LEARNING GOAL

By attending and participating in this Small Group Problem Solving Session, you will be able to describe the process by which bacterial cells regulate the conversion of genetic code into protein. This will provide you with a basic understanding of genetic regulatory mechanisms used by pathogens to cause disease.

ENABLING OBJECTIVES

To achieve this goal, you will be able to:

- use your knowledge of transcription initiation to investigate how certain pathogens induce resistance to tetracycline;

- combine your knowledge of genome organization, two-component signal transduction and regulation of transcription initiation to examine how the gram-negative pathogen *Shigella* controls its expression of certain virulence factors;

- use your knowledge of mechanisms of transcription initiation to explore the consequences of certain mutations on the ability of a pathogen to cause disease.

DEVELOPED BY

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GENETICS

PRETEST

Instructions: Complete *BEFORE* coming to this small group problem solving session.

DEFINE:

1. sigma factor
2. operon
3. promoter
4. open complex
5. repressor
6. RNA polymerase
7. activator
8. inducer
9. regulon
10. polycistronic message

11. State the difference between:
   A. inducer & activator
   B. regulator & regulon
   C. activator & repressor
   D. inducer & co-activator
CASE 1

Many antibiotic resistance genes are regulated. One type of regulation is repressor-mediated regulation of efflux-type tetracycline resistance genes. The gene encoding the repressor (tetR) and the gene encoding the structural gene (tetB) are transcribed from divergent and overlapping promoters (i.e., they face in opposite directions). Note that tetB encodes an efflux pump. In the absence of tetracycline, the repressor TetR is made. It totally represses transcription from the gene tetB and most of the transcription from its own gene tetR.

Fig. 1. The effect of tetracycline on the transcription of tetR and tetB. R = TetR repressor, T = tetracycline, TetB = tetracycline efflux pump, P = promoter, bent arrow = transcription initiation site, wavy lines = mRNA. By Alan J. Wolfe.
1. What is the generic name applied to the sequence of DNA to which proteins like TetR bind?

2. How does the addition of tetracycline affect TetR? Describe the transcriptional consequence of this effect.

3. What other small molecule, that you have encountered in lecture, does tetracycline resemble in its ability to affect transcription?

4. Describe the consequences of a mutation in tetR that decreases the affinity of the repressor, TetR, for its ligand, tetracycline.

5. Describe the consequences of a mutation in the TetR binding site (i.e., the operator) that causes a significant increase in its affinity for TetR.
CASE 2

Many of the genes necessary for virulence of *Shigella flexneri* are carried on a large plasmid. Most of the structural genes necessary for adherence, invasion, and cell-to-cell spread are located on this virulence plasmid. Some virulence genes are located on the chromosome: they tend to be regulatory in nature. The following figure represents a very simplified schematic of this system.

**Fig. 2.** Regulatory circuit for the *Shigella* virulence. Triangles show the location of the plasmid-encoded genes *virF* and *virB* and the *ipa* operon. P indicates the promoter and bent arrows indicate the transcription initiation site. *vacC, envZ ompR, virR* and *fur* are located on the chromosome. By Alan J. Wolfe
The operon *ipaADCB*, located on the virulence plasmid, encodes proteins involved in adherence.

The genes *virB* and *virF*, also located on the plasmid, encode DNA-binding proteins that function as regulators. VirF positively regulates transcription of *virB*, while VirB positively regulates transcription of the *ipa* operon. Note: Activation by VirB is essential for *ipa* transcription.

The chromosomal gene *virR* encodes a repressor of *virF*. High temperature (i.e., 37°C) inactivates VirR.

The chromosomal genes *envZ* and *ompR* encode a two-component signal transduction system that senses osmolarity. EnvZ is a histidine kinase, while OmpR is the response regulator. High osmolarity ultimately results in the phosphorylation of OmpR, which acts as a positive regulator of the *ipa* operon.

The chromosomal gene *vacC* also acts as a positive regulator of the *ipa* operon.

Finally, the chromosomal gene *fur* acts as a positive regulator of other chromosomal genes required for the sequestration of iron from the host.

1. Researchers have demonstrated that cells of *Shigella* can exchange this plasmid. Name the most likely method of transfer.

2. How many distinct types of mRNA transcripts would you expect RNA polymerase to transcribe from the *ipa* operon?

3. Draw a detailed schematic demonstrating how cells of *Shigella* activate transcription in response to the increased osmolarity encountered upon entering the host’s gastrointestinal tract.

4. Mutants that lack a functional *fur* gene are avirulent. Explain the reason.

5. State whether the *ipa* operon is transcribed under the following conditions

   a) wild-type cells at low temperature  
   b) wild-type cells at high temperature  
   c) *virR* mutants at low temperature  
   d) *virR* mutants at high temperature

6. At least 3 different positive regulators directly control the transcription of the *ipa* operon. Describe possible reasons for this apparent redundancy.
CASE 3

The following figure depicts a cascade of events that culminates in the synthesis of 2 virulence factors, Vir1 and Vir2. RNAP = RNA polymerase holoenzyme; Act1 and Act2 = transcriptional activators. Each works independently and additively. Act1 contributes 20% of the total act2 transcription, while Act2 contributes the remaining 80%.

Fig. 3. Regulation of the vir1 and vir2 genes. By Alan J. Wolfe.
1. State the fundamental reason that activators enhance transcription initiation.

2. How does Act2 affect its own expression? List the potential benefits to a pathogen that can regulate act2 transcription initiation in this manner.

3. What would happen to the synthesis of Vir1 and Vir2 if a transposon inactivated the act1 gene?

4. What would happen to the synthesis of Vir1 and Vir2 if a transposon inactivated the act2 gene?

5. What would happen to the transcription initiation from the act2 promoter if a transposon inactivated the act2 gene?

6. Describe how you would determine the consensus sequence for the Act2 binding site.

7. What would happen to the synthesis of Vir1 and Vir2 if Act2 represses its own transcription rather than activating it?