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Perspective

Chloroplasts in living cells and the string-of-grana concept of chloroplast structure revisited

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Abstract

In 1980, Wildman et al. proposed a three-dimensional model for chloroplast structure whereby the grana were arranged in non-overlapping rows, like beads on a string. This string-of-grana model was developed from phase microscope analysis of living cells and partially disrupted, isolated chloroplasts. However, models based on analyses by various electron microscope (EM) techniques (which inevitably encompass a relatively small fraction of the whole chloroplast) indicated that grana are interconnected in all directions by intergranal lamellae and not just along a single 'string'. Hence the string-of-grana model was not widely accepted. Recently, confocal laser scanning microscopy (CLSM) of both living and fixed cells, which gives views of the three-dimensional disposition of grana by imaging Photosystem II fluorescence over much larger sample volumes, that is, the entire chloroplast, has revealed that, although many grana are apparently not in any discernible arrangement, some are indeed present in strings of varying lengths in a range of taxa. The topic therefore warrants revisiting, using techniques, for example, such as EM tomography to assess the degree of variation in the geometry of intergranal connections in whole chloroplasts, and its possible functional consequences and developmental origins.

Abbreviations: CLSM – confocal laser scanning microscopy; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EM – electron microscopy; SDS – sodium dodecyl sulfate

Historical background

In 1962, Spencer and Wildman compared the appearance of *in situ* spinach chloroplasts in living cells with those isolated from the same batch of leaves. Figure 1, copied from Plate I of their publication, shows a large number of chloroplasts in a living mesophyll cell where a Zeiss 100X, Neofluar, oil-immersion objective and a 12.5X ocular had been employed to produce a total microscopic magnification of 1250X. Translucent jackets of stroma surround many of the chloroplasts in the living cell and grana with dimensions of ∼0.5 µm in diameter and varying numbers of larger starch grains are also clearly evident. In the

living cell, the stromal jacket may constantly undergo changes in shape with amoeboid movements. Occasionally, the stromal jacket forms long protuberances that stretch out into the cytoplasm and may even fuse with the stromal jacket of a distant chloroplast. These movements have been recorded by cinemicrography (Wildman et al. 1962) and time-lapse photomicrography (Köhler et al. 1997). The movie also recorded the apparent segmentation of these protuberances to release a particle with a similar appearance and mobility to mitochondria. More recently, the protuberances have been named stromules and their behaviour studied with the aid of green fluorescent protein-labelled chloroplasts (Köhler and Hanson 2000; Arimura et al.
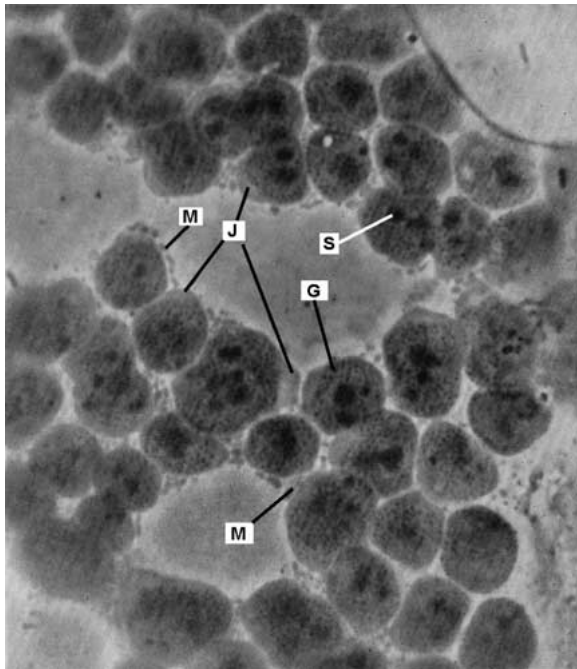
Figure 1. Chloroplasts in a living mesophyll cell of spinach observed by phase microscopy. G – granum; J – chloroplast jacket; M – mitochondrion; S – starch grain (from Spencer and Wildman 1962).
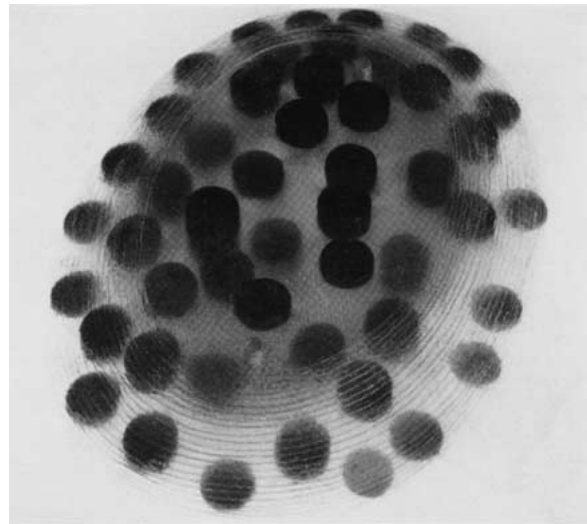

