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# Regulation of Amino Acids on tRNA

# Translational questions

1) How is translation initiated and give examples of antibiotics that can inhibit this process
2) During polypeptide synthesis, how does the process of chain elongation and termination occur. Give examples of drugs that can inhibit these processes
3) What happens to a newly synthesised polypeptide chain?

# Key concepts in translation

Genetic information transcribed from DNA to mRNA as a nonoverlapping, degenerate triplet code

1 codon = 1 amino acid but 1 amino acid $\geq$ 1 codon

2 key molecules responsible for decoding nucleotide sequence into amino acid sequence are tRNAs and aminoacyl-tRNA synthetases

3 base anticodon in tRNA allows base-pairing with corresponding sequence in mRNA

20 specific aminoacyl-tRNA synthetases present

Both pro and eukaryotic ribosomes have a large and small subunit

# What is translation?

mRNA directed synthesis of polypeptides

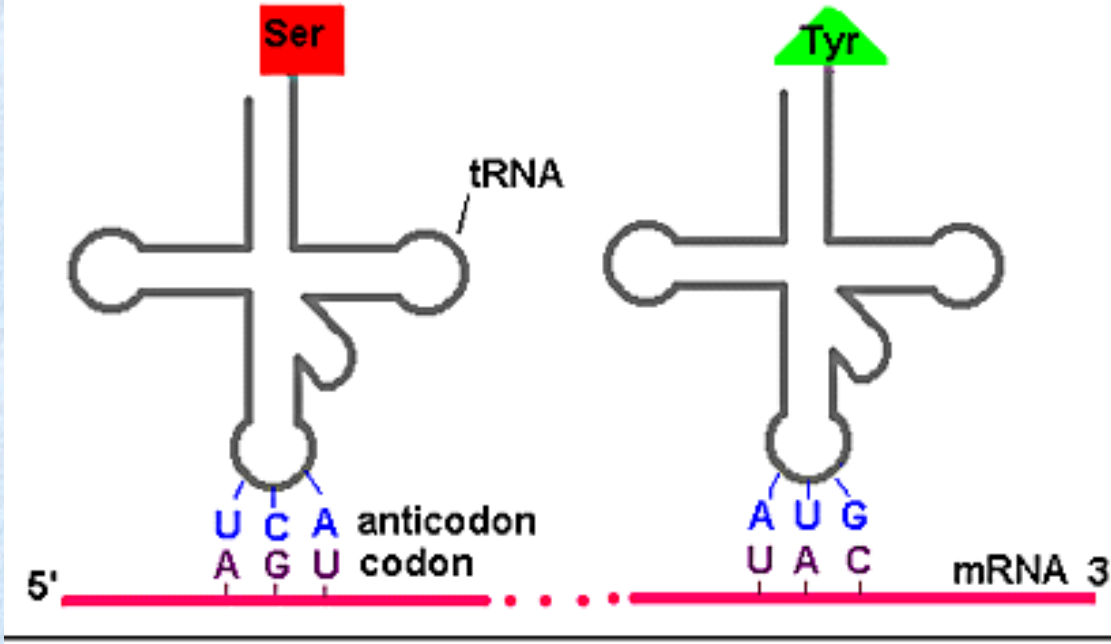
Translates DNA sequence information into proteins

Genetic code dictates translation of specific RNA triplet codons to amino acids

Occurs in the cytosol

# Genetic code

- Triplet code
- Degenerate – more than 1 triplet may encode same amino acid
- Non overlapping
  E.g AUGCGTACT
- Start codon mainly AUG (rarely GUG)
- Stop codons are UAG, UGA, UAA




2nd base in codon

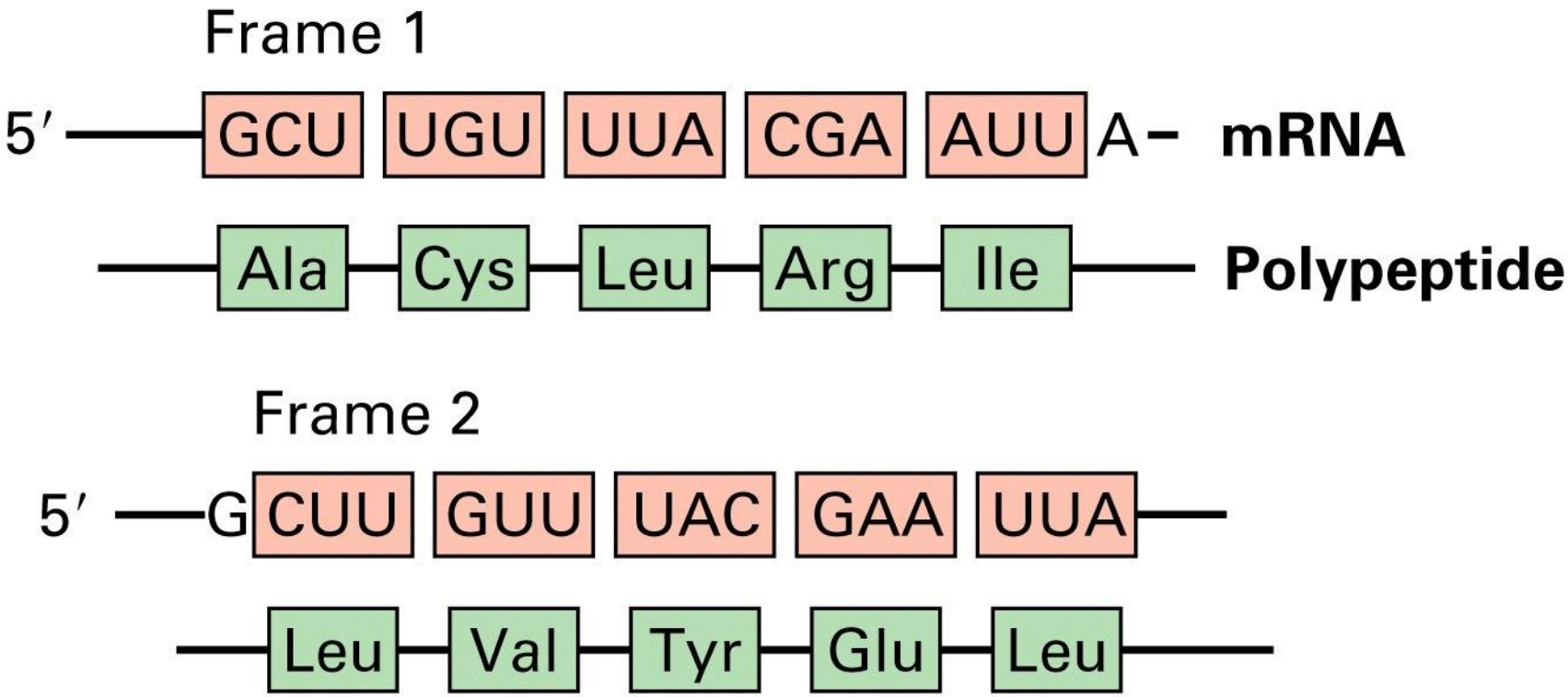
| 1st base in codon | U | C | A | G | 3rd base in codon |
|---|---|---|---|---|---|
| U | Phe | Ser | Tyr | Cys | U |
| | Phe | Ser | Tyr | Cys | C |
| | Leu | Ser | STOP | STOP | A |
| | Leu | Ser | STOP | Trp | G |
| C | Leu | Pro | His | Arg | U |
| | Leu | Pro | His | Arg | C |
| | Leu | Pro | Gln | Arg | A |
| | Leu | Pro | Gln | Arg | G |
| A | Ile | Thr | Asn | Ser | U |
| | Ile | Thr | Asn | Ser | C |
| | Ile | Thr | Lys | Arg | A |
| | Met | Thr | Lys | Arg | G |
| G | Val | Ala | Asp | Gly | U |
| | Val | Ala | Asp | Gly | C |
| | Val | Ala | Glu | Gly | A |
| | Val | Ala | Glu | Gly | G |

# Exceptions!

| CODON | UNIV CODE | UNUSUAL CODE | ORGANISM |
|---|---|---|---|
| UGA | Stop | Trp | mycoplasma, mitochondria (some spp) |
| CUG | Leu | Thr | Yeast mitochondria |
| UAA, UAG | Stop | Gln | Paramoecium, Tetrahymena etc |

# Open Reading frames (ORF)

Uninterrupted sequence of codons in mRNA (from start to stop codon) that is translated into amino acids in a polypeptide chain



