Instructor Profile

Dr. Stefanie H. Chen

Research Areas:
- DNA repair mechanisms
- Regulated gene expression
- Protein characterization
- Microbial metabolism/resistance

Techniques:
Molecular cloning, recombinant protein expression in bacteria, quantitative PCR, ChIP, SDS-PAGE, Western blot, yeast two-hybrid, EMSA, fluorescence polarization, fluorescence microscopy, AFM, affinity chromatography, directed evolution, genomic sequencing

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.

Characterization of *radD* is focused on both *in vivo* expression profiling and *in vitro* protein assays. Unlike most genes in the cell, the *radD* gene is predicted to be under the control of the σ^{54} promoter, a stress condition-specific transcriptional regulator involving binding of the rpoN subunit of RNA polymerase. Using purified RpoN protein and segments of the *radD* promoter DNA, a student will characterize binding of the subunit to this genomic regulatory element, potentially documenting the first incidence of the σ^{54} promoter responding to DNA damage.

A second focus of my research is the function of the isolated RadD protein. RadD contains the conserved domains of 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 student will use atomic force microscopy (AFM) through the imaging facility on campus to directly visualize RadD and SSB binding to DNA substrates.

Students involved in this research will contribute to the broad understanding of microbial metabolic pathways, particularly DNA repair, while refining their research and communication skills.