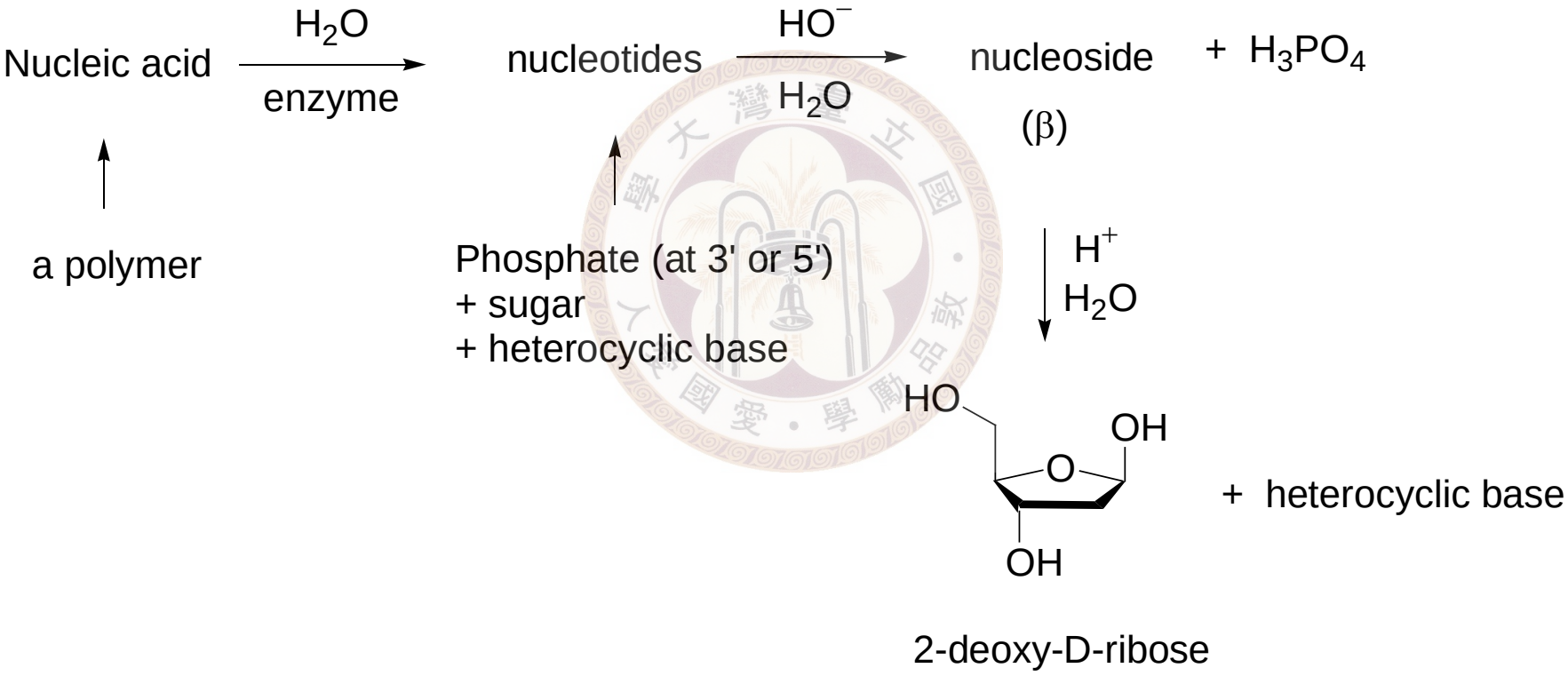
台灣大學開放式課程

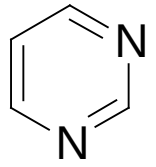
有機化學乙

蔡蘊明　教授

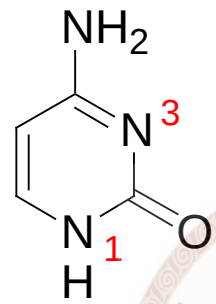
Chapter 25 Nucleic acids



◎ The base

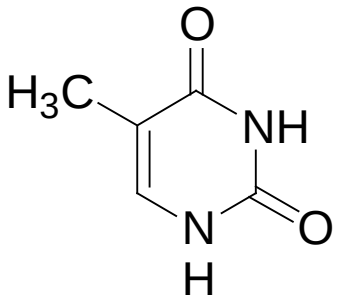


pyrimidine

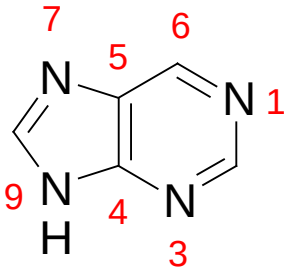


cytosine

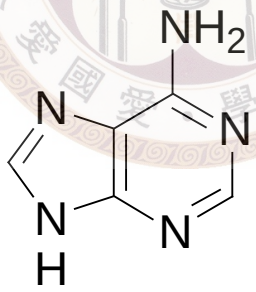
uracil (RNA)



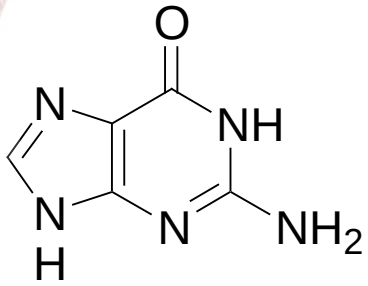
thymine (DNA)



