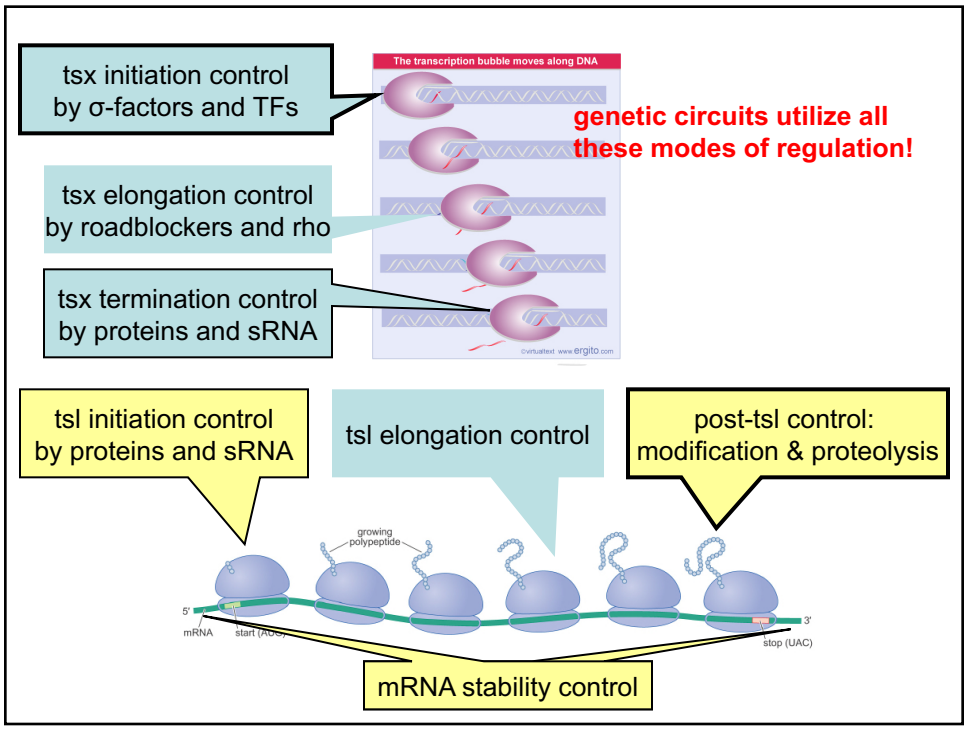


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## Topic 3: Post-transcriptional control

A. Transcriptional elongation and termination
   1. Basic models of tsx elongation and termination
   2. mechanisms of elongation (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
   4. effect on gene regulation

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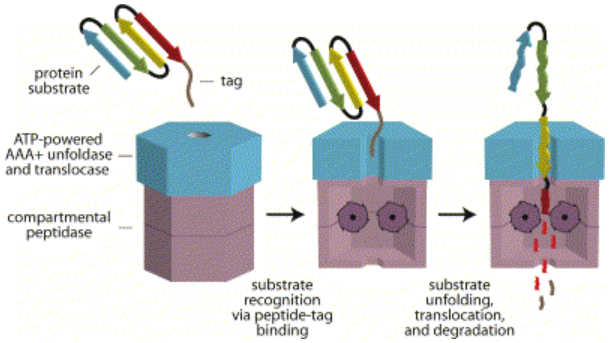
# E. Protein Degradation and Control

- most bacterial proteins are stable; their concentrations are governed by 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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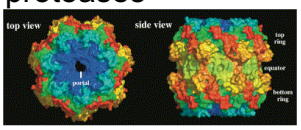
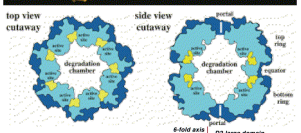
# 1. Proteolytic machinery: ATP-dependent proteases

