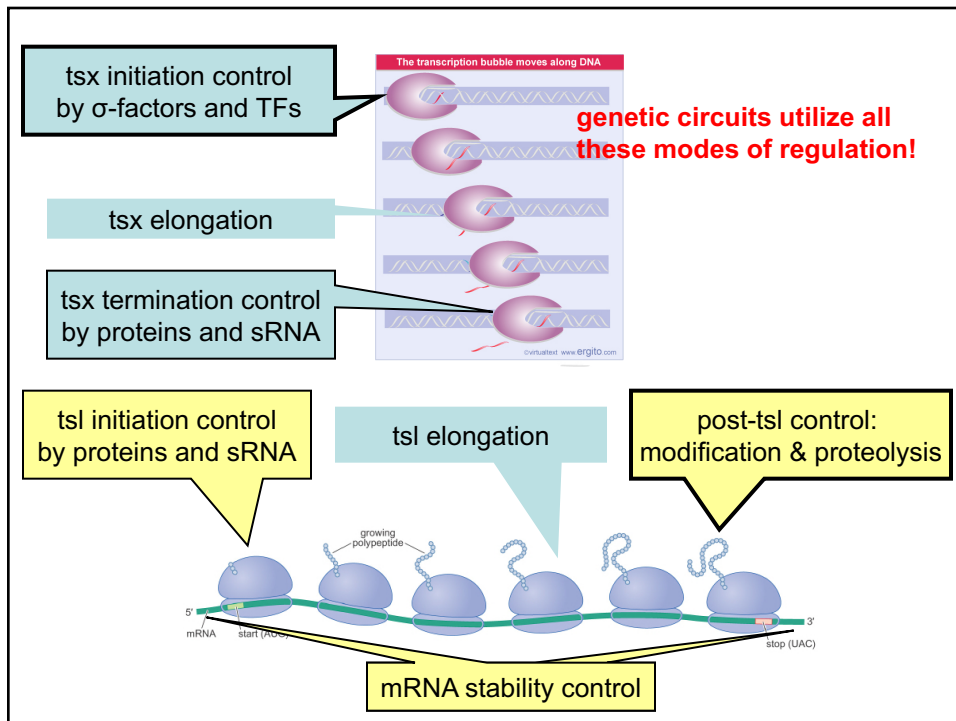


1



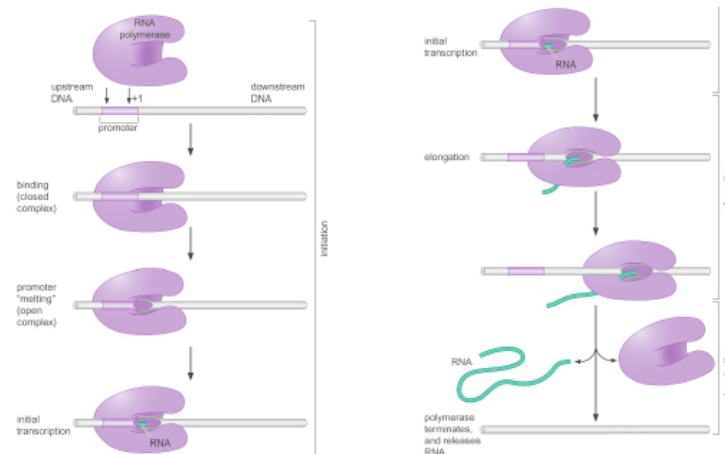
2

### 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

3

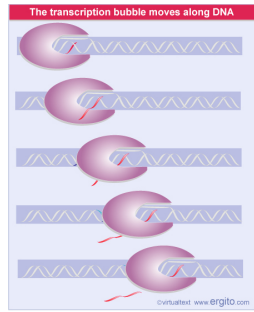
#### A. Transcriptional elongation and termination (post-tsx-initiation control)



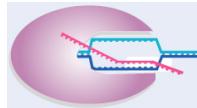
- “normal” termination at end of an operon
- **premature termination** within or even at the beginning of an operon  
→ control mechanism (antitermination)

