Paper

Readings from *Molecular Biology of the Cell* (pp. 239–240, 242–244, 250–251)
- DNA Replication Mechanisms
- Base-Pairing Underlies DNA Replication and DNA Repair
- The High Fidelity of DNA Replication Requires Several Proofreading Mechanisms
- A Strand-Directed Mismatch Repair System Removes Replication Errors That Escape from the Replication Machine

Relevant Techniques
- Transgenic/fluorescent proteins (p. 502)
- Fluorescence microscopy (pp. 536–537, 542–546)
- Yeast genetics assays used in Table 1:
  - For the hom3-10 reversion assay, these strains contain a frameshift mutation that inactivates the *hom3* gene; yeast with this mutation require the presence of threonine in the medium. The assay looks for mutations that will revert this frameshift mutation back to a functional *hom3* gene, and allow the yeast to grow on medium that is lacking threonine.
  - Canavanine is a toxic form of an amino acid that is incorporated erroneously in place of arginine. In this assay, it is added to the yeast growth-medium plates, and only yeast that contain the *CAN1* gene are resistant to canavanine and can grow. They start with yeast cells that are resistant, and they are looking for the rate at which the *CAN1* gene is mutated to inactivate the gene and therefore make the cells sensitive to canavanine.

Questions
1. What was the motivation for their study?
2. What strategy did they use to observe mismatch repair (MMR) proteins in yeast cells? What was important to consider when using this strategy? What is an alternative strategy they could have used, and what is an advantage/disadvantage of that strategy?
3. What hypothesis and prediction did the authors have for the first experiment in Figure 1? Did their data support their hypothesis? Explain. What additional experiment could have strengthened this result?
4. How is the Msh2-Msh6 complex recruited to sites of DNA replication? What data did they present to support this conclusion?

5. The authors suggest that, based on previous work, there is likely some redundancy in the role of Exo1 in MMR. What is meant by redundancy, and what are the authors able to contribute to our understanding of Exo1 function based on their data in Figure 3?

6. The authors state that mutations that cause increased misincorporation of bases into the lagging strand during replication are synergistic with a mutation in Exo1. What does it mean to say that there is a synergistic effect, and what does it suggest about the role of Exo1 in MMR?

7. What evidence do the authors present to show that Pms1 foci represent a short-lived intermediate in MMR?

8. What additional questions do you have after reading this paper?