Central Dogma + Regulation

- tsx initiation control by transcription factors (TF)
- tsl initiation control by sRNA and RNA-binding proteins
- tsx termination control by anti-terminators (e.g., protein, sRNA)
- control of mRNA and protein degradation

Coupled to environmental signals

Genetic circuits utilize all these modes of regulation!
**Topic 3: Post-transcriptional control**

**A. Transcriptional elongation and termination**
1. kinetic model of tsx elongation and termination
2. mechanisms of termination (intrinsic vs rho-dependent)
3. regulation of anti-termination

**B. Protein synthesis and translational control**

**C. Protein degradation and post-translational control**

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**A. Transcriptional elongation and termination**
(post-tsx-initiation control)

- “normal” termination at end of an operon
- premature termination within or even at the beginning of an operon
  → control mechanism (antitermination)
1. kinetic model of tsx elongation/termination:

\[ \begin{align*}
\text{terminated} & \xrightarrow{k} \text{paused} \\
\text{paused} & \xrightarrow{1/\tau} \text{N-1} \\
\text{N-1} & \xrightarrow{v_0} \text{N} \\
\text{N} & \xrightarrow{v_0} \text{N+1}
\end{align*} \]

Termination efficiency

\[ T = \frac{1}{1 + \frac{v_0}{f} \left( \frac{1}{\tau k} \right)} = \frac{1}{1 + \frac{v_0}{f\tau k}} \]

normal site: \( v_0 \sim 100 \text{ s}^{-1} \) \( \tau \sim 1 \text{ s} \) \( f \sim 0.1 \text{ s}^{-1} \) \( k \leq 0.1 \text{ s}^{-1} \) \( \Rightarrow T < 10^{-4} \)

termination site: \( v_0 \sim f \sim 100 \text{ s}^{-1} \) \( \tau = 1 \sim 10 \text{ s} \) \( k = 1 \sim 5 \text{ s}^{-1} \) \( \Rightarrow T = 0.2 \sim 1 \)

Need for anti-termination (AT):

• long genes: prob. not terminating = \( (1 - T)^{\text{length}} \ll 1 \)
• regulation: extra layer of control for downstream transcription

2. Mechanisms of termination
(a) intrinsic termination

E. coli: 50% of mRNA have intrinsic terminators at their end
70% of non-coding RNA

optimal terminator:

• hairpin with GC-rich stem followed by runs of U’s
• 7-9nt separating hairpin and U’s
• downstream sequence inductive to TEC pausing
**Model of intrinsic termination**

- destabilization of TEC requires simultaneous disruption of HBS and RBS
  - hairpin formation displaces RNA from UBS (stimulated by NusA, part of TEC)
  - stretch of U’s provides weakest HBS
- pausing at terminator promotes hairpin formation and is essential for termination (depends on downstream sequence)

![Diagram of Model of intrinsic termination](image)

[Nudler & Gottesman, 2002]

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**(b) rho-dependent termination**

- rho binds to ~40nt stretch of unstructured, C-rich mRNA (≈ RUT)
- translocation of rho requires energy
- actual termination site not well-defined
  - up to 120nt distal to RUT
  - generally correlate with pause sites

![Diagram of (b) rho-dependent termination](image)
Polarity effect:
nonsense mutation affects tsx of downstream genes

- enforces tsx-tsl coupling → no tsx of untranslated mRNA
  (quality check on translation?)
  → similar effect may arise at on-set of starvation (a.a. shortage)
  or upon exposure to translation-inhibiting antibiotics?

Quantitative study of transcription processivity

- wildtype: processive
- stop codon: 50% loss per 500 bases
- similar but weaker effect from sub-inhibitory dose of translation-inhibiting drug (Cm)
Quantitative study of transcription processivity

[Quantitative data and analysis]

→ effective termination by rho requires tsx pause site following stop codon

3. Regulated anti-termination (diverse mechanisms)

(a) via protein-mRNA interaction

usually involves intrinsic terminator

alternative hairpin stabilized by RNA-binding protein BglG

positive feedback: increases the amplitude of fold-changes
3. Regulated anti-termination (diverse mechanisms)

General scheme: stabilize alternative mRNA 2nd structures

alt structure stabilized by complementary small RNA

alt structure stabilized by metabolite

(b) via coupling to translation ("translational attenuation")

e.g., trp operon of E.coli (biosynthesis of Trp)

Trp level high: ribosome fast → termination
Trp level low: ribosome stalls → antitermination
Topic 3: Post-transcriptional control