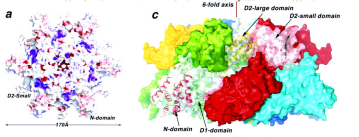
- ClpAP/XP family

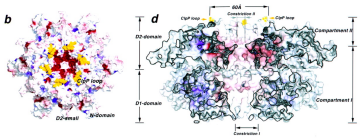
ClpP (peptidase)
-- two 7-membered rings
-- small pore (~10A) allow entry of only short, unstructured peptide
-- active site: serine protease (proteolysis does not require ATP)

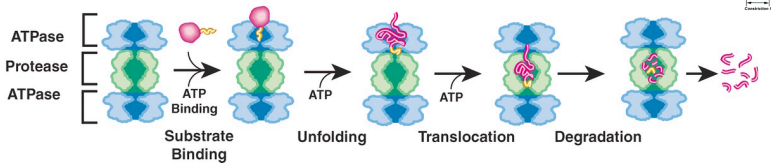
ClpA and ClpX (ATPase)
-- assemble into hexameric rings and sandwiches ClpP to form AP, XP or XAP
-- positioned over the entrance to the proteolytic chamber
-- unfold and translocate tagged proteins in ATP-dependent process
-- ClpA/X have different substrate specificity

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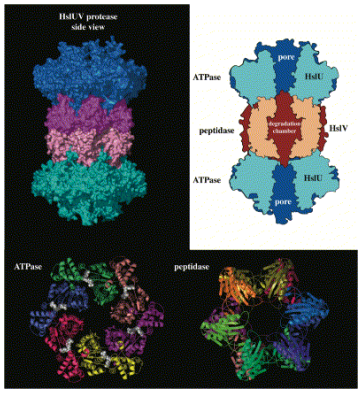
- HslUV family
  - hexameric rings for both HslV(peptidase) and HslU (ATPase)
  - active site: threonine

- 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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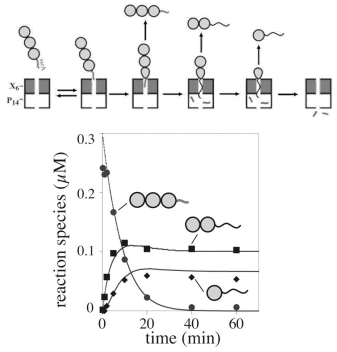
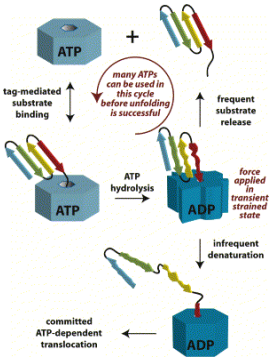
## 2. Protein unfolding

- 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 per 100 residue protein (for ClpXP)
- FtsH cannot degrade very stable proteins

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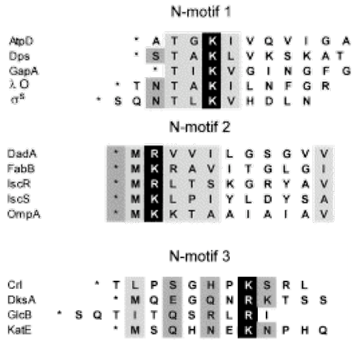
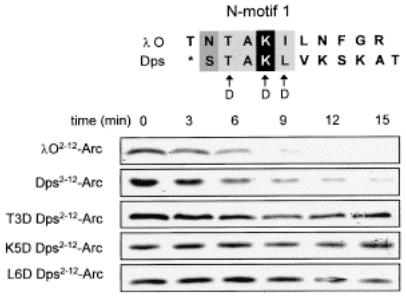
## 3. Substrate selection

