How does the cytoskeleton read the laws of geometry in aligning the division plane of plant cells?

CLIVE W. LLOYD

Department of Cell Biology, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK

Summary

Since Robert Hooke observed the froth-like texture of sectioned plant tissue, there have been numerous attempts to describe the geometrical properties of cells and to account for the patterns they form. Some aspects of biological patterning can be mimicked by compressed spheres and by liquid foams, implying that compression or surface tension are physical bases of patterning.

The 14-sided semi-regular tetrakaidecahedron encloses a given volume most efficiently and packs to fill space. However, observations of real plant tissue (and of soap bubbles) in the first half of this century established that plant cells only rarely form this mathematically ideal figure composed predominantly of 6-sided polygons. Instead, they tend to form a topologically transformed variant having mainly pentagonal faces although there is variability in the number of sides and the angles formed. But the one irreducible component of normal cell and tissue geometry is that only three edges meet at a point in a plane. In solid space, this gives rise to tetrahedral junctions and it is from this that certain limitations on sidedness flow. For three edges to meet at a point means that there must be an avoidance mechanism which prevents a new cell plate from aligning with an existing 3-way junction. Sinnott and Bloch (1940) saw that the cytoplasmic strands which precede the cell plate, predicted its alignment and also avoided 3-way junctions in unwounded tissues. Recently, F-actin and microtubules have been detected in these pre-mitotic, transvacuolar strands. The question considered here is why those cytoskeletal elements avoid aligning with the vertex where a neighbouring cross wall has already joined the mother wall. An hypothesis is discussed in which tensile strands – against a background of cortical re-organization during pre-mitosis – tend to seek the minimal path between nucleus and cortex. In this way, it is suggested that unstable strands are gradually drawn into a transvacuolar baffle (the phragmosome) within which cell division occurs. Vertices are avoided by the strands because they constitute unfavoured longer paths. The demonstrable tendency of tensile strands to contact mother walls perpendicularly would seem to account for Hofmeister's and Sachs' rules involving right-angled junctions. As others have discussed, such right-angled junctions give way to co-equal 120° angles between the three walls during subsequent cell growth. It is this asynchrony of cell division – where attachment of a cell plate causes the neighbouring wall to buckle – that forms a vertex to be avoided by subsequent pre-mitotic strands in that neighbouring cell. In this way, successive division planes would not co-align. It is therefore suggested that the exceptional formation of 4-way junctions in wounded tissue results from the fact that adjacent cells divide simultaneously; the lack of pre-buckling of a common wall under these circumstances means that there is no vertex to be avoided by the minimal path mechanism.

Key words: plant cells, packing patterns, cytoskeleton, geometry.

The form of an object is a 'diagram of forces', D'Arcy Thompson (1942)

Honeycombs, compressed spheres (such as lead shot and pea seed), solid and liquid foams and plant cells in a tissue show fascinating, attractive and rather similar packing patterns. Some – like the honeycomb – appear to be highly regular, composed of identical cells. Others may be less well ordered, composed of units of different size and shape. But the ways in which cells (in the wider sense) can pack is limited and the limitations have been distilled over the years, into various rules suggesting a basic conformity. The geometrical constraints on filling space apply as much to plant cells as to inanimate objects. However, although patterns may be homologous, and reflect the same underlying laws, the ways in which they are achieved may be quite different. Plant cells are more than just compressible bodies and it is their ability to regenerate packing patterns by the process of cell division that is of interest here.

Up until about 1950, there was great interest in describing cellular geometry by scientists such as, D'Arcy Thompson, 1942; Lewis, 1923–1943; Matzke, 1939, 1945; Marvin, 1939. Most of this work dealt with

comparing real cellular polyhedra with mathematically ideal figures, and with soap bubbles which readily exemplified principles of surface tension in achieving stability. In a sense, the living, biological dimension was missing from such descriptions, since more emphasis was placed on the shape achieved than on the act of placing a cell plate across a mother cell. Nineteenth century continental scientists did attempt to crystallize their observations on wall placement, into rules. As we shall see, such rules are not always adhered to, but the fact that they are at least good approximations suggests the action of a common underlying cellular mechanism. Because daughter cells conform to the restrictions of packing space, the mechanism for dividing the cells must be sensitive to, and somehow read, those restrictions. The empirical rules provide no clue to the cytoplasmic mechanism of establishing the cell plate but the important and central work of Sinnott and Bloch in the 1940s (1940, 1941a,b) did show that the alignment of the plate could be predicted by the orientation of pre-mitotic cytoplasmic strands. The question is therefore, how is the behaviour of cytoplasmic strands compatible with the laws of solid geometry that lead to efficient cell packing?

A reasonable description can now be proposed for how actin filaments and microtubules, (particularly those in transvacuolar cytoplasmic strands), reorganize and predict the plane of cell division. The aim of this essay is to set these recent advances against the geometrical pre-occupations of the first half of this century.

The efficient packaging of space: polygons into polyhedra

The sphere represents the most efficient way (surface area/volume ratio) of enclosing space but a collection of spheres is not, because of the gaps between. But solid bodies can be compressed together to eliminate the interstices, and bubbles aggregate. As they do, their circular cross-sections are deformed to make polygons. The solid bodies thus constructed pack together but what forms do these polyhedra take and which represents the most efficient packaging of space?

By joining regular polygons at their edges, without distorting them, five regular convex polyhedra can be produced. Four triangles combine to form a tetrahedron, eight triangles form the octahedron, twenty triangles can be joined to form the icosahedron. Respectively, these regular polyhedra have 3-way, 4-way and 5-way corners. Six squares can be joined to form the cube (with three-way corners) and twelve regular pentagons can be combined to form a dodecahedron with 3-way corners. Space cannot be enclosed by combining only 6- or 7- or 8-sided polygons and thus there are only 5 simple polyhedra. As long as the polygons, and the angles they form, are kept regular, there can be no more than 5. These regular solids were known to the Greeks and are referred to as the Platonic bodies. Joining regular plane figures of more than one type – but keeping the corners or vertices the same – yields semi-regular polyhedra. Excluding prisms, only

14 such Archimedean bodies can be made (Stevens, 1976). Of these, the tetrakaidecahedron is of the greatest interest. It has eight hexagonal and six square sides and is perhaps most easily conceived as a cube with extra facets produced by shaving the corners (a truncated octahedron).

