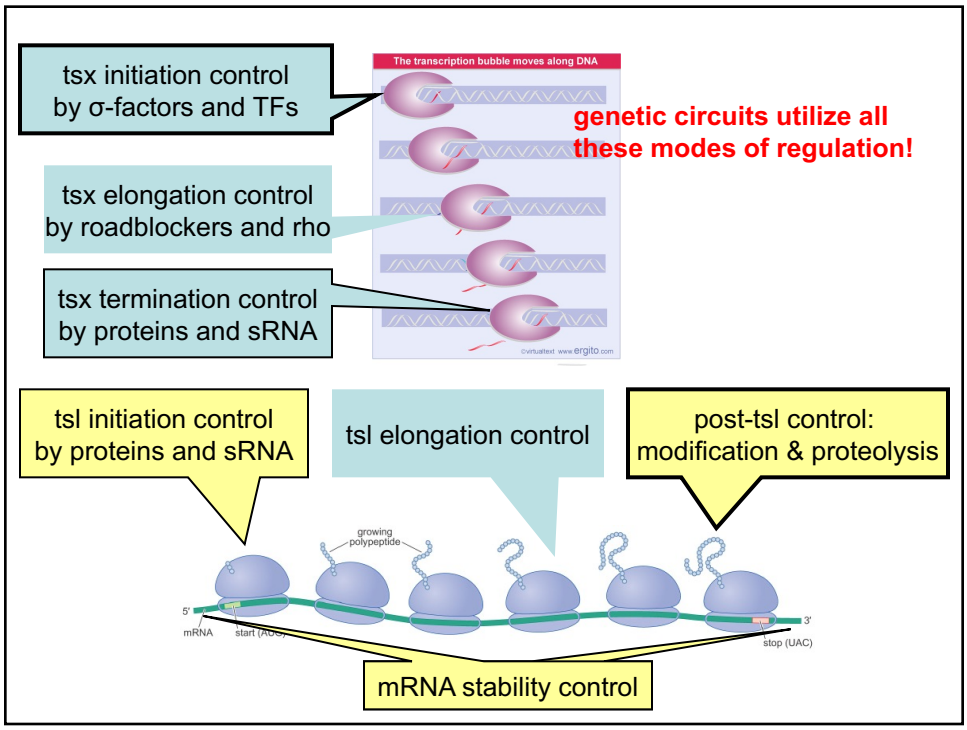


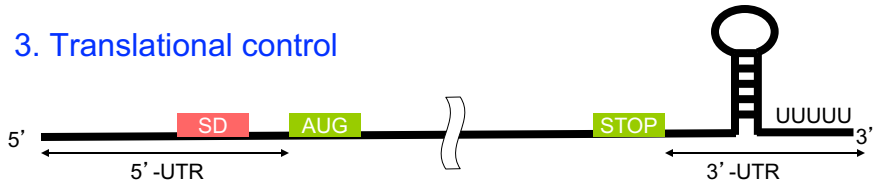
1

Topic 3: Post-transcriptional control

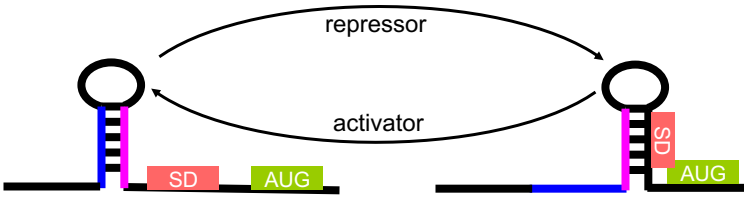
A. Transcriptional elongation and termination
B. Protein synthesis and translational control
   1. tRNA and the genetic code
   2. translational mechanisms (initiation, elongation, termination)
   3. translational control
C. Protein degradation and post-translational control

2

3. Translational control



- Shine-Dalgarno (SD) seq: AGGAGGNNNNNNAUG
  [strong translation from 4 consecutive matches + appropriate spacing to AUG]
- key control strategy:
  access of ribosome to ribosomal binding site (RBS or SD)

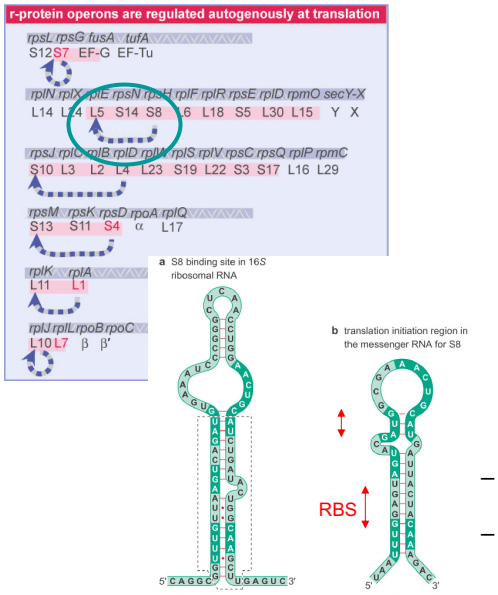


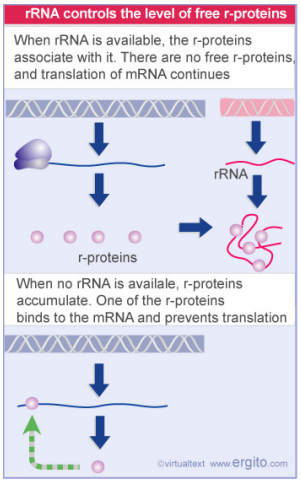
-- analogous to tsx termination control
-- can be modulated by RNA-binding proteins, small RNAs, metabolites

