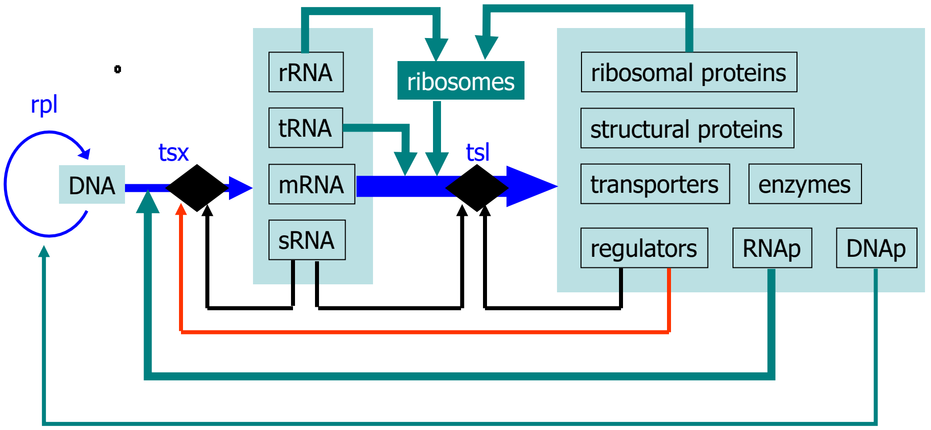
central dogma + regulation



rpl

DNA

tsx

rRNA

tRNA

mRNA

sRNA

ribosomes

tsl

ribosomal proteins

structural proteins

transporters

enzymes

regulators

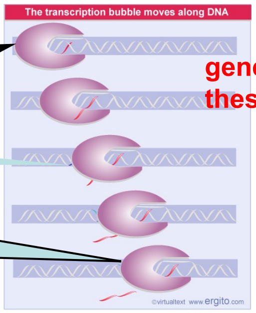
RNAp

DNAp

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

} coupled to environmental signals

tsx initiation control by σ-factors and TFs



genetic circuits utilize all these modes of regulation!

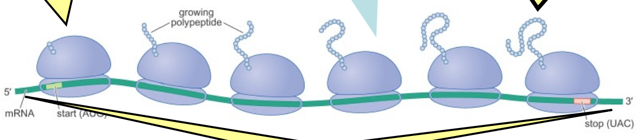
tsx elongation

tsx termination control by proteins and sRNA

tsl initiation control by proteins and sRNA

tsl elongation

post-tsl control: modification & proteolysis



mRNA stability control

Topic 3: Post-transcriptional control

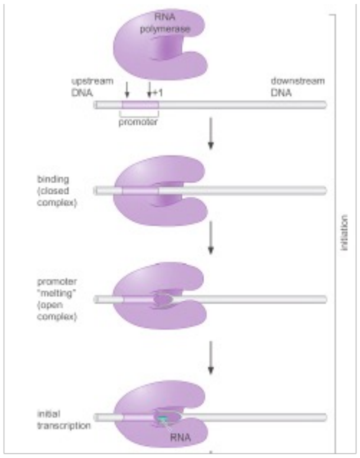
A. Transcriptional elongation and termination
  1. kinetic model of tsx elongation and termination
  2. mechanisms of termination (intrinsic vs rho-dependent)
  3. regulation of anti-termination

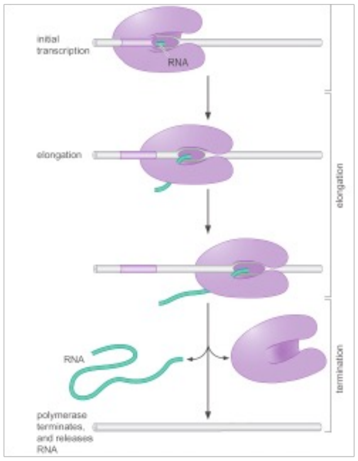
B. Protein synthesis and translational control

C. Protein degradation and post-translational control

A. Transcriptional elongation and termination

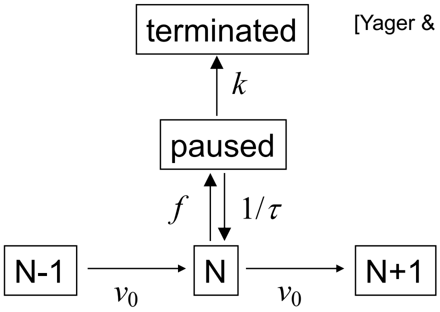
(post-tsx-initiation control)