In discussing the theory of polyhedra, Euler's Law (see Thompson, 1942; Gibson and Ashby, 1988) has a central importance. This states that for every polyhedron, the sum of the faces and corners outnumbers the edges by two. Various corollaries flow from this: no polyhedron can exist without a certain number of triangles, squares or pentagons in its composition; and no polyhedron can be constructed entirely of hexagons. Furthermore, it follows from Euler's Law that an irregular 3-connected aggregation of polyhedra has an average of six sides per face. A 5-sided face can only be introduced if a 7-sided face is created, for balance.

As D'Arcy Thompson (1942) states, the broad general principle to be learned is that we cannot group as we please any number and sorts of polygons into a polyhedron, but that the number and kind of facets in the latter is strictly limited to a narrow range of possibilities.

## Soap films illustrate the juncture angles of space-filling bodies

The apparent similarity between soap bubbles in a foam and plant tissue has long been noted, and gives rise to some interesting demonstrations.

By sandwiching three drawing pins (thumb tacks) between two plates of glass (Fig. 1), soap films are observed to form between the pins when the whole apparatus is removed from a soap solution (see Stevens, 1976). The films meet at co-equal angles of 120° (Fig. 1A). Surface tension drives the films to seek the minimal path and V-shaped and Δ-shaped figures do not form since they would use significantly more material.

A fourth thumb tack can be added, defining the vertices of a square (Fig. 1B). When removed from the soap solution, the film does not enclose three sides of
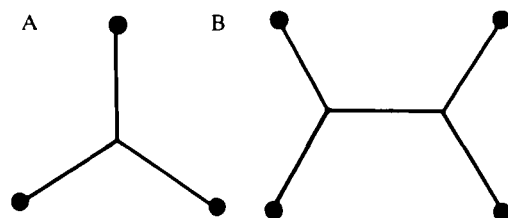
Fig. 1. Soap films formed by thumb tacks sandwiched between two glass plates then dipped into a soap solution. In A, the films do not form a triangle around the boundary but meet in the 3-way junctions of 120°; this uses less material. Even when the shape of the triangle is altered by moving the pins, the 3-way junction remains the same. The second illustration (B) demonstrates that soap films naturally form double 3-way junctions within a 4-pin arrangement. Again, this uses less material than any path around the edges (see Stevens, 1976).
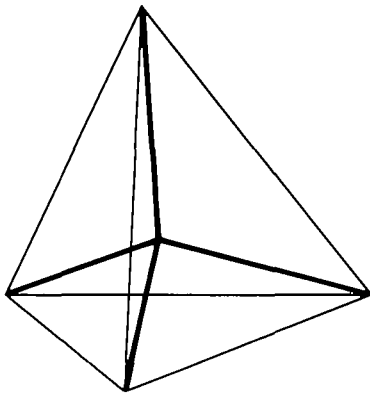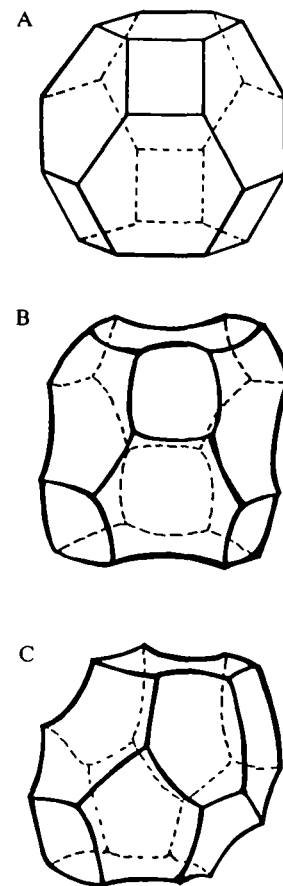
Fig. 2. The Belgian physicist Plateau (1801–1883) demonstrated how soap films spanned the contour of various wire cages, forming a minimal surface of least area. The above illustrates the arrangement formed within a tetrahedron. This shows that only 3 films meet at an edge, and that only 4 fluid edges meet at a point – all at co-equal angles. As D'Arcy Thompson (1942) noted, this symmetry applies for any close-packed tetrahedral aggregate of co-equal spheres: a froth of soap suds or a parenchyma of cells.

Fig. 3. (A) Lord Kelvin demonstrated that the semi-regular polyhedron – the tetrakaidecahedron – most efficiently divided space with minimal partitional area, *ie* it encloses a given volume with minimal surface area and close-packs to fill space. The figure has three pairs of equal and opposite quadrilateral faces, and four pairs of equal and opposite hexagonal faces. This figure – in which all the facets are plane – is the ortho-tetrakaidecahedron. However, this is not a minimal figure. (B) shows such a minimal 14-hedron in which the quadrilaterals have curved edges and the hexagons have slightly curved surfaces. This curvature meets the stability requirements demonstrated by froths of bubbles, such that it forms the tetrahedral angle of 109° 28' upon packing. Although these figures represent mathematical solutions, they do not embody the sidedness displayed by real plant tissues. (C) shows Williams' (1968) β-tetrakaidecahedron produced by topologically transforming the Kelvin body. This has a predominance of pentagonal faces.

the square, nor does it form an X. The X – if formed – would use 6 % less material than needed to enclose the square on 3 sides. Instead, the film unerringly forms a double, 3-way junction (see Fig. 1B) which is more economical still, in using 9 % less material. Formation of 3-way junctions by elements capable of exerting equal tension is a crucial process in biological pattern formation, as we shall see later.

This sandwich system of films is essentially two-dimensional, demonstrating that three films meet a point at equal angles of 120°. Plateau's beautiful demonstration (see D'Arcy Thompson, 1942) shows what happens when films meet in three dimensions (Fig. 2). He constructed a tetrahedral wire cage and dipped it into soap solution. When withdrawn, six films could be seen to meet in threes, at four edges. This demonstrates the principles of minimal area that only three films meet at an edge, and no more than four edges meet at a point. The four edges meet at co-equal angles which, in this case, are 109°28' 16". This is the tetrahedral angle which runs throughout the simple homogeneous partitioning of three-dimensional space.

