第6章
蛋白质的生物合成

(K/M/O/P/Q)

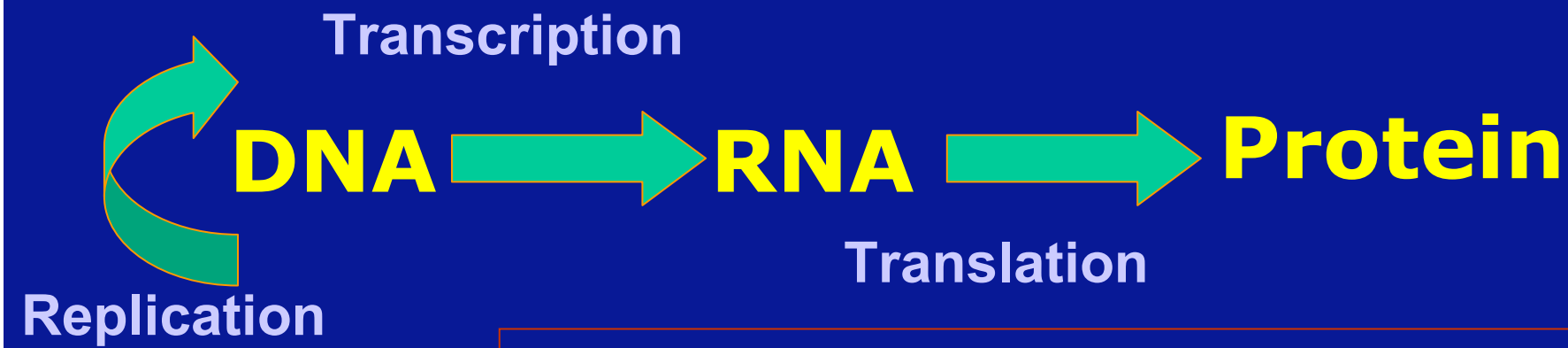
Section P

THE GENETIC CODE & tRNA

- P1 THE GENETIC CODE
- P2 tRNA STRUCTURE AND FUNCTION

THE GENETIC CODE

The Central Dogma



1. Genetic information transfer from polynucleotide chain into polypeptide chain.
2. Take place in ribosomes.
3. tRNAs recognize codons.

- **Genetic code is a triplet code**

(three nucleotide encode one amino acid)

The way in which the nucleotide sequence in nucleic acids specifies the amino acid sequence in proteins.

The triplet codons are nonoverlapping and comma-less.

---UCU UCC CGU GGU GAA---

- **Genetic code is degenerate (简并):**
  - Only 20 amino acids are encoded by 4 nucleotides in triplet codons (4^3 =64 of amino acids could potentially be encoded). Therefore, more than one triplet are used to specify a amino acids, and the genetic code is said to be degenerate, or to have redundancy.
  - Codons specifying the same amino acid are called synonyms (同义密码子).

Deciphering

cell-free protein synthesizing system
from *E. coli*

1. DNase treated cell lysate to prevent new transcription
2. Add homopolymeric synthetic mRNAs [poly(A)] + 19 cold (non-labeled) and one labeled aminoacids
3. In vitro translation
4. Analyze the translated polypeptides

poly(U) ---UUU--- polyphenylalanine

poly(C) ---CCC--- polyproline

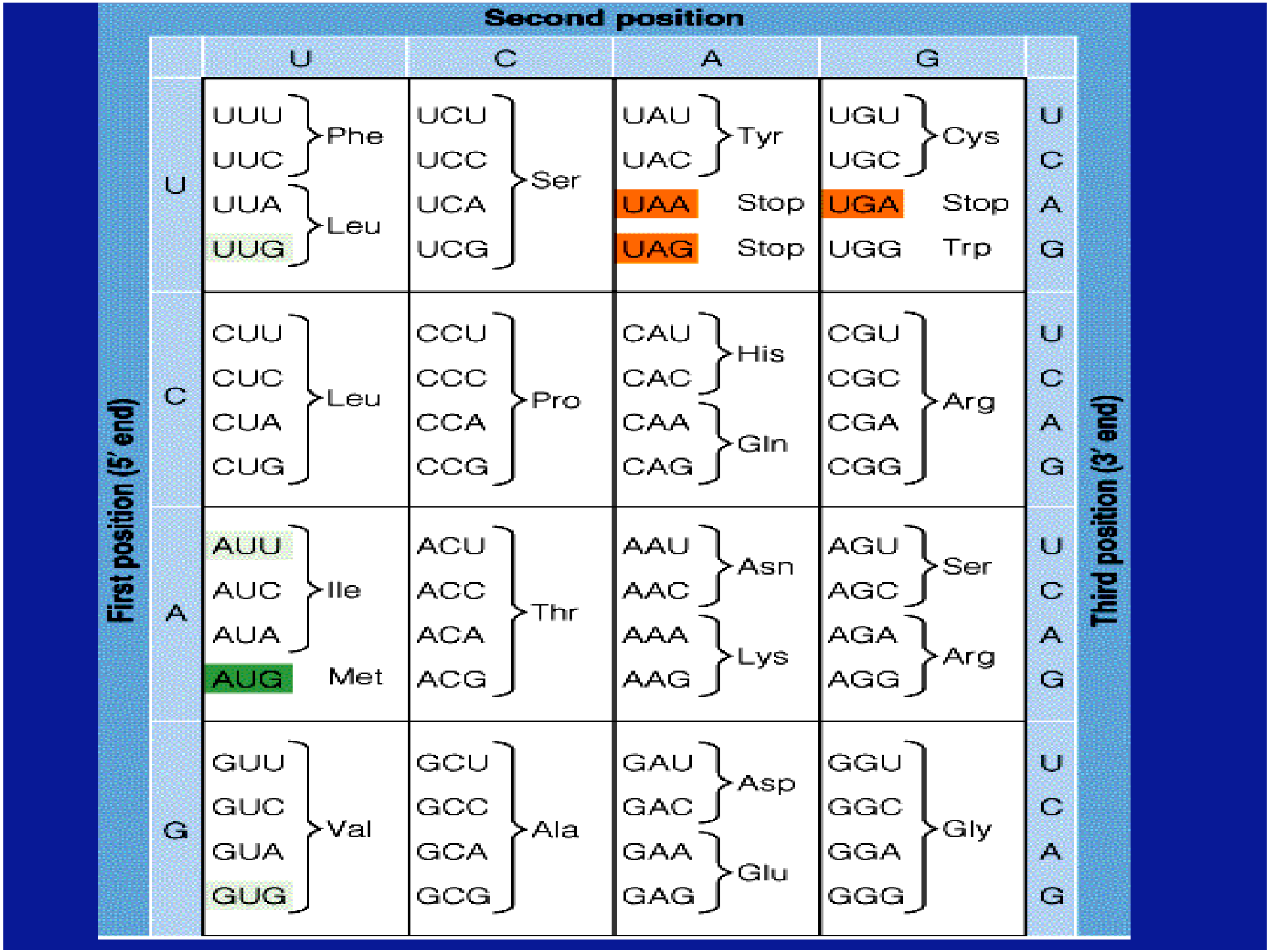
poly(A) ---AAA--- polylysine

poly(G) --- did not work because of the complex secondary structure

Random co-polymers were also used as mRNAs

Synthetic trinucleotides (late 1960s) could assign specific triplets unambiguously to specific amino acids.

Synthetic trinucleotides attach to the ribosome and bind their corresponding aminoacyl-tRNAs from a mixture. Upon membrane filtration, the trinucleotides bound with ribosome and aminoacyl-tRNA would be retained.


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

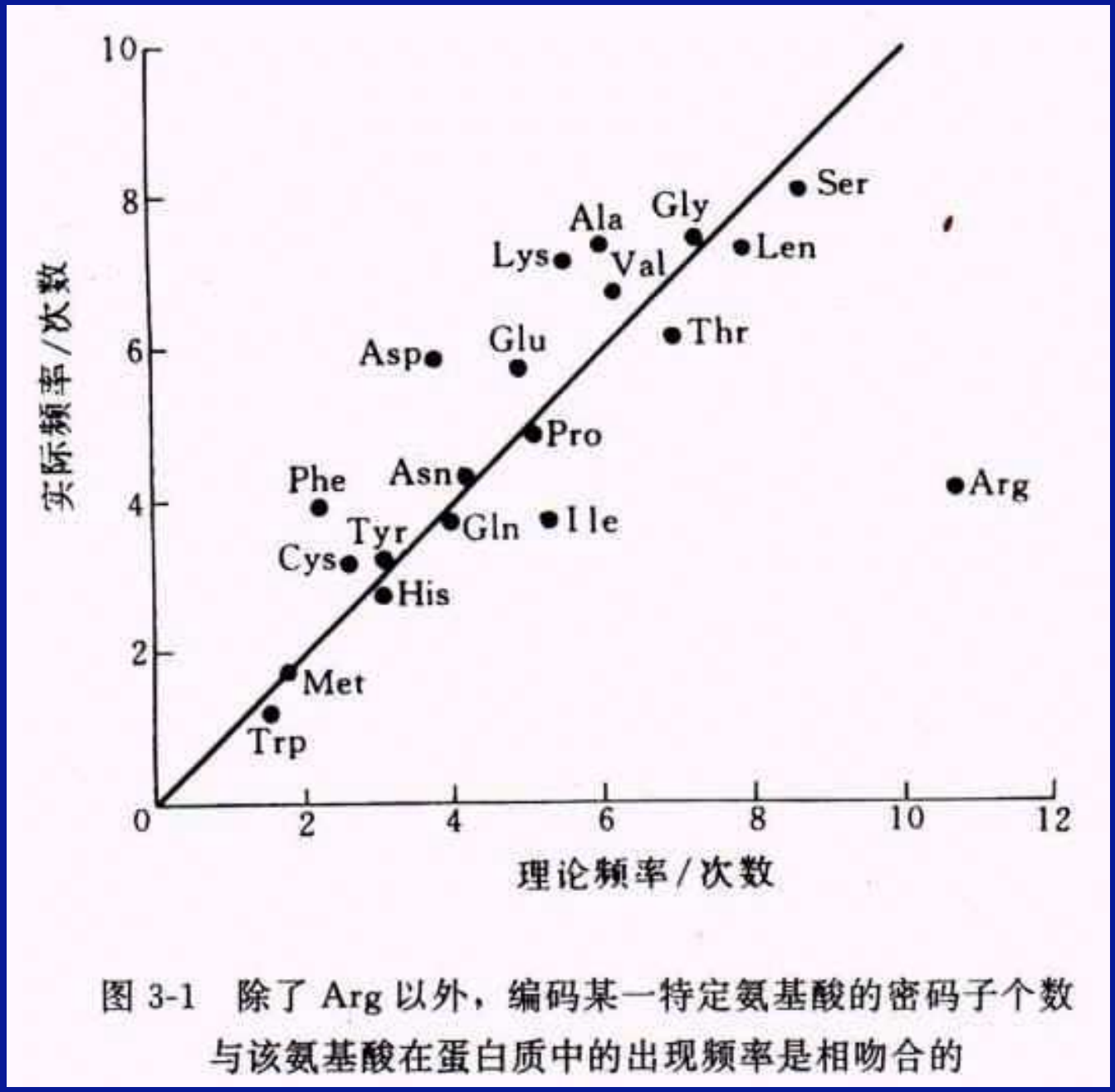


图 3-1　除了 Arg 以外，编码某一特定氨基酸的密码子个数与该氨基酸在蛋白质中的出现频率是相吻合的

只有精氨酸是个例外，因为在真核生物中CG双联子出现的频率较低，所以尽管有4个密码子同时编码，蛋白质中精氨酸的使用率仍不高。

Genetic code

- **Synonymous codons**:

  Those (more than one) encode the same amino acids (18 out of 20).

  the third position:

  pyrimidine ----synonymous (all cases)

  purine ----synonymous (most cases)

  the second position:

  pyrimidine ----hydrophilic amino acids

  purine -----polar amino acids

Effect of Mutation

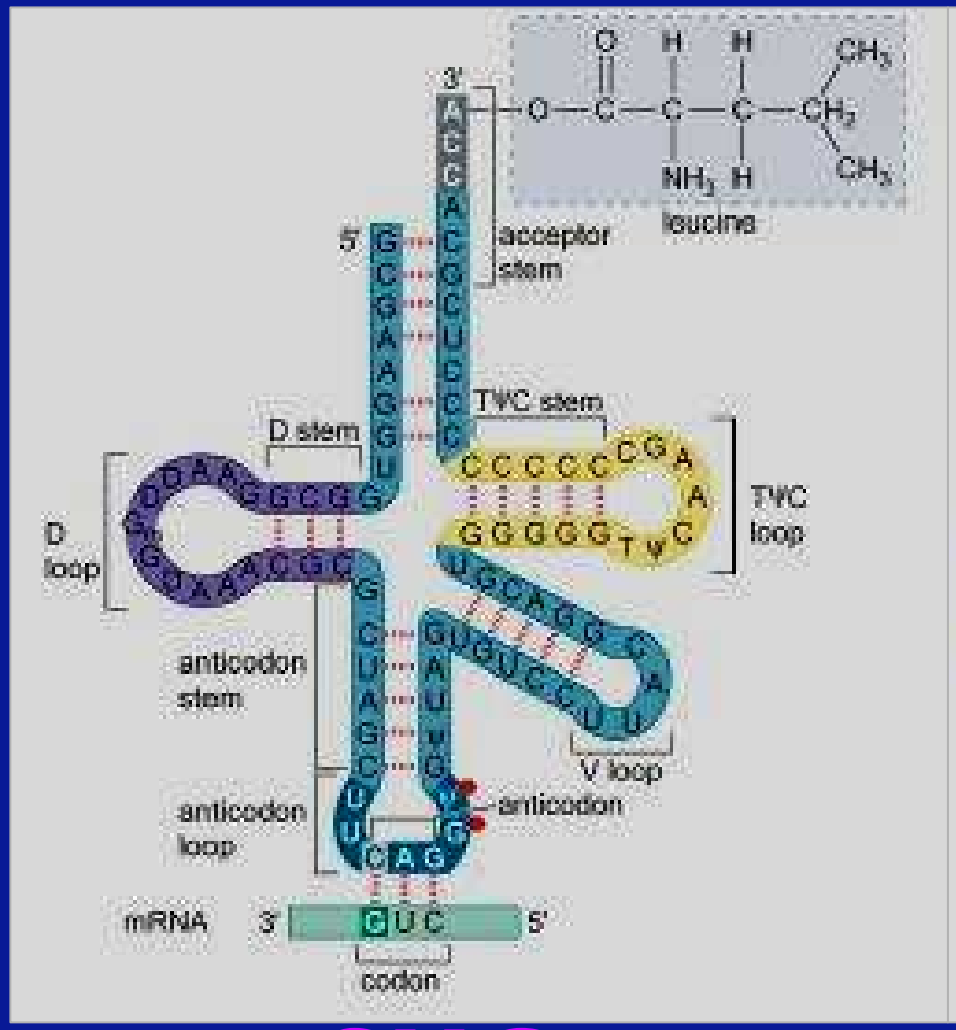
- **Transition**: the most common mutation in nature

  changes from purine to purine, or pymidine to pymidine

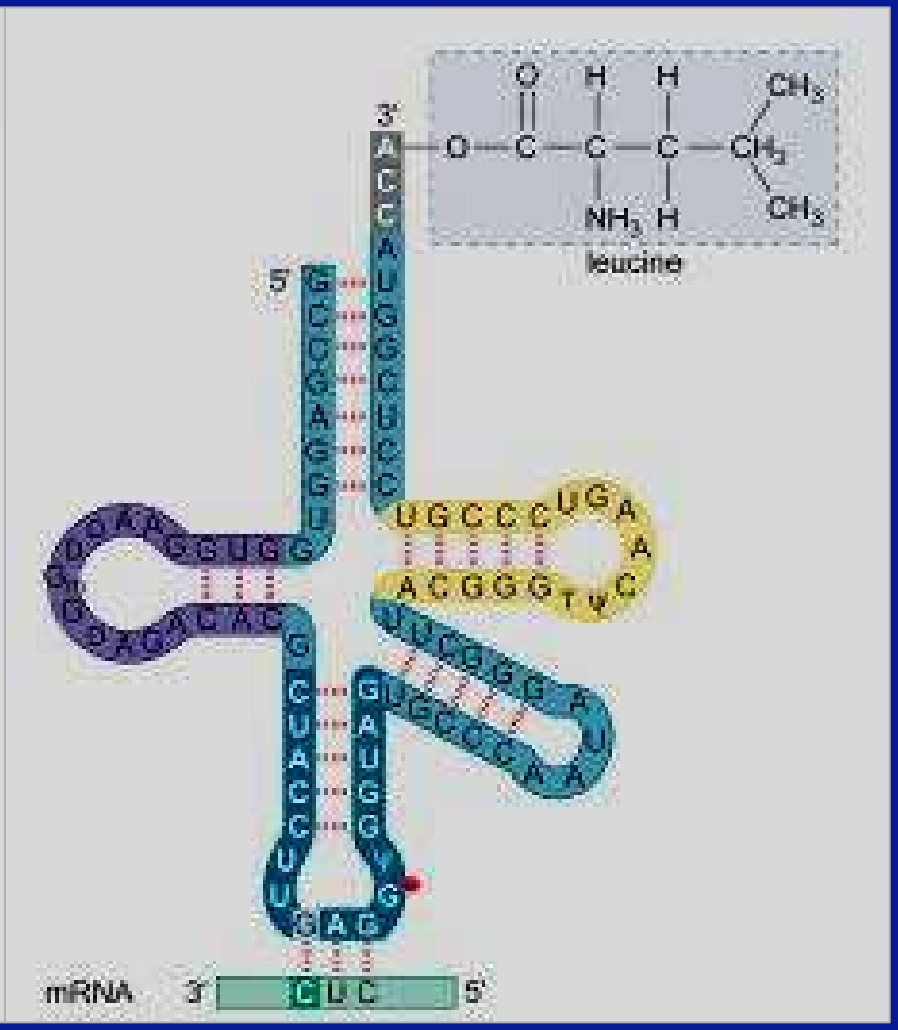
At third position: no effect except for

Met ⇔ Ile; Trp ⇔ stop

second position: results in similar chemical type of amino acids.



CUG



CUC

Codon-anticodon
pairing of two tRNA Leu moleculars

- **Transversions:**

  purine ⇔ pymidine

  At **third position:** over half have no effect and result in a similar type of amino acid. (Example: Asp ⇔ Glu)

  At **second position:** change the type of amino acid.

- In the **first position**, mutation (both transition and transvertion) specify a similar type of amino acid, and in a few cases it is the same amino acid.

Thus, natural triplet codons are arranged in a way to minimize the harmful effect of an mutation to an organism.

Universality

- The standard codons are true for most organisms, but not for all.

Codon	Usual meaning	Alternative	Organelle or organism
AGA AGG	Arg	Stop,Ser	Some animal mitochondria
AUA	Ile	Met	Mitochondria
CGG	Arg	Trp	Plant mitochondria
CUN	Leu	Thr	Yeast mitochondria
AUU GUG UUG	Ile Val Leu	Start	Some protozoans
UAA UAG	Stop	Glu	Some protozoans
UGA	Stop	Trp	Mitochondria,mycoplasma

ORFs

Open reading frames (ORFs) are suspected coding regions starting with ATG and end with TGA,TAA or TAG identified by computer.

When the ORF is known to encode a certain protein, it is usually referred as a coding region.

Overlapping genes

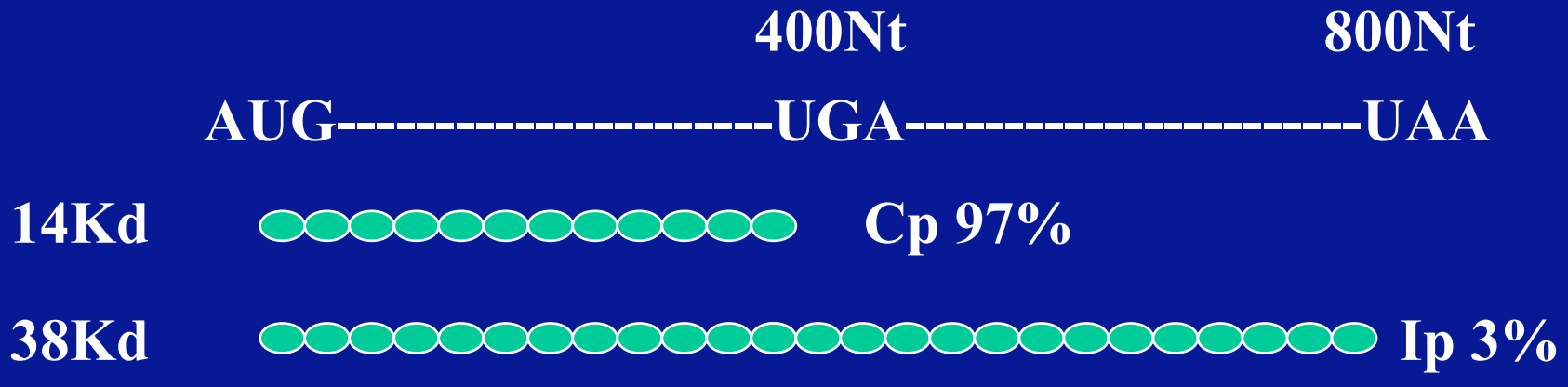
- Generally these occur where the genome size is small (viruses in most cases) and there is a need for greater information storage density.
- More than one start codons in a DNA sequence are used for translate different proteins.
- A way to maximize the coding capability of a given DNA sequence.

- 1973年Weiner和Weber发现大肠杆菌的一种RNA病毒中，有两个基因从同一起点开始翻译，一个在400bp处结束，生成较小的蛋白质，而在少数情况下（3%），翻译可以一直进行到800bp处碰到双重终止信号才结束，合成较大相对分子量的蛋白质。但是当时他们认为后者含量少，不予重视，没有进一步研究，就这样他们和重叠基因的发现失之交臂。

- 1977年Sanger在测定ΦX174全部核苷酸序列的时候发现

- 不同终止位点

(*Qβ RNA virus* 1973. A. Weiner)

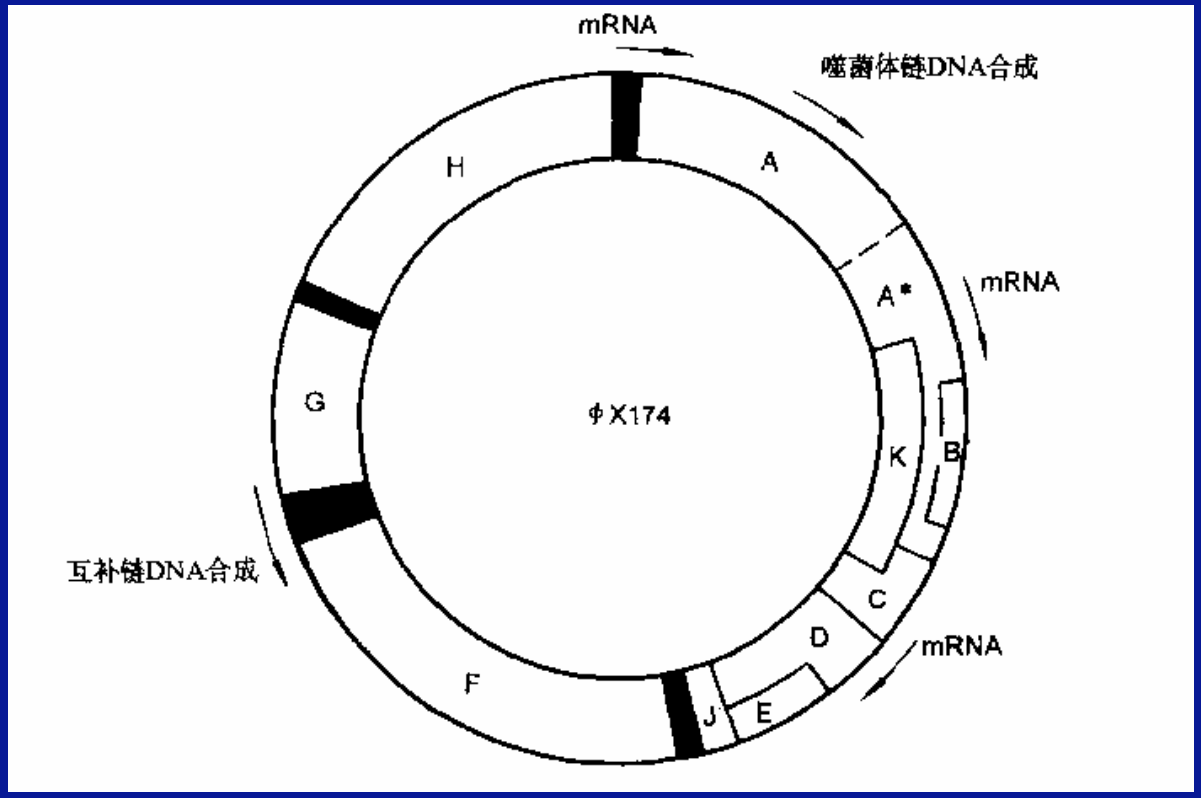


UGA, UAG　易被漏读，错读

UAA 能严格终止

- 不同的阅读框

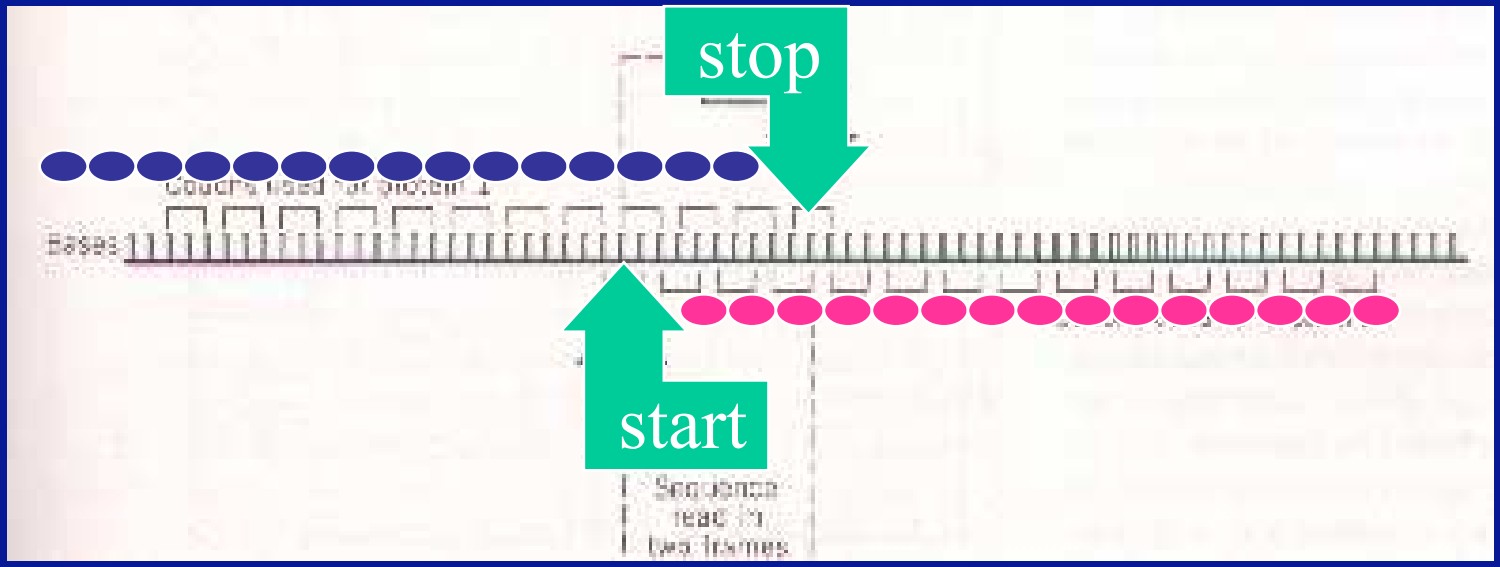
ϕX174 （F. Sanger, 1977）



A ---ATG-----//------AATGCC ----//---ATAACG---//--TAA----

B ATGCCN----NNATAA

不同的阅读框



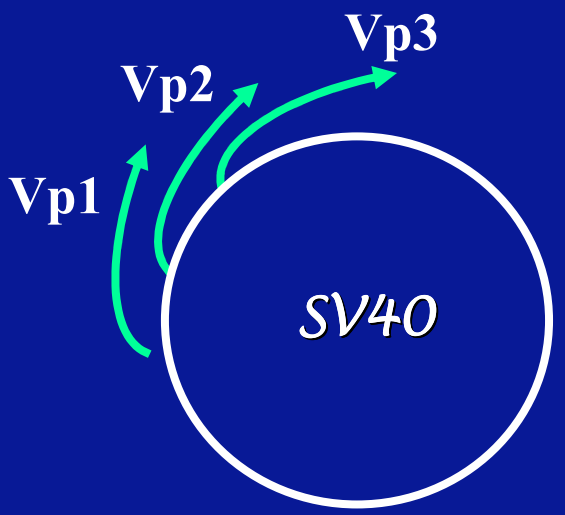
Stop

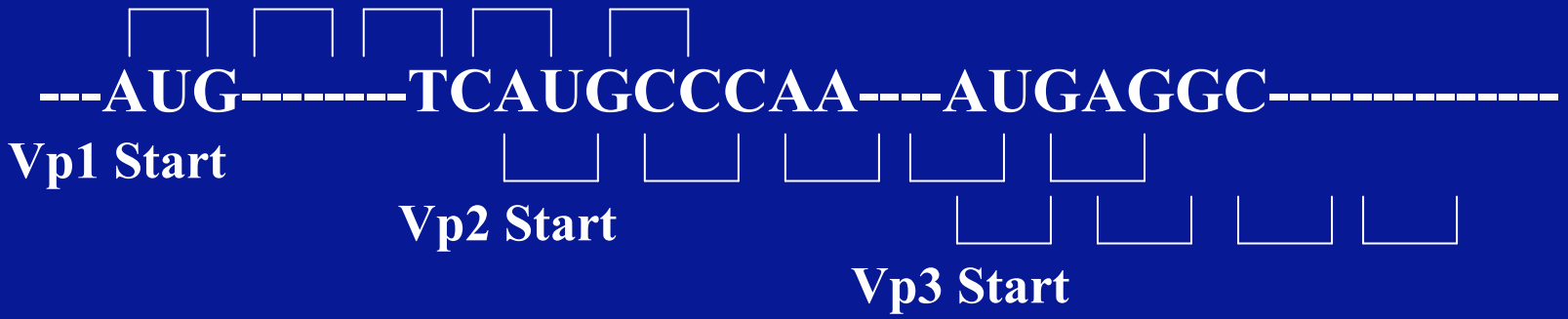
----------TCAUGCCCAAACUAGGC----------

Start

- 选择不同的起始和终止

(Simian Virus 40　SV40)





tRNA STRUCTURE AND FUNCTION

- tRNA primary structure
- tRNA secondary structure
- tRNA tertiary structure
- tRNA function

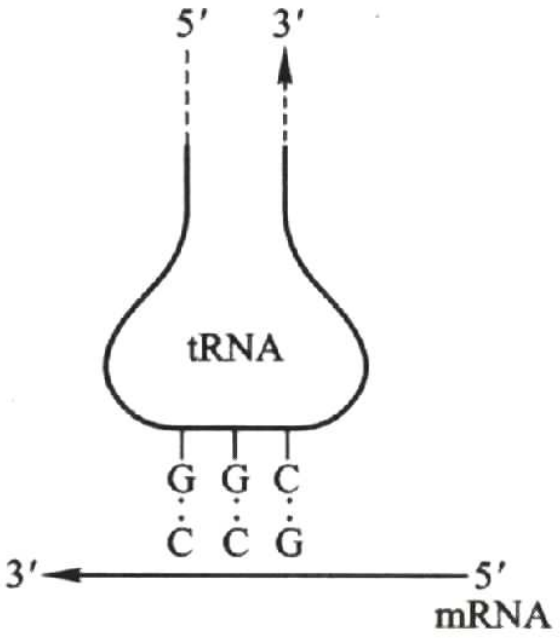


图 38－6　密码子与反密码子的识别

tRNA primary structure

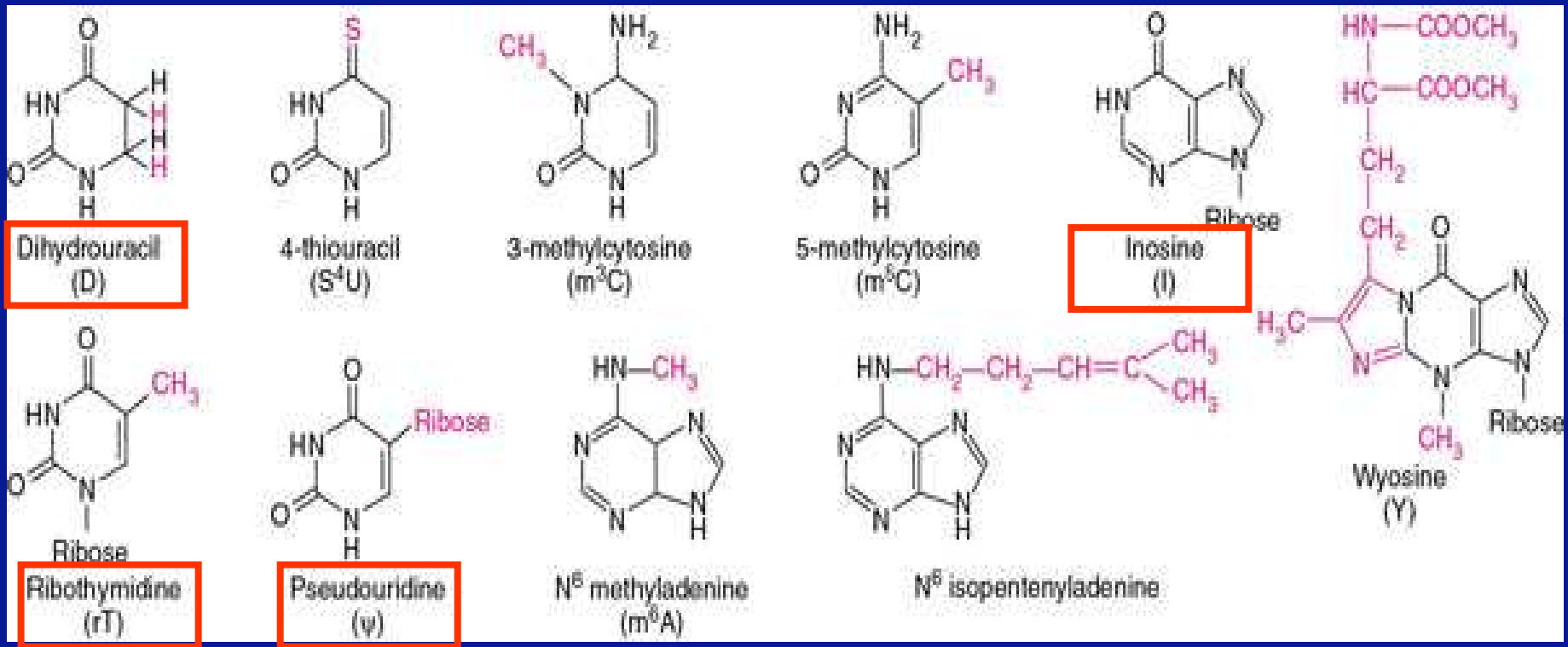
- **tRNA** are the adaptor molecules that deliver amino acids to the ribosome and decode the information in mRNA.

