Living Cells:
Structure, Function and Diversity"

Viewer's Guide to the DVD and Background Information

"....if we wish to understand life, we must start by understanding cells...." C. de Duve, "Blueprint for a Cell", 1991.

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CHAPTERS:

NOTES:
> 1)   Prior to viewing this DVD with a video projector, we recommend that the levels on the projector be checked.   If the projector has been previously used for Power Point presentations, its

Brightness, Contrast and/or Colour levels are often left very high. If the settings are not reduced to normal levels, video images may be coarse and garish.

2)   To keep this Guide short, the references cited are limited and given when they may be difficult to find. Most subjects are covered comprehensively in the many excellent text books on cell biology.

STUDYING CELLS : MICROSCOPY.

Live cells can be studied by light microscopy (LM) which has inherent limitations. Specimens have to be thin and transparent. Thus, single cells are particularly suitable for LM. Live tissues are very difficult to examine. Many tissue cells can only be studied live by culturing them on glass slides or coverslips.

Many cellular processes are slow and benefit from time-lapse recording. The approximate duration of the sequences or time-lapse rates are indicated in the commentary or in these notes.

Living cells are usually transparent and complex imaging techniques are needed to generate contrast. The two techniques most often used in this DVD are :

i)   phase contrast; and

ii)   differential interference contrast (DIC) which gives a shadowed effect; the appearance of side illumination is artificial!

Other types of images are generated by brightfield microscopy, (e.g., the Paramecium in Chap. 1), darkfield microscopy (e.g., Volvox in Chap.1) and polarization microscopy for birefringent structures (Chaps. 9, 15).

Electron microscopy (EM) gives much higher resolution images than those from LM.   In transmission electron microscopy (TEM), a beam of electrons is passed through the specimen which therefore has to be exceedingly thin.   For scanning electron microscopy (SEM), specimens are scanned by a beam of electrons and an image is constructed from the reflected electrons. SEM shows the whole cell and its surface features.

Immunofluorescence Light Microscopy uses fluorescently-tagged antibodies to localise specific cellular components (usually proteins such as actin). Examples of Immunofluorescent labelling are given in Chaps. 10, 12. As a convention on this DVD: tubulin is stained green , actin red and DNA, (nucleus or chromosomes), blue.   The technique is often extended by computer-generated Three-Dimensional Reconstructions using the Confocal Microscope. Some examples are given in Chaps. 11, 13.

In summary:

LM can give images of live cells which are of limited resolution; tissues are difficult to study live. SEM gives surface views of dead cells or tissues.   TEM gives high resolution, 2-dimensional images of structure in dead cells altered by fixation; microscopists have to interpret what might have been happening in living cells.

CHAP. 1. INTRODUCTION

This chapter introduces viewers to a range of microscopic cells and organisms, many collected from the wild. Water sampled from ponds or the ocean are frequently disappointing as planktonic organisms are usually very dilute and few will be encountered on a slide. A plankton net or other filtration apparatus is a good investment for concentrating the organisms.

Sequences in order. All in RT unless indicated otherwise.

TLR = Time Lapse Rate;   RT = real time;   Duration = approx. duration of the sequence in real time.

- (Opening cells) Micrasterias sp.
- Tadpole of Xenopus
- Epidermis of Tradescantia
- Chara sp., (green alga); antheridium and oogonium

- Cultured newt lung epithelial cells; TLR = 25x
- Marine plankton, mostly diatoms, from Port Phillip Bay
- Fresh-water pond sample; diatoms, protozoa, green alga
- unidentified Amoeba; TLR = 25x
-    "   "   (Acanthocystis ?); TLR = 25 x
- Paramecium sp., slowed down in viscous medium
- Eutreptiella sp., a euglenoid flagellate from Port Phillip Bay
- Ceratium hirundinella, a dinoflagellate
- Micrasterias lux (desmid, green alga) from Kakadu National Park
- Chaetoceros sp., a diatom from Port Phillip Bay
- Dictyocha octinaria, a silicoflagellate from Port Phillip Bay
- Stentor sp., feeding on green algae
- Volvox sp., a colonial green alga
- Synura sp., a colonial chrysophyte
- Bacillaria paradoxa, a colonial marine diatom; TLR = 2 x
- Straurastrum sp., desmid (green alga) and unidentified bacteria
- Oedogonium cardiacum, green alga; fertlization; TLR = 2x
- Closterium sp. (desmid, green alga); fertilization; duration = 3 hrs. approx.
- cultured newt lung epithelial cells; TLR = 25x

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CHAP. 2.   CELL MEMBRANE

Living cells control their internal environment by the cell membrane or plasmalemma. Control is physical, by containing the cellular components, and chemical, by selectively allowing certain ions and metabolites through the barrier, while keeping the rest out.

The cell membrane also rigidly defines and protects identity. Cells display extreme resistance to fusion even when they have just divided.   Normally, cell fusion only occurs during sexual reproduction, when it is controlled with exquisite precision. The rituals of sexual behaviour - whether the mating dances of unicells or humans - ultimately serves to bring into contact specialised regions of the cell membrane in the gametes. Fertilization (e.g., Chap. 17) marks the fleeting moment when this resistance to cell fusion is overcome.

