protein synthesis and the ribosome
Central dogma of biology

DNA codes for DNA
- DNA polymerase
- Primase
- Helicase

DNA codes for RNA
- DNA template
- RNA polymerase
- RNA message
- mRNA
- Growing polypeptide chain
- Ribosome

RNA codes for proteins
- RNA
- mRNA
- Protein

not surprisingly, many points for regulation of the process
RNA codes for proteins

genetic code is very redundant

64 combinations of codons for 20 amino acids + 1 stop codon

redundancy typically in third base of codon

start codon is also Met - all proteins begin with this amino acid
Peptide bond formation - process takes place at the heart of the ribosome in its peptidyl transferase center (PTC)
ribosome structure

composed of two subunits, large and small

large: 23S + 5S rRNA + 31 proteins (bacteria)

small: 16S rRNA + 21 proteins (bacteria)

large + small: 50S + 30S = 70S?? (bacteria) or 60S + 40S = 80S?? (eukaryotes)

catalytic core formed at center, RNA only (ribozyme?)

2009 Nobel Prize (chemistry)
large subunit contains **exit tunnel** for polypeptide as it’s being made

some nascent proteins target the ribosome to the membrane, where they insert directly from tunnel to channel

**exit tunnel** prevents polypeptide from looping back on itself during synthesis

it’s also the site where a large class of antibiotics bind (macrolides)
The first high-resolution structures came in 2000.

We now have hundreds of structures of ribosomes in different conditions.

Structures from bacteria, archaea, yeast, fruit flies, humans, mitochondria (!), chloroplasts (!!), and others have been determined.

The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath "for studies of the structure and function of the ribosome."
Increase in ribosome complexity

Although main elements of ribosome are conserved (particularly the catalytic core), new features are layered on top in higher-order species for additional regulation.

Additions include:
- RNA expansion segments
- Longer existing proteins
- More ribosomal proteins

Increase in ribosome complexity

what defines a human?

Having the most complex ribosome! (according to Loren Williams)

Translation by the ribosome

Figure reproduced from: Schmeing and Ramakrishnan (2009) What recent ribosome structures have revealed about the mechanism of translation. *Nature.* 461:1234.
transfer RNA (tRNA)

Wobble base pairing (non-canonical) allows ~45 tRNAs to match 61 codons.

tRNAs bring each amino acid to the ribosome.

Peptide Synthesis

three sites: A, P, and E; peptide-bond formation happens at P.
molecular basis of fidelity

(ribosome) \[ R + T_{\text{corr}} \rightleftharpoons RT_{\text{corr}} \rightarrow \text{elongation} \]

(tRNA) \[ k_{c+} \]

\[
\frac{d[RT_{\text{corr}}]}{dt} = k_{c+}^c[R][T_{\text{corr}}] - k_{c-}^c[RT_{\text{corr}}] = 0
\]

Similar form for wrong tRNA (error), just with different rates

\[
\frac{p_{\text{err}}}{p_{\text{corr}}} = \frac{[RT_{\text{err}}]}{[RT_{\text{corr}}]} \approx \frac{K_{d_{\text{corr}}}}{K_{d_{\text{err}}}}
\]

thermodynamic specificity

correct vs. incorrect tRNA different by a single hydrogen bond (~ 2-4 \( kT \)), giving an error rate of 0.13

however, the known error rate is 0.0001, a factor of 1000 less!
High specificity requires energy

Additional proofreading step

\[ R + T_{\text{corr}} \rightleftharpoons RT_{\text{corr}} \rightleftharpoons RT^*_{\text{corr}} \rightarrow \text{elongation} \]

Energy-consuming proofreading step causes a conformational change that alters off rates, mainly by delaying accommodation of tRNA.

Proofreading carried out by GTP hydrolyzing EF-Tu, an elongation factor.

\[
f = \frac{[RT^*_\text{err}]}{[RT^*_\text{corr}]} \approx f_0 \frac{k_d}{p_d}
\]

Error rate is equal to original rate scaled by new off rates.

PBoC 21.5.1
The ribosome rocks

• Ratchet-like intersubunit rotation was imaged by cryo-EM [1,4], smFRET [3]
• P-site peptidyl-tRNA locks the ribosome [2]
• ratcheting moves tRNAs through A, P, and E sites (classical states); intermediate states known as hybrid
• ratcheting requires energy from GTP hydrolysis

Several nascent chains have been found to regulate their own translation at the ribosome.

Most examples identified thus far involve nascent chains that cause ribosome stalling under certain conditions.

There are known regulatory nascent chains in prokaryotes, eukaryotes, and viruses.

Some examples:
- cat, cmIA, ermC (regulation of expression of antibiotic resistance genes)
- SecM (sensor of protein translocation)
- TnaC (regulation of Trp degradation)

Stalling through binding of erythromycin + nascent peptide
ErmCL in exit tunnel

Regulation of the tna operon

Low [Trp]:
- Transcription termination by Rho factor
  ⇒ No expression of structural genes (tnaA/tnaB)

High [Trp]:
- Translational stalling leads to transcription antitermination
  ⇒ Induction of expression of structural genes (tnaA/tnaB)

TnaA
CAP = catabolite activating protein
RNA polymerase
Transcription initiates
Transcription pauses
Ribosomal subunits 50S 30S
Translation terminates
Transcription resumes
Rho factor
mRNA
TnaC + Trp stall the ribosome
Stop + Trp stall the ribosome
Translation terminates
High [Trp]


Discovered by Yanofsky and colleagues
SecM regulates expression of translocase SecA via a negative feedback loop. SecM is translated until the stalling point is reached. Transcription pauses, repressor helix unfolds, and secA is translated. SecA pulls (?) on SecM, permits translation to continue, and repressor helix refolds.
Closeup of SecM in the exit tunnel (MDFF-fitted structure)

- Hydrophobic interaction between R163 and A2062
- Base stacking between W155 and A751 at L4/L22 constriction site

Structure is static! We need dynamics to determine importance of observed interactions

R163 acts to restrain A2062

SecM compresses A2062 against tunnel wall, reducing its fluctuations

Role of A-site tRNA$^\text{Pro}$

tRNA$^\text{Pro}$ shifts downward when placed where A-site tRNA$^\text{Pro}$ is in stalled ribosome, reduces N-C bonding distance significantly

Gumbart et al. (2012)

*Biophys. J.* 103: 331-341.
Macrolides: Antibiotics That Target the Bacterial Ribosome

- Have mild side effects and broad spectrum
- Azithromycin is one of the most prescribed antibiotics
- Bind in the protein exit tunnel and prevent protein synthesis

Resistance due to ribosomal modifications is a growing problem

Novel azithromycin derivatives mimic SecM interactions

- Indole derivatives mimic π-stacking between W155 and A751
- Surprisingly, improved activity was only observed for the less polar derivatives

Summary

Protein synthesis, translation, is done by the ribosome.

During elongation process, codons of mRNA are matched with anti-codons of tRNA, carrying the correct amino acid.

The matching is very accurate due to additional proofreading by EF-Tu.

Some peptides can stall the ribosome during their translation, giving yet another way to control gene expression.