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Membrane geometry of "open" prolamellar bodies

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Summary. The "open" type of prolamellar body in etiplasts was examined by electron microscopy to characterise its three-dimensional organisation. As in more compact forms of prolamellar body, its basic geometric unit is a tetrahedrally branched tubule. In the "open" type, these lie smoothly confluent with one another at the vertices of 5- and 6-membered rings which circumscribe the faces of three kinds of polyhedra: pentagonal dodecahedra (with 12 pentagonal faces), 14-hedra (2 opposite hexagonal faces joined by two circlets of six pentagonal faces), and 15-hedra (3 hexagonal and 12 pentagonal faces). These polyhedra join confluently in their turn, sharing faces with one another in at least one recognisable superstructure which accounts for the appearance of "open" prolamellar bodies in many ultrathin sections. In this organisation, columns of pentagonal dodecahedra are arranged at 120° to one another in the x–y-plane of the lattice. They do not fill the plane but intersect so as to delimit voids in the form of hexagonally arranged 14-hedra (with hexagonal rings in the x–y-plane). Strata of this type alternate with strata made of face-sharing 15-hedra (with their hexagonal rings normal to x–y), which also delimit 14-hedra. The 14-hedra thus lie in register in the z-axis in hexagonally arranged columns, normal to the alternating strata. Other possible organisations cannot be excluded and local variations and dislocations certainly occur, but many micrographs that display elements of symmetry in "open" prolamellar bodies can be matched to thin slices through such a model. Its geometry is like that of the cages of water molecules in type IV (*sensu* Jeffrey = type III *sensu* O'Keeffe) clathrate-hydrates, point group P6/mmm, but about two orders of magnitude larger.

Keywords: Clathrate; Etioplast; Membrane tubule; Minimal surface; Prolamellar body.

Introduction

Etioplasts or etio-chloroplasts arise when normal chloroplast development is curtailed by lack of light. Instead of the arrangement of grana and stroma lamellae seen in light-grown chloroplasts, the thylakoid membranes assume the form of semicrystalline prolamellar bodies consisting of branched, interconnected tubules bearing protochlorophyllide and NADPH-protochlorophyllide oxidoreductase enzyme. Lütz (1986), A. Wellburn (1987), von Wettstein et al. (1995), Gunning and Steer (1996), Sundqvist and Dahlin (1997), and Reinbothe et al. (1999) review aspects of prolamellar body structure and the initial photochemical reactions of the greening process that produce chlorophyllide, rapidly destroy the symmetry of the tubular membrane system, and initiate utilisation of prolamellar body tubules during formation of relatively flat thylakoids.

The membrane architecture of prolamellar bodies has been analysed in detail by means of ultrathin sectioning (Wehrmeyer 1965a–c, 1967; Ikeda 1968; Gunning 1965; Gunning and Steer 1975, 1996; Hyde et al. 1997), freeze fracture (Osumi et al. 1984, Murakami et al. 1985), X-ray diffraction (Williams et al. 1998), and mathematical analysis (Israelachvili and Wolfe 1980, Charvolin and Sadoc 1996, Hyde et al. 1997). The most common geometries are analogues of zincblende and wurtzite crystal lattices, first described in detail by Wehrmeyer (1965b, c) and Ikeda (1968) and consisting of tetrahedrally branched tubular membranes (Fig. 7a–c). A form based on hexahedrally branched tubules has also been detected, but the original idea (Gunning 1965) that sectioning it in various planes could account for the views now known to be based on tetrahedral units was wrong. Introduction of pentagonal dodecahedra (also composed of tetrahedrally branched units but branching at the pentagonal angle of 108° rather than the tetrahedral angle, 109°28′) creates additional options. In centric prolamellar bodies (Wehrmeyer 1965a) 20 tetrahedral blocks of

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membrane lattice of the zincblende form radiate from each vertex of a central pentagonal dodecahedron, joined smoothly to one another by interfaces of the wurtzite form (Murakami et al. 1985, Gunning and Steer 1996, Charvolin and Sadoc 1996). Potentially this organisation could produce perfectly symmetrical icosahedral prolamellar bodies consisting of 20 equal sectors surrounding one pentagonal dodecahedron. Such, however, probably do not exist; the sectors are either irregular or, more often, the energy cost of the distortions that are inherent in the organisation, which must increase as the system grows, are eased by production of multicentric forms containing several to many pentagonal dodecahedra at vertices of relatively small intervening tetrahedral blocks of zincblende-wurtzite lattice (Gunning and Steer 1996).

Prolamellar bodies are the most consistently formed and distinctive of the many forms of curved membranous sheets and tubules that occur in plant and animal cells (see Hyde et al. 1997 for a comprehensive survey). They can be interpreted in structure-function terms as stores of a large surface area of lipid-rich membrane (composed predominantly of mono- and di-galactosyldiacylglycerols) in an unstacked conformation in a low-energy state and in a small space, in a geometry that can readily serve as prefabricated substrate for transformation to flat thylakoids containing many additional proteins, thus aiding rapid development of active photosynthesis upon illumination. Up to about 50 μm^2 of membrane is packed into each cubic micrometer of prolamellar body (Gunning and Steer 1975), which is probably the highest density of packing of membrane in an unstacked conformation achieved in any cells.