4

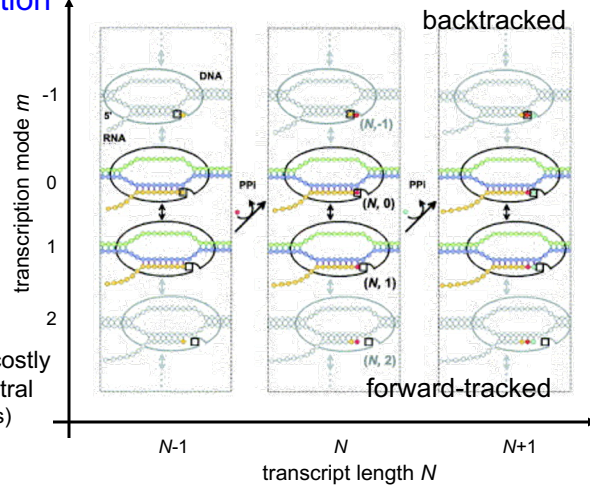
# 1. Model of tsx elongation



- forwardtrack: energetically costly
- backtrack: 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)



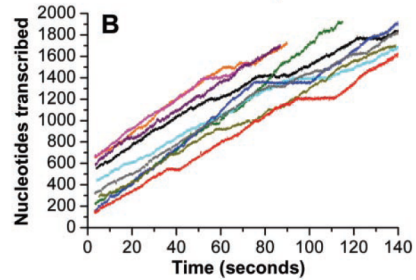
## energetics of tsx elongation

$$\Delta G_{N,m} = \Delta G_{N,m}; \text{DNA bubble} + \Delta G_{N,m}; \text{RNA-DNA hybrid} + \Delta G_{N,m}; \text{RNAP binding}$$

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## kinetics of tsx elongation:

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



Adelman et al. 2002

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

Stabilized Pause	Backtrack-Stabilized	Regulator-Stabilized	Hairpin-Stabilized	Downstream DNA-Stabilized
<b>Example</b>	HIV-1 +62 pause	ops pause	his leader pause	Both his leader & HIV-1 pauses
<b>RNAP</b>	human RNAPII	E. coli RNAP	E. coli RNAP	
<b>Function</b>	allows TAR formation at +62	recruitment of RfaH	allows ribosome loading to synchronize attenuation control	

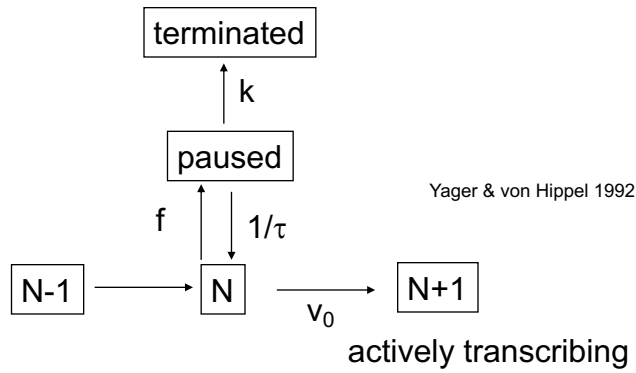
typ. few-tens sec.

in vitro also arrest

Landick 2006

6

kinetic model:



Termination efficiency  $T = \frac{1}{1 + \frac{v_0}{f} \left(1 + \frac{1}{\tau \cdot k}\right)} \approx \frac{1}{1 + \frac{v_0}{f \tau \cdot k}}$

'normal' site:  $v_0 \sim 100/s$ ,  $f \sim 0.1/s$ ,  $\tau \sim 1s$ ,  $k < 0.1/s$        $T < 10^{-4}$

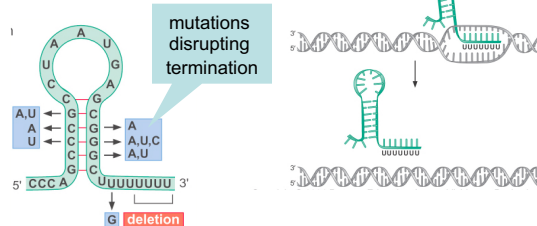
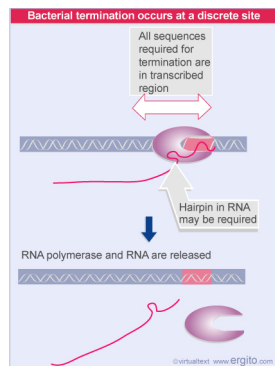
termination site:  $v_0 \sim f \sim 100/s$ ,  $\tau \sim (1-10) s$ ,  $k \sim 1-5/s$        $T \sim 0.2-1$

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## 2. Mechanisms of termination