Figure 2. Model of grana arrangement. Plastic scale model of a chloroplast showing grana as dark truncated cylinders (from Spencer and Wildman 1962).

2001; Gray et al. 2001). In contrast to the stroma phase of the chloroplast, the thylakoid system with its associated grana does not undergo rapid changes in shape.

Based on phase contrast microscope observations of *in situ* chloroplasts in living cells and the appearance of *in vitro* swollen chloroplasts, together with data from the literature displaying electron micrographs of ultra-thin chloroplast sections, a three-dimensional, plastic model of chloroplast structure was constructed; it is reproduced in Figure 2 (from Spencer and Wildman 1962). In 2003 hindsight, however, the authors had failed to realize how extremely thin spinach chloroplasts are in living cells. The thinness should have been discerned because several instances of overlapping chloroplasts can be seen in Figure 1. In the areas where the edges of two or more chloroplasts overlapped, the grana in the upper and lower edges remained in almost the same plane of focus at the highest magnification of the phase microscope attesting to the thinness of the overlapping organelles. More attention to the overlaps might have suggested that these spinach chloroplasts were in the range of 1–2 μm in thickness. In retrospect, it was probably the thinness of these chloroplasts, and those

of *Nicotiana tabacum* and *N. excelsior* used in later studies, which made them particularly suitable for study by phase contrast microscopy.

These earlier observations on living cells had been performed with pieces of tissue held in willow pith with slices being made by hand with a razor blade. Soon after, it was found that compressed, high density styrofoam was a superior supporting medium whereby 20 to 30 highly uniform sections could be cut with a sliding microtome, each section containing hundreds of cells displaying cytoplasmic streaming as proof that the cells were still healthy. This improvement made possible a more intensive examination of the structural organization of grana in chloroplasts in living cells as observed by phase contrast and fluorescence microscopy.

