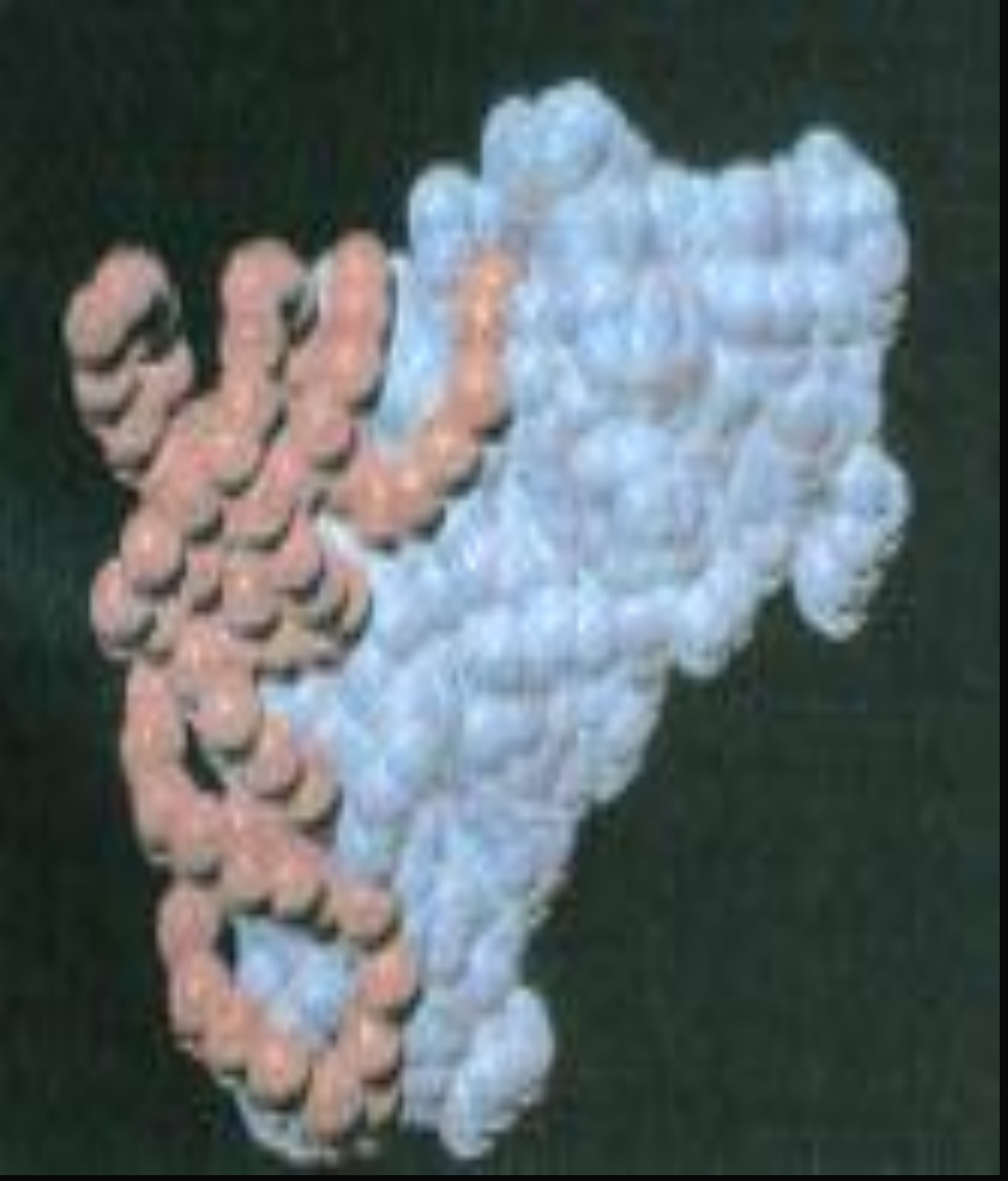


第五章

蛋白质翻译

来源：不详

5.1. 基本元件

5.2. Genetic Code

5.3. peptide synthesize

5.4. 保证peptide准确翻译的机制

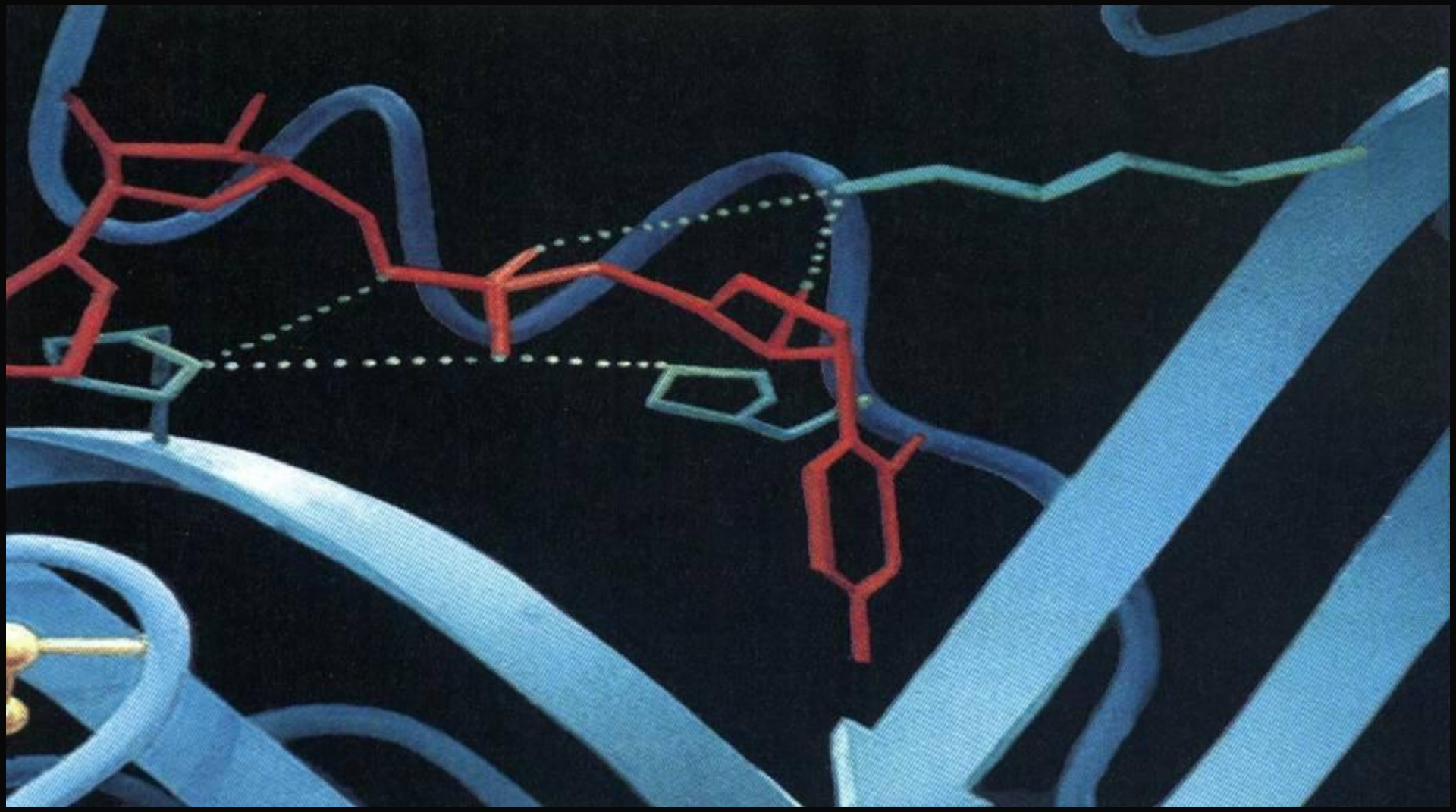
5.5. Central Dogma 的发展

5.1.基本概念

- **the second step of gene expression**
- **Multiple & complex assessment**
- **tRNA as a adapter for codon & amino acid**
- **tRNA loading aa by aa-tRNA^{aa} synthetase (AARS) & paracodon**
- **tRNA recognition codon by anti-codon**

- codon; universal triplex codon
  two of three reading codon
  paracodon
  codon in codon
- codon degeneracy; wobble hypothesis
  isoacceptor
- codon usage (codon bias)
- mechanism of accurate translation
  initiation, loading, elongation, proofreading

5.2. 基本元件



(Source:*Irving Geis/Peter Arnold,Inc.)*

5.1.1. tRNA

- mini RNA, 4s, (70-80 Nt)
- tRNA ^{phe}, 77Nt cloverleaf form (1964 Holly R.)
- Nt more modified by methylation
- 5 arms & 4 loops

KUNGL. VETENSKAPS AKADEMIEN

Nobelpriset i kemi 2009

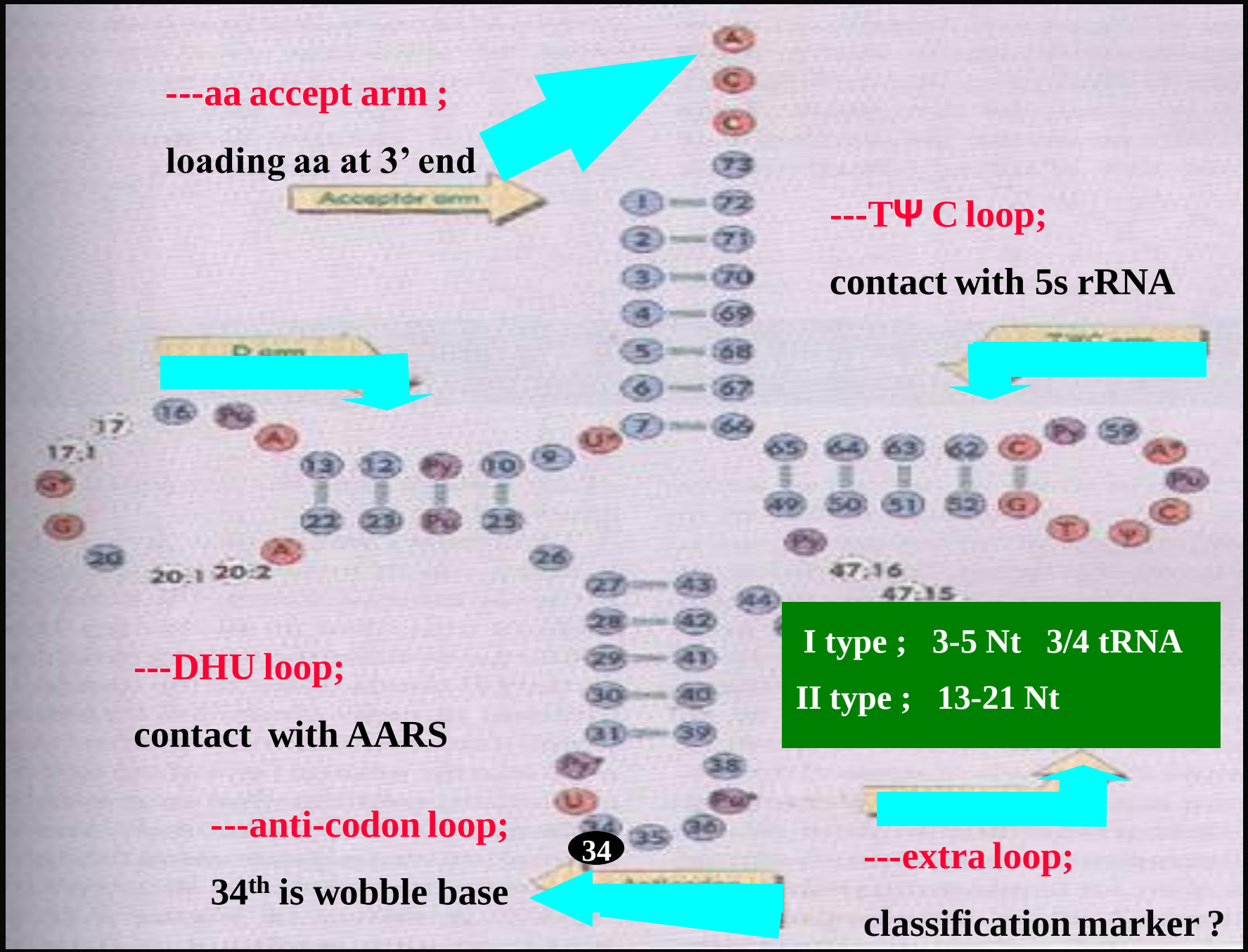
Venkatraman Ramakrishnan
MRC Laboratory of Molecular Biology, Cambridge, UK

Thomas A. Steitz
Yale University, New Haven, CT, USA

Ada E. Yonath
Weizmann Institute of Science, Rehovot, Israel

"for studier av ribosomens struktur och funktion"

"for studies of the structure and function of the ribosome"



(来源：分子生物学（2007），郑用琏，第179页)

- Capping
  - ✓Cap 0: $m^7GpppXpYp$
  - ✓Cap 1: $m^7GpppXmpYp$
  - ✓Cap 2: $m^7GpppXmpYmp$
  - ✓Help the splicing of the first intron
- Pre-RNA tailing
  - ✓A poly(A) tail (50-200±) be added at -20 Nt ± tailing signal (AAUAAA) from 3'-end of Pre-RNA
  - ✓Specific endonulcease recognizes AAUAAA and the following GUGUGUG, cuts within the sequence, adding poly(A)s at 3'-end

- RNA internal methylation
  - m^5C
  - m^6A
  - Modifications of tRNAs
- Pre-RNA splicing
  - ✓ Introns be classified into I, II, III by junction sequence
  - ✓ Group I splicing model: 5'---exon----U ------intron------G ----exon-----3'
    - ✓ CCS (central core sequence)
    - ✓ Internal guide sequence (IGS): within the intron close to 5' junction seq
    - ✓ 3 times of trans-esterification between G & U
    - ✓ RNA auto-splicing as Ribizyme
  - ✓ Group II splicing model: 5'----exon----- GUGCG---------B.S----Py AU ----exon---3'
  - ✓ Group III splicing model: 5'---exon--- GU--------intron--------AG ------exon----3', SnRNAs

Chapter 5 Protein translation

- tRNA
  - ✓mini RNA, 4s, (70-80 Nt)
  - ✓Nt more modified by methylation
  - ✓tRNA ^{phe}, 77Nt cloverleaf form
    - ✓ Aa accept arm, DHU loop (contact with AARS), anti-codon loop, TΨC loop (contact with 5S rRNA), extra loop
    - ✓ Paracodon: a number of Nts, on tRNA, contact with AARS

- **Paracodon**

  ---由若干Nt组成，存在于tRNA不定位置上

  ---与AARS侧链基团的分子发生特异的“契合”

  ---成为tRNA准确负载氨基酸的机制之一

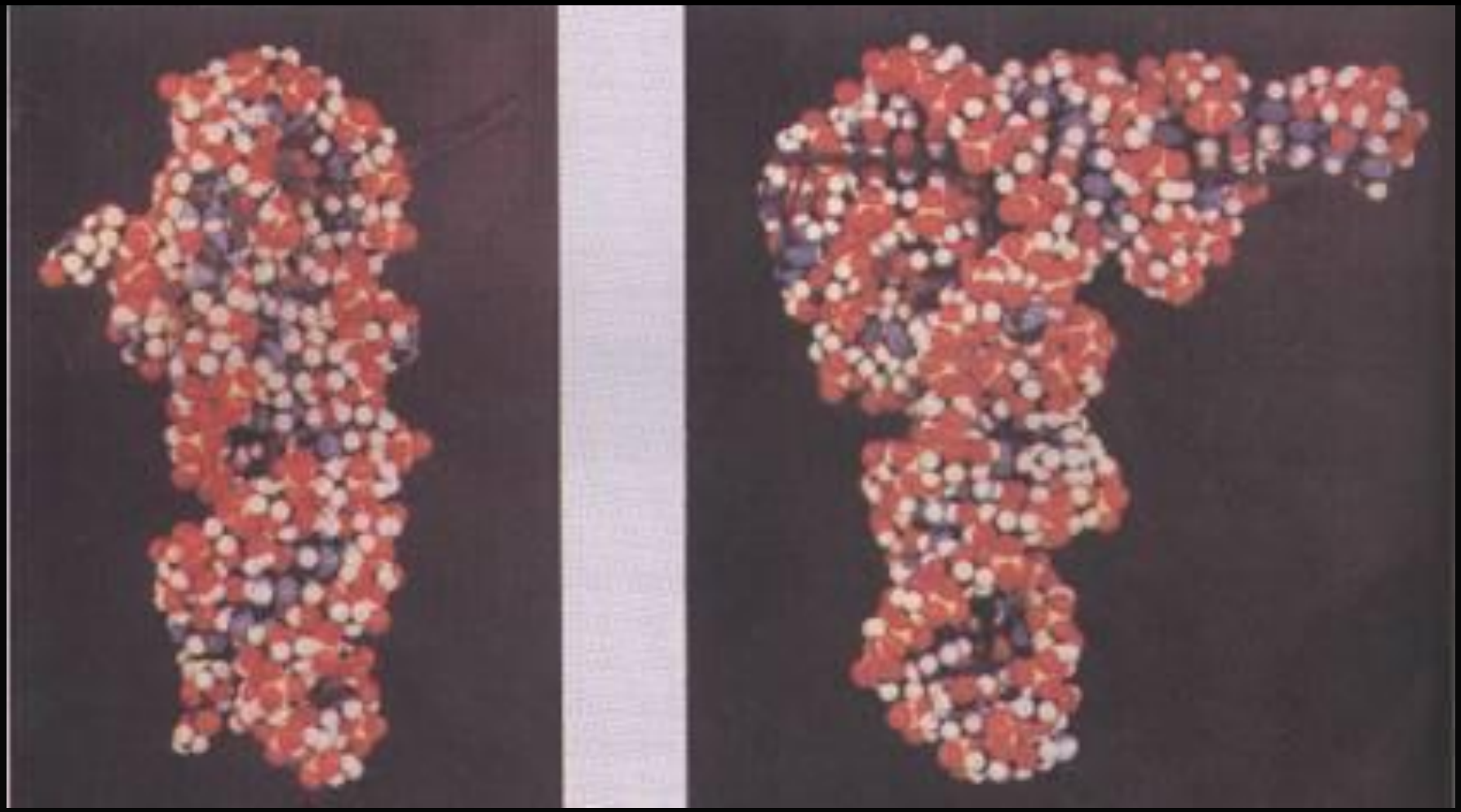
- **tRNA的”L”三维结构与功能**

  “L”构型的结构力

  ---二级结构中双链区的碱基堆积力和氢键

  ---二级结构中非双链区在“L”结构中，形成氢键结合

tRNA的”L”三维结构

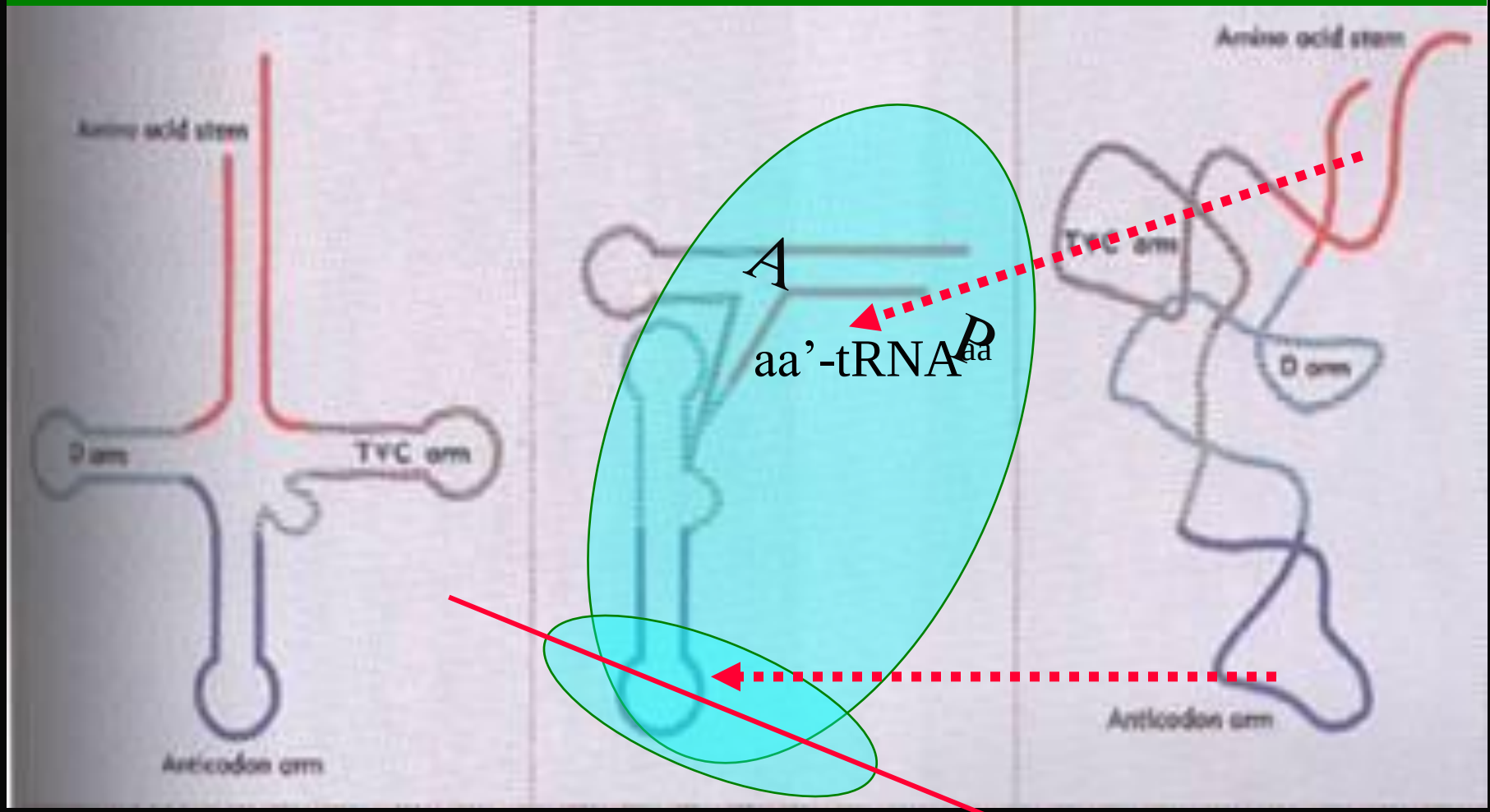


(来源：分子生物学（2007），郑用琏，第180页)

---aa accept arm 位于“L”的一端，契合于核糖体的肽基转移酶结合位点 P ——— A，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

“L”结构域的功能



(来源：不详)

--- 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

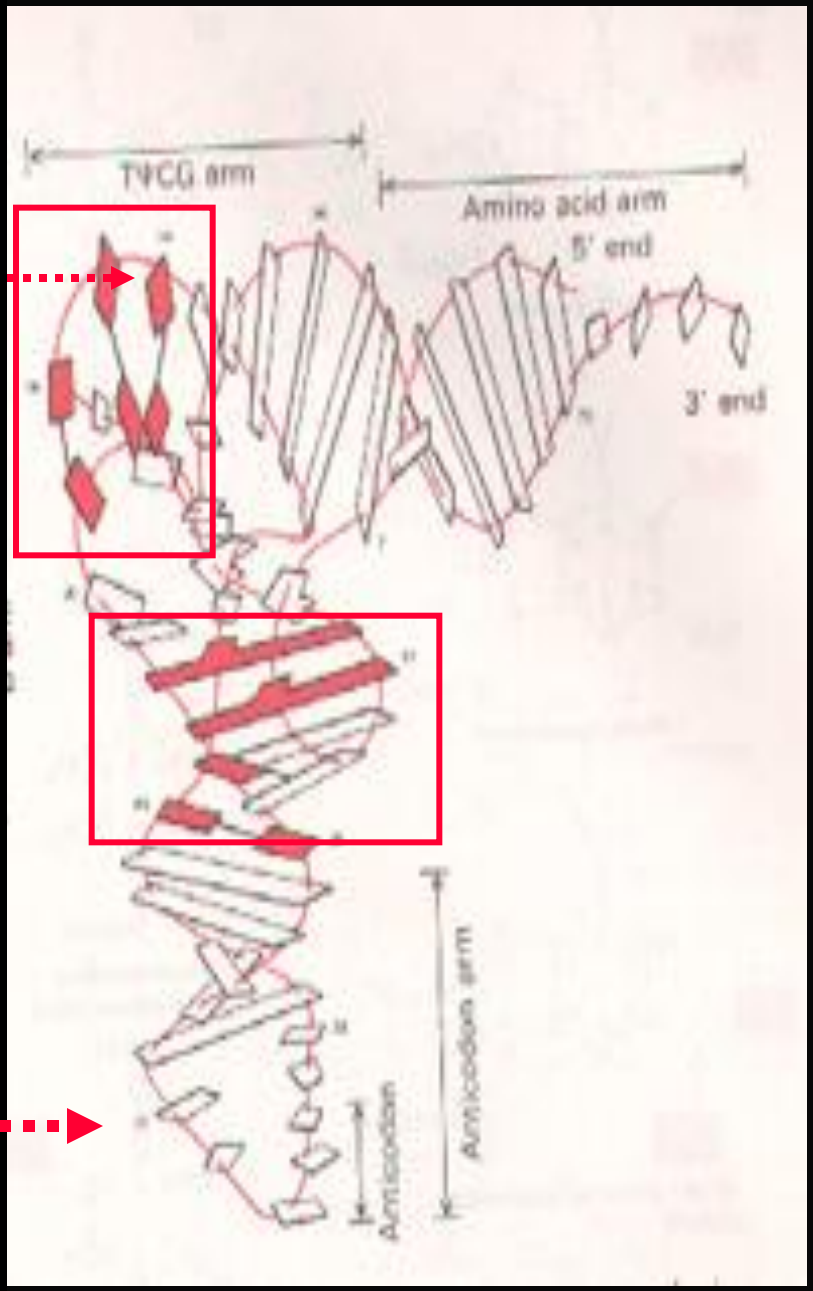
wobble base

位于“L”结构末端

堆积力小

自由度大

使碱基配对摇摆



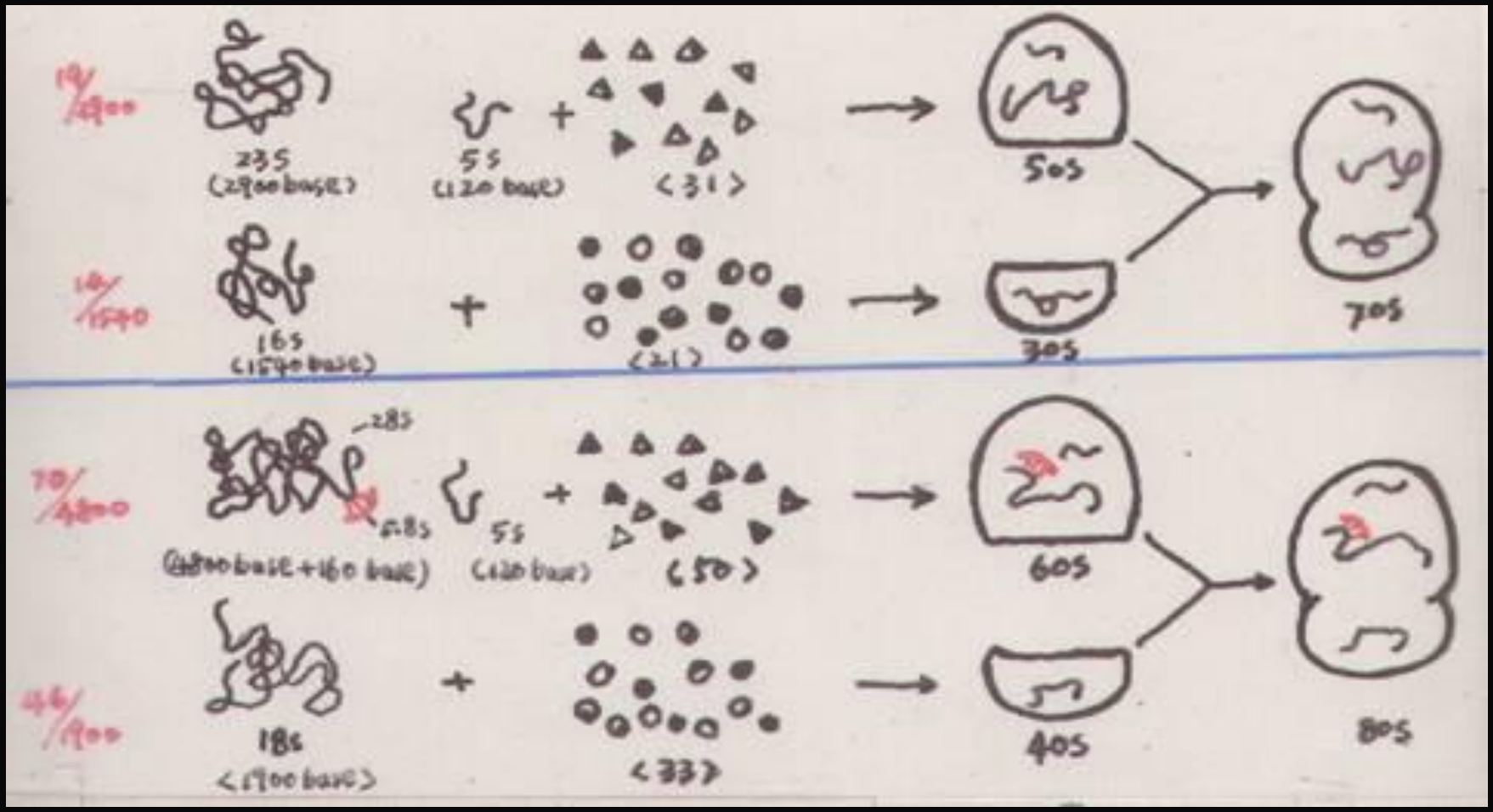
Source:Quigley,G.J,Structural domains of transfer RNA moleculars,Science 194:197

5.1.2. rRNA

Ribosomal genes (rDNA) are different in several ways from other nuclear gene

- *have GC content of 60% & Rich methylation*
- *each cell contains from several hundred to over 20,000 copies of rDNA gene*
- *rRNA synthesized in nucleolus and was stimulated by low ionic strength & Mg^{+2}*

- **Prokaryote** 23s, 16s, 5s / **Eukaryote** 28s-5.8s, 18s, 5s
- Rich methylation (m^2U, m^3A, m^3U, m_2^6A(二甲基)…)



(来源：不详)

- 5s RNA 与TΨ C loop of tRNA部分互补，并可配对
- In Prok.