3

(a) via RNA-binding proteins

(e.g., ribosomal proteins)



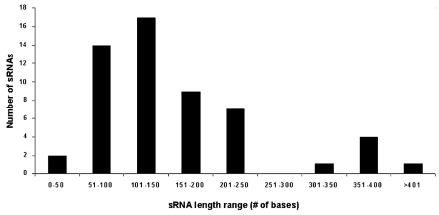


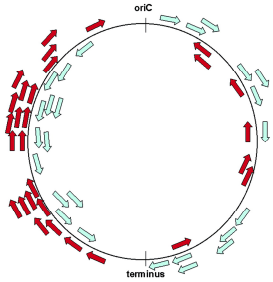
– weak 2nd structure stabilized by ribosomal protein (-ve feedback)
– rRNA itself regulated by (p)ppGpp (stringent response)

5

(b) via small RNA

length and location of sRNA



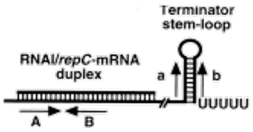


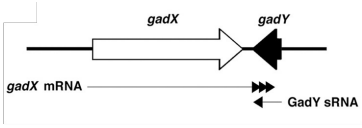
sRNA-mediated control found in

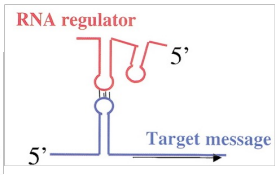
-- transcriptional termination (plasmic copy # control)

-- mRNA stability control

➔ translational inhibition/activation & mRNA degradation







7

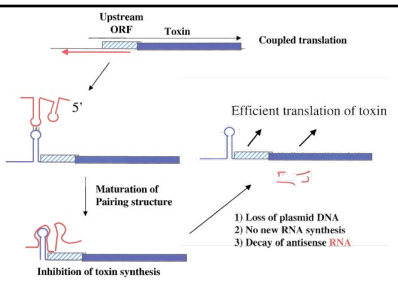
cis-acting

e.g., hok/sok (toxin-antidote system)

-- inhibits translation

-- unique target, spatially localized

-- rapid sRNA decay



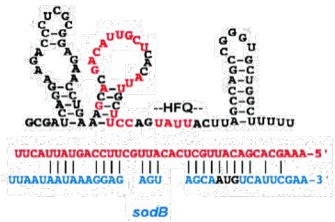
trans-acting

- Regulation of iron metabolism by the repressor Fur

Iron → tsx repressor (Fur) ⊣ Iron acquisition genes

Iron → RyhB ⊣ Iron storage genes, Iron consuming genes, oxidative stress relief, …

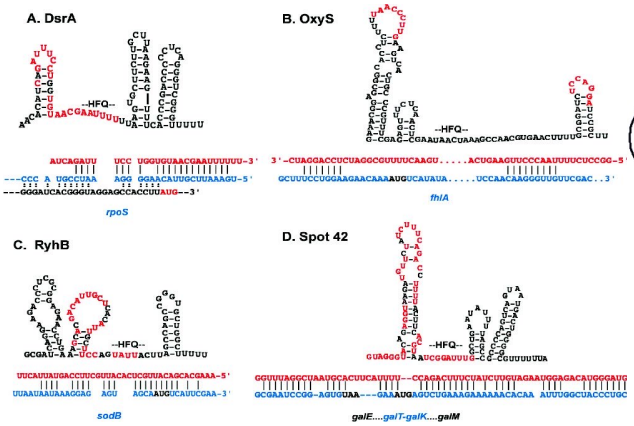
-- RyhB binds to translational initiation regime of mRNA (sodB, sdh, …)

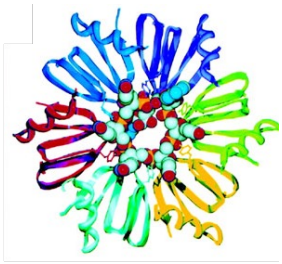


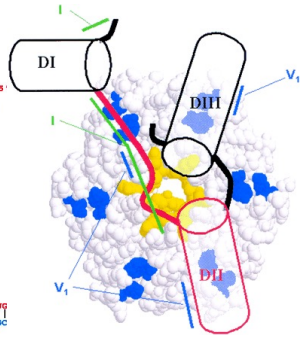
8

Mechanism(s) of sRNA-mediated regulation

- many require Hfq (e.g., DsrA, OxyS, Spot42, RyhB)
  - -- hexameric protein forming a ring
  - -- homologous to eukaryotic Sm-like proteins that function in RNA splicing
  - -- binds A/U-rich single-stranded RNA next to stem-loop region







