

mRNA — tRNA —> Amino acid.

RNA code characteristics:

- 4 nucleotides code for 64 codons ( $4^3$ ), 61 of which code for 20 amino acids.
- Codon coding is degenerate: multiple codon codes for a single amino acid.
- Code is universal (4 nucleotides), but meaning assigned to the codons are different (e.g. human and mitochondria)

tRNA characteristics:

- Structure: 5' end D loop, 3' end T loop, 3' end CAA sequence binding to amino acid
- More than 100 species, synthesised by RNA polymerase III.
- Each amino acid attached to the CAA site with a high energy bond catalysed by 1 of 20 amino acyl synthetase.  
(ATP —> AMP)
- Wobble phenomenon:
  - Due to loose structure of ribosome at mRNA 3' end and low specificity, extra pairing is allowed
  - Reading from 5' to 3' direction, 1st position of tRNA is the wobble position
  - At wobble position, G can pair with U, while Inosine (deaminated A) from tRNA able to pair with all other nucleotides except Guanine

• Prokaryotic translation initiation

- Shine Dalgarno sequence GGAGG around 10 base upstream of first mRNA codon
- 16S rRNA + protein = 30S small subunit have E, P, A, sites
- Initiation factor 3 (IF3) bind to E site, prevent large subunit binding.
- IF1&2 duplex + GTP bind to A site
- 30S subunit complex recognise Shine-Dalgarno sequence, bind with AUG site
- f-Met is recruited to P-site, IF3 detach, allow 50S binding
- 23S + 5S + protein = 50S large subunit binding, form 70S complex
- Binding of 50S, IF1&2&GTP induce GTP hydrolysis, release IF1&2 from A site, allow elongation to start

• Prokaryotic translation elongation

- Elongation factor Tu (EF-Tu) responsible for selecting correct amino acyl tRNA to enter A site
- Elongation factor G (EF-G) responsible for translocation of ribosome down 1 unit in 5' —> 3', synthesising in 5' to 3' direction (polypeptide not antiparallel). Protein synthesised in the N-terminus - C terminus direction.
- Amino acyl tRNA enter at A-site, form peptide bond with polypeptide chain at P site, move to P site, P site tRNA exit via E site.

• Prokaryotic termination

- Three termination factors (RF1,2,3)
- RF1&2 bind to A site at STOP codon, stop tRNA entry, hydrolyse polypeptide chain
- RF3 proofreads the STOP codon, facilitate dissociation of ribosome subunit and RF 1&2

• Eukaryotic translation preinitiation

- Eukaryotic initiation factor (eIF) 1 and 3 bind to E site, 1A bind to A site of the 40S small subunit (18S + protein).
- eIF2-GTP-Met bind to P site, eIF5 bind to E site, form 43S complex
- eIF4 complex containing many subunits bind to 5' end of mRNA, also bring poly-A tail to a loop
- 43S complex bind to eIF4 complex at 5' end

• Eukaryotic translation initiation

- 43S complex leaves 4E subunit of eIF4 at 5' end and scan along the mRNA sequence, until recognising Kozak sequence (5'A AUGG3')
- eIF2-GTP hydrolyse, all initiation factor except 1A dissociates, 60S subunit (28S + 5.8S + 5S + protein = 60S) binds form 80S, eIF 5B-GTP binds to eIF-1A at A site.
- Hydrolysis of eIF5B-GTP, release of eIF5B and 1A, expose A site, elongation starts.
- Eukaryotic elongation
  - eEF1 $\alpha$ -GTP bring tRNA to A site, hydrolysis cause ribosomal conformational change, catalyse peptide bond formation
  - eEF2-GTP bind, hydrolysis cause translocation of ribosome towards 3' mRNA, release 1 tRNA.
- Eukaryotic termination
  - At STOP codon, eRF1 and eRF3-GTP bind to A site, prevent tRNA entering.
  - eRF3-GTP hydrolyse, cleave polypeptide
  - ABCE (ATP binding cassette) dissociates 60S subunit, exchange eRF1 with eIF1, 1A and 3, prevent 60S binding, resets the cycle.
- In eukaryotes, ribosome dissociate and assemble again around the mRNA loop created by eIF4 complex binding 5' end and poly A tail
- Antibiotics targeting translational mechanisms:
  - Tetracycline - prevent tRNA entry to A site
  - Streptomycin- prevent transition between initiation and elongation stages
  - Chloramphenicol - block peptidyl transferase
  - Erythromycin - Blocks E site

Prokaryotes couples transcription - translation

Eukaryotes couple transcription - processing in nucleus - export - translation



mRNA code combinations

tRNA structure, characteristic, synthesis

Wobble phenomenon

Prokaryotic initiation, elongation, termination

Eukaryotic initiation, elongation, termination

Antibiotic targets

Central dogma in Pro/Eu