  3’-end of 16s rRNA rich CCU conservative seq.

  complementary with

  5’ leading seq. of mRNA

  Shine-Dilgarno seq. of rich AGG

(来源：分子生物学（2007），郑用琏，第183页)

● 23s rRNA

--- 6 domains

---有的与对抗生素的抗性有关

---2660±Nt region α -I loop (alpha Sarcin)

binding with complex of aa-tRNA^{aa}~(EF)-Tu~GTP

(引起核糖体变构！！)

$G_{2661} \rightarrow C$ ， aa-tRNA^{aa} into A site go down

---G_{2252}, G_{2253}双突变为C, 将对转肽酶的活性产生抑制

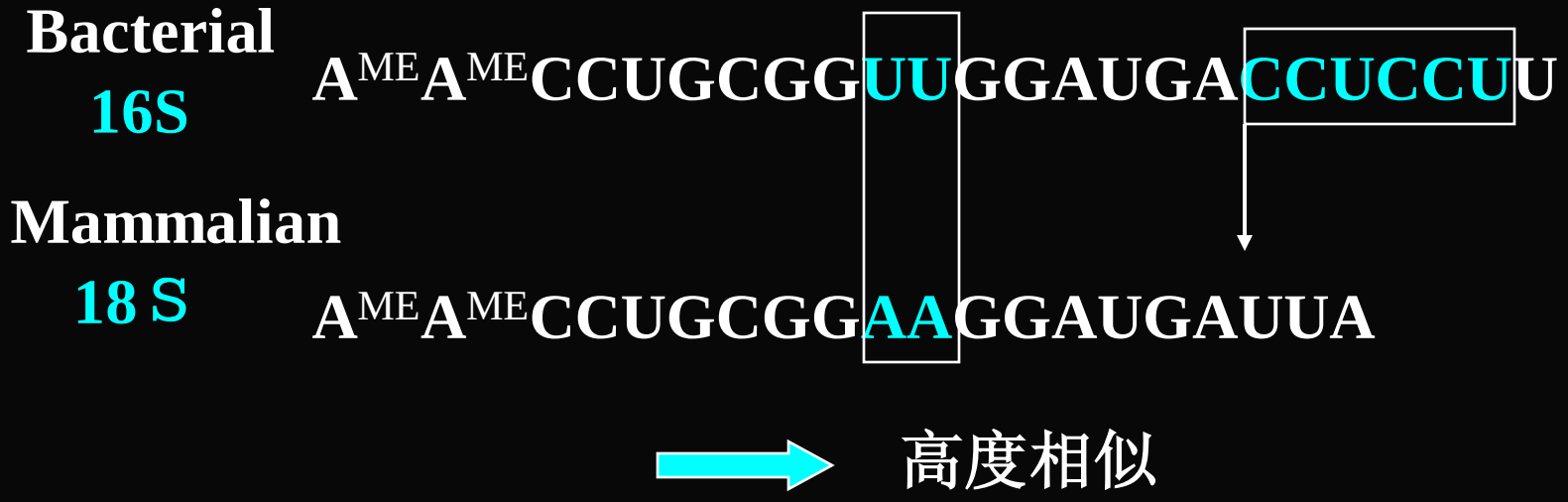
In Euk.

3’-end of 18s rRNA 与原核生物高度相似,

但无与 S.D.seq.互补的保守序列

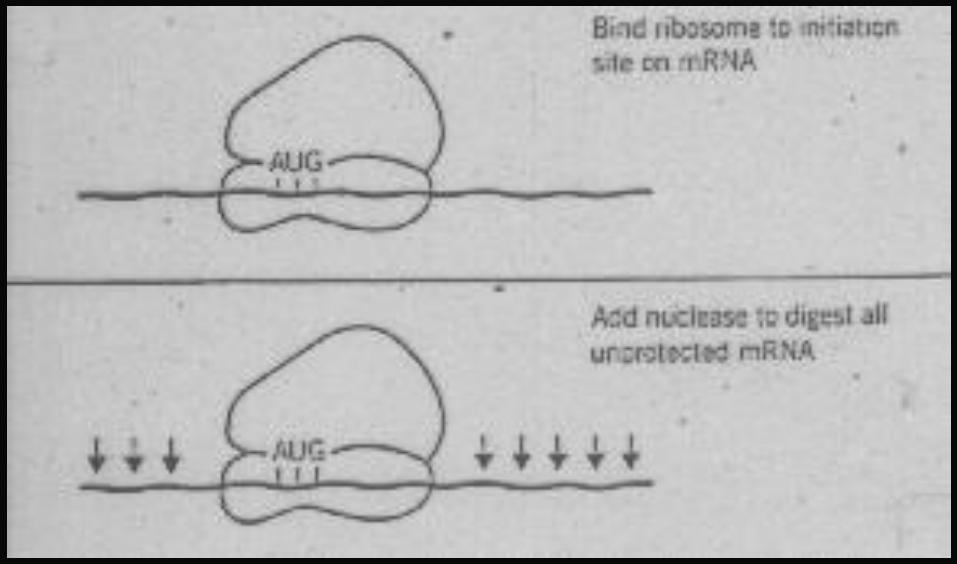
在 mRNA的AUG上游存在CCACC核糖体scanning seq

成为核糖体识别第一个 AUG的信号



5.1.3. mRNA

- In Prokaryote



(Source:Molecular Biology(2002),Robert F.Weaver,Page539）

5’-end; 300±Nt leading seq. (A/G----------AUG)

Shine-Dalgarno seq. (S.D seq) GGAGG

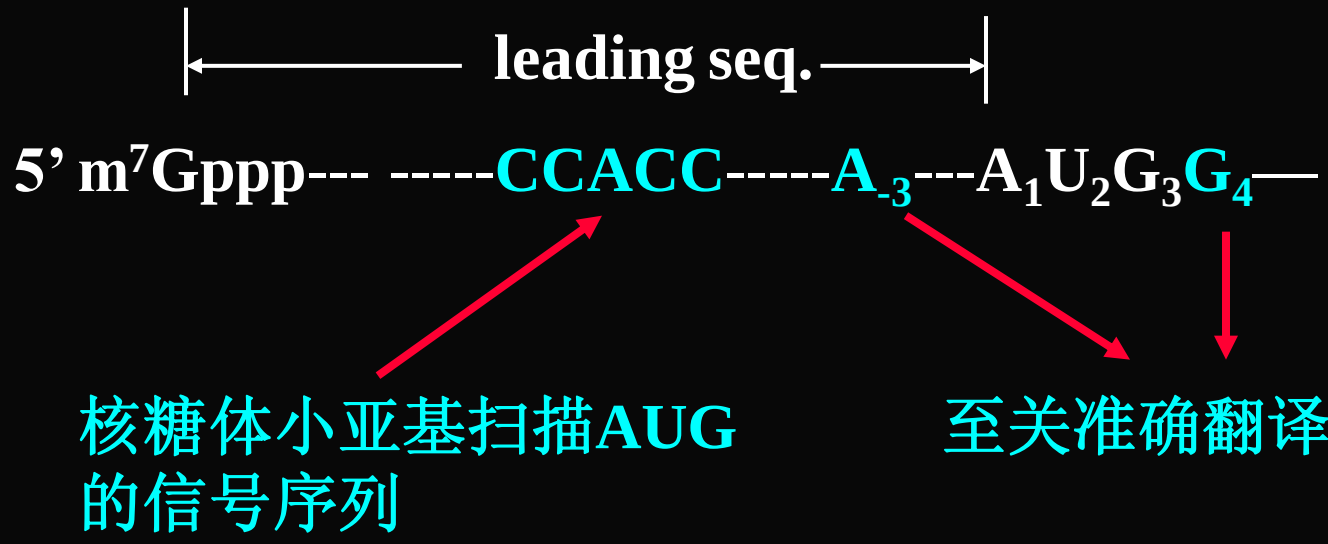
S.D seq----------AUG

7－9Nt better

rich A,U, → G mut. translation go down

poly-cistron

- **In Eukaryote mono-cistron**

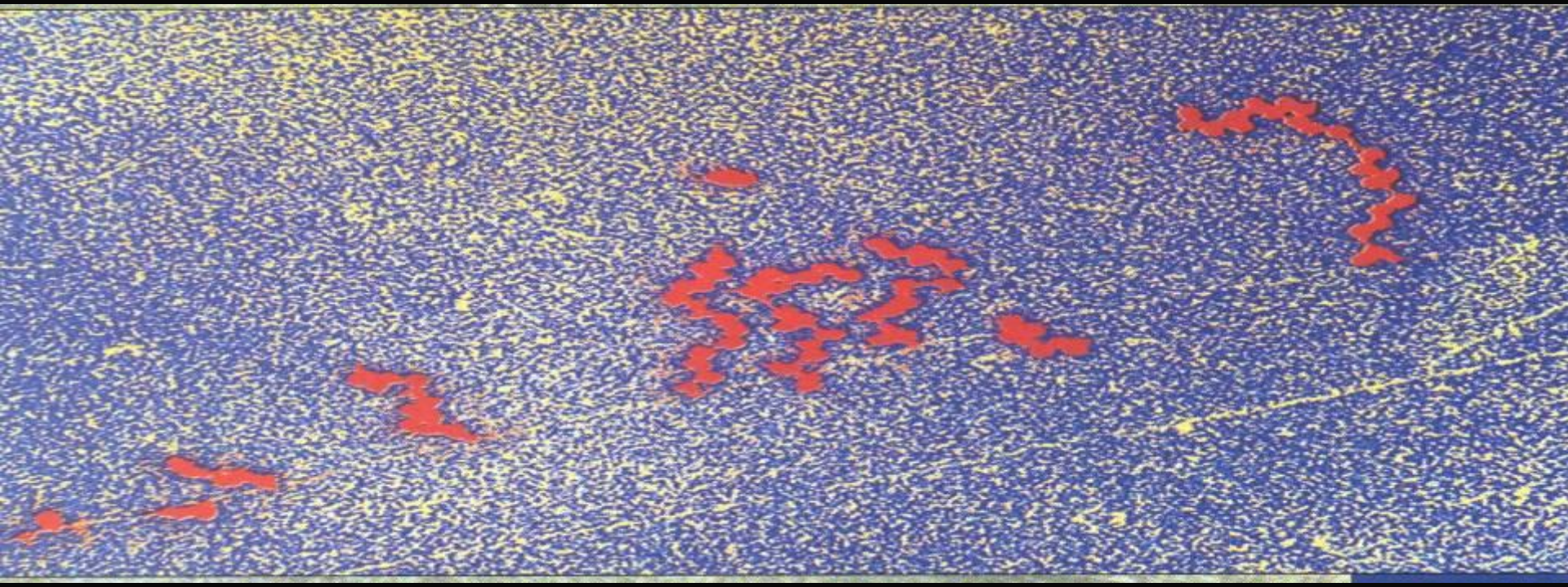


But mRNA of chloroplast shows similarities to prokaryote

type1; S-D seq. with greater secondary structure in L. S.

type2; rich AU with little secondary structure in L. S. polycistron

5.2. Genetic Code



Source:*Oscar Miller/SPL/Photo Researchers,Inc*

5.2.1. Universal triplet codon

codon的特征

- codon是mRNA 上连续排列的三个核苷酸序列，并编码一个氨基酸信息 的遗传单位
- codon具有四大生物系统的通用性与保守性（除mt）
- 在一个基因序列中 codon具有不重叠性和无标点性

密码子的破译 (1968. nobel prize)

Marshall Nirenberg (1961)

In vitro **Poly(U) → poly(Phe) peptide**

Poly(C) → poly(Pro) peptide

Poly(A) → poly(Lys) peptide

Poly(G) → poly(Gly) peptide

But

poly(UCUCUC…) → poly(Ser-Leu-Ser-Leu…)

UCU/CUC → Ser/Leu ?

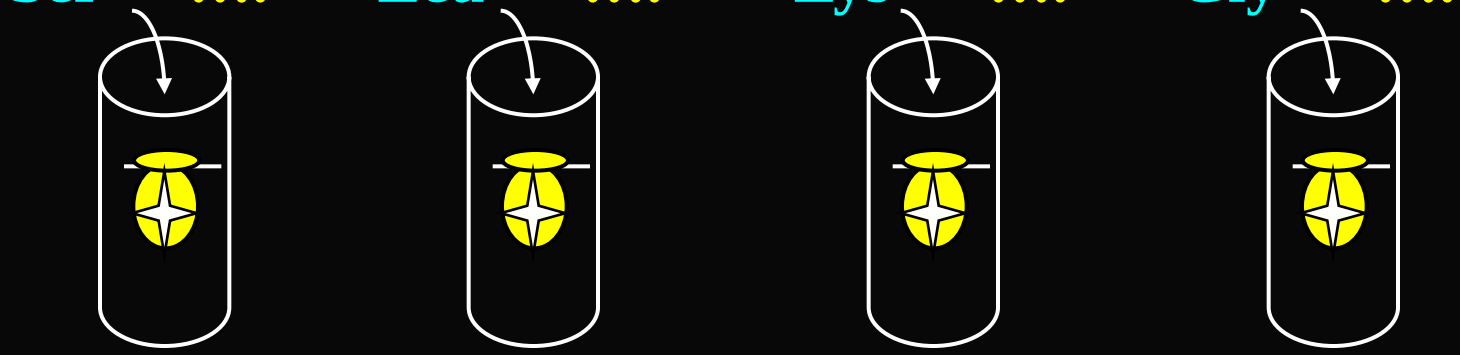
M. Nirenberg & P. Leder (1964. Science 145;1399)

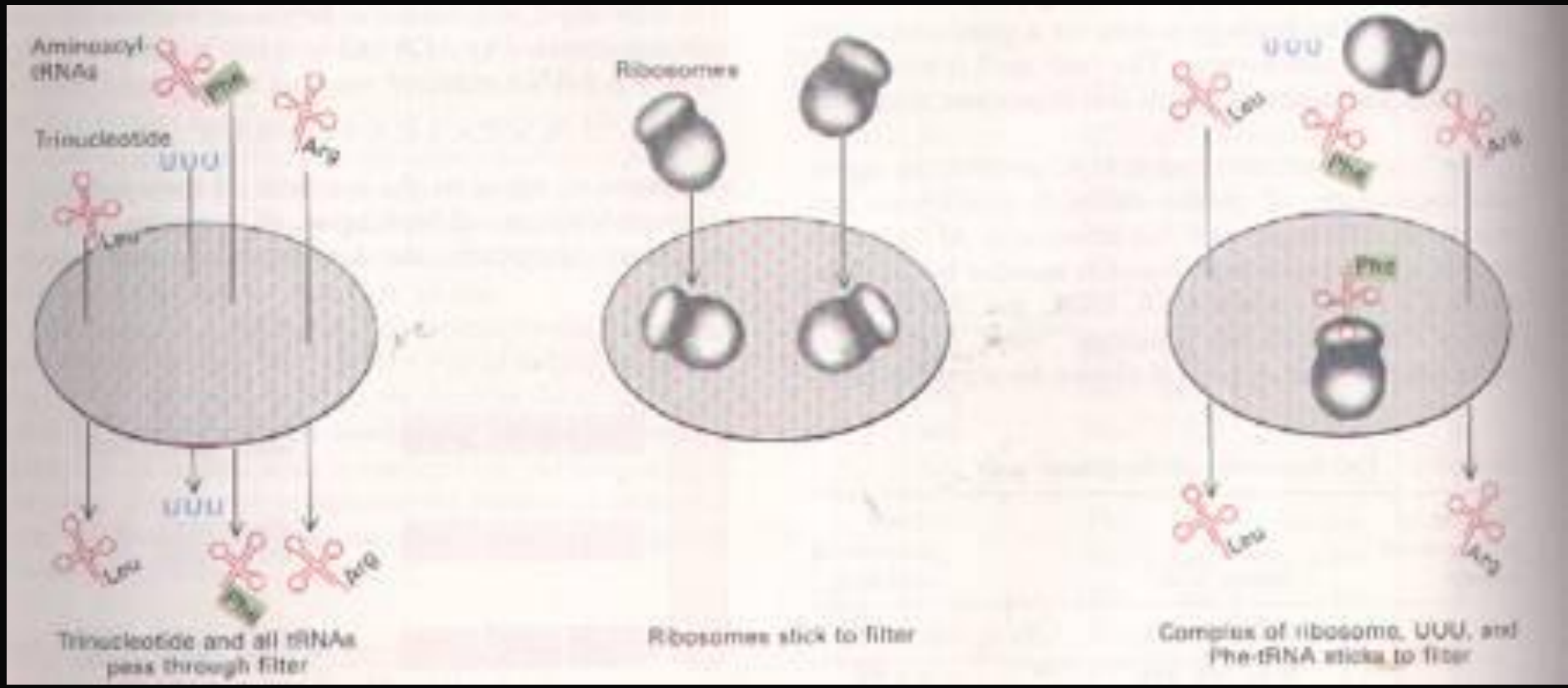
In vitro

UCU
(trinucleotides)

- Ser-^{C14}, Leu, Lys, Arg,…
- Ser, Leu^{-C14}, Lys, Arg,…
- Ser, Leu, Lys^{-C14}, Arg,…
- ……

→ **tRNA^{aa}**
Ribosome
Nitrocellulose filter





(来源：分子生物学（2007），郑用琏，第188页)

61个codons被破译，(仅剩UAA,UAG,UGA？)

Stop codon 的证实

Brenner (1961)

获得：T4 phage 头部蛋白基因的琥珀突变（amber）

证明：突变体头部蛋白较野生型的变短

推测：头部蛋白基因发生了终止突变，使蛋白质合成中断。

Garen (1965)

获得： *E.coli* 碱性磷酸酯酶基因（*phoA*）Amber突变株的大量回复突变株

分析：回复突变株中对应“回复”的氨基酸

Stop codon 的证实

aa and codon in back mutant

发生终止突变的原氨基酸 Trp (UGG)

- Ser : UAG UCC, UCA, UCU, AGU, AGC
- Leu : UAG UUA, CUU, CUC, CUA, CUG
- Tyr : UAU, UAG
- Lys : UAG AAA
- Gln UAG CAA
- Glu UAG GAA

证明：终止突变密码为

UAG (amber 琥珀突变)　X Y Z ?! U G G

UAA (ocher 赭色突变)

UGA (opal 蛋白石突变)

Genetic codon

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

(来源：分子生物学（2007），郑用琏，第190页)

5.2.2. Degeneracy of codon (密码子的简并现象)

a) 简并现象的概念；

---一种氨基酸受2个以上codon编码的遗传现象

---编码一种aa的4个codon间, 仅3rd Nt 不同,
称为 **codon family**

例： Ser (6 codons) 1 codon family & 2 extra codons

b) 简并现象的机理；

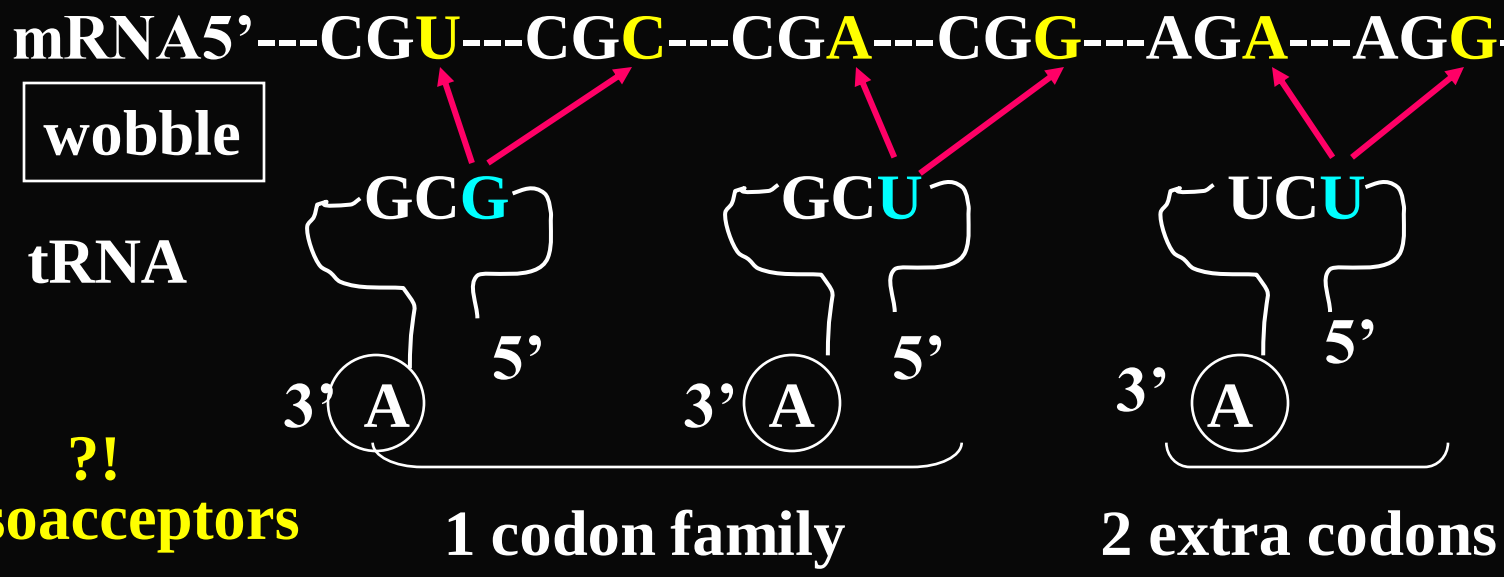
- **Isoacceptor;** 负载同一氨基酸，但识别不同密码子的不同tRNA

- **Wobble hypothesis;**

反密码子：密码子

$1^{th}(Nt^{34})$ ： 3^{rd}-Nt

} 在一定范围内的可选择配对现象



简并现象的机理；

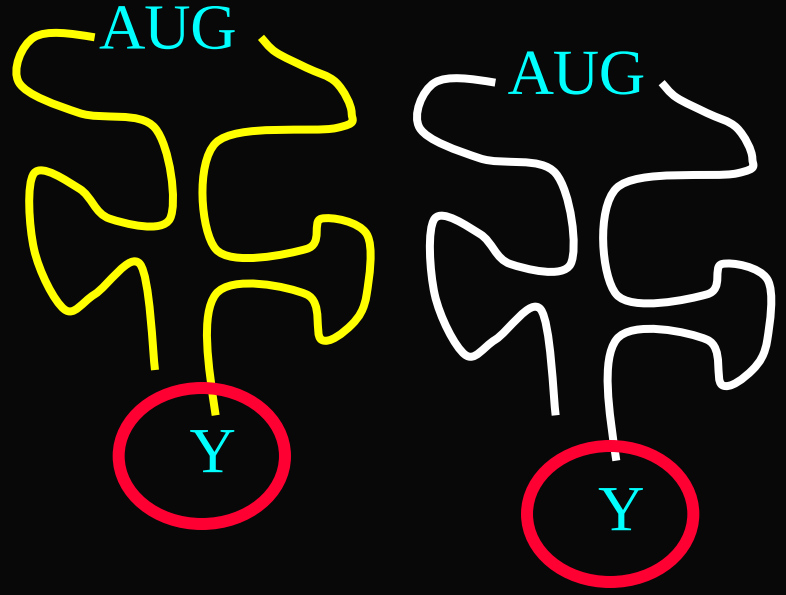
- **Isoacceptor；**负载同一氨基酸，但识别不同密码子的不同**tRNA**

负载同一氨基酸，识别相同密码子的不同**tRNA**？！

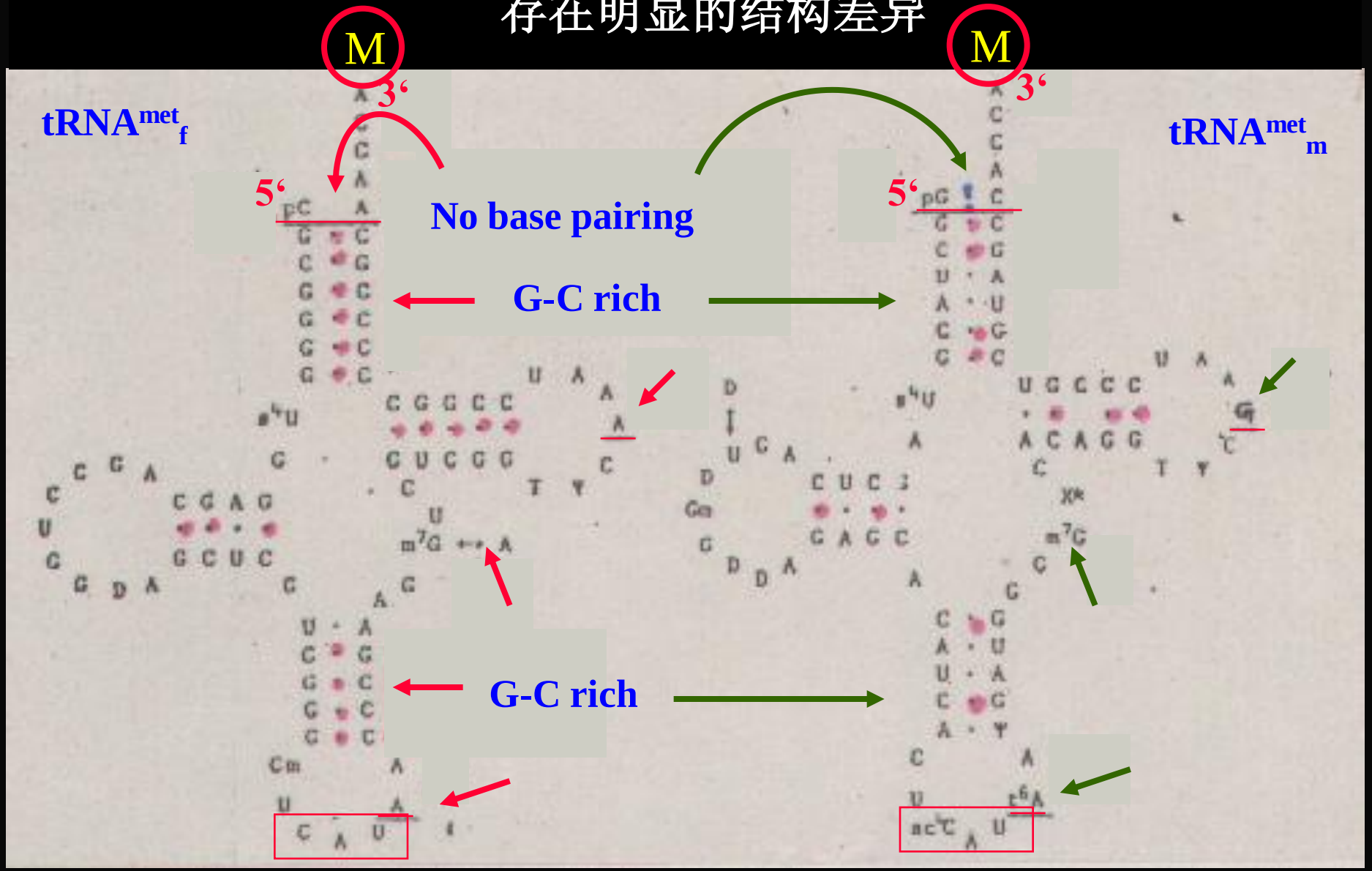
Tyr codon: UAC

AUG

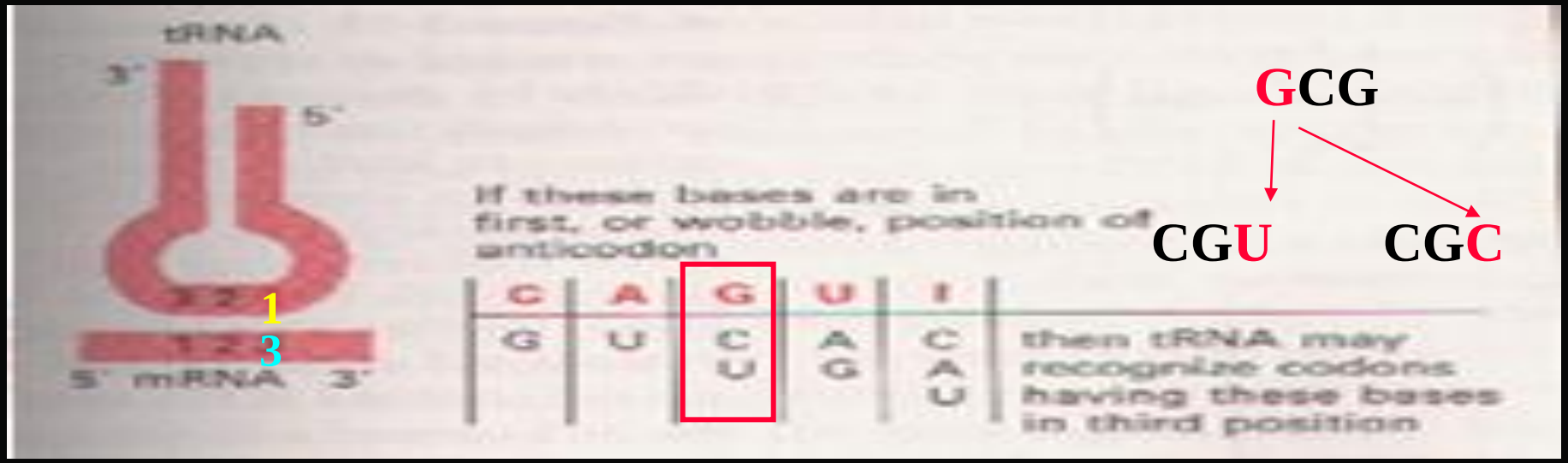
识别UAC Codon负载Try的tRNA有两个，但结构向差较大。



$tRNA^{met}_i$ & $tRNA^{met}_e$; $tRNA^{met}_f$ & $tRNA^{met}_m$
存在明显的结构差异



(来源：分子生物学（2007），郑用琏，第196页)



(来源：分子生物学（2007），郑用琏，第193页)

- **Wobble base的摇摆配对原则**

Genetic codon

FIRST BASE	SECOND BASE U	SECOND BASE C	SECOND BASE A	SECOND BASE G	THIRD BASE
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA TERM	UGA TERM	A
	UUG Leu	UCG Ser	UAG TERM	UGG Trp	G
A	AUA Met	ACA	AAA Lys	AGA Arg	A
	AUG Met	ACG	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

GUG（*val*）的第一Nt会以较低频率与$tRNA^{met}_{f}$

反密码子(CAU)发生“摇摆”配对，而作为起始密码.

