Molecular Crystals and Liquid Crystals

ISSN: 1542-1406 (Print) 1563-5287 (Online) Journal homepage: http://www.tandfonline.com/loi/gmcl20

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To cite this article: John Lydon (2016) Microtubules – nature's universal mesogens, Molecular Crystals and Liquid Crystals, 632:1, 29-48

To link to this article: http://dx.doi.org/10.1080/15421406.2016.1185569

MOLECULAR CRYSTALS AND LIQUID CRYSTALS
2016, VOL. 632, 29–48
http://dx.doi.org/10.1080/15421406.2016.1185569

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Microtubules – nature's universal mesogens

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ABSTRACT

The roles of microtubules in living systems are outlined - and the ways in which they can both create and exploit liquid crystalline structures are stressed. It is argued that ordering in many biological systems arises from the director fields of liquid crystalline regions within the cells.

Two biological processes are discussed - cell division by mitosis and the alignment of cellulose microfibrils in plant cell walls. In both cases microtubules are the key components and the events take place within what, as far as the cell is concerned, are global director fields. A link between these two widely disparate processes is identified, in terms of the mechanism of cell division in some dinoflagellates. A possible solution is offered to the long-standing problem of how plant cells are able to dictate the complex architecture of secondary walls through the plasma membrane.

KEYWORDS

Liquid crystals; Microtubules; Mitosis; Spindle; Plant cell wall

1. Preface

In higher animals, the major nerve axons are wrapped in electrically-insulating layers of lipid, known as myelin. When these axons are dissected and placed in water, a remarkable swelling process occurs as large quantities of water molecules are drawn into the material between the lipid bilayers and an expanded liquid crystalline phase is formed. The structure swells rapidly. In a matter of minutes, writhing 'myelin figures' extend. When it was found that artificial myelin-like figures could be produced from soap/water mixtures, the early investigators concluded that perhaps liquid crystals had 'life' of their own [1].

This paper is more or less the converse argument. It suggests that many of the complex self-ordering processes in living systems are explicable in terms of the known properties of liquid crystalline systems which can be demonstrated outside living systems. In particular, the properties of tubulin units are invoked. The liquid crystalline phase formed by concentrated solutions of microtubules have been extensively studied *in vitro* [2]. When commanded by the appropriate chemical messages, these small protein molecules polymerise to form microtubules and hence dictate the formation of regions of long-range nematic or cholesteric ordering. They have a remarkably wide range of roles in living systems. They can act as 'intelligent' cytoskeletal elements, helping to determine the structure and changes of structure of cells. They can direct the production of cilia, and by 'treadmilling', they can enable cells to crawl across surfaces. They provide cellular tramways along which motor enzymes travel, drawing with them the vital merchandise of the cell.

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This paper is dedicated to Professor R D Preston (1908 – 2000), pioneer of plant cell wall studies.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/gmcl.

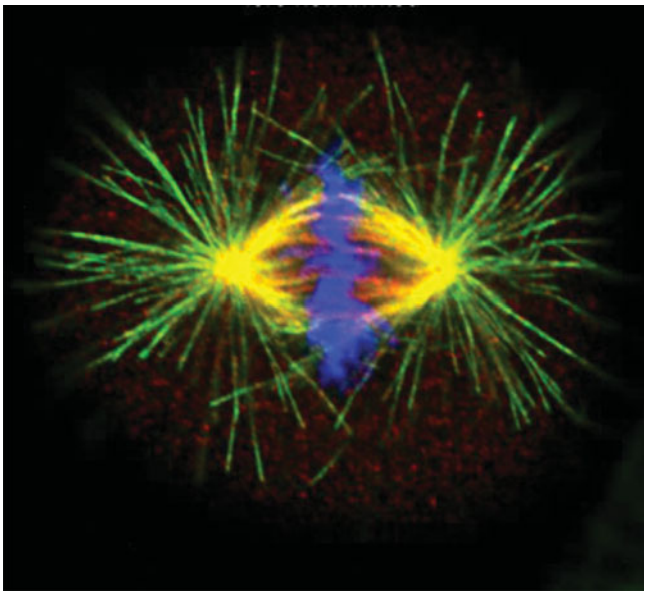

Figure 1. Modern optical microscopy techniques, using fluorescent labels, have produced some beautiful images of dividing cells. In this picture of the mitotic spindle in a human cell, the microtubules are shown in green and yellow, and the chromosomes in blue—from wikipedia 'kinetochore' commons [3].

The involvement of microtubules in two particular processes is discussed. The first is the spectacular series of events which occur during cell division - when almost the entire contents of the cell are transformed into a liquid crystalline 'spindle' (shown in Figure 1) which controls the separation and equal distribution of the genetic material between the two daughter cells. The second concerns the way in which the alignment of layers of cellulose microfibrils in the plant cell wall is directed.

2. Introduction

This introductory section places liquid crystalline phases within a biological, context - and lists some *in vitro* properties of liquid crystalline phases which will be invoked later. It argues that, in general, lyotropic liquid crystalline phases occur within an energy range appropriate for living systems (Figures 2 and 3). In terms of the energy changes for dynamic physical changes in molecular organisation within biosystems, it is only the liquid crystal phase that can provide a suitable environment. For typical biomolecular weights, isotropic liquid phases would only exist at high temperatures, well above those capable of supporting life. Molecular organisation in a liquid crystal phase can result in a match between the thermal energy available (kT) and the energy necessary to effect changes in translation, diffusion, or rotational motion or molecular conformation. Furthermore, the detail observed in spontaneously-formed director field patterns occurs in a size range corresponding to subcellular structures such as the spindle of the dividing cell (Figures 4 and 5) [4]. The characteristic Zeppelin shape of nematic tactoids is shown in Figure 6 [5,6 and 7] and textures of nematic and cholesteric mesophases confined within a cylindrical tube are shown are shown in Figures 7 and 8 and [7–10].

In this paper it is argued that some structure-forming processes in the living cell are accomplished *via* liquid crystalline phases and that the director field of the mesophase is the initial

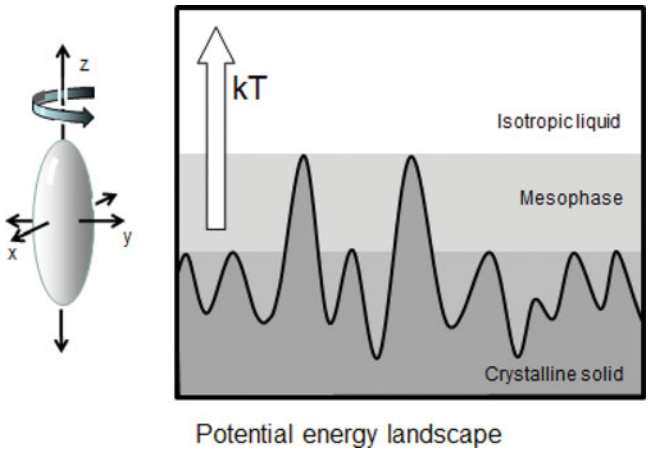

Figure 2a. This stylised sketch depicts the energy 'landscape' for a compound which forms a crystalline solid, a mesophase (say a nematic state) and an isotropic liquid. The vertical axis is the thermal energy of the molecules. The various degrees of freedom (rotational, translational or internal coordinates) are plotted on the horizontal axis. In the crystalline solid the thermal motion is not able to overcome the energy barriers restricting translation and rotation of the molecules – and they are only able to rattle around in their potential energy wells. In the mesophase, the increased thermal motion is able to overcome many of these barriers, but a few remain, restricting the molecules to a more-or-less parallel alignment, with rotation allowed only about the long axis. At the transition to the isotropic liquid, all of the energy barriers are overcome and both translational and rotational motion of the molecules are now unrestricted.

template. A key factor in this argument is the widespread occurrence of liquid crystalline phases in both biological material extracted from living systems, and synthetic analogues. This is true for the four major categories of biological compounds: proteins, nucleic acids, polysaccharides and lipids. For the cases of mitosis and plant cell wall synthesis it is the *in vitro* liquid crystalline phase of extracted microtubules which is relevant [2].