The plasmalemma recycles quickly in some cells. During secretion, some products are made within membrane-bound vesicles which fuse with the plasmalemma, adding to its area as their contents are discharged (Chap. 6). Alternatively, vesicles pinched off the plasmalemma transport extracellular material into the cell, shrinking the area of the plasmalemma. This pinocytosis is brought about by tiny vesicles. Cells remain the same size during secretion or pinocytosis, and the membrane recycling mechanisms are not evident in living cells.

Sequences in order
-cultured mouse epithelial cells; TLR = 50X
        - cultured newt lung epithelial cells; TLR = 25x
        -    "   "   "   "   " ; TLR = 50x
        -    "   "   "   "   " ; TLR = 50x
- cultured mouse epithelial cells TLR = 50X
        - Tradescantia, stamen hair cell; TLR = 25x

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CHAP. 4   NUCLEUS and NUCLEOLUS

All eucaryotic cells contain at least one nucleus. Many larger plant, protistal and fungal cells are multinucleate, containing hundreds or even thousands of nuclei. The nucleus is enclosed by a membrane, the nuclear envelope, continuous with the endoplasmic reticulum.

Division of the nucleus is called mitosis. In animal and higher plant cells, the nuclear envelope

disappears during early mitosis, and later a new nuclear envelope reforms around the new nuclei (Chap. 10). However, in many protist and fungal cells, the nuclear envelope stays intact during mitosis.

The nucleus contains the cell's DNA, organised into chromosomes, varying from to several hundred per cell. When the cell is not dividing (during interphase), the DNA is usually dispersed. Prior to mitosis, this dispersed DNA, called chromatin, condenses into individual chromosomes. In a few cells (e.g., dinoflagellates, euglenoid flagellates), the chromatin remains condensed during interphase.

The ability of the cell to organise, replicate and then segregate its DNA into two exactly equal complements during division is astounding.   Human cells contain 46 chromosomes averaging about a meter of DNA. The human body contains about 10 quadrillion cells, and thus about 460 quadrillion meters of DNA. The moon is 200 billion meters away. Thus the DNA in a human body assembled into a single thread would extend to the moon and back over a million times (Margulis & Sagan, 1986: p.118).

The nucleus contains one or more nucleoli, the site(s) of synthesis of ribosomal RNA. The nucleoli usually disperse during mitosis and reform on certain chromosomes. However, in certain cells (e.g., Spirogyra; some dinoflagellates), nucleolar material coats the chromosomes; in others (e.g., Euglena, some fungi) it is divided approximately in half as the chromosomes separate (Pickett-Heaps, 1970) and nucleolar components have been detected on mitotic chromosomes in animal cells.

Margulis, L. & Sagan, D. (1986). Microcosmos. Four billion years of evolution from our microbial ancestors. Summit Books, N.Y.
Pickett-Heaps, J.D. (1970). The behaviour of the nucleolus during mitosis in plants. Cytobios. 6: 69-78.

Sequences in order
- cultured newt lung epithelial cells; TLR = 25x
- Amoeba sp., TLR = 25x
- Spirogyra sp. (green alga)
-    "   " (larger species); TLR = 10x
- cultured newt lung epithelial cells; TLR = 25-100x
- Closterium sp., (green alga); duration = 1 hr. approx.
- cultured LLC (mammalian) cells; duration = 1.5 hrs. approx.
- Spirogyra   sp., TLR = 1.25 hrs. approx.
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CHAP. 4.   MITOCHONDRIA

Most eucaryotic cells contain one or more mitochondria. They are often long and thin, rapidly changing their shape; they may become branched as they are pulled in different directions by invisible cytoplasmic elements like microtubules (MTs: Chap. 7).   They divide and sometimes fuse with one another. Small protistal cells have a single mitochondrion in the form of a complex three-dimensional network.

Mitochondria, like chloroplasts (Chap. 24), are symbiotically derived organelles, evolved from aerobic bacteria . Symbiotic bacteria are encountered in protists even today.

Sequences in order
- cultured PtK cells; computer processed image; RT (2 sequences)
- cultured newt lung epithelial cells; TLR = 25x (2 sequences)
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CHAP. 5.   ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is a complex network of closed membranous elements that permeates the cytoplasm.   Its area may be considerable and is critical to its biosynthetic and transport functions. Gunning and Steer (1975) calculate that the human liver contains about 100 sq. meters of ER.

The ER is involved in protein synthesis and TEM shows that its cytoplasmic surface is covered with ribosomes. It is thin and transparent and so is difficult to detect in vivo (Allen & Brown, 1988) but certain dyes stain it (e.g., Sanger et al., 1989). In many living cells, the ER tends to retract, needing to interact with the MT skeleton to remain dispersed. A few images on this DVD show mitochondria superimposed on elements of the ER; both organelles were probably interacting with the same MTs. One preparation uses onion epidermal cells (Knebel et al., 1990), to visualize ER in vivo.