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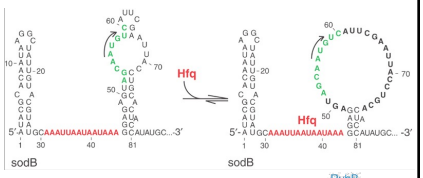
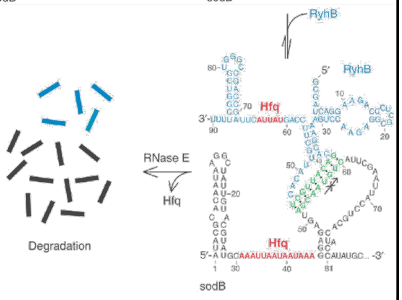
- effect of Hfq
  - – can stabilize sRNA (half-life > 30 min)
  - – can also bind to target mRNA (and induce alternative 2nd structure)
  - – can stimulate sRNA/target mRNA pairing (e.g., acting as RNA chaperone)



- effect of sRNA/mRNA pairing
  - – can change ribosome accessability
  - – can lead to rapid mRNA degradation
  - – can lead to rapid sRNA degradation (stoichiometric rather than catalytic)



- effect of RNase E
  - – required in mRNA and sRNA degradation
  - – recognition motif similarly to Hfq (dissociation of Hfq upon pairing?)
  - – mRNA degradation must be blocked in some cases; how? (e.g., Spot42 regulation of *galK* and DsrA stimluation of *rpoS*)

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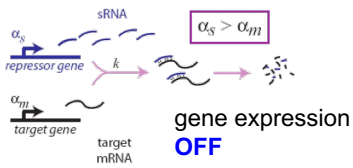
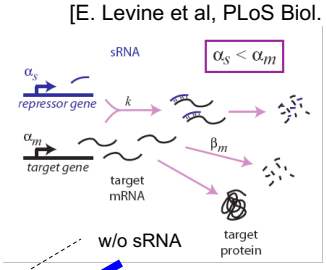
sRNA-mediated gene silencing

[E. Levine et al, PLoS Biol. 2007]

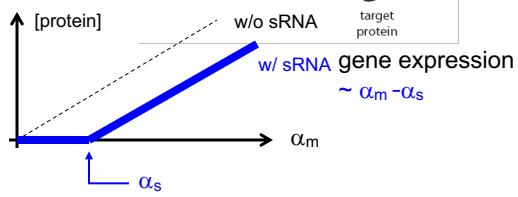


gene expression **OFF**



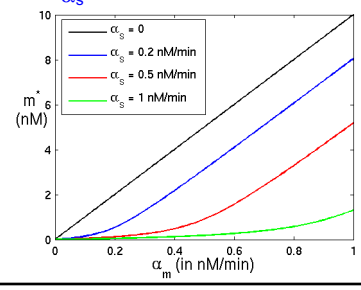
- qualitative expectation:

  threshold-linear response
  - tight repression for $\alpha_m \lesssim \alpha_s$
  - weak repression for $\alpha_m \gg \alpha_s$



- quantitative prediction:

$$\begin{cases} \dfrac{dm}{dt} = \alpha_m - \beta_m m - k \cdot m \cdot s \\ \dfrac{ds}{dt} = \alpha_s - \beta_s s - k \cdot m \cdot s \end{cases}$$



parameters characterized for RyhB/sodB,
with $\beta_m^{-1} \approx 6$ min; $\beta_s^{-1} \approx 30$ min
and $k^{-1} \sim 50$ nM-min (diffusion-limited)

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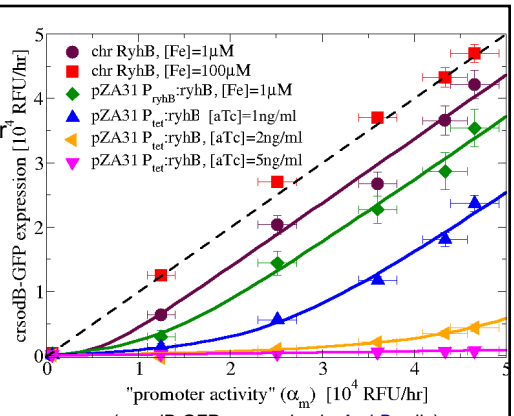
Expt' l characterization

plasmid-encoded inducible GFP reporter

translationally fused with truncated *sodB*

various sources of *ryhB*



(crsodB GFP expression in *ΔryhB* cells)

➔single-parameter fit to the expected threshold-linear form

further verification by

- direct mRNA measurement: similar to GFP
- mutant crsodB: lose nonlinear response
- compensating mutation in RyhB: restores threshold-linear response

