PROTEIN SYNTHESIS

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- THE GENETIC CODE

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THE GENETIC CODE

WHAT DOES "CODE" MEANS ?

A coded language is incomprehensible unless it is translated into comprehensible language using a code.

Genetic information of an individual is stored in the form of "coded language" – stretches of nucleotides. It has to be translated into "comprehensible language" – stretches of amino acids which correspond to phenotypic characters of the individual. Each codon, composed of 3 nucleotides, encodes one amino acid.

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

* *Also used as initiator codon*

The codon:amino acid correlation is expressed in the Genetic Code

The Genetic Code has some characteristics : degenerate, universal, noninterrupted and nonoverlapped and unidirectional

SOME CHARACTERISTICS OF THE GENETIC CODE

THE CODE IS DEGENERATE

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

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With 3 out of 4 possible nucleotides encoding 1 amino acid, the total number of nucleotide combinations = 4^3 = 64 possible combinations. Each combination of 3 nucleotides is called codon.

In the table above, 3 out of 64 codons correspond to chain-terminating signals. The remaining 61 encode 20 amino acids → 1 amino acid is encoded by more than 1 codon. This characteristic of the Genetic Code is called degeneracy.

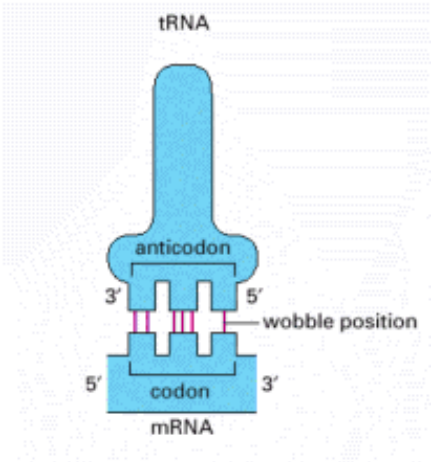
Degeneracy concerns mostly the nucleotide of the third position. Nevertheless, in some cases with great generacy e.g for Arg, Leu, Ser, the first nucleotide can also vary.

WHAT IS THE BIOLOGICAL MEANING OF DEGENERACY ?

Degeneracy limits the the harmful effect of point mutations in an individual : (1) mutations in the third nucleotide do not change the encoded amino acid, (2) codons having a pyrimidine at the second position mostly encode hydrophobic amino acid whereas those having a purine at this position encode hydrophilic amino acids and because transitions (A:T ↔ G:C) are the most common types of point mutations, mutations at this position do not have serious consequence.

Basepairing between codon and anticodon constitutes the basis of protein synthesis. According to Watson-Crick basepairing, there must be 61 anticodons corresponding to 61 codons. In fact, the number of anticodons found is inferior to 61 → 1 anticodon can recognize more than 1 codon.

HOW COULD IT BE POSSIBLE ?



1st base in Anticodon	3rd base in Codon
G	U /G
C	G
A	U
U	A/G
I	A/U/G

WOBBLE CONCEPT

Francis Crick proposed the Wobble Concept (1966) to explain this phenomenon : the 5'base of the anticodon is more flexible than the two others and can basepair with bases not classically matching it. This flexibility is however restricted to some combinations (figure in the left). The Wobble rules only allow the pairing between purines and pyrimidines, except for I:A pairing, because purine-purine or pyrimidine-pyrimidine pairings would be respectively too long or too short compared to classical basepairing.

An unusual base exists in tRNAs bearing anticodons, Inosine (I), which can basepairs with A, U or G.

No single tRNA can recognize more than 3 codons

SOME CHARACTERISTICS OF THE GENETIC CODE (continued)

THE CODE IS UNIVERSAL

The proposed universality of the Genetic Code was firmly confirmed by recent findings from genome sequencing programs of different organisms.

The Genetic Code is actually used by nearly all living organisms which denotes a common origin of Life on the Earth.

But as people said "Exceptions confirm the rules", some codons can be read differently than what determined by the Standard Code. Examples can be found mostly in mitochondria of many species, in some prokaryotes and even in certain eukaryotes. For example, UGA – the stop codon in the Standard Code – encodes Trp in *Mycoplasma*, *Spiroplasma* and mitochondria of many species ; UAA/UAG – the two other stop codons in the Standard Code – encode Gln in *Acetabularia*, *Tetrahymena*, Paramecium, …

The universality of the Code makes possible our investigation about the Nature of Life as well as the Evolution of Life on the Earth. It is also largely exploited in Genetic engineering to manipulate and to produce foreign recombinant proteins in any experimental organisms.

THE CODE IS NON-INTERRUPTED AND NON-OVERLAPPED

This means that a stretch of nucleotides is translated as a chain of successive codons, without any gaps. This implies that a frameshift mutation can not be isolated in a defined region and thus will affect the whole genome from the point of deletion or insertion.

The reading frame of a genome is determined by the intiation codon and will end with a stop codon.

SOME CHARACTERISTICS OF THE GENETIC CODE (continued)

THE CODE IS UNIDIRECTIONNALLY READ

A coding sequence, mRNA, is always read in 5'-3' direction.

Thus, the sequence 5'-GCUACUUUA-3' codes for the tripeptide Ala-Thr-Leu

A CLASSIC EXPERIMENT (1966) VALIDATING THE GENETIC CODE

- **A mutant gene of phage T4 encoding lysozyme, a cell wall degrading enzyme, was constructed. This mutant bore a pair of deletion/insertion mutations which mutually suppress each other.**
- **The wild-type and mutant polypeptide sequences when compared showed difference in a stretch of 5 amino acids : Ser-Pro-Ser-Leu-Asn- (wild-type) and –Val-His-His-Leu-Met- (mutant)**
- **This experiment postulated that if the Genetic Code is valid, one can deduce the sets of mutant codons bearing a deletion and an insertion compared to the wild-type, this was proved :**

NH2 – Lys Ser Pro Ser Leu Asn Ala - COOH Wild-type

5' - AAA AGU CCA UCA CUU AAU GC - 3'

5' - AAA GUC CAU CAC UUA AUG GC - 3'

NH2 - Lys Val His His Leu Met Ala - COOH Mutant

Furthermore, this experiment permitted the verification of some codon assignments as well as the degeneracy of the Code, e.g for Serine, Leucine or Histidine. Lastly, experimental results corresponded to one possibility of reading direction, 5' to 3'.

DECODING THE GENETIC INFORMATION

ꕤ In 1961, synthetic mRNA combined to cell-free systems for *in vitro* translation were used to elucidate the Code.

Synthetic homopolymers such as poly-U, pol-A, poly-C and poly-G can be made. Translation of the homopolymers established the first codon assignments : UUU for phenylalanine, CCC for proline and AAA for lysine ; poly-G was not translatable due to secondary structure.

With different combinations of mixed polymers, additional codon assignments were found. Two kinds of experiments were performed :

ꕤ Copolymers with known sequences were synthesized.

For example : – UGUGUGUGU – which were translated in -Cys–Val-Cys-Val- ; the question is "Does UGU corresponds to cystein or valine ? idem for GUG ?".

Another copolymer was then synthesized – UGGUGGUGG- which can be translated in three reading frames UGG UGG UGG, GGU GGU GGU or GUG GUG GUG giving three different polypeptides composed of polytryptophan, poly-glycine and poly-valine.

Combining the two experiments, GUG assignment was establihed.

ꕤ Trinucleotides, aminoacyl-tRNAs and ribosomes were used.

For example, when a trinucleotide UUU was mixed with ribosomes and 20 types of aminoacyl-tRNAs, only phenyl-tRNA can bind to the trinucleotide and was retained by the ribosome forming a codon-aminoacyl-tRNA-ribosome complex. By separately testing all the possible codons, Nirenberg *et al* (1964) deciphered almost all codons.

TRANSLATION (PROTEIN SYNTHESIS)

COMPONENTS OF THE TRANSLATIONAL MACHINERY

WHAT ARE THE FUNDAMENTAL DIFFERENCES BETWEEN TRANSLATION AND TRANSCRIPTION OR REPLICATION ?

➔ The amino acid side chains, especially non polar or aromatic chains, have little or no affinity with bases of the mRNA. Thus, direct interactions between the template and the synthesized product is not favorized as in the case of transcription or replication.