- **Linear length**:
  60-95 nt (commonly 76)

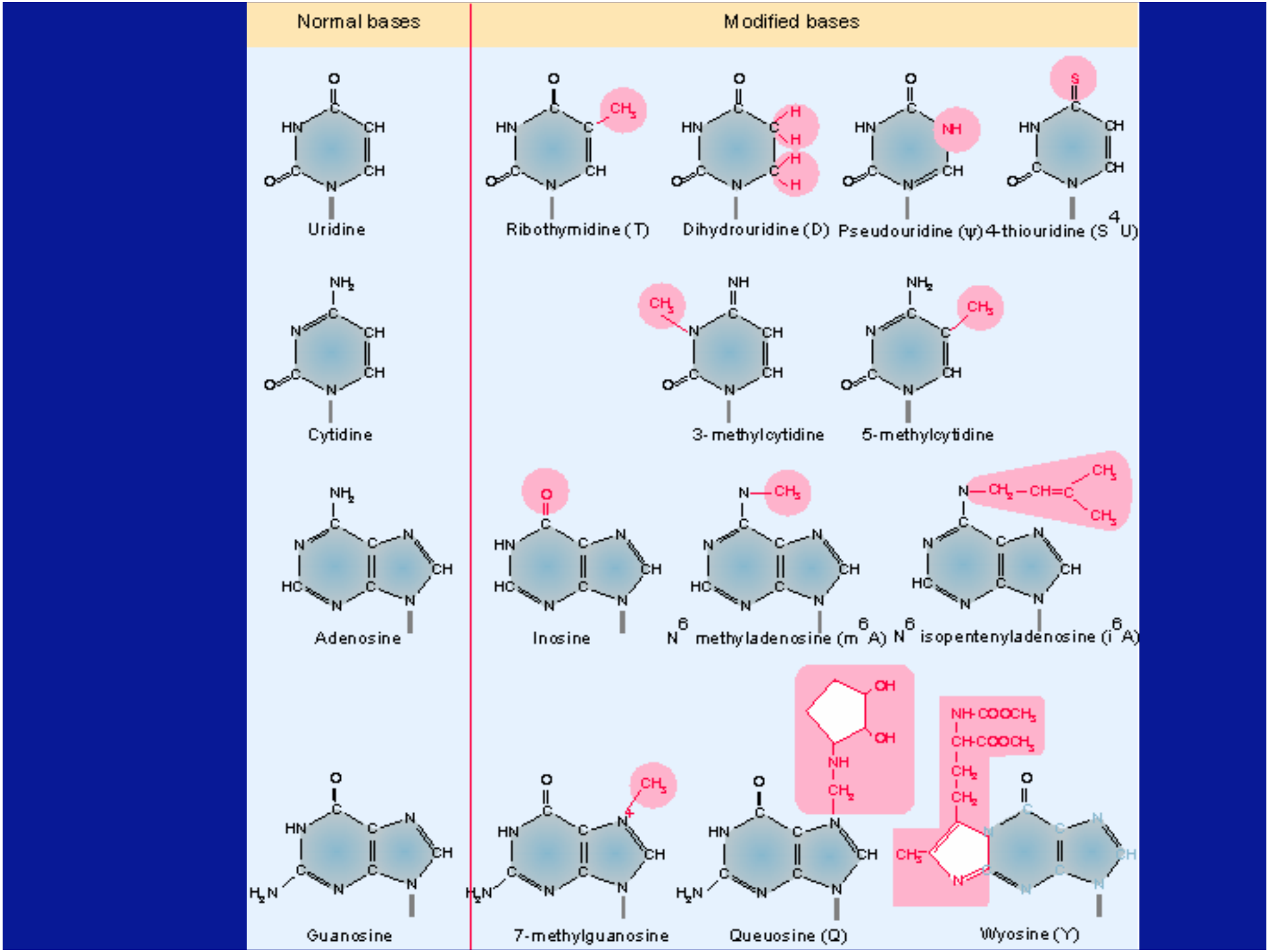
- **Residues:**
  15 invariant and 8 semi-invariant .The position of invariant and semi-variant nucleosides play a role in either the secondary and tertiary structure.

- **Modified bases:**

  Sometimes accounting for 20% of the total bases in any one tRNA molecule.Over 50 different types of them have been observed.

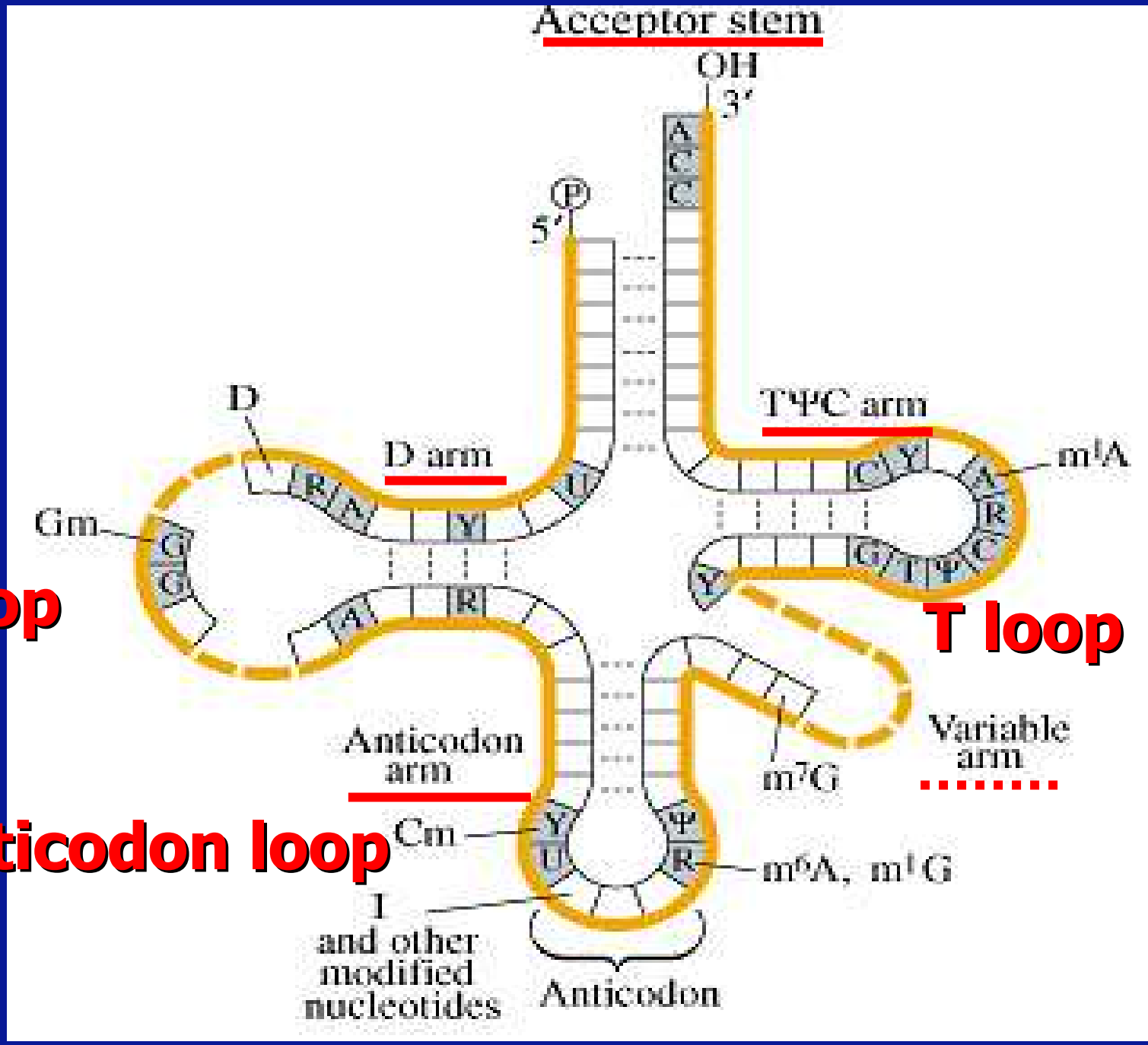


Modified nucleosides in tRNA


Normal bases
Modified bases
Uridine
Ribothymidine (T)
Dihydrouridine (D)
Pseudouridine (ψ)
4-thiouridine (S^4U)
Cytidine
3-methylcytidine
5-methylcytidine
Adenosine
Inosine
N^6 methyladenosine (m^6A)
N^6 isopentenyladenosine (i^6A)
Guanosine
7-methylguanosine
Queuosine (Q)
Wyosine (Y)

tRNA secondary structure

- The cloverleaf structure is a common secondary structural representation of tRNA molecules which shows the base paring of various regions to form four stems (arms) and three loops.



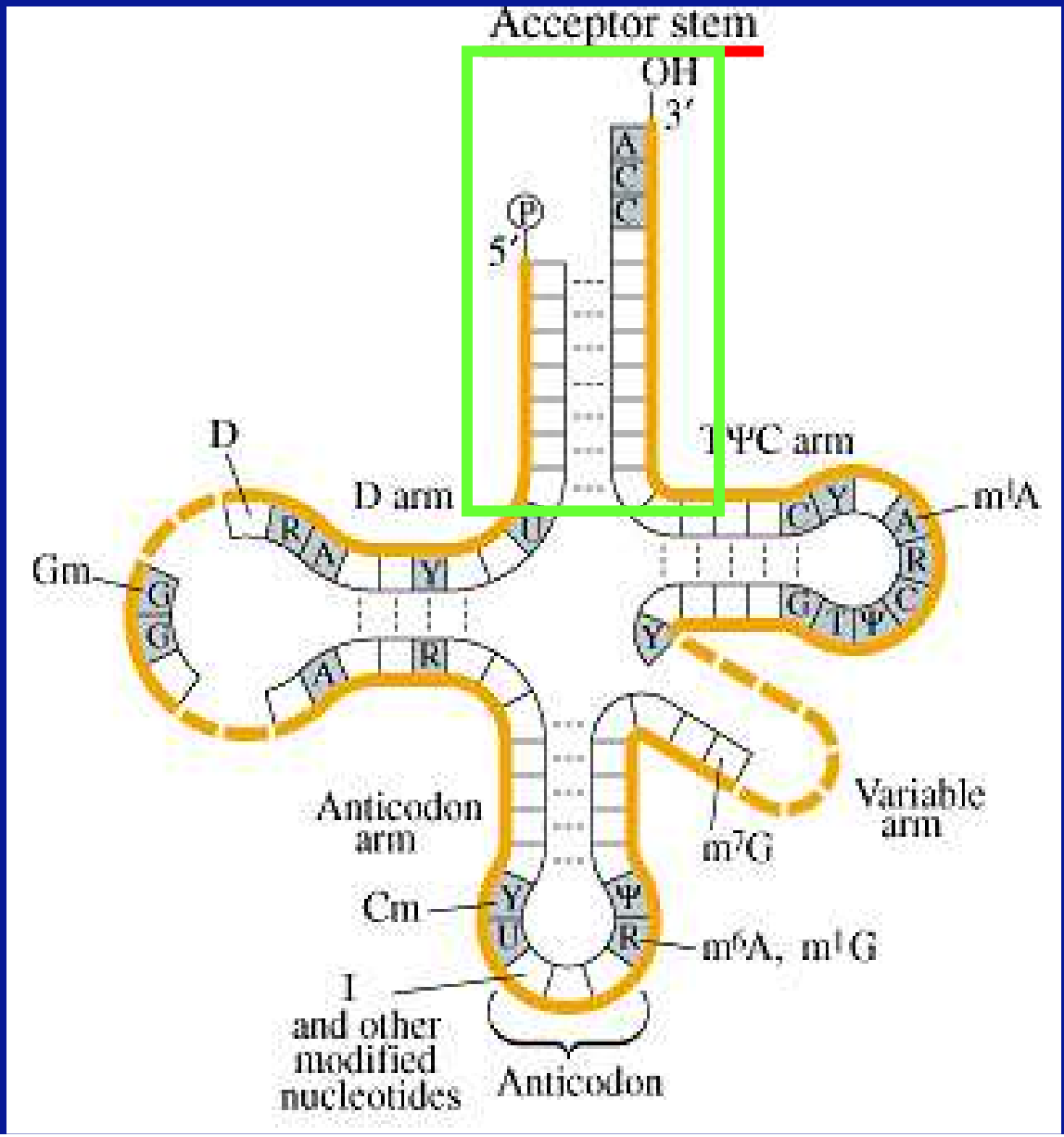
D loop

Anticodon loop

T loop

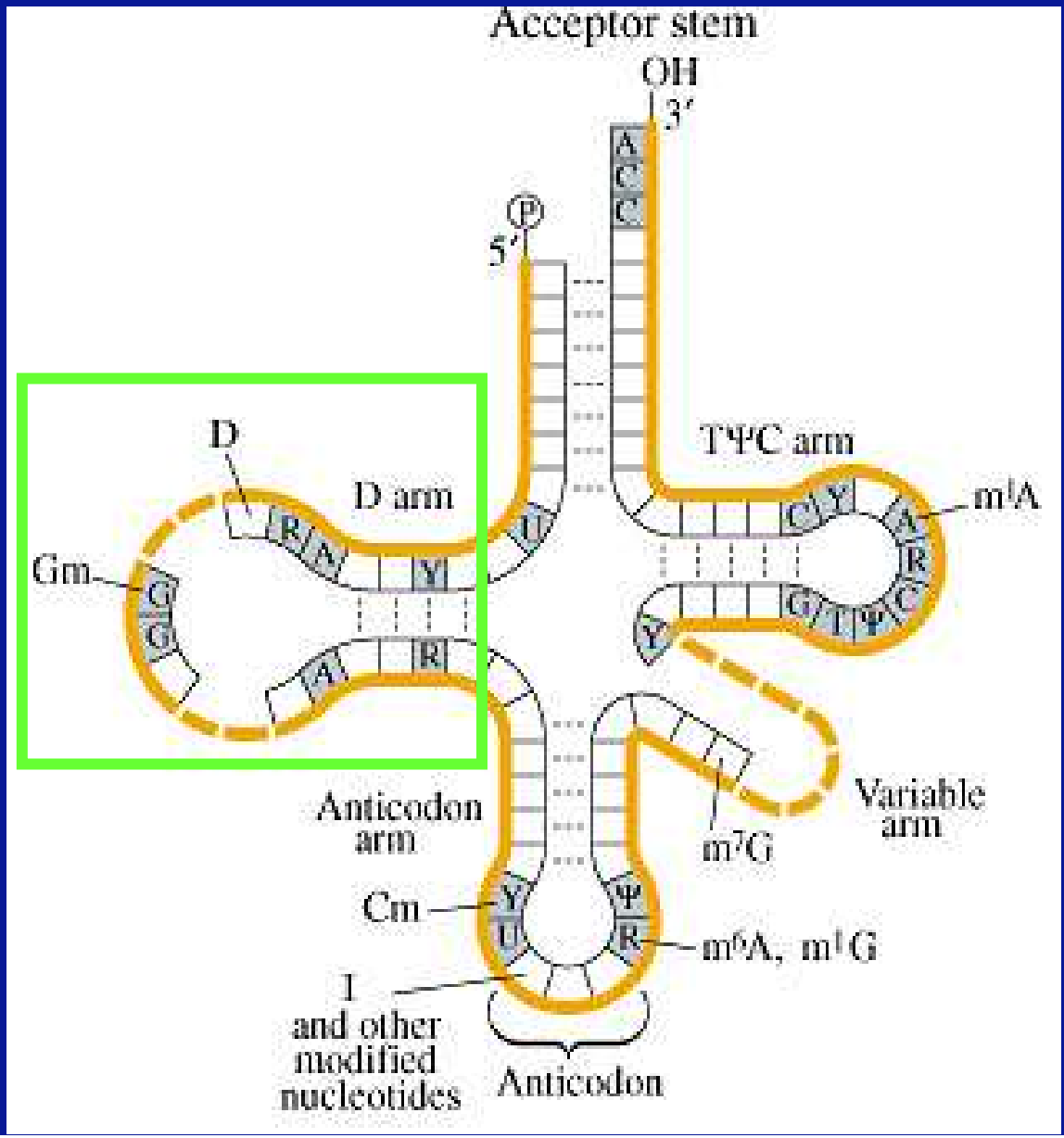
•Amino acid acceptor stem:

- The 5'-and 3'-end are largely base-paired to form the amino acid acceptor stem which has no loop.



- **D-arm and D-loop**

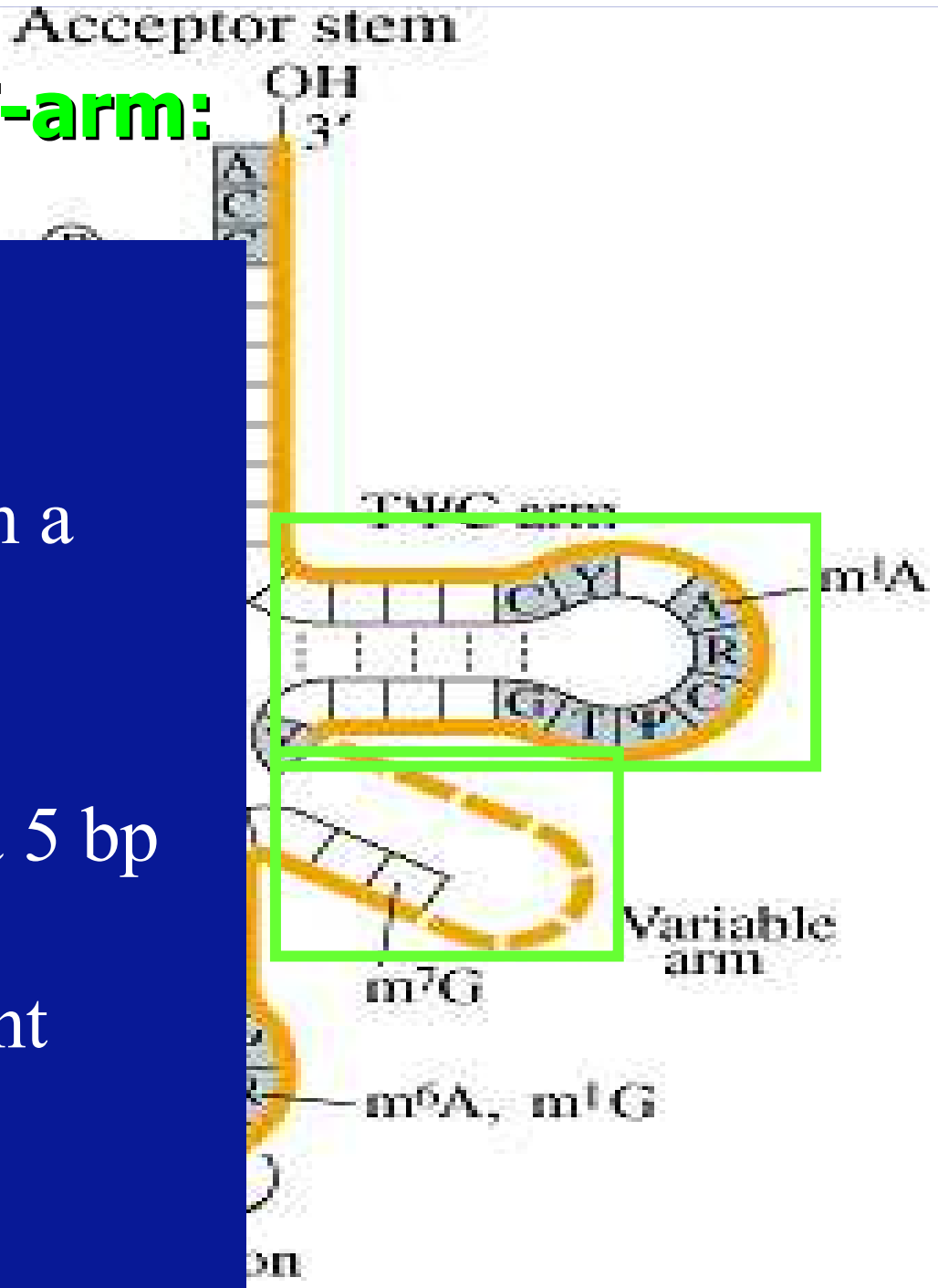
Composed of 3 or 4 bp stem and a loop called the D-loop (DHU-loop) usually containing the modified base dihydrouracil.



- **Variable arm and T-arm:**

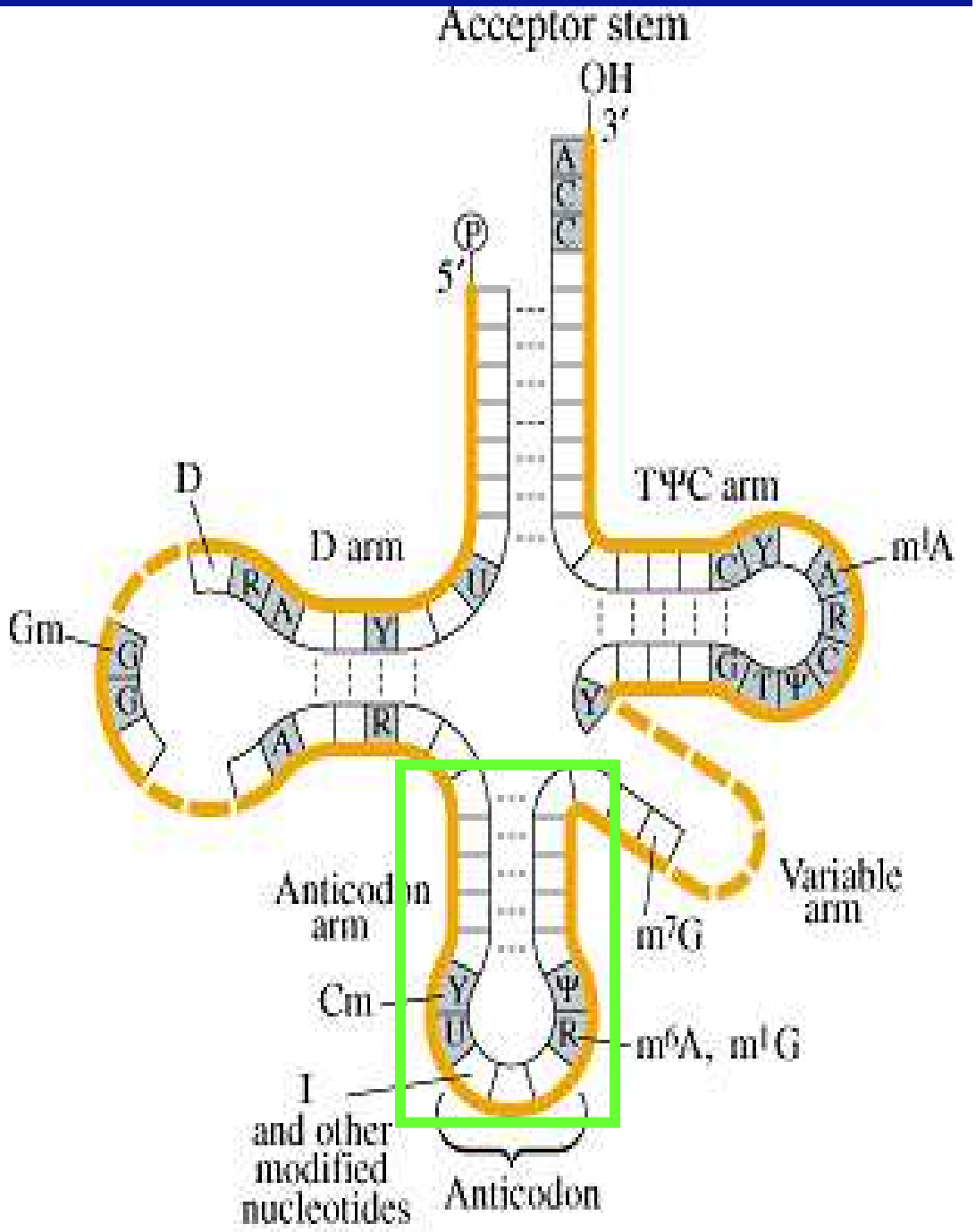
Variable arm: 3 to 21 residues and may form a stem of up to 7 bp.

T-arm is composed of a 5 bp stem ending in a loop containing the invariant residues GTΨC.



Anticodon loop:

Consisting of a 5 bp stem and a 7 residues loop in which there are three adjacent nucleosides called the anticodon which are complementary to the codon sequence (a triplet in the mRNA) that the tRNA recognize.



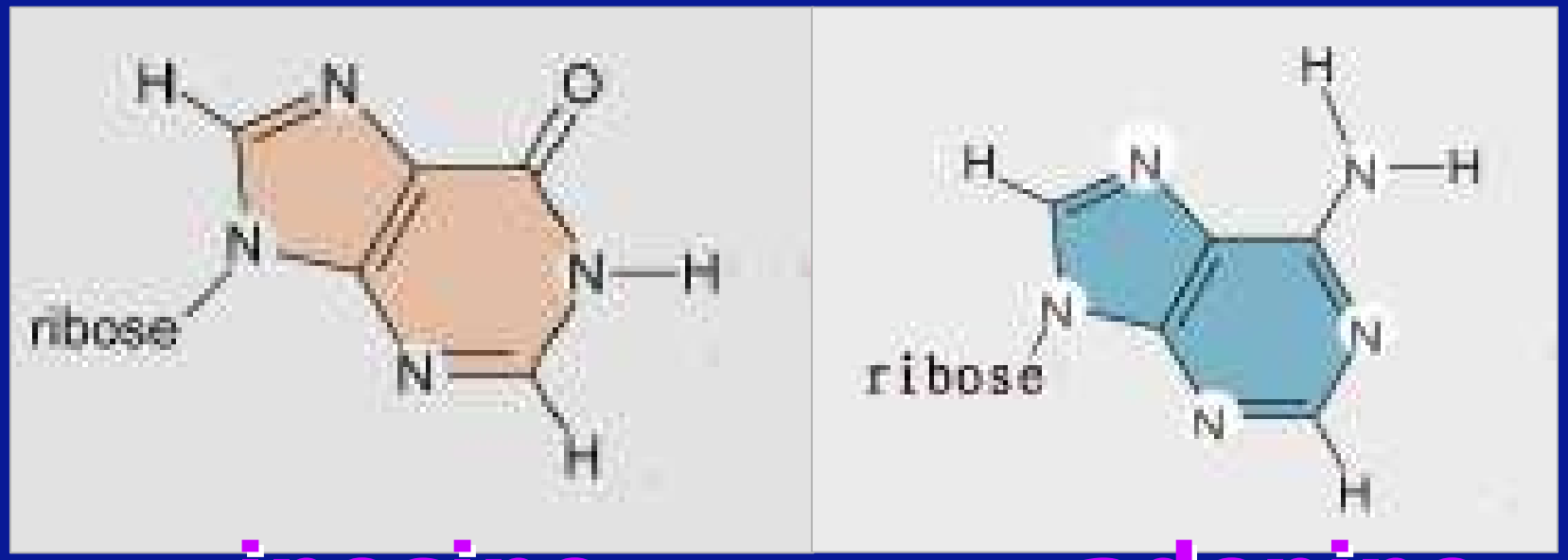
Wobble in the Anticodon

Question: Is there a specific tRNA for every codon? (If it was true, at least 61 different tRNAs would exist.)

The answer is NO

- **Some tRNA could recognize several different codons**
- **Inosine is present in the anticodon loop as a fifth base**

Inosine



inosine adenine

Inosine arises through enzymatic modification of adenine

Wobble Concept

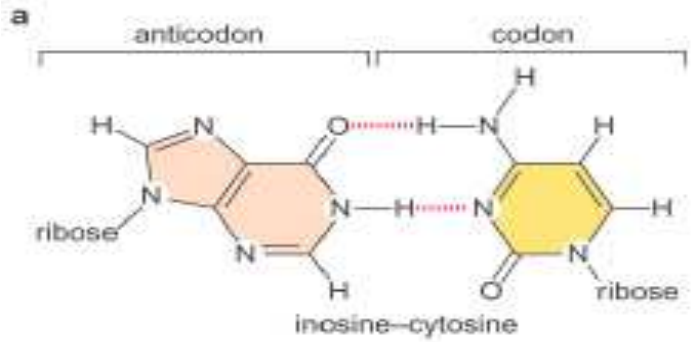
In 1966, Francis Crick devised the wobble concept. It states that the base at the 5′ end of the anticodon is not as spatially confined as the other two, allowing it to form hydrogen bonds with more than one bases located at the 3′ end of a codon.