Allen, N.S. & Brown, D.T. (1988). Dynamics of the endoplasmic reticulum in living onion epidermal cells in relation to microtubules, microfilaments and intracellular particle movement. Cell Motil. Cytoskeleton 10: 153-163.
Gunning, B.E.S. & Steer, M.W. (1975). Ultrastructure and the Biology of Plant Cells. Edward Arnold, London.
Knebel, W., Quader, H. & Schnepf, E. (1990). Mobile and immobile endoplasmic reticulum in onion bulb epidermis cells: short- and long-term observations with a confocal laser scanning microscope. Europ. J. Cell Biol. 52: 328-340.
Sanger, J.M., Dome, J.S., Mittal, B., Somlyo, A.V. & Sanger, J.W. (1989). Dynamics of the endoplasmic reticulum in living non-muscle and muscle cells. Cell Motil. Cytoskeleton 13: 301-319.

Sequences in order
- cultured PtK cells; computer processed image (2 sequences); RT
- cultured newt lung epithelial cells; TLR = 25x (2 sequences)
- onion epidermal cells (2 sequences); TLR = 5x

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CHAP. 6.   GOLGI BODIES

Most eucaryotic cells contain Golgi bodies, named after Camillo Golgi who discovered them in 1883. Their structure can only be resolved by TEM and SEM. They consist of a stack of flattened, membranous sacs (cisternae), surrounded by vesicles and usually associated with ER Many animal cells have a single Golgi body near the centrosome while plant and algal cells often have numerous smaller Golgi bodies (dictyosomes). Proteins synthesised in the ER are transported to the Golgi bodies for further biochemical modification. Then the product may be carried to the cell membrane for secretion.

Golgi bodies, being small and transparent, are invisible in most live cells and their secretory function is impossible to observe directly. However, they are identifiable in cells such as desmids and diatoms from equivalent TEM images. Many algae have walls of layers of elaborate polysaccharide scales. In marine coccolithophorids, these (Brown & Romanivicz, 1976) may become heavily calcified, in which event they are called coccoliths. Coccoliths have accumulated into massive sedimentary layers and fossil coccoliths are of great importance to geologists. Scales and coccoliths are assembled in Golgi cisternae and large coccoliths are visible in live cells. Cisternae containing mature coccoliths detach from the Golgi body and migrate to the flagellar base where the coccolith is discharged into the layer on the cell surface. At peak secretory activity, a scale is secreted every 1-2 mins. Gunning & Steer (1975) calculate that the Golgi body delivers an area of membrane up to the area of the cell every hour.

Many algae display a variety of scales. For example, some Pryamimonas (Chap. 15) have six different types of scales (e.g., Pienaar & Aken, 1985) and each type is deployed into a predetermined locality on the surface or on flagella; in total, there can be several thousand scales per cell. The terminal spines of Mallomonas (Chap. 19) are formed, secreted, reoriented and then the base of each spine is attached to a particular base-plate scale. Thus, each scale and spine must have an `address' directing it to the correct site, and a docking mechanism that ensures its correct orientation. How such efficient distribution systems work is unknown.

Brown, R.M. & Romanovicz, D.K. (1976). Biogenesis and structure of Golgi-derived cellulosic scales in Pleurochrysis. I. Role of the endomembrane system in scale assembly and exocytosis. Appl. Polymer Symp. 28: 537-585.
Gunning, B.E.S. & Steer, M.W. (1975). Reference in Chap. .5

Pienaar, R.N. & Aken, M.E. (1985). The ultrastructure of Pyramimonas pseudoparkae sp. nov. (Prasinophyceae) from South Africa. J. Phycol. 21: 428-447.

Sequences in order
    - Dividing Micrasterias thomasiana (desmid, green alga); RT
    - Surirella robusta, a diatom; 3 sequences; TLR = 25x
    - Hantzschia amhpioxys, a diatom; TLR = 2x
    - Pleurochrysis carterae, a coccolithophorid; 5 sequences; TLR = 5-15x

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CHAP. 7.   INTERPHASE MICROTUBULES

Two major cytoskeletal components are microtubules (MTs) and actin microfilaments. These filamentous organelles are different chemically and in appearance under the TEM; they have different, but sometimes overlapping functions. For a comparative discussion, see Mitchison (1992). MTs are 24nm in diameter, below the theoretical resolving power of LM; however, DIC optics combined with computer image processing techniques can reveal them, while single MTs are visible in vitro in darkfield.

In living cells, many MTs extend from Microtubule Organising Centers (MTOCs), dense and often ill-defined bodies which may control their spatial deployment; the centriole is an MTOC,when it templates the precise arrangement of 9+2 flagellar MTs Chap. 15). Other MTOCs include more ill-defined centrosomes which in some cells contains a pair of centrioles. Whether all MTs are directly controlled by MTOCs is unknown.

While some MTs are stable, assembly, disassembly and reformation of the MT cytoskeleton is of great functional significance. During cell division (Chaps. 10, 11), the interphase MT skeleton is broken down and the tubulin reassembled into the mitotic spindle; after division, the spindle in turn is disassembled and the tubulin deployed into a new interphase array. Many MTs continuously vary their length by addition and removal of tubulin from their (+) end, distal to the MTOC. This "dynamic instability" (Mitchison & Kirschner, 1984a,b) can sometimes be observed in live cells. Dynamic instability of spindle MTs is particularly significant during early mitosis (Chap. 10) where their probing of the massed chromosomes ensures that each kinetochore has the opportunity to attach to the mitotic spindle.