- 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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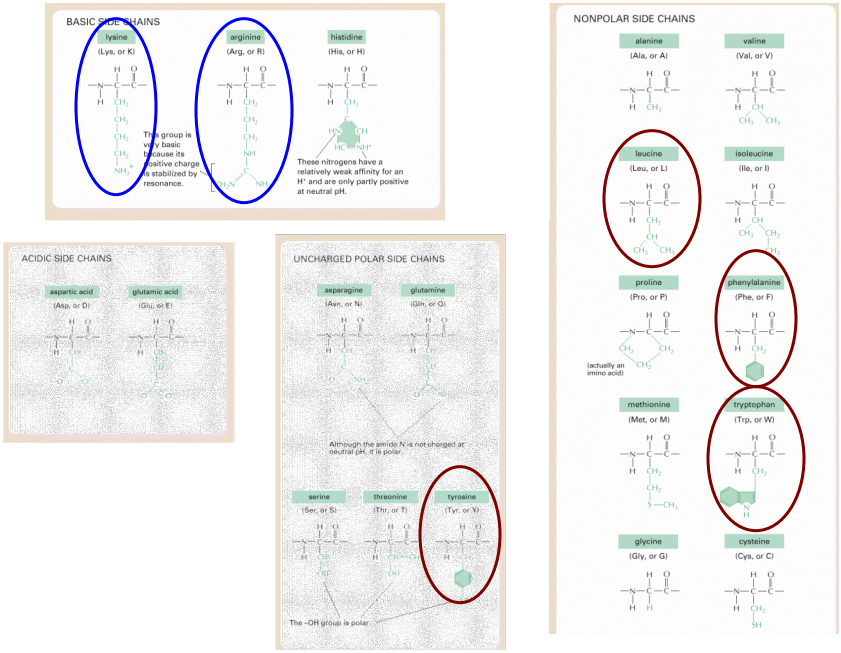


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• C-terminal recognition signals (last 3-4 aa's) for ClpX [Flynn, …, Baker, 2003]

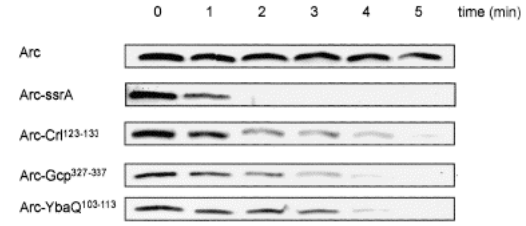
C-motif 1

| | |
|---|---|
| ssrA | LAA-COOH |
| YdaM* | LAA |
| GlpD | LAS |
| Crl*† | LTA |
| PepB | LTA |
| Tpx | LKA |
| RplU | ISA |
| DksA | MAG |
| IscR | LRA |
| RplJ | EAA |
| LldD* | NAA |
| Gcp† | PAA |
| KatE | IPA |
| AldA | LQS |
| NrdH | ASA |
| CysA | QSA |
| AcnB | TAV |
| GlyA | VKA |
| CheW | EVA |
| Fnr | NVA |
| YcbW | QAV |
| ExbB | RAG |

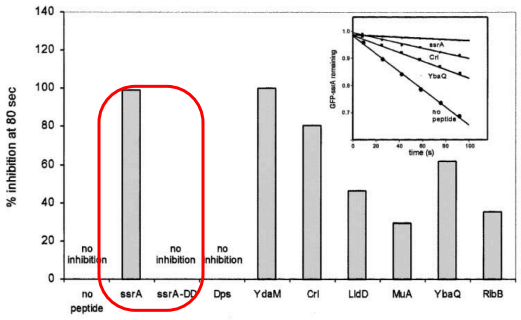
C-motif 2

| | |
|---|---|
| MuA | RRKKAI-COOH |
| YbaQ*† | RAKKVA |
| RlbB* | HERKAS |
| PncB | HIKKAS |
| Rsd | RVKHPA |
| PaaA | HARKVA |

– fusion protein has reduced life time




– inhibition of degradation by synthetic peptides

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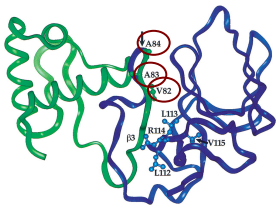
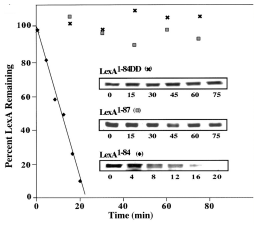
❖ cryptic recognition signals:
recognition signal exposed upon unfolding, dissociation, cleavage