# Mutations

MAN CAN FLY- correct sequence

DAN CAN FLY – substitution

DAC ANF LY - frameshift mutation

# Main classes of mutations

Deletions or Insertions: 1bp to several Mbp

Single base substitutions

Missense mutations: replace one amino acid codon with another

Nonsense mutations: replace amino acid codon with stop codon

Splice site mutations: create or remove exon-intron boundaries

Frameshift mutations: alter the ORF due to base substitutions

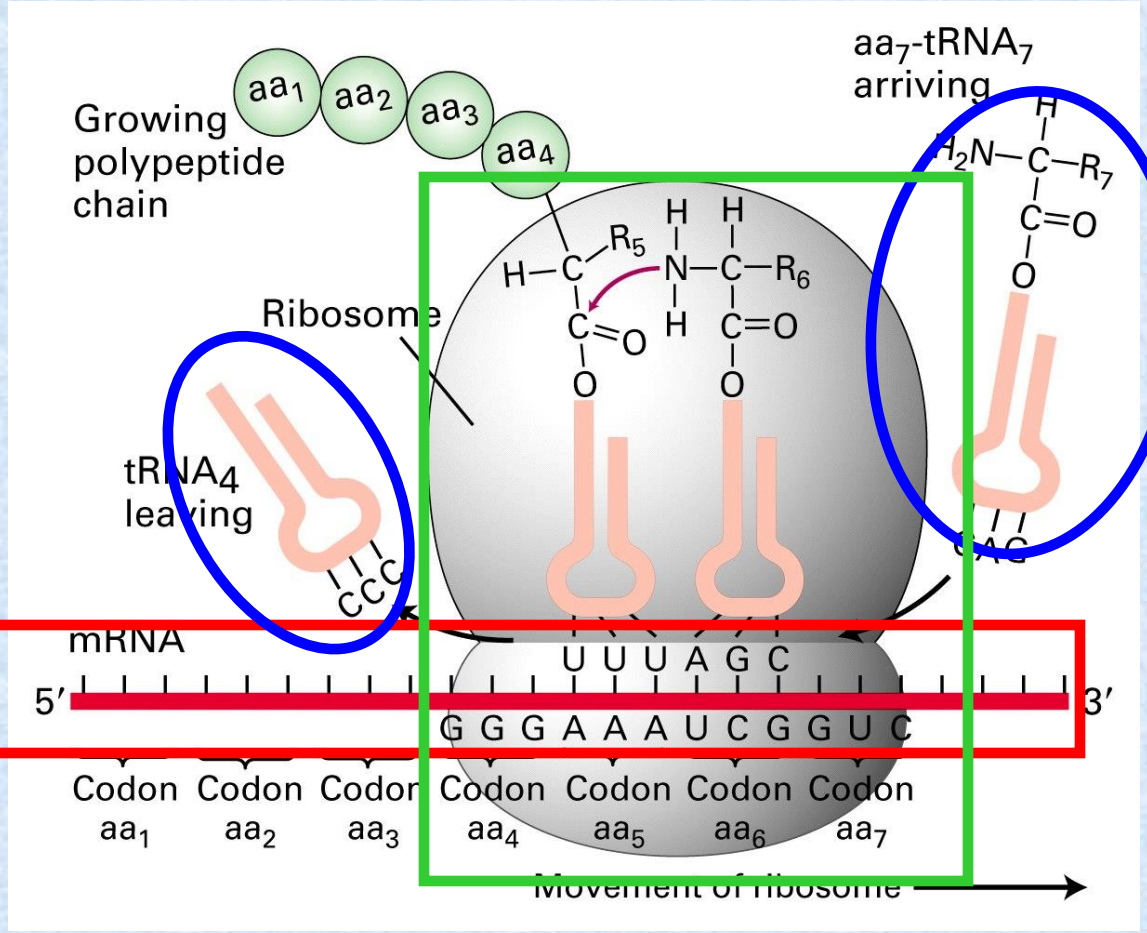
Dynamic mutations: changes in the length of tandem repeat elements

# Translation requires…..

**1) mRNA**

**2) Aminoacyl- transfer RNA (aatRNA)**

**3) Ribosomes**



# 1) Messenger RNA (mRNA)

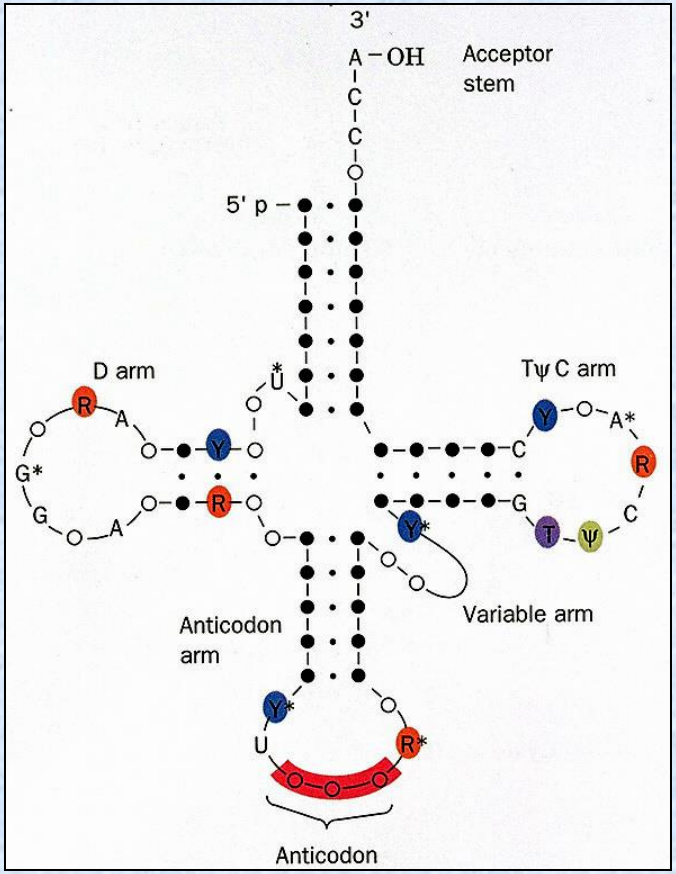
This class of RNAs are the genetic *coding templates* used by the translational machinery to determine the order of amino acids incorporated into an elongating polypeptide in the process of *translation*.

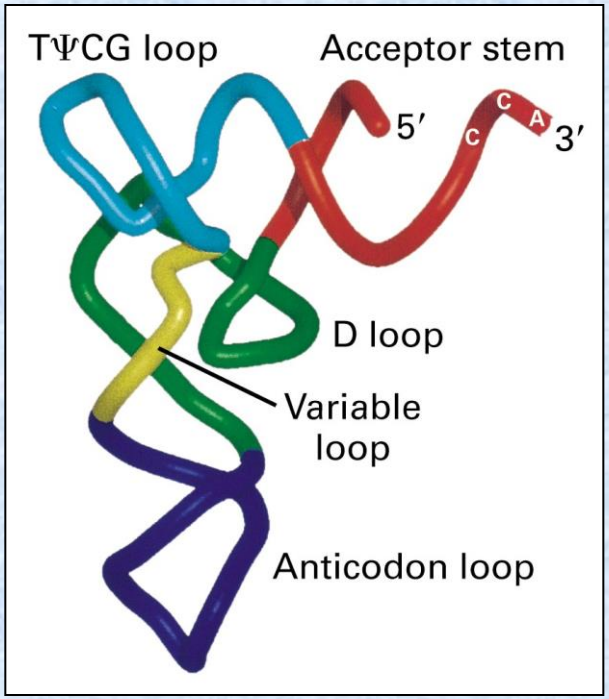
# 2) Transfer RNA (tRNA)

class of small RNAs

form covalent bonds to amino acids

allows correct insertion of amino acids into the elongating polypeptide chain.



# 3) **Ribosomes**

**Ribosomal RNA (rRNA)** assembled, together with numerous ribosomal proteins, to form the ribosomes.

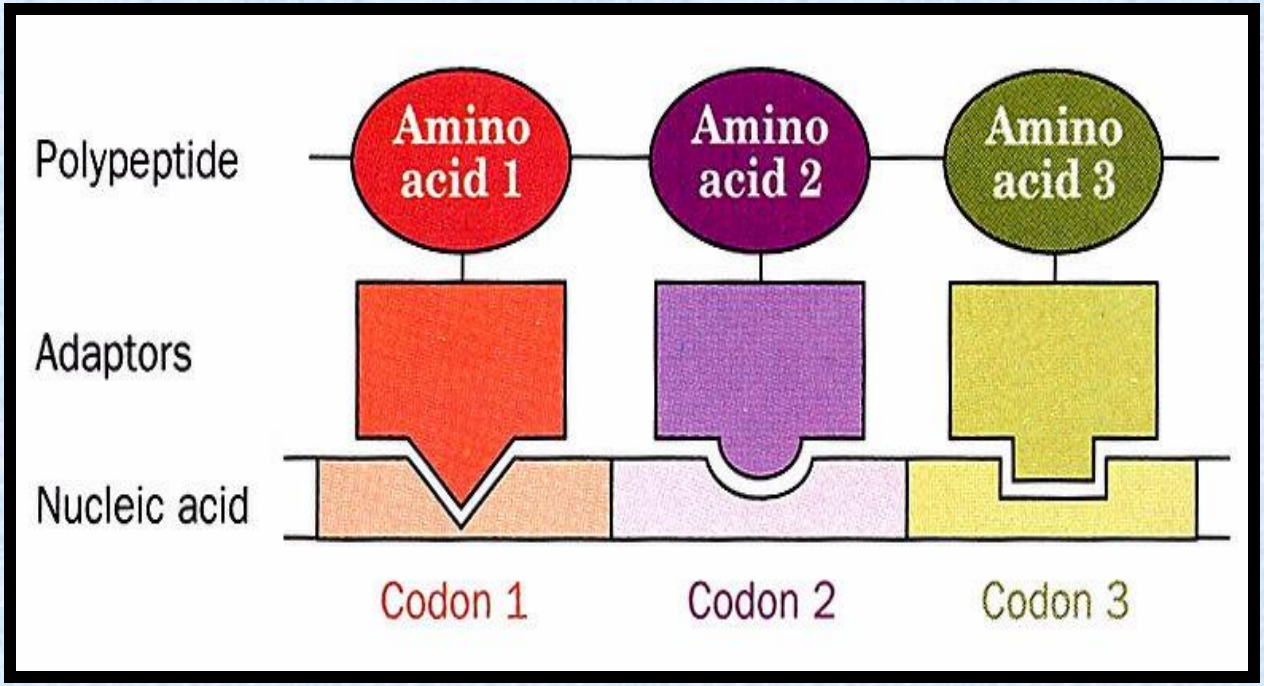
**Ribosomes** engage the mRNAs and form a catalytic domain into which the tRNAs enter with their attached amino acids. The proteins of the ribosomes catalyze all of the functions of polypeptide synthesis