purine

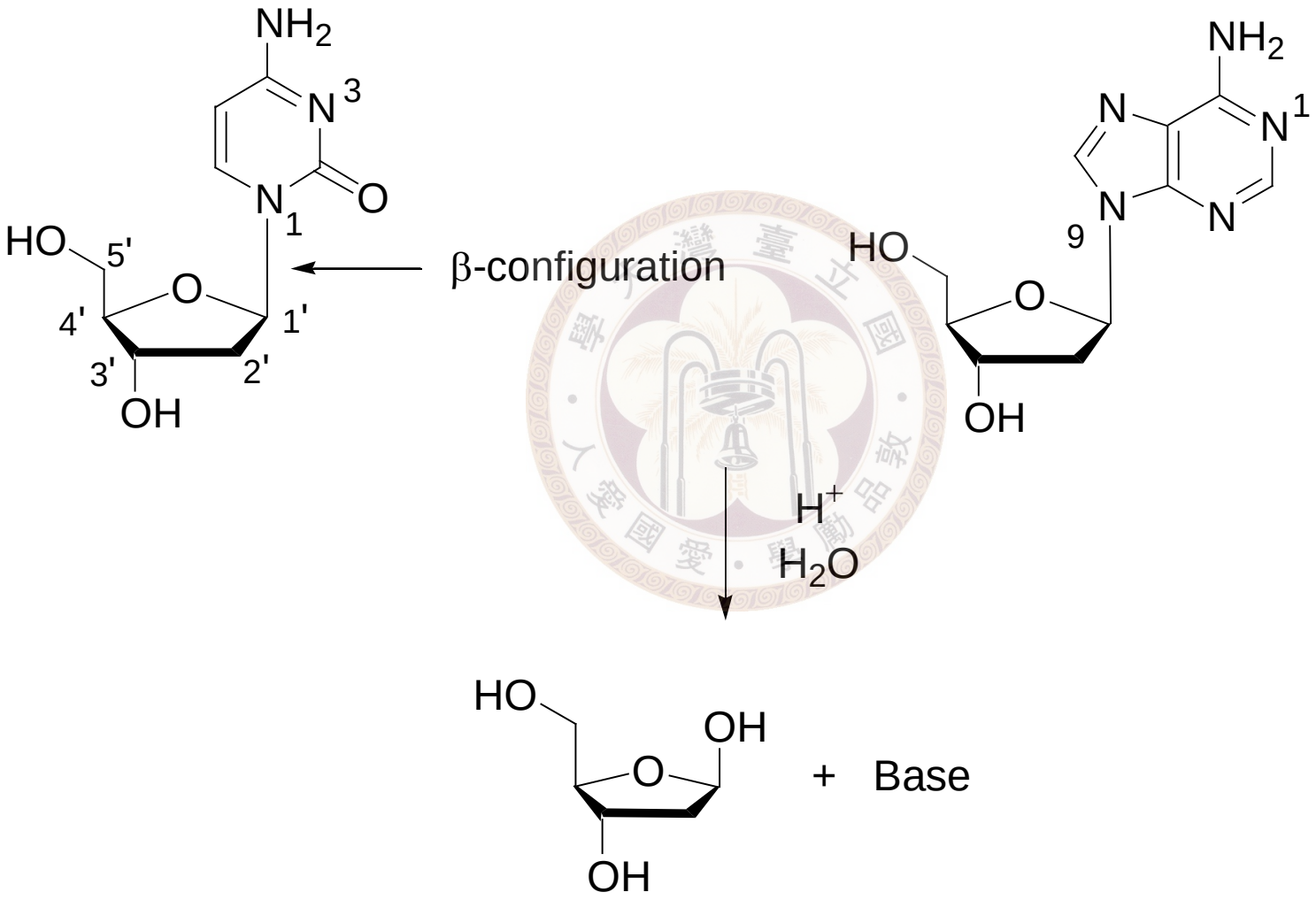


adenine

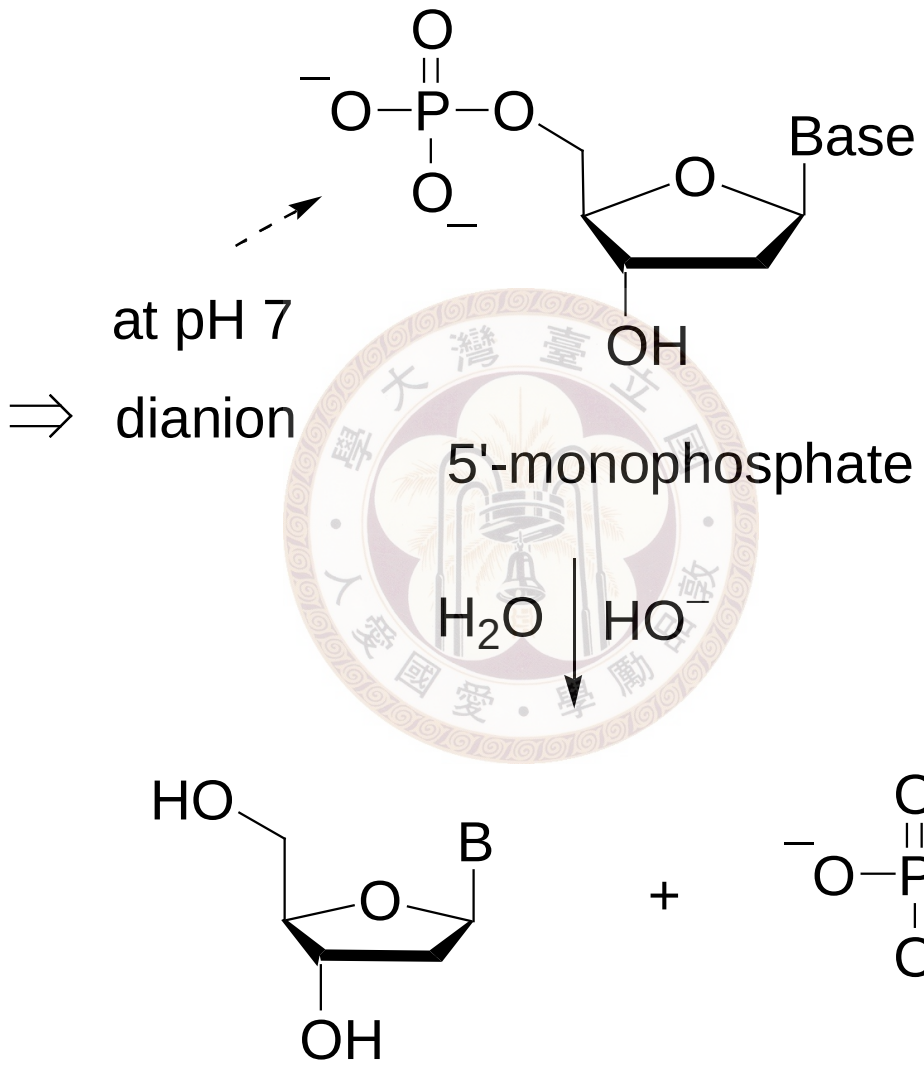


guanine

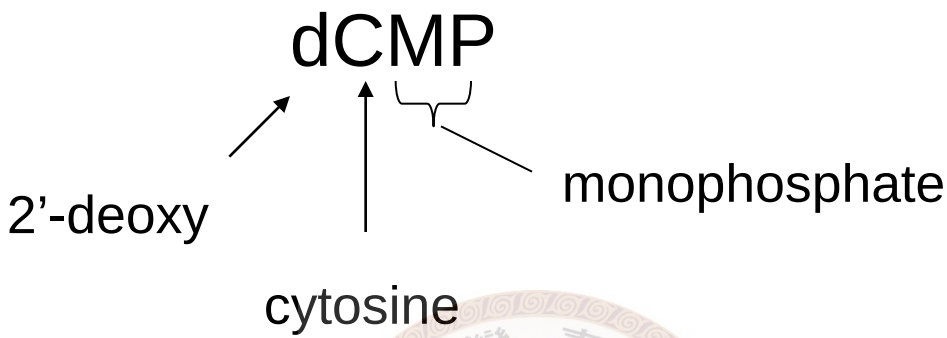
◎ The nucleosides: *N*-glycosides



◎ The nucleotides



✓ Nomenclature



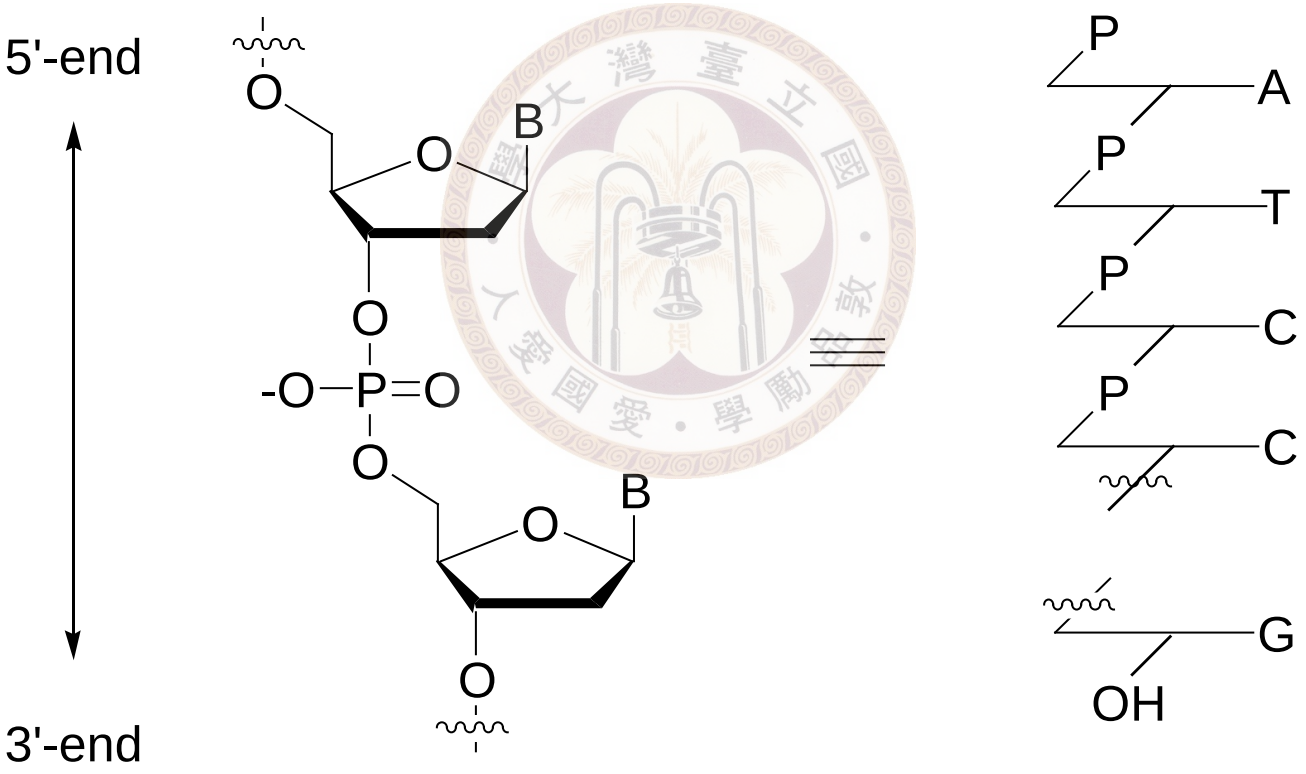
2’-Deoxycytidine-5’-monophosphate

— adenosine—
— thymidine—
— guanosine—
— uridine —

※ Structure of DNA

Primary structure

read from the 5’ end to the 3’ end



◎ Secondary structure —— The double helix

A/T (1:1 ratio) and G/C (1:1 ratio) in DNA

The Watson-Crick model

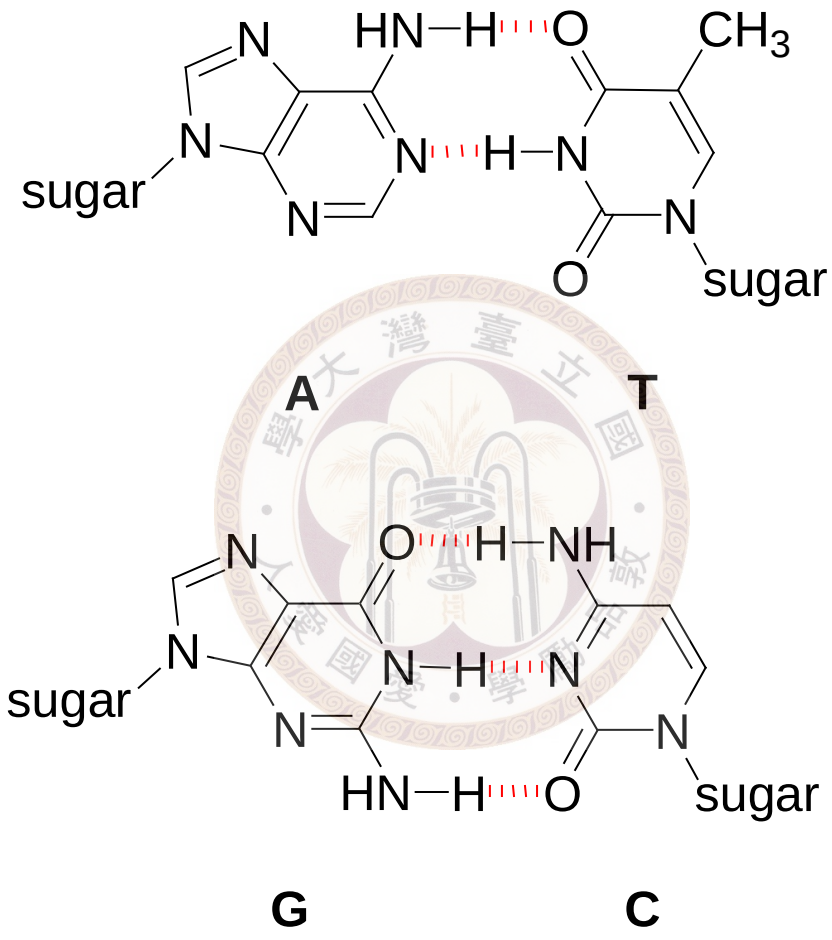