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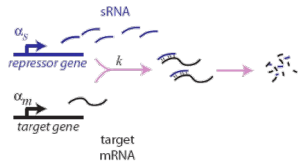
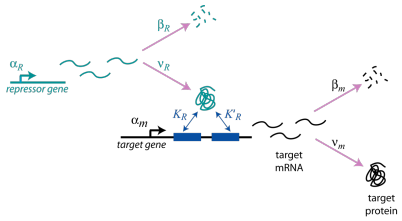
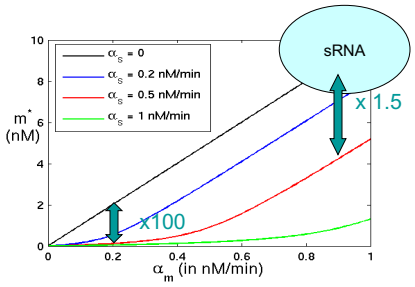
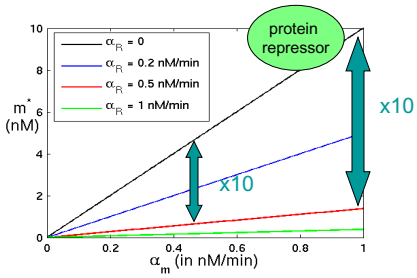
Compare to protein regulators









sRNA-mediated tsl regulation
➔ fold-repression depends sensitively on α_m

protein-mediated tsx regulation
➔ fold repression independent of promoter activity (α_m)

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Topic 3: Post-transcriptional control

A. Transcriptional elongation and termination
B. Protein synthesis and translational control
C. Protein degradation and post-translational control
   1. proteolytic machinery
   2. protein unfolding
   3. substrate selection
   4. effect on gene regulation

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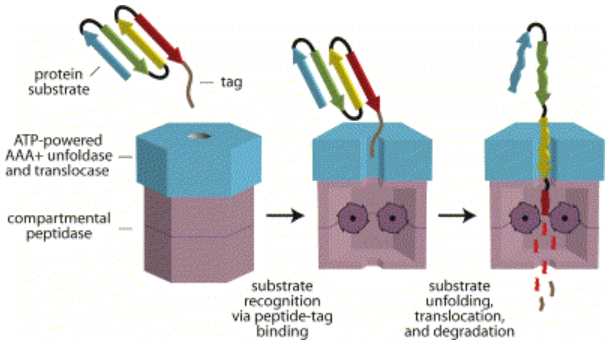
E. Protein Degradation and Control

- most (95%) of bacterial proteins are stable during exp growth; their conc are governed by the balance of synthesis and dilution (due to cell growth)
- selected proteins are targets of proteolysis (half-life ~ minutes)
  - – keep basal level low (e.g., to avoid accidental trigger due to small changes in synthesis)
  - – rapid change in protein conc. (usually coordinated with change in synthesis)
  - – opportunity for combinatorial control
- overview



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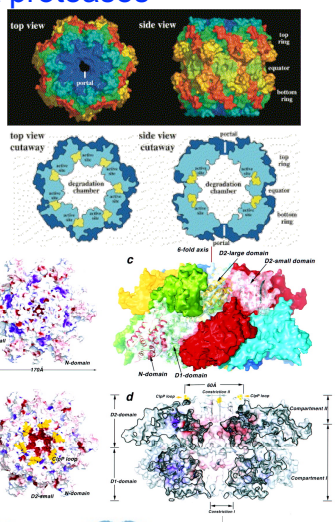
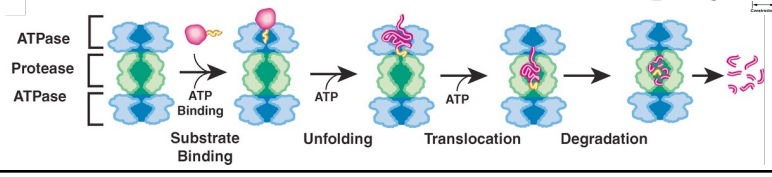
1. Proteolytic machinery: ATP-dependent proteases

- ClpAP/XP family

ClpP (peptidase)
-- two 7-membered rings
-- small pore (~10A) allow entry of only short, unstructured peptide
-- active site: serine protease (proteolysis does not require ATP)

ClpA and ClpX (ATPase)
-- assemble into hexameric rings and sandwiches ClpP to form AP, XP or XAP
-- positioned over the entrance to the proteolytic chamber
-- unfold and translocate tagged proteins in ATP-dependent process
-- ClpA/X have different substrate specificity





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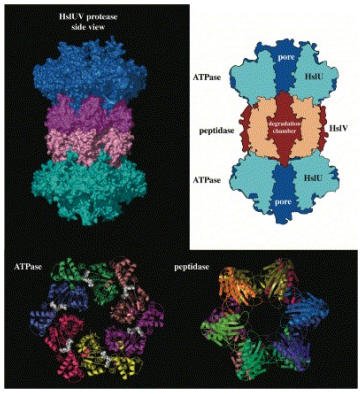
- HslUV family
  - hexameric rings for both HslV(peptidase) and HslU (ATPase)
  - active site: threonine