- "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:



[Yager & von Hippel 1992]

Termination efficiency

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

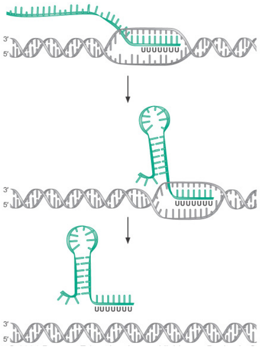
normal site: $v_0 \sim 100\ s^{-1}$ $\tau \sim 1\ s$ $f \sim 0.1\ s^{-1}$ $k \lesssim 0.1\ s^{-1}$ $\Rightarrow\ T < 10^{-4}$

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

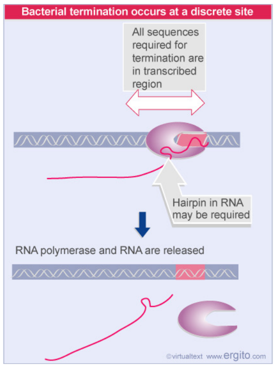
Need for anti-termination (AT):

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

2. Mechanisms of termination
(a) intrinsic termination



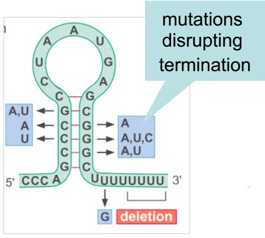
E. coli: 50% of mRNA have intrinsic terminators at their end
70% of non-coding RNA


Bacterial termination occurs at a discrete site

All sequences required for termination are in transcribed region

Hairpin in RNA may be required

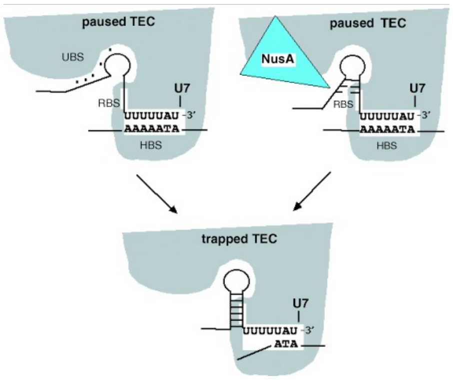
RNA polymerase and RNA are released


mutations disrupting termination

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

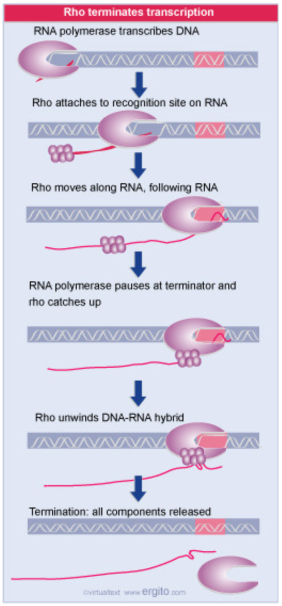
Model of intrinsic termination

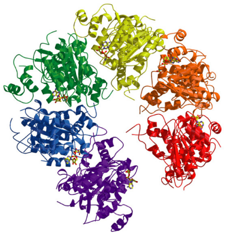


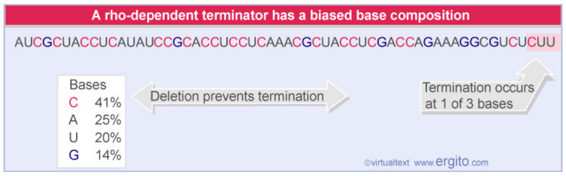
[Nudler & Gottesman, 2002]

- 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)

(b) rho-dependent termination







A rho-dependent terminator has a biased base composition

AUCGCUACCUCAUAUCCGCACCUCCUCAAACGCUACCUCGACCAGAAAGGCGUCUCUU

Bases	
C	41%
A	25%
U	20%
G	14%

Deletion prevents termination

Termination occurs at 1 of 3 bases

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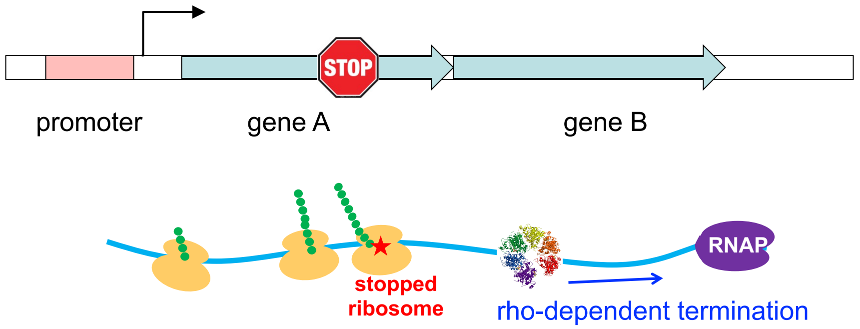
- rho binds to ~40nt stretch of unstructured, C-rich mRNA (= RUT)
- translocation of rho requires energy
- actual termination site not well-defined
  - up to 120nt distal to RUT
  - generally correlate with pause sites