# Adaptor hypothesis

tRNA acts as a ‘shuttle’ linking amino acid to nucleic aid

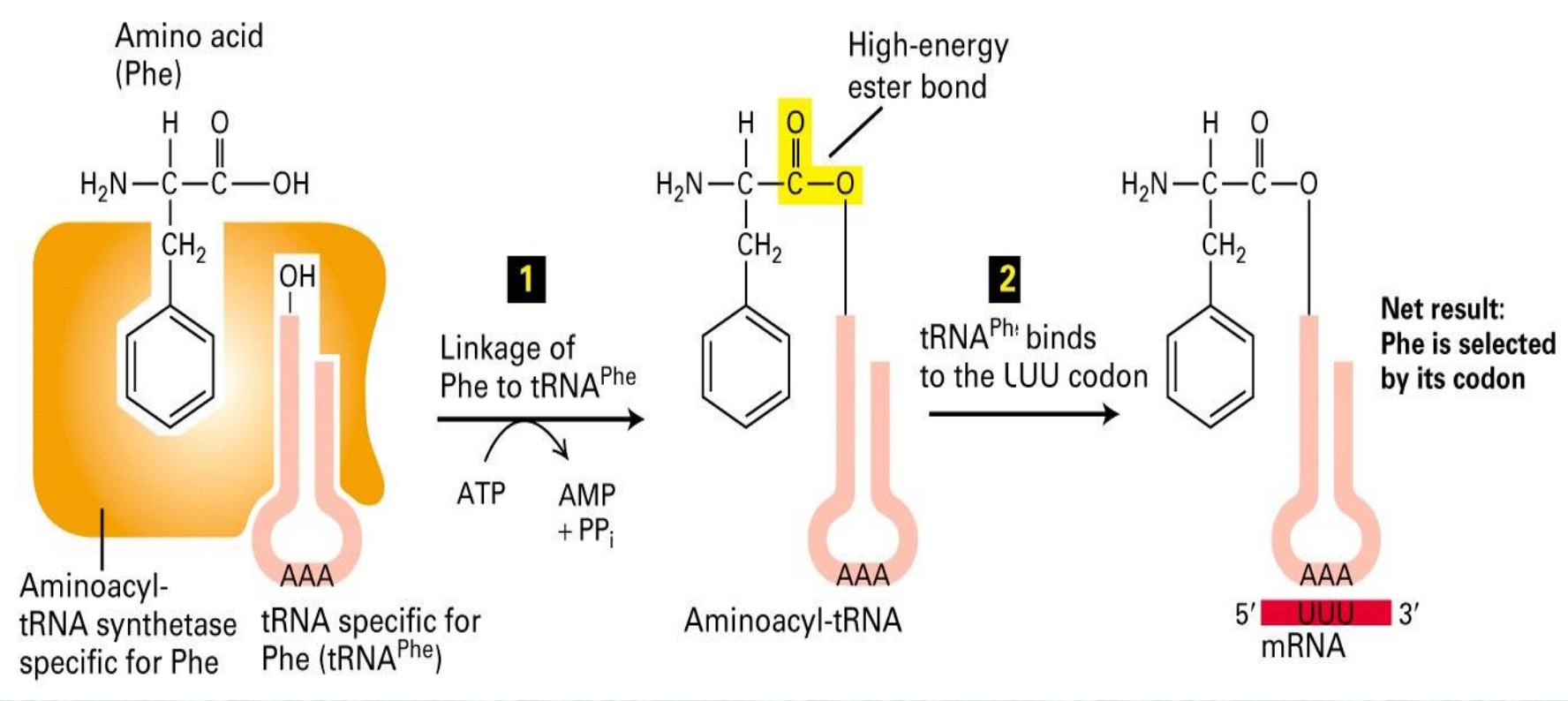
Aligns correct amino acids to form a polypeptide

One tRNA per amino acid



# Translation has 2 important recognition steps

1. **Correct aminoacylation ('charging'):** Covalently attach the correct amino acid to tRNA (specified by anticodon)
2. **Select the correct charged tRNA** as specified by mRNA



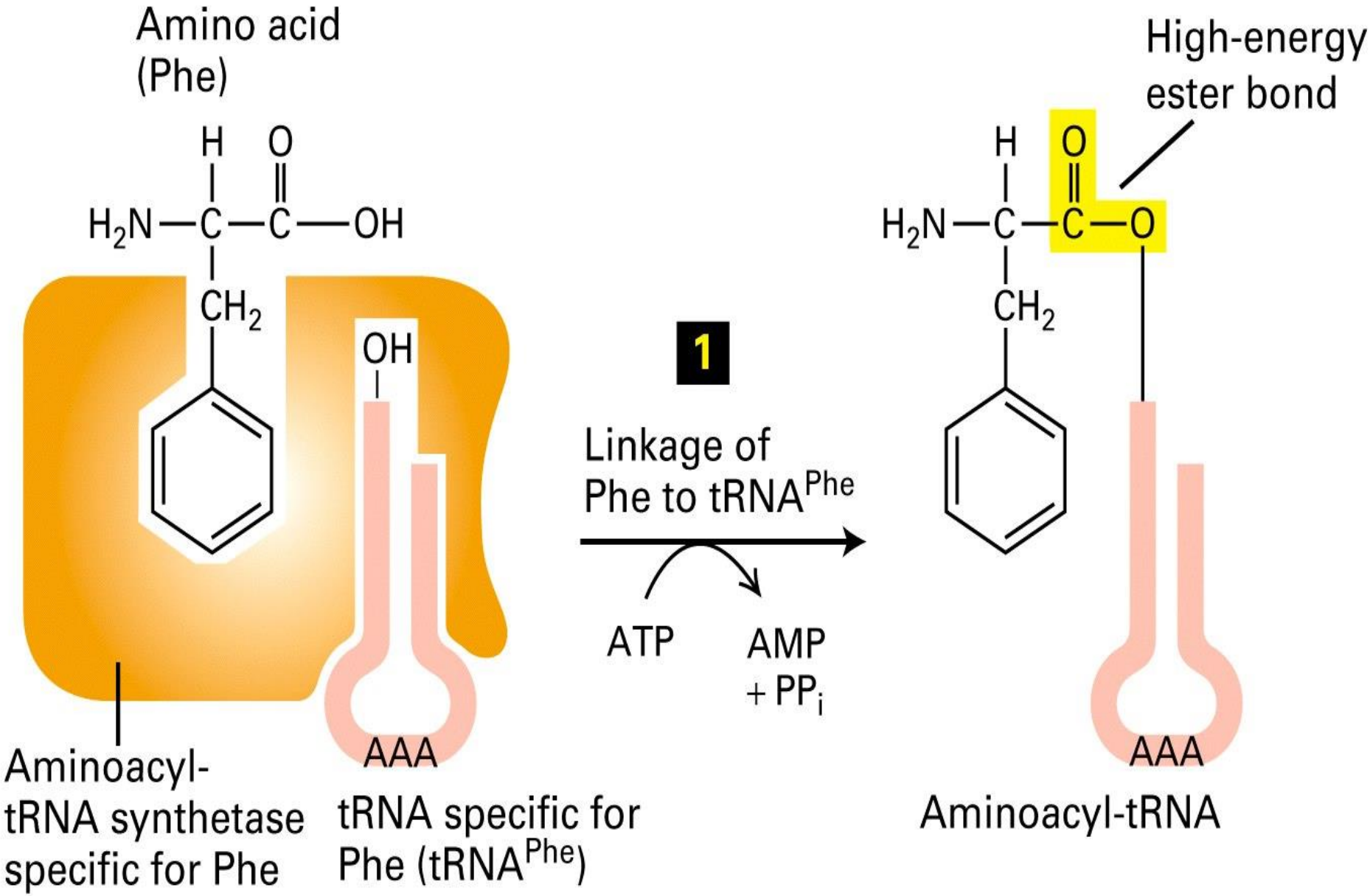
# 1 Aminoacylation of tRNA ('charging')

