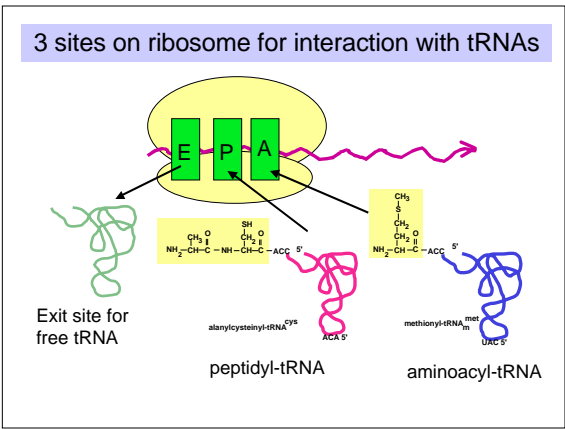
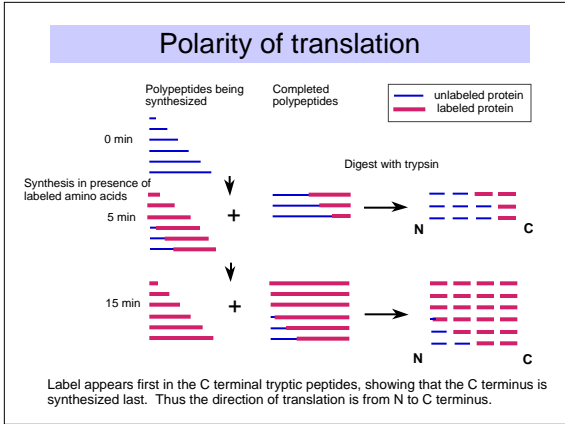
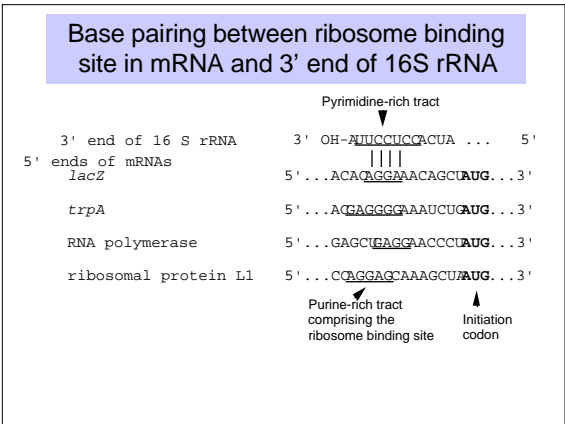


# Mechanics of Translation

Initiation  
Elongation  
Termination



- ## Initiation
- mRNA binds to small ribosomal subunit such that initiator AUG is positioned in the precursor to the P site
  - In eubacteria, such as *E. coli*, the positioning of the initiator AUG is mediated by base pairing between the ribosome-binding site in the 5' untranslated region and the 3' end of the 16S rRNA

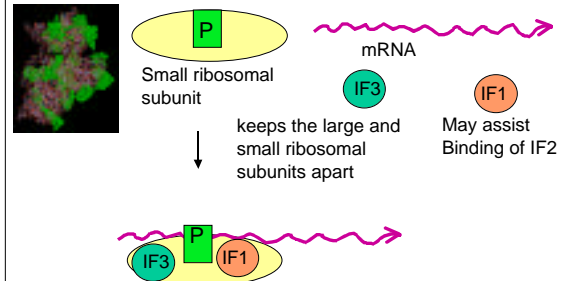


- ## Translation factors
- Translation **factors** are proteins used at only one step of the translation process
  - They are not permanent components of the ribosome, but cycle on and off.