(E.coli GUG / AUG =1/30)

（Source:Molecular Biology(2002),Robert F.Weaver,Page570）

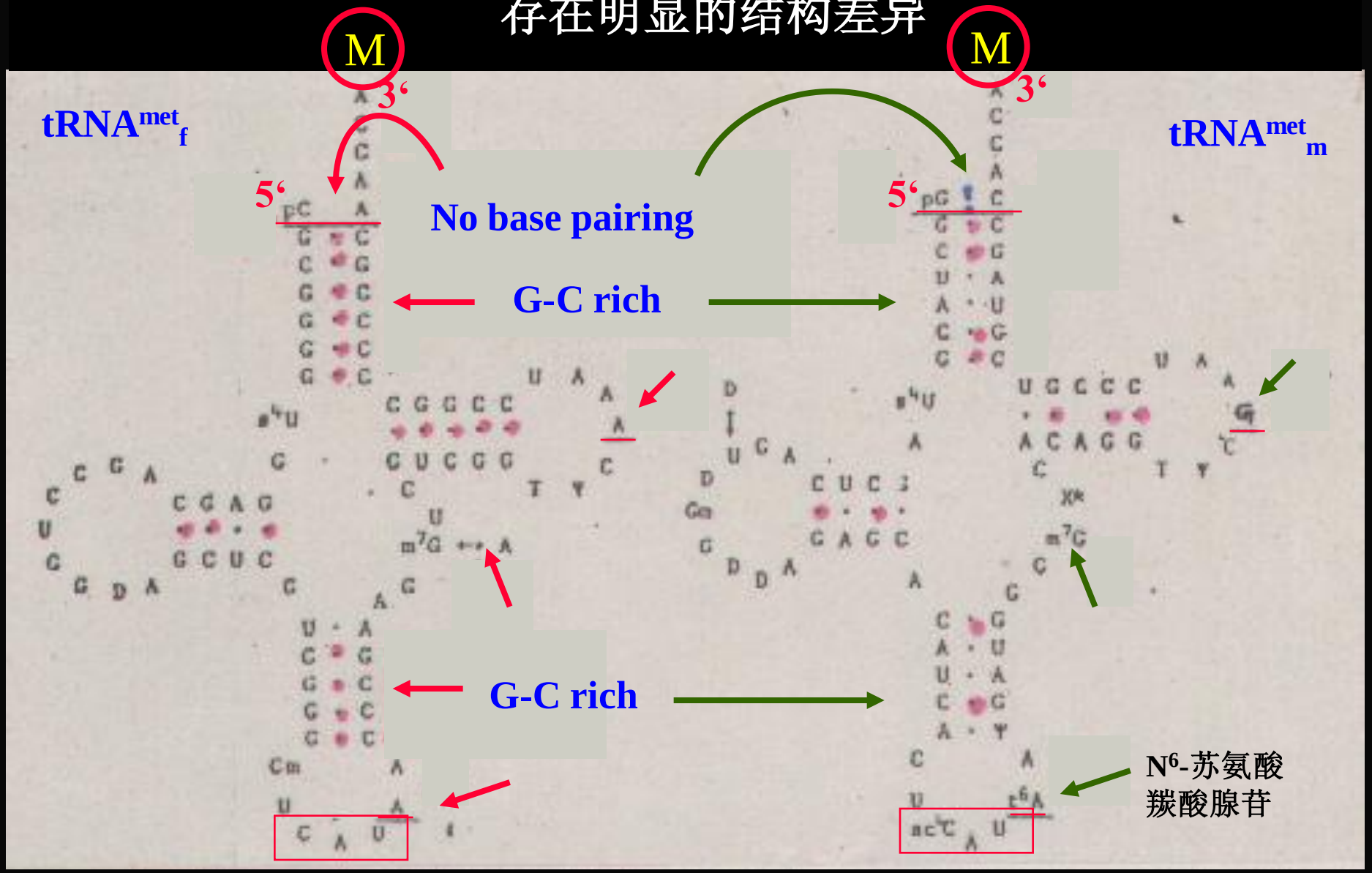
mRNA (^{1}GUG)（*val*）作为起始密码. 与$tRNA_f^{met}$的反密码子(CA^{3}U)配对，不是真正意义上的”摇摆”.

由于$tRNA^{met}_{f}$中反密码子下游第一个Nt(37)为未修饰的A，而其他tRNA第37个Nt几乎为较大的烷化修饰的Nt

例如$tRNA^{met}_{m}$第37个Nt为$t^{6}A$

(N6-苏氨酸羰酸腺苷)

$tRNA^{met}_i$ & $tRNA^{met}_e$; $tRNA^{met}_f$ & $tRNA^{met}_m$ 存在明显的结构差异



(来源：分子生物学（2007），郑用琏，第196页)

意味着反密码子边序碱基修饰对限制错读的机制

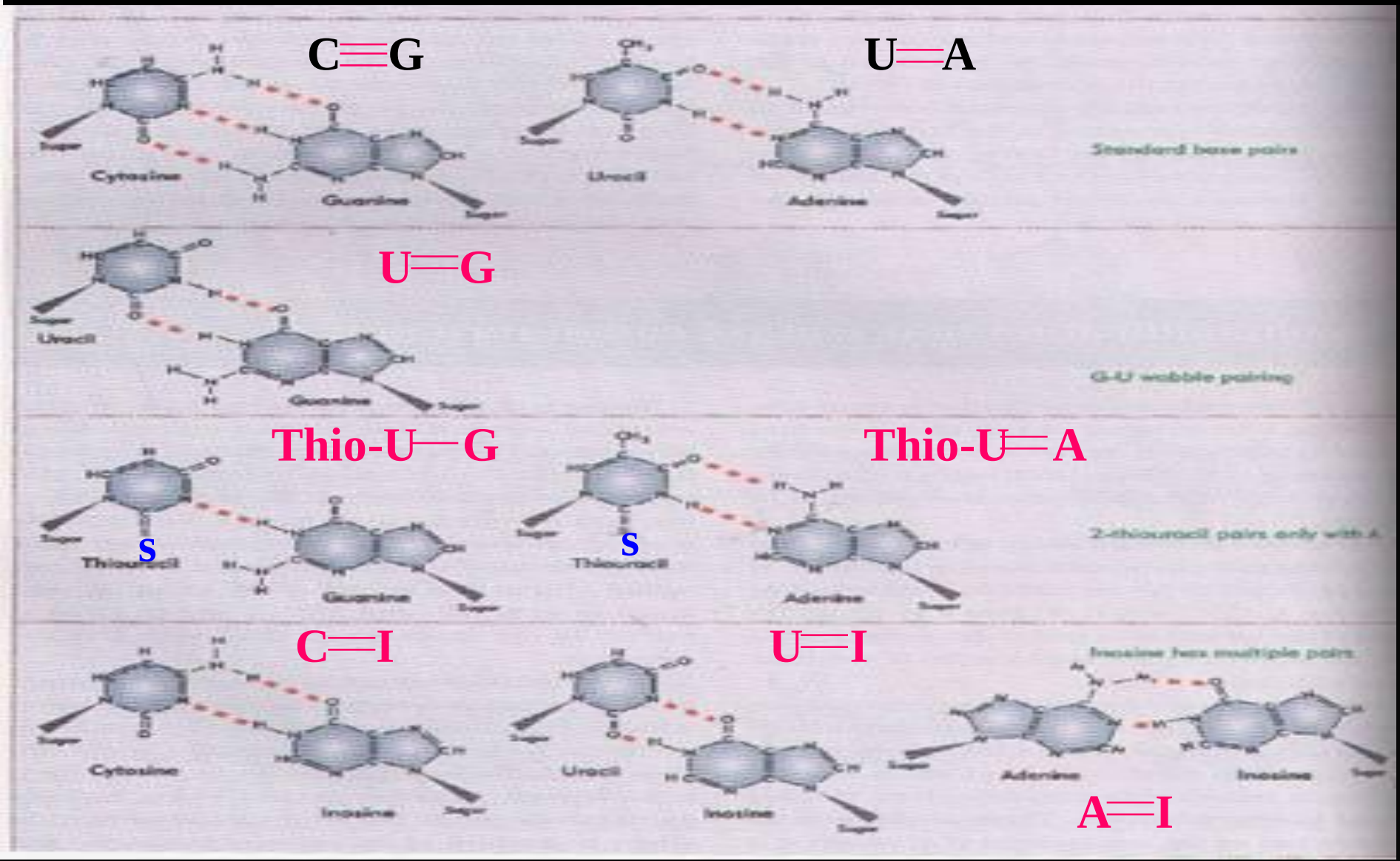
mRNA **^{1}GUG** **AUG**

tRNA$^{met}_{f}$ **CA^{3}UA（37）** **CA^{3}UA（37）**

AUG

tRNA$^{met}_{m}$ **CAUt^{6}A（37）**

碱基摇摆配对的方式



(Source:Molecular Biology(2002),Robert F.Weaver,Page570）

- **Wobble base摇摆配对的机理**

--- tRNA的拓扑空间结构

34th摇摆位点位于拓扑结构的末端，

碱基堆积力小，

选择性配对的自由度大

--- 34th摇摆位点被修饰的频率高

导致配对原则的改变

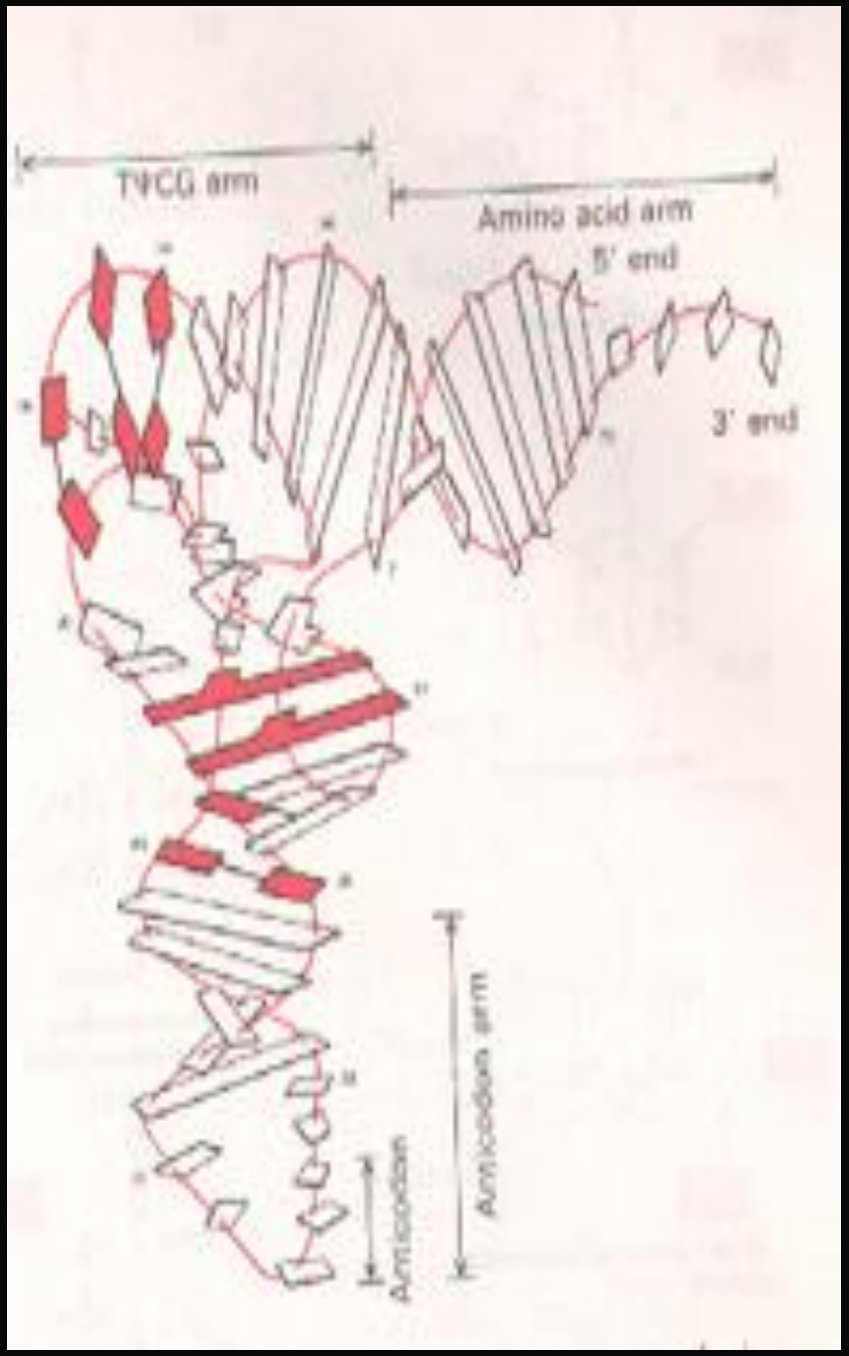
尤以$A^{34} \rightarrow I$ ⟹ I = A / I = C / I = U

--- 34th几乎无A

--- 线粒体中

U^{34} = N(any)

when $U^{34} \rightarrow U^*$ = A/G only



Source:Quigley,G.J,Structural domains of transfer RNA moleculars,Science 194:197

5.2.3. Anti-codon及其两侧碱基修饰对密码子解读的生物学意义

a) Methylated Nt at anti-codon and flanked

Xo^5U (5-羟基尿苷)

$Cmnm^5U$ (5-羧甲基氨甲基尿苷)

mCm^5U (5-甲氧基羰甲基尿苷)

Xm^5s^2U (5-甲基-2硫代尿苷)

K^2C (2-赖氨酸胞苷)

Com^5U (5(2)-羟羧甲基尿苷)

I (Inosine次黄嘌呤)

m^7G (7-甲基尿苷)

m^5C (5-甲基胞苷)

m^6A (6-甲基腺苷)

s^2C (2-硫代胞苷)

Ψ （假尿苷）

t^6A (N6-苏氨酸羰酸腺苷)

Q (Queuosine辫苷)

b) 被修饰的Nt^{34}的配对能力

Nt^{1} of anti-codon → $Nt^{3}$of codon

U (mt,ct)	**A,U,C,G**
$CmO^{5}U$ (5(2)-羟羧甲基尿苷)	**A,G,U**
$Cmnm^{5}U$ (5-羧甲基氨甲基尿苷)	**A,G**
$mCm^{5}U$ (5-甲氧基羰甲基尿苷)	**A,G**
Um (2'-O-甲基尿苷)	**A,G**
$Xm^{5}S^{2}U$ (5-甲基-2硫代尿苷)	**A**
Q (Queuosine)	**U,C**
I (Inosine）	**U,C,A**

c) tRNA中anti-codon碱基修饰的意义

- 限制对密码识读的随意性，以保证遗传的稳定

$U \rightarrow A/G$　　$Cm^5S^2U \rightarrow A$ (only) 在特定表达细胞中

$S^2U \rightarrow A$

- 提高摇摆能力，防止突变效应，以保证遗传的稳定

$A \rightarrow U$　　A ⇒ $I \rightarrow A/C/U$

$U \rightarrow A/G$　　U ⇒ $CmO^5U \rightarrow A/G/U$

5.2.4. tRNA abundance

&

codon usage (codon bias)

Codon usage Observed for *E.coli* Ribosome Protein

Second Position

First Position (5′ End)	U	C	A	G	Third Position (3′ End)
U	10 UUU, 23 UUC Phe; 1 UUA, 2 UUG Leu	18 UCU, 18 UCC, 1 UCA, 1 UCG Ser	3 UAU, 13 UAC Tyr; UAA Stop; UAG Stop	1 UGU, 6 UGC Cys; UGA Stop; 3 UGG Trp	U C A G
C	4 CUU, 3 CUC, 0 CUA, 79 CUG Leu	3 CCU, 0 CCC, 4 CCA, 36 CCG Pro	3 CAU, 15 CAC His; 9 CAA, 33 CAG Gln	48 CGU, 26 CGC, 0 CGA, 0 CGG Arg	U C A G
A	13 AUU, 51 AUC, 0 AUA Ile; 30 AUG Met	36 ACU, 26 ACC, 3 ACA, 0 ACG Thr	3 AAU, 42 AAC Asn; 90 AAA, 24 AAG Lys	1 AGU, 12 AGC Ser.; 1 AGA, 0 AGG Arg	U C A G
G	54 GUU, 6 GUC, 40 GUA, 16 GUG Val	93 GCU, 10 GCC, 45 GCA, 28 GCG Ala	17 GAU, 45 GAC Asp; 61 GAA, 16 GAG Glu	49 GGU, 34 GGC, 0 GGA, 0 GGG Gly	U C A G

1209 codons

(来源：分子生物学（2007），郑用琏，第197页)

Codon usage in the genes of Animals

First Position (5' End)	U	C	A	G	Third Position (3' End)
U	13 UUU, 28 UUC — Phe 2 UUA, 9 UUG — Leu	16 UCU, 18 UCC, 9 UCA, 2 UCG — Ser	10 UAU, 23 UAC — Tyr UAA — Stop UAG — Stop	10 UGU, 13 UGC — Cys UGA — Stop 12 UGG — Trp	U C A G
C	9 CUU, 27 CUC, 7 CUA, 47 CUG — Leu	14 CCU, 17 CCC, 10 CCA, 5 CCG — Pro	10 CAU, CAC — His 10 CAA, 28 CAG — Gln	8 CGU, 11 CGC, 4 CGA, 5 CGG — Arg	U C A G
A	11 AUU, 24 AUC, 4 AUA — Ile 16 AUG — Met	15 ACU, 28 ACC, 11 ACA, 6 ACG — Thr	8 AAU, 28 AAC — Asn 19 AAA, 49 AAG — Lys	12 AGU, 21 AGC — Ser 8 AGA, 10 AGG — Arg	U C A G
G	9 GUU, 21 GUC, 5 GUA, 33 GUG — Val	2 GCU, 38 GCC, 14 GCA, 6 GCG — Ala	16 GAU, 24 GAC — Asp 21 GAA, 34 GAG — Glu	22 GGU, 32 GGC, 16 GGA, 11 GGG — Gly	U C A G

2244 codons

(来源：分子生物学（2007），郑用琏，第198页)

tRNA abundance & codon usage (codon bias)

生物GC%不等 → 各种codon的频率不等

↓

进化过程

中度重复基因tRNA的拷贝数与codon使用频率的对应

--- 识别同一氨基酸的不同tRNA(isoacceptor)量不等

--- 不同生物间同一isoacceptor的量不等

tRNA abundance ； codon usage (codon bias)
是进化中形成的基因表达调控机制之一

tRNA abundance ~ 正相关 ~ codon usage

a）需要量多的蛋白质（除mRNA转录速率高外）

进化中形成的蛋白质翻译调控机制（modulator ）

关键aa的codon usage 低 → 相应tRNA量少

b） codon 与anti-codon间的作用强度

→ codon usage

G ⋯ U 弱氢键配对 ⟹ aa-tRNA^{aa}

需较长时间 ⇓ 以求结合稳定

into A site of ribosome

G ≡ C 强氢键配对 ⟹ aa-tRNA^{aa}

融解温度高 ⇓ 需较长时间

Out P site of ribosome

自然选择codon/anti-codon 间适度结合强度的codon usage
以保证最佳的蛋白质合成速率

In prok. Gly (GGG) usage = 0 Pro (CCC) usage = 0

Phe (UUC) > (UUU) Tyr (UAC) > (UAU)

Anti-codon AAG[1] AUG[1]

23 / 1209 > 10 / 1209 13 / 1209 > 3 / 1209

共性: codon/anti-codon 间适度结合强度

个性: G/C含量不同, tRNA丰度各异

the seq.of codon in usage 1 2 -- 3 1 2 --- 3

in general UU G AA C

GG U CC A

5.2.5. two of three codon-reading in mitochondrial

a) 线粒体中具有与通用密码不同的编码信息

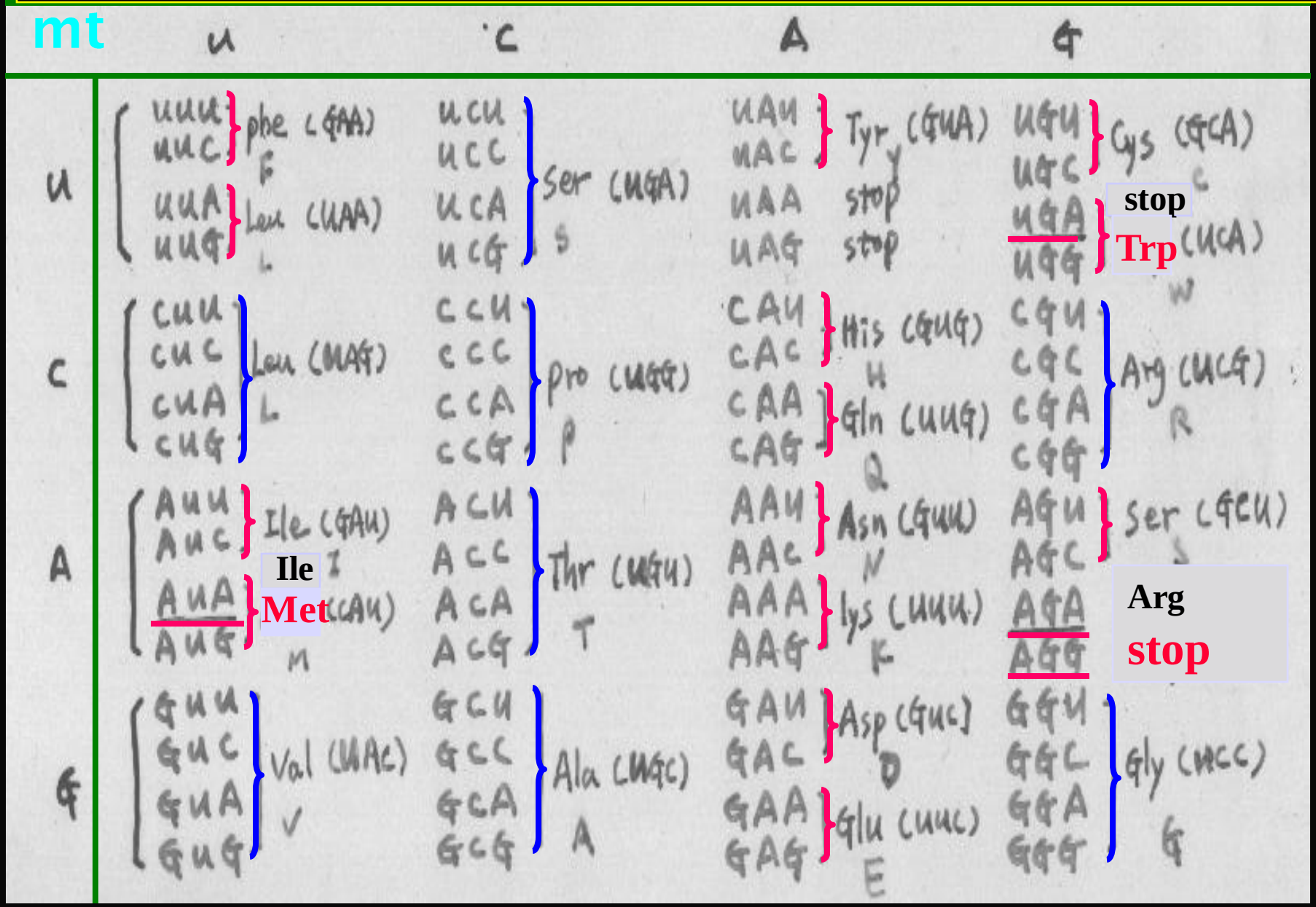
- 线粒体codon较为整齐（均为2/4/6）

  2 codon; F, I, Y, H, Q, N, E, k, D,W, M, C
  4 codon; V, P, T, A, R, G, (family) & stop codon
  6 codon; L, S (2 isoacceptors each)

- In mt 22 tRNA only (32 tRNA in universal code)

  线粒体“三中读二”方式可减少tRNA

Codons Comparing between in usual and in mt



(来源：不详)

Changes occur in the mitochondrial genetic code

Organism	Codon	Meaning in Mitochondrion	Usual Meaning
Common Mammal	UGA AG$^{A}_{G}$	tryptophan termination	termination arginine
Mammal Fruit fly Yeast	AUA AUA AUA	Met (initiation) Met (initiation) Met (elongation)	isoleucine isoleucine isoleucine
Yeast Fruit fly	CUA AGA	threonine serine	leucine arginine

(来源：不详)

● Codon-reading

For codon family; two of three reading

codon **UCU-----UCA-----UCG-----UCC**

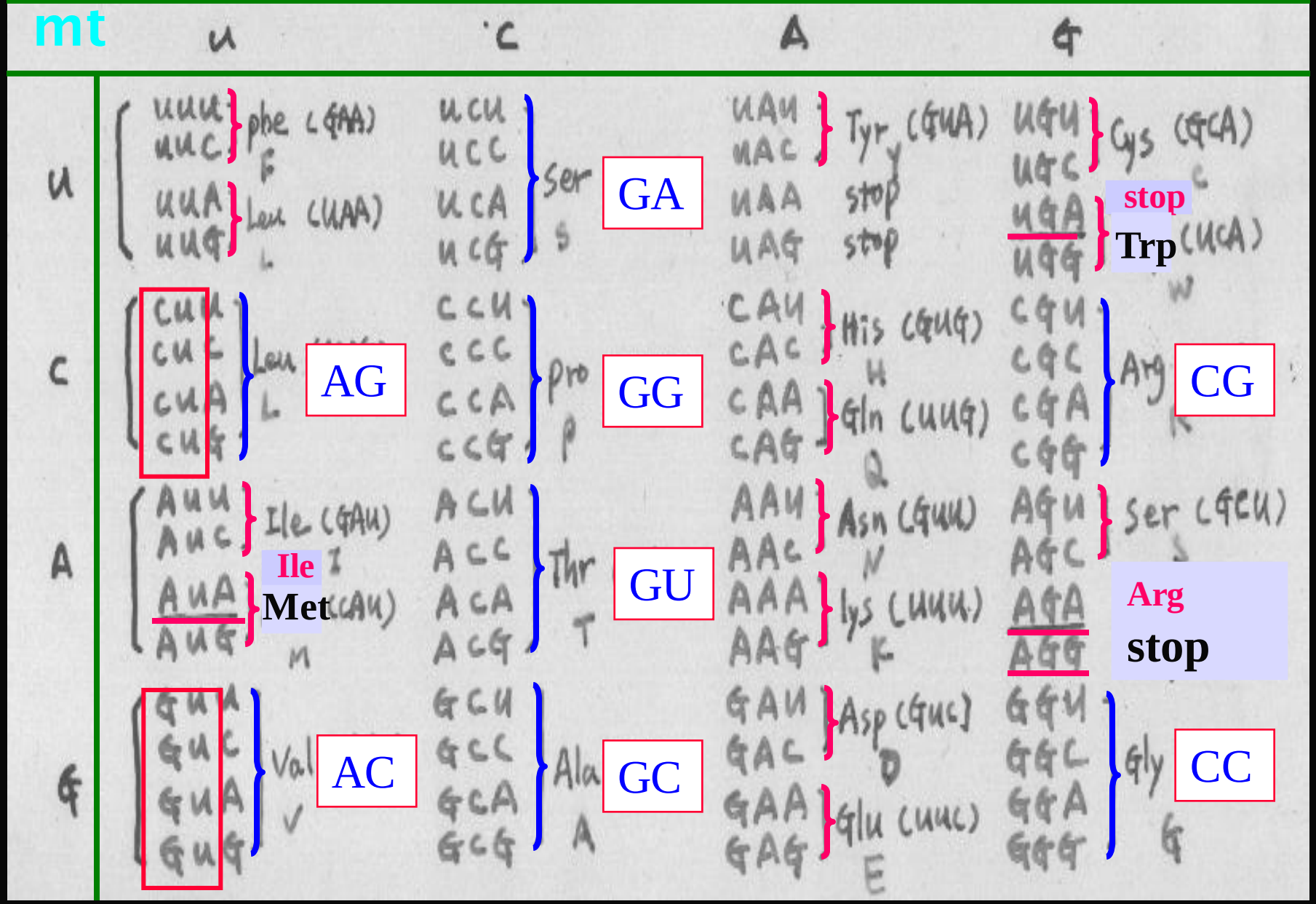
anti-codon **UAG**

UC → Ser codon

N^{34} (U) ⟶ A/U/C/G

仅起将codon隔开的作用

Codons Comparing between in usual and in mt



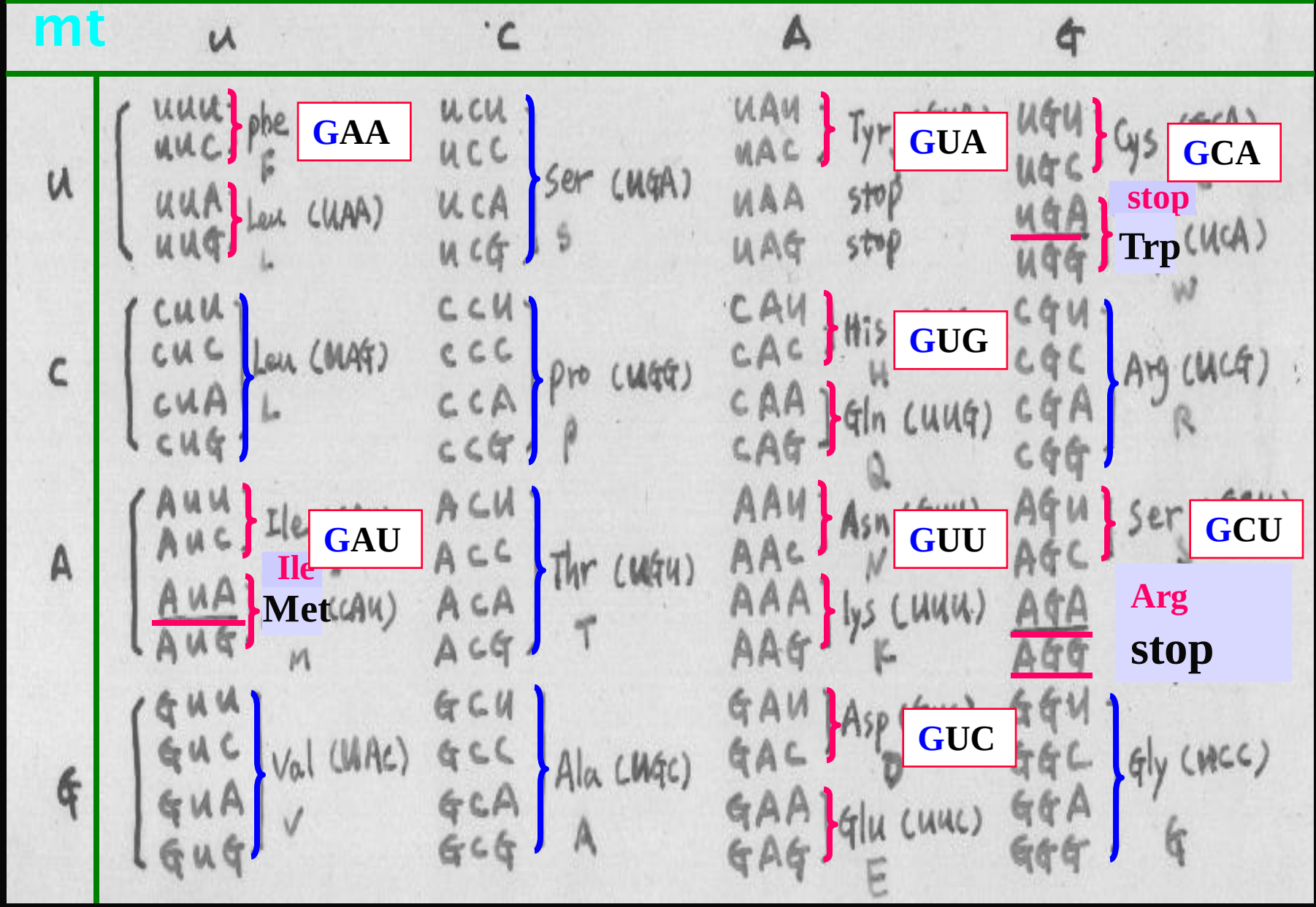
(来源：不详)