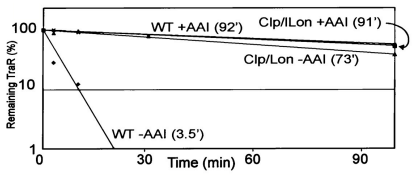
– LexA self-cleavage activated by DNA damage (initiates SOS)
exposes C-terminal (**VAA**) targets it to ClpXP




– MuA/MuB association protects disassembly of MuA by ClpX
– λO stabilized in DNA replication complex
– ribosomal proteins unstable if unable to assemble into ribosome
– quorum sensing regulators (LuxR, LasR, TraR, …) stable only in the presence of the respective autoinducer (folding/dimerization requires AI)

➔ Use-it-or-lose-it!

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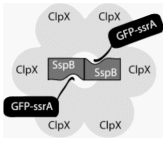
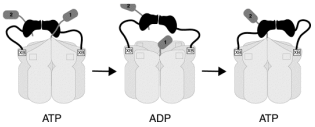
❖ regulation of proteolysis by adaptors

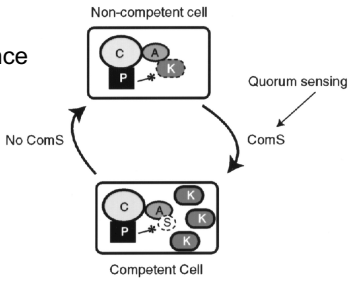
• SspB dimer binds both to ssrA-tagged substrate and ClpX
➔ efficient degradation even at low conc




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

• competence control in *B. subtilis*:
  – ComK is the master regulator of competence
  – MecA mediates degradation of ComK
  – ComS rescues ComK from degradation
  ➔ competence (DNA exchange) only at sufficiently high quorum (activation signal for ComS)




• tethering signals in these adaptors are similar and bind to a common site on ClpX

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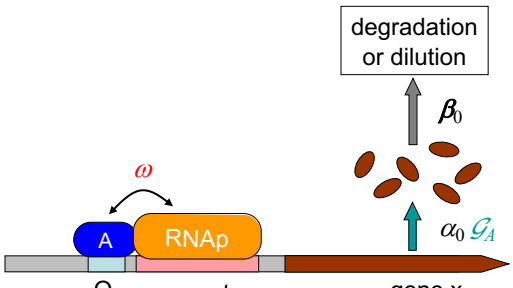
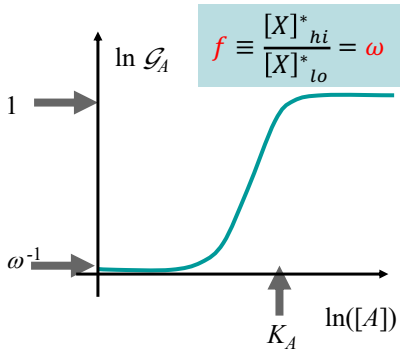
4. Effect of proteolysis on gene regulation $\frac{d}{dt}[X] = \alpha_0 \cdot \mathcal{G}_A - \beta_0 \cdot [X]$

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




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

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

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

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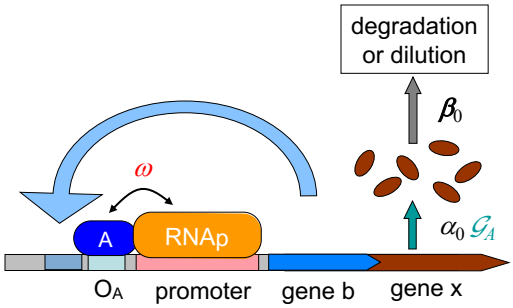
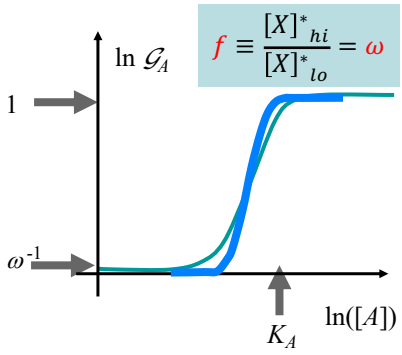
## 4. Effect of proteolysis on gene regulation