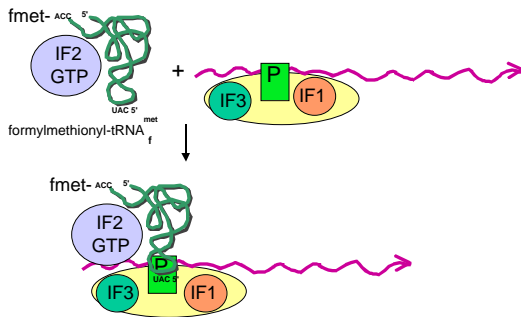
### Factors and components needed for **initiation**

- Small ribosomal subunit
- mRNA
- IF3 = initiation factor 3: keep ribosomal subunits apart
- IF1= initiation factor 1: assist binding of IF2?
- IF2 = initiation factor 2
  - In complex with GTP, it brings fmet-tRNA<sub>f</sub> to the partial P site on the small subunit.
  - Activates a GTPase activity in the small subunit, which allows dissociation of IF2, IF3, and IF1.
- fmet-tRNA<sub>f</sub>
- GTP
- Binding of large subunit to the initiation complex gives a complete ribosome ready for elongation

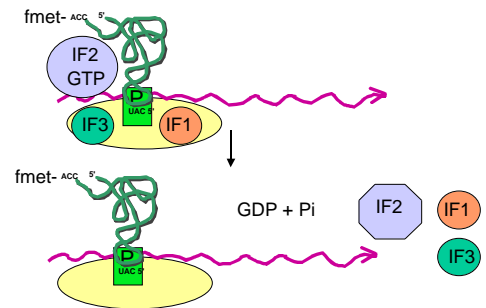
### Initiation: mRNA binds to small subunit



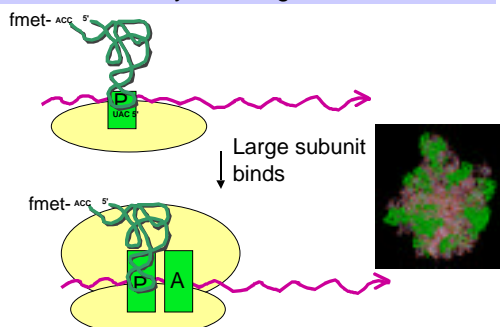
### fmet-tRNA<sub>f</sub> binds to small subunit:mRNA