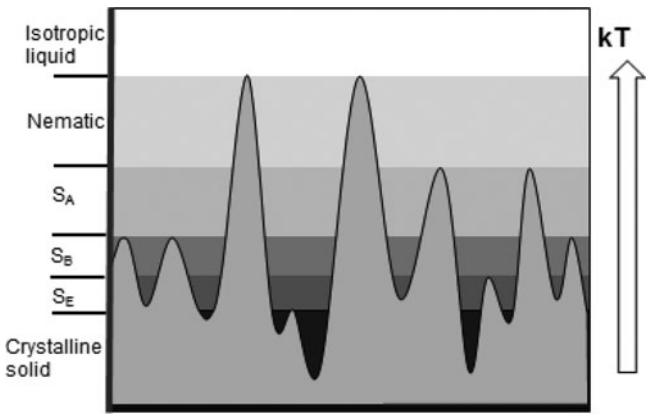

Figure 2b. This stylised sketch shows the energy 'landscape' for a mesogen, which on heating, forms a sequence of smectic phases and a nematic phase before clearing to the isotropic liquid. The figure shows the stepwise loss of order (and gain in degrees of freedom) as the temperature is raised. The various valleys in the 'landscape' represent the different geometrical arrangements of the mesogen molecules. The peaks dividing them indicate the barriers to the modes of translational and rotational motion. Note that synergistic relationships between different modes of motion (i.e. degrees of freedom) lead to numbers of peaks clustering at the same level.

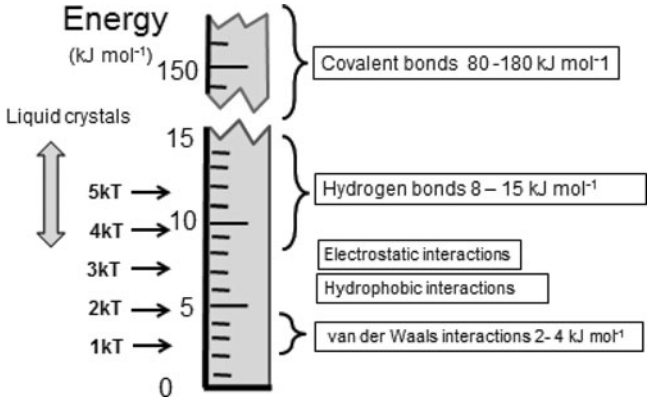

Figure 3. A comparative energy scale. The vertical rule is graduated in units of thermal energy. Typical energy ranges for van der Walls interactions and covalent bonds are shown. It is difficult to assign meaningful values for hydrophobic and electrostatic interactions (of the dipole/dipole type) - but in biological materials they are generally taken to be in the 5–10 kJ^{-1} range. The kT values listed on the left are for a temperature of 300 K. The energy range for liquid crystals indicated on the left refers to the thermal energy required for the creation of the biologically relevant state (i.e. its lower temperature threshold).

3. Microtubules

Microtubules are present in the cells of all multicellular organisms [11].They are hollow cylinders formed by polymeric strands of the globular protein, tubulin. The outer diameter is 24 nm and the inner diameter is about 12 nm. They are formed by the polymerization of dimers of two homologous protein molecules, α and β tubulin They occur throughout the cytoplasm of the cell and are major components of the cell's cytoskeleton. Microtubules are dynamic rather than static structures, in the sense that they are continually growing and then shrinking - enabling a rapid reorganization of the cytoskeleton when it is required.

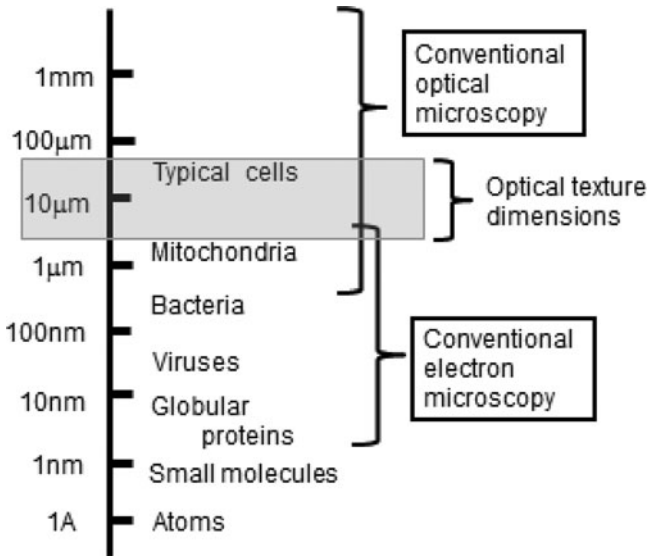

Figure 4. A dimensions scale, showing the typical size ranges of atoms, molecules and sub-cellular features. Note that the size range of the detail found within the optical textures of mesophases coincides with the typical dimensions of many self-ordered structures found in living systems - see Figure 5.

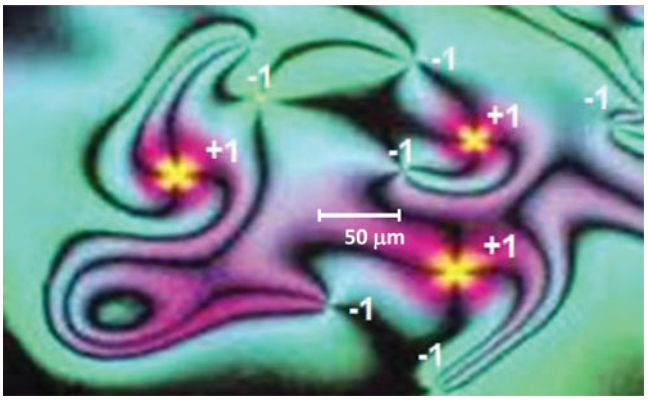

Figure 5. A typical optical texture of a nematic phase, with the signs and strengths of the disclinations indicated. Spontaneous relaxation in the director field causes disclinations of the same sign to repel each other and those of opposite signs to attract [4]. A texture of this kind therefore represents an equilibrium state where the various attractive and repulsive forces are balanced. Note that in general, optical textures do not resemble fractal patterns - and in practice it is usually found that the distances between singularities lie in a relatively narrow band between 40 μm and 80 μm, which is more-or-less the range of the self-ordered patterns (such as the separation of the spindle poles) which appear in biological structures. Presumably this is a consequence of the fairly narrow ranges of the absolute and relative values of elastic constants of nematic phases at ambient temperature.

Microtubules are crucial components in a range of cellular processes. Together with actin microfilaments and the loose collection of other polymeric structures, collectively known as intermediary fibrils, they make up the cytoskeleton of cells. They are involved in maintaining the structure of the cell. They also make up the internal structure of cilia and neurons. They provide 'tram tracks' for intracellular transport of vesicles and organelles. They are also involved in establishing the mitotic spindle, in chromosome separation in the later stages of mitosis, and in creating the complex architecture of plant cell walls. The structural hierarchy of a microtubule is sketched in Figure 9. The basic structural unit is the $\alpha\beta$ dimer, and chains of these build up the hollow cylindrical structure. Microtubules are polar and the rates of growth are different at the two ends (Figure 10).