Evidence for grana being arranged in strings

Although grana in living cells appeared to be more or less evenly distributed over the chloroplast surface in the examined species, closer inspection showed that they were not scattered at random but tended to be arranged in strings of varying length, like beads on a necklace. This concept was first put forward by Wildman et al. (1980) and later in a computer simulation of the arrangement of grana in a single chloroplast (Jope et al. 1980). The *N. excelsior* chloroplast in Figure 3(A) and the spinach chloroplast in
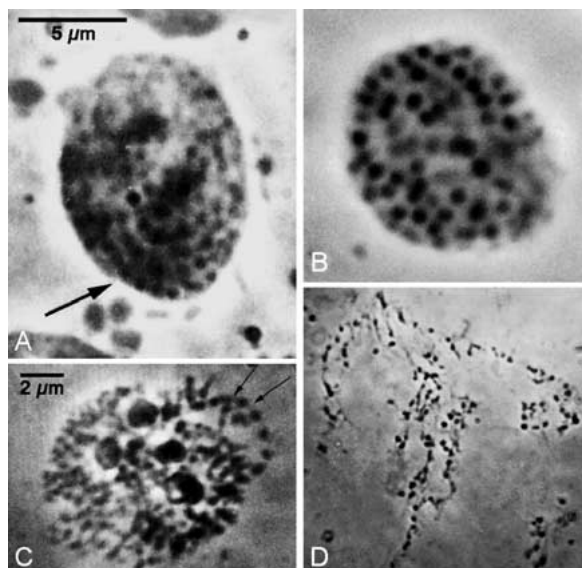
Figure 3. Phase contrast microscope views of chloroplasts. (A) High magnification photomicrograph of an *N. excelsior* chloroplast in a living mesophyll cell. The arrow points to a discontinuity where the spirally arranged string may have terminated. (B) Photomicrograph of a chloroplast in spinach mesophyll cell viewed by the highest magnification of a phase microscope. (C) An isolated *N. excelsior* chloroplast partially disrupted by the shear forces generated by surface tension when a 1 µl drop of a chloroplast suspension was mounted between the slide and a large coverslip. The four large objects are starch grains. (D) An isolated *N. excelsior* chloroplast extensively destabilized by addition of 0.5% SDS to a chloroplast suspension. All the illustrations are scans of original prints of figures that had been previously published in Wildman et al. (1980) except for 3D, which is an unpublished photomicrograph provided by SGW.

Figure 3(B) show many such strings. In addition, the overall pattern of the grana in the *N. excelsior* chloroplasts occasionally conveyed the impression of an extensive string-of-grana folded into a coherent spiral configuration.[1] The arrow in Figure 3(A) points to a perceptible discontinuity at the edge of the chloroplast that may be a terminus of a string-of-grana that seem to be traceable as a continuous string almost to another terminus in the middle of the chloroplast.

Support for the string-of-grana concept also came from observations on isolated chloroplasts (Wildman et al. 1980). *N. excelsior* chloroplasts can be rapidly

[1]SGW regrets very much not having been aware of a prior publication by M.C. de Rezendo-Pinto, which had appeared in 1948 in Portugalie Acta Biol. (A) 2:111–115, entitled 'Sur la structure hélicoïdale des chloroplasts' until having been notified by the author, and apologizes for this omission because Rezendo-Pinto's illustrations do convey the impression of a spiral arrangement of grana.

isolated from intact leaves by placing a drop of water on the leaf surface and making a series of closely spaced cuts with a razor blade through the droplet and epidermis and mesophyll layers. *N. excelsior* chloroplasts isolated in this way are remarkably stable and closely resemble the chloroplasts in the living cell. In sharp contrast, chloroplasts of spinach and other species become seriously disorganized following this treatment. When a small (1 µl) aliquot of the resultant *N. excelsior* suspension is placed on a coverslip, inverted, and lowered gently on to a slide, surface tension shear forces cause partial disruption of some chloroplasts. One such chloroplast is shown in Figure 3(C). The general outline of the chloroplast, along with four starch grains, is still evident. However, the shear forces generated between the coverslip and slide surfaces have caused about 15 grana to become sufficiently detached as to form a loop extending away from the main body of the chloroplast. Six grana in the loop are very conspicuous, and two arrows point to fine threads connecting grana just at the limit of resolution of the phase microscope.

This partial disruption of isolated chloroplasts can be taken a stage further by substituting 0.5% sodium dodecyl sulfate for water when making a leaf extract from *N. excelsior* as outlined above. The leaves are composed of ~90% water so that a significant dilution of the SDS concentration occurs during the release of the chloroplasts and other cellular contents. When observed in a hanging drop, the numerous isolated chloroplasts resembled 'tadpoles' where most of the chloroplast remained intact to form a 'head', while a 'tail' consisting of a string of 10–20 grana dangled away from the 'head'. These chloroplasts were highly destabilized and impossible to keep in focus in a hanging drop long enough to photograph. When a large drop of this extract was placed on a coverslip that was then inverted onto a slide and excess fluid removed with a filter paper wick, the 'tadpoles' disintegrated into configurations such as that shown in Figure 3(D). Fluorescence microscopy showed that the black objects contained chlorophyll that fluoresced brilliantly whereas no fluorescence emanated from the threads holding the strings of grana together.