The fact that the membrane surface is 100% accessible to the etioplast stroma phase may indicate that this interface is important during the initial photo-conversion reactions of greening. This is where the extrinsic membrane enzyme NADPH-protochlorophllide oxidoreductase and its associated pigment is located (von Wettstein et al. 1995, Sundqvist and Dahlin 1997, Reinbothe et al. 1999), although it is present in higher concentration in prothylakoids than in prolamellar bodies (Lütz 1986, Staehelin 1986, von Wettstein et al. 1995). It participates in the initial reactions (Reinbothe et al. 1999), whereas chlorophyll synthetase, which is active in a later step, is an intrinsic membrane protein (von Wettstein et al. 1995, Sundqvist and Dahlin 1997). It is also relevant to the unstacked nature of prolamellar body membranes that the factors that bring about stacking in grana in later stages of development are absent in the etiolated state.

Tightly curved tubules do provide a large interfacial area, as described above, but they might be thought to be an inefficient form of storage for a surface that is destined to transform quickly into flat sheets. It has been argued that the very high content of monogalactosyldiacylglycerol (which is "cone-shaped"; Murphy 1986, Bruce 1998) in prolamellar bodies brings about pronounced curvature (Lütz 1986, Staehelin 1986) and indeed this and related lipids do form hexagonal phase tubular micelles in vitro (Sprague and Staehelin 1984). However, one of the chief features of the tetrahedral "units" of prolamellar body membranes of all types is that at every point on the surface the curvatures along any pair of axes normal to each other are nearly equal and opposite (most closely so in the compact wurtzite and zincblende forms; Hyde et al. 1997). Thus compression of constituent molecules in one axis is almost matched by relaxation normal to that axis, leading to the unintuitive conclusion that the prolamellar body membrane is, in toto, a surface of low net curvature and hence very suitable as a store of prefabricated progenitor of flat thylakoids, convertible without requiring energy-consuming exchanges between the two sides of the membrane (Israelachvili and Wolfe 1980). Given the low net curvature, the importance of the cone shape of the major constituent lipid therefore is conjectural. Introduction of anisotropic proteins can also cause flat membranes to fold into saddle shapes with opposing curvatures (see Luzzati 1997). It should be noted, however, that low net curvature is not of itself a raison d'être for tetrahedrally branched tubules, for there are many other membrane surfaces which share the same feature, though many are multilayered, unlike prolamellar bodies (see surveys by Hyde et al. 1997, Luzzati 1997).

All forms of prolamellar body are variations on categories of infinite periodic minimal surfaces (Gunning and Steer 1975, 1996; Larsson et al. 1980; Murakami et al. 1985; Hyde et al. 1997). Given the conditions necessary for initiating the geometry, one aspect of which is discussed later, the periodicity could propagate automatically with no requirement for additional morphogenetic information until the essential constituent molecules are used up. Analysis of mutants and transformants has shown that among the molecular prerequisites for prolamellar body formation are protochlorophyllide pigment and NADPH-

protochlorophyllide oxidoreductase (the major protein of prolamellar bodies, although present in small amounts) (see Lütz 1986, von Wettstein et al. 1995, Sundqvist and Dahlin 1997, Sperling et al. 1998). No doubt other membrane lipids (including, obviously, mono- and di-galactosyldiacylglycerols) and proteins are also essential.

One form of prolamellar body stands apart from the rest by virtue of the less compact packing of its constituent membrane tubules. It has never been analysed to the same extent as the wurtzite, zincblende, and centric types and has been described variously as a spongy tubular system (Wehrmeyer 1967), the atypical lattice (Gunning and Jagoe 1967), the open prolamellar body (Weier and Brown 1970), wide-spaced tubules (Henningsen and Boynton 1969, 1970), noncrystalline prolamellar body (Berry and Smith 1971), and open lattice (Gunning and Steer 1975, 1996). As pointed out by Rosinski and Rosen (1972) these may all have the same structure. It can occur in the same species as the more regular forms and indeed in the same etioplasts (Weier et al. 1996; Weier and Brown 1970; Gunning and Steer 1975, 1996; Walles and Hudak 1975; Cooke et al. 1975) and may transform to (and from) the more compact forms during leaf aging (Henningsen and Boynton 1969). It is, however, relatively infrequent except in *Hordeum vulgare*, where it predominates in young etiolated leaves (Berry and Smith 1971), but it has also been observed in other species of monocotyledons, dicotyledons, and gymnosperms.

The present paper shows that some (not necessarily all) "open" prolamellar bodies consist of confluent tetrahedrally branched tubules delimiting pentagonal dodecahedra and larger 14- and 15-hedra, which in turn are combined by face-sharing in a lattice that has counterparts in certain clathrate-hydrate inclusion compounds.

Material and methods

The micrographs selected for inclusion here are from a large collection taken on several different transmission electron microscopes. They show etiolated *Avena sativa* (oat) or *Hordeum vulgare* (barley) first-leaf mesophyll etioplasts, processed either as in Gunning (1965) or as in that protocol but embedded in Spurr's resin. Figures 3 and 4 are from a batch taken at 400 kV with an AEI EM7 high-voltage instrument in Oxford University.

Computer models were made with the software package 3D Studio Max.