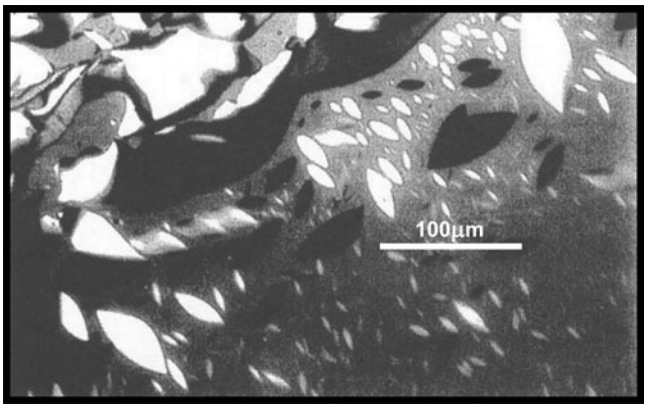

Figure 6. Tactoids (reproduced from W. R. Kenchington, PhD thesis, Leeds) [5] This optical micrograph shows the appearance of tactoids of silk precursor in the spinning gland of a spider. Virtually identical images were obtained from concentrated solutions of tobacco mosaic virus, by Bernal and Fankuchen [6] - and these prompted Bernal's suggestion that the mitotic spindle is a tactoid [7].

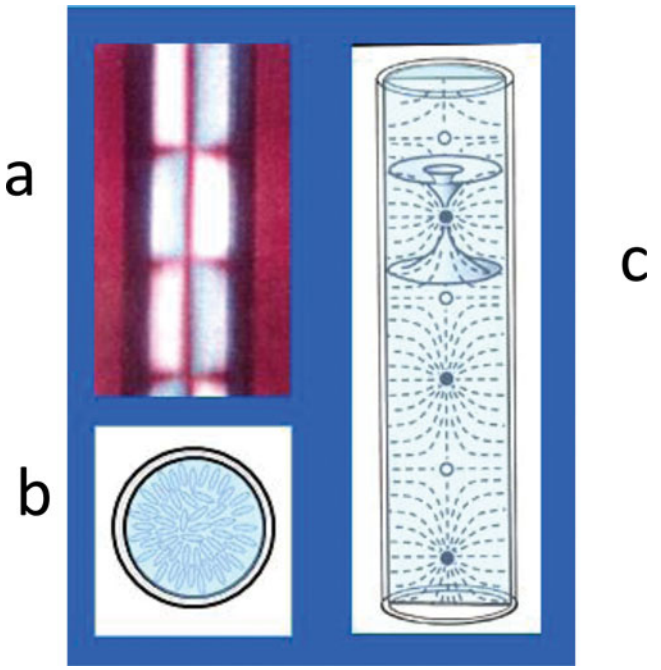

Figure 7. The cellular texture adopted by nematic phases in cylindrical tubes. (reproduced from Bunning and Lydon [8]). The dimensions of the tube are not particularly critical. In the example shown here, the tube had an internal diameter of 50 μm. The optical micrograph (a) shows the texture spontaneously adopted by a nematic phase in a glass capillary. This sample is a nematic discotic phase of a polyflourinated lyotropic mesogen which forms flat pancake-shaped micelles - but the texture appears to be general for any nematic system either calamitic or discotic - and either thermotropic or lyotropic [9]. The director orientation at the wall is normal and as shown in (b), when this alignment is projected into the centre of the sample, the molecules encounter increasing splay stress as they approach the axis. The sample reduces the splay distortion by 'escaping into the third dimension' as shown in (c). In this cellular texture, the escape direction alternates between up and down (so that there is no bulk flow of fluid as it is formed). The director field drawn in (c) shows the way in which the curvature of the phase varies with its position. In this sense, a molecule can be said to 'know' where it is positioned within the sample.

As shown in Figure 9, the assembly of tubulin monomers into dimers and of dimers into protofilaments, requires the presence of molecules of the small organic compound, guanosine triphosphate (GTP). These molecules are incorporated at two sites. Those bound to α-tubulin are held firmly and are stable. On the other hand, those bound to the β units can be hydrolyzed subsequent to assembly, to give guanosine diphosphate (GDP). This weakens the bonding but the microfilaments do not fall apart in the middle, instead they unravel rapidly from the β end if this is not protected. The rapid disintegration of microtubules is known as a 'catastrophe'.

The graph shown in Figure 11 shows the dynamics of 'microtubule growth. As described in Figures 9 and 10, there are different energy barriers to be overcome for addition (and removal) of dimer units at the two ends. As a consequence, the critical concentration required for addition at the β end (Cc^{β}) is lower than that at the α end (Cc^{α}). There is also a difference in the rates of addition and loss of dimer units at the two ends, with the plot of growth rate against concentration being steeper for the β end.

At concentrations between the two critical values, there is 'treadmilling' - i.e. addition at the β end and erosion at the α end. The particular situation where the rate of gain at the β end balances the rate of loss from the α end is termed 'steady-state treadmilling'. Note that to maintain this non-equilibrium steady-state, a constant input of energy is required. As an

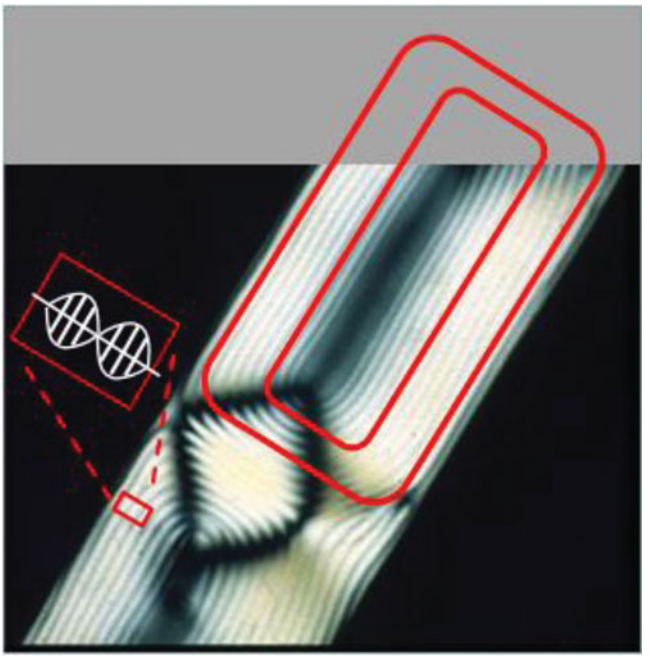

Figure 8. The appearance of a cholesteric solution (of virus particles) held in a cylindrical tube. (Reproduced from Dogic *et al.* [10] with annotations added). The structure, which is spontaneously adopted, consists of concentric shells of helicoidal mesophase. The small box drawn on the left outlines two half-pitches of the sample. The director field of cylindrical shell outlined in red, corresponds to that postulated for the microtubules in the cortex of the plant cell, which act as a template for cellulose microfibril orientation - see Figure 22.

analogy, this situation could be compared to a motorist holding a car stationary on a steep hill by controlling the clutch rather than applying the hand brake.

4. Mitosis

The process of normal, (asexual) division of a cell into two identical daughter cells is called *mitosis* to distinguish it from more the more complex process, *meiosis*, which is involved in sexual reproduction, Chromosomes are so-called, not because they are intrinsically coloured (which they aren't) but because they can easily be made visible by selective staining. A section of onion root tip is shown in Figure 12 (which dates from 1900). This had been stained with the dye haematoxolin, which makes the chromosomes very obvious. The various stages of mitosis involve their movement into the equatorial plane of the cell and their division into daughter chromatids which are then segregated to opposite ends of the cell. It is clearly a carefully orchestrated performance, but the early optical microscope studies gave little indication of how this operated, since it did not indicate what was happening in the apparently featureless cytosol matrix surrounding the chromosomes.

