

Chapter II: Ribosomal Steps of Protein Synthesis

I. Initiation

- A. General**
- B. Initiation in Bacteria**
- C. Initiation in Eukaryotes**

II. Elongation

- A. General**
- B. Mechanisms in Bacteria**
- C. Inhibition of ribosomal steps of prokaryotic protein synthesis**
- D. Mechanism in Eukaryotes**

III. Termination

I. Initiation

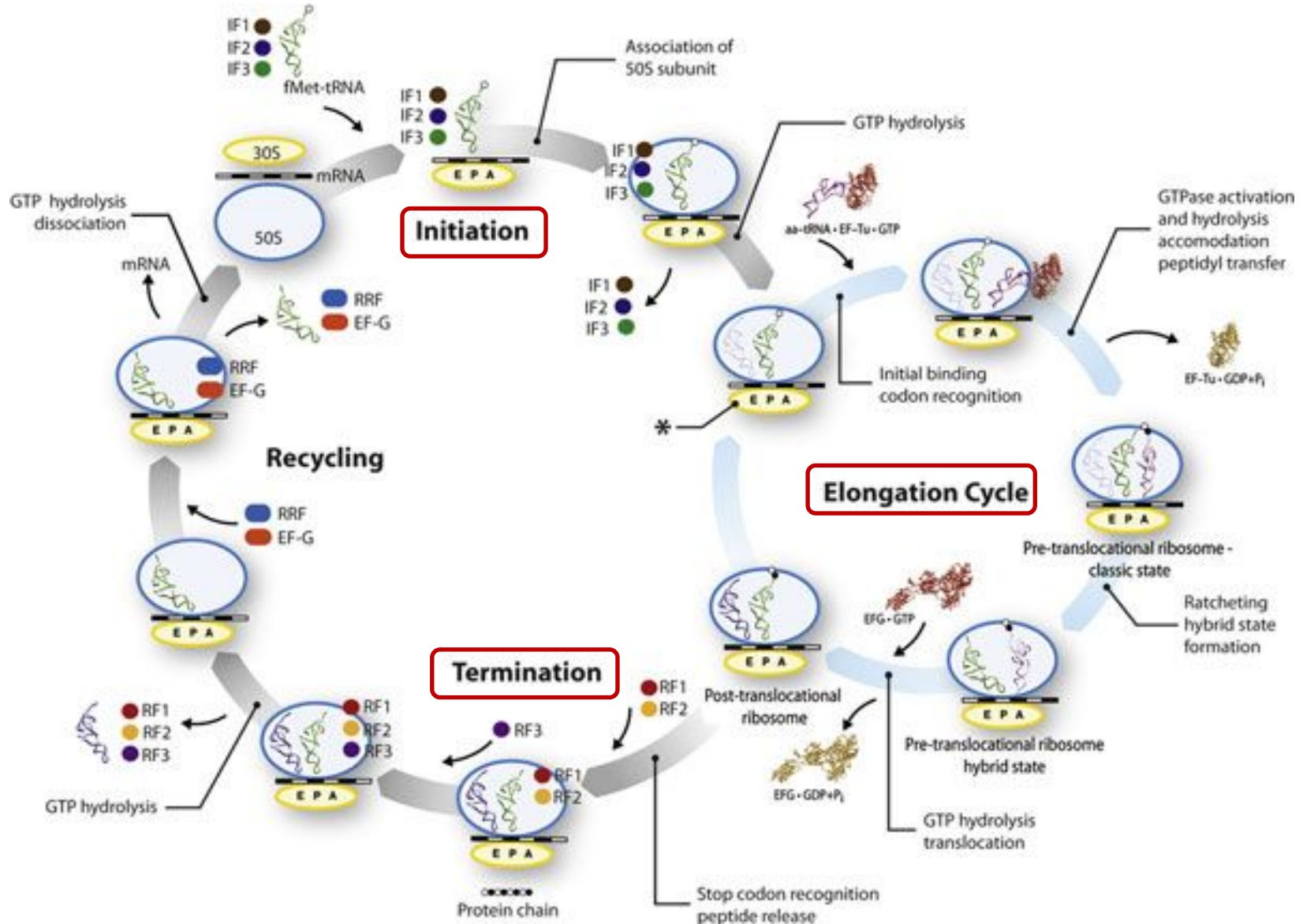
A. Généralités

Review | Published: 13 April 2010

From DNA to proteins via the ribosome: Structural insights into the workings of the translation machinery

[Xabier Agirrezabala](#) & [Joachim Frank](#)

[Human Genetics](#) 4, Article number: 226 (2010) | [Cite this article](#)



I. Initiation

A. General

Initiation must:

lead to the formation of the ternary complex: ribosome • tRNA^{iMet} • mRNA

Elongation

It is a cyclic repetition of several steps:

- Fixation of aa-tRNA
- Transpeptidation
- Translocation

The Termination

 The ribosome is not sufficient on its own to ensure this cyclic incorporation of the aa, **it needs accessory proteins called translation factors** which are of 3 types

- Initiation factors (IF)
- Elongation factors (EF) that are of 2 types: those that allow the binding of aa-tRNA in the A site and those that allow translocation

 **There are no factors for transpeptidation**

- Release factors (RF)

I. Initiation

A. General

Aa-tRNA binding sites in the ribosome

- A-site = **A**minoacyl-tRNA site
- P-site = **P**eptidyl-tRNA site
- E-site = **E**xit site

 There is a 4th site which is the entry site of the aa-tRNA

B. Initiation in Prokaryotes

1. General mechanism according to Gualerzi and description of the components involved

a. Initiation factors

- Gualerzi is an Italian researcher who works on understanding the different ribosomal stages of protein synthesis and more specifically on initiation
- **In bacteria there are 3 initiation factors:**
 - IF3** = 20.7 kDa whose gene (infC) is part of an operon. This protein is well characterized, its function and structure are known.

I. Initiation

B. Initiation in Prokaryotes

IF2 = encoded by the *infB* gene which can give 2 translation products:

- From the AUG initiation codon to the STOP which allows the **IF2 α = 97.2 kDa** protein to be obtained, which binds to the GTP to give IF2•GTP and which participates in protein synthesis by bringing fMet-tRNA^{Met} to the 30S subunit
- From a 2nd initiation codon, GUG, located inside the ORF which allows synthesis of a smaller protein **IF2 β = 79.7 kDa** which also binds to the GTP to give IF2•GTP and which also participates in protein synthesis by bringing fMet-tRNA^{Met} to the 30S subunit

 **The 2 versions of IF2 (α and β) exist in bacteria and are active for MS but it is not known why there is a long and short version of IF2**

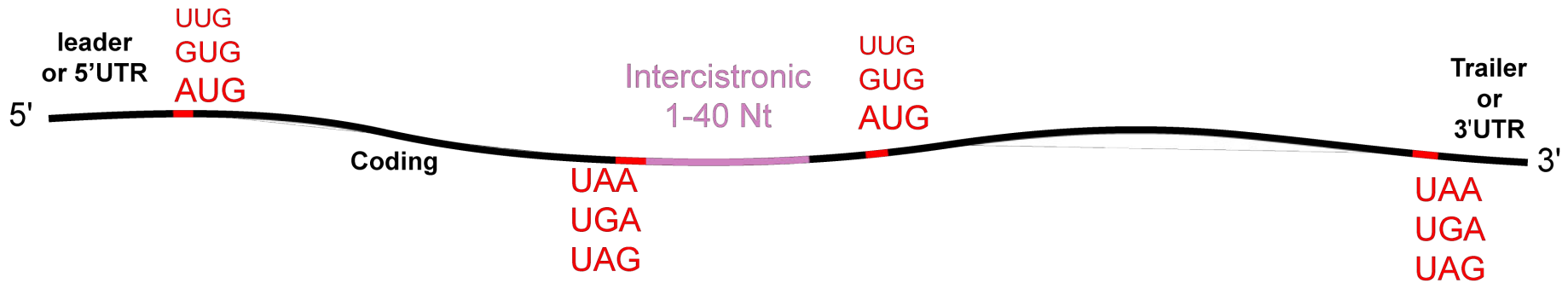
IF1 = 8 kDa prevents access to ribosome A-site for the fMet-tRNA^{Met}

I. Initiation

B. Initiation in Prokaryotes

b. mRNA

In bacteria, mRNAs are often **polycistronic**, i.e. they are the product of the transcription of an **operon** that groups genes coding for several Prot

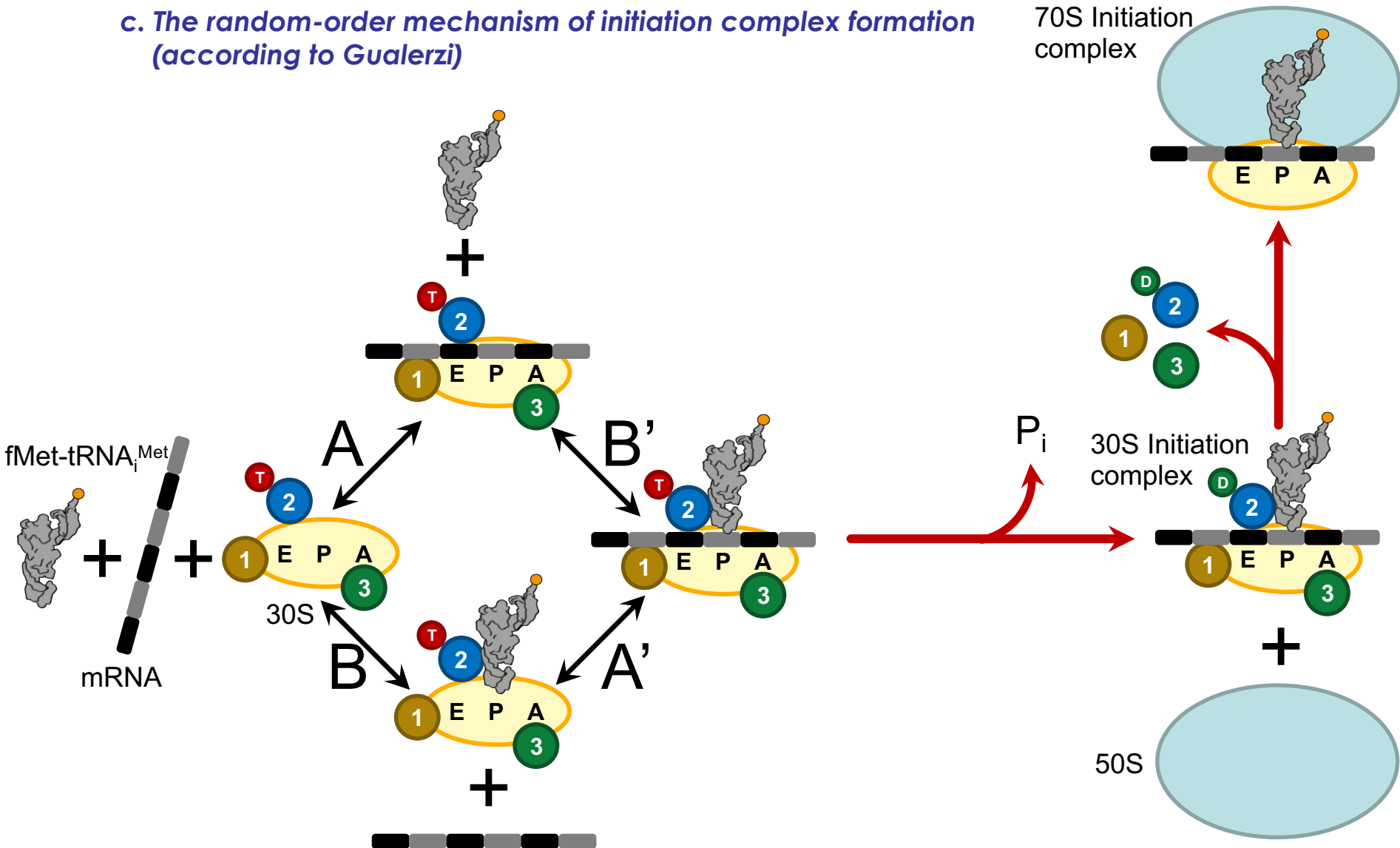


Schematically, the mRNA consists of: 5'UTR-initiation codon-ORF1-STOP codon-Intercistronic region-5'UTR-initiation codon-ORF2-STOP codon-3'UTR

I. Initiation

B. Initiation in Prokaryotes

c. The random-order mechanism of initiation complex formation (according to Gualerzi)



I. Initiation

B. Initiation in Prokaryotes

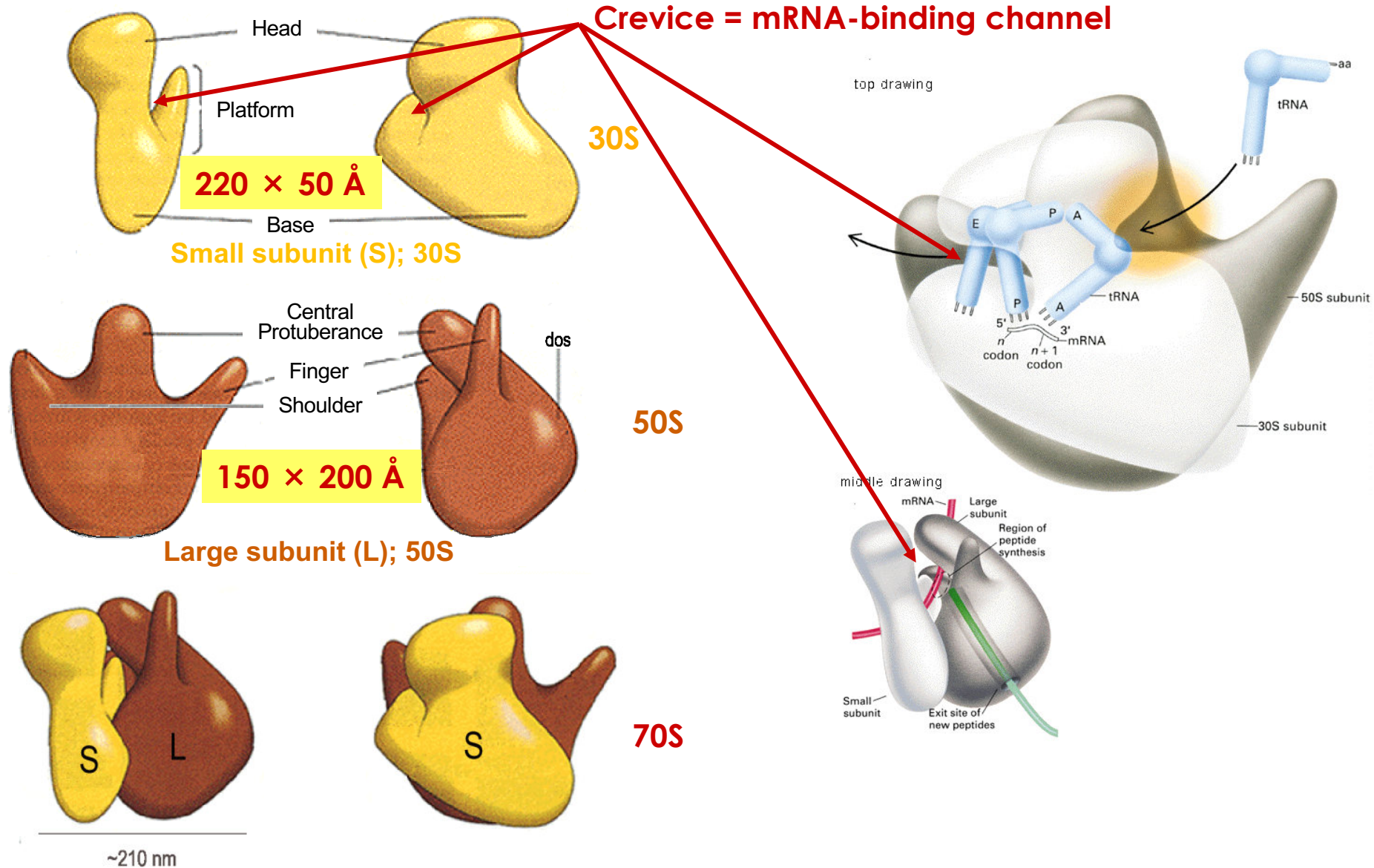
According to Gualerzi: when the ribosome reaches the end of the translation of an mRNA, it dissociates and then

- 1st step: fixation of the 3 initiation factors on the small (30S) su of the ribosome in the crevice where the mRNA will bind
- IF1 blocks entry of the A-site
- IF2 brings GTP
- This complex is stable because the 3 IFs have a very strong affinity for the small su of the ribosome
- As the 2 separated su of the ribo can assemble automatically, it is necessary to prevent the reassociation of the 2 su before the mRNA attaches to the small su
 - The 1st role of IF3 is to prevent this reassociation which, as long as it is bound to the small su, prevents the association of the large su = anti-association
 - Its 2nd role is that it is involved in the binding of mRNA to the small su at the level of the crevice

I. Initiation

B. Initiation in Prokaryotes

d. The ribosome



I. Initiation

B. Initiation in Prokaryotes

The *E. coli* ribosome contains three RNA molecules and 52 proteins.

