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### **Proposal Title**

The Predicted Role of a Long Noncoding RNA in Plant Flavin Homeostasis

### **Proposal Description**

All cells must have the capacity to exchange electrons to survive- flavins are some of the cofactors that facilitate this. Flavins, comprising riboflavin and its derivatives flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), are water soluble metabolites with a unique structure that allows for the exchange of one or two electrons making them key in numerous biological reactions. Humans and other animals are able to synthesize FMN and FAD in situ- however, they must consume the precursor to both of these, riboflavin, through the diet. On the human side, riboflavin deficiency is one of the major vitamin deficiencies in developing nations, linked to anemia, brain disfunction, cancer, and other health issues<sup>1</sup>. On the animal side, ~80% of synthesized riboflavin is diverted to animal feed<sup>2</sup> (~2,400 tons per year). Because of this, increasing flavin levels in crops is of great interest. The first step to accomplishing this is to understand the basis of flavin metabolism. Unfortunately, while much of the flavin biosynthetic pathway has been elucidated, its regulation is still a mystery.

Flavin levels are tightly regulated in the plant through a currently unknown method. However, work in our lab has led us to a long noncoding RNA (lncRNA), At2g05995, that appears to have a role in maintaining flavin homeostasis in plants. lncRNAs are transcripts greater than 200bp that do not code for protein and have many identified regulatory roles in plants including maintaining flowering time, acting as scaffolds for protein complexes under heat stress, helping the plant respond to phosphate starvation, and more<sup>3</sup>. We have generated transgenic plants with decreased levels of this lncRNA. Excitingly, our preliminary results show that these transgenic plants have a statistically significant increase in seed flavin levels; approximately 10-times increased riboflavin levels compared to wild-type, and 2- to 3- times increased FMN and FAD levels. Our next steps in the lab are to determine how this lncRNA is functioning- we will be examining where it localizes in the cell (key to lncRNA function), and if it is functioning by interacting with DNA, RNA, or protein and drawing up a scheme for how exactly this lncRNA works. This project has implications both in the research world by providing a novel function for plant lncRNAs and beginning to chip away at the long-standing question of flavin homeostasis, along with real-world implications for generating riboflavin-enriched crops for regions that would otherwise be at risk for deficiency-related illnesses.

This grant will allow me to communicate this research at the 2024 national American Society of Plant Biologists (ASPB) meeting in Honolulu, HI from June 22-26. This year (2023) I received a WSU IBC travel grant that allowed me to present preliminary research at the meeting in Savannah, GA. At this

meeting I competed in the Science Communication Hackathon, where my team and I won first place; this prize includes free registration and hotel stay at the meeting next year. This research week grant would cover the cost of the flight and allow me to present next year's research. At the 2024 meeting I will be presenting this research on the main stage during the 3-minute theses competition, and am also applying to give a research talk in addition to presenting a poster. This will allow me to network with other scientists in the field to gain connections for after graduation, and promote research at WSU.

<sup>1</sup>Edwards et al, 2014. Methods Mol Biol

<sup>2</sup>Ji et al, 2014. BMC Plant Biology

<sup>3</sup>Wu et al, 2020 Plants

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