Studies of dividing cells using polarised light give a significantly different picture. They show very clearly that, during the metaphase stage in the middle of mitosis, the whole inner region of the cell is occupied by a spindle-shaped birefringent area [13] but the movement of chromosomes within the spindle shows that they are in a fluid environment. It would appear therefore that, by definition, the spindle has a liquid crystalline state - and that the region between the poles has a director field like that of the Zeppelin-shaped tactoids which Bernal

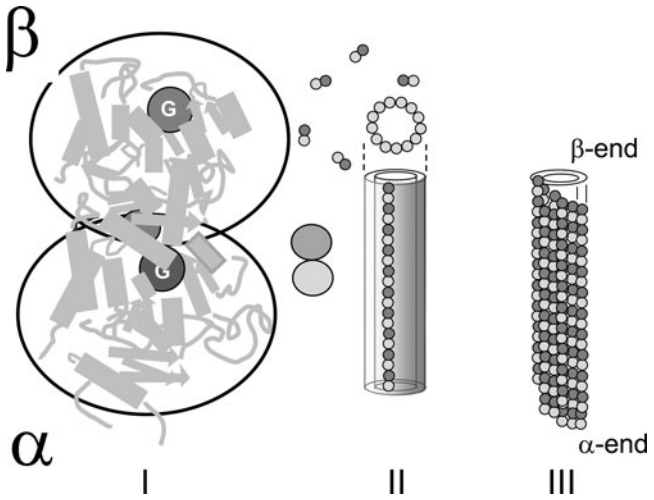

Figure 9. The structural hierarchy of a microtubule (redrawn from Molecular Biology of the Cell, 5th edition) [11]. **(I)** This stylized sketch shows the structure of the heterodimer of α and β tubulin subunits. Each of these is a globular protein composed of a single polypeptide chain. The shaded rectangles represent lengths of alpha helix and the broad arrows represent strands of beta structure. The darker circles indicate the sites where molecules of GTP (guanosine triphosphate or GDP (guanosine diphosphate) are bound. Note the strand of protein chain crossing the site on the α subunit. **(II)** A protofilament - formed from α β heterodimers linked end-to-end. **(III)** A microtubule - a hollow cylinder, usually composed of 13 protofilaments. Microtubules are polar in the sense that one end is not the same as the other. At one end the β subunit is exposed, and at the other, the α subunit. Because of this, growing, microtubules extend more rapidly from the β end.

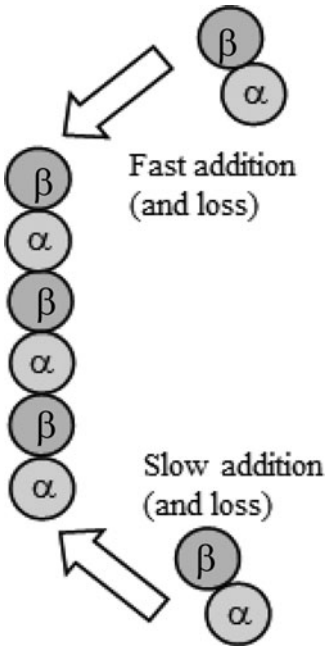

Figure 10. The asymmetric growth of microtubules This sketch depicts the growth of a microtubule protofilament by addition of $\alpha\beta$ dimers to its ends. Addition at the α end requires a conformational change of the terminal subunit, whereas addition at the β end does not. There are two consequences of this. Firstly, for the β end, the critical dimer concentration (Cc^{β}) at which addition of subunits begins, is significantly lower. Secondly, the rate at which subunits are added (or lost) is higher.

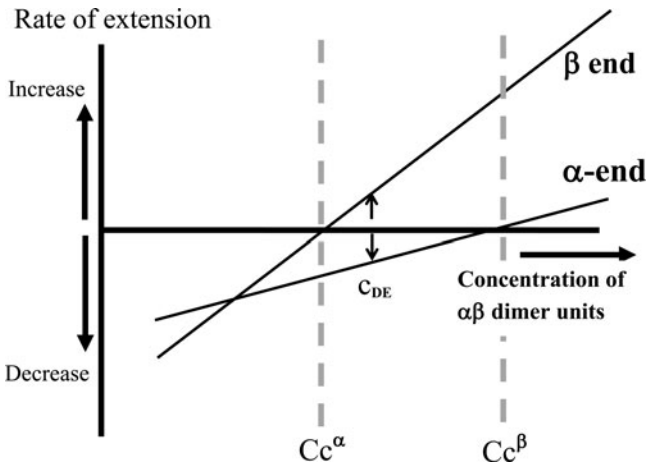

Figure 11. The dynamics of microtubule growth If other factors are constant, the growth or shrinkage of a microtubule is dependent on the concentration of tubulin $\alpha\beta$ dimer subunits in the surrounding cytosol. $\mathbf{Cc^{\alpha}}$ is the critical concentration required for the addition of these units to the α end of a microtubule. $\mathbf{Cc^{\beta}}$ is the critical concentration required for the addition at the β end. At concentrations between these critical values, there is 'treadmilling' - i.e. addition at the β end and erosion at the α end. Note that there is a specific concentration $\mathbf{c_{DE}}$ between the two critical values where there is a state of dynamic equilibrium, with the rate of addition at one end equaling the rate of loss at the other.

had encountered in his study of tobacco mosaic virus [6] and [7]. However, in spite of his scientific status at that time, his suggestion that the dividing cell used a liquid crystalline region to shepherd chromosomes was never generally accepted in biological circles.

Later investigations showed the importance of microtubules in the mitotic process, and with specific fluorescent labels very beautiful optical micrographs can be obtained with the microtubules clearly highlighted (Figure 1).

The sequence of distinct stages in the mitotic process (in mammalian cells) is sketched in Figure 13 - and the different roles which microtubules are called upon to play, are indicated in Figure 14.

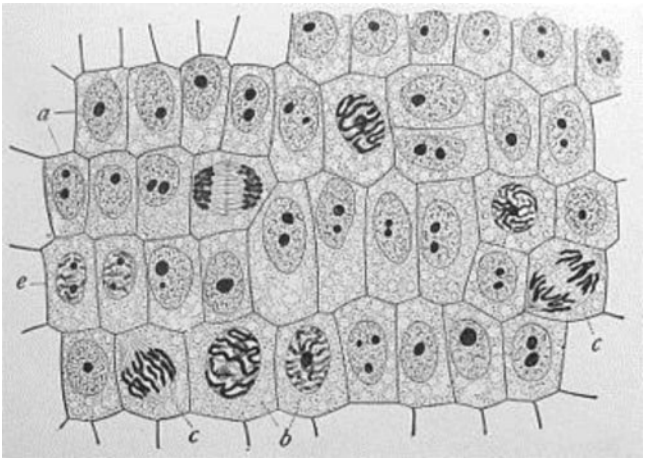

Figure 12. A section of onion root tip, with cells at a variety of stages of mitosis from E. B. Wilson 1900 [12], a. non-dividing cells b. nuclei preparing for division c. dividing cells showing mitotic figures e. pair of daughter-cells shortly after division (for some reason, there are no cells labelled 'd')

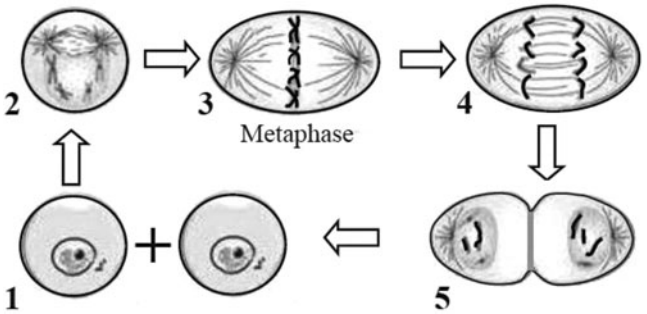

Figure 13. The sequence of events in mitosis - redrawn from [14]. In the 'resting' state of the cell (1), the chromosomes are fully unwound and are not visible with ordinary light microscopy. In preparation for mitosis, each chromosome produces an identical copy of itself and the two are joined together at the centromeres. The chromosomes condense by coiling and supercoiling until they become clearly visible X-shaped bodies (2). The nuclear membrane then disappears and microtubules attach themselves to the centromeres of the chromosomes. At *metaphase* (3) the chromosomes are all now positioned in the equatorial plane of the spindle. At some signal, the bonds which hold the two chromosomes together break and the separated chromatids are pulled towards the poles of the spindle. The spindle becomes unclear as the microtubules disintegrate. A groove then forms around the equator, indicating that the division of the cell into two is about to occur (5). In the final stage, two daughter cells are formed, each with the same number of chromosomes as the parent cell.

