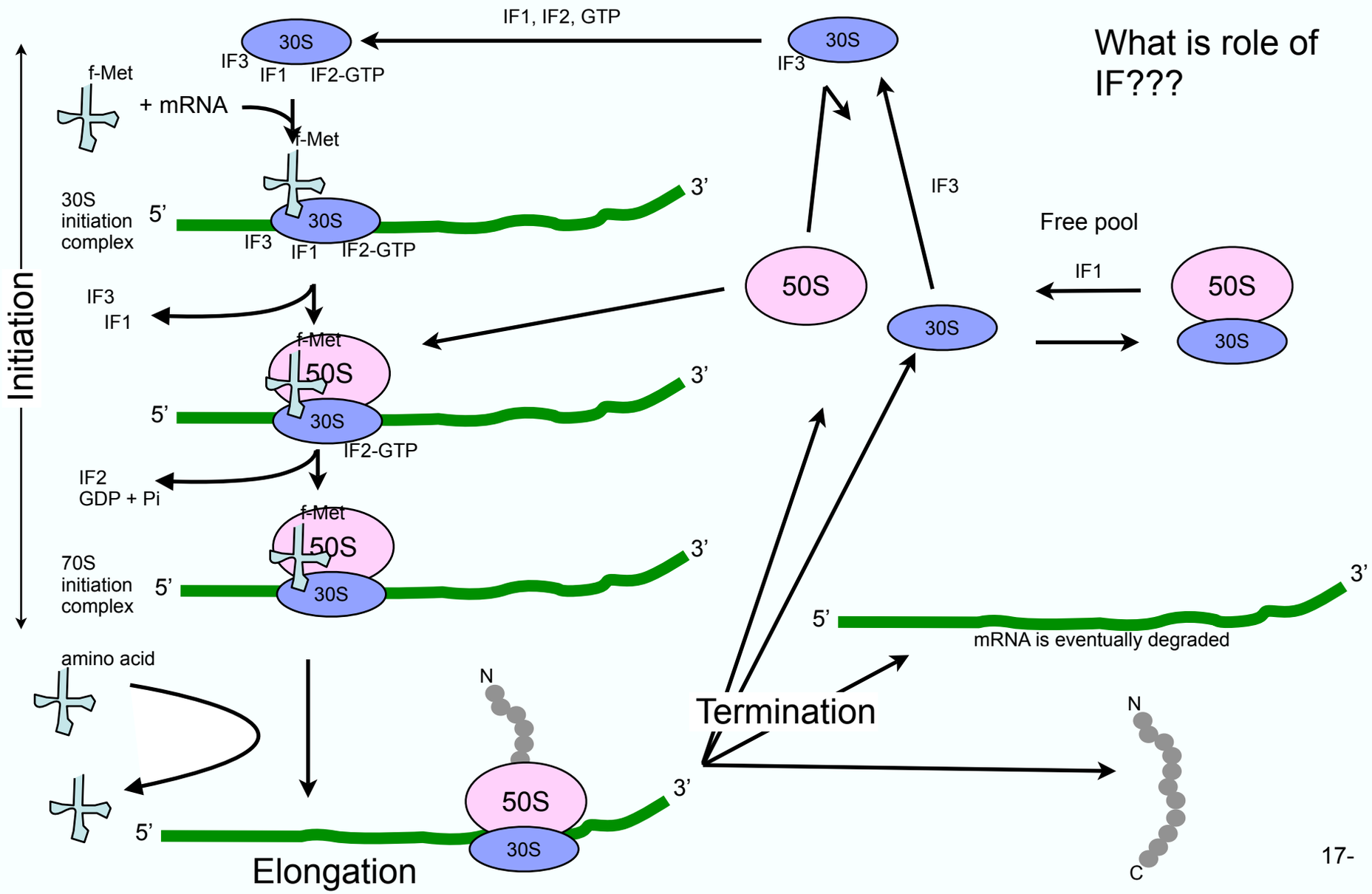
Chapter 17
The Mechanism of Translation I: Initiation

Focus only on experiments discussed in class.
Completely skip Figure 17.36

Read pg 521-527 up to the sentence that begins "In 1969, Joan Steitz ..."
Scan pg 527 "In 1969, Joan Steitz ..." - pg 528 column 1 "... stearothermophilus)."
Read pg 528 "These findings stimulated ..." - pg 529 second column "... no ribosome binding occured." to pg 529.
Scan pg 529 first paragraph 2nd column - pg 534
Read pg 534 Summary - Pg 543 through the summary
Scan pg 543 Structure and Function of eIF3 - pg 547 After the second summary
Read pg 547 17.3 Control of initiation - pg 459 End of the first column
Scan pg 549 column 2 - pg 551
Read pg 551 Eukaryotic Translational Control - pg 556 after the summary
Scan pg 556 Stimulation of an mRNA-binding protein - 557 before the summary
Read 557 Blockage of Translation Initiation by an mIRNA - End of Chapter

Prokaryotic Translation



Three phases: Initiation, Elongation and Termination
GTP is hydrolyzed after the 50S subunit joins the 30S complex to form the 70S initiation complex
This GTP hydrolysis is carried out by IF2 in conjunction with the 50S ribosomal subunit
Hydrolysis purpose is to release IF2 and GTP from the complex so polypeptide chain elongation can begin

1.IF1 influences dissociation of 70S ribosome to 50S and 30S
2.Binding IF3 to 30S, prevents subunit reassociation
3.IF1, IF2, GTP bind alongside IF3
4,Binding mRNA to fMet-tRNA forming 30S initiation complex
    Can bind in either order
    IF2 sponsors fMet-tRNA
    IF3 sponsors mRNA
5.Binding of 50S with loss of IF1 and IF3
6.IF2 dissociation and GTP hydrolysis

For the students to read

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How is the start codon identified?

- Shine-Dalgarno sequence - a sequence preceding the start codon that base pairs with the 3' end of the 16S rRNA

```
      SD            start codon
5'--GCAGG-------AUG--3'   mRNA
    |||||
3'--CCUCC------------5'16S rRNA
```

