Outline

GS/M411 Last Lecture!!! March 11, 2009

1. What is global regulation?
2. σ factors on so many levels
3. Two component regulatory systems
4. Small RNAs
5. Cross talk: CpxR effects on *ompF/C*

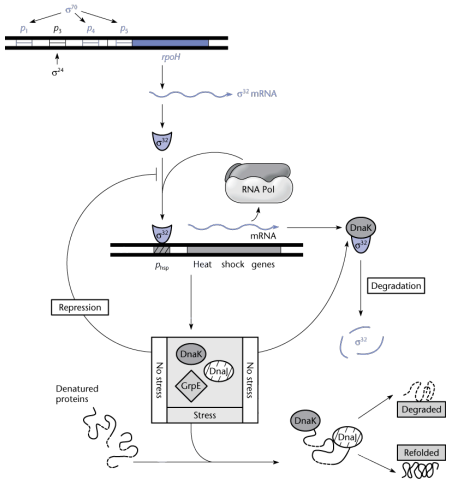
Sensing and responding to the environmental changes

- Variations in the local environment lead to changing growth rates, nutrient availability, and stresses.
- In response, bacteria vary the timing for initiation of DNA replication, adjust the number of ribosomes, and change their gene expression profiles.
- Coordination of broad changes in gene expression is key to bacterial adaptation.

Controlling σ^{32} levels

- While *rpoH* gene (for σ^{32}) not essential, deletion mutants don't grow above 20°C.
- Transcription of *rpoH* largely controlled by σ^{70} holoenzyme; transcription at similar levels under heat stress, but stability of σ^{32} regulated by a protein (DnaK) that senses the heat shock. Binding of σ^{32} by DnaK occurs under normal growth, leads to immediate degradation of σ^{32}.
- When DnaK distracted by heat shock, σ^{32} is not proteolyzed by FtsH and activates σ^{32}-dependent promoters.

Figure 13.13

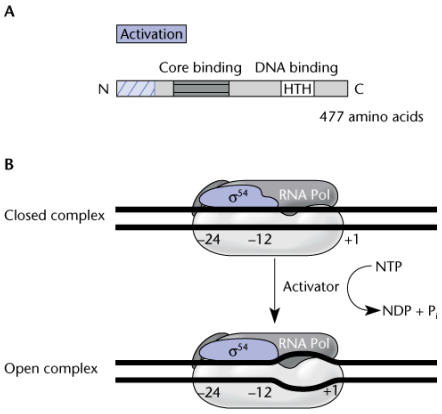


Regulation by variation in σ factors

Different forms of RNA pol holoenzyme will initiate transcription at very different promoters. Some σ factors are not routinely "available."

- Availability of σ factor can be controlled by proteolysis: σ^{32} is degraded in absence of "heat shock" stress by protease FtsH; stress increases [σ^{32}] 15x and allows transcription of 30+ genes needed for stress response
- Functioning of σ factor controlled by other proteins: nitrogen regulation and σ^{54}

Nitrogen regulation

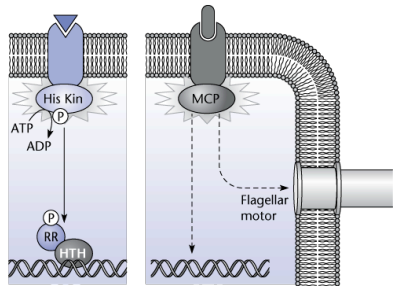


Box 13.3

- Unlike other forms of RNA pol holoenzyme, RNA pol with σ^{54} binds to nitrogen-regulated promoters, but depends on a transcriptional activator to form open complex and make RNA.
- Activator must hydrolyze ATP, then causes conformational change in σ^{54}

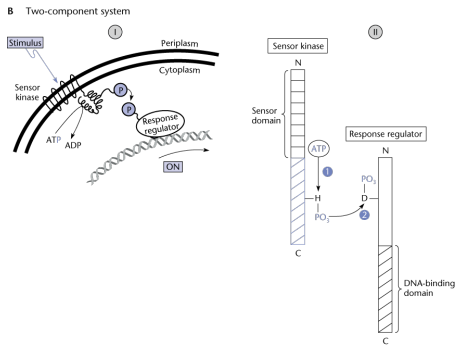
Two components systems

- Allow bacteria (and some plants) to sense and respond to environmental changes
- Affect motility, gene expression, etc. Can be up to 200+ pairs of proteins in a bacterial species.
- Rely on "sensor His kinase," which interacts with "response regulator"



Box 13.4A

Altering gene expression via "typical" two component systems



Box 13.4B

- Sensor kinase receives signal (usually unknown) and auto-phosphorylates at conserved His.
- Sensor transfers its -Pi to conserved Asp on Response regulator, changing its DNA binding characteristics and altering gene expression.

