Investigating partner enzyme interactions with cytochrome P450 71AV1 in *A. annua* and their role in artemisinin biosynthesis.

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Research Abstract

Artemisinin, from the plant *Artemisia annua,* is the most available and cost-effective medicinal treatment for malaria. Its availability is, however, threatened by inconsistencies in plant production, varying in content from 1.2% to as low as 0.1%. Thus, uncovering the genes involved in its biosynthesis is critical for enhancing it within plants and synthetic systems. Cytochrome P450 71AV1 (CYP71AV1) is one of the key enzymes which is implicated in the conversion of amorpha 4, 11-diene into artemisinin precursors and artemisinin itself. Its efficacy is reliant on partner enzymes such as cytochrome reductase 1 (CPR1), cytochrome B5 (CYB5), cytochrome c, and possibly CPR2. Overexpressing *CYP71AV1* and these associated genes in *A. annua* plants was reported to increase artemisinin levels. While this confirms the synergistic effects of these genes on artemisinin production, the exact nature of these protein-protein interactions remains to be fully understood. Assessing the way in which these enzymes compete with and enhance each other's activity is critical to the advancement of metabolic engineering in planta and within synthetic systems. I intend to explore these enzymatic qualities using a combination of genetic, biophysical, and microscopy approaches. First, by characterizing CPR2's involvement in the CYP system through complementation experiments in tobacco, and then by using super plasmon resonance (SPR) to determine the kinetic parameters of the CPR1, CPR2, and CYB5 enzymes with CYP71AV1. Using confocal microscopy and other microscopic techniques, I will visualize the localization and organization of these proteins. This data will help inform computer modelling on these and related proteins and ultimately contribute to increasing global artemisinin production.