

BICH/GENE 431 KNOWLEDGE OBJECTIVES

Chapter 14 - Translation

Signals on mRNA: codons (know start and stop codons); meaning of an open reading frame (ORF); ribosome binding site (RBS or Shine-Delgarno sequence) on bacterial mRNA just upstream of start codon base-pairs to 16S rRNA; Kozak sequence on euk. mRNA surrounds AUG start codon; 5' cap recruits ribosome that slides to start codon; 3' polyA tail helps to recycle ribosomes from end

Bacterial mRNAs may be polycistronic; eukaryotic mRNAs are monocistronic

Transfer RNAs (tRNAs) discovered by Zamecnik and Hoagland in 1957

tRNA structure: highly conserved secondary and tertiary structures; know basic secondary structure (cloverleaf) with names of stems and loops and why named that way; CCA on acceptor arm is site of attachment of amino acid; 3D structure determined by Klug and Rich labs in mid-1970s: L-shaped structure with acceptor arm and anticodon loops on ends

aminoacyl tRNA synthetases catalyze charging to tRNAs with amino acids

- one aminoacyl tRNA synthetase per amino acid, but can charge isoacceptor tRNAs
- amino acid covalently attached to CCA 3' end of tRNA with acyl linkage
- two major classes: Class I and Class II
- two steps in charging reaction: adenylation reaction adds AMP moiety from ATP to carboxyl group of amino acid; tRNA charging transfers amino acid to either 3' OH or 2' OH of tRNA with release of AMP
- must be very accurate (second genetic code) – specificity determinants on tRNA located in acceptor arm (discriminator base) and anticodon stem/loop

composition of ribosomes in prokaryotes and eukaryotes

prokaryotes: 70S ribosome; 50S large subunit with 23S rRNA and 5S rRNA plus many proteins; 30S subunit with 16S rRNA plus many proteins

eukaryotes: 80S ribosome; 60S large subunit with 28S rRNA, 5.8S rRNA, and 5S rRNA plus many proteins; 40S subunit with 18S rRNA plus many proteins

large subunit of ribosome contains peptidyl transferase center; small subunit contains decoding center where tRNAs basepair with mRNA

translation divided into initiation, elongation, termination phases

translation in prokaryotes occurs on mRNA while being synthesized by RNA

polymerase – rate of translation ~20aa/sec; rate of transcription ~50-100 nt/sec

translation in eukaryotes uncoupled from transcription because of nucleus/cytoplasm polysomes cover mRNA with ~80 nt per ribosome

peptidyl transferase reaction transfers growing polypeptide chain to amino acid on incoming tRNA – does not require direct input of energy from ATP or GTP

three tRNA binding sites in ribosome: A site for aminoacylated tRNA, P site for peptidyl tRNA (contains growing polypeptide chain), E site for exiting tRNA after peptidyl transferase reaction

3D structures of ribosomes determined by three labs in early 2000s: Tom Steitz (Yale), Venkatraman Ramakrishnan (MRC) and Ada Yonath (Weizmann) – received Nobel Prize for this work in Fall 2009

Most RNA on inside of structure and most proteins on outside

Initiation of translation requires initiation factors (IFs)

Special initiator tRNAs are charged with N-formyl-methionine (fMet) in prokaryotes or Met in eukaryotes and bind to P site of ribosome

Formyl group from fMet often removed after translation by deformylase enzyme or sometimes one or more translated amino acids removed from protein by aminopeptidase enzyme(s)

Know IFs and pathway of initiation in prokaryotes (Fig. 14-25)

Do not need to know details of initiation in eukaryotes, but know basic differences compared to prokaryotes (charged initiator tRNA binds to small subunit before binding mRNA and scans from 5' cap in 3' direction until encounters first AUG; different eIF factors can bind separately to ribosome and mRNA, and then all form big complex)

eukaryotic mRNAs are circularized via proteins that bind to 5' cap and 3' polyA tail
eIF4G (that interacts with cap binding eIF4E) binds to polyA binding protein (PAP)

this circularization facilitates ribosome recycling from termination to new initiation

Elongation phase of translation

- EF-Tu plus GTP binds aminoacyl tRNA and escorts to A site in ribosome; GTP hydrolysis to GDP results in release of EF-Tu~GDP
- Peptidyl transferase center in large subunit of ribosome composed of RNA only; appears that both rRNA and tRNAs participate in catalysis
- EF-G plus GTP binds ribosome; upon GTP hydrolysis it triggers translocation of tRNAs; then release of EF-G~GDP
- EF-Ts needed to displace GDP from EF-Tu, so that it can be reloaded with GTP
- Energy requirement (ATP, GTP) for elongation: know how much and where used

Termination of translation

- know stop codons and release factors needed for each stop codon in prokaryotes
- two release factors used (RF1 or RF2, plus RF3); role of GDP/GTP
- functions of RRF (ribosome recycling factor), EF-G and IF3 to dissociate tRNAs and ribosome after termination

Many antibiotics function to inhibit translation – know some examples, but not mechanisms, except know how puromycin works; remember cycloheximide as an inhibitor of eukaryotic translation

Regulation of translation: understand how ribosomal protein translation is autorepressed by ribosomal protein in prokaryotes; understand how ferritin translation is controlled by IRE/IRP interaction in eukaryotes