MTs have numerous cytoskeletal functions. They direct certain forms of motility, particularly during mitosis. They are often important in morphogenesis when cells transform their shape and internal organization during differentiation. The MT and actin cytoskeletons often interact. As shown in the pseudopodia of the giant amoeba Reticulomyxa, the vigorous streaming is generated over a MT-based cytoskeleton containing actin filaments (Koonce et al., 1986). Transport is mediated by MTs (Travis & Bowser, 1986) which define tracks along which particles move (Travis & Bowser, 1988). In foraminifera, actin and MTs in concert generate the traction forces that move the organism.

Interphase higher plant cells have a cytoskeleton of MTs oriented transversely just inside the cell membrane. These control the deposition of wall microfibrils which in turn controls the shape of growing cells (Gunning & Steer, 1975; Williamson, 1991. Complexes of enzymes in the plasmalemma (often called rosettes) spin out molecules of cellulose which crystallise into microfibrils. The oriented movement of these complexes may be directly influenced by the wall MTs. In protists and fungi, MTs are major cytoskeletal elements controlling cell shape and organization. Anti-MT drugs such as colchicine and nocodazole rapidly and often reversibly break down MTs in living cells.

Gunning, B.E.S. & Steer, M.W. (1975). Reference in Chap. 6.
Koonce, M.P., Eutenauer, U., McDonald, K.L., Menzel, D. & Schliwa, M. (1986). Cytoskeletal architecture and motility in a giant freshwater amnoeba, Reticulomyxa. Cell Motil. Cytoskelet. 6: 521-533.
Mitchison, T.J. (1992). Compare and contrast actin filaments and microtubules. Mol. Biol. Cell 3: 1309-15.
Mitchison, T.J. & Kirschner, M. (1984a). Microtubule assembly nucleated by isolated centrosomes. Nature 312: 232-237.

Mitchison, T.J. & Kirschner, M. (1984b). Dynamic instability of microtubule growth. Nature 312: 237-242.
Travis, J.L. & Bowser, S.S. (1986). A new model of reticulopodial motility and shape: evidence for a microtubule-based motor and an actin cytoskeleton. Cell Motil Cytoskelet. 6 2-14.
Travis, J.L. & Bowser, S.S. (1988). Optical approaches to the study of foraminiferan motility. Cell Motil. Cytoskelet. 10: 126-136.
Williamson, R.E. (1991). Orientation of cortical microtubules in interphase plant cells. Int. Rev. Cytol. 129: 135-206.

Sequences in order
- cultured newt cell, TLR = 50x
- Surirella robusta, a diatom; TLR = 10x approx.
- PtK (mammalian) cell , TLR = 25x
- dividing lung epithelial cell (newt); 2 sequences; TLR = 25x approx.
- microtubules in vitro on microscope slide
- newt lung epithelial cell (computer enhanced); 2 sequences
- giant amoeba Reticulomyxa; 4 sequences; RT
- dividing lung epithelial cell (newt); TRL = 25x approx.
- Confocal reconstruction

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CHAP. 8   MICROTUBULES: CHROMATOPHORES

Some animals can change their color using specialised epidermal cells called chromatophores. In scales of fish, some chromatophores can emerge from the scale and can be cultured on coverslips. Chromatophores contain thousands of pigment granules which are synchronously dispersed or aggregated, often quite rapidly. In the living fish, synchronous activity is controlled by the nervous system. Erythophores in culture (e.g., as shown on this DVD) often pulse and can be aggregated by epinephrine and dispersed with cAMP or caffeine. Dispersion is slow and irregular. Particles oscillate along the MTs with "saltatory" movement, a type common elsewhere, for example, in the asters of the spindle (Chap. 10). Aggregation is smooth and relatively rapid. Other organelles (nucleus, mitochondria etc.) do not move with the pigment.

Movement is generated over a radial cytoskeleton of MTs (Bickle et al., 1966) controlled by a central MTOC (centrosome). The MTs act as guides for granule motion. In pseudopodial extensions, the MTs are of uniform polarity. If such a pseudopod is cut off, the MTs within it reoganise into a radial array in the absence of the centrosome (McNiven & Porter, 1988). The mechanisms of movement are poorly understood (Porter & McNiven, 1982; Porter et al., 1983). Actin does not appear to play a major role. Cytochalasin does not affect movement even though it breaks down the actin cytoskeleton. The MT cytoskeleton is stable and nocodazole and cold treatment is needed to break it down whereupon the cell loses its organization and ability to move pigment. However, the cytoskeleton and granule movement are rapidly restored once these inhibitory conditions are removed. The cell membrane can be stripped off to probe the physiology of movement (e.g., McNiven & Ward, 1988).

