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

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

A. Transcriptional elongation and termination
   1. Basic models of tsx elongation and termination
   2. mechanisms of termination (intrinsic vs rho-dependent)

B. Control of termination (=anti-termination or AT)
   1. AT at a single termination site (various mechanisms)
   2. processive AT (Q, N, Nus)

C. Translational mechanisms (initiation, elongation, termination)

D. Translational control
   1. RNA-binding protein
   2. riboswitch
   3. small regulatory RNA

E. Protein degradation and post-translational control
   1. proteolytic machinery
   2. protein unfolding
   3. substrate selection

• “normal” termination at end of an operon
• premature termination within or even at the beginning of an operon
→ control mechanism (antitermination)
1. Model of tsx elongation

- forward-track: energetically costly
- back-track: energetically neutral (even favored if tsx error occurs)

- bubble prefers A/T-rich stretches of DNA
  (AT: weaker basepair, DNA:DNA stronger than DNA:RNA hybrid)

energetics of tsx elongation

\[
\Delta G_{N,m} = \Delta G_{N,m}^{DNA} + \Delta G_{N,m}^{RNA-DNA hybrid} + \Delta G_{N,m}^{RNA binding}
\]

kinetics of tsx elongation:

- elemental pause
  -- freq: 1 every 10s
  -- duration: ~1s

- longer pauses
  -- freq: 1 every 100s
  -- duration: 1~10s

- pauses can be stabilized (and prolonged) by several mechanisms:

<table>
<thead>
<tr>
<th>Example</th>
<th>RNAP</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 +02 pause</td>
<td>human RNAPII</td>
<td>allows TAR formation at +02</td>
</tr>
<tr>
<td>ops pause</td>
<td>E. coli RNAP</td>
<td>recruitment of RsfA</td>
</tr>
<tr>
<td>his leader pause</td>
<td>E. coli RNAP</td>
<td>allows ribosome loading to synchronize attenuation control</td>
</tr>
<tr>
<td>Bulb his leader &amp; HIV-1 pauses</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adelman et al. 2002

Landick 2006
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)

[Nudler & Gottesman, 2002]

(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
• polarity: nonsense mutation affects the expression of downstream genes

- 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 tsx processivity

Dai, Zhu, TH (Nat Microb 2019)

T₀: IPTG

T₁

T₂

Glucose (DT: 45 min)

Glucose FA 0.25 µg/mL (DT: 200 min)

Glucose FA 0.1 µg/mL (DT: 100 min)

Glucose 8 µM Cm (DT: 140 min)

Position-dependent waiting time

x-intercept: (~10s) tsx init time

slope: tsx speed (47.6 nt/s)

Quantitative study of tsx processivity

Dai, Zhu, TH (Nat Microb 2019)
Quantitative study of tsx processivity

Dai, Zhu, TH (Nat Microb 2019)

- Similar effects for different drugs and starvation
- Gradual loss of tsx processivity: 50% loss per 1-2 kb
- Abrupt drop (~4x) at the end of lacZ gene

Slope vs. Probe location

Glucose
FA 0.1
FA 0.25
Cm8
C-Starvation
Quantitative study of tsx processivity

- similar effects for nonsense mutation (no physiological perturbation)
- 50% loss per 0.5 kb

Dai, Zhu, TH (Nat Microb 2019)

- effective termination by rho requires tsx pause site following stop codon
- intended function of rho: termination vs quality control
B. Control of termination: Anti-termination

- ~10% of genes in E. coli are controlled by AT
- two types:
  - control of a single termination site
  - processive AT (controls many terminators)
- many different mechanisms

1. AT at a single terminator
   (a) via protein-mRNA interaction
      (e.g., bgI operon)
   A

   B alternative structure

   usually involves intrinsic terminator

   alternative hairpin stabilized
   by RNA-binding protein BglG

   positive feedback: increases
   the amplitude of fold-changes
(b) via sRNA-mRNA interaction

**time window** for sRNA action

![Diagram showing sRNA-mRNA interaction](image)

[Gerhard Wagner lab]

(c) via small molecule-RNA interaction (riboswitch)

![Diagram showing small molecule-RNA interaction](image)
(d) 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

---

translational attenuation

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Table 16.1 Leader Peptides of Attenuator-Controlled Operons Containing Genes for Amino Acid Biosynthetics

<table>
<thead>
<tr>
<th>Operon</th>
<th>Amino Acid Sequence of Leader Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>Met Lys Ala Ile Phe Val Leu Lys Gyr Arg Thr Ser</td>
</tr>
<tr>
<td>Threonine</td>
<td>Met Lys Arg Val Ser Thr Thr Thr Thr Ser Thr Thr Ser Ser</td>
</tr>
<tr>
<td>Alanine</td>
<td>Met Thr Arg Val Gin Thr Thr Thr Thr Thr Thr Thr Thr Thr</td>
</tr>
<tr>
<td>Valine</td>
<td>Met Thr Gin Val Thr Thr Thr Thr Thr Thr Thr Ser Ser</td>
</tr>
<tr>
<td>Leucine</td>
<td>Met Sor His Ile Val Gin Gin Gin Gin Gin Gin Gin</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Met Lys His Ile Pro Val Pro Pro Pro Pro Pro Pro Pro</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Met Thr Thr Sor Met Sor Sor Sor Sor Sor Sor Sor</td>
</tr>
</tbody>
</table>

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very different implementation of the same ‘idea’ in *B. subtilis*

\[ \text{trp RNA-binding attenuation protein} \]
bound TRAP stabilizes terminator conformation
no TRAP bound: AT more stable