Amino acid + tRNA + ATP

Aminoacyl-tRNA synthetases (aaRSs)

aminoacyl-tRNA + AMP + PPi

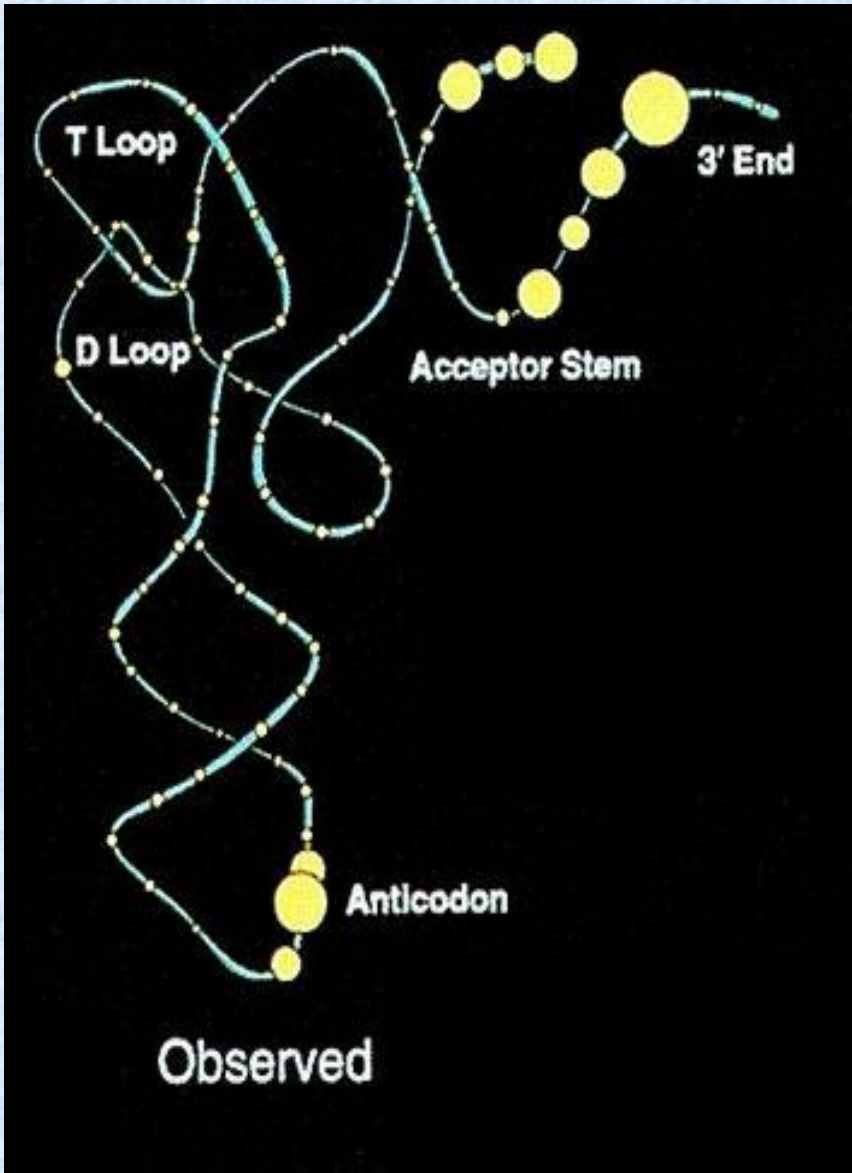
# Aminoacylation of tRNA ('charging')



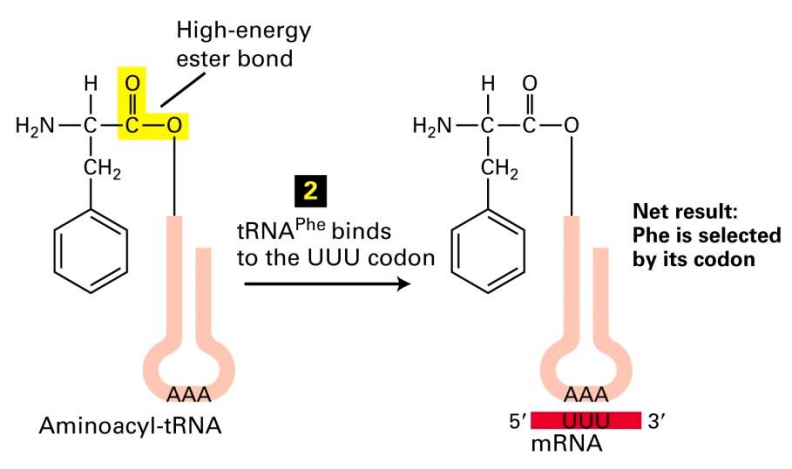
# How does the aaRSs select the right tRNA to be acylated especially since most tRNAs are structurally similar?

By recognising specific tRNA identifiers present on the acceptor step & anticodon loop

e.g. AlaRSs recognise G3·U70 bp



# 2 Select the correct charged tRNA as specified by mRNA

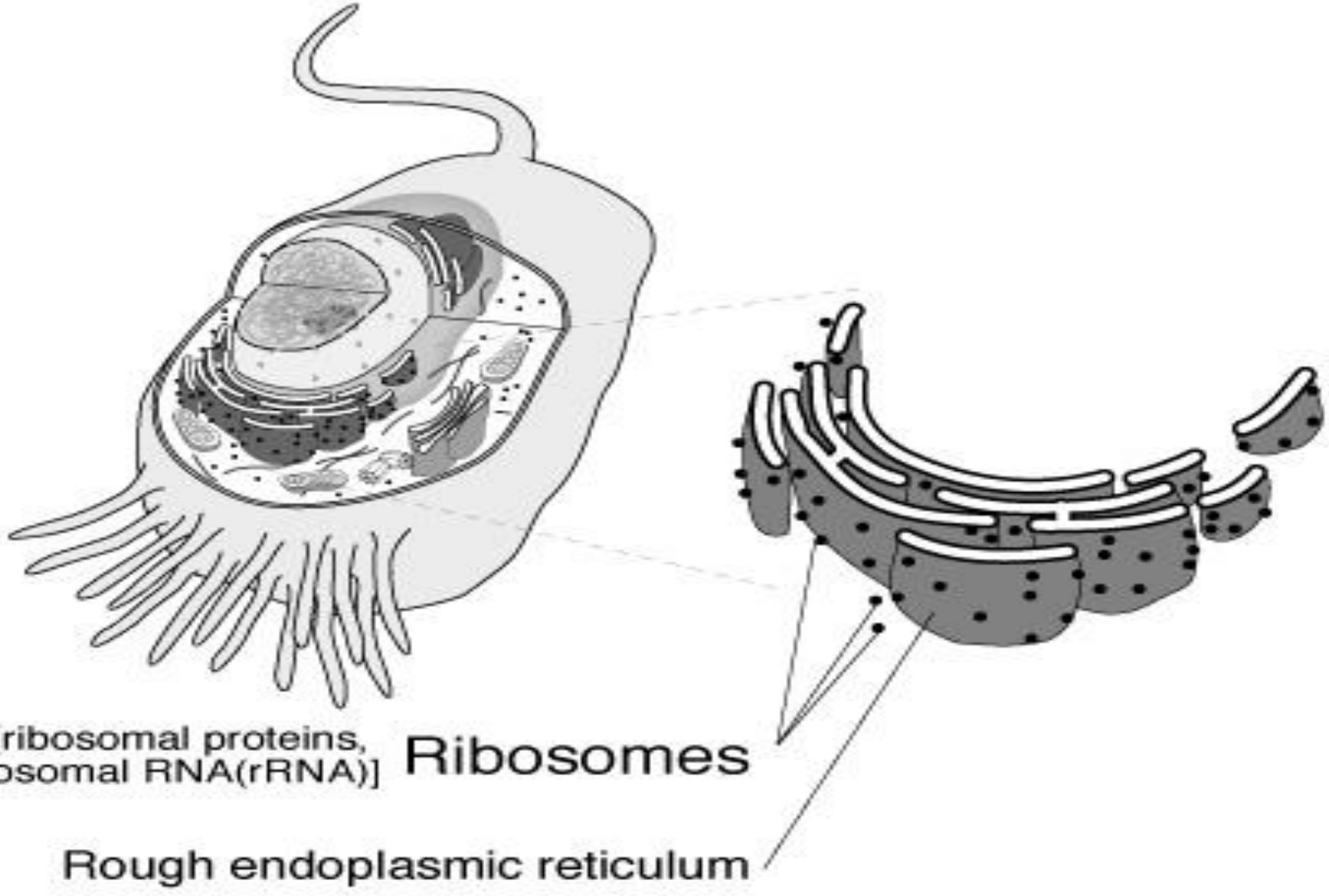



- Less than 61 tRNAs found in cells
- Ribosomes select aa-tRNA based only on their codon – anticodon interactions
- This pairing is antiparallel and the base in the third position forms non standard base pairing (Wobble hypothesis)

tRNA anticodon 3’-A A G-5’ or 3’-A A G-5’

mRNA codon 5’-U U C-3’ 5’-U U U-3’