- Lon family
  - encoded by a single polypeptide
  - works similar to Clp and Hsl
  - primary quality control protease (degrades abnormal proteins)

- FtsH family
  - encoded also by a single polypeptide
  - anchored to the inner-membrane (but can degrade cytoplasmic proteins)
  - only essential protease



➔ while these proteases have different specificity, they also share many target substrates

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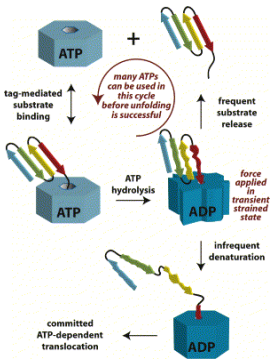
2. Protein unfolding

- unfolded proteins are rapidly degraded in vivo
- thermodynamically very stable proteins can be unfolded in few minutes
  - ATPase first unfold structure adjacent to the degradation tag
  - global denaturation of single-domain proteins follows due to cooperativity

- mechanism of enzymatic unfolding: forced denaturation by pulling thru pore
- energy cost: 10-500 ATPs/100 residue (for ClpXP)
- FtsH cannot degrade very stable proteins



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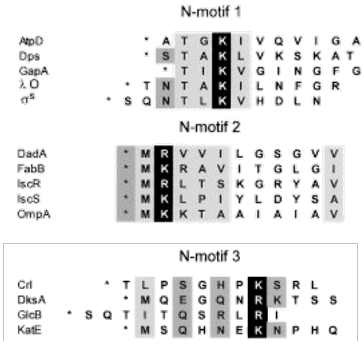
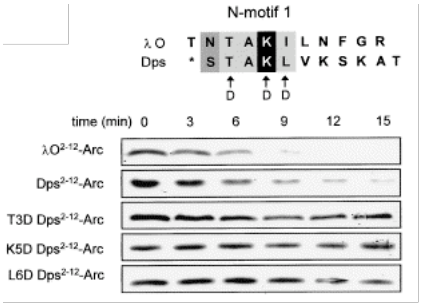
3. Substrate selection

(a) Intrinsic recognition signals:

– sequence necessary and sufficient to direct protein degradation
– many found to reside at protein termini

- N-terminal recognition signals
  – N-end rule (for ClpAP):
    half-life ~2min if N-terminal residue is replaced by **F**, **L**, **W**, **Y**, **R**, **K**
  – N-motifs (within first 11 aa's) for ClpXP: mostly **nonpolar** and **basic**





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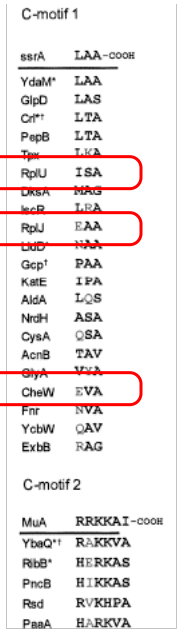
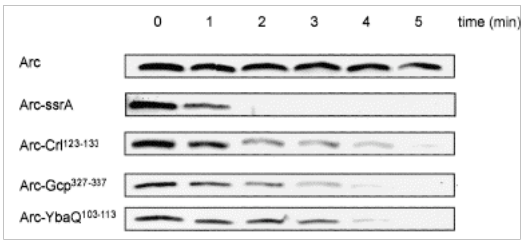
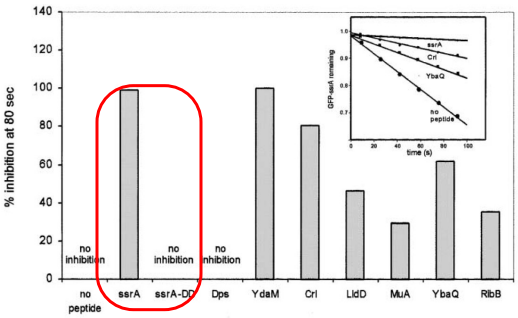
- C-terminal recognition signals (last 3-4 aa's) for ClpX [Flynn, …, Baker, 2003]



– fusion protein has reduced life-time



– inhibition of degradation by synthetic peptides



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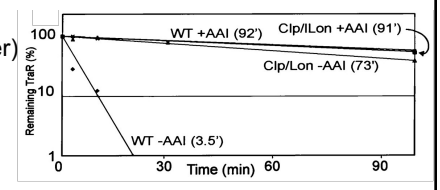
(b) recognition signal exposed upon unfolding, dissociation, cleavage

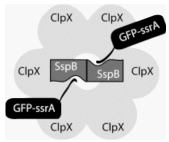
- ribosomal proteins unstable if unable to assemble into ribosome
- λO stabilized in DNA replication complex
- quorum sensing regulators (LuxR, LasR, TraR, …) stable only in the presence of the respective autoinducer (folding/dimerization requires autoinducer)

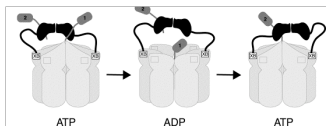
➔ Use-it-or-lose-it!