⟹ Two strands coiled in a double helix

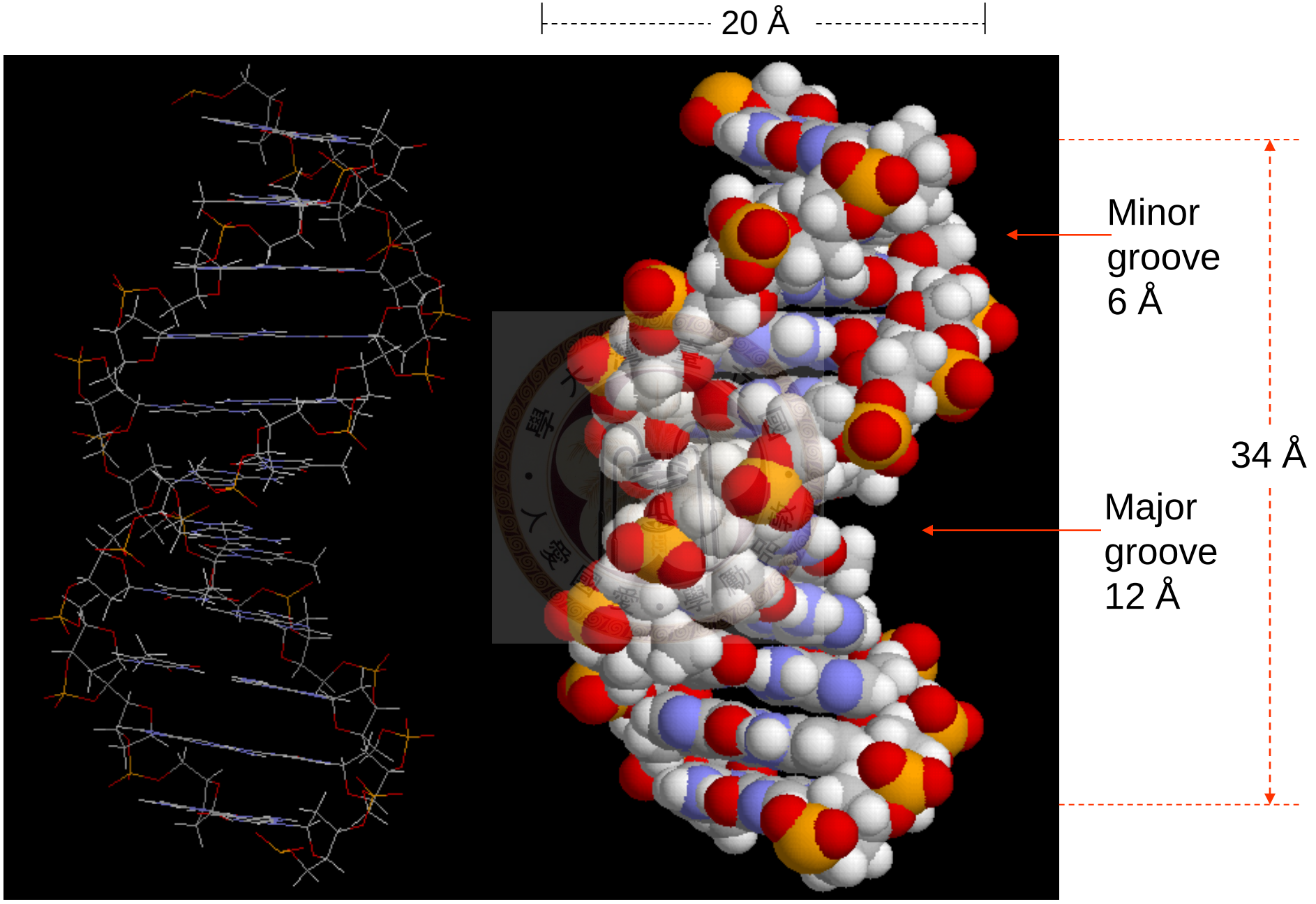
⟹ Base pairing by hydrogen bonding

A ⁞⁞ T

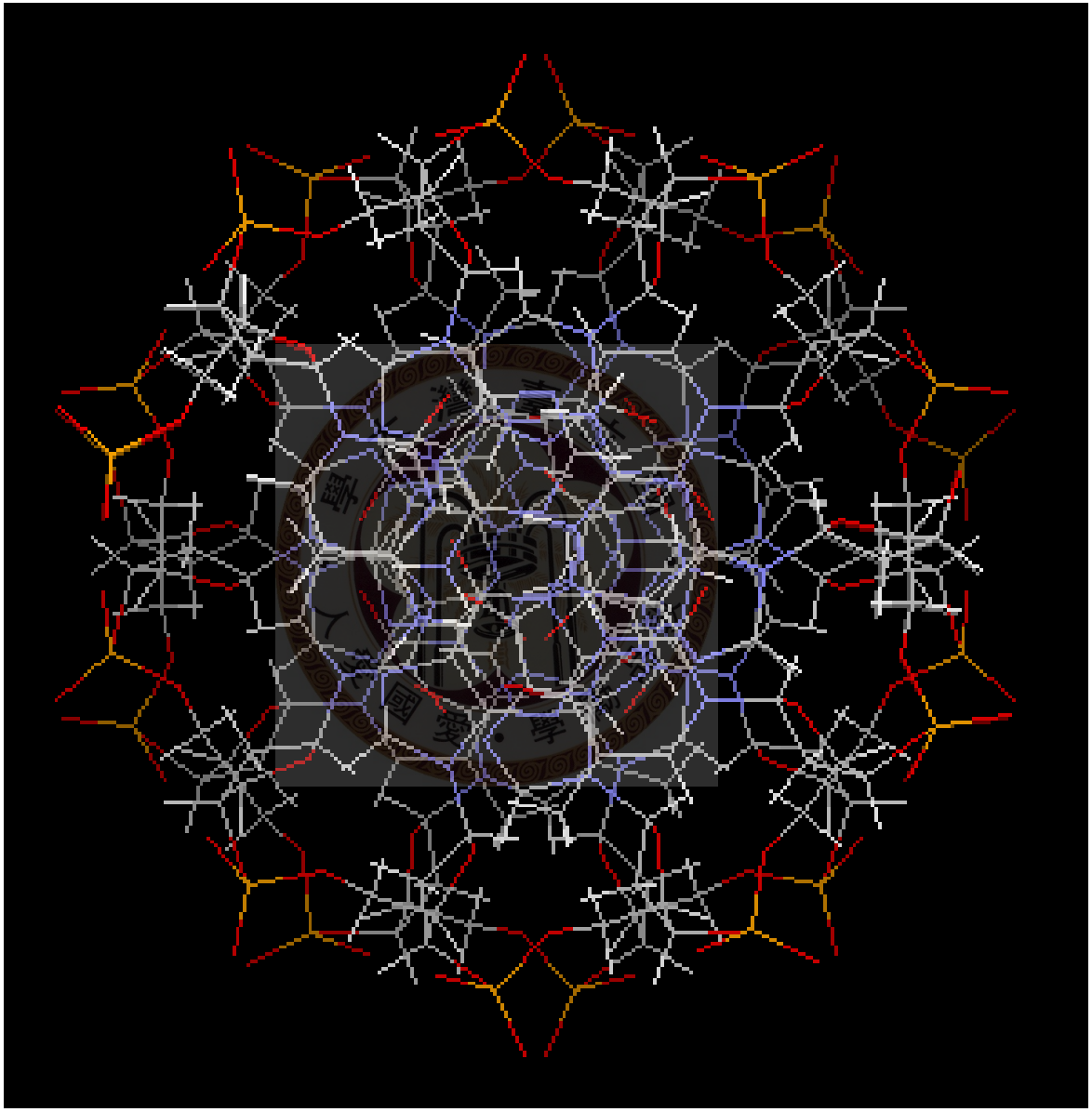
G ⁞⁞⁞ C

(U for T in RNA)


HN–H
O
CH3
N
N
sugar
N
H–N
N
N
O
sugar
A
T
O
H–NH
N
N
sugar
N–H
N
N
N
HN–H
O
sugar
G
C


20 Å
Minor
groove
6 Å
34 Å
Major
groove
12 Å

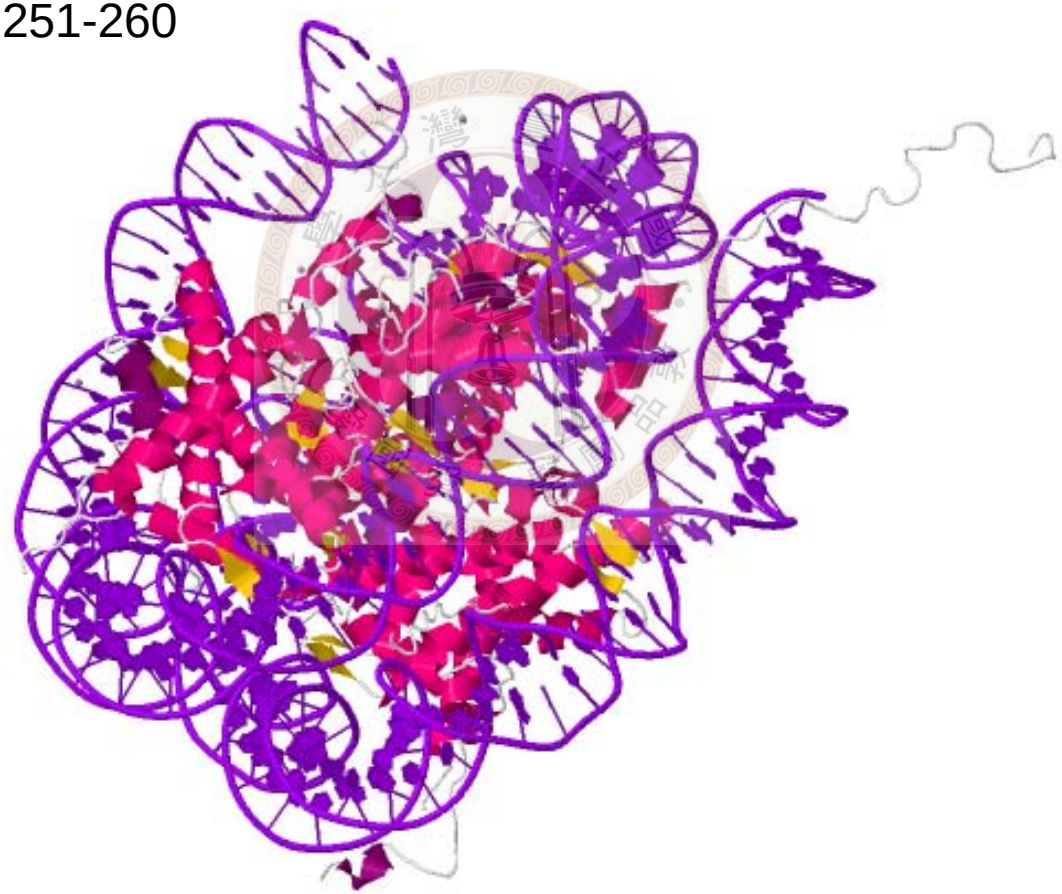
Top view



◎ Tertiary structure

supercoiled

COMPLEX BETWEEN NUCLEOSOME CORE PARTICLE (H3,H4,H2A,H2B) AND 146 BP LONG DNA FRAGMENT
Crystal structure of the nucleosome core particle at 2.8 A resolution (1997) Nature **389:** 251-260