# Ribosomes


[ribosomal proteins, ribosomal RNA(rRNA)]
Ribosomes
Rough endoplasmic reticulum

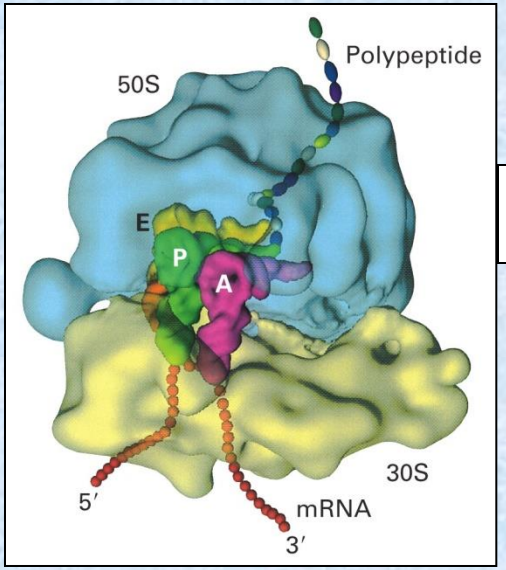
# Ribosomes

Made of ***rRNA*** **&** ***ribosomal proteins***

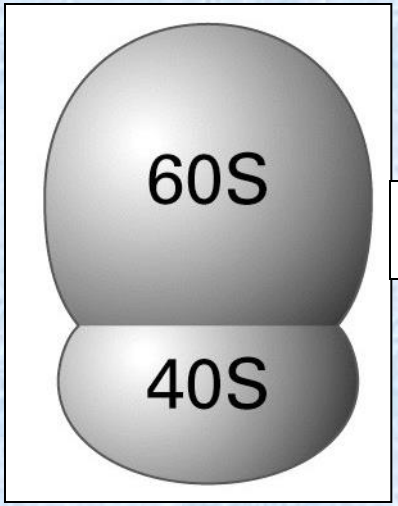
2 subunits – large and small

Subunits are self assembling

combine only in the presence of mRNA and a charged (aminoacylated) tRNA

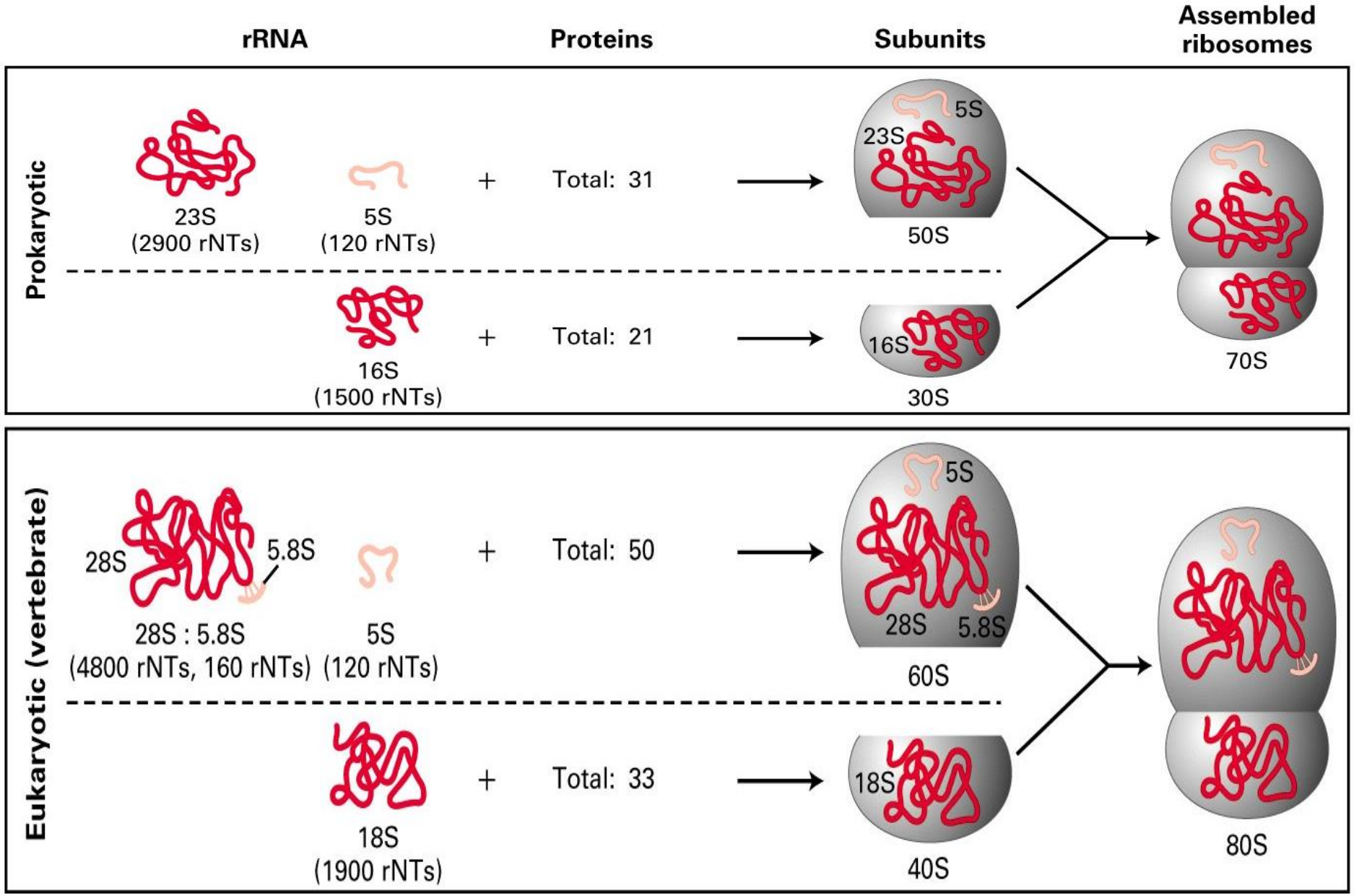



***E.coli***




***eukaryote***

# Ribosomes


rRNA
Proteins
Subunits
Assembled ribosomes
Prokaryotic
23S
(2900 rNTs)
5S
(120 rNTs)
+
Total: 31
5S
23S
50S
16S
(1500 rNTs)
+
Total: 21
16S
30S
70S
Eukaryotic (vertebrate)
28S
5.8S
28S : 5.8S
(4800 rNTs, 160 rNTs)
5S
(120 rNTs)
+
Total: 50
5S
28S
5.8S
60S
18S
(1900 rNTs)
+
Total: 33
18S
40S
80S