Bubbles, unencumbered by frames, are free to form foams; these coarsen with time, demonstrating the constant readjustment of fluid walls as surface tension constantly strives to compartmentalize maximum space with minimal surface area. Although we can talk about limits that define the numbers of edges and the size of angles, such foams are heterogeneous. Contemplating the difficulty, in nature, of making a regular foam, Lord Kelvin (Sir W. Thomson, 1887; 1894) proposed the tetrakaidecahedron to be the ideal volume-containing form with space packing properties (Fig. 3A). Of the space-filling polyhedra with plane sides of a *similar* shape, the rhombic dodecahedron possesses the minimal surface area/volume ratio but this is improved

upon by the semi-regular tetrakaidecahedron. However, no *planar* polyhedron that packs to fill space also satisfies the stability requirement that juncture angles should be 109°28'. From observing other experiments of Plateau, showing that congruent soap films gently curve, Lord Kelvin proposed the minimal tetrakaidecahedron (Fig. 3B). By distorting this 14-hedron so that it has doubly curved hexagonal faces, and quadrilateral faces with bowed edges, the so-called Kelvin body can be made to satisfy the requirements of 109°28' juncture angles.

But plant cells are not ideal figures

Of the regular and semi-regular polyhedra, the dodeca-hedron and the tetrakaidecahedron are the most efficient in packing space. However, there has been a long history in attempting to define whether any such ideal figure is actually the one that nature uses to compartmentalize cellular space.

Stephen Hales, in 1727, compressed pea seed in a pot to form pretty regular dodecahedrons. This, of the regular polyhedra with identical faces, has the least surface area/volume ratio, but Matzke (1939, 1950) could not confirm the observations of this frequently cited classical experiment. Matzke suggested that Hales might have been influenced by the stacking of cannon balls, where 12 surround a central one. Compressing such an aggregation (without slippage) would produce a rhombic dodecahedron, providing that the regular units were first stacked in that configuration and not, as presumably was the case for Hales' peas, poured into the container. Marvin (1939) and Matzke (1939) repeated this experiment with lead shot and found that regular units, when stacked like cannon balls, did compress to form dodecahedra, but that poured shot produced irregular 14-sided bodies. Mixing large and small shot produced bodies with more or less than 14 sides, the average being less than 14. This agreed with Lewis' (1923) detailed observations of undifferentiated plant tissue, showing an average of 14 faces.

In a similar investigative vein, Matzke (1945) re-examined bubbles in a foam and found an average of 13.7 contacts. Just as he could not find a single cell with the 6–0–8 formula (numbers of 4-, 5- and 6-sided polygons respectively) required by the Kelvin body, neither did he find a single central bubble amongst 600 to have the ideal configuration. Indeed, the commonest was 1–10–2, revealing a predominance of pentagonal faces. As Stevens (1976) discusses, polygons meeting at the tetrahedral juncture angle of 109°28' 16" must have 5.1 edges, and in order to make a polyhedron, 13.394 of those polygons must join at 22.789 corners. These are the geometrical constraints necessary to make continuous three-dimensional networks. Matzke's direct observations established that the theoretical ideals are approached in averages – an average of 5–6 edges per face, 13–14 faces and 22–23 corners in a froth of bubbles. Clearly, bubbles cannot be stacked, bubbles are of different sizes, and unlike pre-arranged cannon balls, bubble walls slip and adjust as the foam degrades. Similarly, plant cells are produced asynchronously, cells are not of equal size, the network expands and such growth is often polar. For plant cells, therefore, the *history* of divisions is an important aspect of shape since the majority of cell facets are inherited by daughters, from the mother cell. Plant walls too, are not produced as a compression of spheres but by the deposition of an initially non-rigid cell plate.

Searching for minimal tetrakaidecahedra amongst leaf parenchymal cells, in order to record the curvature of the walls, Macior and Matzke (1951) had great difficulty in identifying minimal figures. Again, although the average number of cell faces tended

towards 14, the authors illustrate great variation in achieving the average. And again, the occurrence of 5-sided faces was stressed. Williams (1968, 1979) collected these examples, together with those from uniform bubbles, mixed bubbles and β-brass grains to illustrate the point that 5-edged faces occur most frequently. This detracts from Kelvin's tetrakaideca-hedron as a paradigm but Williams did show that this figure could be topologically transformed to another 14-sided figure – the β-tetrakaidecahedron – with space packing properties (Fig. 3C). The β-figure has two quadrilateral, eight pentagonal and four hexagonal faces. This has an average of 5.143 sides per face. This accommodates the tendency towards 5-sidedness observed in the faces of naturally packed polyhedra although it requires about 4 % more surface than the Kelvin body to enclose the same volume.

The message for plant material is that there is no one way of enclosing space; cells may tend to have 14 sides but they vary in their number of edges, five edges being preferred to six. There are several reasons why vegetable polyhedra are variable but the way in which they divide is likely to be an important aspect of this. In the next section, the impact that division has on the patterning of plant cells is examined.

Some feature of cell division is concordant with cell packing principles

Lewis (1926) studied the anticlinal division of cells in the epidermal mosaic of cucumber epidermis. Epidermal cells are different from those of internal tissue, for the absence of external neighbours replaces four facets with a free surface, giving an average of 11 instead of 14 sides. Lewis considered the two-dimensional epidermal mosaic as being composed, basically, of hexagons. Although it is clear that the number of cell sides and cell sizes is variable, Lewis was engaged in explaining how division tended to restore the hexagonal average. In epidermis, periclinal divisions are generally suppressed, inhibiting stratification, and so anticlinal (radial) divisions are visible – like hedges across a patchwork of fields – as they divide cells in a mosaic. When a hexagonal polygon divides (Fig. 4), avoiding forming 4-rayed intersections, it forms two pentagons. Two adjacent cells are facetted in this process – acquire an extra face – and become heptagons. This maintains the average of six sides.