http://www.rcsb.org/pdb/101/motm_disscussed_entry.do?id=1aoi

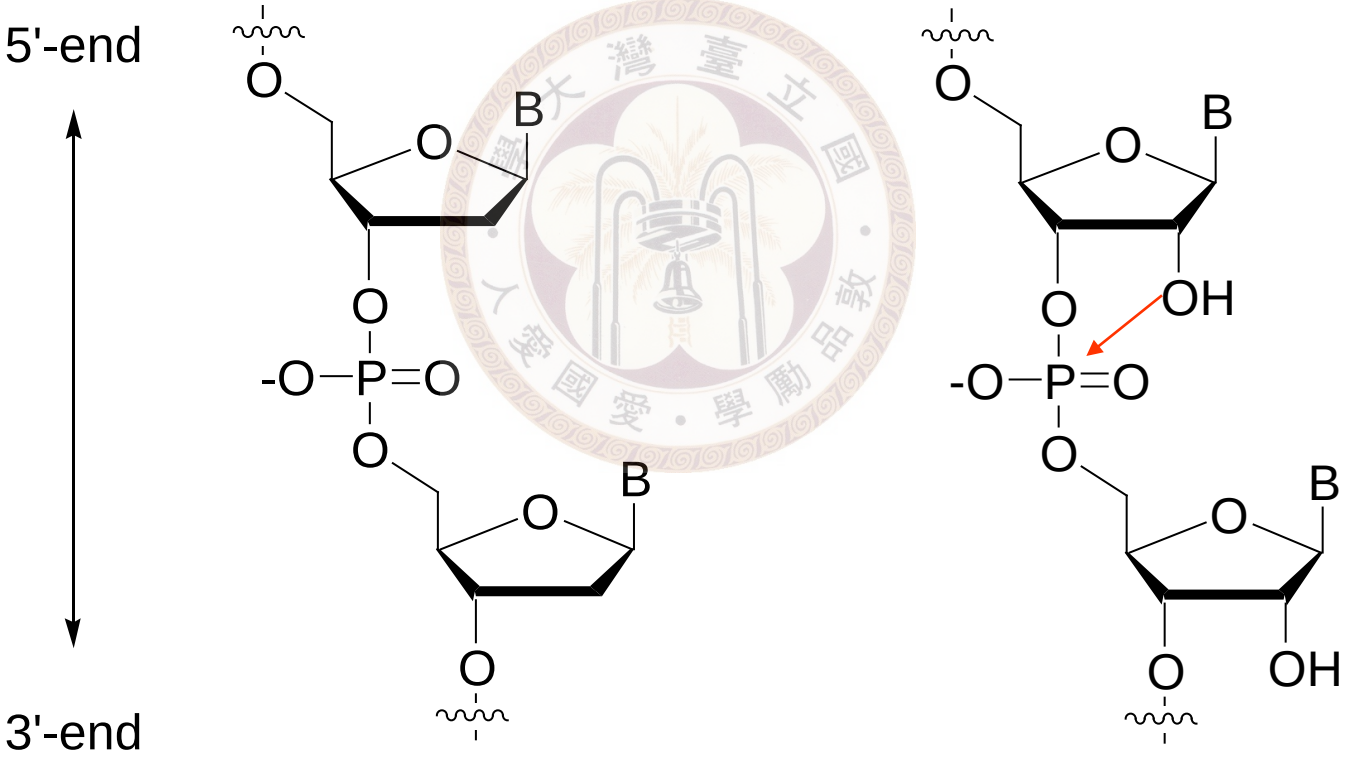
※ RNA

1. β-D-Ribose
2. A=U, G≡C
   ↑
   U for T
3. Single strand

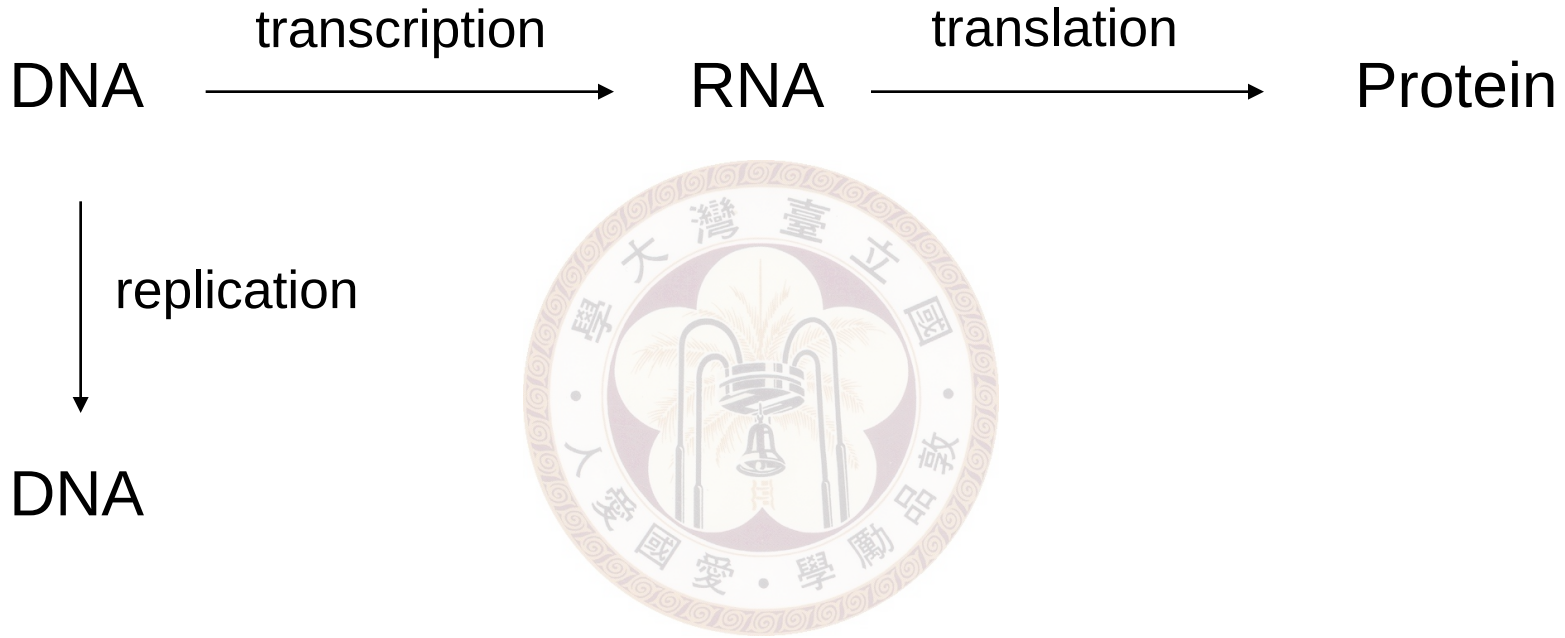
RNA is less stable

DNA as genetic material
→ More stable

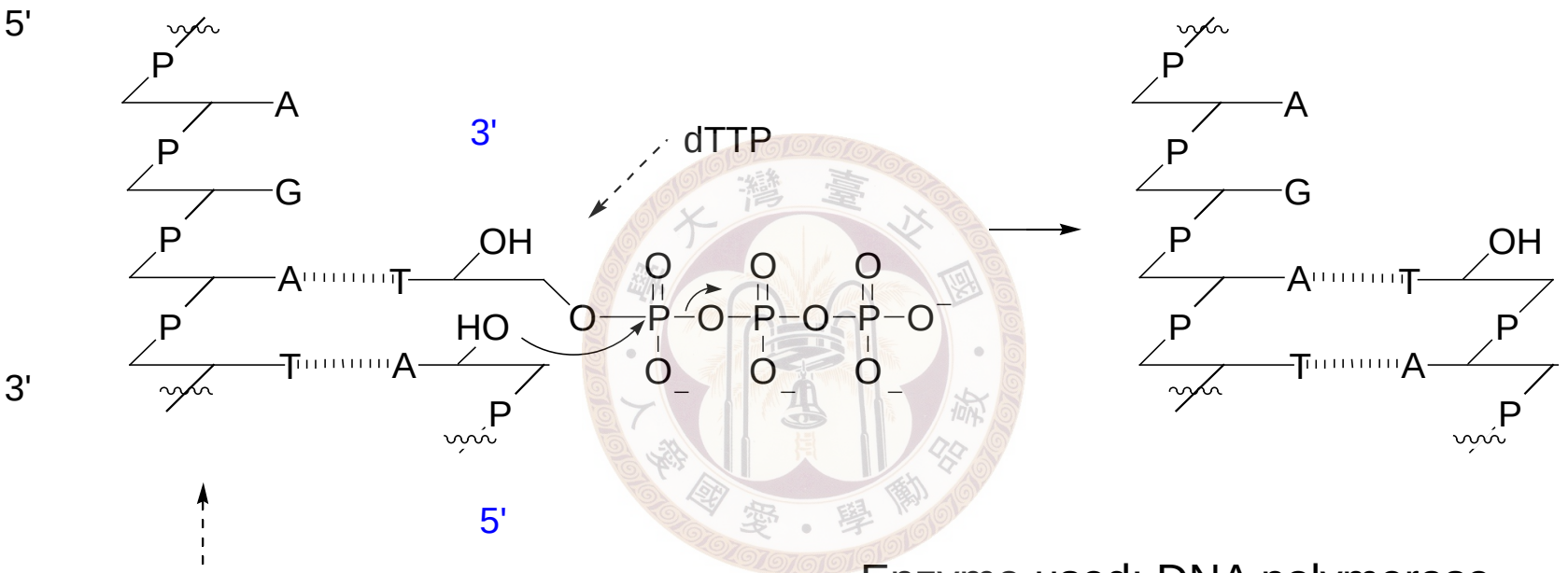
RNA has 2’-OH
→ May attack 3’-phosphate



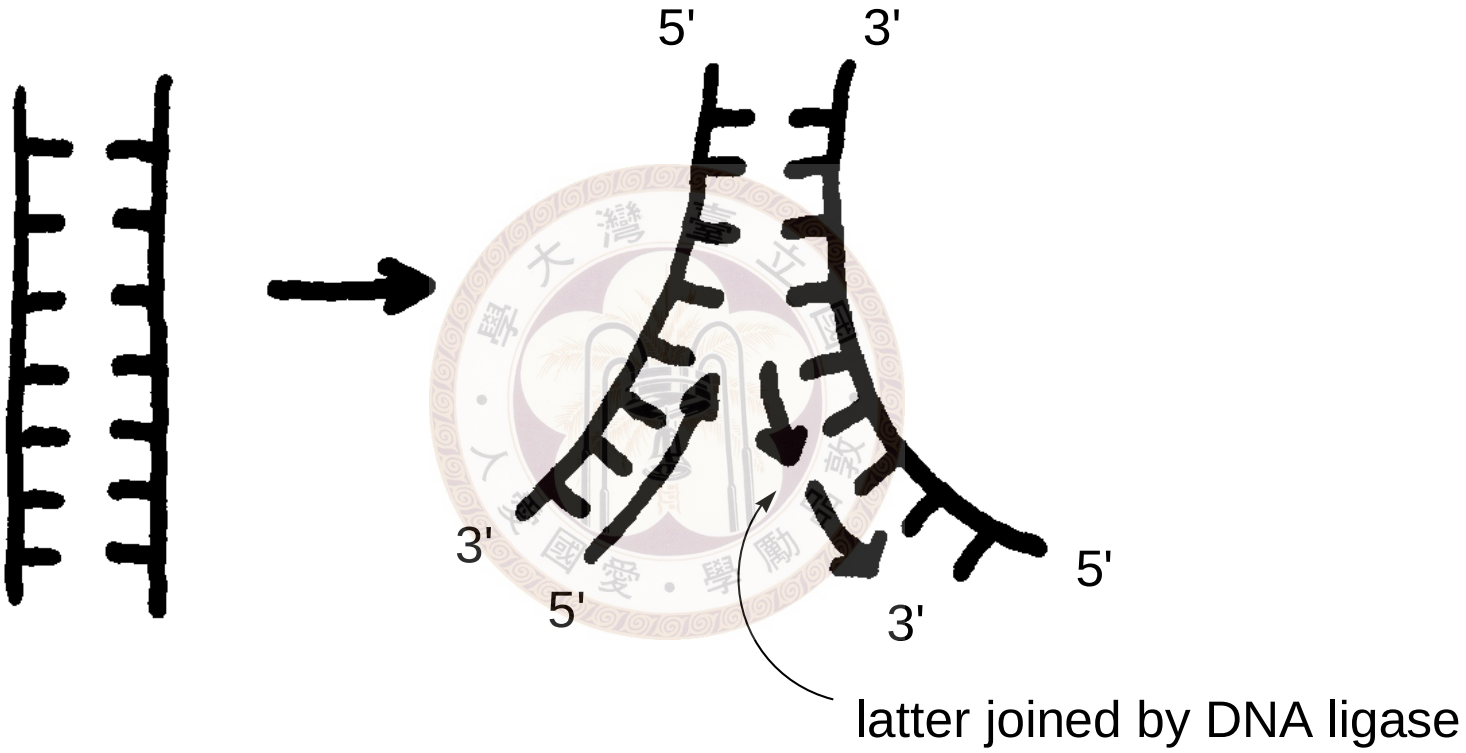
※ Function


DNA
transcription
RNA
translation
Protein
replication
DNA

※ Replication of DNA



Enzyme used: DNA polymerase


5'
3'
3'
5'
5'
3'
latter joined by DNA ligase

※ Transcription

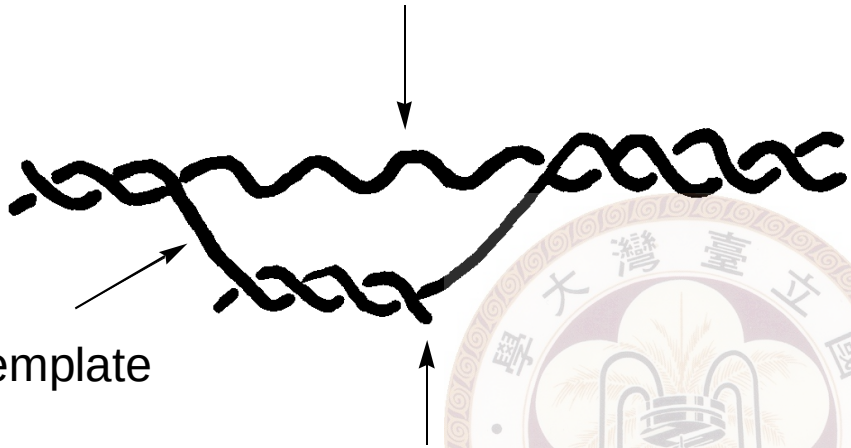
Formation of smaller and single strand RNA

Grow from 5’ to 3’

Use: RNA polymerase

Only this strand has sense: the informational strand

$\Longrightarrow$ contains the gene



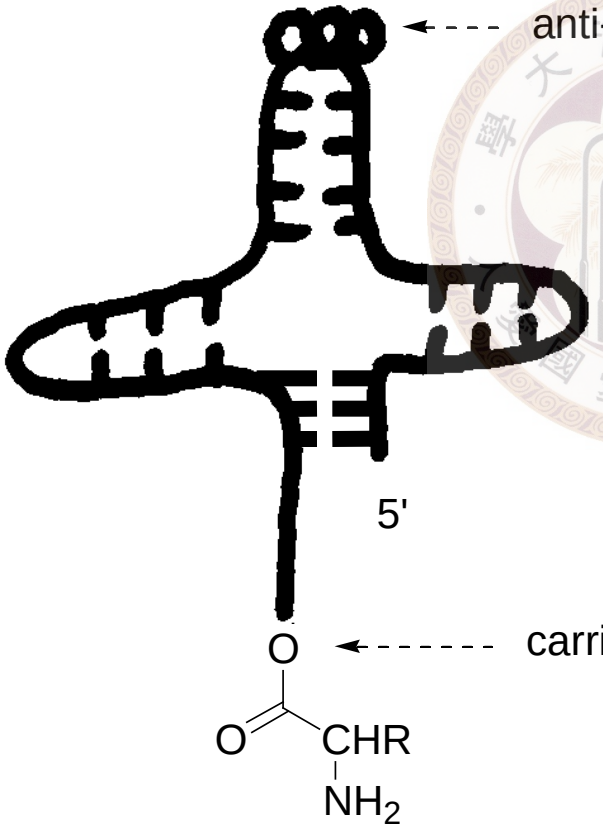
The template

mRNA: A copy of the informational strand

Only difference is T & U

RNA

- mRNA: use three-nucleotide codes to specify protein sequence
- rRNA: ribosomal RNA → the site of protein synthesis
- tRNA: transfer RNA



$4^3 = 64$ possible combination

Representing 20 AAs
(three for stop signs)

carries amino acid at the 3'-end

Phenylalanyl tRNA (yeast)

Data retrieved from: http://www.rcsb.org/pdb/101/motm_disscussed_entry.do?id=4tna