➔ Each amino acid is specified by three nucleotides. This creates a spatial problem for the formation of peptide bonds between two adjacent amino acids.

Francis Crick (1955) proposed the existence of a molecule called adaptor which circumvents problems of direct interactions between the mRNA and the polypeptide chain. This adaptor interacts with the growing polypeptide through amino acid binding to one end and, at the same time, recognizes corresponding codons on the mRNA. His hypothesis was verified and the adaptor was called tRNA.

The three main components of the translational machinery are :

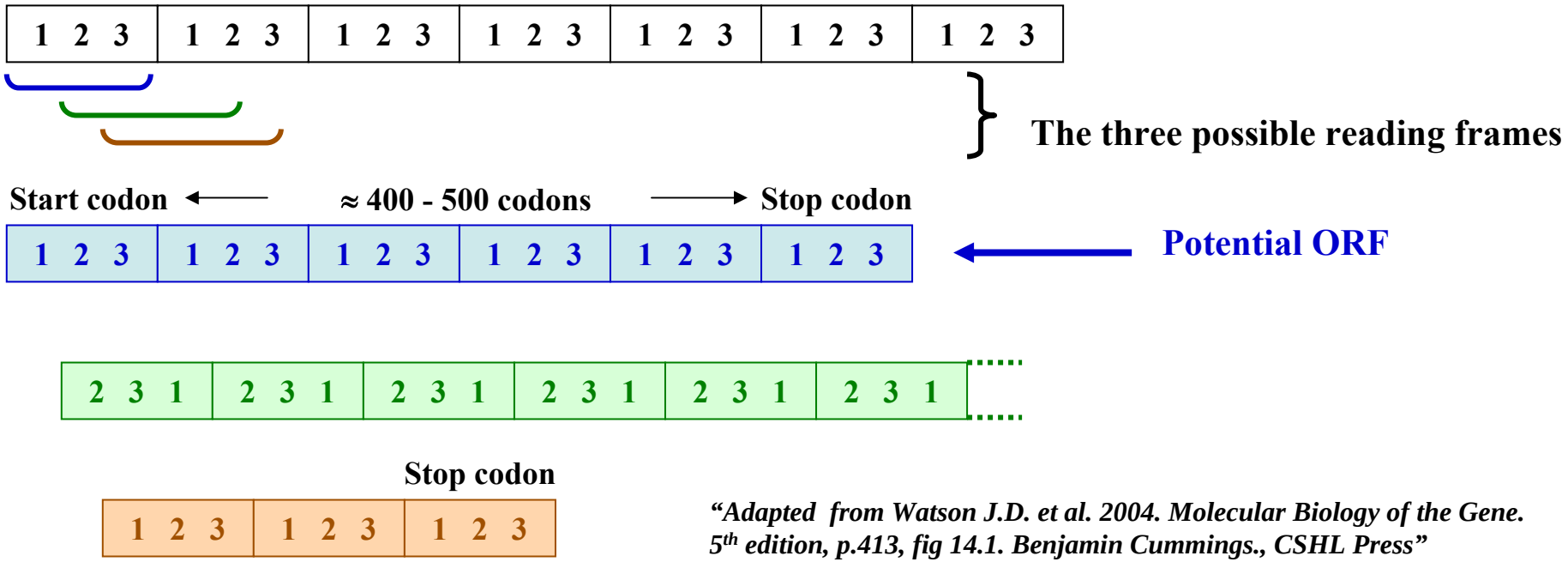
- **mRNA : intermediate molecule which conveys genetic information of one or a group of genes stored in DNA to the translational machinery. Genetic information is presented as stretch of codons.**
- **tRNA : adaptor molecule, transporting incoming amino acids to the translational machinery and forming the bridge between mRNA and the growing polypeptide**
- **Ribosome composed of rRNA and proteins provides the contact point of all components of the translational machinery and catalyzes the formation of new peptide bonds**

COMPONENTS OF THE TRANSLATIONAL MACHINERY – mRNA

☙ Messenger RNA (mRNA)

Only a portion of an mRNA is translated. This portion, composed of codons, is called open reading frame (ORF) and is delimited by a start codon at the 5' end and a stop codon at the 3'end. The term "open reading frame" implies that the RNA sequence is translatable ("open" → open to translation) and is "read" in a defined "frame". A potential ORF posesses a start codon and a stop codon ; these codons are separated by a distance corresponding to a normal polypeptide size (~300 codons in prokaryotes, 400-500 in eukaryotes)

Example of reading frames and potential ORFs :

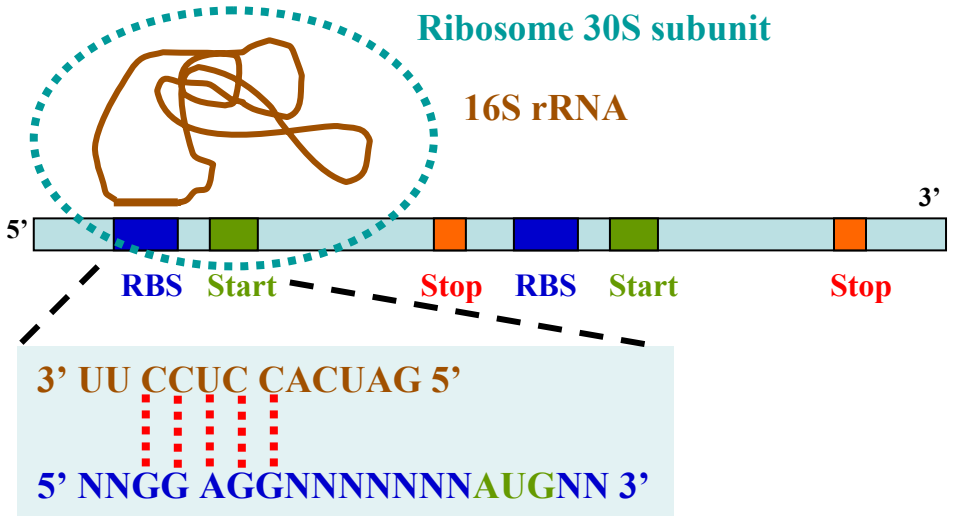


"Adapted from Watson J.D. et al. 2004. Molecular Biology of the Gene. 5th edition, p.413, fig 14.1. Benjamin Cummings., CSHL Press"

Polycistronic mRNAs in prokaryotes contain many ORFs whereas eukaryotic monocistronic mRNAs usually encode one ORF.

COMPONENTS OF THE TRANSLATIONAL MACHINERY - mRNA

The structure of prokaryotic and eukaryotic mRNAs are presented below :



"Adapted from Watson J.D. et al. 2004. Molecular Biology of the Gene. 5th edition, p.414, fig 14.2. Benjamin Cummings., CSHL Press"

In the polycistronic prokaryotic mRNA, each ORF is composed of a start and stop codon which define the coding region, and an upstream ribosome binding site (RBS), also called Shine-Dalgarno sequence after the scientists who discovered it.

The RBS has two functions : (1) it basepairs with a 16S rRNA fragment → correctly position the 30S ribosome subunit at the beginning of the ORF, (2) contributes to determine the start codon which is an AUG situated 5-9 bp downstream of the RBS