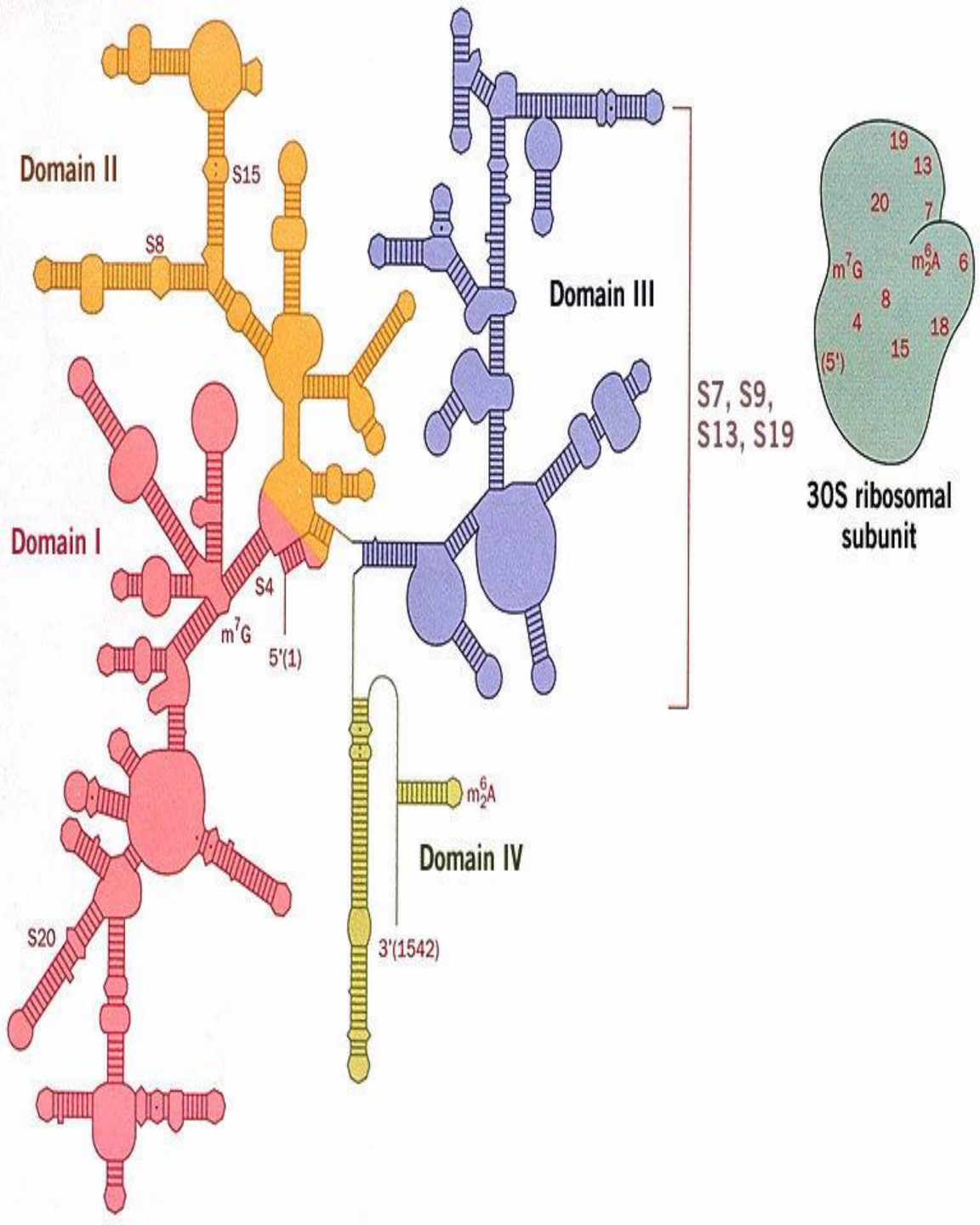
# rRNA

Key component of ribosome

Responsible for

- Ribosome structure
- tRNA positioning
- Catalytic function?

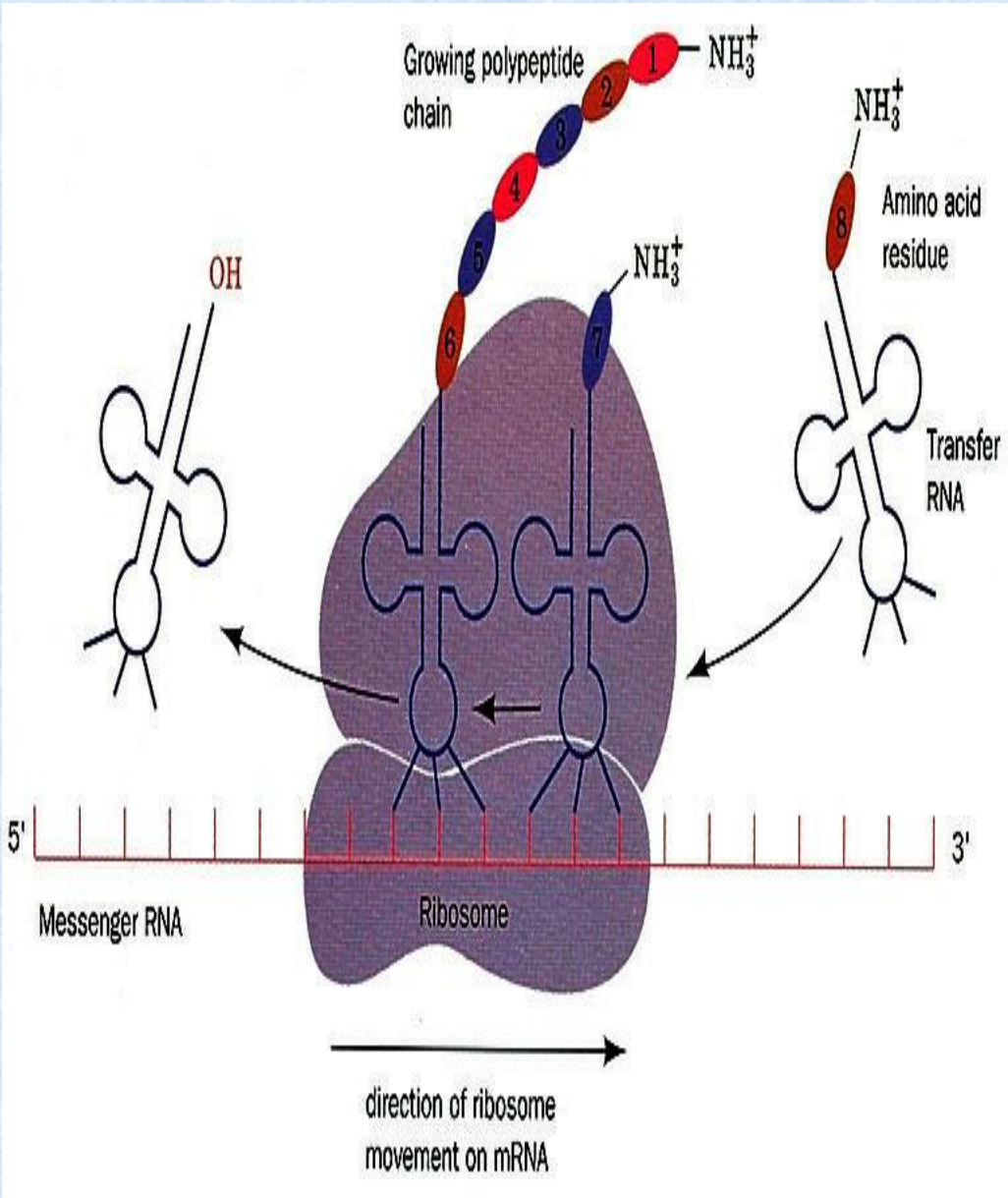
Structure provides evolutionary clues about different organisms



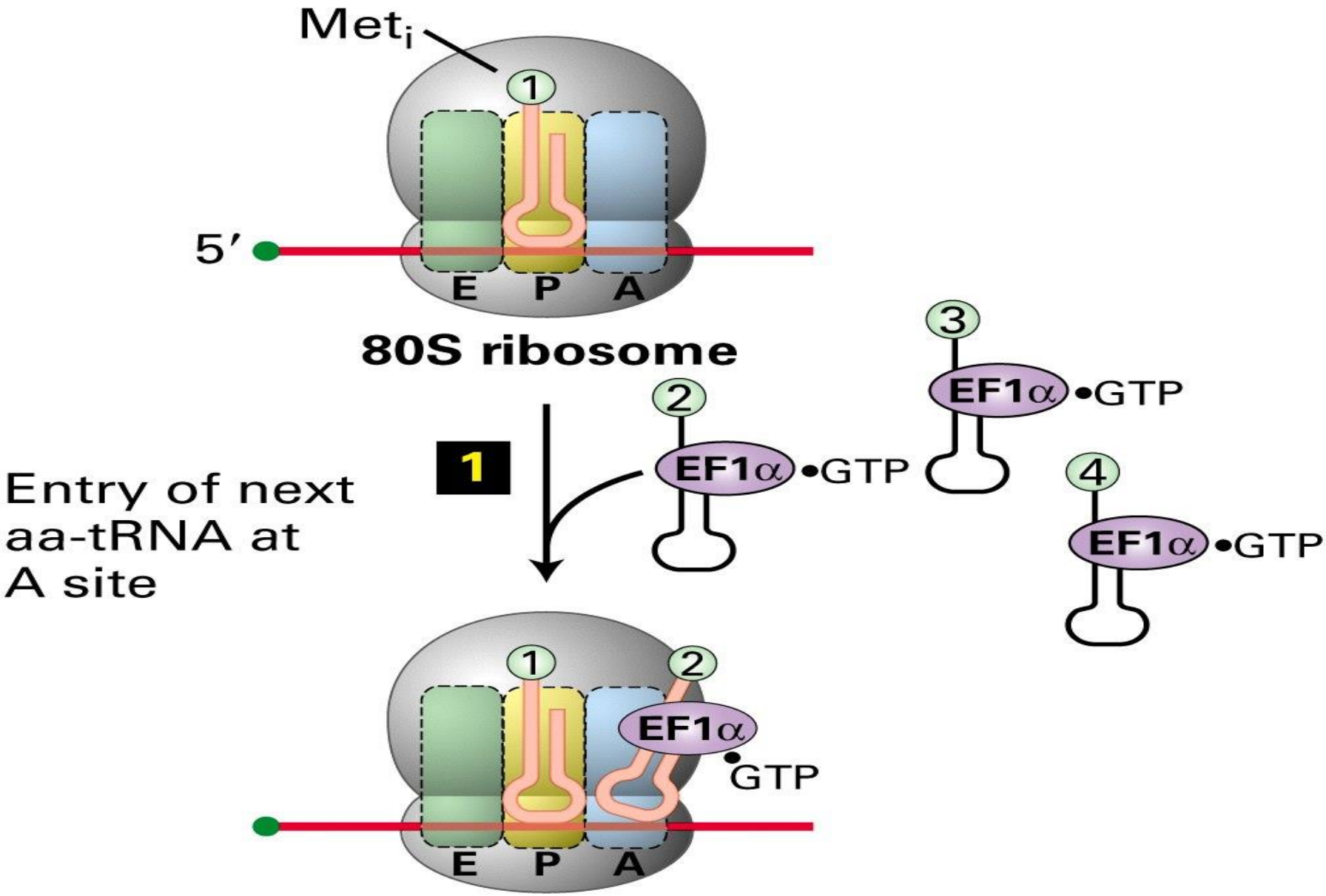
# Polypeptide synthesis (overview)

