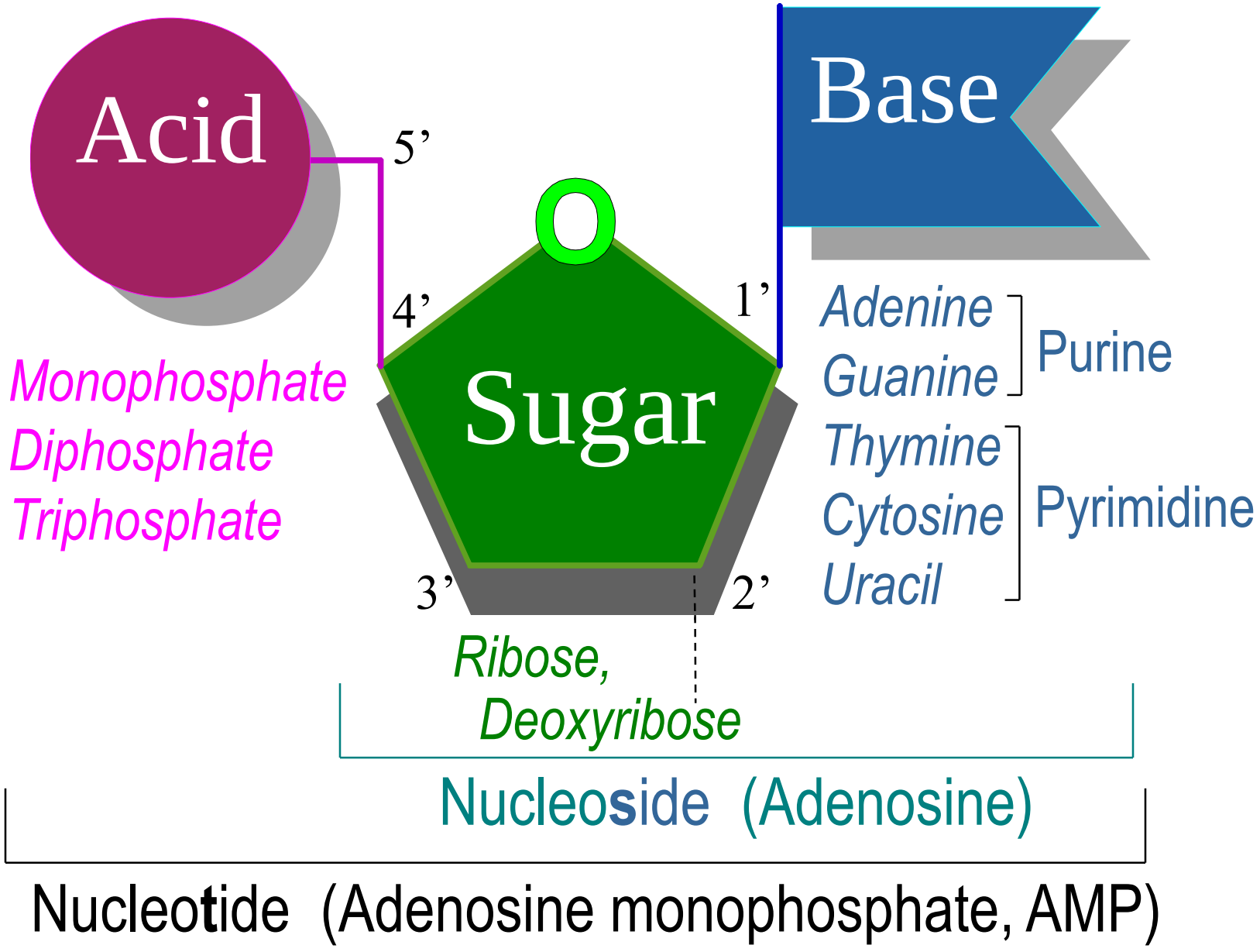
Bukti; DNA sbg materi genetik



Nucleic Acids

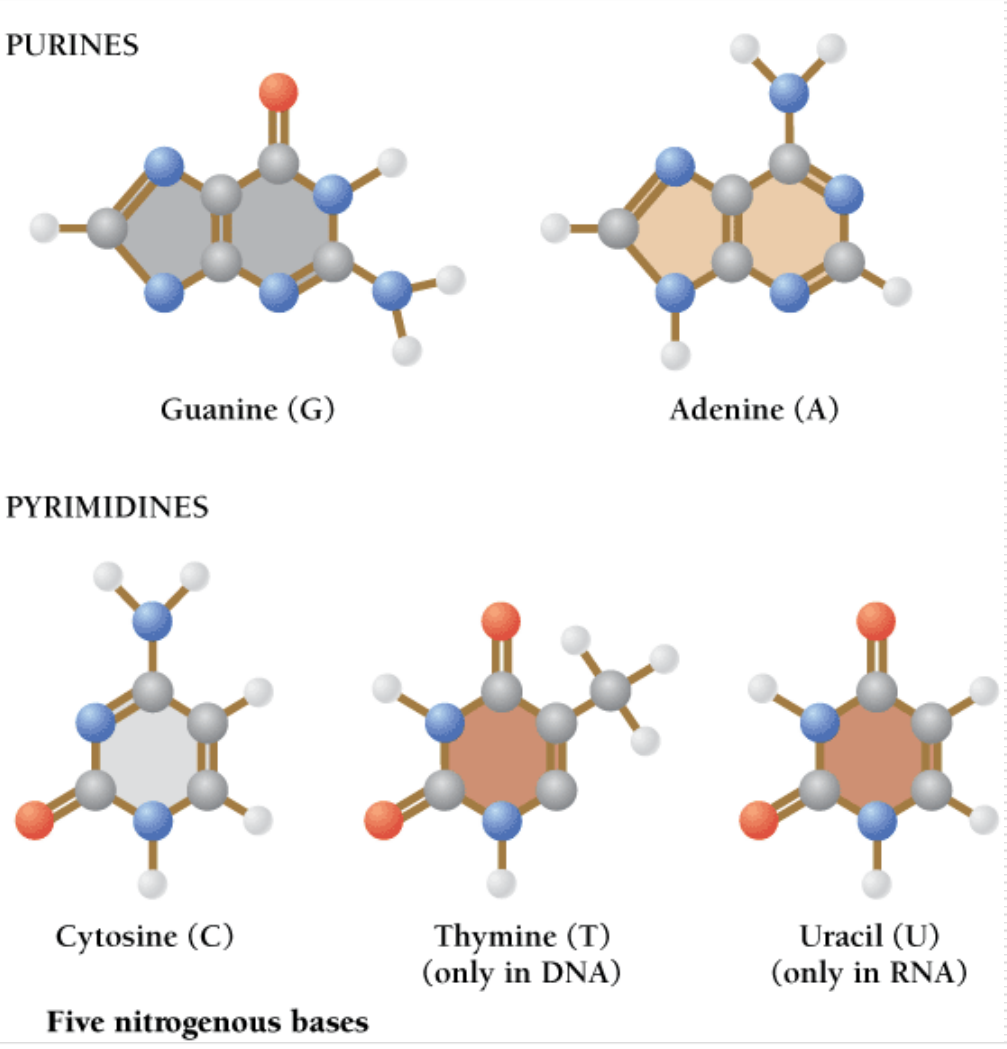
- Structure
  - Subunits — nucleotides
  - 3 building blocks for each nucleotide:
    - Sugar
    - Phosphate
    - Nitrogenous base





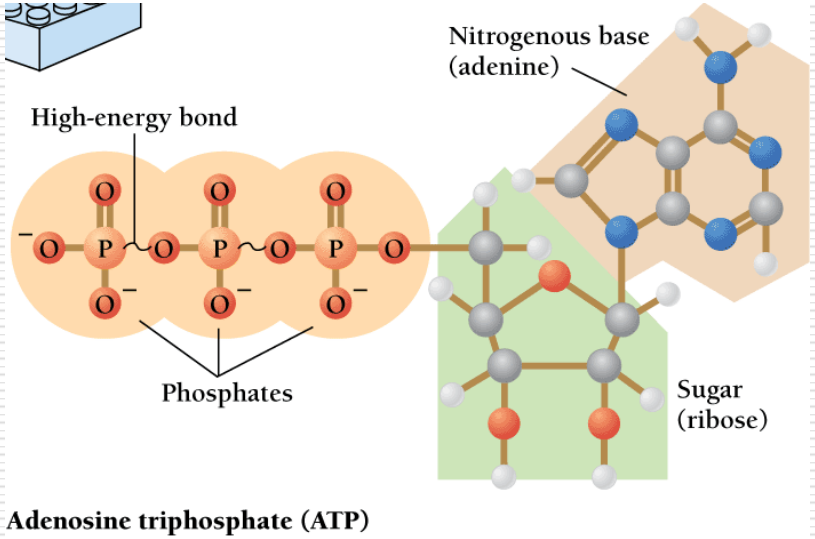
Komponen

- Nitrogenous Base
  - Pyrimidines, single ring
  - Purines, 2 rings
  - Differ in functional groups attached to the rings
- 5 carbon sugars
  - Ribose in RNA
  - Deoxyribose in DNA
- Phosphate groups link nucleotides together

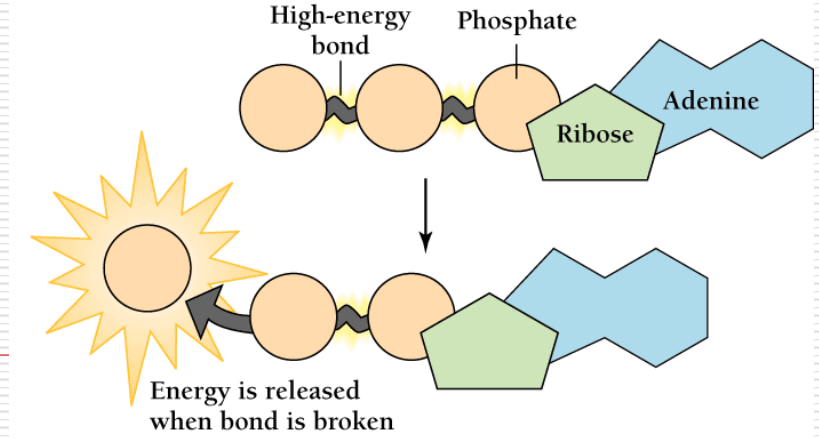


Pembentukan asam nukleat

- Diikat melalui proses kondensasi dehidrasi
- Ikatan yang terbentuk dinamakan Ikatan **Fosfodiester**

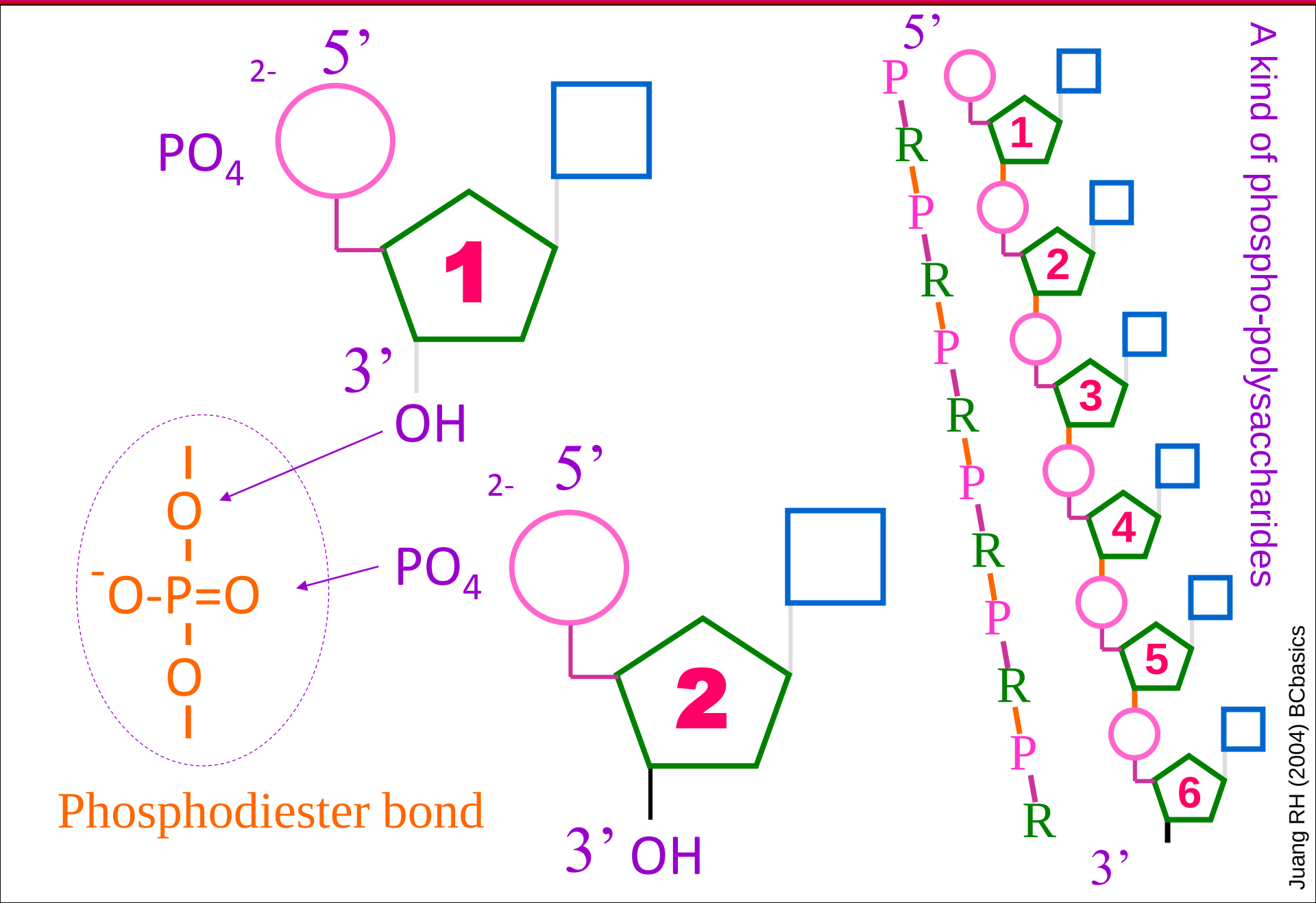


Adenosine triphosphate (ATP)



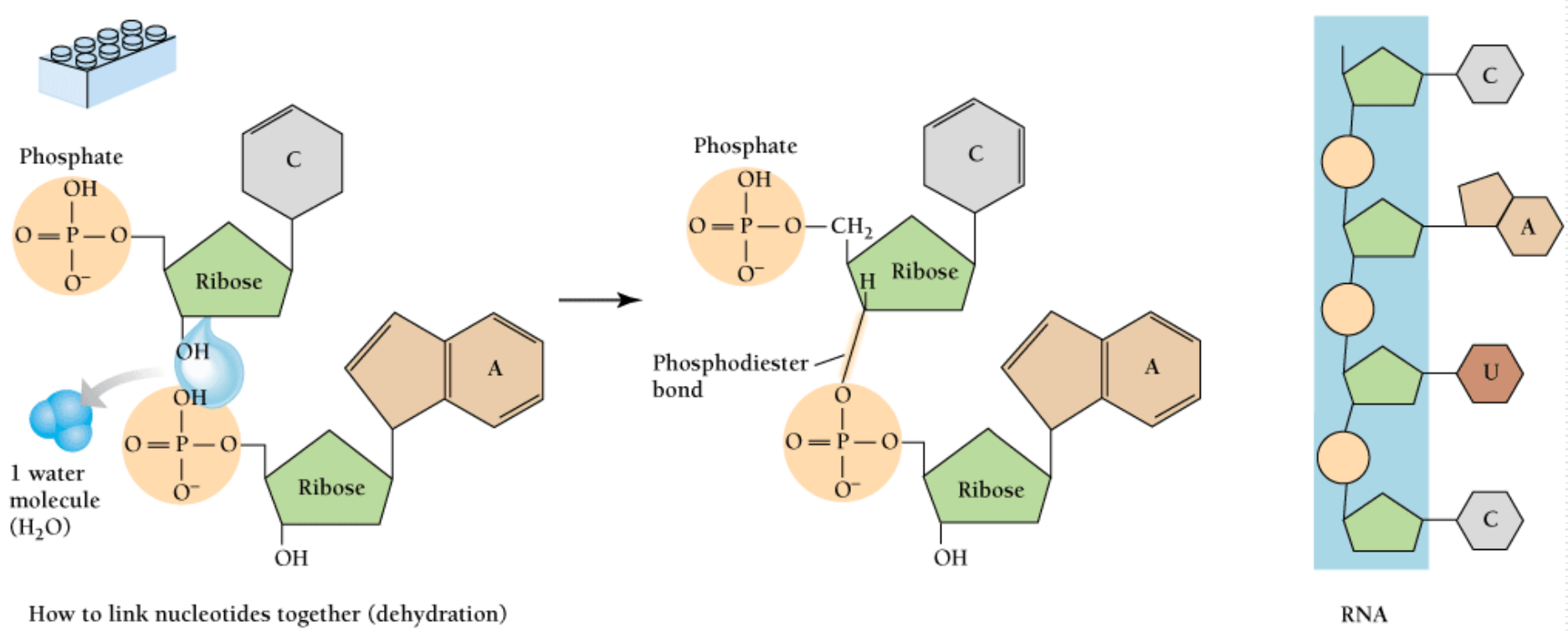
Adenosine diphosphate (ADP)

Nucleotides Linked by Phosphodiester Bond



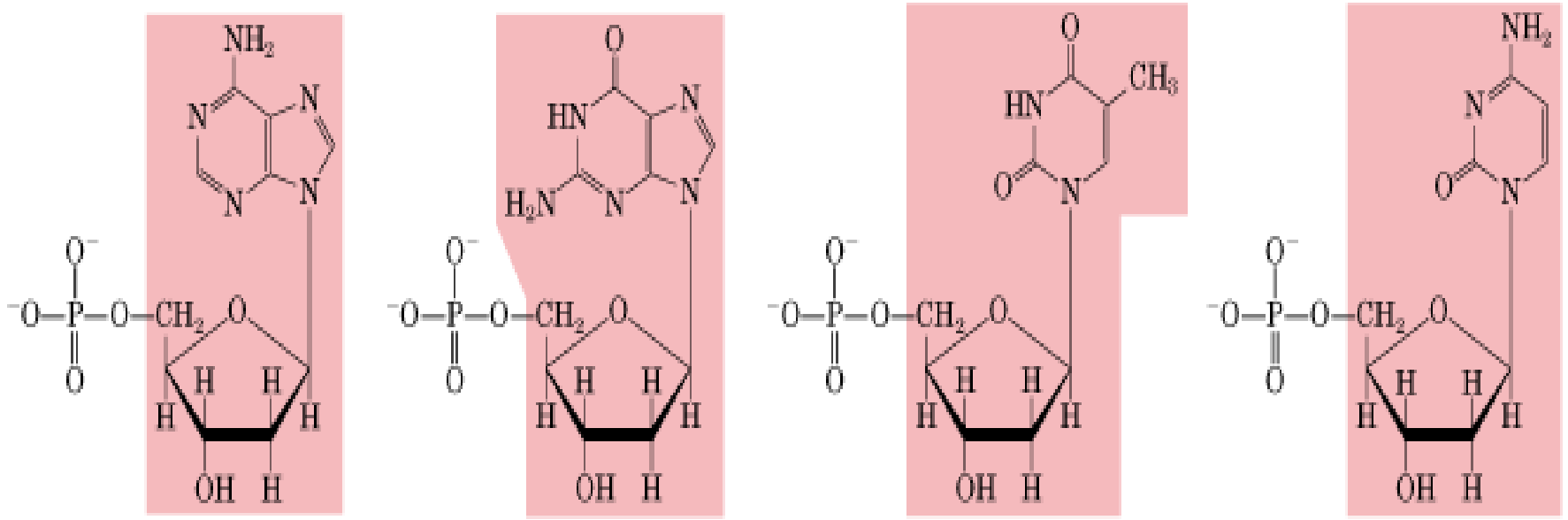
Juang RH (2004) BCbasics

Nucleic Acid Structure



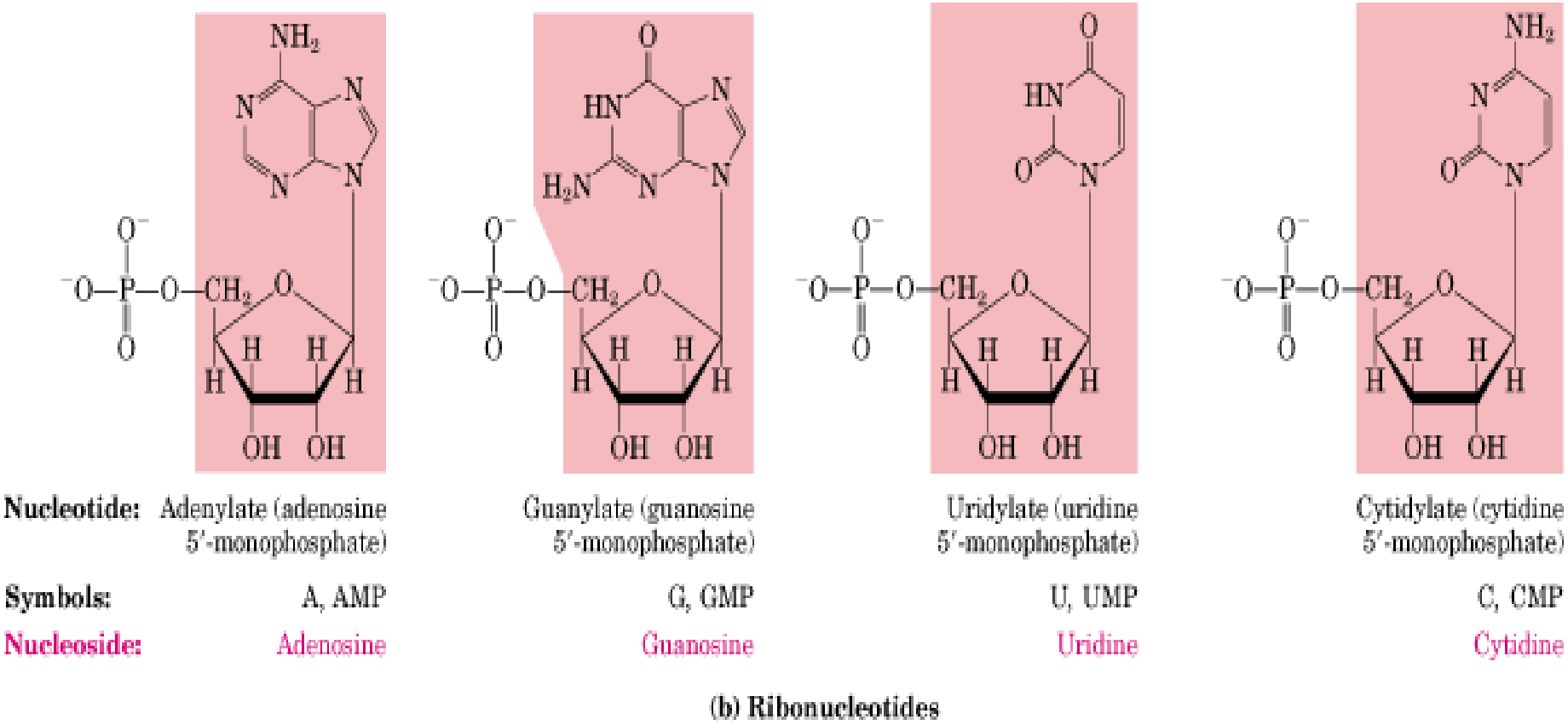
How to link nucleotides together (dehydration)

RNA



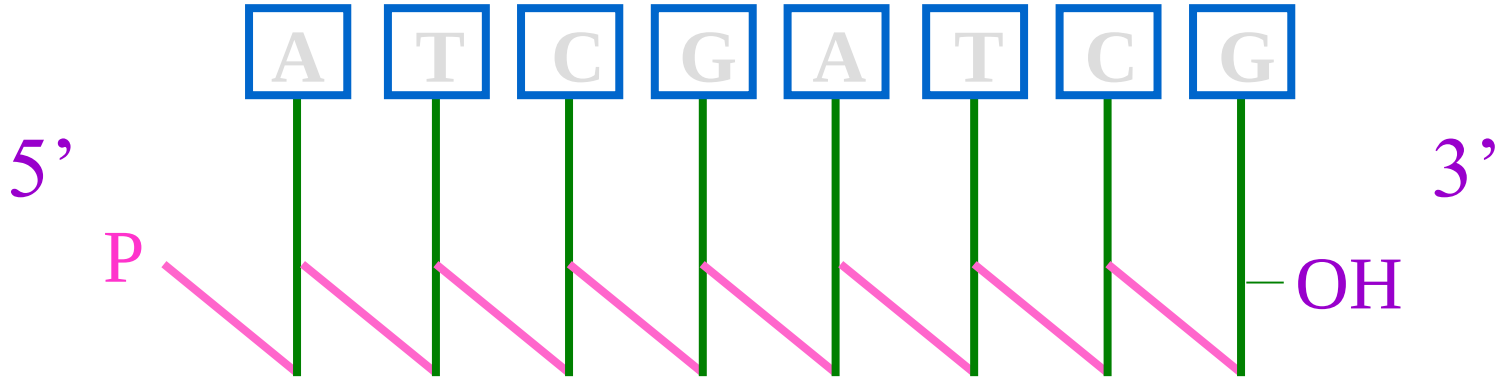
Nucleotide:	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

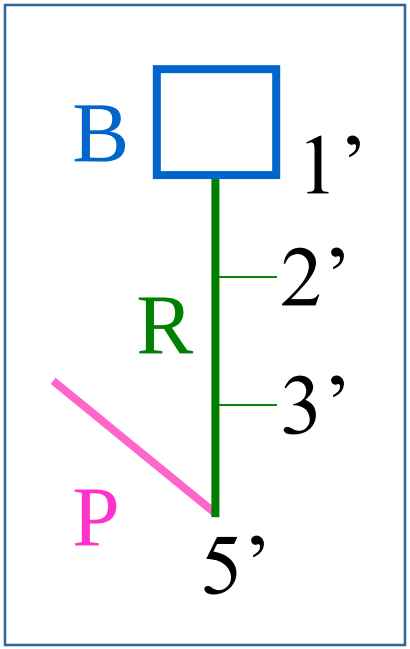
(a) Deoxyribonucleotides



(b) Ribonucleotides

The Notation for Nucleic Acids



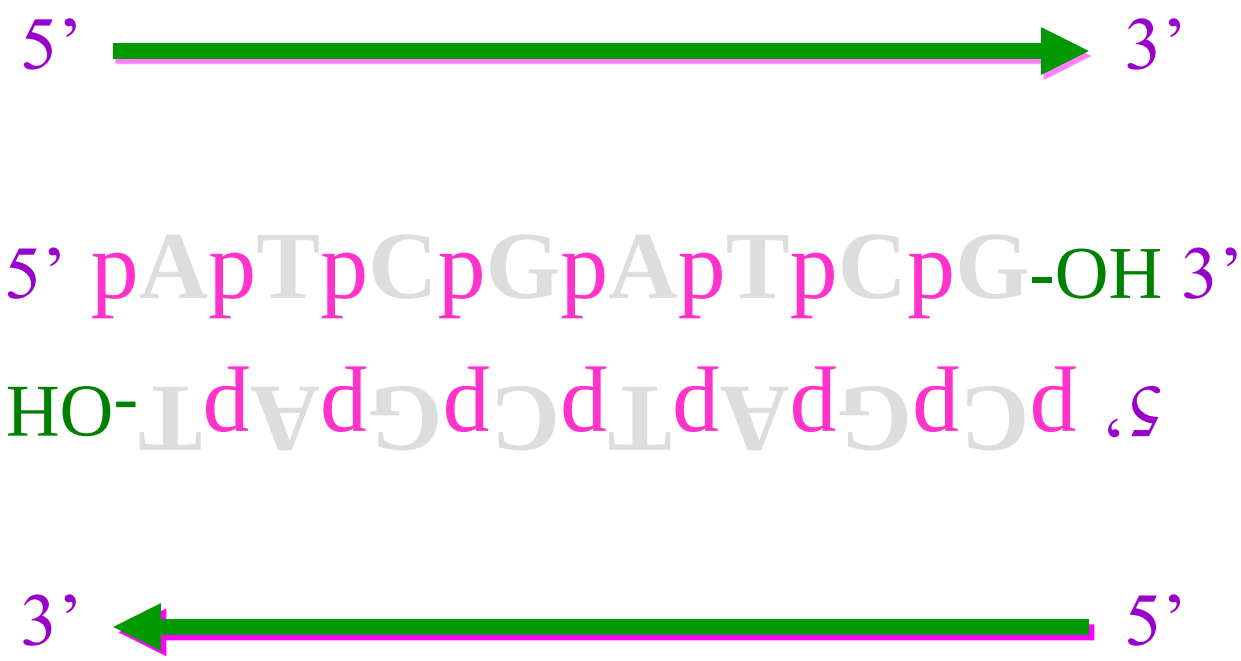


5’ pApTpCpGpApTpCpG-OH 3’

