Elucidation of a BNM2 BURP domain biosynthetic pathway in *Ziziphus jujuba*

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Third Year Seminar
Medicinal Chemistry Program
February 16, 2023

Ribosomally synthesized and post-translationally modified peptide (RiPP) natural products have been attractive compounds for pharmaceutical discovery as they exhibit broad biomedical activities and are highly diversifiable. RiPPs follow a biosynthetic logic in which the RiPP sequence is directly encoded in a structural gene. The corresponding precursor peptide is post-translationally modified in a core peptide motif and proteolytically processed to the mature RiPP. Many RiPPs exhibit their bioactivity due to macrocyclization of the peptide backbone. Recently, the Kersten group has identified that plant-specific BURP-domain proteins (named after founding members BNM2, USP, RD22, and PG1β) act as peptide cyclases in the biosynthesis of bioactive plant peptides such as anticancer moroidins and sedative cyclopeptide alkaloids. Known BURP-domain cyclases catalyze macrocyclization of amino acid side chains via tyrosines and tryptophans in a copper-dependent autocatalytic reaction. Genome mining of BURP-domain gene loci shows that BURP-domain genes of the BNM2 class colocalize with separate repetitive genes, which show similarity to core peptide domains of characterized autocatalytic BURP domain cyclases of the RD22. In particular, a gene cluster of BNM2 BURP-domain genes and separate repetitive genes encoding for putative cyclopeptide alkaloid precursor peptides have been identified in the genome of Chinese date tree (*Ziziphus jujuba*), which belongs to a genus producing many bioactive cyclopeptide alkaloids such as sedative sanjoinine A and anti-coronaviral jubanine H. The split genetic structure of BNM2 BURP domains and their candidate substrates suggests that these BURP-domain proteins could be peptide cyclases for *in trans* core peptide macrocyclization similar to canonical RiPP macrocylization without the need for substrate-enzyme-fusion. The BURP domain ZjeBURP2 was identified in close proximity to three putative RiPP precursor genes; with one (ZjePrec-FPIY) being adjacent to the BURP domain. Utilizing ZjeBURP2 and ZjePrec-FPIY, this project aims to elucidate and establish the biosynthetic assembly of the mature RiPP product FPIY. Elucidating this system provides a potential route to accessing and diversifying cyclopeptide alkaloids. The outlook of this project is to use these BNM2 BURP domains to develop a library of cyclopeptide alkaloid analogs of known products with interesting bioactivities, like jubanine H.