An octagon sometimes replaces two heptagons and as division proceeds, the active division of cells and the passive acquisition of facets by neighbours tends to restore the primary hexagonal form.
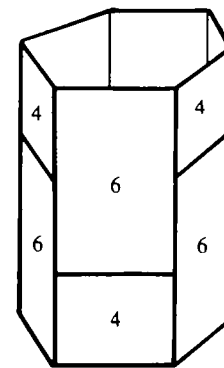
This relates to division of polygons within a two-dimensional mosaic but Lewis (1928) does, parenthetically, extend the case to solid bodies in a tissue. Division of a 14-hedron adds 14 surfaces to the assemblage of polyhedra. Each half of the divided 14-hedron has 11 surfaces, and 6 adjacent polyhedra each receive one added surface; 22+6−14=14. A related observation, known as the Aboav/Weaire Law (see Gibson and Ashby, 1988), and derived from soap bubble honeycombs, is that cells with more sides than
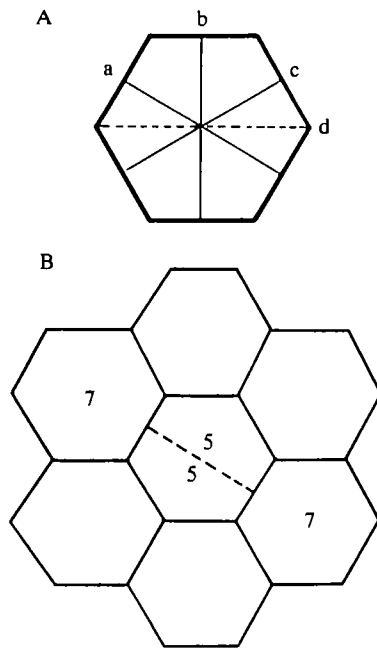
Fig. 4. From a study of cucumber epidermis (A), Lewis (1926) concluded that a hexagonal cell was just as likely to divide in planes a, b or c but not in plane d, which would result in a 4-way junction. When such a cell divided (B) it produced two daughters with pentagonal outer faces. Attachment of the cell plate to two neighbouring cells divided the attached walls, giving each an extra facet. One division therefore produced two pentagonal and two heptagonal cells, maintaining the hexagonal average.

Fig. 5. Lewis' (1936) prismatic tetrakaidecahedron. The ⅓, ⅔ alternation of lateral cross walls shows maximal avoidance of 4-way junctions (which would form an encircling belt) and maintains the original cell proportions.

average have neighbours with fewer sides than average. Gibson and Ashby use Euler's Law and the Aboav/ Weaire Law in characterizing cellular solids such as foams, but they also make use of a rule ascribed to Lewis (1943): that the area of a cell varies linearly with the number of edges. These latter two rules help describe the effects of coarsening in foams but biological cells can mitigate the effects of coarsening by adding new walls, *ie* biological cells can regenerate. The message to be extracted from this is that the passive acquisition of facets, by cells surrounding a dividing cell, increases the size of neighbours. And, according to Lewis (1943), the epidermal cells studied were more likely to divide perpendicular to the free surface, the more sides they possessed; the net result of dividing large cells was to reduce their number of sides towards the average.

Lewis (1936, 1943) also developed ideas on how elongating cells in a shaft divide in a manner that retains particular cell proportions and relationships with neighbours. He considered a 14-hedral elongate cell as a hexagonal prism (Fig. 5). The edges on the lateral faces alternate 1/3, 2/3 etc. around the cell thus making alternating pairs of rectangular hexagons and quadrilaterals. The important feature of this particular configuration is that it demonstrates a uniform and maximum avoidance of 4-rayed vertices within the figure itself *ie* corners are only made as the meeting of three edges. Plotting the level at which transverse walls are actually

attached in 100 cells, Lewis found that this 1/3, 2/3 alternation on opposite sides of a cell did exist and it is claimed that this 1/3, 2/3 succession is the only pattern in which bisection maintains hexagonally-sectioned cells in their original proportions. And since division affects the sidedness of neighbours, some rhythm would appear to be required in the timing of divisions if the original proportions are to be maintained during growth.

As we have seen, such a prismatic tetrakaideca-hedron of the 6-0-8 formula (a distorted version of the Kelvin body) does not seem to be favoured by real cells. Nevertheless, the principle demonstrated by this illustration seems to hold (and was later taken up by Sinnott and Bloch, 1941*a*), namely, that formation of 14-hedra and their maintenance during subsequent divisions, requires the maximum avoidance of 4-rayed intersections. The latter, if produced, would lead to a checkerboard of cells in ranks and files, instead of the bonded brick appearance of cells packed (in section) with 3-way junctions.

Framing the rules for division plane alignment

Three laws or rules have been drawn from direct observation of the way that cell plates meet existing walls. The first belongs to Hofmeister (1863) who stated that the partition wall always stands perpendicular to what was previously the principal direction of cell growth, *ie* generally perpendicular to the long axis of the cell. Sachs (1878) proposed that the cell tends to divide equally, and each new plane of division tends to be perpendicular to the previous plane. Errera (1888) further proposed that the cell plate is the minimal area for halving the cell's volume.
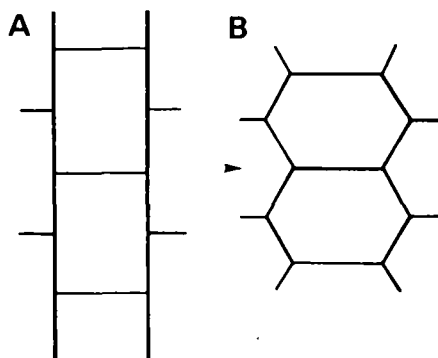
In a series of papers, Korn re-examined the utility of these rules – a valuable exercise which placed the mechanics of cell plate alignment in centre stage rather than seeing cell shape as the expression of packing forces or mathematical inevitabilities. In 1974, Korn modelled cell growth by cutting three-dimensional blocks according to two rules only: Hofmeister's rule that cell division is perpendicular to the long axis, and a rule ascribed to Sinnott and Bloch (1941*a*), that cells

divide to form only 3-rayed vertices even if it requires unequal division. Using these rules, blocks could be cut repeatedly to yield cells whose sides closely correspond to those observed in real tissue. Since the application of these two rules was sufficient to yield data comparable to those derived from real tissues, Korn concluded that proposing further shaping forces was unnecessary. Although he dismissed 'mutual pressure' as a shaping force in plant tissue, Korn did muse upon why the geometry of cells formed by progressive halving, and those of soap bubbles contoured by mutual pressure forces and slippage of films, should both average 14 sides.

Perpendicular attachment of cell plates and the generation of 120° angles

A common strand running through the laws of Hofmeister, Sachs and Errera is the perpendicularity of the cell plate to existing walls. How does this give rise to the 120° edge angles approximated when three mature walls meet? D'Arcy Thompson (1942) explained this on the basis of relative tensions (Fig. 6). A new partition wall meets an old wall at 90° because its tension is small compared to what theirs has become. It is not the meeting of three co-equal partners that occurs when bubble walls meet. However, with subsequent growth, the tension of the walls becomes equal, realigning the juncture to co-equal angles of 120°.