✓ Codon assignments of base triplets

First base	Second	Third base (3' end)			
(5' end)	base	U	C	A	G
U	U	**Phe**	**Phe**	**Leu**	**Leu**
	C	**Ser**	**Ser**	**Ser**	**Ser**
	A	**Tyr**	**Tyr**	**Stop**	**Stop**
	G	**Cys**	**Cys**	**Stop**	**Trp**
C	U	**Leu**	**Leu**	**Leu**	**Leu**
	C	**Pro**	**Pro**	**Pro**	**Pro**
	A	**His**	**His**	**Gln**	**Gln**
	G	**Arg**	**Arg**	**Arg**	**Arg**
A	U	**Ile**	**Ile**	**Ile**	**Met***
	C	**Thr**	**Thr**	**Thr**	**Thr**
	A	**Asn**	**Asn**	**Lys**	**Lys**
	G	**Ser**	**Ser**	**Arg**	**Arg**
G	U	**Val**	**Val**	**Val**	**Val**
	C	**Ala**	**Ala**	**Ala**	**Ala**
	A	**Asp**	**Asp**	**Glu**	**Glu**
	G	**Gly**	**Gly**	**Gly**	**Gly**

Amino acid	Sequence
Ala (A)	GCA
	GCC
	GCG
	GCU
Arg (R)	AGA
	AGG
	CGA
	CGC
	CGG
	CGU
Asn (N)	AAC
	AAU
Asp (D)	GAC
	GAU
Cys (C)	UGC
	UGU
Gln (Q)	CAA
	CAG

Amino acid	Sequence
Glu (E)	GAA
	GAG
Gly (G)	GGA
	GGC
	GGG
	GGU
His (H)	CAC
	CAU
Ile (I)	AUA
	AUC
	AUU
Leu (L)	CUA
	CUC
	CUG
	CUU
	UUA
	UUG

Amino acid	Sequence
Lys (K)	AAA
	AAG
Met (M)	AUG
Phe (F)	UUU
	UUC
Pro (P)	CCA
	CCC
	CCG
	CCU
Ser (S)	AGC
	AGU
	UCA
	UCG
	UCC
	UCU

Amino acid	Sequence
Thr (T)	ACA
	ACC
	ACG
	ACU
Trp (W)	UGG
Tyr (Y)	UAC
	UAU
Val (V)	GUA
	GUG
	GUC
	GUU
Ini.	AUG
Term.	UGA
	UAA
	UAG

◎ rRNA (以細菌為例)