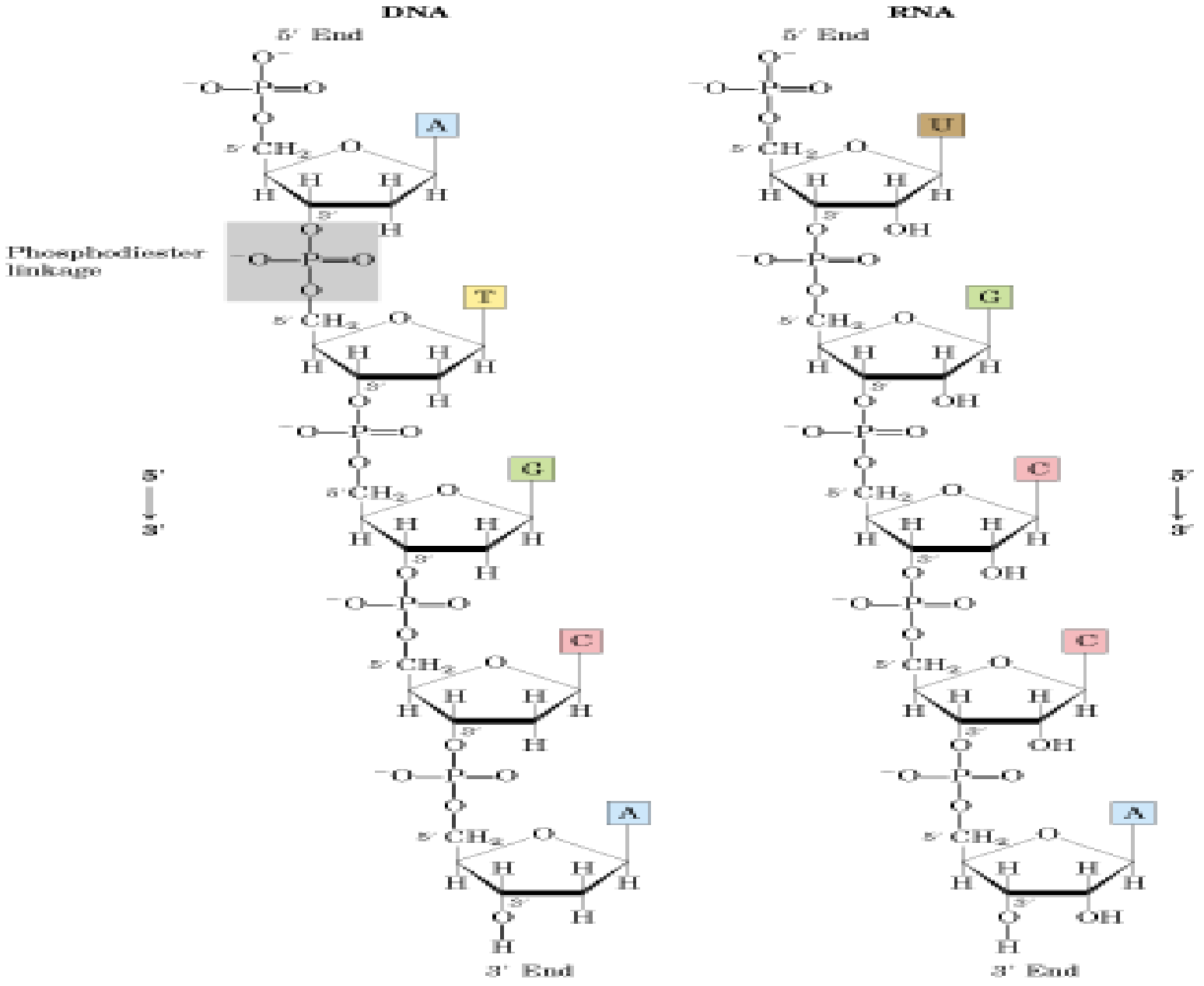
5’ pATCGATCG-OH 3’

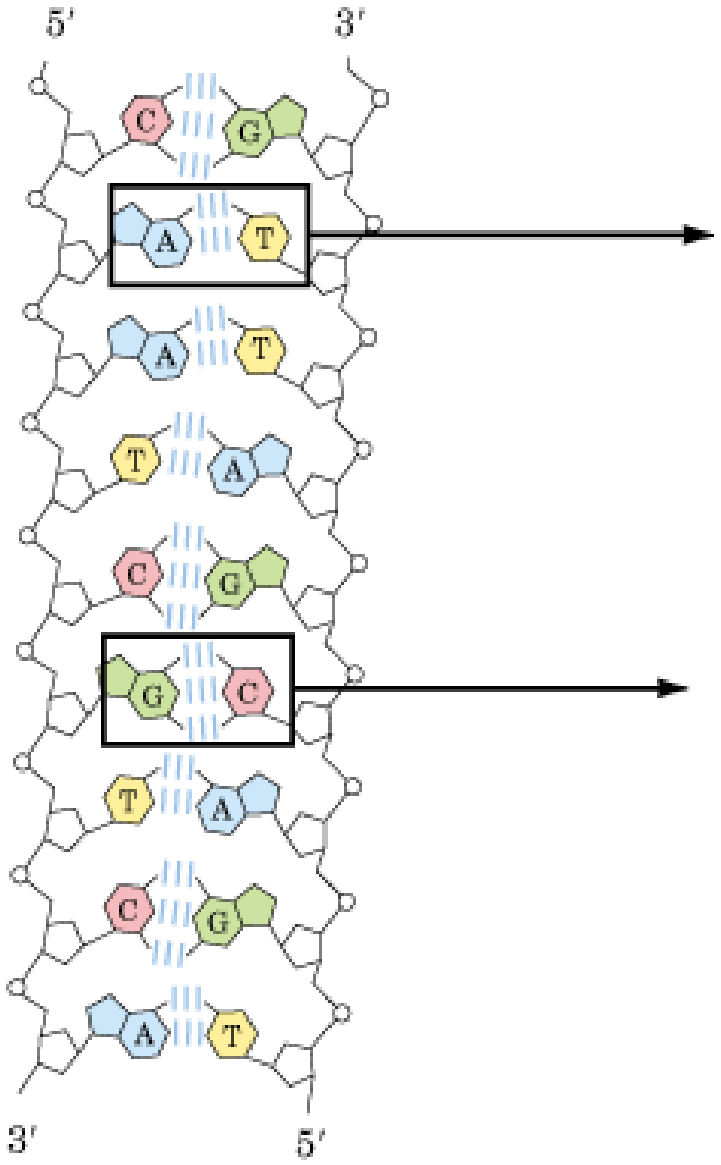
ATCGATCG

The Two Chains of DNA Are Antiparallel



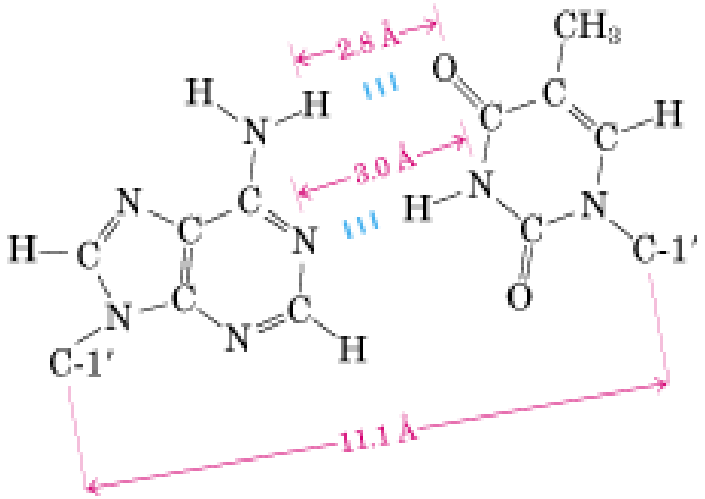
Asam Nukleat, Sintesis Protein, Pangan Transgenik; ITP 240





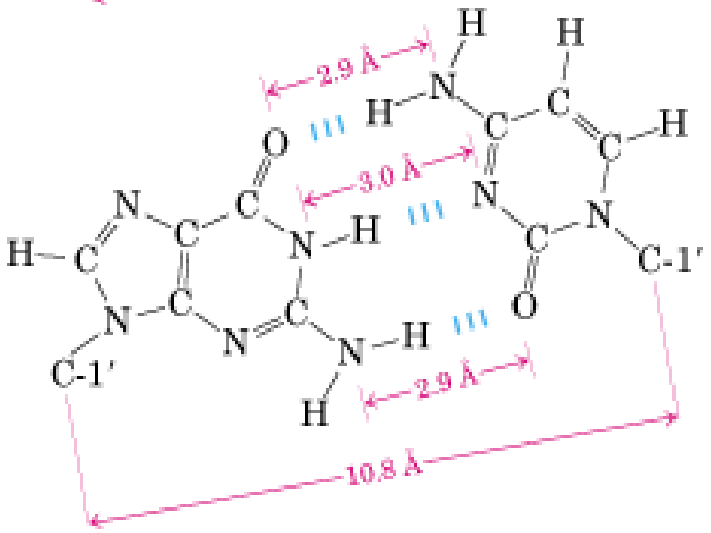
Adenine

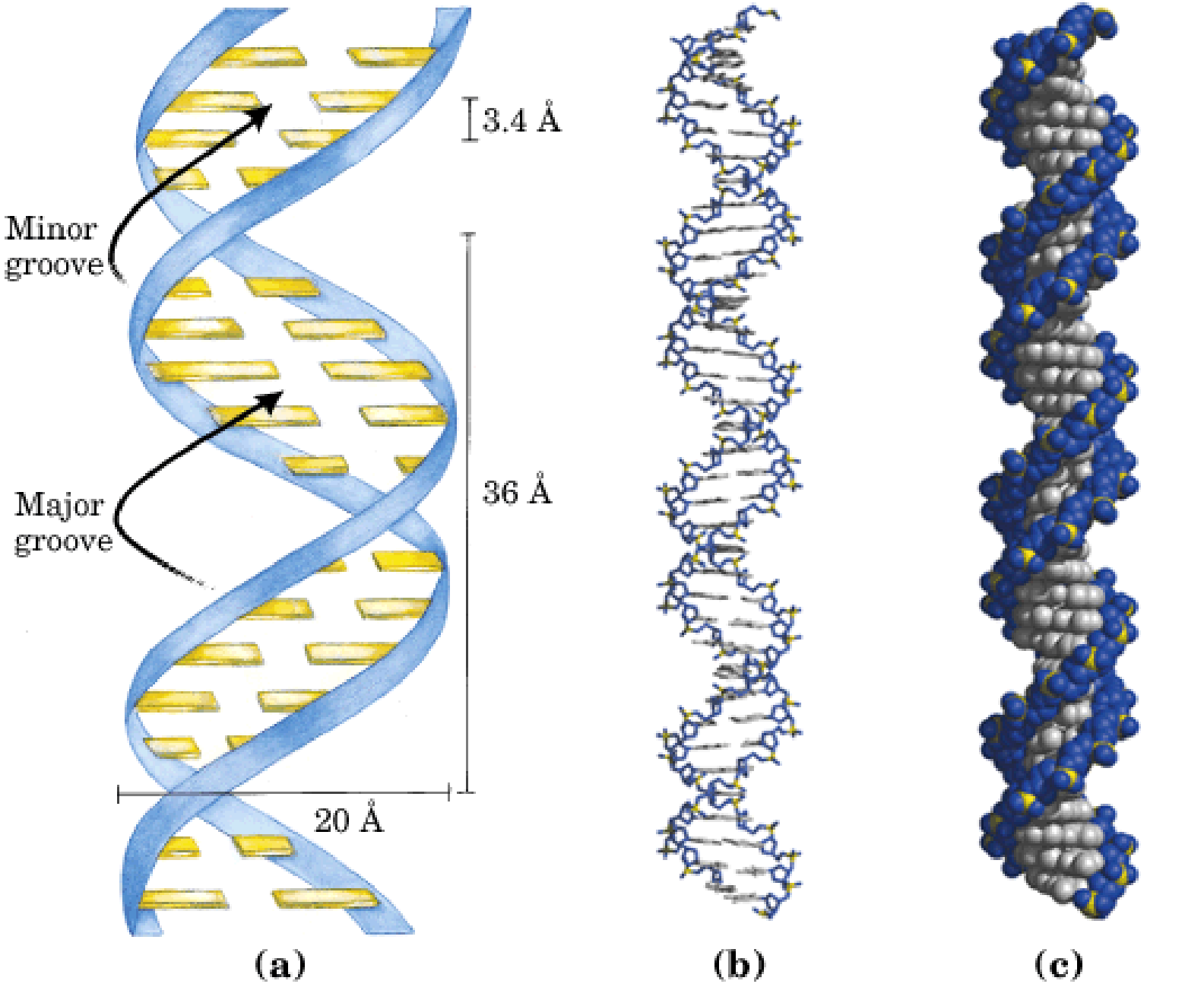
Thymine



Guanine

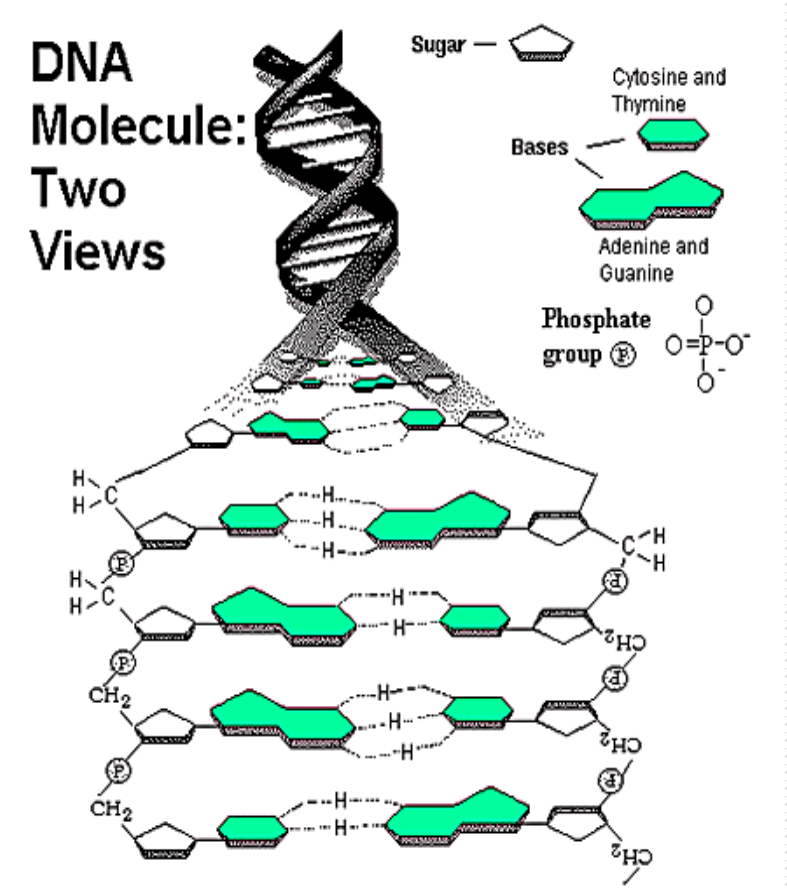
Cytosine



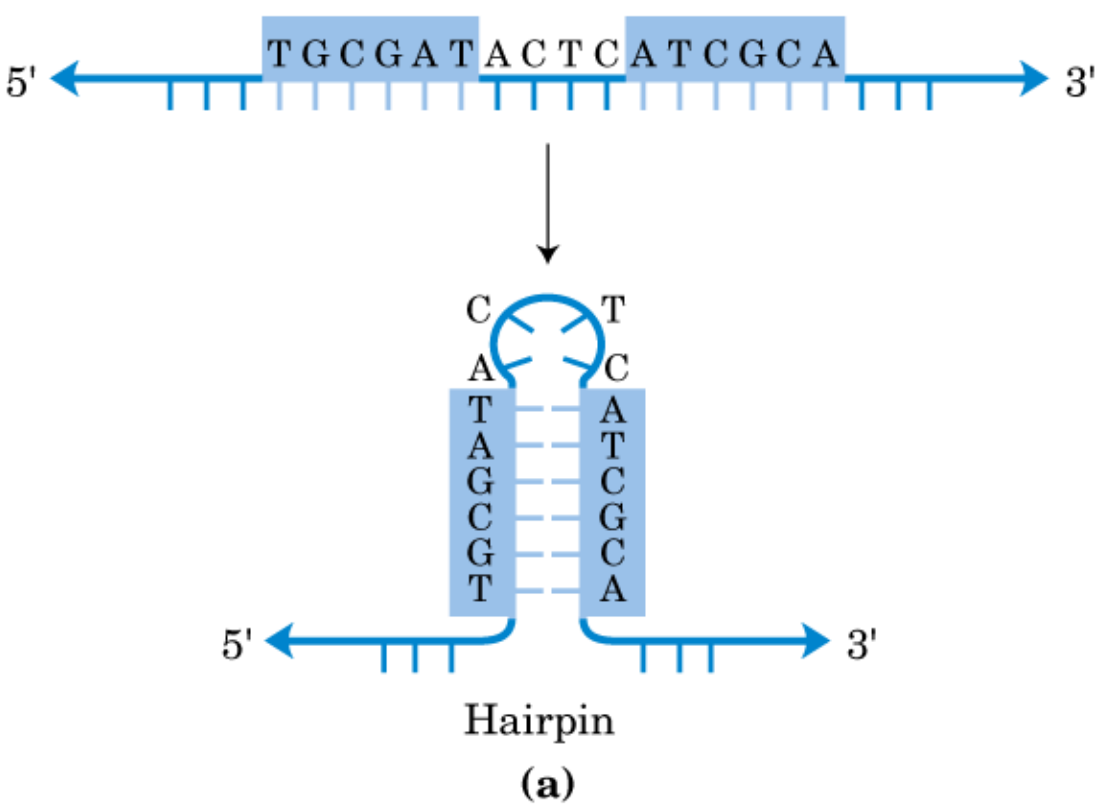

3.4 Å
Minor groove
Major groove
36 Å
20 Å
(a)
(b)
(c)

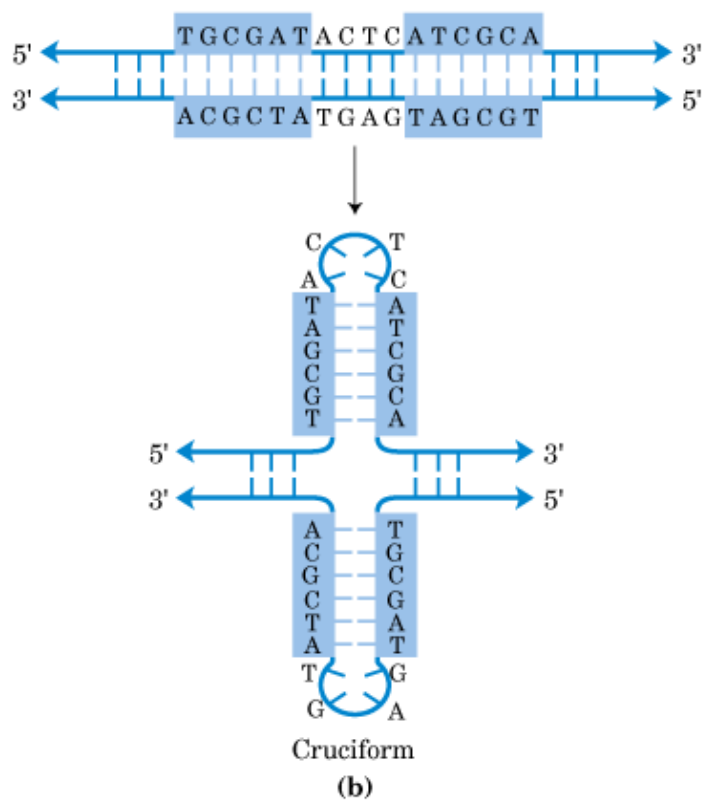
Catatan Penutup tentang DNA

- Kerangka gula/fosfat di bagian luar, basa nitrogen mengarah ke dalam
- Basa N menumpuk di atas sesamanya
- Polinukleotida dibaca dari arah 5’ ke ujung 3’. Orientasi kedua rantai (pasangan) adalah **antiparalel** : arah 5’-3’ berlawanan
- Ikatan hidrogen antara basa nitrogen kedua rantai bersifat spesifik, A dengan T, G dengan C



Untai Tunggal (mis RNA)





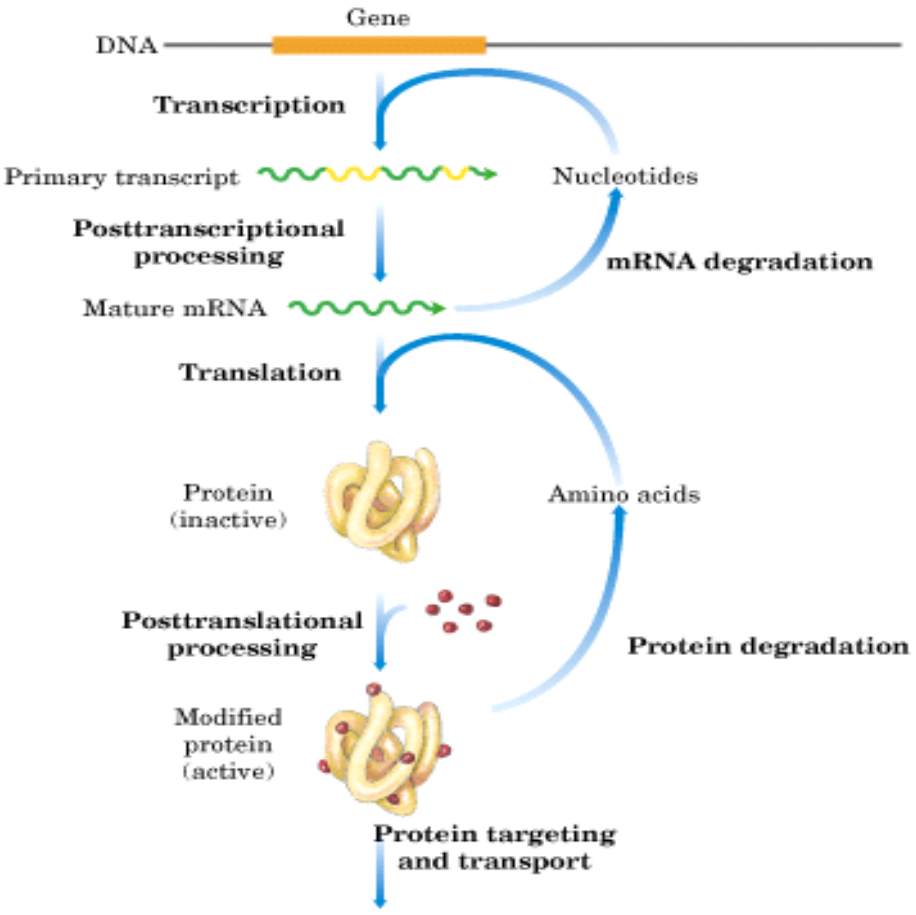
Terminologi Penting

- Gen : Area spesifik pada DNA yang memiliki fungsi yang spesifik pula; kode untuk sintesis protein
- Ekspresi Gen : sintesis protein yang dimulai dari menterjemahkan kode gen
- Ekson : bagian DNA yang menyandi protein
- Intron : bagian DNA yang tidak menyandi protein
- Replikasi : Penggandaan DNA ke sel anak
- Transkripsi : Penggandaan bagian tertentu dari gen menjadi messenger RNA (mRNA)
- Other RNA : ribosomal, transfer

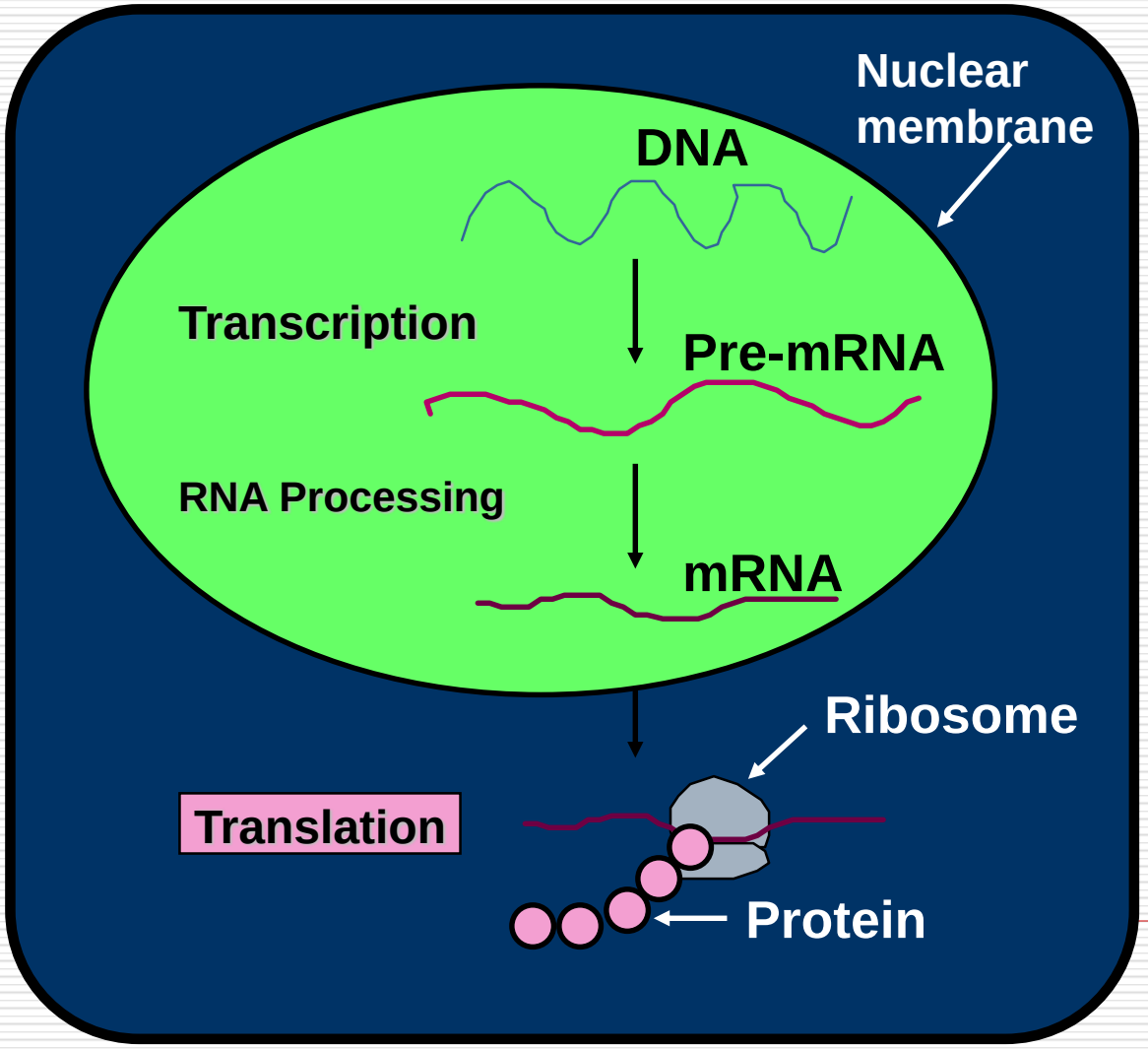
Sintesis Protein

- Transkripsi
- Pematangan RNA
- Aktivasi asam Amino; kode genetic
- Translasi
- Elongasi
- Terminasi