In animal cells, the centrosomes have a prominent role in establishing the spindle and they would appear to be vital parts of the mitotic machinery. Bearing this in mind, it is most strange that they do not occur in plant cells – which seem to be able replicate themselves perfectly satisfactorily without them. The inference is that perhaps centrioles are not always the prime agents in establishing the spindle – and that in some cases they merely sit at the poles and reinforce the director fields around them. In this context perhaps the structure shown in Figure 7 is relevant. Here a director field has been established with a line of point defects (poles) lying along the axis of the cell. Their creation is solely due to the elastic constants of the mesophase and the boundary conditions. Nothing has been placed in the cell other than the mesophase. In this situation, the poles are *positions* rather than *objects*.

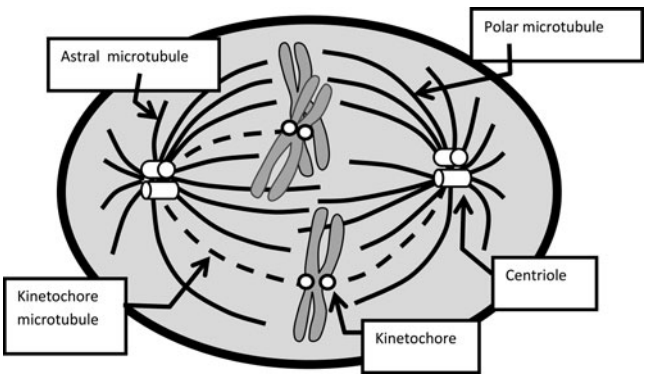

Figure 14. The principal components of the mitotic spindle. This stylised sketch shows the dividing cell at the stage immediately before metaphase. The three distinct kinds of microtubules are indicated – **polar microtubules** which form the central spindle, **astral microtubules** which radiate from the centrioles but are not part of the spindle assembly and **kinetochore microtubules** (shown in broken lines) which are attached to kinetochore regions of the chromosomes.

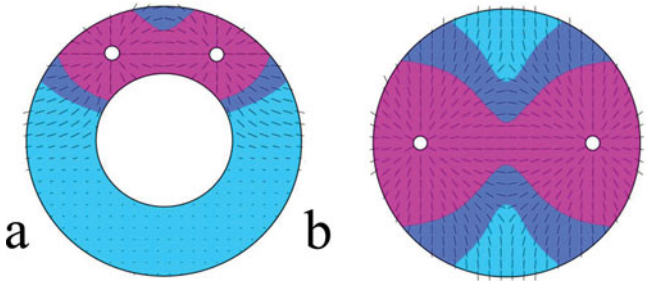

Figure 15. A liquid crystal model for the assembly of the spindle from D. Miroshnychenko, N. A. Hill, N. J. Mottram and J. E. Lydon [15] This figure shows a slice through a model of a three-dimensional spherical cell in the plane which contains the centre of the cell, the nucleus and the two centrosomes. The rods depict the director field and show the local average orientation of microtubules in the cytoplasm. The small circles represent the centrosomes. The colours indicate the degree of mesogenic ordering (identified with the level of tubulin polymerisation). Red indicates a highly ordered liquid crystalline state, and blue indicates a region of a low order, almost isotropic, fluid. The highest level of ordering occurs around the centrosomes and along the polar axis joining them, within the spindle. The chromosomes are not shown in this depiction. **Figure (a)** shows an early stage of mitosis. The asters radiating from the centrosomes are beginning to create a spindle-like pattern. The central circle represents the nuclear envelope. **Figure (b)** shows a slice through the cell immediately before metaphase, when the centrosomes are in position and the spindle is fully formed. The structure now has full rotational symmetry around the axis joining the poles and the nuclear membrane has disappeared.

In 1986, Kirschner and Mitchison proposed that microtubules use their dynamic properties of growth and shrinkage to probe for the centromeres of chromosomes [16, 17 and 18]. In the early stages of mitosis, microtubules extending from a spindle pole become attached to chromosomes before shepherding them into the equatorial plane of the spindle. This 'search and capture' process involves the periodic phases of microtubule growth and shrinkage, as depicted in Figure 16.

This mitotic process begins with the expansion of the asters. Microtubules extend outwards from the centrosomes in all directions 'searching' the cell. Whenever the growing end of a microtubule encounters the centromere region of a chromosome, a bond is established and the chromosome is 'captured'. This bond remains unbroken until the final stage of mitosis. When a periodic wave of catastrophe occurs, those microtubules which have not found a centromere, have their vulnerable (β) ends exposed. They rapidly disintegrate and shrink back towards the centrosome. Unprotected microtubules have a half-life of 5–10 minutes, whereas captured microtubules can survive for a number of hours. When this contraction stage is over, microtubules begin to extend once again, 'searching' for the centromeres of the remaining uncaptured chromosomes.

An additional process has been proposed. It is suggested that the centromeres take a more active role in the process - creating asters of their own, as shown in Figure 16 (d). This would considerably increase the effective target area for capture.

It would appear that the action of the two centromeres on a chromosome are linked, and that it is not until the second microtubule had been attached that the kinetochores can come into action and trigger the contraction of the chromosomal microtubules. When this has occurred, the bonds which have held the chromatids together can break and the two daughter chromatids are free to be drawn towards the opposite poles by the contraction of the microtubules.

Pictures of mitosis shown in textbooks usually relate to cell division in higher animals. But lower organisms have far less genetic material to separate and they can operate with a much

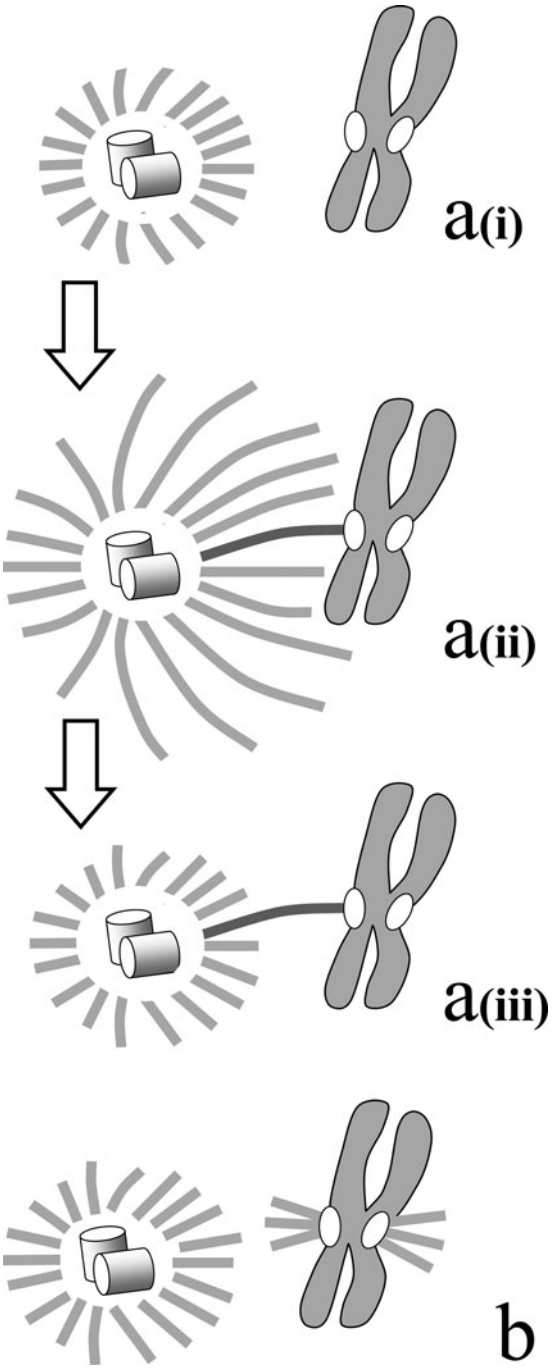

Figure 16. The 'search and capture' of chromosomes by microtubules. [16, 17 and 18]. **a(i)** During a phase of microtubule growth, an aster extends from the centrosome. **a(ii)** When a microtubule encounters the centromere region of a chromosome it forms a strong attachment. **a(iii)** During a subsequent phase of microtubule shrinkage, the 'capturing' microtubule remains attached to the chromosome, whilst the others shrink back towards the centrosome. This cycle of growth and shrinkage is repeated until all of the chromosomes have been 'captured'. **b** It has also been proposed that the centromere region may take a more active role in the process - and act as a microtubule organising centre, sending out its own aster of microtubules, aiding the capture process by increasing the target area.