### (a) intrinsic termination

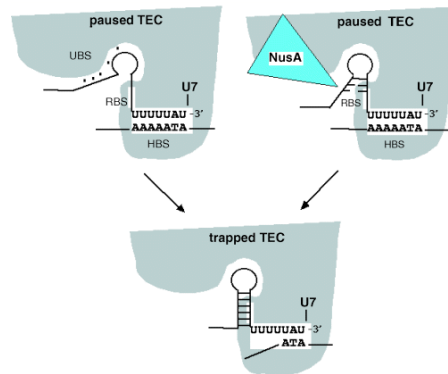
*E. coli*: 50% of mRNA have intrinsic terminators at their end  
70% of non-coding RNA



- 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

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## Model of intrinsic termination

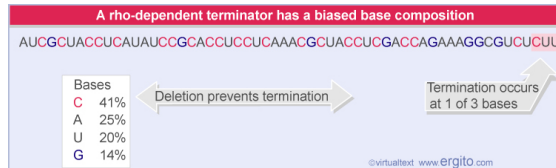
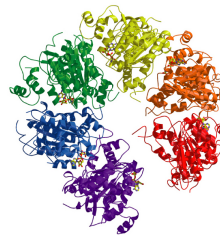
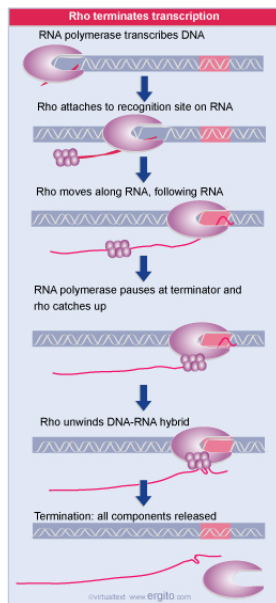


[Nudler & Gottesman, 2002]

- 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)

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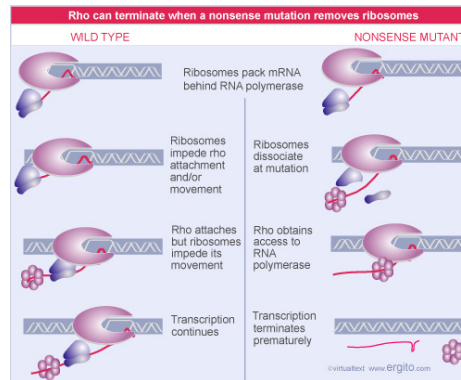
## (b) rho-dependent termination



- 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

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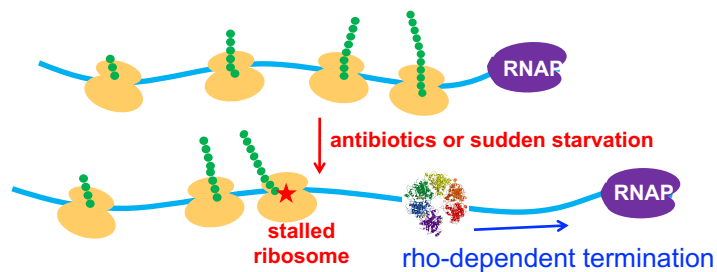
- polarity: nonsense mutation affects the expression of downstream genes



- 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?