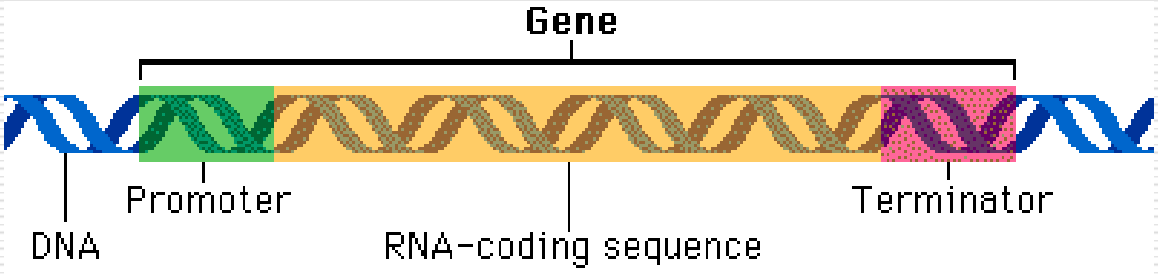
- Animasi Sintesis Protein

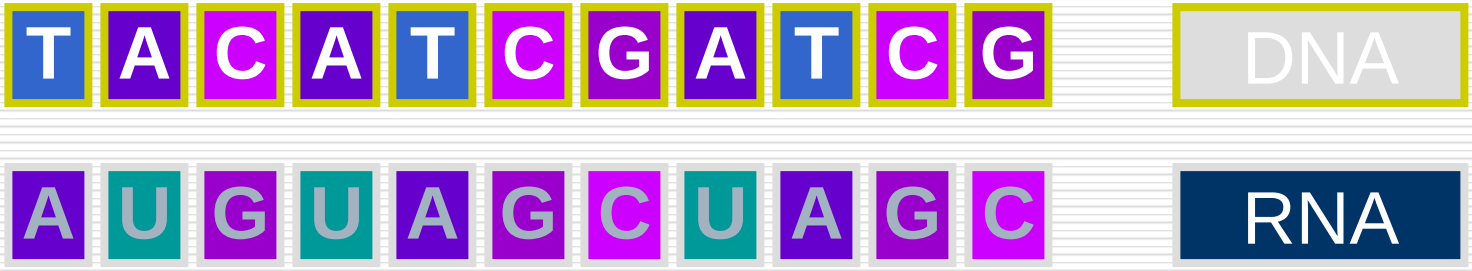


Tahap 1 : Transkripsi

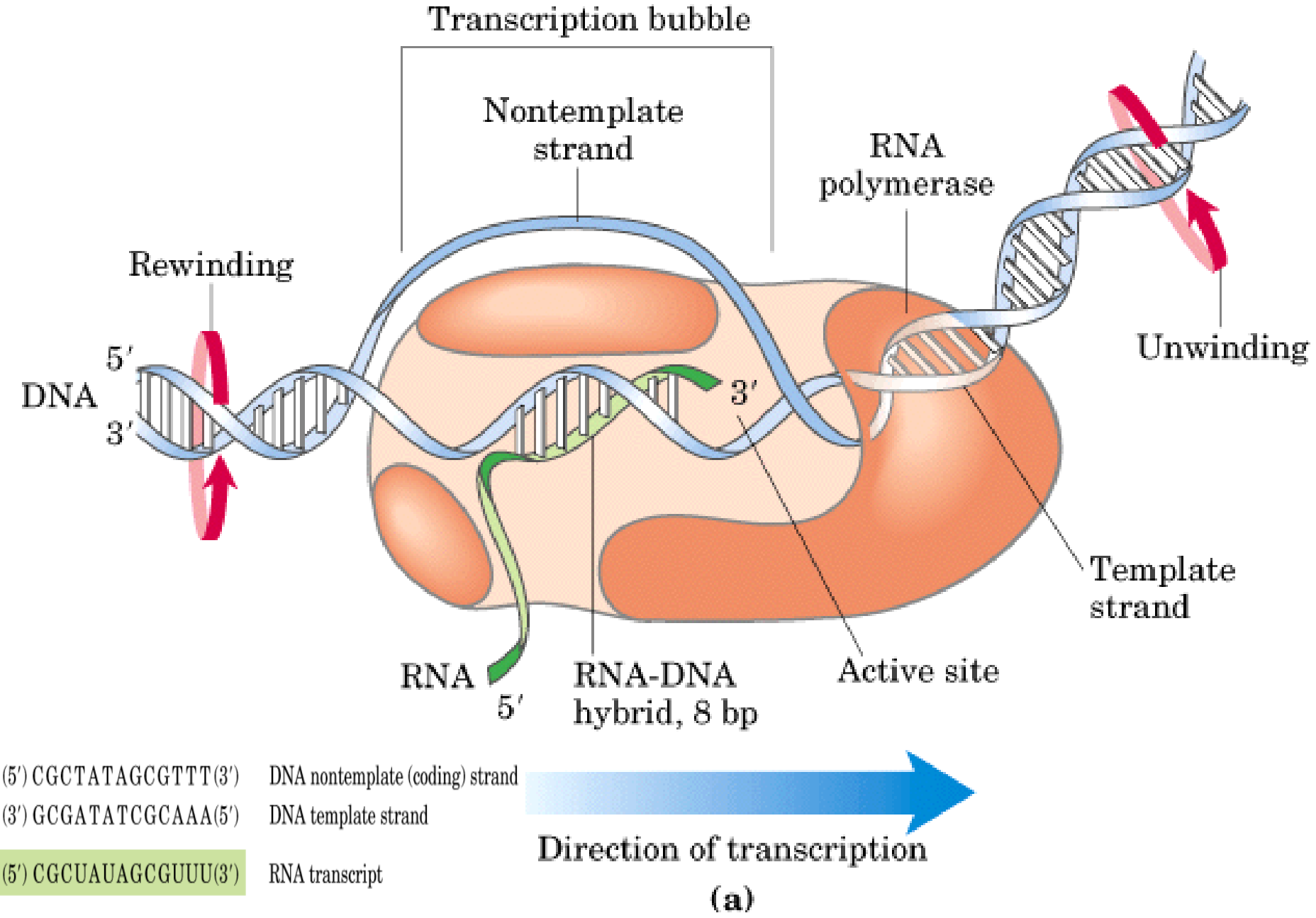


Transcription = Transkripsi

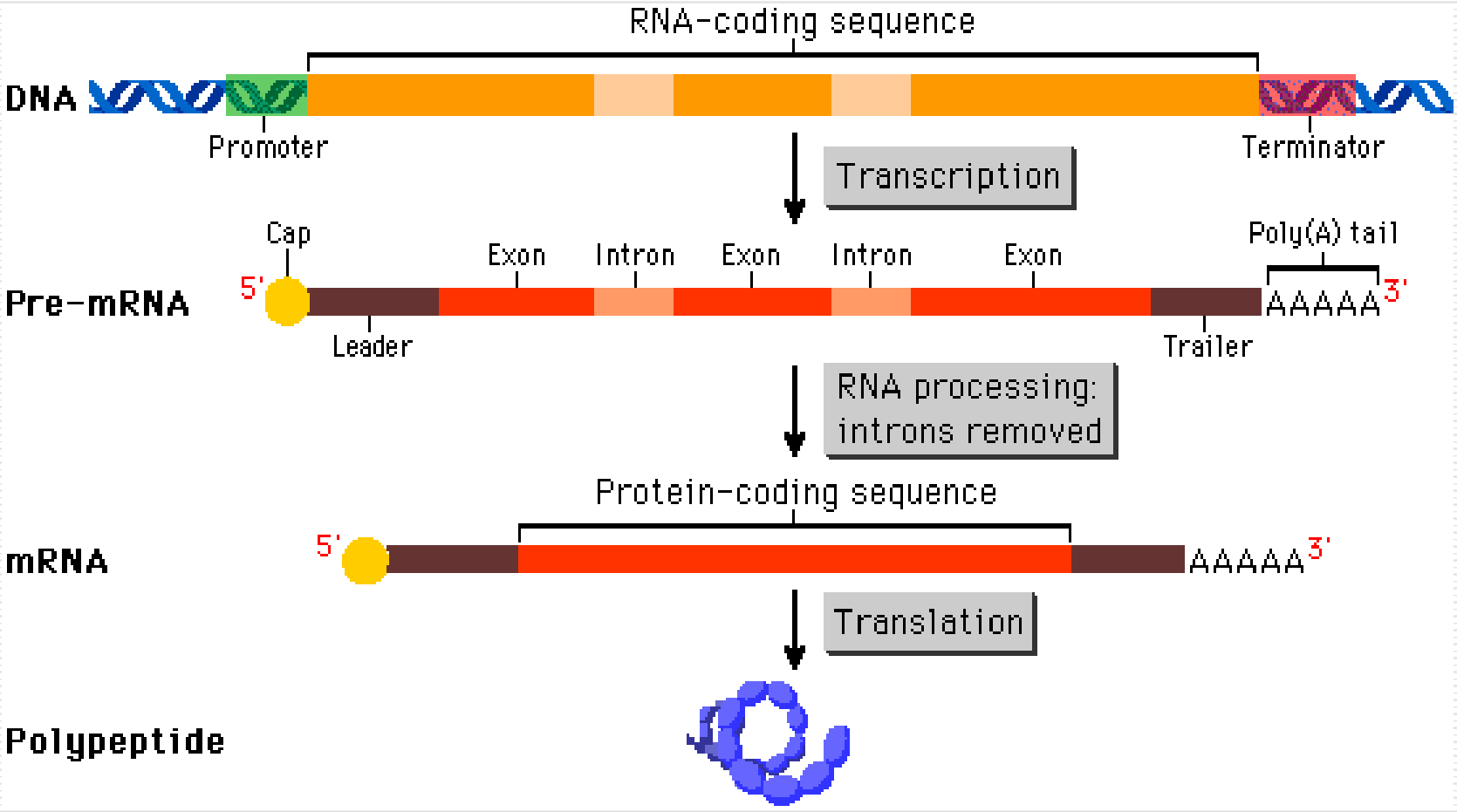




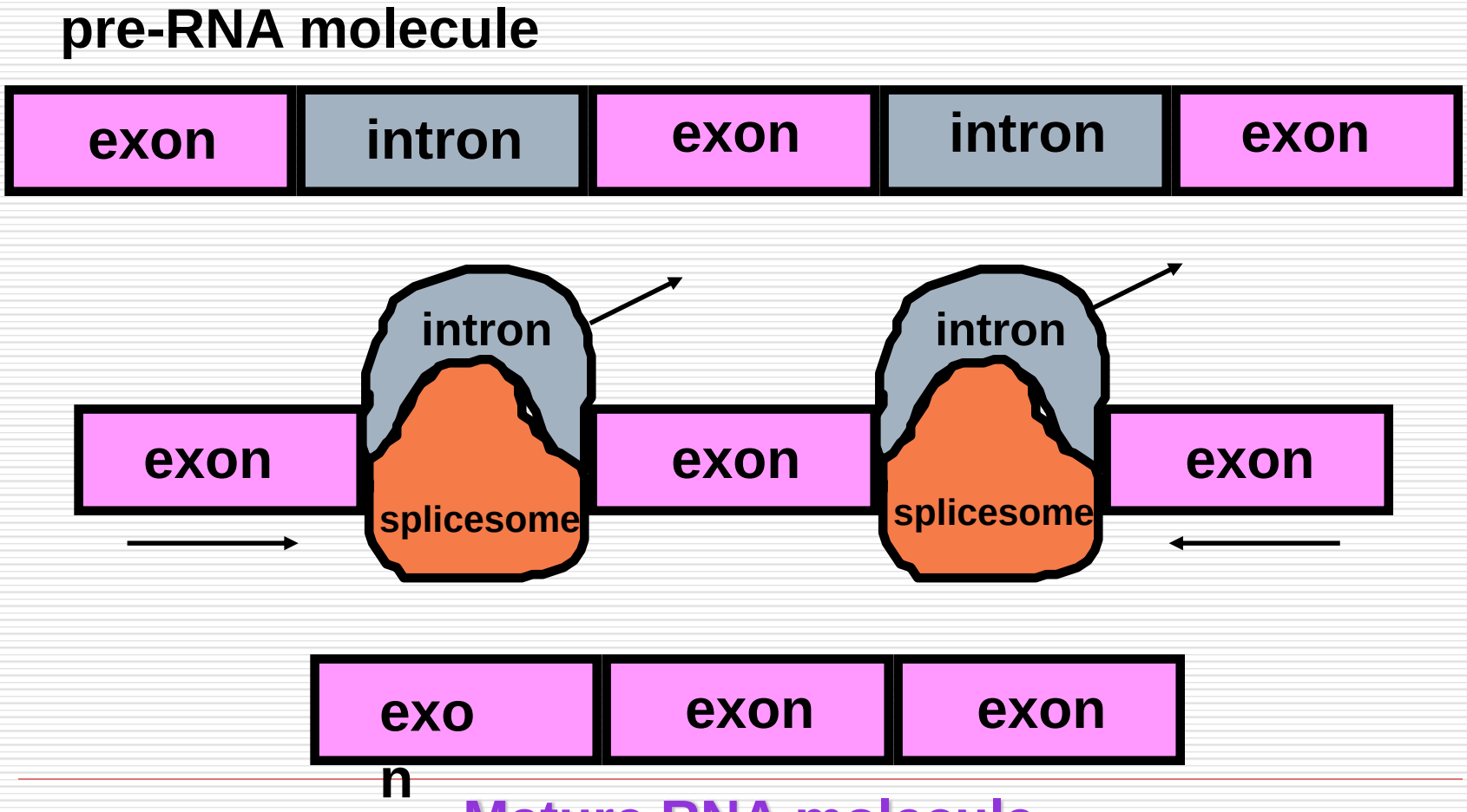
- The transfer of information in the **nucleus** from a **DNA** molecule to an **RNA** molecule.
- Only **1 DNA** strand serves as the **template;** Starts at **promoter DNA** (TATA box), Ends at **terminator DNA** (stop)
- When complete, **pre-RNA** molecule is released.


Transcription bubble
Nontemplate strand
RNA polymerase
Rewinding
Unwinding
DNA
5′
3′
3′
Template strand
RNA
5′
RNA-DNA hybrid, 8 bp
Active site
(5′) CGCTATAGCGTTT (3′)
DNA nontemplate (coding) strand
(3′) GCGATATCGCAAA (5′)
DNA template strand
(5′) CGCUAUAGCGUUU (3′)
RNA transcript
Direction of transcription
(a)

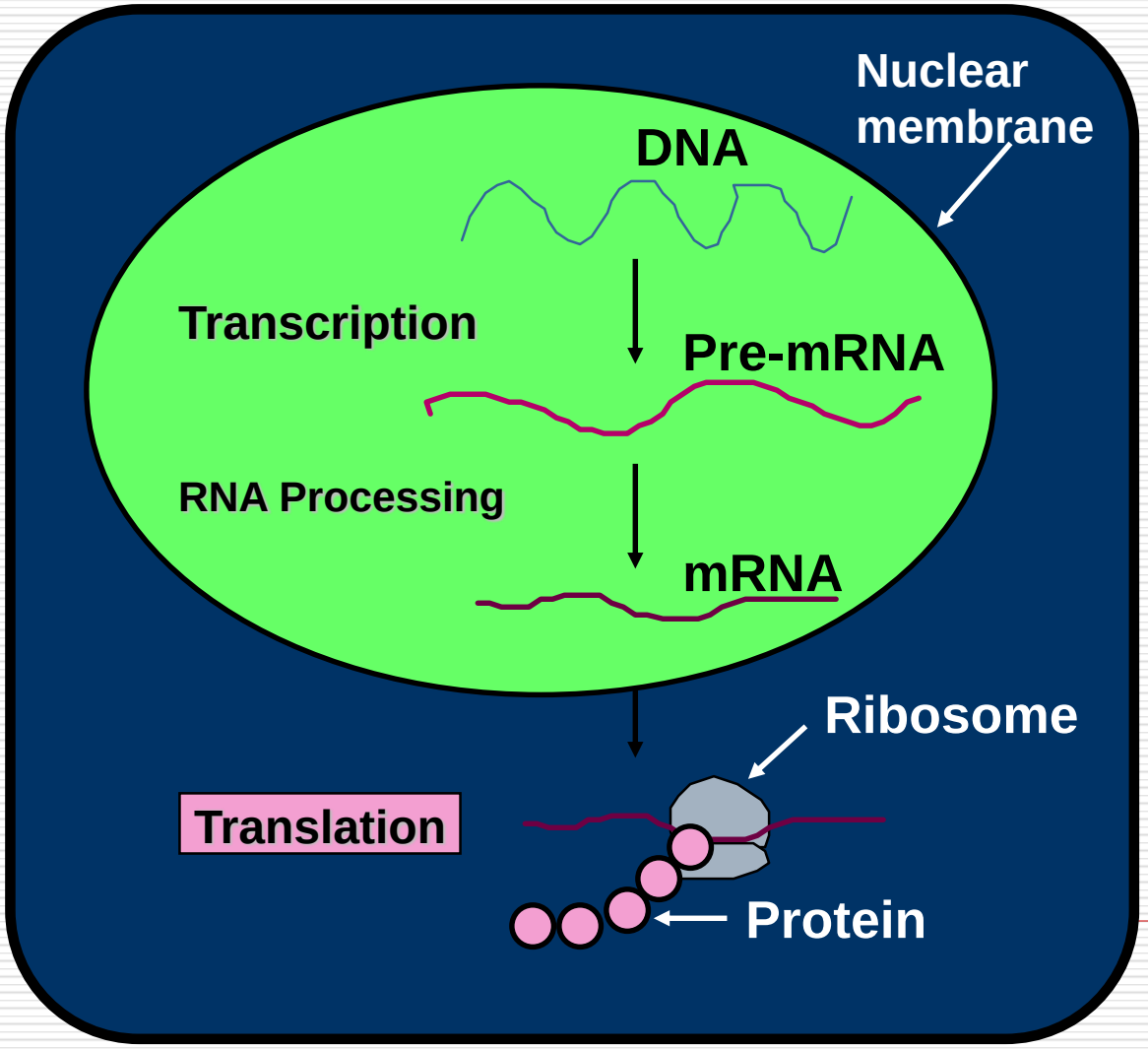
Post-transcriptional Modification



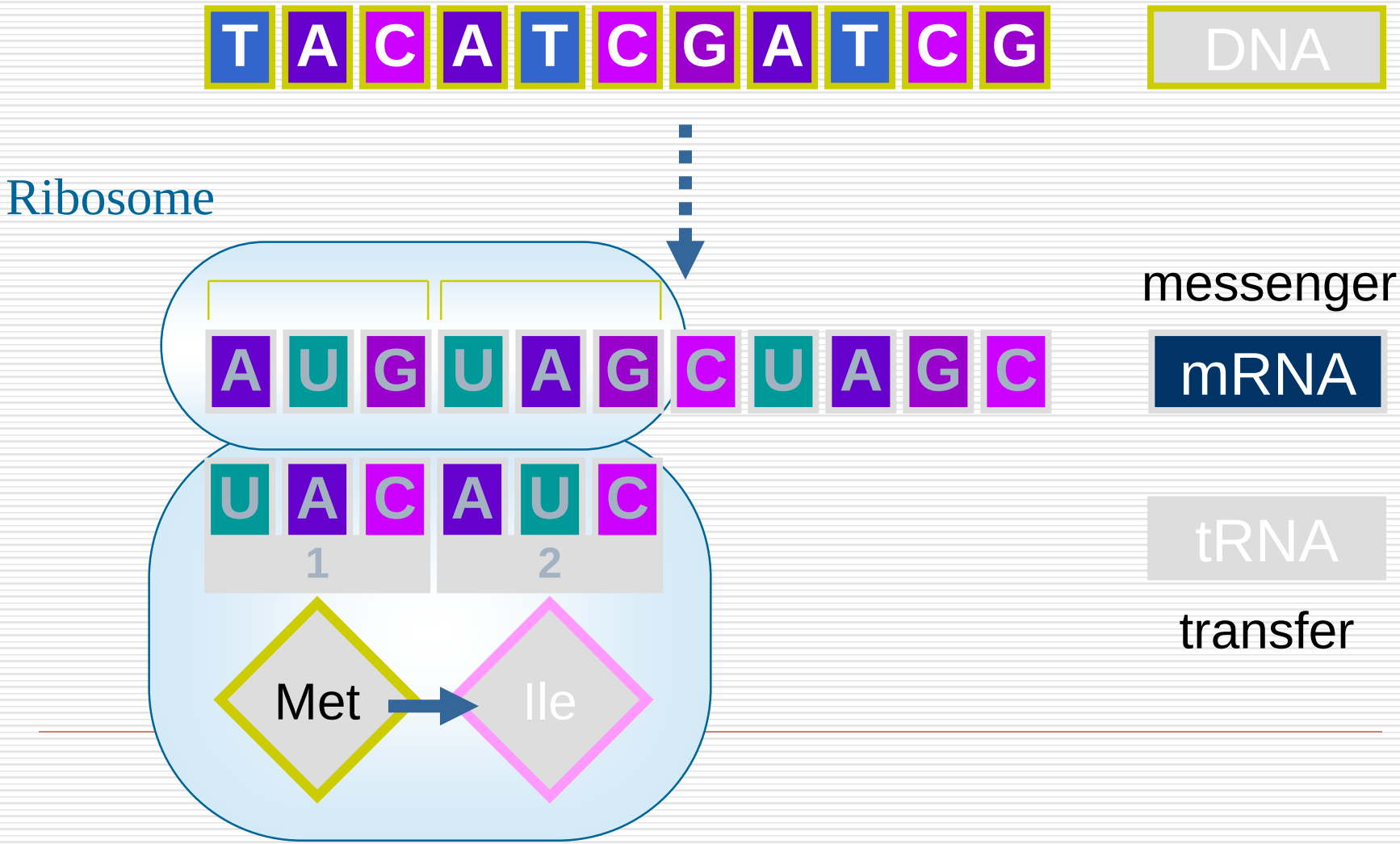
mRNA - Processing



Tahap 2 : Translasi



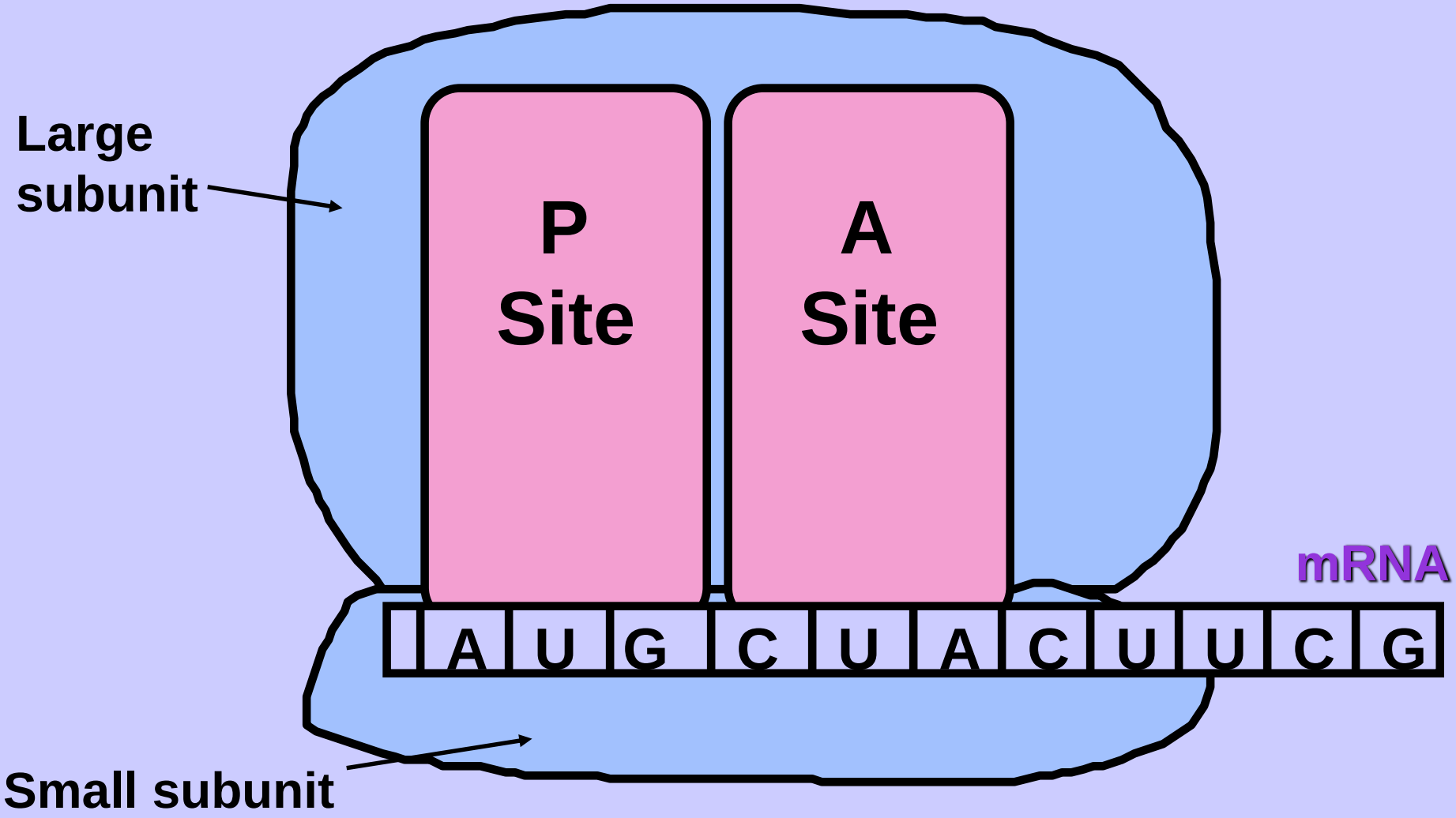
Translasi


T A C A T C G A T C G
DNA
Ribosome
messenger
A U G U A G C U A G C
mRNA
U A C A U C
1
2
tRNA
transfer
Met
Ile

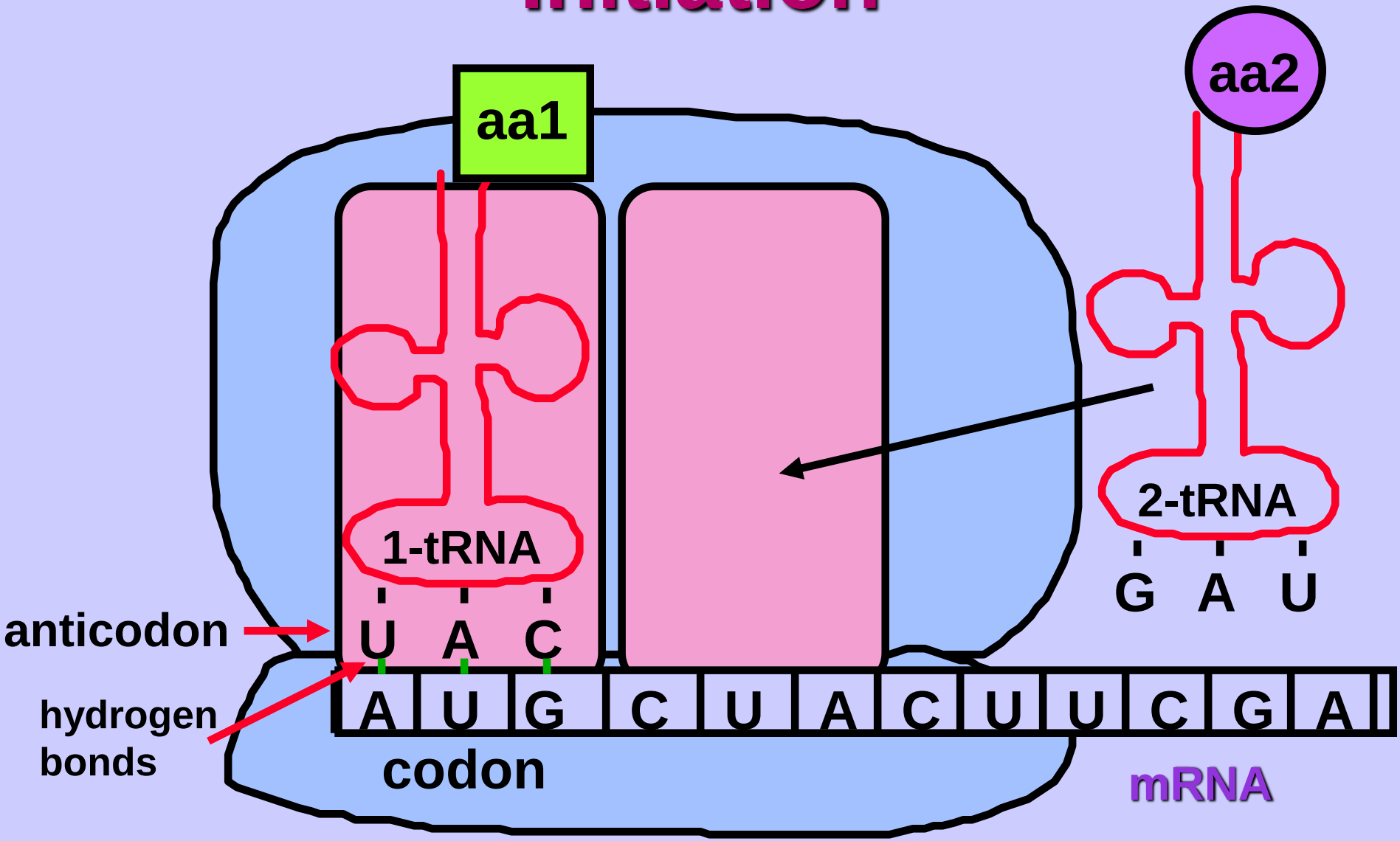
Komponen dan Tahapan

- Involves the following:
  1. mRNA (codons)
  2. tRNA (anticodons)
  3. rRNA
  4. ribosomes
  5. amino acids
- Three parts:
  1. initiation: start codon (AUG)
  2. elongation: gabungan asam amino
  3. termination: stop codon (UAG)