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

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

➔ how to increase capacity & sensitivity?




some possible mechanisms of amplification:

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

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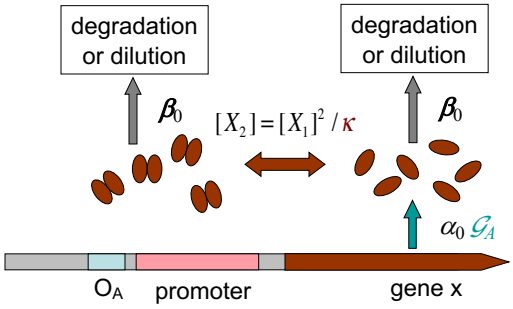
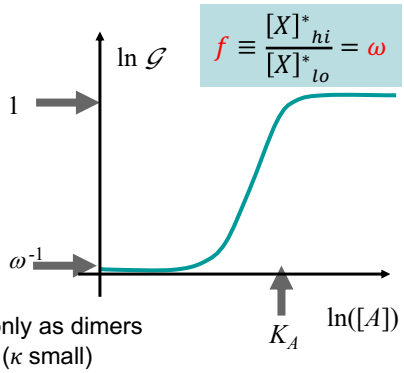
## 4. Effect of proteolysis on gene regulation

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

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

➔ how to increase capacity & sensitivity?




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

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

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

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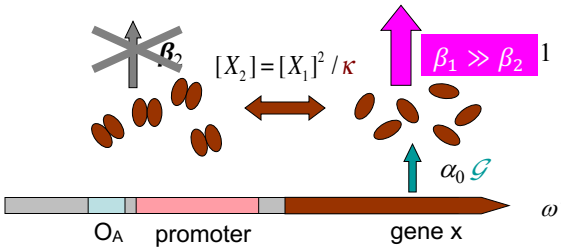
## 4. Effect of proteolysis on gene regulation

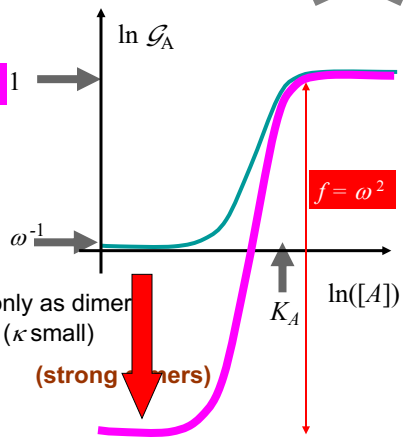
➔ how to increase capacity & sensitivity?

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

dilution

rapid degradation




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

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

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

- **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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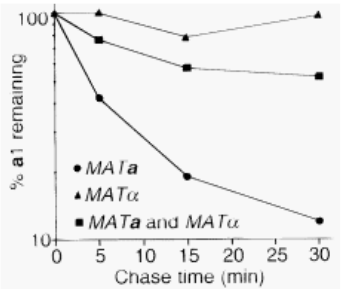
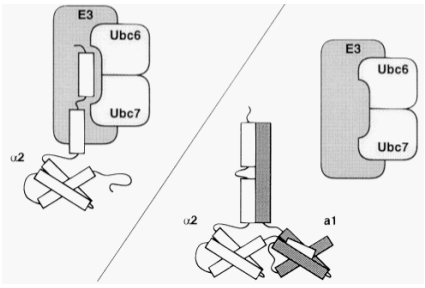
## Nonlinear degradation of oligomers