function is to position mRNA in ribosome

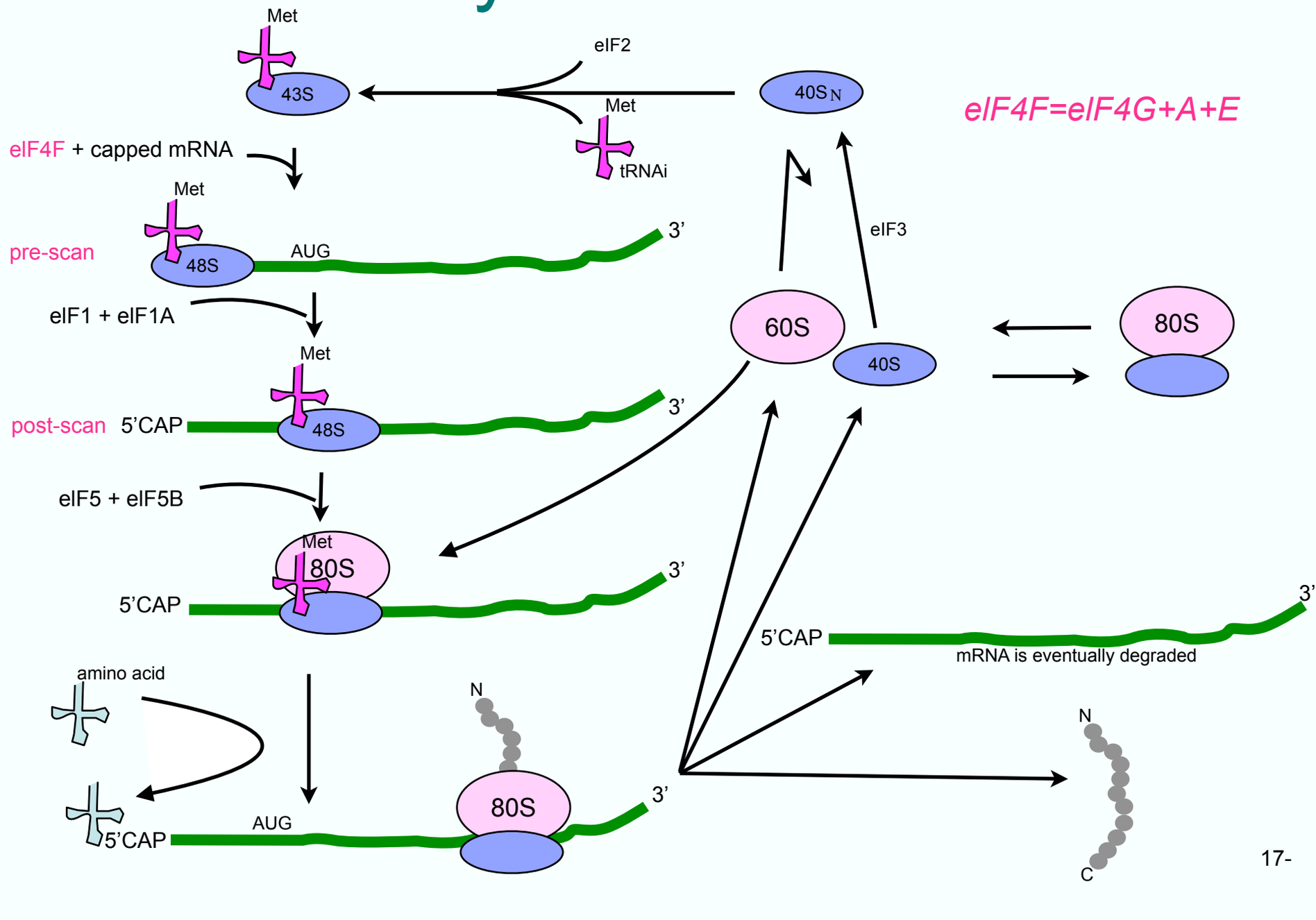
- The AUG is also very important

Binding of the Shine-Dalgarno sequence with the complementary sequence of the 16S rRNA is mediated by IF3
Assisted by IF1 and IF2
All 3 initiation factors have bound to the 30S subunit at this time

17.2 Initiation in Eukaryotes

- Eukaryotic
  - Begins with methionine
  - Initiating tRNA not same as tRNA for interior
  - No Shine-Dalgarno
  - mRNA have caps at 5'end
- Bacterial
  - N-formyl-methionine
  - Shine-Dalgarno sequence to show ribosomes where to start

Eukaryotic Translation



Scanning Model of Initiation

- Eukaryotic 40S ribosomal subunits locate start codon by binding to 5'-cap and scanning downstream to find the 1st AUG in a favorable context.
- Kozak's Rules are a set of requirements
- Best context uses A of ACCAUGG as +1:
  - Purine in -3 position
  - G in +4 position
- 5-10% cases ribosomal subunits bypass 1st AUG scanning for more favorable one