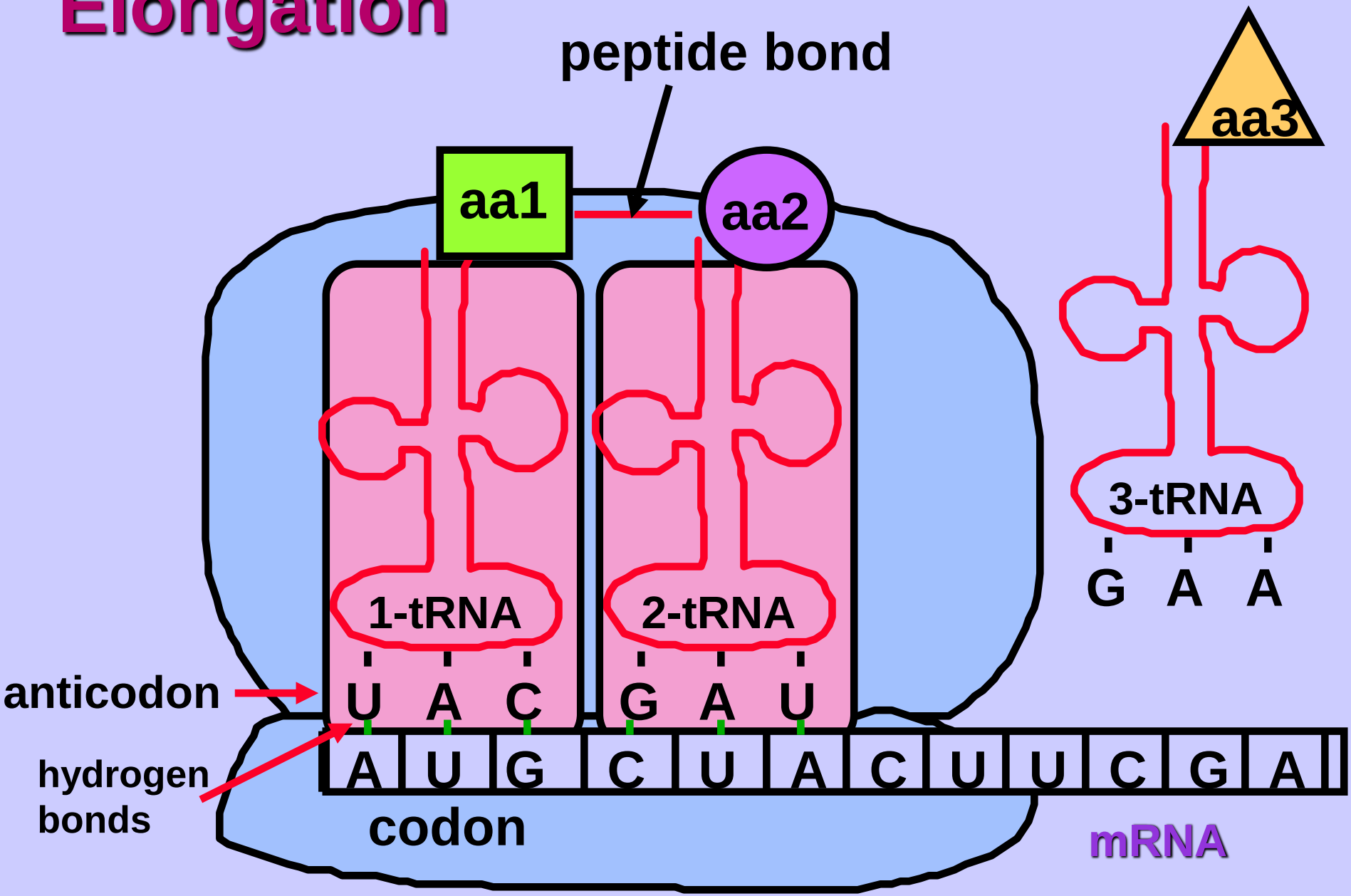
Translation at glance

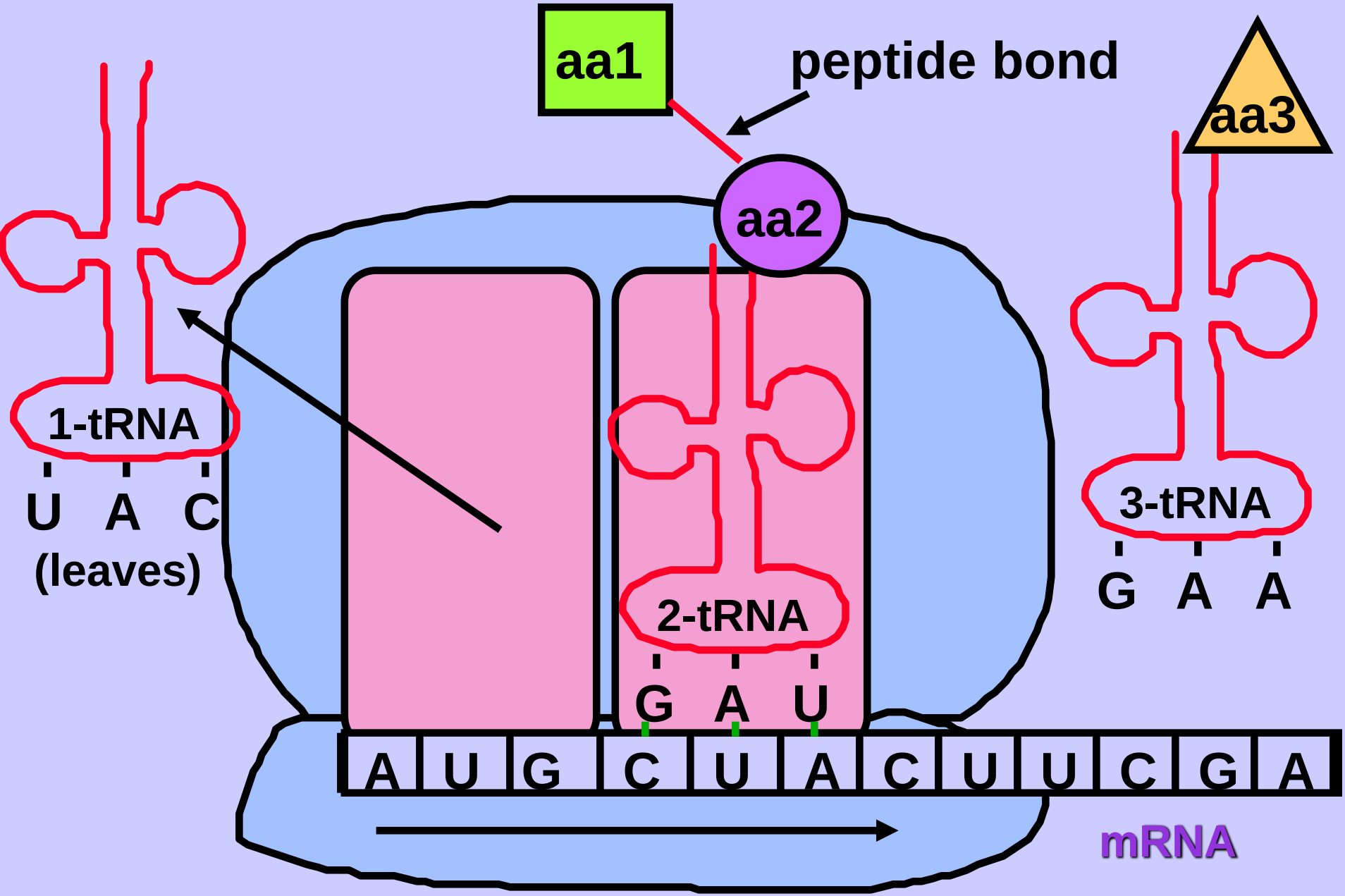


Initiation

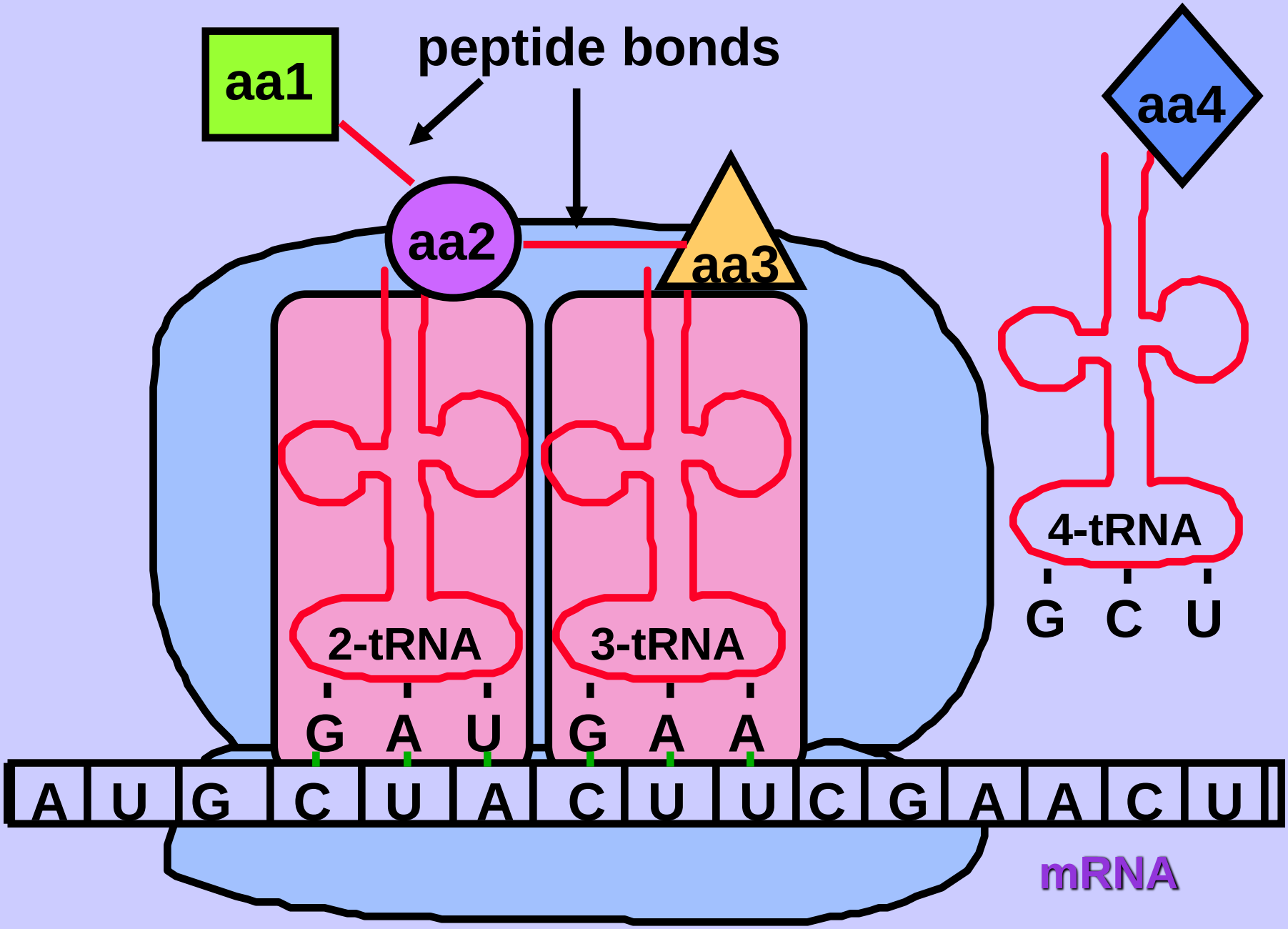

aa1
aa2
1-tRNA
2-tRNA
anticodon
U A C
G A U
hydrogen bonds
A U G C U A C U U C G A
codon
mRNA

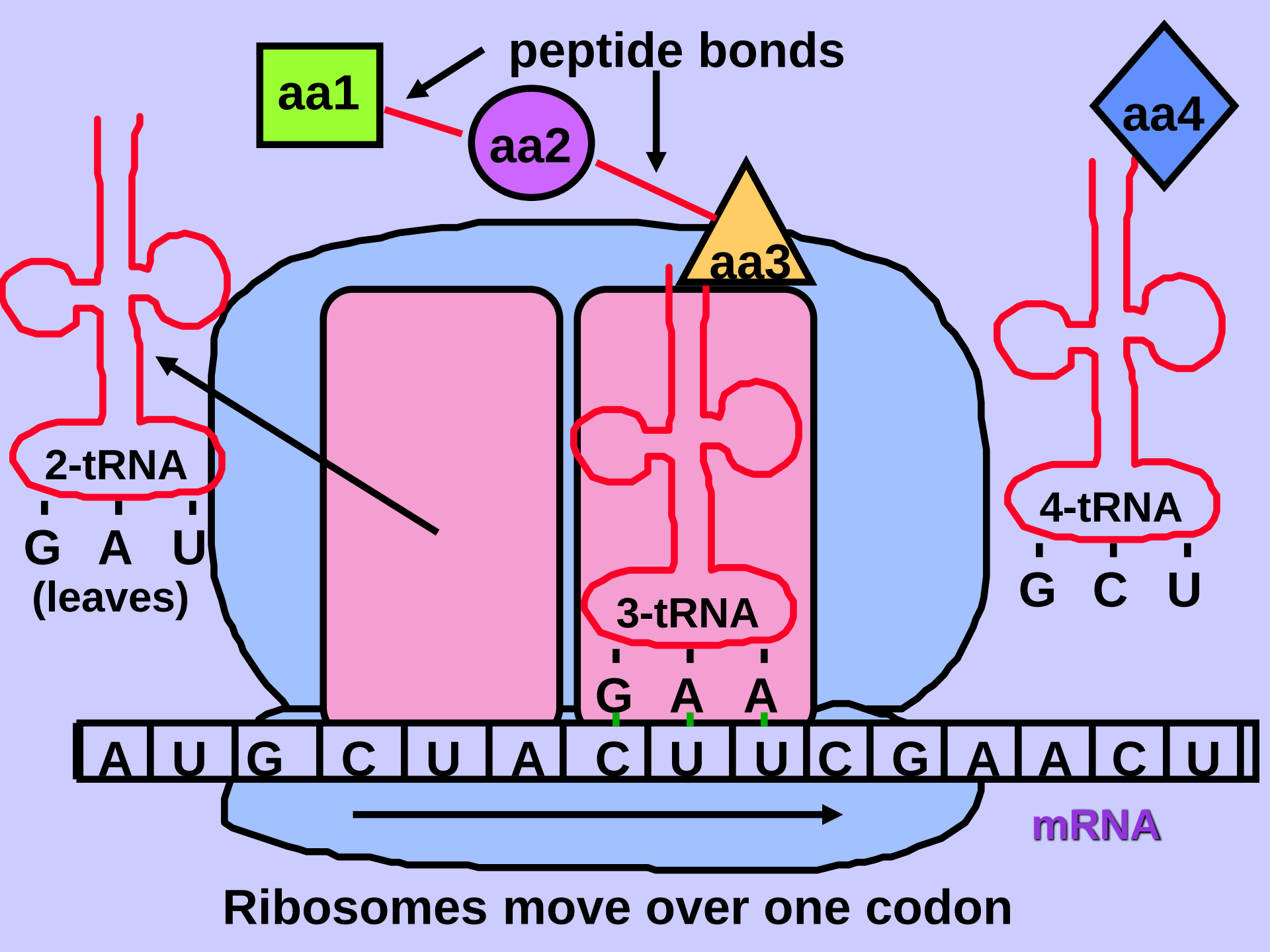
Elongation

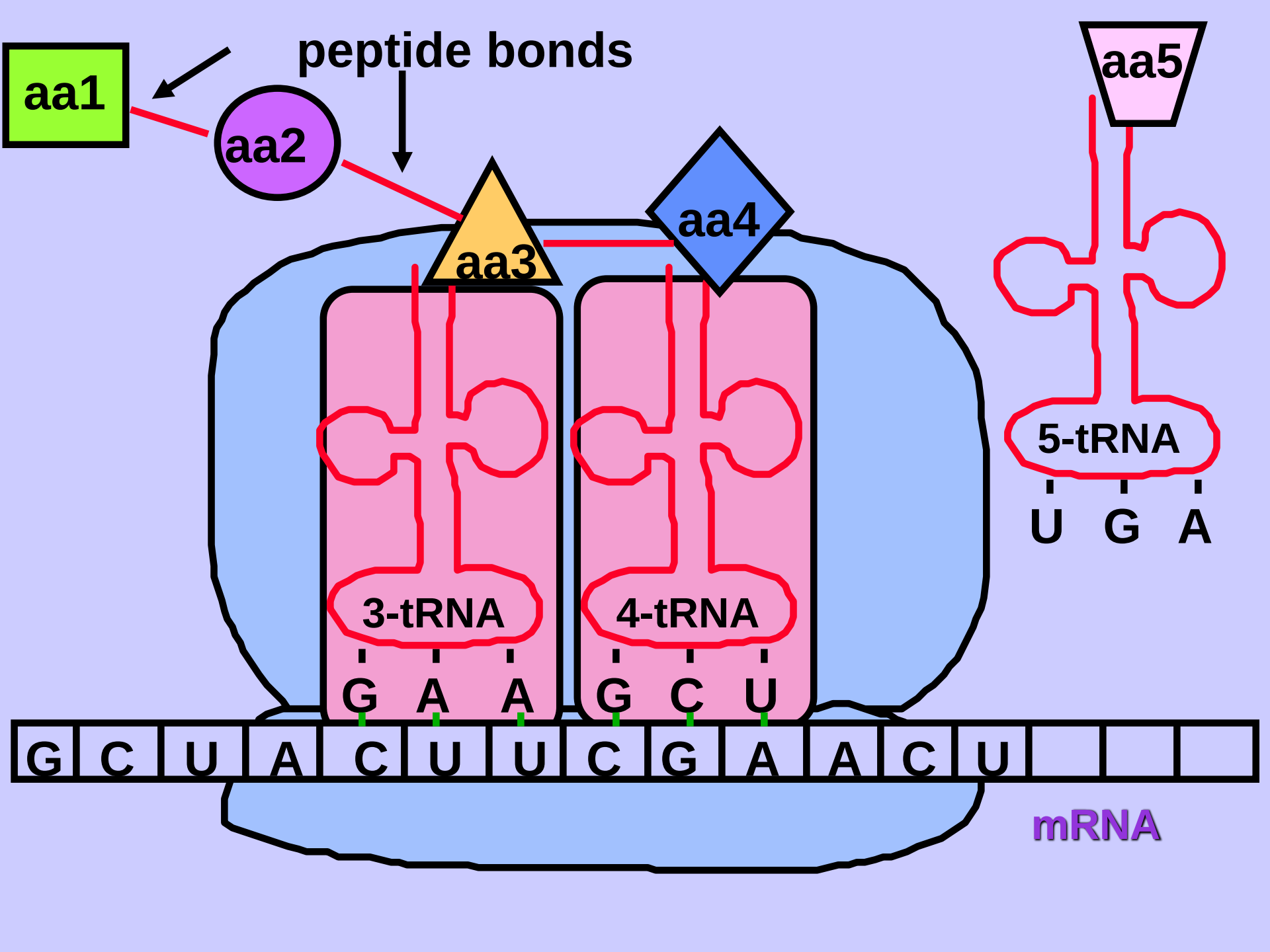

peptide bond
aa1
aa2
aa3
1-tRNA
2-tRNA
3-tRNA
G A A
anticodon
U A C
G A U
hydrogen bonds
A U G C U A C U U C G A
codon
mRNA

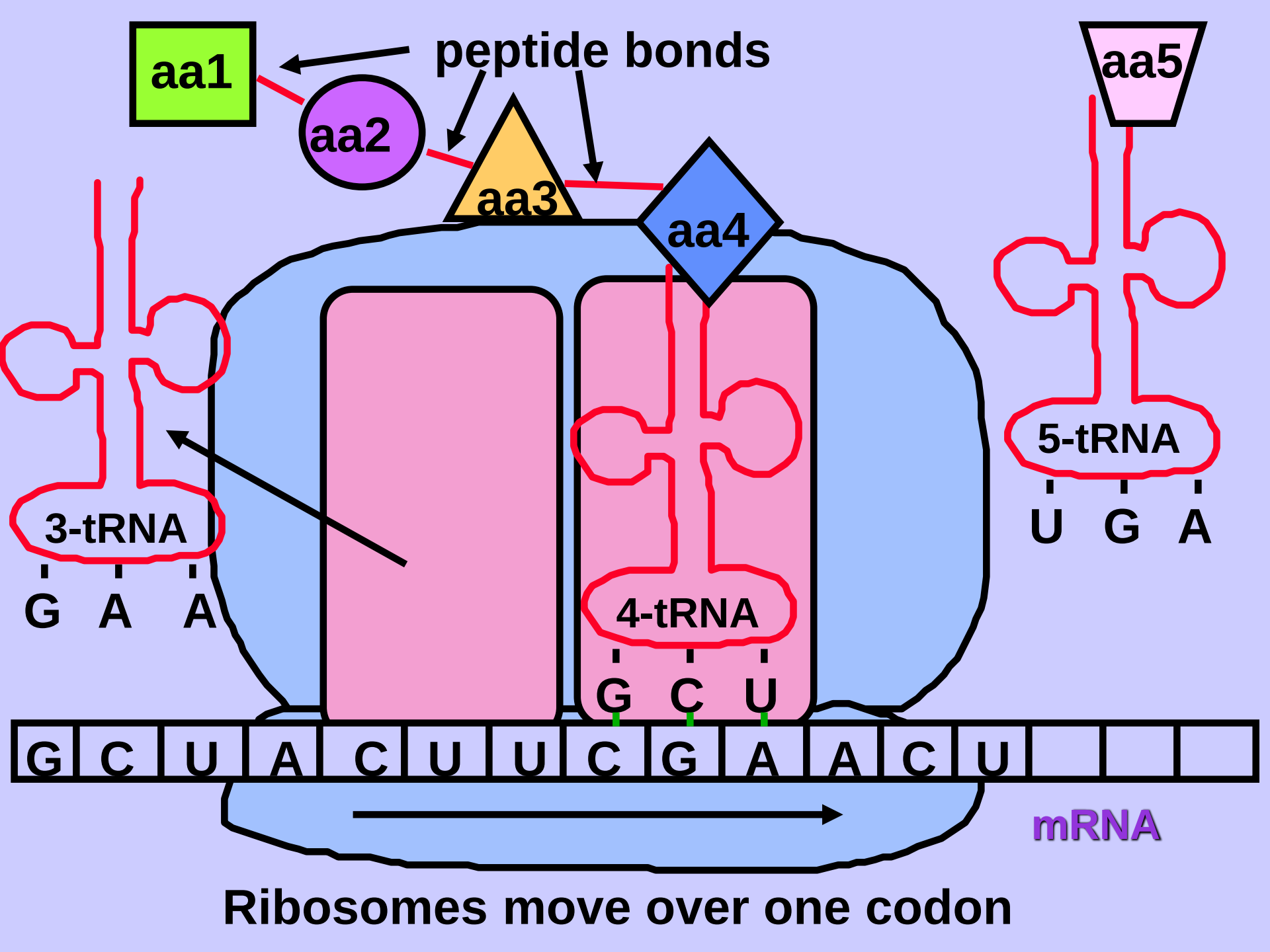


Ribosomes move over one codon


aa1
peptide bonds
aa4
aa2
aa3
4-tRNA
G C U
2-tRNA
3-tRNA
G A U
G A A
A U G C U A C U U C G A A C U
mRNA

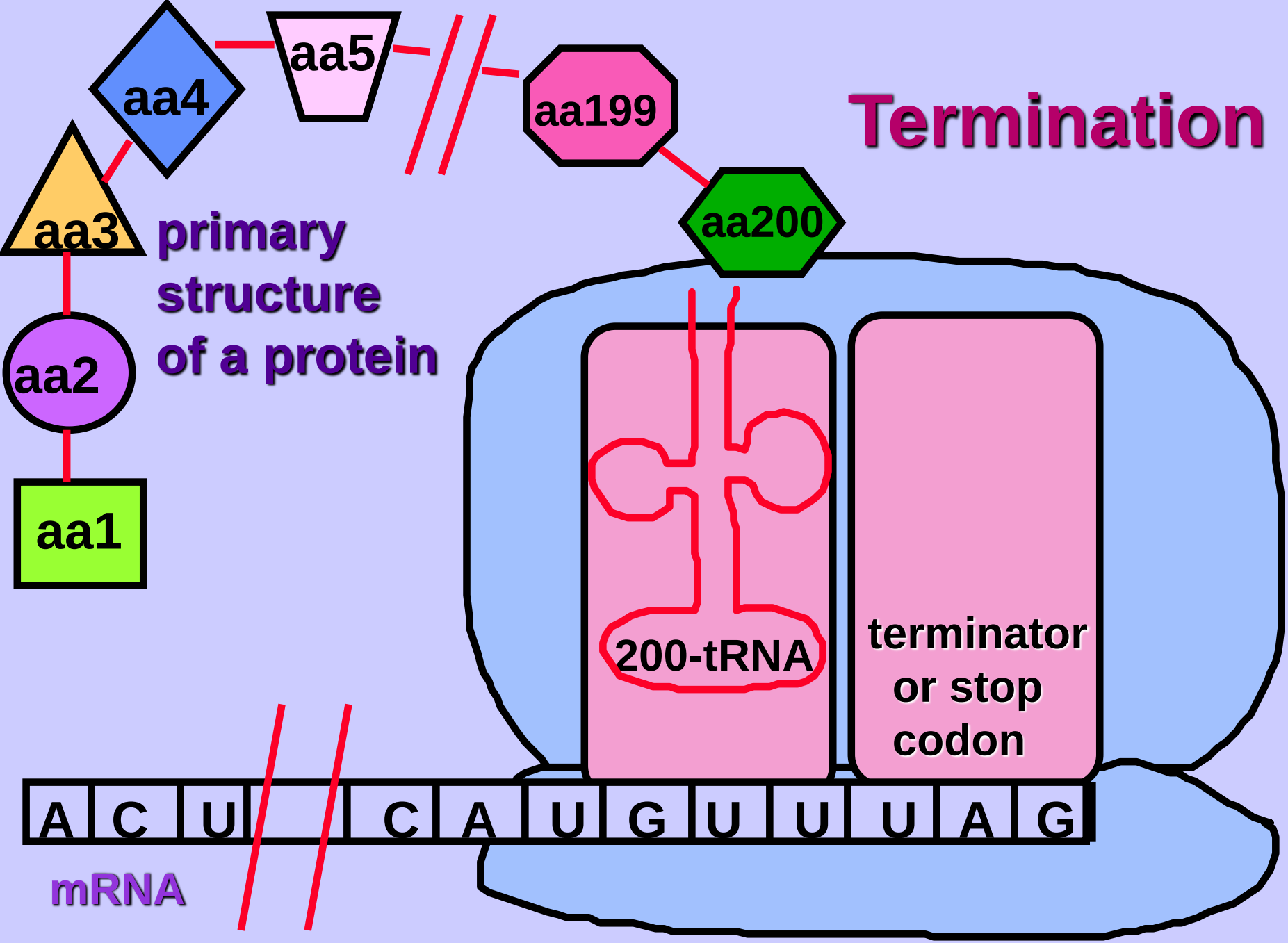

peptide bonds
aa1
aa2
aa3
aa4
2-tRNA
G A U
(leaves)
3-tRNA
G A A
4-tRNA
G C U
A U G C U A C U U C G A A C U
mRNA
Ribosomes move over one codon


peptide bonds
aa1
aa2
aa3
aa4
aa5
3-tRNA
4-tRNA
5-tRNA
G A A
G C U
U G A
G C U A C U U C G A A C U
mRNA



