Spotlight

Cryo-electron microscopy delineates the *in situ* structure of the thylakoid network

Lu-Ning Liu[1,2,*], Yu-Zhong Zhang[1,3,4]

[1]College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China
[2]Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom
[3]State Key Laboratory of Microbial Technology, Marine Biotechnology Research Center, Shandong University, Qingdao 266237, China.
[4]Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

[*]Correspondence: Lu-Ning Liu (luning.liu@liverpool.ac.uk)

Photosynthesis is conceivably the most important biological process on Earth. By performing oxygenic photosynthesis, cyanobacteria, algae, and plants can use solar energy to power their metabolism and produce sugars and oxygen for life on earth. These photosynthetic organisms have evolved a specialized intracellular membrane system − the thylakoid membrane − inside the cytoplasmic membrane to carry out the reactions of photosynthesis. The key players in the thylakoid membrane for efficient photosynthetic electron flow are a series of membrane-spanned multi-subunit complexes with hundreds of noncovalently-bound chlorophyll and carotenoid pigments, including photosystem I (PSI), photosystem II (PSII), cytochrome b_6f, and ATP synthase (ATPase). In addition, the membrane-associated antenna in cyanobacteria and red algae, known as the phycobilisome, and the membrane-embedded light-harvesting complexes (LHC) in algae and plants play decisive roles in enhancing the light-harvesting capacity of photosystems. Recent development and application of cryo-electron microscopy in photosynthesis studies have substantially empowered our toolkit for elucidating the macromolecular structures and functions of individual photosynthetic proteins and supercomplex assemblies.

Despite a great amount of information on the atomic structures and spectroscopic properties of individual photosynthetic pigment-protein complexes, we lack an extensive understanding about how thylakoid membranes are formed and structurally defined in cells, as well as the spatial organization and dynamics of photosynthetic complexes in thylakoids (Liu, 2016). A recent paper by Engel, Nickelsen, and colleagues (Rast et al., 2019) reported a cryo-electron tomography (cryo-ET) study on the subcellular architecture of thylakoids in a model cyanobacterium *Synechocystis* sp. PCC 6803. Cryo-ET has demonstrated its extraordinary power in performing *in situ* high-precision observations of biological structures in a near-physiological context, inside cells frozen without chemical fixation. Based on the cryo-ET observations, here we discuss the recent advances in understanding the biogenesis, networking and supercomplex organization of cyanobacterial thylakoid membranes.

Thylakoid membrane convergence and continuity

Numerous studies have indicated that thylakoid membranes in cyanobacterial cells converge at several sites of the cell periphery (Nevo et al., 2007; van de Meene et al., 2006). These convergence regions, termed “thylakoid centers”, were previously described as cylindrical, rod-like structures, acting as the sites where PSII biogenesis commences (Stengel et al., 2012). The recent cryo-ET images revealed that the thylakoid convergence regions are membrane tubules and vary in structure (Rast et al., 2019).

At the thylakoid convergence regions, distinct layers of thylakoid stacks interconnect with each other to form a continuous membrane network (Figure 1A). The highly connected thylakoid membrane network appears as a conserved feature in not solely cyanobacterial species (Nevo et al., 2007; Rast et al., 2019; Ting et al., 2007), but also other photosynthetic organisms. Similar structures have also

been visualized by cryo-ET for the thylakoid membranes in chloroplasts of *Chlamydomonas* and plants (Daum and Kuhlbrandt, 2011; Engel et al., 2015), and the photosynthetic membranes of purple photosynthetic bacteria, such as the vesicular intracytoplasmic membranes (ICMs) of *Rhodobacter sphaeroides* (Noble et al., 2018; Scheuring et al., 2014) and the lamellar ICMs of *Blastochloris viridis* (Konorty et al., 2008). Interconnection between different photosynthetic membrane layers could potentially facilitate diffusion of constituents between adjacent thylakoid lumens and within the whole membrane network.