	Ribosome	Small Subunit	Large Subunit
Sedimentation rate Mass (daltons)	70S 2,520,000	30S 930,000	50S 1,590,000
Major RNAs Minor RNAs RNA mass RNA proportion	 1,664,000 66%	16S = 1541 bases 560,000 60%	23S = 2904 bases 5S = 120 bases 1,104,000 70%
Protein number Protein mass Protein proportion	 857,000 34%	21 polypeptides 370,000 40%	31 polypeptides 487,000 30%

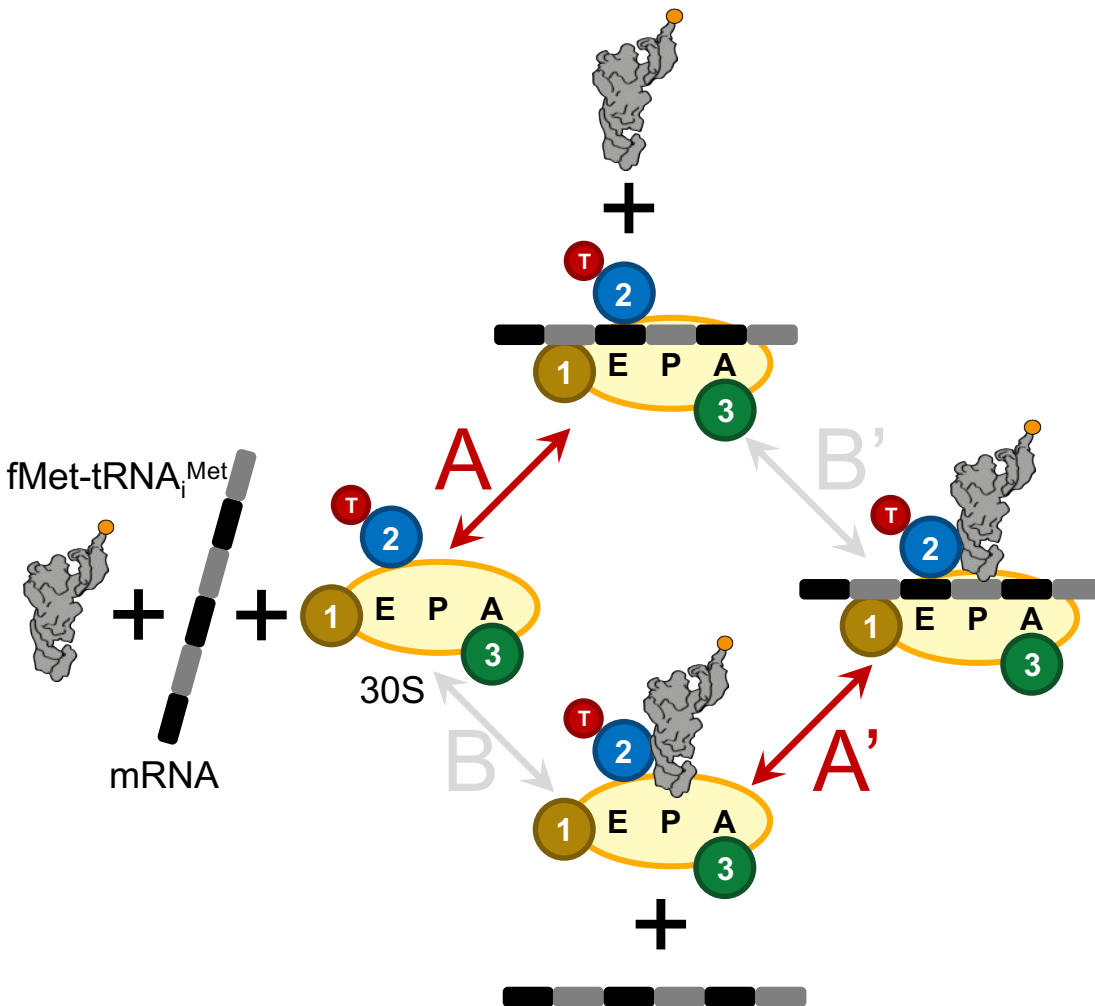
The small subunit proteins are numbered S1–S21. The large subunit proteins are numbered L1–L34, because of some early misassignments.

I. Initiation

B. Initiation in Prokaryotes

According to Gualerzi:

- If the mRNA binds in the crevice and nothing happens, the complex is not stable enough and can re-dissociate (A and A')



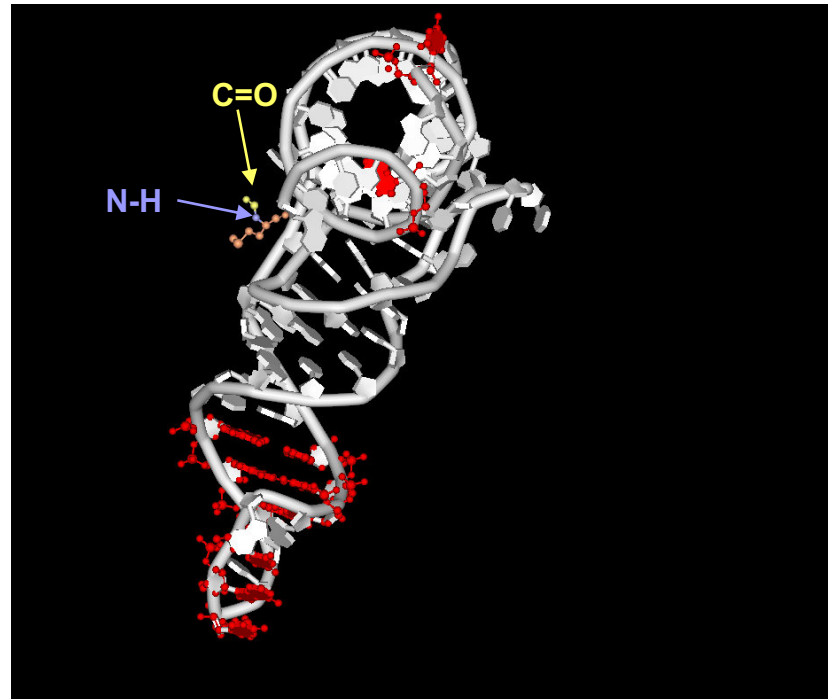
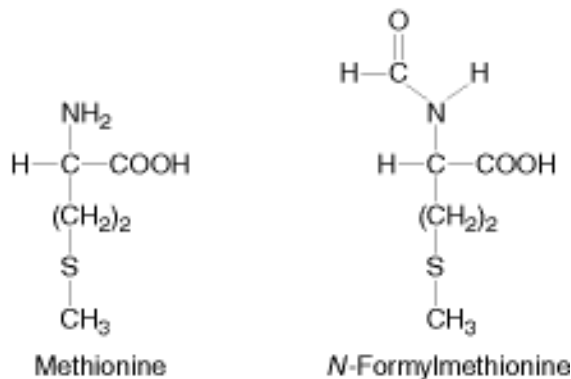
I. Initiation

B. Initiation in Prokaryotes

According to Gualerzi:

e. The 30S-fMet-tRNA^{iMet} interaction

- The initiator tRNA in bacteria is a tRNA^{Met} carrying the CAU @codon and carrying in 3' a Met to which a **formyl (CHO) group** (fMet-tRNA^{iMet}) is attached, it **binds to the P-site** of the 30S su and the 70S ribosome by codon-@codon interaction.

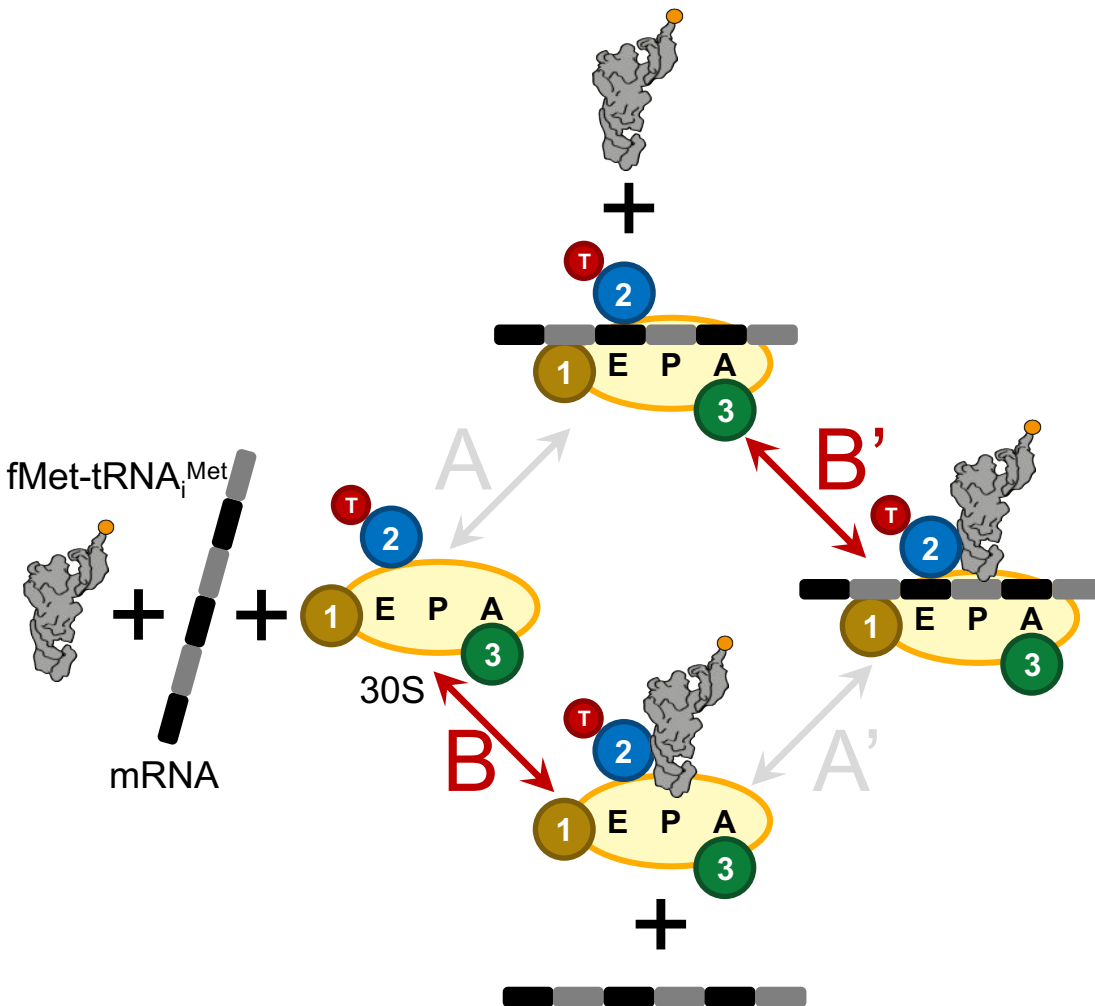


I. Initiation

B. Initiation in Prokaryotes

According to Gualerzi:

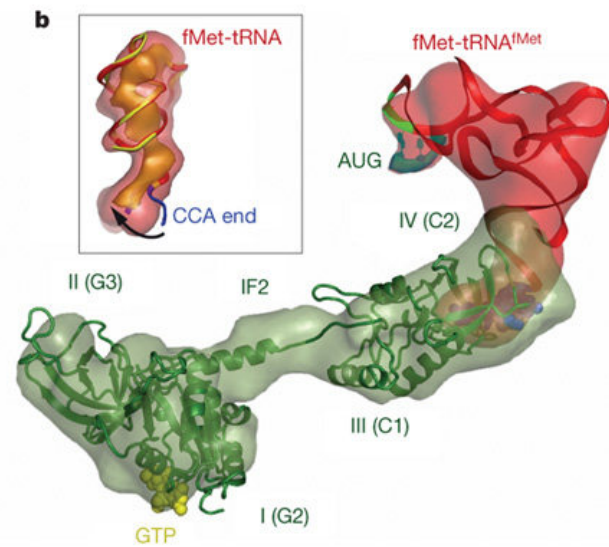
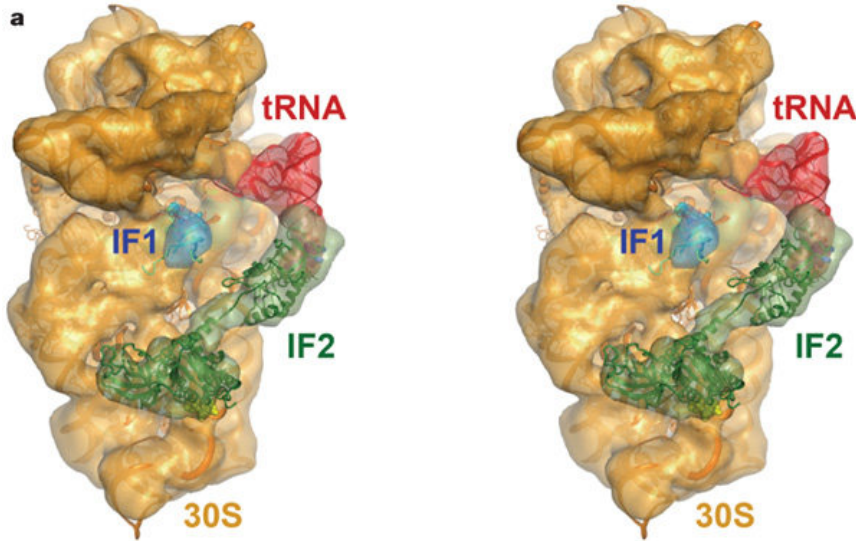
- The molecule allowing the binding of mRNA and fMet-tRNA_i^{Met} is **IF2-GTP**, but the 30S initiation complex is not very stable and can re-dissociate (B and B')



I. Initiation

B. Initiation in Prokaryotes

IF2-GTP Fitting and map interpretation of the 30SIC.