Even where expansion is asymmetric, such that the cells markedly elongate to form rectangular hexagons, D'Arcy Thompson illustrates local curvature around the triple junction in an attempt to meet – if only microscopically – the 120° angles. The 120° and 90° angles are found in a wide variety of materials. D'Arcy Thompson (1942) notes that fine veins on a dragonfly's wing meet in threes at 120°, whereas such fine veins meet the stronger ribs at 90°. Similarly, wax walls in a honeycomb meet each other at 120°, but meet the wooden walls of the hive at 90°. Soap films meet in
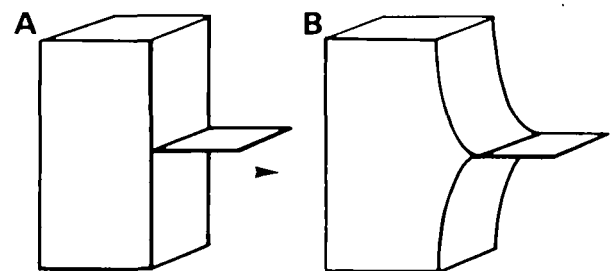
threes at 120°, but contact the containing vessel at 90°. These angles are also the ones in which materials tend to crack. Elastic materials such as rock and mud form cracking patterns based on sudden production of 3-way joints (Stevens, 1976). By contrast, inelastic material, such as the glaze on a piece of pottery, cracks to relieve the stress first in one direction and then at right angles to relieve the perpendicular stress. This latter, essentially orthogonal pattern, is produced by sequential formation of new cracks perpendicular to older ones. This returns us to D'Arcy Thompson's conclusion for biological materials, that Sachs' rule is limited to cases where one wall grows stiff or solid before another impinges upon it. The difference in strength between the mother wall and the cell plate is suggested to yield perpendicular junctions, which, when the new plate gains strength (or can exert equal tension), allows the three facets to re-arrange, forming co-equal angles of 120°.

Korn (1980) offers a variant of this explanation (Fig. 7). He finds that the cell plate does not expand for one generation. Certainly, the new cell plate is callosic such that its initial lack of expansion might be attributed to its slow conversion to a regular cellulosic wall. A cell becomes marked by the external attachment of a cell plate deposited across a dividing neighbour. As the cell expands, its wall in contact with the cell plate becomes facetted, that is, the non-expanding plate causes the older wall to hinge at that junction. Perpendicular junctions thereby change, and planar surfaces become curved as the cell plate acts as a brake on its faster-expanding neighbour.

According to Korn (1980) therefore, this change in juncture angles occurs because of differential expansion while the plate is immature, not when the plate becomes strong enough to exert equal tension.

Whatever the reason, lack of expansion or lack of toughness, the curvature caused by the recession of the cell plate away from the centre of the expanding neighbour provides one explanation for the curvature of leaf parenchymal cells illustrated by Macior and Matzke (1951), and required of minimal, as opposed to orthic, polyhedra.

Fig. 6. The 120° angles of Fig. 1 depend upon the meeting of 3 films of equal tension. D'Arcy Thompson (1942) observed (A) that the new partition walls generally meet mother walls perpendicularly because the tension in the former is smaller. However, as the new wall strengthens (B) it is able to exert equal tension and the network adjust to form co-equal angles of 120°.

Fig. 7. Korn (1980) measured new cell walls and concluded that they did not expand for one generation. Meanwhile, other cell walls continued to expand (A–B), causing them to curve away from the non-expanding attached wall. This 'buckling' depends upon asynchrony of division between neighbours. It is suggested to generate the curvature required for minimal space-packing polyhedra.

Interim summary

Although the minimal 14-hedron represents the ideal solution for cellular packing, plant cells evidently fall short of the ideal. Ideal figures can be produced by compressing regular stacked spheres but lack of regularity and asynchronous division frustrate a mathematical best fit.

Cell plates tend to attach to mother walls perpendicularly, but this subsequently changes, if only locally, to form co-equal angles of 120°.

A key feature of actual cell packing is the formation of 3-way junctions. Avoidance of attaching a cell plate to an existing vertex, where three facets already meet, is therefore crucial in generating and maintaining cellular geometry.

Avoidance of 4-way junctions

Minimally-packed figures have curved edges that meet the angular stability conditions in liquid films. Observing curved edges and facets in plant tissue, Macior and Matzke (1951) mooted that cell walls must be laid down in a liquid or semi-liquid state. The argument against a liquid-film-like explanation for division plane alignment is that the structure that precedes and predicts the cell plate is not a continuous structure and should not therefore be confused with analogues such as bubble walls (although, as we shall see, similar principles do apply). Two structures are known to foretell the position of the cell plate: the phragmosome and the preprophase band. Only a brief description of these structures can be given here; their history and cytoskeletal composition are fully referenced elsewhere (Lloyd, 1991).

*The transvacuolar phragmosome predicts the division plane*

Sinnott and Bloch (1940) described the phragmosome as a pre-mitotic fusion of transvacuolar strands that supported the central nucleus across the vacuole and predicted the eventual division plane. Importantly, they emphasized that cell plate alignment could be predicted prior to mitosis. Furthermore, they noted that the phragmosome avoided aligning with transverse walls in adjacent cell files. These authors made use of wounded tissue since nucleus-anchoring strands could be seen clearly in large vacuolated cells where the nucleus, in preparation for mitosis, was induced to migrate into the centre of the cell. In wounded tissue, however, groups of cells are induced to divide synchronously in contrast to the asynchronous mitosis in normal tissue. A corollary of the synchronous divisions is that cell plates in this case line up from cell to cell, parallel to the cut made in the tissue. In normal tissue this is avoided in favour of staggered 3-way junctions. Sinnott and Bloch (1941a) offered the tentative explanation that if the phragmosome tended to carry on, at its point of attachment to the wall, an active exchange of nutrients between its own cell and the next, then the junction with the adjacent transverse wall, would form a 'blind spot' to be avoided. Korn (1980) later proposed that

diffusion of some substance, *ie* from the vertex, inhibited placement of the cell plate there.