- **Codon-reading**

For 2 codon type;

Nt^{34} wobble base G $\rightarrow$ C/U

Codons Comparing between in usual and in mt



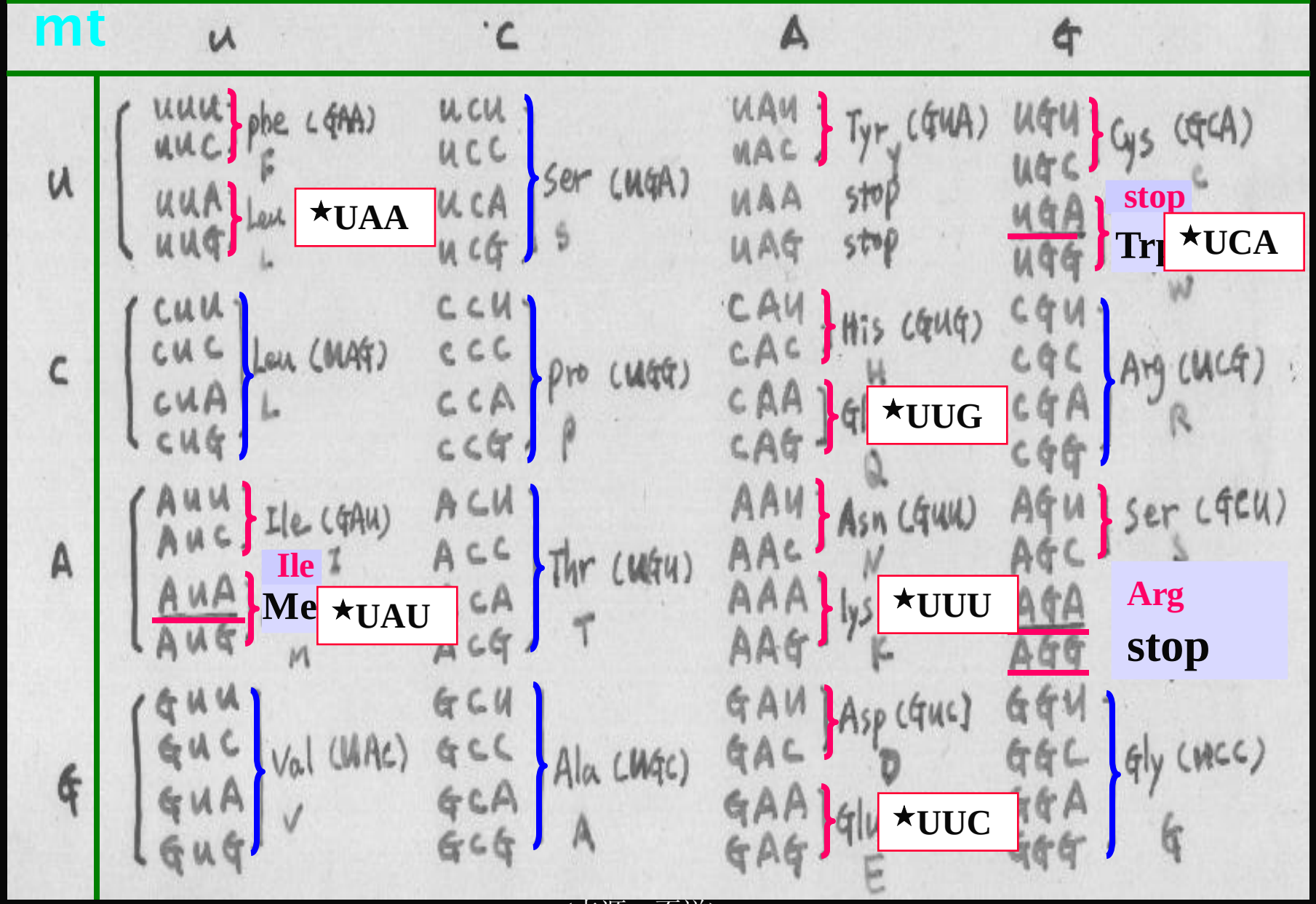
(来源：不详)

- **Codon-reading**

For 2 codon type;

Nt^{34} wobble base * U → G/A

Codons Comparing between in usual and in mt



(来源：不详)

● Codon-reading in mt (Nt^{34} : U/U*/G)

For codon family; two of three reading

codon UCU-----UCA-----UCG-----UCC

anti-codon UAG

UC → Ser codon , N^{34}(U) 仅起将codon隔开的作用

For 2 codon type; Nt^{34} wobble base U* → G/A

G → C/U

5.2.6. codon in codon or general genetic codon (GGC 广义密码子)

生物体除具有标准的通用密码保证蛋白质的准确翻译外

同时存在**GGC**

⇒ 转录的模糊性（非转录错误）

⇒ 生物的适应性

一种GGC 编码几种氨基酸 ⇒ 蛋白质性质不变

a) codon / anti-codon间的缔合能分析

- 2^{ed} Nt of codon （$N_1N_2N_3$）

  对codon/anti-codon的缔合能贡献最大

  凡2^{ed}Nt相同的codon

  codon/anti-codon间的缔合能相似

- 对缔合能的贡献

  ^{2}Nt ＞ 1thNt ＞ 3rdNt

b) codon对氨基酸性质的决定

2edNt of codon 对氨基酸性质和蛋白质空间结构的决定度较大

NUN → 非极性疏水性氨基酸

α-helix & β-sheet的形成者

位于蛋白质分子内部

NAN → 极性亲水性氨基酸,

位于蛋白质分子外部

N（G/C）N → 编码的氨基酸极性居中

Codon in codon (依 2edNt of codon预测氨基酸的性质)

不同方法测定aa的亲水性和分子量结果

F.J.R.Taylor 1989 Bio-Systems 22.p177-187

(1) $N_1N_2N_3$		(2) $N_1N_2N_3$		$N_1N_2N_3$	
GGN	疏	UGU/C	疏	GGN	MW 75 kd
CUN		UUU/C		GCN	小
AUA/C		AUA/C		UCN	
GUN		GUN		CCN	
GCU		CUN		GUN	
UUU/C		AUG		CAN	
UGU/G		UGG		UGU/C	
AUG		CAU/C		CUN	
ACN	中	UAG/C	中	AUA/C	中

UCN	中	GCN	中	GAU/C	中
UGG		GGN		AAU/C	
UAU/C		ACN		GAA/G	
CAA/G		UCN		CAA/G	
AAA/G		CCN		AAA/G	
AAU/C		CGN		AUG	
GAA/G		AAU/C		CAU/C	
CAU/G		CAA/G		UUU/C	
GAU/C	亲	GAA/G		CGN	
CGN		GAU/C	亲	UAU/C	
		AAA/G		UGG	大 204 kd

2ed Nt = U (hydrophobic aa)

A (hydrophilic aa)

G/C (neutral aa)

c) Nt of codon 对蛋白质功能的决定

- **1^{th} & 3^{rd} Nt的摇摆**

  对蛋白质的结构与功能影响不大

- **2^{ed}Nt不能摇摆**

2^{ed}Nt of codon对氨基酸的编码特征
即为GGC or codon in codon

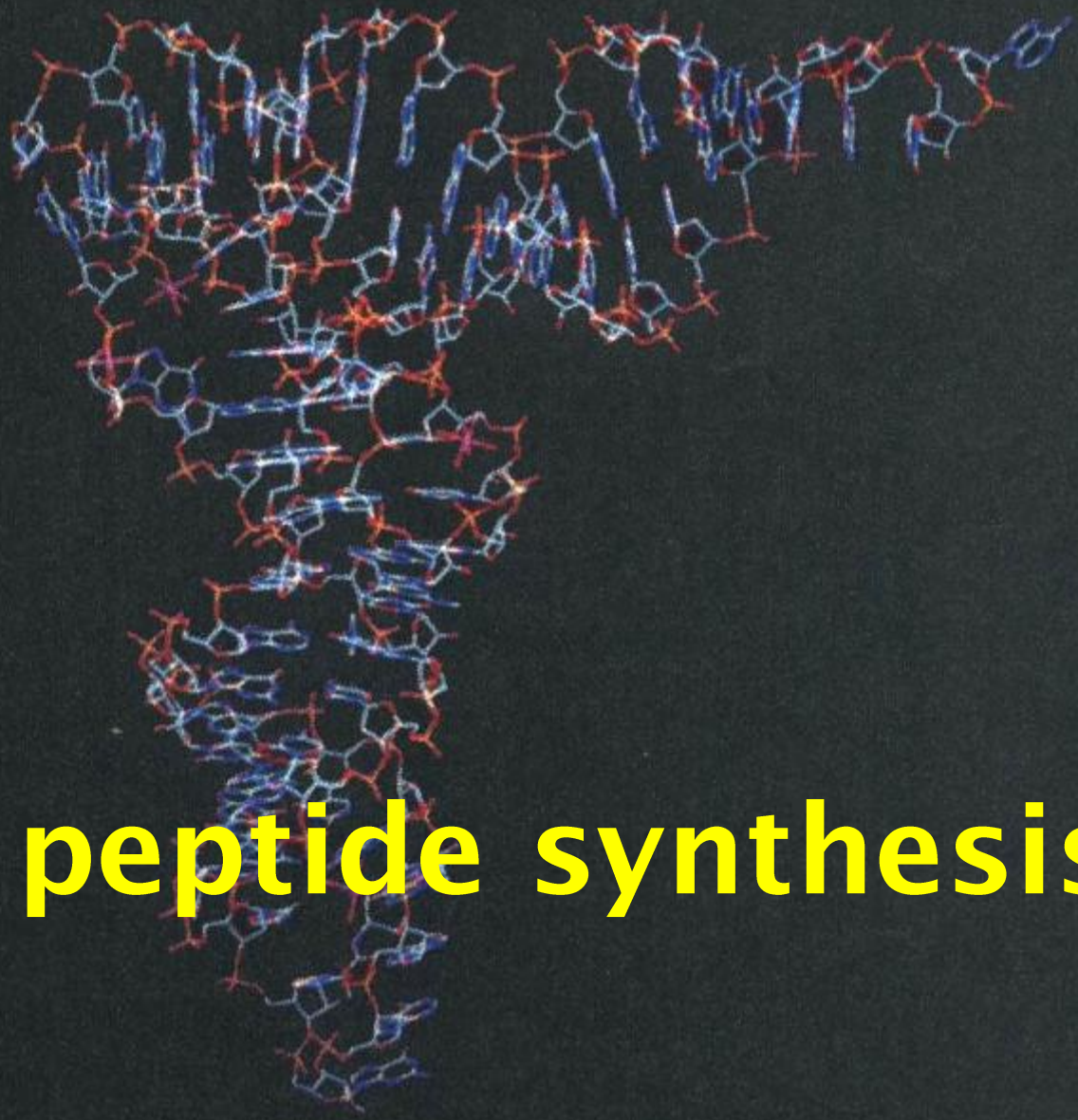
d) 生物学意义

- 保证遗传的稳定
- 依据codon in codon （2^{ed}Nt of codon）

→ 判断蛋白质的性质

- 蛋白质性质预测
  蛋白质定点诱变
  蛋白质改造

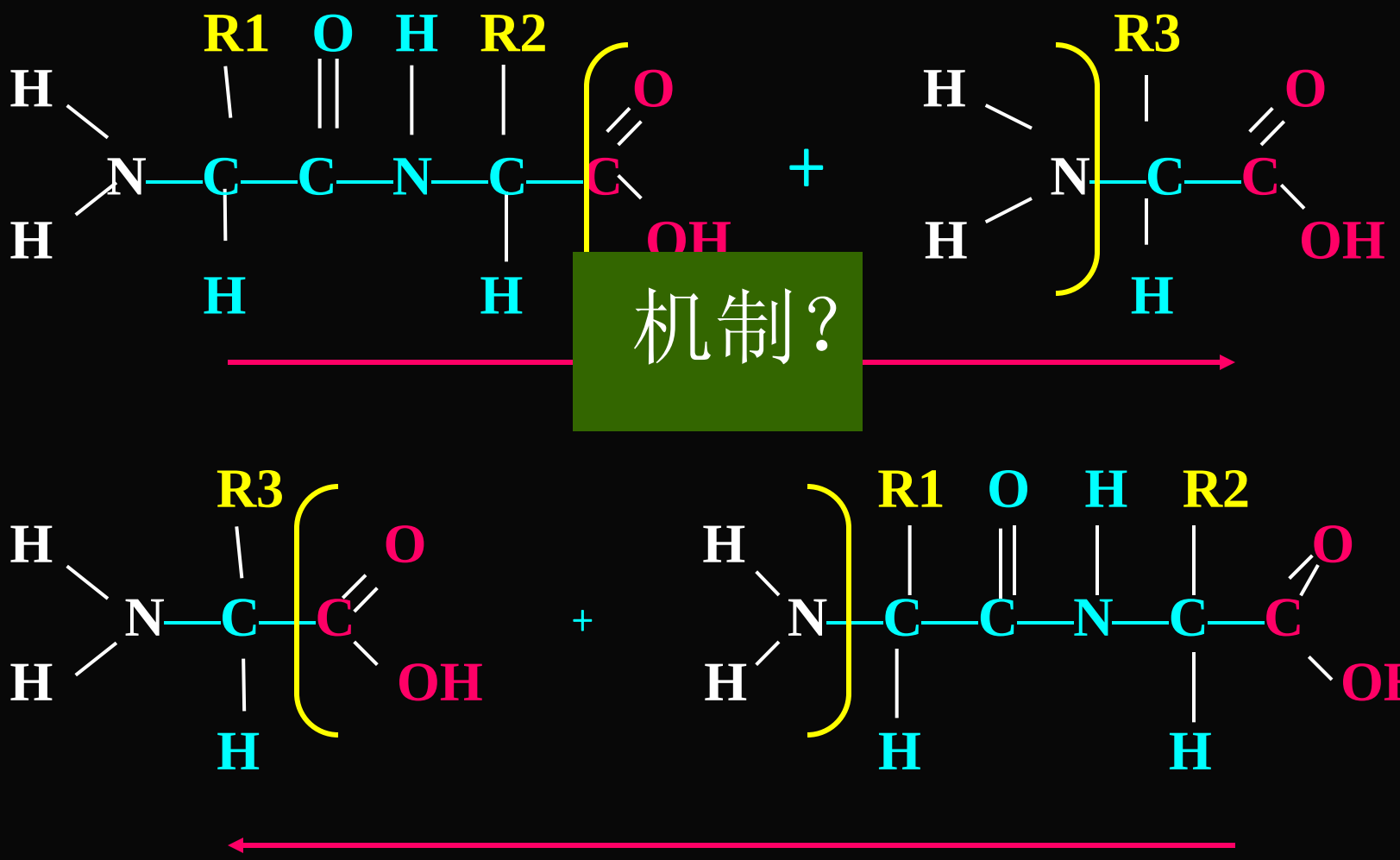
（蛋白质工程）

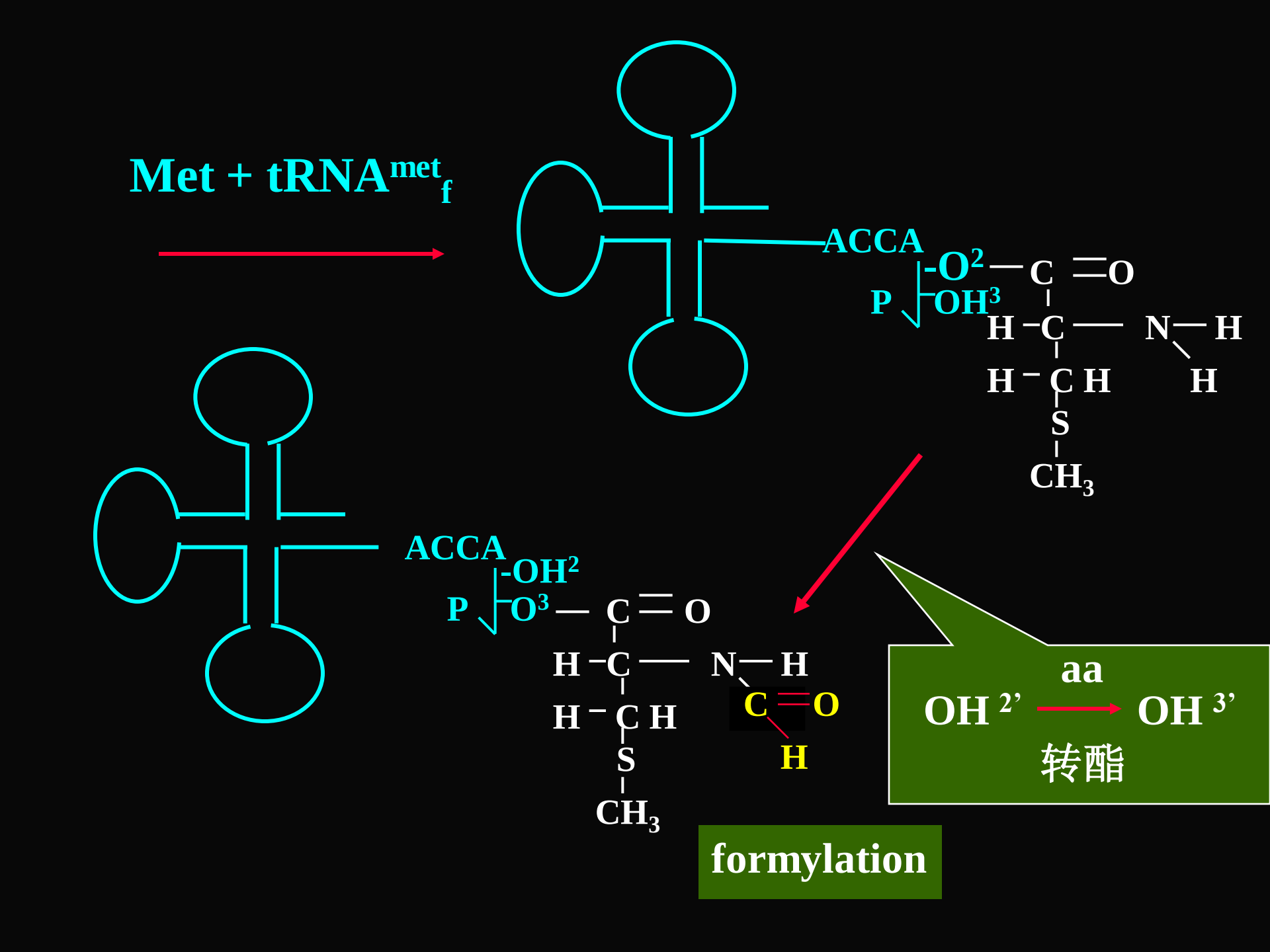


5.3. peptide synthesis

(Source:Tripos Associates/*Peter Arnold,Inc.*)

5.3.1. direction of peptide elongation N'→ C'




Met + tRNA$^{met}_{f}$
ACCA
-O^{2}
P
OH^{3}
C
O
H
C
N
H
H
C H
H
S
CH$_3$
ACCA
-OH^{2}
P
O^{3}
C
O
H
C
N
H
H
C H
C
O
H
S
CH$_3$
aa
OH 2’
OH 3’
转酯
formylation

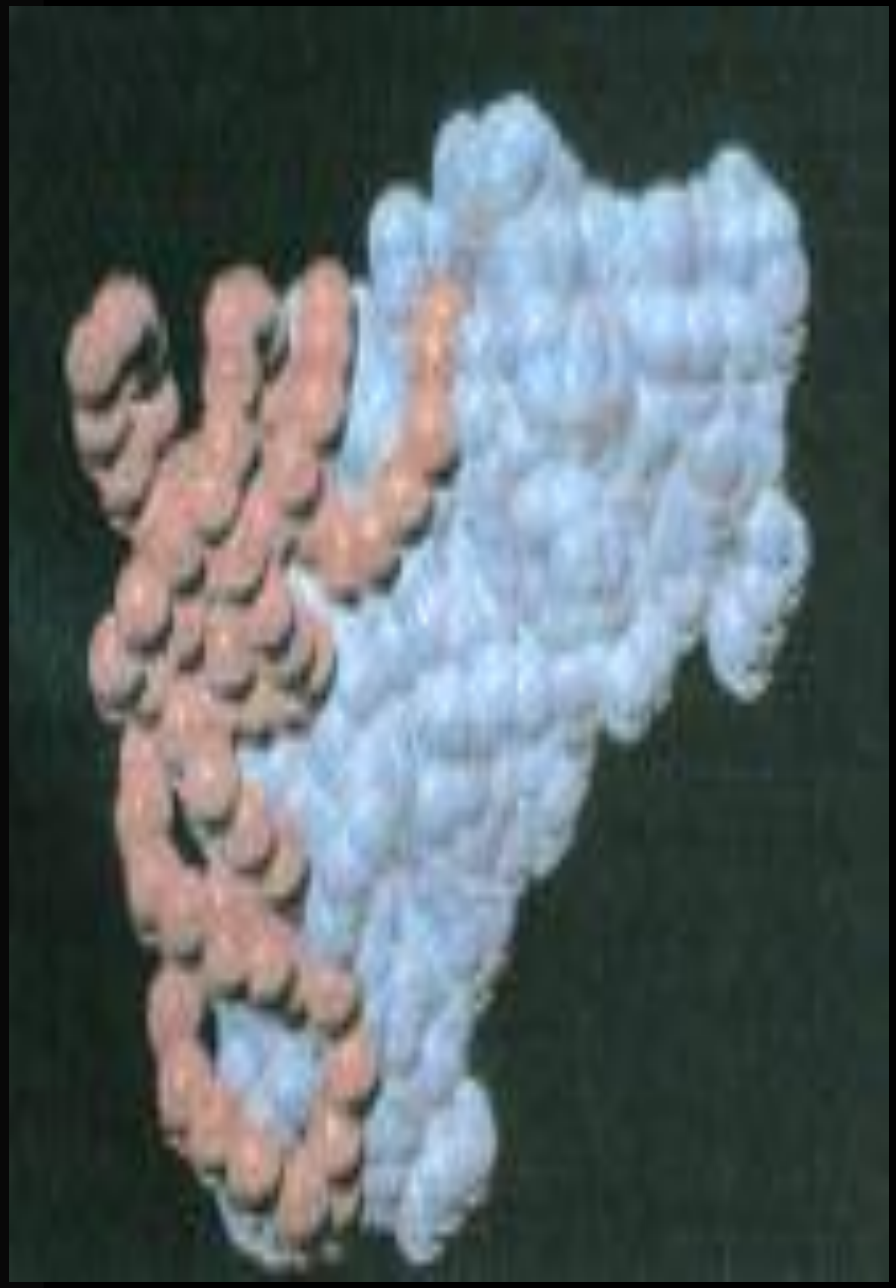
5.3.2. Aminoacyl—tRNA^{aa}

in Prok.

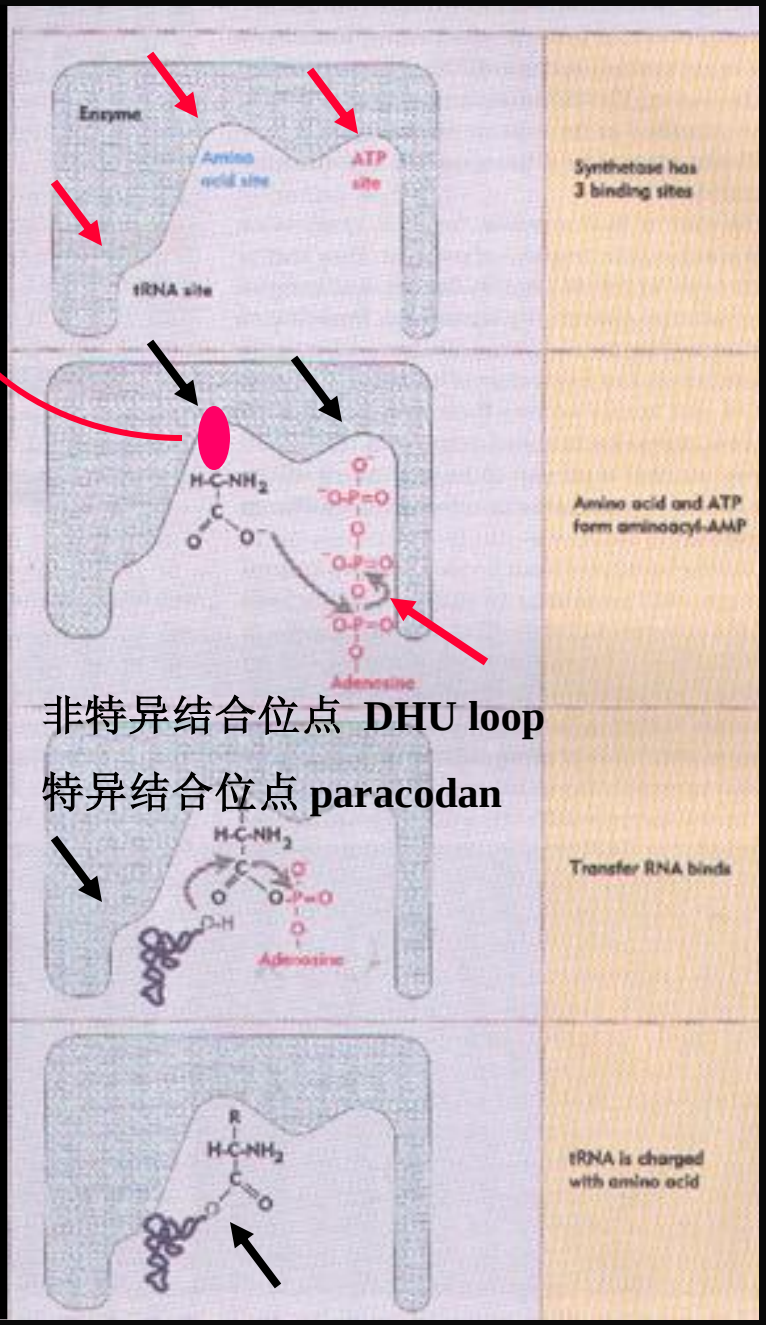
f **Met—tRNA** $^{met}_{f}$ **&** **Met--tRNA** $^{met}_{m}$

in Euk.

Met—tRNA $^{met}_{I}$ **&** **Met--tRNA** $^{met}_{e}$



R



非特异结合位点 DHU loop

特异结合位点 paracodan

(来源：分子生物学（2007），郑用琏，第211页)

5.3.3. peptide synthesis

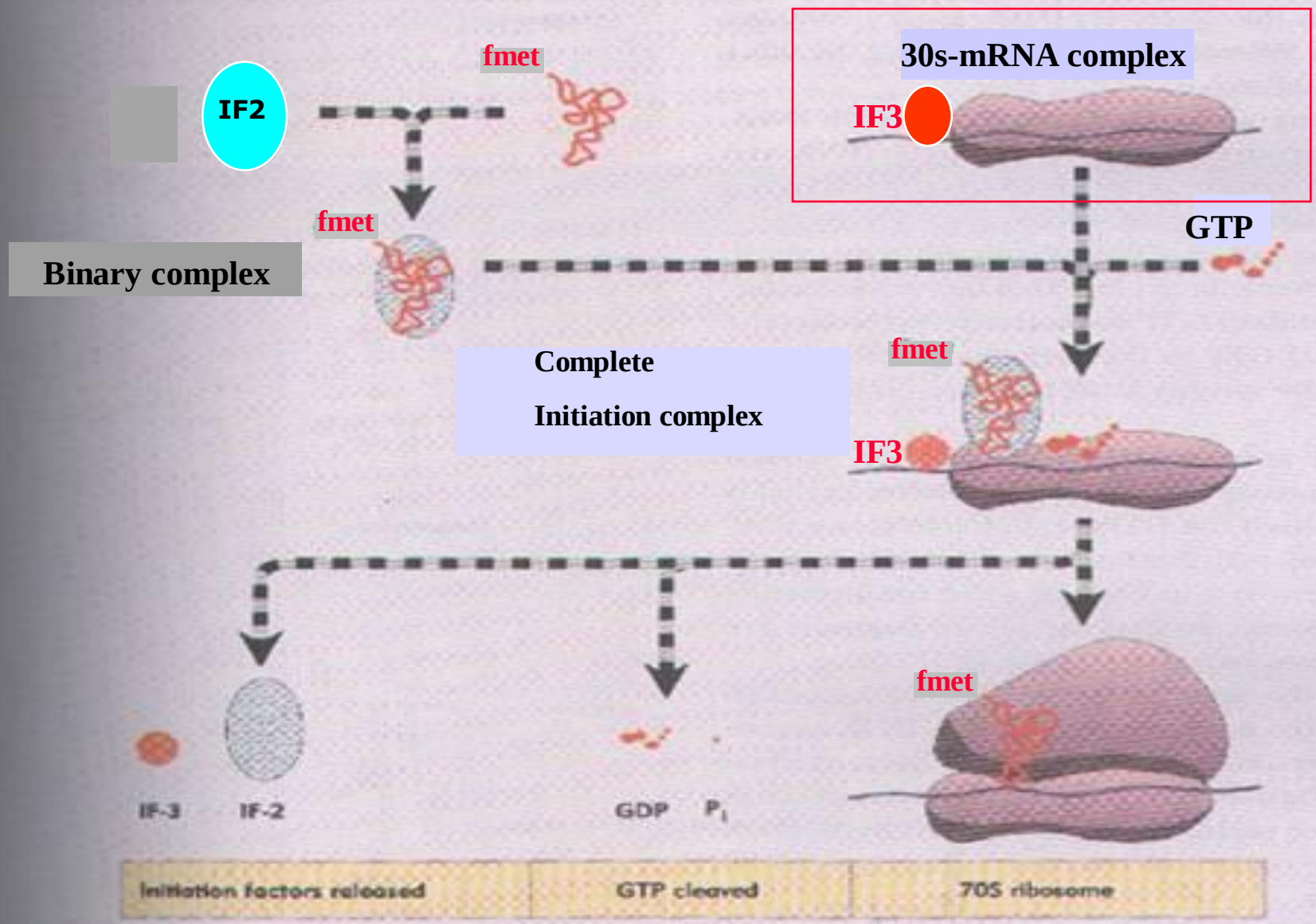
a. Initiation Enzyme of translation in Prok.