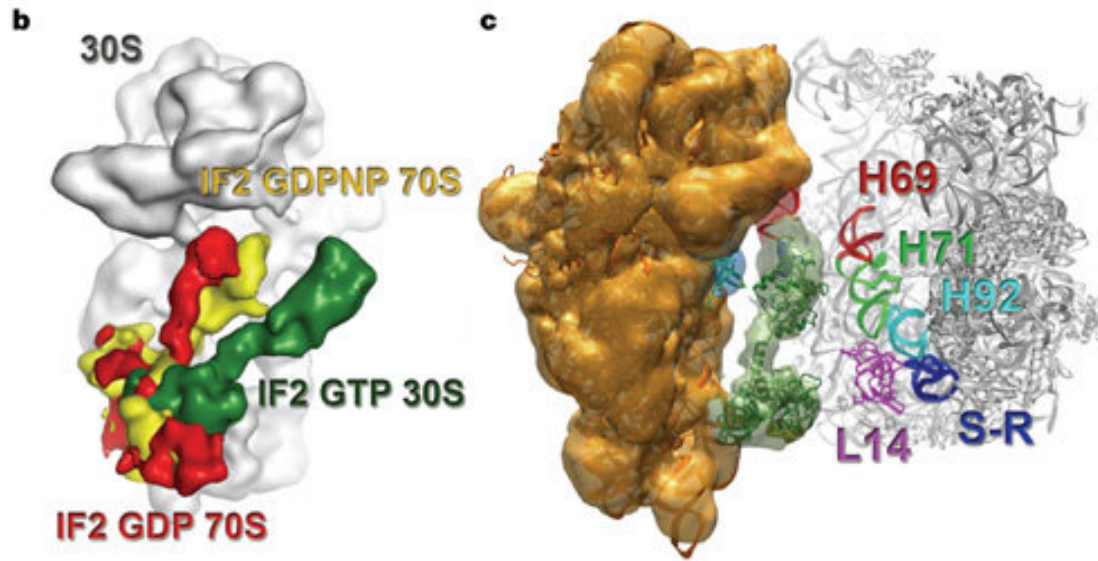
The map and the derived molecular model of the 30SIC disclosing the positions and interactions of IF1, IF2 and fMet-tRNA^{fMet} on the 30S subunit are shown. For map interpretation and domain assignments, the crystal structure of the 30S/IF1 complex¹¹, of the tRNA¹⁰ and the IF2 homology model¹³ derived from the aIF5B crystal structure¹², have been fitted in the cryo-EM map of set 1. **a**, A stereo representation of the structure of the 30S–mRNA–fMet-tRNA^{fMet}–IF1–IF2–GTP complex and assignment of the components (global view from subunit interface). The density parts corresponding to fMet-tRNA^{fMet}, IF1 and IF2 are coloured in red, blue and green, respectively. **b**, View of the IF2–fMet-tRNA^{fMet} sub-complex (view rotated to the left compared to **a**). Domain assignment of IF2 and analysis of its interaction with the fMet-tRNA^{fMet} is shown. The tRNA decoding stem is bent towards the mRNA (the anti-codon is shown in green; the residual density next to it corresponds to the AUG start codon shown in dark green). Notably, the 3' CCA end of the tRNA (insert, view from 50S side) is linked towards IF2 domain IV (C2) compared to a classical tRNA (in yellow), as seen from differential protein/RNA density contouring levels (shown in pink and orange).

A Simonetti *et al. Nature* **455**, 416–420 (2008) doi:10.1038/nature07192

I. Initiation

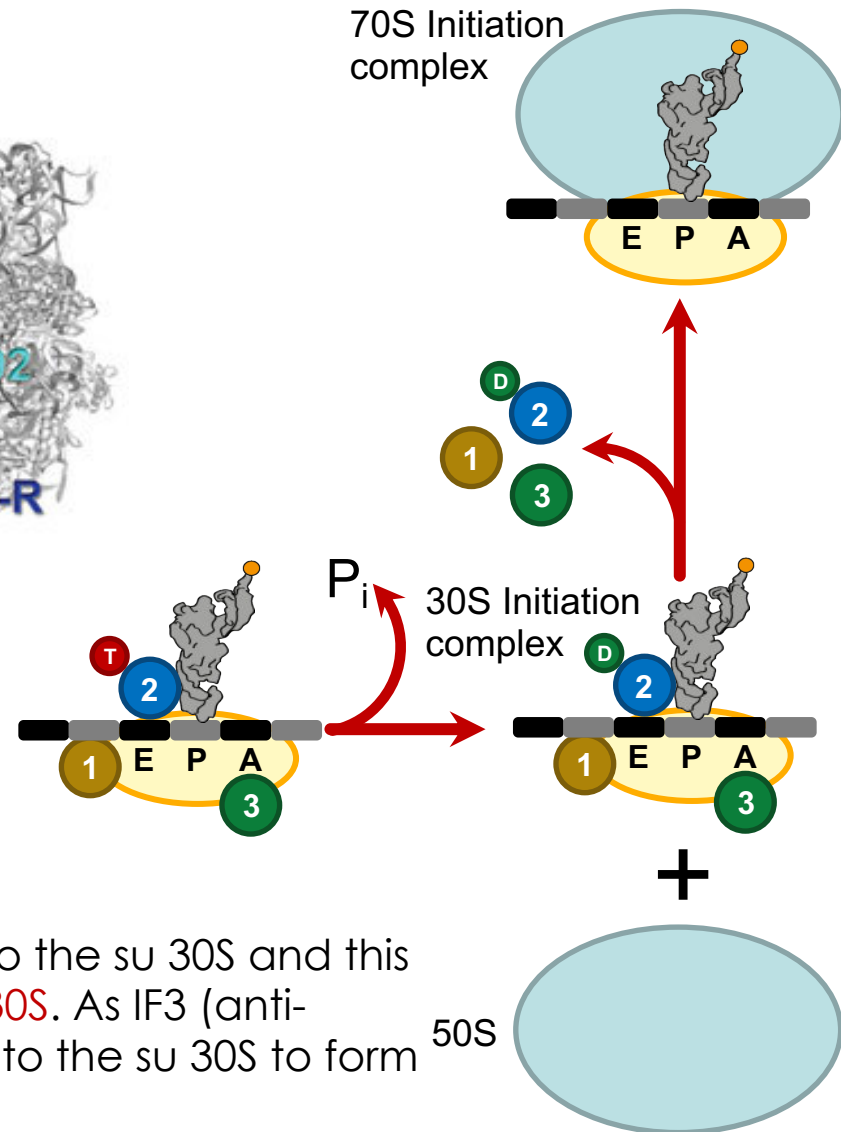
B. Initiation in Prokaryotes

IF2 & GTP Hydrolysis



This step is the **rate-limiting step** of initiation and requires energy which is provided by the **hydrolysis of GTP $\rightarrow$ GDP + Pi**. This energy allows a **change in the conformation of the 30S subunit**

Then initiation factors lose their binding affinity to the 30S and this is why IF1, -2-GDP and 3 dissociate from the 30S. As IF3 (anti-associant) has dissociated, the 50S can bind to the 30S to form the 70S ribosome



I. Initiation

B. Initiation in Prokaryotes

- There is a controversy regarding Gualerzi's theory: IF2 does not bind itself to the su 30S but **first interacts with the fMet-tRNA^{fMet} to bring it to the ribosome**

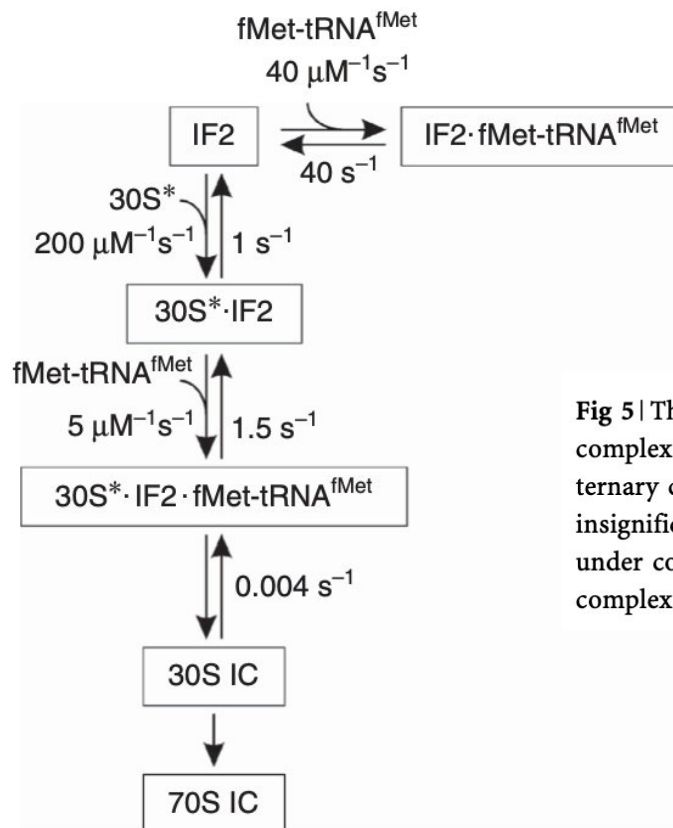


Fig 5 | The pathway of IF2 · GTP and fMet-tRNA^{fMet} binding to the 30S* complex. The main pathway is shown vertically. The formation of the ternary complex IF2 · GTP · fMet-tRNA^{fMet}, which is probably an insignificant side reaction under normal conditions, might take place under conditions of 30S subunit shortage, perhaps acting as a storage complex. IC, initiation complex; IF, initiation factor.

I. Initiation

B. Initiation in Prokaryotes

2. Shine and Dalgarno Sequence

Discovery of the sequence by the **Toeprint** technique. The protected sequence includes the initiation codon as well as a purine-rich sequence

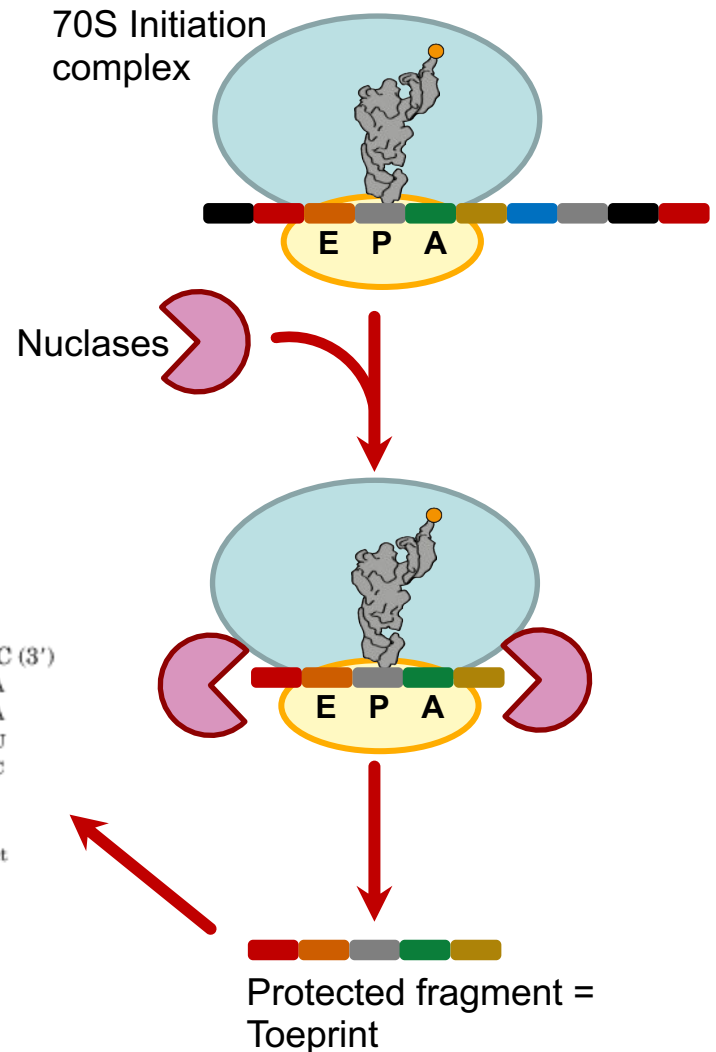
5'AGGAGGU 3'

<i>E. coli trp A</i>	(5') A G C A C G A G G G G A A A U C U G A U G G A A C G C U A C (3')
<i>E. coli ara B</i>	U U U G G A U G G A G U G A A A C G A U G G C G A U U G C A
<i>E. coli lac I</i>	C A A U U C A G G G U G G U G A A U G U G A A A C C A G U A
ϕ X174 phage A protein	A A U C U U G G A G G C U U U U U U A U G G U U C G U U C U
λ phage cro	A U G U A C U A A G G A G G U U G U A U G G A A C A A C G C

Shine-Dalgarno sequence;
pairs with 16S rRNA

Initiation codon;
pairs with fMet-tRNA^{fMet}

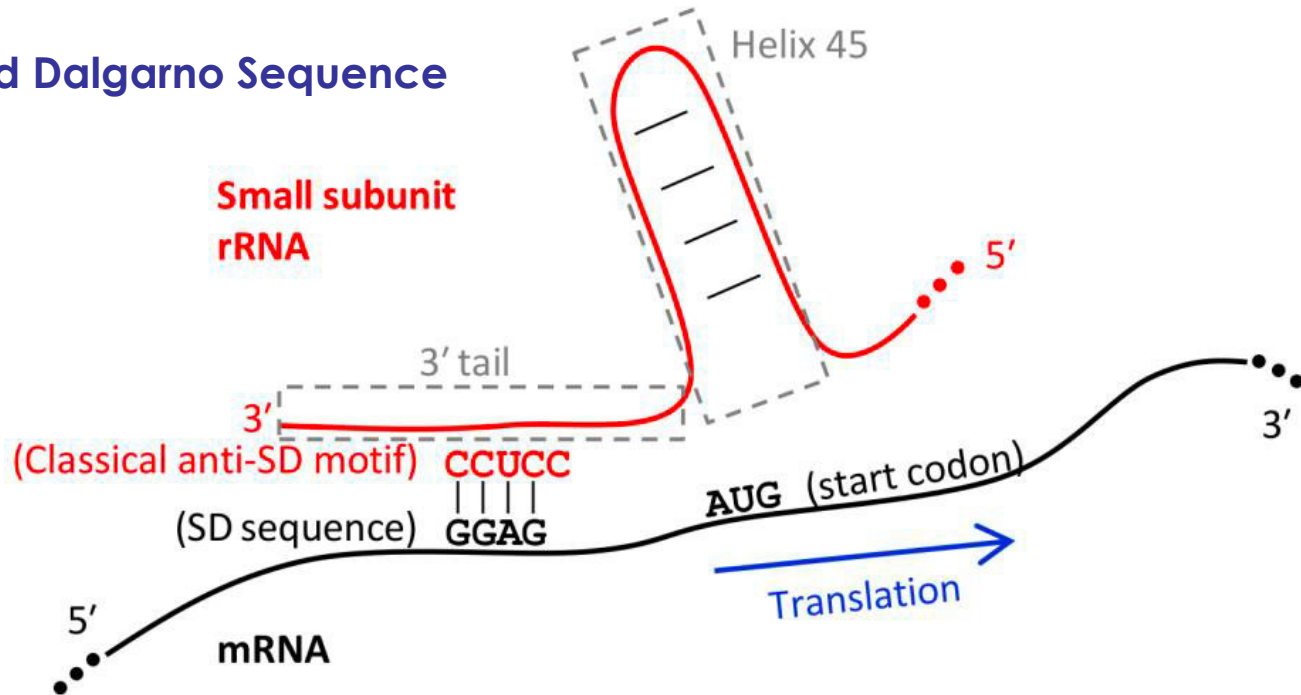
(a)



I. Initiation

B. Initiation in Prokaryotes

2. Shine and Dalgarno Sequence



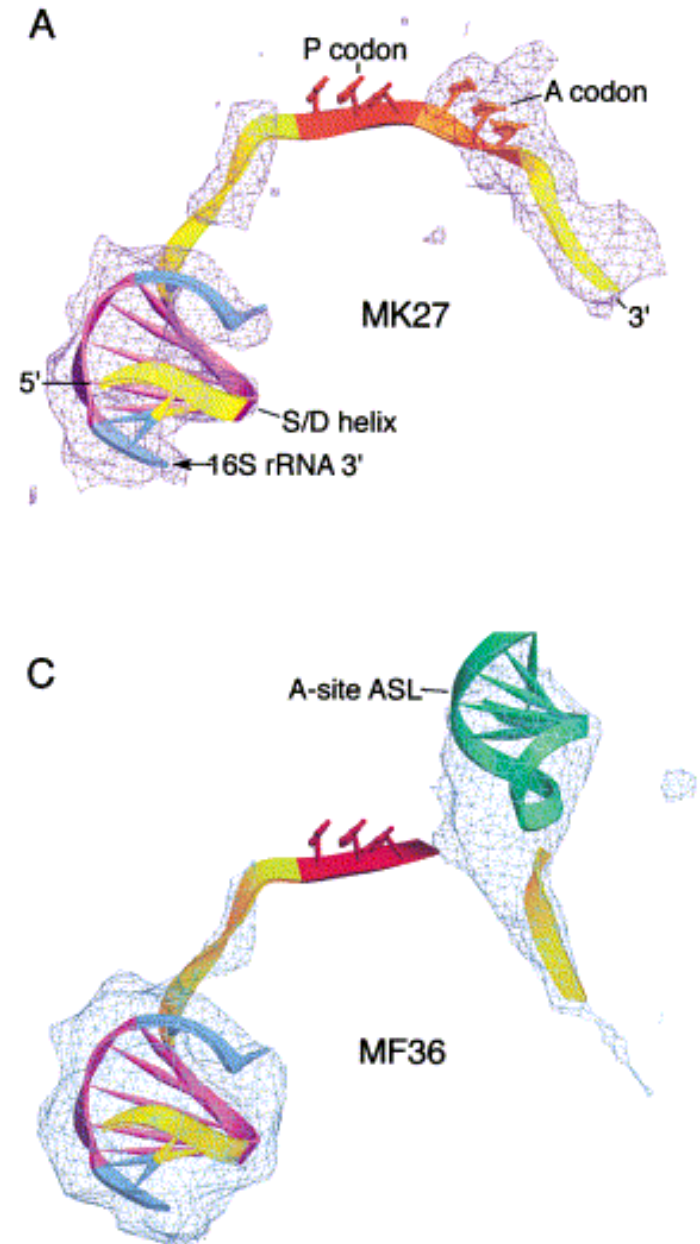
By studying all the mRNAs that could be recognized by the prokaryotic ribosome, we observe that all of them present this sequence rich in purines from 3 to 10 Nt upstream of the AUG. The consensus sequence is **AGGAGGU** = SD sequence that binds a pyrimidine-rich sequence called the **@SD sequence (5' CCUCC 3')** located at 3'-end of 16S rRNA.