Polarity effect:

nonsense mutation affects tsx of downstream genes

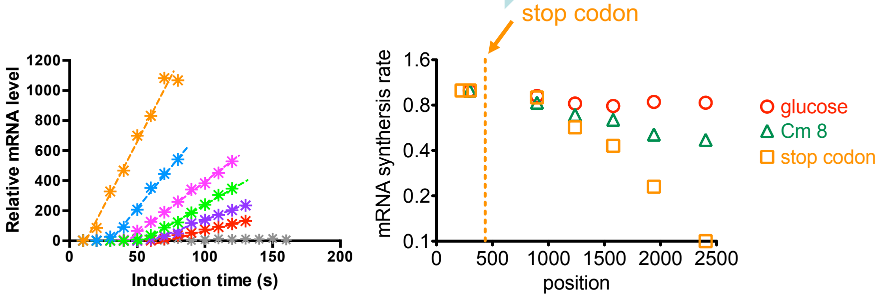


- enforces tsx-tsl coupling → no tsx of untranslated mRNA
  (quality check on translation?)

→ similar effect may arise at on-set of starvation (a.a. shortage) or upon exposure to translation-inhibiting antibiotics?

Quantitative study of transcription processivity

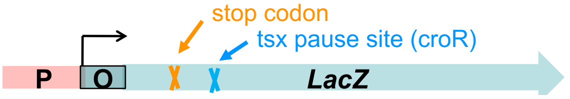
[Dai, Zhu, TH (Nat Microb 2019)]

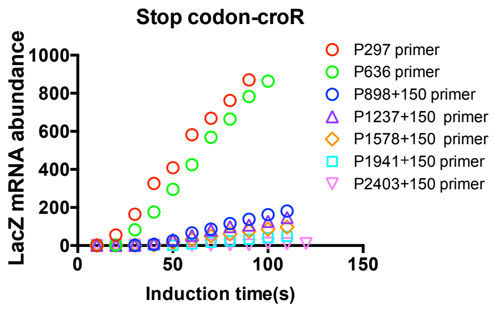


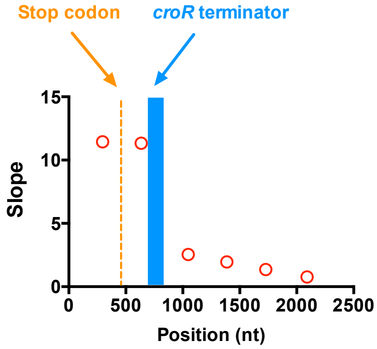
- wildtype: processive
- stop codon: 50% loss per 500 bases
- similar but weaker effect from
  sub-inhibitory dose of translation-inhibiting drug (Cm)

Quantitative study of transcription processivity

[Dai, Zhu, TH (Nat Microb 2019)]





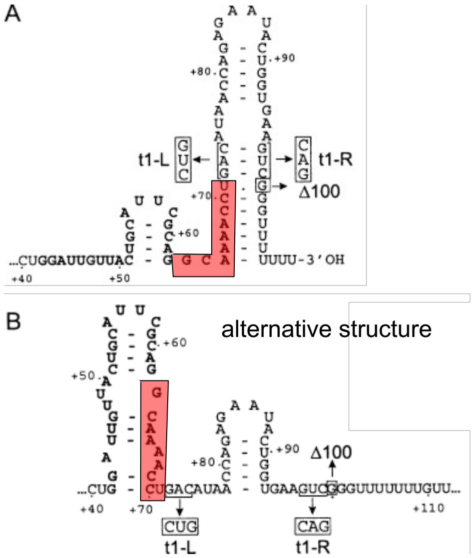


➔ effective termination by rho requires tsx pause site following stop codon

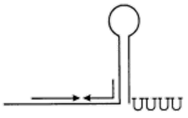
3. Regulated anti-termination (diverse mechanisms)