Results

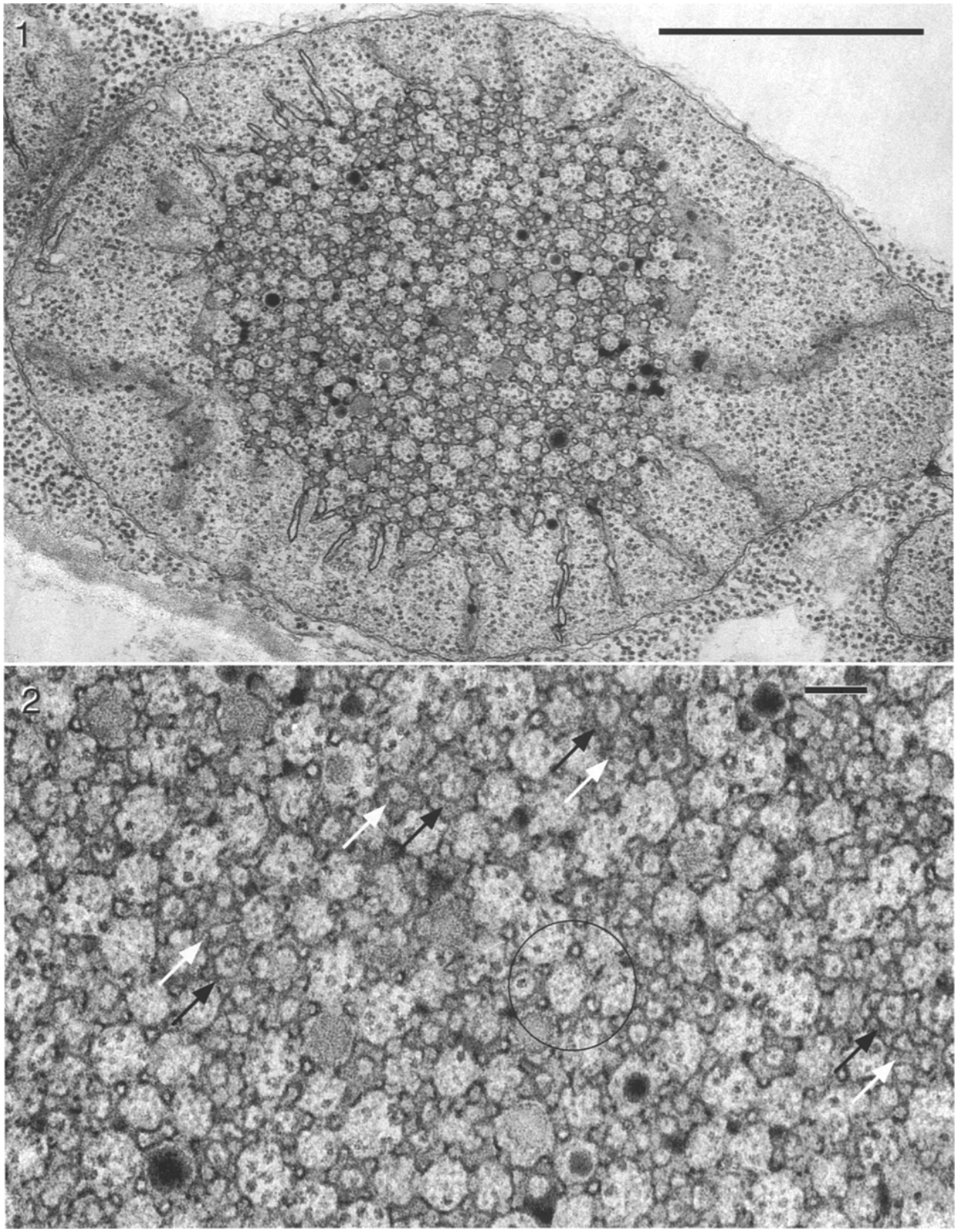
Introductory micrographs

Figure 1 shows a prolamellar body of the "open" type in an *A. sativa* etioplast, to illustrate the lack of order that is the first impression gained from most planes of section. It consists of 5- and 6-membered ring structures built from tetrahedrally branched units whose arms, in cross section, are seen to be membrane tubules. Pentagonal rings greatly outnumber hexagonal rings in most views (Fig. 2). Voids of various sizes, circumscribed by tubule profiles, are filled with etioplast stroma. The frequency of ribosomes in the stroma compartment of the prolamellar body is much the same as it is in the largely membrane-free stroma between the prolamellar body and the etioplast envelope.

The impression of disorder in most micrographs arises because the void diameters in the prolamellar body considerably exceed the thickness of ultrathin sections, and because the large size of the polyhedral units of construction (relative to the more compact types of prolamellar body) reduces the chance that a given plane of section will reveal meaningful symmetry. Scanning transmission electron microscopy and high-voltage transmission electron microscopy of sections up to 0.5 μm thick were both tried in efforts to overcome this problem. Figures 3 and 4 show micrographs taken at 400 kV. Figure 3 shows evidence of regular substructures arranged in linear order, and Fig. 4 shows regions where there are hexagonal arrangements of ring structures, each with a lucent centre, interconnected by membrane tubules. In the clearest portion of Fig. 4 one of the dense ring

Fig. 1. An *A. sativa* etioplast containing an open prolamellar body sectioned in no obvious plane of symmetry and therefore giving an impression of a disordered aggregate of pentagonal rings (mostly) and occasional hexagonal rings, surrounding larger voids in which stroma components are seen, including ribosomes and plasto-globuli. Bar: 1.0 μm