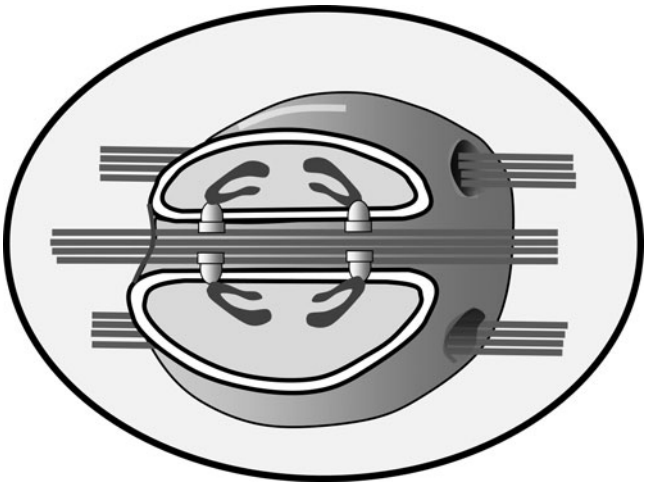

Figure 17. The stage in the mitotic cell division of a family of unicelluar dinoflagellates, which corresponds to metaphase in higher organisms [19]. The nuclear membrane remains intact throughout the process of dividing the genetic material, and the nucleus divides into two equal halves only during the final stage of cell division. In this figure, the horizontal rods represent microtubules which are able to direct the separation of the daughter chromatids on the other side of the membrane, presumably *via* motor proteins and transmembrane proteins.

simpler process. One can visualize the stepwise evolution from the earliest unicellular organisms with simple loops of nucleic acid, towards more complex forms of life requiring increasingly large quantities of genetic material. The process of cell division becomes progressively more exacting and requires more and more complex machinery.

In some unicellular organisms there are interesting modifications to the process of cell division, which improve its effectiveness. In particular, there is group of dinoflagellates where separation of the chromosomes occurs within an unbroken nuclear membrane but is directed by microtubules outside the nuclear membrane. Perhaps this gives a glimpse of the first stage in the long process of development towards the sophistication of mitosis in mammalian cells, where the properties of microtubules are increasingly brought into play.

The picture shown in Figure 17 depicts the state corresponding to metaphase for a species of dinoflagellate [19]. Note that the nuclear membrane remains intact throughout the process of segregating the chromatids and that the chromosomes are manipulated through the plasma membrane by motor proteins running along microtubule tram tracks on the other side. [This concept of transmembrane proteins ploughing transversely through a membrane, being driven by motor proteins running along microtubule tram tracks, will be invoked again in a proposed mechanism for the alignment of cellulose microfibrils in plant cell walls.]

It is not intended to imply that dinoflagellates necessarily lie in the direct line of descent of mammals. It is only argued that the necessary assembly of components of the cellular apparatus (i.e. microtubules, motor proteins and transmembrane units) postulated for transmitting the orientation of microtubules through the plasma membrane, is credible.

5. Plant cell wall formation

In land plants, the cellulose wall is the major structural element. It enables the plant to hold its shape and grow - and gives protection against mechanical injury. Less obviously, by enabling

the cells to maintain an internal turgor pressure, it prevents them exploding with uncontrolled water intake by osmosis.

In many plant tissues, the cell wall is a flexible structure, unable on its own to maintain a fixed shape. The apparent rigidity of the tissues is actually due to the internal pressure from the cell contents which are constrained by the plasma membrane. As an analogy, think of the cell wall as a flexible wickerwork basket held rigid by the pressure of an inflated balloon inside it. Provided the balloon holds its pressure, the structure remains rigid, but if it is deflated, the structure loses its rigidity and will droop. See Cosgrove [25].

When plant cell walls were first imaged by electron microscopy, their appearance came as a considerable surprise. Neither of the previously used techniques for studying them (optical microscopy and X-ray diffraction), had hinted that there would be layer upon layer of apparently endless cellulose fibrils arranged in such complex patterns. And as so frequently happens, the subsequent extensive study of plant cell walls generated more questions than it answered.

The turgor pressure inside the cells is appreciable. It can range from 100 to 1,000 atmospheres and this creates problems for cell growth. In their early stages of development, before there is any secondary wall thickening, many plant cells undergo enormous extension - xylem vessels for example, can increase their volume by a factor of 1,000. How can a load-bearing wall be softened enough to be able to extend without weakening it and risking it bursting with its internal pressure? The answer appears to be a process of carefully regulated 'polymer creep'. The growing plant cell wall has a structure like fibreglass, with cellulose microfibrils embedded in a matrix of complex polysaccharides (pectins and hemicelluloses) together with some protein. In some way, a selective microfibril-by-microfibril process occurs, loosening, shifting and re-establishing the load-bearing linkages between microfibrils in the matrix, without weakening the entire assembly [23].

Where secondary wall growth occurs, it does so after this extension process is over, and when the cell has more-or-less grown to its final external dimensions. The deposition of reinforcing layers of microfibrils inside the primary wall occurs within a more controlled cell geometry [23, 24 and 25].

A typical arrangement of cellulose microfibrils in a plant cell wall is sketched in Figure 18. The wall is synthesised from the inside. The first layer to be laid down is termed the primary wall. It usually has a fairly random pattern of microfibrils. In contrast, the secondary wall consists of aligned layers of microfibrils, as shown in figure 19.

Note the appearance of Figure 19. The way in which the microfibrils are aligned in each sheet suggests some long-range interactions are responsible - but there is an appreciable level of short range deviation from the consensus orientation. For example, occasional microfibrils can be seen, which start with one orientation and then curve away to follow another. In other instances, microfibrils in adjacent layers can be seen interleaving like the threads in a piece of woven cloth. The overall picture gives the impression that the microfibrils within each layer have been laid down individually and this has occurred within a director field which covers the whole area of the cell surface.

The initial layer of the secondary wall (S1) usually has hoops of microfibrils lying in the equatorial plane of the cell. Subsequent layers usually lie in regular repeating sequences of orientation of some kind. The way in which these patterns are orchestrated is not obvious. In general, they do not lie at geometrically simple angles (of, for example, 90°, 45° or 60°) to each other or to the cell axis. Perhaps the most telling pattern sometimes found is that where there is a small, apparently constant, angular offset between one layer and the next giving a helicoidal structure (see Figures 20 and 21).

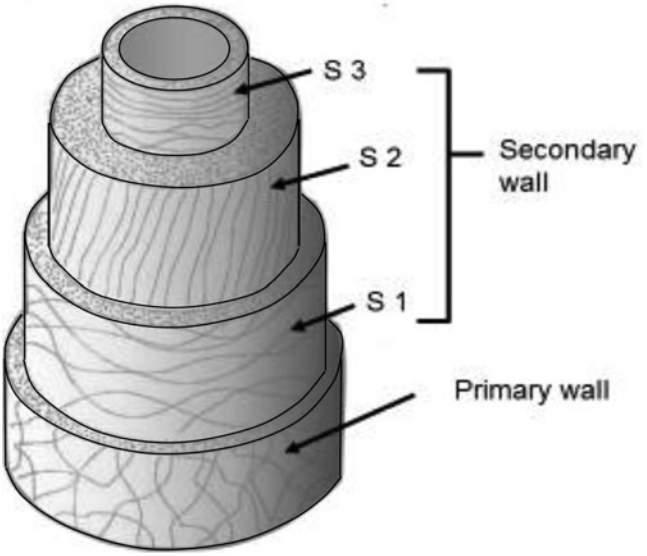

Figure 18. A typical arrangement of cellulose microfibrils in a plant cell wall [20]. The first layer to be laid down is termed the primary wall. It usually has a fairly random pattern of microfibrils. In contrast, the secondary wall consists of aligned layers often in a simple repeating sequence.