Bikle, D., Tilney, L.G. & Porter, K.R. (1966). Microtubules and pigment migration in the melanophores of Fundulus heteroclitus. Protoplasma 61: 322-345.
McNiven, M.A. & Porter, K.R. (1988). Organization of microtubules in centrosome-free cytoplasm. J. Cell Biol. 106: 1593-1605.
McNiven, M.A. & Ward, J.B. (1988). Calcium regulation of pigment transport in vitro. J. Cell Biol. 106:111-125.
Porter, K.R., Beckerle, M. & McNiven, M. (1983). The cytoplasmic matrix. Mod. Cell Biology (A.R.Liss, N.Y.) 2: 259-302.
Porter, K.R. & McNiven, M.A. (1982). The cytoplast: a unit structure in chromatophores. Cell 29: 23-32.

Sequences in order
- scale from squirrelfish TLR = 100x
- the squirrelfish Holocentrus

- scales,   " ;
- ",   " ; higher magnification, 3 sequences; TLR = 5 – 15x
- erythrophore, xanthophore; RT
-    " ; 2 sequences; TLR = 10x – 25x approx.
-    " ; (2 drug treatments); TLR = 10x, 25x
- melanophores from the fish Fundulus; 2 sequences; 25x approx.

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CHAP. 9   MICROTUBULES: AXOPODIA in HELIOZOA

Heliozoa are amoeboid that possess very long, thin axopodia, extensions of the cytoplasm used in prey capture . Axopodia support transport of cytoplamic particles. Food organisms adhere to them and are transported to the cell body whereupon cytoplasm flows out, enclosing the prey in a food vacuole (Hausman & Patterson, 1982) which kill and digest the prey. Some prey are captured by the adhesion of their flagellar membrane to the axopod and unicells such as Chlamydomonas can escape by shedding their flagella.

The core of the axopod is a bundle of tightly organised MTs (e.g., Tilney & Porter, 1965: Febre-Chevalier, 1990) and so is highly birefringent. Axopodia are very sensitive to anti-MT agents such as colchicine (Tilney, 1968). Particle movement along axopodia does not seem to be typical of other MT-based motility (Chaps. 7, 10).   Edds (1975a, b) pushed a glass microneedle through a cell, creating an artificial axopodium over which normal particle transport continued.

Edds, K.T. (1975a). Motility in Echinosphaerium nucleofilum. I. An analysis of particle motions in the axopodia and a direct test of the involvement of the axoneme. J. Cell Biol. 66: 145-155.
Edds, K.T. (1975b). Motility in Echinosphaerium nucleofilum. II. Cytoplasmic contractility and its molecular basis.J.Cell Biol. 66: 156-164.
Febvre-Chevalier, C. (1990). Phylum Actinopoda; Class Heliozoa. In Handbook of Protoctista (Margulis, L., Corliss, J.O., Melkonian, M., Chapman, D.J.,eds.); Jones & Bartlett, Boston; Chap. 20b, pp.347-379.
Hausmann, K. & Patterson, D.J. (1982). Pseudopod formation and membrane production during prey capture by a Heliozoon (Feeding by Actinophrys, II). Cell Motility 2: 9-24.
Tilney, L.G. (1968). Studies on the microtubules in Heliozoa. IV. The effect of colchicine of the formation and maintenance of the axopodia and the redevelopment of pattern in Actinosphaerium nucleofilum (Barret). J. Cell Sci. 3: 549-562.
Tilney, L.R. & Porter, K.R. (1965). Studies on the microtubules in heliozoa. I. The fine structure of Actinosphaerium nucleofilum (Barret), with particular reference to the axial rod structure. Protoplasma 60: 317-344.

Sequences order
     - The organisms illustrated are probably Actinophrys sp.
     - first 5 sequences: TLR = 10 – 25x
     - capture of Chlamydomonas: total time = 25 mins. approx.
     - "   "   protozoan cell; total time = 8 mins.

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CHAP. 10   MICROTUBULES and MITOSIS: ANIMAL CELLS

The stages of mitosis as described in text books are, in real life, part of a continuum of very complex activity. During the transition from prophase to prometaphase , the nuclear envelope breaks down and astral fibres (MTs) from the centrosomes penetrate amongst the chromosomes which immediately start moving. Some chromosomes initially move toward either pole, as kinetochores slide along polar MTs. They oscillate unstably until each pair sooner or later connects to both poles and the balancing of the two polewards forces moves the chromosome to the middle of the spindle. Metaphase is reached slowly as the cell waits till all chromosome becomes correctly oriented. These complex manoeuvres are vital for allowing each chromosome to split and segregate to the poles correctly during anaphase.

At some stage during prometaphase, polar MTs become attached to individual kinetochores by terminating in them, after which chromosomal movement is coupled with changes in their length. During anaphase, as the chromosomes move to the poles, these MTs shorten. The changes in length are probably controlled by the kinetochore, although what is happening is not clear. In addition, during early mitosis some fibres from one aster interact with some from the other, creating overlapped, "continuous" fibres that run from pole to pole. These fibres later form the basis of the midbody that arises at the cleavage furrow during cytokinesis.

The mechanisms that move chromosomes are still poorly understood. Chromosome movement in many cells is very slow (often 1 uM/min.), and the minimum theoretical power needed to move them at this speed is very low (Nicklas, 1975); in practice, the force exerted is considerably higher than the minimum necessary (Nicklas, 1983).