Connection between the thylakoid and cytoplasmic membranes

It remained unclear whether cyanobacterial thylakoid membranes physically connect with cytoplasmic membranes and whether the biogenesis of cyanobacterial thylakoids correlates with the cytoplasmic membrane contact sites. Conventional electron tomography using high-pressure freezing and freeze substitution has proposed that there are direct connections between cytoplasmic and thylakoid membranes in cyanobacteria (van de Meene et al., 2006). Likewise, connections between thylakoids and the inner chloroplast envelope in *Chlamydomonas* (Engel et al., 2015) and invaginations between ICMs and cytoplasmic membranes in purple photosynthetic bacteria (Konorty et al., 2008; Noble et al., 2018) have been discerned. The recent Cryo-ET views of *Synechocystis* 6803 cells validated the presence of contact sites, with a distance of 2−4 nm between the cytoplasmic membrane and the thylakoid convergence region facing the cytoplasmic membrane (Figure 1B)(Rast et al., 2019). Such tight contacts appear to be mediated by special protein-based bridges. Strikingly, Cryo-ET did not show physical fusions of thylakoid and cytoplasmic membranes, in agreement with previous results (Liberton et al., 2006). Given the limited sample volume and the "local" view of current cryo-ET methodology, one could not make a conclusive statement whether the fusion of two membranes exist, or is highly dynamic depending on the conditions of the external environment and cell growth. Indeed, the connections between the ICMs and cytoplasmic membranes in *Rhodobacter sphaeroides* have been proved to be dynamic during the aging process (Noble et al., 2018).

Heterogeneity of the thylakoid membrane

Like other biological membranes such as plant chloroplasts, cyanobacterial thylakoid membranes are highly dynamic and exhibit heterogeneity in membrane shape, protein distribution and function. Pigment–protein complexes that functionally coordinate are prone to be in close proximity to and interact with each other, leading to the formation of functionally distinct thylakoid membrane domains. Cryo-ET revealed that the thylakoid convergence regions of *Synechocystis* 6803 are structurally distinct from other thylakoid regions (Figure 1B)(Rast et al., 2019). Membrane-associated ribosomes, but not phycobilisomes, are present on the convergence membrane surface, indicating the biogenic role of the convergence membrane region. In addition, thylakoid membranes that face the internal cytosol harbor the majority of membrane-associated ribosomes, indicating that these regions are the

primary sites of membrane protein biogenesis. In contrast, thylakoid membranes facing other thylakoids accommodate a high content of phycobilisomes and very low levels of ribosomes, suggesting the photosynthetic activities of these thylakoid regions. In addition to the spatial segregation of phycobilisomes and ribosomes, previous work using atomic force microscopy (AFM) and live-cell confocal fluorescence microscopy has described the heterogeneous distribution of PSI, PSII, cytochrome b_6f, and ATPase throughout cyanobacterial thylakoid membranes (Casella et al., 2017).

The thylakoid surface appears discontinuous, with perforations observed in thylakoid sheets (Engel et al., 2015; Nevo et al., 2007). These membrane apertures may aid the transport of molecules/proteins through thylakoid sheets. The thylakoid perforations were not explicitly delineated in the cryo-ET study of *Synechocystis* 6803 (Rast et al., 2019). However, the 3D architecture of the convergence membranes may allow diffusion of cytosolic molecules around the thylakoid network.

In summary, *in situ* cryo-ET has opened fascinating opportunities for visualizing cells and subcellular compartments in three dimensions with molecular details. The recent cryo-ET observations of cyanobacterial cells by Engel and colleagues (Rast et al., 2019) have provided new insight into the biogenesis, continuity and protein organization of thylakoid membranes. Advances in tomographic imaging and data analysis gained in the study could be extended to *in situ* structural characterizations of plant chloroplasts, mitochondria, and other biological organelles, to yield important information about their physiological structures and functions in a near-physiological context. These molecular views will have an impact on the rational design and generation of artificial photosynthetic systems. Further efforts may focus on visualizing the biosynthesis, spatial distribution, and dynamics of photosynthetic complexes in thylakoids, using cryo-ET of sectioned cell or intact cells in combination with complementary techniques, such as AFM and super-resolution live-cell fluorescence microscopy. Cryo-ET imaging at sequential timepoints will allow evaluation of organelle dynamics and structural flexibility in variable physiological states, to explore extensively the adaptive strategies of the photosynthetic machinery in response to environmental changes.