Scanning (or Kozak) Model for Translation Initiation in Eukaryotes

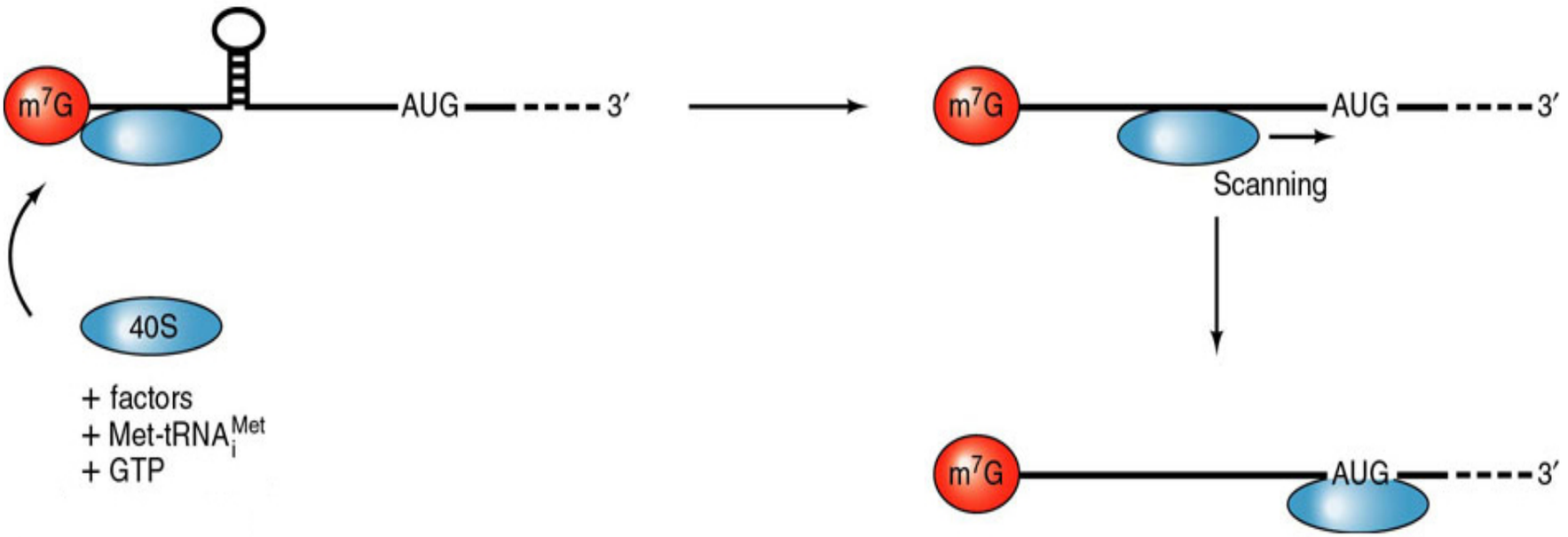
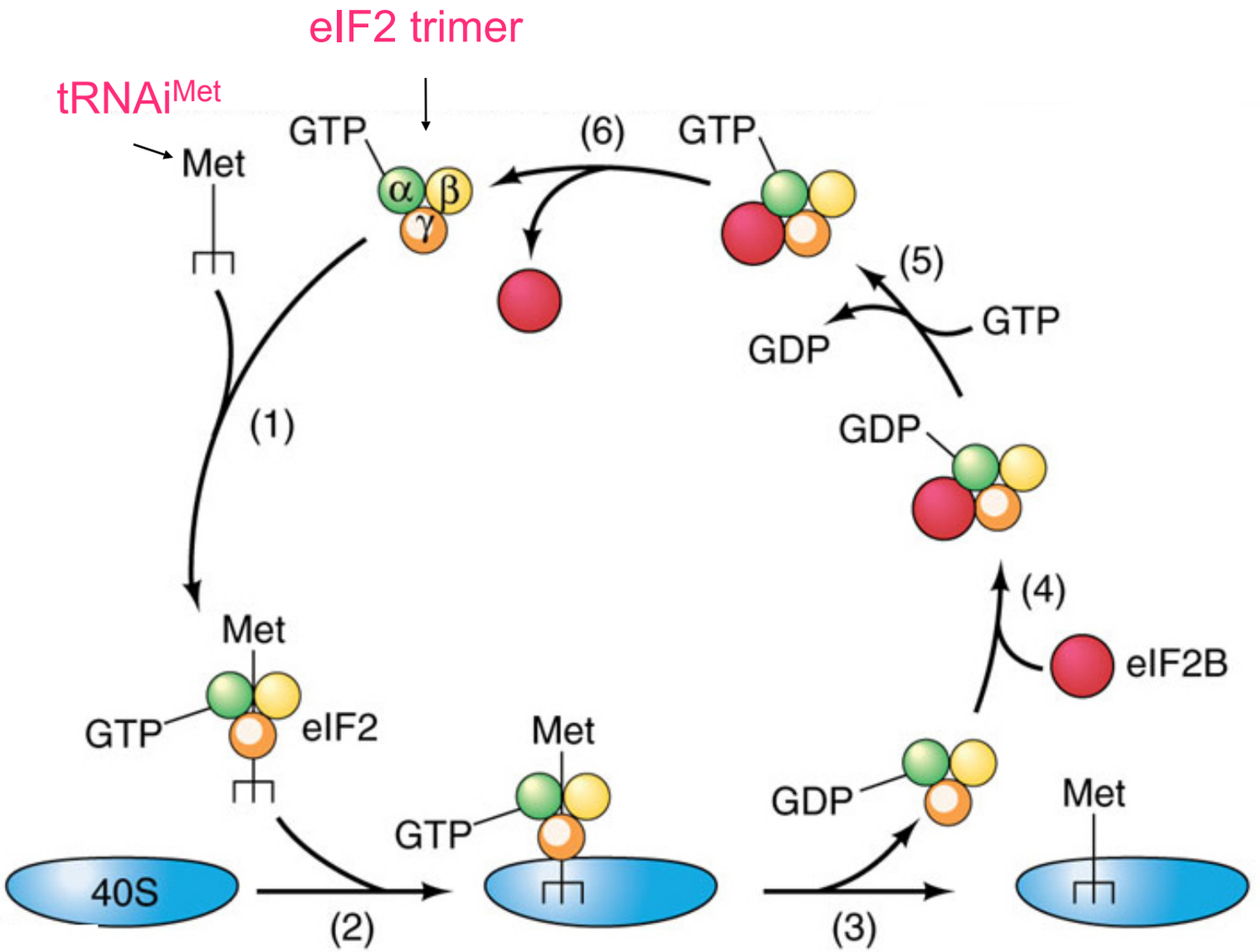
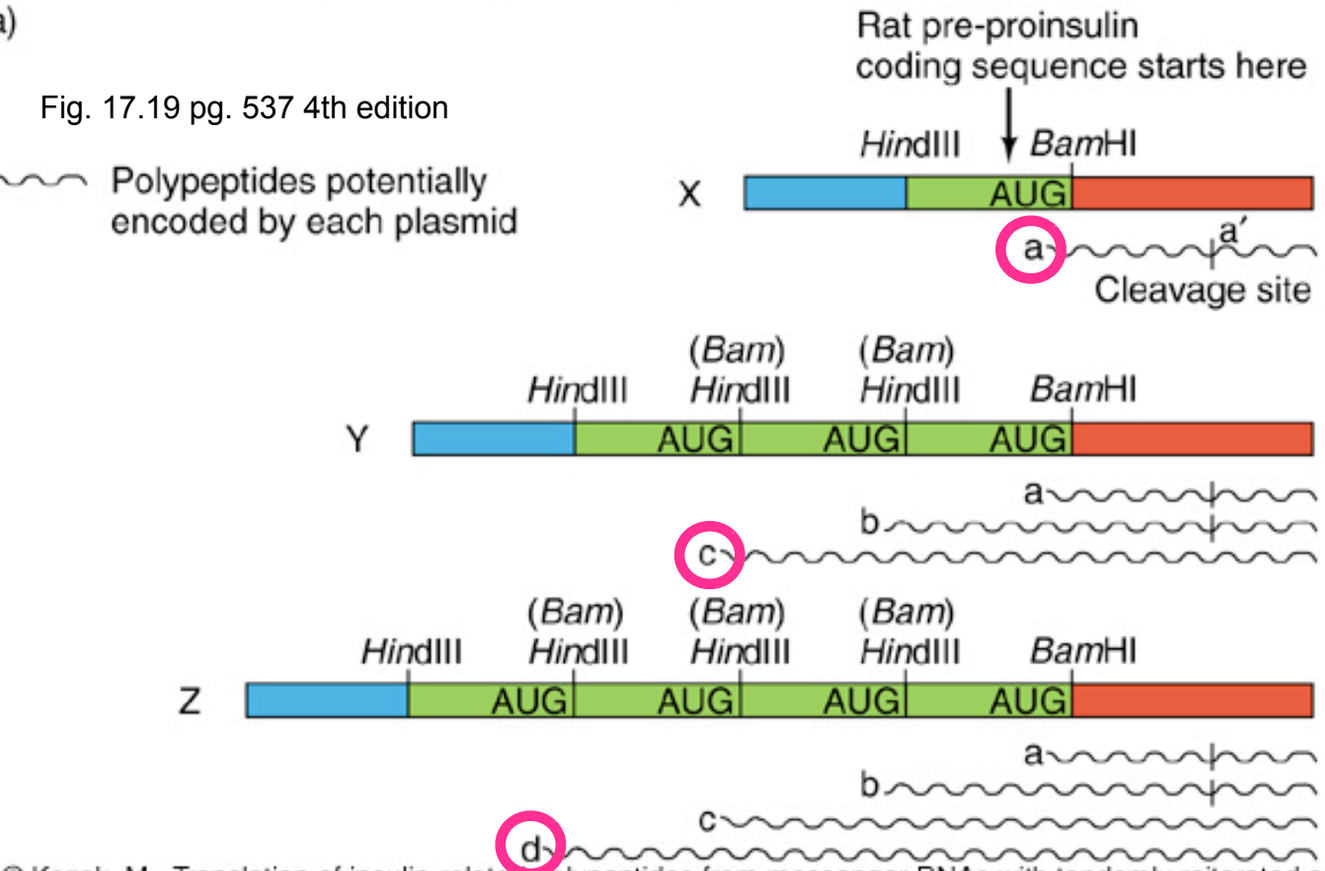


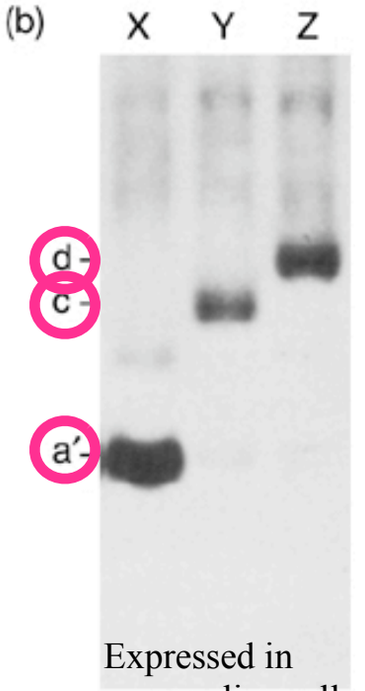
Fig.17.16 pg 535 4th edition


eIF2 trimer
tRNAiMet
Met
GTP
α
β
γ
(6)
GTP
(5)
GDP
GTP
GDP
(1)
Met
GTP
eIF2
(4)
eIF2B
Met
GTP
GDP
Met
40S
(2)
(3)

EXPERIMENT
Is the first AUG really favored?

Fig. 17.19 pg. 537 4th edition





Expressed in mammalian cell, radiolabelled, immunoppt, run on gel.

