Is Pleased to Present a Seminar
Presented By

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Thursday, September 12, 2019
At 3:15 pm
NWC 1313

Deciphering the Mechanisms Underlying CRISPR Biology and their Repurposing as Biotechnological Tools

Even the smallest of organisms, bacteria and archaea, have developed complex immune systems to ward off phage infections. CRISPR-Cas systems, involving RNA-guided nucleases that sequence-specifically target and cleave intruder DNA or RNA, provide adaptive immunity to bacteria and archaea against phage infections. Interestingly, CRISPR-Cas mechanisms are being repurposed for gene therapy applications and developing biotechnological tools.

My laboratory’s approach has been to characterize the fundamental mechanisms involved in several aspects of CRISPR biology to facilitate the development of biotechnological tools. Since starting my independent research career at OU in August 2014, my lab has focused on four distinct aspects of CRISPR-Cas systems. We use a combinatorial approach of molecular biology, biochemistry, bioinformatics, biophysics, cell biology and structural biology for our research.

(1) Determine the stringency of Cas proteins in target DNA cleavage. Prior to our work, it was believed that RNA-mediated activation of Cas proteins is a prerequisite for DNA cleavage. Work in our laboratory discovered an unprecedented RNA-independent DNA cleavage by Cas9 and Cas12a under specific conditions. This promiscuous activity can cause unwarranted effects in gene therapy applications. We are characterizing the associated mechanism(s) to develop protein variants that are devoid of such activity.

(2) Elucidate Cas protein mechanisms to improve stringency of DNA cleavage. We focused on identifying the role of an arginine-rich bridge helix (BH) that is conserved across several Cas proteins used in biotechnology applications. We showed for the first time that varying the amino acids in the BH imparts selectivity in target DNA cleavage by Cas9 and Cas12a. Our finding is unique and significant because it can be translated to all Cas proteins with BH to develop stringent variants, and avoids cumbersome individual ortholog-specific manipulations of entire multidomain proteins. (continued on back)

Refreshments will be served at 3:00 pm
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(3) **Determine the mechanism of CRISPR adaptation.** Bacteria become immune to phage infections by inserting a piece of intruder DNA into the CRISPR locus in a process called adaptation. The inserted DNA produces the guide RNA required for targeted DNA cleavage during secondary infections. Our work has uncovered protein-DNA interactions that dictate site-specific insertion of foreign DNA into the CRISPR locus. Using this information, we are developing CRISPR adaptation proteins as tools to insert DNA tags at any desired genomic location.

(4) **Identify mechanisms of CRISPR-mediated bacterial pathogenicity.** Cas9 has been implicated with virulence in several bacteria, the mechanisms of which are currently unknown. This is an area of CRISPR biology that is currently under-studied. We have identified in vitro interactions of Cas9 with other cellular proteins that may enable pathogenicity. The knowledge gained will facilitate novel anti-microbial approaches based on Cas9 modulations.