2. Processive antitermination
- requires special proteins (AT complex) which associate and travel with RNAP
- loading of ATC upstream of terminator
- can read through multiple terminators over many thousands of bases
- well-studied examples:
  - N and Q from phage lambda
  - rRNA operons

\[ \rightarrow \text{Q and N necessary for transcribing long operons (Q: 23,000nt)} \]
- N recruited by Nut site in RNA
- Q recruited by *qut* site in DNA

- mechanism: stabilizes paused TEC

For rRNA synthesis:
why need AT? why Rho?
AT of rRNA

- rRNA: no amplification by translation - one transcript one (RNA) product (cf mRNA ~20 protein products per transcript, transcription rate ~1/minute)
- demand for rRNA at fast growth:
  -- E. coli @ 20 min/doubling contains ~70,000 ribosomes/cell
  -- one rRNA transcript per ribosome
  -- must make 2100 rRNAs per minute
- 7 copies of \( rrn \) genes on chromosome (~36 copies @ 20 min/doubling)
  \[ \text{60~70 transcripts per operon per minute = one succ txs initiation/sec} \]
- size of RNAP = 30-50nt; elongation speed: 100 nt/s
  \[ \text{dense RNAP traffic; traffic jam?} \]

Traffic model of rRNA transcription

- RNAP = point particle on a 1d lattice
- makes forward steps with rate \( v_0 \) if site not occupied
  (Asymmetric Simple Exclusion Process
  – standard model for non-equilibrium phase transitions)

\[ J = v_0 \cdot \rho \cdot (1 - \rho) \]
\[ u \equiv J / \rho = v_0 \cdot (1 - \rho) \]
- maximum density \( \rho_{\text{max}} = 1/2 \)
- maximum tsx rate \( J_{\text{max}} = v_0 / 4 \)

\[ \text{tsx init attempt rate } \alpha : \rho \leftarrow \alpha / v_0 \text{ for } \alpha < \alpha_c \equiv \rho_{\text{max}} \cdot v_0 \]
\[ \text{avg tsx rate } J = \alpha \cdot (1 - \alpha / v_0) \text{ for } \alpha < \alpha_c \equiv \rho_{\text{max}} \cdot v_0 \]

\[ \text{due to asynchronous nature of RNAP movement} \]

[Klumpp & TH, PNAS 2009]

heuristic derivation:
let particle density = \( \rho \)
then avg transcription rate
avg elongation rate

[Derrida et al, 1993]
Traffic model of rRNA transcription

- RNAP = point particle on a 1d lattice
- makes forward steps with rate $v_0$ if site not occupied
  (Asymmetric Simple Exclusion Process
  – standard model for non-equilibrium phase transitions)

$L$-mer generalization
[Shaw, Zia, Lee, 2003]

$$v_0 \approx 100\text{nt/s}$$

meets physiological demand on rrn tsx rate: 60-70/min

\[ J(\alpha) = \frac{\alpha \cdot (v_0 - \alpha)}{v_0 + \alpha \cdot (L - 1)} \quad \text{for } \alpha < \alpha_c = \frac{v_0}{1 + \sqrt{L}} \]

with $J_{\text{max}} = \frac{v_0}{1 + \sqrt{L}} \approx 1.5 / \text{sec} = 90 / \text{min}$

\[ J_{\text{max}} = \frac{v_0}{1 + \sqrt{L}} \approx 1.5 \approx 90 / \text{min} \]

\[ \alpha \]

\[ v_0 \]

Traffic model of rRNA transcription

- meets physiological demand on rrn tsx rate: 60-70/min
- but not sufficient when including tsx pausing

avg tsx rate

due to traffic jams!

\[ J(\alpha) = \frac{\alpha \cdot (v_0 - \alpha)}{v_0 + \alpha \cdot (L - 1)} \quad \text{for } \alpha < \alpha_c = \frac{v_0}{1 + \sqrt{L}} \]

\[ J_{\text{max}} = \frac{v_0}{1 + \sqrt{L}} \approx 1.5 / \text{sec} = 90 / \text{min} \]
Ribosomal antitermination

• ribosomal AT: similar to N AT, RNA sequences recruits a complex of Nus factors + r-proteins
• suppresses rho-dependent, but not intrinsic termination

• ribosomal AT suppresses pauses
  → increases maximal transcription rate

<table>
<thead>
<tr>
<th></th>
<th>without AT</th>
<th>with AT</th>
<th>fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>elongation rate</td>
<td>52 nt/s (*)</td>
<td>80 nt/s</td>
<td>1.5</td>
</tr>
<tr>
<td>pause duration</td>
<td>1.16 s</td>
<td>0.26 s</td>
<td>4.5</td>
</tr>
<tr>
<td>tsx rate</td>
<td>38.5 min(^{-1})</td>
<td>76 min(^{-1})</td>
<td>2</td>
</tr>
</tbody>
</table>

max growth  30 min/dbl  20 min/dbl

⇒ important function of AT for rrrn is anti-pausing

Ribosomal antitermination

[Klumpp & TH, PNAS 2009]

• the puzzle of rho-dependent termination on rRNA genes: possible function?
  -- AT is not 100% efficient
  -- a few non-AT\(^{-}\)d RNAPs can cause traffic jams

⇒ rho-dependent termination removes slow non-AT\(^{-}\)d RNAPs;
⇒ restores RNAP traffic for small fraction of non-AT
Final remarks

- many factors control elongation
- Termination/AT often regulates gene expression

- elongation/termination coupled

- role of elongation control less clear
- appear often to have multiple functions

- clear case: dense RNAP traffic on rrn, elongation control enables high tsx rates