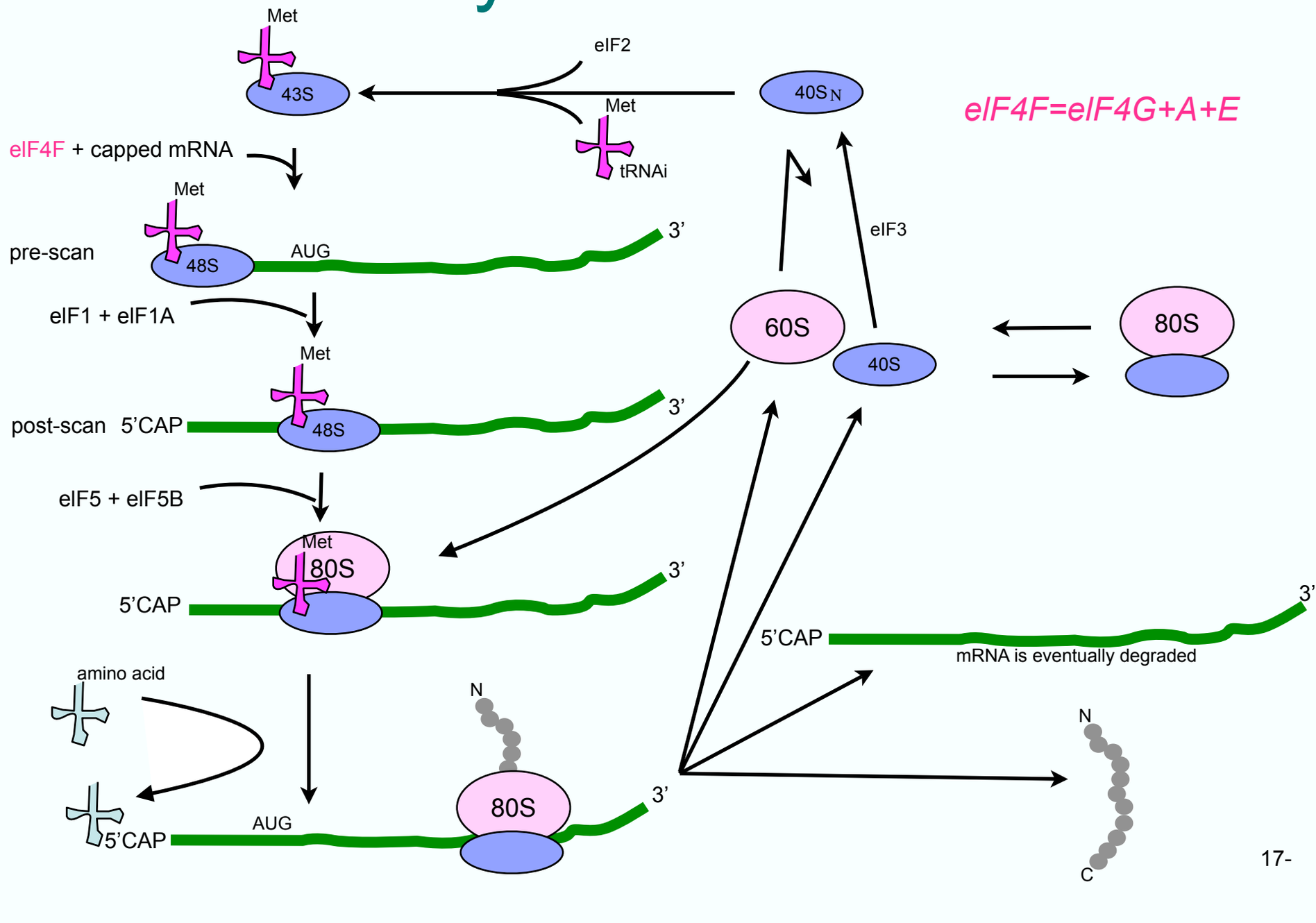
Translation Initiation in Eukaryotes

pg 539 4th ed

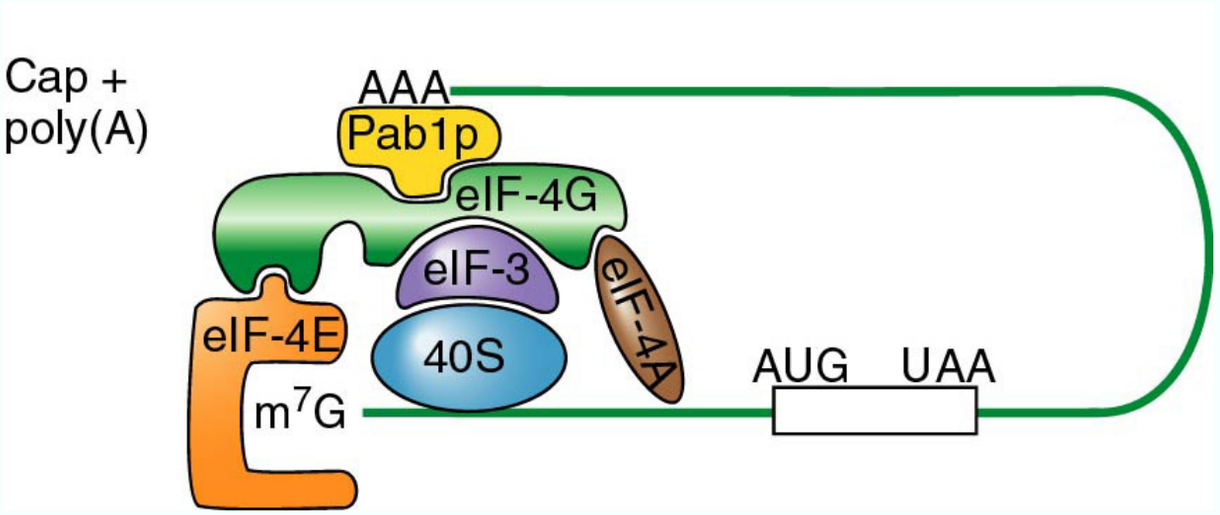
Eukaryotic initiation factors and general functions:

- eIF2 binds Met-tRNA to ribosomes
- eIF2B activates eIF2 replacing its GDP with GTP
- eIF1 and eIF1A aid in scanning to initiation codon
- eIF3 binds to 40S ribosomal subunit, inhibits reassociation with 60S subunit
- eIF4 is a cap-binding protein allowing 40S subunit to bind 5'-end of mRNA
- eIF5 encourages association between 60S ribosome subunit and 48S complex
- eIF6 binds to 60S subunit, blocks reassociation with 40S subunit

Eukaryotic Translation



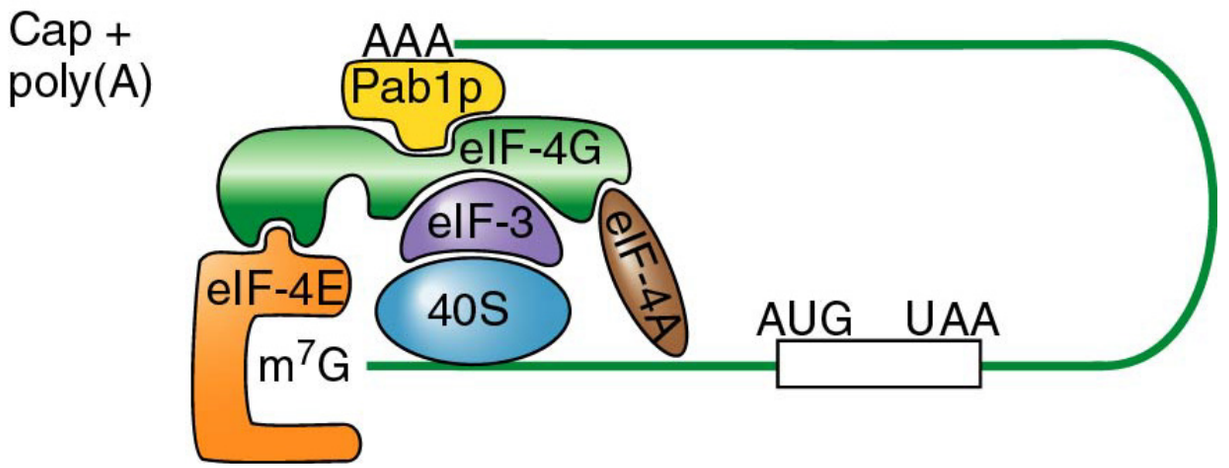
Function of eIF4



- eIF4 is a cap-binding protein
- This protein is composed of 3 parts:
  - eIF4E, 24-kD, has actual cap binding activity
  - eIF4A, a 50-kD polypeptide
  - eIF4G is a 220-kD polypeptide
- The complex of the three polypeptides together is called eIF4F