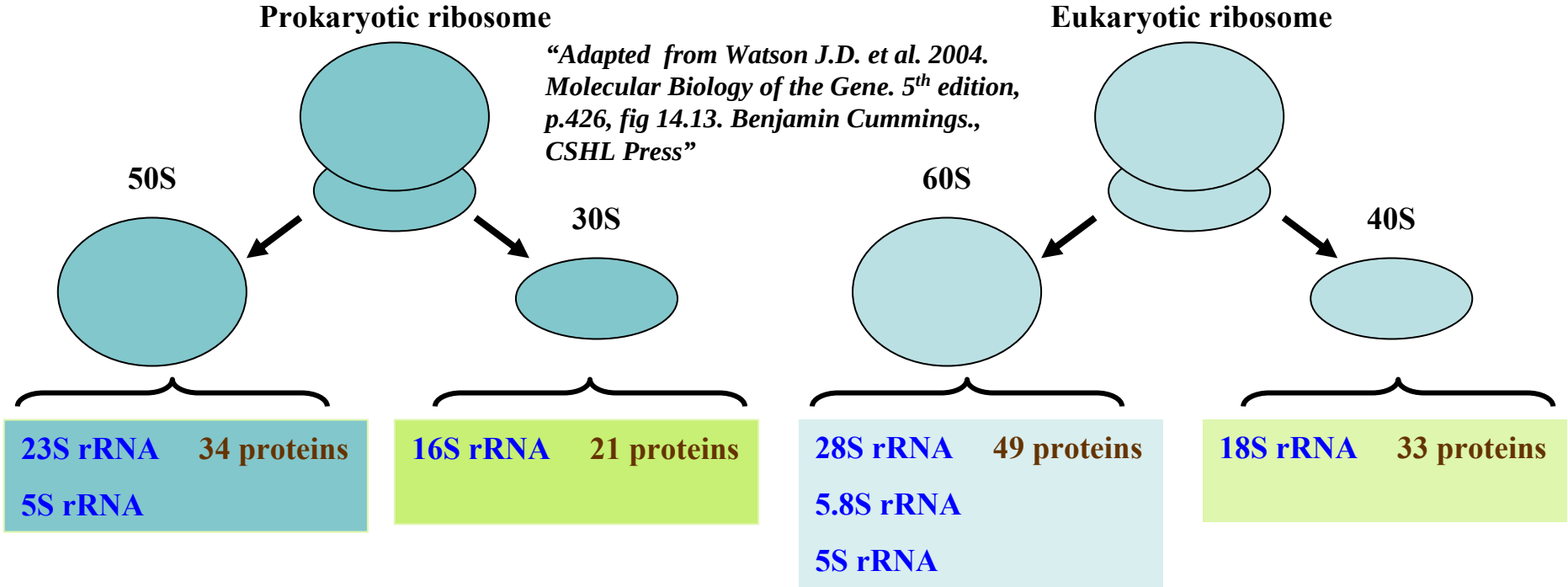
- **Each monocistronic eukaryotic mRNA has a 5'cap, a methylated guanine, which recruits ribosome to the mRNA**
- **Some eukaryotic mRNAs possess a sequence called Kozak sequence, after the scientist who identified it. The Kozak sequence is composed of 3 bases upstream and a G downstream of the start codon – 5'G/ANNAUGG – and interacts with the initiator tRNA.**

COMPONENTS OF THE TRANSLATIONAL MACHINERY – RIBOSOME

The ribosome

The ribosome is composed of a small and a large subunits. They are the 30S and 50S subunits in prokaryotes, the 40S and 60S subunits in eukaryotes. The small and large subunits are named according to their sedimentation rate which depends on their size and shape. S (Svedberg, after the inventor of the ultracentrifuge) is the measure unit for sedimentation rate.

The large subunit has peptidyl transferase activities which creates peptide bonds. The small subunit contains the decoding center where aminoacyl-tRNA basepairs with the appropriate codon. The ribosomal subunits are composed of many rRNAs and ribosomal proteins :



"Adapted from Watson J.D. et al. 2004. Molecular Biology of the Gene. 5th edition, p.426, fig 14.13. Benjamin Cummings., CSHL Press"

The two ribosomal subunits exist in separate free form in the cytoplasm between two translational cycles.

COMPONENTS OF THE TRANSLATIONAL MACHINERY - RIBOSOME

The ribosome has three binding sites for tRNAs : (1) the A site binds the aminoacyl-tRNA, (2) the P site is the binding site for the tRNA bearing the growing polypeptide – the peptidyl-tRNA, (3) the E site is the binding site for the free tRNA ready to exit the ribosome after it has transferred the growing polypeptide to the following aminoacyl-tRNA.

An mRNA which is translated by multiple ribosomes is called a polysome. The existence of polysomes explains the number of about 38,000 ribosomes and 1,500 mRNA found in a *E. coli* cell, since an mRNA can be simultaneously read by many ribosomes.

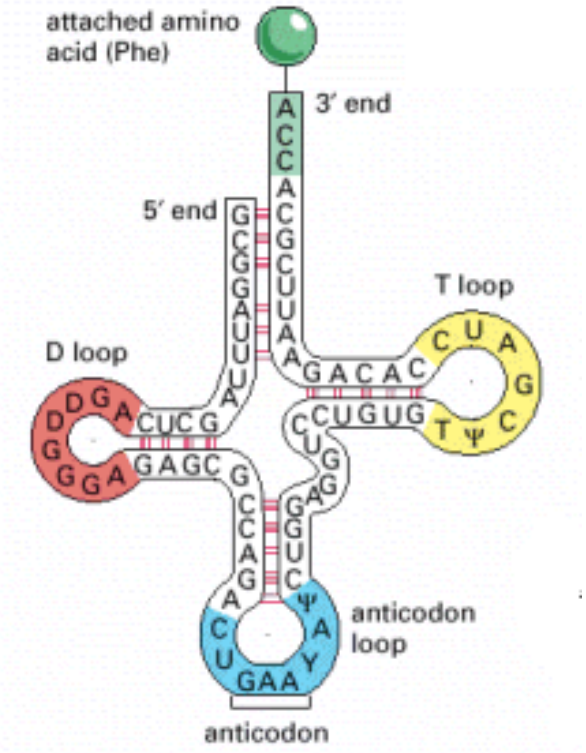
The synthesis rate during translation is about 2 – 20 amino acids/second, much more smaller than DNA replication rate which is 200 – 1,000 nucleotides/second.

In prokaryotes, since transcription and translation are coupled, translation rate – about 20 amino acids/second – corresponds to transcription rate which is 50 - 100 nucleotides /second. On the other side, translation in eukaryote which occurs in different space and time to transcription, has a low speed of 2 – 4 amino acids/second

COMPONENTS OF THE TRANSLATIONAL MACHINERY - tRNA

Transfer RNA (tRNA)

Linear sequence of phenylalanyl-tRNA



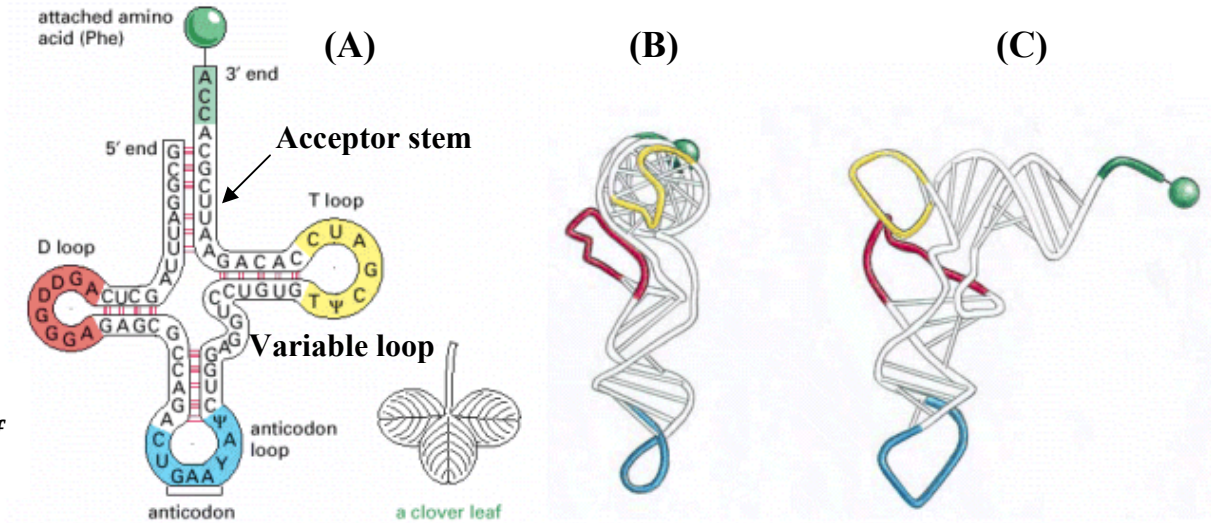
Cloverleaf structure of phenylalanyl-tRNA

All tRNAs have a length about 75 – 95 nucleotides anh have some common characteristics :

- **They have the same trinucleotide 5'-CCA-3' at the 3'end.**
- **They contain unusual bases which are the products of post-transcriptional modifications of the normal bases, such as pseudouridine (ψU), dihydrouridine (D), inosine, 1-methylguanosine, .. They seem to influence the efficiency of tRNA activities.**
- **They have a same shaped secondary structure – a cloverleaf-like structure**

COMPONENTS OF THE TRANSLATIONAL MACHINERY - tRNA

Structure of a phenylalanyl-tRNA. (A) Cloverleaf-like structure with the stem and loops. (B), (C) L-shaped three-dimensional structure.