Ribosomes move over one codon

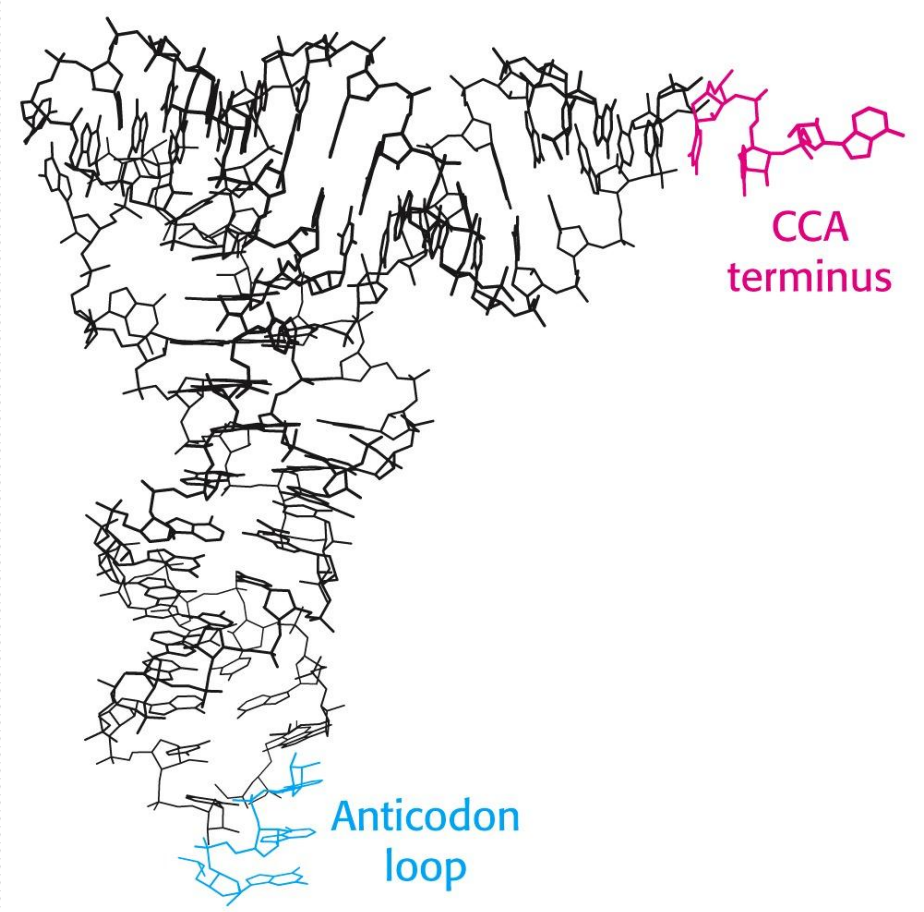
Termination

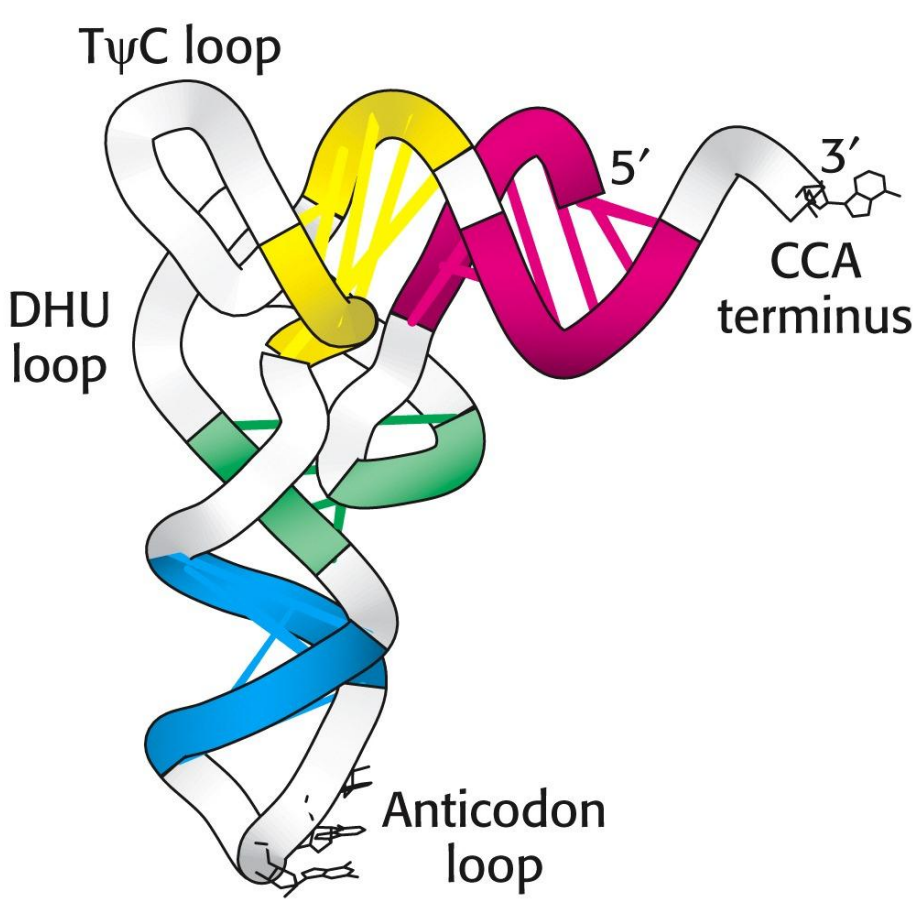


GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	
A	R	D	N	C	E	Q	G	H	I	
UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
L	K	M	F	P	S	T	W	Y	V	

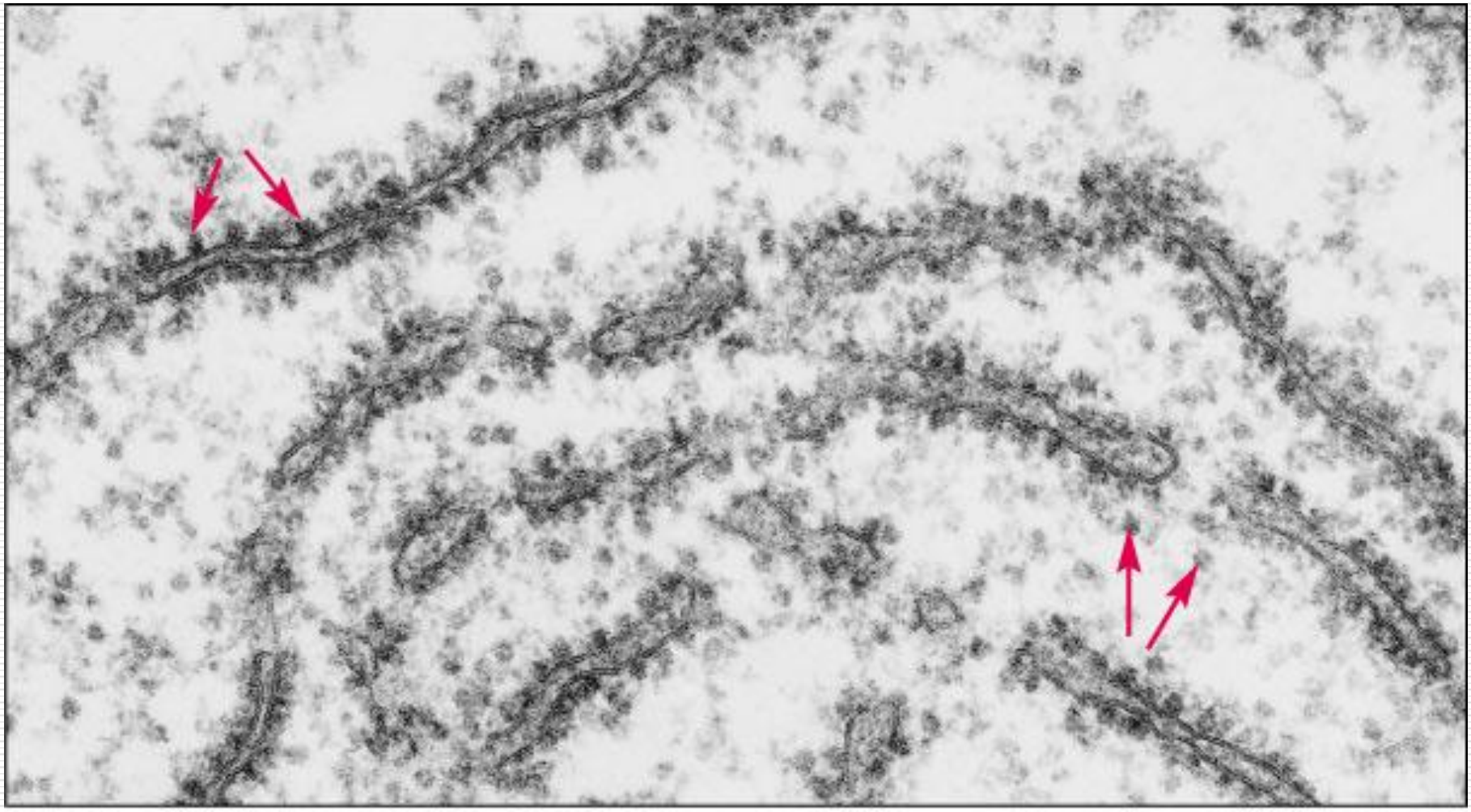
Figure 6–50. Molecular Biology of the Cell, 4th Edition.

Struktur

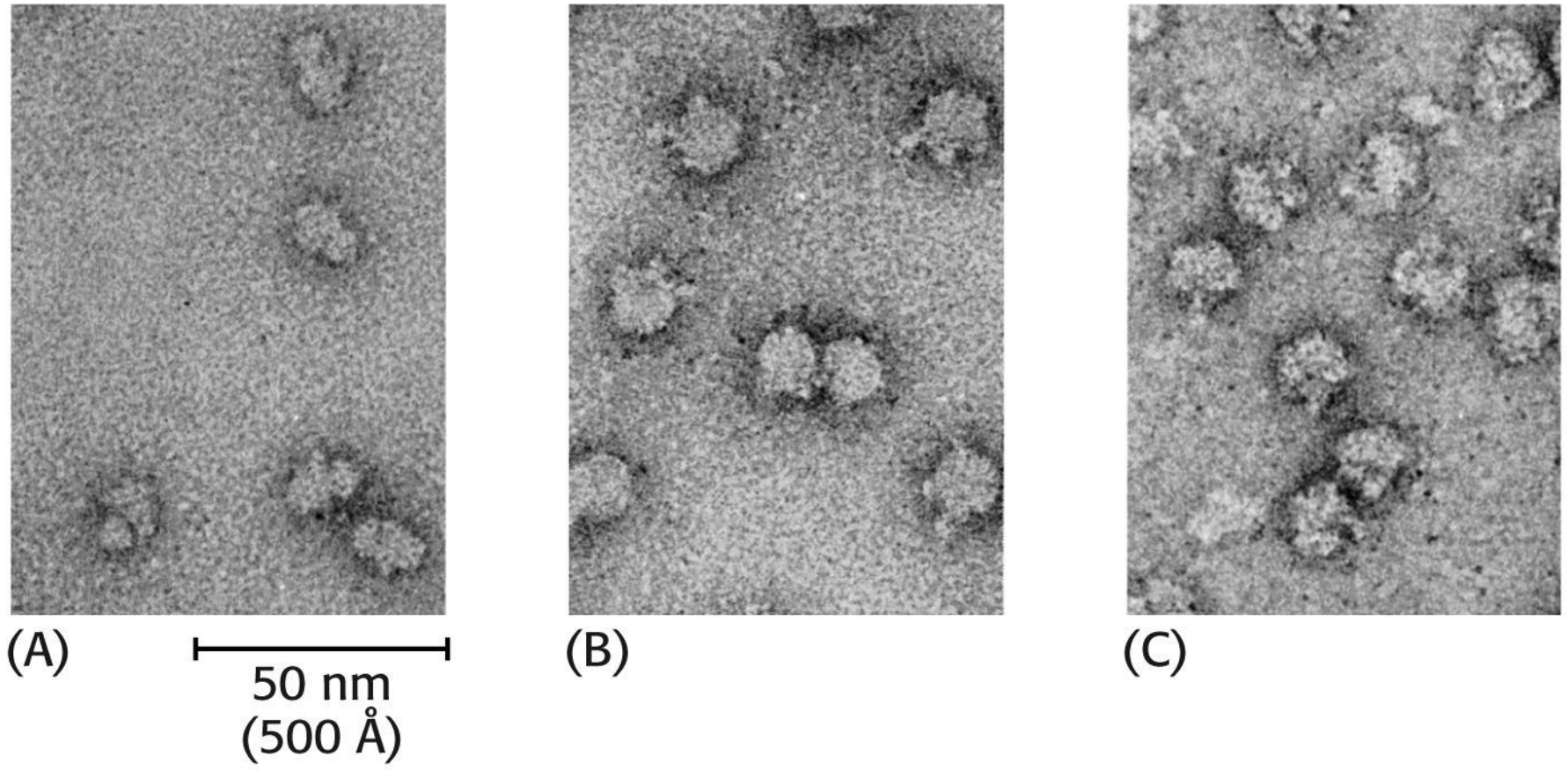

CCA
terminus
Anticodon
loop

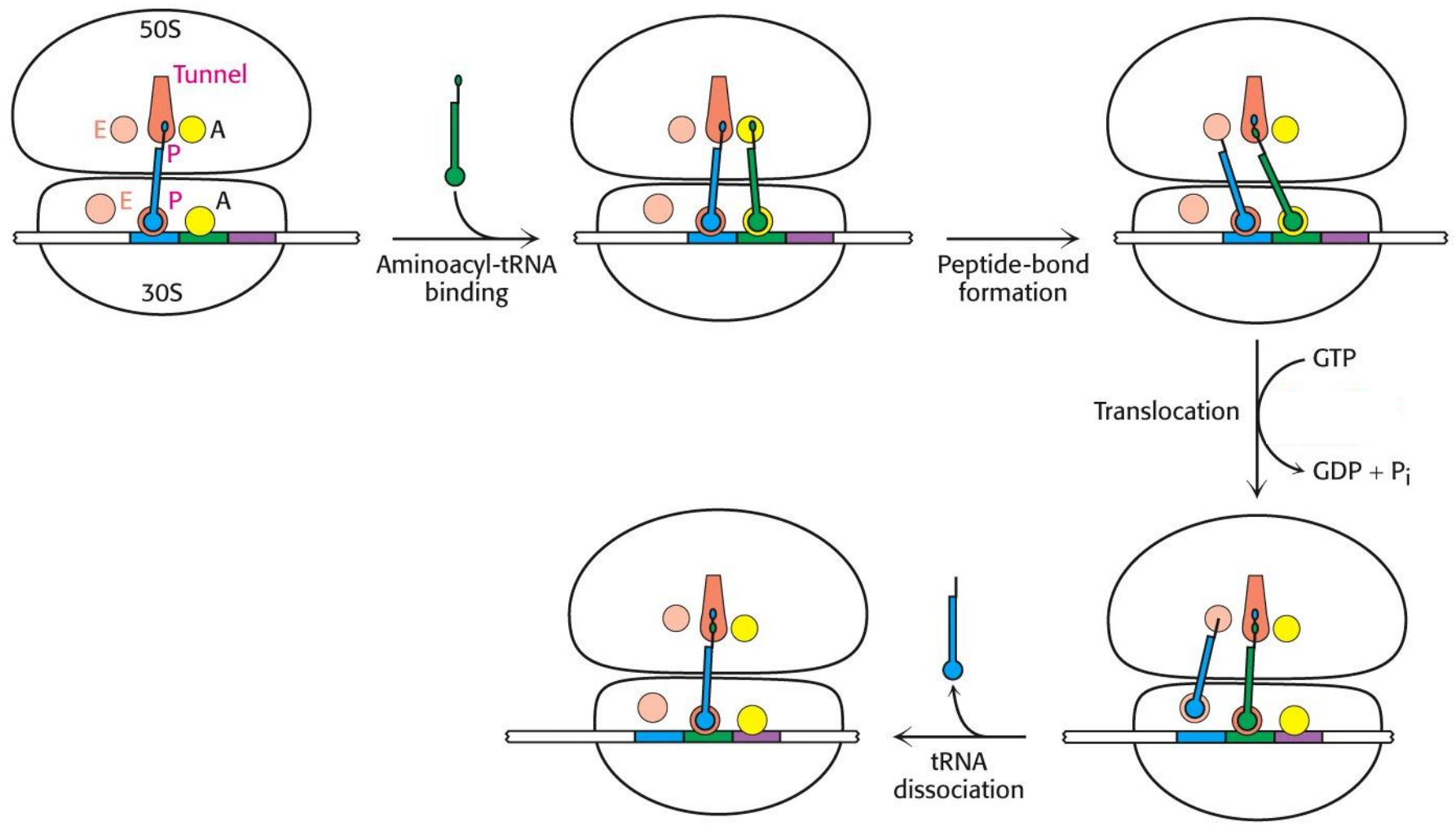

TψC loop
5′
3′
DHU
loop
CCA
terminus
Anticodon
loop

Protein synthesis occurs on ribosomes



Protein synthesis occurs on ribosomes




50S
Tunnel
E
A
P
E
P
A
30S
Aminoacyl-tRNA binding
Peptide-bond formation
GTP
Translocation
GDP + Pi
tRNA dissociation

Peptide bond formation is catalyzed by the large subunit rRNA

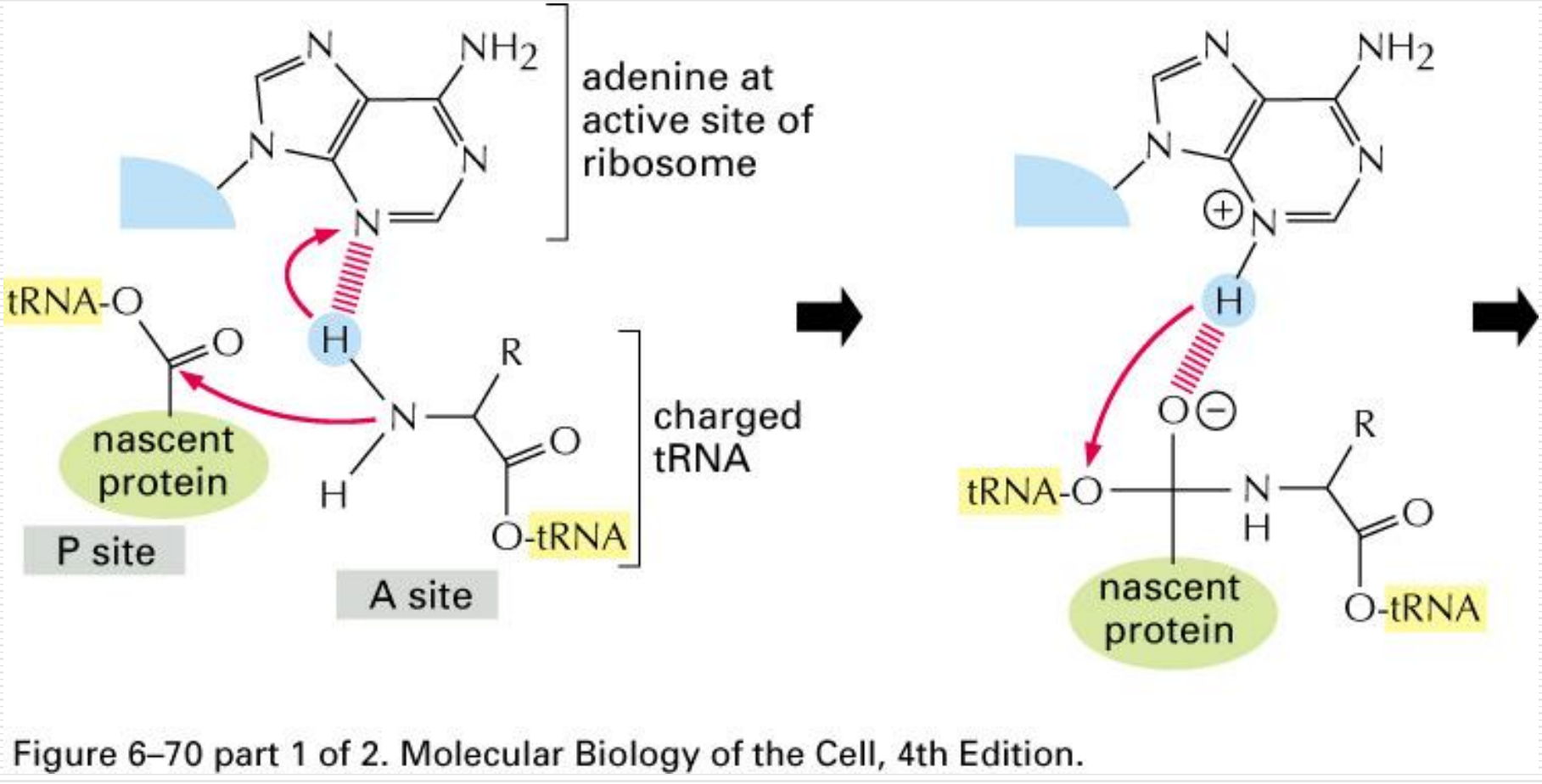


Figure 6–70 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Peptide bond formation is catalyzed by the large subunit rRNA