I. Initiation

B. Initiation in Prokaryotes

2. Shine and Dalgarno Sequence

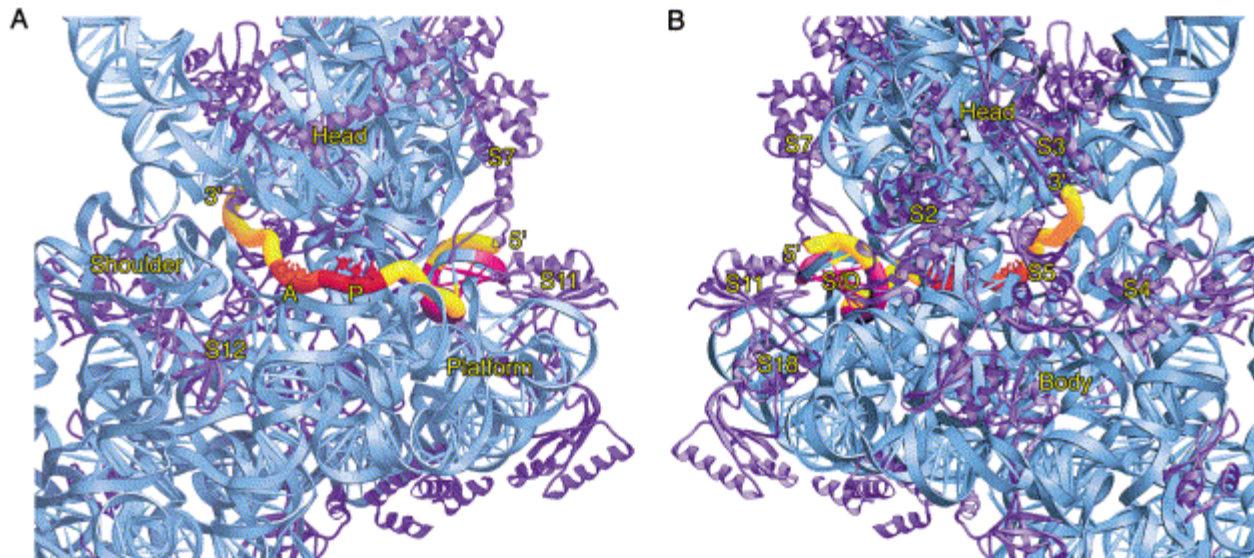
Fourier Difference Maps of mRNAs **(A)** 7 Å Fourier difference map of MK27 mRNA with the mRNA model (yellow) docked, showing the position of the Shine-Dalgarno (S/D) helix (magenta) and the positions of the A- and P-site codons (orange and red, respectively), viewed from the top of the 30S ribosomal subunit. **(B)** Difference map of the MF36 mRNA, showing a four-base-pair tetraloop helix (A-site helix) fitted to the extra density at the A site. **(C)** Same as for **(B)**, except that the A-tRNA anticodon stem-loop (green) is shown in the position observed experimentally in the A-tRNA difference map (Yusupov et al., 2001), in place of the A-site mRNA helix. **The five-nucleotide (GGAGG/CCUCC) core of the Shine-Dalgarno interaction is shown in magenta,** and the rest of the 16S rRNA tail in cyan



I. Initiation

B. Initiation in Prokaryotes

2. Shine and Dalgarno Sequence



Path of mRNA through the 30S Ribosomal Subunit **(A) Interface** and **(B) solvent** views of the mRNA in the 30S ribosomal subunit. A, P; the A- and P-site codons. 5', 3'; the 5' and 3' correspond to positions -15 and +15 of the mRNA model. The head, platform, shoulder, and body of the subunit, and ribosomal proteins S2, S3, S4, S5, S7, S11, S12, and S18 are indicated. The ribosomal proteins are shown in dark blue, 16S rRNA in cyan and **the mRNA is colored as in previous figure.**

I. Initiation

B. Initiation in Prokaryotes

2. Shine and Dalgarno Sequence

- In an mRNA with 3 coding regions, each coding region must be preceded by an SD

The genes of the tRNA-dependent amidotransferase of *B. subtilis*

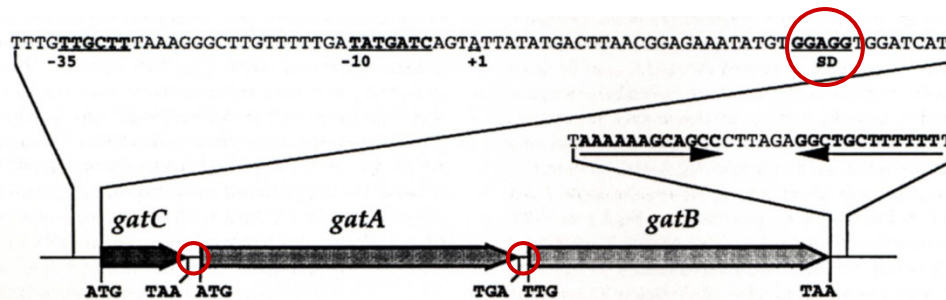


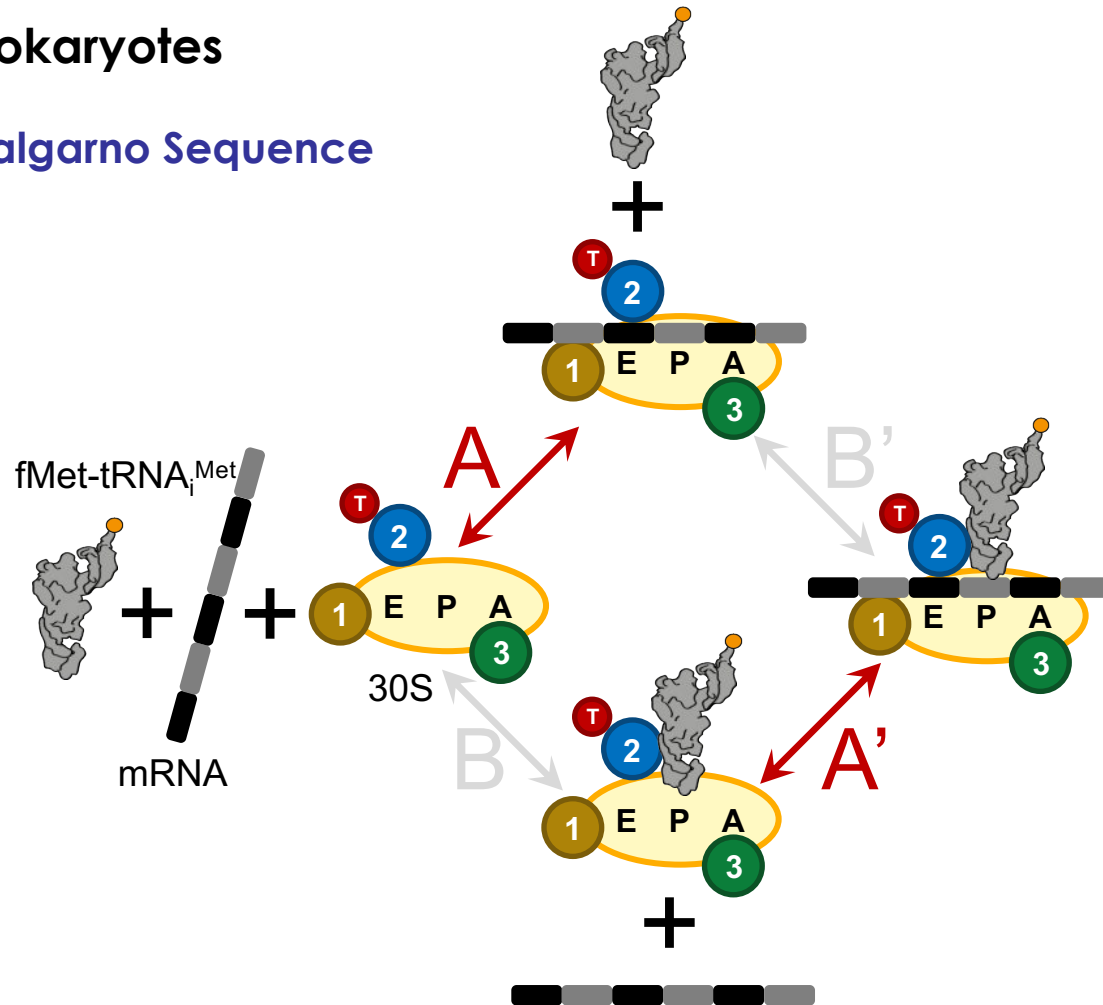
FIG. 2. Glu-AdT gene arrangement. The genomic organization of the Glu-AdT operon is shown. This includes -35 and -10 regions of the putative promoter, the Shine-Dalgarno (SD) sequence, and the putative transcription start +1.

- SD allows the interaction between the 3' part of the rRNA (@SD) and the SD sequence thanks to IF3 which, through its interaction with the su 30S, makes the @SD region of the rRNA accessible

I. Initiation

B. Initiation in Prokaryotes

2. Shine and Dalgarno Sequence



- As this SD•@SD interaction is made of 5 to 6 bps, it is not very stable and allows the re-dissociation of the complex if nothing happens (see steps A and A' of the Gualerzi mechanism)

I. Initiation

B. Initiation in Prokaryotes

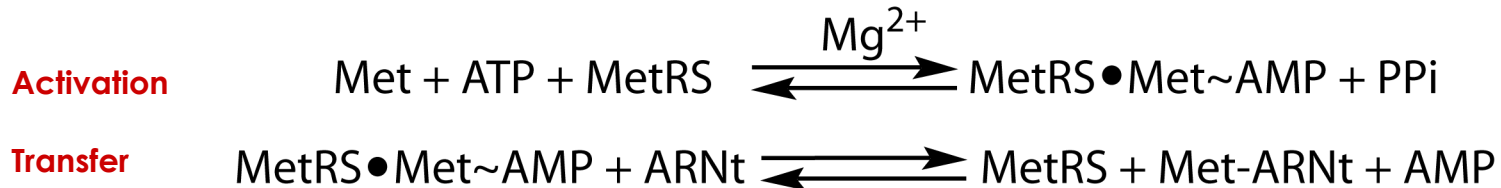
3. $\text{tRNA}_i^{\text{Met}}$ & fMet

- Since there is only **one Met codon** (AUG), this codon can be used as both an **initiation codon** and an **elongation codon**, → they must be distinguishable

- **Initiator** AUG ← **fMet-tRNA_i^{Met}**

- **Élongateur** AUG ← **Met-tRNA_e^{Met}**

- The 2 tRNA^{Met} s are aminoacylated by a single enzyme: **methionyl-tRNA synthetase (MetRS)** which does not differentiate between $\text{tRNA}_e^{\text{Met}}$ and $\text{tRNA}_i^{\text{Met}}$

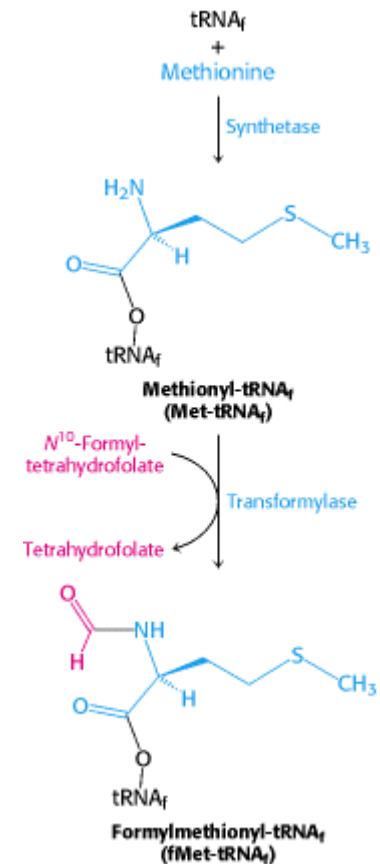
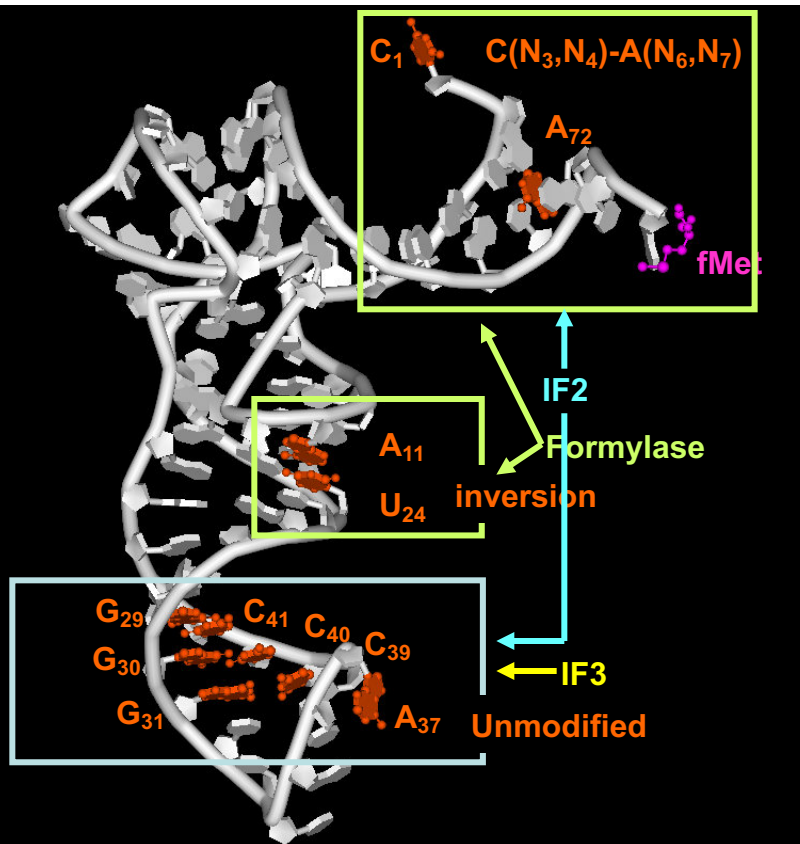


I. Initiation

B. Initiation in Prokaryotes

3. $\text{tRNA}_i^{\text{Met}}$ & fMet

- The 2 tRNAs are aminoacylated in the same way but the $\text{tRNA}_i^{\text{Met}}$ has structural features that the $\text{tRNA}_e^{\text{Met}}$ does not have and which make it the substrate of an enzyme (Ez) **formylase** which adds a **formyl** gp to the Met's NH_2 gp, thus releasing the $\text{fMet-tRNA}_i^{\text{Met}}$