Chloroplast structure as observed by confocal microscopy

In the late 1980s, the advent of a new procedure for optical sectioning of plant tissues using the
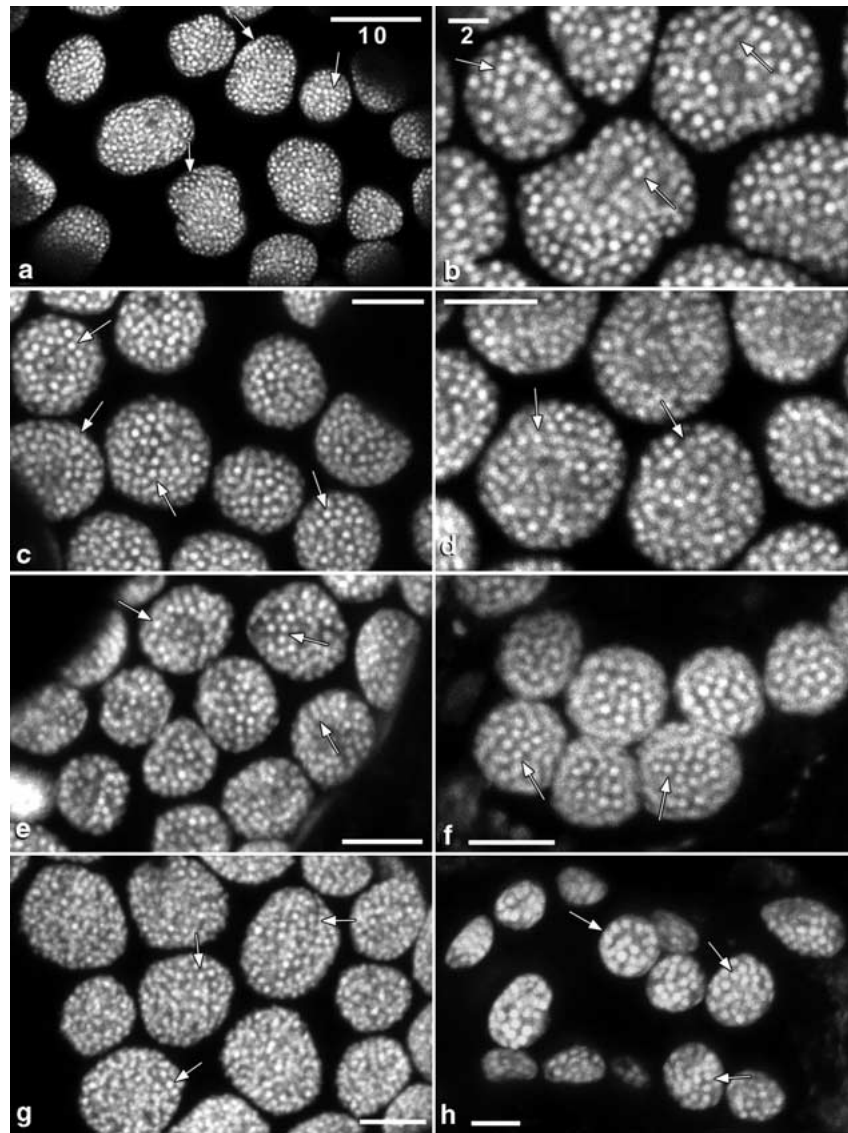
Figure 4. CLSM views of chloroplasts in living mesophyll cells from six different plant species. The distribution of grana is revealed by the autofluorescence of chlorophyll. All species show the string-of-grana arrangement at least in some regions of some chloroplasts. The arrows highlight some of the most obvious granal strings in the chloroplasts. Frames a and b, spinach chloroplasts at two magnifications; c and d, tobacco chloroplasts at two magnifications; e, tomato; f, Begonia; g, pea; h, Schefflera. The various images, except frame b, are complete projections of 10–15 optical slices with increments of $0.4 \, \mu m$. The image in frame b is of a single optical slice. The scale bars represent $10 \, \mu m$, except where otherwise indicated.

