Mechanism of Vipp1 aggregation in the green alga *Chlamydomonas reinhardtii*

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The thylakoid membrane, found within most cyanobacteria and all chloroplasts, is the site of the light reactions of oxygenic photosynthesis and is essential for the creation of the proton gradient which powers ATP synthesis. Therefore, its integrity is required to ensure the optimal function of both photosynthetic electron transport and downstream carbon fixation. Vesicle-inducing protein in plastids 1 (Vipp1), an ESCRT-III family protein, regulates thylakoid remodeling and homeostasis, but its mechanisms of action and aggregation are poorly understood in vivo. While we have observed aggregation kinetics and regulation in a relatively simple cyanobacterium, eukaryotic systems have more complex thylakoid architecture and we sought to investigate domain effects on aggregation kinetics. By inducing controlled light damage to the thylakoid membrane in the green alga *Chlamydomonas reinhardtii* and immediate spatiotemporally resolved confocal imaging of GFP-tagged Vipp1, we were able to resolve repair priorities. In this system, Vipp1 aggregates to damage sites within the thylakoid membrane over timescales of minutes, much as in cyanobacteria. However, when multiple damage sites are stimulated, the protein prioritizes repair to a certain location depending on the damage site's distance to the pyrenoid. This research demonstrates the importance of the pyrenoid as a major regulator for the spatiotemporal distribution of Vipp1 and how damage repair is prioritized within the thylakoid membrane. The protection of the pyrenoid from proton leakage, and thus the protection of carbonic anhydrase-mediated carbon fixation, is prioritized over other regions of the chloroplast.