(c) regulation of proteolysis by adaptors

SspB dimer binds both to ssrA-tagged substrate and ClpX
➔ efficient degradation even at low conc





(but SspB inhibits ClpAP from degradation of ssrA-tagged substrate)

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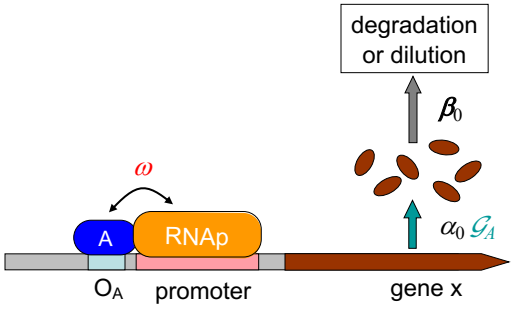
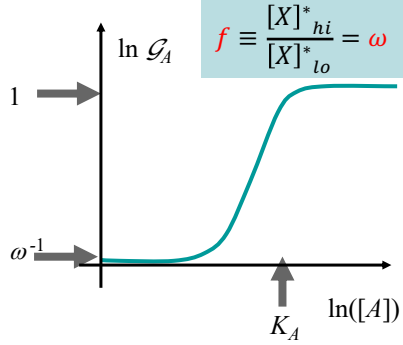
4. Effect of proteolysis on gene regulation

$$\frac{d}{dt}[X] = \alpha_0 \cdot \mathcal{G}_A - \beta_0 \cdot [X]$$
$$[X]^* = \alpha_0 \mathcal{G}_A / \beta_0$$



$$f \equiv \frac{[X]^*_{hi}}{[X]^*_{lo}} = \omega$$



$$\frac{d}{dt}[m] = \alpha_m \cdot \mathcal{P}([A]) - \beta_m \cdot [m] \xrightarrow{\frac{d}{dt}[m]=0} [m] = \alpha_m \alpha_p \mathcal{P}([A])/\beta_m$$

$$\frac{d}{dt}[X] = \alpha_p \cdot [m] - \beta_0 \cdot [X] = \alpha_0 \cdot \mathcal{G}_A - \beta_0 \cdot [X]$$

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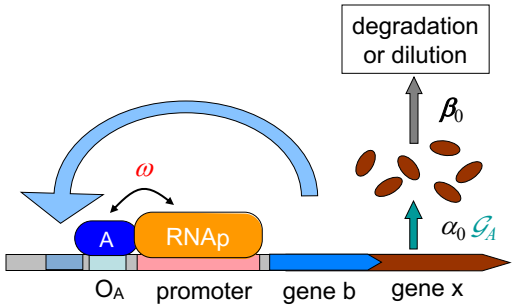
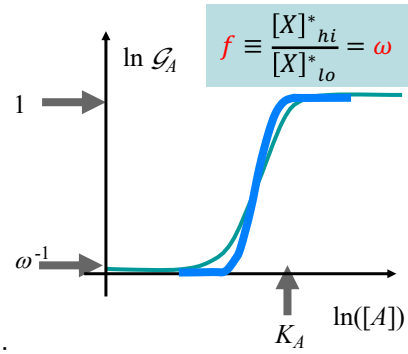
4. Effect of proteolysis on gene regulation

➔ how to increase capacity & sensitivity?

$$\frac{d}{dt}[X] = \alpha_0 \cdot \mathcal{G}_A - \beta_0 \cdot [X]$$

$$[X]^* = \alpha_0 \mathcal{G}_A / \beta_0$$





some possible mechanisms of amplification:

- **positive feedback** (transcriptional or post-transcriptional)
  - sensitivity increased but still has $f = \omega$
  - requires an additional gene/protein for <u>each</u> promoter (very expensive!)

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4. Effect of proteolysis on gene regulation

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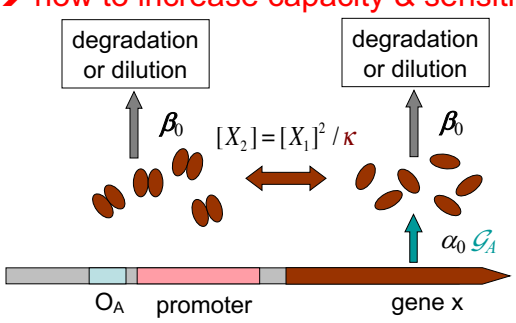