Clearly, there is some mechanism at work which orchestrates the process of cell wall synthesis and, over the years since microfribrils were first seen in the electron microscope, this problem has occupied the minds of generations of botanists and a number of theories have been proposed – but none of these would explain the helicoidal structure. However, helicoidal structures suggest cholesteric phases; it is tempting to propose that that there is a potentially cholesteric liquid crystalline phase operating somewhere in the microfibril-aligning mechanism.

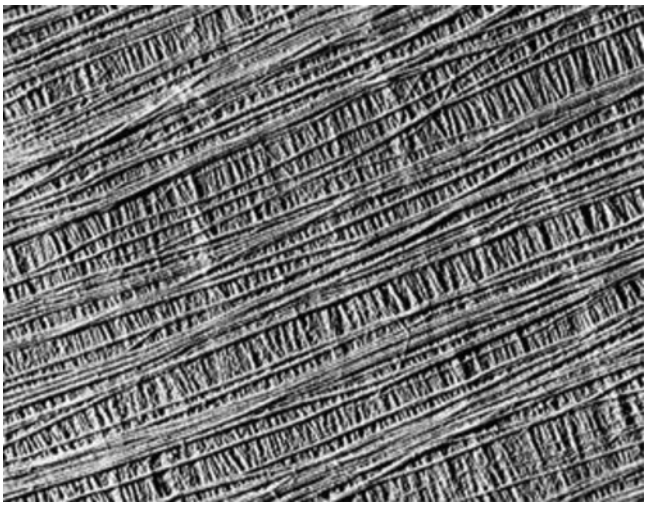

Figure 19. Electron micrograph showing cellulose microfibrils in a plant cell wall [20]. This shows two layers of microfibrils in the secondary wall of a plant cell. Note the characteristic type of pattern, with good long-range orientation of the microfibrils but with appreciable short-range meandering, away from and then back towards the consensus alignment direction

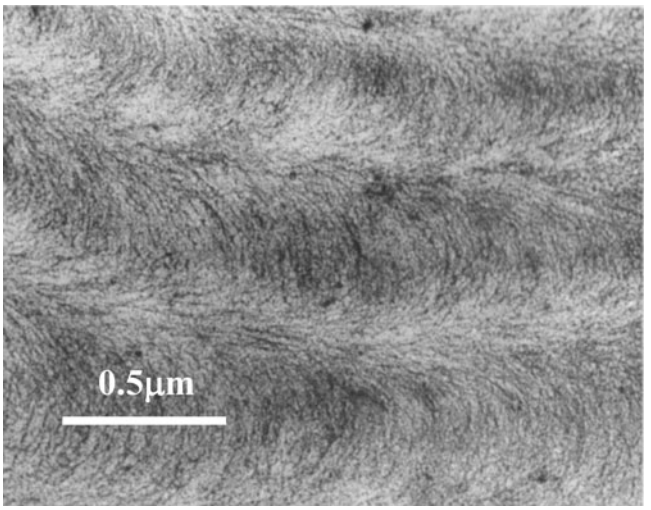

Figure 20. An electron micrograph of a plant cell wall (*Nitella*, a fresh water alga) showing a Bouligand pattern of nested arcs [21, page 70]. This is the characteristic appearance of an oblique section through a helicoidal array of fibrils. See Figure (21).

In the definitive and richly illustrated text - *Plant cell walls - from chemistry to biology* by Albersheim *et al.*[23] there is a brief mention of the helicoidal arrangement of plant cell walls, concluding with words -

> *"At present we have no way of predicting when or why the cell forms a helicoidal wall, and much remains to be learned about these remarkable structures."*

Note that, rather than being a complex and puzzling arrangement, the helicoidal state is the default liquid crystalline structure of chiral polymer solutions, found in polypetide and nucleic acid solutions. A plausible explanation for its origin in plant cell walls may throw some

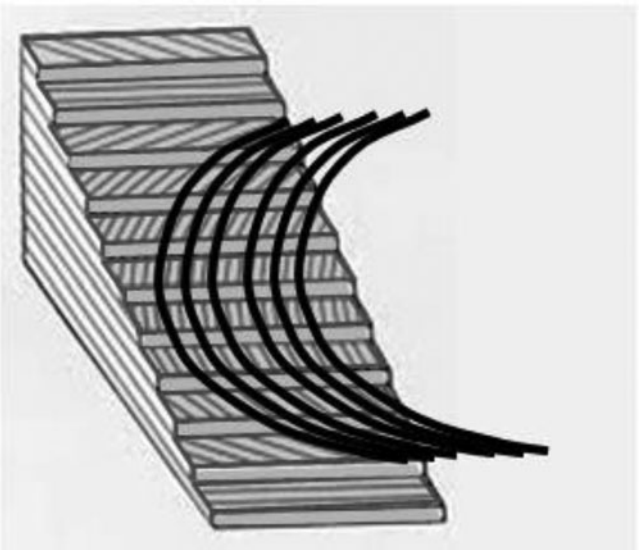

Figure 21. The origin of Bouligand patterns (nested arcs) in an obliquely- cut section of a helicoidal structure [22].Note that the apparent curvature is an illusion. The microfibrils lie in parallel straight lines within each sheet.

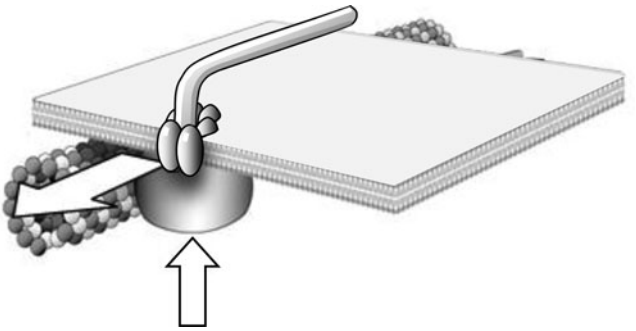

Figure 22. A model for the production of microfibrils in the secondary cell walls of plants. [23, 24 and 25] redrawn from [23]. This schematic sketch depicts the cellular apparatus involved in the production of a cellulose microfibril. The horizontal slab represents the plasma membrane, with the cytoplasm below and the cell wall above. The vertical arrow represents the cellulose precursors entering a rosette. The object behind this arrow is the motor complex which is driving the rosette along a microtubule to the left, as it leaves the microfibril extending towards the right. In the synthesis of the primary wall, the rosettes are not coupled to microtubules, and they wander randomly through the plasma membrane, producing a disorganised layer of microfibrils.

light on the whole problem of cell wall architecture. If a mechanism for forming this structure actually occurs, then only small modification would be required to explain the repeating patterns commonly seen in secondary walls.

It was 30 years after the first discovery of cellulose microfibrils when improvements in electron microscopy led to the discovery that their alignment follows that of microtubules on the other side of the plasmalemma. (This lengthy delay was largely due to the early techniques for sample preparation which gave clear images of cellulose microfibrils but destroyed microtubules.) This observation led to the model for cell wall construction shown in Figure 22.

As electron microsopy techniques improved, it became clear that that there are small hexagonal structures visible on the plasma membrane surface that might be responsible for microfibril synthesis. Subsequent studies have confirmed that these 'rosettes' are the sites of a cellulose microfibril production.