(a) via protein-mRNA interaction

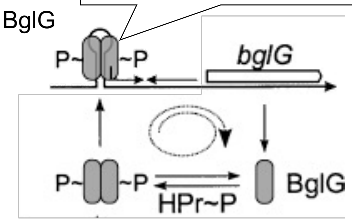
(e.g., bgl operon)



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)

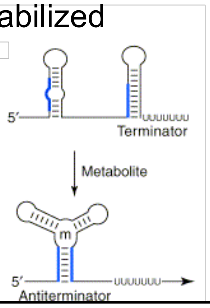
General scheme: stabilize alternative mRNA 2nd structures

default
alternative

alternative
default

alt structure stabilized
by complementary small RNA

alt structure stabilized
by metabolite

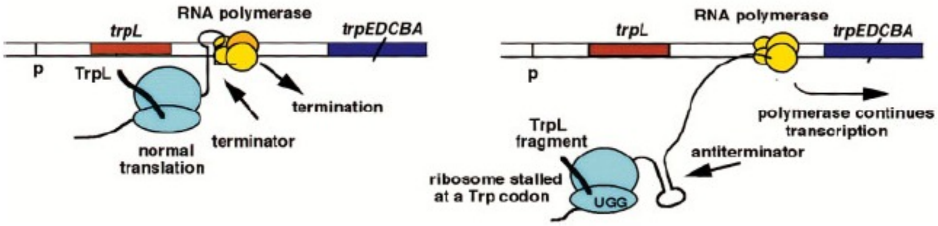


(b) via coupling to translation ("translational attenuation")

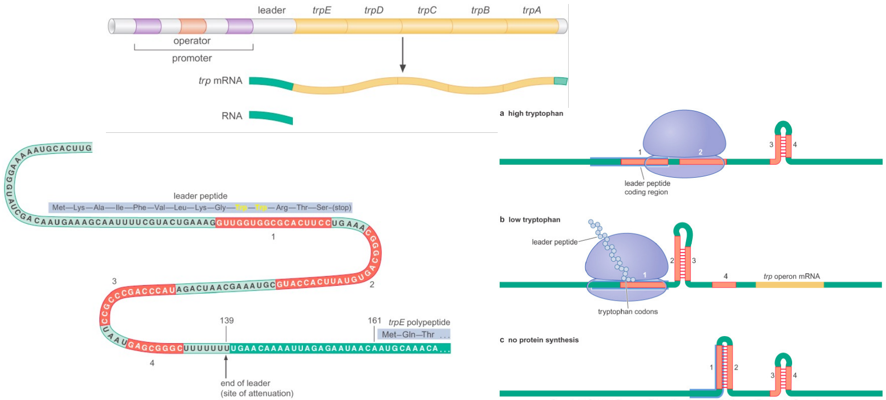
e.g., trp operon of E.coli (biosynthesis of Trp)

Trp level high:
ribosome fast → termination

Trp level low:
ribosome stalls → antitermination



translational attenuation



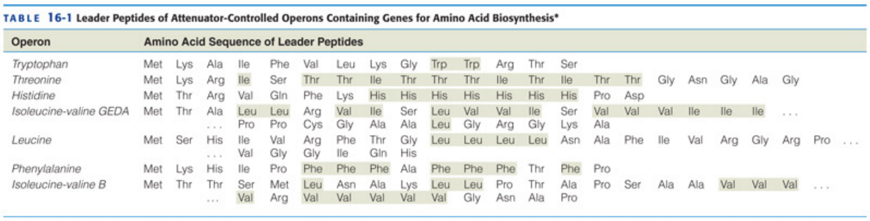


TABLE 16-1 Leader Peptides of Attenuator-Controlled Operons Containing Genes for Amino Acid Biosynthesis*

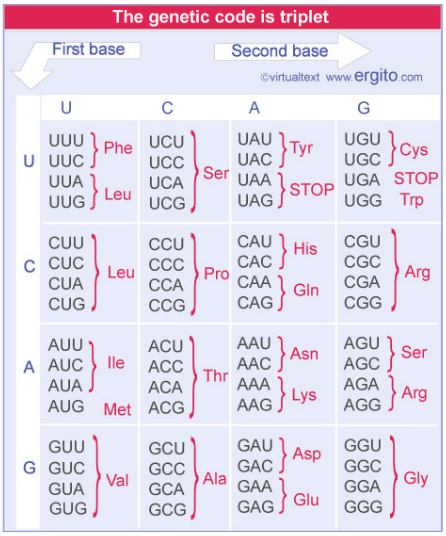
Operon	Amino Acid Sequence of Leader Peptides
Tryptophan	Met Lys Ala Ile Phe Val Leu Lys Gly Trp Trp Arg Thr Ser
Threonine	Met Lys Arg Ile Ser Thr Thr Ile Thr Thr Thr Ile Thr Ile Thr Thr Gly Asn Gly Ala Gly
Histidine	Met Thr Arg Val Gln Phe Lys His His His His His His His Pro Asp
Isoleucine-valine GEDA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