A. Transcriptional elongation and termination

B. Protein synthesis and translational control
   1. tRNA and the genetic code
   2. translational mechanisms (initiation, elongation, termination)
   3. translational control

C. Protein degradation and post-translational control
B. Intro to protein synthesis

1. tRNA and the Genetic code

<table>
<thead>
<tr>
<th>First base</th>
<th>Second base</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUU</td>
<td>UUC</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>UUA</td>
<td>UUG</td>
<td>Leucine</td>
</tr>
<tr>
<td>CCA</td>
<td>CGG</td>
<td>Arginine</td>
</tr>
<tr>
<td>GUG</td>
<td>GUU</td>
<td>Valine</td>
</tr>
</tbody>
</table>

The genetic code is triplet:

- **First base**
- **Second base**
- **Third base**
- **Amino Acid**

Secondary structure of tRNA<sub>Val</sub><sup>UAC</sup>

"Wobbled" pairing at the 3<sup>rd</sup> position

Wobble pairing at 3rd codon position:

- G-U pairs form at the third codon base
- Standard base pairs occur at all positions
- G-U wobble pairing occurs only at third codon position

Third bases have least meaning:

- UCU
- UCC
- UCA
- UCG
- AUU
- AUC
- ACG

Distinguishing the 3<sup>rd</sup> base involves tRNA base modification
strong codon bias exists even for those with single tRNA species?? (related to AT/GC bias?)

2. Translational mechanisms
(a) translational initiation

- dependence on distance between RBS and ATG

The complete SD sequence

A: SD-ATG spacing series D,

5'...TAAGAUGT............ATG

SD

D1: TCGA7GCGTCATATG
D2: TCGA7GCGTCATATG
D3: TCGA7GCGTCATATG
D4: TCGA7GCGTCATATG
D5: TCGA7GCGTCATATG
D6: TCGA7GCGTCATATG
D7: TCGA7GCGTCATATG
D8: TCGA7GCGTCATATG
D9: TCGA7GCGTCATATG
D10: TCGA7GCGTCATATG
D11: TCGA7GCGTCATATG
D12: TCGA7GCGTCATATG

- broad range of allowed spacing

• dependence on 5’-UTR sequence

\[ \Delta G^\text{p} \] (ribosomal protein S1)

30S + U \rightleftharpoons 30S\cdot U \rightarrow \text{translation}

\[ \Delta G_{30S}^\text{slow} \]

⇒ quantitatively accounted for by thermodynamics RNA pairing

⇒ translational efficiency is highly tunable; often explored in synthetic biology

⇒ but endogenous genes mostly have high translational efficiency (later)
(b) translational elongation

- **tRNA charging**
  - associates the correct a.a. to the tRNA
  - uses a dedicated tRNA synthetase for each a.a. (and all isoacceptors)
  - consumes ATP
  - aa-tRNA recognition not necessarily dependent on anticodon

- **formation of tRNA-aa•EF-TU•GFP ‘ternary complex’**
  - tRNA-aa unstable otherwise
  - almost all tRNA-aa present in ternary complex
  - large demand for EF-TU (~40kD)
  - most abundant protein in fast growing cells (~5x no. ribosomes; sets the total tRNA amount)

- **ribosomal incorporation of tRNA as ternary complex**
  - note: spends energy (GTP)
  - translocation via the help of EF-G again spends energy (GTP)

- **total energy:**
  4ATP/peptide bond
• translational accuracy?
  – translational error rate = $10^{-3}$ to $10^{-4}$
  – but thermo probab of base mismatch much larger

kinetic proof reading (Hopfield, Ninio)

spend energy to enhance specificity

$5\cdot10^{-3}$ to $5\cdot10^{-4}$

but thermo probab of base mismatch much larger

amento and Peptidyl transfer

Table 1. Parameters of aa-tRNA Discrimination

<table>
<thead>
<tr>
<th>Codon</th>
<th>Initial Selection</th>
<th>Procooming</th>
<th>k_equ/k_u + k_h</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUU</td>
<td>190 ± 20</td>
<td>2.0 ± 0.6</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>CUC</td>
<td>0.4 ± 0.1</td>
<td>3.25 ± 0.1</td>
<td>260 ± 40</td>
</tr>
</tbody>
</table>

~1000x discrimination against near-cognate!

(c) translational termination

release of peptide  recycling of ribosome  rescue of broken mRNA