### GTP hydrolysis allows dissociation of factors

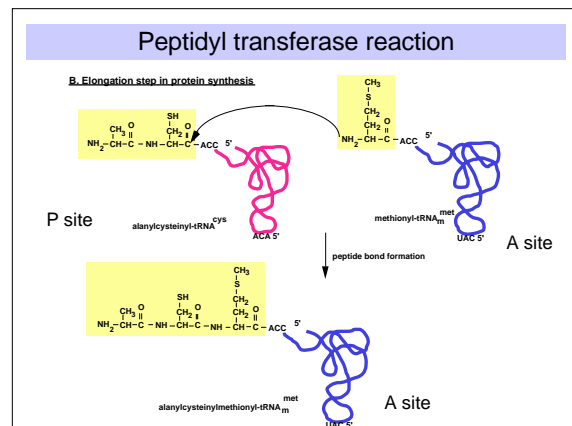
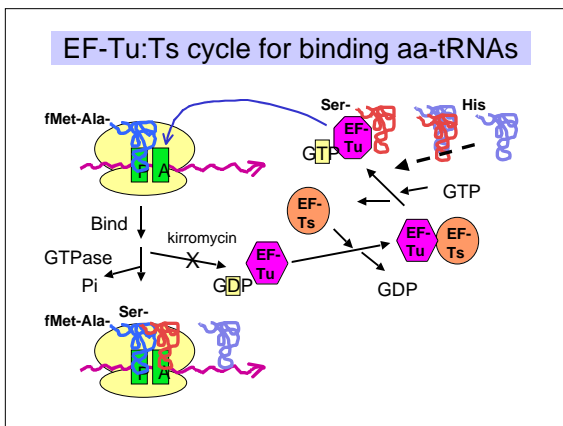
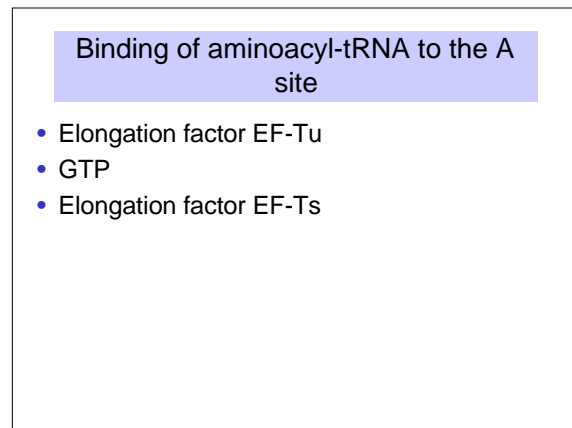
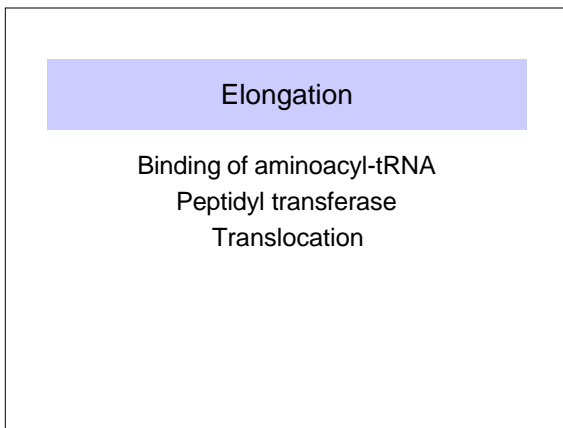
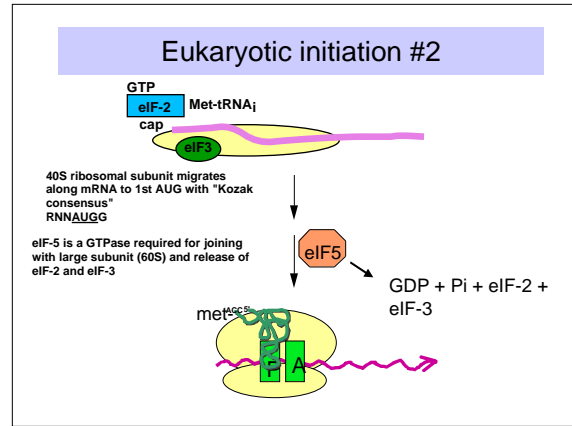
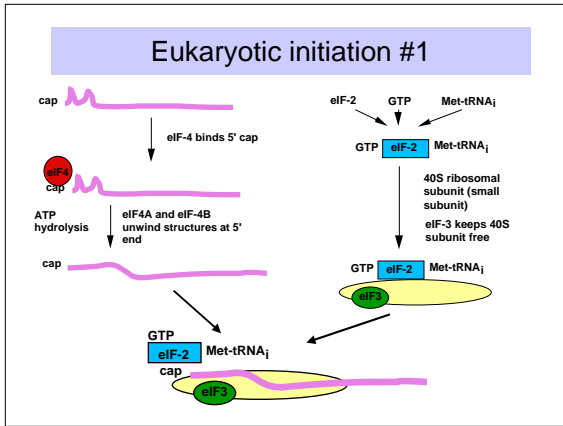


### Binding of large subunit produces ribosome ready for elongation



### Identification of initiator AUG in eukaryotes

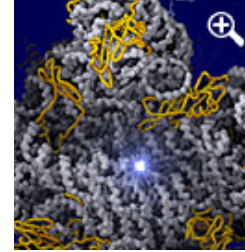
- Bases around AUG influence the efficiency of initiation: **RNNAUGG**
- Eukaryotic initiation factor eIF4 scans along mRNA from cap to find initiator AUG



## Peptidyl transferase is a ribozyme: LS rRNA

- No protein has peptidyl transferase activity.
- Large subunit rRNA plus some remnants of protein is catalytic.
- Some mutations that confer resistance to antibiotics that block peptidyl transferase map to large subunit rRNA genes.
- Crystal structure of large subunit: active site is RNA. The closest protein is far away (20 Å).
- Can select *in vitro* for ribozymes capable of catalyzing peptide bond formation.