- 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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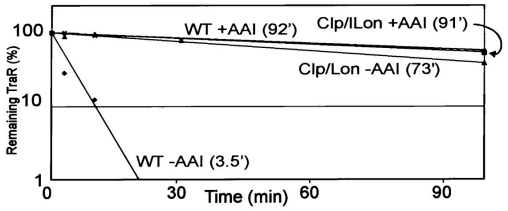
## Nonlinear degradation of homodimers

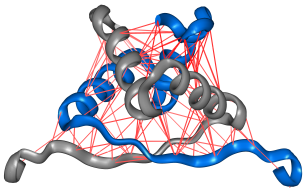
• quorum-sensing regulators (e.g., LuxR, LasR, TraR)

-- 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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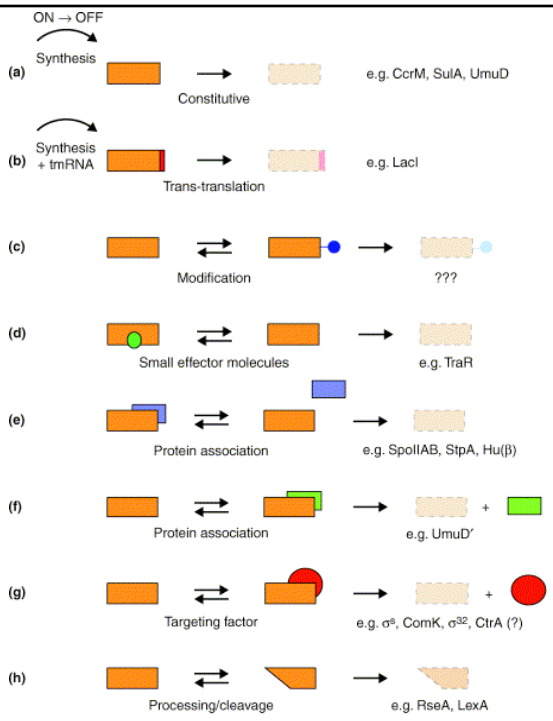
**Summary of modes of proteolytic control**

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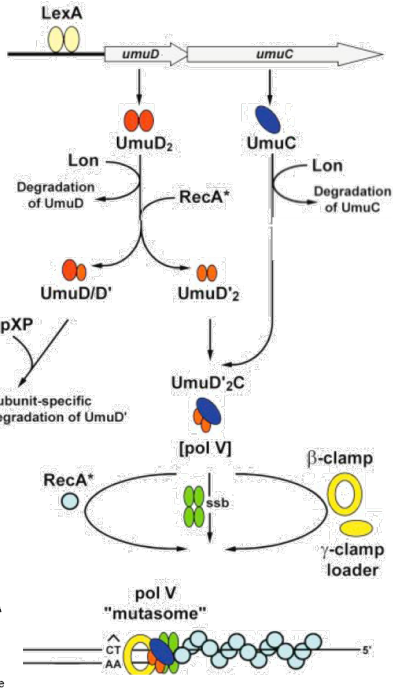
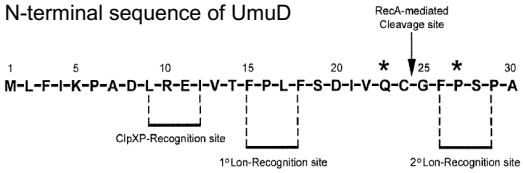
- turning off error-prone replication in SOS:
  - expression of umuDC operon mutagenic
  - tsx repressed by LexA
  - $UmuD_2$ and UmuC degraded by Lon
  - DNA damage activates RecA, which cleaves first 24 aa of UmuD to UmuD'
  - UmuD/D' heterodimerizes (D/D' has much stronger affinity than either D/D 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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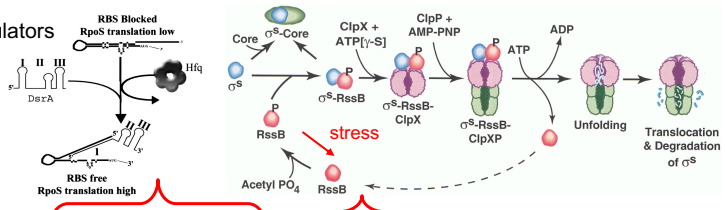
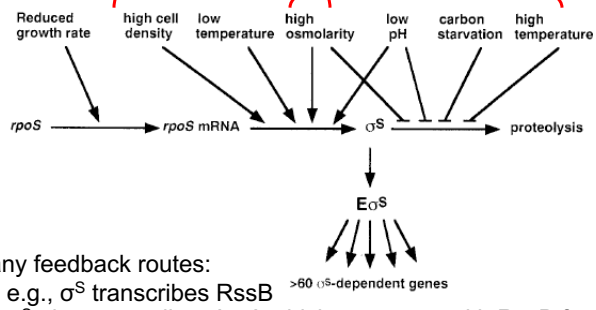
- control of stress response:
  - RssB-P adaptor mediates degradation of $\sigma^S$
  - RssB/RssB-P balance dictated by degree of stress (most mediators unknown)
  - regulations at all levels: tsx, tsl, proteolysis

