Outline

1. What is global regulation?
2. $\sigma$ 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 $\sigma^{32}$ levels

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

Regulation by variation in $\sigma$ factors

Different forms of RNA pol holoenzyme will initiate transcription at very different promoters. Some $\sigma$ factors are not routinely “available.”

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

Unlike other forms of RNA pol holoenzyme, RNA pol with $\sigma^{54}$ 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 $\sigma^{54}$

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”

Altering gene expression via “typical” two component systems

- 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 among 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^\prime),\ \text{ompF}\ (21^\prime),\ \text{ompB}\ (74^\prime)\.

OmpF- mutations map to \text{ompF}; mutations that decrease OmpF (but don’t completely block) map to \text{ompB}.

Fine structure genetics showed that \text{ompB} contained two genes.

Expression of OmpF/C

Silhavy and colleagues used \textit{lacZ} transcriptional fusions to study regulation of \textit{ompC} and \textit{ompF}.

- \text{Tn5} insertion in \text{envZ} prevents \textit{ompC}/allows minimal \textit{ompF} expression (white/pink on Lac MacConkey)
- \text{OmpR2}/\text{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!

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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 stabilize 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