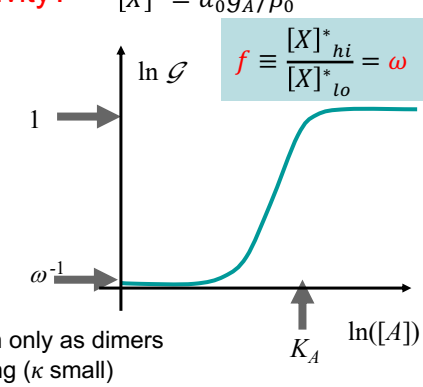

- many active proteins are **dimers** and function only as dimers
  - but typical dimerization affinity very strong (κ small)

$$[X] = [X_1] + 2 \cdot [X_2] = \cancel{\sqrt{\kappa \cdot [X_2]}} + 2 \cdot [X_2] \quad \textbf{(strong dimers)}$$

$$\Rightarrow \quad [X_2^*] \approx [X^*]/2 \propto \mathcal{G}_A, \quad \text{still } f = \omega$$

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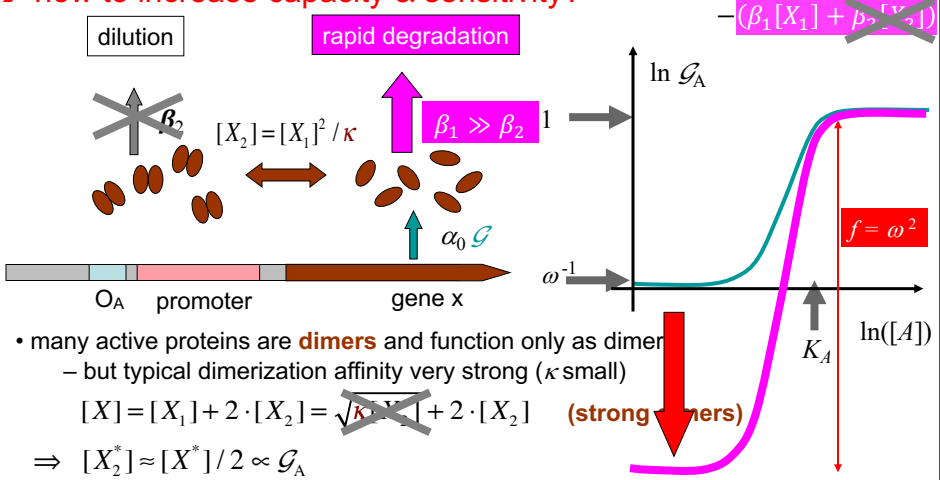
4. Effect of proteolysis on gene regulation

➔ how to increase capacity & sensitivity?

$$\frac{d}{dt}[X] = \alpha_0 \mathcal{G}_A - (\beta_1[X_1] + \cancel{\beta_2[X_2]})$$



- many active proteins are **dimers** and function only as dimers
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$$[X]=[X_1]+2\cdot[X_2]=\cancel{\sqrt{\kappa[X_1]}}+2\cdot[X_2] \quad \textbf{(strong dimers)}$$

$$\Rightarrow \quad [X_2^*]\approx[X^*]/2 \propto \mathcal{G}_A$$

- **nonlinear degradation** $[X_1^*]\propto\mathcal{G}_A$, thus $[X_2^*]\propto\mathcal{G}_A^2$

[Buchler et al, PNAS 2005]

➡ amplify gene expression by suppressing the basal level

➡ effective dynamic mechanism of cooperativity ("cooperative stability")

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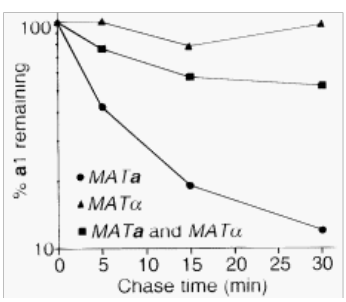
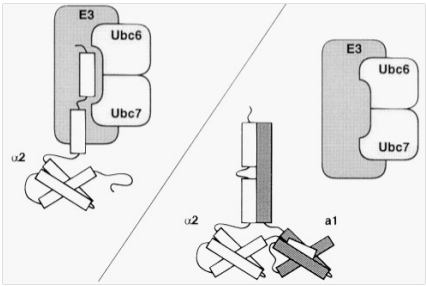
Nonlinear degradation of oligomers

- many examples where subunits of multimeric protein complexes degrade faster when alone, e.g., components of ribosome [Gottesman & Maurizi, 92]
- quantitative characterization for some heterodimers, e.g., yeast MATa1:α2 heterodimers 15x more stable than the monomers

[Johnson et al, 1998]





suggested mechanism: degradation signal protected by dimerization

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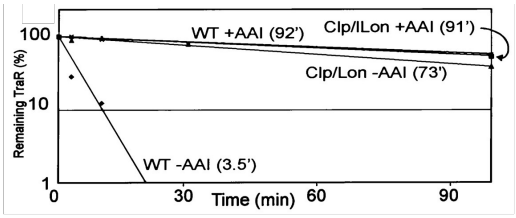
Nonlinear degradation of homodimers

- quorum-sensing regulators (e.g., LuxR, LasR, TraR)

  -- fold only in the presence of the auto-inducers

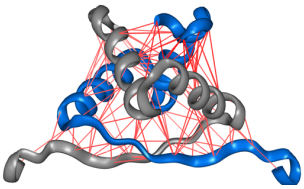
  -- unfolded molecules rapidly degraded