Implications of this structure

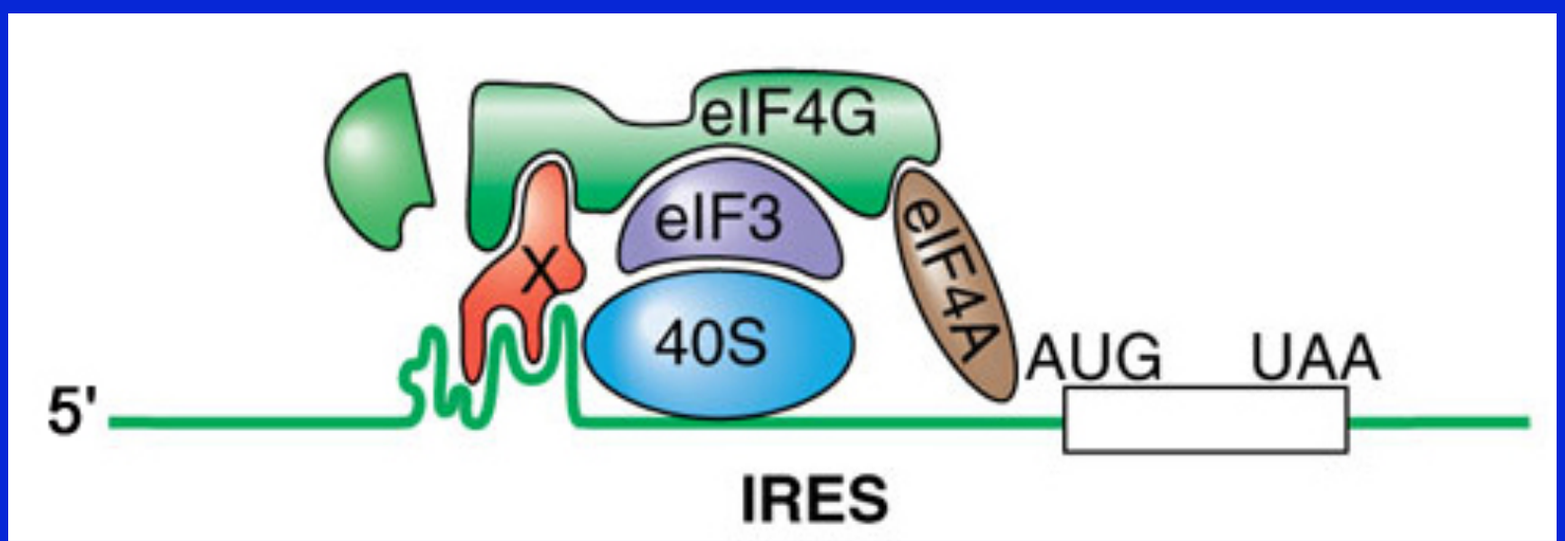
Why interact with both Cap and polyA-tail?



Observation: Some **viral** mRNAs (such as Polio virus) are not capped, yet are preferentially translated. Some are also translated via internal ribosome entry sites (**IRES**) (apparently without scanning to them).

Mechanism: Viral protease clips off N-terminus of eIF4G, so it can't bind eIF4E. eIF4G binds a viral protein (X), that binds to the IRES, promoting translation of the uncapped viral mRNAs.

Result: Don't translate host mRNAs as well as the viral mRNAs.



17.3 Control of Initiation

- Given the amount of control at the transcriptional and post-transcriptional 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

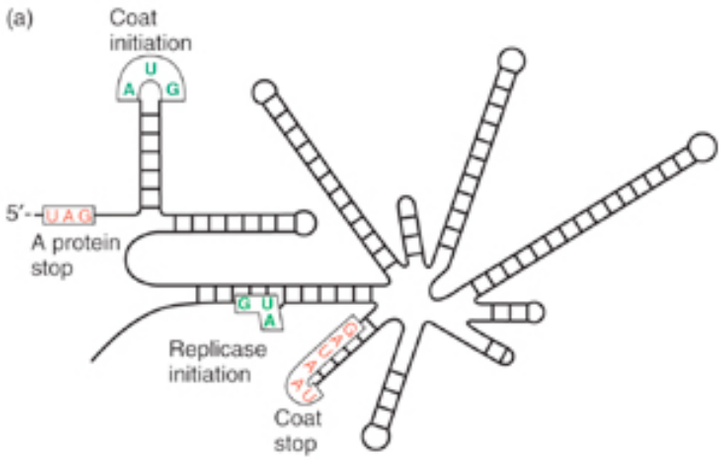
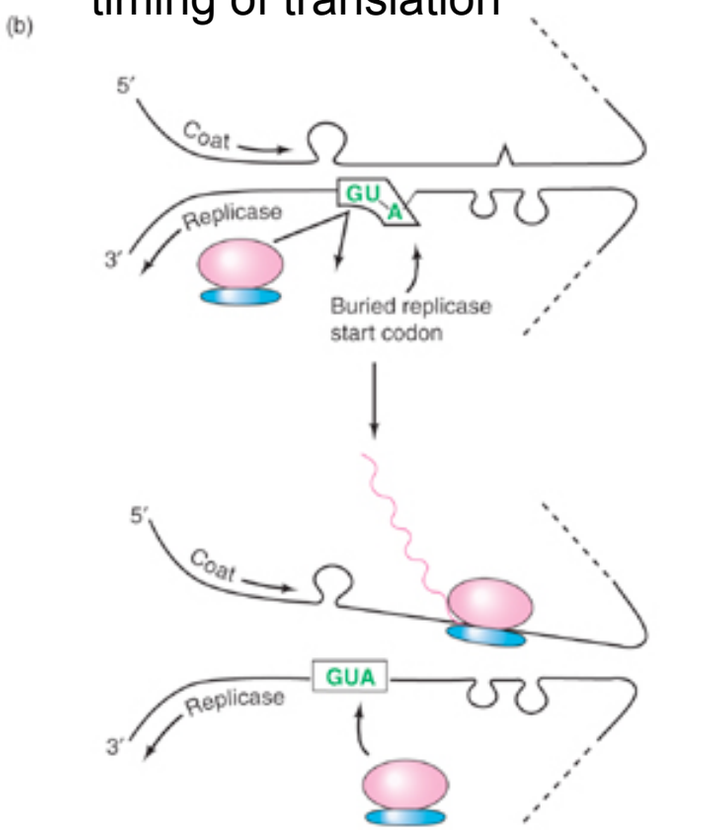
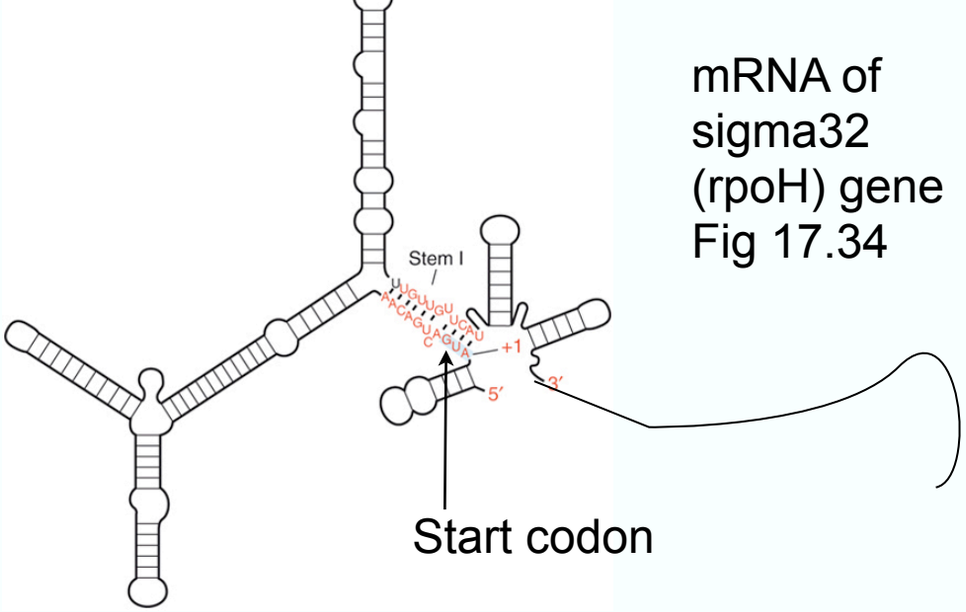