Fig. 2. Higher magnification of portion of the prolamellar body shown in Fig. 1, showing many pentagonal rings (e.g., white arrows) and fewer hexagonal rings (black arrows). Where the section shows the two types of ring side by side, they are seen to share a common edge. Many of the void areas, occupied by stroma components, have hexagonal outlines, often with sectioned tubules at the vertices and often with portions of pentagonal rings at their edges. Bar: 0.1 μm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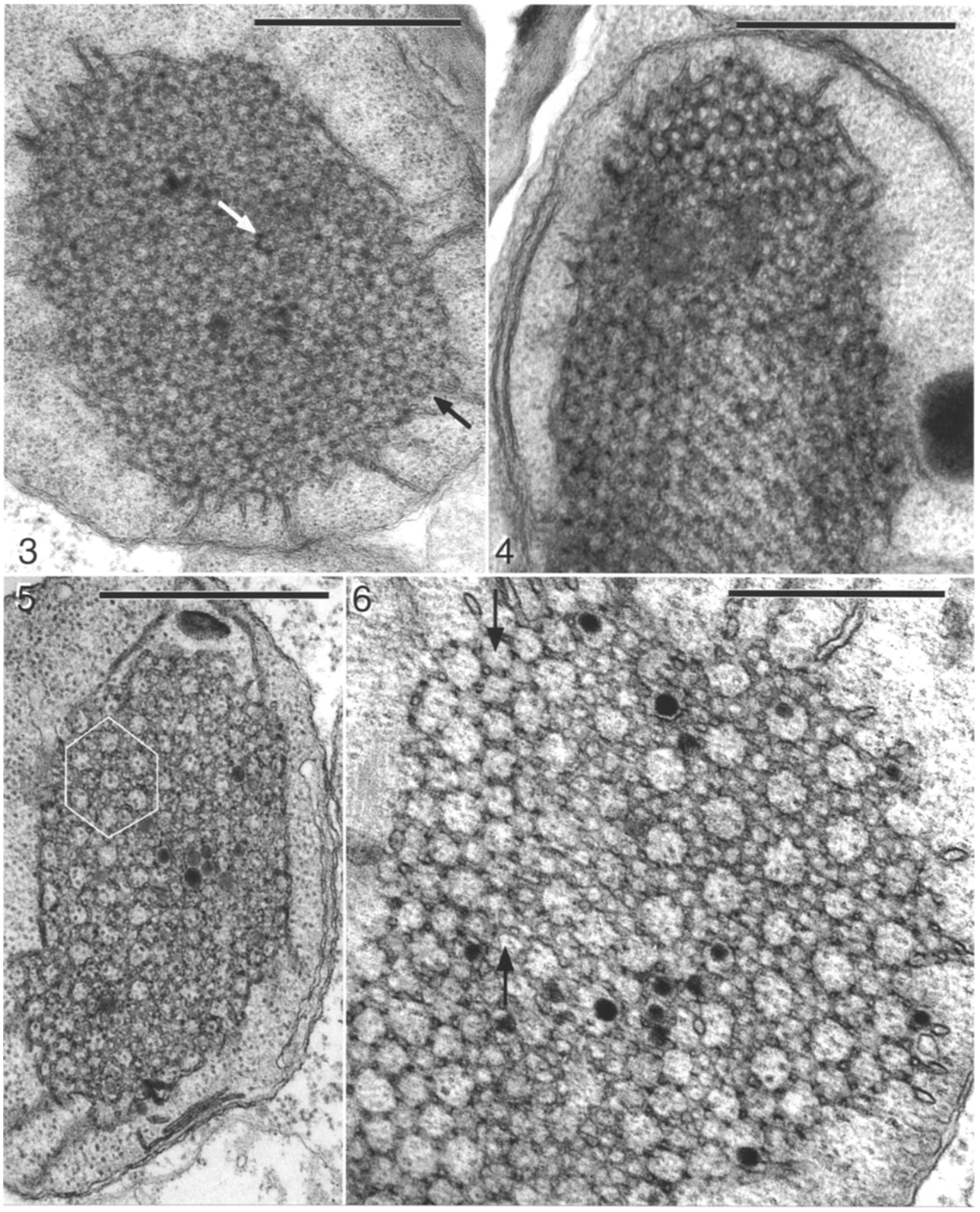
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elements is surrounded by 12 small pentagonal rings. This suggests that the ring structure element is a polyhedron with three- or sixfold symmetry surrounded by other polyhedra with pentagonal faces. It is not possible to say from this micrograph whether the hexagonal arrangement occurs in three dimensions or whether the image represents a slice at approximately right angles to hexagonally arranged columns of ring structures. Images described later favour the latter view, and even here it is noticeable that columns of polyhedra are apparent in another part of the same micrograph. The more compact forms of prolamellar body are often made up of sectors with differing orientation, and the same may well apply to the open type.

Figures 5 and 6 show ultrathin equivalents of the hexagonally patterned region of Fig. 4, confirming the occurrence of circlets of 12 pentagonal rings around voids which are themselves arranged hexagonally. The arrangement of neighbouring pentagonal rings is similar to that seen on the surfaces of the pentagonal dodecahedra that lie at foci of centric prolamellar bodies.

Model

A model based in part on the above micrographs is presented here, followed by additional micrographs which are interpreted in relation to the model. Model building was done first with chemistry construction kits of tetrahedral 4-armed "carbon atoms" of the sort that can be used to build wurtzite and zincblende lattices (Gunning and Steer 1975, 1996). Computer-generated models similar to the physical models are used for illustration purposes (Fig. 7) and to confirm interpretations of micrographs.

A convenient introduction is to model the hexagonal pattern of Figs. 4–6. Pentagonal dodecahedra cannot alone fill space, but they can be assembled so as to circumscribe voids with 14, 15, or 16 faces (Williams 1979). The hexagonal pattern is readily mimicked by pentagonal dodecahedra sharing faces to make linear columns which intersect at 120° angles (Fig. 7 d, e). This requires small (approximately 3°) distortions in the angles between the faces from those in regular pentagonal dodecahedra (Williams 1979). Every contiguous set of six face-sharing pentagonal dodecahedra has a gap in the centre. The gap is actually two back-to-back cup-shaped halves of 14-hedra (each complete 14-hedron has 2 opposite hexagonal faces joined to one another by two circlets of six pentagonal faces). If the array shown in Fig. 7 d and e were to be extended laterally and sectioned in the plane of the array, the result would duplicate Figs. 4 and 6, the void in the centre of each of the hexagonally arranged ring structures in those micrographs being part of the 14-hedron "cup" and the 12 pentagons around it being adjacent faces of the six surrounding pentagonal dodecahedra. From this point on the "ring-shaped structures" of Figs. 4–6 are referred to as 14-hedra.

