Identification of an early assembly factor for Photosystem II biogenesis

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Photosystem II (PSII) initiates photosynthesis in oxygen-evolving photosynthetic organisms, including cyanobacteria, algae, and plants, by catalyzing light-driven electron transfer from water to plastoquinone. PSII is a large thylakoid membrane-localized complex composed of at least 20 protein subunits and many cofactors including chlorophylls, hemes, quinones, and the unique Mn₄CaO₅ cluster. Its homodimer forms supercomplexes with a variable number of light-harvesting complexes II depending on the light intensity. Cytochrome b₅₅₉ (Cyt b₅₅₉) is an essential component of PSII that is required for PSII assembly and function, likely by participating in secondary electron transfer pathways and preventing photoinhibition (Chiu and Chu 2022). The reaction center complex, consisting of D1, D2, Cyt b₅₅₉, and PsbI, is the first transiently accumulating complex during the early phase of PSII biogenesis (Nickelsen and Rengstl 2013; Figure, C). Cyt b₅₅₉ is a heme-bridged heterodimer of the α and β subunits, encoded by the chloroplast genes, psbE and psbF, respectively (Figure, B). The heme is non-covalently bound by a histidine residue from each of the subunits. Although numerous studies have been conducted on the structure and function of Cyt b₅₅₉, its assembly pathway and the exact function in oxygenic photosynthesis remain enigmatic.

In this issue, Li-Ping Che and coauthors (Che et al. 2024) show that Arabidopsis thaliana RPH1 is an assembly factor of Cyt b₅₅₉ and is thereby crucial for PSII assembly. RPH1 was originally identified as a conserved chloroplast protein required for plant disease resistance against oomycete pathogens, such as Phytophthora brassicae and the late blight-causing Phytophthora infestans (Belhaj et al. 2009). Che et al. found that PSII abundance is severely reduced in rph1 mutants compared to the wild-type, likely a causal factor in the drastic retardation in plant growth and development and higher photosensitivity together with elevated levels of reactive oxygen species observed in the rph1 mutant. Based on immunoblot analyses and pulse-chase labeling, the authors attribute the reduction in PSII abundance in the rph1 mutant to a defect in Cyt b₅₅₉ formation. The authors ruled out alterations in psbE and psbF gene expression and protein synthesis in rph1 mutants as the transcript levels and ribosome occupancies were similar to the corresponding wild-type levels.

A mutant of the α subunit in the green alga Chlamydomonas reinhardtii can no longer bind heme to form Cyt b₅₅₉, but can still assemble PSII supercomplexes to 15–20% of the wild-type levels (Hamilton et al. 2014). As the PSII supercomplexes in rph1 are similarly reduced, Che et al. measured the heme level from the dithionite-reduced minus ferricyanide-oxidized optical difference spectra. The heme level of rph1 PSII supercomplexes was severely reduced compared to that of wild-type PSII supercomplexes. Hence, RPH1 appears to facilitate heme insertion during Cyt b₅₅₉ formation. Accordingly, localization studies indicated RPH1 to be an intrinsic thylakoid membrane protein with four predicted transmembrane helices, and the N-terminus was found to be exposed to the stroma based on protease digestion of the wild-type thylakoids followed by immunoblotting. Moreover, RPH1 directly interacts with both the Cyt b₅₅₉ subunits as evident from split-ubiquitin two-hybrid assays and bimolecular fluorescence complementation. Interestingly, apo-Cyt b₅₅₉ was specifically detected in rph1 but not in the

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wild-type or any of the several other PSII mutants analyzed (Figure, A). Therefore, the authors propose that RPH1 mediates heme insertion for efficient assembly of Cyt\textsubscript{b559} during PSII assembly in chloroplasts.

In summary, RPH1, initially identified to be required for pathogen resistance, has now been found to be a putative molecular chaperone for heme insertion into apo-Cyt\textsubscript{b559} to form the functional holo-Cyt\textsubscript{b559} during PSII assembly in Arabidopsis chloroplasts (Belhaj et al. 2009; Che et al. 2024; Figure). This key finding not only paves the way for studying the exact mechanism of heme insertion during Cyt\textsubscript{b559} biogenesis but also stimulates further investigations on other immune-related proteins that are chloroplast localized, as they could be critical for chloroplast function as well as immune response.

This key finding paves the way for uncovering the mechanism of heme insertion during Cyt\textsubscript{b559} biogenesis and further exploration of its essential role in photoprotection and its relationship to plant immunity.

![Figure](https://www.rcsb.org/structure/5MDX) was adapted using ChimeraX (Meng et al. 2023).

**References**