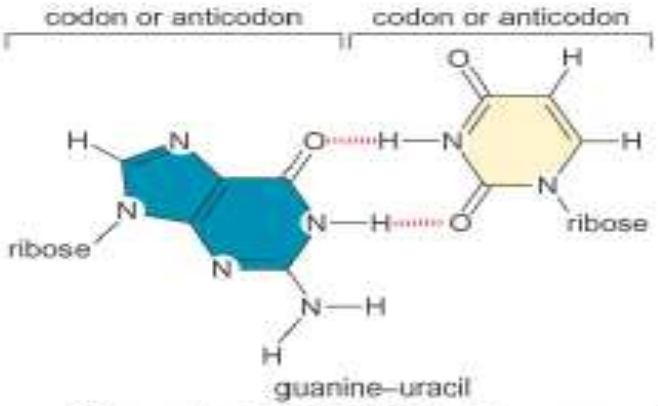
Pairing Combinations with the Wobble Concept

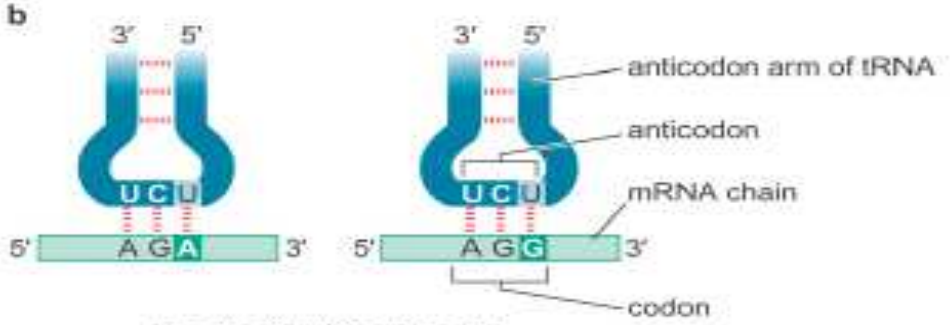
Base in 5' Anticodon	Base in 3' Codon
G	U or C
C	G
A	U
U	A or G
I	A, U, or C

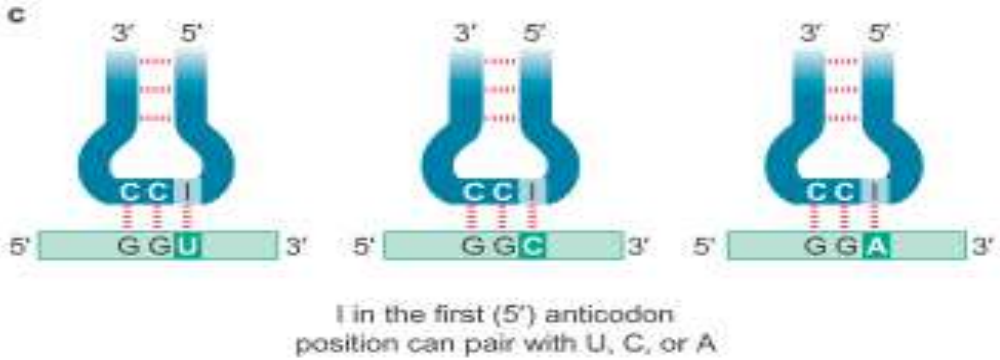
The Wobble Rules

- The pairings permitted are those give ribose-ribose distances close to that of the standard A:U or G:C base pairs.
- The ribose-ribose distances:
  - Purine-purine: too long
  - Pyrimidine-pyrimidine: too short









The ribose-ribose distances for the wobble pairs are close to those of A:U or G:C base pairs

Wobble base pairing

tRNA tertiary structure

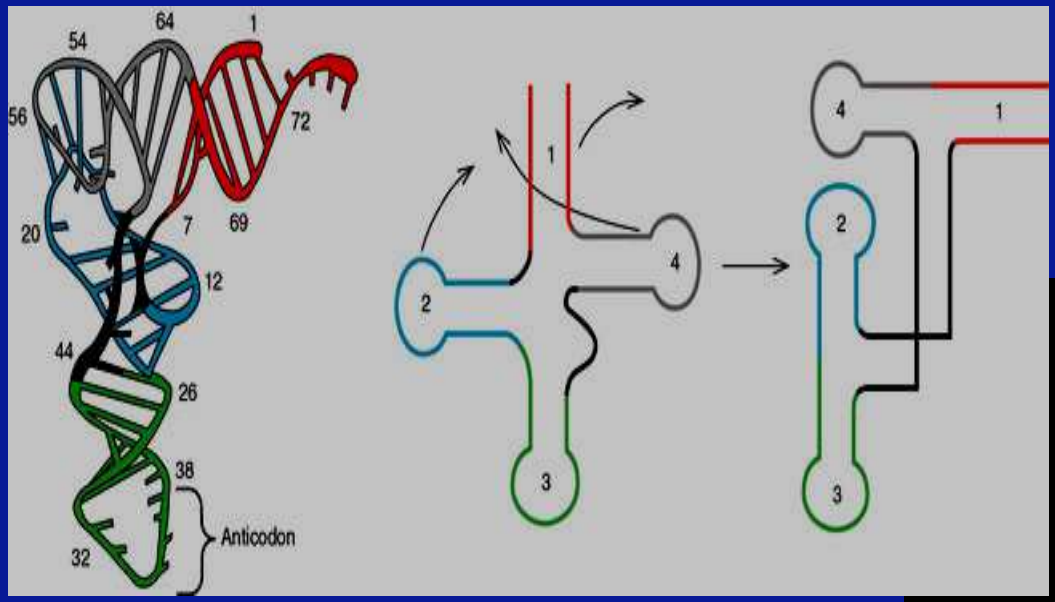
- **Formation:**

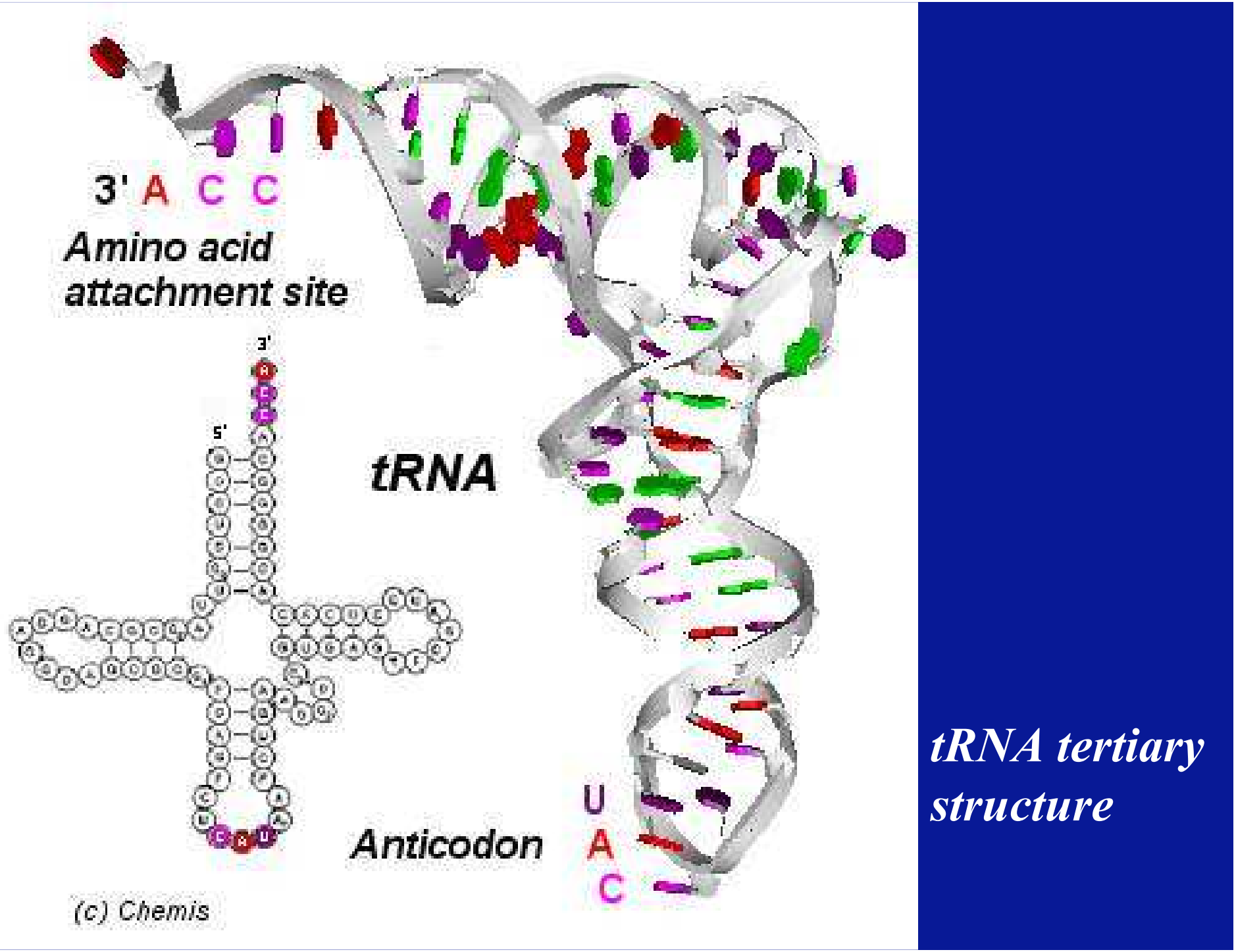
  9 hydrogen bones (tertiary hydrogen bones).

- **Hydrogen bonds:**

  Base pairing between residues in the D- and T-arms fold the tRNA molecule over into an L-shape, with the anticodon at one end and the amino acid acceptor site at the other. It is strengthened by base stacking interactions.

tRNA tertiary structure



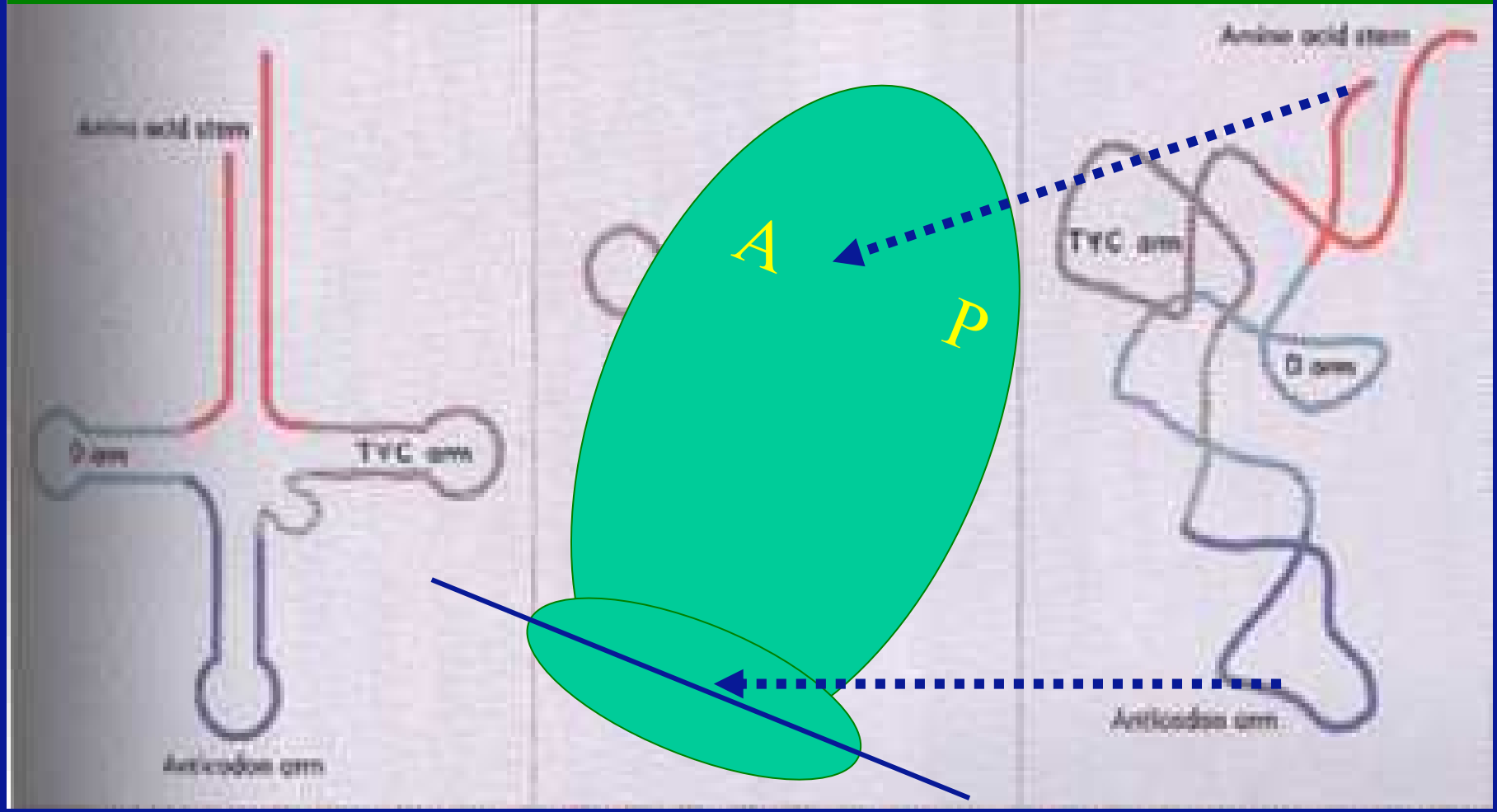


tRNA tertiary structure

“L”结构域的功能

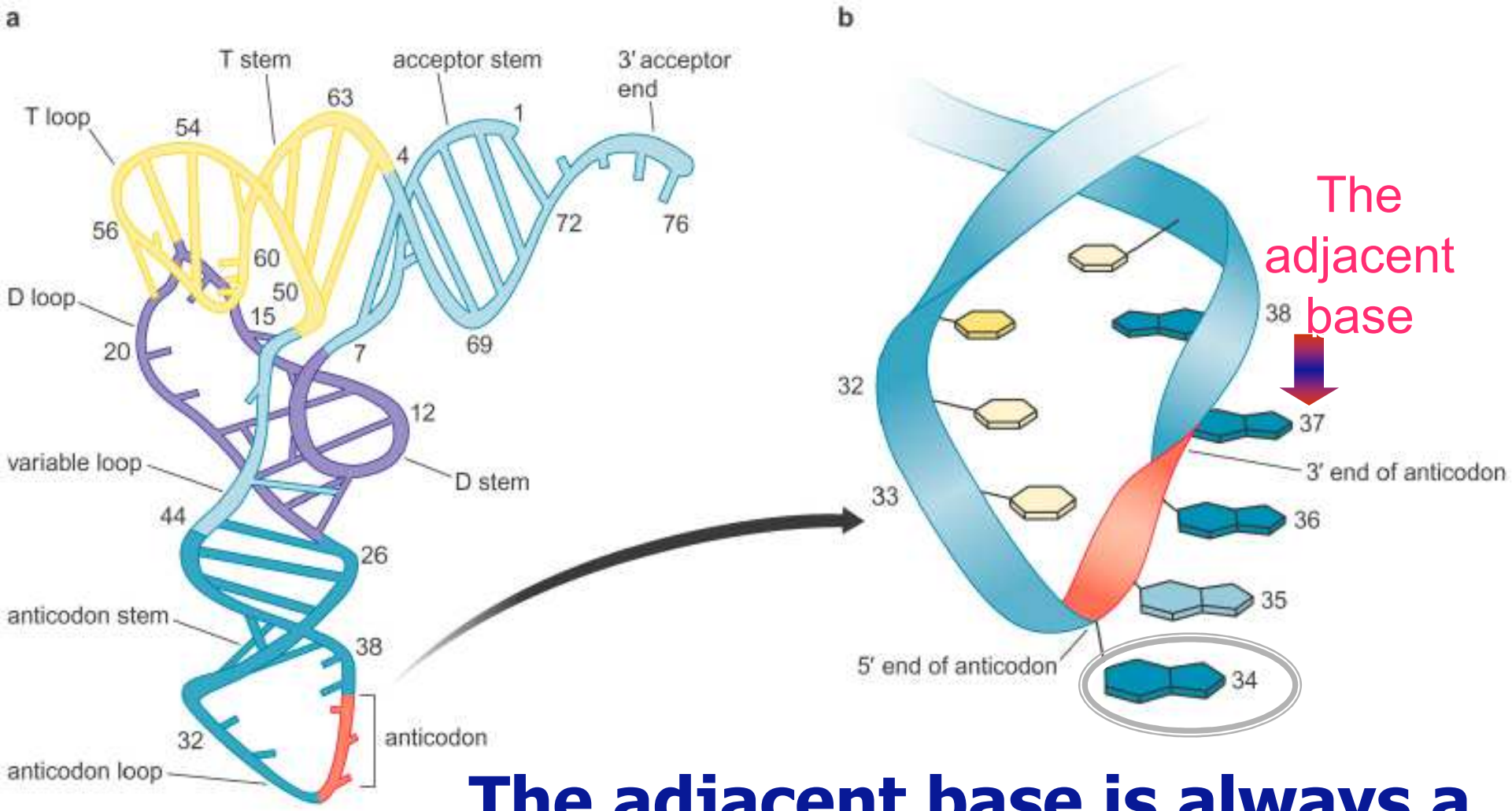
---aa accept arm 位于“L”的一端，契合于核糖体的P位点和A位点，以利肽键的形成

---anti-codon arm 位于“L”另一端，与结合在核糖体小亚基上的codon of mRNA配对



Why wobble is allowed at the 5′ anticodon

- The 3-D structure of tRNA shows that the stacking interactions between the flat surfaces of the 3 anticodon bases + 2 followed bases position the first (5′) anticodon base at the end of the stack, thus less restricted in its movements.
- The 3′ base appears in the middle of the stack, resulting in the restriction of its movements.



The adjacent base is always a bulky modified purine residue.

Structure of yeast tRNA(Phe)

--- TΨC loop & DHU loop

位于“L”两臂的交界处，
利于“L”结构的稳定

---“L”结构中碱基堆积力大

使其拓扑结构趋于稳定

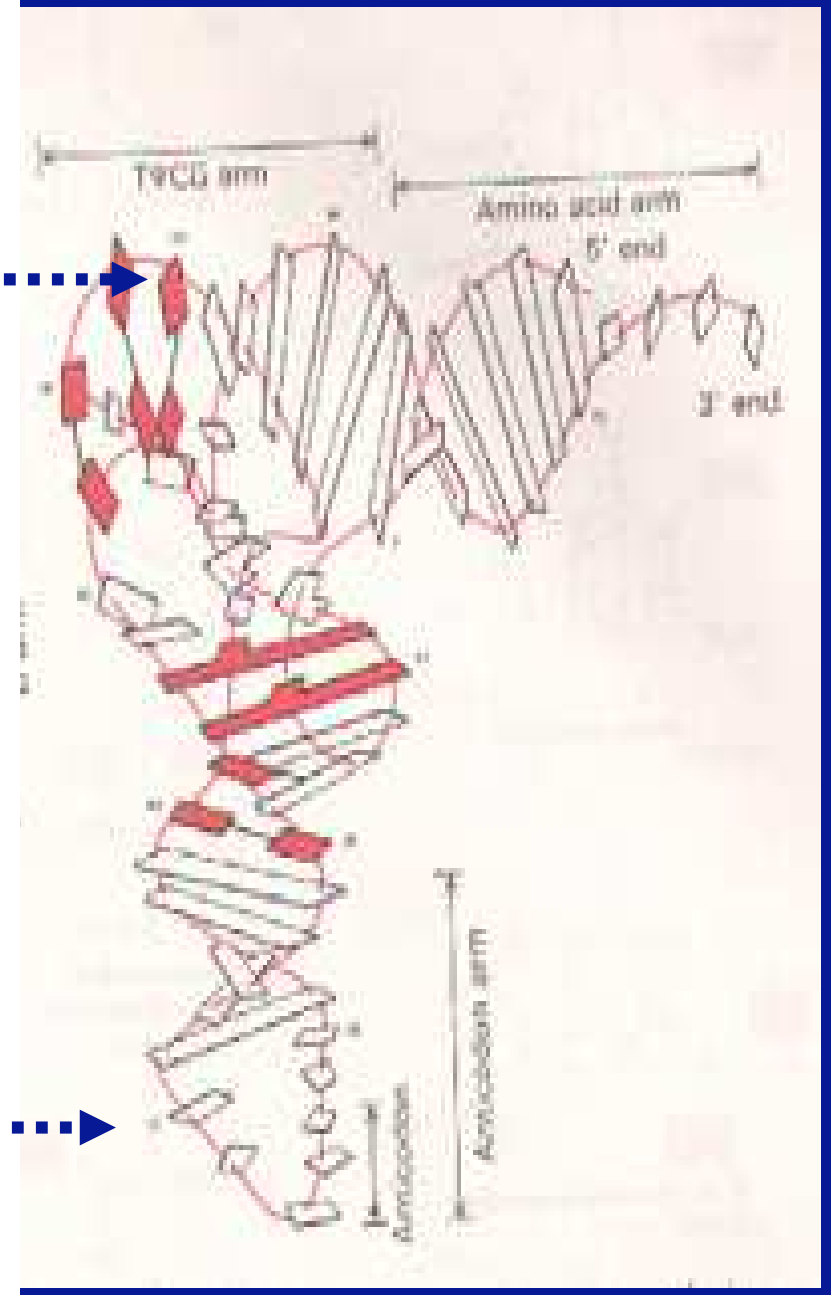
wobble base

位于“L”结构末端

堆积力小

自由度大

使碱基配对摇摆



tRNA function

- When charged by attachment of a specific amino acid to their 3’-end to become aminoacyl-tRNAs, tRNA molecules act as adaptor molecules in protein synthesis.

Aminoacyl-tRNA synthetases

catalyze amino acid-tRNA joining reaction which is extremely specific.

- ***Nomenclature of tRNA-synthetases and charged tRNAs***

Amino acid: serine

Cognate tRNA: $tRNA^{ser}$

Cognate aminoacyl-tRNA synthetase:

seryl-tRNA synthetase

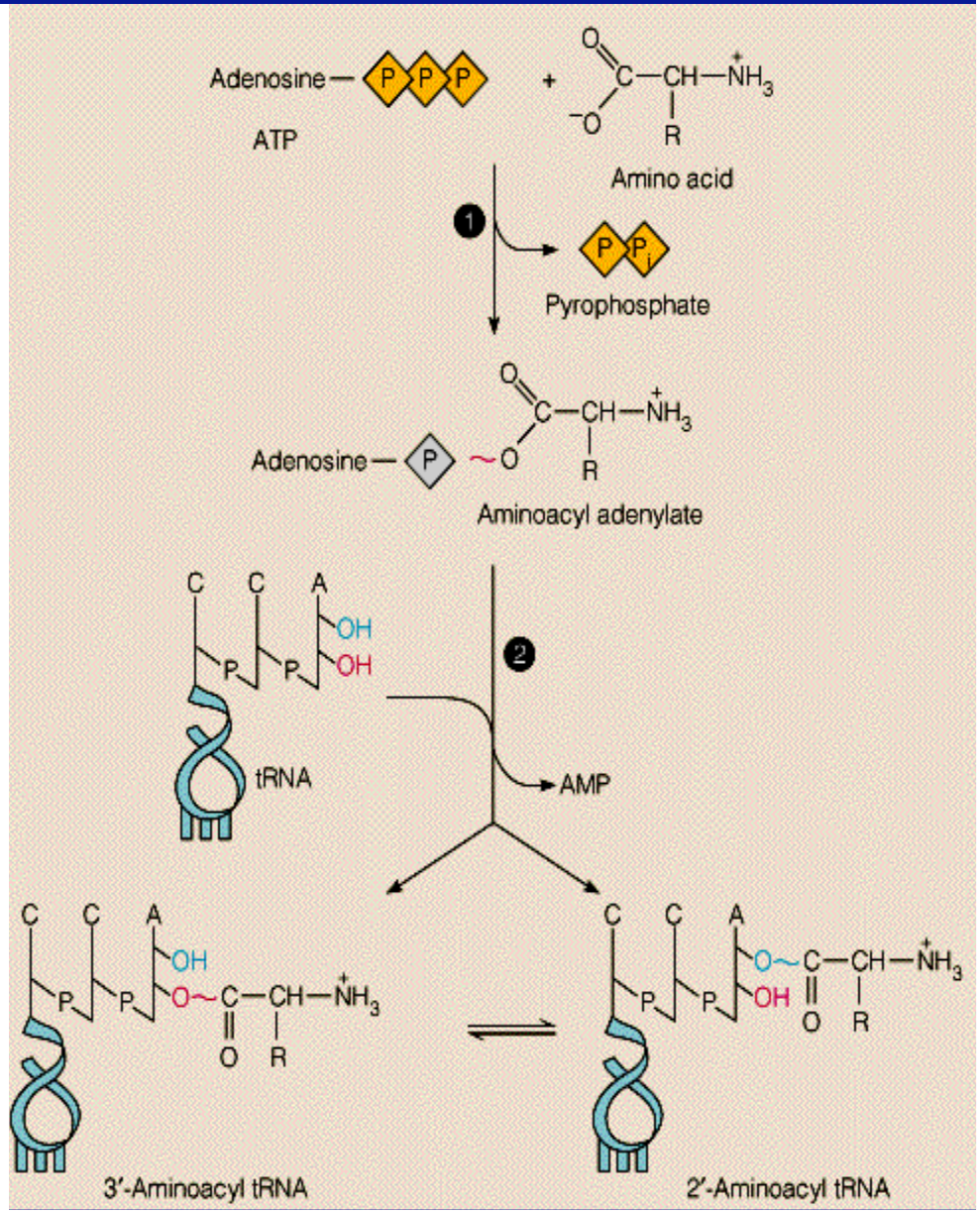
Aminoacyl-tRNA: seryl-$tRNA^{ser}$

Aminoacylation of tRNAs

- **Reaction step:**

First, the aminoacyl-tRNA synthetase attaches AMP to the -COOH group of the amino acid utilizing ATP to create anaminoacyl adenylate intermediate.

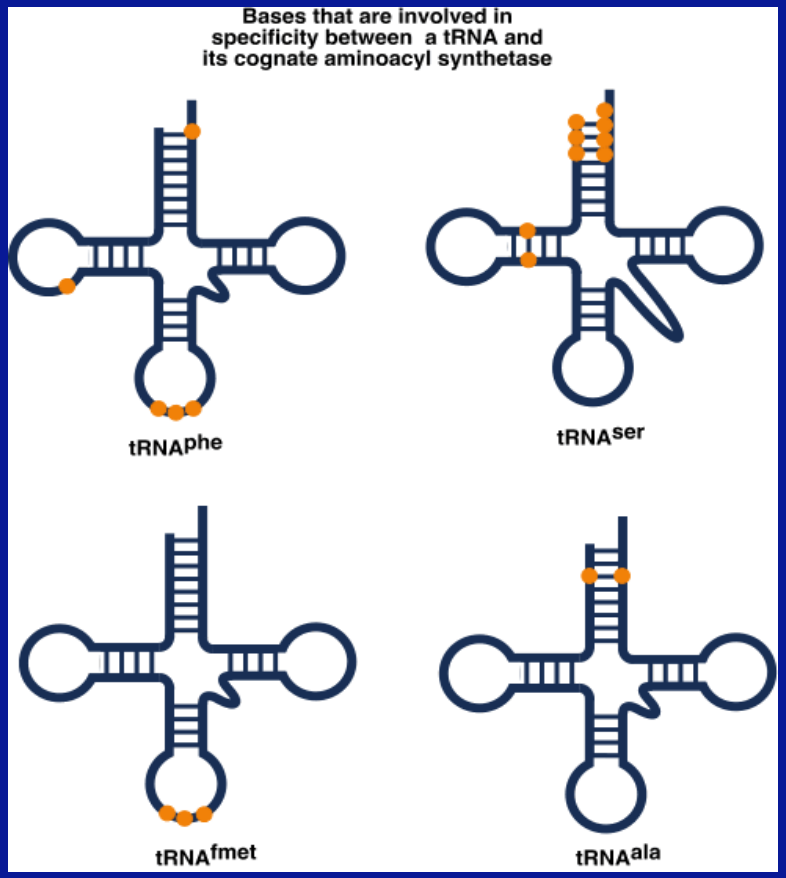
Then, the appropriate tRNA displaces the AMP.



Aminoacyl-tRNA synthetases

- The synthetase enzymes are either monomers, dimers or one of two types of tetramer.They contact their cognate tRNA by the inside of its L-shape and use certain parts of the tRNA, called identity elements, to distinguish these similar molecules from one another.

Identity elements in various tRNA molecules



- **Identity element:**

  They are particular parts of the tRNA molecules. These are not always the anticodon sequence,but base pair in the acceptor stem. If these are swapped between tRNAs then the synthetases enzymes can be tricked into adding the amino acid to the wrong tRNA

1）氨酰－tRNA合成酶帮助使氨基酸结合到特定的tRNA上，氨酰－tRNA合成酶参与氨基酸与tRNA结合的二步反应。

a、氨基酸＋ATP $\rightleftharpoons$ 氨酰－AMP＋Ppi （活化）

b、氨酰－AMP＋tRNA $\rightleftharpoons$ 氨酰－tRNA＋AMP

氨酰－tRNA合成酶催化的反应是可逆的，其作用在于：

a、氨基酸与tRNA分子的结合使得氨基酸本身被活化，利于下一步肽健形成的反应。

b、tRNA可以携带氨基酸到mRNA的特定部位，使氨基酸能够被掺入到多肽链的合适位置。(反应的专一性）

2）每一个氨酰－tRNA合成酶可以识别一个特定的氨基酸和与此氨基酸对应的tRNA的特定部位。

酶与底物的选择性主要由氢健来决定的。

例子：酪氨酰－tRNA合成酶与反应中间物酪氨酰－腺苷复合物的晶体结构解析模型。反应中间物结合在酶分子的一个深沟里，二者之间形成11个氢键。6个氢键涉及AMP部分，5个涉及酪氨酰部分。

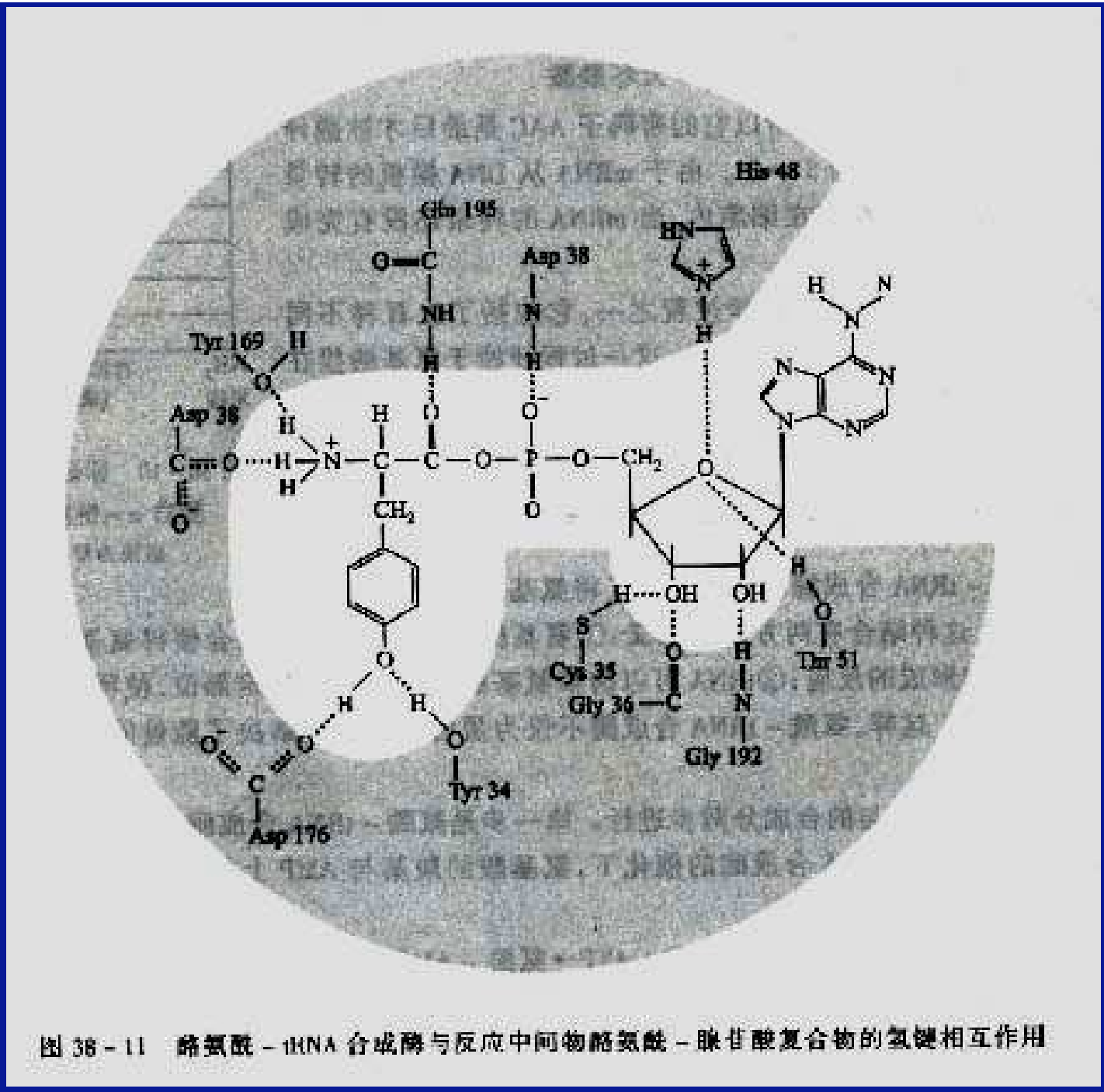
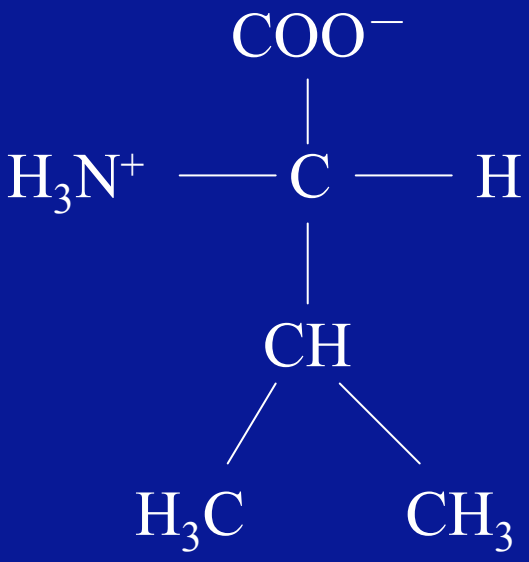


图 38－11　络氨酰－tRNA 合成酶与反应中间物酪氨酰－腺苷酸复合物的氢键相互作用

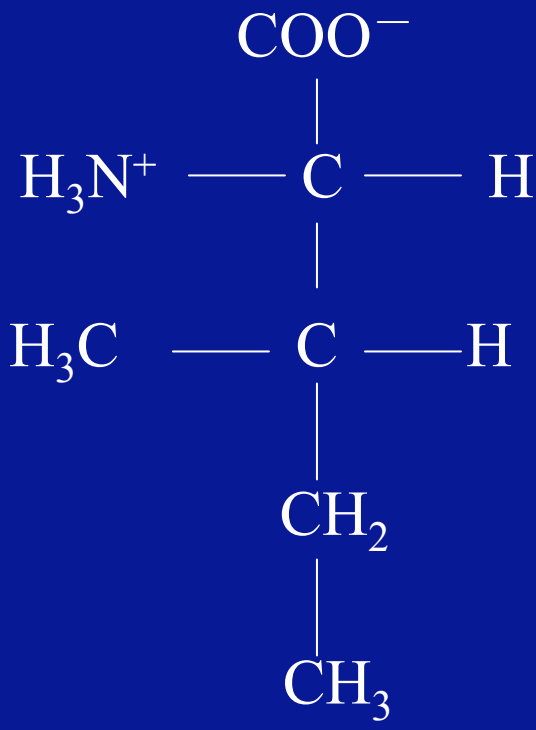
3）氨酰－tRNA合成酶能够纠正酰化的错误

氨酰－tRNA合成有校正某些错误的功能，可以水解非正确组合的氨基酸和tRNA之间形成的共价联系。通过氨酰化部位以及校正部位的共同作用，可使翻译过程的错误频率小于万分之一。



缬氨酸

（Val. V）



异亮氨酸

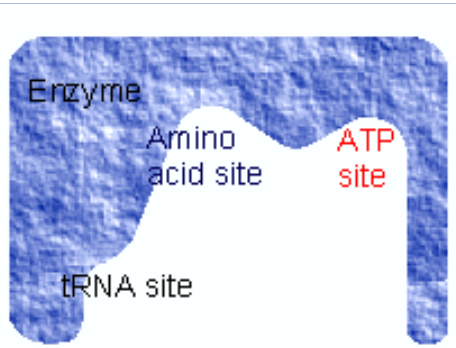
（Ile. I）

异亮氨酰tRNA合成酶

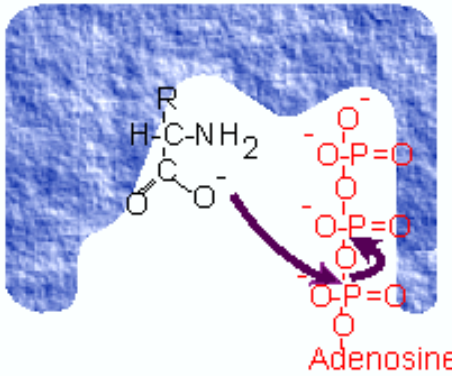
缬氨酰－$tRNA^{ILe}$ $+H_2O$ → 缬氨酸＋$tRNA^{ILe}$

