

Prokaryotic Translation (Protein Synthesis)

1 Initiation

IF = initiation factor

Occurs after termination
Something to do with preventing the 30S subunit with bonding to the 70S subunit without RNA, forming a disfunctional ribosome

1 IF1 and IF3 binds to a free 30S subunit - role of IF1 still not clear

2 IF2 binds to fMet-tRNA [see earlier], and they together bind to the complex from (1) [NEEDS GTP]

The fMet-tRNA then base-pairs RIBOSOME BINDING SITE or SHINE-DLAGARNO SEQUENCE (complementary to the 3' end of the 16S RNA) near the initiating codon (which is ALWAYS AUG)

This does not necessarily have to be at the very start of the RNA molecule!

3 (which is ALWAYS AUG)

The 50S subunit binds, GDP is hydrolysed and the aminoacyl tRNA ends up in the P site

4

One of the key consequences of this is that this tRNA ends up in the P and not the A site

Ribosomes

Prokaryotic ribosomes are different to eukaryotic ones

They have "70S" RNA, consisting of

- A 50S subunit
 - 31 proteins
 - 23S RNA
 - 5S RNA
- A 30S subunit
 - 21 proteins
 - 16S RNA

rRNA (ribosomal RNA) is exceptionally well conserved, across species and kingdoms

rRNA have a complex secondary structure, which forms a "scaffold" over which proteins present in the ribosomes can be assembled

The structure, however, is mostly dominated by rRNA - a vestige of an old RNA world

The ribosome has two major catalytic sites

- The P site
- The A site

2 Elongation

EF = Elongation factor

The aminoacyl-tRNA corresponding to the next codon is delivered to the site by EF-Tu

This leads to the hydrolysis of GTP by EF-Tu, and the EF-Tu remains bound with a molecule of GDP

EF-Ts displaces GDP from EF-Tu

It is itself displaced by GTP

The new Ef-Tu bound with GTP is ready to bind another tRNA

The EF-Tu must be regenerated by the EF-Tu - EF-Ts EXCHANGE CYCLE

Aminoacyl tRNA delivery

The INITATOR tRNA is UNABLE to form a complex with EF-Tu

The P site and A site now contain two adjacent amino acids in close proximity

The PEPTIDYL TRANSFERASE activity of the 50S subunit can now form a peptide bond between them

Peptide bond formation

NOTE: This energy is from the tRNA in the "P" site

No further energy is required, because energy was already input when CHARGING the tRNA

A complex of EF-G (TRANSLOCASE) and GTP binds to the ribosome

The discharged tRNA is ejected from the P site

The peptidyl-tRNA is moved from the A site to the P site

The mRNA moves one codon relative to the ribosome

In an energy consuming step

Translocation

It first moves to an "E-site" (EXIT SITE)

It is only ejected when the next aminoacyl tRNA binds

This ensures that the ribosome is constantly bound to mRNA via 6 bp, which minimises frame shifts

Recent evidence suggests that in PROKARYOTES, the discharged tRNA does not exit immediately

3 Termination

RELEASE FACTORS (RFs) recognise STOP codons and bind to these in the vacant A sites

- RF1 recognises UUA and UAG
- RF2 recognises UAA and UGA
- RF3 helps the other two

They make PEPTIDYL TRANSFERASES transfer the polypeptide chain to WATER rather than to the usual aminoacyl tRNA

Requires hydrolysis of GTP

Finally, EF-G together with the RFs release the uncharged tRNA and mRNA

IF3 can now bind the small subunit to prevent inactive ribosomes forming

Dealing with truncated mRNA

A special RNA, called tmRNA frees STALLED ribosomes

If a ribosome has stalled (no valid codon in A-site), tmRNA binds

First behaves as tRNA, delivering ALANINE

Then, behaves as mRNA, adding 10 extra codons in total to the protein and releasing it

The 10 codons added at the carboxy end target the released damaged protein for rapid degradation

Antibiotics

No cells can survive without proteins - the translation machinery is essential to life, and ribosomal mutants which block translation are lethal

Anything else which blocks translation will kill the cell

- CHLOROAMPHENICOL - inhibits the peptidyl transferase
- PUROMYCIN - premature release of the peptide chain (it's quite similar in structure to aminoacyl tRNA)