3 distinct steps

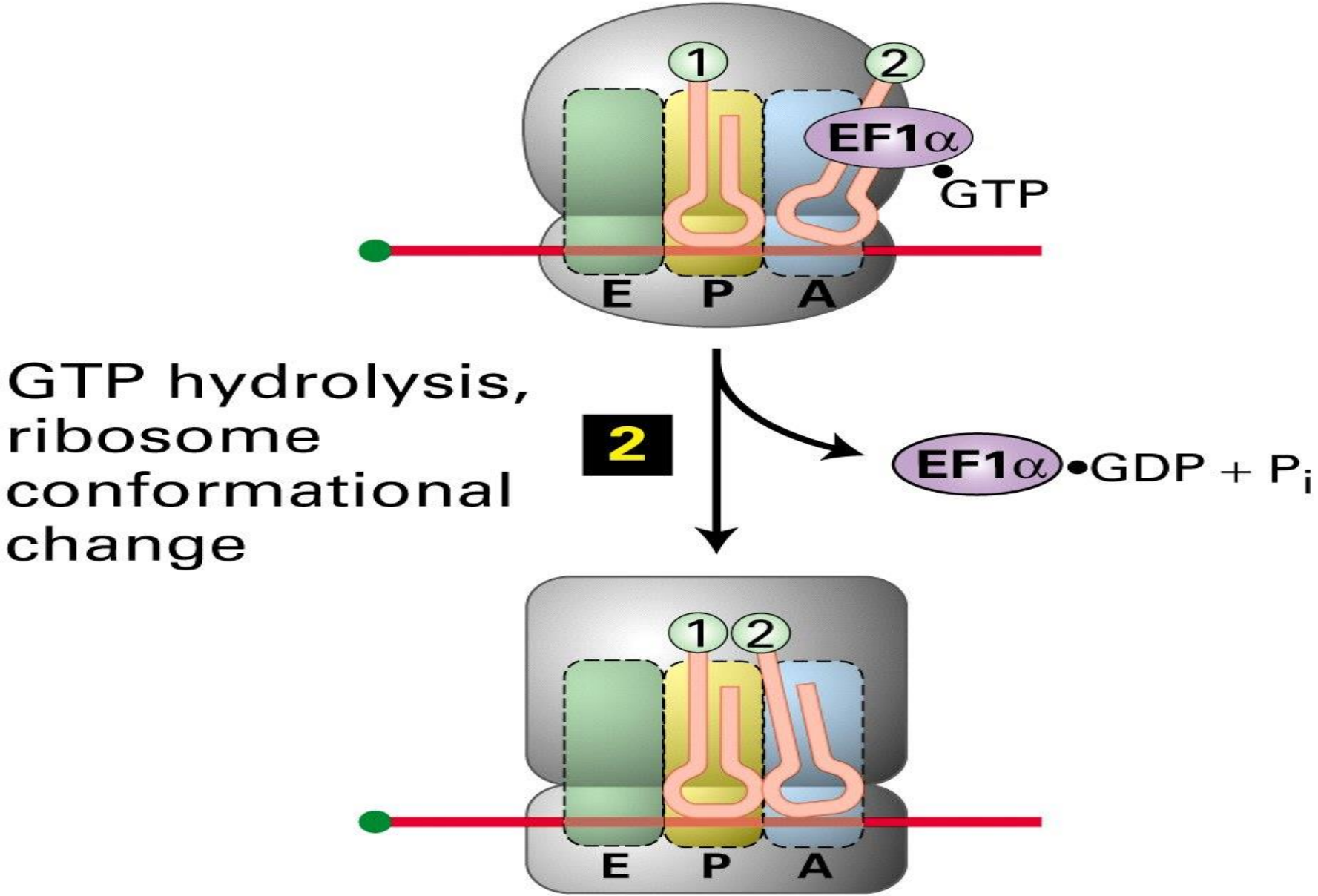
1. Chain initiation
2. Chain elongation
3. Chain termination