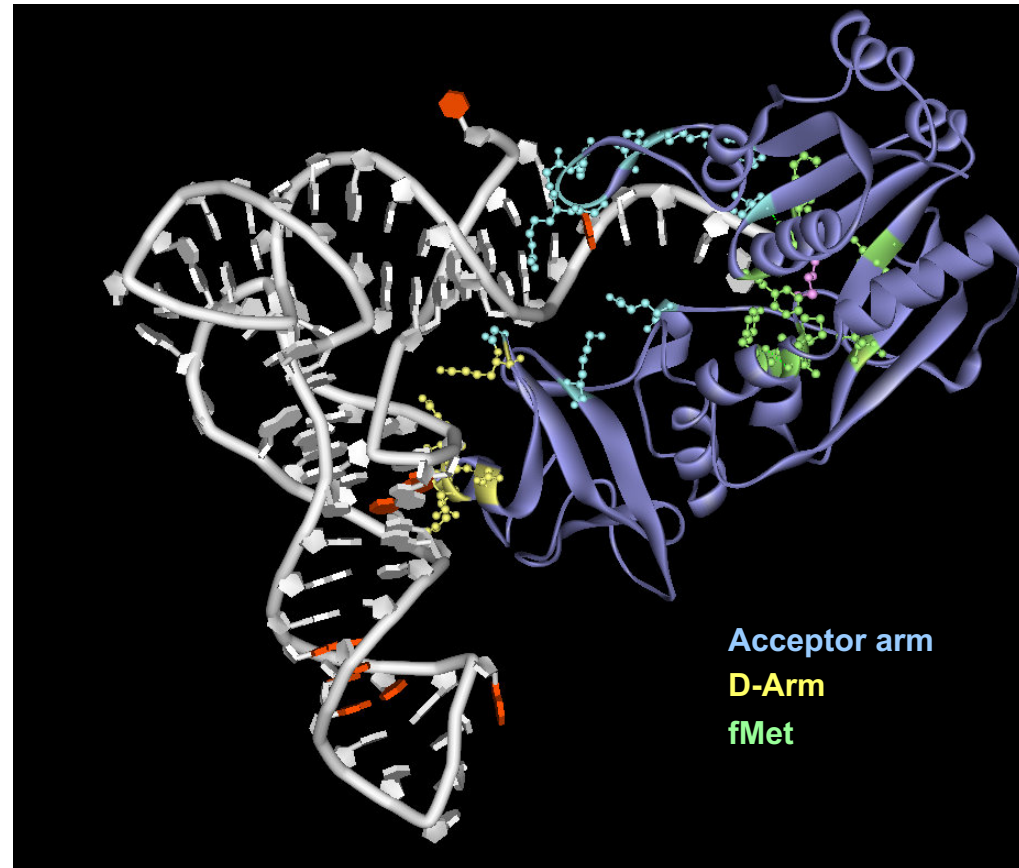
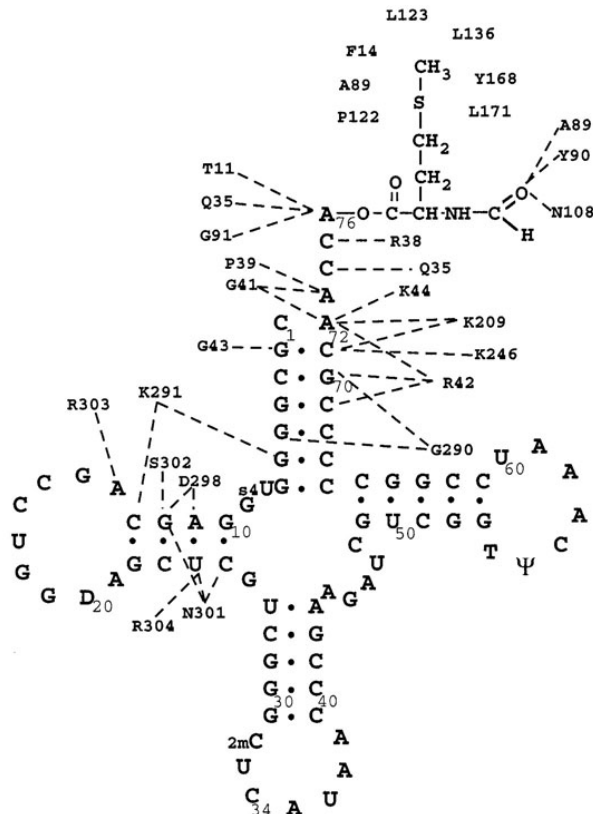


I. Initiation

B. Initiation in Prokaryotes

3. tRNA^{Met} & fMet

- The 2 tRNAs are aminoacylated in the same way but the tRNA_i^{Met} has structural features that the tRNA_e^{Met} does not have and which make it the substrate of an **enzyme (Ez) formylase** which adds a **formyl** gp to the Met's NH₂ gp, thus releasing the fMet-tRNA_i^{Met}



I. Initiation

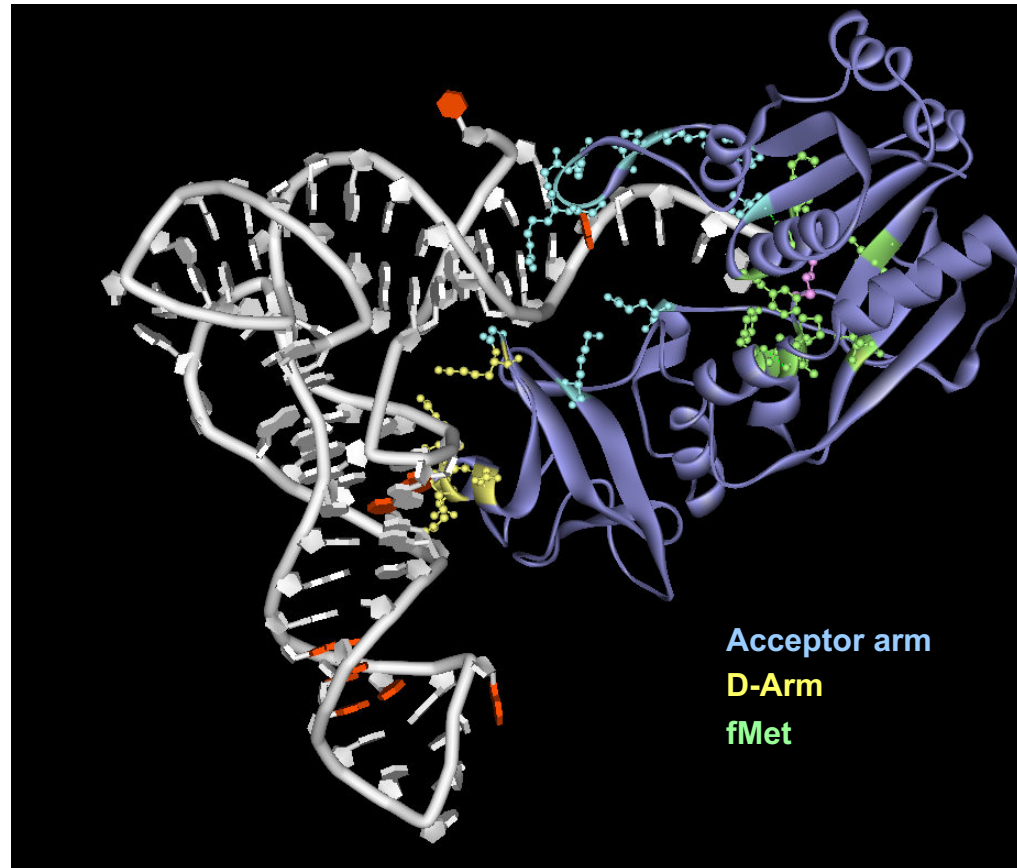
B. Initiation in Prokaryotes

3. tRNA_i^{Met} & fMet

- The 2 tRNAs are aminoacylated in the same way but the tRNA_i^{Met} has structural features that the tRNA_e^{Met} does not have and which make it the substrate of an **enzyme (Ez) formylase** which adds a **formyl** gp to the Met's NH₂ gp, thus releasing the fMet-tRNA_i^{Met}



Comparison of various tRNAs with the formylase-bound tRNA^{fMet}. Free tRNA^{Phe} (green), EF-Tu bound tRNA^{Phe} (blue) and GlnRS bound tRNA^{Gln} (pink) were superimposed to tRNA^{fMet} (yellow) complexed to the formylase (gray). Superimposition was based on the fitting of the phosphate atoms of tRNAs, excluding those of the acceptor arms. The Calpha trace of the enzyme and the phosphate backbone traces of tRNAs were drawn using Setor (Evans, 1993).



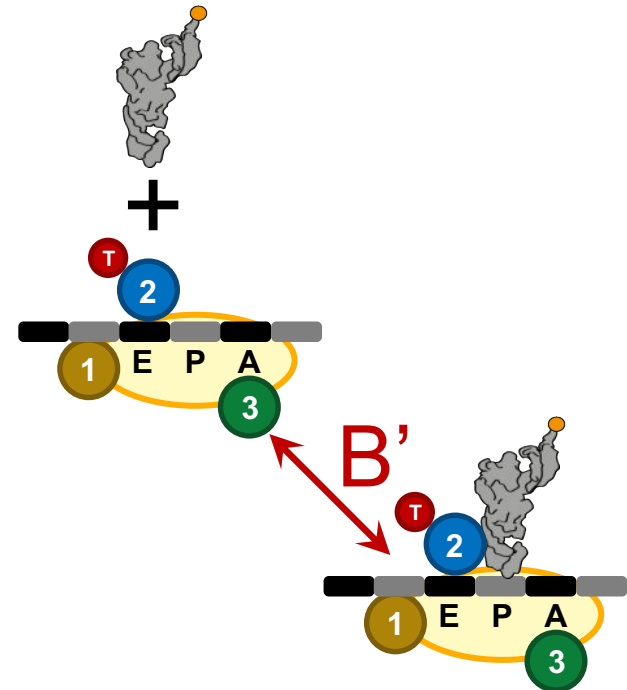
I. Initiation

B. Initiation in Prokaryotes

3. tRNA_i^{Met} & fMet

- Once the fMet-tRNA_i^{Met} is formed, it binds to IF2-GTP attached to the 30S su, and IF2 **specifically recognizes only the fMet-tRNA_i^{Met}** and not the Met-tRNA_e^{Met}

Here again the complex is not stable enough to avoid re-dissociation. To avoid this, **there must be codon•@codon interaction**



I. Initiation

B. Initiation in Prokaryotes

4. IF3

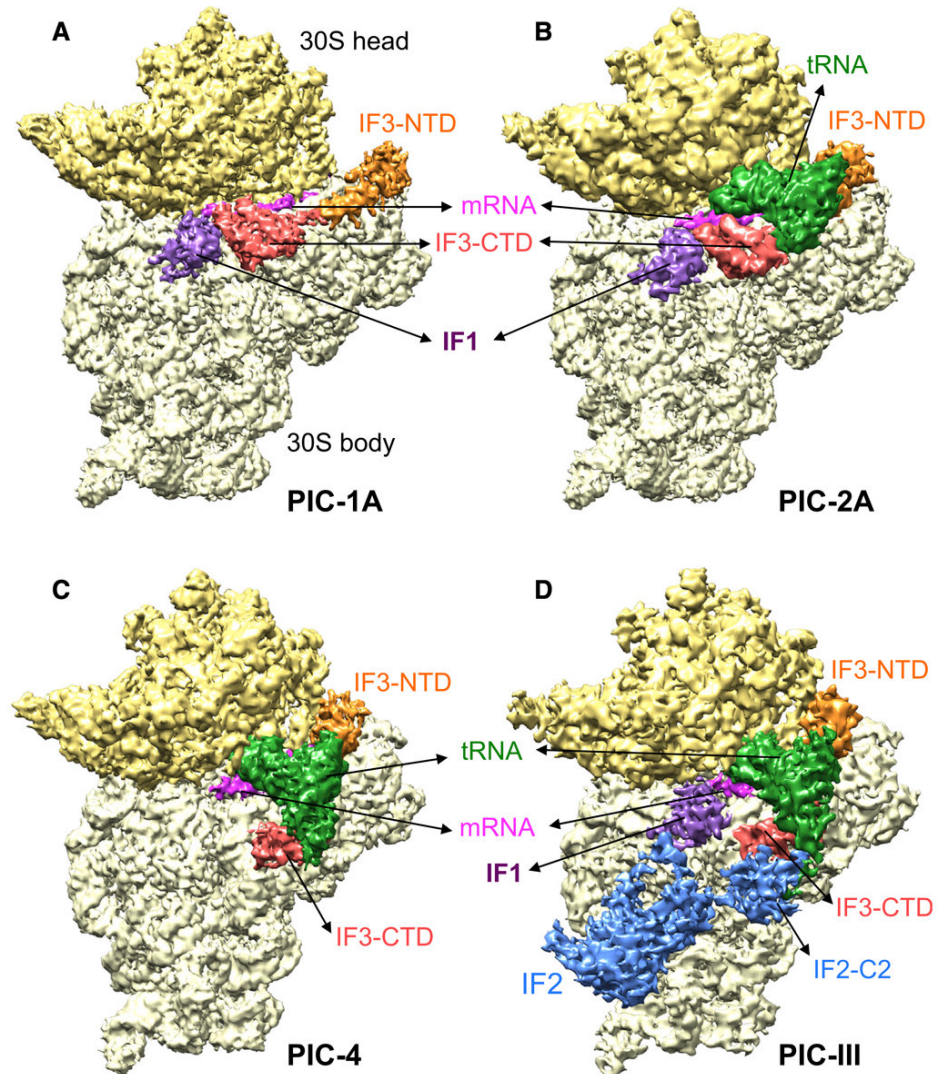
This is done thanks to **IF3** correctly orienting the tRNA^{iMet} with respect to the mRNA. **IF3** recognizes the @codon arm of the tRNA^{iMet} at a very rigid region of 3' pdb G≡C. IF3 binds to the RNAt^{iMet} and brings the tRNA^{iMet} into a good position for codon• interaction@codon

Hussain et al., 2016, Cell 167, 133–144

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<http://dx.doi.org/10.1016/j.cell.2016.08.074>

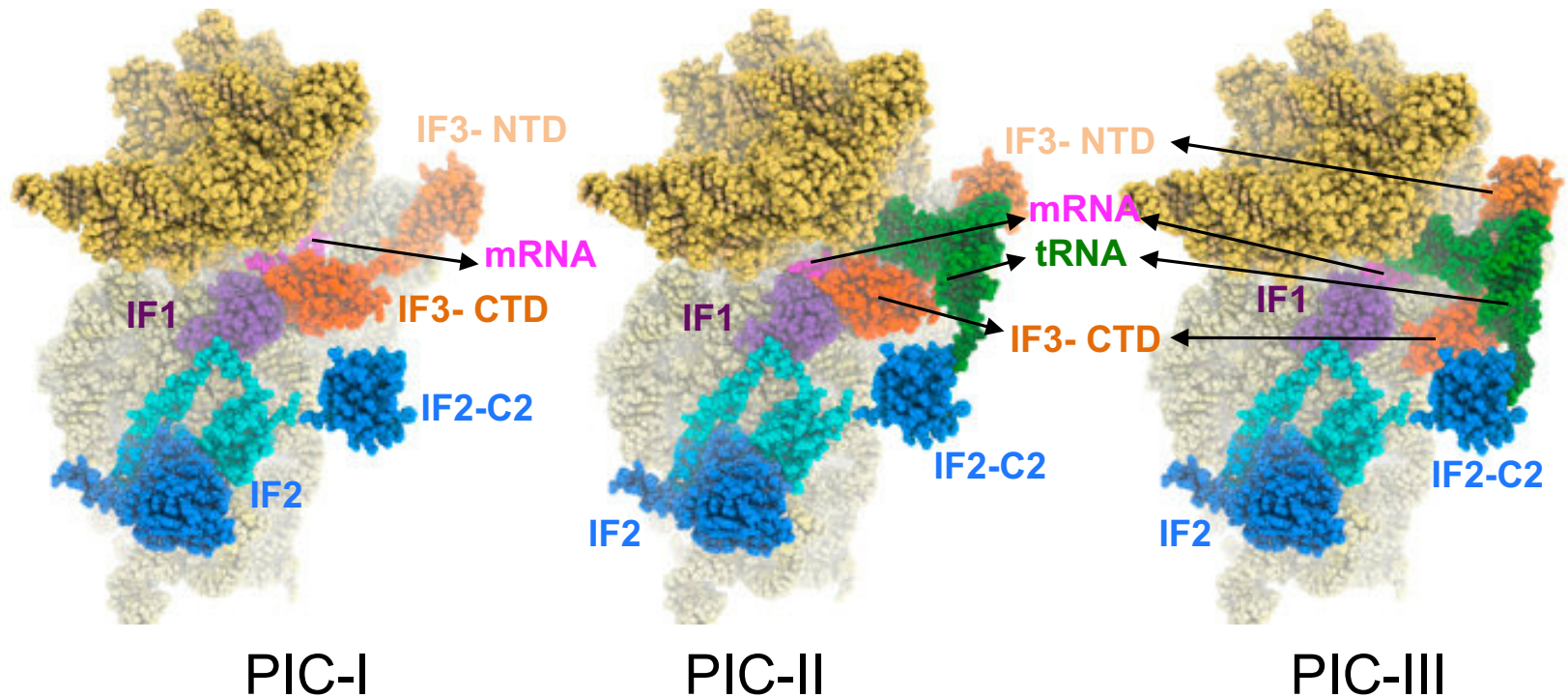
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I. Initiation