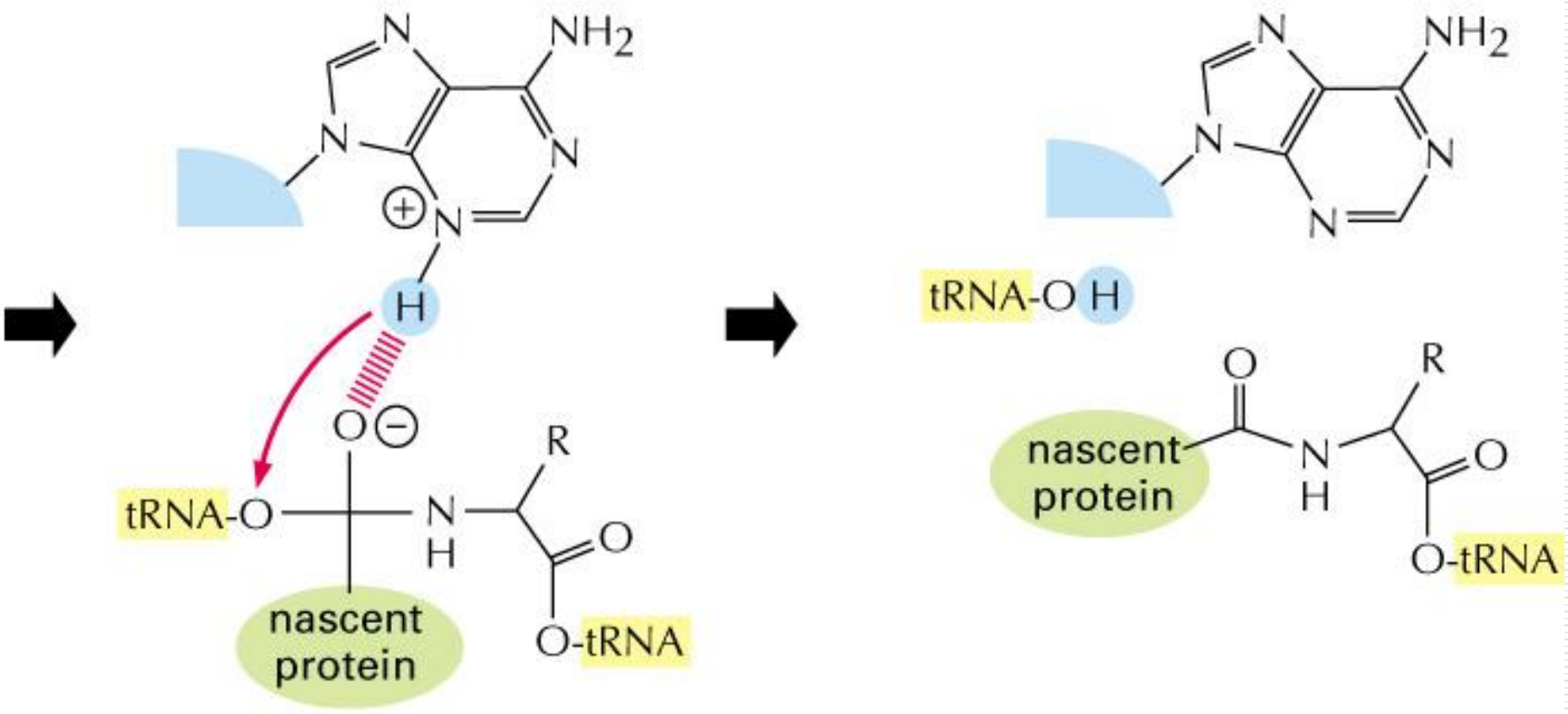


Figure 6–70 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

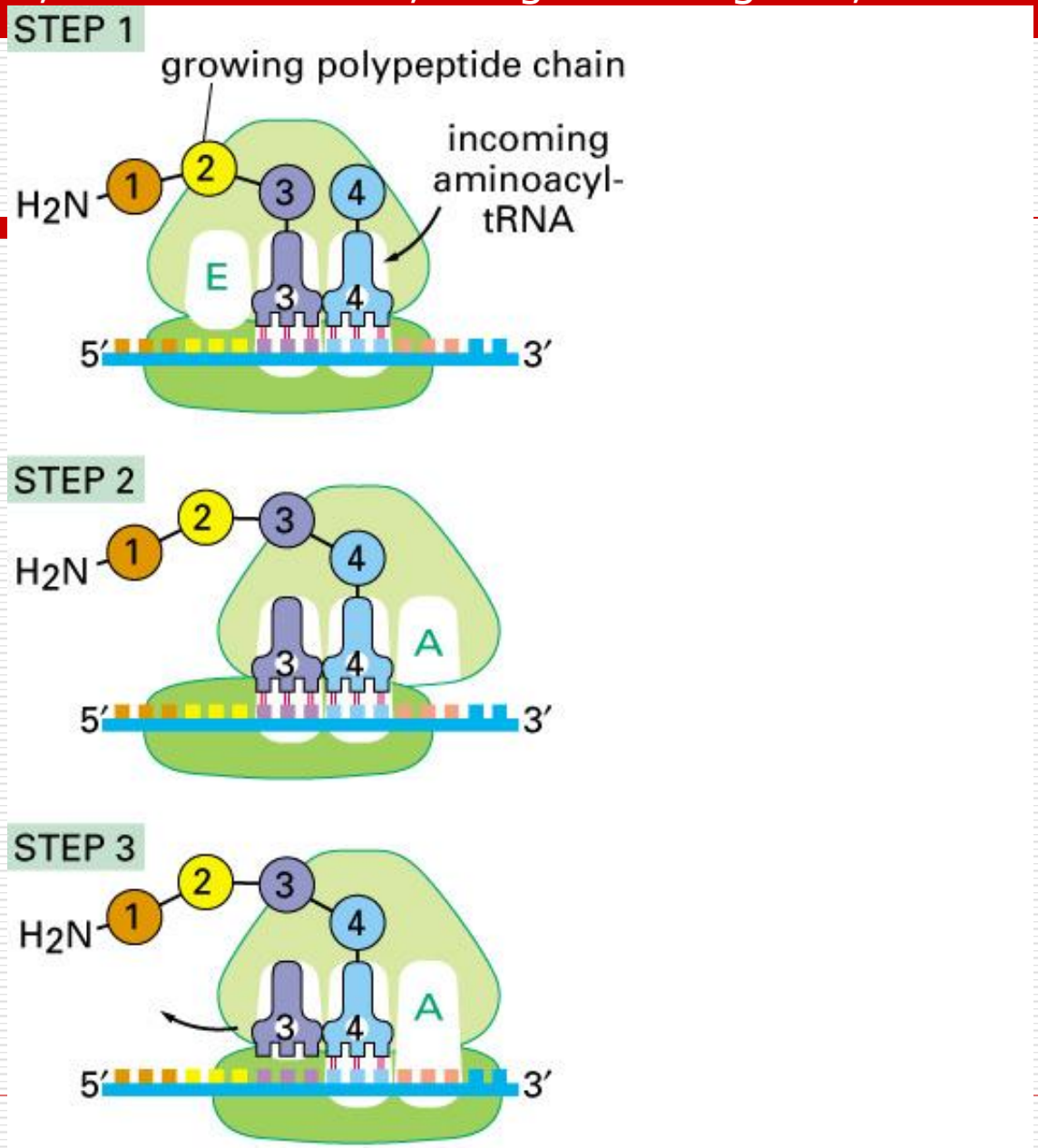


Figure 6–65 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

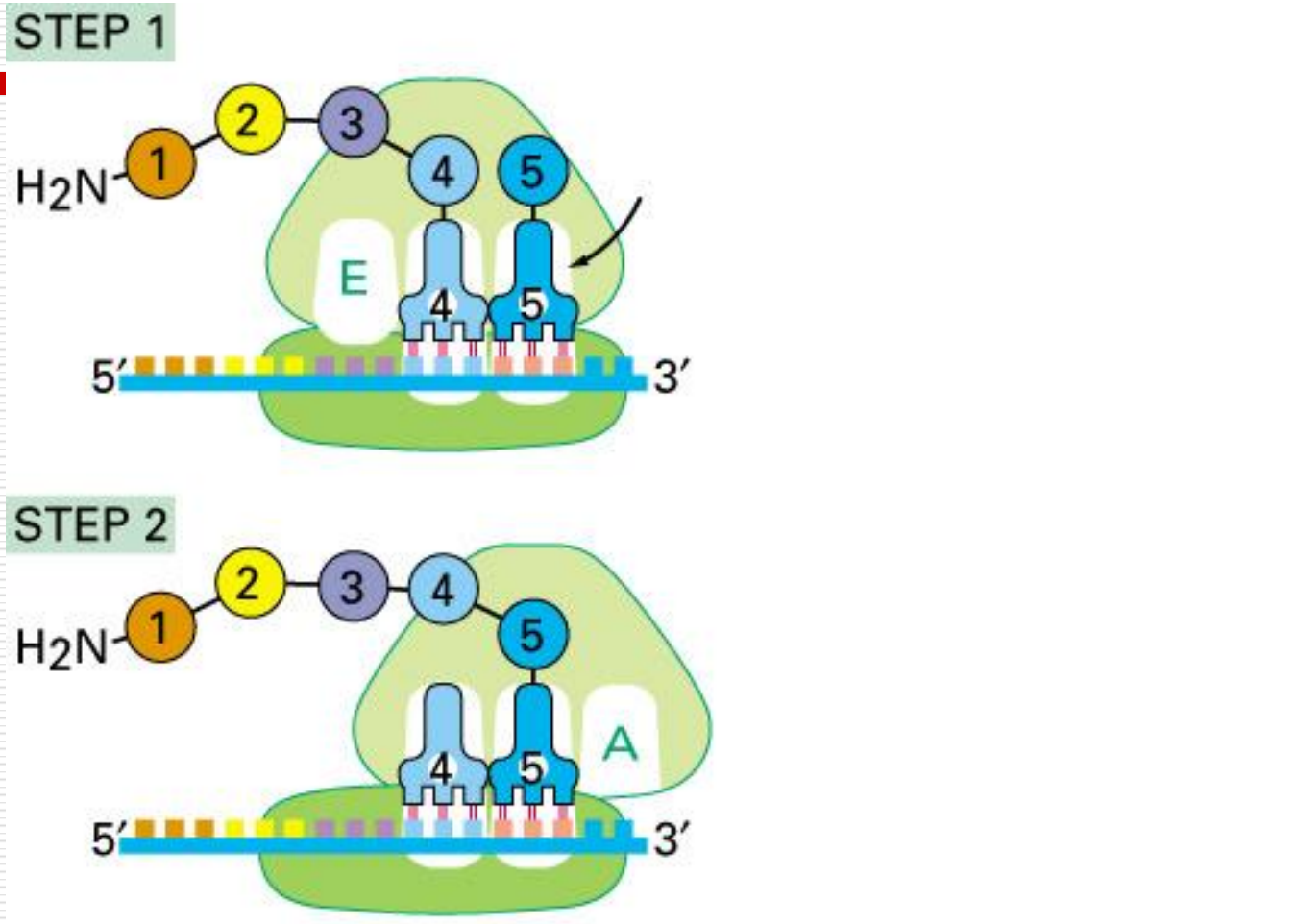


Figure 6–65 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

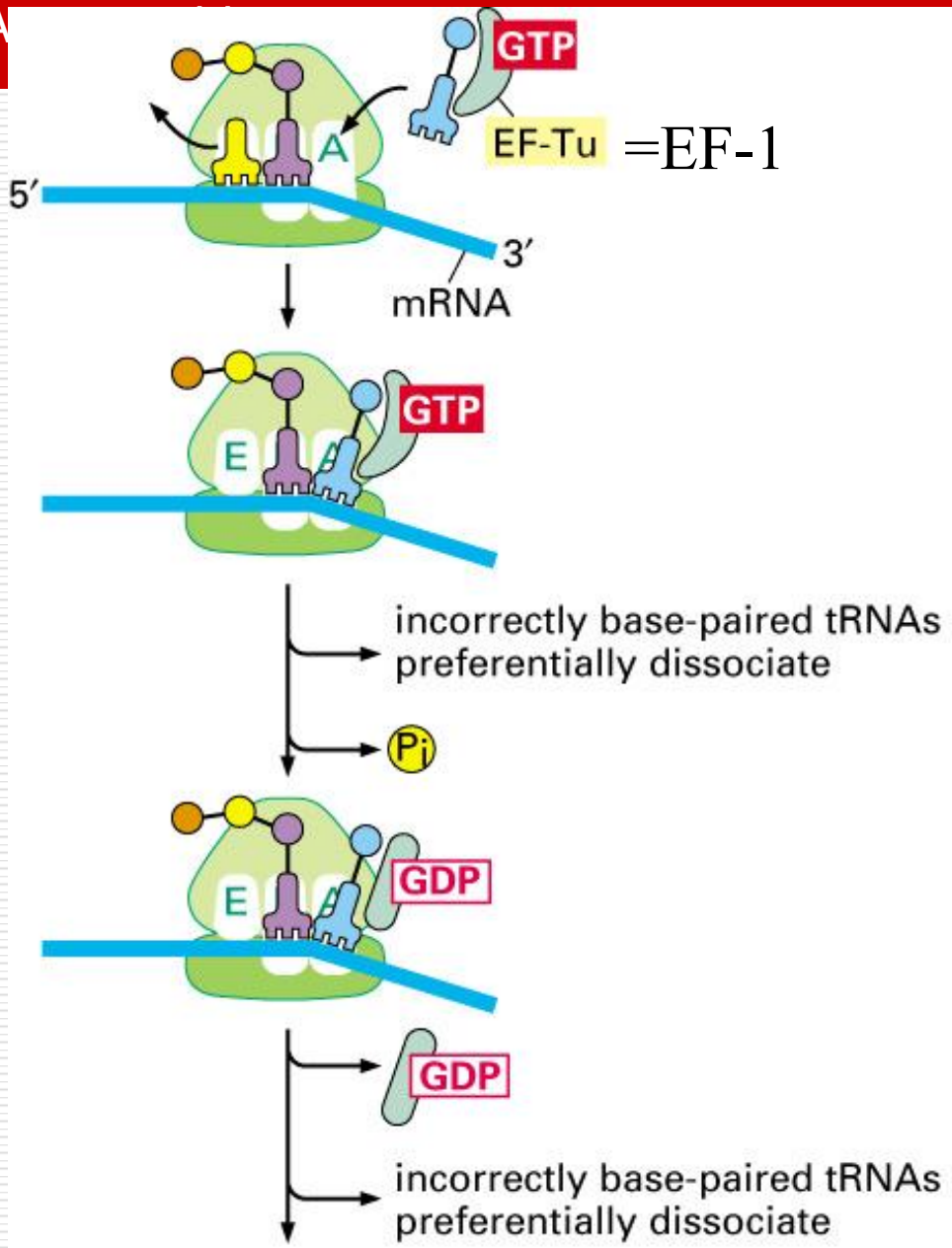


Figure 6–66 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Proper reading of the anticodon is the second important quality control step ensuring accurate protein synthesis

Elongation factors Introduce a two-step "Kinetic proofreading"

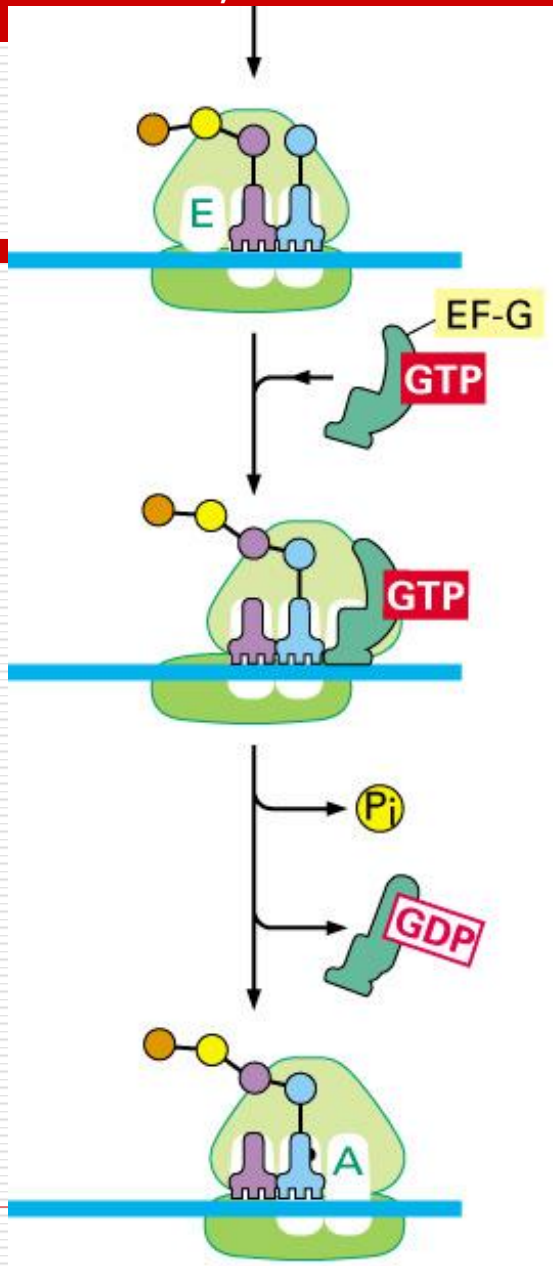


Figure 6–66 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

A second elongation factor EF-G or EF-2, drives the translocation of the ribosome along the mRNA

Together GTP hydrolysis by EF-1 and EF-2 help drive protein synthesis forward

Termination of translation is triggered by stop codons

Release factor enters the A site and triggers hydrolysis the peptidyl-tRNA bond leading to release of the protein.

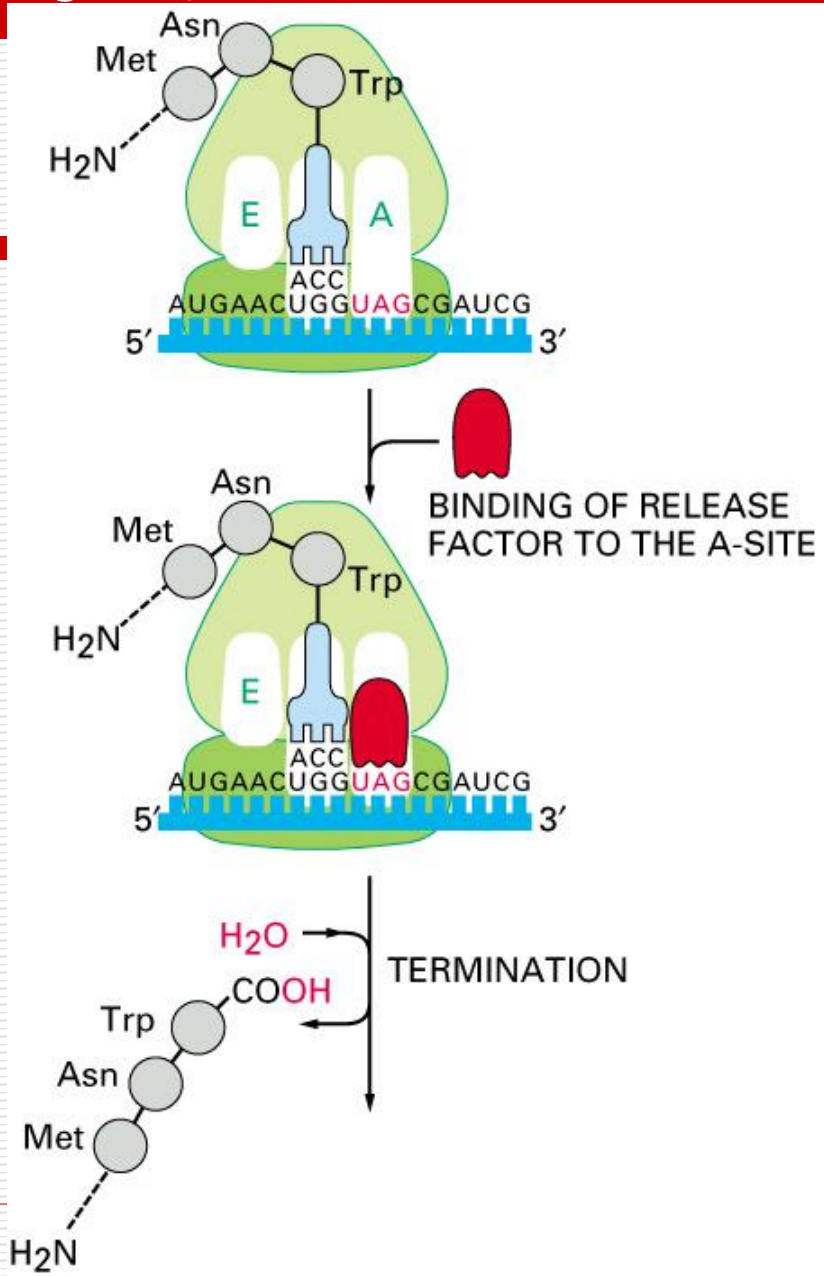


Figure 6–73 part 1 of 2. Molecular Biology of the Cell, 4th E

Release of the protein causes the disassociation of the ribosome into its constituent subunits.

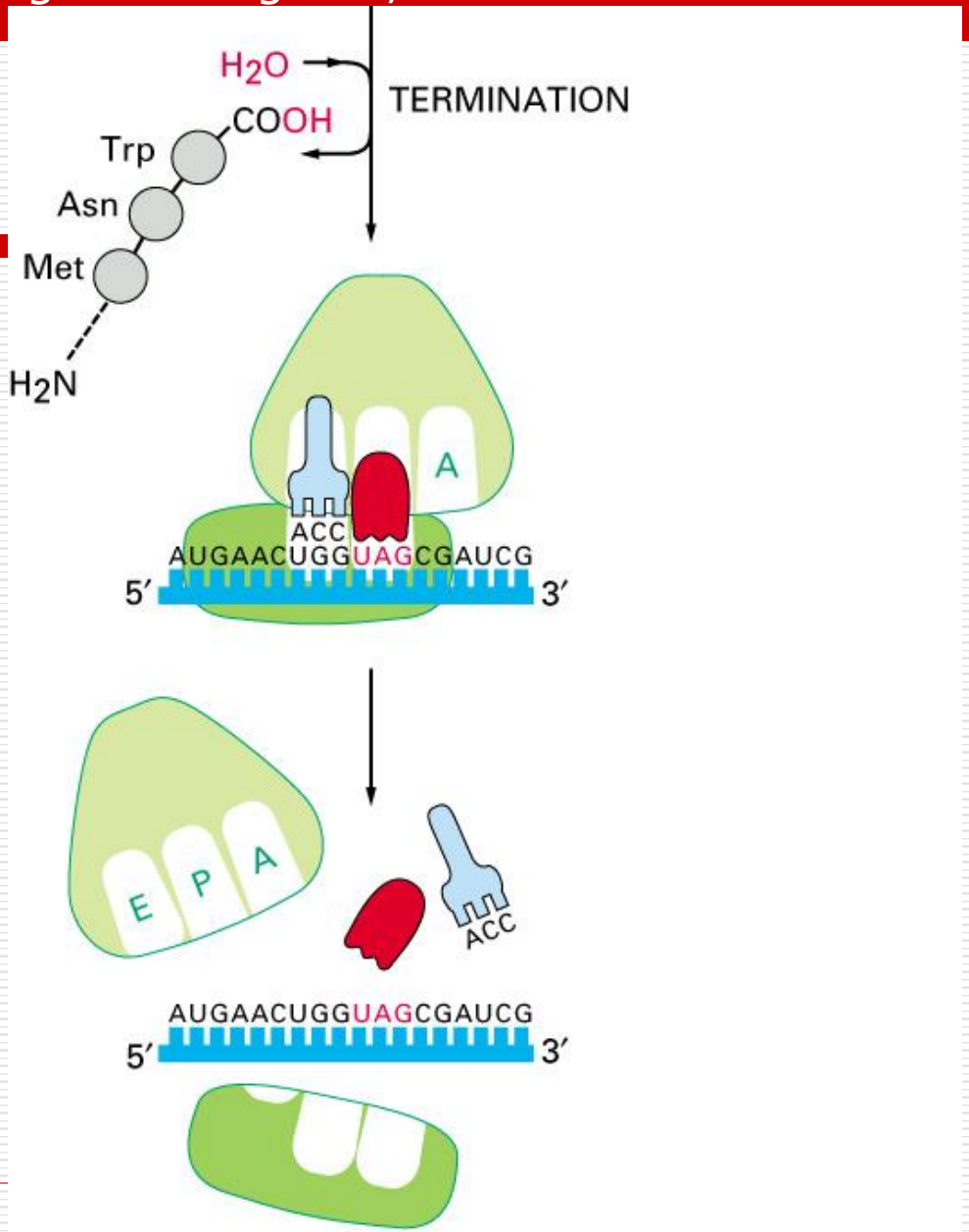
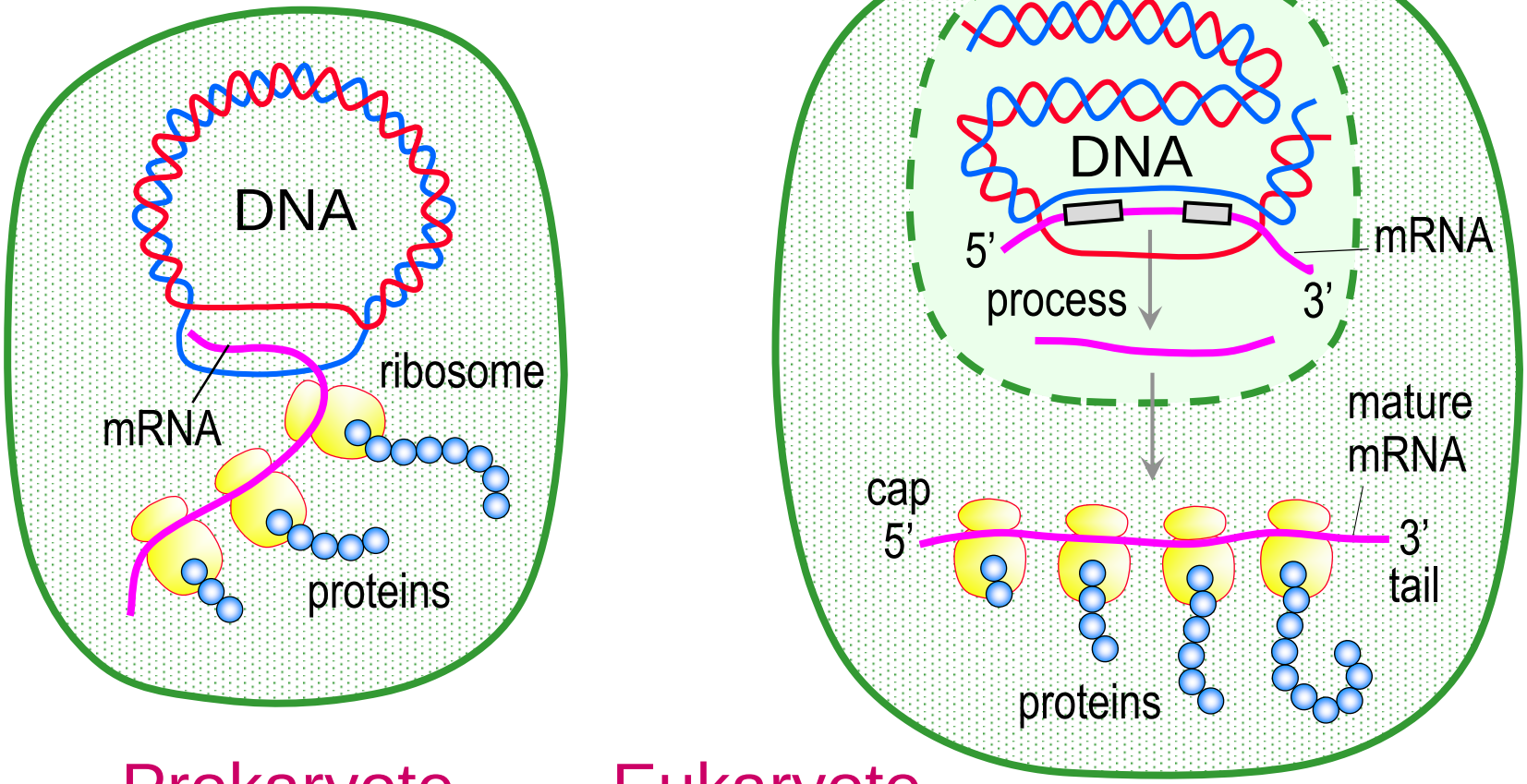


Figure 6–73 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Final Version of Cellular Genetic Mechanism

DNA replaced RNA becoming the major genetic material

RNA shifted its role to protein biosynthesis



Prokaryote

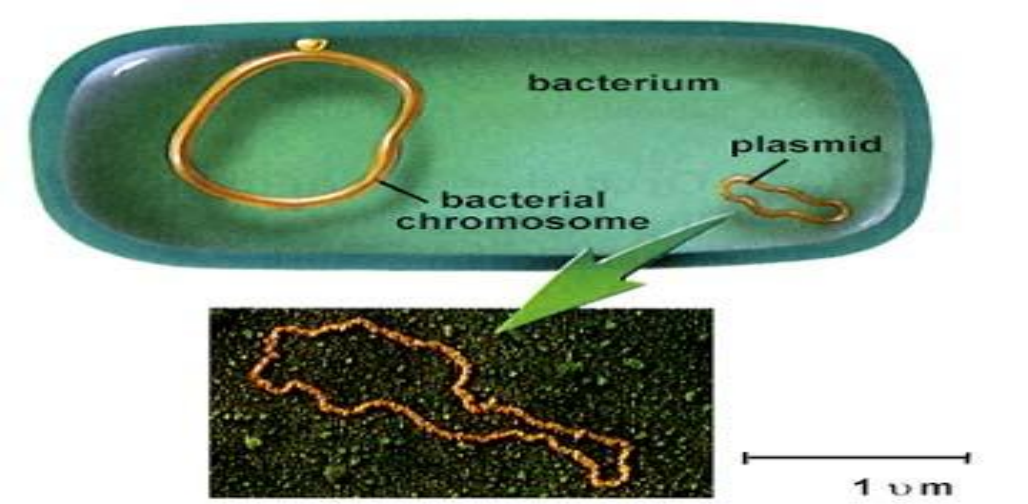
Eukaryote

Student dormitory

Furnished apartment

Juang RH (2004) BCbasics

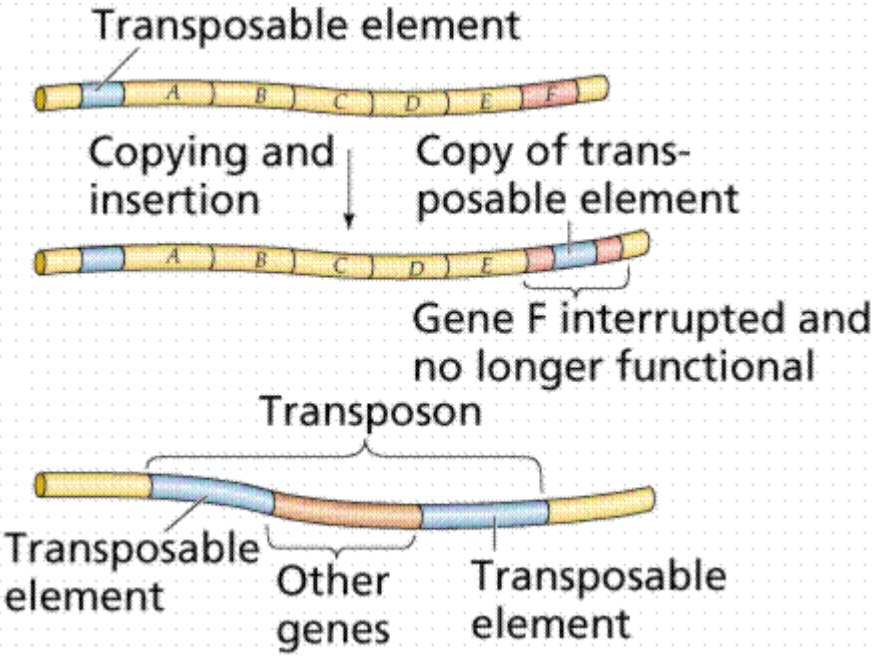
Teknologi Gen; Plasmid



- DNA (sirkular) di luar kromosom
- Biasanya mengandung sifat penciri, seperti ketahanan terhadap antibiotika

- Plasmid hasil rekayasa dipakai sebagai vektor/kendaraan pembawa gen asing di dalam kloning.
- Pada plasmid rekayasa ditambahkan beberapa lokasi penting, seperti tempat yang bisa dikenali oleh beberapa enzim pemotong DNA (Endonuklease restriksi)

Teknologi Gen; Transposon



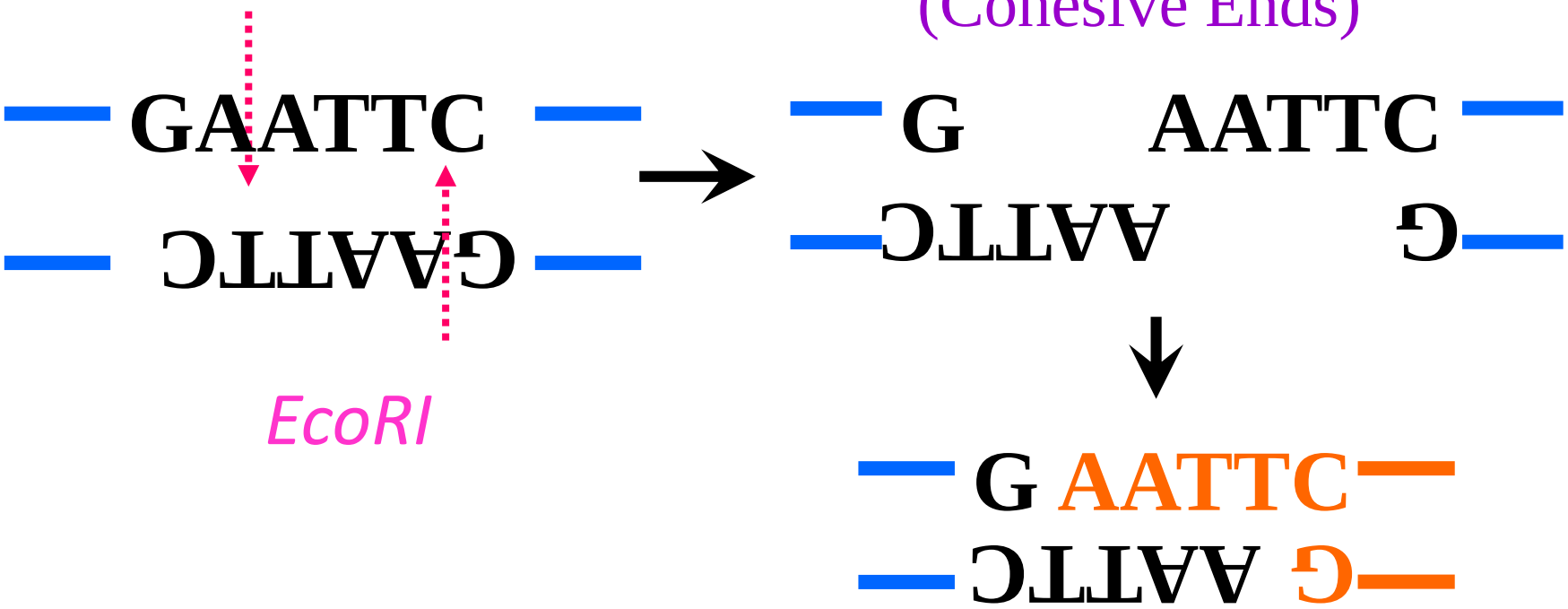
- Elemen lompat, mengandung gen penyandi enzim untuk menyisipkan elemen ini pada sisi baru.
- Apabila tempat yang disisipi ini merupakan gen fungsional, maka gen ybs. akan menjadi tidak berfungsi.
- Mengandung sekuen berulang

Teknologi Gen; Restriksi dan Ligasi

Arber, Nathans, Smith (1978)

CIVIC, Madam

Sticky Ends
(Cohesive Ends)