The secondary cloverleaf-like structure included :

- **An acceptor stem bearing the 3'end trinucleotide 5'-CCA-3' which is the attachment site for the amino acid**
- **A ψU (or T) loop containing the unusual base ψU**
- **A D loop containing some dihydrouridines (D)**
- **An anticodon loop which contains the anticodon**
- **A variable loop having variable size.**

The cloverleaf-like secondary structure is folded into an L-shaped tertiary structure.

The tertiary structure can be seen in profile as an upside down L with a branch corresponding to the acceptor stem and the stem of ψU loop ; the other branch is constituted of the anticodon stem and the stem of D loop.

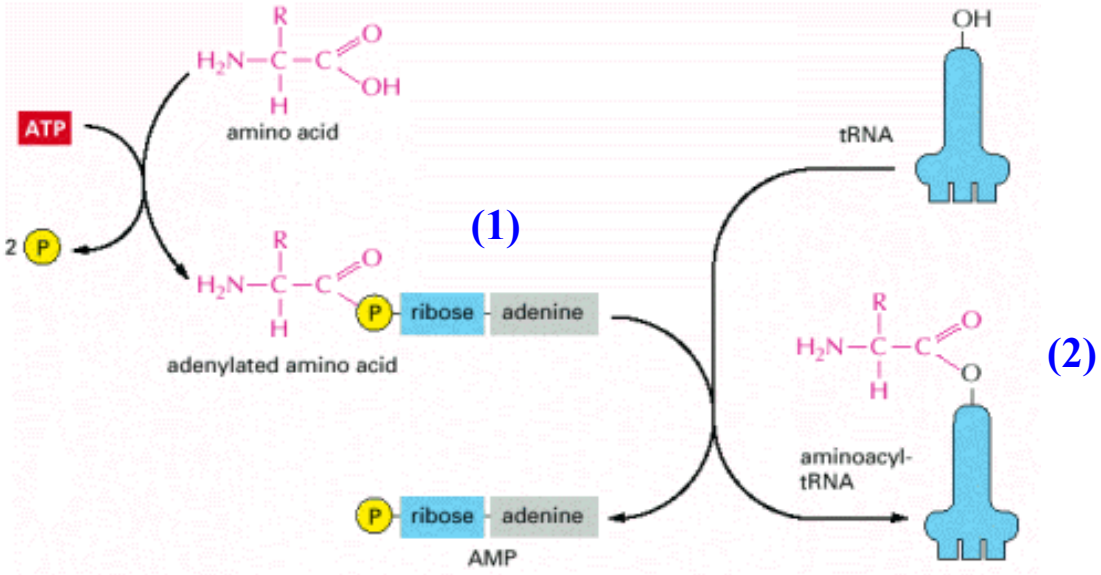
AMINO ACID ACTIVATION

Before being used for protein synthesis, amino acids are activated by attachment to their tRNAs with high-energy bonds. This is accomplished by enzymes called aminoacyl-tRNA synthetases.

An aminoacyl-tRNA synthetase attaches an amino acid to a tRNA in a two-step reaction :

(1) Adenylylation of amino acid : amino acid + ATP → aminoacyl-AMP + pyrophosphate (adenylylated amino acid)

(2) tRNA charging : aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP



There are 20 aminoacyl-tRNA synthetases in most organisms, each of them is responsible of charging one type of amino acid on all the corresponding tRNAs

AMINO ACID ACTIVATION (continued)

The ribosome has no mechanism to selectively recruit correctly charged tRNAs. As long as an aminoacyl-tRNA exhibits correct codon-anticodon basepairing, it will be accepted for translation whatever the amino acid it bears.

For this reason the charging of tRNA by aminoacyl-tRNA synthetases has a crucial role for the accuracy of translation. The enzymes have three binding sites, for the amino acid, for the tRNA and for the ATP molecule.

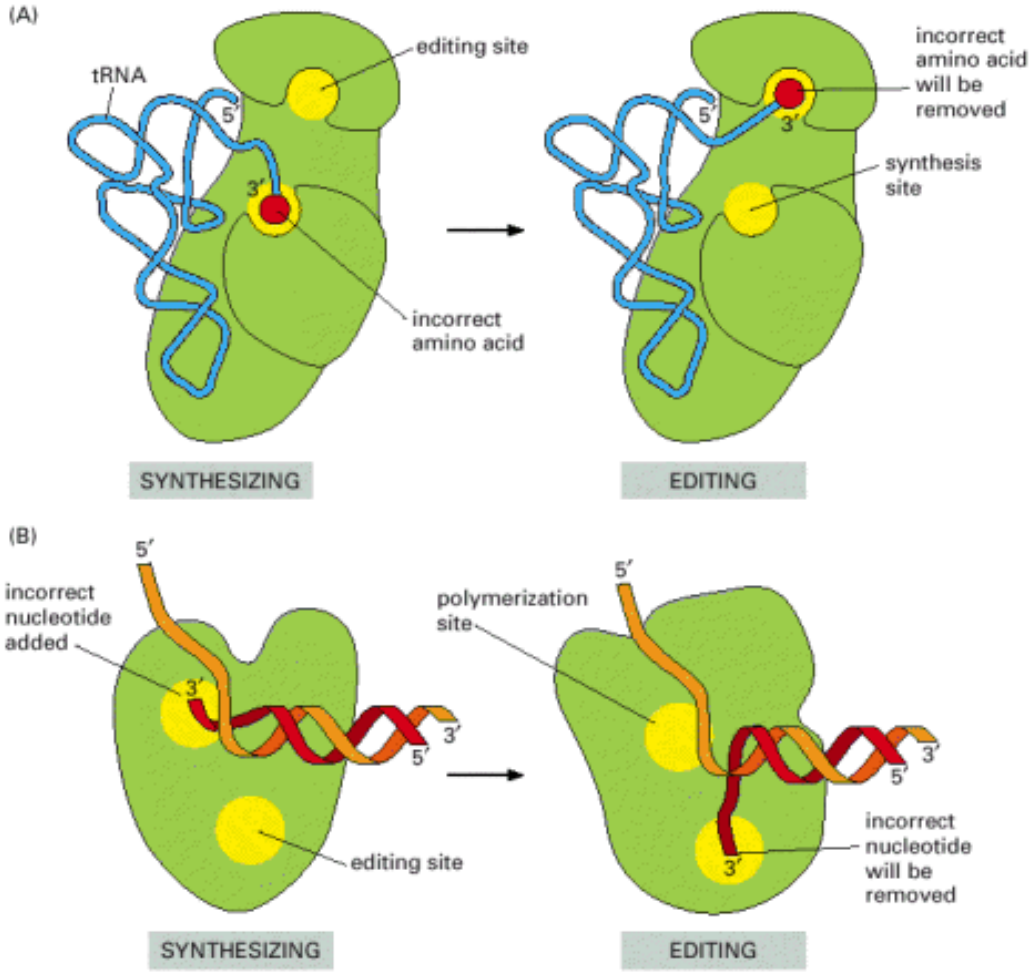
➔ Aminoacyl-tRNA synthetases have to recognize all the tRNAs for a particular amino acid and charge them with the correct one. This recognition is based on the anticodon loop, and most importantly, on the acceptor stem of the tRNAs. Since codons for an amino acid can greatly vary, the corresponding anticodons are also different from each other. Thus, some determinants other than the anticodon are used for a proper recognition.

➔ The enzymes have to properly recognize the amino acid to be attached. The charging reaction is highly accurate - < 1/1,000 tRNAs is charged with an incorrect amino acid. Some examples concerning very similar amino acids show how this accuracy could be obtained :

- **The two aromatic amino acids phenylalanine and tyrosine differ from each other by only one -OH group. The discrimination is made through the presence or absence of hydrogen bonding between the -OH group and an active group of the binding site.**

- **In the case of valine and isoleucine which differ from each other by a $-CH_3$ group. It is easy to understand that isoleucin can not enter the small binding site of valyl-tRNA synthetase. But how about the inverse process ? In fact, van der Waals interactions between the extra $-CH_3$ group of isoleucine favorize the binding of isoleucine to its binding site more than the binding of valine**

ADENYLYLATED AMINO ACID EDITING



Some aminoacyl-tRNA synthetases use editing activities to proofread the charged tRNA (A).