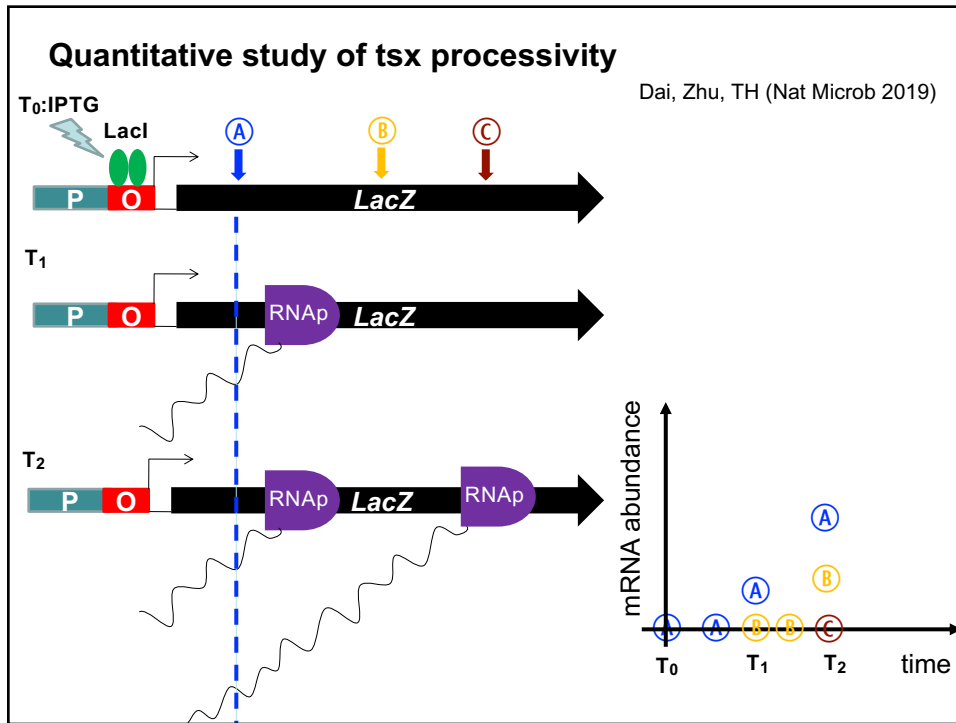
11

- polarity: nonsense mutation affects the expression of downstream genes

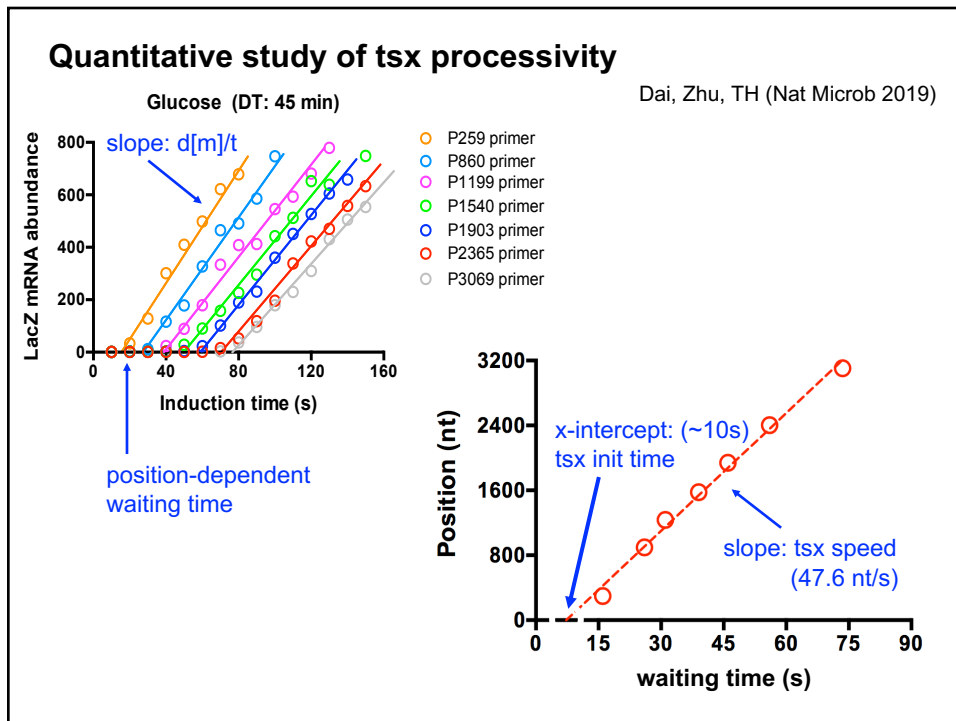


- tsx-tsl coupling → no tsx of untranslated mRNA  
(quality check on translation?)
- similar effect may arise at on-set of starvation (a.a. shortage) or upon exposure to translation-inhibiting antibiotics?