Fig 17.9 MS2 phage timing of translation

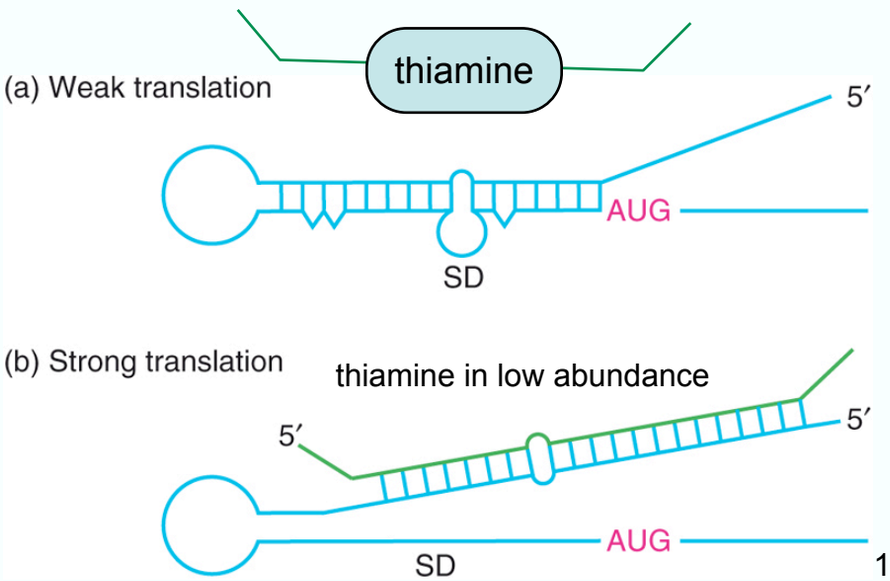




mRNA of sigma32 (rpoH) gene Fig 17.34

Start codon

Riboswitch (aptamer) fig 17.35



encoded protein involved in thiamine synthesis

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

Small RNAs with proteins can affect mRNA 2° structure to control translation initiation
Riboswitches can be used to control translation initiation via mRNA 2° structure
5'-untranslated region of *E. coli thiM* mRNA contain a riboswitch
This includes an aptamer that binds thiamine and its metabolite
Thiamine phosphate
Thiamine pyrophosphate (TTP)

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

Eukaryotic Translational Control

Eukaryotic mRNA lifetimes are relatively long, so there is more opportunity for translation control than in bacteria

- Examples to be discussed:
  - eIF2 - heme regulation of globin synthesis, interferons
  - eIF4E - modulation by mTOR + 4E-BP1 (PHAS-1)
  - eIF4E - Maskin
  - Protein can bind 5'UTR and act as a roadblock (Ferritin mRNA translation)
  - miRNA - Let7

- Skip details of virus stuff this time. Here it is for later.
- Interferons are anti-viral proteins induced by viral infection
- Repress translation by triggering phosphorylation of eIF2α
- Kinase is called **DAI**, for double-stranded-RNA- (dsRNA)-activated inhibitor of protein synthesis
- dsRNA can triggers the same pathway (mimics virus)

Role: Block reproduction of the virus

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- Examples:
  - eIF2 - heme regulation of globin synthesis, interferons
    - eIF2 α-subunit is a favorite target for translation control in general
    - Heme-starved reticulocytes activate HCR (heme-controlled repressor)
      - » Phosphorylates eIF2α
      - » Inhibit initiation
- When heme is low, RBC's pause in the production of globin.