The preprophase band contains actin and MTs that radiate from the central nucleus

The preprophase band (PPB) is a cortical ring of MTs that anticipates where the cell plate will fuse with the maternal wall (Pickett-Heaps and Northcote, 1966). It does this for meristematic cells but, importantly, also predicts the division plane in vacuolated cells that are induced, by wounding, to divide outside the normal developmental programme. A puzzling feature of the PPB is that it disappears well before the cytokinetic apparatus deposits the cell plate along the predetermined division plane. However, recent studies using rhodamine–phallotoxins to label actin filaments in dividing suspension cells have indicated that these filaments remain in the division plane throughout; they anchor the central nucleus and guide the outgrowing phragmoplast to the cortical site once marked by the PPB (Traas *et al.* 1987; Kakimoto and Shibaoka, 1987; Lloyd and Traas, 1988). One model proposed from observations on carrot suspension cells (Lloyd, 1989) is that the actin filaments, radiating from the nucleus by means of transvacuolar strands associate with MTs at the cortex. As the cortical MTs bunch up during PPB formation, the radial strands re-align so that they occupy a plane defined at its perimeter by a PPB of MTs and actin filaments. The cortical cytoskeleton depolymerizes by metaphase, but the radial F-actin component of the transvacuolar phragmosome remains to mark the division plane until cytokinesis. This model is now revised to accommodate the more recent observations that MTs also radiate from the pre-mitotic nucleus (Flanders *et al.* 1990; Katsuta *et al.* 1990). This indicates that PPBs may be constructed, at least in part, from nascent MTs. However, these radiating MTs, within the transvacuolar strands, share the same fate as the cortical MTs of the PPB – both classes disappear by metaphase, leaving the radial actin strands to memorize the division plane.

As discussed, the characteristic texture of unwounded plant tissue is heavily dependent upon the avoidance of forming intercrossing septa, and now that actin filaments and microtubules radiating from the nucleus are established as being part of the predictive system, the question reduces to how these cytoskeletal elements avoid lining up with existing 3-way junctions. In *Datura stramonium* stem epidermal cells, the pre-mitotic cytoskeletal strands, which gradually re-align to form the phragmosome, certainly do avoid contacting vertices. The pattern of strand alignment in different cells provides additional clues. Stem epidermal cells form two kinds of pattern, containing cells of different shape. In one, elongated cells occur in files with transverse cross walls forming a pattern like a path of bonded bricks. In the other, isodiametric cells form an irregular patchwork with cross walls at various angles to the stem axis (Flanders *et al.* 1989). Both display avoidance of 4-way junctions but in one, directional cell expansion is accompanied by transverse partitions,
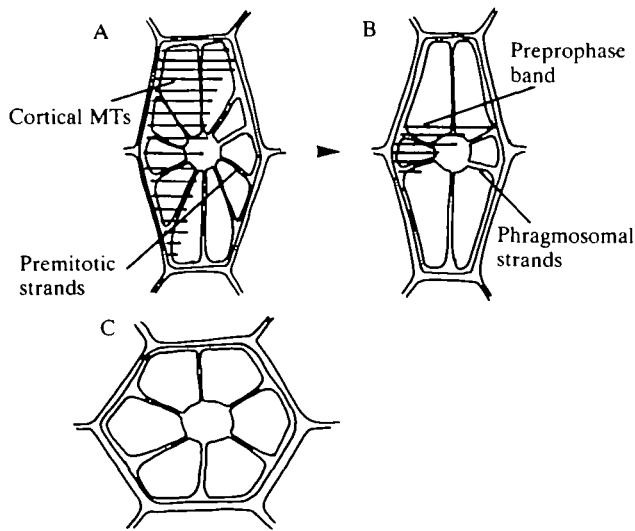
Fig. 8. Summarizing the observations of Flanders *et al.* (1990). (A) During pre-mitosis, the nucleus adopts a central position in elongated, vacuolated stem epidermal cells of *Datura stramonium*. The nucleus is suspended by strands containing MTs and actin filaments. As the cortical cytoskeleton re-organizes to form the preprophase band (B), the strands radiating from the nucleus re-align. Those to the end walls remain as polar strands, whilst those within the confines of the PPB establish the transvacuolar phragmosome. Phragmosomal strands avoid contacting 3-way junctions. In epidermal cells of hexagonal section (C), the strands align with the mid-edge. These strands also avoid vertices but, being equi-axed, constitute more potential division planes than in elongated cells (compare with Fig. 4).

whilst in the other cells expand isotropically with no apparent preferred plane of division.

In the case of the elongated cells, the MT-containing strands radiate in all directions from the central pre-mitotic nucleus. Interphase MTs wind transversely around such cells (Flanders *et al.* 1989) and during this pre-mitotic stage, form initially broad transverse PPBs. But as the PPB bunches up to form a tight transverse ring (Fig. 8), so the radial strands that contacted the cortex within the confines of the initially broad PPB also re-align. Eventually, most of the radial strands come to lie within the plane outlined by the PPB, leaving only a few polar strands to run perpendicularly away to end walls or distant locations on side walls (Fig. 8B).

Such elongated cells occasionally form double PPBs that straddle the plane that would – if selected – bring the new cell plate in alignment with cross walls between cells in neighbouring files. This is not easy to comprehend in terms of PPB placement *per se*, since cortical interphase MTs previously lined these facetted cell edges without showing signs of avoidance. But focusing upon the underlying radial, MT-containing strands reveals that they avoid contacting the existing vertices where neighbouring walls attach. If band placement depends upon the alignment of underlying strands (particularly if new MTs pass out from the nuclear surface, along the strands, to contribute to the

PPB), then, again, an understanding of division plane alignment may be more readily gained from understanding strand behaviour.

The isodiametric cells are intriguing because examples are readily found in which strands radiate from the central nucleus to the mid-edge of subtending walls (Fig. 8C). This appears to be a stable configuration, for strands remain in this star-like configuration instead of forming a transverse phragmosome with perhaps only two polar strands, as can occur in elongated cells. The PPBs in such cells are weak, not dense and tight; they may even be angled on the outer epidermal wall, following non-diametrical radii. This irregularity cannot be attributed to the isodiametric nature of the cells, since non-elongate orthogonal cells form tight PPBs with the majority of the transvacuolar strands in a corresponding phragmosome. It seems instead to be due to an inability of planar PPBs to form across opposite cell edges with non-parallel sides; either because the underlying radial strands do not massively re-organize into a single plane and/or because of the geometrical difficulty of constructing a band around polyhedra without a defined long axis.