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



The genetic code is triplet

First base / Second base

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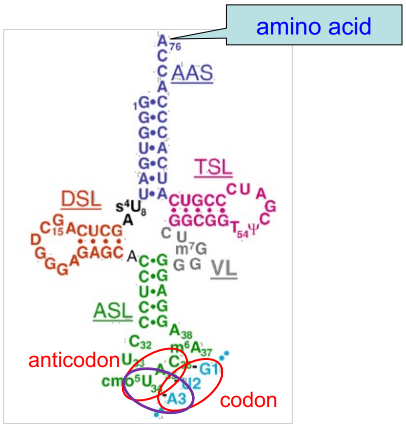
First base	U	C	A	G
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA STOP UAG STOP	UGU Cys UGC Cys UGA STOP UGG Trp
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly

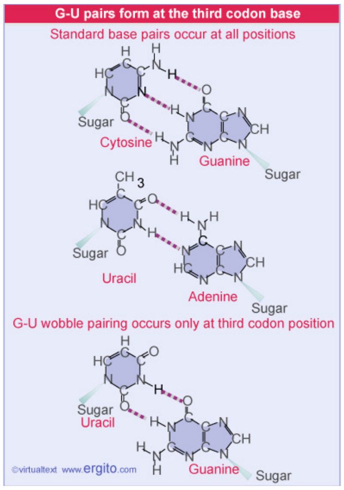
secondary structure of $\text{tRNA}^{\text{Val}}_{\text{UAC}}$



"wobbled" pairing at the 3rd position

---

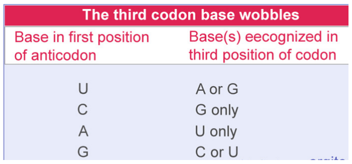
wobble pairing at 3rd codon position



G-U pairs form at the third codon base

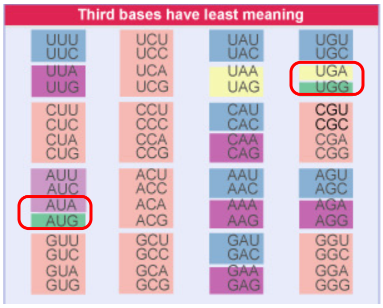
Standard base pairs occur at all positions

G-U wobble pairing occurs only at third codon position



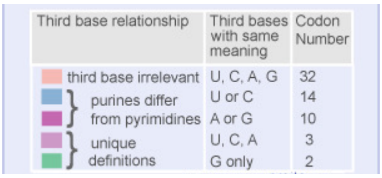
The third codon base wobbles

Base in first position of anticodon	Base(s) eecognized in third position of codon
U	A or G
C	G only
A	U only
G	C or U



Third bases have least meaning

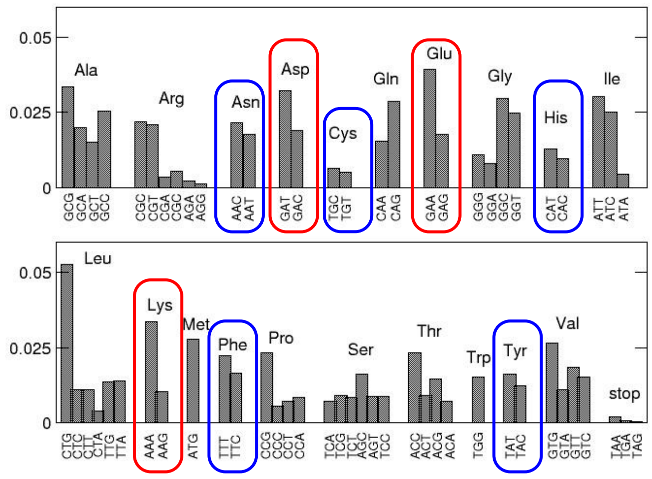
UUU UUC UUA UUG	UCU UCC UCA UCG	UAU UAC UAA UAG	UGU UGC UGA UGG
CUU CUC CUA CUG	CCU CCC CCA CCG	CAU CAC CAA CAG	CGU CGC CGA CGG
AUU AUC AUA AUG	ACU ACC ACA ACG	AAU AAC AAA AAG	AGU AGC AGA AGG
GUU GUC GUA GUG	GCU GCC GCA GCG	GAU GAC GAA GAG	GGU GGC GGA GGG