表 3-4　活化 $tRNA^{Ile}$ 合成酶的准确性受双重控制

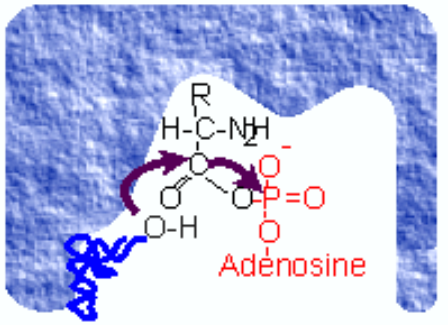
阶　　段	错　误　率
缬氨酸活化	1/225
缬氨酸-$tRNA^{Ile}$的释放	1/270
总误差率	1/225×1/270＝1/60 750



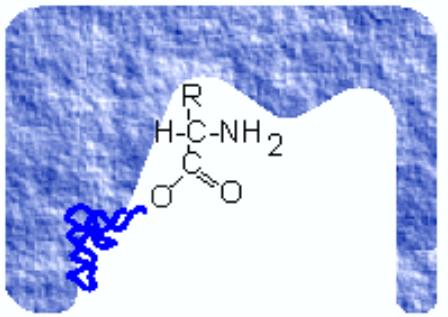
Synthetase has 3 binding sites



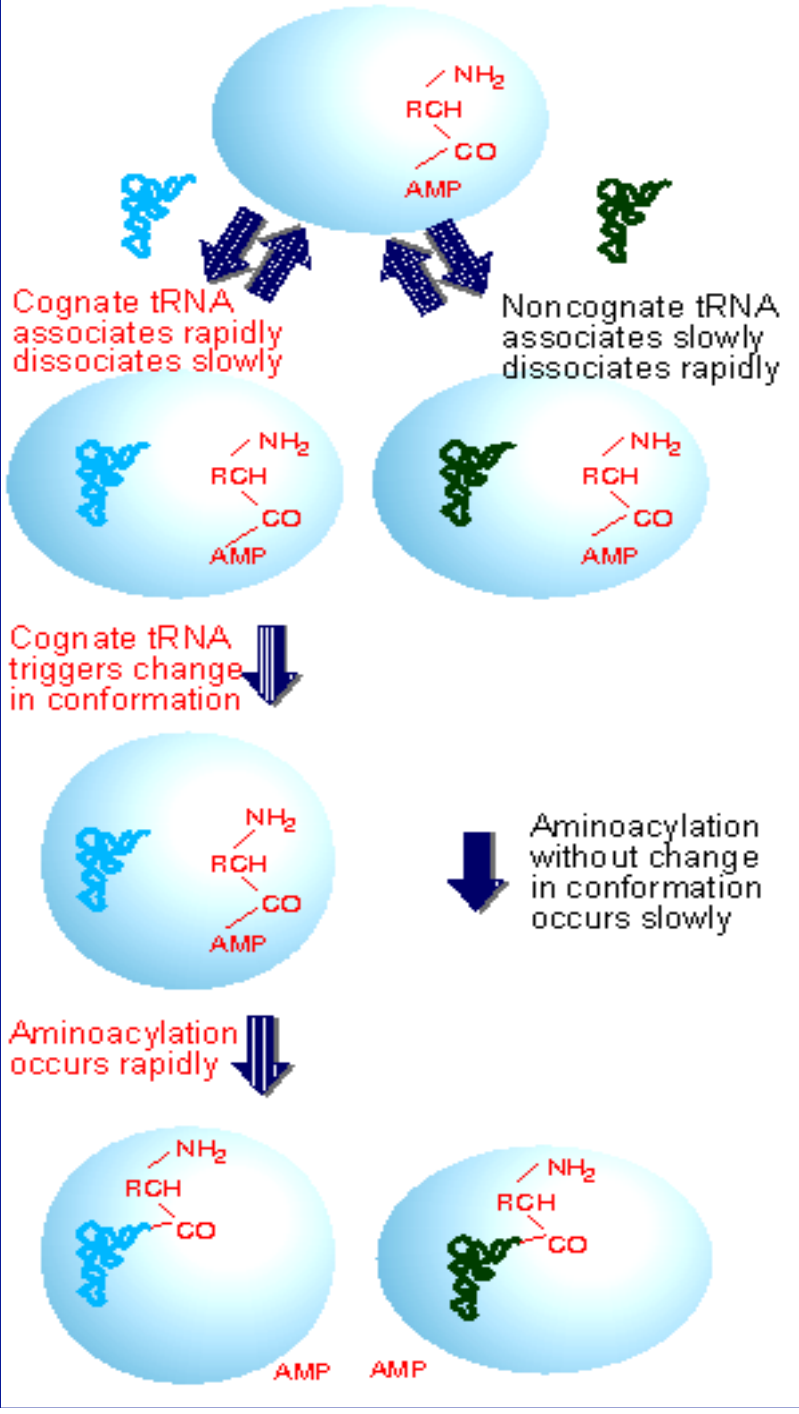
Amino acid and ATP form aminoacyl-AMP



Transfer RNA binds



tRNA is charged with amino acid



Proofreading

- Proofreading occurs at step 2 when a synthetase carries out step 1 of the aminoacylation reaction with the wrong, but chemically similar, amino acid.
- Synthetase will not attach the aminoacyl adenylate to the cognate tRNA, but hydrolyze the aminoacyl adenylate instead.

tRNA的识别只与反密码子有关，而与**tRNA**上携带的氨基酸无关。

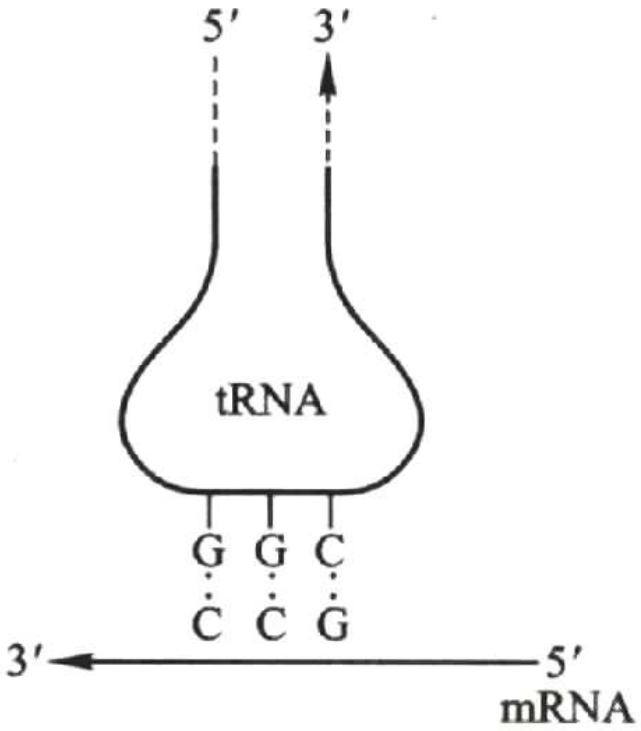


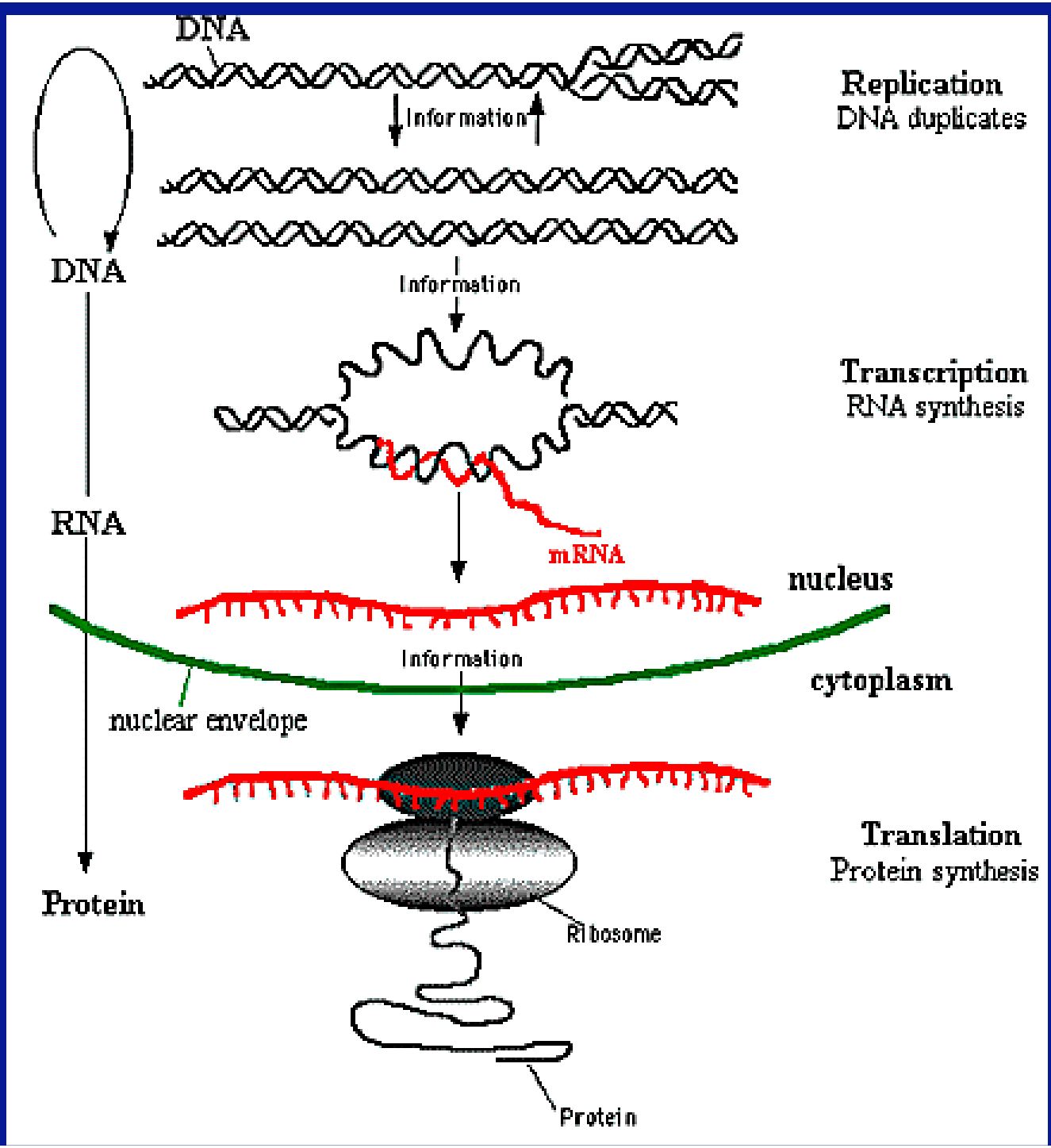
图 38－6　密码子与反密码子的识别

- Chapeville 和 Lipmann的试验：

将放射性同位素标记的半胱氨酸在Cys-tRNA合成酶催化下与$tRNA^{cys}$形成Cys-$tRNA^{cys}$。然后用活性镍作催化剂，使半胱氨酸转变成丙氨酸，形成Ala-$tRNA^{cys}$，然后将它放到网织红细胞无细胞体系中进行蛋白质合成，结果发现，丙氨酸插入到原半胱氨酸的位置中了。

第6章
蛋白质的生物合成

(K/M/O/P/Q)


DNA
Information
Replication
DNA duplicates
DNA
Information
Transcription
RNA synthesis
RNA
mRNA
nucleus
Information
cytoplasm
nuclear envelope
Translation
Protein synthesis
Protein
Ribosome
Protein

Q Protein synthesis

Q1. Aspects of protein synthesis

Q2. Mechanism of protein synthesis

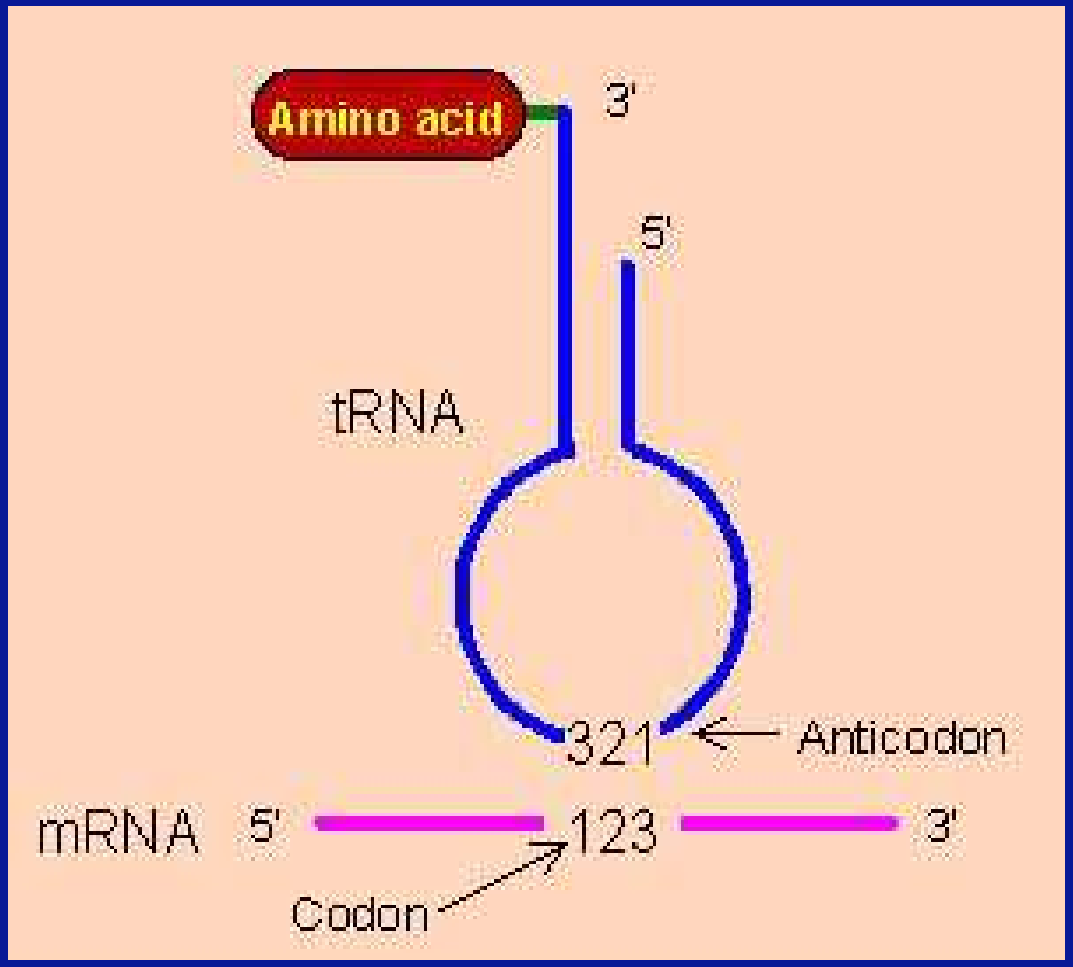
Q3. Differences between prokaryotes and eukaryotes

Q4. Translational control and post-translational events

Aspects of protein synthesis

- Codon-anticodon interaction
- Wobble
- Ribosome binding site
- Polysomes
- Initiators tRNA

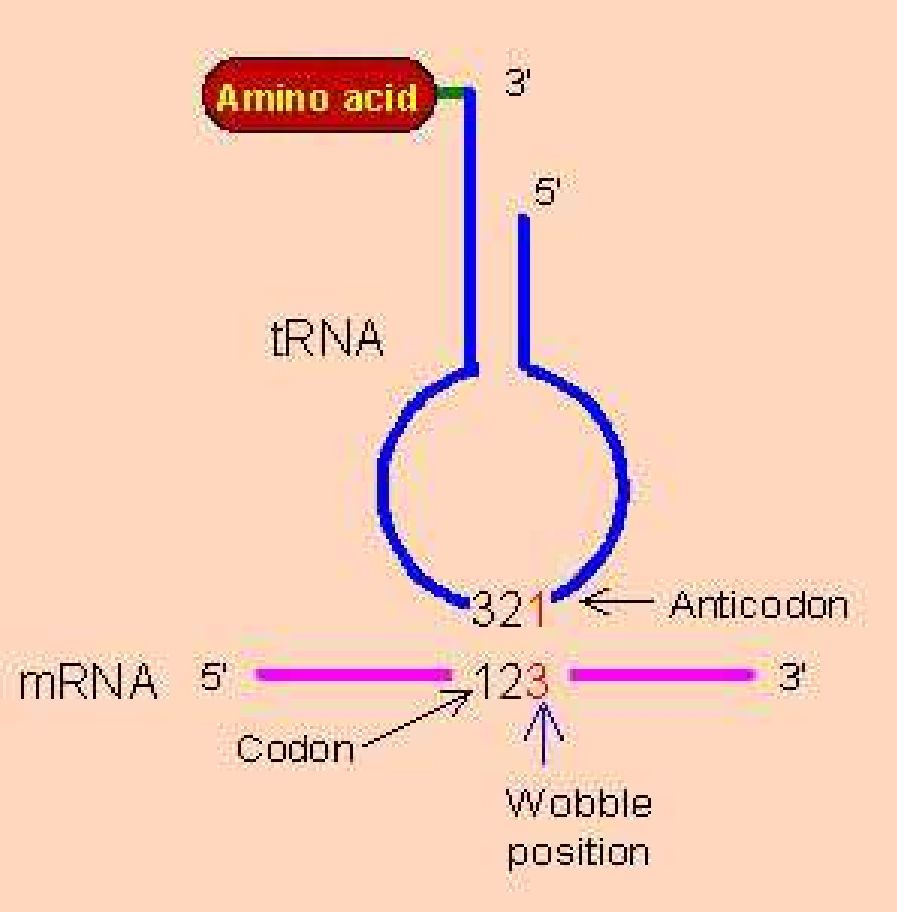
Codon-anticodon interaction



In the cleft of the ribosome , an anti-parallel formation of three base pairs occurs between the codon on the mRNA and the anticodon on the tRNA.

WOBBLE

To explain the redundancy of the genetic code. 18 aa are encoded by more than one triplet codons which usually differ at 5'-anticodin base



5'-anticodon base is able to undergo more movement than the other two bases and can thus form non-standard base pairs as long as the distances between the ribose units are close to normal.

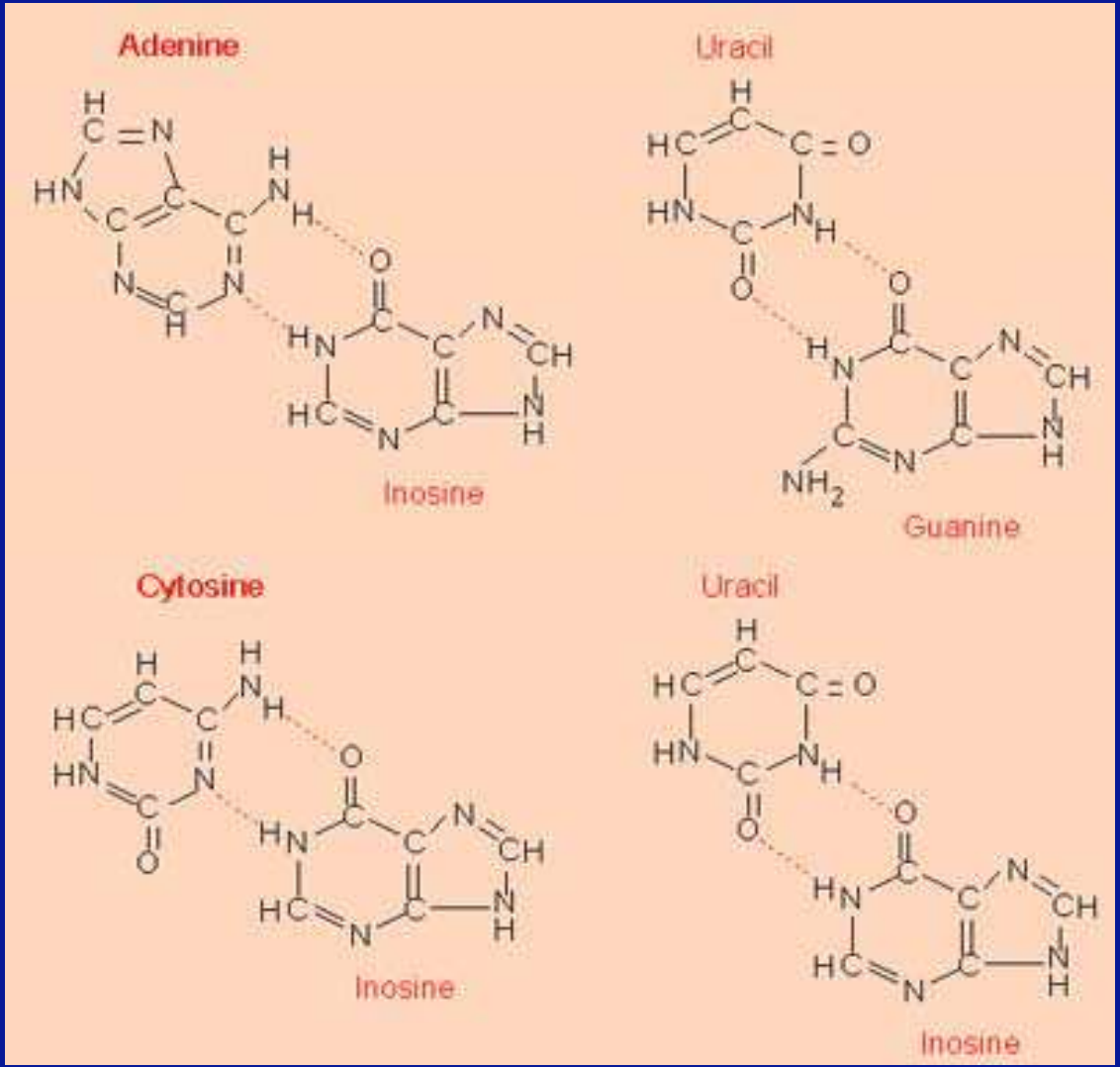
All possible base pairings at the wobble position

No purine-purine or pyrimidine-pyrimidine base pairs are allowed as ribose distances would be incorrect.

	Wobble bases				
tRNA	C	A	G	U	I
mRNA	G	U	C U	A G	C A U

	Wobble bases			
mRNA	C	A	G	U
tRNA	**G** **I**	U I	C U	A G I

Wobble pairing



Ribosome binding site
(Shine-Dalgarno sequence)

- Solely for prokaryotic translation
- A purine-rich sequence usually containing all or part of the sequence 5'-AGGAGGU-3'
- Upstream of the initiation codon in prokaryotic mRNA
- To position the ribosome for initiation of protein synthesis

SD序列

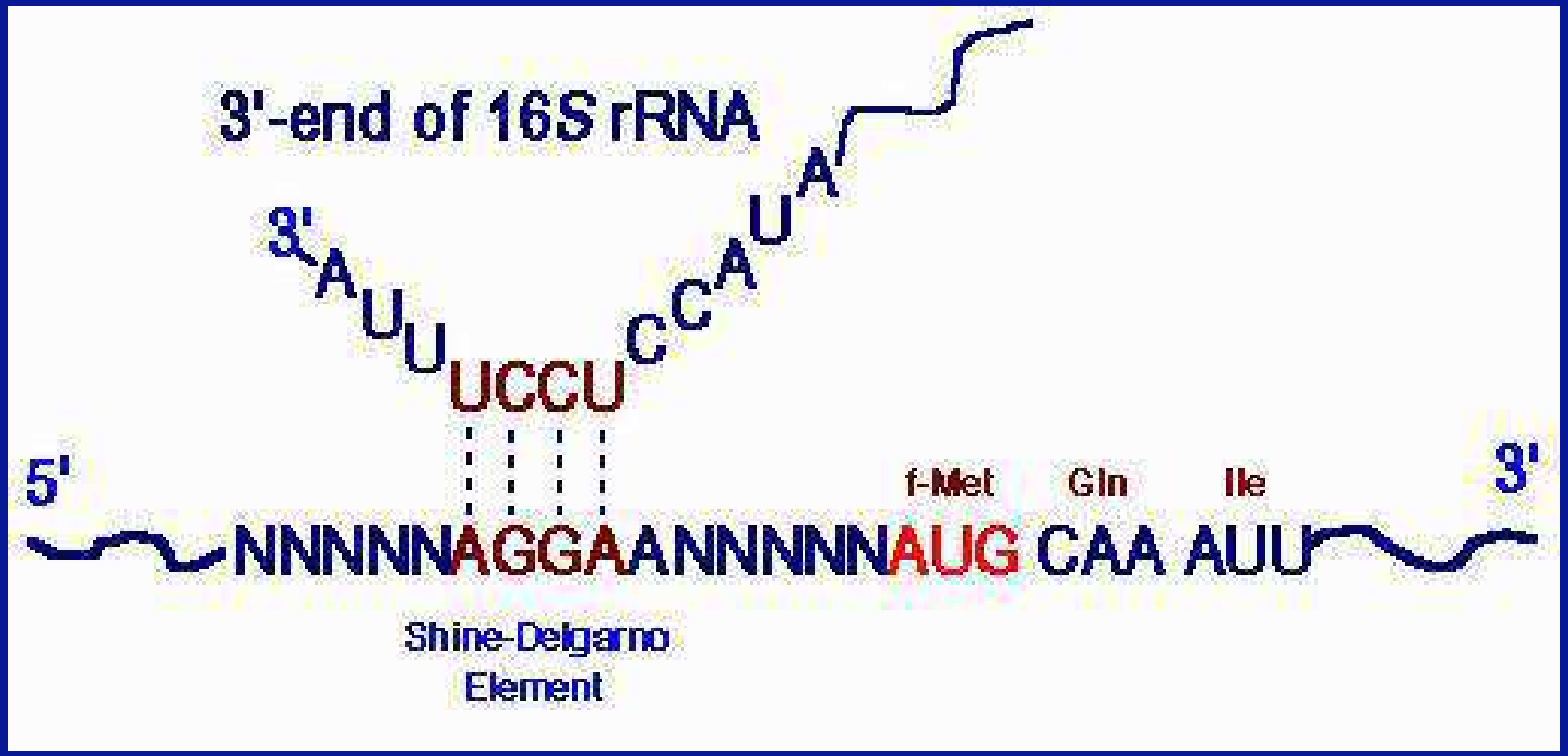
原核生物的mRNA中核糖体的结合位点，即AUG起始密码子之前的多聚嘌呤序列AGGAGGU的部分或者全部，与16S rRNA的3‘末端互补。

大肠杆菌 16*S* rRNA 与 SD 序列的识别

	与 SD 序列互补的嘧啶碱基富含区
16*S* rRNA	3′···HO AUUCCUCCACUA···5′
lacZ mRNA	5′···ACACAGGAAACAGCUAUG···3′
trpA mRNA	5′···ACGAGGGGAAAUCUGAUG···3′
RNA polymerase βmRNA	5′···GAGCUGAGGAACCCUAUG···3′
r-Protein L10 mRNA	5′···C C AGGAGCAA AGCUAAUG ···3′

富含嘌呤碱基的SD序列　起始密码子

Shine-Delgarno element



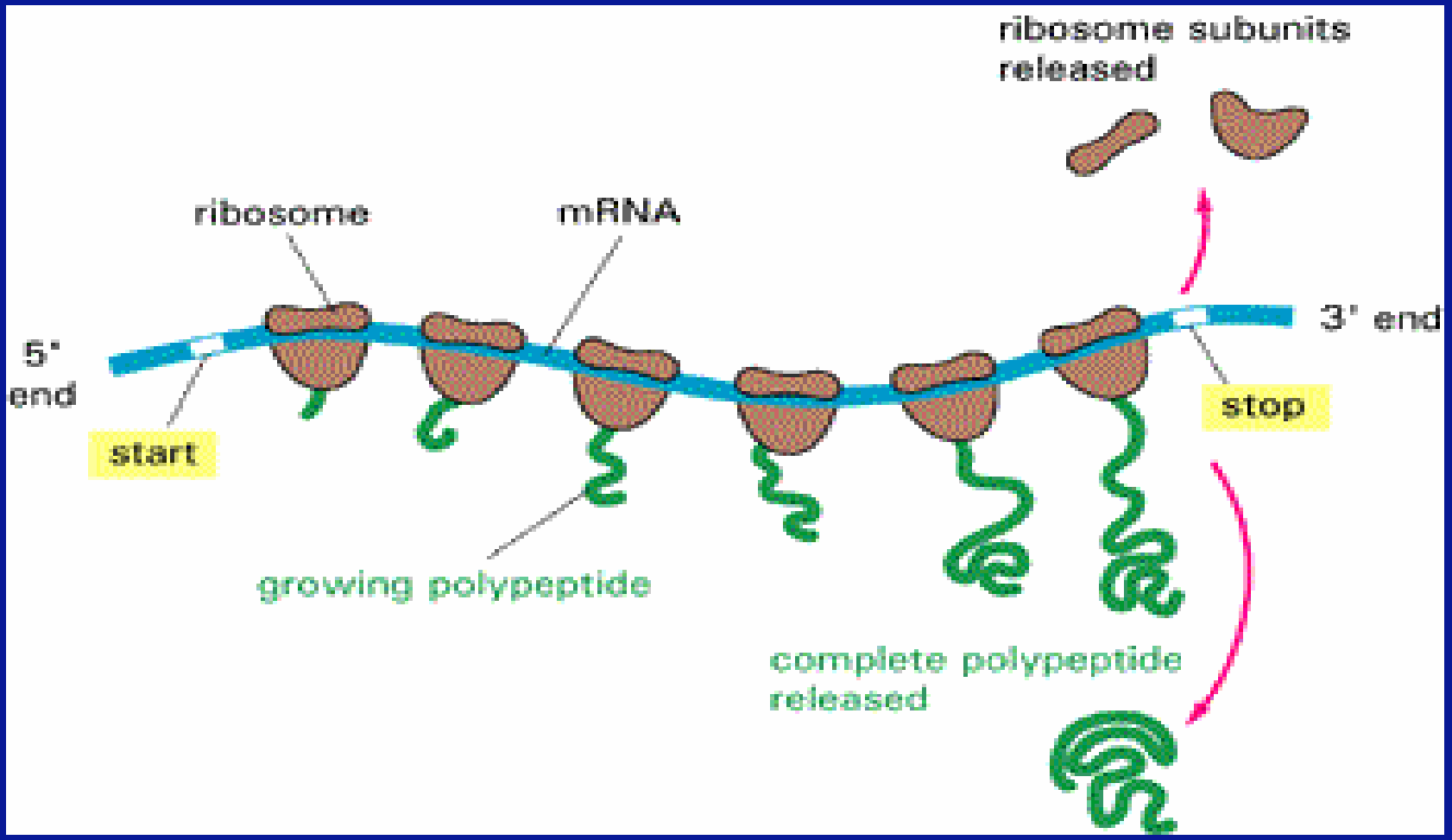
SD序列的重要性

- 细菌毒素colecin E3 可通过核酸酶的活性特异的在16S rRNA3’端切下50个碱基左右的片段，使得核糖体小亚基中的16S rRNA失去了与mRNA上SD序列互补的可能，由此抑制了细菌蛋白质的合成。由于原核和真核生物在合成蛋白质机制上的差异， colecin E3 不影响真核生物的蛋白质合成。

Polysomes

- Each mRNA transcript is read simultaneously by more than one ribosome.
- A second, third, fourth, etc. ribosome starts to read the mRNA transcript before the first ribosome has completed the synthesis of one polypeptide chain.
- Multiple ribosomes on a single mRNA transcript are called polyribosomes or polysomes.
- Multiple ribosomes can not be positioned closer than 80 nt.