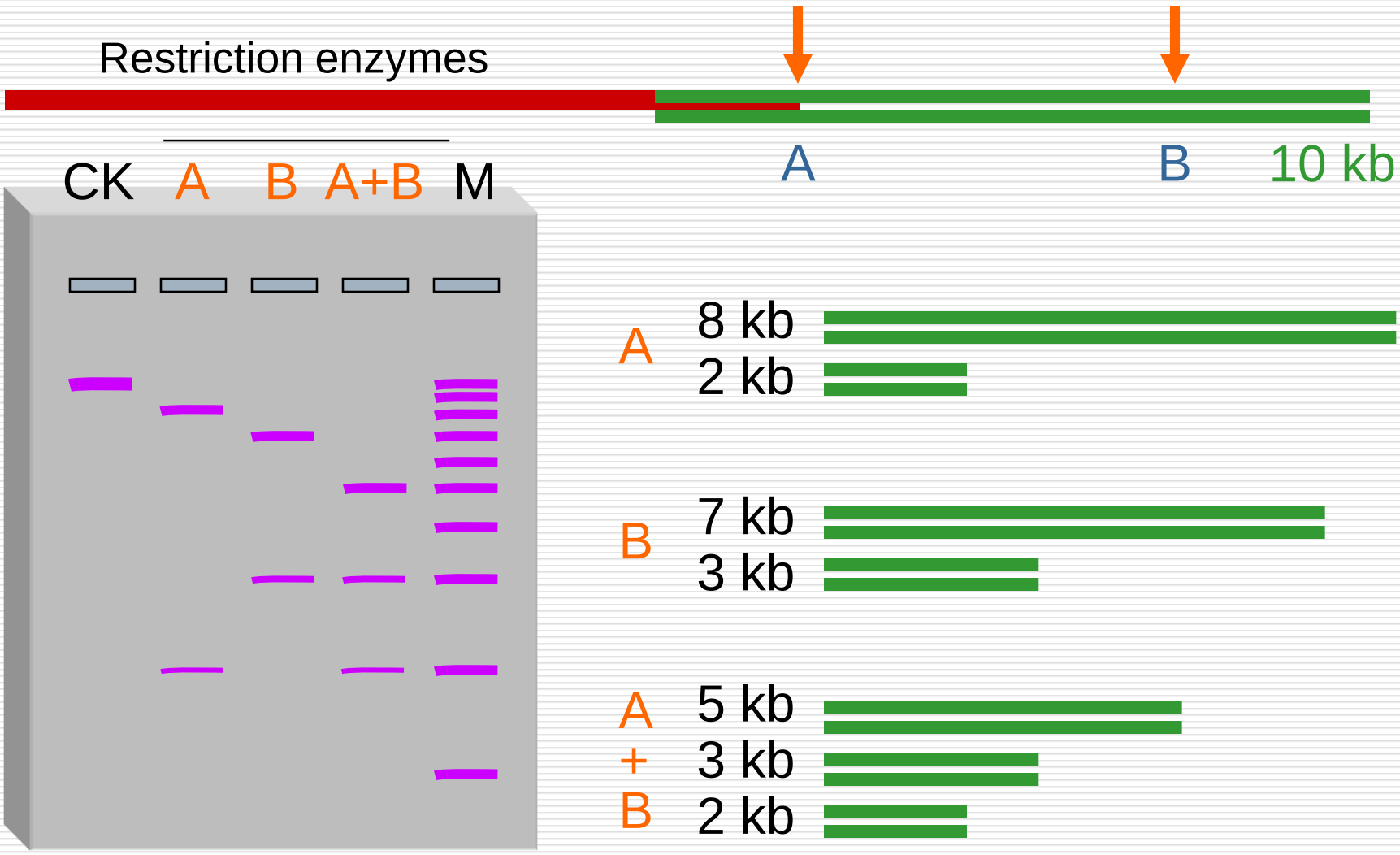


EcoRI

Get An Apple To The Class

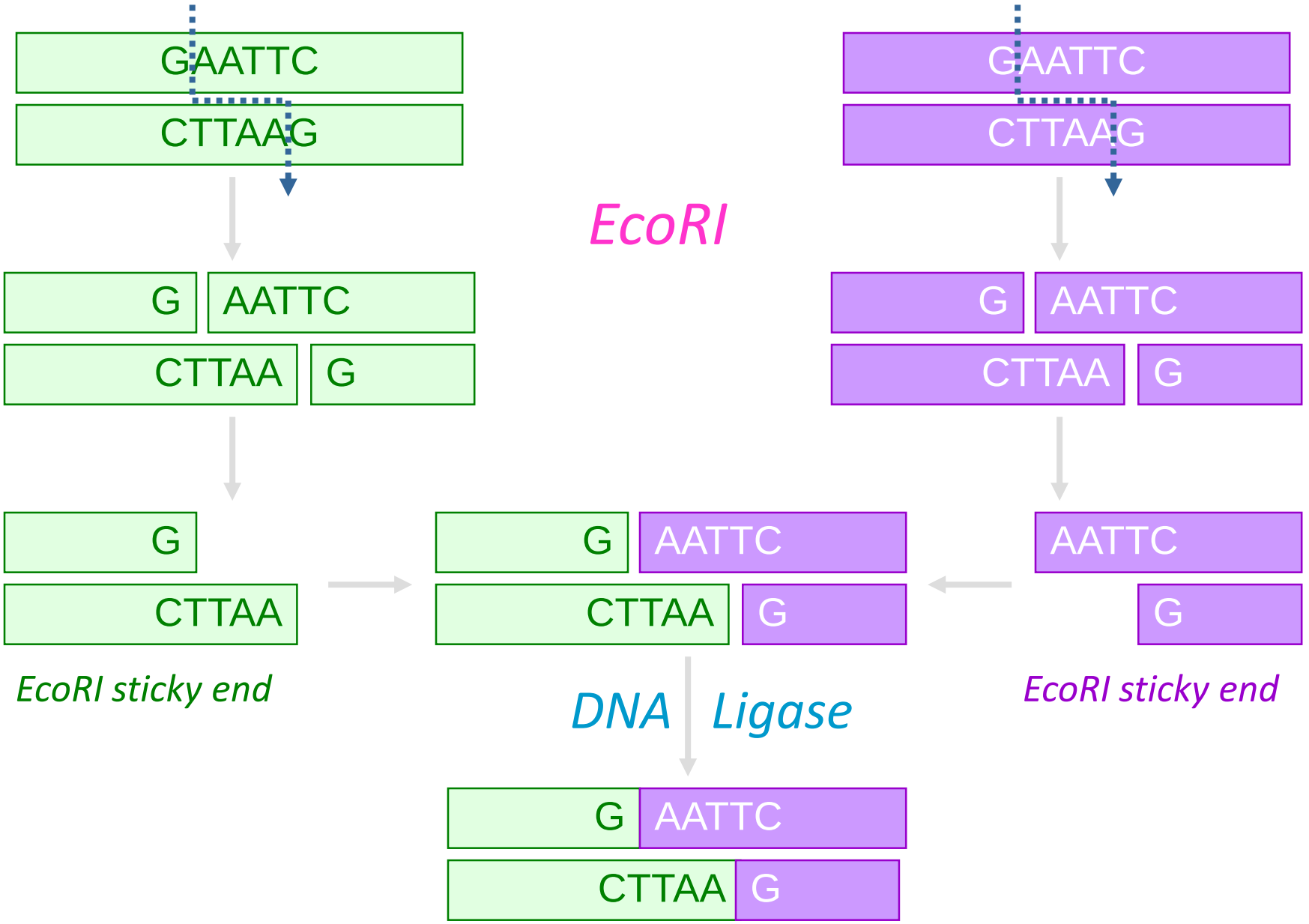
Juang RH (2004) BCbasics

Restriction Mapping of DNA



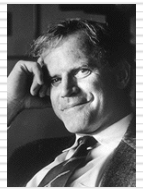
Juang RH (2004) BCbasics

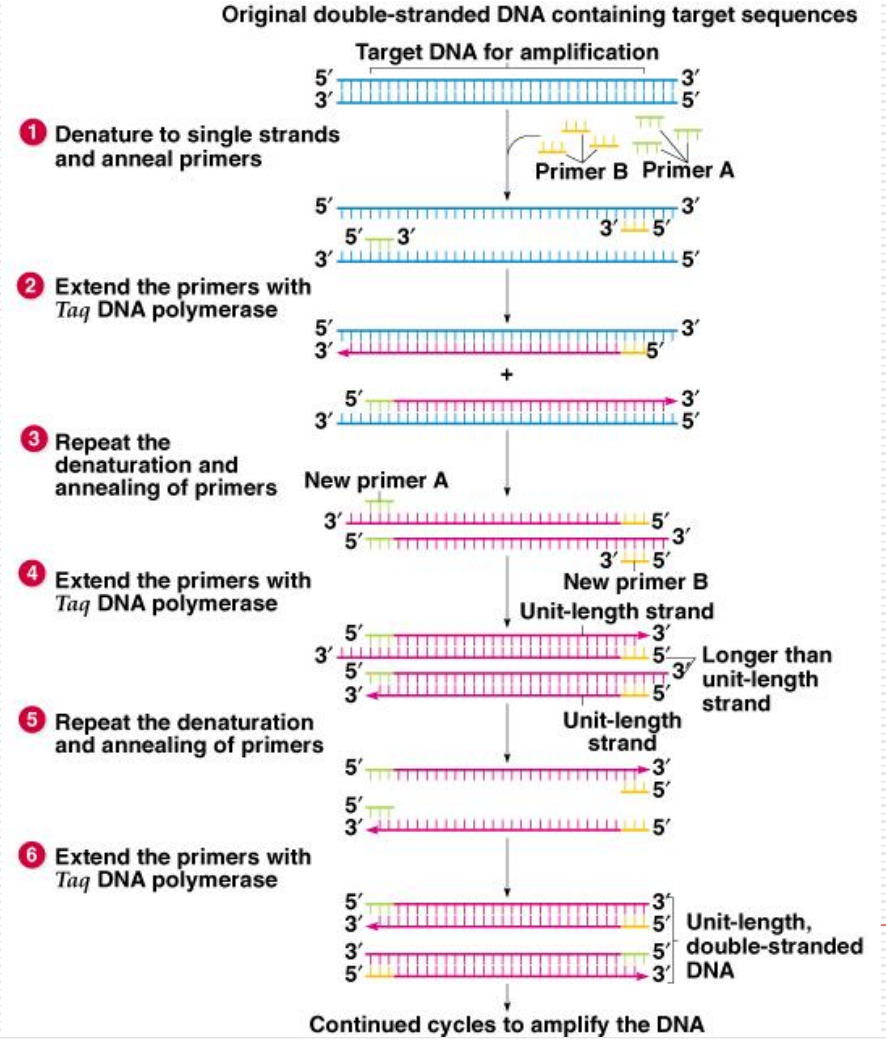
The Specific Cutting and Ligation of DNA



Juang RH (2004) BCbasics

PCR: Polymerase Chain Reaction

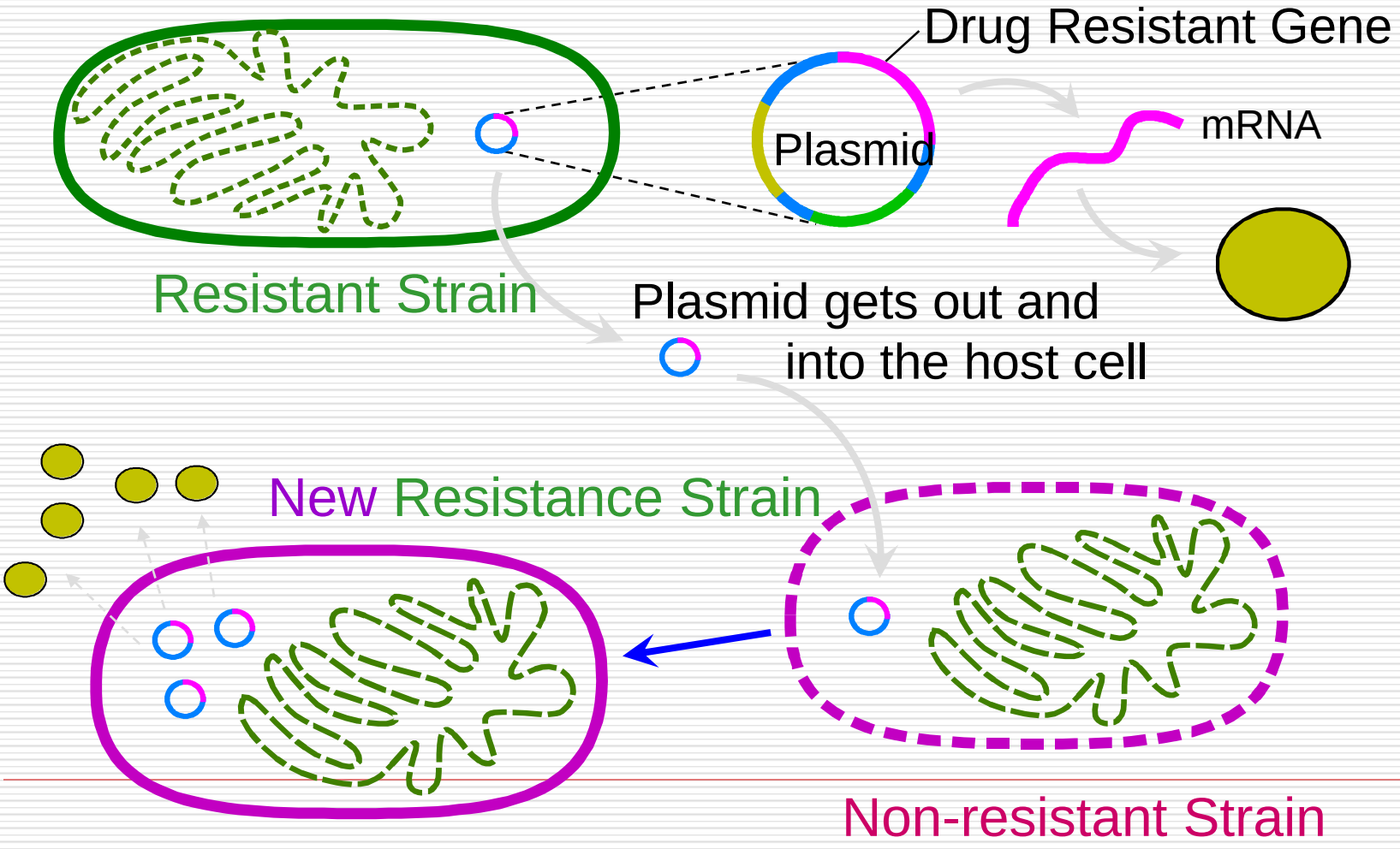




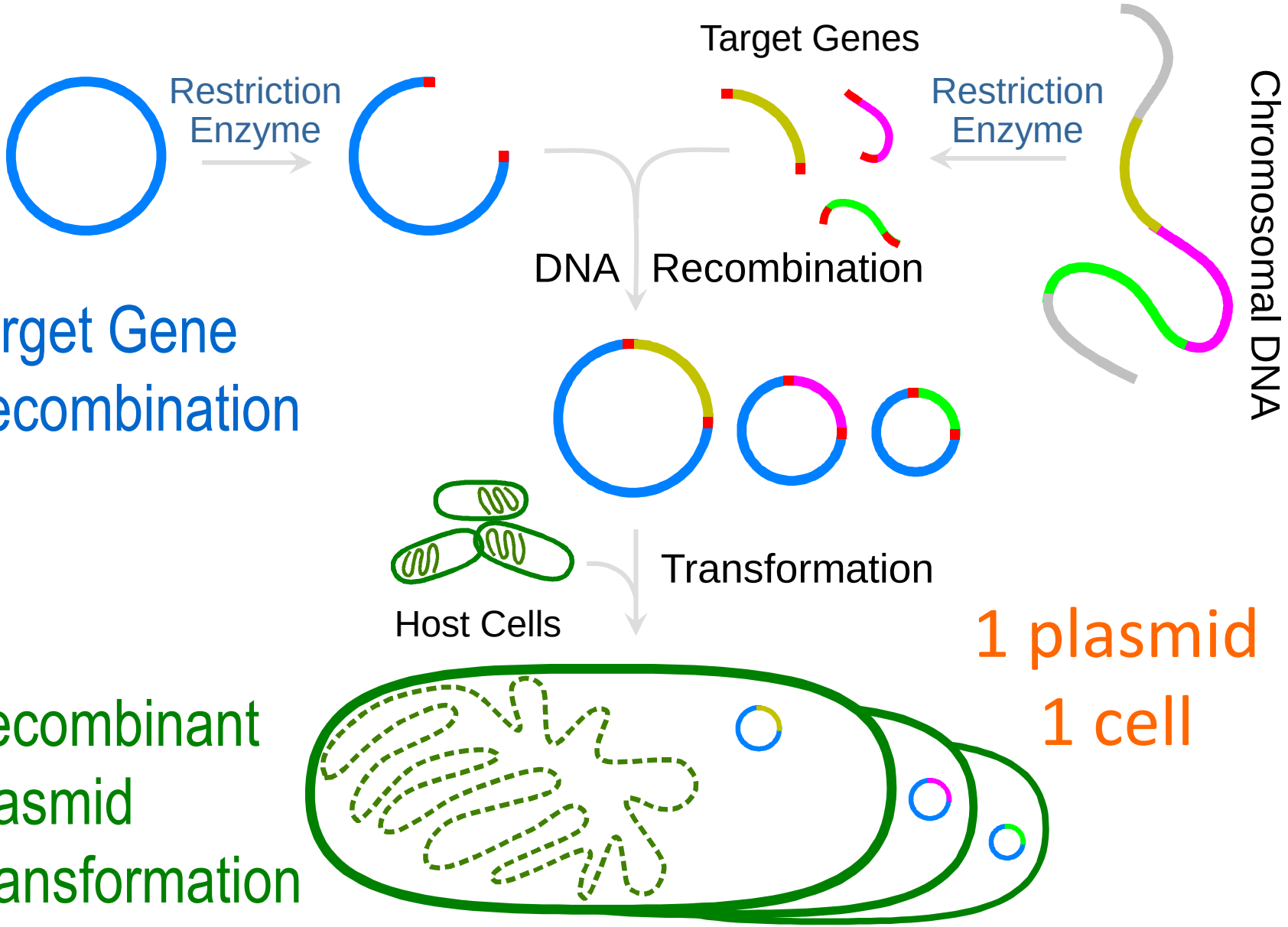
- "discovered" in 1983 by Kary Mullis,
- enables the amplification (or duplication) of millions of copies of any DNA sequence with known flanking sequences.
- Requires only simple, inexpensive ingredients and a couple hours
  - DNA template
  - Primers (anneal to flanking sequences)
  - DNA polymerase
  - dNTPs
  - Mg2+
  - Buffer
- Can be performed by hand or in a machine called a thermal cycler.
- 1993: Nobel Prize for Chemistry

- Animasi

Teknologi Gen; Cloning

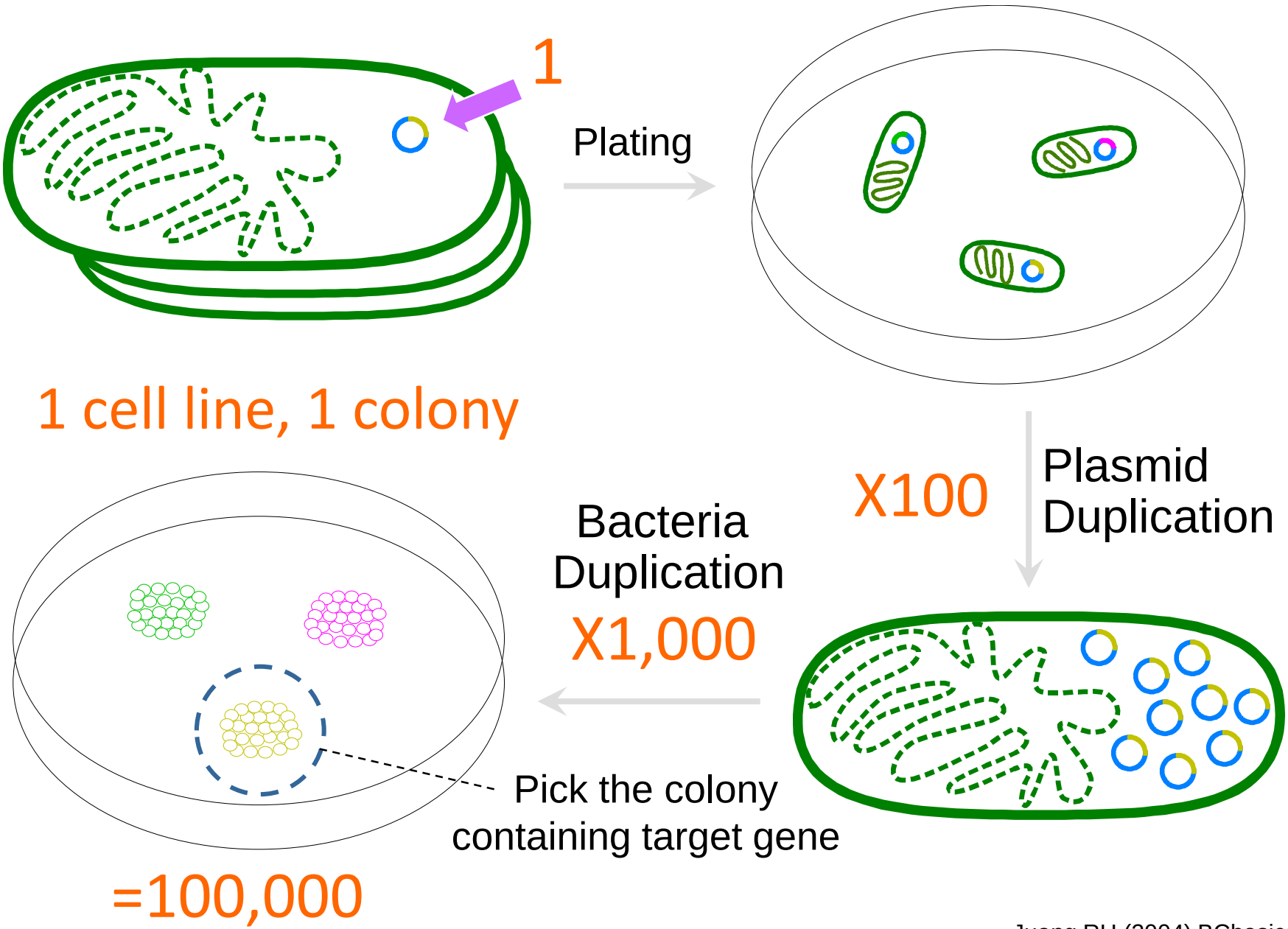


Target Genes Carried by Plasmid



Juang RH (2004) BCbasics

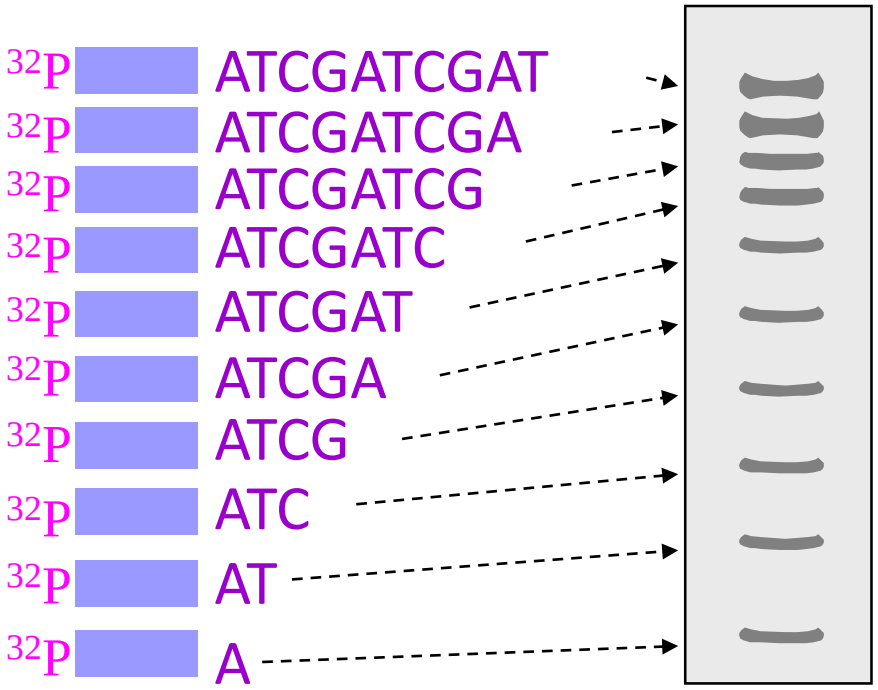
Amplification and Screening of Target Gene



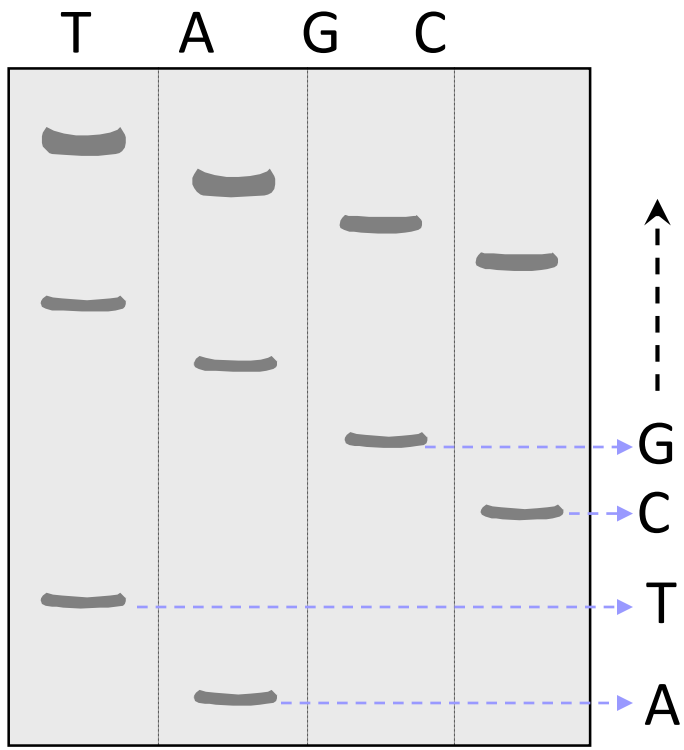
Juang RH (2004) BCbasics

How DNA Sequence Is Determined?