12



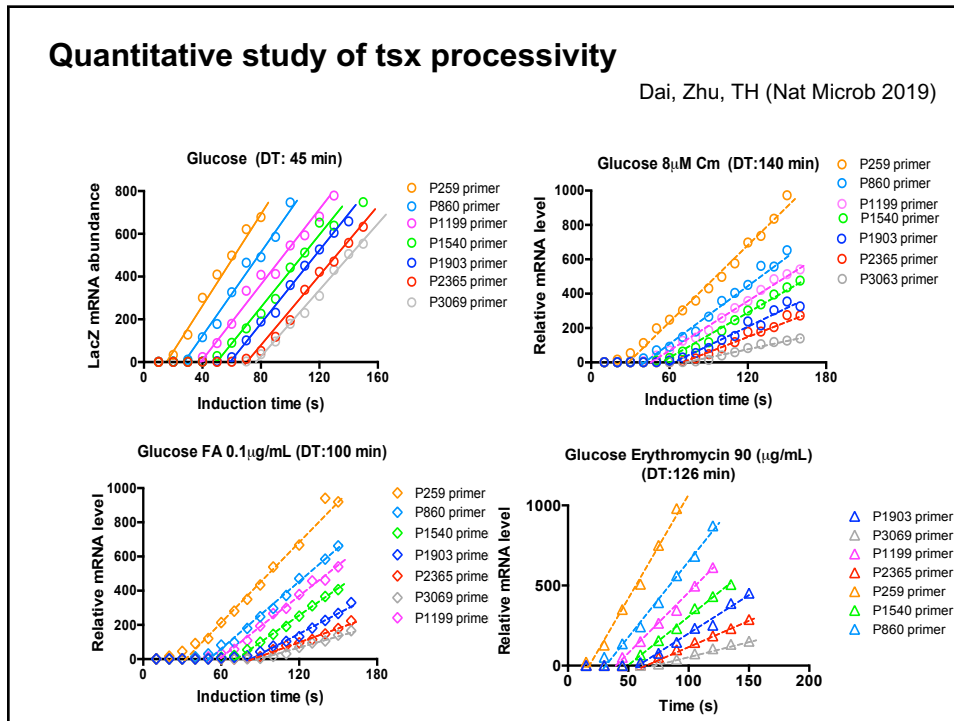
13



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## Quantitative study of tsx processivity

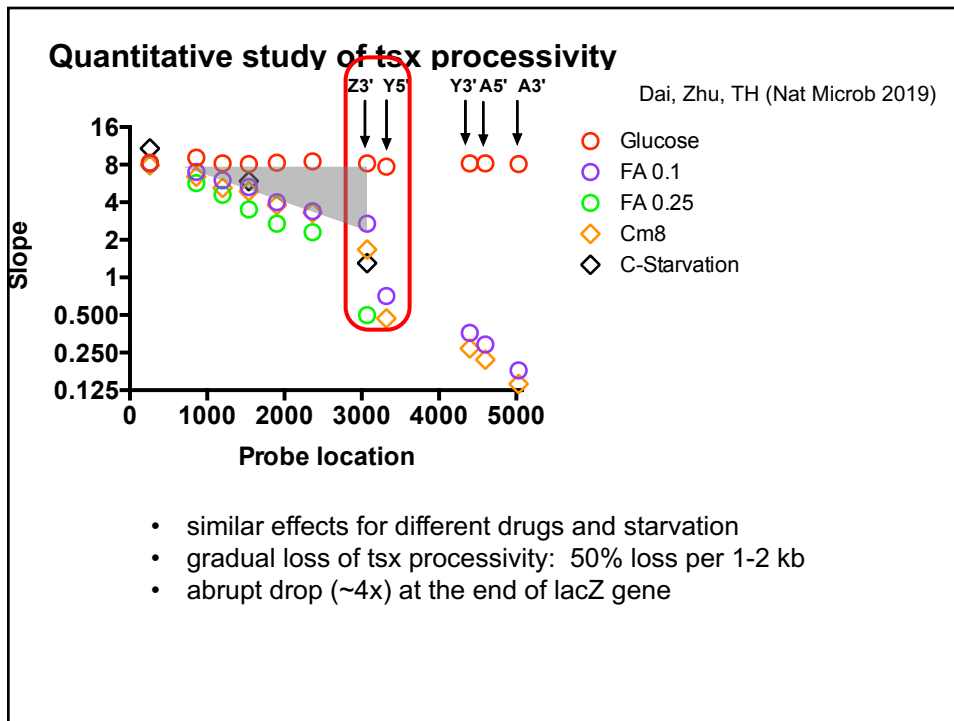
Dai, Zhu, TH (Nat Microb 2019)



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## Quantitative study of tsx processivity

Dai, Zhu, TH (Nat Microb 2019)



