

Insights into the complex 3-D architecture of thylakoid membranes in the unicellular cyanobacterium *Cyanothece* sp. ATCC 51142

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In cyanobacteria and chloroplasts, thylakoids are the complex internal membrane system where the light reactions of oxygenic photosynthesis occur. In plant chloroplasts, thylakoids are differentiated into a highly interconnected system of stacked grana and unstacked stroma membranes. In contrast, in cyanobacteria, the evolutionary progenitors of chloroplasts, thylakoids do not routinely form stacked and unstacked regions, and the architecture of the thylakoid membrane systems is only now being described in detail in these organisms. We used electron tomography to examine the thylakoid membrane systems in one cyanobacterium, *Cyanothece* sp. ATCC 51142. Our data showed that thylakoids form a complicated branched network with a rudimentary quasi-helical architecture in this organism. A well accepted helical model of grana-stroma architecture of plant thylakoids describes an organization in which stroma thylakoids wind around stacked granum in right-handed spirals. Here we present data showing that the simplified helical architecture in *Cyanothece* 51142 is left-handed in nature. We propose a model comparing the thylakoid membranes in plants and this cyanobacterium in which the system in *Cyanothece* 51142 is composed of non-stacked membranes linked by fret-like connections to other membrane components of the system in a limited left-handed arrangement.

Thylakoid Organization in Plants and Cyanobacteria

In cyanobacteria, algae and plants, the light reactions of photosynthesis are performed by large protein complexes embedded in the thylakoid membranes. In eukaryotic plants and algae, thylakoid membranes reside in chloroplasts, semi-autonomous organelles of prokaryotic origin. An early cyanobacterium, or cyanobacterial ancestor, was almost certainly the evolutionary precursor of chloroplasts via an endosymbiotic event involving the engulfment of a photosynthetic cell by a mitochondria-containing eukaryote.¹ Despite this common ancestry, over evolutionary time the photosynthetic membrane systems in plants and cyanobacteria have evolved into architecturally dissimilar forms. Understanding in detail the morphology and the evolutionary history of these extraordinary membrane systems, upon which oxygen-requiring life depends, is a topic of outstanding interest in cell biology.

In higher plant chloroplasts, thylakoid membranes have a distinct architecture, forming an intricate network of stacks of flattened or appressed lamellae called grana that are connected by unstacked stroma thylakoids that traverse the chloroplast stroma matrix. The details of the three-dimensional arrangement of grana and stroma thylakoids have been debated for years, and different models have been proposed (reviewed in refs. 2 and 3). The foremost of these, the helical model, was originally based on serial-section electron microscopy data collected over decades

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and describes stroma thylakoids as joining grana at numerous frets or junctional slits or connections, forming a stair-step arrangement in which multiple right-handed helices of stroma thylakoids wind around a granum. Together, the grana and stroma thylakoids enclose a single luminal space.^{2,4,5} Recent studies and discussions have focused on the precise details of the architecture of this system. Importantly, some recent studies have taken advantage of significant technical advances in 3D data acquisition and analysis, employing electron tomography followed by computer-aided model generation. In particular, Shimoni et al.⁶ analyzed electron tomographic data of chloroplast thylakoid membranes in lettuce leaves and proposed a model significantly at odds with the helical model: grana membranes are formed from bifurcations of stromal sheets, so that entry and exit of the stroma membranes from the grana occur in the same plane, with no helical component. This study was followed by a refinement of the helical model,⁷ and subsequent discussions of the validity of the two models.^{8,9} Most recently a study by Austin and Staehelin¹⁰ examining tomographic data of multiple complete grana stacks and surrounding stroma thylakoids has resolved some of this controversy by confirming the basic tenants of the helical model. From these data, it is apparent that stroma thylakoids are analogous to threads on a screw that coil in right-handed helices around the central column, the granum.