Polysomes

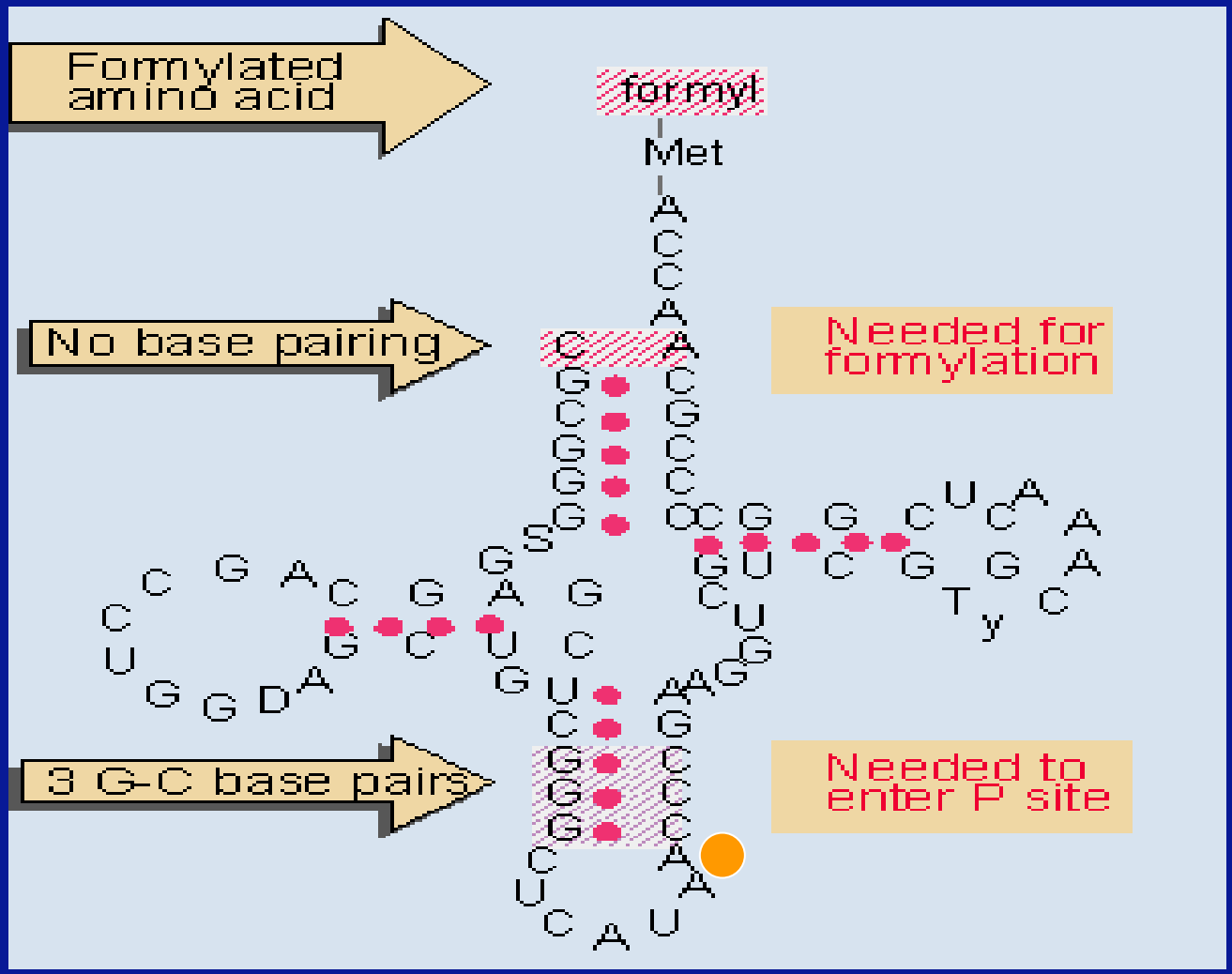

ribosome subunits
released
ribosome
mRNA
5'
end
start
3' end
stop
growing polypeptide
complete polypeptide
released

- Electron micrographs of ribosomes actively engaged in protein synthesis revealed by "beads on a string" appearance.

Initiator tRNA

- Methionine is the first amino acids incorporated into a protein chain in both prokaryotes (modified to N-formylmethionine) and eukaryotes.
- Initiator tRNAs are special tRNAs recognizing the AUG (GUG) start codons in prokaryotes and eukaryotes.
- Initiator tRNAs differ from the one that inserts internal Met residues.

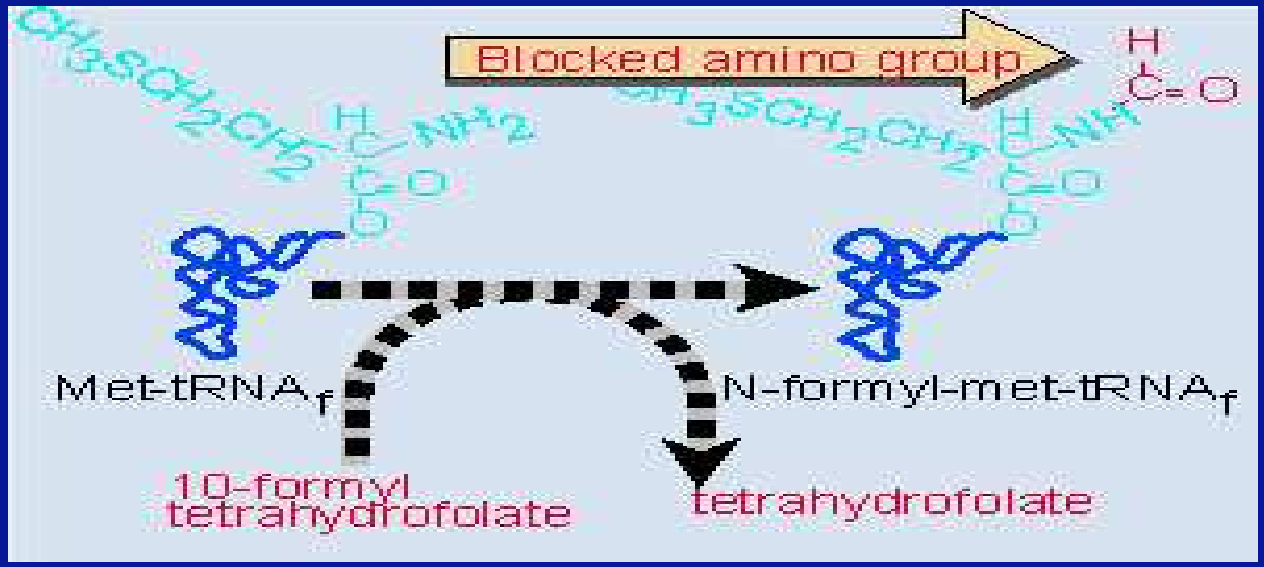
Initiator tRNA, fMet-tRNA^{fMet} in *E. coli*



Lacking alkylated A endorses more flexibility in recognition in base pairing (both AUG and GUG).

Initiator tRNA formation in *E. coli*

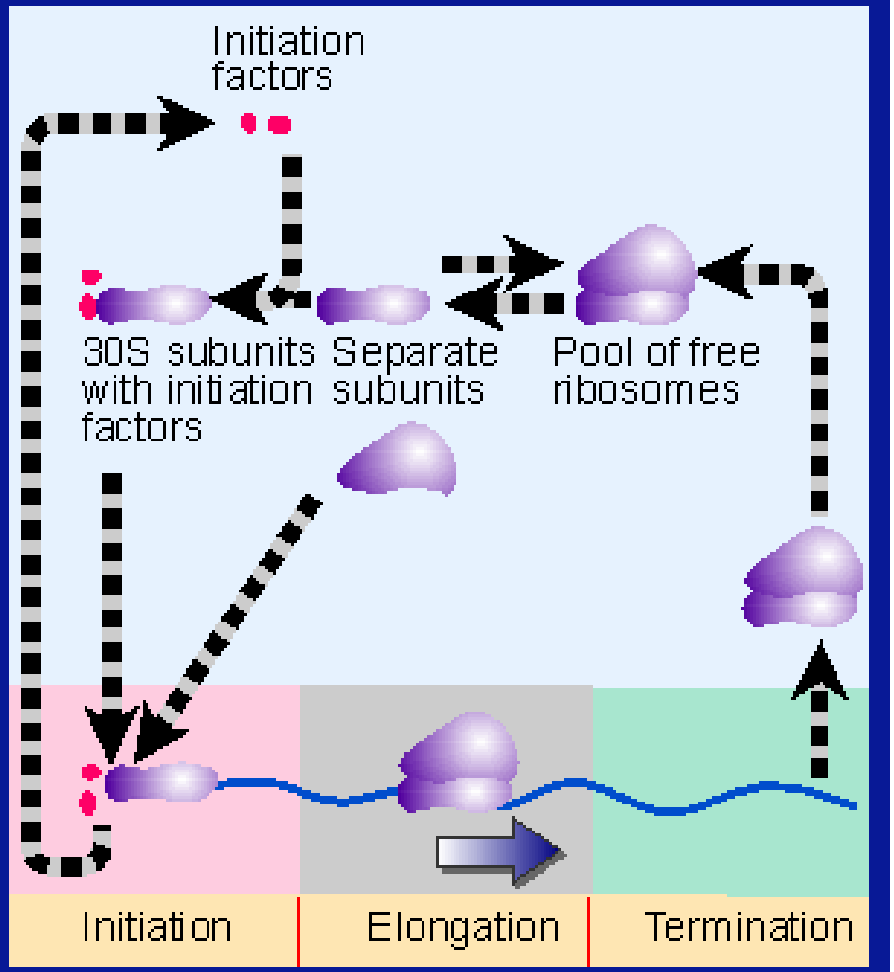
1. Both initiator tRNA and noninitiator $tRNA^{met}$ are charged with Met by the same methionyl-tRNA synthetase to give the methionyl-tRNA
2. Only the initiator methionyl-tRNA is modified by transformylase to give N-formylmethionyl-$tRNA^{fmet}$.



Mechanism of protein synthesis

Protein synthesis falls into three stages .

1. **initiation**-the assembly of a ribosome on an mRNA molecule.
2. **elongation**-repeated cycles of amino acid addition.
3. **termination**-the release of the new protein chain.

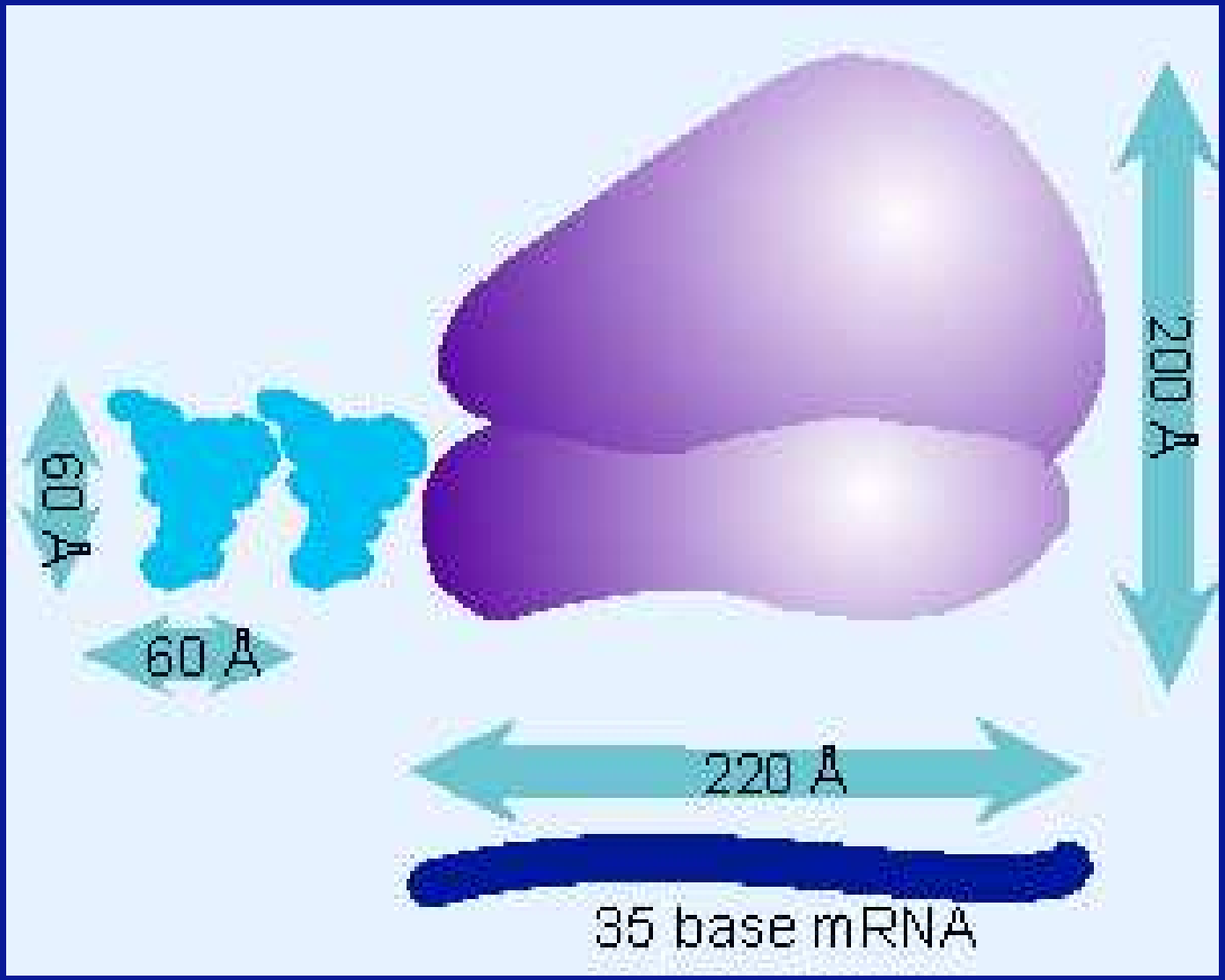


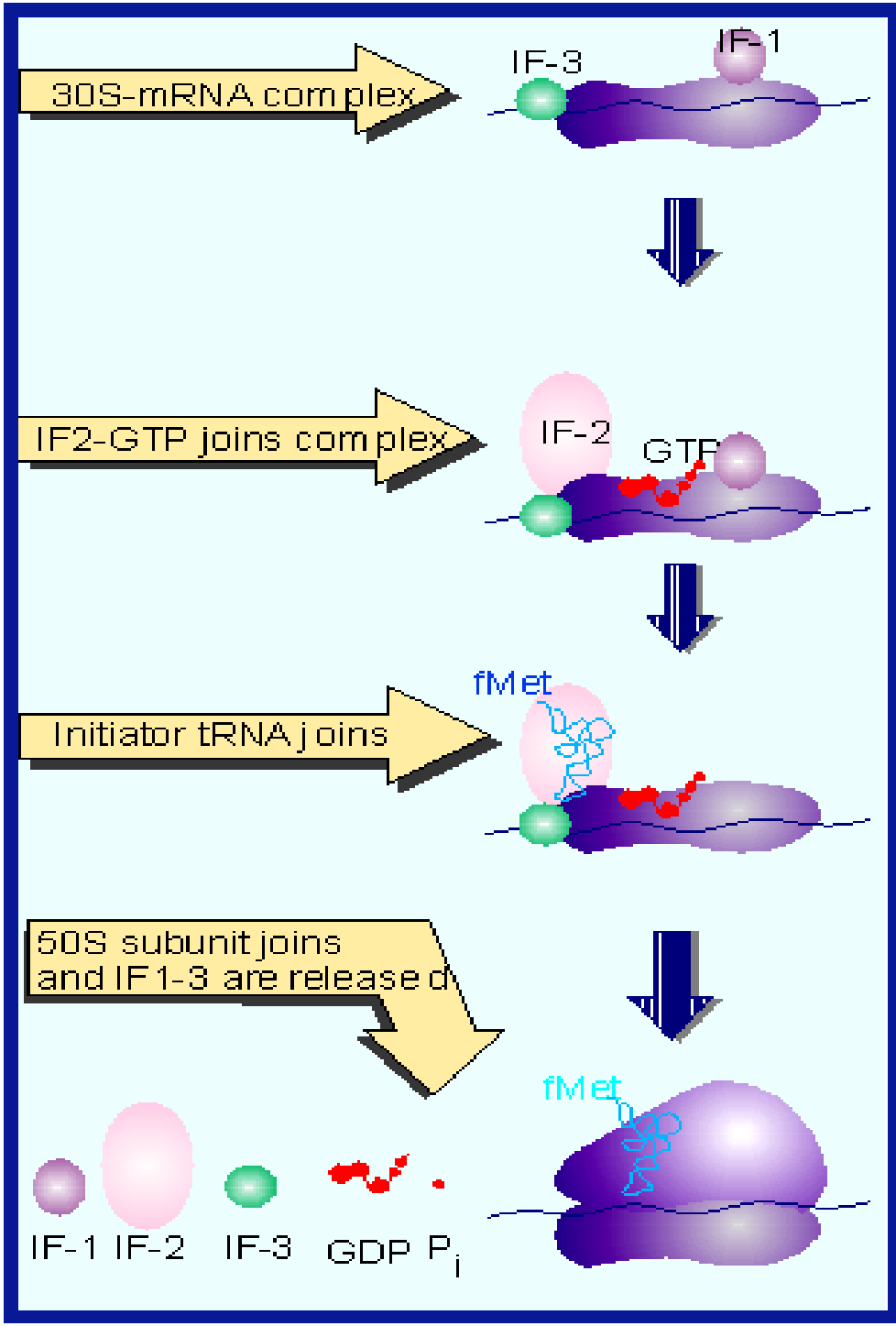
Initiation

In prokaryotes, initiation requires

- large and small ribosome subunits,
- mRNA
- initiator tRNA (fMet-tRNA ^{fMet})
- three initiation factors (IF) & GTP

Size comparisons show that the ribosome is large enough to bind tRNAs and mRNA.



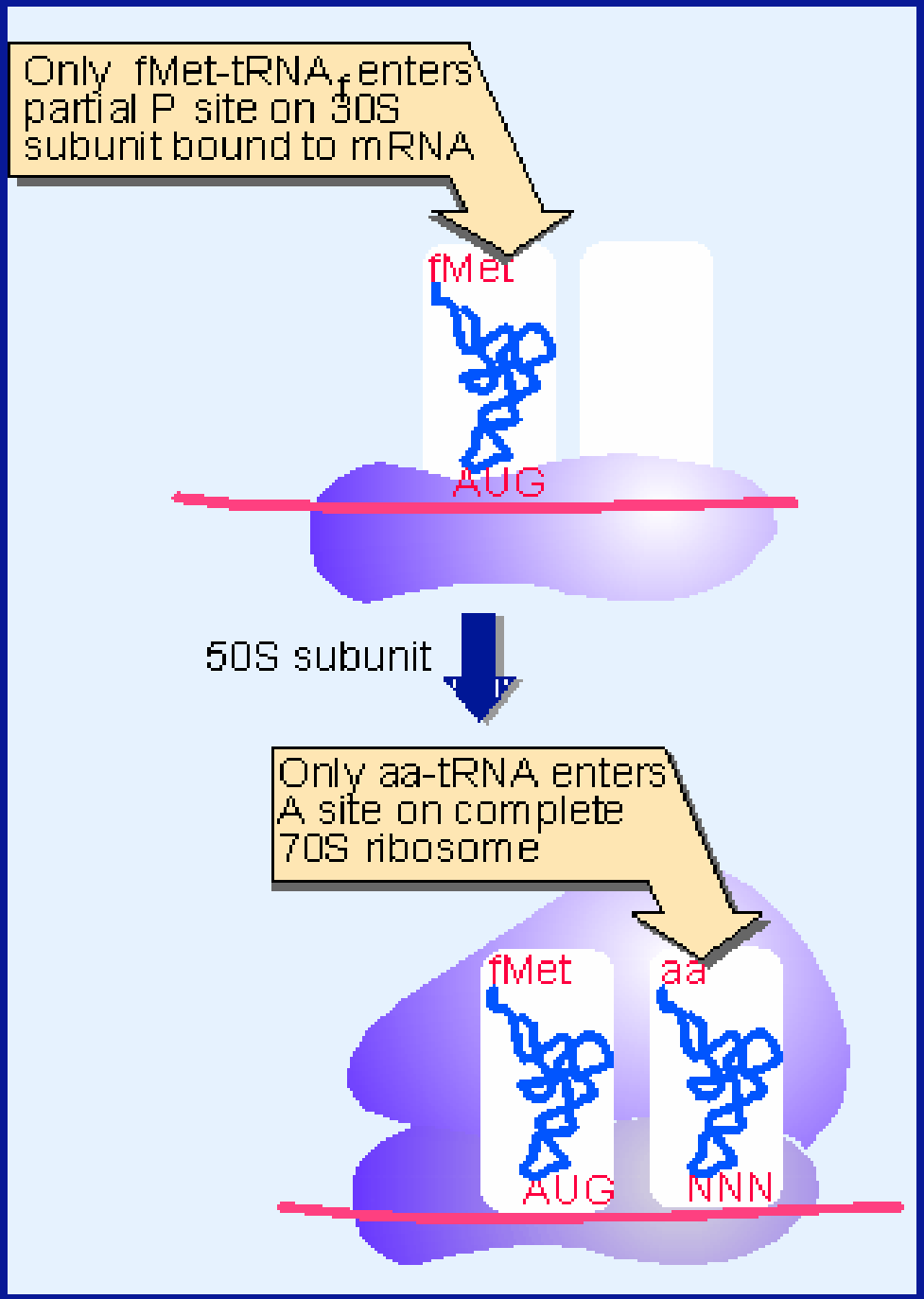


IF1 and IF3 bind to a free 30S subunits.

IF2 complexed with GTP then bind to the small subunits, forming a complex at RBS.

The initiator tRNA can then bind to the complex at the P site paired with AUG codon.

The 50S subunits can now bind. GTP is then hydrolyzed and IFs are released to give the **70S initiation complex**



The assembled ribosome has two tRNA-binding sites, which are called A- and P-site, for aminoacyl and peptidyl sites respectively.

Only fMet-tRNA^{fMet} can be used for initiation by 30S subunits; all other aminoacyl-tRNAs are used for elongation by 70S ribosomes.

起始复合物都需要起始因子

IF-3 为**30S**亚单位特异性结合到**mRNA**起始位点所必需；

IF-2 结合一个特定的起始**tRNA**，并控制其进入核糖体；

IF-1 结合**30S**亚单位，只是作为完全起始复合物的一部分，可能与复合物的稳定而不是与识别任何特异性组分有关。

起始需要30s亚基与IF-3结合

IF-3具有双重功能

- IF-3稳定游离的30S亚基，保持大小亚基分离；
- IF-3促使30S起始复合物同mRNA结合。

Elongation

With the formation of the 70S initiation complex, the elongation cycle can begin.

Elongation involves the three factors, EF-Tu, EF-Ts, EF-G, as well as GTP, charged tRNA and the 70S initiation complex.

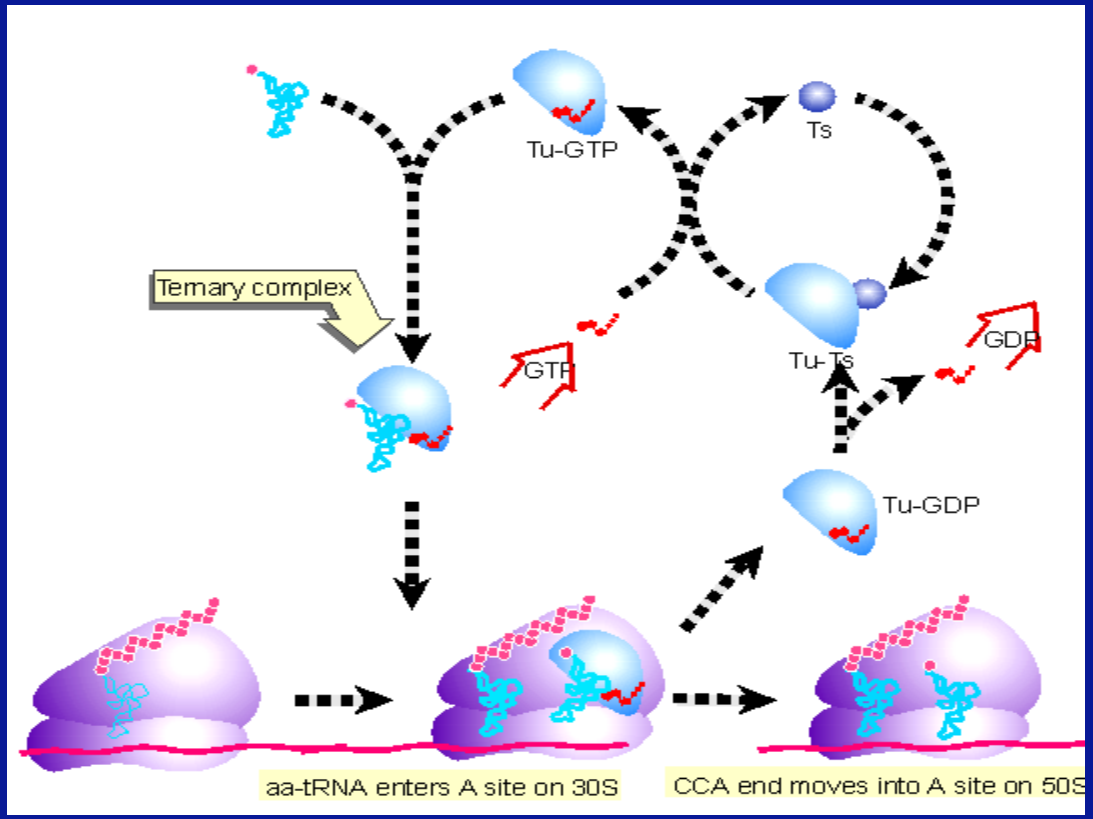
The three steps of elongation

1. Charged tRNA delivery
2. Peptide bond formation
3. Translocation

The first step of elongation

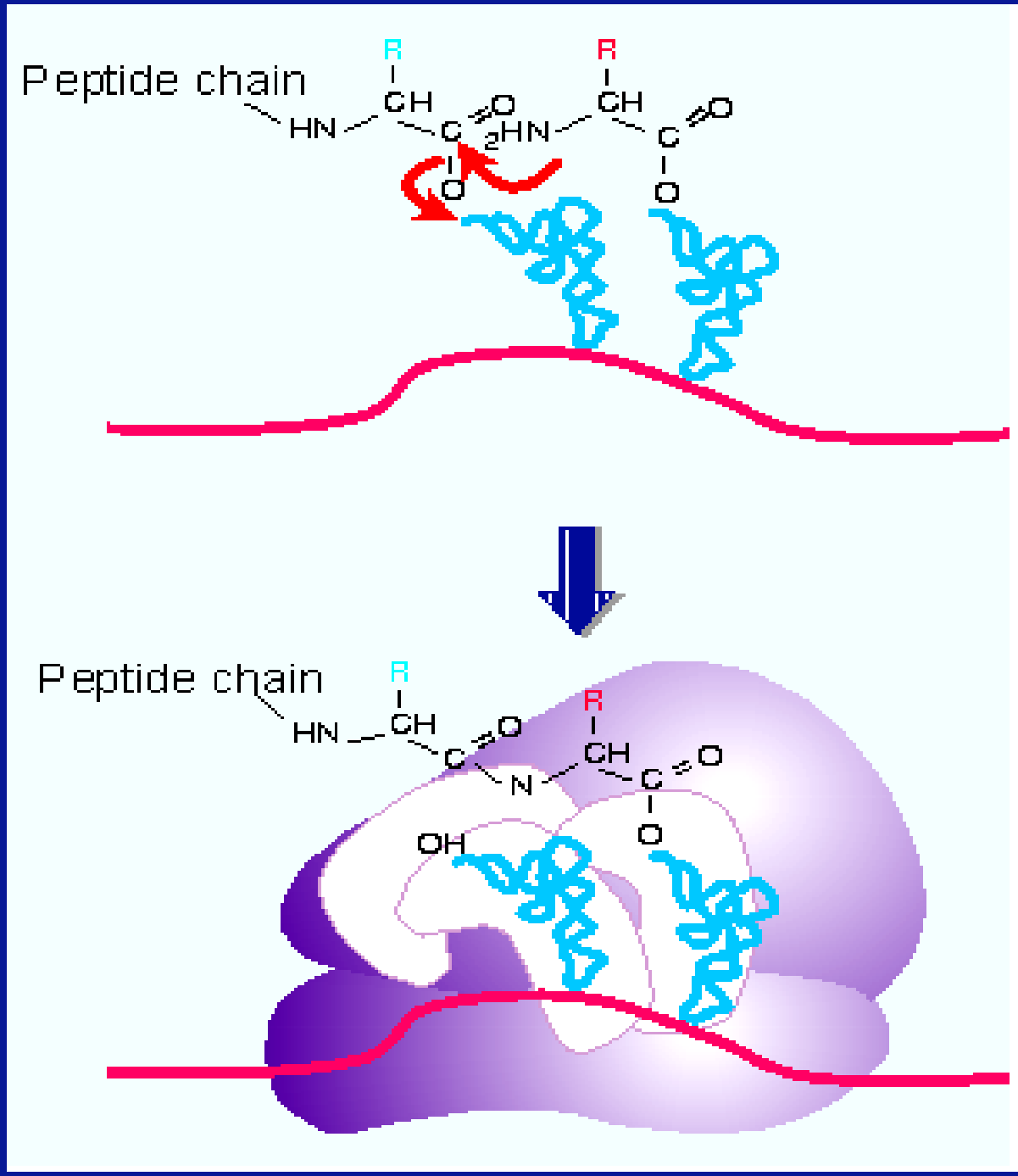
1. Charged tRNA is delivered as a complex with EF-Tu and GTP.

EF-Tu-Ts exchange cycle



The second step of elongation

2. Peptidyl tranferase (50S ribosomal subunit) makes a peptide bond by joining the two adjacent amino acid without the input of more energy.



Peptide bond formation takes place by reaction between the polypeptide of peptidyl-tRNA in the P site and the amino acid of aminoacyl-tRNA in the A site.

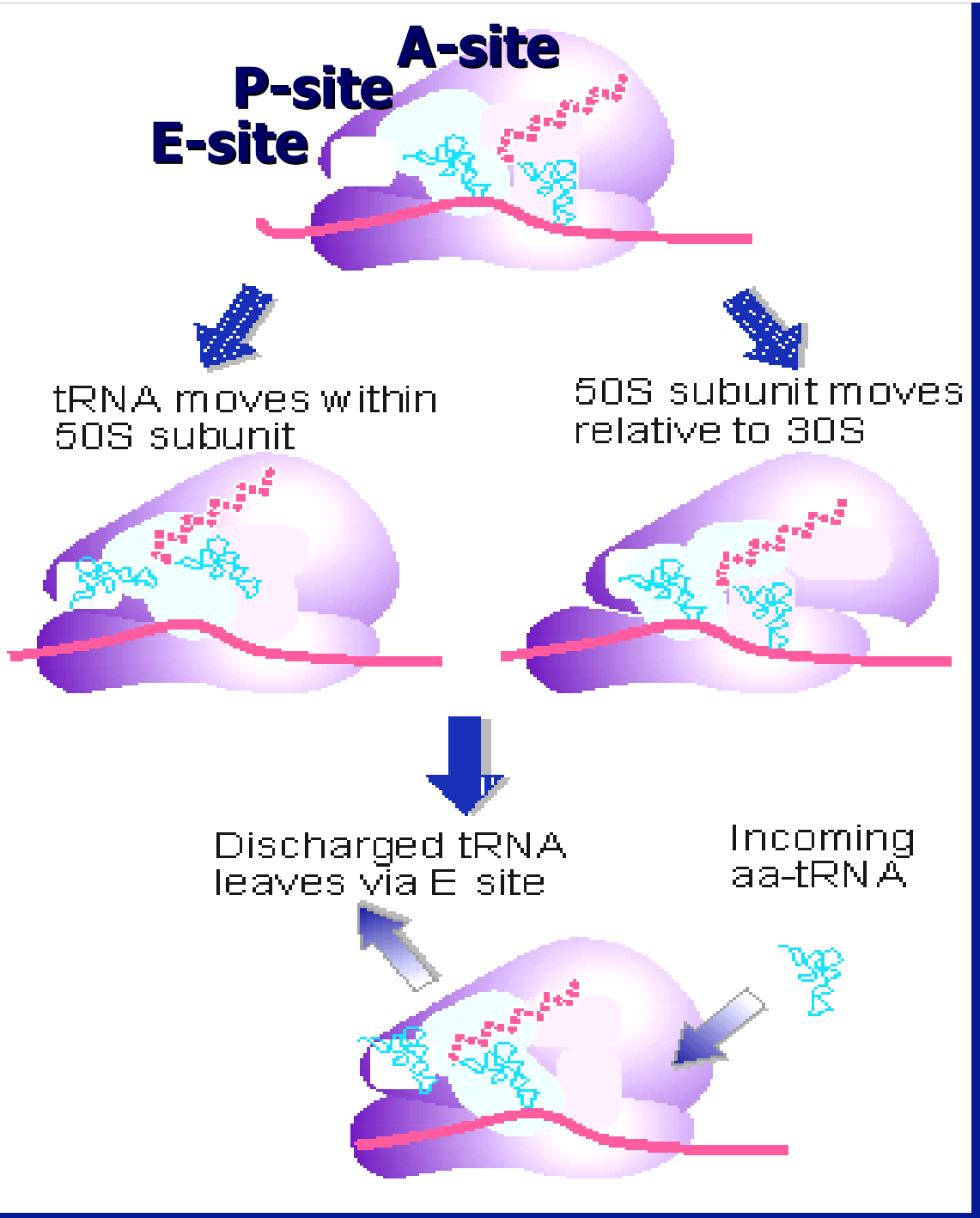
The third step of elongation

3. Translocase (EF-G), with the energy from GTP, moves the ribosome one codon along the mRNA, ejecting the uncharged tRNA and transferred the ribosome peptide from the mRNA.

Translocation

- In bacteria, the discharged tRNA leaves the ribosome via another site, the E site.
- In eukaryotes, the discharged tRNA is expelled directly into the cytosol.
- EF-G (translocase) and GTP binds to the ribosome, and the discharged tRNA is ejected from the P-site in an energy consuming step.
- the peptigly-tRNA is moved from A-site to P-site and mRNA moves by one codon relative to the ribosome

Translocation in *E. coli*



Termination

Protein factors called release factors interact with stop codon and cause release of completed polypeptide chain.

RF1 and RF2 recognizes the stop codon with the help of RF3

The release factors make peptidyl transferase transfer the polypeptide to H_2O, and thus the protein is released

Release factors and EF-G: remove the uncharged tRNA and release the mRNA,.