confocal laser scanning microscope (CLSM) provided an exciting new tool for the study of chloroplast structure in living and intact cells. van Spronsen et al. (1989) presented some of the first observations on plant tissue using this recently developed procedure. To achieve their excellent images, they had simply placed whole pieces of leaf tissue, immersed in a saturated solution of DCMU (to suppress photosynthesis and enhance chlorophyll fluorescence), between coverslips and then performed the confocal observations. They concluded that their optical sections from *Synnema triflorum*, an aquatic plant, and *Aspidistra*, both species growing under low light conditions, presented a 'stack of coins' view of granal arrangement rather than a spiral. This is a surprising conclusion in view of the fact that their Figure 2 shows a chloroplast

with many examples of long strings of grana. In 1999, Mehta and co-authors used CLSM to investigate the structure of chloroplasts in six different plants, both C_4 and C_3, and concluded that the grana did indeed conform to a 'beads-on-a-string' model.

In 1998, we began a series of CLSM observations of chloroplasts on living cells of a still wider range of plant species. These experiments were done with the aid, advice, and equipment of Professor Brian Gunning. CLSM observations were made of chloroplasts in leaf cells that had first been determined by phase or bright field microscopy to be in the living condition, as evidenced by vigorous cytoplasmic streaming. These observations confirmed the widespread occurrence of strings of grana in all the species examined. A selection of these CLSM images for six different plant species is shown in Figure 4. The frequency with which strings of grana of varying length are observed in living cells of a wide range of species establishes this as a fairly general, but not omnipresent, arrangement of grana within the chloroplasts of higher plants. As noted above, there is, in addition, often an indication of an overall spiral arrangement of these strings (Figure 4). This tendency to form linear arrays of grana is revealed more clearly when a succession of serial optical sections from the CLSM are examined. Figure 5 shows a series of 11 such sections made at $0.4\,\mu m$ increments through a mesophyll cell of a sorghum leaf. Many strings of grana can be followed through a number of consecutive sections and in one chloroplast (boxed in Figure 5(e)), an almost continuous spiral arrangement is apparent. The current techniques do not permit us to resolve whether this arrangement is due to the formation of one continuous chain of grana or to collections of short chains forming one or more spirals. It should be noted that the images in Figure 5, unlike those in Figures 3(A), (B) and 4, were obtained from a leaf section that had been fixed in 2.5% glutaraldehyde.

In the living cell, the immobility of the grana contrasts markedly with the high degree of mobility of the peripheral stroma (Spencer and Wildman 1962; Wildman et al. 1962; Köhler et al. 1997), suggesting that the whole of the system, comprising the grana and associated intergranal membranes, forms a highly interconnected, fairly rigid, network. Because of lack of autofluorescence in the intergranal spaces (and the limits to resolution in the light microscope), CLSM microscopy cannot cast more light on the fine structure of the material connecting grana to each other in either the string arrangements or in the less organized areas.