IF-1	9.5kd	加强IF-2，IF-3的酶活
IF-2	95kd-117kd	促使$fMet\text{-}tRNA_f^{met}$选择性的结合在30S亚基上
IF-3	20kd	促使30S亚基结合于mRNA起始部位

(识别$tRNA_f^{met}$中富含GC的反密码子臂, way in P site ?!)

具有解离30S与50S亚基的活性

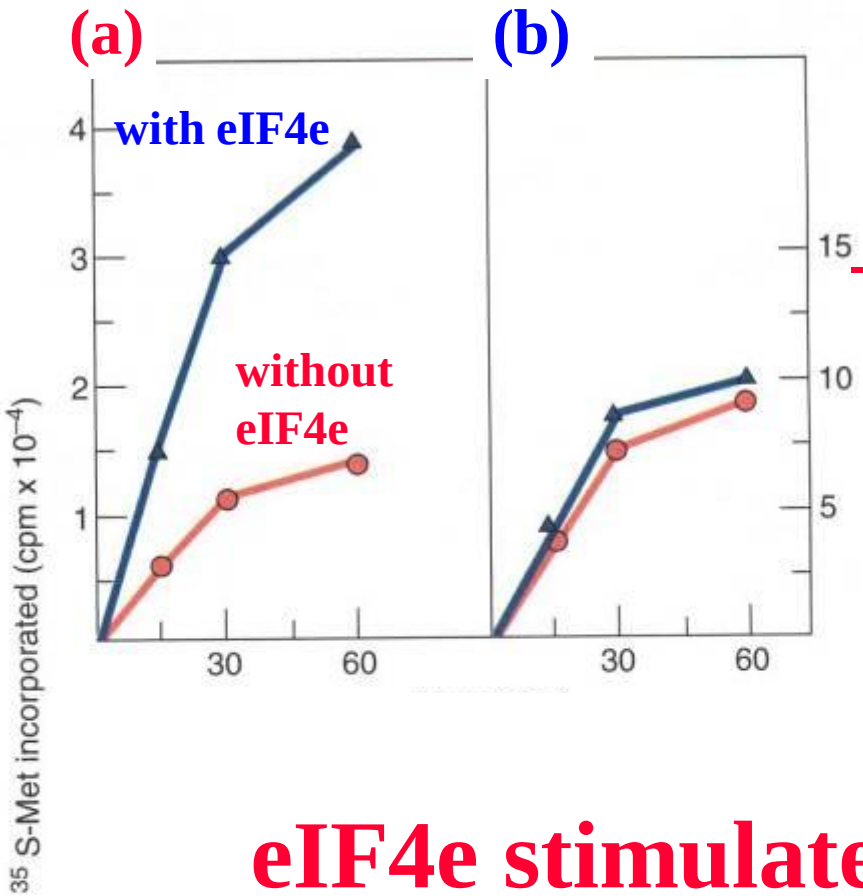
Initiation of translation in Prok.



(来源：不详)

b. Initiation Enzyme Of translation in Euk.

eIF2	3种亚基	形成三元起始复合体（eIF2, GTP, tRNA)
eIF2-A	65kd	促使$Met\text{-}tRNA_i^{met}$与40S亚基结合
eIF1	15kd	促使mRNA与40S亚基结合
eIF3	＞500kd	促使mRNA与40S亚基结合
eIF4b	80kd	促使mRNA与40S亚基结合
eIF4a	50kd	促使与mRNA,GTP结合
eIF4C	19kd	促使两亚基结合
eIF5	150kd	释放eIF2, eIF3
eIF4e	(eIF4f 的亚基)	与5‘端帽子结合



(a) : translation of Capped *Sindbis* virus mRNA

(b) : translation of Uncapped *picona* virus mRNA

eIF4e stimulates translation of capped, but not uncapped,

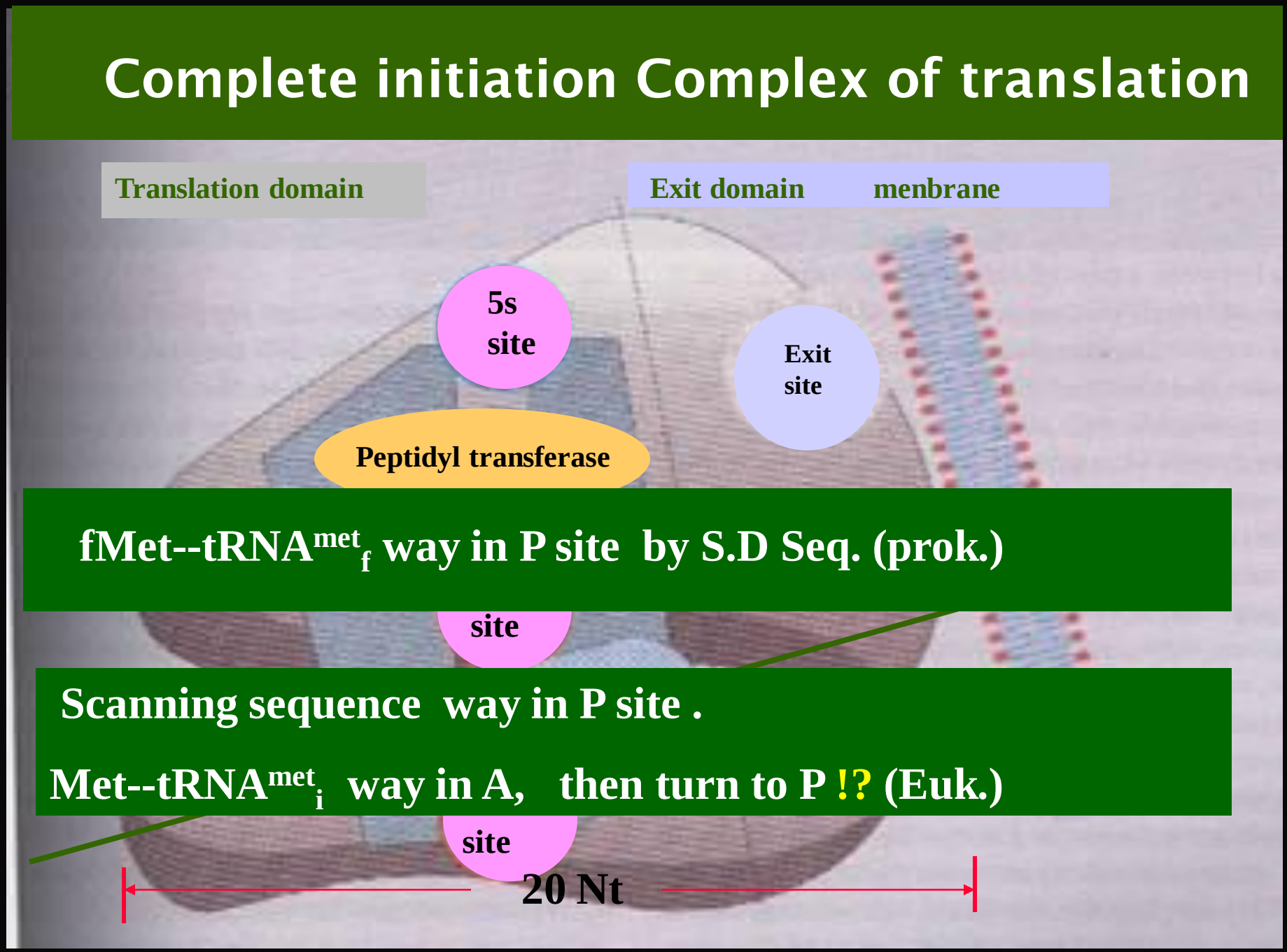
(Source:Shatkin,Differential stimulation of capped mRNA translation in vitro by cap-binding protein Nature 285:331, 1980.

c. Initiation ribosome complex

including 8 activation sites & occupy 20± Nt

- **P site (peptidyl attachment site)**
- **A site (Aminoacyl binding site)**
- **E site (Exit site of tRNA)**
- **5s rRNA site (5s rRNA + TΨC loop)**
- **转位因子EF /G binding site**
- **mRNA biding site**
- **peptididyl transferase binding site**
- **延伸因子复合体EF-Tu-aa-tRNA^{aa} binding site**

Complete initiation Complex of translation



fMet--tRNA$^{met}_{f}$ way in P site by S.D Seq. (prok.)

Scanning sequence way in P site .

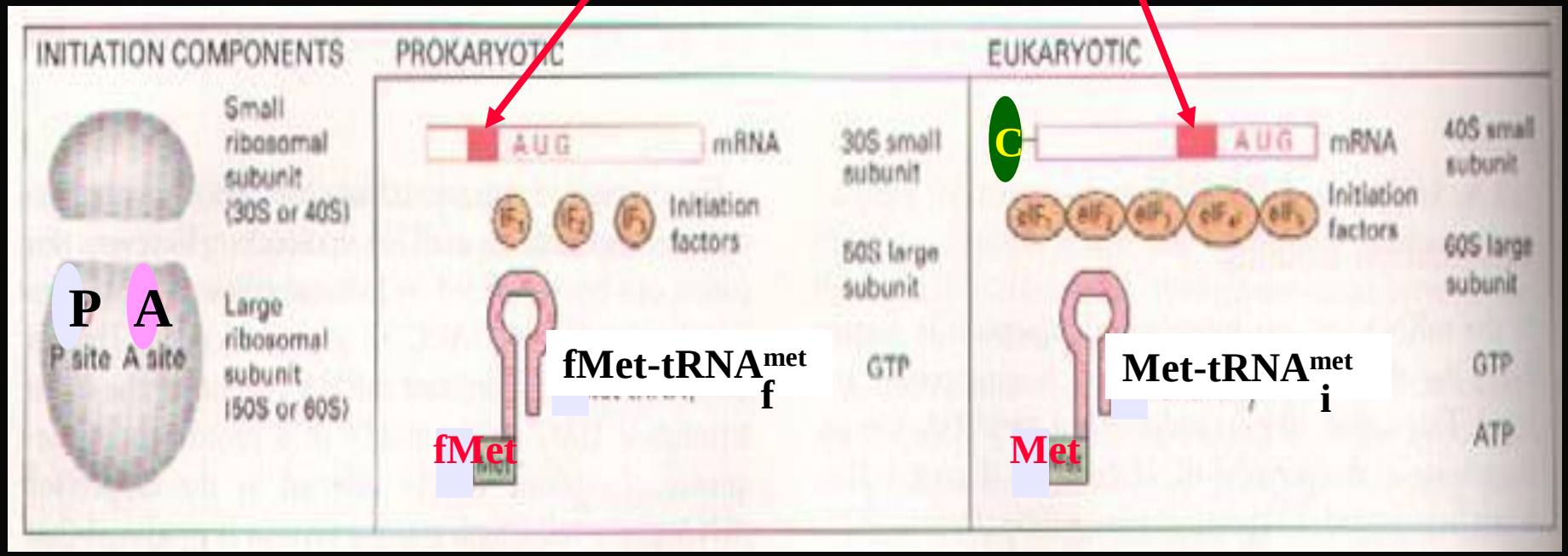
Met--tRNA$^{met}_{i}$ way in A, then turn to P !? (Euk.)

(来源：分子生物学（2007），郑用琏，第185页)

d. Processing initiation

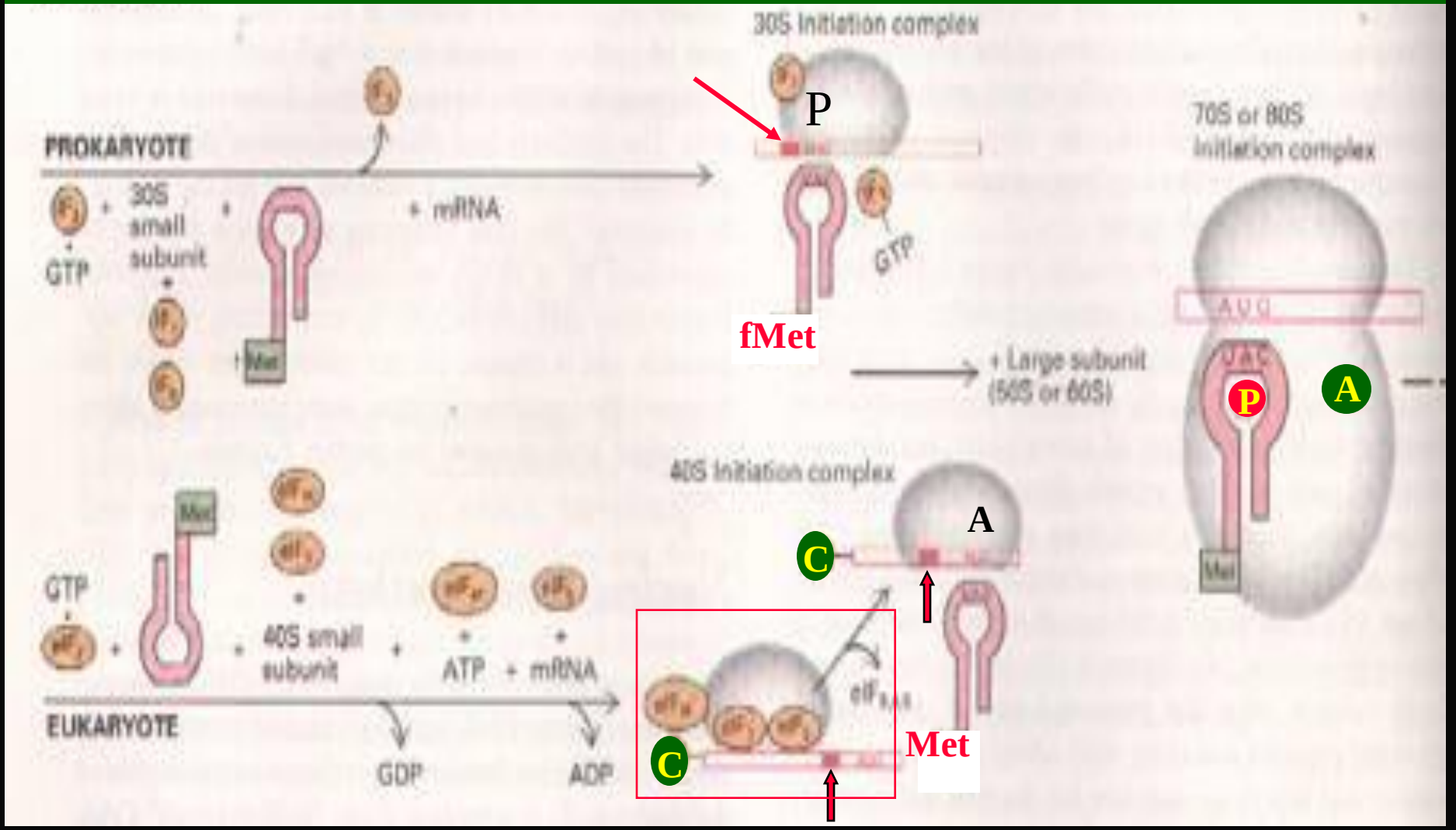
S.D. Sequence ?

Scanning sequence ?

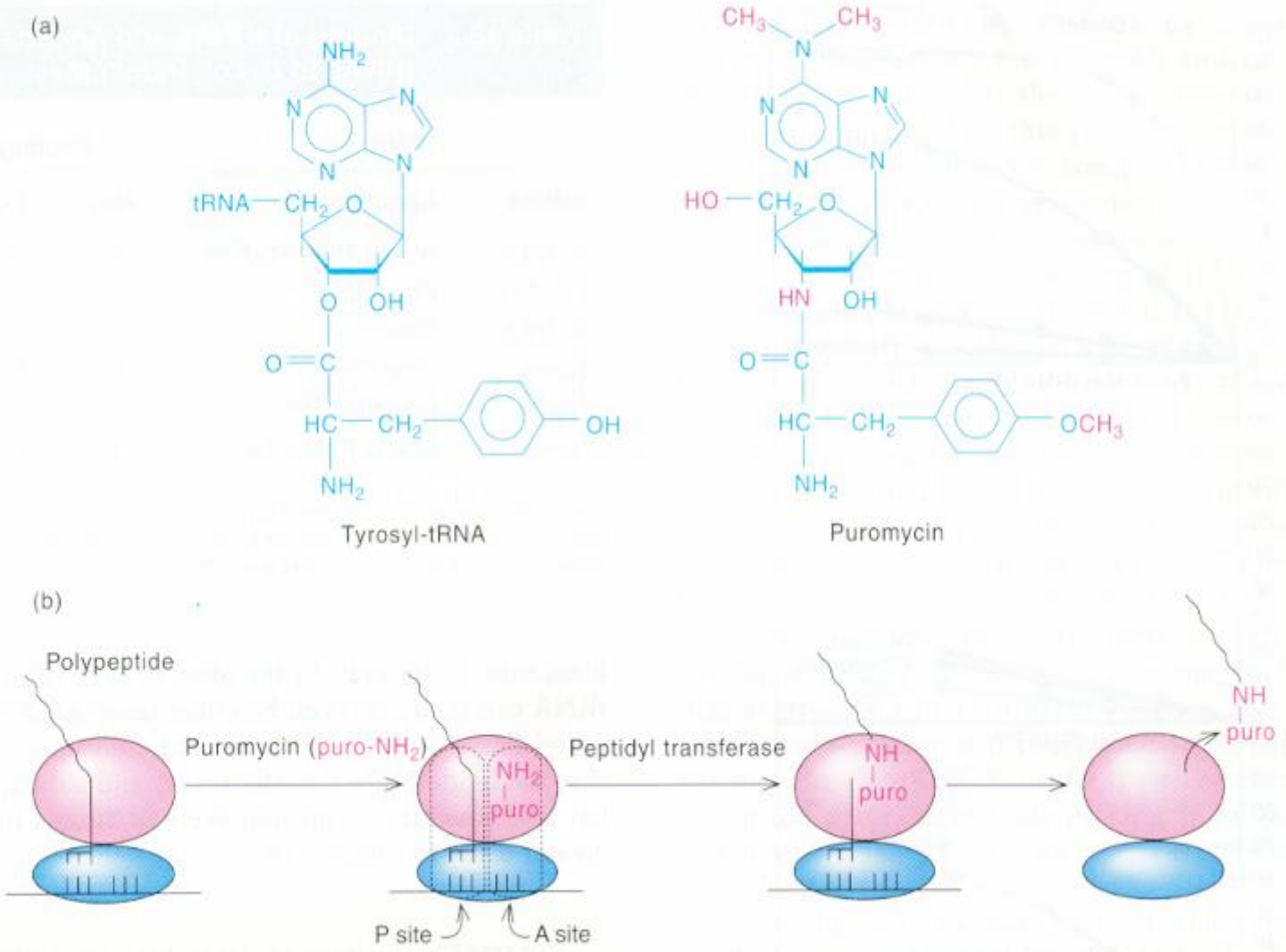


(来源：不详)

Initiation



(来源：分子生物学（2007），郑用琏，第205页)



(Source:Molecular Biology(2002),Robert F.Weaver,Page575）

Chapter 5 Protein translation

- tRNA

  mini RNA, 4s, (70-80 Nt)

  Nt more modified by methylation

  tRNA phe, 77Nt cloverleaf form

  - Aa accept arm, DHU loop (contact with AARS), anti-codon loop, TΨC loop (contact with 5S rRNA), extra loop
  - Paracodon: a number of Nts, on tRNA, contact with AARS

- rRNA

  High GC-content, rich methylation, high copy number, synthesized in nucleolus

  Pro: 23S + 5S, 16S; Euro: 28S/5.8S + 5S, 18S

- mRNA
  - Pro: Shine-Dalgarno seq. (S.D seq) GGAGG
  - Euro: 5’ m7Gppp--- -----CCACC-----A-3---A1U2G3G4—
  - Degeneracy of codon
  - Codon family
  - Mechanism of codon degeneracy
    - Isoacceptor: different tRNA that load the same aa, but recognize different/same codon
    - Wobble hypothesis: 34th Nt in tRNA
- Codon usuage/bias

- mRNA

  Pro: Shine-Dalgarno seq. (S.D seq) GGAGG

  Euro: 5’ m7Gppp--- -----CCACC-----A-3---A1U2G3G4—

  Degeneracy of codon

  Mechanism of codon degeneracy

  - Isoacceptor: different tRNA that load the same aa, but recognize different/same codon
  - Wobble hypothesis: 34th Nt in tRNA

- Codon usuage/bias

  Different condons are used at different frequency by a species

- Peptide synthesis

  Direction of peptide elongation

  Aminoacyl—tRNA^{aa}, Initiation and elongation

  AARS

  - three sites: tRNA site, AA site, ATP
  - DHU loop, nonspecific; paracodon, specific

  Enzymes for translation in Prok

  - IF1: separate 50S and 30S subunits, help other factors
  - IF2: for the binding of fMet-tRNA $_{f}^{met}$ to 30S
  - IF3: for the binding of mRNA to 30S

- Peptide synthesis

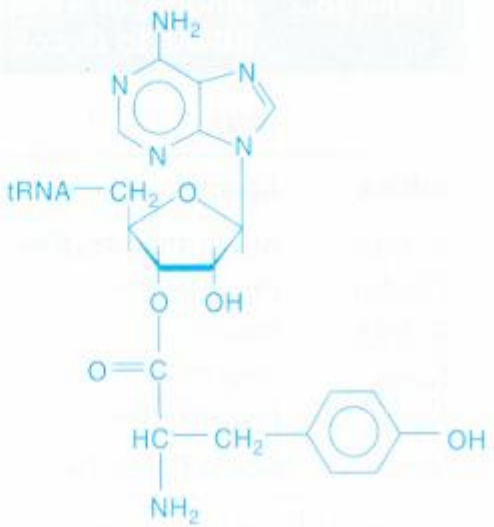
  Enzymes for translation in Eu

  - eIF4e, cap binding factor
  - eIF4e stimulates translation of capped, but not uncapped mRNA
  - *Met-tRNA*^{Met} occupies ribosomal P site & Initiation translation
  - Tu and Ts

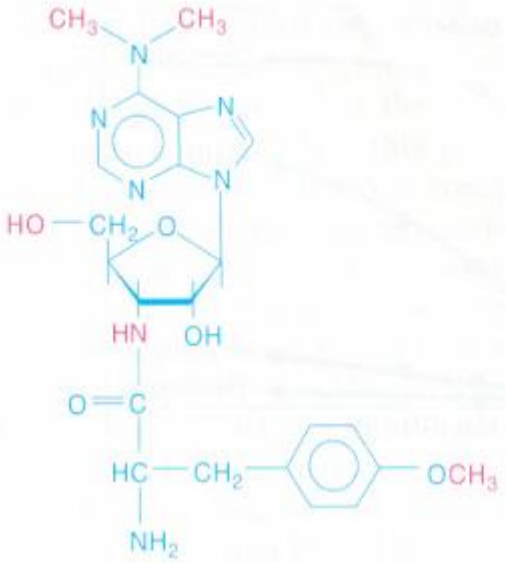
A Three-Site Model of Ribosome

- Puromycin
  - Resembles an aminoacyl-tRNA
  - Can bind to the A site
  - Couple with the peptide in the P site
  - Release it as peptidyl puromycin
- If peptidyl-tRNA is in the A site, puromycin will not bind to ribosome, peptide will not be released
- Two sites are defined on the ribosome:
  - Puromycin-reactive site (P)
  - Puromycin unreactive site (A)
- 3rd site (E) for deacylated tRNA bind to E site as exits ribosome

(a)

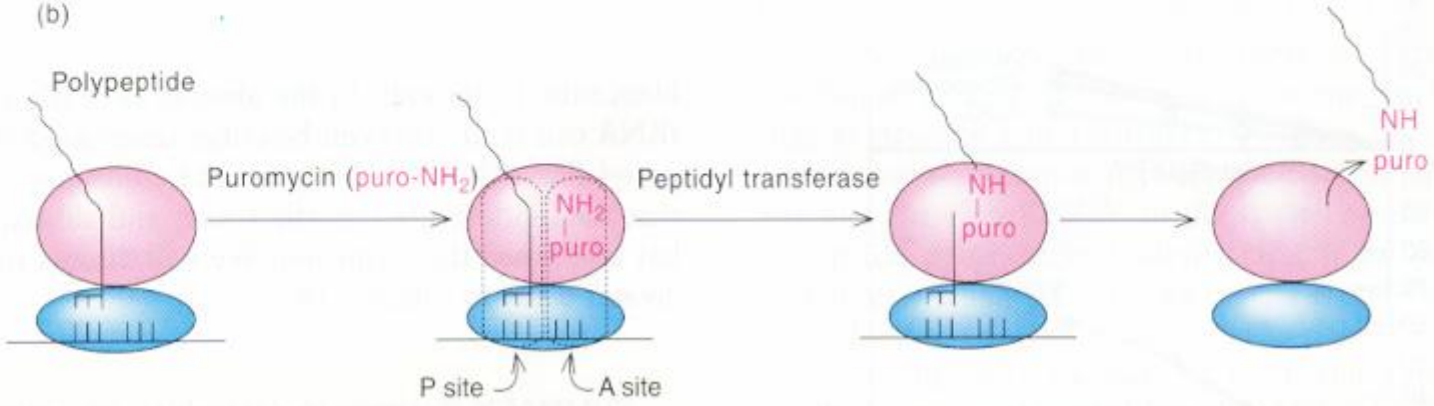


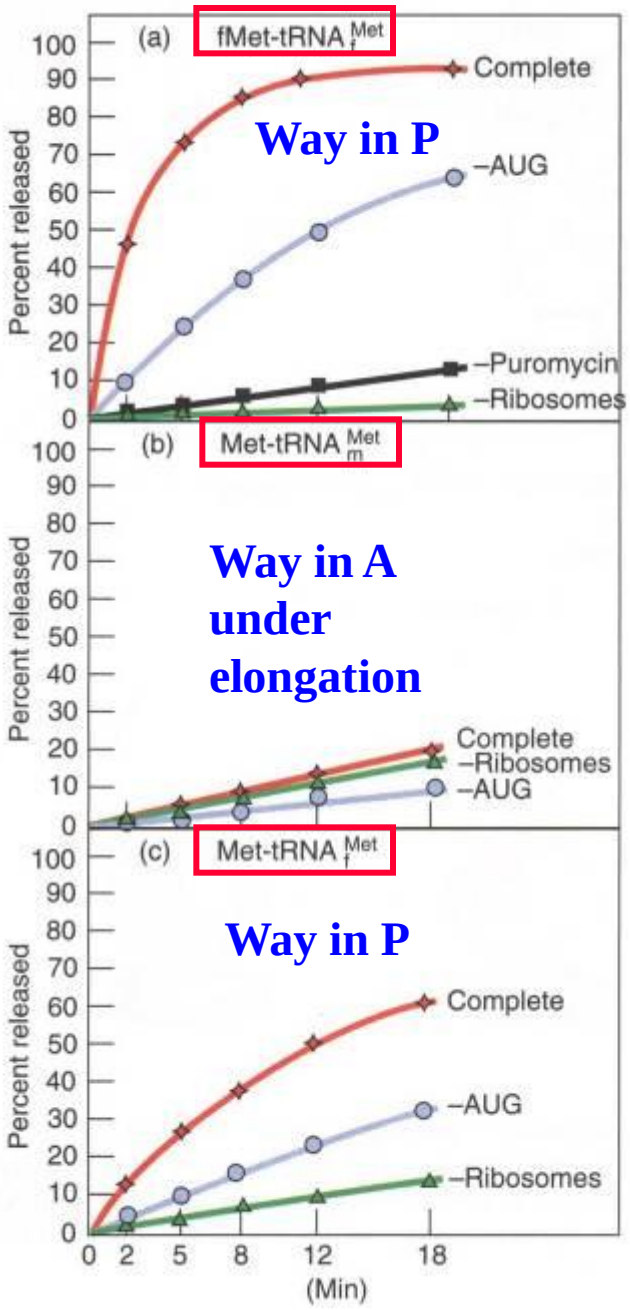
Tyrosyl-tRNA



Puromycin

(b)





• Mixed [^{35}S]fMet-tRNAf^{Met} with ribosomes, AUG, and puromycin(嘌呤酶素).