Logistical Aspects of Mitosis. Replication of DNA has to be extremely accurate to preserve the integrity of the cell's genetic material over millions of cell divisions (about 1 mistake per billion bases replicated: Wolfe; 1993: p. 964). Chromosome replication in eucaryotic cells poses great logistical problems. The two copies of replicated DNA have to be assembled into two adjacent chromatids without tangling. In humans, for example, 45 chromosomes each containing on average a meter of DNA are packed into the tiny nucleus (Chap. 3). Next, these chromosomes (paired chromatids) have to be individually organised so that each new cell will inherit one, and only one, chromatid from each chromosome. To put these logistics in perspective: at any moment, hundreds of millions of cells are undergoing mitosis in your own body and every division must be absolutely accurate. The mitotic spindle is a machine of astounding reliability and accuracy. Mitosis is reviewed very frequently; see McIntosh & McDonald (1989) on the structure of the spindle and McIntosh & Koonce (1989) on its physiology.

McIntosh, J.R. & Koonce, M.P. (1989). Mitosis. Science 246: 622-628.
McIntosh, J.R. & McDonald, K.L. (1989). The mitotic spindle. Scientific American 261.Pt.4: 26-35.
Wolfe, S.L. (1993). Molecular and Cellular Biology. Wadsworth, Inc., Belomont, Calif.: 1145 p.

Sequences in order
all newt epithelial cells
- confocal images
     -(TEM is of Oedogonium)
     - first squence: total time = 4 hrs. approx.
     - second sequence: 1.5 hrs. approx
     - third sequence: 1.5 hrs. approx.
     - fourth sequence: TLR = 25X

CHAP. 11 MICROTUBULES and MITOSIS: HIGHER PLANT CELLS

The mitotic spindle of higher plant cells is morphologically different to that of animal cells. Sexual reproduction in most land plants no longer relies on flagellated sperm cells and the ability to form centrioles and flagella has been lost. The spindles do not have asters; instead, the poles are broad and unfocussed. More primitive land plants (e.g., mosses, liverworts, ferns, cycads and even Ginkgo) still use flagellated sperm during sexual reproduction. The spindle in vegetative cells is typical of higher plants; however, during spermatogenesis, centrioles appear de novo and are then associated with spindle poles. The spindle in these spermatogenous cells now becomes astral, like that of animal cells. Thus, the centriole is non-essential for spindle structure and function (Pickett-Heaps, 1969) and astral and non-astral spindles are functionally similar.

For cytokinesis, higher plants do not cleave. Instead, the phragmoplast arises amongst the remnant spindle MTs between the daughter nuclei. These MTs proliferate and guide components such as Golgi-derived vesicles into the forming cross-wall or cell plate. The out-growing edge of the phragmoplast then locates the division site previously occupied by the "Preprophase Band" (next para.). Green land plants were derived from green algae which cleave . The alga Spirogrya illustrates how the phragmoplast could have evolved (Fowke & Pickett-Heaps, 1969).

Plane of cell division: the Preprophase Band of Microtubules

Plant tissues consist of organized, often geometric patterns of cells. Bounded by rigid walls, these cells cannot move and thus the geometry of tissues is established by their cell divisions (Gunning, 1982; Gunning et al., 1978). At the cytological level, higher plant cells display the enigmatic Preprophase Band of Microtubules (PPB), a gathering derived from wall MTs (Chap. 7) which assembles prior to prophase and disappears by prometaphase. Its position indicates exactly where the phragmoplast will join up with existing cell walls, and thus it predicts the plane of cytokinesis (Pickett-Heaps & Northcote, 1966). Actin also appears to be present in the PPB (Li et al., 2006; Palevitz, 1987). The response of asymmetric divisions to cytochalasin, shown in the DVD, suggest that the MT and actin cytoskeletons are both involved in morphogenesis. The role of the PPB remains to be determined (Gunning & Wick, 1985).

These asymmetric divisions illustrate "polarization". The first asymmetric division requires an epidermal cell to respond to an unknown general morphogenetic gradient, while the formation of subsidiary cells attests to the powerful, highly localised polarization induced by the Guard Mother Cell. Expanding phragmoplasts curve markedly as they seek out the site earlier occupied by the PPBs which appear to have left some imprint in the cell cortex.

Fowke, L.C. and J.D. Pickett-Heaps (1969). Cell division in Spirogyra. II. Cytokinesis. J. Phycol. 5: 273-281.
Gunning, B.E.S. (1982). The cytokinetic apparatus: its development and spatial regulation. In The Cytoskeleton in Plant Growth and Development (C.W. Lloyd, ed.), Acad. Press, N.Y.; pp. 229-292.
Gunning, B.E.S., Hughes, J.E. & Hardham, A.R. (1978). Formative and proliferative cells divisions, differentiation and developmental changes in the meristem, of Azolla roots.. Planta 143:121-144.
Gunning, B.E.S. & Wick, S.W. (1985). Preprophase bands, phragmoplasts and spatial control of cytokinesis. J. Cell Sci. Suppl. 2: 157-179.
Li, C-L., Chen, Z-L. & Yuan, M. (2006). Actomyosin is involved in the organization of the microtubule preprophase band in Arabidopsis suspension cells. J. Intergrative Plant Biol. 48: 53-60.
Palevitz, B.A. (1987). Actin in the preprophase band of Allium cepa. J. Cell Biol. 104: 1515-1519.
Pickett-Heaps, J.D. (1969). The evolution of the mitotic apparatus: an attempt at comparative ultrastructural cytology in dividing plant cells. Cytobios 3: 257-280.
Pickett-Heaps, J.D. and D.H. Northcote (1966). Cell division in the formation of the stomatal complex in the young leaves of wheat. J. Cell Sci. 1: 121-128.

