Instructor Profile

Dr. Stefanie H. Chen

Research Areas:

- DNA repair
- Regulated gene expression
- Protein characterization

Techniques:

Quantitative PCR, chromatin immunoprecipitation, PCR, SDS-PAGE, Western blot, yeast two-hybrid, fluorescence polarization, fluorescence microscopy, recombinant protein expression in bacteria, molecular cloning

Despite being the most studied organism in the world, there are still many genes in the *E. coli* genome with unknown functions. A recent screen for genes needed for recovery from ionizing radiation damage identified eight previously uncharacterized genes as having important functions in the repair of radiation-induced damage (Byrne *et al*, 2014). One of these genes, *radD* (previously *yejH*), is the focus of my research.

One project is to identify the conditions under which *radD* is expressed. Unlike most genes in the cell, the *radD* gene is not normally expressed because it is under the control of the σ^{54} promoter, a condition-specific transcriptional regulator. Although we have evidence that *radD* is needed for DNA repair, the other genes controlled by the σ^{54} promoter are involved in a variety of other aspects of metabolic stress, including nitrogen assimilation, motility, detoxification, and alternative carbon sources. Using quantitative PCR, a student will study the conditions under which the *radD* gene is expressed, potentially documenting the first incidence of the σ^{54} promoter responding to DNA damage. This student will gain experience with microbial cell growth, RNA isolation, quantitative PCR, and stress response systems.

A second project is to study the function of the RadD protein. RadD appears to be a superfamily 2 helicase, although no helicase activity has been observed, and the protein directly interacts with SSB, a central organizer of DNA repair functions. A variety of biochemical and genetic methods will be employed to explore the interaction of RadD with other cellular components, including other DNA repair proteins and various forms of DNA. This student will gain experience with recombinant protein expression, protein purification, SDS-PAGE, and potentially other methods including fluorescence anisotropy, fluorescence microscopy, far Westerns, and yeast two hybrid.