While the organization of thylakoid membranes into grana and stroma regions is universally observed in mature land plant chloroplasts, cyanobacterial thylakoid membranes have not been found to form grana stacks connected by stroma thylakoids, and appression has been noted in only a few strains. In fact, only within the past few years has work using tomographic data shown that thylakoid membranes are connected by channels or bridging membranes in some cyanobacterial strains.^{11,12} Our recent work¹³ examining the thylakoid membrane system in *Cyanothece* 51142 depicted, for the first time, a membrane system in a cyanobacterium with strong similarities to that in plants: a highly interconnected network composed by branching

membranes enclosing a single luminal space. Furthermore, by examining the arrangement of branch points within the cell, we discovered a rudimentary helical architecture in the thylakoid membranes of *Cyanothece* 51142. Here we describe our additional findings regarding this helical architecture, and discuss its implications in cyanobacteria.

Handedness of the Thylakoid Arrangement in *Cyanothece* 51142

To examine the thylakoid membranes of *Cyanothece* 51142 cells, we prepared samples for electron microscopy by high-pressure freezing followed by freeze substitution, and used serial electron tomography and montaging to examine and model the components of cyanobacterial cells. A region of the thylakoid membrane system from a cell harvested at the D10 time-point (after 10 h of growth in dark) of the diurnal cycle and prepared in this manner is shown in **Figure 1**. We found that the majority of membranes were separated from each other by approximately 50 nm, and did not form stacks or appressed areas, but could be closely associated with inclusion bodies. This architecture is similar to what we reported in our examination of cells from the L10 time point.¹³ In tomographic slice images, the thylakoid membranes appeared sheet-like and the areas where the membranes branched could be clearly observed (**Fig. 1A–E**). In order to show the helical arrangement in the tomographic slices, membrane regions were labeled with numbers and letters; in **Figure 1A–E**, the regions on the left are numbered and the regions on the right are lettered. By following the rearrangements to the letter-number pairs while moving through the tomogram, it was apparent that the membrane regions to the left (numbered) advanced upward relative to the regions on the right (lettered) (see arrows in **Fig. 1C**).

We used the IMOD software to model the membranes and inclusion bodies in this area to more clearly illustrate this organization (**Fig. 1F and G**). We rendered the numbered membranes in green and the lettered membranes in orange; however, spatial overlapping obscured some of the details of how the green and

orange regions were arranged in the complete model. We therefore pared down the model to only the relevant membranes (**Fig. 1H**), and next showed only the numbered membranes connected to membrane “b” (**Fig. 1I**) and the numbered membranes connected to membrane “a” (**Fig. 1J**). In the case of both membranes “a” and “b,” the green numbered membranes are connected by thin flattened membrane tubules (designated by the asterisks). These tubules appear similar to the junctional connections between stroma thylakoids and granum thylakoids, also being highly variable in size as recently found to be the case in plants.¹⁰ It was apparent that these tubules join the green numbered membranes in a stair-step arrangement that ascends to the left, as in a limited left-handed spiral (**Fig. 1I and J**). Our analysis of eight such regions in *Cyanothece* 51142 showed that all of them had the same left-handedness. These examples encompassed multiple cells from different time points and from independently grown cultures, suggesting that this architecture is widespread in *Cyanothece* 51142.

We then compared these data to previous studies examining the helical arrangement of chloroplast membranes. It has been shown that the association of stroma thylakoids with grana forms only right-handed helices,⁴ so that stroma thylakoids always ascend to the right when spiraling around grana stacks. In such data, the membrane regions to the right always advanced upward when moving through subsequent slice images.^{5,10} Hence, *Cyanothece* 51142 membranes have the opposite helical arrangement compared to chloroplast membranes.

We have created schematic models to compare the morphology of chloroplast and *Cyanothece* 51142 thylakoid membranes (**Fig. 2**). In chloroplasts, stacked thylakoids of the granum are connected to stroma thylakoids that spiral around the granum, ascending to the right (**Fig. 2A**). In *Cyanothece* 51142, thylakoid membranes are not stacked, but are connected by membrane tubules, analogous to junctional slits, arranged like stair steps ascending to the left (**Fig. 2B**).