B. Initiation in Prokaryotes

4. IF3

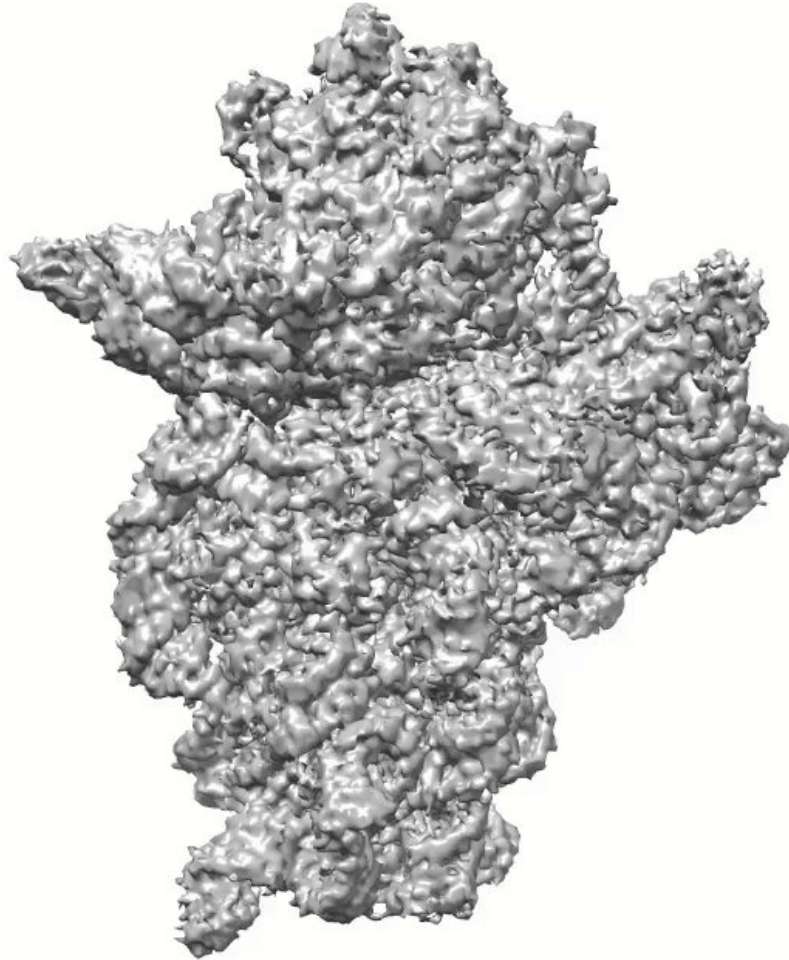


I. Initiation

B. Initiation in Prokaryotes

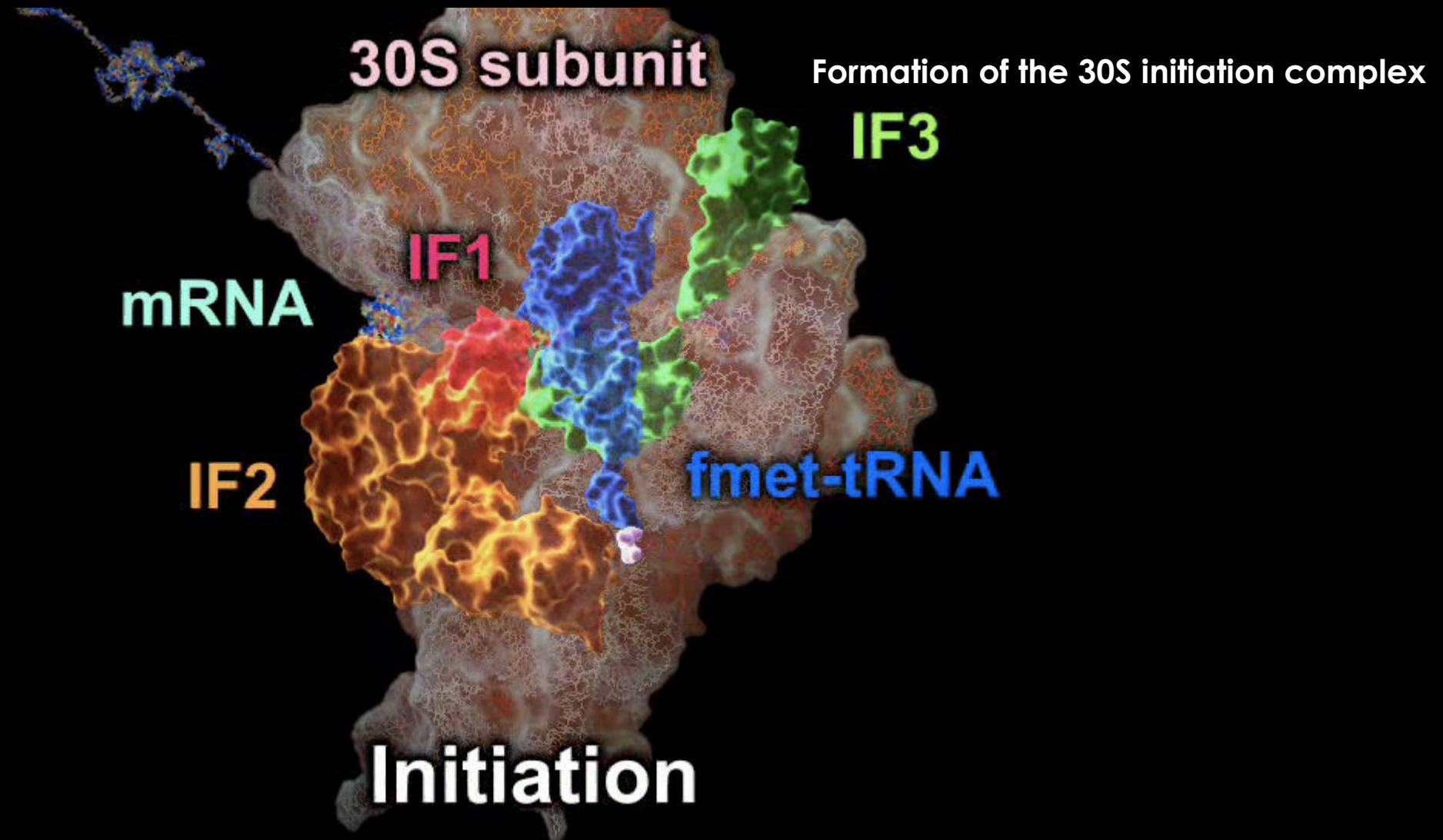
4. IF3

PIC-1A



I. Initiation

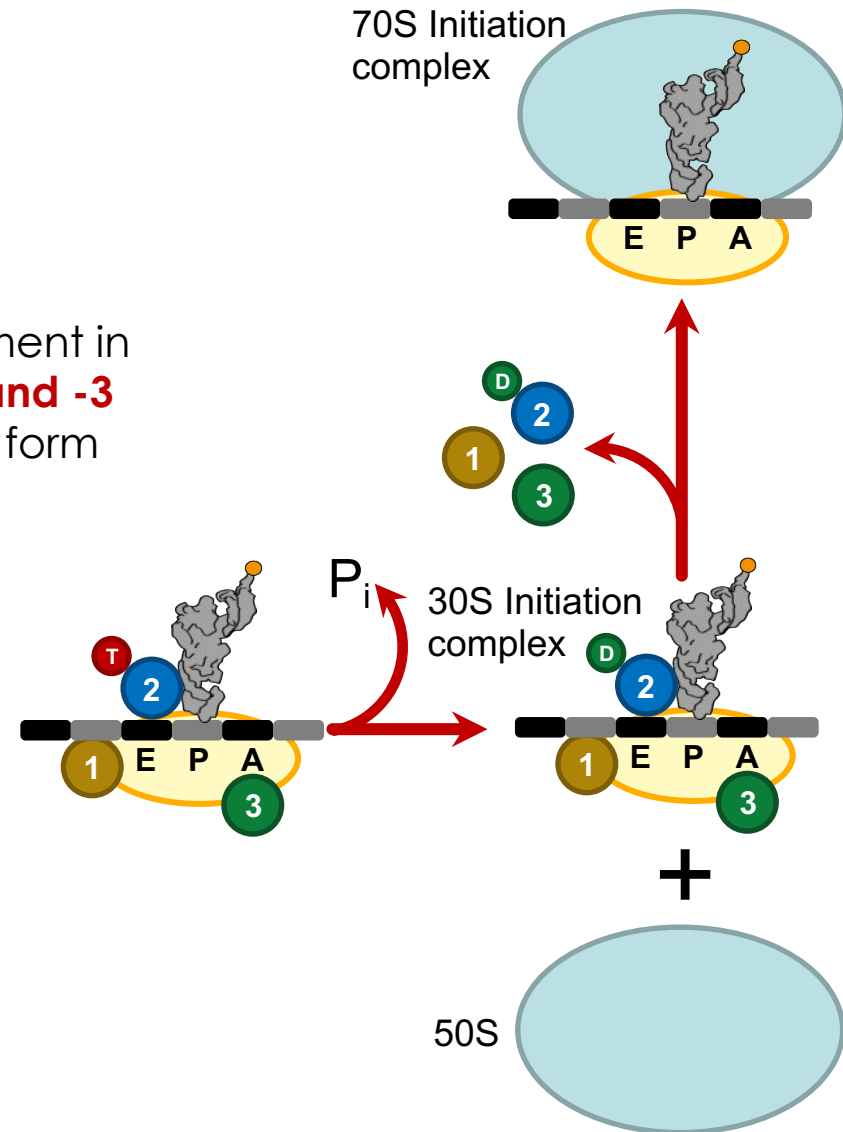
B. Initiation in Prokaryotes



I. Initiation

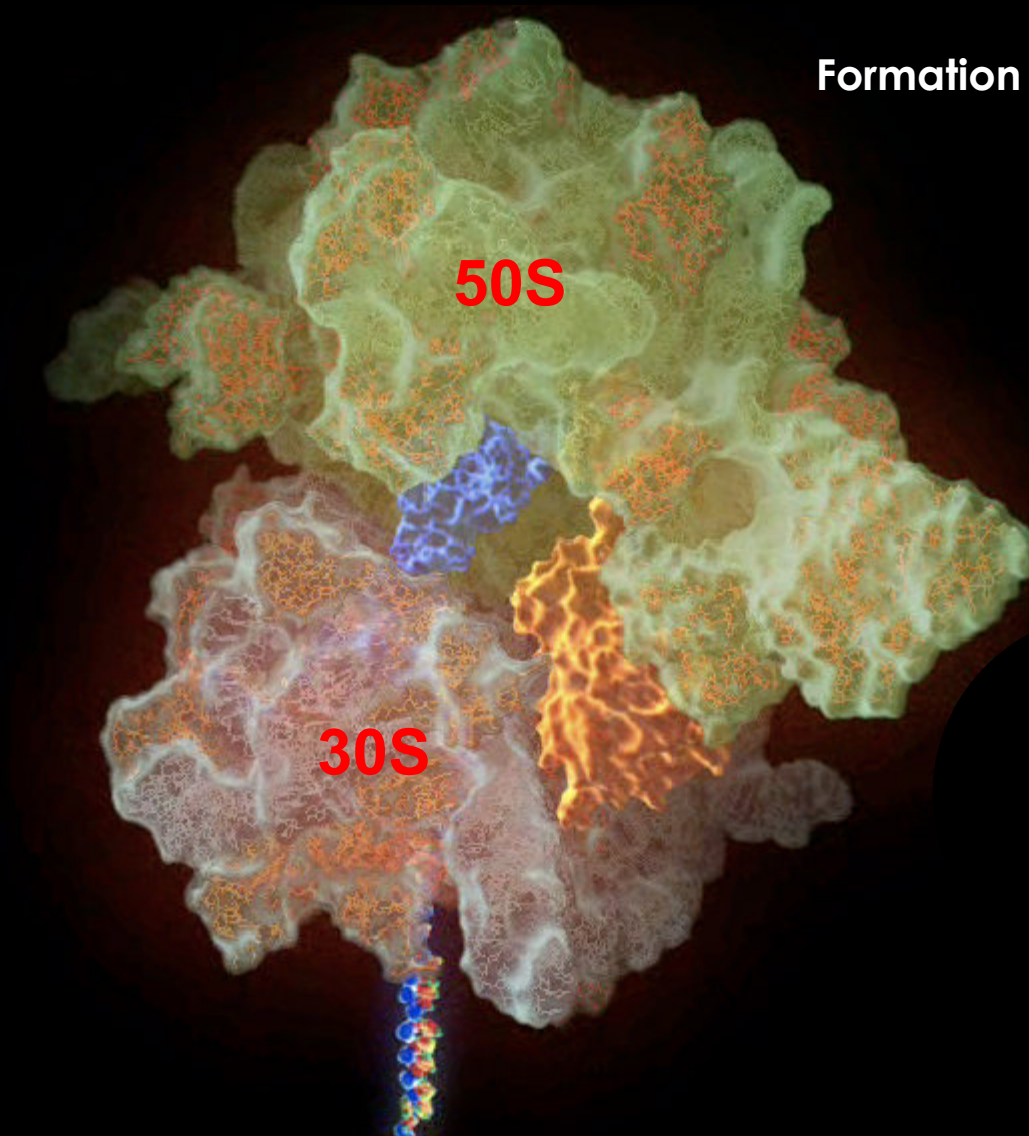
B. Initiation in Prokaryotes

Once the complex is formed there is a **conformational change induced by the hydrolysis of the $GTP \rightarrow GDP + P_i$** , this movement in the 30S su triggers **release of IF1, -2-GDP and -3** and then allows the 50S su to bind and to form the **70S initiation complex**



I. Initiation

B. Initiation in Prokaryotes

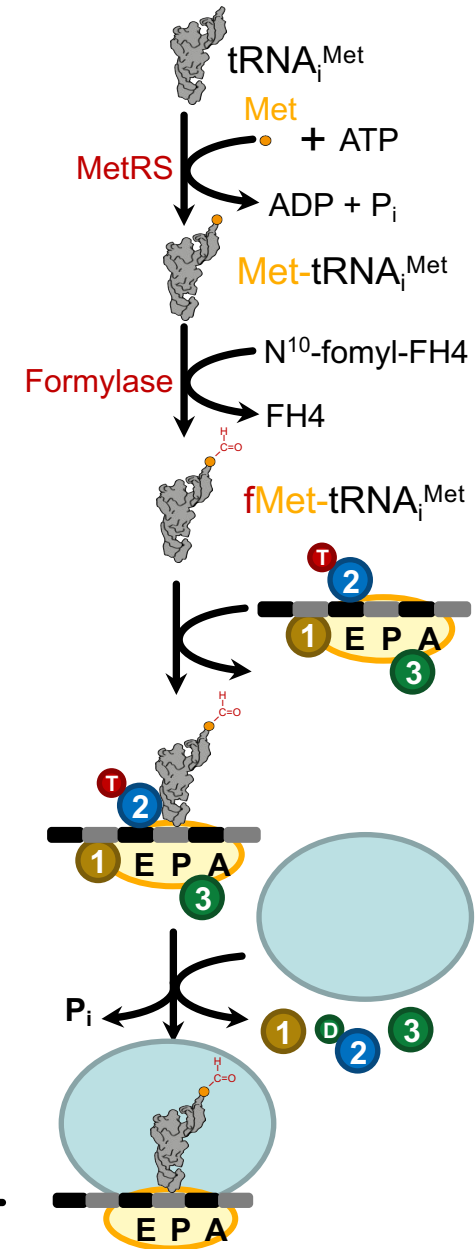
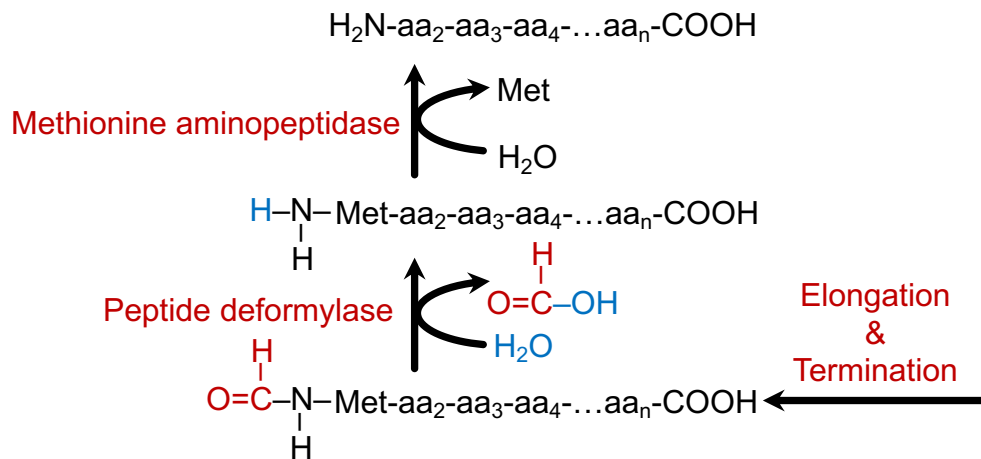