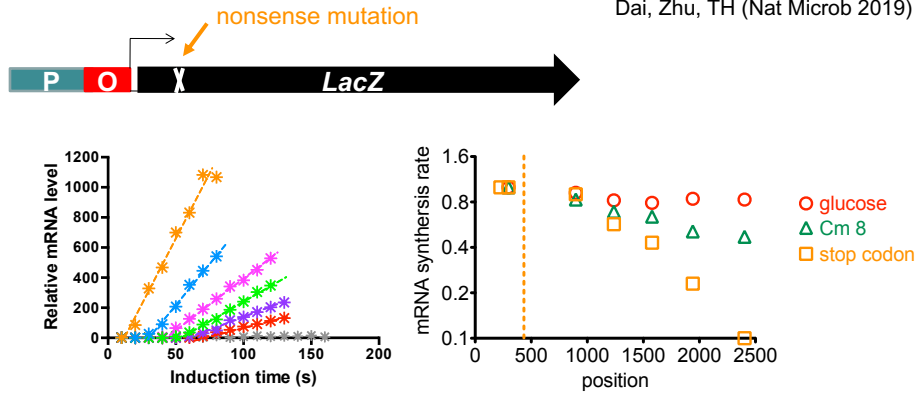
- similar effects for different drugs and starvation
- gradual loss of tsx processivity: 50% loss per 1-2 kb
- abrupt drop (~4x) at the end of lacZ gene

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## Quantitative study of tsx processivity

Dai, Zhu, TH (Nat Microb 2019)

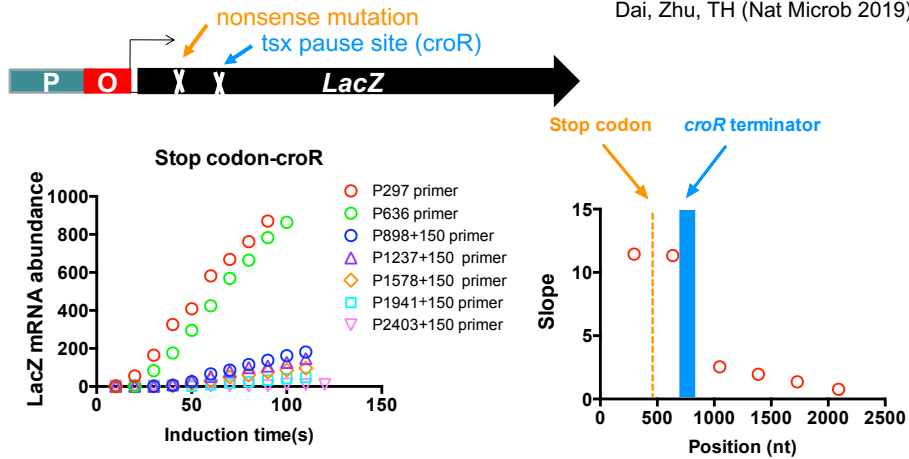


- similar effects for nonsense mutation (no physiological perturbation)
- 50% loss per 0.5 kb

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## Quantitative study of tsx processivity

Dai, Zhu, TH (Nat Microb 2019)



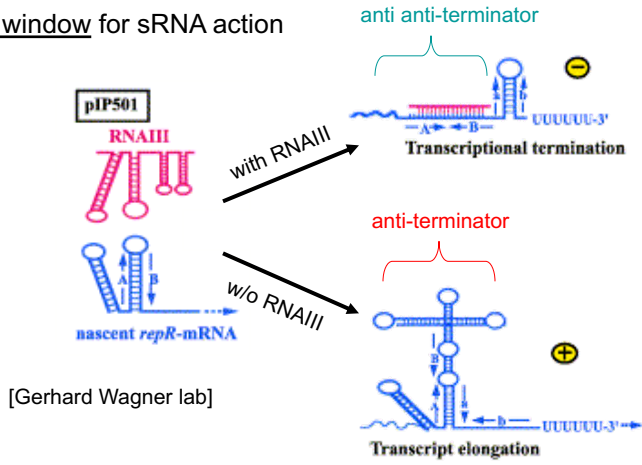
- ➔ effective termination by rho requires tsx pause site following stop codon
- ➔ intended function of rho: termination vs quality control

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(b) via sRNA-mRNA interaction