Funding

This project is supported by the Royal Society to L.-N.L. (UF120411 and URF\R\180030), the UK Biotechnology and Biological Sciences Research Council to L.-N.L. (BBSRC, BB/R003890/1), the National Science Foundation of China (91851205), the National Key R & D Program of China (2018YFC1406700), and AoShan Talents Cultivation Program funded by Qingdao National Laboratory for Marine Science and Technology (2017ASTCP-OS14).

References

Casella, S., Huang, F., Mason, D., ZHAO, G.Y., Johnson, G.N., Mullineaux, C.W., and Liu, L.N. (2017). Dissecting the native architecture and dynamics of cyanobacterial photosynthetic machinery. Mol Plant 10:1434-1448.

Daum, B., and Kuhlbrandt, W. (2011). Electron tomography of plant thylakoid membranes. J Exp Bot 62:2393-2402.

Engel, B.D., Schaffer, M., Kuhn Cuellar, L., Villa, E., Plitzko, J.M., and Baumeister, W. (2015). Native architecture of the Chlamydomonas chloroplast revealed by in situ cryo-electron tomography. Elife 4:e04889.

Konorty, M., Kahana, N., Linaroudis, A., Minsky, A., and Medalia, O. (2008). Structural analysis of photosynthetic membranes by cryo-electron tomography of intact *Rhodopseudomonas viridis* cells. J Struct Biol 161:393-400.

Liberton, M., Howard Berg, R., Heuser, J., Roth, R., and Pakrasi, H.B. (2006). Ultrastructure of the membrane systems in the unicellular cyanobacterium Synechocystis sp. strain PCC 6803. Protoplasma 227:129-138.

Liu, L.N. (2016). Distribution and dynamics of electron transport complexes in cyanobacterial thylakoid membranes. Biochim Biophys Acta 1857:256-265.

Nevo, R., Charuvi, D., Shimoni, E., Schwarz, R., Kaplan, A., Ohad, I., and Reich, Z. (2007). Thylakoid membrane perforations and connectivity enable intracellular traffic in cyanobacteria. EMBO J 26:1467-1473.

Noble, J.M., Lubieniecki, J., Savitzky, B.H., Plitzko, J., Engelhardt, H., Baumeister, W., and Kourkoutis, L.F. (2018). Connectivity of centermost chromatophores in *Rhodobacter sphaeroides* bacteria. Mol Microbiol 109:812-825.

Rast, A., Schaffer, M., Albert, S., Wan, W., Pfeffer, S., Beck, F., Plitzko, J.M., Nickelsen, J., and Engel, B.D. (2019). Biogenic regions of cyanobacterial thylakoids form contact sites with the plasma membrane. Nat Plants 5:436-446.

Scheuring, S., Nevo, R., Liu, L.N., Mangenot, S., Charuvi, D., Boudier, T., Prima, V., Hubert, P., Sturgis, J.N., and Reich, Z. (2014). The architecture of Rhodobacter sphaeroides chromatophores. Biochim Biophys Acta 1837:1263-1270.

Stengel, A., Gugel, I.L., Hilger, D., Rengstl, B., Jung, H., and Nickelsen, J. (2012). Initial steps of photosystem II de novo assembly and preloading with manganese take place in biogenesis centers in Synechocystis. Plant Cell 24:660-675.

Ting, C.S., Hsieh, C., Sundararaman, S., Mannella, C., and Marko, M. (2007). Cryo-electron tomography reveals the comparative three-dimensional architecture of *Prochlorococcus*, a globally important marine cyanobacterium. J Bacteriol 189:4485-4493.