Formation of the 70S initiation complex

I. Initiation

B. Initiation in Prokaryotes

5. Fate of Formyl-Methionine



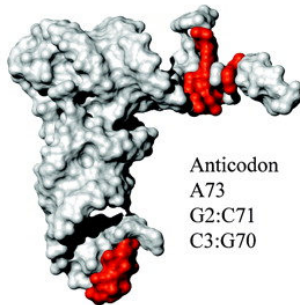
I. Initiation

B. Initiation in Prokaryotes

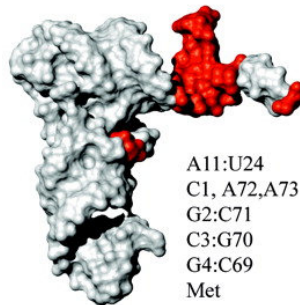
In summary: the tRNA_{Met} is "substrate" of 4 proteins and of the ribosome

A

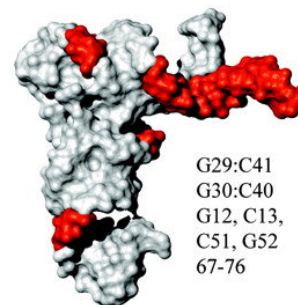
Interactions with MetRS



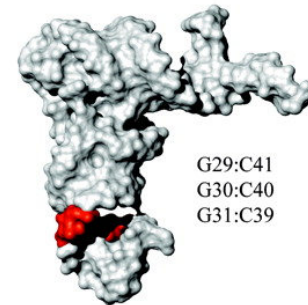
Interactions with MTF



Interactions with IF2

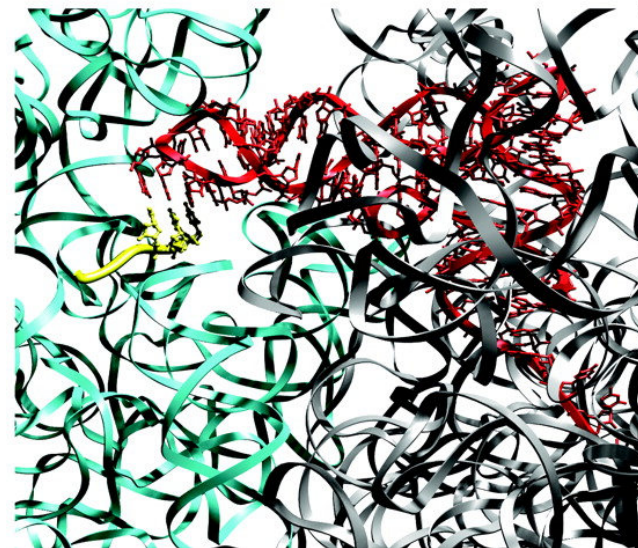
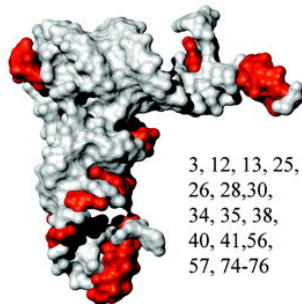


Interactions with IF3



B

Interactions with the 70S ribosome



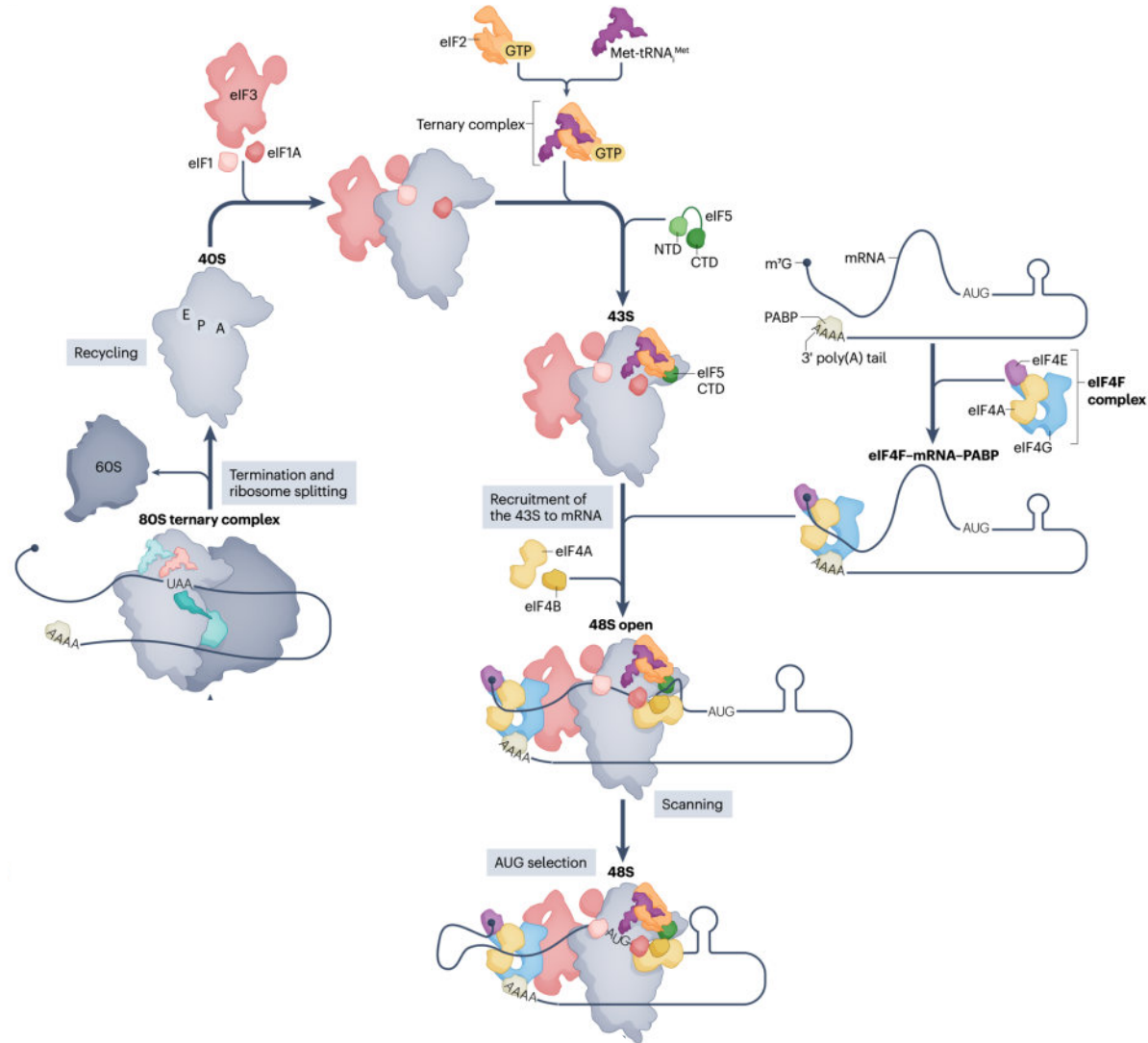
I. Initiation

C. Initiation in Eukaryotes

In eukaryotes as in prokaryotes the cycle is the same:
Dissociation of the 2 su → mRNA and Met-RNAⁱMet binding → initiation complex.

BUT

- i) the composition of the ribosome is different,
- ii) the recruitment of the mRNA is different,
- iii) the positioning of the initiating AUG in the 1/2 site P is done in a different way and
- iv) the number of initiation factors is significantly higher.



I. Initiation

C. Initiation in Eukaryotes

High-Resolution Structure of the Eukaryotic 80S Ribosome

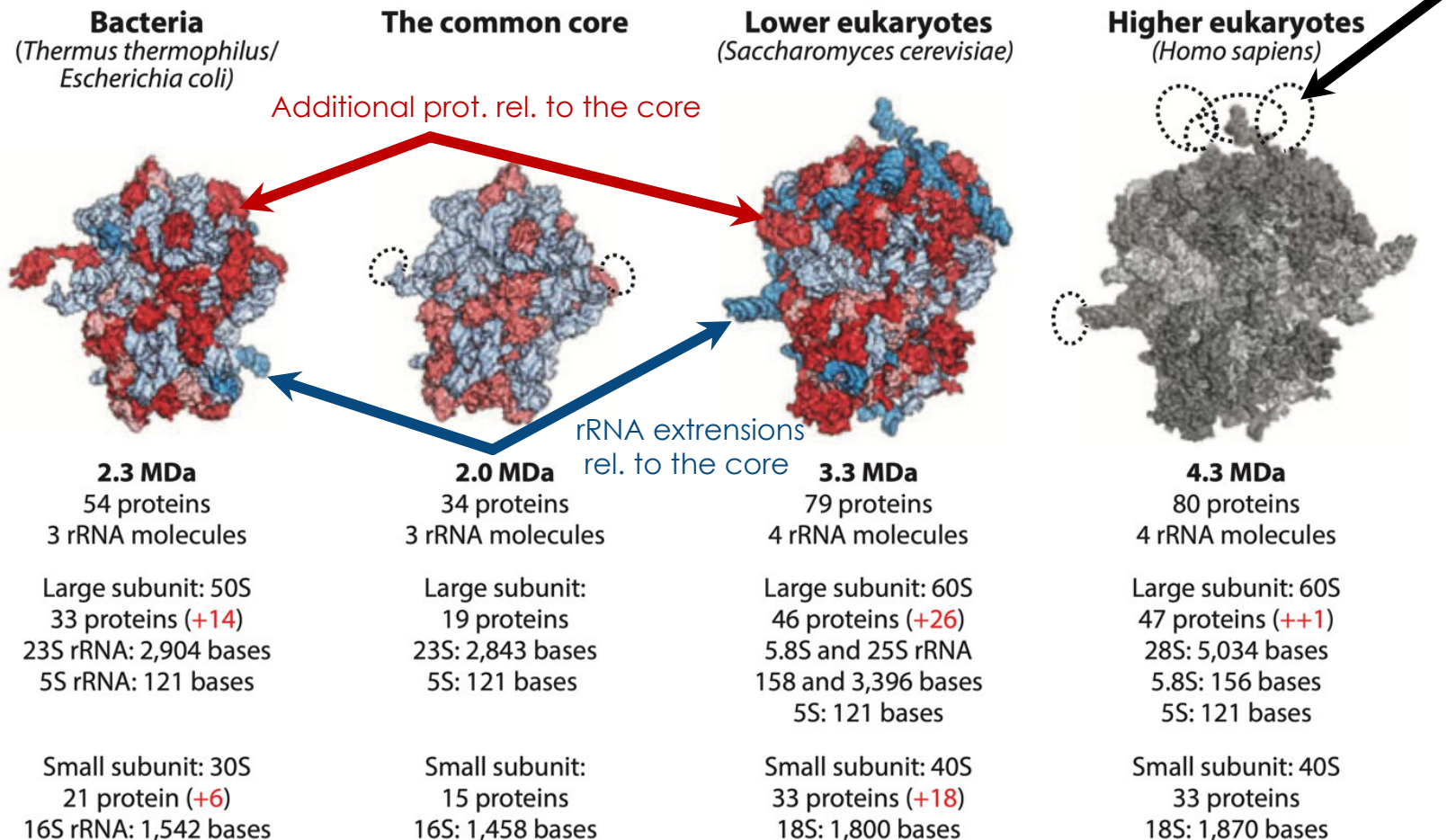
Annu. Rev. Biochem. 2014. 83:467–86
First published online as a Review in Advance on February 21, 2014
The Annual Review of Biochemistry is online at biochem.annualreviews.org
This article's doi: 10.1146/annurev-biochem-060713-035445

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rRNA extensions
rel. to lower Euk.



I. Initiation

C. Initiation in Eukaryotes

1. Description of the components

a. The ribosome

- The eukaryote ribo is bigger: 80S instead of 70S
- It is made up of 2 su: **40S = ARNr 18S + 33 prot**
 - **60S = ARNr 28S + 5,8S + 5S + 46-47 prot***

* This number can vary because there are prots that are not an integral part of the ribo but are associated with the ribosome

 **There is a molecule common to the 2 ribo = 5S rRNA which is the only RNA whose function is not known but which is nevertheless necessary for prot Synthesis**

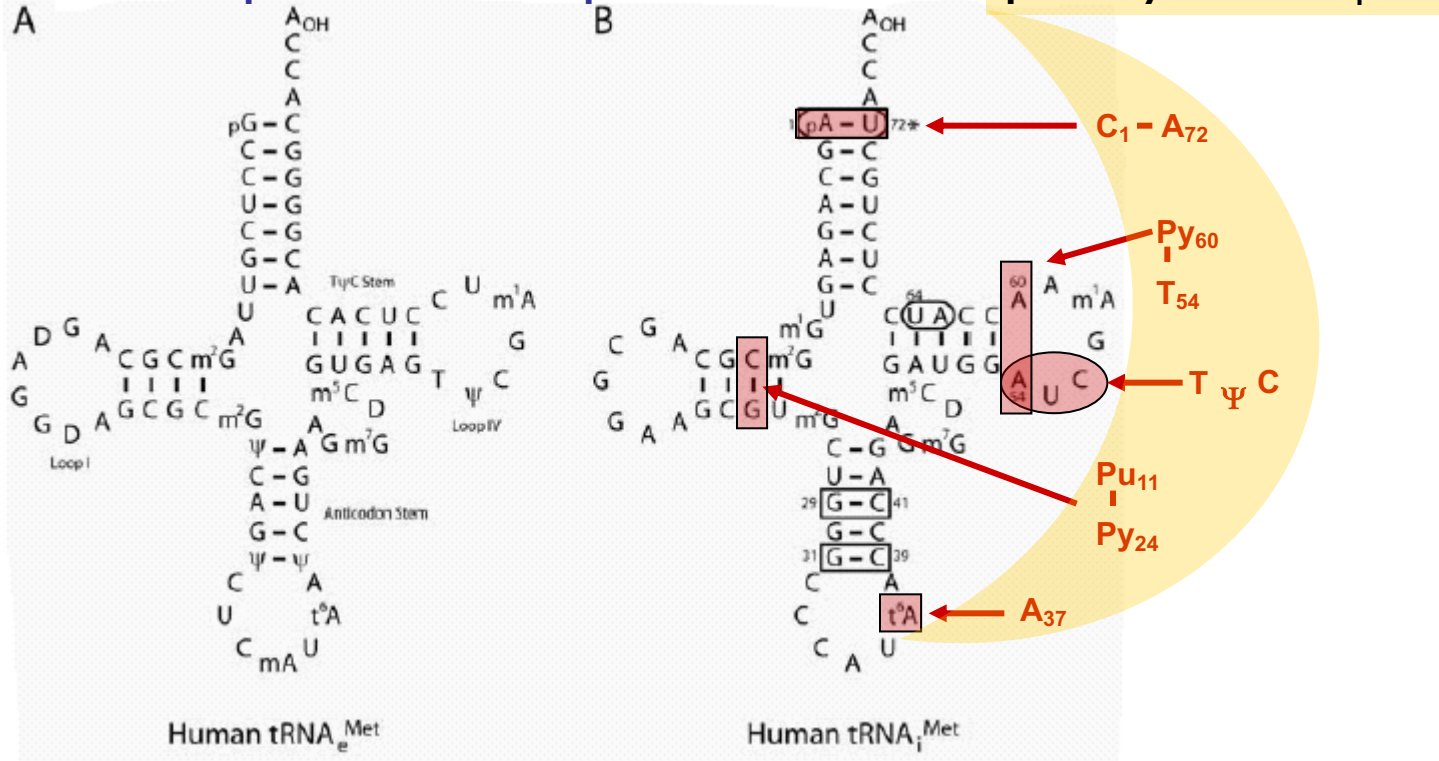
b. The $tRNA_i^{Met}$

- As there is only one Met codon (AUG), this codon can be used as both an initiation codon and an elongation codon, → it is necessary to be able to distinguish them, the distinction is made thanks to 2 different $tRNA^{Met}$ s
 - **initiator AUG** ← **Met- $tRNA_i^{Met}$**
 - **elongator AUG** ← **Met- $tRNA_e^{Met}$**