Third base relationship	Third bases with same meaning	Codon Number
🟧 third base irrelevant	U, C, A, G	32
🟦 purines differ from pyrimidines	U or C	14
🟪 purines differ from pyrimidines	A or G	10
🟪 unique definitions	U, C, A	3
🟩 unique definitions	G only	2

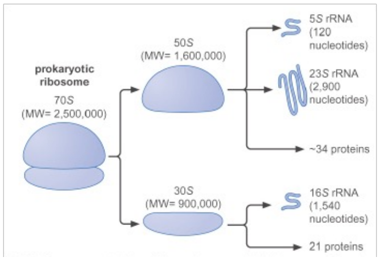
→ distinguishing the 3rd base involves tRNA base modification

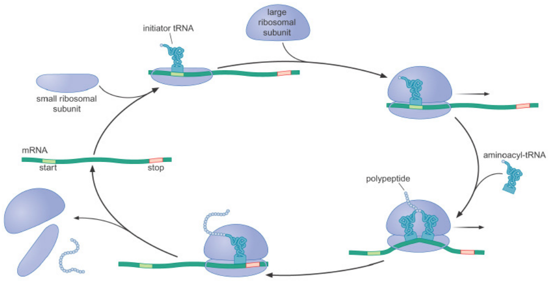
Codon frequency in the E. coli genome

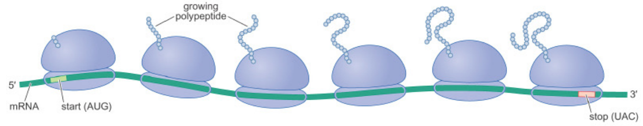


strong codon bias exists even for those with single tRNA species??
(related to AT/GC bias?)

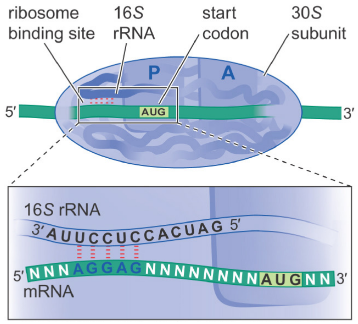
2. Translational mechanisms

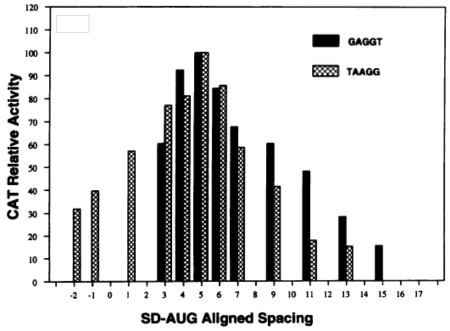






(a) translational initiation





- broad range of allowed spacing

- dependence on distance between RBS and ATG

The complete SD sequence

A: SD-ATG spacing series D_n

5′...**TAAGGAGGT**'..............ATG

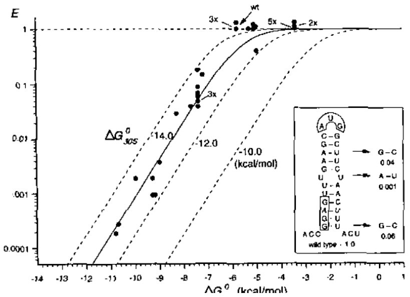
	SD
D_3	TCGAT**GAGGT**CATATG
D_4	TCGAT**GAGGT**CCATATG
D_5	TCGAT**GAGGT**CACATATG
D_6	TCGAT**GAGGT**CTTCATATG
D_7	TCGAT**GAGGT**CATTCATATG
D_9	TCGAT**GAGGT**CATTATCATATG
D_{11}	TCGAT**GAGGT**CTTATTATCATATG
D_{13}	TCGAT**GAGGT**CTATTATTATCATATG
D_{15}	TCGAT**GAGGT**CATTATTATTATCATATG