DNA fragments having a difference of one nucleotide can be separated on gel electrophoresis



Polyacrylamide Gel Electrophoresis

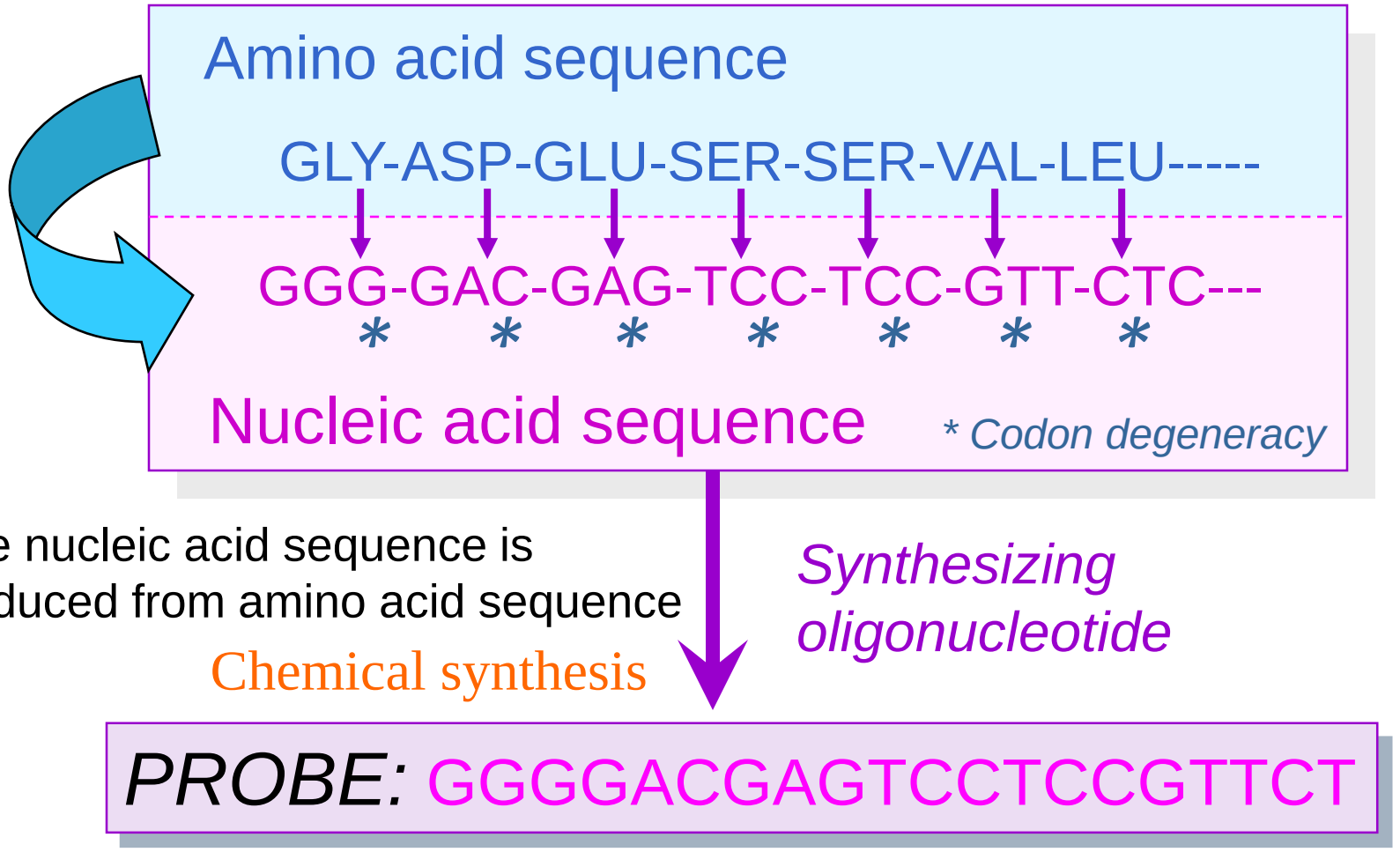


But these bands can't tell us the identity of the terminal nucleotides

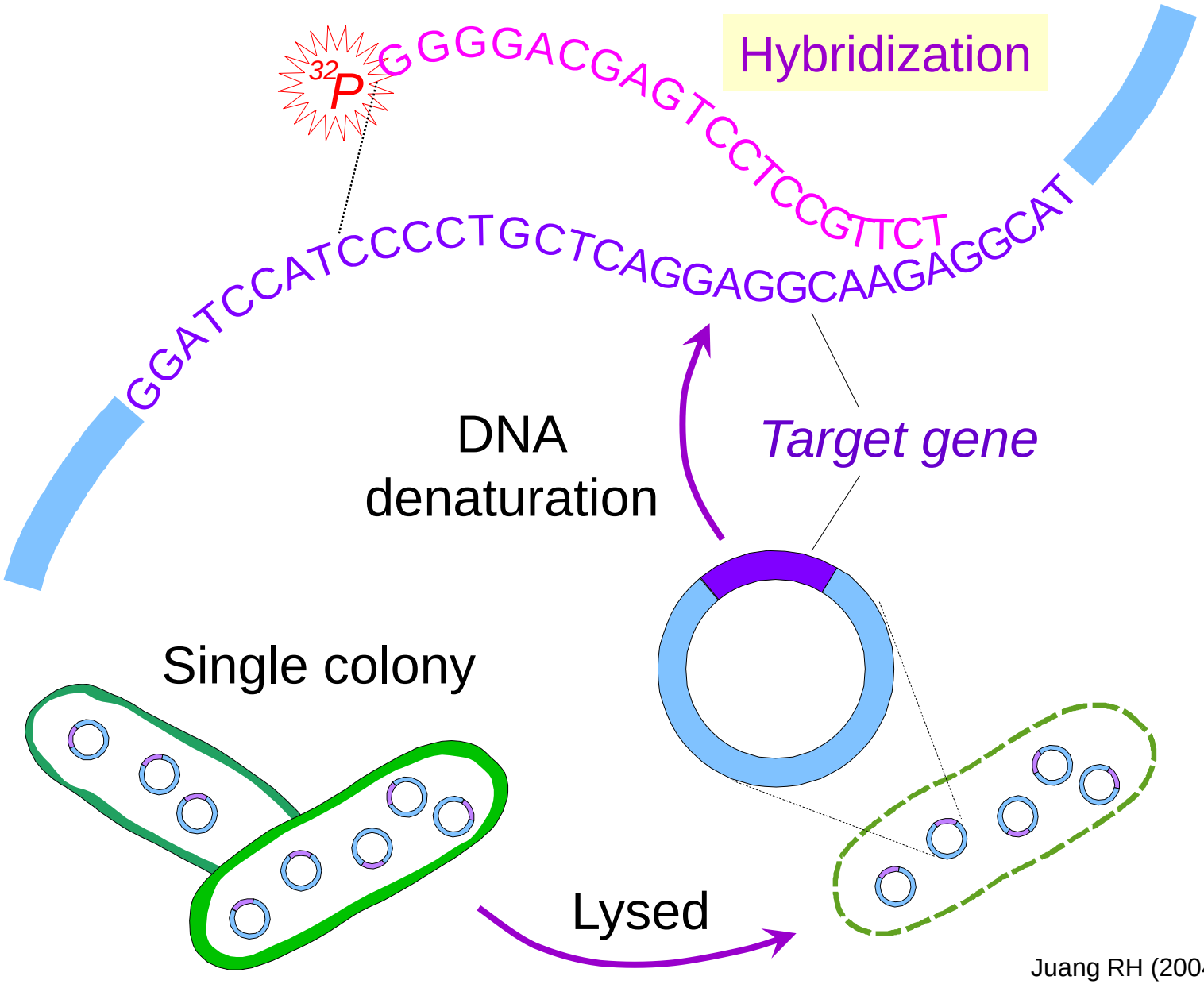
If those band with the same terminal nucleotide can be grouped, then it is possible to read the whole sequence

Juang RH (2004) BCbasics

Preparation of Traditional Nucleic Acid Probe

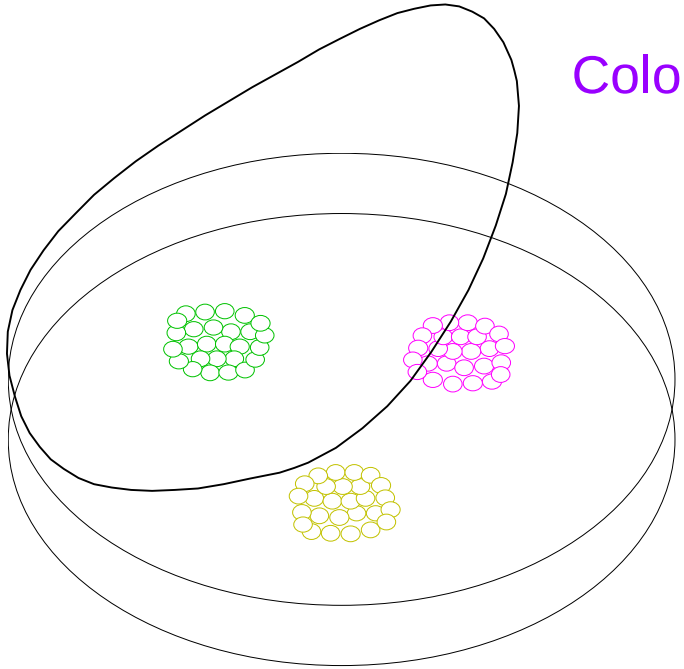


Probe is labeled with radioactive ^{32}P



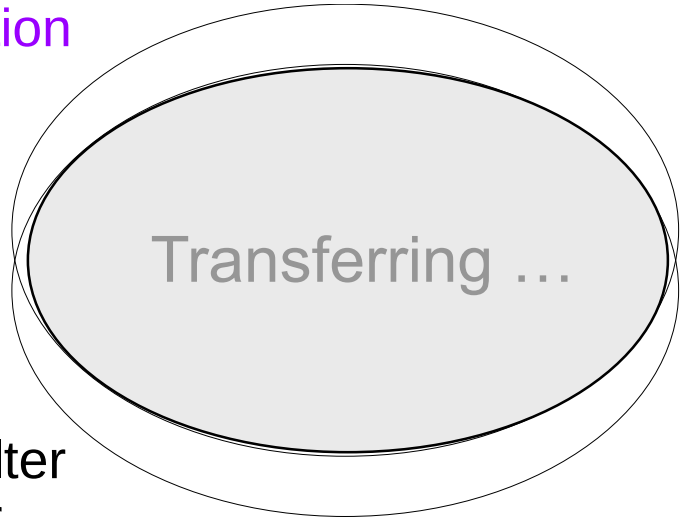
Juang RH (2004) BCbasics

Colony Is Screened by Hybridization with Probe



Colony hybridization

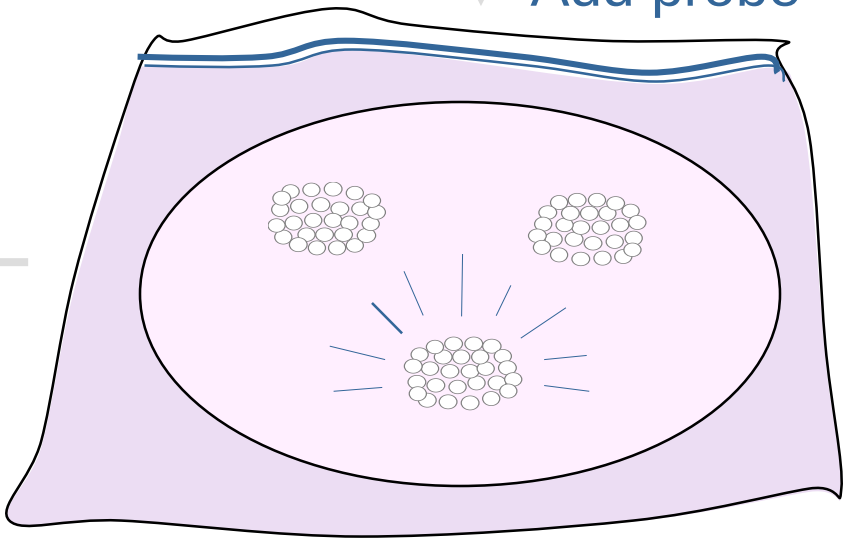
Cover with filter paper



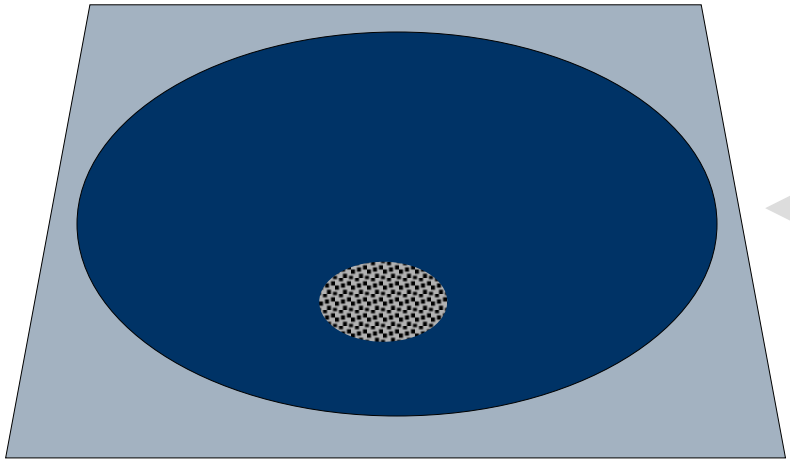
Collect filter paper

Dissolve cell | DNA denatured

Add probe



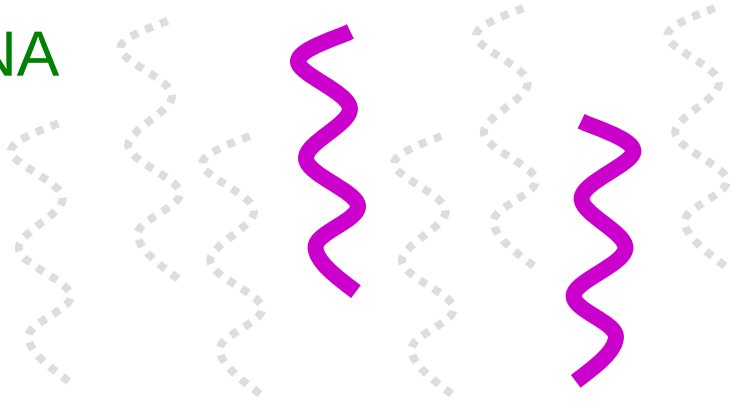
Autoradiography



Biochip Based on Hybridization

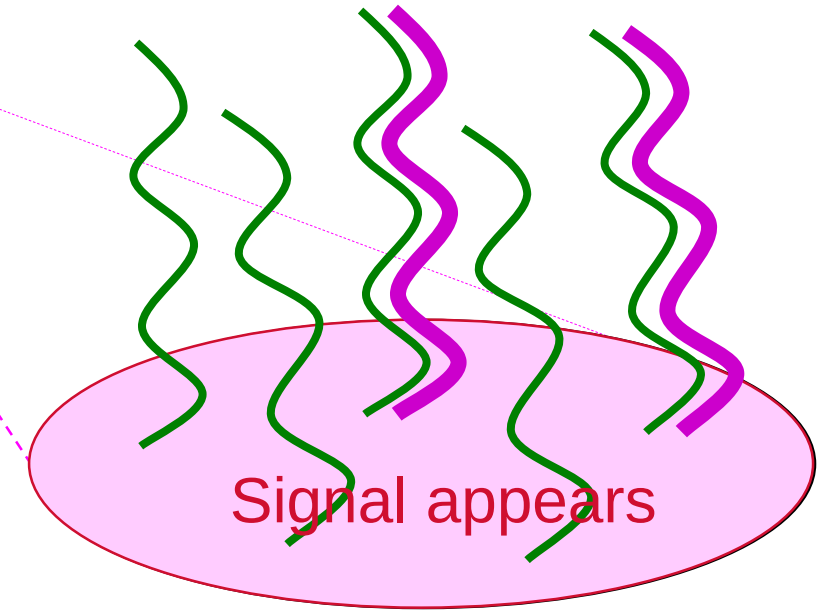
Biochip

Sample DNA



Complementary DNA hybridize

Each spot contains known DNA



Schena (2000) Microarray Biochip Technology, p. A31

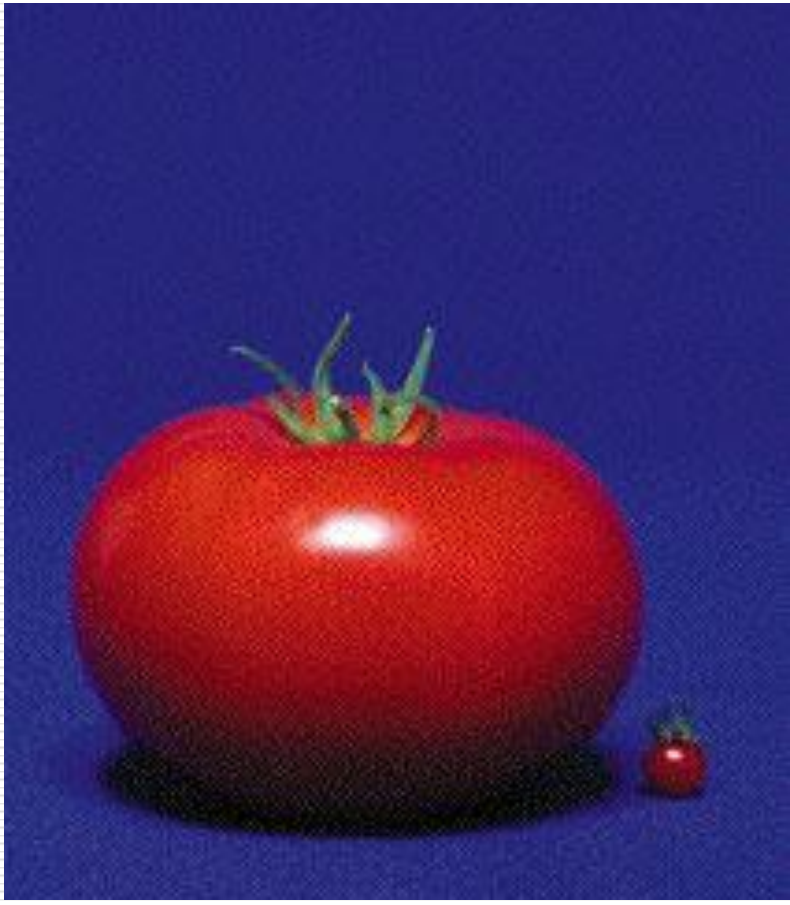
Juang RH (2004) BCbasics

Pangan Transgenik

- Transgenik : ditambah bahan genetik asing (transgen); bisa juga dihilangkan
- Bahan pangan dimasukkan transgen dengan tujuan tertentu
- Transgen : sekuens DNA yang menyandikan sintesis protein tertentu
- Background Information
  - 1982 - First genetically modified plants produced
  - 1986 - First field trials
    - More than 40,000 field trials, on 60 crops, in at least 50 countries, performed during last 14 years
  - 1993 - First GM food product commercialised
  - 1996 - Roundup Ready soybeans adopted by US farmers

What is a genetically modified food?

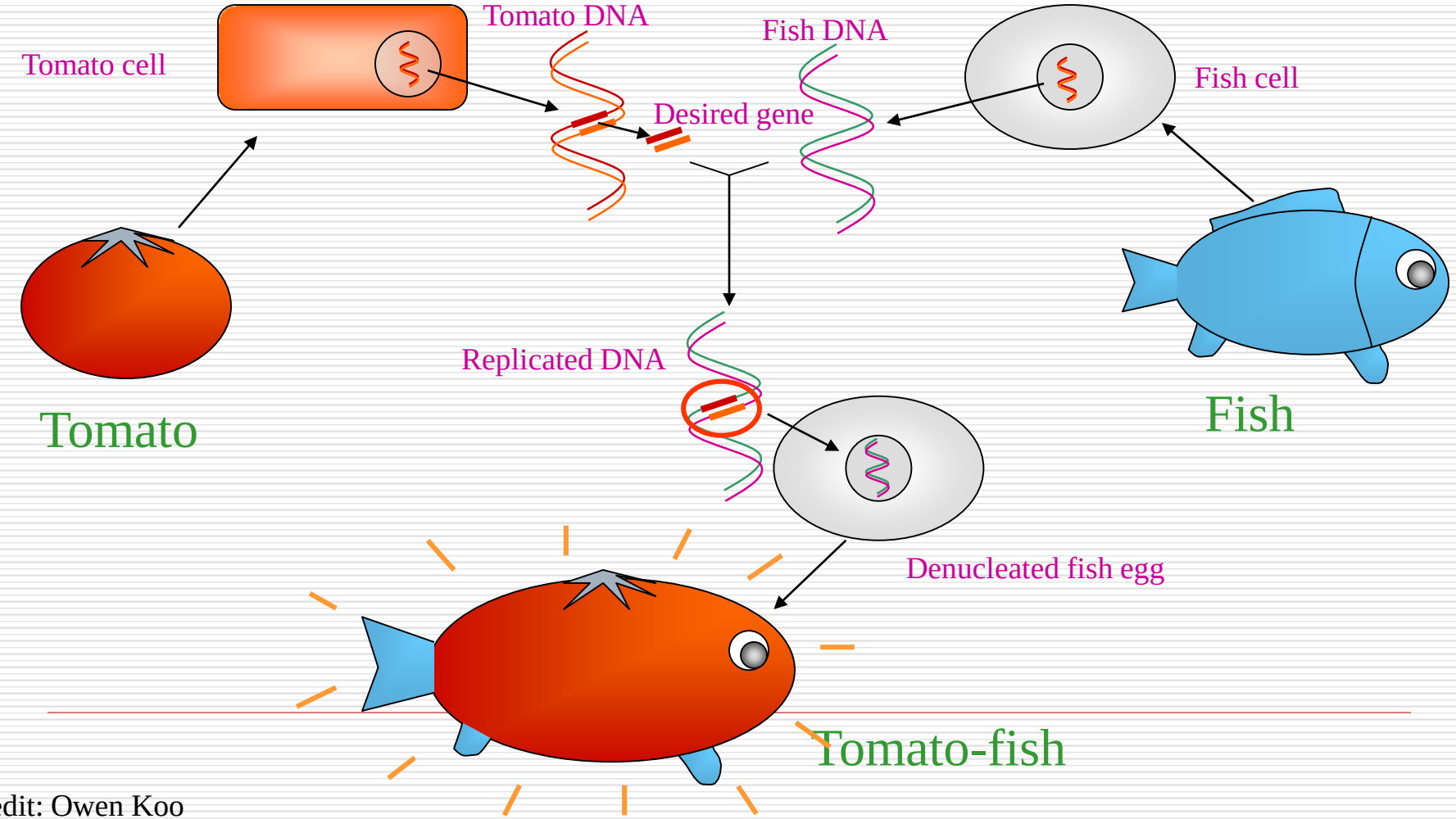
- The term GM foods is commonly used to refer to crop plants created for human or animal consumption using the latest molecular biology techniques.
- These plants have been modified in the laboratory to enhance desired traits, such as, increased resistance to herbicides or improved nutritional content.



How to Produce GM Food? [1]

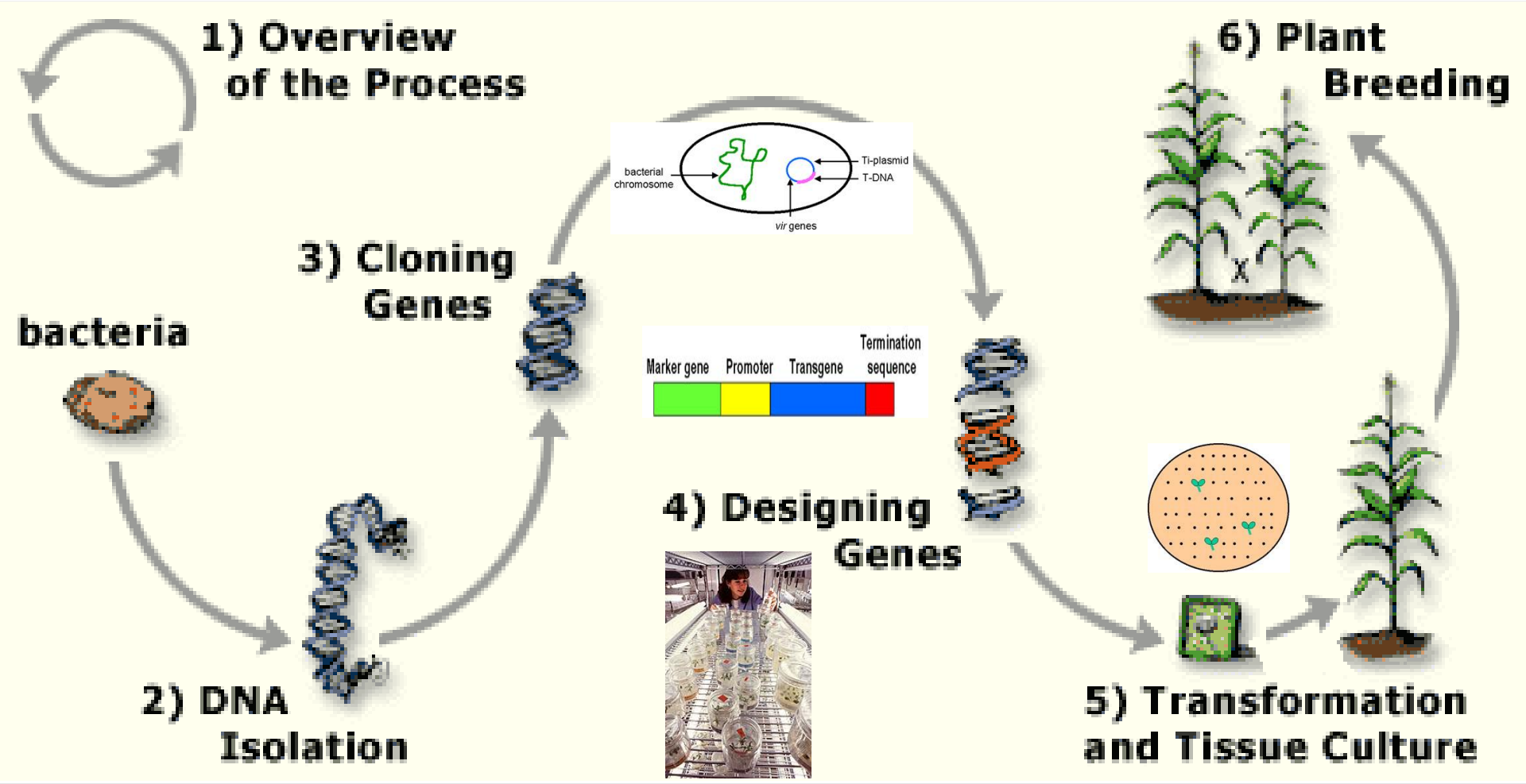
- Concept
  - Convert existing gene(character) of or add new gene (character) to target species
- Practice
  - Extract an desirable gene from source species and introduce to target species

How to Produce GM Food? [2] - *Example*



Credit: Owen Koo

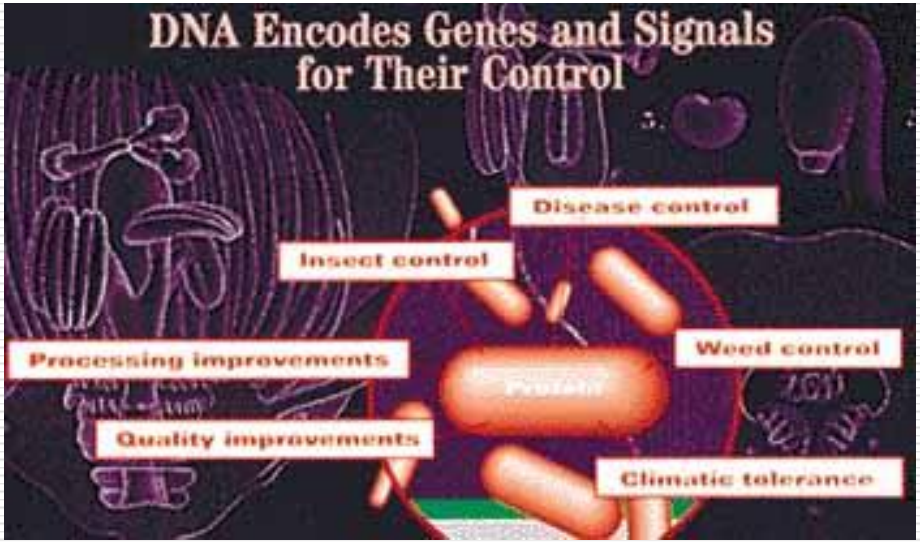
Tanaman Transgenik

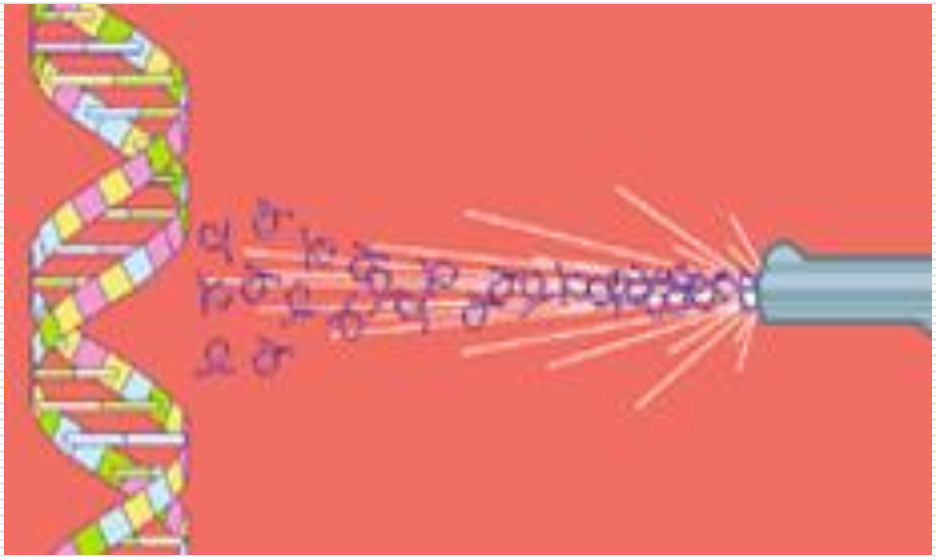


Contoh : Kedele Roundup Ready®

- Sebelum dan Setelah penyemprotan herbisida golongan glifosat (Monsanto)

Prinsip Pembuktian transgenik





- Keberadaan hasil ekspressi gen yang disisipkan
- Keberadaan sekuens gen yang disisipkan

Aturan dan Kontroversi

- Keamanan Pangan
  - Pengujian jangka pendek vs jangka panjang
- Keamanan Lingkungan
  - Loncatan gen ke lingkungan
- Perang Dagang (US vs EU)
  - Perselisihan di WTO; 1998 - 2007
- Isu-isu politik global
  - Utara vs Selatan; Maju vs Berkembang