Controlling expression of OM proteins

- Ability of Gram negative bacteria to live is dependent on movement of solutes across their outer membrane (OM). While some nutrients cross OM via specific pores (porins; e.g., maltodextrins), most movement occurs via “general porins” such as *E. coli* OmpF, OmpC
- General porins do not discriminate aomng solutes that are hydrophilic, 500 Da, and not highly charged.
- Approximately 10^5 general porin molecules/cell!!
- *E. coli* responds to osmotic environment by varying OmpC : OmpF content of OM, using two component regulatory system of OmpR/EnvZ.
- OmpC has smaller pore, higher abundance at high osmolarity; OmpF with larger pore….

Regulating expression of OmpC and OmpF

Mutants identified that altered expression of OmpF and OmpC in outer membrane. Three loci identified: *ompC* (47’), *ompF* (21’), *ompB* (74’).

OmpF$^-$ mutations map to *ompF*; mutations that decrease OmpF (but don’t completely block) map to *ompB*.

Fine structure genetics showed that *ompB* contained two genes.

Expression of OmpF/C

Silhavy and colleagues used *lacZ* transcriptional fusions to study regulation of *ompC* and *ompF.*

- Tn5 insertion in *envZ* prevents *ompC*/allows minimal *ompF* expression (white/pink on Lac MacConkey)
- OmpR2/OmpR3 constitutive mutants allow expression of only OmpF or OmpC, consistent with + regulation
- OmpR-Pi required for optimal expression of both genes; differential affinity of OmpR-Pi to diff promoters doesn’t explain all the data, suggesting other inputs!

TABLE 13.3 Phenotypes of *envZ* and *ompR* mutations

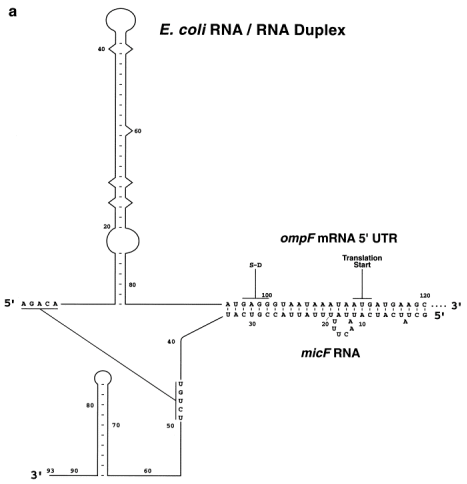
Genotype	Phenotype
envZ$^+$ *ompR*$^+$	OmpC$^+$ OmpF$^+$
envZ$^+$ *ompR1*	OmpC$^-$ OmpF$^-$
envZ(null) *ompR*$^+$	OmpC$^-$ OmpF^{+-a}
envZ$^+$ *ompR2*(Con)	OmpC$^-$ OmpF$^+$ (low osmolarity)
	OmpC$^-$ OmpF$^+$ (high osmolarity)
envZ(null) *ompR2*(Con)	OmpC$^-$ OmpF$^+$ (low osmolarity)
	OmpC$^-$ OmpF$^+$ (high osmolarity)
envZ$^+$ *ompR3*(Con)	OmpC$^+$ OmpF$^-$ (low osmolarity)
	OmpC$^+$ OmpF$^-$ (high osmolarity)
envZ$^+$ *ompR3*(Con)/ *envZ*$^+$ *ompR*$^+$	OmpC$^+$ OmpF$^-$ (low osmolarity) OmpC$^+$ OmpF$^-$ (high osmolarity)

[a] + − indicates that OmpF levels are reduced but not eliminated.

Table 13.3

Antisense RNA input to OmpF levels

- Found that multiple copies of *ompC* plasmid repressed OmpF level in cell.
- Effect unrelated to *ompC* but due to small upstream ORF, codes for multiple inhibiting copy RNA for OmpF expression, MicF
- MicF (+ Hfq) blocks translation, leads to degradation of *ompF* RNA



Antisense RNA control

- Many known examples in bacteria of small regulatory RNAs (sRNA); OmpC synthesis affected by MicC RNA
- Limited RNA-RNA homology compensated for by accessory proteins like Hfq, which stablize RNA duplexes while regulation occurs. Hfq is ubiquitous in bacteria.
- sRNA genes largely missed by standard genetics, but can represent significant coding capacity of genome.

More 2 component regulatory systems

Sensing status of environment and OM also done via CpxR/CpxA: these proteins also affect OmpC:OmpF

- *ompF-yfp* and *ompC-cfp* transcriptional fusions used to assay other inputs with Tn library
- ↑*ompC* /↓↓*ompF* dues to *cpxA*::Tn
- While CpxA/CpxR are set, CpxR needed for effect on *ompC/ompF* regulation, so CpxR likely getting Pi-group from another sensor kinase.

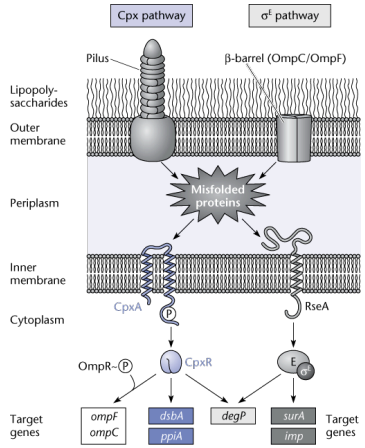


Figure 13.15