In apparent contradiction to some of these light microscope observations, there is strong evidence from many EM studies that intergranal connections radiate out in many directions from each granum to other grana; this has become the generally accepted model. These EM studies included fixed and embedded tissue (for example, Heslop-Harrison 1963; Gunning and Steer 1975; Paolillo and Rubin 1980; Mustárdy 1996), the freeze-fracture images of Staehlin (1986), and the negatively stained preparations of Wehrmeyer (1964). This is quite the opposite of what would be expected from the frequency with which linear strings of grana are seen in living cells and in isolated chloroplasts by light microscope procedures. Is there a conflict between the EM and the light microscope observations? We believe that this is not necessarily the case. It cannot be claimed from the images in Figures 3–5 that all, or, in some cases, even a majority of grana are arranged in strings in all chloroplasts. Within any one chloroplast, short strings seem to co-exist with grana that seem to lie in no readily discernible pattern. The strings only become apparent (particularly along chloroplast margins) because of the large chloroplast volumes that can be mapped by the optical sectioning technique of confocal microscopy and other light microscope systems. By contrast, all of the EM techniques, of necessity involve samples of much smaller chloroplast volumes. For example, the detailed reconstruction by Paolillo and Rubin (1980) is of ultrathin serial sections within a $0.5\,\mu m$ slice through the midregion of a chloroplast; comparable constraints apply to the other EM data referred to above. All these EM studies show small though no doubt representative numbers of grana. Nevertheless, these investigations collectively show that intergranal membranes can radiate in all directions from one granum to another. That conclusion is not in dispute, and in the present state of knowledge, all that can be said about the CLSM images is that, with larger samples sizes, EM methods might also have revealed strings of grana, possibly with differently arranged interconnections. In view of the light microscope observations with isolated, partially disrupted chloroplasts, one can only speculate that in isolated chloroplasts, the intergranal connections in the string-of-beads configuration are less readily disrupted than those joining grana in non-linear fashion. This may be the result of the presence of slightly different forms of fret structure (such as tubes versus sheets) between grana in the 'string' regions. It is possible also that the connections in the linear arrays of grana may play an important role in
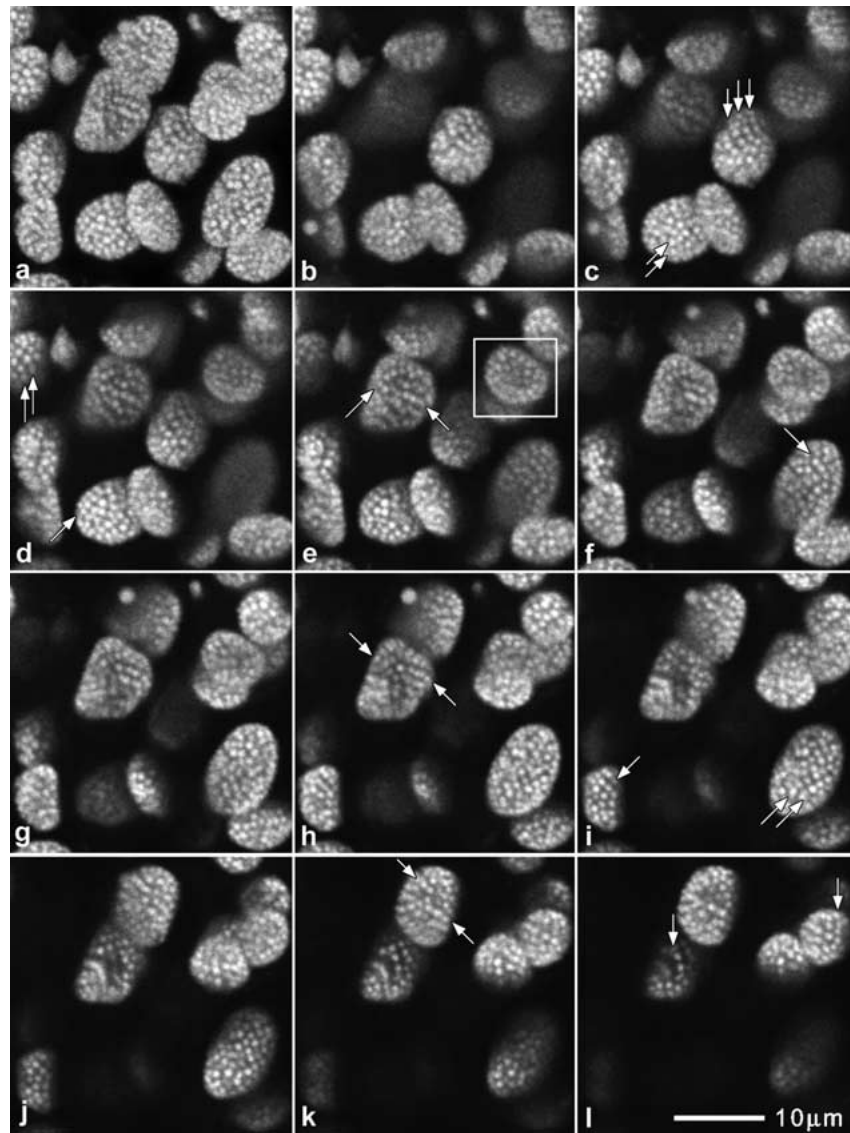
Figure 5. Confocal images of chloroplasts in a sorghum mesophyll cell. The image in frame a is a projection of 11 consecutive optical sections at increments of $0.4\,\mu m$. The remaining images (b–i) show the 11 successive optical slices that make up this projection. The arrows indicate some of the linear arrays of grana, which can be followed through a number of adjoining optical slices. The boxed area in frame (e) highlights a chloroplast in which grana can be traced in a continuous linear string through five successive optical slices. This leaf section was fixed in a solution containing 2.5% glutaraldehyde and 0.05 M phosphate, pH 6.5, prior to analysis by CLSM.