Initiation in eukaryotes

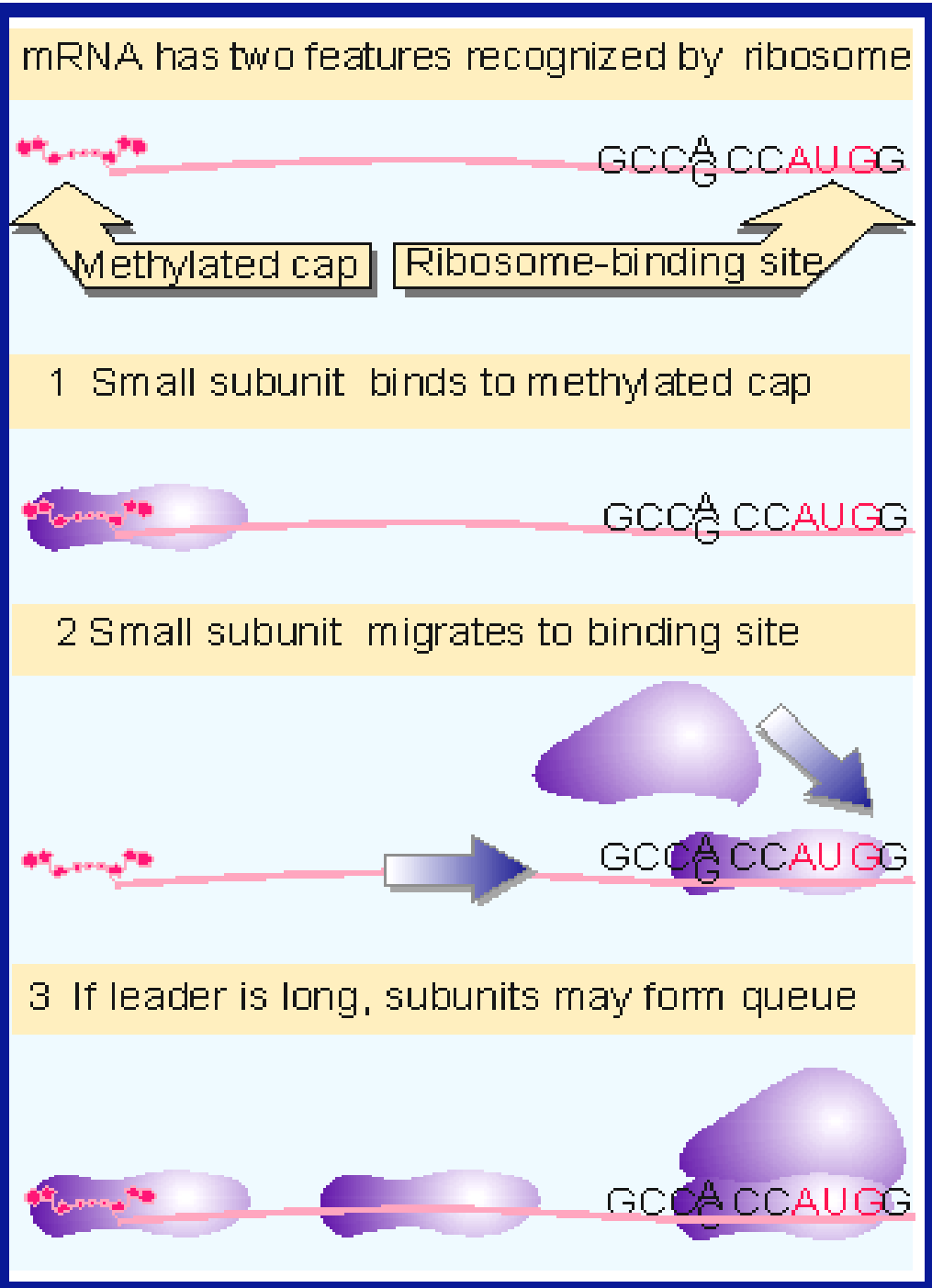
Most of the differences in the mechanism of protein between prokaryotes and eukaryotes occur in the initiation stage, where **a greater numbers** of eIFs and **a scanning process** are involed in eukaryotes.

The eukaryotic initiator tRNA does not become **N-formylated**.

prokaryotic	eukaryotic	function
Initiation factor		
IF1/IF3	eIF3 eIF4c eIF6 eIF4B eIF4F	Bind to ribosome subunits Bind to mRNA
IF2	eIF2B eIF2	Initiator tRNA delivery
	eIF5	Displacement of other factors
Elongation factor		
EF-Tu	eEF1 α	Aminoacyl tRNA delivery
EF-Ts	eEF1 β γ	Recycling of EF-Tu or eEF1 α
EF-G	eEF2	Translocation
Termination factors		
RF1		Polypeptides Chain release
RF2	eRF	
RF3		

Scanning

The eukaryotic 40s ribosome subunit complex bind to the 5′cap region of the mRNA and moves along it, scanning for an AUG start codon.



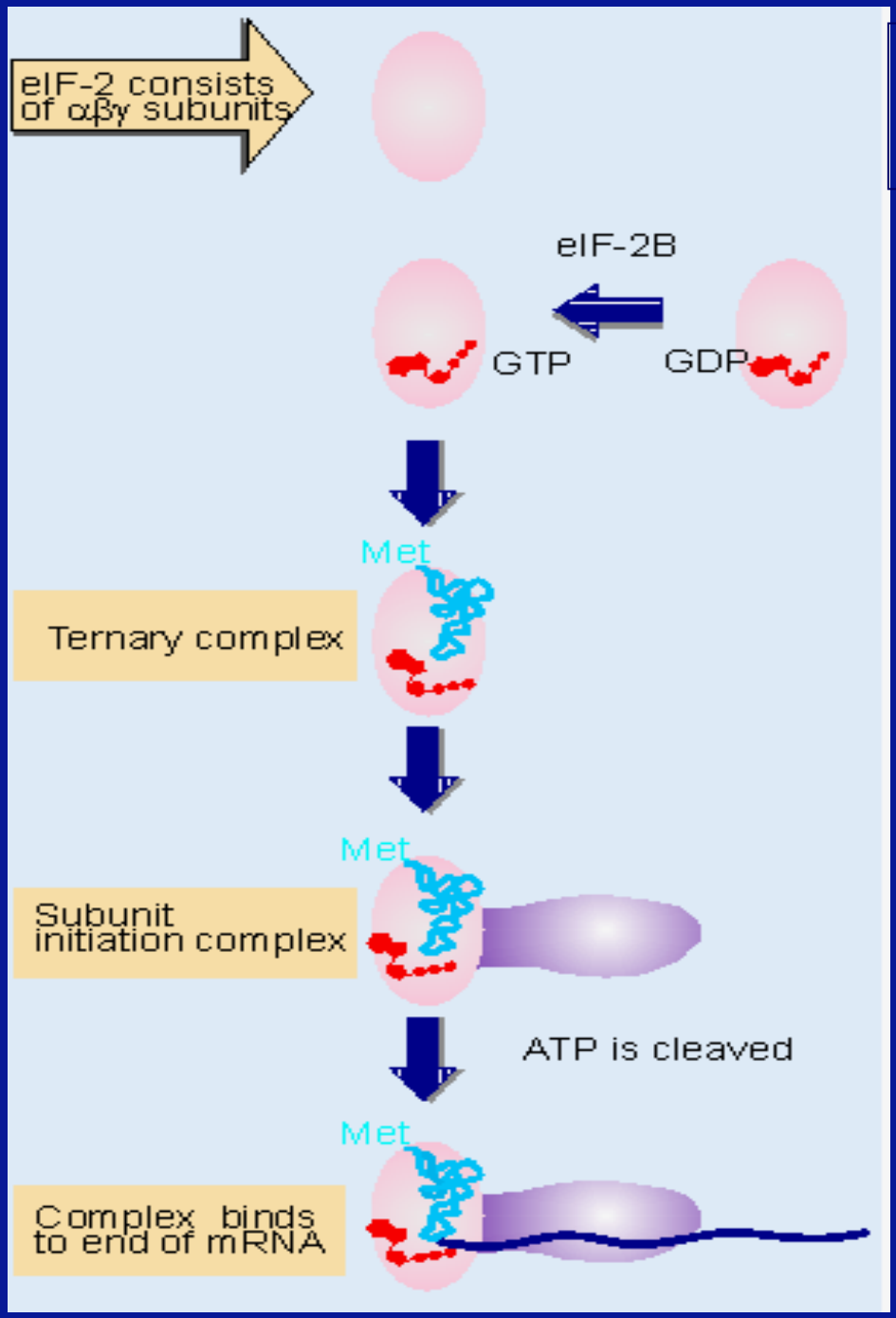
Eukaryotic ribosomes migrate from the 5' end of mRNA to the ribosome binding site, which includes an AUG initiation codon.

Initiation

In contrast to the events in prokaryotes, initiation involves the initiation tRNA binding to the 40S subuits before it can bind to the mRNA. Phosphorylation of eIf2, which delivers the initiation tRNA, is an important control point.

The initiation factor can be grouped to their function as follow

Binding to ribosomal subunits	eIF6 eIF3 eIF4c
Binding to the mRNA	eIF4B eIF4F eIF4A eIF4E
Involved in initiation tRNA delivery	eIF2 eIF2B
Displace other factors	eIF5



Initiator tRNA+eIF2+GTP

eIF3+4C+ 40S

Ternary complex + 43S ribosome complex

43S preinitiation complex

ATP

+mRNA+eIF4F +eIF4B

ADP+Pi

48S preinitiation complex

Scanning

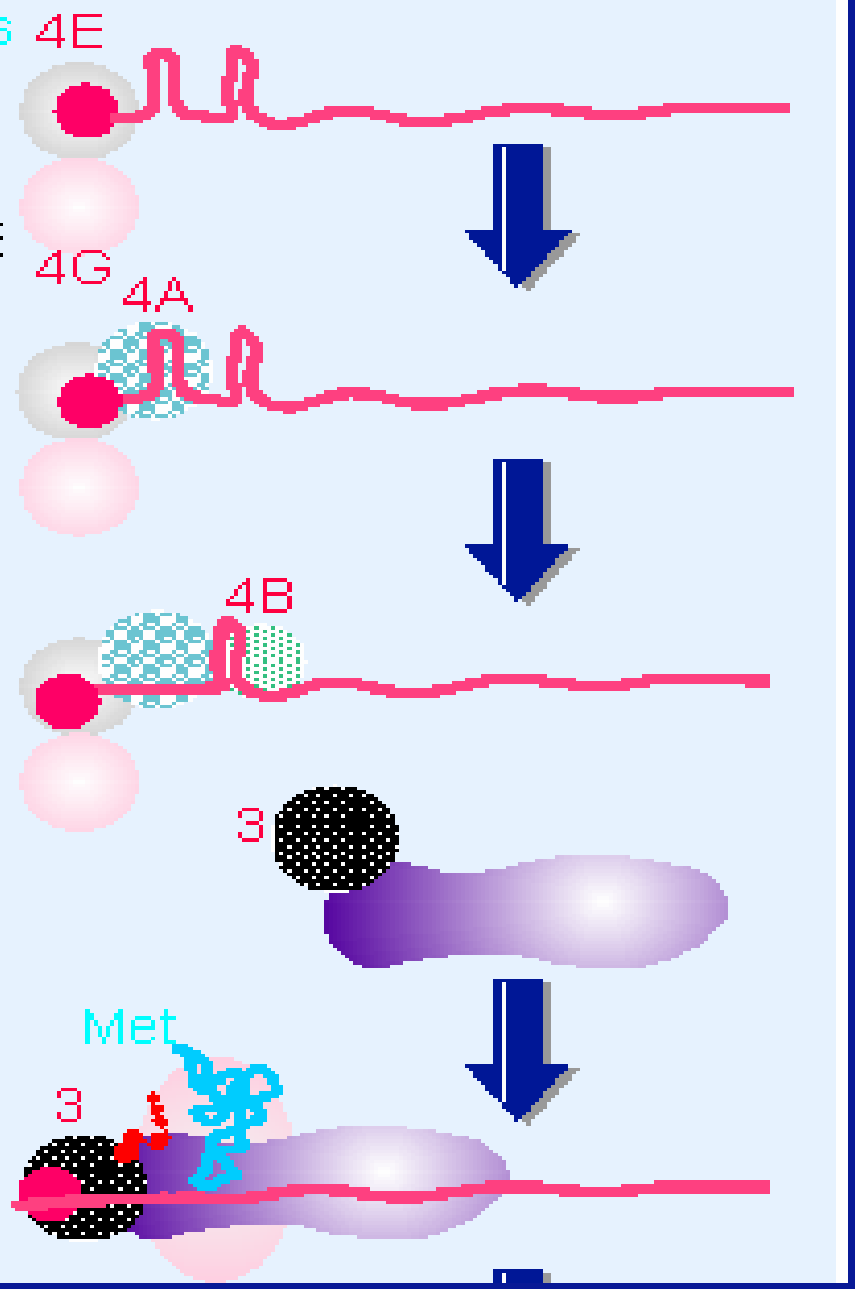
eIF-4F includes
eIF-4E
binds to 5' cap
EIF-4G
binds to eIF-4E

eIF-4A
unwinds
structure
at 5' end

eIF-4B
assists further
unwinding

eIF-3
maintains free
40S subunits

eIF-3
required for
40S subunit
with ternary
complex to



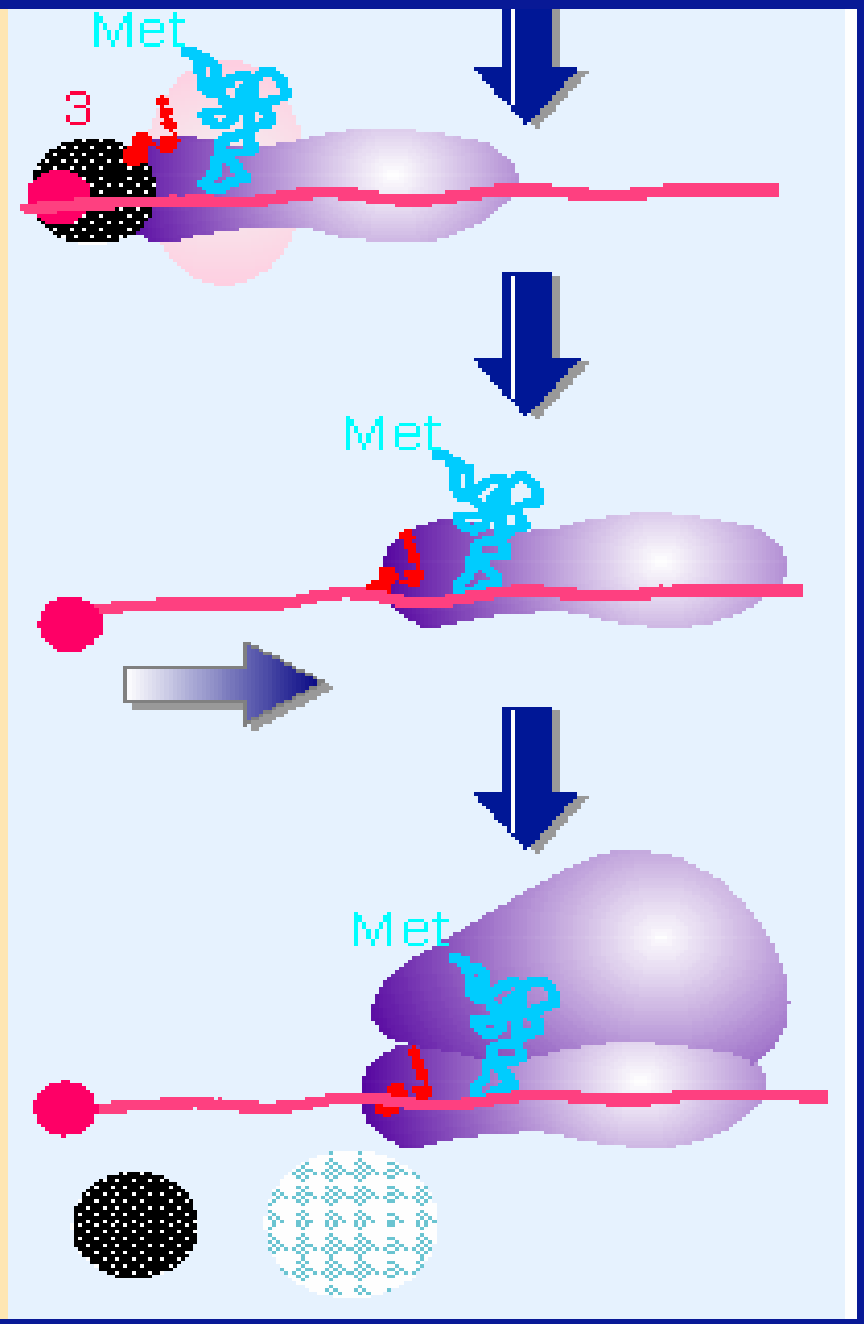
More factors
involved

Scanning to find AUG

eIF-3
required for 40S subunit with ternary complex to bind to 5' end

40S subunit migrates along mRNA to AUG codon

eIF-5
GTPase required for 60S joining, release of eIF-2 & eIF-3



Elongation

The protein synthesis elongation cycle in prokaryotes and eukaryotes is quite similar.

The factors EF-Tu EF-Ts EF-G have direct eukaryotic equivalents called eEF1 α eEF1 β γ eEF2

Termination

Eukaryotes use only one release factors eRF, which requires GTP,recognize all three termination codons.

Termination codon is one of three (**UAG**, **UAA**, **UGA**) that causes protein synthesis to terminate.

Translational control and post-translational events

- Translational control
- Polyproteins
- Protein targeting
- Protein modification
- Protein degradation

Translational control

- In prokaryotes, the level of translation of different cistrons can be affected by:

  (a) the binding of short antisense molecules,

  (b) the relative stability to nucleases of parts of the polycistronic mRNA ,

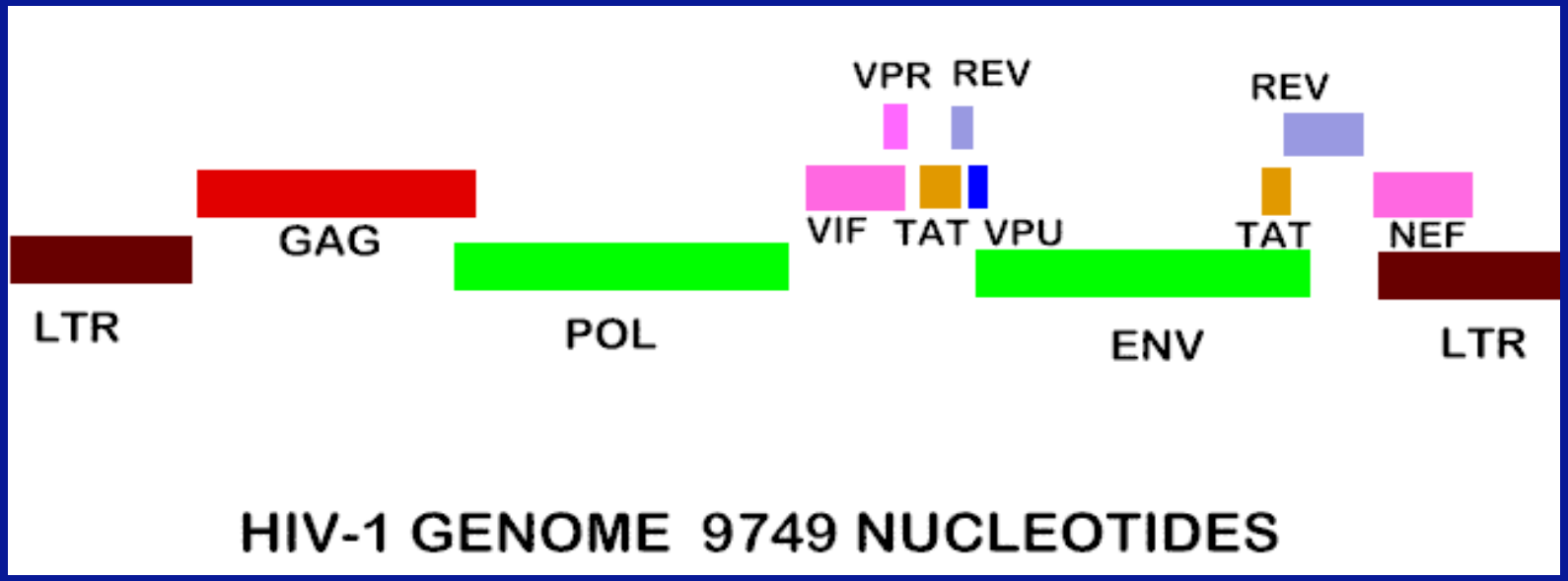
  (c) the binding of proteins that prevent ribosome access.

In eukaryotes

- protein binding can also mask the mRNA and prevent translation,
- repeats of the sequence 5'-AUUUA -3' can make the mRNA unstable and less frequently translated.

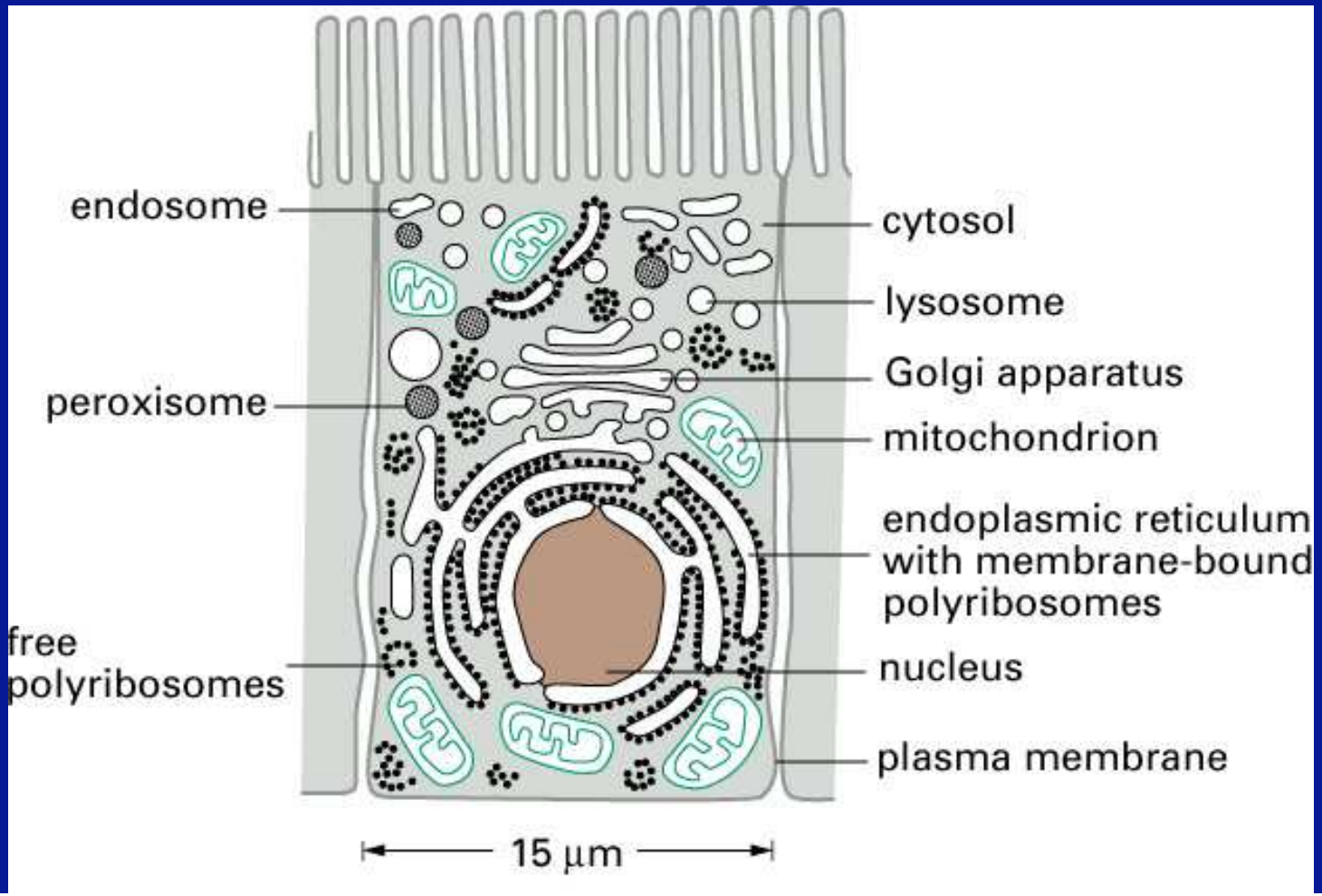
Polyprotein

- A single translation product that is cleaved to generate two or more separate proteins is called a polyprotein. Many viruses produce polyprotein.



Protein Targeting

The cell cytoplasm contains many different specialized compartments

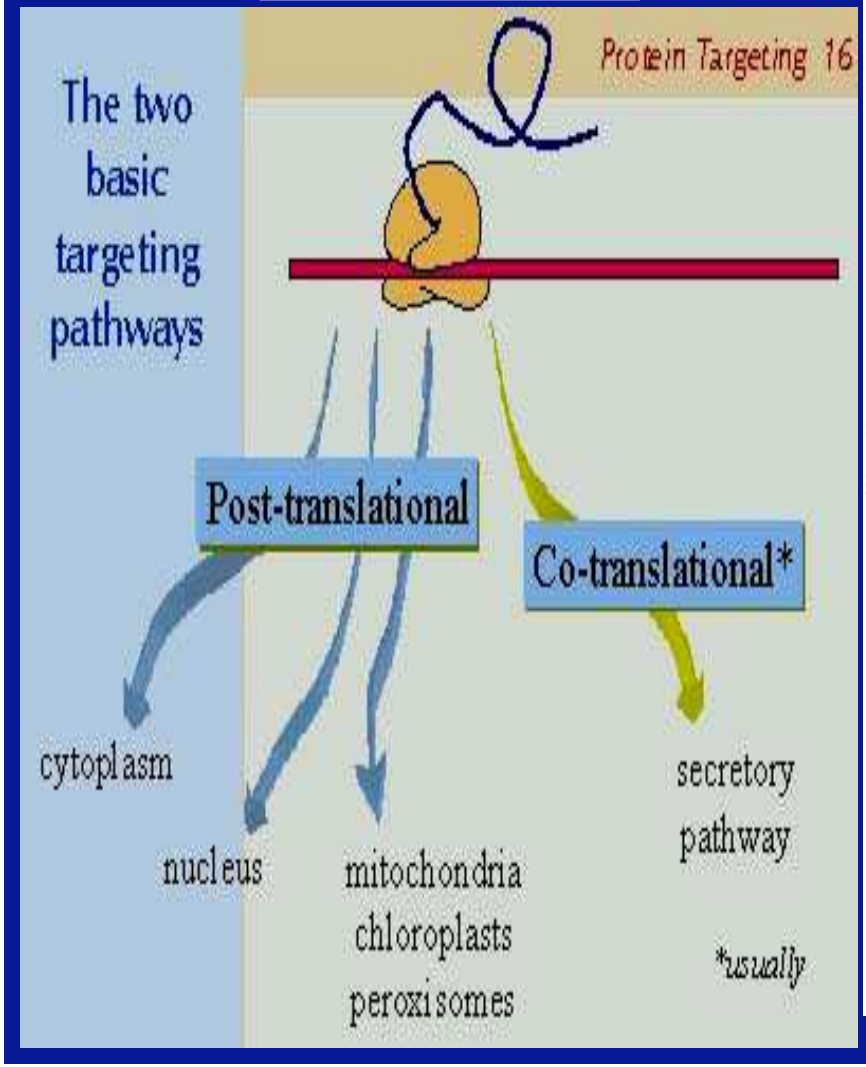


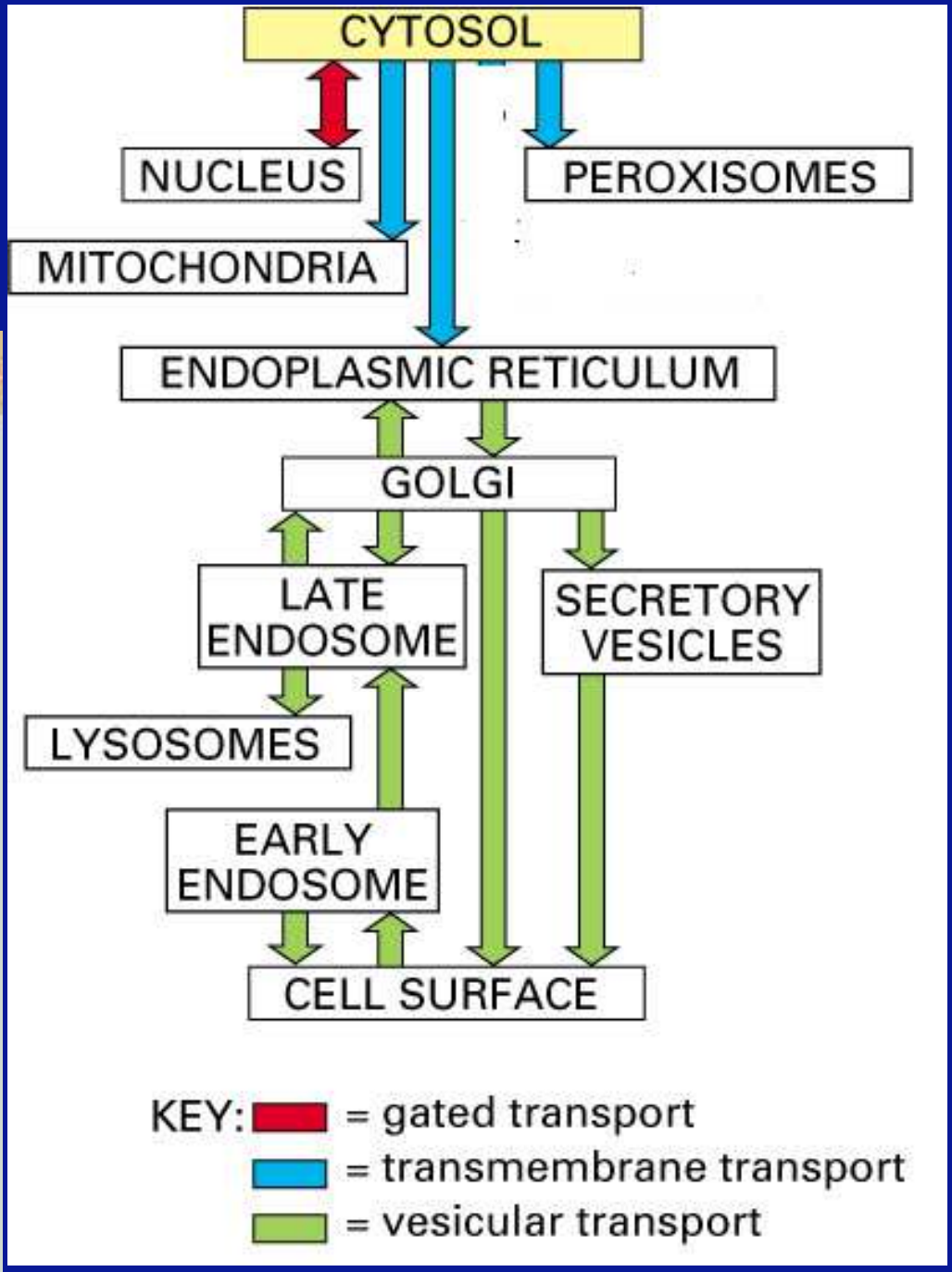
-Maintenance of specialized cellular architecture and function requires that cellular proteins be arranged properly within the cell

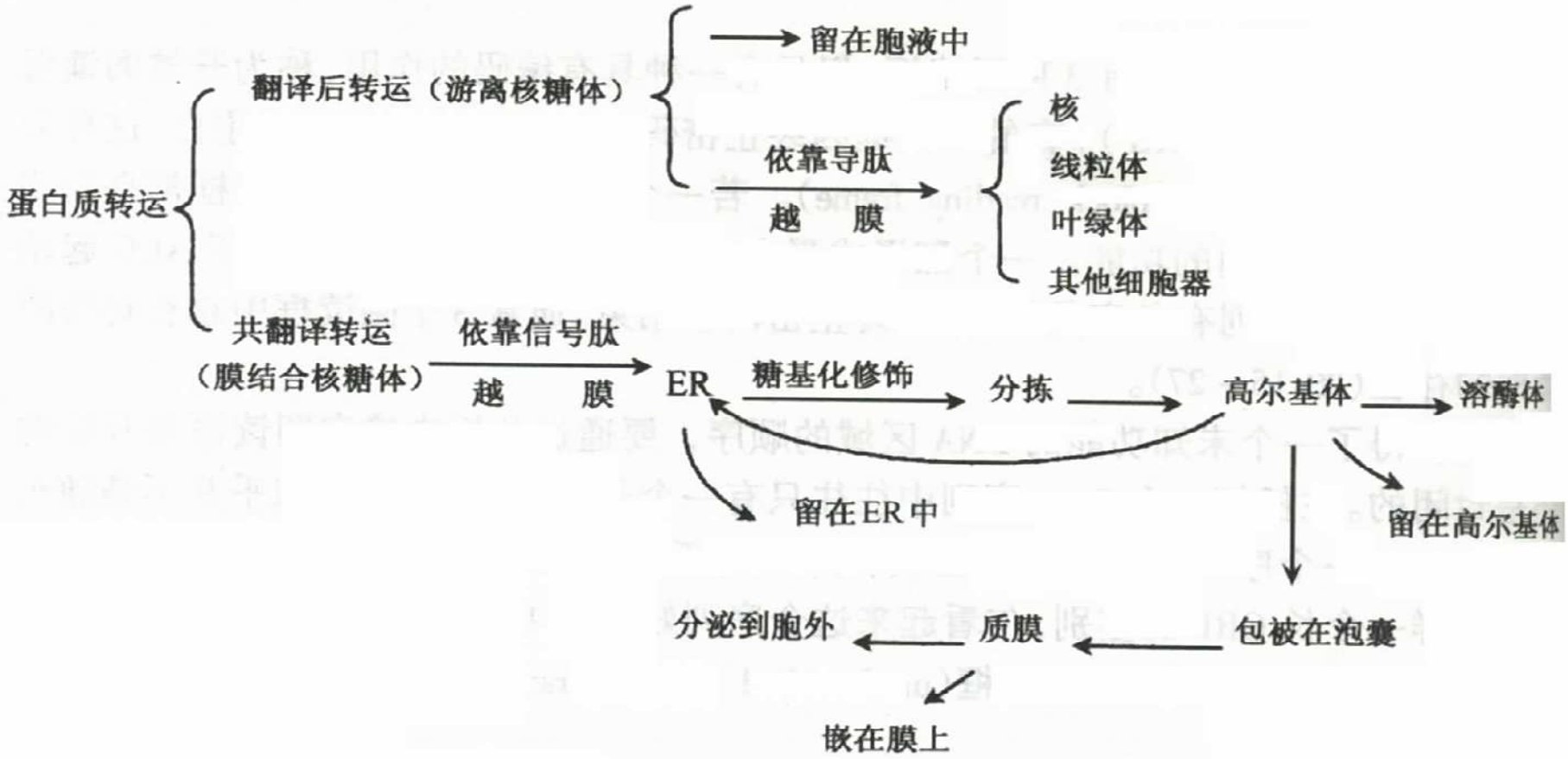
-Regulation of where a given protein functions within a cell can be as important as the function of the protein itself.