Reticulocytes
make hemoglobin

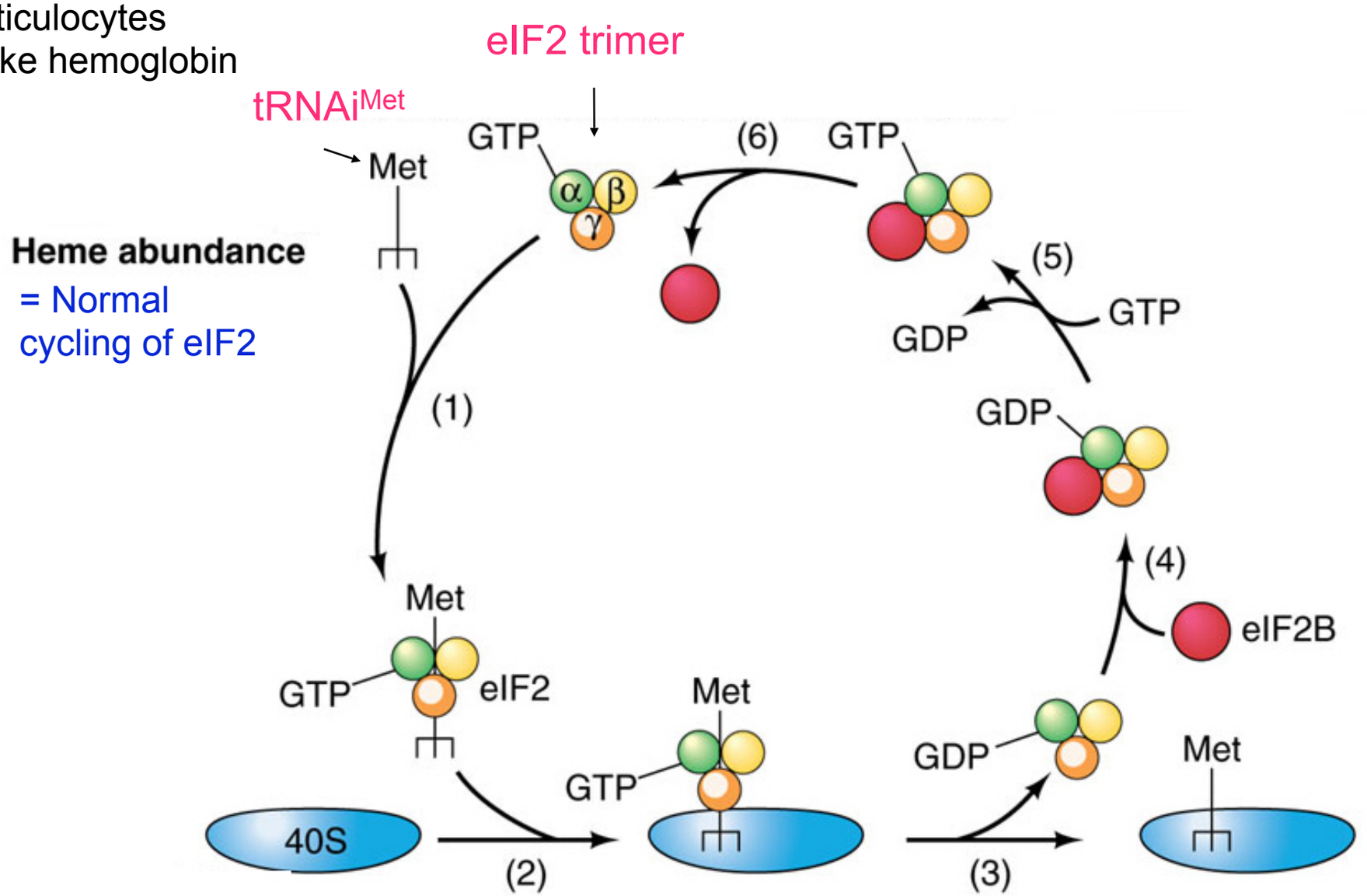
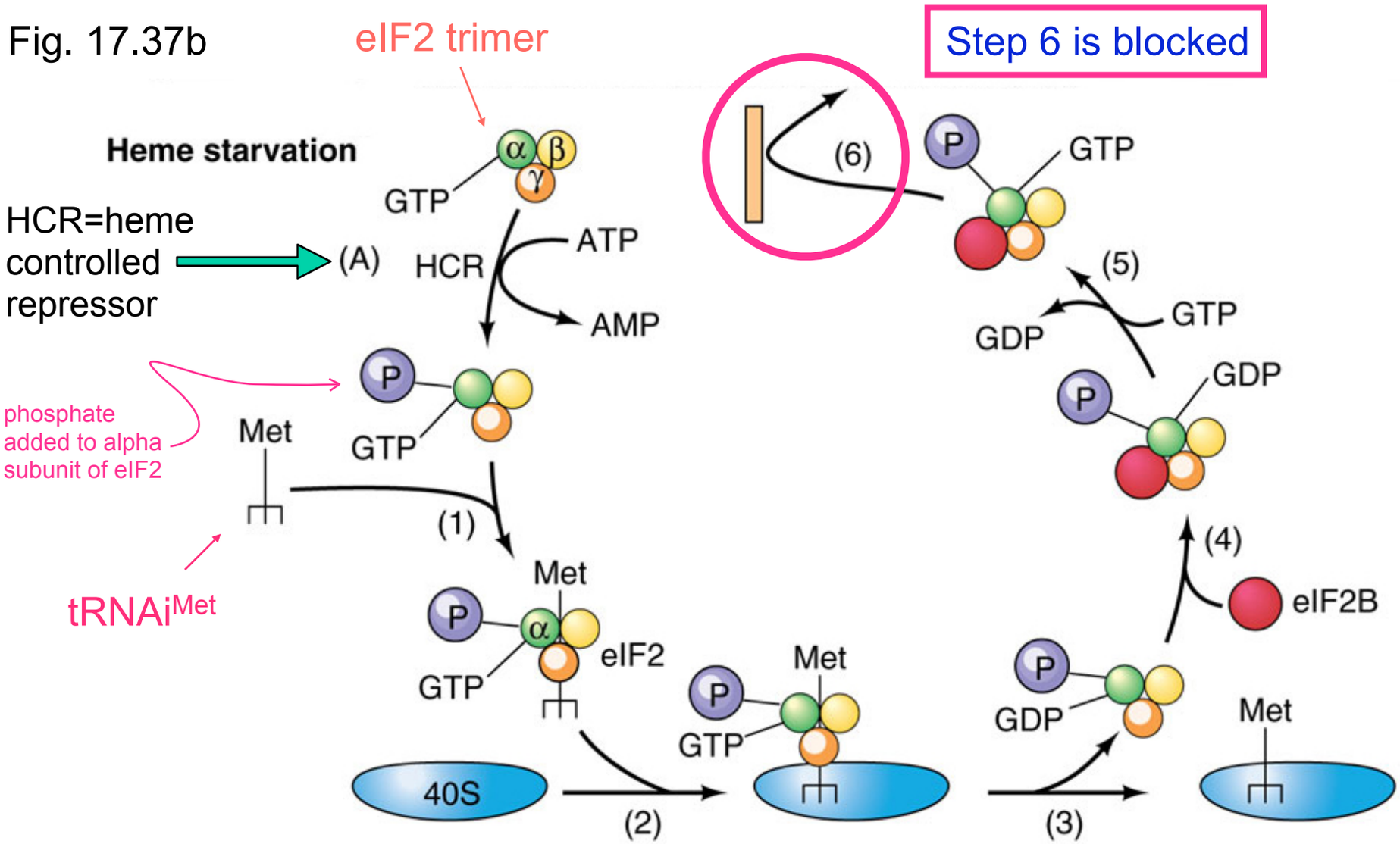


Fig. 17.37a

Fig. 17.37b



1. If heme is limiting, a protein kinase (**HCR**, heme-controlled repressor) phosphorylates eIF2α
2. Phosphorylated eIF2 binds more tightly to eIF-2B. It doesn't release and eIF2 can't recycle
3. The function is to prevent wasteful synthesis of globin

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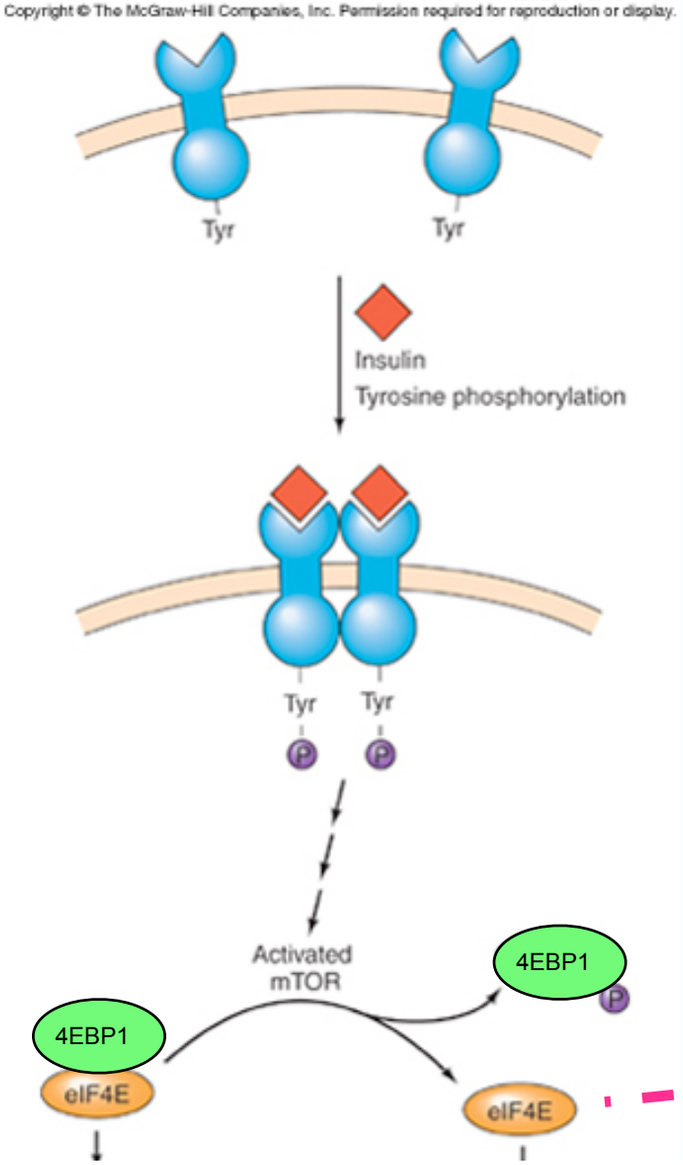
Phosphorylation of an eIF4E-Binding Protein



- Insulin and a number of growth factors stimulate a pathway involving a protein kinase known as mTOR
- mTOR kinase's target protein is
  - 4E-BP1 in humans - eIF4E binding protein #1
  - (PHAS-1 in rats)
- Once phosphorylated by mTOR
  - This protein dissociates from eIF4E
  - Releases it to participate in active translation initiation

