Reply: On Three-Dimensional Models of Higher-Plant Thylakoid Networks: Elements of Consensus, Controversies, and Future Experiments

The rapid technical advancement of electron microscopy tomography (EMT), and of data acquisition and analysis, opened new vistas in revealing complex membrane structures (Frey and Mannella, 2000) and in the understanding of the three-dimensional (3D) organization of the thylakoid membranes of vascular plants, in particular. As often happens with new techniques, EMT not only settled important issues but brought up some controversies, as highlighted in the Perspective by Mustárdy et al. (2008a) and the Letter to the Editor by Brumfeld et al. (2008) in this issue of The Plant Cell.

EMT provided solid evidence that the attractive models, still often used in textbooks, which depict the stroma thylakoid membranes as long flat tubes interconnecting the grana, have no structural basis and thus should now be abandoned, as argued previously (Mustárdy and Garab, 2003). With dual-axis EMT, Shimoni et al. (2005) could see that the stromal compartments are defined by wide lamellar sheets (not narrow tubes); the attractive models, still often used in textbooks, which depict the stroma thylakoid membranes as long flat tubes interconnecting the grana, have no structural basis and thus should now be abandoned, as argued previously (Mustárdy and Garab, 2003). With dual-axis EMT, Shimoni et al. (2005) could see that the stromal compartments are defined by wide lamellar sheets (not narrow tubes); data by Mustárdy et al. (2008b) are fully consistent with this picture. It is equally

References


important to stress, again revealed by both data sets, that the membrane network is far less regular than implied by earlier models, based on serial sections, that depict a helical organization of the stroma membranes around the granum and a strict periodicity of the structure. Shimoni et al. (2005) and Brumfeld et al. (2008) reject the helical model outright and offer a pairwise organization model with stroma membranes perpendicualr to the granum. Mustárdy et al. (2008a, 2008b) propose a modified version of the helical model, which retains the tilt and spiral organization of the stroma thylakoids around the granum stacks, but without strict helical symmetry, since they feel that abandoning the basic attributes of the helical models ignores too much electron microscopy (EM) and EMT data.

In particular, the observations from serial sections and EMs that stroma thylakoids tend to step up and down (i.e., shift in opposite directions on opposite sides, as the helical thread on a screw) are consistent with the helical model(s), although do not strictly rule out the pairwise organization model. However, shifts (steps) of more than one level, as observed in serial section EM and EMT (cf. Mustárdy et al., 2005) in most cases find the stroma lamellar sheets intersecting the grana roughly perpendicular to the granum cylinder. However, the plasticity of the membranes, the rippling of these membranes in and out of the section plane (Shimoni et al., 2005), and the limits to resolution in the two data sets (cf. McEwen and Marko, 2001) may not allow the precise determination of intersection angles for the respective planes. The situation is different for the near meridian sections, where the rippling of the stroma membrane is restricted by its junction to the granum thylakoid. In this case, all the edges of the stroma lamellae appear at well-defined tilts with respect to granum pillars (e.g., see Figure 3B in Mustárdy and Garab, 2003). In serial sections, the tilt angles on opposite sides of the grana have reversed signs, as on a screw, equal to about +20° or −20°, respectively (Paolillo and Reighard, 1967; Brangeon and Mustárdy, 1979; Mustárdy et al., 2008a). This pronounced feature, seen in thin section EMs, in serial sections, and in ETMs, should be taken into consideration in all models.

Another point of disagreement between the two models is the bifurcation of the membrane vesicles, as opposed to a mechanism governed mainly by fusion and overlapping of the thylakoid vesicles. This question is topological on the one hand, and on the other hand, is closely related to the possible mechanism of the self-assembly of the granum-stroma thylakoid membrane system. From a topological point of view, it would be essential to trace the continuity of the membranes and of the lumenal phases at the granum-stroma interface. Positing contiguity of the thylakoid vesicles is warranted by the openings (slits) in the margins of the granum. The precise determination of the topology of the junction between the stroma and granum thylakoid vesicles requires high-resolution EMT on thin samples with well-discriminable lumenal phases. The required contrast can be achieved either by optimizing staining protocols (Austin et al., 2006) or, ideally, by switching to frozen-hydrated samples that, though more difficult to prepare, retain the inherent contrast between aqueous compartments and surrounding membranes (Hsieh et al., 2002). EMT on thin samples can lead to a satisfactory solution since the granum-stroma thylakoid membrane assembly contains repeating structures. In other words, because there are multiple junctions around the rim (margin) of the granum, EMT thin sections will contain the critical structural elements. For the quasihelical model, one should expect to see more clearly the transitions of the stroma thylakoids from one layer to the other of the granum pillar; and for the pairwise organization model, the proposed bifurcation of the stroma thylakoid vesicle into two granum thylakoid vesicles, pairwise bendings of the thylakoids, and fusion within the granum body should be possible to observe at high resolution.

Regarding the mechanism of self-assembly, we need to learn more about the nature of structural reorganizations displayed by thylakoid membranes (Consoli et al., 2005; ChUARTZMAN et al., 2008) and determine the structure of thylakoid networks in developing chloroplasts and in mutants with altered self-assembly capacities. Most certainly, EMT will be an indispensable technique to solve these questions.

REFERENCES