The arrangement shown in the x–y-plane (i.e., the plane of the paper in Fig. 7 d and the upper part of Fig. 7 e) can propagate in two but not three dimensions. However, a three-dimensional lattice can be formed if successive layers of the structure shown in Fig. 7 e are joined together in the z-axis by interposing additional layers of tetrahedral units. A simple way to picture this is to make a structure similar to that of Fig. 7 e but starting with three pairs of 15-hedra (each of which has 12 pentagonal and three hexagonal faces; Fig. 7 f). This structure (Fig. 7 g) also circumscribes

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Figs. 3 and **4.** Micrographs of approximately 0.5 μm thick sections of *A. sativa* etioplasts taken at 400 kV. Bars: 1.0 μm

Fig. 3. The section is not in a readily defined plane relative to the symmetry of the prolamellar body, but nevertheless there is sufficient material in the 0.5 μm thick section to reveal the presence of ring-shaped elements, sometimes seen in linear arrays (e.g. between arrows). These elements appear in other figures, and as they are later identified as 14-hedra, they are referred to as such in subsequent figure legends

Fig. 4. The upper part of the prolamellar body clearly shows a hexagonal arrangement of 14-hedra, interconnected by arrays of much smaller pentagonal rings. This region corresponds to the x–y-plane of the model in Fig. 7 j

Fig. 5. *Avena sativa* etioplast sectioned in a plane that is slightly tilted with respect to the x–y-plane of the model (Fig. 7 j), as shown by apparent slight lateral compression of the hexagonal arrangement of 14-hedra. Bar: 1.0 μm

Fig. 6. A barley etioplast sectioned nearly in the x–y-plane of the model (Fig. 7 j), showing circlets of 12 pentagonal rings around each of the sectioned 14-hedra (right-hand side of the micrograph). At the left-hand side of the micrograph the tilt in the plane of section relative to x–y has taken the section into less regular profiles, but a column of polyhedra (pentagonal dodecahedra) arranged on the same axis as one of the lines of symmetry at the right-hand side is seen (between arrows). Bar: 0.5 μm