Lewis (1926) figured the possible division planes of a cell which – like isodiametric *D. stramonium* epidermal cells – tended to form regular hexagons in section (Fig. 4A). He suggested that whereas planes a,b,c were equally likely, plane d was unlikely because: (1) it would give rise to unstable tetrahedral angles at either end; (2) it would contravene Errera's rule of least area; or (3) it would place the poles of the mitotic spindle in a short axis of the cell.

Modelling this situation with springs or bubbles in flexible hexagonal frames produces another answer (Flanders *et al.* 1990).

Soap films model the avoidance of 4-way junctions exhibited by transvacuolar strands

In Fig. 9, glycerol-stabilized soap bubbles are held in a frame arranged as a rectangular hexagon (A). When the central pin-joints down the long edges are pulled slightly outwards (B) to form vertices, bubble walls slide away from the vertices. When the frame is distorted further (C) into a regular hexagon, bubble walls take up the maximal avoidance configuration in which they attach to each mid-edge. Although bubble walls meet in the centre of the frame, in threes, at co-equal angles of 120°, a bubble wall tends to meet the stiffer edge of the frame, perpendicularly (as was discussed earlier for the walls of a honeycomb). The other feature of minimal area systems demonstrated here is that elements under tension adopt short paths to the perimeter, not the longer paths required to meet a vertex. In Fig. 9C, for example, it can readily be seen that the bubble walls to the mid-edge constitute radii of an in-circle (of which the edges are tangents) whereas a larger radius would be required to reach the vertices which lie on the circum-circle. Where cells divide asynchronously, the attachment of a cell plate to a neighbouring wall causes that wall to buckle or to form a facet as the wall (but not the cell plate) subsequently
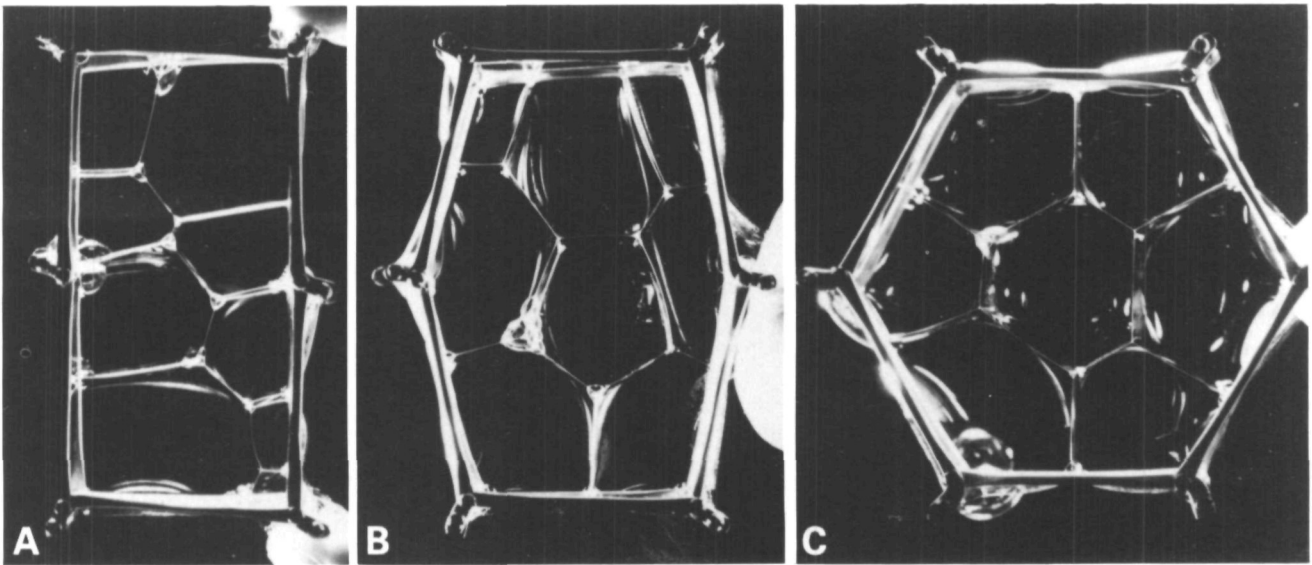
Fig. 9. Modelling the behaviour of cytoplasmic strands with bubble walls. Glycerol-stabilized bubbles are held in a flexible, prismatic hexagonal frame. In A, the frame forms a rectangular hexagon. In B, the long side walls are pulled slightly outwards to form vertices. The bubble walls now avoid the pin-joints at the vertices, just as they avoid making contact with the outer corners of the frame. As the frame is pulled into an isodiametric hexagon, bubble walls show maximum avoidance of vertices by aligning perpendicularly with the mid-edges of the frame. A similar demonstration was shown (Flanders *et al.* 1990) using springs in a frame. The *minimal path avoidance hypothesis* is based on the fact that tensile elements, which are free to move, will tend to avoid the long path to a vertex and to contact side walls perpendicularly. In asynchronously dividing tissue, the pre-buckling of a cell by a neighbouring cross wall (see Figs 6 and 7) forms a vertex to be avoided when that buckled cell later divides. Thus successive cross walls would not touch. However, this does occur in wounded tissue where cross walls are deposited simultaneously and should also occur in un-wounded tissue where lack of expansion has failed to generate the necessary buckling. (Reproduced from Flanders *et al.* 1990, by permission of the Journal of Cell Biology).

expands (Thompson, 1942; Korn, 1980). This feature of asynchronous division to form vertices, together with the tendency for elements under tension (which are free to move) to seek a minimal path, provides a mechanism for the avoidance of forming 4-way junctions. Such a mechanism, which tends to form tetrahedral angles in 3-D, is sufficient to account for the quasi-14-hedral geometry that follows from such an arrangement. Although strands that connect the nucleus to the cortex are not continuous like bubble walls, the similarity between plant tissues and foams is not fortuitous, for both depend upon minimal path mechanisms. But what evidence is there that pre-mitotic strands are under tension? They are certainly free to move and this has been observed by time lapse microscopy by Goodbody and Lloyd (1990) in wound-stimulated *Tradescantia* epidermal cells. Hahne and Hoffman (1984) used laser microsurgery to demonstrate that transvacuolar strands, holding the central nucleus in *Hibiscus* protoplasts, are under tension and cause in-pullings on the protoplast surface. We have recently confirmed (K. C. Goodbody, C. J. Venverloo and C. W. Lloyd, unpublished observations) that the pre-mitotic radial strands in epidermal cells stimulated to divide by explantation are also under tension: laser ablation of a strand between nucleus and cortex causes the nucleus to recoil towards the opposite cortex and the cut ends of the strand to retract.