Sequences in order
- confocal images
- stamen hair, Tradescantia; total time = 45 mins. approx.
- endosperm, Haemanthus; total time = 3 hrs. approx.
-     "   "   "   "   = 1.75 hrs. "
- stamen hair, Tradescantia; total time = 40 mins. approx.
- (LM of root tip, stem)
- stomatal complexes : all of Tradescantia; mitoses each last about 1 hr.
- confocal reconstructions

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CHAP. 12 ACTIN: CYTOPLASMIC MOVEMENT

Actin is abundant, up to 15% of the total protein in some cells. Actin filaments are the second major cytoskeletal components and share certain properties with MTs (Chap. 7) which is confusing. For example, some MT-based movement (Chap. 7) resembles actin-generated streaming (Chap. 13). Like MTs, actin filaments are polar, assembly/disassembly occurring mainly at one end. Actin-associated proteins confer various properties. Myosin complexed with actin generates motility and is the major constituent of muscle. Tropomyosin strengthens actin filaments laterally, filamin cross-links them into a gel, and spectrin links them to the plasmalemma. In cultured mammalian cells, actin bundles are called stress fibres.

Like MTs, some actin filaments are dynamic; however, the flux of subunits is steady and unidirectional,

unlike the dynamic instability of MTs. This cycling is easiest seen in microspikes growing from the cell margin as bundles of actin filaments steadily assemble at their tip ("+" end) and concurrently disassemble in the cell body. Microspikes and filopodia may be very long (e.g., up to 50 uM in growth cones) and enable the cell to explore its surroundings; when they stick to the substrate, they can move the cell. Ruffling of cell margins (Chap. 12) is generated by actin cycling.

In many naked animal or protistal cells, locomotion is generated by the actin cytoskeleton via amoeboid movement . The clear edges of pseudopodial outgrowths often contain only a meshwork of actin filaments. Actin is involved with other activities, such as rotation of the chloroplast in the alga Mougeotia.

A family of drugs called the cytochalasins specifically disrupts actin cytoskeletons. Different cytochalasins act in different ways. Some stop polymerization of actin into filaments while disassembly continues. Comparison of the effects of anti-microtubule and anti-actin drugs reveals the relative contribution of these cytoskeletal systems to cell organization and behaviour. For example, the rotation of the chloroplast in Mougeotia, illustrated in this DVD, is stopped by cytochalasin (Wagner et al., 1972).

Wagner, G., Haupt, W. & Laux, A. (1972). Reversible inhibition of chloroplast movement by cytochalasin in the green alga Mougeotia. Science 176: 808-809.

Sequences in order
- confocal images, then cultured newt lung epithelial cells; 2 sequences; TLR = 25-50x
- protozoan (Penardia sp.?); TLR = 25x
- amoeboid protozan; TLR = 25x
-     "   " ; TLR = 25x
- coelomocytes (from sea urchin embryos); three sequences
- Spirogyra (green alga); duration = 4 hrs. approx.
- Mougeotia (green alga); 2 sequences; duration = 15 mins. approx.
_____

CHAP. 13:   ACTIN: STREAMING in PLANT CELLS

In small cells, diffusion is adequate to distribute the myriad molecules needed for life throughout the living cytoplasm but the rate of diffusion falls off markedly as cell get bigger. Plant cells are usually much bigger than animal cells and diffusion is augmented by active stirring of cytoplasm brought about by cytoplasmic streaming. Long fine filaments associated with particles can sometimes be visualised in streaming cytoplasm of some large plant cells; movement is sensitive to cytochalasin. LM using suitably fixed and stained cells reveals a cytoskeleton that is highly variable in morphology.

Giant algal cells in the alga Chara are often 1-2 cms. long, and are wonderful subjects for illustrating cytoplasmic streaming.   In the largest cells, the streaming cytoplasm is organised into one continuous band oriented longitudinally, spiralling slightly. This rapid cyclosis stirs the vacuole , slowly swirling the many crystalline bodies in it. The motive force is generated over a uniform layer of chloroplasts attached to the wall. At this interface is the actin cytoskeleton of parallel bundles of filaments of uniform polarity. These bundles can be preserved for TEM and LM.

These cells are favored objects for experimentally investigating streaming and the activity of the actin observed in vitro (Higashi-Fujime, 1980; Kamiya, 1981). Sheetz & Spudich (1983) slit open the cells while keeping the actin cytoskeleton intact on the chloroplasts; myosin-coated beads placed on these filaments in ATP moved along the filaments like particles in vivo.

Higashi-Fujime, S. (1980). Active movement in vitro of bundles of microfilaments isolated from Nitella cells. J. Cell Biol. 87: 569-578.
Kamiya, N. (1981). Physical and chemical basis of cytoplasmic streaming. Ann. Rev. Plant Physiol. 32: 205-236.
Sheetz, M.P. & Spudich, J.. (1983). Movement of myosin-coated fluorescent beads on actin cables in vitro. Nature 303: 31-35.