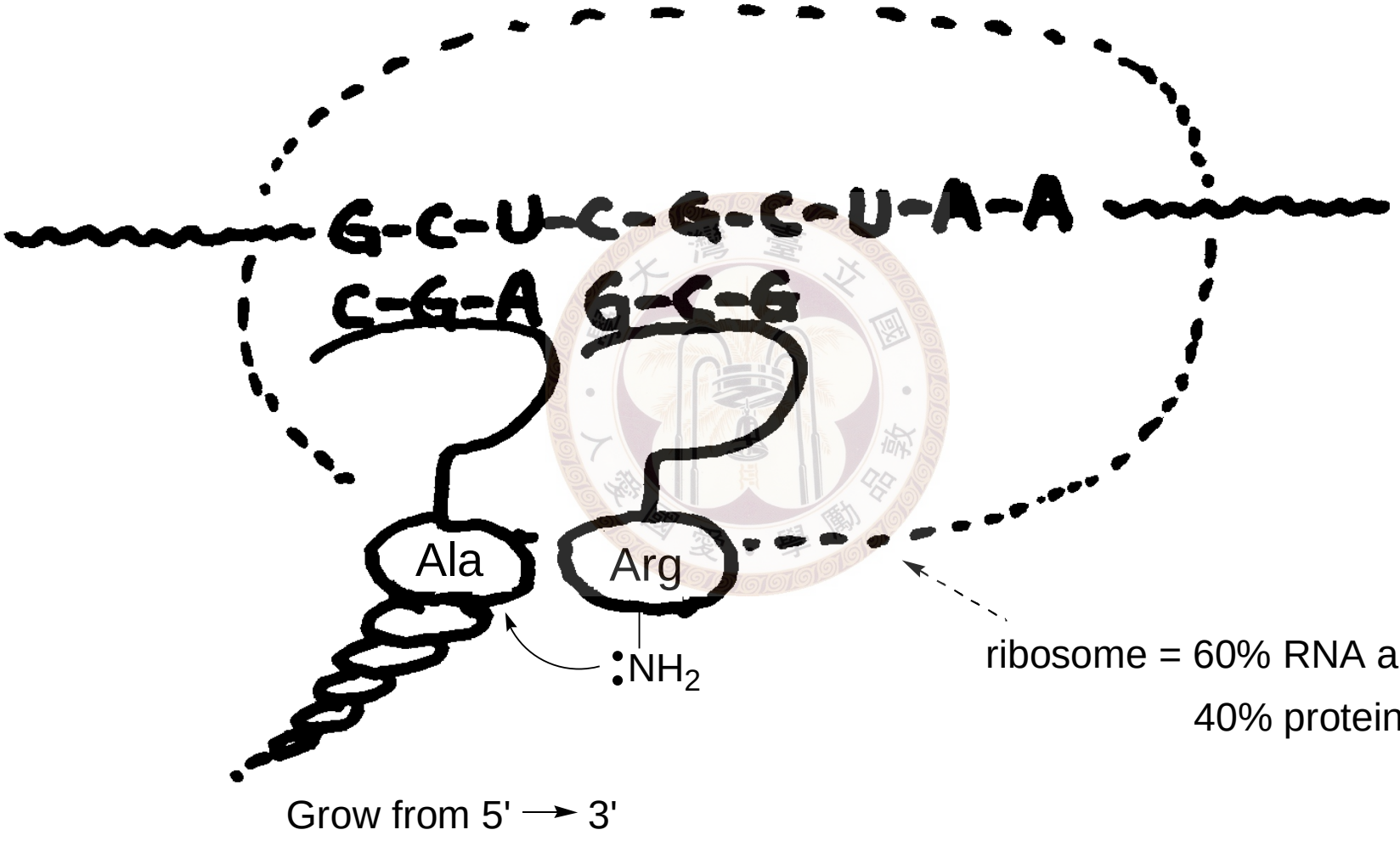
細菌的核糖體(70S)是由一個小的30S單元與一個大的50S單元所組成

30S的分子量為800,000
50S的分子量為1,500,000

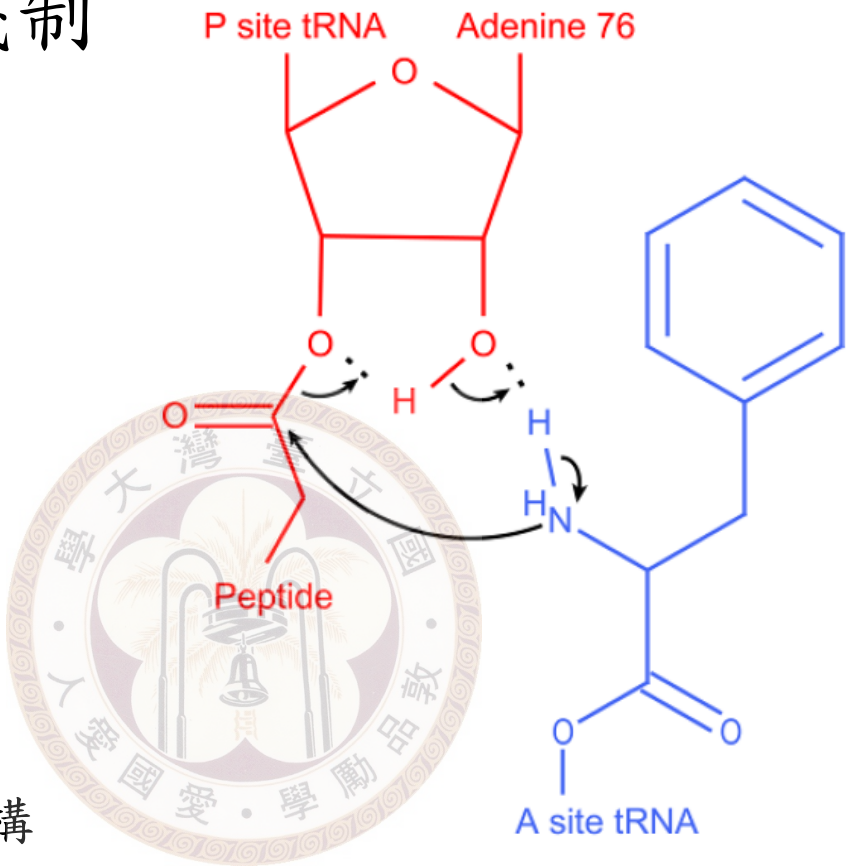
30S單元是由約 20 種不同的蛋白質與一個由約 1600 個核糖酸單元所組成核糖酸序列(rRNA), 16S

50S單元是由約 33種不同的蛋白質與一個23S rRNA (約 2900核糖酸單元), 以及另一個 5S rRNA (約 120 核糖酸單元)

◎ Translation

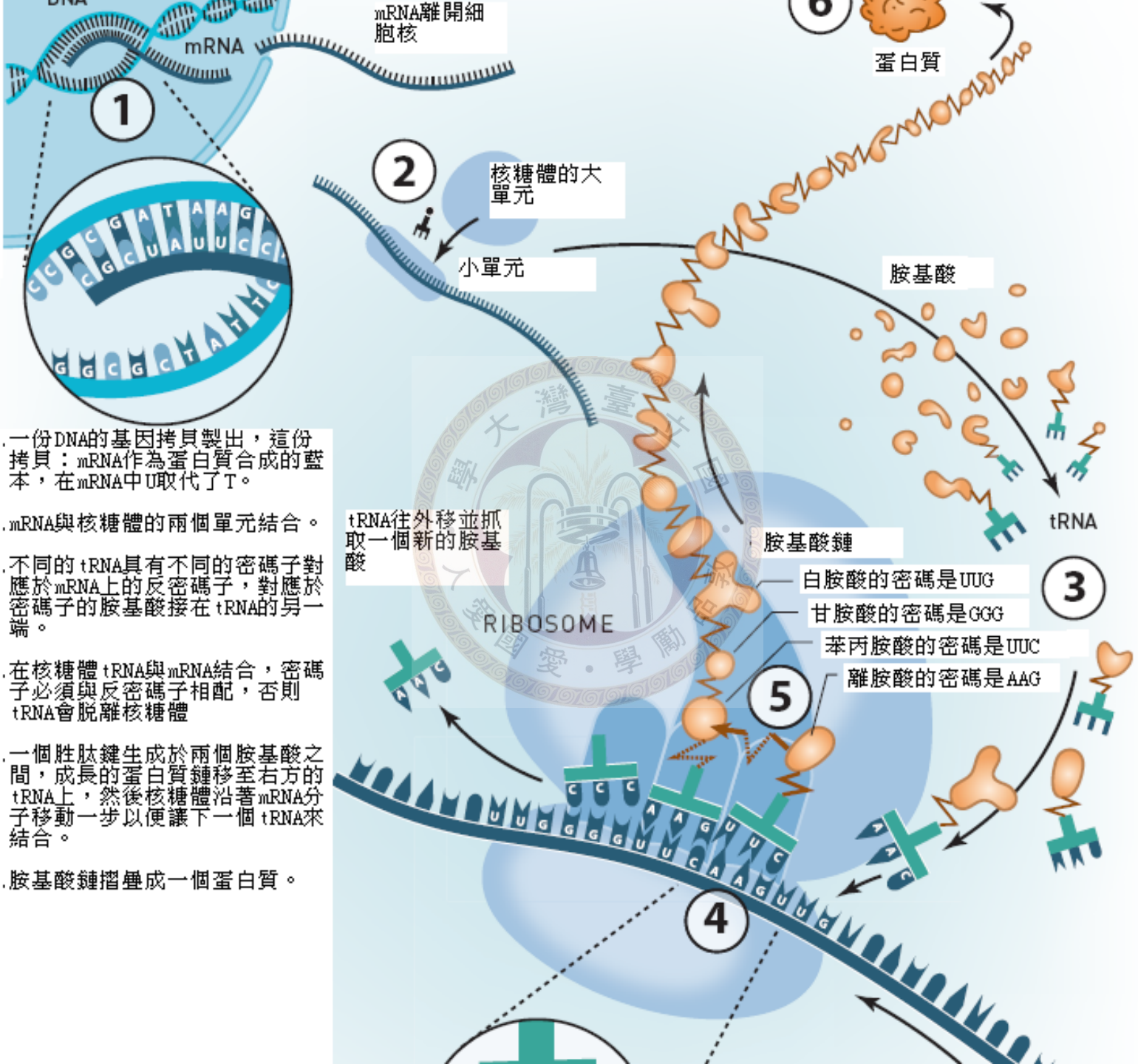


胜肽鍵生成的機制



基於Steitz發表的50S結構

The mechanism of peptide bond formation on the ribosome. The α-amino group of aminoacyl-tRNA in the A site (blue) attacks (black arrow right to left) the ester bond of the peptidyl-tRNA in the P site (red). A proton is shuttled via the OH' group of A76 in peptidyl-tRNA in the P site (black arrows left to right), aided by an H-bond network (**Trobro and Åqvist, 2005**) established with the help of **23S rRNA bases** and water molecules (not shown).