maintaining the integrity of granal thylakoids during stacking and de-stacking, which has been modelled by Arvidsson and Sunby (1999).

Although the sometimes drastic effects of EM fixatives on subcellular fine structures have been well documented (e.g., Hongladarom et al. 1968; Mersey and McCully 1978), this does not appear to be a significant factor in the CLSM studies presented here, as evidenced by the preservation of extensive granal strings in glutaraldehyde-treated cells (Figure 5). It

seems probable, therefore, that strings of grana would be preserved under comparable conditions during fixation for EM studies.

Closing comments

The wide range of chloroplast sizes in expanding leaf cells has been reported earlier (Honda et al. 1971). In the cells of the apical dome where leaf primordia ori-

Figure 6. (Left) *left to right*: Sam Wildman, Ann Hirsch and Stefan Kirchanski. (Right) Donald Spencer.

ginate, the first objects to acquire chlorophyll are the size of a single granum (Wildman and Jope 1977). As the cells in a leaf primordium expand and differentiate to form leaf cells, the chloroplasts keep increasing in size and number per cell. The string-of-grana concept makes it easier to visualize how a population of different sized chloroplasts could appear in the same cell. Beginning as a single granum at different times during the differentiation and expansion phases of the leaf cell, the chloroplasts increase in surface area by accretion of grana. The single granum-chloroplast that appears first would inevitably grow into the largest chloroplast in the population of that cell. Other single grana appear later at different times during leaf expansion and continue their accumulation of grana at the same rate as the first granum-chloroplast. Hence, the string-of-grana model offers flexibility for changing the configuration of grana during development and most likely under different light conditions as well.

Finally, it is hoped that this paper may prompt further appraisal of current models of chloroplast structure, particularly in light of recent improvements in the methods of visualizing subcellular structure and dynamics. EM tomography has resulted in exciting new views of the structure of mitochondria (see Frey and Manella 2000). The use of such methods could help to provide more details and resolve some of the apparent contradictions in current observations on the organization of grana within the chloroplast. It seems reasonable to argue that any future models of the total organization of grana and stroma lamellae within the chloroplast should be consistent with the patterns seen in the living, streaming cell. Currently, these patterns are only available from light microscope observations.

We end this perspective with photographs of the authors. Figure 6(Above) shows Wildman, Hirsch and Kirchanski; Spencer is shown in Figure 6(Right).

