Rational Engineering of Hybrid RiPP Biosynthetic Pathways

Brandon Burkhart and Douglas A. Mitchell

Ribosomally synthesized and posttranslationally modified peptides (RiPPs) are a rapidly growing class of structurally diverse natural products and have potential as new drug leads. These complex molecules begin as unstructured peptides, and then distinct RiPP biosynthetic enzymes form heterocycles, macrocyclic structures, or other modifications to conformationally restrict these peptides and endow them with various bioactivities. Because of this biosynthetic logic, genetic engineering of precursor peptide sequences and varying the modifying enzymes within a gene cluster allow for the creation of new natural products. Indeed, nature has already diversified peptide sequences and mixed-and-matched biosynthetic enzymes in different pathways. Based on this inspiration and recent advances in our understanding of precursor peptide recognition, an engineered biosynthetic system was designed with a hybrid peptide to enable combination of modifying enzymes from different RiPP pathways to create artificial, hybrid natural products. Using this strategy, there is potential to rationally combine selected RiPP biosynthetic enzymes, which do not act together in nature, to produce novel peptide scaffolds that can be screened for unique bioactivities.