An important difference between these two systems is the extent of the helical

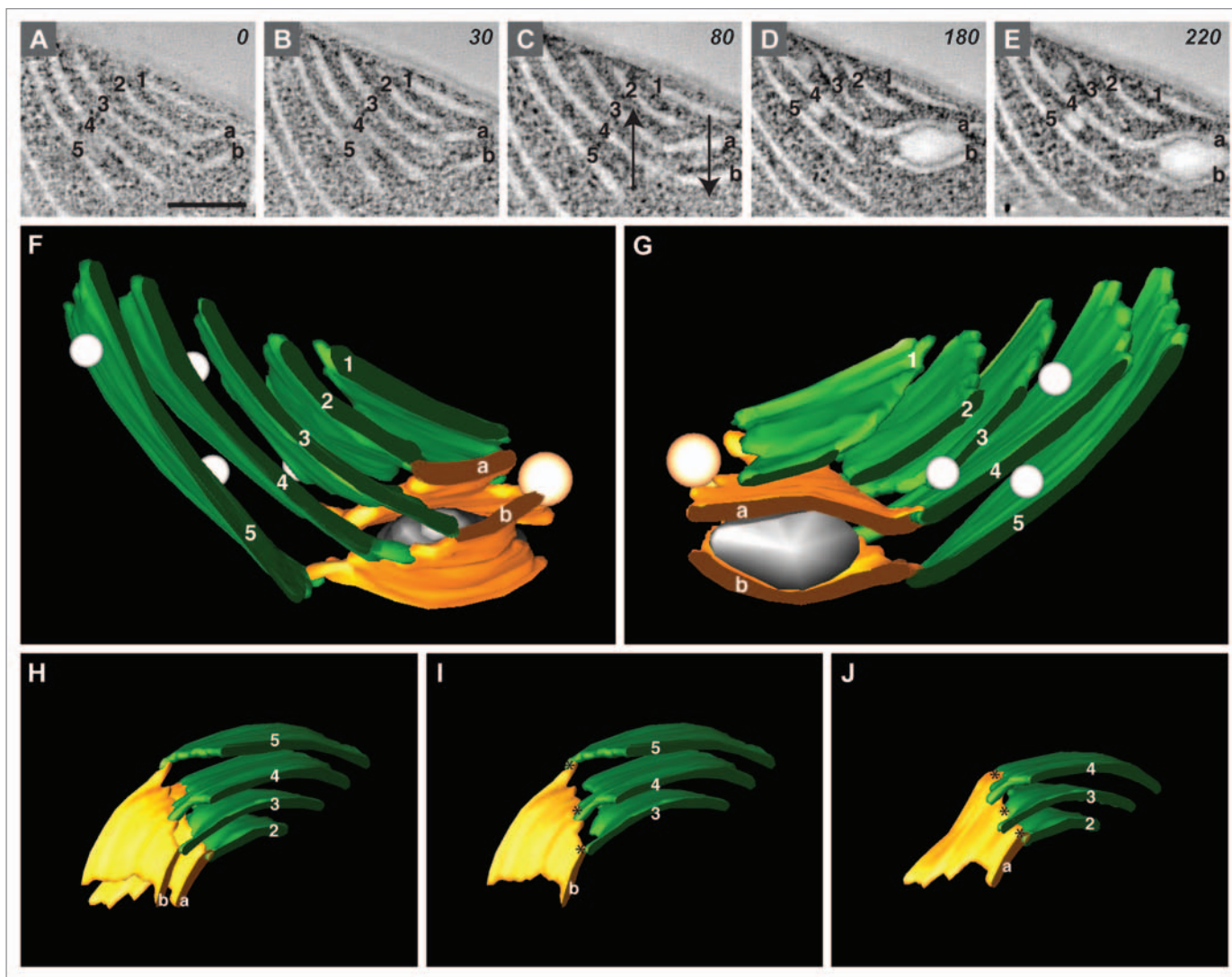


Figure 1. Handedness of thylakoid membranes in *Cyanosethece* 51142. (A–E) Tomographic slice images (composed from 3 superimposed serial 2 nm slices) taken through the tomogram at $z = 0, 30, 80, 180$ and 220 slice intervals. Membrane regions are labeled (1–5) and (a and b). Arrows in (C) show the direction of the membrane rearrangements through the tomogram. Bar = 200 nm. (F and G) Model generated from the tomographic data shown in (A–E); (G) is rotated 180 degrees relative to (F). (H) Model derived from the model shown in (F and G); the model has been rotated and inclusion bodies have been removed for clarity. (I and J) Dissected images of the model shown in (H); (I) shows only the numbered membrane regions connected to membrane “b;” (J) shows only the numbered membrane regions connected to membrane “a.” Asterisks designate the junctional connections.