1. 一份DNA的基因拷貝製出，這份拷貝：mRNA作為蛋白質合成的藍本，在mRNA中U取代了T。

2. mRNA與核糖體的兩個單元結合。

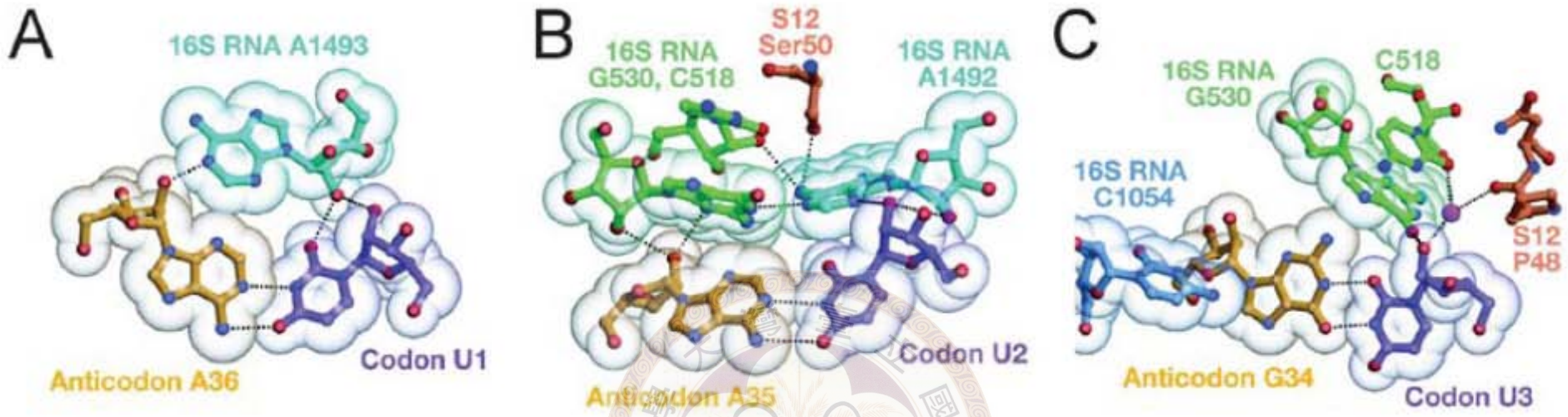
3. 不同的tRNA具有不同的密碼子對應於mRNA上的反密碼子，對應於密碼子的胺基酸接在tRNA的另一端。

4. 在核糖體tRNA與mRNA結合，密碼子必須與反密碼子相配，否則tRNA會脫離核糖體

5. 一個胜肽鍵生成於兩個胺基酸之間，成長的蛋白質鏈移至右方的tRNA上，然後核糖體沿著mRNA分子移動一步以便讓下一個tRNA來結合。

6. 胺基酸鏈摺疊成一個蛋白質。

精準度的問題



How the ribosome increases the intrinsic selectivity, *d*, of codon recognition. (A) The geometry of base pairing between U1 in first codon position and A36 in the anticodon is monitored by A1493. (B) The geometry of base pairing between U2 in second codon position and A35 in aminoacyl-tRNA is monitored by A1492 and G530, while the geometry of the base pairing in third codon position (U3:G34) is less stringently monitored, explaining the wobble hypothesis (From (Ogle and Ramakrishnan, 2005)).

從高解析度的結構發現，當tRNA在A區結合時，會導致30S rRNA結構改變，形成一種監控機制

http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2009/advanced.html

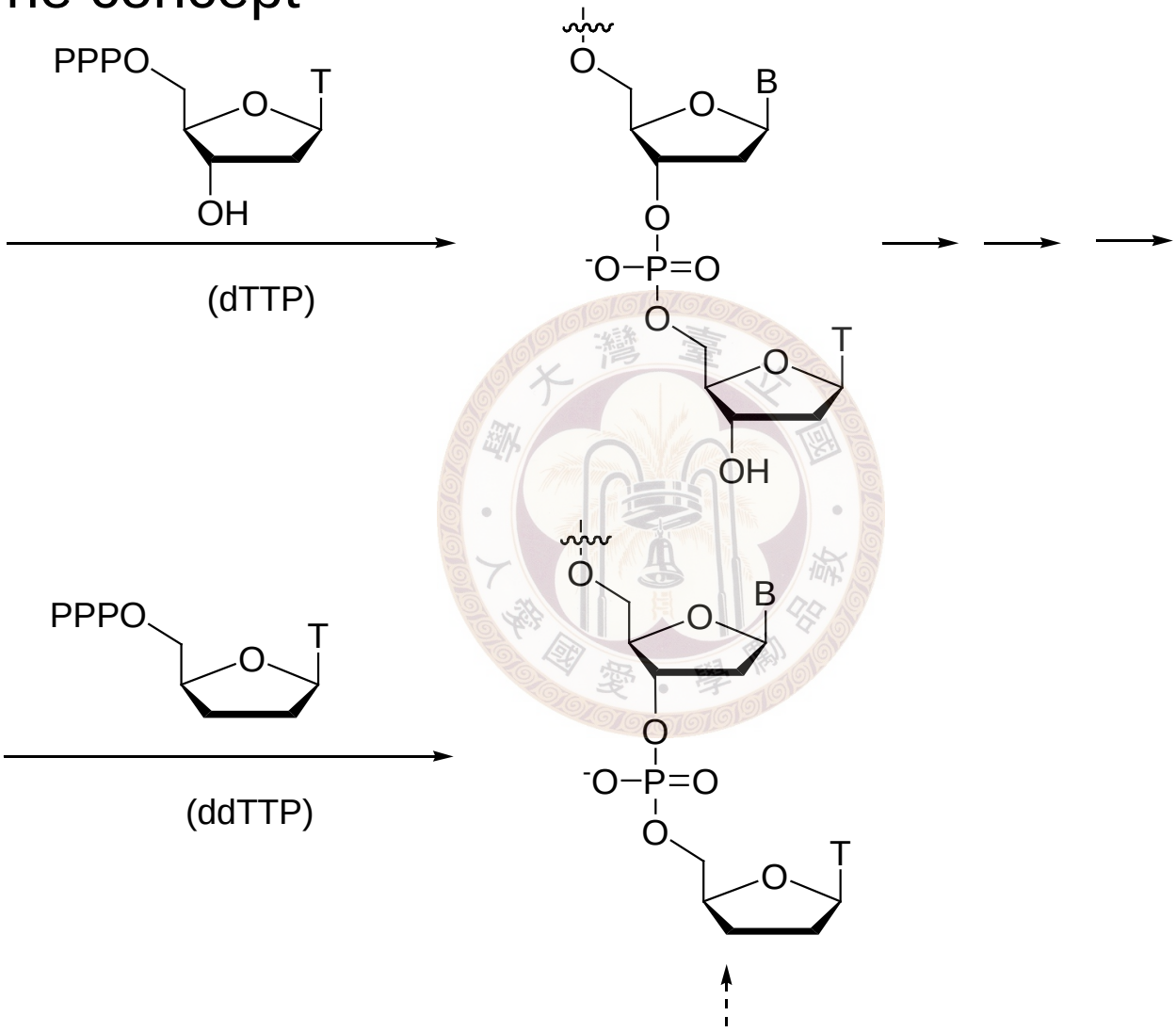
※ DNA Sequencing

1. First cleaved at specific points into smaller fragments using restriction nucleases. There are more than 200 such enzymes.

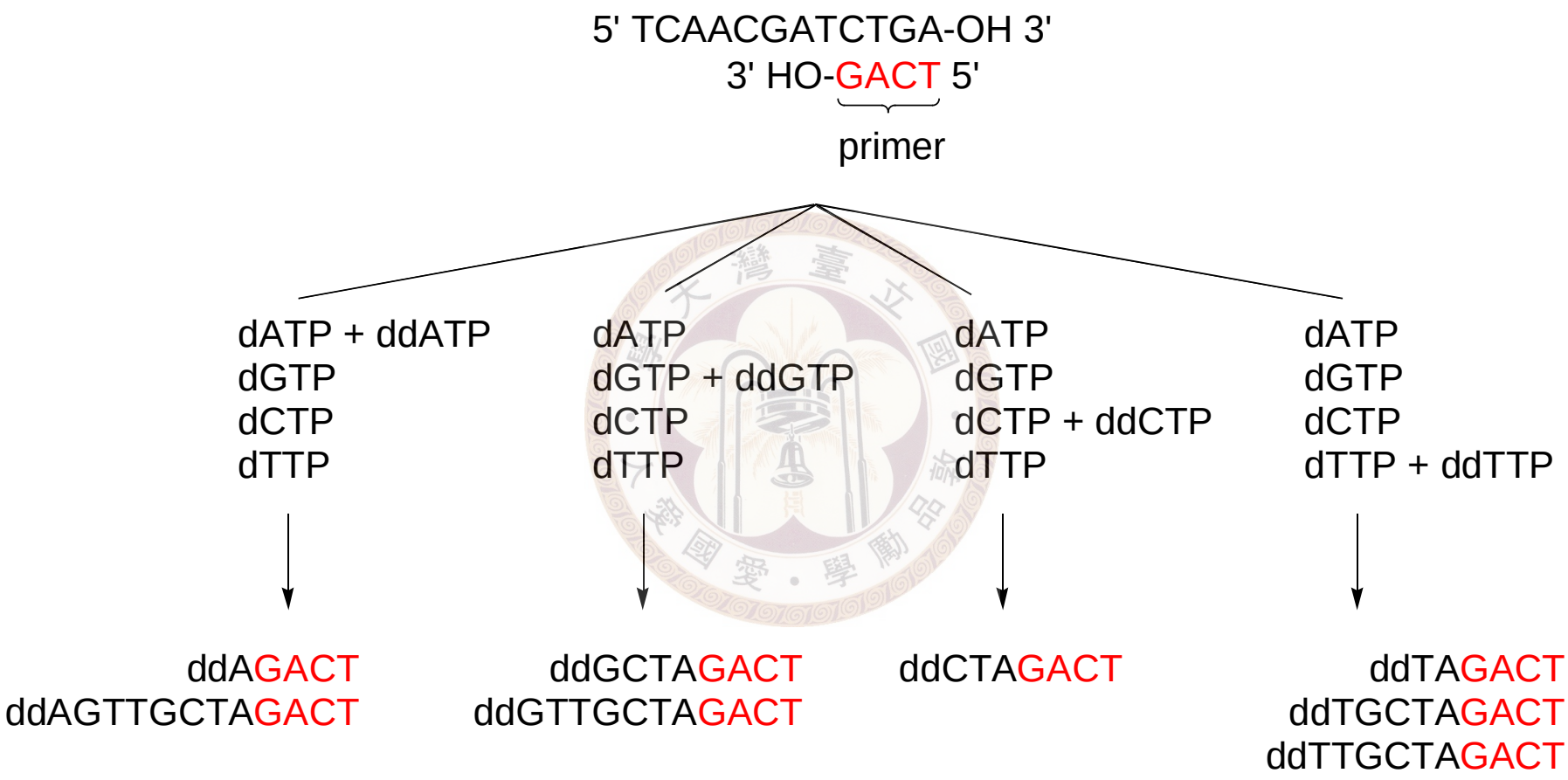
2. The sequence of each fragment can then be determined by a chemical (Gilbert-Maxam) or enzymatic (Sanger) procedure.