This process is very similar to the proofreading reaction of DNA polymerase during DNA replication (B).

These aminoacyl-tRNA have an editing site in addition to the synthesis site. The incorrectly attached amino acid fits into the editing site and is cleaved whereas the correct one has larger size and can not enter the editing site, thus is retained.

Consequently, aminoacyl-tRNA synthetases guarantee the accuracy of translation by two mechanisms : (1) selective charging tRNAs, and (2) eliminating improperly charged products.

TRANSLATIONAL PROCESS

- **INITIATION OF TRANSLATION IN PROKARYOTES AND EUKARYOTES**
- **ELONGATION OF TRANSLATION**
- **TERMINATION OF TRANSLATION**

The initiation of the translation requires some crucial events : (1) the recruitment and correct positioning of the first ribosome on the mRNA and (2) the binding of the first aminoacyl-tRNA to the P site.

Even sharing these common features, the initiation of translation in prokaryotes and eukaryotes differs from each other by some details.

TRANSLATION INITIATION IN PROKARYOTES

(1) THE RECRUITMENT AND POSITIONING OF THE FIRST RIBOSOME ON THE mRNA

The small ribosomal subunit is recruited to the mRNA. The basepairing between the Shine-Dalgarno sequence on the mRNA and a sequence of the 16S rRNA correctly positions the ribosome so that the start codon lies at the P site.

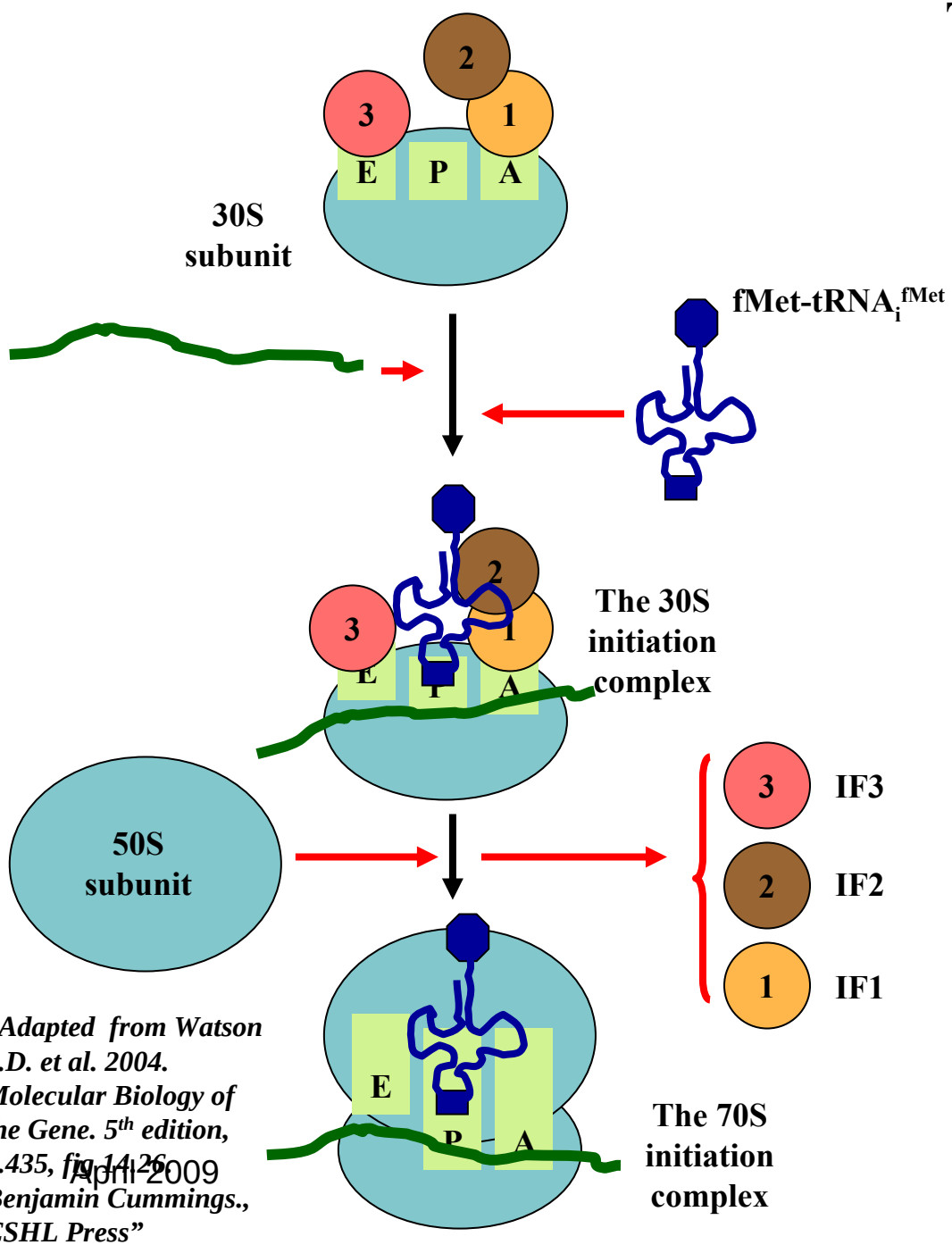
(2) THE BINDING OF THE INITIATOR AMINOACYL-tRNA TO THE START CODON

Since the P site is dedicated to peptidyl-tRNA, it accepts the initiator tRNA as the only aminoacyl-tRNA.

The prokaryotic initiator tRNA bears a N-formyl methionine which is a methionine formylated after its attachment to the tRNA. This formyl group is later removed by a deformylase. The first methionine can be even be eliminated from the polypeptide by aminopeptidases. The initiator tRNA is named $tRNA_i^{fMet}$.

HOW COULD THESE EVENTS OCCUR IN A COORDINATED MANNER ?

TRANSLATION INITIATION IN PROKARYOTES



"Adapted from Watson J.D. et al. 2004. Molecular Biology of the Gene. 5th edition, p.435, fig.14.26. Benjamin Cummings., CSHL Press"

The initiation of translation is mediated by three initiation factors – IF1, IF2 and IF3 :

- **IF1 blocks the A site to prevent its binding by charged tRNAs**
- **IF2, associated with GTP, binds to IF1 and facilitates the binding of fMet-tRNA$_i^{fMet}$ to the P site.**
- **IF3 binds to the future E site and prevents a premature association of the small and large subunits.**

Once the charged initiator tRNA is bound to the complex mRNA/small ribosomal subunit at the P site, IF3 is released. This allows the large subunit to associate with the small subunit complex. This association induces the release of IF2 and IF1, freeing the A site for the next step.

TRANSLATION INITIATION IN EUKARYOTES

The initiation of translation in eukaryotes have similarities of the same process in prokaryotes : formation of the small initiation complex with the participation of initiation factors, association of the large subunit to form the active ribosome.

