FINAL TECHNICAL REPORT

Steve Rodermel: Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA

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PURPOSE OF RESEARCH

The *immutans* (*im*) variegation mutant of Arabidopsis is an ideal developmental system to gain insight into the poorly understood mechanisms of chloroplast biogenesis. This process entails the differentiation of chloroplasts from proplastids in the leaf meristem or from etioplasts in illuminated dark-grown seedlings. Previous DOE-sponsored research from our lab showed that the *IM* gene codes for a plastid membrane-localized plastoquinol oxidase (PTOX) that bears functional and structural similarity to the alternative oxidase (AOX) class of mitochondrial inner membrane ubiquinol terminal oxidases. We also discovered that PTOX functions as a terminal oxidase in redox regulation of developing and mature thylakoids by transferring electrons from plastoquinol (PQH_2) to molecular oxygen, forming water and plastoquinone (PQ). In this role it participates in several processes that are crucial for photosynthesis: the desaturation reactions of carotenoid biosynthesis, chlororespiration, cyclic electron flow around PSI, and protection from oxidative stress.

In the present grant we turned to second-site suppressor analysis to identify genes that modify the *im* variegation phenotype. This type of analysis can provide information about PTOX function and activity *per se* (e.g., by analyzing interaction suppressors), as well as knowledge about factors and pathways that act early in chloroplast biogenesis, but which might not be easily accessible by other means (bypass suppressors). ***Our purpose was to gain insight into mechanisms of PTOX function, im variegation and chloroplast biogenesis. Or goal was to gain much-needed information about the mechanisms that regulate photosynthesis, and that compensate for a loss of photosynthetic activity in a plant.***

RESEARCH PERFORMED & RESULTS

In our search for *im* suppressors, we were intrigued by observations of Rédei in the 1960's that "vigorous vegetative growth" of *im* could be obtained by crossing it with *gigantea (gi*), a late-flowering "supervital" mutant he had recently isolated. Unfortunately, Rédei did not describe the chloroplast phenotype of the *imgi* double mutants, nor their phenotype during development; he assumed the traits acted independently. However, it has since become apparent that "supervitality" is one of many strikingly diverse, and seemingly unrelated, phenotypes caused by a *GIGANTEA* deficiency. These phenotypes also include alterations in period lengths of circadian rhythms; resistance to paraquat; impairment in phytochrome B signaling; enhanced accumulation of starch and anthocyanins; elevated expression of detoxifying enzymes such as SOD and APX in the presence of paraquat; and increased sensitivity to freezing. *GI* was cloned nearly forty years after Rédei's initial description of *gi*, and molecular analyses since that time have revealed that *GIGANTEA* acts as a scaffold protein for the assembly of complexes that mediate protein-protein stability/activity via the 26S proteasome. It also interacts with a number

of components of the circadian clock-associated flowering pathway in Arabidopsis and plays an important role in photoperiodic flowering.

To assess how *gi* suppresses *im* variegation, we undertook an integrated approach to characterize the *im gi* double mutants: ***our aim was to determine which defect(s) in gigantea are responsible for im suppression and, hence, which are important factors that compensate for a lack of PTOX and capable of controlling photosynthetic activity.*** These included a classical genetic approach in which mutants perturbed in each of the downstream effects of *gi* were crossed with *im* to see whether variegation is suppressed. A second approach involved an investigation of how photosynthetic activity is affected in an *imgi* background, using techniques of chlorophyll fluorescence, gas exchange, and molecular biology -- techniques we have used to characterize a number of other photosynthetic mutants.

Our results showed that *gi* suppresses *im* variegation during a late stage of plant development; at this stage, newly-emerging leaves are all-green, not variegated. We found that this suppression is due to a developmental-specific reduction in giberrelin (GA)-signaling late in *gi* development that de-represses cytokinin-signaling. This conclusion was verified by showing that the cytokinin and GA- signaling pathways are integrated by the GA response inhibitor, SPINDLY (SPY). Importantly, the de-repression of cytokinin signaling late in *gi* development reprograms processes required for chloroplast differentiation, resulting in an enhancement of photosynthetic rates and an elevated ROS scavenging capacity. These observations make it likely that when *gi* is combined with *im*, these processes act in concert to relax excitation pressures of developing *im* thylakoids (i.e., *im* has overreduced membranes), thereby promoting the formation of photosynthetically-competent chloroplasts and suppression of the *im* variegation defect.

Interestingly, the present research also showed that the suppression phenotype of *imgi* can be mimicked by crossing *im* with the starch accumulation mutant, *sex1*, perhaps because *sex1* utilizes pathways similar to *gi.*

In conclusion, the present DOE-sponsored research has provided a direct genetic linkage between GIGANTEA and chloroplast biogenesis, providing insight into how the plant growth cytokinin and GA affect the photosynthetic process.