Transport pathways control the movement of proteins into and out of particular intracellular compartments

Road Map of Transport Routes in the Cell







蛋白质合成后的转运，定位及分泌。

几类主要蛋白质的运转机制

蛋白性质	运转机制	主要类型
分泌	蛋白质在结合核糖体上合成，并以翻译－运转同步机制运输	免疫球蛋白、卵蛋白、水解酶、激素等
细胞器发育	蛋白质在游离核糖体上合成，以翻译后运转机制运输	核、叶绿体、线粒体、乙醛酸循环体、过氧化物酶体等细胞器中的蛋白质
膜的形成	两种机制兼有	质膜、内质网、类囊体中的蛋白质

Common Features of Transport Mechanisms

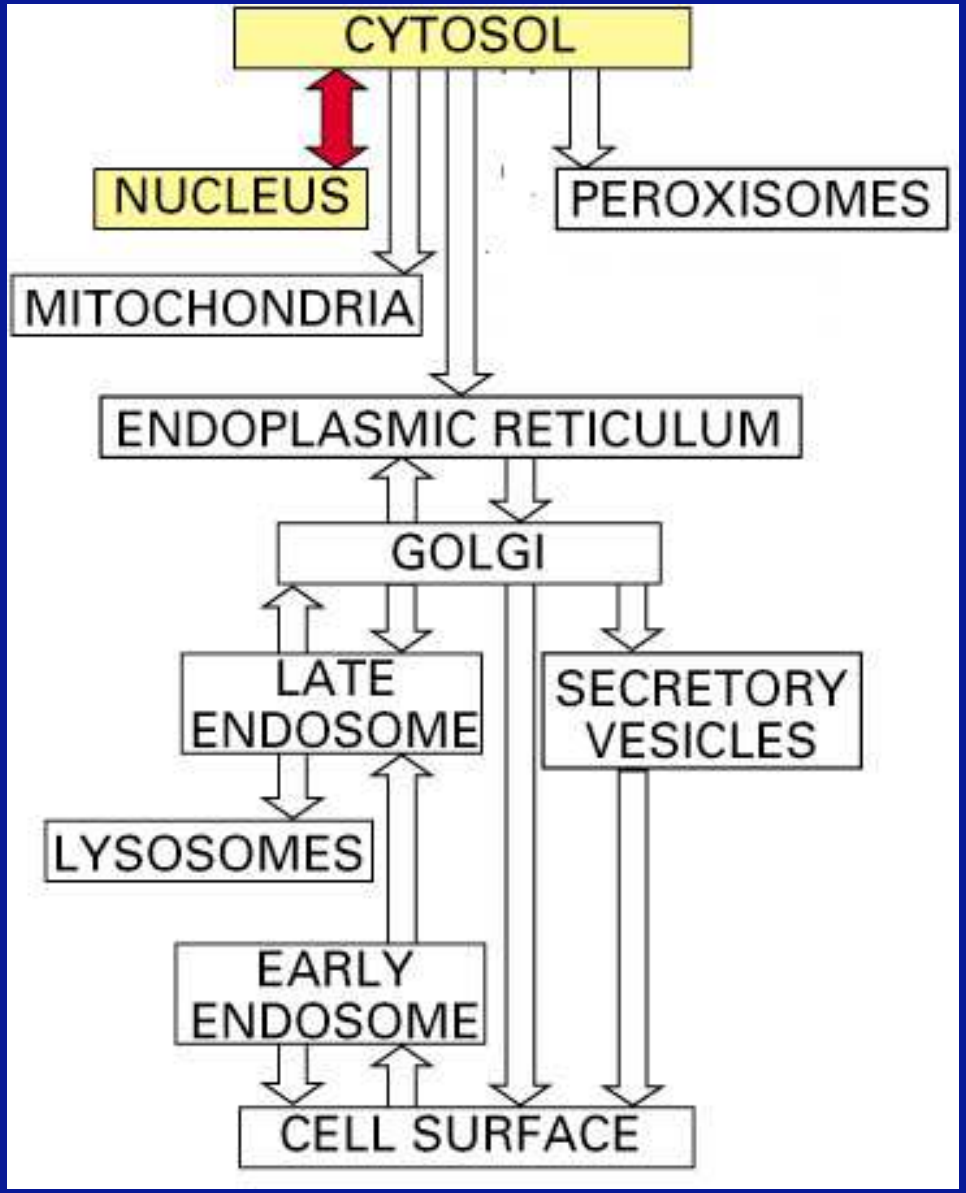
Signal sequences --

Short regions of a protein that act as targeting signals to direct the protein to specific subcellular localization

Receptors that recognize particular signal sequences

Require energy (ATP or GTP)

Nuclear transport involves the passage through a “gate” that separates two aqueous compartments, the cytoplasm and the nucleoplasm.



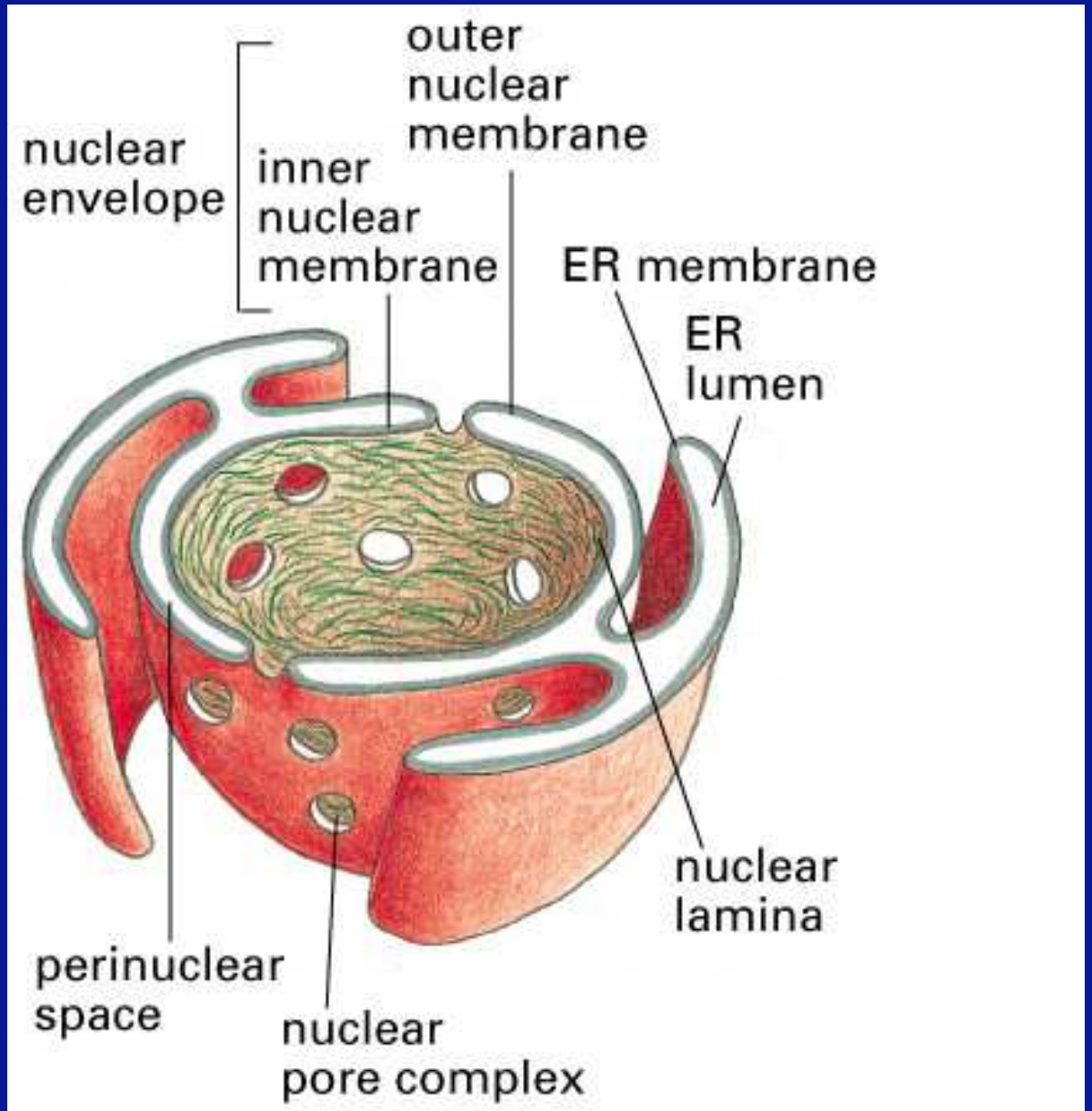


Figure 12–9. Molecular Biology of the Cell, 4th Edition.

The nucleus is bounded by double membrane, the Nuclear Envelope, that is continuous with the ER.

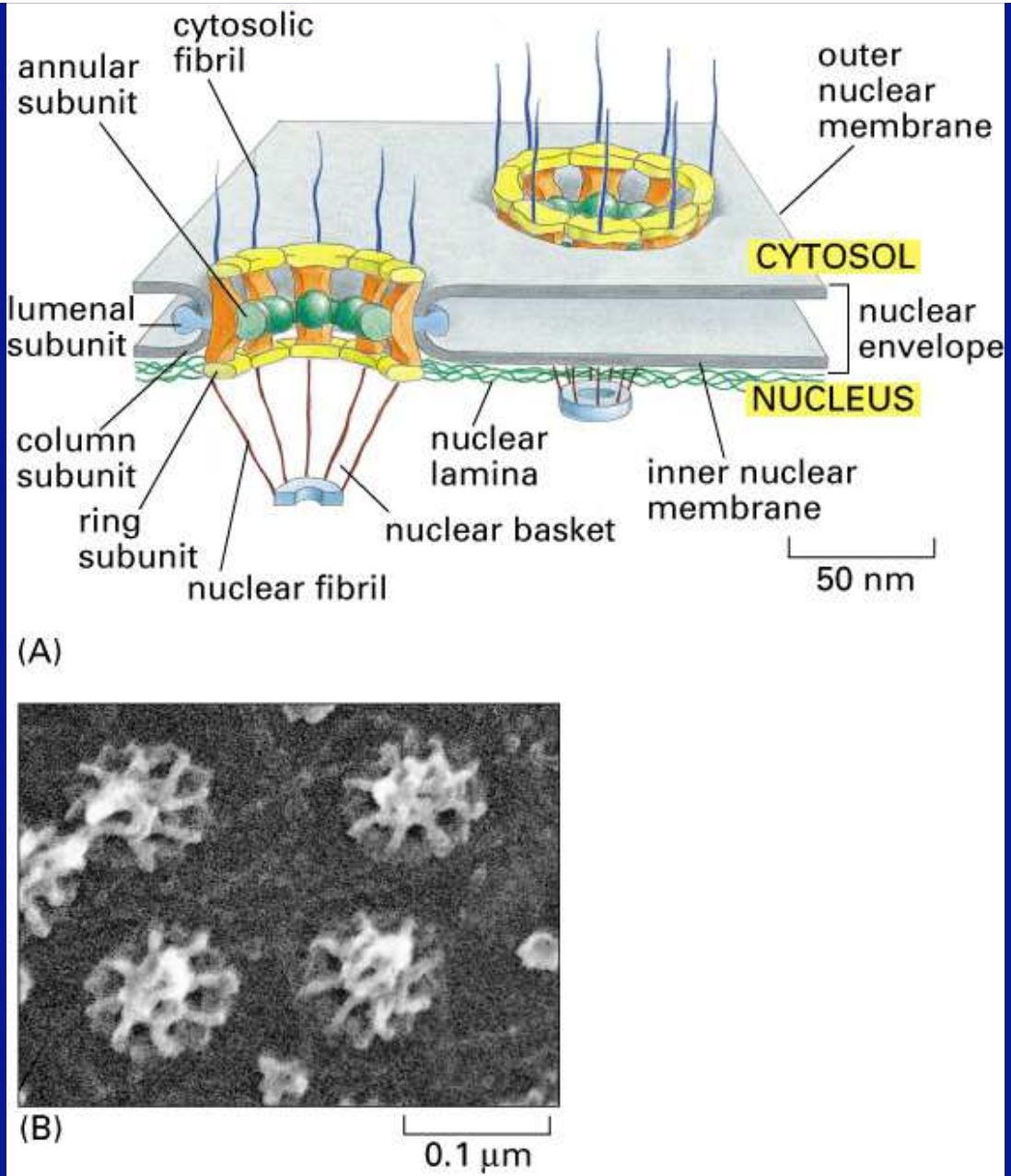
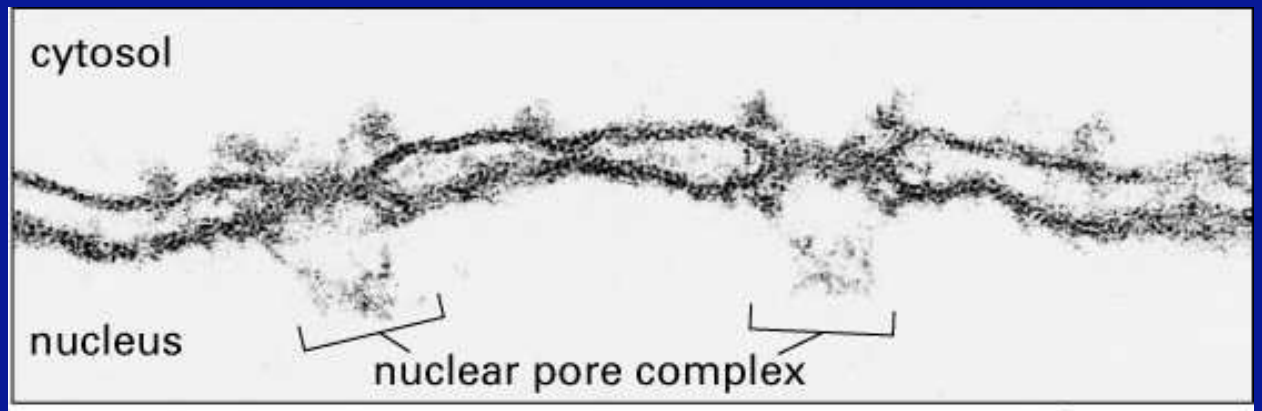


Figure 12–10 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

The openings in the Nuclear Envelope that allow the passage of material in and out of the nucleus are called Nuclear Pores



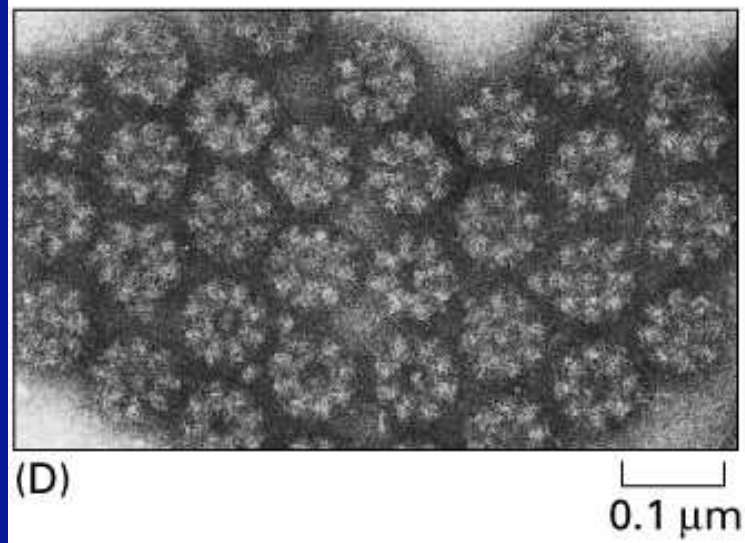


Figure 12–10 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

The openings in the Nuclear Envelope that allow the passage of material in and out of the nucleus are called Nuclear Pores

Small Molecules can diffuse freely through the Nuclear Pore, Larger molecules require active transport

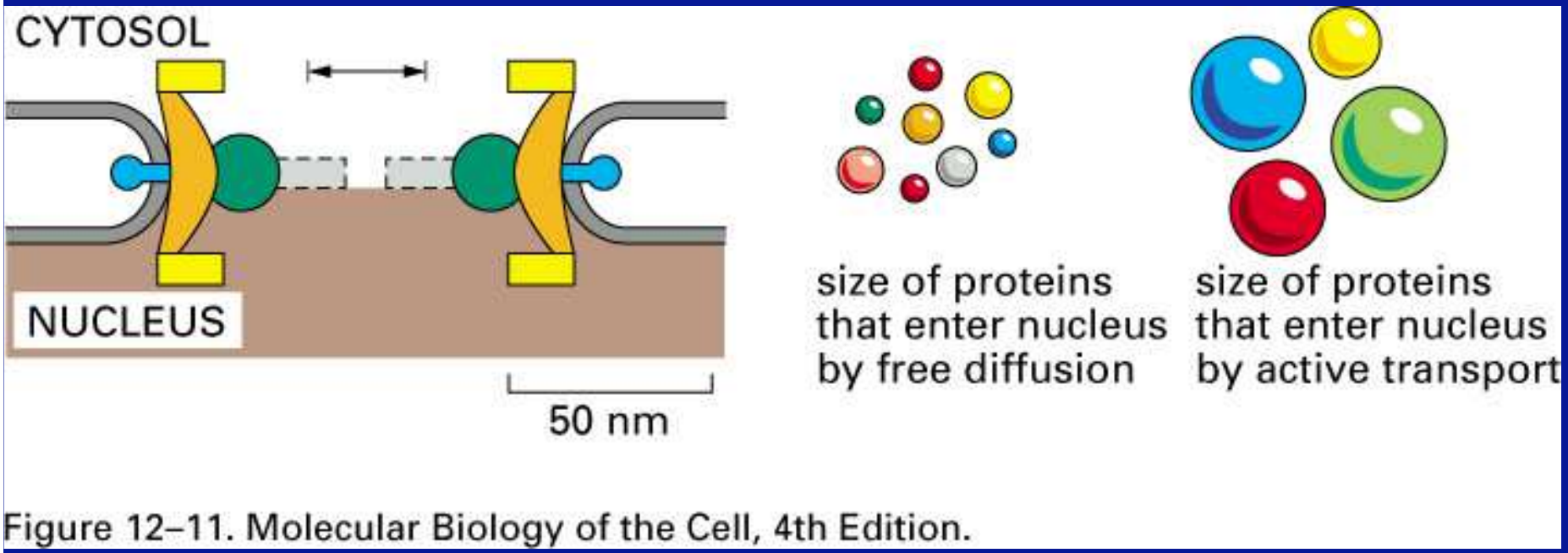
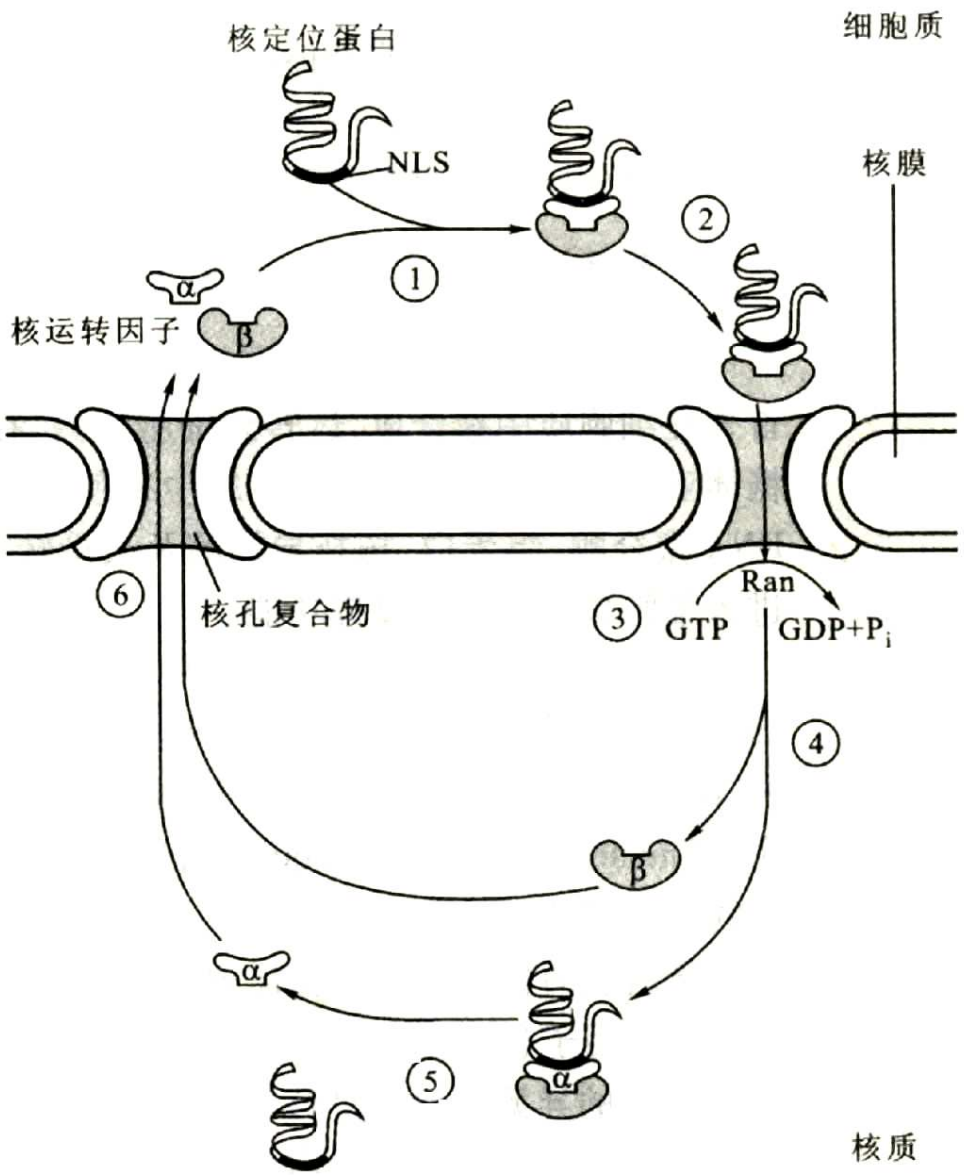


Figure 12–11. Molecular Biology of the Cell, 4th Edition.



核定位蛋白跨细胞核膜运转过程示意图

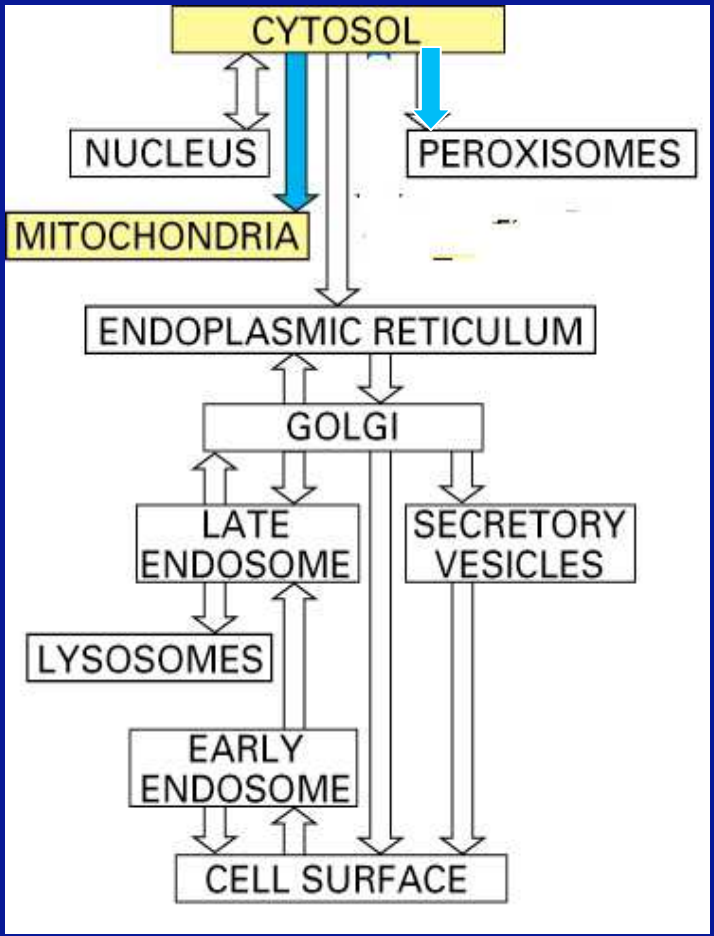
Transport Across Membranes

Into the peroxisome, the mitochondria or the endoplasmic reticulum (ER)

Common features of transport across membranes :
ATP driven
Requires an aqueous channel through the membrane
Protein is unfolded as it passes through the channel

Transmembrane transport can be
post-translational
Mitochondria & Peroxisomes
or co-translational:
ER

Post-translational translocation



An amphipathic alpha helix at the Amino-terminus of a protein can act as a Mitochondrial Targeting Sequence

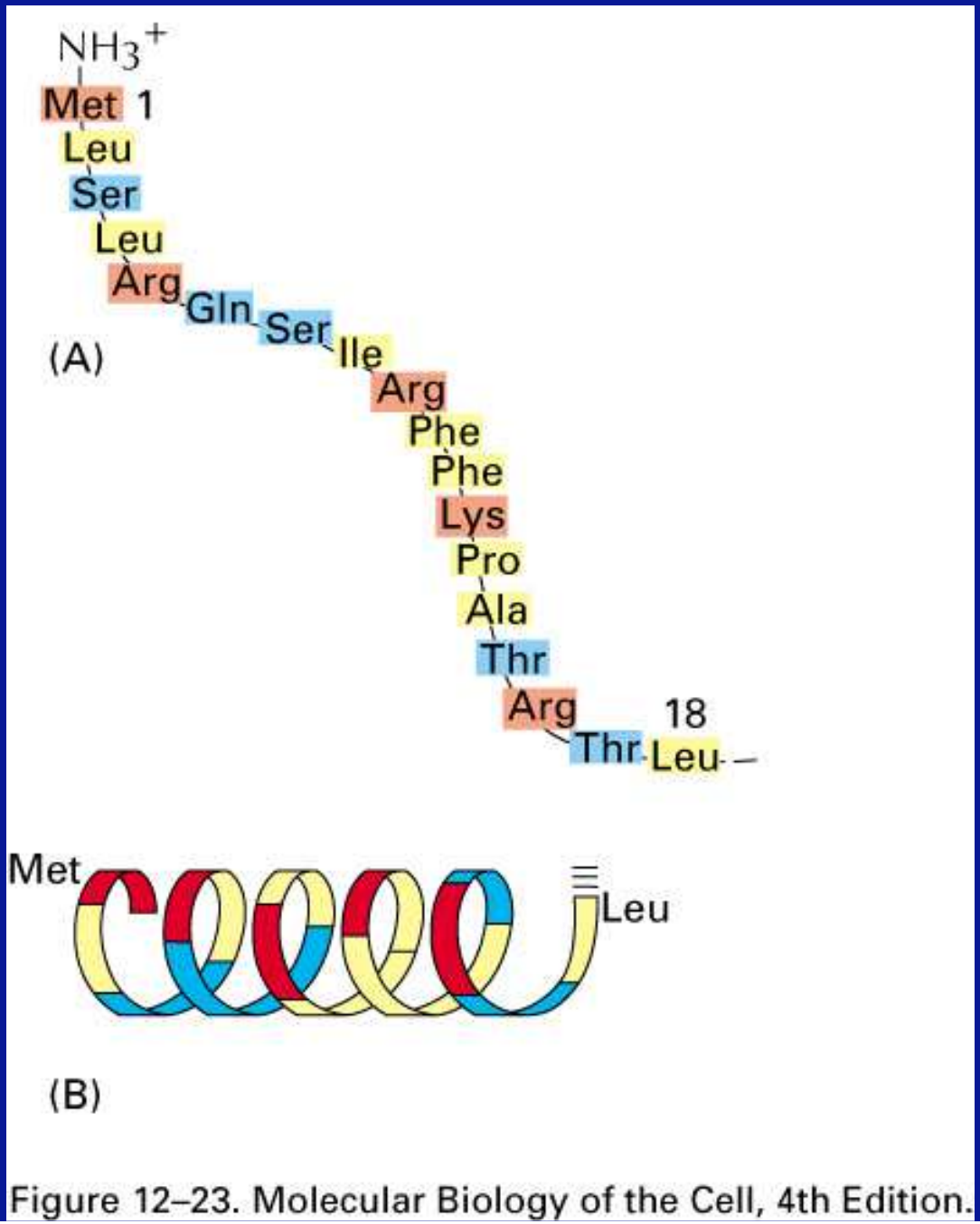


Figure 12–23. Molecular Biology of the Cell, 4th Edition.

Transport from the Cytoplasm to the Mitochondrial Matrix requires two distinct translocation complexes Tom & Tim.

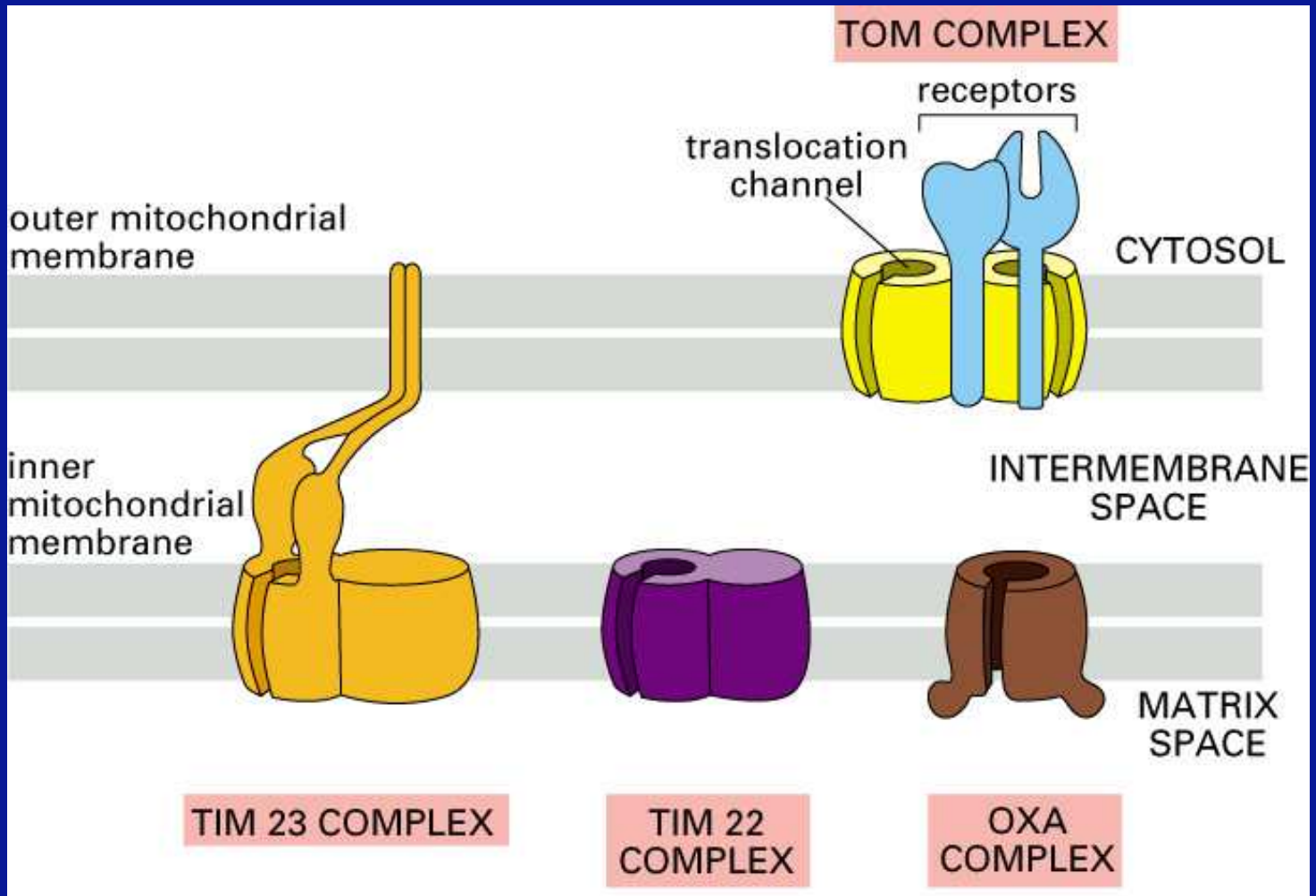


Figure 12–24. Molecular Biology of the Cell, 4th Edition.

