

Question. 1 (5 points)

A. Describe how transcription and translation differ between eukaryotic cells and prokaryotic cells and how these differences lead to differences in gene regulation.

Prokaryotic translation is coupled to transcription; eukaryotic is not.

Transcription rates can influence RNA secondary structure thereby regulating the ribosomes ability to translate.

B. Describe the similarities and differences between bacterial type III and type IV secretion

Type III and Type IV both secrete effector proteins into eukaryotic cells.

Type III is homologous to flagella and delivers only proteins.

Type IV is homologous to the conjugation Pilus and delivers either proteins or DNA.

Question. 2 (10 points)

A. Name some strategies and give examples of the strategies bacteria use to avoid killing by the lysosome.

1. Avoid phagocytosis (capsules or inactivation of uptake mechanisms) *E. coli* EPEC, *Yersinia*
2. Inhibit fusion of phagosome with lysosome. (*Mycobacteria*)
3. Escape into the cytosol. (*Listeria*, *Rickettsia*)
4. Survive the lysosome (*Coxiella*)
5. Acquire characteristics of a more hospitable compartment (*Legionella*, *Chlamydia*)

B. A novel organism is isolated and sequenced. Its genome encodes a large number of basic proteins ($pI > 7$). During growth in eukaryotic cells the organism is very sensitive to Gentamicin. Propose what cellular compartment it is likely to thrive in and describe experiments to further define its cellular niche.

(Hint: Gentamicin is an antibiotic that is not permeable to eukaryotic membranes, but is small enough to be actively pinocytosed by eukaryotic cells).

Basic proteins are protonated (H^+), acting as a proton sink, suggesting an acidic environment.

Gentamicin is pinocytosed and distributed throughout the fluid phase of the endocytic compartment. This organism is extremely sensitive to Gent, suggesting it is in a compartment accessible to the fluid phase (endosomes, lysosomes). Its extreme sensitivity also suggests that this compartment is

highly fusogenic, maintaining a high concentration of Gent, perhaps an up regulation of fluid-phase uptake.

Another possibility is that the organism increases the permeability of the cell letting the Gent gain access to unusual compartments, such as the cytosol.

This compartment could be tested by EM or Fluorescent microscopy looking at co-localization with known endocytic markers.

Fluorescent microscopy could be used to probe the pH of the compartment, and its accessibility to fluid phase markers.

The bug could be directly labeled with probes that can be used to report on the nature of its surrounding. (Ca^{++} , H^+ , Na^+ , K^+) among other things.