Micro201
Rudner Lecture 1: Replication and its control
February 26th, 2019

Overview:

In this class we will discuss DNA replication and its control. The background reading is focused on replication initiation and we will spend some time at the beginning of class discussing the mechanics of initiation (the biochemistry/molecular biology) as well as how it is regulated. How does the cell ensure that all origins fire synchronously and once and only once per cell cycle? How does the bacterial cell coordinate replication with growth? We have answers to some of these questions. Others are interesting and outstanding questions to think about and answer in the future.

The first paper ((**Lu, Campbell, Boye, Kleckner 1994**) seeks to identify factors that ensure that when "replication initiation potential" is high origins fire once and only once. We will only discuss the first section of the results: the genetic "selection" for mutants that prevent sequestration and Table 1. Make sure you understand the strategy, the logic behind the selection, and how they (or you) might have followed up to confirm you had a true sequestration mutant. What would the most efficient method be? What would you do next?

We will then focus our attention on an unusual level of replication control: regulation of replication elongation. The paper (**Wang, Sanders, and Grossman 2007**) discusses how *B. subtilis* controls DNA replication in response to amino acid starvation and the induction of ppGpp (also called "magic spot").

Most of the primary data in the Wang paper is not shown. Instead, the authors present their results in graphical format (so-called "derived data"). Read the methods (and figure legends) carefully so you can reconstruct the actual experiments. I recommend building flow charts. If you don't understand these unusual techniques – look them up. I will definitely ask you to go to the board and walk us through the experiments (maybe in even greater detail than Tom). There are many tools for studying DNA replication and understanding them will help you critically evaluate the paper and think of follow up experiments. Once you've solidified the experiments in your mind, ask yourself if you find them convincing. Would you want more? If yes, what?? When might *B. subtilis* need such a control mechanism?

The background reading: 1) A comprehensive review on the regulation of replication initiation (principally focused on *E. coli*). 2) A perspective on how cell cycle events (DNA replication, segregation, and division) might be coupled to nutrient availability and metabolic status. 3) A snapshot of the replisome from Mike O'Donnell's lab.

1) Leonard and Grimwade, 2011
2) Wang and Levin, 2009
3) Yao and O'Donnell 2010