MACROMOLECULAR ORGANIZATION OF FLAVONOID BIOSYNTHESIS IN *ARABIDOPSIS THALIANA*.

by

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Living cells manufacture and degrade thousands of chemical compounds *in vivo*. To do this cells rely on the activities of thousands of different protein catalysts distributed in aqueous interior compartments. Over the past several decades studies have shown that the thermodynamic and kinetic properties of most proteins, including enzymes, are different *in vivo* as compared to *in vitro*. Based on *in vitro* studies metabolic pathways have traditionally been thought to consist of intermediates randomly diffusing between soluble enzymes and are still portrayed as such in many biochemistry textbooks. A large number of metabolic pathways however are now known to exist as enzyme complexes due to molecular crowding effects *in vivo*. These differences have contributed to the controversy that surrounds explanations of how metabolic pathways are spatially organized and regulated in the living cell. The organization of enzymes *in vivo* is now thought to play a significant role in normal cellular physiology but evidence of this role, beyond intermediate channeling, is lacking. The long term goal of this work is to develop an experimental model and test the validity of theories concerning the spatial arrangement of enzymes in regulating metabolic pathways.

The studies described in this dissertation have been focused on understanding how living cells organize metabolic pathways. I have examined some of the theoretical aspects of enzyme-enzyme interactions by modeling the complex formed by mitochondrial malate dehydrogenase and citrate synthase. These studies show that MDH and CS may bind in a specific orientation that facilitates the direct transfer of oxaloacetate from MDH to CS through a molecular channel. During these studies it was determined that *A. thaliana* does not encode stilbene synthase (STS), which catalyzes the first step in a pathway that competes with flavonoid biosynthesis in other plant species. Moreover, it was shown that flavonols are not required for pollen viability in *A. thaliana* as they are in maize and petunia. I also describe a novel method to clone fragments of DNA without ligase using the polymerase chain reaction (PCR). To establish an experimental model I have used a variety of techniques to analyze interactions between enzymes in the well-characterized flavonoid biosynthetic pathway in *Arabidopsis thaliana*. Evidence is presented that indicates that the first four enzymes in this pathway form a complex. Collectively this work suggests that the structural organization of enzymes into complexes is an important aspect of cellular metabolism and might directly impact the relative levels of specific compounds that are synthesized *in vivo*.