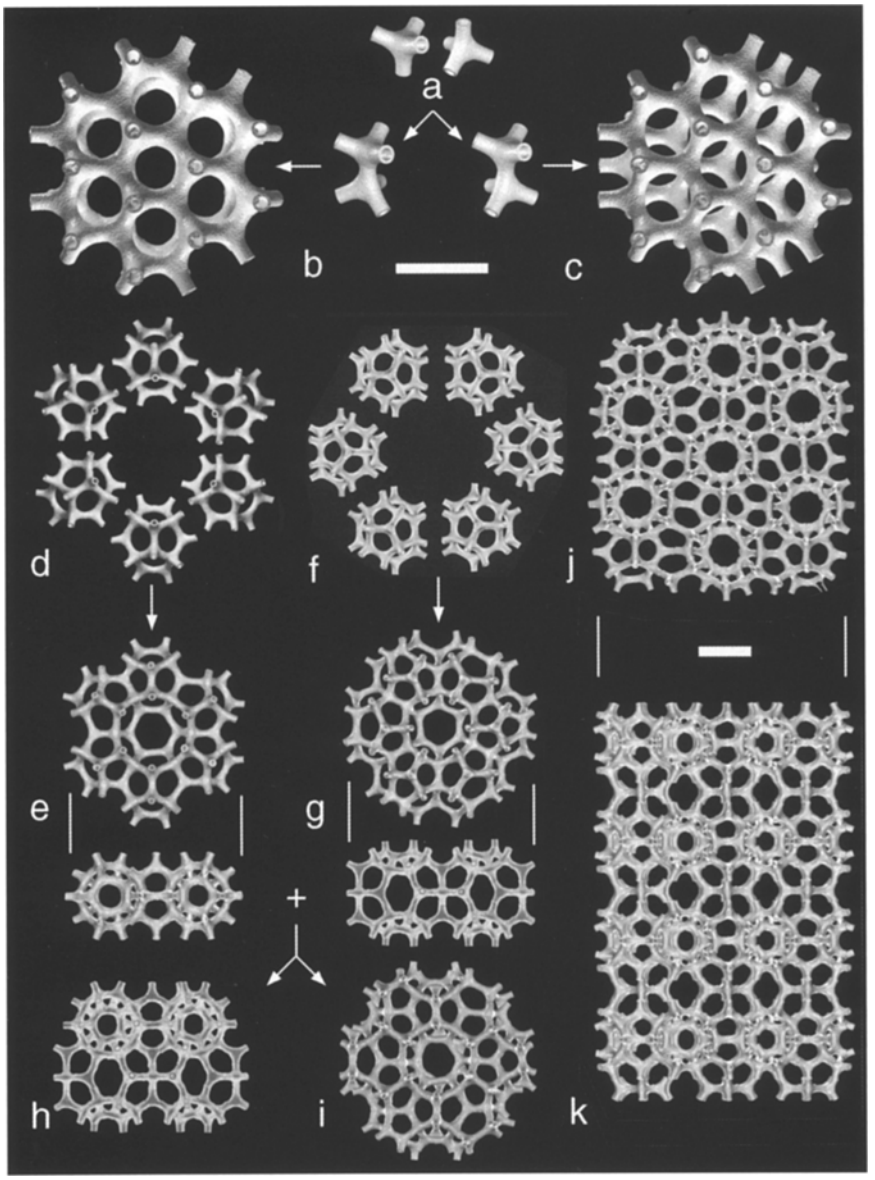


Fig. 7. Views of three-dimensional models generated with 3D Studio Max software to illustrate the proposed structure of the open prolamellar body (d–k), compared with the more compact wurtzite and zincblende forms (b and c). **a** Tetrahedrally branched units, common to all prolamellar bodies shown. **b.** and **c** Wurtzite (**b**) and zincblende (**c**) forms and the isomeric arrangements of tetrahedral units that give rise to the differences between them. **d** Arrangement of pentagonal dodecahedra (with 12 pentagonal faces), each in edge view. **e** The same set after they have been made to share adjacent faces; a hexagonal ring is formed at the centre, and above and below it are two cup shapes facing opposite directions, each being half of a 14-hedron. When they are completed, each 14-hedron has two opposite hexagonal faces joined by two edge-sharing circlets each with six pentagonal faces. **f** and **g** A similar situation but starting with 15-hedra in vertex view (these have three hexagonal faces which are shown in profile view and 12 pentagonal faces). The aggregates **h** and **i** of the structures in **e** and **g** are shown fitted together by face-sharing in side (**h**) and face (**i**) views. This composite structure now has a completed 14-hedron at its centre connected in a linear file to two more half 14-hedral "cups" facing outwards. **j** and **k** The composite structure of **h** and **i** can propagate in x, y, and z axes, as shown in **j** (face, or x–y, view) and **k** (side, or x–z, view). Bars: between b and c, 0.1 μm (for b and c only); between j and k, 0.1 μm (for j and k only)

two half 14-hedra, just as does its counterpart made from pentagonal dodecahedra. When the two structures shown in Fig. 7e and g are oriented as shown and superimposed upon one another, they can share "upper" and "lower" faces respectively, as in Fig. 7h ("side" view) and i ("face", or x–y-view). The composite structure now contains a central column consisting of one complete 14-hedron in the centre joined in line (in the z-axis) to two outward-facing half 14-hedra. Furthermore, the composite structure can propagate in three dimensions (Fig. 7j, k) to make a repeating lattice composed of hexagonally arranged columns of 14-hedra circumscribed by strata of intersecting columns of pentagonal dodecahedra alternating with strata made of face-sharing pairs of 15-hedra. The crystallographic point group is P6/mmm.

Matching micrographs to the model

Many micrographs of open prolamellar bodies show elements of symmetry which provide crucial tests for the above model. Examples selected to illustrate features of different planes of section are analysed here.

The simplest matches between the model and the micrographs arise when sections lie in or close to the x–y-plane shown in Fig. 7j, as in parts of Figs. 4–6. Figure 7j is a projection of many superimposed layers, but if a thin slice of the model is cut so as to mimic

an ultrathin section the correspondence is clearer (compare Fig. 6 with Fig. 8). The centre–centre spacing of the 14-hedra in the x–y-plane is 0.17 μm. Another view of the x–y-plane is presented below.

Views at right angles to the x–y-plane of Fig. 7j, i.e., in a z-plane such as Fig. 7k, should show columns of 14-hedra (provided that they are included within the thickness of the section), and, normal to those columns, alternating strata of polyhedra of two sizes. Figure 10 is in one such plane. The main feature is a vertical column of 14-hedra. Alternating horizontal columns interpreted as pentagonal dodecahedra and larger 15-hedra are also present, as predicted.

Figure 11 also approximates to a z-plane and contains two vertical linear columns of 14-hedra, though the plane of section is sufficiently oblique that only short columns are seen. The alternating layers of 15-hedra appear here as successive pairs from left to right, with intervening columns of pentagonal dodecahedra, in this view seen as individual profiles rather than continuous horizontal columns. Two horizontal pairs of 15-hedra are marked. Reference to Fig. 7d–g shows that a section passing through a pair of 15-hedra (Fig. 7g) cannot also pass along a column of pentagonal dodecahedra (Fig. 7e), for the two are at 30° to one another. One pentagonal dodecahedron in Fig. 11 has half of its edge tubules within the section thickness; the vertices are marked to show that the view is as for the pentagonal dodecahedra in Fig. 7d and e. With respect to the x–y-plane of Fig. 7j, the z-section of Fig. 11 passes (at approximately right angles to the page) from one column of 14-hedra to another plus 1 in x and plus 3.5 in y, passing through pairs of 15-hedra half way between. Figures 10 and 11 therefore both accord with predictions derived from the model about the appearance of z-planes.