Acknowledgements

References

Arimura S, Hirai A and Tsutsumi H (2001) Numerous and highly developed tubular projections from plastids observed in tobacco epidermal cells. Plant Sci 160: 449–454

Arvidsson P-O and Sunby C (1999) A model for the topology of the chloroplast thylakoid membranes. Aust J Plant Physiol 26: 687–694

Frey TG and Mannella CA (2000) The internal structure of mitochondria. Trends Biochem Sci 25: 319–324

Gray JG, Sullivan JA, Hibberd JM and Hansen MR (2001) Stromules: mobile protrusions and interconnections between plastids. Plant Biol 3: 223–233

8

Gunning BES and Steer WM (1975) Ultrastructure and the Biology of Plant Cells. Arnold, London

Heslop-Harrison J (1963) Structural features of the chloroplast. Sci Prog Oxford 54: 519–541

Honda SI, Hongladarom-Honda T, Kwanyuen P and Wildman SG (1971) Interpretations on chloroplast reproduction derived from correlations between cell and chloroplasts. Planta 97: 1–15

Hongladarom T, Honda SI and Wildman SG (1968) Swelling of spinach chloroplasts induced by HCHO and $KMnO_4$, an effect partially hidden by chloroplast size. Plant Cell Physiol 9: 159–168

Jope CA, Atchison BA and Pringle RC (1980) A computer analysis of a spiral, string-of-grana model of the three-dimensional structure of chloroplasts. Bot Gaz 141: 37–47

Köhler RH and Hanson MR (2000) Plastid tubules of higher plants are tissue-specific and developmentally regulated. J Cell Sci 113: 81–89

Köhler RH, Cao J, Zipfel WR, Webb WW and Hanson MR (1997) Exchange of protein molecules through connections between higher plant plastids. Science 276: 2039–2042

Mehta M, Sarafis V and Critchley C (1999) Thylakoid membrane architecture. Aust J Plant Physiol 26: 709–716

Mersey B and McCully ME (1978) Monitoring of the course of fixation in plant cells. J Microscopy 114: 49–76

Mustárdy L (1996) Development of thylakoid membrane stacking. In: ■■■ Oxygenic Photosynthesis: The Light Reactions, pp 59–69. Kluwer Academic Publishers, Dordrecht, The Netherlands

Paolillo DJ Jr and Rubin G (1980) Reconstruction of the grana-fretwork system of a chloroplast. Am J Bot 67: 575–584

Spencer D and Wildman SG (1962) Observations on the structure of grana-containing chloroplasts and a proposed model of chloroplast structure. Aust J Biol Sci 15: 599–610

Staehelin LA (1986) Chloroplast structure and supramolecular organization of photosynthetic membranes. In: Staehelin LA and Arntzen CJ (eds) Photosynthesis III. Photosynthetic Membranes and Light Harvesting Systems, pp 1–84. Springer Verlag, Berlin

van Spronsen EA, Sarafis V, Brakenhoff GJ, van der Voort HTM and Nanninga N (1989) Three-dimensional structure of living chloroplasts as visualized by confocal scanning laser microscopy. Protoplasma 148: 8–14

Wehrmeyer W (1964) Zur Klärung der strukturellen Variabilität der Chloroplastengrana des Spinats in Profil und Aufsicht. Planta 62: 272–293

Wildman SJ and Jope CA (1977) Origin of chloroplasts the size of single grana in apical meristems. Plant Cell Physiol ■■■ (special issue) 385–401

Wildman SG, Hongladarom T and Honda SI (1962) Chloroplast and mitochondria in living cells: cinephotomicrographic studies. Science 138: 434–436

Wildman SG, Jope CA and Atchison BA (1980) Light microscope analysis of three-dimensional structure of higher plant chloroplasts. Position of starch grains and probable spiral arrangement of stroma lamellae and grana. Bot Gaz 141: 24–36