## Peptidyl transferase on 50s subunit



Active site for peptidyl transferase on 50S ribosomal subunit

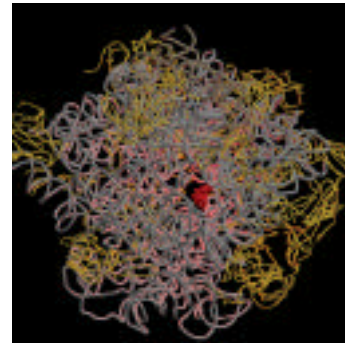
## Active site for peptidyl transferase on rRNA: binding of antibiotics



Interaction of chloramphenicol with peptidyl transferase cavity in 50S subunit. Erythromycin and relatives bind to different nts in same site to block exit of peptides.

Nature 413, 814 - 821 (2001)  
Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria  
FRANK SCHLÜNZEN...FRANÇOIS FRANCESCHI

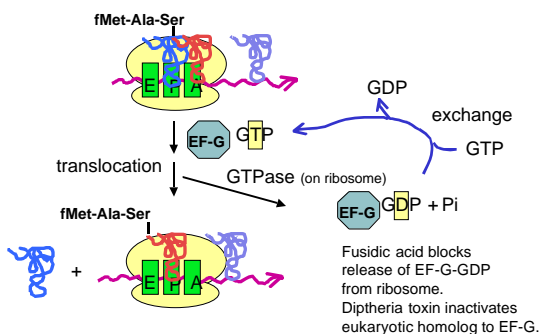
## Tunnel for peptide exit



Top view of the *D. radiodurans* 50S subunit showing erythromycin (red) bound at the entrance of the tunnel. Yellow, ribosomal proteins; grey 23S rRNA; dark grey, 5S rRNA.

Nature 413, 814 - 821 (2001)  
Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria  
FRANK SCHLÜNZEN...FRANÇOIS FRANCESCHI

## EF-G:GTP for translocation



## Elongation rates

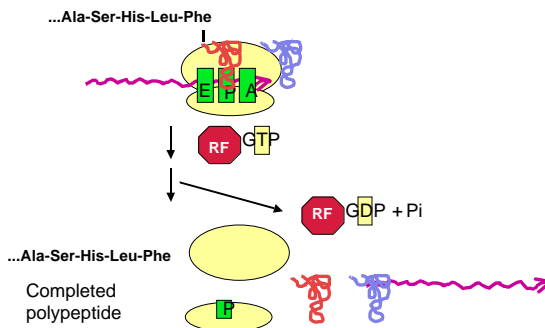
- Bacteria
  - Translation: add 15 amino acids per sec
  - Transcription: add about 50 nucleotides per sec
  - Translation and transcription can occur simultaneously and at roughly the same rates
  - Recall replication is much faster: 1000 nucleotides per sec
- Eukaryotes:
  - Translation elongation is about 2 amino acids per sec

## Termination

## Release factors (RF)

- Bacterial
  - RF1 recognizes UAG and UAA
  - RF2 recognizes UGA and UAA
- Eukaryotic eRF recognizes all 3 termination codons
  - Requires GTP to bind.
  - Hydrolysis probably promotes dissociation of eRF.
- Need peptidyl tRNA in P site and termination codon in A site for RFs to act.

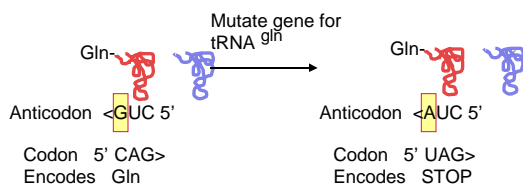
## Termination leads to dissociation of new protein, ribosome and mRNA



## Suppressor mutations

- Mutations at a second site that can overcome the effects of a missense or nonsense mutation are **suppressors**.
- Can be in the same gene (but affecting a different codon) OR can be in a different gene.
- Isolation of suppressor mutations in other genes indicates that the product of the other gene **interacts** with the product encoded by the gene with the original mutation.

## Mutant tRNAs can act as suppressors



The mutant Gln-tRNA<sup>gln</sup> will insert a Gln at a UAG. If the UAG were a premature stop (i.e. *nonsense* mutant), then the mutant tRNA would **suppress** the nonsense mutant.