Cytoplasmic proteins cross both the outer and inner mitochondrial membranes in a single step

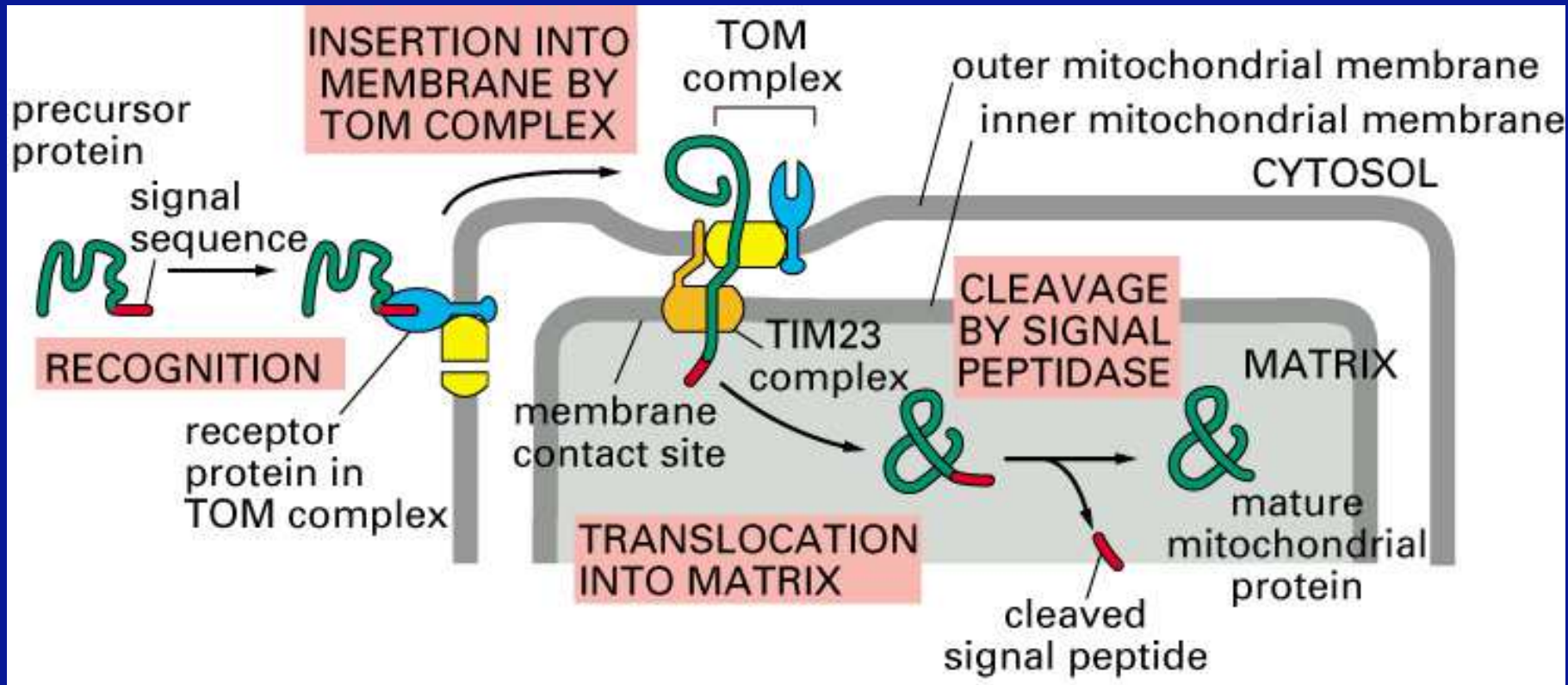


Figure 12–26. Molecular Biology of the Cell, 4th Edition.

The electrochemical gradient across the inner mitochondrial Membrane helps lead the protein through the translocation pore

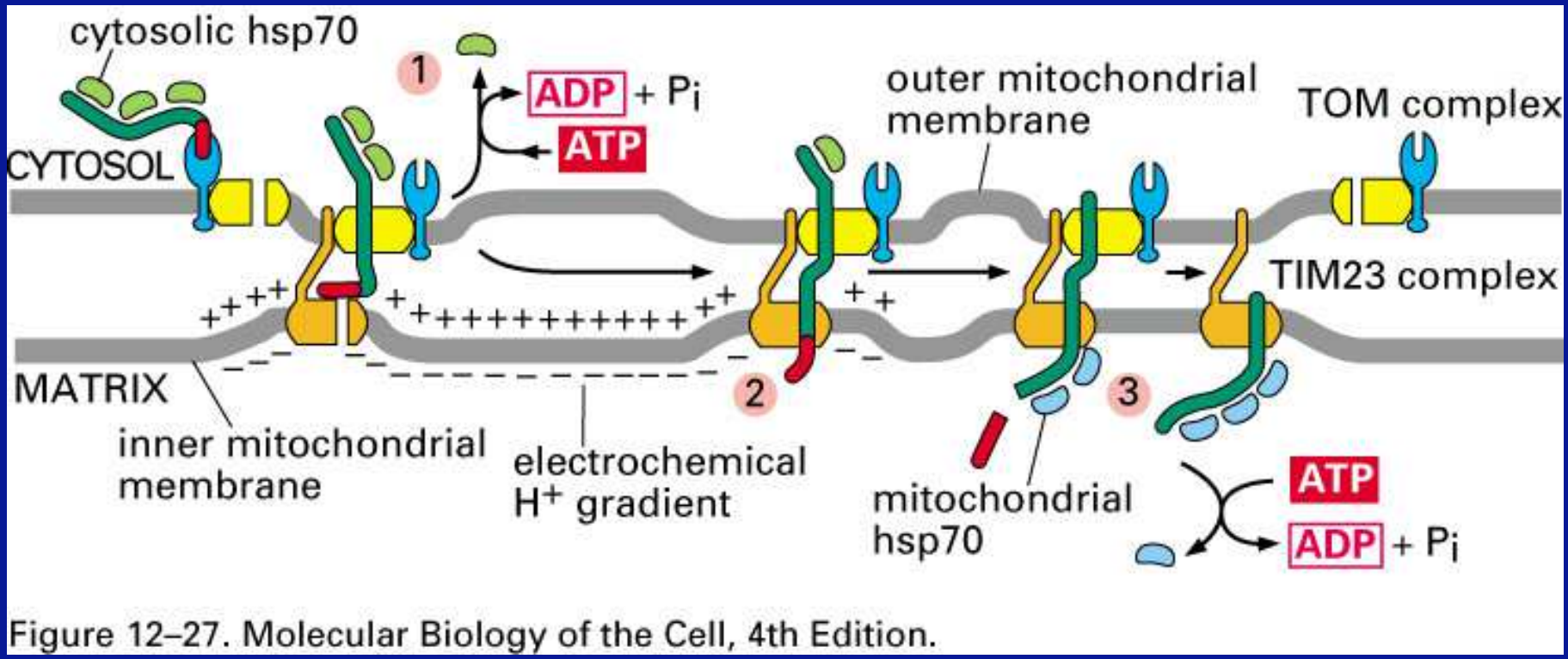
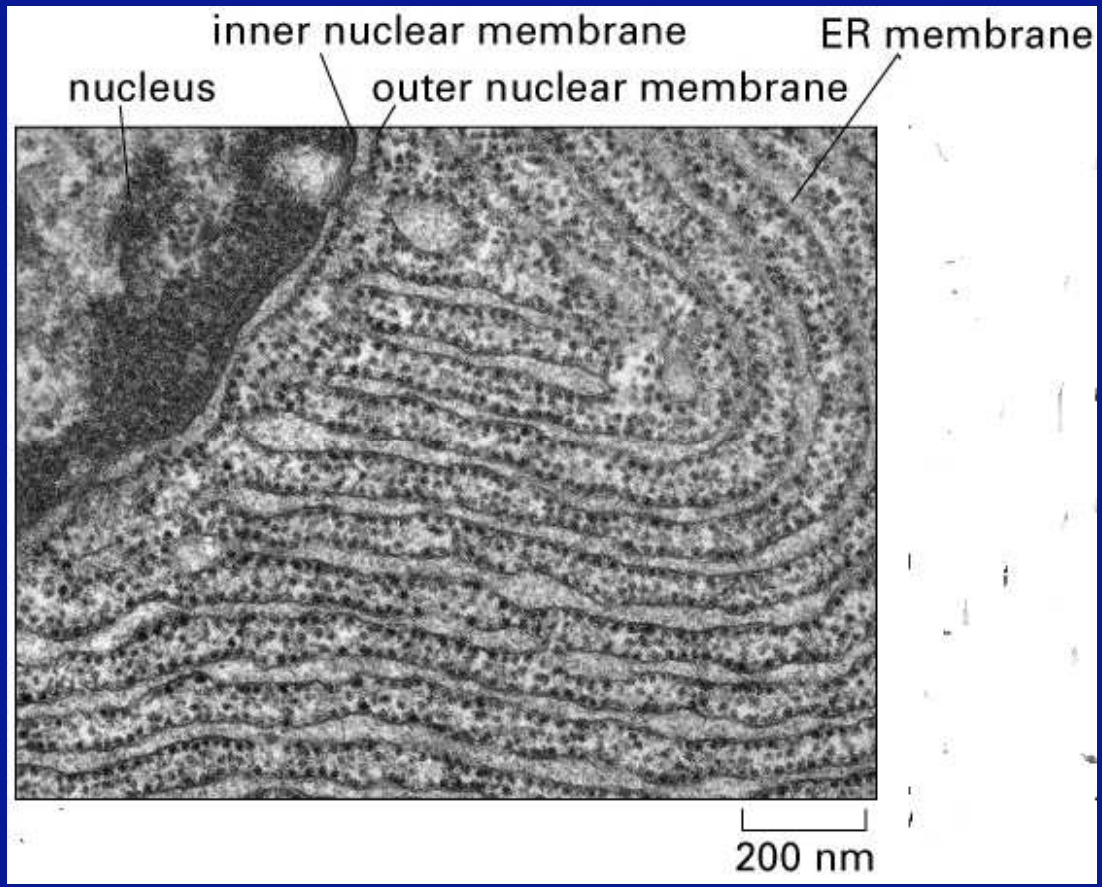


Figure 12–27. Molecular Biology of the Cell, 4th Edition.

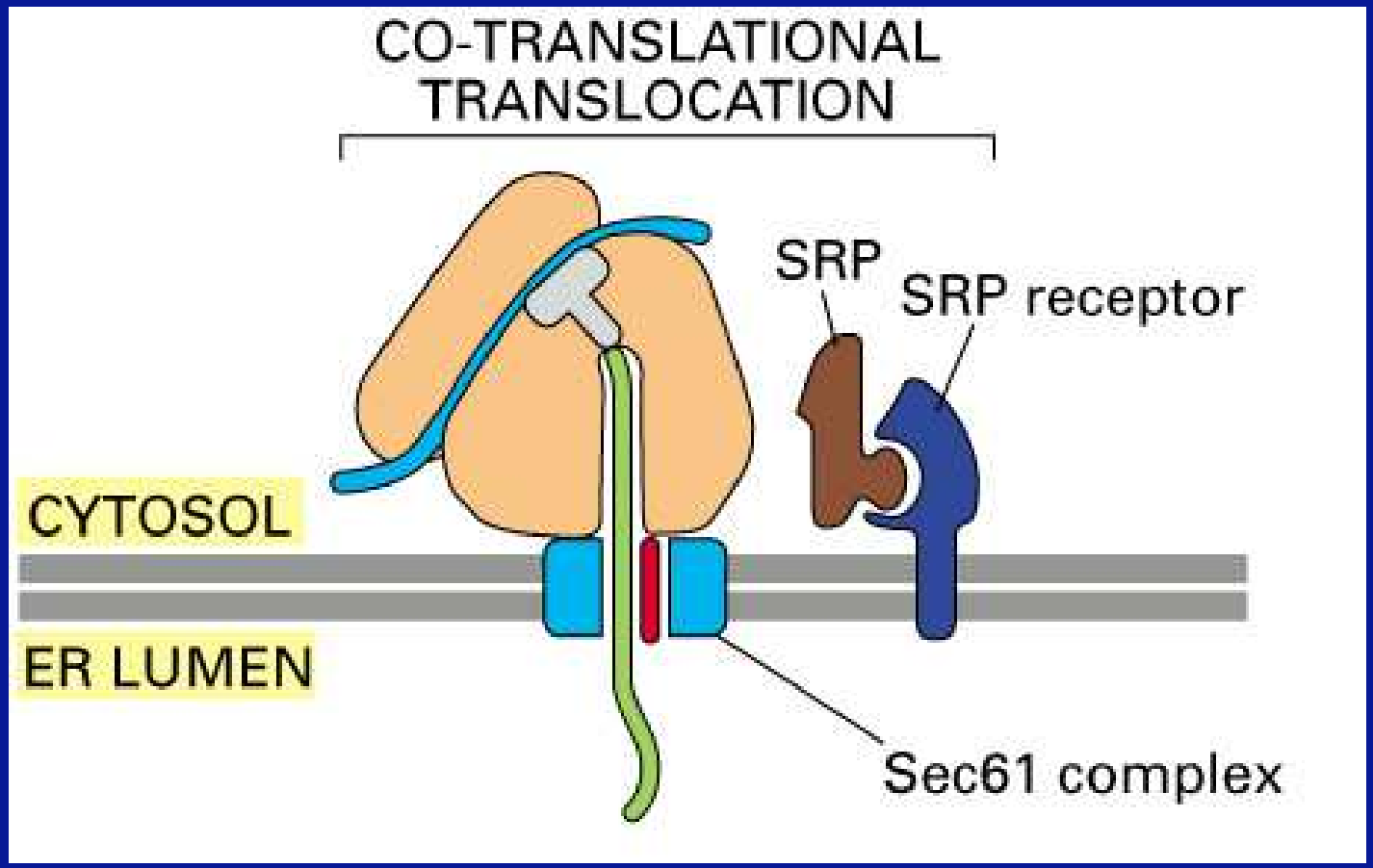
Co-translational translocation

Transmembrane transport into the Endoplasmic Reticulum
(Gateway to the Secretory Pathway)

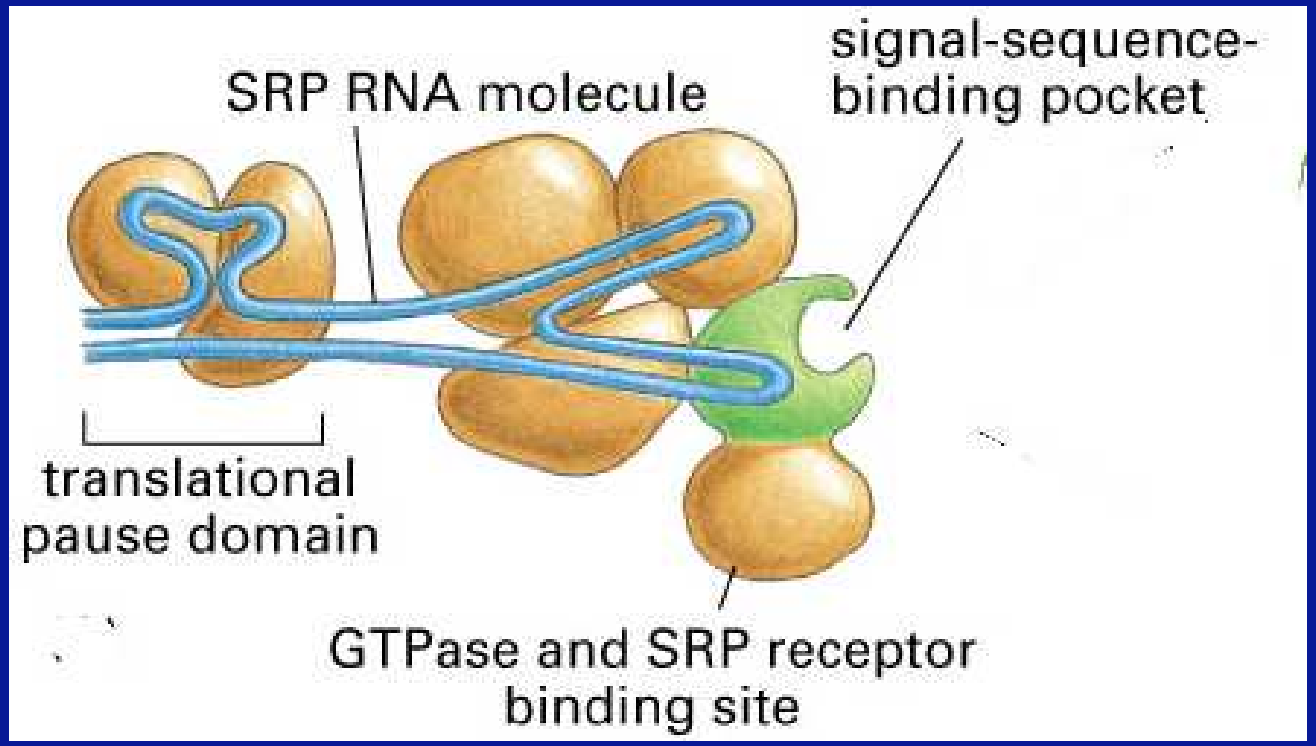
The "Rough ER" - Endoplasmic Reticulum with ribosomes attached is the site of co-translational translocation of proteins into the ER



In co-translational translocation, the nascent protein crosses the ER membrane as it leaves the ribosome



ER targeting signals are recognized by SRP (Signal Recognition Particle)



信号肽序列的一些特征

1）信号肽在多肽链的N端，也有在中间部位的。

2）这个序列一般为10－40氨基酸残基，氨基端至少有一个带正电荷的氨基酸。

3）序列中部一般有一段长度为10－15个具有高度疏水性的氨基酸序列，这段序列对多肽的跨膜起了决定性作用。

4）在信号肽的C端有一个可被信号肽酶识别的位点，此位点上游有一段疏水性较强的5肽。

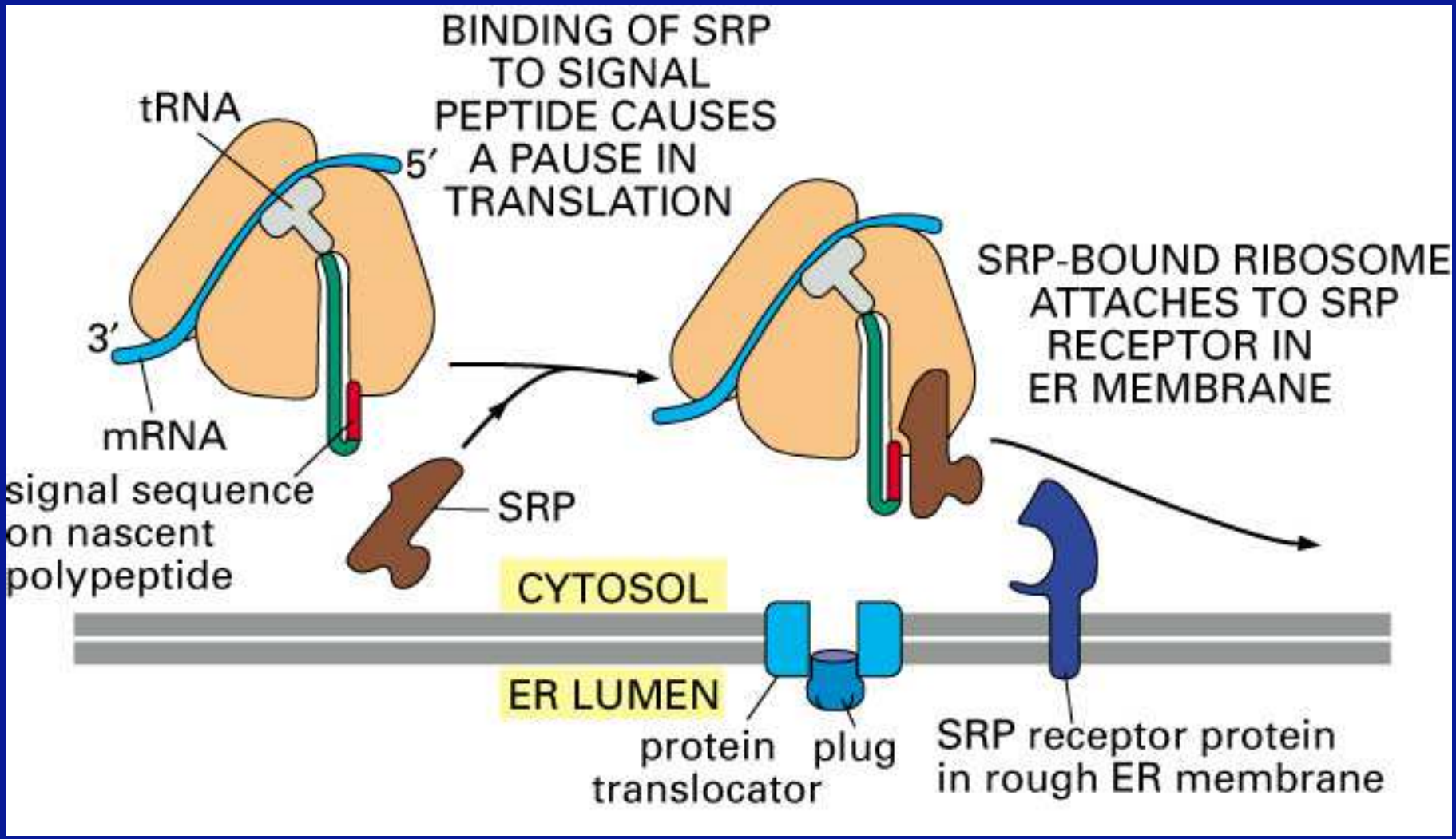
5）酶切位点上游的第一个及第三个氨基酸常为具有一个小侧链的氨基酸如丙氨酸。

Examples of different types of signal sequence

一些真核细胞多肽氨基端的信号肽结构

疏水氨基酸残基以加重线字母表示，碱性氨基酸残基用带网纹字母表示

SRP recognizes signal sequences as they come off the ribosome and carries the mRNA-ribosome-nascent polypeptide complex to the ER



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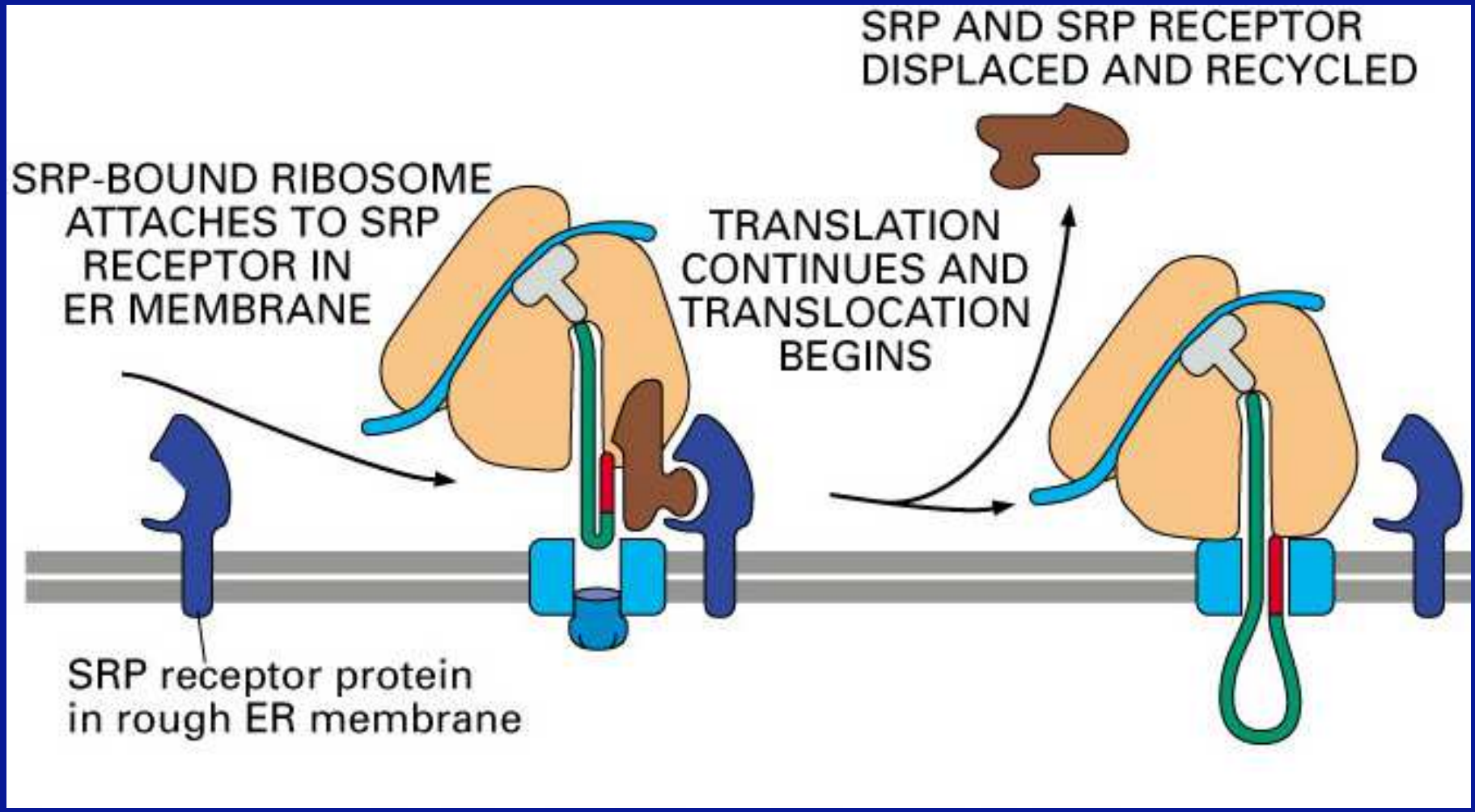
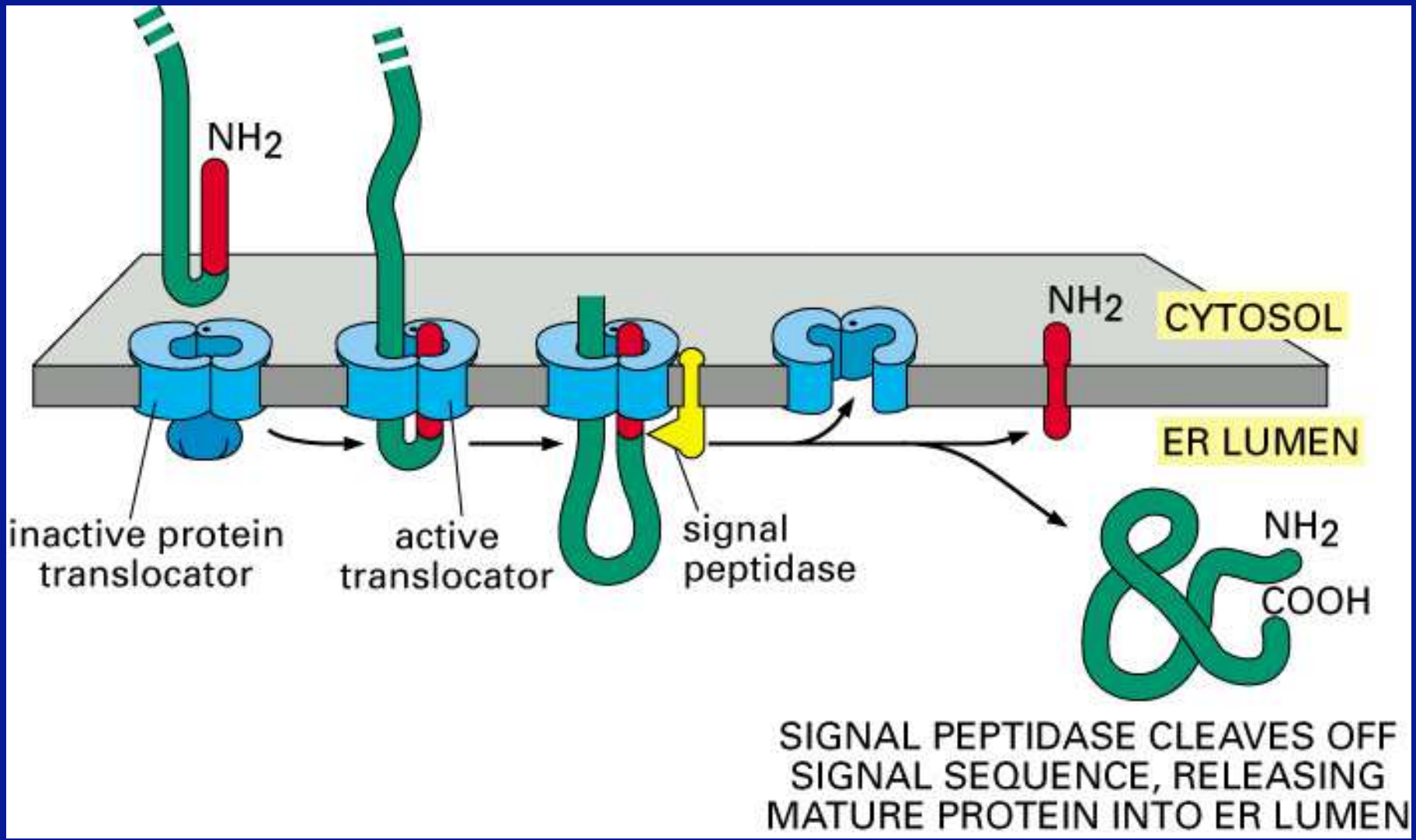


Translocation of a soluble protein into the ER lumen

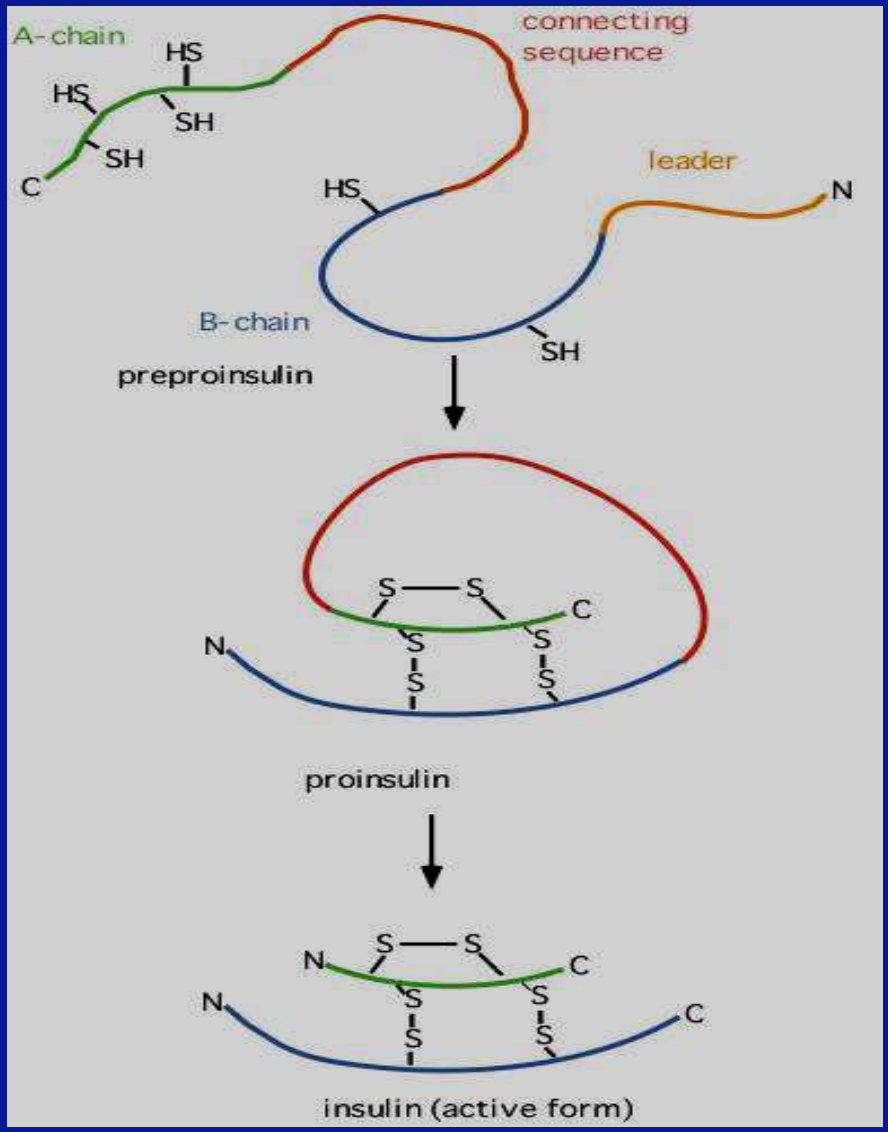


Once proteins have entered the endoplasmic reticulum they can be move on to other compartments of the secretory pathway or out of the cell entirely. But in addition to acting as a port of entry for proteins into the secretory pathway, two additional important functions for protein targeting take place in the ER.

- N-linked glycosylation of proteins
- Protein folding

Protein modification

- Cleavage:
  - To remove signal peptide
  - To release mature fragments from polyproteins
  - To remove internal peptide as well as trimming both N- and C-termini



- Covalent modification:
  - Acetylation;
  - Hydroxylation;
  - Phosphorylation;
  - Methylation;
  - Glycosylation;
  - Addition of nucleotides.

Protein degradation

- Different proteins have very different half-lives. Regulatory proteins tend to turn over rapidly and cells must be able to dispose of faulty and damaged proteins.

Protein degradation: process

- Faulty and damaged proteins are attached to ubiquitins (ubiquitinylation).
- The ubiquitinylated protein is digested by a 26S protease complex (proteasome) in a reaction that requires ATP and releases intact ubiquitin for re-use.

- In eukaryotes, it has been discovered that the N-terminal residue plays a critical role in inherent stability.
  - 8 N-terminal aa correlate with stability:
    Ala Cys Gly Met Pro Ser Thr Val
  - 8 N-terminal aa correlate with short $t_{1/2:}$
    Arg His Ile Leu Lys Phe Trp Tyr
  - 4 N-terminal aa destabilizing following chemical modification:
    Asn Asp Gln Glu