• ***If AUG attracted fMet-tRNA^{Met} to the P site, then the labeled fMet should have been able to react with puromycin(in A site), releasing labeled fMet-puromycin.***

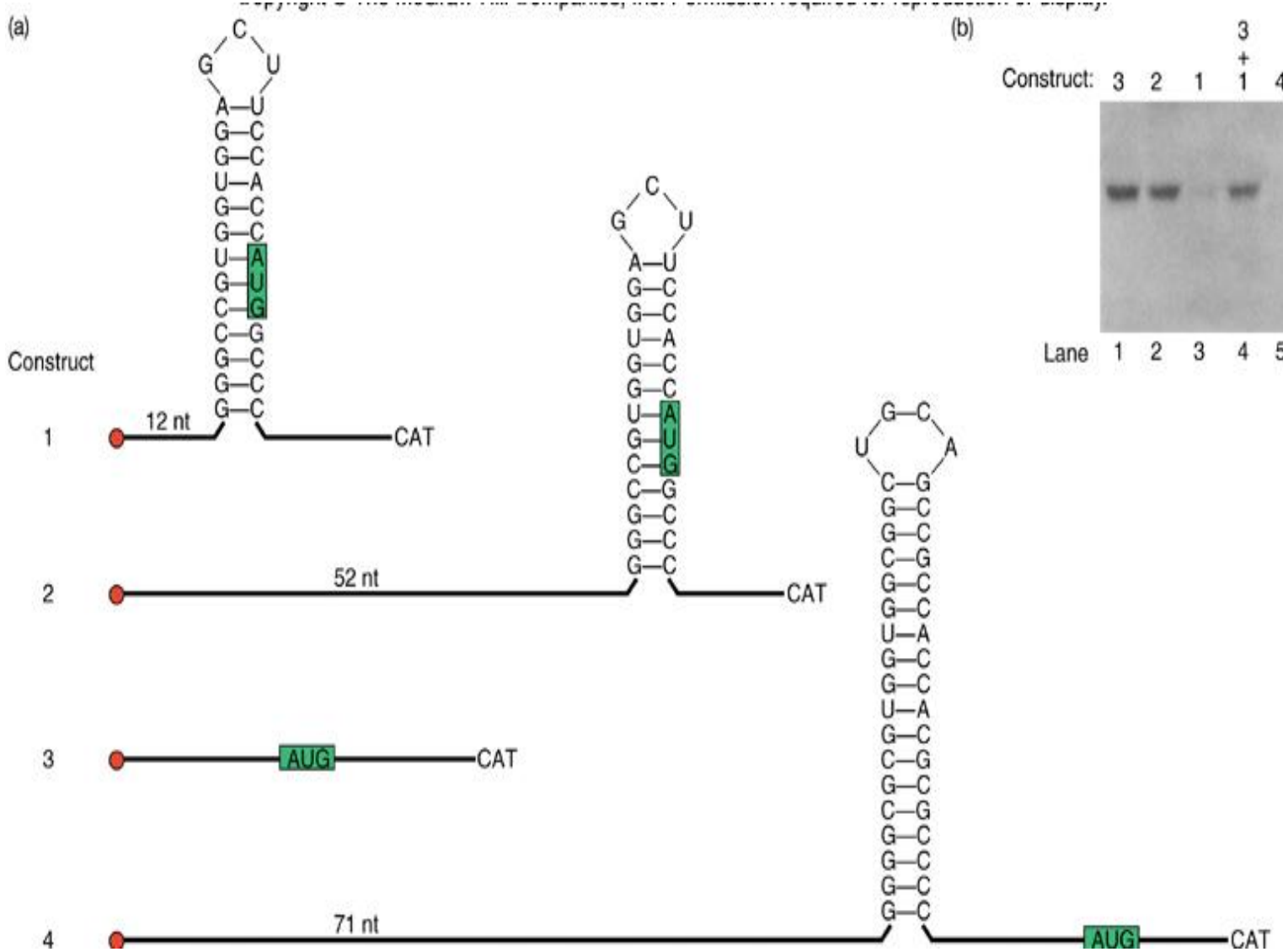
• If the *fMet-tRNA^{Met}* went to the **A** site, puromycin should **not** have been able to bind, so **no release** of labeled amino acid should have occurred.

• ***fMet-tRNA^{Met}*** **occupies ribosomal P site & Initiation translation**

(Source:Bretscher and Marcker Nature 211:382-3, 1966)

Effects of mRNA Secondary Structure

- **Secondary structure near the 5'-end of an mRNA can have either positive or negative effects**
- **Hairpin just past an AUG can force a pause by ribosomal subunit and stimulate translation**
- **Very stable stem loop between cap and initiation site can block scanning and inhibit translation**


(a)
Construct
1
12 nt
CAT
2
52 nt
CAT
3
AUG
CAT
4
71 nt
AUG
CAT
(b)
Construct: 3 2 1 3+1 4 3+4
Lane 1 2 3 4 5 6

Control of Initiation

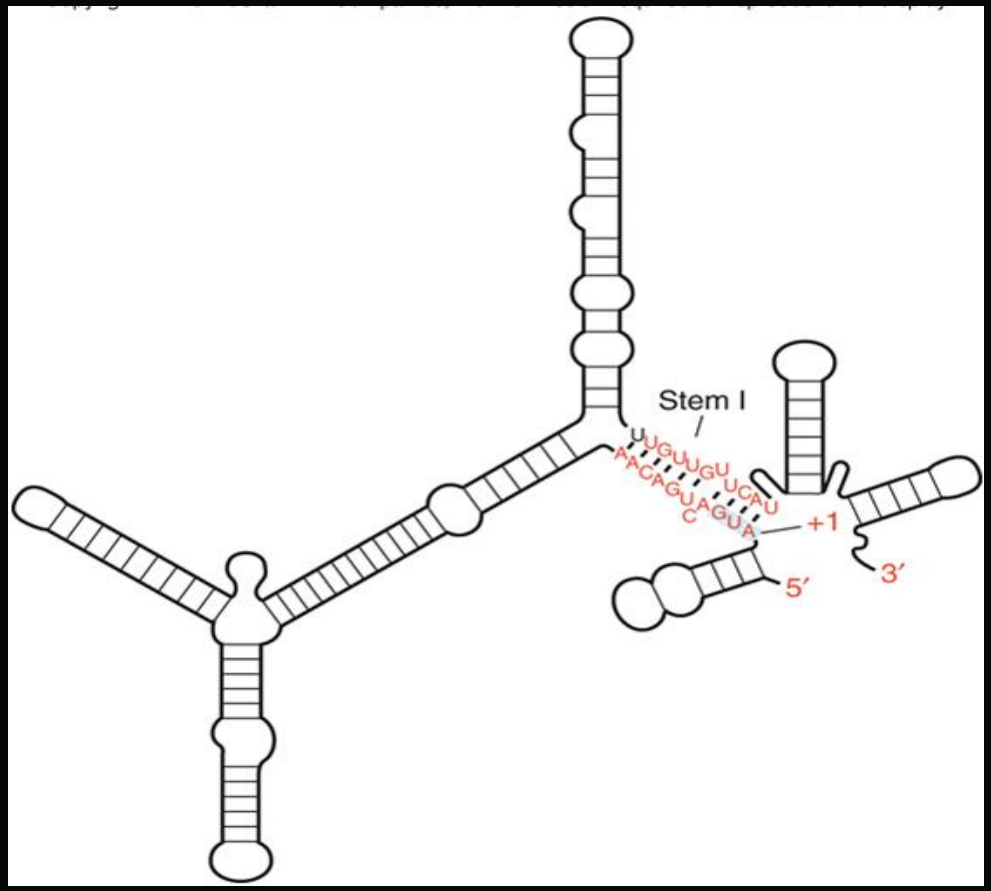
- Given the amount of control at the transcriptional and posttranscriptional levels, why control gene expression at translational level?
- Major advantage = speed
  - New gene products can be produced quickly
  - Simply turn on translation of preexisting mRNA
    - Valuable in eukaryotes
    - Transcripts are relatively long
    - Take correspondingly long time to make
  - Most control of translation happens at the initiation step

Bacterial Translational Control

- Most bacterial gene expression is controlled at transcription level
- Majority of bacterial mRNA has a very short lifetime
  - Only 1 to 3 minutes
  - Allows bacteria to respond quickly to changing circumstances
- Different cistrons on a polycistronic transcript can be translated better than others

Shifts in mRNA Secondary Structure

- mRNA secondary structure can govern translation initiation
  - Replicase gene of the MS2 class of phages
    - Initiation codon is buried in secondary structure until ribosomes translating the coat gene open up the structure
  - Heat shock sigma factor, σ^{32} of *E. coli*
    - Repressed by secondary structure that is relaxed by heating
    - Heat can cause an immediate unmasking of initiation codons and burst of synthesis



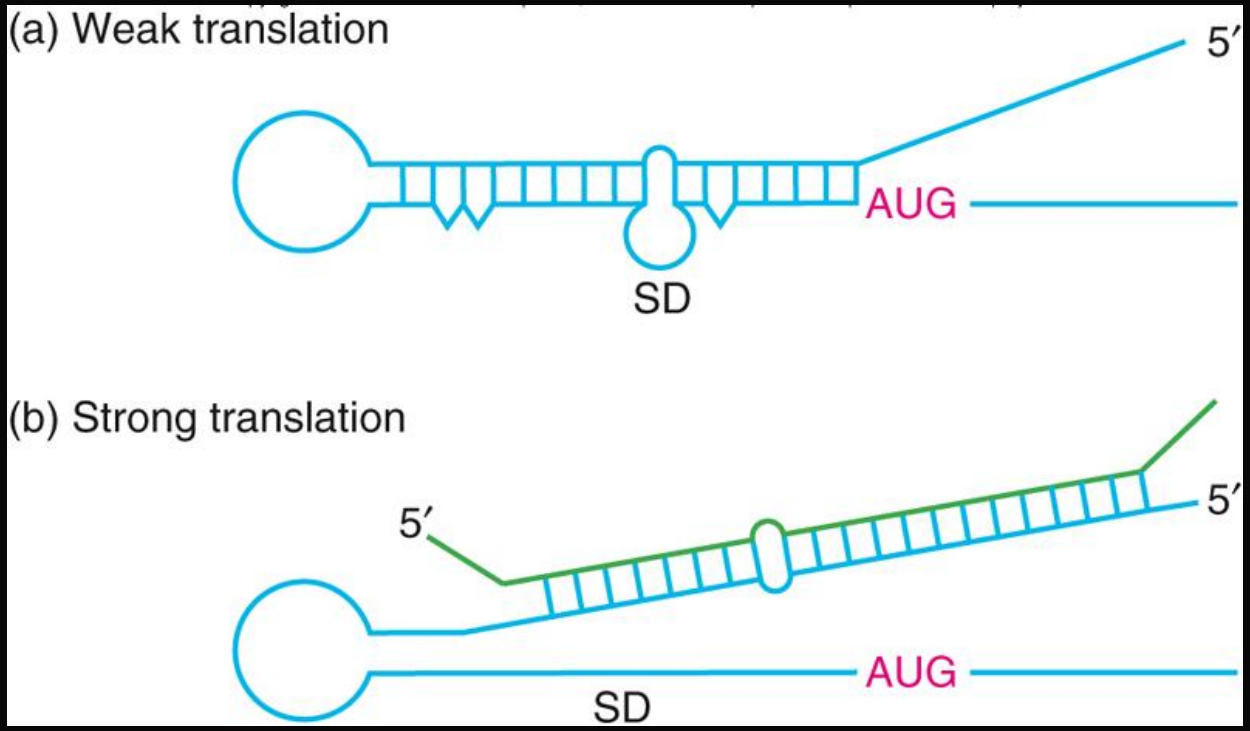
Shift from σ^{70} to σ^{32} at temperature higher than 37° C

当突变使茎I的碱基配对增强时，高温诱导作用减弱：如+5的C变为A，诱导作用由3.5倍降低为1.4倍

当突变使茎I的碱基配对减弱时，高温诱导作用增强

mRNAs Induce mRNA Secondary Structure Shifts

- Small RNAs with proteins can affect mRNA secondary structure to control translation initiation



Stimulation by an mRNA-Binding Protein

- Ferritin mRNA translation is subject to induction by iron
- Induction seems to work as follows:
  - Repressor protein (aconitase apoprotein) binds to stem loop iron response element (IRE)
  - Binding occurs near 5’-end of the 5’-UTR of the ferritin mRNA
  - Iron removes this repressor and allows mRNA translation to proceed

Elongation: Protein Factors and Peptide Bond Formation

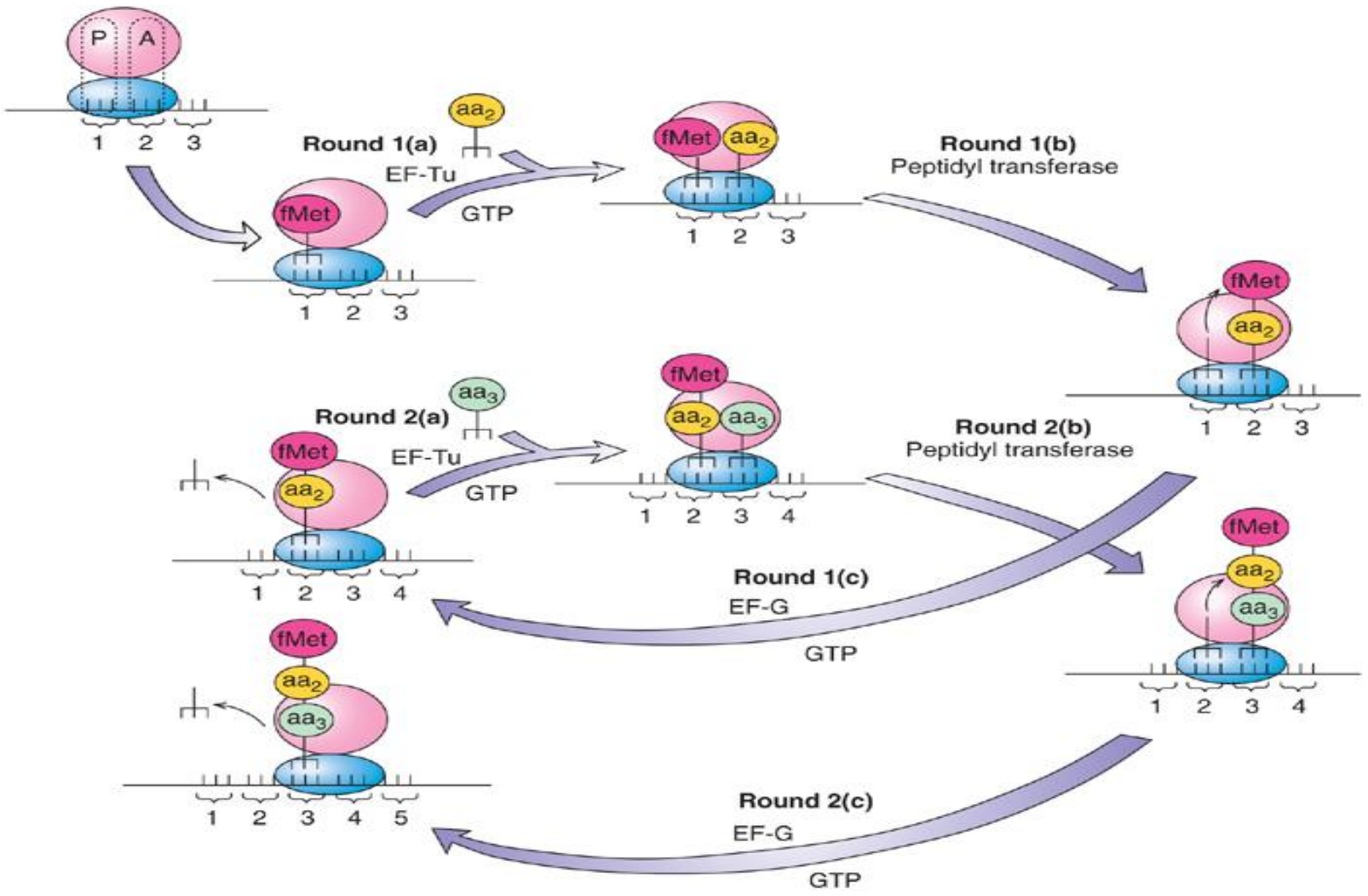
- One factor is T, transfer
  - It transfers aminoacyl-tRNAs to the ribosome
  - Actually 2 different proteins
    - Tu, u stands for unstable
    - Ts, s stands for stable
- Second factor is G, GTPase activity
- Factors EF-Tu and EF-Ts are involved in the first elongation step
- Factor EF-g participates in the third step

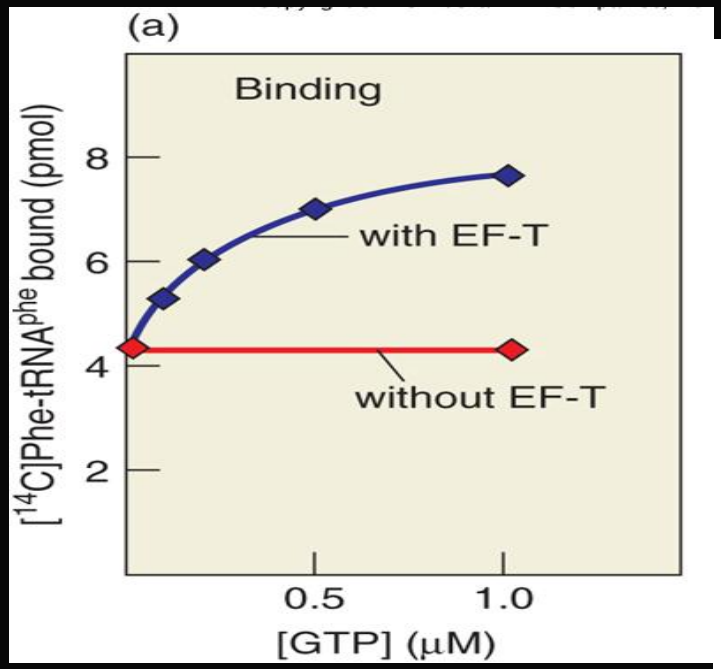
Elongation: The Mechanism

Elongation takes place in three steps:

1. EF-Tu with GTP binds aminoacyl-tRNA to the ribosomal A site
2. Peptidyl transferase forms a peptide bond between peptide in P site and newly arrived aminoacyl-tRNA in the A site

   Lengthens peptide by one amino acid and shifts it to the A site
3. EF-G with GTP translocates the growing peptidyl-tRNA with its mRNA codon to the P site


P
A
1 2 3
Round 1(a)
EF-Tu
GTP
aa2
fMet
Round 1(b)
Peptidyl transferase
Round 2(a)
aa3
Round 2(b)
Peptidyl transferase
Round 1(c)
EF-G
GTP
Round 2(c)
EF-G
GTP



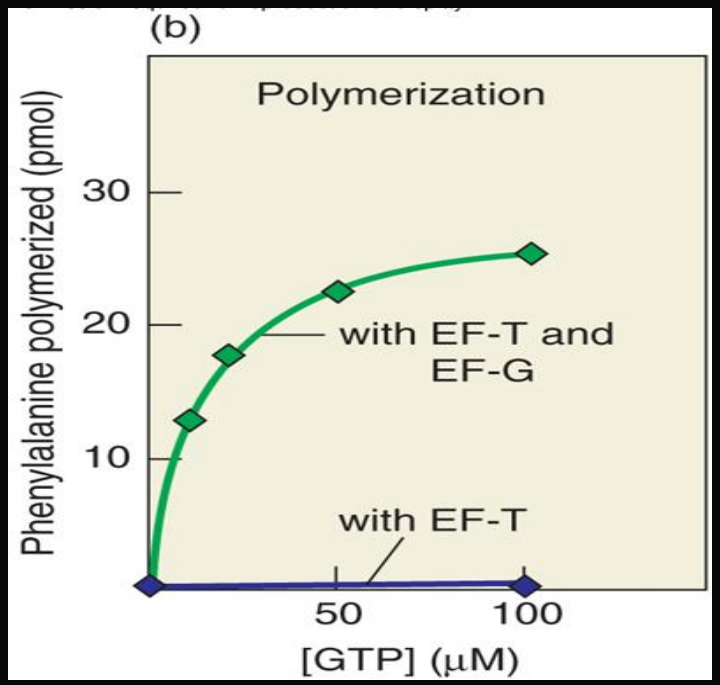
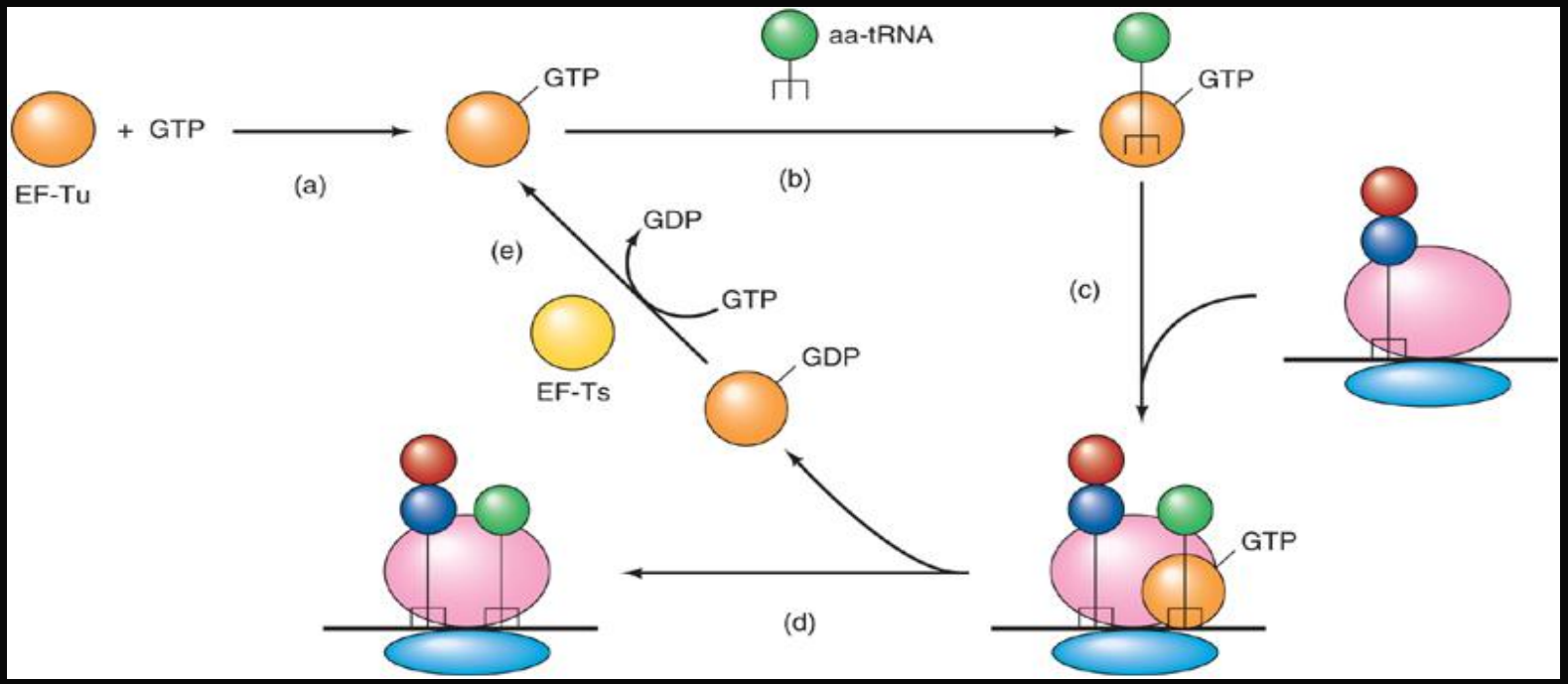


Figure 18.13 Effects of EF-T and GTP on Phe-tRNA^{Phe} binding to ribosomes and on poly-Phe synthesis. **(a)** Binding Phe-tRNA^{Phe} to ribosomes. Ravel mixed ^{14}C-Phe-tRNA^{Phe} with washed ribosomes and various concentrations of GTP in the presence or absence of EF-T. She measured Phe-tRNA^{Phe}–ribosome binding by filtering the mixture and determining the labeled Phe bound to the ribosomes on the filter. Considerable nonenzymatic binding occurred in the absence of EF-T and GTP, but the EF-T-dependent binding required GTP. **(b)** Polymerization of phenylalanine. Ravel mixed labeled Phe-tRNA^{Phe} with ribosomes, EF-T, and various concentrations of GTP in the presence and absence of EF-G. She measured polymerization of Phe by acid precipitation as follows: She precipitated the poly(Phe) with trichloroacetic acid (TCA), heated the precipitate in the presence of TCA to hydrolyze any phe-tRNA^{Phe}, and trapped the precipitated poly(Phe) on filters. Polymerization required both EF-T and EF-G and a high concentration of GTP. (*Source:* Adapted from Ravel, J.M., Demonstration

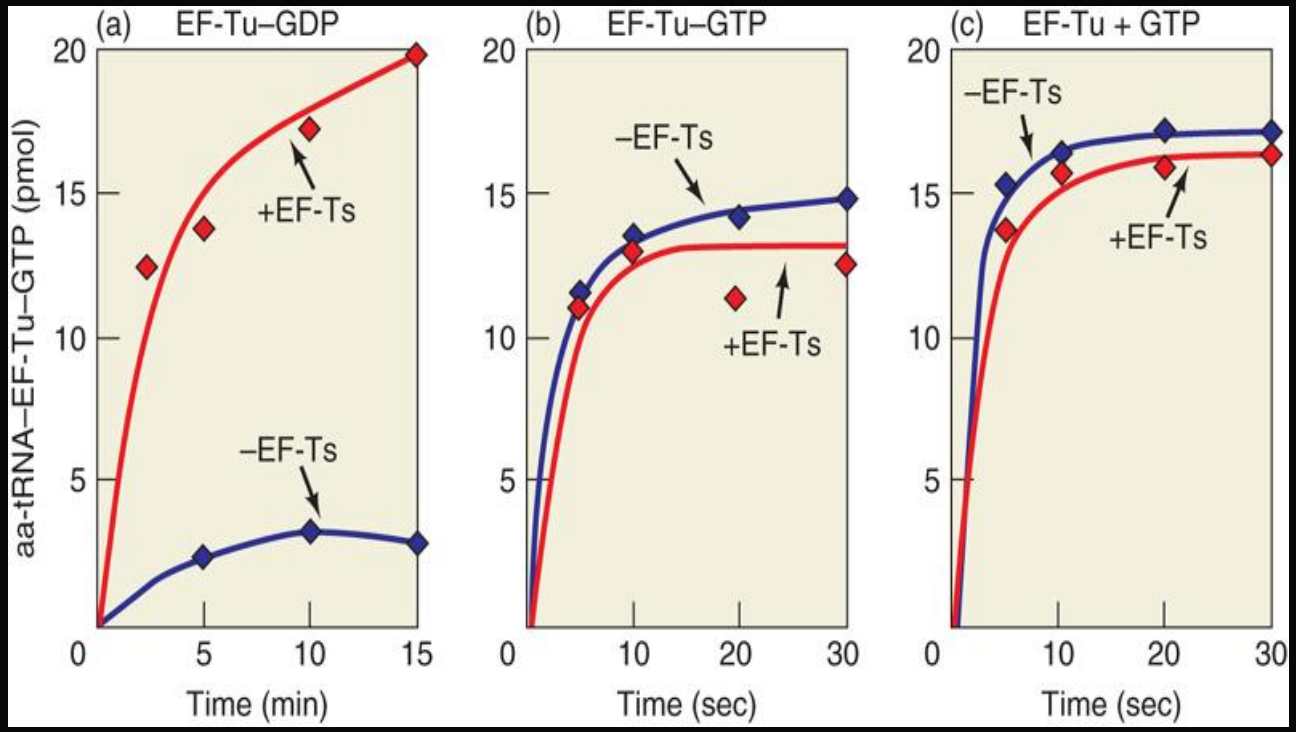
EF-T dependent binding of charged tRNA to ribosome required GTP

Polymerization required both EF-T and EF-G and a high concentration of GTP

The cycle of T (Tu and Ts)



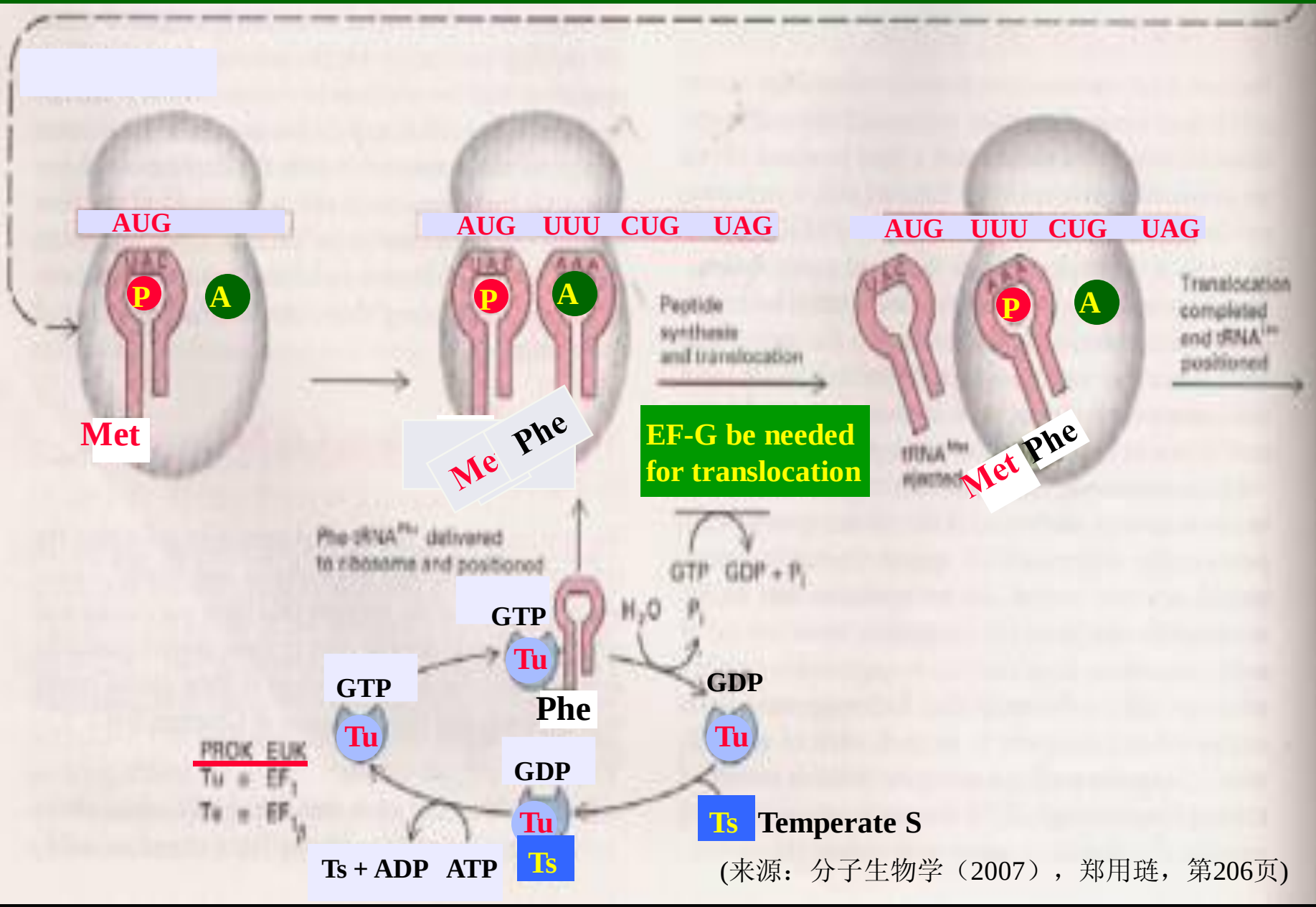
(a) EF-Tu与GTP结合形成二元复合物
(b) 进一步与aminoacyl-tRNA 形成三元复合物
(c) 三元复合物与P位点已有peptidyl-tRNA的核糖体结合
(d) GTP被水解，形成 EF-Tu－GDP复合体，从核糖体上解离，在A位点留下新的aminoacyl-tRNA
(e) EF-Ts exchanges GTP for GDP on EF-Tu，生成新的 EF-Tu－GTP复合体



EF-Ts在以**EF-Tu–GDP** 为底物时能够促进aminoacyl-tRNA 、Tu、GTP三元复合物的形成 **(panel a).**

EF-Tu–GTP (panel b) or EF-Tu+GTP (panel c) 能够不依赖于**EF-Ts**自发形成三元复合物**.**

Elongation



(来源：分子生物学（2007），郑用琏，第206页)

Termination of peptide

---When stop codon into ribosome A site

↓

Release factor $_{1/2}$ (or transpeptidase or RF or rRNA ribozyme ?)