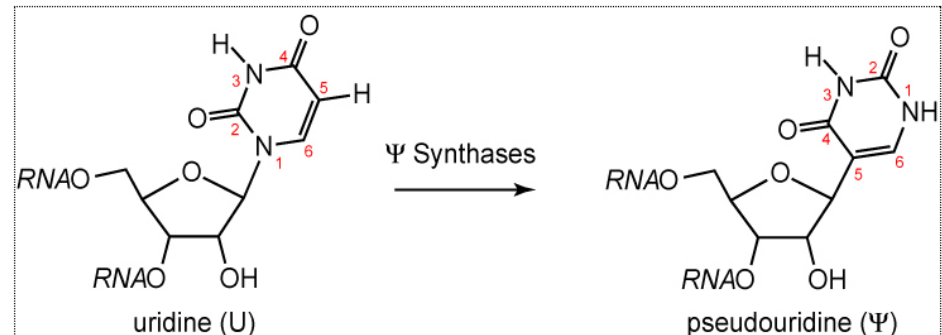
I. Initiation

C. Initiation in Eukaryotes

1. Description of the components



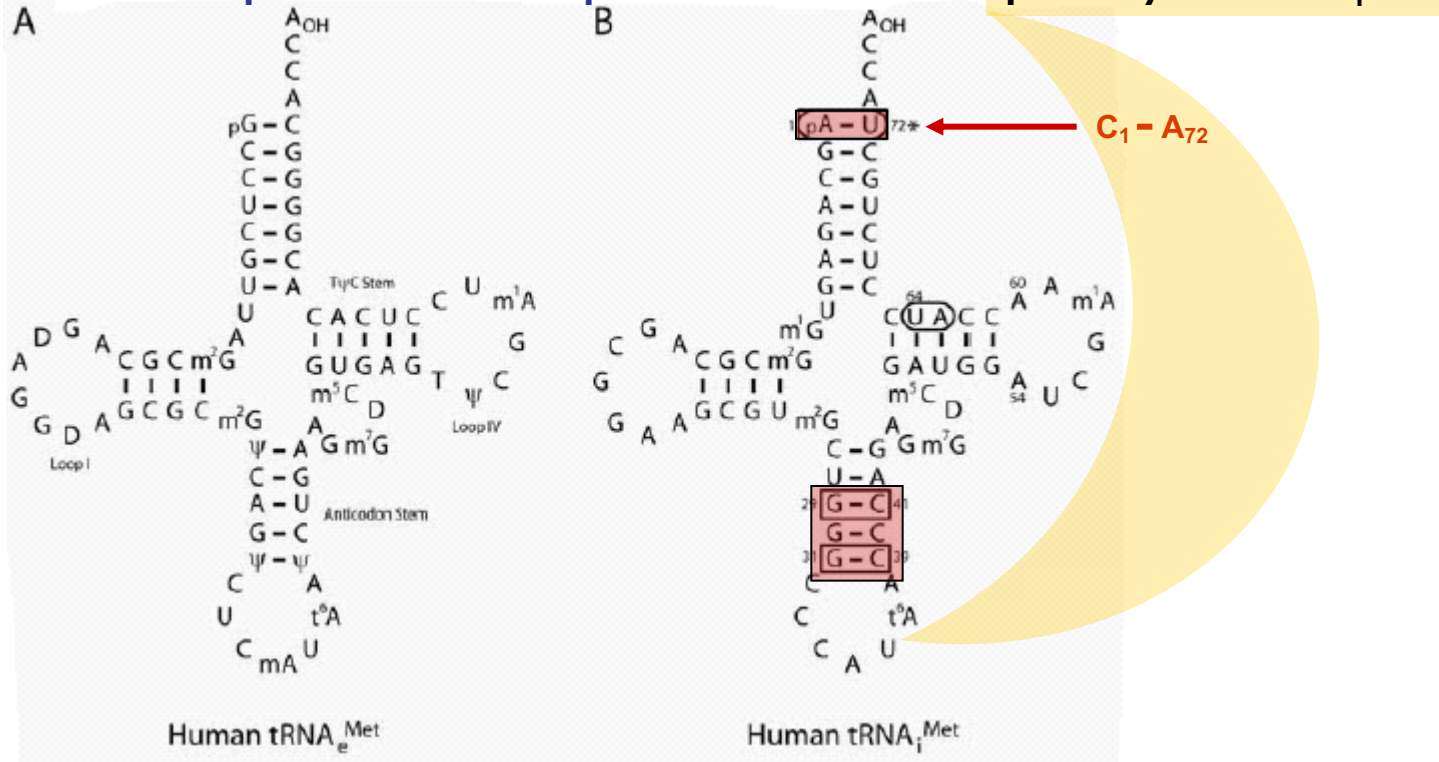
Pseudouridine (abbreviated Ψ) is the C-glycoside isomer of the nucleoside uridine, and it is the most prevalent of the over one hundred different modified nucleosides found in RNA. Ψ is found in all species and in all classes of RNA except mRNA. Ψ is formed by enzymes called Ψ synthases, which post-transcriptionally isomerize specific uridine residues in RNA



I. Initiation

C. Initiation in Eukaryotes

1. Description of the components



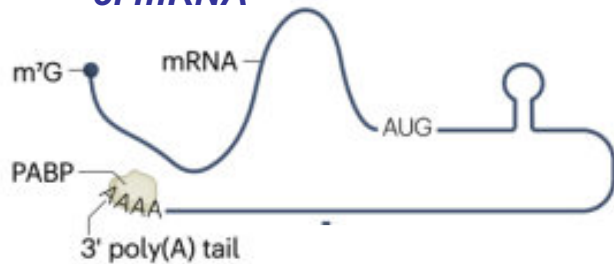
- The **absence of the C1-A72 mismatch** results in eukaryotic Met-tRNA_i^{Met} **not being formylated** although formylase exists in these organisms
- The 3 **G≡C bp** in the **@codon arm** play the same role in eukaryotes

I. Initiation

C. Initiation in Eukaryotes

1. Description of the components

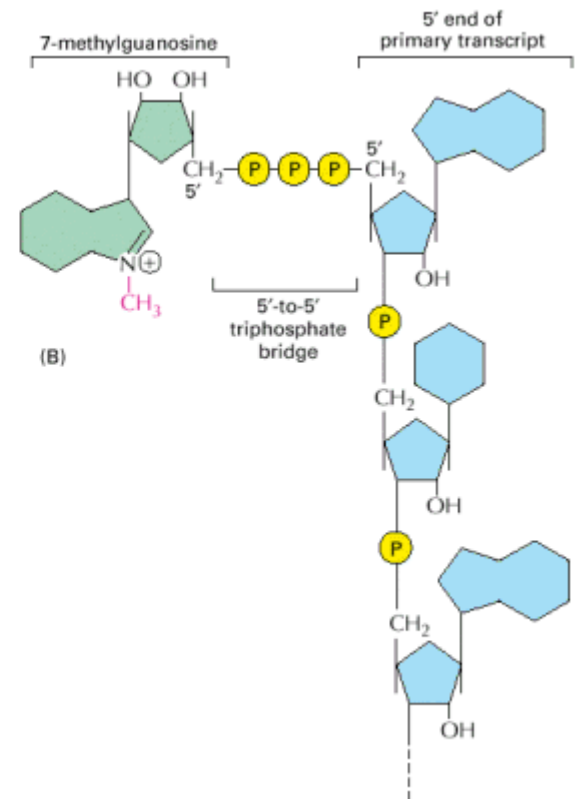
c. mRNA



- In prokaryotes, the mRNA is **polycistronic**
- In eukaryotes, mRNA is **monocistronic**, but there are polycistronic mRNAs that respond to different initiation mechanisms

- In **prokaryotes**, the 5' end of the mRNA = NTP
- In eukaryotes the 5' end of the mRNA is called a **cap** = **Guanosine methylated in N7** bound to the next Nt by a **5'-5' TriPhosphate bond**, the 2nd Nt being a **methylated Guanosine** in the 2' position

- In eukaryotes there **AUG → ORF → STOP** codon (UAA, UAG, UGA) followed in all euk mRNAs by a **polyA sequence of 100 to 150 A** inside the 3' UTR



I. Initiation

C. Initiation in Eukaryotes

1. Description of the components

c. mRNA

- euk. mRNAs do not have an SD sequence
- These euk. mRNAs are **ribonucleoprotein** complexes since 2 regions are systematically complexed to proteins = **CAP and PolyA**
 - CAP = **CAP Binding Proteins (CBP)**, one of which is an IF
 - PolyA = **PolyA Binding Proteins (PABP)** which completely cover the polyA sequence and form a kind of chapelet
- These prots have several roles:
 - **Protection** of mRNA from degradation by nucleases
 - They are involved in the **nucleocytoplasmic transport** of mature mRNA
 - **Initiation of PSis** because there is interaction between the prots attached to the 5' end (CBP) and those attached to the 3' end (PABP) which leads to **circularization of mRNA**

I. Initiation

C. Initiation in Eukaryotes

1. Description of the components

c. mRNA

- **Circularization** of eukaryotic mRNAs

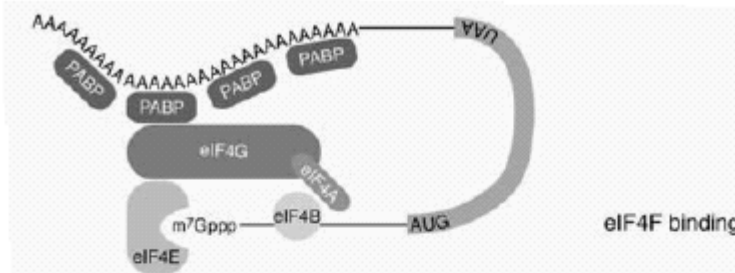


Figure: The initiation phase of translation. Shown is a eukaryotic mRNA with the two typical end modifications: the 5' cap structure (m7Gppp) and the 3' poly(A) tail (AAA). The protein-coding open reading frame is marked by a start and stop codon. An early step in initiation is the binding of the eIF4F complex, consisting of eIF4E, eIF4G, and eIF4A, to the cap structure. eIF4A, stimulated by eIF4B, unwinds secondary structure in the 5' UTR. The interaction of the poly(A)-binding protein PABP with eIF4G leads to circularization of the mRNA.

c. Eukaryotic initiation factors (eIF)

- In the prokaryotes there were 3
- In eukaryotes, the situation is much more complex since there are more of them and they also assemble into complexes

I. Initiation

C. Initiation in Eukaryotes

1. Description of the components

d. Eukaryotic initiation factors (eIF)

eIF4 (eIF4F)

The eIF4 initiation factors include eIF4A, eIF4B, eIF4E, and eIF4G.

eIF4F = eIF4A + eIF4B + eIF4E + eIF4G.

eIF4G is an architectural protein that interacts with eIF3, -4E, -4A, 4B, and PABP

eIF4A – is an RNA helicase – removes secondary structures from the mRNA

eIF4E binds the 5' CAP of mRNAs and this binding is the limiting step of CAP-dependent translation

eIF4B contains 2 RNA-binding domains – 1 is mRNA-specific, the other binds to 18S.

This factor acts as an anchor for the mRNA on the small subunit, and is an important cofactor of eIF4A.

eIF1 and eIF3

eIF1, eIF1A, and eIF3, bind to the complex formed between the small su and the mRNA. They prevent The big one binds to the small one before the ribosome is ready to enter the elongation phase.

eIF1 & -1A participate in the scanning and are involved in the selection of the initiating AUG

eIF3 is the largest structurally active initiation factor (13 su (α -m), 750 kDA). It controls the assembly of the 40S su on mRNAs that have a 5' CAP or an IRES

eIF2

eIF2 is a GTP-binding protein that delivers Met-tRNA^{iMet} to the P site of the pre-initiation complex. When it releases the Met-tRNA^{iMet} by hydrolysis of its GTP, it causes the departure of eIF1, -1A and -3 and allows the association of the big su. eIF2 = eIF2- α , β , and γ .

eIF5, eIF5A and eIF5B

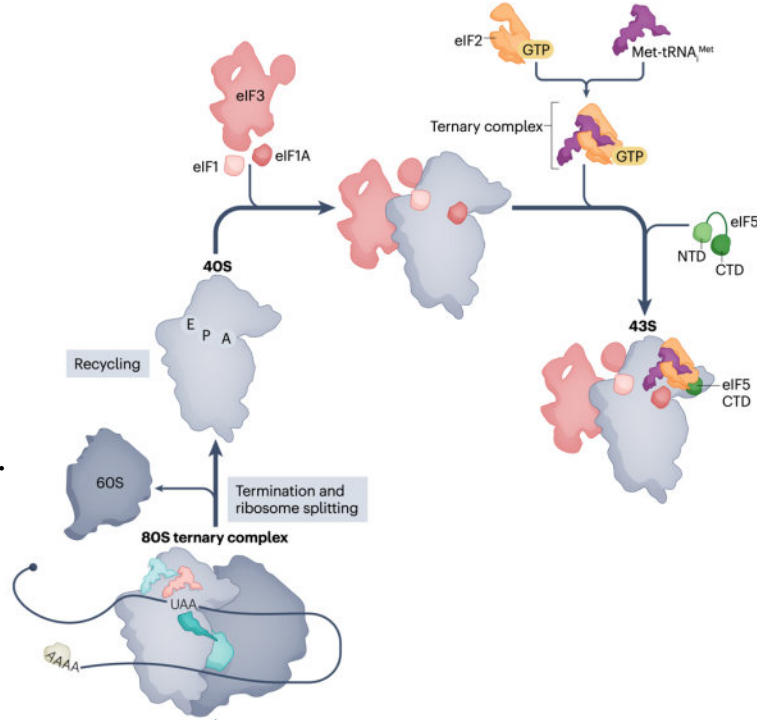
eIF5 is an assembly factor and helps in the hydrolysis of GTP by eIF2. eIF5A is a GTPase activating protein, which helps the large su to associate with the small one. eIF5B is a GTPase, and is involved in the assembly of the entire ribosome

I. Initiation

C. Initiation in Eukaryotes

CAP-dependent Initiation

- After a translation cycle, the ribosome is released and dissociates.
- The small su binds **eIF3**, **eIF1**, **eIF1A** forming a complex ready to receive the 1st ligand of protein synthesis which is Met-tRNA_i^{Met}. Binding of **eIF3**, **eIF1**, **eIF1A** to the 40S su prevents binding of the 60S su.
- The Met-tRNA_i^{Met} is recognized by **eIF2**•GTP thus forming a **ternary complex** (Met-tRNA_i^{Met}•eIF2•GTP) that binds the 40S su. Then **eIF5** binds 40S and its CTD is located next to the GTP-binding site of **eIF2-GTP**.



I. Initiation

C. Initiation in Eukaryotes

CAP-dependent Initiation