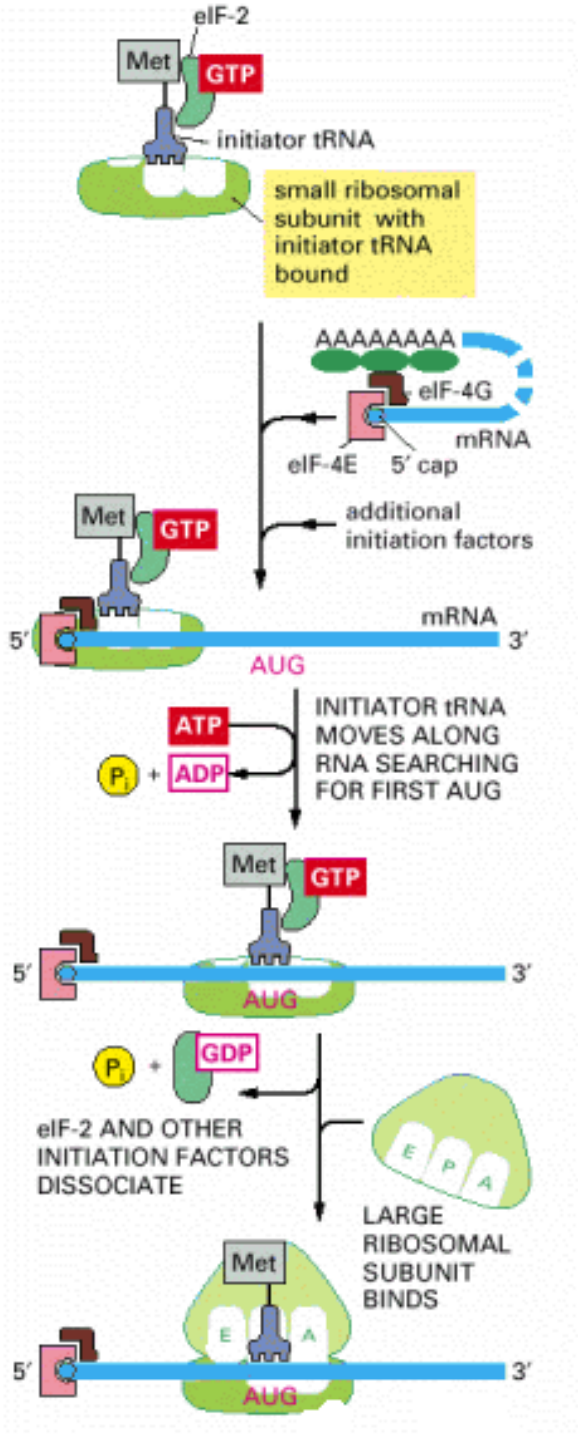
Nevertheless, the two processes have some differences :

- **The small subunit is always associated to the initiator tRNA which is a Met-tRNA$_i^{Met}$, before it is recruited to the mRNA**
- **The correct positioning of the ribosome is made through a scanning process : the "small subunit-tRNA-initiation factors" complex scans the mRNA until it encounters the first AUG located in a correct context, e.g in a Kozak sequence.**

In fact, the recognition of the correct start codon is made through basepairing between anticodon on the tRNA and codon on the mRNA (instead of mRNA and rRNA as in prokaryotes).

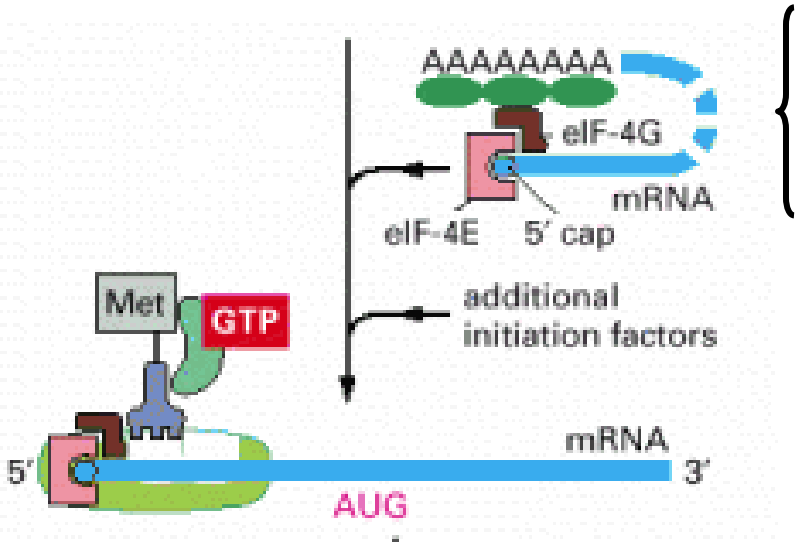
- **The initiation step in eukaryotes require more than 30 factors. Some of them correspond to their prokaryotic homologs such as eIF1, eIF2, eIF3.**

TRANSLATION INITIATION IN EUKARYOTES



- The small subunit is blocked by **eIF1A** and **eIF3**
- The charged initiator tRNA is recruited and positioned on the future P site of the small subunit by **eIF2** and **eIF5B**. The **43S initiation complex** is formed
- On the other side, mRNA associates with two factors : (1) **eIF4F** which binds to the 5'cap and (2) eIF4B which removes undesired secondary structures at the 5'end of the mRNA
- The 43S initiation complex correctly associates with the mRNA through interaction between the eIF4F and eIF3, forming a "small subunit-tRNA-mRNA" assembly
- This assembly begins the scanning step until it reaches the potential start codon. Codon-anticodon correct basepairing induces the release of eIF2 and eIF3, allowing the large subunit to join the complex. Binding of the large subunit catalyzes the release of all the remaining initiation factors. The **80S initiation complex** is formed, ready for the elongation stage.

TRANSLATION INITIATION IN EUKARYOTES – *RECYCLING OF THE RIBOSOMES*



Circularization of the mRNA through interactions of initiation factors and poly-A binding factors

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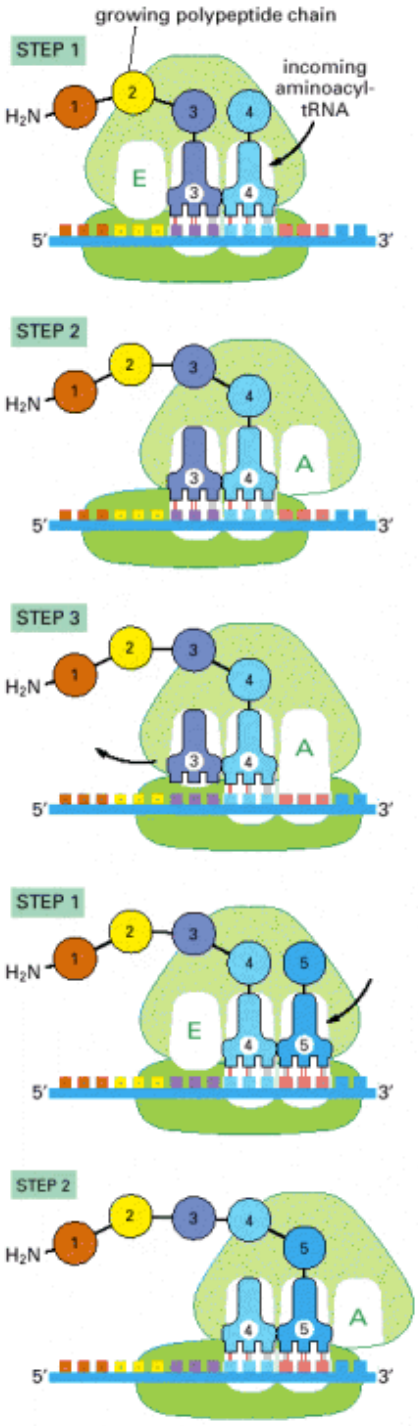
The initiation factors bind to the 5'end of the mRNA, and in the same time, are associated with its 3'end. This is the results of interactions between the eIF4F and the poly-A binding proteins (PABP) which are associated to the poly-A tail. The mRNA becomes a "closed" circle. This configuration of the mRNA facilitates the rapid re-use of the ribosomes which just finished a polypeptide synthesis in a new translational cycle

THE ELONGATION OF TRANSLATION



The elongation of translation is very similar in prokaryotes and eukaryotes. The presentation below is thus focused on prokaryotes.

The translation elongation process includes three steps :

(1) An aminoacyl-tRNA is recruited to the A site

(2) A peptide bond is formed between the incoming amino acid and the initiator methionine

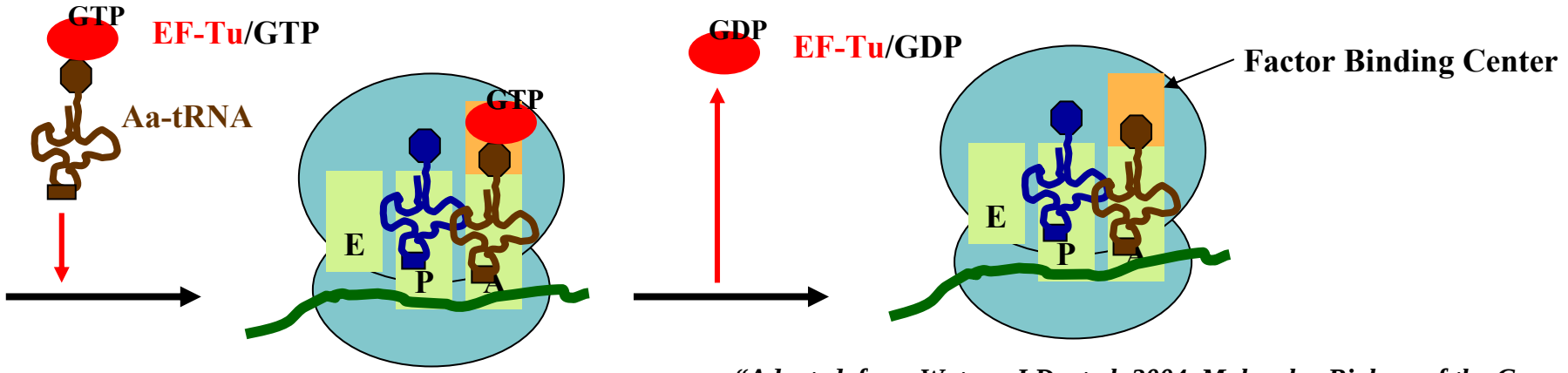
(3) The peptidyl-tRNA translocates from A to P site.