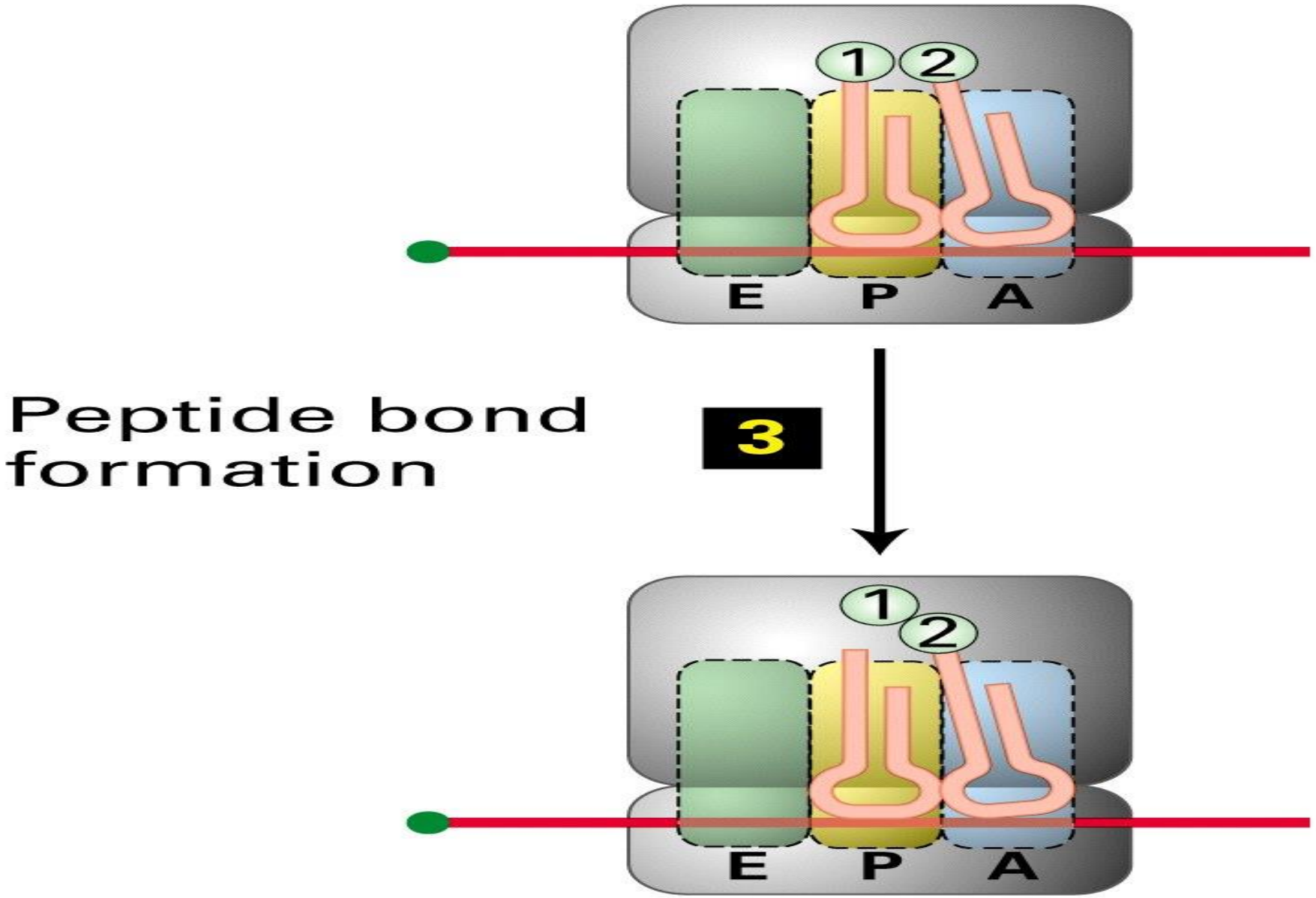
# Step 1 : aatRNA binding



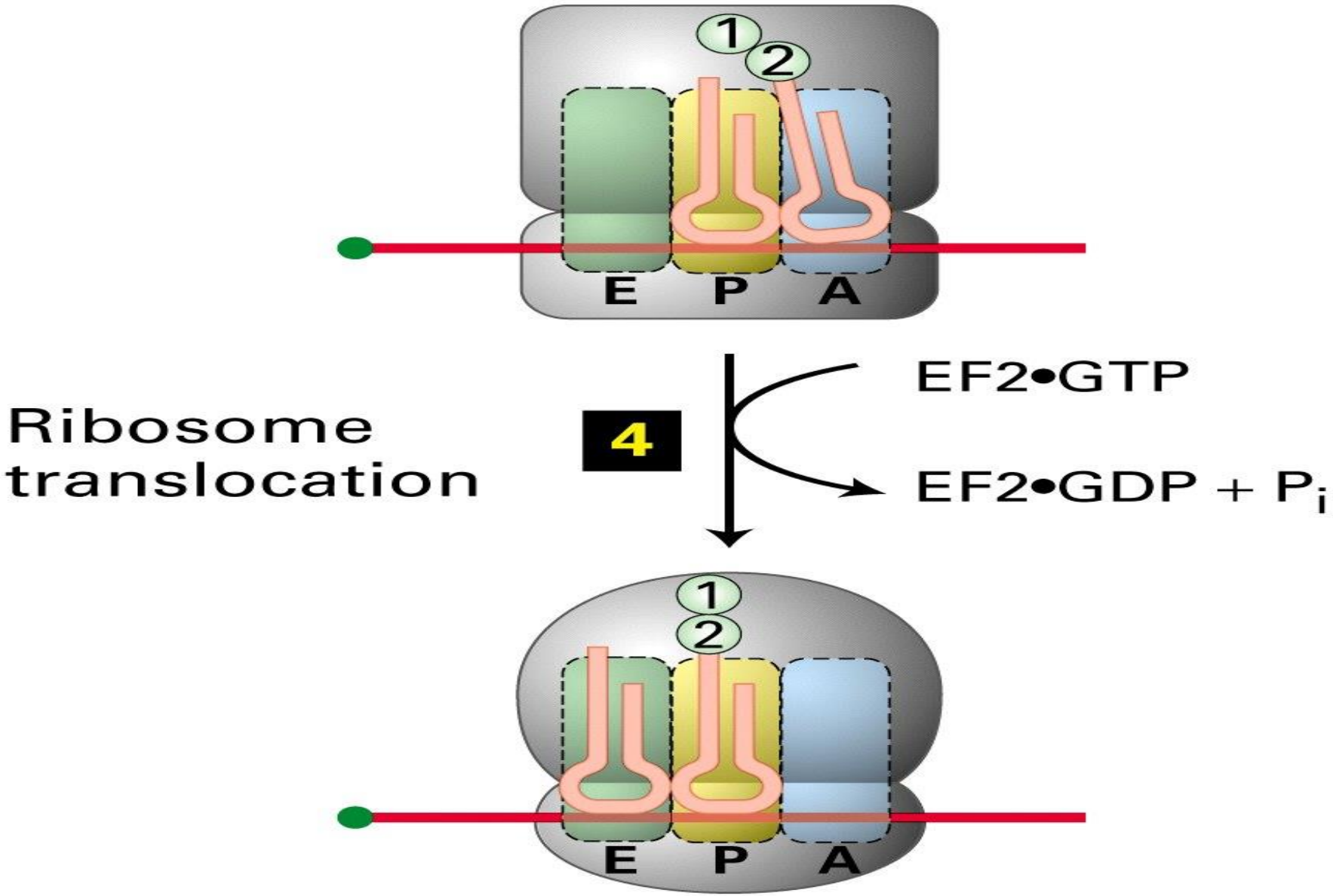
# Step 2: conformational change



# Step 3 : Transpeptidation



# Step 4 : Translocation



# termination

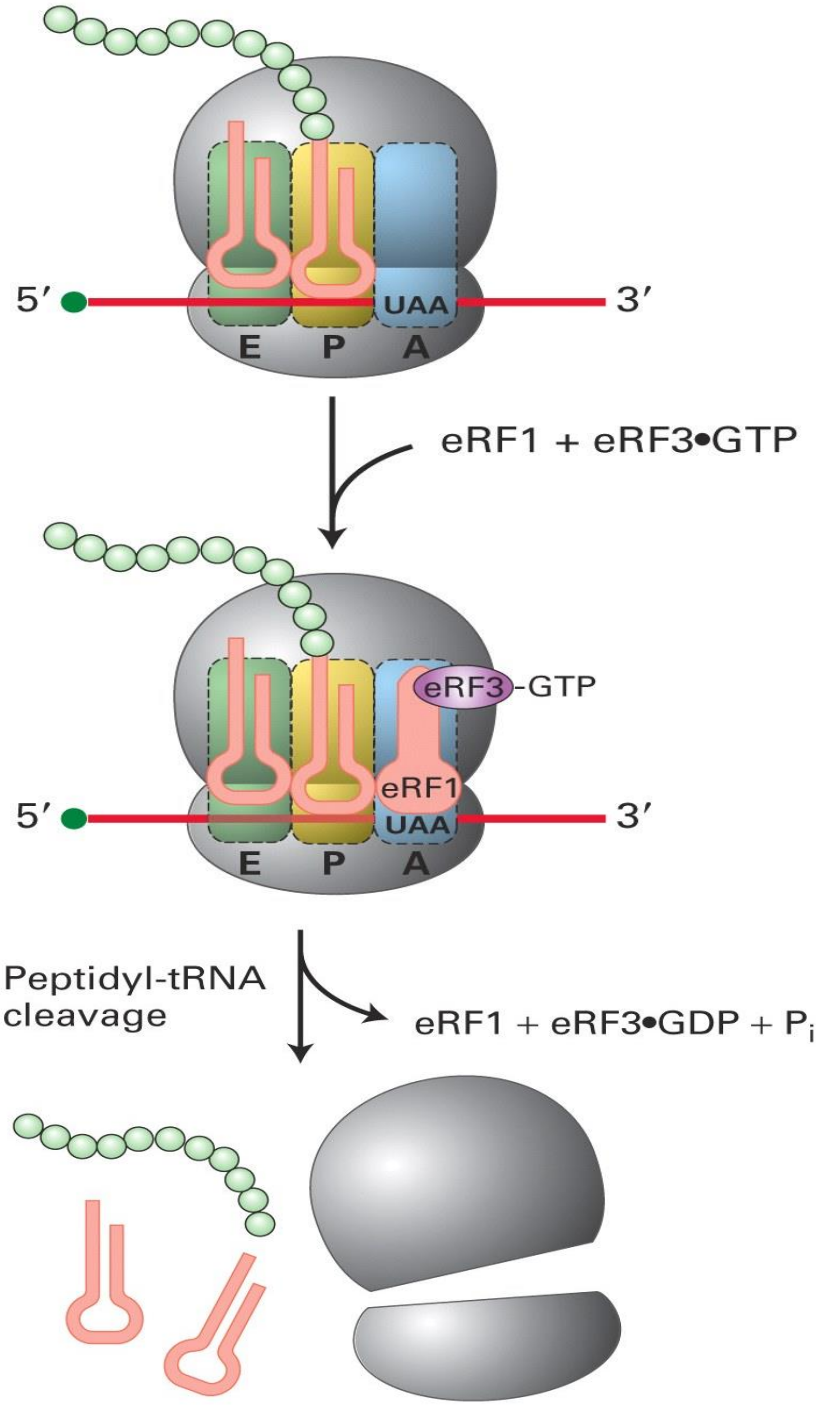
***Release factors*** (eRFs) recognise and bind to stop codons

This induces *peptidyl transferase* to transfer peptidyl group to *water* instead of aatRNA

Uncharged tRNA released from ribosome

Inactive ribosome then release mRNA

**Typically the entire process takes 30-60sec!!**



# Some antibiotics inhibit translation

## Only prokaryotes

- Streptomycin prevents initiation-elongation
- Chloramphenicol blocks peptidyltransferase

## Only eukaryotes

- Cycloheximide blocks translocation

## Both

- Puromycin causes premature release of polypeptide

# Post translational modifications

## Protein folding

- Nascent protein is folded and/or modified into mature, functional forms
- Amino acid sequence determines its folding into specific 3-D conformation
- This folding is mediated by molecular chaperones (e.g. Hsp70) or chaperonins (Hsp60 complexes)

## Covalent modification

- Various chemical groups (e.g acetyl, phosphoryl, hydroxyl, glycosyl etc) are added to the NH2 or COOH terminal or internal residues of the polypeptide
- These modifications are essential and dictate the activity, life span or the cellular location of proteins.

## Proteolytic cleavage

Activates some inactive precursors

E.g. caspases, zymogens etc

# Death of proteins

Proteins that are misfolded, denatured, in excess or extracellular in origin are targeted for degradation within lysosomes

Another pathway is by the addition of *ubiquitin* to lysine residues, which is recognised are destroyed by the *proteosome* complex.

Degradation of proteins can be a part of normal cell processes (cell cycle) or may be implicated in disease, especially neurodegenerative diseases (Parkinsons, Alzheimers)