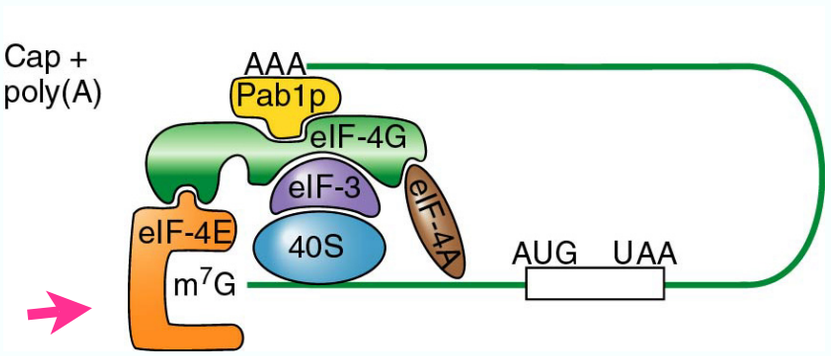


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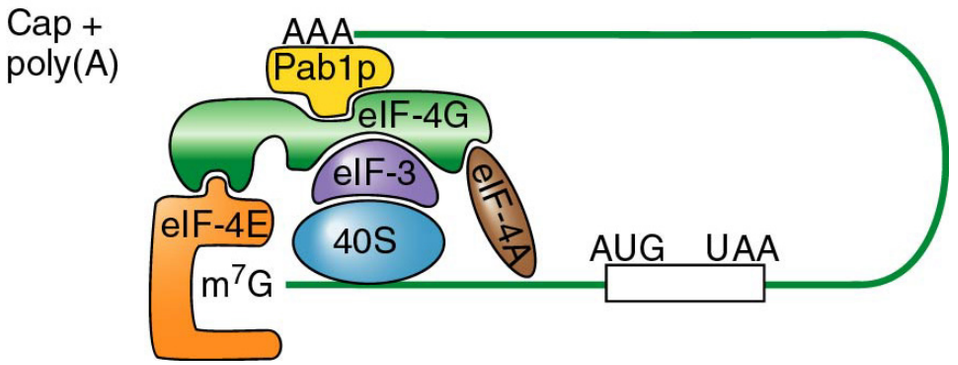
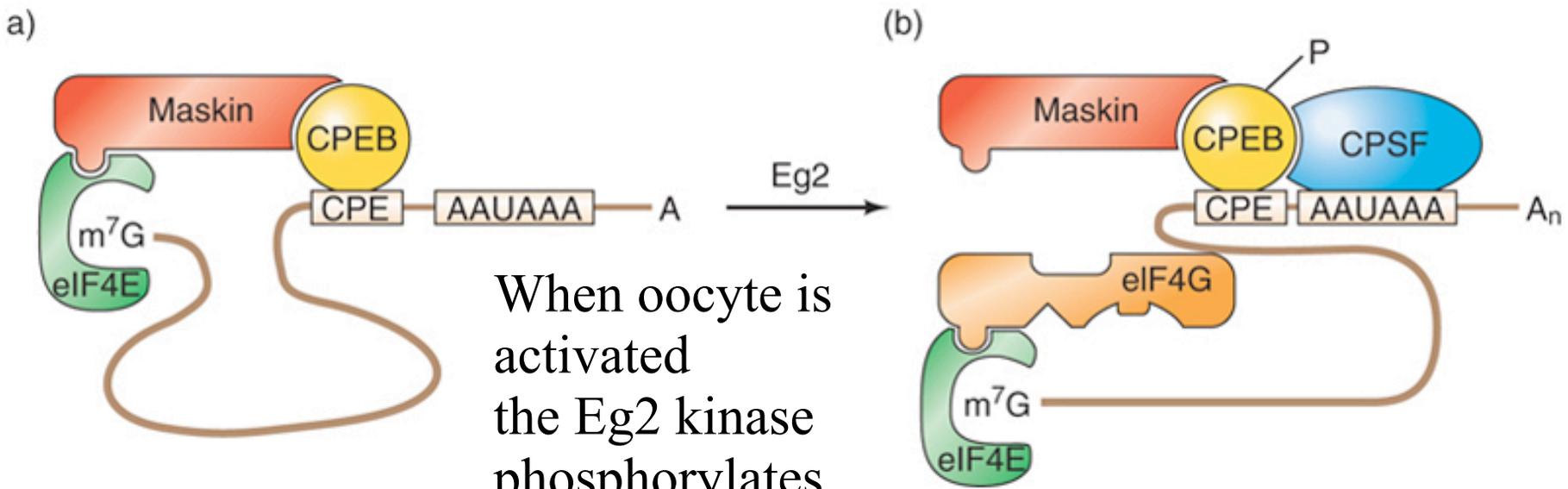
Fig. 17.43

Normally-->



- In *Xenopus* oocytes, Maskin binds to eIF4E and to CPEB (cytoplasmic polyadenylation element binding-protein) translation is now inhibited



When oocyte is activated the Eg2 kinase phosphorylates CPEB

Fig. 17.43

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Role: Block reproduction of the virus

Induction by removal of a 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

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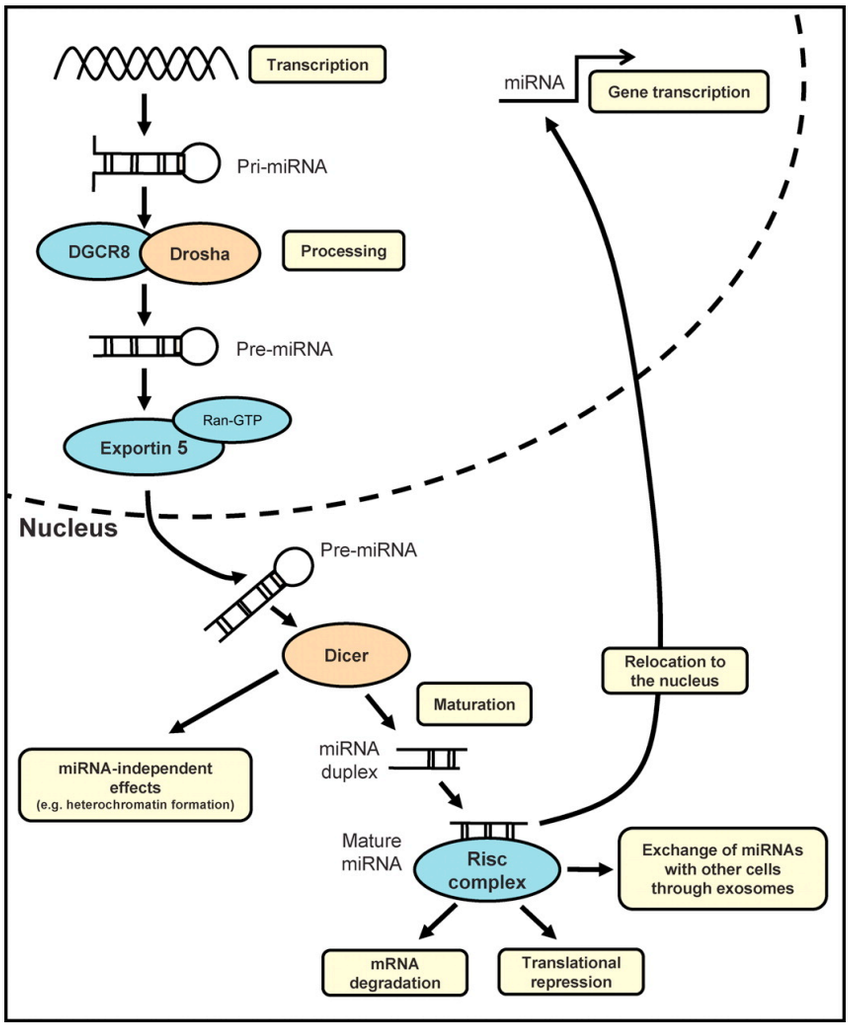
MiRNA biogenesis



Urbich, C. et al. Cardiovasc Res 2008 79:581-588; doi:10.1093/cvr/cvn156

Blockage of Translation Initiation by an miRNA

- The let-7 miRNA shifts the polysomal profile of target mRNAs in human cells toward smaller polysomes
  - This miRNA blocks translation initiation in human cells
- Translation initiation that is cap-independent due to presence of an IRES, or a tethered initiation factor, is not affected by let-7 miRNA
  - This miRNA blocks binding of eIF4E to the cap of target mRNAs in the human cell

End