Theses steps are repeated many times until a termination signal is encountered.

Elongation is controlled by elongation factors (EFs).

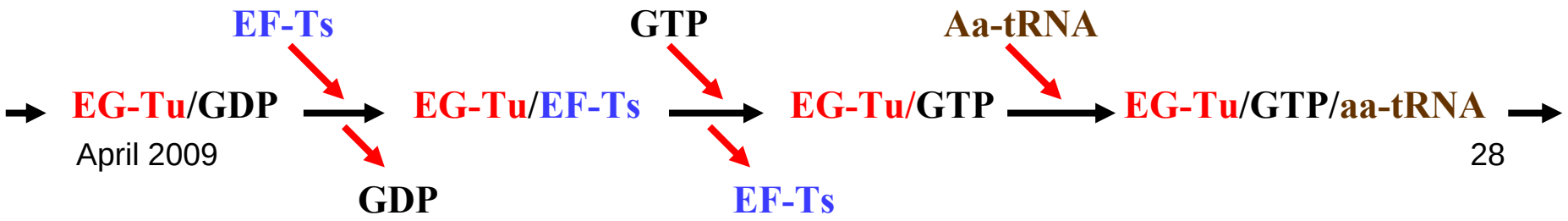
TRANSLATION ELONGATION (continued)

(1) RECRUITMENT OF AN AA-tRNA TO THE RIBOSOME. An elongation factor, EF-Tu associated with GTP, brings the aminoacyl-tRNA to the ribosome. When EF-Tu/GTP has positioned the aminoacyl-tRNA on the A site, it gets into contact with the adjacent factor binding center. This center activates the hydrolysis of GTP, releasing EF-Tu/GDP from its bound aa-tRNA.



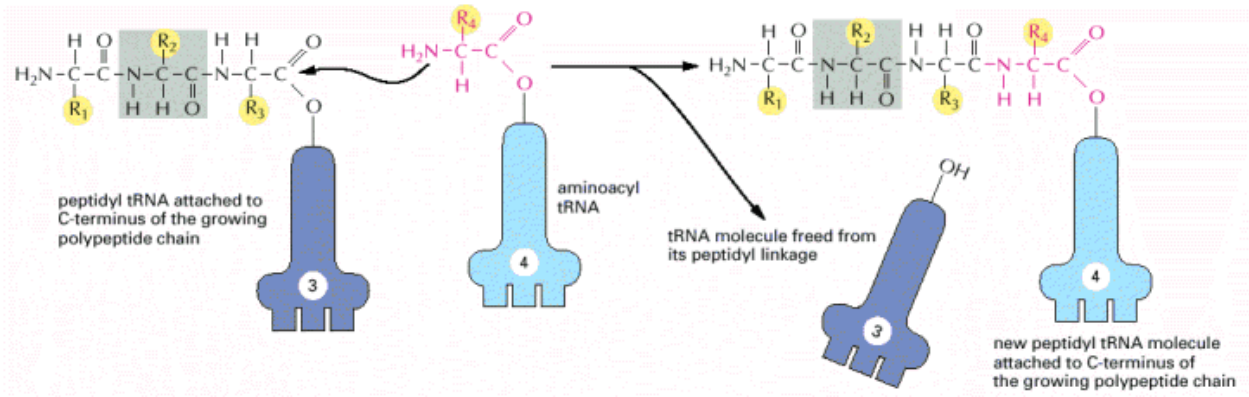
"Adapted from Watson J.D. et al. 2004. Molecular Biology of the Gene. 5th edition, p.442, fig 14.31. Benjamin Cummings., CSHL Press"

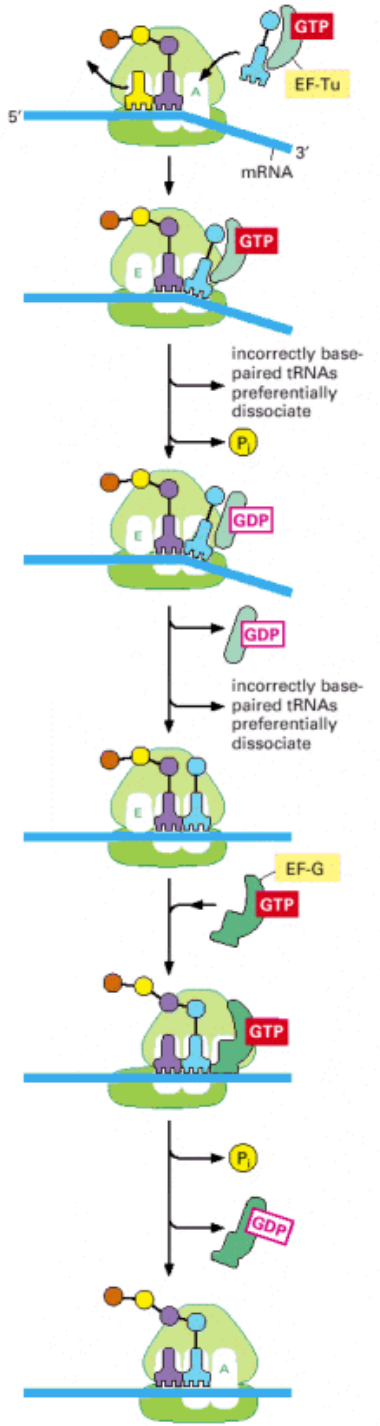
The released EF-Tu/GDP, to be reused in another translational cycle, has to exchange the bound GDP for a GTP. This exchange is catalyzed by an elongation factor named EF-Ts.



TRANSLATION ELONGATION (continued)

(2) FORMATION OF PEPTIDE BOND. Once the aminoacyl-tRNA is introduced into the A site, it follows rotative movement to present its amino acid in a position favorable for peptide bond formation. This event is called accomodation. A peptide bond is then formed between the two amino acids located one in the P site and the other in the A site. This reaction is catalyzed by peptidyl transferase activities of the 23S rRNA component of the large subunit.





TRANSLATION ELONGATION (continued)

(3) TRANSLOCATION OF tRNA AND mRNA. After the polypetide has been transferred from the P site-tRNA to the A site-tRNA, the translational machinery moves to the next codon. An elongation factor, EF-G complexed with GTP, induces the translocation.

