

Micro201 PROBLEM SET 3

**due: Tuesday April 25th 2017**

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Instructions:

Kindly: no more than 4 single-spaced pages; clearly and concisely written; with appropriate (but not excessive) technical detail. And please work independently.

1. Russell and Zynder discovered that *oriC* plasmids (mini-chromosomes) isolated from a DamA+ strain transform DamA- strains very inefficiently. This was the key observation that led Kleckner's group to discover the "sequestration" protein SeqA.

A) Describe the fate of the plasmid upon uptake by the DamA- strain. (i.e. what happens to it upon uptake and how does this lead to a reduction in transformation efficiency?) What happens (at the molecular level) in those rare cases when you get colonies in this transformation?

B) Describe the fate of the same plasmid upon uptake by a DamA+ strain. (What happens to it and how does this lead to efficient transformation?)

note: simply writing something like "the plasmid gets replicated or the plasmid is not replicated" is not sufficient. If you were a microscopic potato bug: what would you "see" at the molecular level happening to the plasmid?

2. Please read: Dubey and Ben-Yehuda "Intracellular nanotubes mediate bacterial communication" Cell 2011 18:590-600. In this paper, the authors report that proteins and other macromolecules can be shared between bacteria of the same species and even between different species. They further suggest that the transfer of these molecules occurs through nanotubes that connect them. If correct, this implies a radical new view of bacterial communities. As you might imagine, this paper is quite controversial and has been highly scrutinized.

A) What experiment (or experiments) in this paper connects the first observation (sharing) to the second (nanotubes)? Do you find it convincing? why? why not?

B) Come up with another ensemble assay (i.e. population-based) other than the ones presented in the paper to monitor the sharing of proteins. Specifically, find one that allows you to permanently "mark" the recipient cell. I am using the words permanently mark here to mean a change in the genome (gene/chromosome) (i.e. not a change in gene expression).

C) Mutants that *both* prevent sharing and no longer produce nanotubes would put an end to the controversy surrounding this very provocative paper. Describe how you would screen for them. Genetic or Biochemical approaches are welcome. Assume cost and labor are no object. (an important subtlety to consider: if these nanotubes are indeed conduits for sharing molecules, do we know whether or not they are gated? In other words: In designing your screen make sure to deal with the possibility that these tubes once connecting two cells allow passive transfer of proteins into *and* out of both cells.)

3. How does Rtp (replication termination protein) work? And why do cells have proteins to terminate replication? (Why isn't it essential? Are there any phenotypes associated with the mutant? Do they make sense in the context of its role in replication termination?)

4. Burkholder and Grossman proposed two models for the role of DnaA in the transcriptional regulation of *sda*. In the first, DnaA is a repressor of *sda*. In the second, DnaA is an activator.

A) What piece of data is inconsistent with a simple repressor model? How do the authors modify their model (and make it less simple) to deal with this?

B) What information appears to be at odds with the activator model?

C) What would your next 2 experiments be to distinguish between these models? (some details are expected here)

D) A simple interpretation of this paper is that Sda functions to prevent sporulation when replication initiation is blocked. But why have a checkpoint to prevent an event in response to an irreparable problem (loss of an initiation factor)? Perhaps Sda normally functions to "coordinate" replication with sporulation during starvation. In other words, maybe Sda ensures cells enter sporulation at the right time during the replication cycle.

Assuming DnaA indeed acts as transcriptional regulator and does so when it is initiation competent (for example in its ATP bound state) (this is a reasonable assumption based on the known regulatory activities of DnaA), when in the replication cycle would cells be able to initiate sporulation in the two different models? (Put another way: when in the replication cycle would cells be inhibited from entry?)

Does this thinking influence your preferred model? If yes, why? How?

E) Which model do the authors favor? Why? Which do you favor? Why?

5. The bacterium you are studying has a chromosomally encoded *parA-parB* locus and an origin-proximal *parS* site. The genes are homologous to the plasmid partitioning genes *parA* and *parB*. You hypothesize that they are involved in chromosome segregation in your bacterium. You make the knock out and discover that the  $\Delta parAB$  mutant has a low frequency (1-2%) of anucleate. You're a little disappointed as you were hoping for a much stronger phenotype. Undeterred, you hypothesize that there is a redundant mechanism that is allowing for chromosome segregation in the majority of cells and if you knock both systems out the cells will not be viable.

A) You try to do a screen like Hiraga to find mutants that generate anucleated cells at a high frequency but it fails for technical reasons. Despite the urging of your PI you decide to give it one last chance. Describe a genetic screen to identify this putative second chromosome partitioning mechanism. Let your hypothesis (described above) guide your choice of screen.

B) How would you validate the mutants you isolate in your screen?

That is, how would determine whether they play a role in chromosome segregation?

How would you analyze the double mutants?

C) What type of “false-positives” might come out of your screen? (i.e. What other types of mutants might you isolate?)

D) If you fail to identify a single mutant in your genetic screen how might you explain this result?

Give **3 independent** and **non-trivial** explanations.

(trivial explanations are technical ones: the mutagen didn't work or I didn't screen enough cells)

In other words: What might this “negative result” be telling you about the biology of chromosome segregation?

E) As a back-up project you decide to study the *parA* and *parB* genes (and/or the proteins) themselves (in case you don't come up with a redundant mechanism). What is the very first experiment you would do to support or reject the proposed role for these proteins in DNA segregation? i.e. What experiment would you invest in first (this must be an easy one that won't take you too long) before committing more time to this project. (I have a very easy one in mind, but as long as you can make a cogent argument for the one you propose and it won't take you more than 1 month of experiments full credit is yours)

6. What is the finding presented in Figure 1H of the Wang Sanders & Grossman paper (Cell, 2007)? What is the larger (broader) conclusion (and implications) the authors are trying to make from this figure? Describe a more direct experiment to test this idea.

7. In the Ben-Yehuda and Losick paper (Cell, 2001), compelling evidence was presented that the membrane protein SpoIIIE and increased expression of FtsZ were necessary and sufficient to shift medial division to the pole (with a spiral-like FtsZ intermediate). However, the authors acknowledge that other proteins could participate in this landmark morphological event.

Invent (i.e. create) two additional activities that might exist to facilitate an efficient switch from medial to polar division.

8. You are pioneering the study of a new bacterium (see question 9). One of the first things you do is sequence its genome. You discover that this bacterium has an autoinduce-2 (AI-2) (Esperanto) synthase. Based on this information, you hypothesize that this bacterium produces a quorum-sensing molecule. Attempts to detect the molecule by Mass Spec in cell supernatants have failed. What biological experiment(s) would you do to convince yourself that this bacterium does indeed produce an AI-2 factor? (No RNA-seq required). Include a control (or two) in your description.

9. In one paragraph, describe a model bacterium that you plan to develop in your future postdoc. And what it will serve as a model for. Take inspiration from the topics in this class, your general interest in biology/biotechnology. There is no “wrong” answer to this question – just make sure you can clearly articulate the question(s) that this model will be useful for. And why this organism and not another. . .

It has been an honor and a pleasure teaching you in Micro201. Enjoy the rest of the semester.