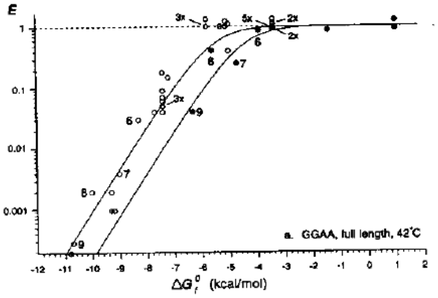
	SD
d_2	TCGAT**TAAGG**CTATG
d_3	TCGAT**TAAGG**CATATG
d_5	TCGAT**TAAGG**CACATATG
d_7	TCGAT**TAAGG**CATTCATATG
d_8	TCGAT**TAAGG**CATTACATATG
d_9	TCGAT**TAAGG**CATTATCATATG
d_{10}	TCGAT**TAAGG**CTATTATCATATG
d_{11}	TCGAT**TAAGG**CTTATTATCATATG
d_{13}	TCGAT**TAAGG**CTATTATTATCATATG
d_{15}	TCGAT**TAAGG**CATTATTATTATCATATG
d_{17}	TCGAT**TAAGG**CATATTATTATTATCATATG

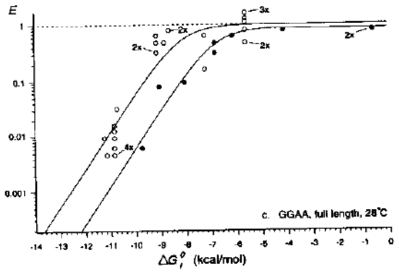
- dependence on 5’-UTR sequence

$$\begin{tikzcd} & \mathbf{F} \arrow[d, shift left, "\Delta G^0_f"'] & & \\ \mathbf{30S + U} \arrow[r, shift left] & \mathbf{30S \cdot U} \arrow[u, shift left] \arrow[l, shift left, "\Delta G^0_{30S}"] \arrow[r, "slow"'] & \mathbf{translation} \end{tikzcd}$$

(ribosomal protein S1)