sRNA regulators




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

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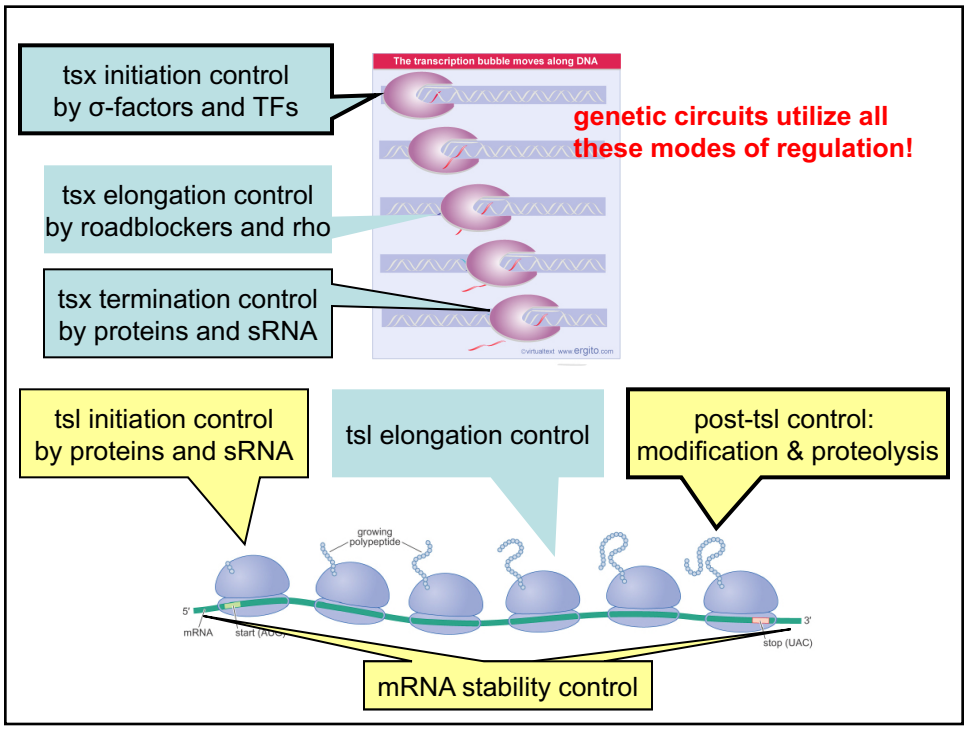
tsx initiation control by σ-factors and TFs
tsx elongation control by roadblockers and rho
tsx termination control by proteins and sRNA
The transcription bubble moves along DNA
genetic circuits utilize all these modes of regulation!
tsl initiation control by proteins and sRNA
tsl elongation control
post-tsl control: modification & proteolysis
growing polypeptide
5'
mRNA
start (AUG)
stop (UAC)
3'
mRNA stability control

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