architecture. In chloroplasts, grana typically contain 10–20 circular membrane layers that are tightly appressed to within ~2.5 nm of each other, and are surrounded by multiple spiraling stroma thylakoids.¹⁴ In *Cyanosethece* 51142, the helical architecture is much more limited. Membranes are not differentiated into flattened discs and sheets, but rather all membranes are sheet-like and are uniformly separated by ~50 nm. Moreover, much fewer membrane sheets are involved, typically only 2–3 membranes forming the grana-like region and 1–2 connecting membranes (see the

green and yellow membrane regions, respectively, in Fig. 1I and J). The tubular membrane connections, however, do seem to be similar in shape and size in the two systems. To date, *Cyanosethece* 51142 is the only cyanobacterium in which any helical architecture has been reported, compared to the universality of grana-stroma architecture in higher plants.

Helical membrane organization might have evolved as a means to efficiently package a large amount of membranes into a limited cellular space. We have shown that the membrane system in *Cyanosethece* 51142

is very dense, with a larger number of membrane layers compared to what has been observed in other cyanobacteria.¹³ It is possible that helical organization originated as a primarily left-handed architecture, followed by the development of grana and the attendant right-handedness of the grana-stroma architecture at a later time. It is tempting to speculate, particularly in light of the recent analysis of stroma thylakoids,¹⁰ that the chloroplast stroma thylakoid arrangement and the thylakoid architecture in *Cyanosethece* 51142 can be compared directly, with the addition

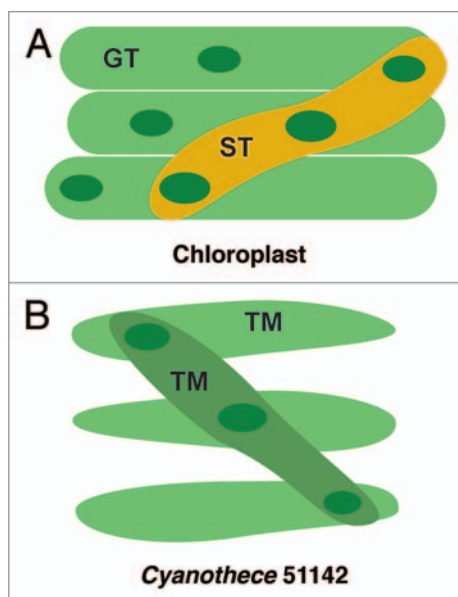


Figure 2. Schematic model of membrane architecture in chloroplasts and cyanobacteria. (A) Stroma thylakoids (orange, ST) associate with grana thylakoids (green, GT) with a right-handed orientation. (B) Thylakoid membranes (TM) in *Cyanothecce 51142* have limited left-handedness. Junctional connections are in dark green.

of grana in chloroplasts. Tomographic data showed that stroma thylakoids were not uniformly sheet-like in nature, nor were they thin strips of membrane that extended from the granum and met in the stroma, but that these membranes have a complex arrangement that has not previously been described.¹⁰ In models based on tomographic data, stroma membranes are sheets of varying widths that often display disruption in their continuity and subsequent fusion to other stroma membranes. Furthermore, stroma thylakoids have a complicated architecture depending on their proximity to neighboring grana stacks.

Collectively, tomographic studies of plant and cyanobacterial thylakoid membrane architecture have shown these membrane systems at a level of detail previously unknown. Further examination of the

thylakoid membranes in both plants and cyanobacteria will doubtlessly reveal additional details in the architecture of these complex membrane systems, and shed light on the complicated evolutionary history of these organisms.

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