To summarize, the reorganization of the cortex at

preprophase allows tensile strands, which radiate from the nucleus, to adopt minimal paths; such paths are generally perpendicular to the cortex. In elongated cells the majority of strands will seek the transverse axis, but will avoid a vertex since this is not a minimal path. In isodiametric cells, strands adopt a favourable configuration on each cell face without the massive long-edge re-distribution observed in elongated cells. This offers alternative axes for division plane alignment as testified by the irregular patchwork arrangement of such cells.

Wounding produces 4-way junctions

Sinnott and Bloch (1941*b*) induced large, vacuolated cells to divide by wounding the tissue. They observed that the new phragmosomes formed parallel to the course of the wound and often lined up, from cell to cell, without staggering the joints. A major difference between this response (which produces 4-way junctions) and normally occurring divisions (producing 3-way junctions) is that the former divisions are synchronous whereas the latter are not. Korn's (1980) concept of cell buckling or facetation depends upon asynchrony: a new cell plate causes the neighbouring attached walls to buckle *before* the neighbouring cells themselves divide. According to the minimal path explanation (Flanders *et al.* 1990) such prior facettation produces a vertex to be avoided subsequently by pre-mitotic strands in neighbouring cells. The synchronicity of wound-induced divisions allows no such pre-buckling

of walls and division planes are free to align across files of un-facetted walls. Why a particular line, parallel to the wound, is selected is a different and much larger question. Goodbody and Lloyd (1990) recently found that prior to division, in leaf epidermal cells surrounding a slit wound, actin cables lined up across groups of the cells. Not only were the cables in the same plane but they aligned, point to point, upon the separating wall. This does suggest the existence of preferred sites for actin filament attachment, shared either side of a common wall. If this proves to be the case, such common junctions would be absent from the site where a cell plate attaches to a side wall, providing another potential explanation for the avoidance by actin strands of 3-way junctions. Alignment of strands parallel to the wound, and the encircling of a punctured leaf epidermal cell by new cell plates (Goodbody and Lloyd, 1990) does imply strain alignment. However, the drastic nature of wounding could equally involve chemical, ionic and electrical gradients. In normally growing tissue, though, such stimuli might be under subtler control. The sensitivity of individual cell division to cell geometry, under un-wounded conditions, does suggest that factors which influence field axiality (*eg* plant growth regulators such as gibberellic acid) will have an in-built effect on division planes.

Conclusion

Plant cells grow in various patterns that only approximate mathematically ideal solutions for packing space. The common feature that unites them, however, with other (minimal) space packing bodies is that only three edges meet at a point on a plane. It is this avoidance of 4-way junctions that imposes conditions of sidedness (although other features that control directional expansion affect the final shape of the figure). Strands that radiate from the central, pre-mitotic nucleus contain actin filaments and microtubules. Against a background of a re-organizing cortical cytoskeleton during pre-mitosis, these radial strands adopt minimal path configurations that can be modelled by bubble walls or springs held in hexagonal frames. Elements under tension move away from the vertex where a neighbouring cross plate previously attached and caused the wall to buckle at that joint. In cells, these elements gradually accumulate into the phragmosome (the device that predicts and constitutes the division plane) and the avoidance of vertices by elements under tension is sufficient to account for the staggering of cross walls between successively dividing cells.

Postscript on isodiametric versus elongated cells

Korn and Spalding (1973) state that although the rules of Hofmeister and Errera do produce appropriate arrangements of cells in tissues, they cannot be held as the initial conditions of spatial order within the cell. In addition, there must be some mechanism by which a cell measures itself into halves, recognizes the long axis and orientates the cell plate by that axis. The two classes of *D. stramonium* epidermal cells illustrate the point: elongated cells demonstrably divide with a heavy bias

towards transverse division whereas isodiametric cells divide in other planes. What is it that (generally) entrains elongated cells to transverse division? Again, Korn (1974) offers a different description of the phenomenon. Cells with some perpendicular adjacent walls are found in tissues expanding in one direction, whereas in cells enlarging in three directions all adjacent walls form angles greater than 90°. This has implications for MT ordering during interphase. In tracing the path of cortical MTs around elongated epidermal cells, using computer-aided microscopy, we (Flanders *et al.* 1989) found that parallel sets of transverse MTs could be traced around the cell until they circumnavigate the transverse axis. Cortical MTs can wrap around the cortex of free-growing elongated cells (such as cotton fibres, hairs and suspension cells) in helices of variable pitch. But this smooth continuous pattern depends upon parallelism between neighbouring MTs and a cellular geometry that allows aggregated sets of MTs to wrap around the cell. This transverse encircling (or en-spiralling) of a cell by MTs is believed to be the basis for cell elongation, since the co-aligned cellulose microfibrils produce a restraining jacket which encourages cell expansion at 90°. Increased interaction between adjacent MTs has been suggested to form a self-cinching device that maintains transverseness of the cortical array (Lloyd, 1984). Even in elongated cells that are facetted by neighbours, MTs can still wind continuously around the cell edges. But in isodiametric epidermal cells, such smooth patterns are not always seen. It would appear that the lack of parallelism between opposite cell faces (the non-orthogonal angles between adjacent faces) causes different sets of parallel MTs to criss-cross upon some surfaces. Criss-crossing, or some such discontinuity, is unavoidable in placing sets of parallel lines upon a continuous surface. In elongated cells with transversely wound MTs, the discontinuities can be pushed to the end walls, but such neat encircling of the cell is considerably more difficult in isotropically expanding cells with non-orthogonal edges. (Consider the ease of wrapping a ribbon around the sides of a tetrakaidecahedron in the form of a prism (Fig. 5) *versus* the difficulty of the Kelvin body (Fig. 3)). Whether PPBs are made by a bunching-up of interphase MTs or as a new structure, the ability to concentrate cortical MTs into a ring, and to bring radial phragmosomal strands into the plane so defined, is more easily achieved in cells that had been elongating in one direction. Unless other factors override, there can be several potential division planes in isodiametric cells. The switch from serial transverse divisions to other division planes might therefore be expected to arise from an enhanced rate of division (which would tend to produce equi-axial cells), or from a failure to elongate.

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