↓

Hydrolysis

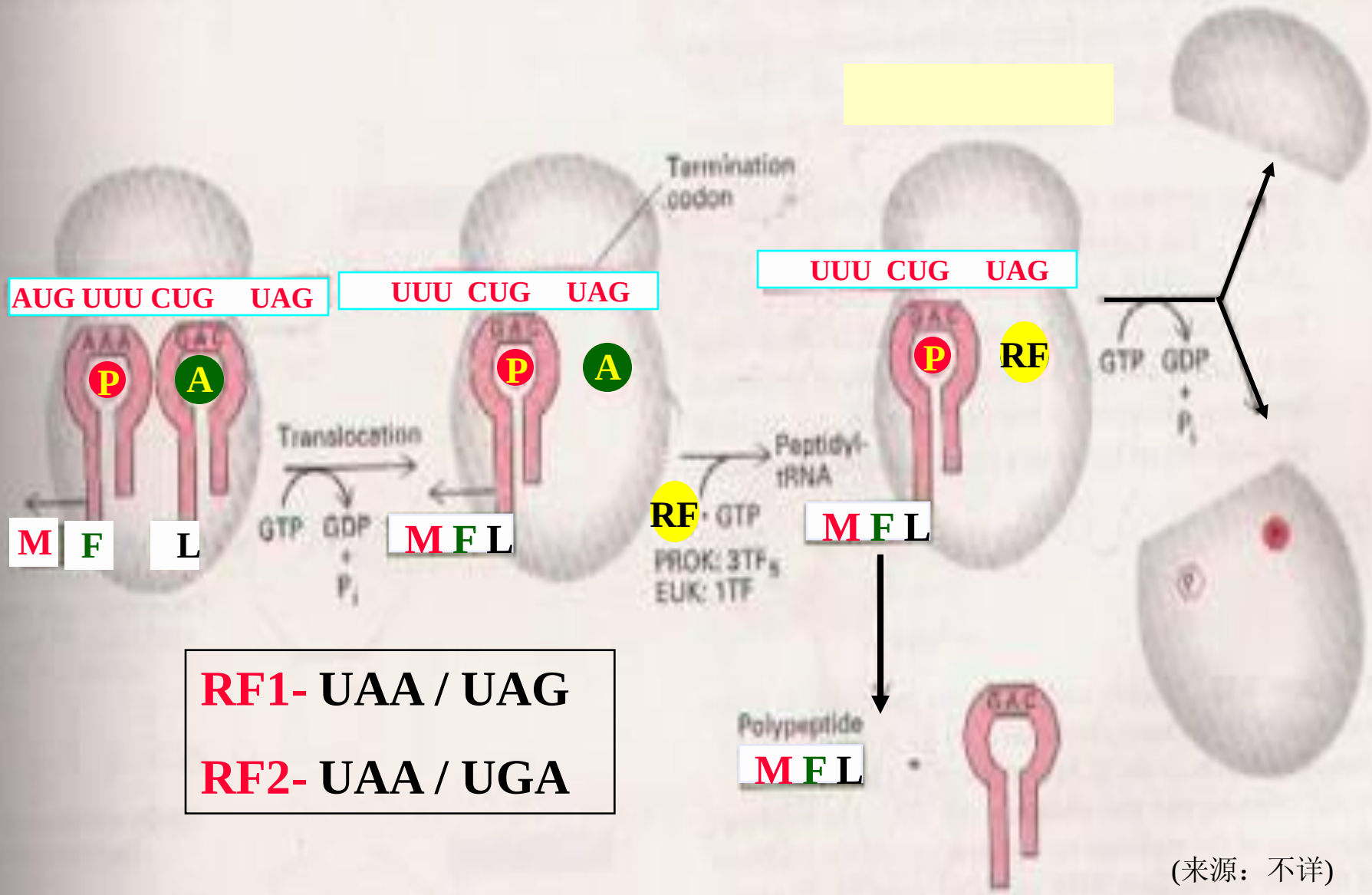
↓

Complex disassemble

↓

peptide + tRNA + mRNA + large & small subunit…

Termination



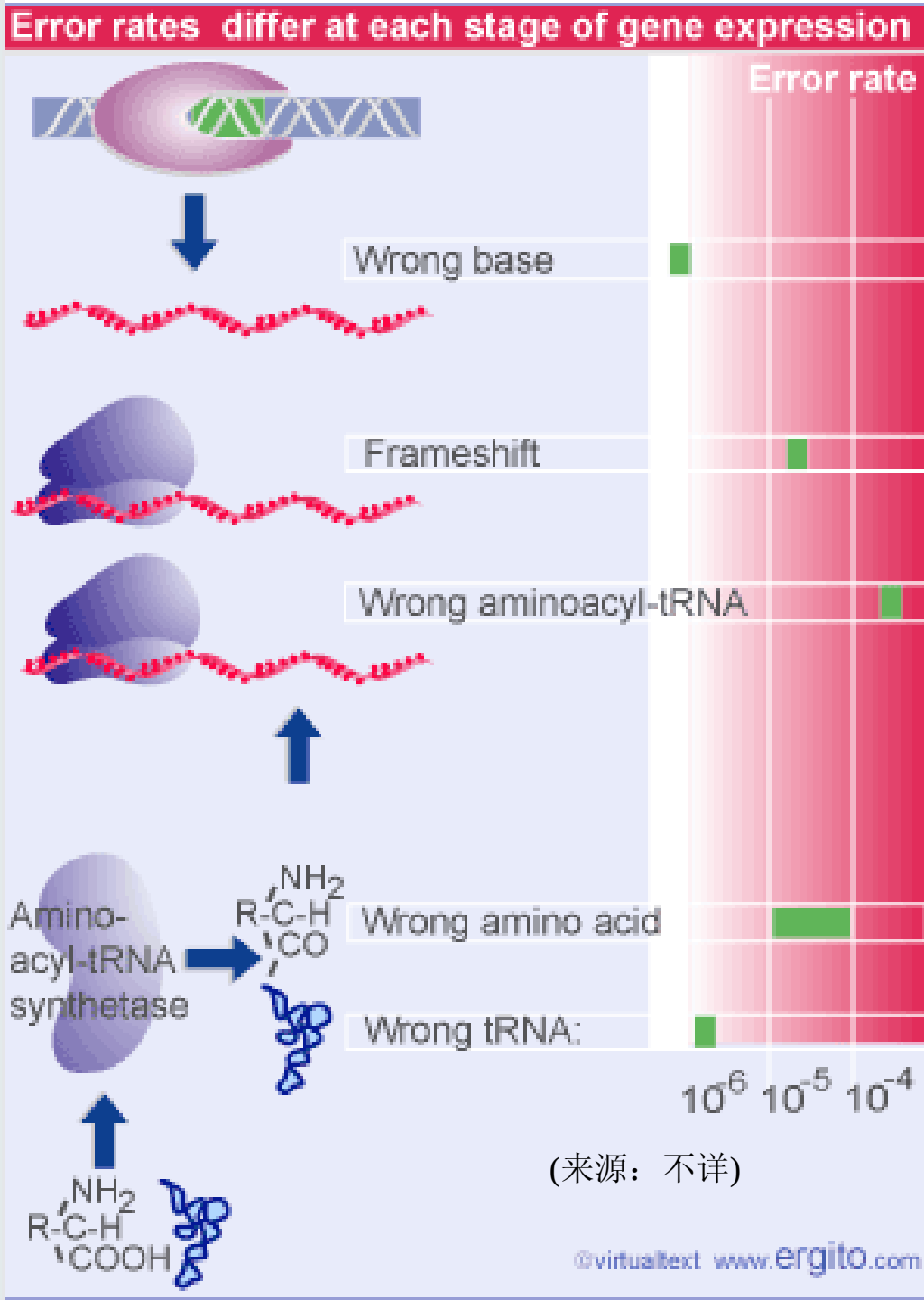
RF1- UAA / UAG

RF2- UAA / UGA

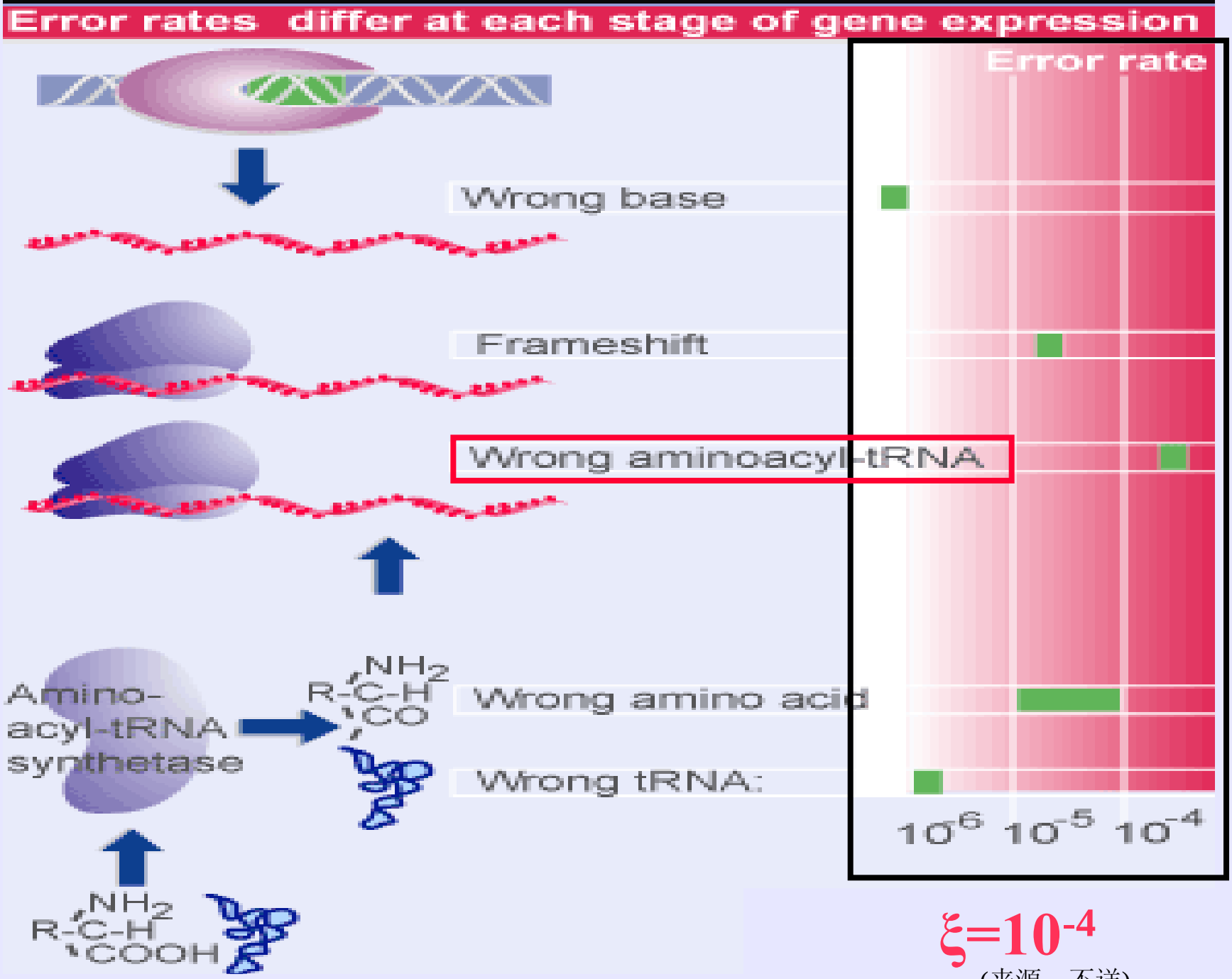
(来源：不详)

5.5.

保证肽链准确翻译的机制



(来源：不详)


Error rates differ at each stage of gene expression
Error rate
Wrong base
Frameshift
Wrong aminoacyl-tRNA
Wrong amino acid
Wrong tRNA:
Amino-acyl-tRNA synthetase
NH2
R-C-H
CO
NH2
R-C-H
COOH
10-6 10-5 10-4

$\xi=10^{-4}$

(来源：不详)

DNA replication $\xi = 10^{-11}$

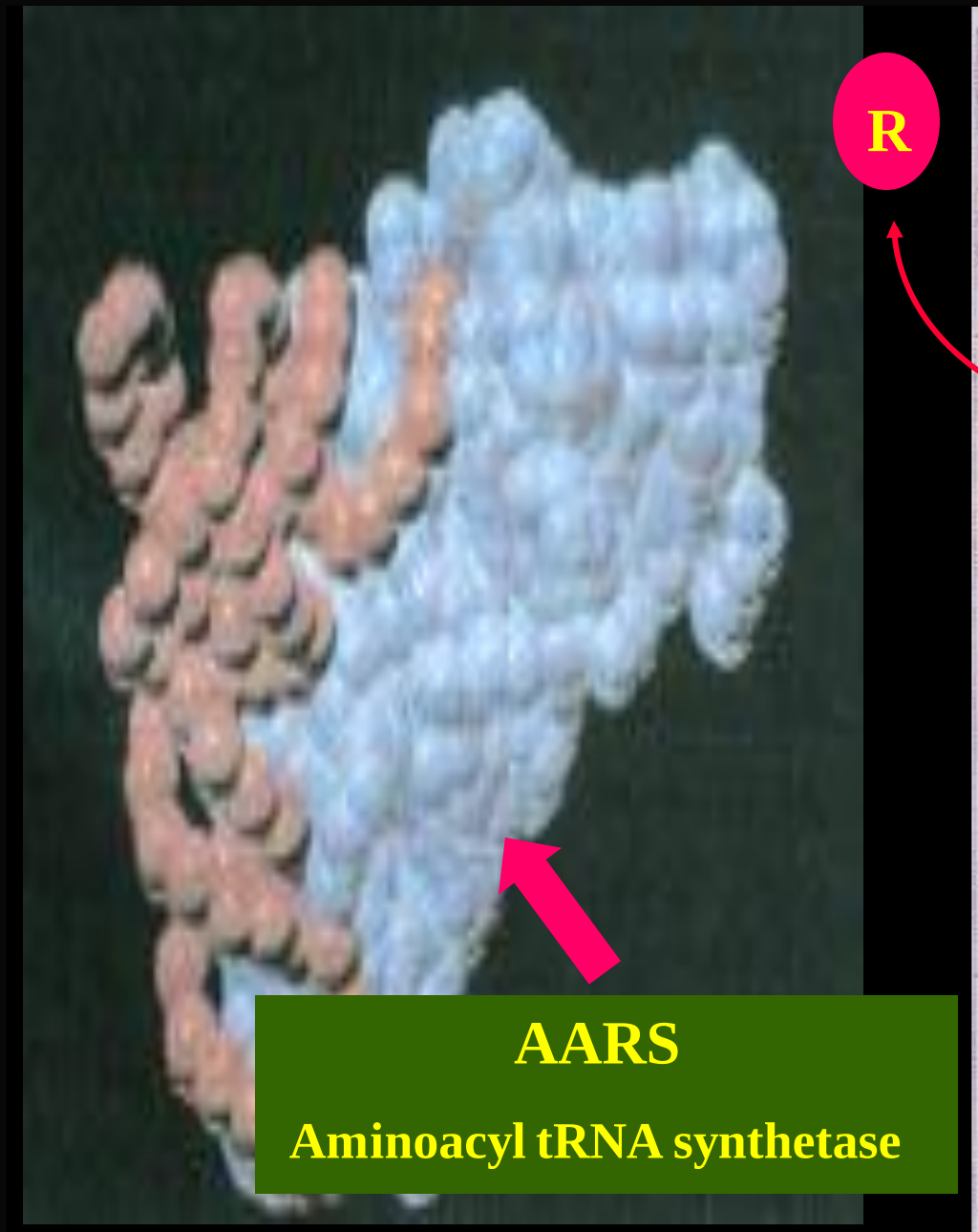
RNA transcription $\xi = 10^{-4}$

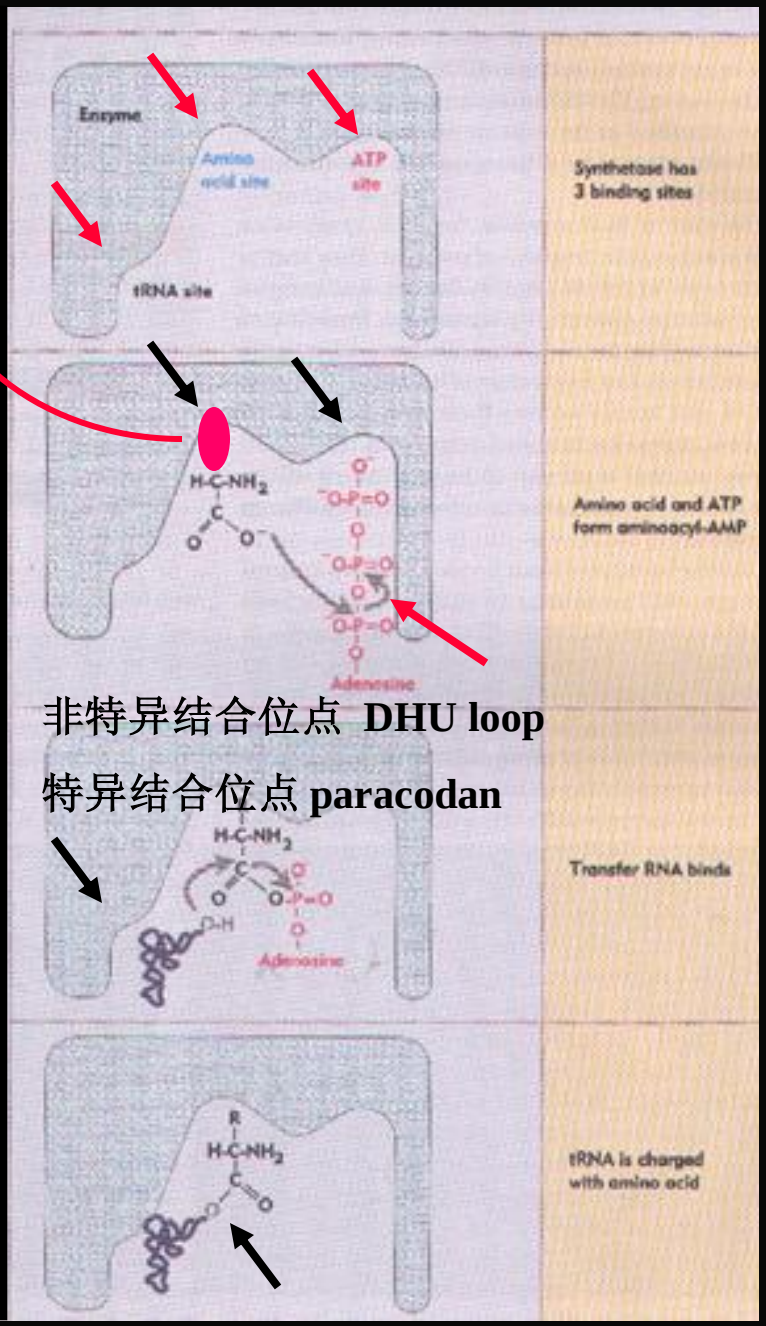
Peptide translation $P(\text{准确率}) = (1-\xi)^{n(\text{氨基酸的数目})}$

N	$P(\xi=10^{-2})$	$P(\xi=10^{-3})$	$P(\xi=10^{-4})$
100	36%	91.5%	99%
200	4.9%	84%	97%
1000	0.004%	36%	90%

√

MACHENISM ?





非特异结合位点 DHU loop

特异结合位点 paracodan

(来源：分子生物学（2007），郑用琏，第211页)

5.5.1. 氨基酸与tRNA间的负载专一性

a) 氨基酰tRNA合成酶（AARS）对氨基酸的特异识别与结合

AARS; aa binding site, tRNA binding site, ATP site

aa binding site 对结构相似的氨基酸的双筛作用

例；Cys $HS—CH_2—CH(NH_2)—COOH$

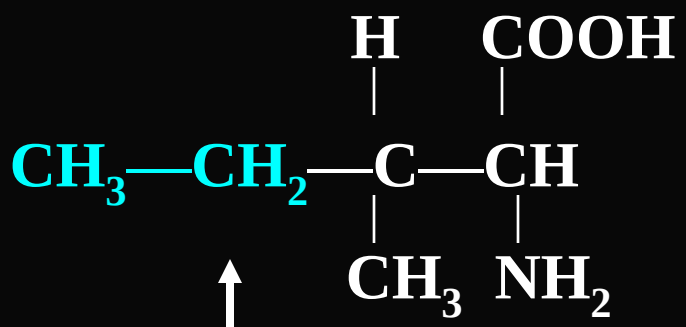
Ala-RS

Ala $H—CH_2—CH(NH_2)—COOH$

结构相似
错误识别
错误负载
错误翻译

In vitro Ile & Val 浓度相等的情况下

Ile

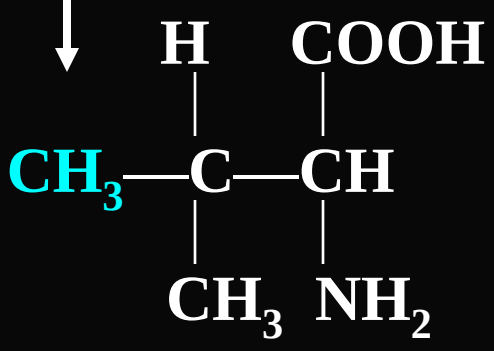


200X

∨ Ile-RS

1X

Val



Val-tRNA^{Ile} 错误负载机率

1/200 !

In vivo

Val : Ile = 5:1

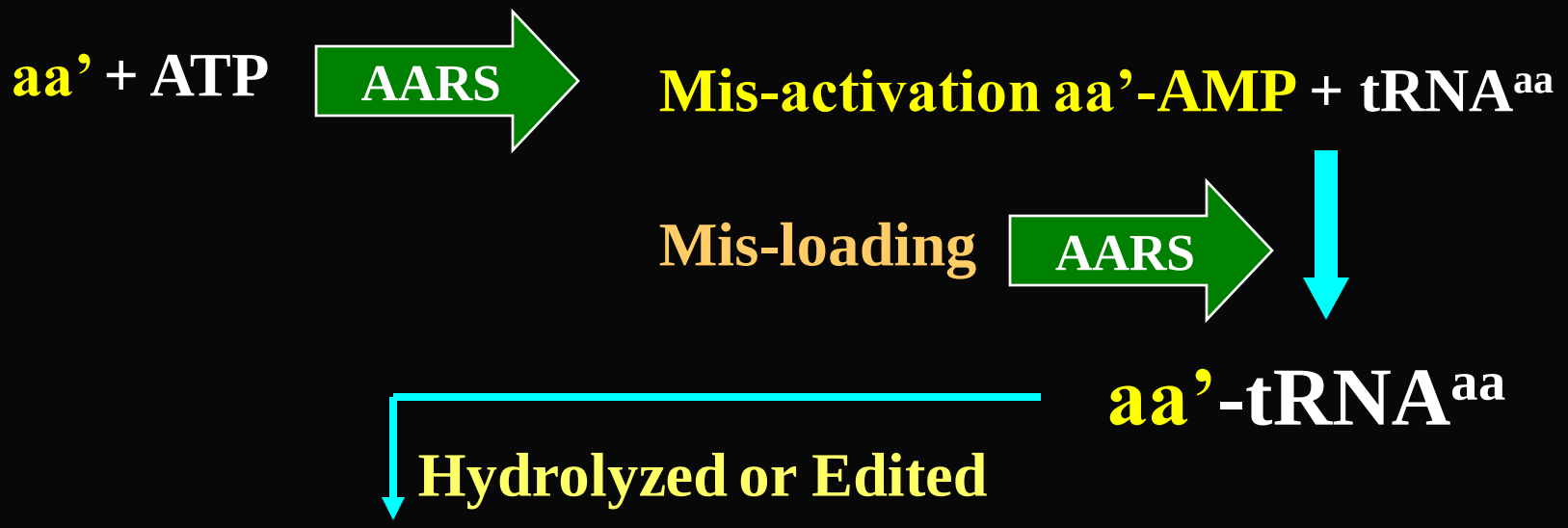
Val-tRNA^{Ile} 错误负载机率

1/40 !!

但实际测定的错译机率仅为1/3000 ?!

Double Sieve effect

How ?



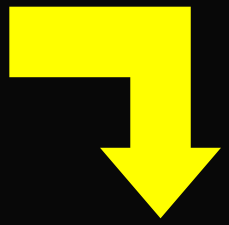
aa binding site具有

结合位点（ **Biding Site or Activation Site)**

水解位点（**Hydrolytic Site or Editing Site** ）

Ile / Val 进入B 位点

Kinetic
Comformational
Chemical



proofreading

发生诱导契合

Ile / Val 进入B 位点

Kinetic
Comformational
Chemical

proofreading → 发生诱导契合

↓

Ile 分子构型大于Val

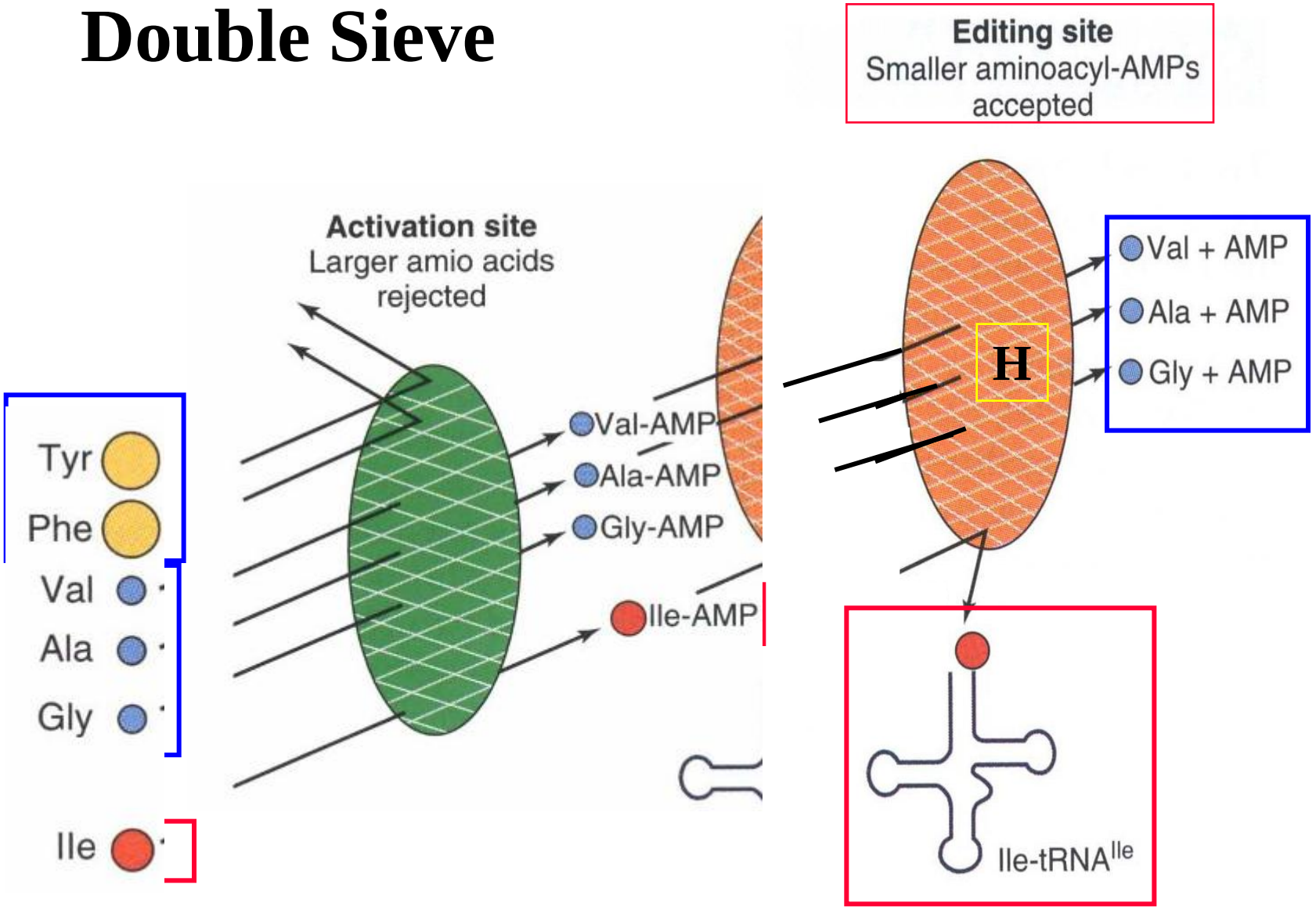
↓

H 位点柔性部位小

↓

- Ile进入B 位点但不能进入H 位点
- Val进入B 位点并进入H 位点而被降解

Double Sieve

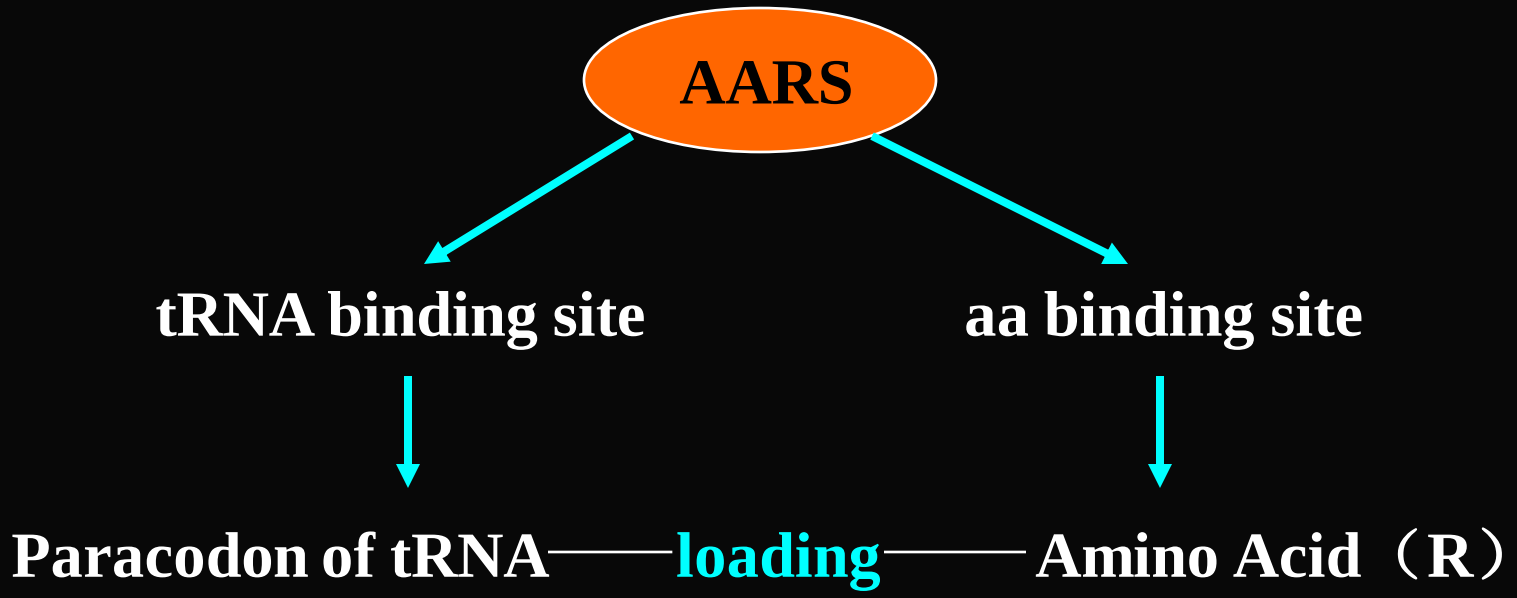


(来源：分子生物学（2007），郑用琏，第212页)