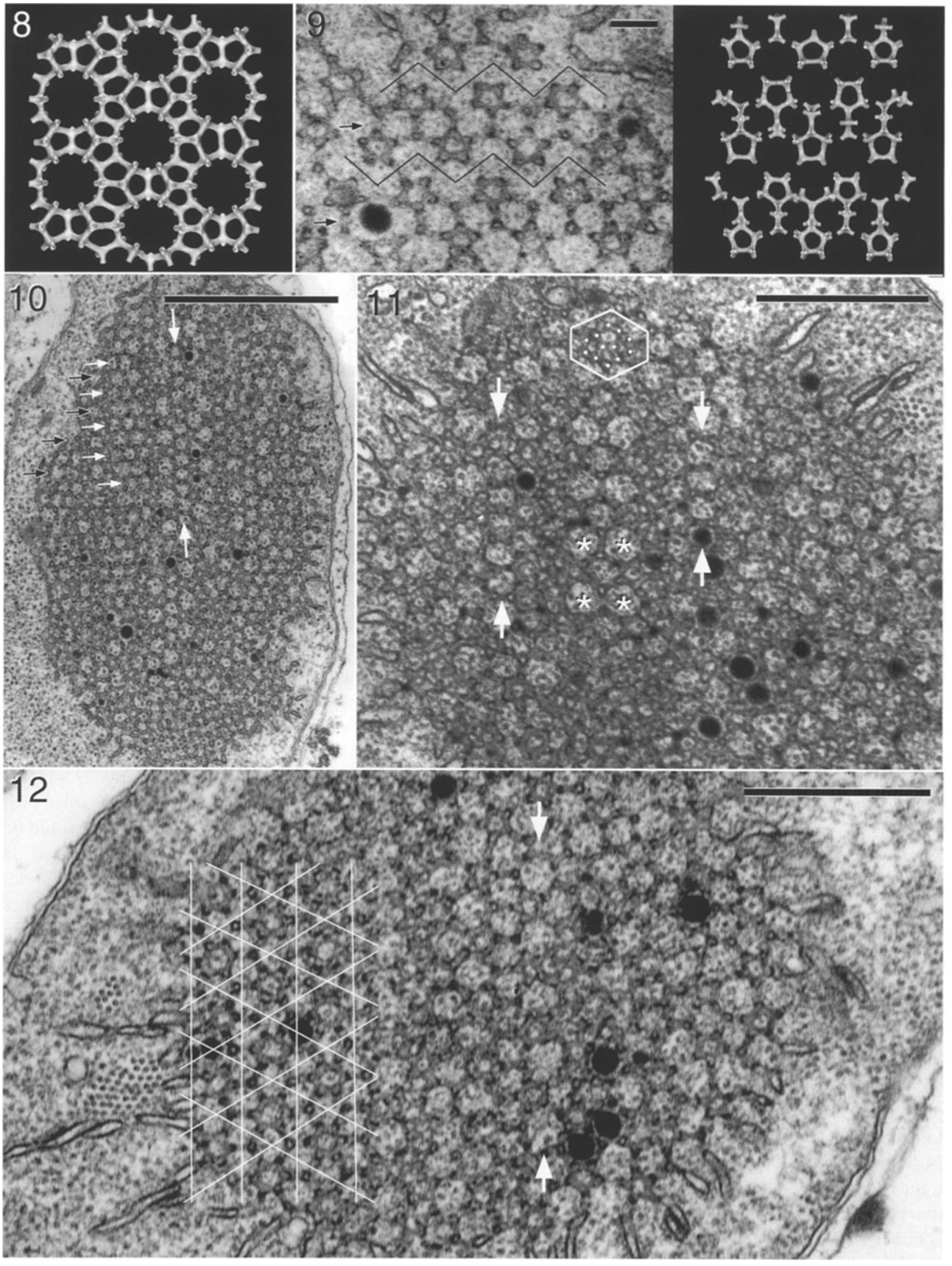
Figure 12 is a return to a plane that is close to x–y. It is strikingly different in appearance from Fig. 6, which nevertheless is also in that plane. Figure 6 shows 14-hedra sectioned across their centres so that the voids (stroma compartment) appear large. The section shown in Fig. 12, by contrast, includes just the hexagonal rings of the 14-hedra. The marked columns of pentagonal dodecahedra intersect in three axes at 120° to each other and delimit the hexagonal faces of the 14-hedra. The section is somewhat oblique, and at the right-hand side of the micrograph has slanted into the adjacent stratum of 15-hedra.

Finally, Fig. 9 shows a still more oblique section, slanting from one column of pentagonal dodecahedra through a zig-zag pattern derived from the arrangement of 15-hedra flanking 14-hedra, and on to the next stratum of pentagonal dodecahedra. In this plane the section includes a striking array of pentagonal rings, each with a particular orientation of its edges and vertices, and all of these details are reproduced in a slice through the model.

Discussion

It is impossible to be sure whether all images of open prolamellar bodies represent one organisation viewed in different ways, or whether there are differing subtypes of orderly structure, or whether some are indeed disordered. However, elements of symmetry that have been observed in a large collection of micrographs do lead to a model, previously introduced by Gunning and Steer (1975, 1996) but without detail or confirmation. The model accounts for numerous points of detail in electron micrographs as well as larger features of symmetry in various planes.

The proposed structure is not unique. It is composed of tetrahedrally branched ultimate units, of similar geometry to those also found in carbon compounds and in aggregated water molecules in structures such as ices and clathrate inclusion compounds. In the latter, tetrahedrally arranged water forms "cages" surrounding guest molecules of various sorts. For example, the clathrate-hydrates trimethylamine decahydrate (Panke 1968) and tetra-iso-amyl ammonium fluoride (with 38 water molecules) (Jeffrey 1984) have the same geometry as described here for the open prolamellar body. The geometry of the clathrate would be transformed into that of the prolamellar body if its dimensions were to be scaled up by nearly 100-fold, its guest molecules replaced by etioplast ribosomes and other components of the stroma phase, and the cage of water molecules, linked by hydrogen bonds, replaced by confluent tetrahedrally branched membrane tubules. There are many other polyhedral clathrates, reviewed by Jeffrey (1984), though it seems that few compounds are in the geometrical category described here, which is his type IV. O'Keeffe (1999) reviews examples of numerous other crystal structures and periodic foams (his type III clathrate equates to the type IV of Jeffrey 1984) based on polyhedra with 12–16 faces. Ultrathin sectioning and transmission electron microscopy could not easily reveal the subtle differences between them, thus re-emphasising that the model presented here for open prolamellar bodies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may be but one of several or even many options. Charvolin and Sadoc (1996) diagram the structural unit of open prolamellar bodies as a tetrahedral arrangement of pentagonal dodecahedra surrounding a central 16-hedron (a space-filling structure described by Williams 1979), but without giving evidence. That arrangement could not give rise to the sections analysed here.