Translocation includes two steps :

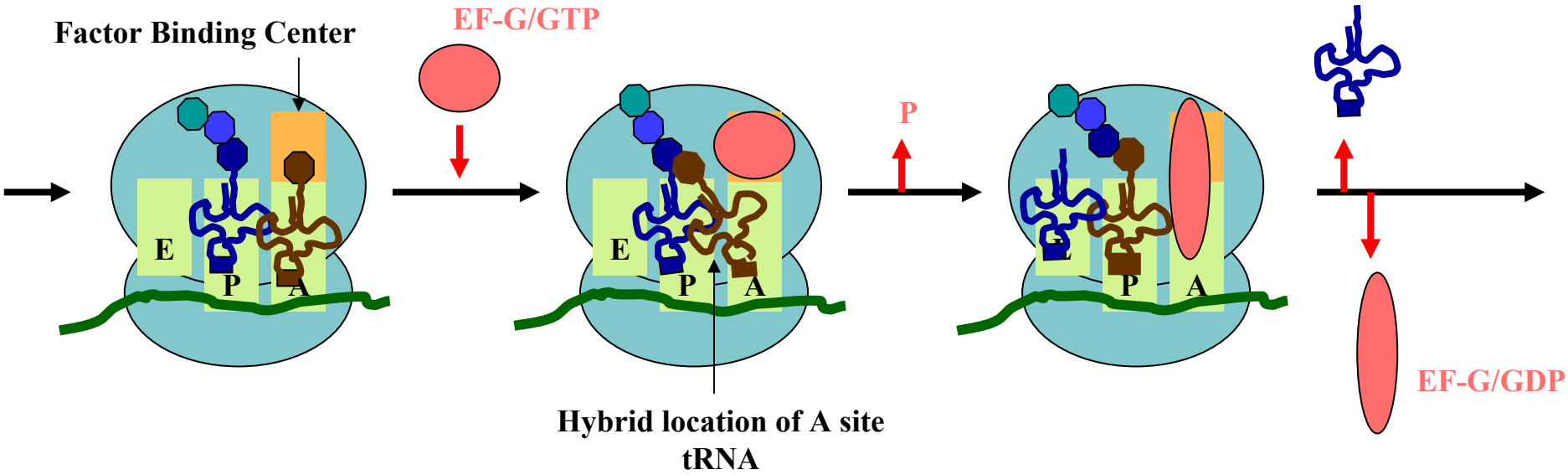
- **When the growing polypeptide is transferred from the P site t-RNA to the A site tRNA, the A site tRNA is simultaneously bound by its 3'end to the P site and bound to the codon at the A site → it has a "hybrid" location**

- **The "hybrid" location of the A site-tRNA leaves a space for the EF-G/GTP to bind to the factor binding center. GTP is hydrolyzed and induces a conformational change of the EF-G/GDP which can binds to the A site, pushing the tRNA from the A site to the P site. Consequently the P site-tRNA is displaced to the E site.**

The A site-tRNA retains the mRNA by codon-anticodon basepairing → its movement causes a similar movement of the mRNA.

EF-G/GDP rapidly drops GDP and rebinds GTP for a new translational cycle.

THE TWO-STEP TRANSLOCATION OF tRNAs



"Adapted from Watson J.D. et al. 2004. Molecular Biology of the Gene. 5th edition, p.445, fig 14.34. Benjamin Cummings., CSHL Press"

Binding of EF-G/GTP to the factor binding center displaces the A site tRNA which, in it turn, displaces the P site tRNA to the E site

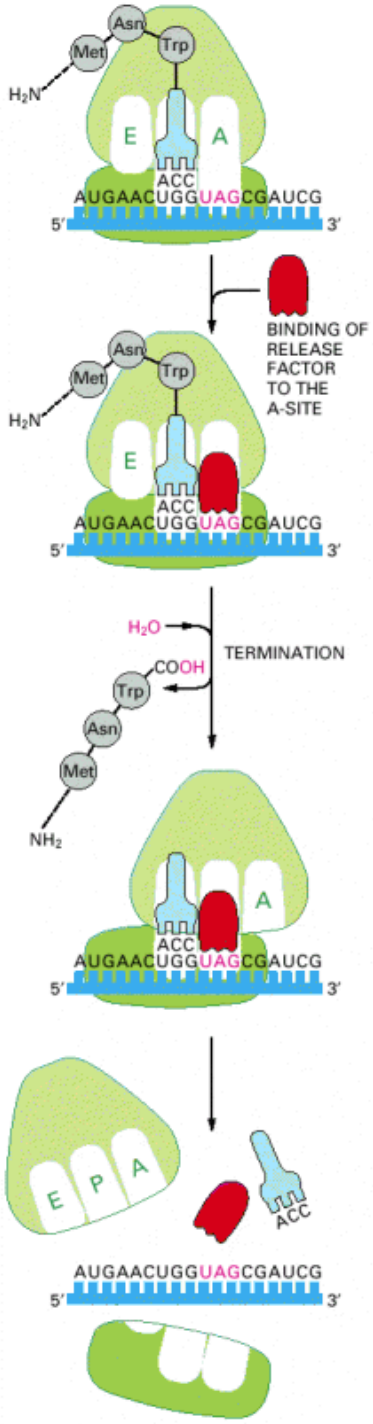
EDITING DURING TRANSLATION ELONGATION

Translation has an error rate of about 10-3 – 10-4. This accuracy is due to three mechanisms which tend to eliminate incorrect basepairing. These mechanisms act at three steps :

(1) CODON-ANTICODON BASEPAIRING : When a correct basepairing occurs, two additional bonds are formed between residues in the anticodon and residues in the 16S rRNA. Incorrect basepaired tRNA, lacking these additional bonds, dissociates more easily and more rapidly.

(2) POSITIONING OF AA-tRNA IN THE A SITE BY EF-Tu/GTP : Correct basepairing correctly positions EF-Tu/GTP in the factor binding center so that GTP can be hydrolyzed and EF-Tu released from the aminoacyl-tRNA. With codon-anticodon mismatches, EF-Tu/GTP complexed with aa-tRNA will not contact the factor binding center in a right manner. GTP is not hydrolyzed and the EF-Tu/GTP/aa-tRNA is removed.

(3) ACCOMODATION : Correct basepairing retains the A site tRNA during its rotation (accomodation) to facilitate the peptide formation with the polypeptide chain. A wrong A site tRNA will dissociate during accomodation



TERMINATION OF TRANSLATION

The termination of translation follows three steps : (1) release of the polypeptide mediated by release factors, (2) release of the release factors, (3) recycling of the ribosome.

(1) RELEASE OF THE POLYPEPTIDE : There are two classes of release factors, class I and class II, acting in a successive manner. When a stop codon comes in the A site, it will be recognized and bound by release factors (RFs) class I (RF1, RF2 in prokaryotes and eRF1 in eukaryotes). These class I RFs catalyze a peptide bond formation between a water molecule and the growing polypeptide, thus release the polypeptide from the ribosome.

(2) REMOVE OF THE RELEASE FACTORS : The class II RF, RF3 (eRF3 in eukaryotes) complexed with GTP, will displace the class I RFs and will be, in it turn, released from the ribosome.

(3) RECYCLING OF THE RIBOSOME : At the final stage, the ribosome remains bound to the mRNA and the two tRNA at P and E sites. A ribosome recycling factor (RRF) binds to the A site and recruits RF-G for translocation. RF-G displaces RRF toward the P site and thus "push away" the two tRNAs out of the ribosome. Finally, EF-F, RRF and the mRNA dissociate from the ribosome. The two subunits of the ribosome, separated by IF3, are ready for a new translational cycle.

SUMMARY

THE GENETIC CODE

The genetic code has some characteristics : degenerate, universal, non-interrupted and non-overlapped, unidirectional. It was decoded by the use of synthetic mRNA and in vitro translation system.

TRANSLATION PROCESS

Translation is a complicated process requiring many components : the mRNA, the ribosome and the tRNA. The mRNA is the intermediate coding element between the genomic DNA and the cytoplasmic translational machinery. The ribosome, composed of rRNAs and proteins, coordinates the process and catalyzes peptide bond formation. The tRNA has a more important role than transporting activated amino acid to the ribosome ; it is the adaptor which circumvents problems of affinity lacking and proximity during the peptide synthesis.

The first event is the activation of amino acid (or the charging of tRNA). Amino acids are linked to appropriate tRNAs by a two-step reaction catalyzed by enzymes called aminoacyl-tRNA synthetases.

Translation include : initiation, elongation and termination. Only the translation initiation shows some differences, the other two stages are similar between prokaryotes and eukaryotes.

ᔓ The initiation of translation requires : (1) the recruitment and correct positioning of the first ribosome on the mRNA and (2) the binding of the first aminoacyl-tRNA to the P site.

SUMMARY (continued)

ᔓ The elongation stage includes : (1) the recruitment of aa-tRNA to the A site (2) peptide formation between the incoming amino acid and the growing polypeptide chain, (3) the translocation of tRNAs and the concordant movement of mRNA so that the next codon can be read.

ᔓ The termination of translation includes : (1) release of the polypeptide catalyzed by release factors, (2) release of the release factors, (3) recycling of the ribosome to be used in a next round.

The translational process can be proofreaded at some stages : during the charging of tRNAs and during the elongation step.