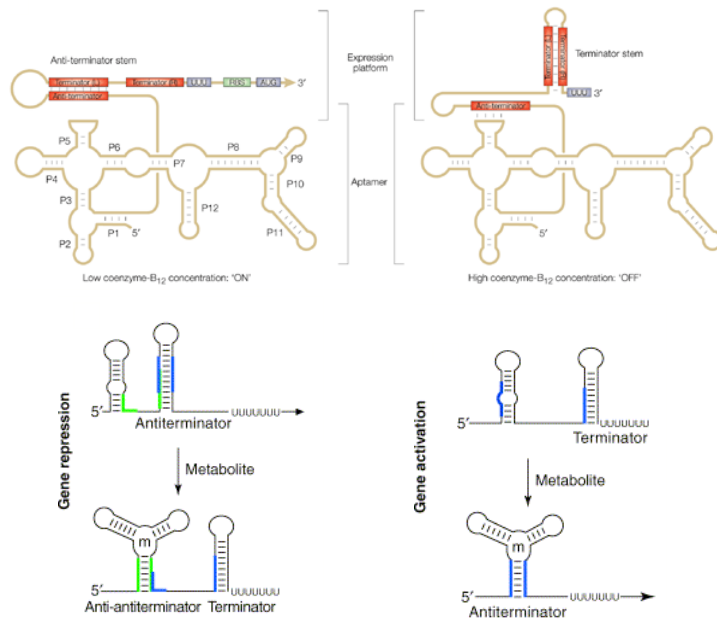
time window for sRNA action



[Gerhard Wagner lab]

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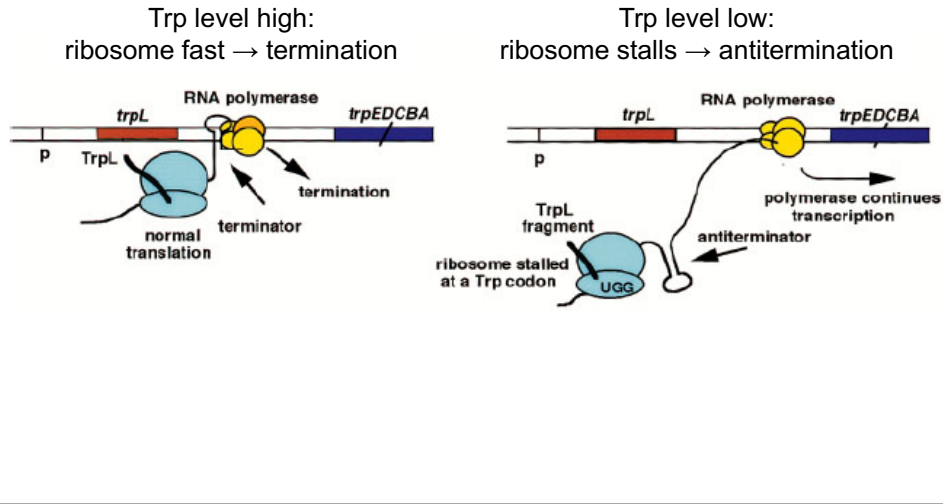
(c) via small molecule-RNA interaction (riboswitch)



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(d) via coupling to translation (= translational attenuation)

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



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### translational attenuation

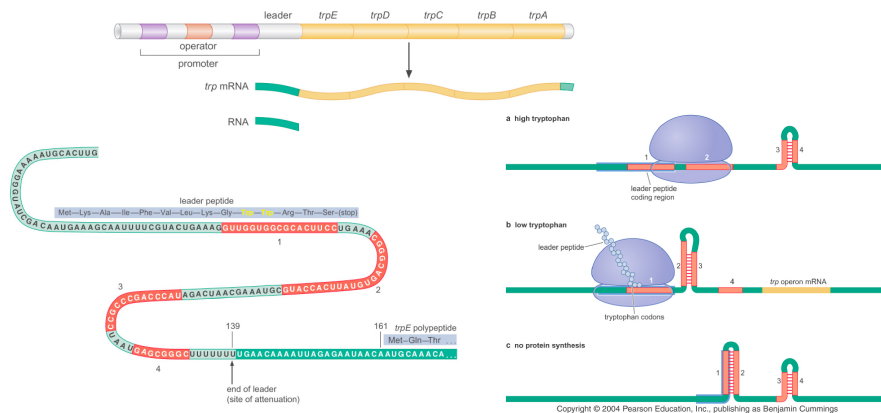
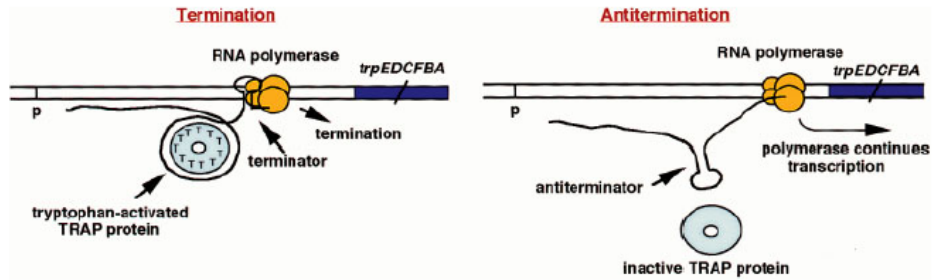


