The Summer Undergraduate Research Fellowship (SURF) Symposium
4 August 2016
Purdue University, West Lafayette, Indiana, USA

Biochemical and structural characterization of Cdc14 phosphatases from pathogenic fungi

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ABSTRACT

Cyclin-dependent kinases (Cdk) drive cell cycle progression and reversal of Cdk phosphorylation is essential for mitotic exit. Cdc14 is a widely conserved family of protein phosphatases that reverse Cdk phosphorylation. Recently, Cdc14 was also found to be essential for pathogenicity of some fungal plant pathogens. Fungal pathogens, like *Ustilago maydis*, decrease agricultural crop yield costing global agriculture by some accounts $60 billion per year. Since Cdc14 is absent in plants, a fungi specific Cdc14 inhibitor could be made to reduce the pathogenicity of *U. maydis* and other fungal plant pathogens to increase crop yields. To guide inhibitor development, a three-dimensional structural model of fungal Cdc14 is needed. Therefore, we recombinantly expressed *Ustilago maydis* Cdc14 (UmCdc14) and a catalytically inactive substrate trapping mutant (UmCdc14^{C318S}) with N-terminal hexa-histidine tags in *E. coli*. Recombinant UmCdc14 and UmCdc14^{C318S} were successfully purified using immobilized metal affinity chromatography. We screened the purified proteins by sitting drop crystallography. Two conditions yielded small crystals for UmCdc14. One condition yielded a crystal for UmCdc14^{C318S}. These conditions were used for large scale crystal growth by hanging drop crystallography. If ideal conditions are found, UmCdc14^{C318S} will be crystallized bound to a peptide substrate to capture the molecular binding determinants that will help guide inhibitor design. Wild-type UmCdc14 will be crystallized bound to small molecule inhibitors identified by a previous high throughput library screen. In the future, we will also explore crystallization of Cdc14 orthologs from other plant pathogens.

KEYWORDS

Cyclin-dependent kinases (Cdk), Mitosis, Cell division cycle 14 (Cdc14)