Structures made of polyhedra of the types found here occur at widely differing scales. For example, there is a lipid-water phase made of aggregated 12- and 14-hedral lipid micelles (the cubic phase Q^{223}; Luzzati et al. 1993), where the polyhedra are about eight times smaller than their counterparts in prolamellar bodies. It resembles the open prolamellar body much more closely than the hexagonal phases formed by monogalactosyl-diacylglycerol in vitro (Sprague and Staehelin 1984, Murphy 1986, Bruce 1998). At a vastly larger size scale 12- and 14-hedra are found in a repeating arrangement in foams (the 12-hedra are slightly distorted pentagonal dodecahedra; Weaire and Phelan 1996). Presumably the common feature at all size scales is the stability of the organisation. Thus the Weaire–Phelan structure is a low-energy state because it has the lowest area of surface film in any foam composed of polyhedral cells of equal volume. Open prolamellar bodies differ fundamentally from such a structure in that their three classes of polyhedra have different volumes (if they are undistorted, the volume ratios of pentagonal dodecahedra, 14-hedra, and 15-hedra would be 1.7 : 2.2 : 2.4). Moreover surface tension of films on the faces of polyhedra is not a factor in prolamellar bodies, where the polyhedra have open faces and the membrane is in confluent tubules lying along the edges of the polyhedra, but nevertheless the geometry is evidently a stable, low-energy state. The structure described here has a ratio of pentagonal to hexagonal rings of just over 8, close to the figure of 8.6, which would make the average angle between tubules equal to the stable tetrahedral angle of 109°28′ (if all faces are planar). To this extent the stresses involved in making pentagonal (108°) and hexagonal (120°) angles largely cancel out. Although this averaging may serve to reduce the free energy of the system, it should be noted that the impression of stability given by static electron micrographs may well be entirely false. It cannot be ruled out that prolamellar body lattices, especially the open type, may undergo dynamic geometrical alterations over short time scales. There is evidence that long-term changes occur during leaf aging (Henningsen and Boynton 1969, Berry and Smith 1971).

Open prolamellar bodies are obviously less efficient than the wurtzite and zincblende forms in packing large expanses of membrane into a small volume within an etioplast. It is therefore not clear why they occur, or why they are so common in barley. Open prolamellar bodies differ from the more compact types in having a more open diffusion path into the inner recesses of the prolamellar body, and in having a much higher ratio of stroma subvolume to intratubule subvolume. These factors may have functional significance for stroma–membrane interactions.

The geometrical analogy to clathrate compounds raises the question of whether the stroma component has a role in generating or maintaining prolamellar body architecture, or whether the determinants of the

Fig. 8. A slice of the model (Fig. 9j) corresponding to Fig. 4 (upper region), part of Fig. 5, and the right side of Fig. 6

Fig. 9. A plane of section showing a distinctive pattern of pentagonal faces connected to horizontal columns of pentagonal dodecahedra (arrows), with intervening zig-zag voids made up of 15-hedra (upper angles of black lines) flanking 14-hedra (lower angles of black lines). A corresponding slice of the model is shown to the right. Bar: 0.1 μm

Fig. 10. *Avena sativa* etioplast, with the section passing through the prolamellar body in a z-plane that reveals columns of polyhedra in two axes at right angles. The most obvious column (between the vertical white arrows) consists of 14-hedra and is oriented in the print so that it runs vertically, corresponding to a view embedded within the projection of Fig. 7k. Other columns lie at right angles to it (small arrows). The small white arrows point to columns of pentagonal dodecahedra. They alternate with columns of 15-hedra (small black arrows). At the lower tip of the prolamellar body the "open" lattice merges with some wurtzite or zincblende lattice. Bar: 1.0 μm

Fig. 11. *Avena sativa* prolamellar body, sectioned in a z-plane such that parts of two columns of 14-hedra are included (between white arrows). The two columns are quite distant (+1 in x, +3.5 in y). Paired 15-hedra lie symmetrically between them (e.g., asterisks), separated in the z-axis (vertical in this micrograph) by strata of pentagonal dodecahedra. One pentagonal dodecahedron is outlined in white at upper centre, with its vertices marked by white dots. See text for further details. Bar: 0.5 μm

Fig. 12. A portion of a barley prolamellar body sectioned in the x–y-plane to show columns of polyhedra intersecting at 120° angles (white lines). These columns delimit the hexagonal faces of 14-hedra. The section is tilted enough to slant into a stratum of 15-hedra (white arrows). Bar: 0.5 μm

morphology reside in the membrane lipids and proteins themselves. The staining properties of the membrane make it the most conspicuous feature of the system, but it may be that packing properties in the stroma compartment create a 3-dimensional contour that is somehow favourable for assembling and/or shaping the membrane. If the membrane could be removed selectively, the surface of the stroma compartment, which is the "dual" of the membrane surface, would have the same geometry. Moreover, in many clathrates the guest molecules do indeed stabilise and shape the cage that encloses them. In prolamellar bodies the most prominent stroma components are ribosomes, which occur singly in most, if not all, unit cells of compact prolamellar bodies (e.g., Gunning and Jagoe 1967, F. Wellburn and Wellburn 1971). By contrast the polyhedral voids of open prolamellar bodies contain multiple ribosomes, as shown by most micrographs presented here. As far as can be seen they are present as mono-ribosomes. Unfortunately electron micrographs of ultrathin sections do not reveal whether they are placed at random or in any particular disposition in the voids. Whether they help to shape the network of tubules that surrounds them (discussed by Gunning and Jagoe [1967] in respect of compact prolamellar bodies) therefore remains no more than a possibility, and indeed the fact that compact prolamellar bodies can be isolated and washed to at least reduce the stroma content suggests that the system of membrane tubules has inherent stability (as discussed by Lütz 1986).

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