◎ Sanger's dideoxy method: chain termination

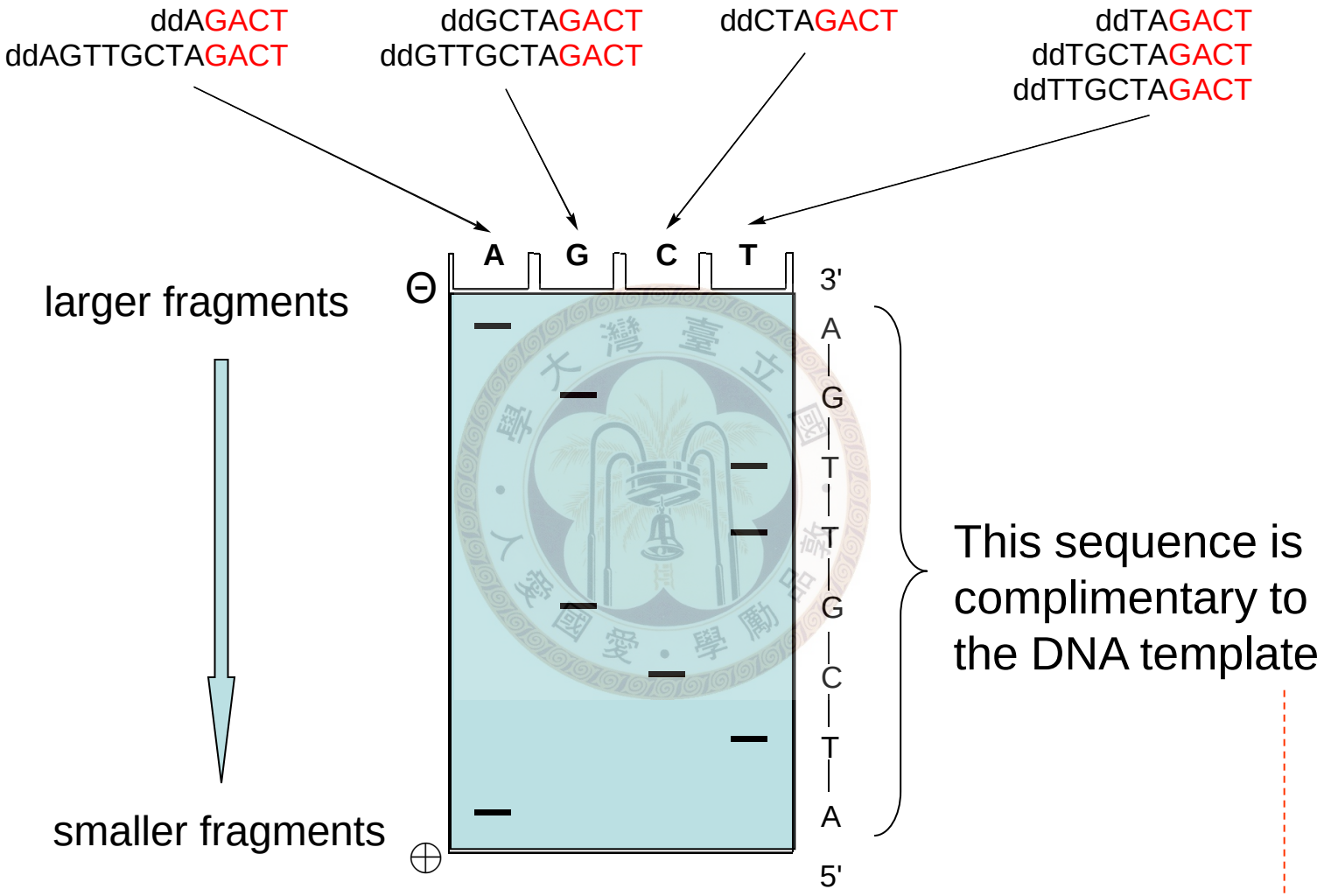
The concept



The experiment



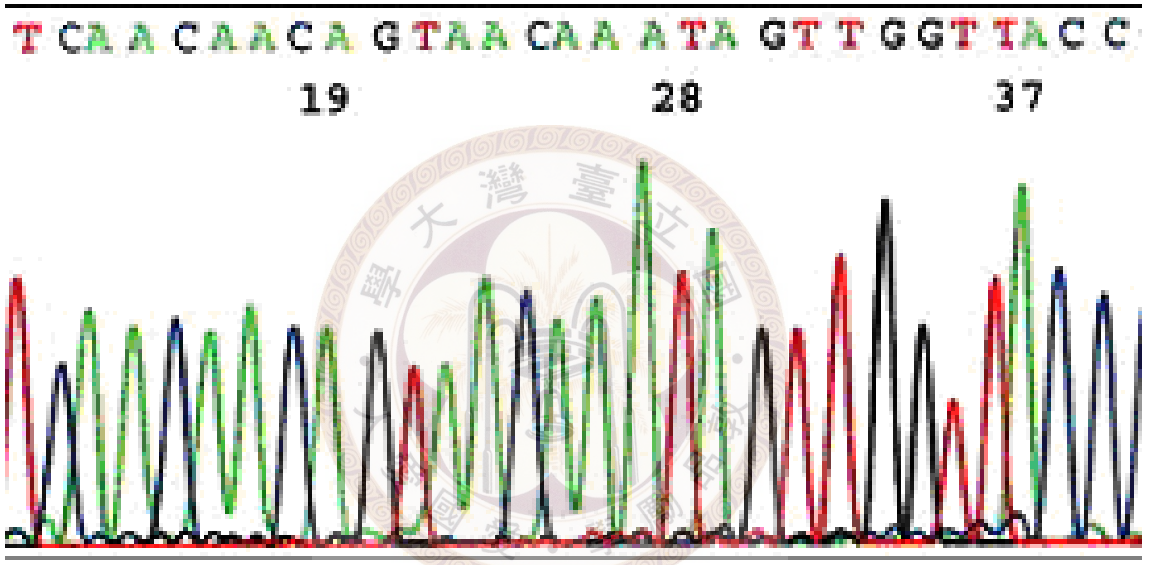
Polyacrylamide gel electrophoresis



5' TCAACGATCTGA-OH 3'

3' HO-AGTTGCTAGACT 5'

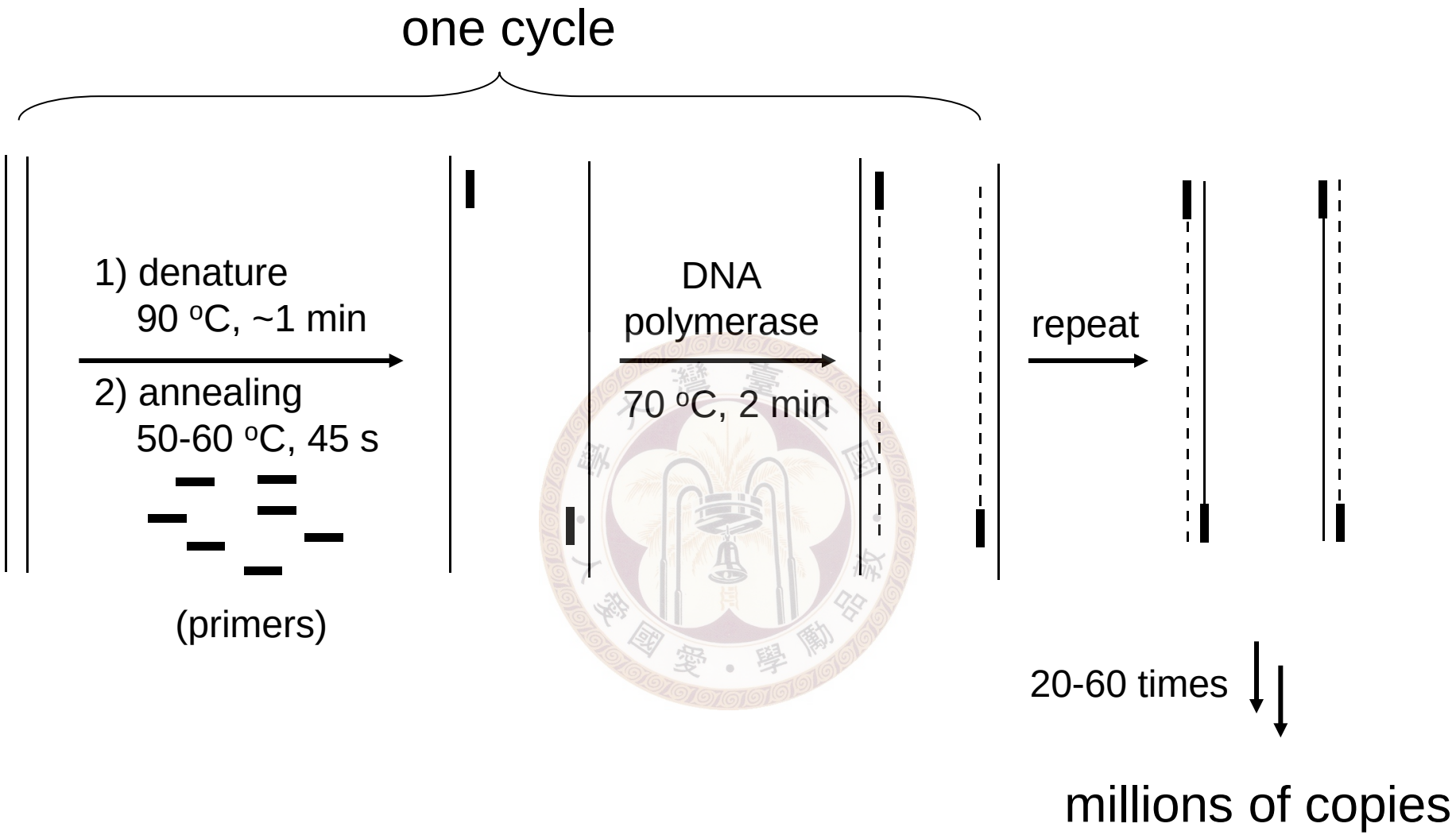
With fluorescence tag



◎ The Polymerase Chain Reaction (PCR)

- An extraordinarily simple and effective method for amplifying the number of copies of a DNA molecule.
  - A single molecule can lead to 100 billion copies in an afternoon
  - The Nobel Prize was awarded to K. Mullis in 1993

- PCR requires:
  - A sample of the DNA to be copied
  - The enzyme DNA polymerase
  - A short ‘primer’ sequence complimentary to the template DNA
  - A supply of A, C, G, and T nucleotide triphosphate monomers
  - A simple device for thermal cycling during the reaction sequence



2^1 = 2 copies, 2^2 = 4 copies,……2^{35} = 34 billion copies