- a large class of candidates: two-state dimers

  [Wright & Dyson, 1999]

  = monomers which do not fold until dimerized (e.g., the Arc repressor)



➡ a generic, versatile mechanism of signal amplification (does not require new proteins)

➡ exploitation of "use-it-or-remove-it" principle

➡ however, quantitative experimental studies lacking…

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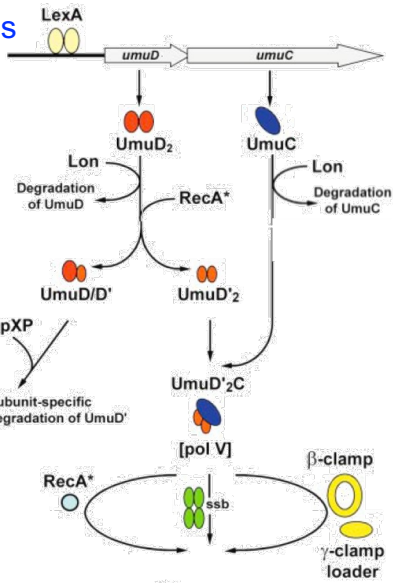
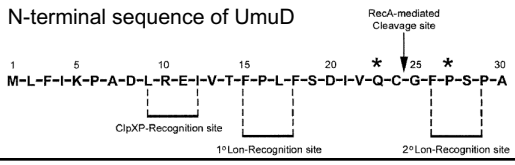
Use of proteolysis for cellular functions

- turning off error-prone replication in SOS:
  - expression of umuDC operon mutagenic
  - tsx repressed by LexA
  - $UmuD_2$ and UmuC degraded by Lon
  - DNA damage activates RecA, which cleaves first 24 aa of UmuD to UmuD'
  - UmuD/D' heterodimerizes (D/D' has much stronger affinity than either D/D or D'/D')
  - subunit specific degradation of UmuD' by ClpXP (trans-signal in UmuD)
  - $UmuD'_2$ form only at very high D'/D ratio
  - forms stable $UmuD'_2C$ complex = DNAp V
  - replaces the normal DNAp III at site of DNA damage (guided by RecA*)

  ➔ sloppy replication of DNA only at damaged sites



N-terminal sequence of UmuD



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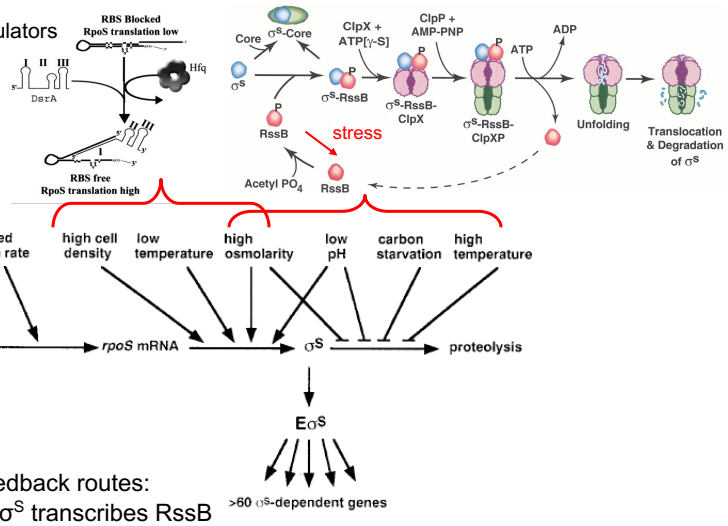
- control of stress response:
  - RssB-P adaptor mediates degradation of σ^s
  - RssB/RssB-P balance dictated by degree of stress (most mediators unknown)
  - regulations at all levels: tsx, tsl, proteolysis

sRNA regulators



- many feedback routes:
  e.g., σ^S transcribes RssB
  σ^S also transcribes ArcA which competes with RssB for P
  ArcA represses rpoS transcription

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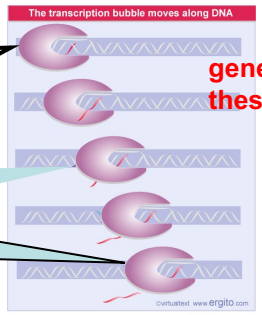
tsx initiation control
by σ-factors and TFs



genetic circuits utilize all these modes of regulation!

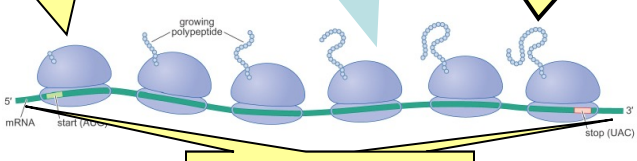
tsx elongation control
by roadblockers and rho

tsx termination control
by proteins and sRNA

tsl initiation control
by proteins and sRNA

tsl elongation control

post-tsl control:
modification & proteolysis



mRNA stability control

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