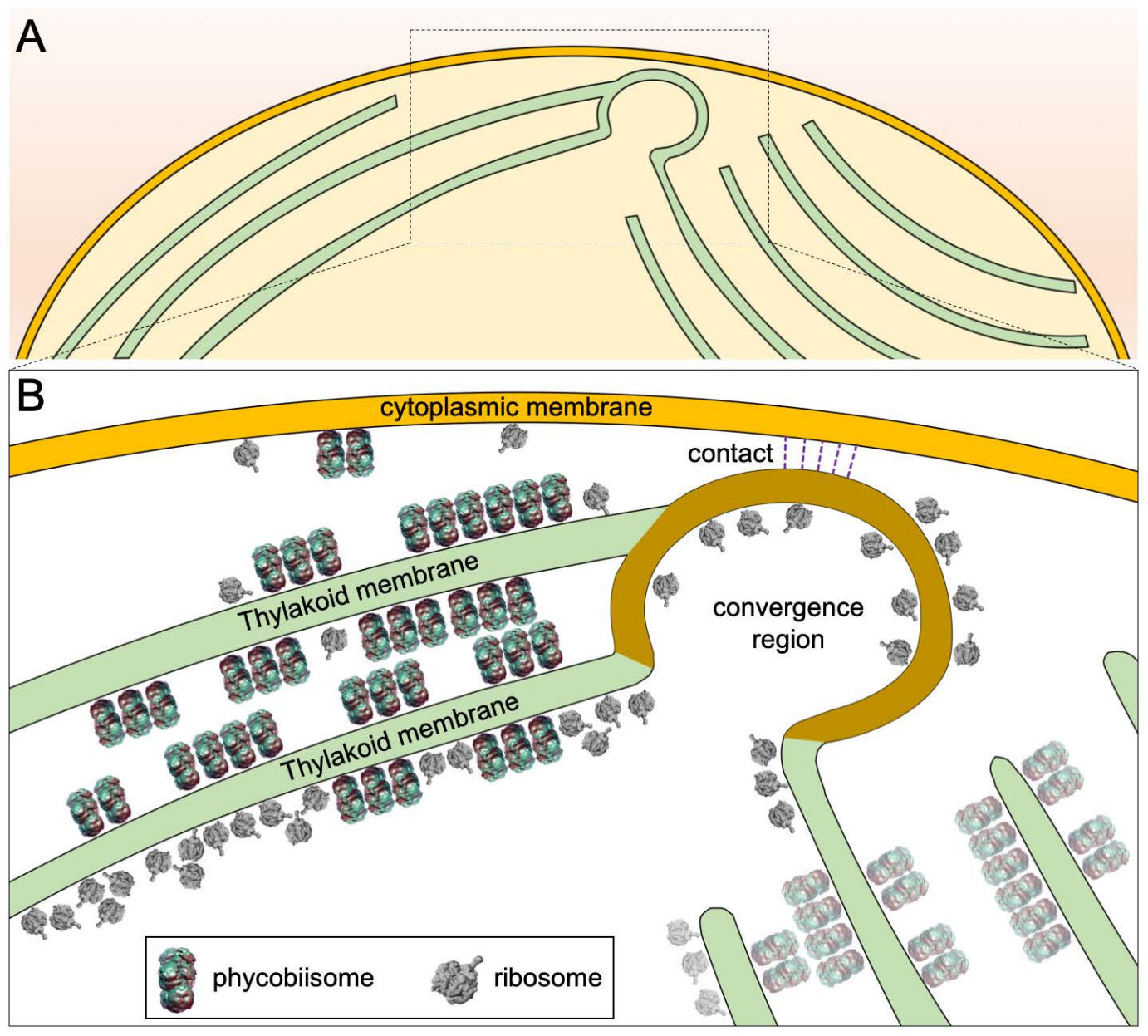
van de Meene, A.M., Hohmann-Marriott, M.F., Vermaas, W.F., and Roberson, R.W. (2006). The three-dimensional structure of the cyanobacterium *Synechocystis* sp. PCC 6803. Arch Microbiol 184:259-270.

Figure Legend

Figure 1. Schematic model of thylakoid membrane organization in the cyanobacterium Synechocystis sp. PCC 6803

(A) Diagram of the *in situ* structure of the thylakoid membrane network determined by cryo-ET (Rast et al., 2019).

(B) Contacts between the thylakoid convergence region and cytoplasmic membrane and the heterogeneous distribution of phycobilisomes and ribosomes on different surfaces of thylakoid and cytoplasmic membranes. Note: the abundance of individual complexes is only an estimation relative to cryo-ET results (Rast et al., 2019) and their exact locations remain speculative.


A
B
cytoplasmic membrane
contact
Thylakoid membrane
Thylakoid membrane
convergence
region
phycobiisome
ribosome