The currently accepted model for the creation of ordered layers of microfibrils in the secondary walls of plants is sketched in Figure 22, [23, 24 and 25]. Unpolymerised cellulose precursors produced in the Golgi apparatus are transported to the outer region of the cytoplasm. Here they reach the microfibril-synthesising rosettes, which penetrate through the membrane. These are linked to the microtubules by motor proteins which drive them along microtubule tram tracks. As the rosettes move, they spew out microfibrils behind them, creating a pattern of oriented layers as directed by the microtubule template. This model is, however, incomplete in that it does not explain what determines the change of orientation from one layer of microfibrils to the next - other than implying that it is probably due to changes in the microtubule pattern.

Recent studies using fluorescent markers. on the cellulose synthesising enzymes and on the tubulin molecules in microtubules, have enabled the direct visualization of both the cellulose synthesising complexes and the microtubules in living cell [24].

> *"It has been possible to follow the movement of rosettes within the plasma membrane and to track them in relation to the underlying microtubules. – This therefore suggests that microtubules do provide guide rails for the movement of cellulose synthases and, together with the finding that the microtubule guide rails rotate with time, suggest a role for microtubules in regulating the variable alignment of cellulose microfibrils.*

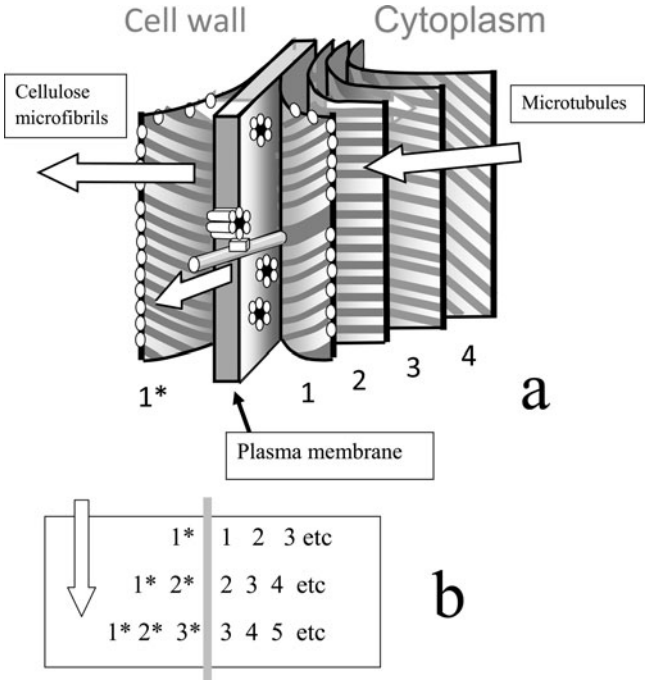

Figure 23. A proposed mechanism for directing the orientation of sheets of cellulose microfibrils in a plant cell wall. The outer layers of the cytoplasm contain microtubules in sufficient concentration to form a liquid crystalline phase (drawn here as a cholesteric phase). As sketched in (a) the outermost layer of microtubules dictates the alignment of microfibrils on the outer face of the plasma membrane. After each microtubule layer has been 'read' it is destroyed and the next moves to take its place, as indicated in (b).

> *…. A major long term problem is what then determines the orientation of the cytoskeletal arrays themselves."*
>
> from page 289 of *Plants Cell Walls* by Albersheim *et al.* [23]

6. A liquid crystal model for microfibril alignment

I propose a possible mechanism for this aspect of the wall-building process. I suggest that the outer layers of the cytoplasm contain microtubules in sufficiently high concentration to form a liquid crystalline cortex. If this is cholesteric, the outer shell of the cytoplasm assumes a helicoidal ordering like that shown in Fig 23 (a). It is the outermost layer of microtubules that dictates the alignment of microfibrils. After each microtubule layer has been 'read', it disintegrates, clearing the way for the next layer of microtubules to take its place, and a new set of contacts is established with the rosettes, as indicated in (b).

The most vulnerable parts of microtubules are the ends – and it seems reasonable to suggest that, as each rosette completes its journey and reaches the terminus, it destabilises the end of the microtubule and causes the structure to disintegrate. There is then a pause in microfibril production as a new set of contacts is established between the rosettes and the next microtubule layer. In recent years the importance of the dynamic state of microtubules in biological systems has become recognised. A recent publication by Brugués and Needleman stresses the active liquid crystalline aspect of the spindle during mitosis [26].

The pattern of cellulose microfibrils in the secondary cell wall is therefore a record of the alignment of the sequence of microtubule layers as they reach the plasma membrane and take their turn at directing microfibril alignment. A loose analogy would be the way in which the reversals in the Earth's magnetic field are recorded in igneous rocks at tectonic fault lines.

7. Summary

Liquid crystalline phases are the supreme exponents of self-ordering. It is argued they are eminently suited for biological roles, since they exist in the same temperature and energy ranges, as those of living systems, and can spontaneously produce *in vitro*, characteristic structures that resemble those found in biological tissues.

The way in which microtubules are able to form polar arrays by treadmilling is outlined - and the role of liquid crystalline phases of microtubules in mitosis and the formation of plant cell walls is discussed. Two specific examples are highlighted. The first is the process of cell division in a group of dinoflagellates where separation of the chromosomes occurs within an unbroken nuclear membrane. The second is the production of helicoidal arrays of cellulose microfibrils in a plant cell wall. From a biological point of view these are remote and totally unrelated. However, in both cases it appears that the crucial factor is the way in which microtubules close to the inner face of the plasma membrane are able to direct the alignment of processes on the outer face. It is suggested that this offers an explanation for the long-unsolved problem of how plant cells control the architecture of their cell walls.

Following the advent of methods for sequencing proteins and DNA, a study of evolution at the molecular level became possible - enabling the course of evolution within families of macromolecules, such as the globins or the cytochromes to be traced. We are now seeing evidence of evolutionary patterns emerging at a higher level within molecular biology, in the context of processes, mechanisms and strategies (rather than the structures and properties of individual macromolecules). In particular, an exciting picture is beginning to emerge of the way in which the complexities of cell division and the production of highly organised structural materials could conceivably have evolved in credible stages since unicellular life first appeared on this planet. In this process, it would appear that the liquid crystal-forming properties of biological materials have been widely exploited.

Throughout this story, there is one continuous theme running through the millennia - the amazing versatility of that innocuous-looking little mesogenic protein, tubulin.

Acknowledgments

I am heavily indebted to Professor Paul Knox for his help concerning the structures of plant cell walls. I fully acknowledge the work of my more mathematically gifted colleagues, Dmitry Miroshnychenko, Nick Hill and Nigel Mottram for the modelling of the mitotic cell. I acknowledge the work of Dr John Bunning on the cellular texture shown in Figure 7. I thank Professor Richard Bushby for his help and advice, and Emma Steer for her help in preparing a readable text.

Glossary

Centrioles– small cylindrical self-replicating organelles which occur in pairs, involved in microtubule growth.

Centromere– the part of a chromosome that links sister chromatids together, and where spindle fibres are attached during mitosis.

Centrosomes– in animals, the main microtubule organizing centres in the cell, containing two orthogonally-arranged centrioles. In mitosis, they separate and migrate to form the poles of the spindle.

Chromatid– one of the two identical strands into which a chromosome separates, prior to cell division.

In vivo– within a the living system.

In vitro– literally 'in glass' (*i e* a test tube) - outside the living system.

Kinetochores– multi-component complexes attached to centromere regions of chromosomes. They come into action at the end of metaphase when the bonds which have held each chromatid pair

together, break and the two daughter chromatids are drawn towards the opposite poles by the contraction of the microtubules.

Mesogen– a compound which is capable of forming a liquid crystalline phase.

Metaphase– the central stage of mitosis, where the chromosomes are all lined up in the equatorial plane of the cell, but have not yet begun to separate into two chromatids.

Plasma membrane– the phospholipid bilayer surrounding the cell.

Tubulin– the small globular protein from which microtubules are assembled.

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