Paracodon (副密码子)的概念：

tRNA中决定负载特定氨基酸的空间密码

tRNA中的特定序列与AARS 的tRNA binding site的特异基团间的分子契合



● paracodon 的特征

--- 为同一种AARS 所识别的一组同功受体具有相同的副密码子(除$AARS_{ala}$外，其他证据不足！！)

$tRNA^{Ala}_{(GGC)}$
$tRNA^{Ala}_{(UGC)}$
} 具有G3 : U70 paracodon

--- paracodon 是为AARS 特定氨基酸所识别的若干Nts

（并非均为一对Nts，也并非仅只有一处的Nts）

--- AARS 对paracodon 的识别与结合是通过氨基酸与碱基之间的连接实现的。属于生物 II 型空间密码

--- paracodon 也是进化进程留下的 footprint

tRNA可能起源于可以携带氨基酸的oligo Nt

由AARS 特异识别 tRNA 中的特定序列

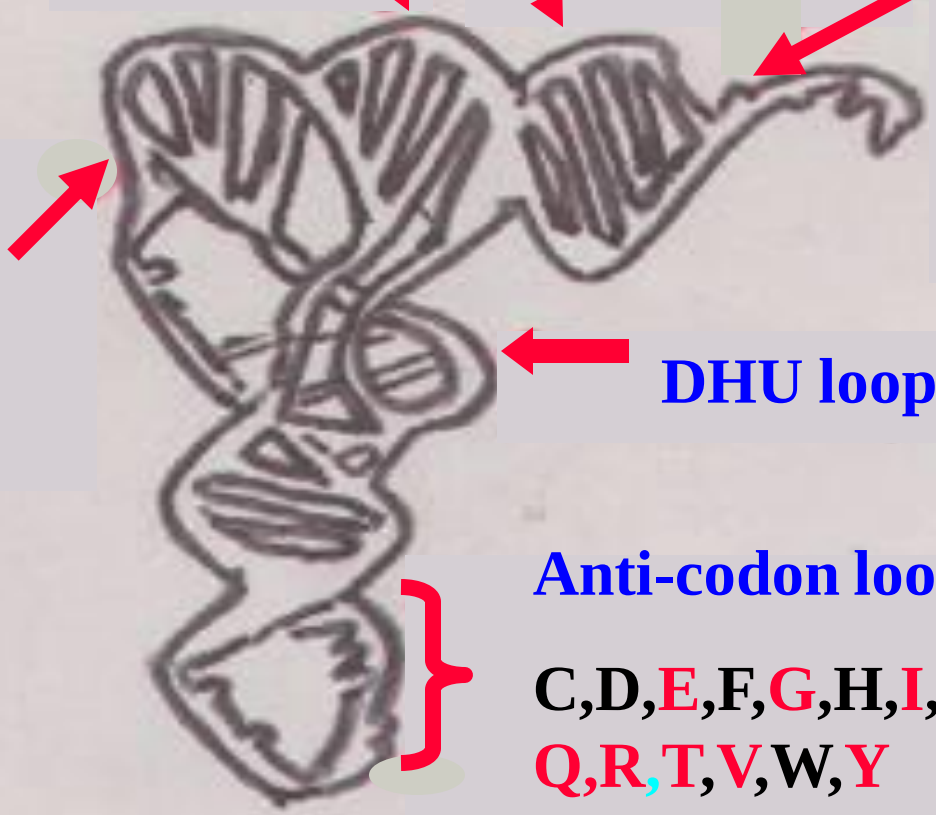
使氨基酸的负载更为准确

成为进化的优势

--- paracodon位于tRNA 的各种环或臂上

不同tRNA 的 paracodon 的定位不同

The position of Paracodon



aa arm

A,D,G,H,N,S,
T,V,W

TφC loop

73th site

A.C.D.E.F.G.
H.I.K.L.M.N.
P.Q.R.S.V.W.
Y.

Extra loop

A, F, L, R

DHU loop

Anti-codon loop

C,D,E,F,G,H,I,K,M,N,P
Q,R,T,V,W,Y

(来源：分子生物学（2007），郑用琏，第212页)

第五章

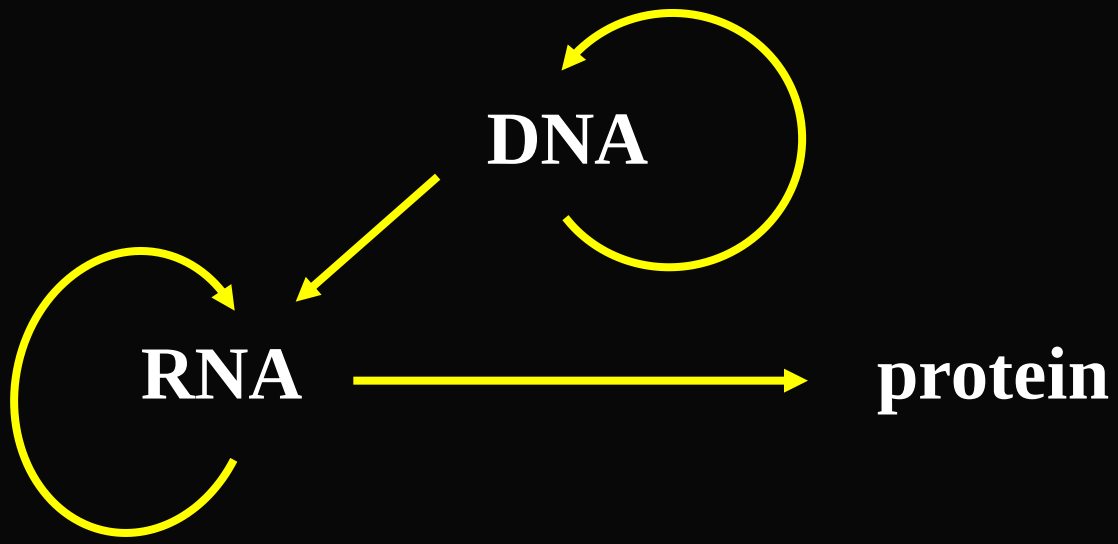
蛋白质翻译

5.5. Central Dogma 的发展

来源：不详

Crick F. H. C On protein synthesis Symp. Soc. Exptl. Biol. 1958 (12) : 138-163

Genetic Central Dogma



中心法则的要点；

• 所谓遗传信息，是指核酸中的碱基序列以及蛋白质中的氨基酸序列。生物的全部遗传信息均包含于 这种大分子的遗传序列的信息中。

• 从DNA 到RNA到蛋白质的遗传信息流是严格的单程路线。信息一旦进入蛋白质，就不可能再行输出。蛋白质是一切性状形成的工作分子。

• 序列假说是中心法则的核心，

中心法则是序列转换的原则

中心法则体现的基本原则；

遗传信息的唯一性

遗传物质的自决性

信息表达的单程性

序列转换的共线性

From 1970s to…..

Reverse transcription

Temin H. m Nature 1970 (226):1211

对分子生物学多年来的最大的一个浪头

Splitting gene

Phillip Sharp . 1977

Crick 更加感到困惑

Untranslated sequence

Huang W.M. Science 1988 (239): 4843

RNA alternative splicing

ChristopherW. Ann.rev.Genet.1989(23) :527

RNA editing

Cech T.R.Cell 1991 (64): 667

Protein as template for peptide synthesis

Lipmann F.Ann. Rev. Biochem1984 (53): 1

Prion

Prusiner S.B. Science 1991 (252): 151

中心法则的发展与修正

科学王国不信奉教义与信条（dogma）

Anti Central Dogma (中心法则的发展)

a. 蛋白质的遗传信息

并不一定来自核酸 !?

DNA/RNA与protein间序列的非共线性

Peptide synthesis by protein as template

短杆菌（*Gramicidin S* GS）⟶ 短杆菌肽（环十肽抗生素）

合成酶体系含轻酶（LE 100kd）重酶（HE 280kd）

LE(消旋化酶)

L-Phe ⟶ D-Phe

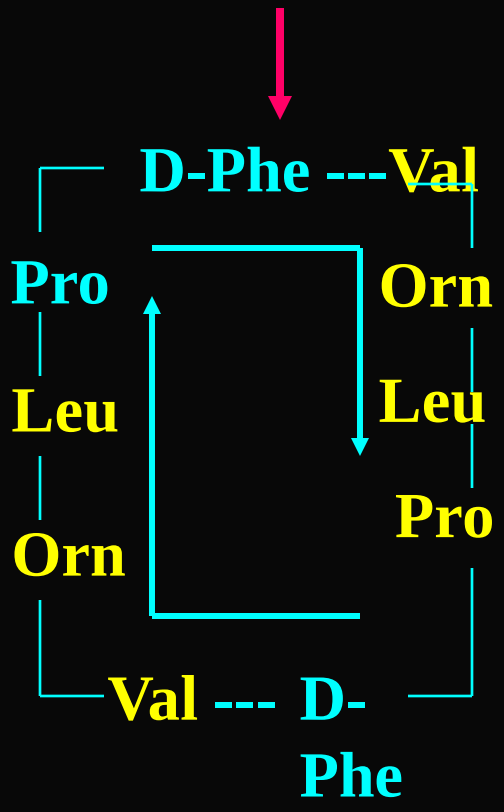
general　　special

HE(聚合酶) 通过转硫醇和转肽反应，将肽链按

D-Phe : Pro : Val : Ornithine (鸟氨酸) : Leu 按1 : 1 : 1 : 1: 1

依次定位聚合并首位相连成环十肽

D-Phe---Val---Orn---Leu--- Pro短五肽 ⟶ 环十肽



短杆菌肽（*Gramicid S*）

其他多肽抗生素

短杆酪肽（ tyrocidin ）

伊短菌素 (edeine)

多粘菌素 (polymyxin)

大肠杆菌素 （colistin）

鹿铃菌素 （surukacillin）

环杆菌素 （circulin）

放线菌素 （actinomycin）

Amanitin……

DNA/RNA与protein间序列的非共线性

Post-translation processing

在中心法则的原则下

modification 修版

Peptide → **Protein**

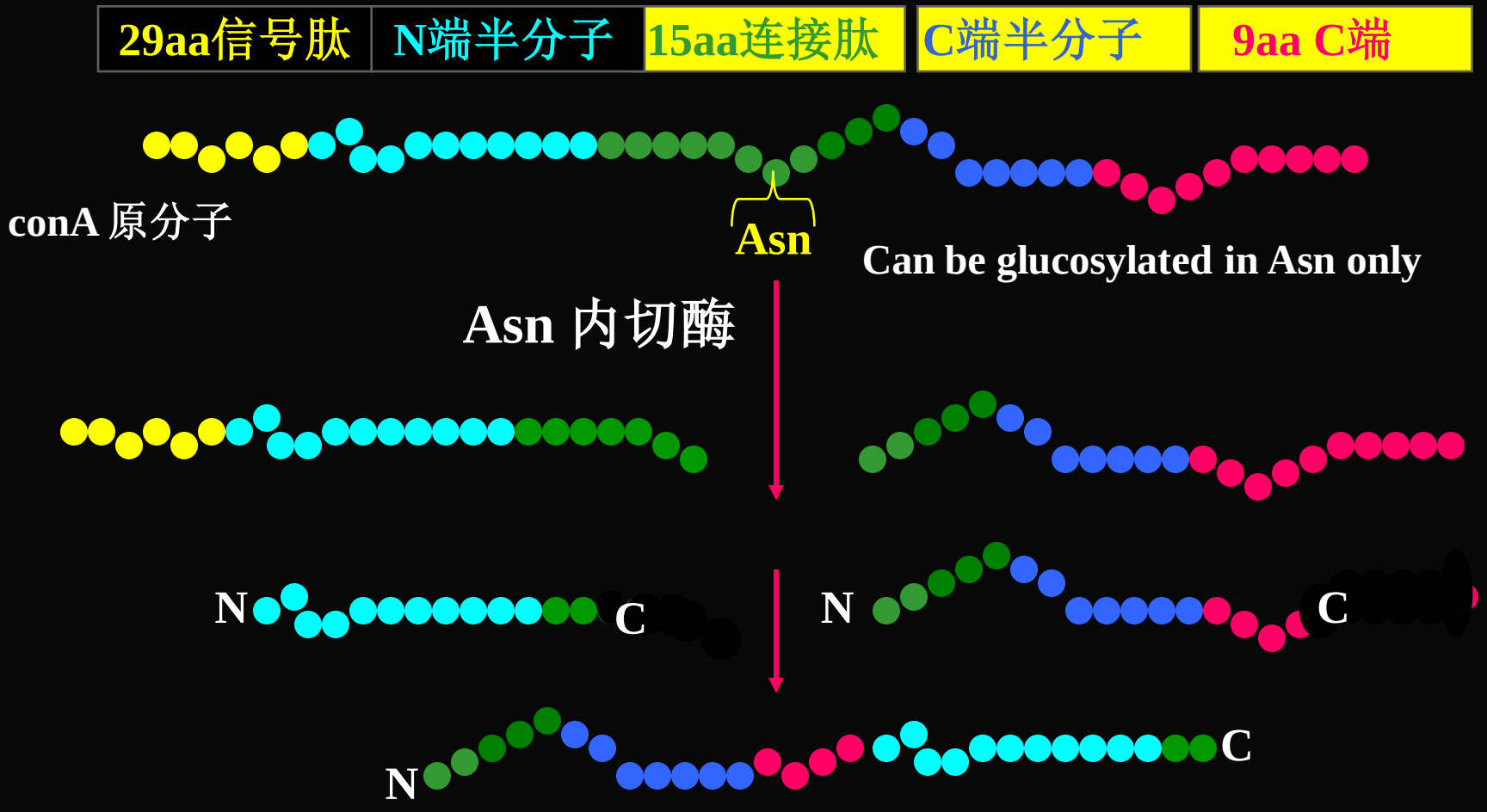
糖基化、乙酰化、磷酸化、烃基化、
intein切除、二硫键形成

但伴刀豆蛋白A(concanavalin A)的合成

氨基酸序列发生了另外意义的改版

背离了中心法则的共线性原则

Post-translation processing



Mature conA: 氨基酸序列被大幅度地剪接重排

完全破坏了与DNA序列的共线性关系

Anti Central Dogma (中心法则的发展)

b. **RNA中的遗传信息**

并不一定来自DNA！？

- **Intron 是中心法则不能包容的序列**
- **Poly(A)与DNA模板无法对应**

在动物线粒体里发现一些poly(A)中有终止密码*Attard G. (1985)*

- **RNA editing (Crypto gene)**

RNA editing 从病毒，原生动物，哺乳动物到植物普遍存在

RNA editing 对基因的编辑幅度可大于序列50%

是否RNA editing 告诉我们一个早期的世界？

生物为什么要进行这样一种明显神秘的RNA成熟方式？

Sollner-Webb. B 1996 Science (273):1182

Anti Central Dogma (中心法则的发展)

c, DNA是遗传信息的主要源头，

但不是唯一的源头

（大江的主流与支流）！？

prion 现象的重要解释（蛋白质是遗传物质吗？）

---Prion是羊痒疫(scrapie), 牛海绵状脑炎BSE (mad cow disease)中央神经系统退化疾病的致病因子

---提纯的prion 证明不含核酸，是动物体内正常存在的一种膜蛋白,

PrP^{C} (MW 33-35kd)，对蛋白酶敏感, 成年动物中组成型表达

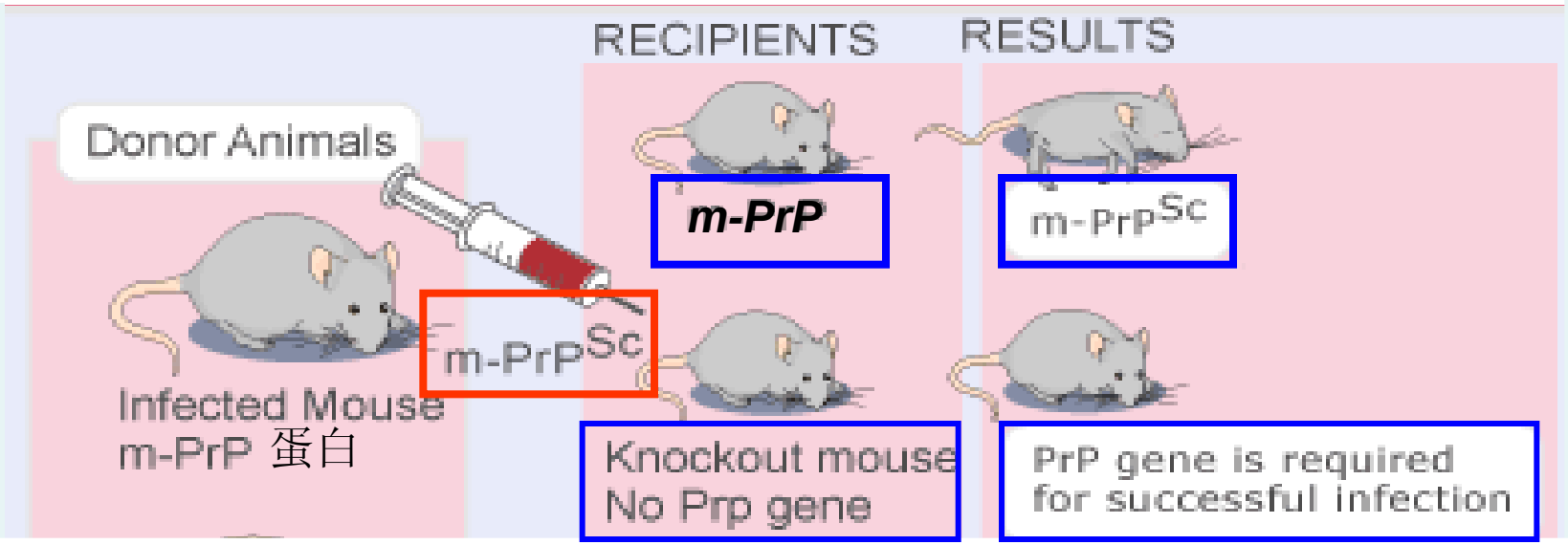
基因定位在 Chrom.$^{20\,S}$ (human) Chrom.^{2} (rat)

病人，病畜体内的PrP^{Sc} (MW 27-30kd)

在N端较PrP^{C} 少67aa, 一级序列相 似，二级结构差异显著

具传染性, 抗蛋白酶

是PrP^{C} 蛋白的 isoform



传染因子**PrP ^{SC}** → 侵染体内 → 迅速催化 → **PrP ^{C} 蛋白**

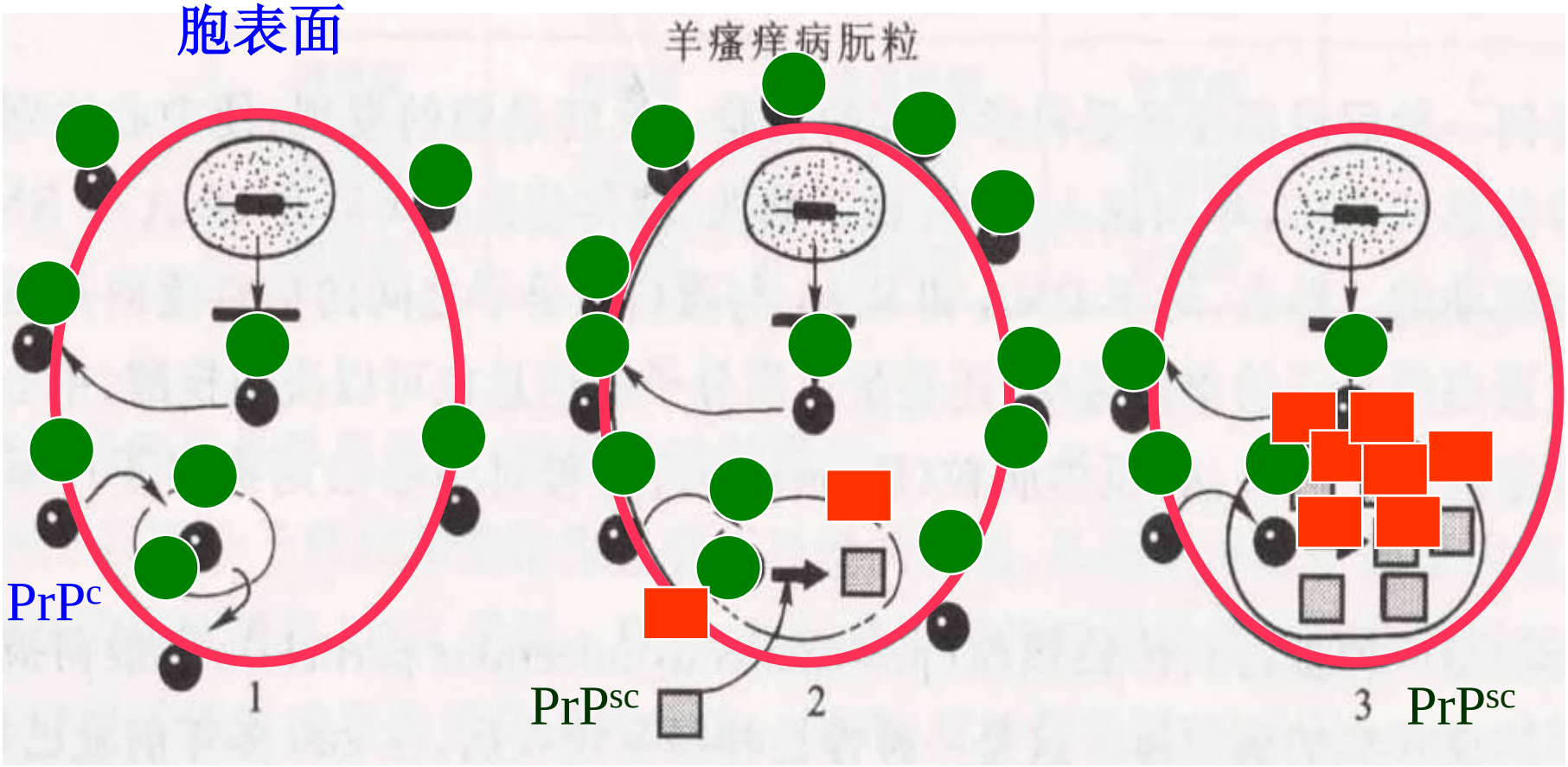
MW 33-35kd

转化 → **MW 27-30kd PrP ^{SC} 蛋白** → 病致

在分子伴侣帮助下
修饰折叠剪短N--端

α-helix	**30%**	**< 42%**
β-sheet	**43%**	**> 3%**

神经细胞表面



Prion 作为蛋白质病毒的繁殖是将自身（PrP^{SC}）的分子结构信息通过与正常蛋白（PrP^{C}）的结合， 在分子伴侣的辅助下，传递给PrP^{C} 并其转化为PrP^{SC}的过程

1997 NP

Stanley B. Prusiner
University of California,
School of Medicine
USA
1942 –

for his discovery of Prions –---
a new biological principle of infection

Griffith (1967) “没有理由惊慌，一种感染性蛋白的存在将打翻分子生物学的整个理论框架”

1997 NP

Stanley B. Prusiner
University of California,
School of Medicine
USA
1942 –

提出了“唯蛋白质”假说，以及对“蛋白质遗传”的肯定，对“中心法则”中关于“蛋白质不能输出遗传信息”的概念是一个严重的挑战

- **DNA 模板并非遗传信息的最初版本（初稿！）**

- **DNA的遗传信息是模糊的（crypto）**

- **DNA的遗传信息是变通的（movable , alternative）**

  transposon : 动态DNA的第一个信号

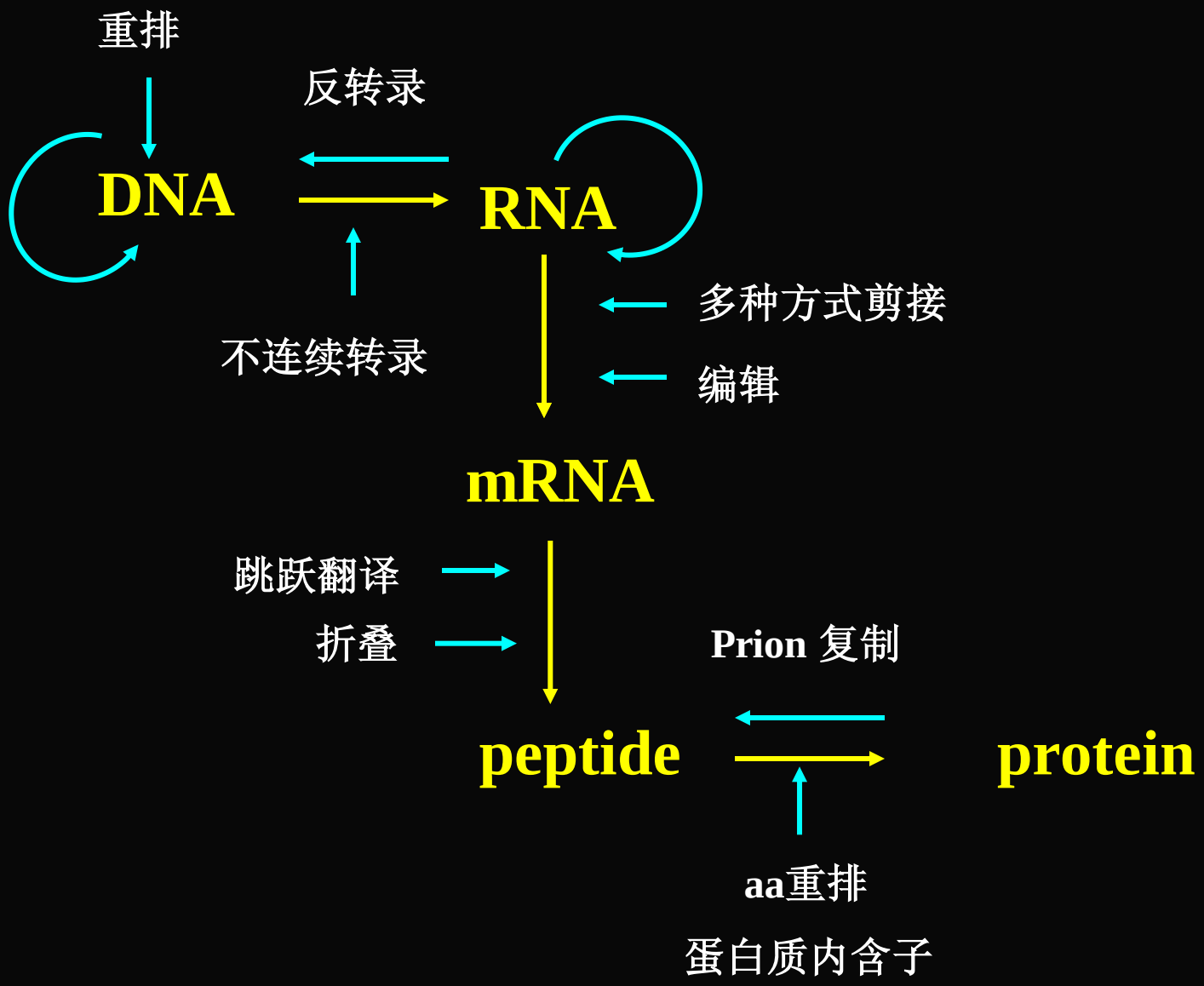
  intron : DNA 上基因不是一个最小的不可分割的功能单位

  DNA rearrangement : Immunoglobulin gene

  RNA tran-splicing

  RNA alternative splicing….

- 从DNA到RNA到肽链不断有新的遗传信息的加入



中心法则以外的遗传信息源于何处？

C 值矛盾的困惑？ N 值矛盾的困惑？

真核生物DNA的序列是否均具有遗传信息含义？

在高度重视基因分析的同时

重视染色体、细胞质、细胞及生物体的研究

中心法则的发展；

基因表达调控的研究是揭示第二遗传信息的重要领域

Non-coding RNA对基因表达的调控