TABLE 16-1 Leader Peptides of Attenuator-Controlled Operons Containing Genes for Amino Acid Biosynthesis\*

Operon	Amino Acid Sequence of Leader Peptides															
Tryptophan	Met	Lys	Ala	Ile	Phe	Val	Leu	Lys	Gly	Trp	Trp	Arg	Thr	Ser		
Threonine	Met	Lys	Arg	Ile	Ser	Thr	Thr	Ile	Thr	Thr	Thr	Ile	Thr	Ile	Thr	Thr
Histidine	Met	Thr	Arg	Val	Gln	Phe	Lys	His	His	His	His	His	His	His	Pro	Asp
Isoleucine-valine GEDA	Met	Thr	Ala	Leu	Leu	Arg	Val	Ile	Ser	Leu	Val	Val	Ile	Ser	Val	Val
				Pro	Pro	Cys	Gly	Ala	Ala	Leu	Gly	Arg	Gly	Lys	Ala	
Leucine	Met	Ser	His	Ile	Val	Arg	Phe	Thr	Gly	Leu	Leu	Leu	Leu	Asn	Ala	Phe
				Val	Gly	Gly	Ile	Gln	His							Ile
Phenylalanine	Met	Lys	His	Ile	Pro	Phe	Phe	Phe	Ala	Phe	Phe	Phe	Thr	Phe	Pro	
Isoleucine-valine B	Met	Thr	Thr	Ser	Met	Leu	Asn	Ala	Lys	Leu	Leu	Pro	Thr	Ala	Pro	Ser
				Val	Arg	Val	Val	Val	Val	Val	Gly	Asn	Ala	Pro		

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

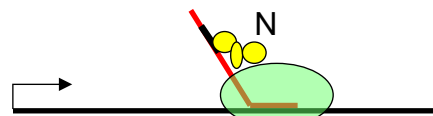


trp RNA-binding attenuation protein  
 bound TRAP stabilizes terminator conformation  
 no TRAP bound: AT more stable

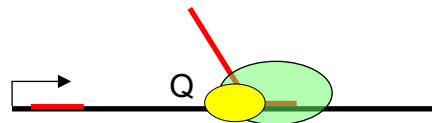
25

## 2. Processive antitermination → Q and N necessary for transcribing long operons (Q: 23,000nt)

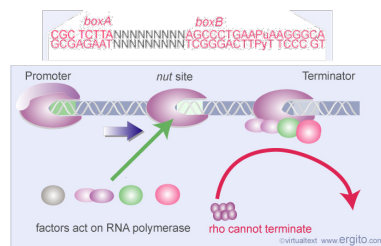
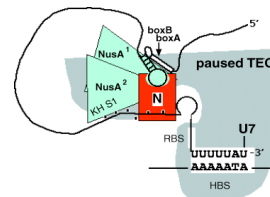
- requires special proteins (AT complex) which associate and travel with RNAP
- loading of ATC upstream of terminator
- can read through multiple terminators over many thousands of bases
- well-studied examples:
  - N and Q from phage lambda
  - rRNA operons



- Q recruited by *qut* site in DNA



- mechanism: stabilizes paused TEC



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Q and N control long operons (Q: 23 kb !!!)

→ probability of spontaneous termination ?

estimate TEC stability using the model of Yager & von Hippel

- termination efficiency at normal site  $\sim 5 \times 10^{-4}$   
(possibly higher in vivo due to rho)
- normal operon (length < 5000 nt): 80 % of initiated transcripts reach end (if no internal termination sites)
- lambda late operon (23000 nt): 32 %

Q: 10-fold stabilization: restored to 90%

→ AT: control of operon + adaptation of TEC to operon length