

ABSTRACT

ANALYSIS OF THE SUBCHLOROPLASTIC DISTRIBUTION OF GENOMES UNCOUPLED 4 AND MAGNESIUM CHELATASE

By

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Chlorophyll is the primary light harvesting pigment for photosynthesis in higher plants and many other organisms that perform oxygenic photosynthesis. Twenty three enzymes contribute to chlorophyll metabolism at different stages of plant growth and development. Tight regulation of chlorophyll biosynthesis is important because chlorophylls and some of their precursors are strong photosensitizers that can produce toxic reactive oxygen species (ROS) if porphyrins that are exposed to bright light collide with molecular oxygen. The GENOMES UNCOUPLED 4 protein from *Arabidopsis thaliana* (hereafter referred to as GUN4) stimulates magnesium chelatase by a mechanism that involves binding the ChlH subunit of magnesium chelatase and its porphyrin substrate and product, the photosensitizing chlorophyll intermediates protoporphyrin IX and magnesium protoporphyrin IX, respectively. We hypothesized that GUN4 stimulates chlorophyll biosynthesis not only by activating magnesium chelatase but also by helping channel protoporphyrin IX into complexes of enzymes that drive chlorophyll biosynthesis on chloroplast membranes—the site of chlorophyll biosynthesis. From this hypothesis, we predicted that the porphyrin-bound form of GUN4 would more stably associate with chloroplast membranes by interacting with chloroplast membrane lipids or enzymes that participate in chlorophyll biosynthesis. Also, by binding protoporphyrin IX and magnesium protoporphyrin IX, GUN4 was previously hypothesized to shield these

porphyrins from collisions with molecular oxygen thereby contributing to photooxidative stress tolerance. To test these hypotheses, I used site-directed mutagenesis to change conserved amino acid residues of GUN4. These amino acid substitutions were previously shown to cause deficiencies in the porphyrin-binding activity and the Mg-chelatase stimulatory activity of a *Synechocystis* relative of GUN4. I found that some of the amino acid substitutions that cause porphyrin-binding defects in the *Synechocystis* relative of GUN4 also cause porphyrin-binding defects in GUN4. I also developed a binding assay that allowed me to show for the first time that GUN4 binds its natural ligands—protoporphyrin IX and Mg-protoporphyrin IX. I used these porphyrin-binding deficient versions of GUN4 to test whether the porphyrin-binding activity of GUN4 that was previously demonstrated for cyanobacterial relatives of GUN4 *in vitro* is also significant *in vivo*. I found that porphyrins promote the association of GUN4 and ChlH with chloroplast membranes and induce Mg-chelatase activity on chloroplast membranes. Additionally, I found that defects in porphyrin binding and defects in ChlH function inhibit the association of GUN4 with chloroplast membranes. Finally, I found that stably transformed *Arabidopsis* plants that express porphyrin-binding-deficient versions of GUN4 exhibit higher expression levels of ROS-inducible genes compared to wild type. Based on these results, I conclude that GUN4 helps channel porphyrins into chlorophyll biosynthesis by binding porphyrins and ChlH on chloroplast membranes and stimulating Mg-chelatase activity. I further conclude that these activities contribute to photooxidative stress tolerance. These findings indicate that the porphyrin-binding activity of GUN4 significantly contributes to chlorophyll biosynthesis and photooxidative stress tolerance *in vivo*.