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Heterologous Expression of the Xantha-L Subunit of MG-Protoporphyrin IX Monomethyl Ester Cyclase from Barley

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Chlorophyll *a* is central to life on this planet because of the central role it plays in the process of photosynthesis. Mg-protoporphyrin IX monomethyl ester (MPE) cyclase catalyzes a poorly understood reaction in chlorophyll *a* biosynthesis that is responsible for formation of the isocyclic ring. The isocyclic ring is a unique and necessary feature that exists in all chlorophyll molecules, including bacteriochlorophyll, primarily because addition of the isocyclic ring shifts absorbance of the chlorophyll into the red absorbance region. This shift in absorbance optimizes the pigment for absorption of light in the energy range of the absorption spectrum used for photosynthesis. The MPE cyclase enzyme is composed of at least three subunits: one soluble and two membrane-associated. This project focuses on one of the membrane-associated components of the cyclase, which is encoded by the Xantha-L locus in barley. Xan-L is a homolog of the *acsF* gene in the photosynthetic bacterium, *Rubrivivax gelatinosus.* The *acsF* locus has been shown to be required for aerobic cyclase function by gene knockout analysis in *R. gelatinosus*. In order to study the Xan-L subunit of the MPE cyclase, it is necessary to express the protein in a soluble form for use in enzyme assays. Previous experiments with heterologous expression of the Xan-L protein led to insoluble protein (Mats Hansson, Carlsberg Research Laboratory, personal communication). In an attempt to express the protein in a soluble form, the Xan-L coding region will be transferred to a set of special expression vectors meant to increase protein solubility when heterologously expressed in *E. coli.* However, moving the cloned Xan-L gene into the vector requires alteration of the sequence by site-directed mutagenesis. Once the mutation is created, the Xan-L gene will be placed into the Variflex plasmids Once the clones in the Variflex vectors have been made, expression of the modified XanL protein will be performed to test whether the protein is soluble.