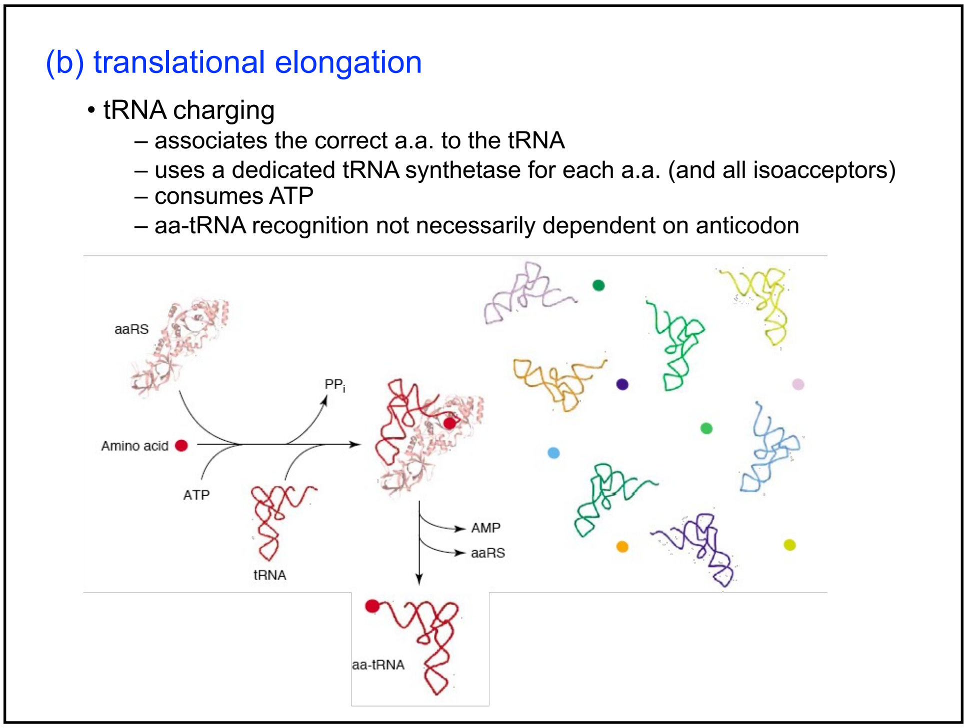




➔ quantitatively accounted for by thermodynamics RNA pairing

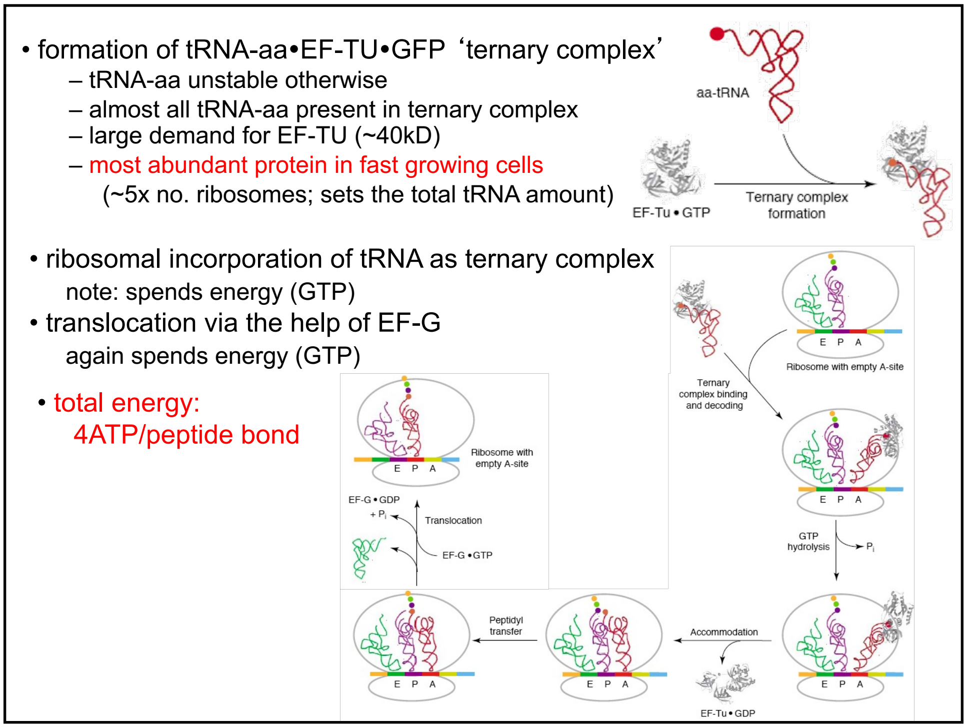
➔ translational efficiency is highly tunable; often explored in synthetic biology

➔ 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

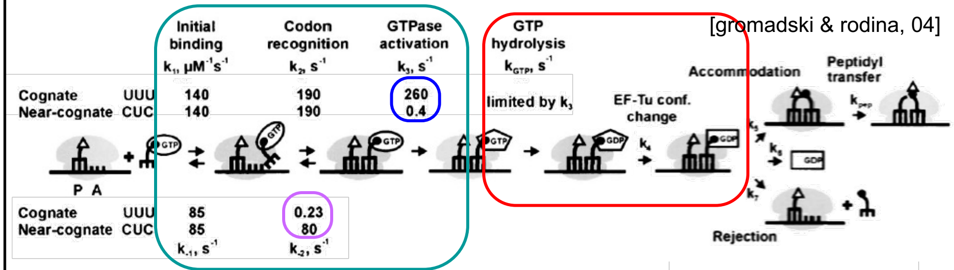


Table 1. Parameters of aa-tRNA Discrimination

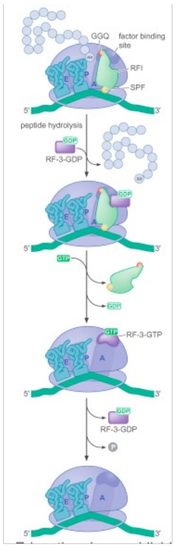
	Initial Selection			Proofreading
Codon	k_{cat}	K_M	k_{cat}/K_M	$k_5/(k_5+k_7)$
UUU	190 ± 20	2.0 ± 0.6	100 ± 20	1.0 ± 0.1
CUC	0.4 ± 0.1	0.25 ± 0.1	1.6 ± 0.5	0.06 ± 0.02
			$F_{initial\ selection} = 60 \pm 20$	$F_{proofreading} = 15 \pm 5$

$\underbrace{\qquad\qquad\qquad}$

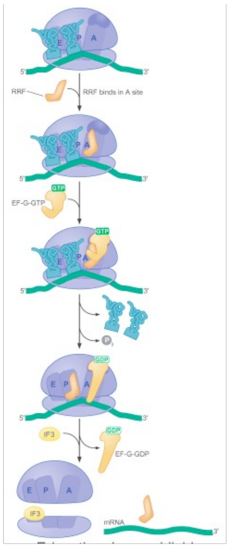
~1000x discrimination against near-cognate!

(c) translational termination

release of peptide



recycling of ribosome



rescue of broken mRNA