Sequences in order
        - Tradescantia stamen hair cells; 2 sequences; RT
         - confocal reconsytuctions
        - Micrasterias thomasiana (desmid, green alga); RT
- Noctiluca scintillans (dinoflagellate); three sequences; first two in reatime, third: TLR = 25x.
next:
        all are of unidentified species of Chara, Nitella ; RT

CHAP. 14 ACTIN: CELL CLEAVAGE

Cytokinesis by cell cleavage usually follows mitosis. The cytoskeletal systems involved in mitosis and cytokinesis are separate but coordinated in time (so cytokinesis follows chromosome separation) and in space (so the cell cleaves between daughter nuclei). The cytokinetic system is set up in prophase when it can often be detected by slight infurrowing. Anaphase triggers contraction in the furrow.

        Cleavage is brought about by ring of actin filaments lining the cell membrane which interacts with myosin concentrated into the site of cleavage . These filaments contract during telophase like a purse-string, pinching the cell in two (Schroeder, 1973, 1976). Cleavage is sensitive to anti-actin drugs. Chandler's review (1992) shows striking views of cleavage .

Chandler, D.E. (1992). The ultrastructure of development: the early cleavages. Microscopy Res. Tech. (1992); Vol. 22, no. 1
Schroeder, T.E. (1973). Actin in dividing cells: contractile ring filaments bind heavy meromyosin. Proc. Nat. Acad. Sci. USA. 70: 1688-1692.
Schroeder. T.E. (1976). Actin in dividing cells: evidence for its role in cleavage but not mitosis. Cold Spring Harbour Conf. Cell Prolif. 3: 265-278.

Sequences in order
        - PtK (mammalian) cell; total time = 25 mins. approx
        - fertilised egg of Xenopus; total time = 4 hrs. approx.
        - Surirella robusta (diatom); TLR = 10x
        - Coscinodiscus (centric diatom); three sequences; TLR = 25 – 50x
        - PtK cells; two sequences; each sequence 30 mins. approx.

CHAP. 15 FLAGELLA and MOTILITY

        Flagella and cilia are equivalent, although the term cilia is often used when they are numerous. They are found in most types of eucaryotic cells and the structure of the flagellum is, apart from trivial details, identical in all organisms (Gibbons, 1981), and extremely conservative during evolution.   The pattern of nine flagellar doublet MTs is templated by the basal body which, by definition, is an MTOC. The flexing of flagella is generated by the sliding of the sets of doublet MTs with respect to each other. The sliding is constrained by radial links between the doublets and thus induces local bending in the whole flagellum. Sliding is generated by a specific MT-associated protein, dynein, that hydrolyses ATP (Summers & Gibbons, 1971).

        A few major groups of organisms do not have flagella. Some (e.g., red algae) appear to have been phylogenetically isolated from the rest of living organisms before the flagellum had evolved . Since these non-flagellated groups display MTs, flagellar must have been a later separate development .   The other non-flagellate groups comprise organisms which primitively did possess flagella but which lost them after dispensing with the need for water and swimming gametes. Examples include most terrestial green plants and fungi such as the ascomycetes and basidiomycetes. A few of these non-flagellated groups (the Zygnematales in the green algae, and the pennate diatoms) appear to have adapted to terrestrial life, losing

their flagella, and then reverting to a life inwater . These groups are thus the algal equivalent of seals and whales.

Flagella usually beat in a "breast stroke" or a planar sinusoidal or helical beat. Basal bodies are attached to an intricate assemblage of fibrous and microtubular elements which control flagellar beat and transmit the stresses of flagellar motility to the cell. The microtubular "rootlet" cytoskeleton thus arising has a precise architecture, diagnostic for each group of flagellates.

In most biflagellate unicells, the flagella differ in length, structure and function but even when they are apparently identical (e.g., in Chlamydomonas), there are functional differences between them (Kamiya & Witman, 1984; Holmes & Dutcher, 1989). Flagellar asymmetry is related to the apparently universal phenomenon of flagellar transformation. In biflagellated cells, one flagellum is the mature form (usually, but not always, the long flagellum). Before cell division, two new immature (short) flagella appear while the parental immature (short) flagellum is transformed into the mature (long) form which may require morphogenetic changes such as addition or removal of flagellar hairs. During cleavage, each cell then gets one short and one long flagellum. Thus, the flagellum takes two cell cycles to become mature. For an example of the two different flagella and their functions, see Epipyxis in Chap. 18; flagellar transformation in this organism is described by Wetherbee et al., (1988). Centrioles (e.g., in mammalian cells) also undergo transformation (thus was not recognised for many years). During the S-phase of the cell cycle, a new, short centriole arises adjacent to each of the parental pair of centrioles. These (immature) centrioles often remain shorter for the remainder of that cell cycle, elongating before the next cycle of cell division.

Many flagella are decorated with fine hairs which increase the effective diameter of the flagellum (Bouck & Rogalski, 1982; Hollwill, 1982) and profoundly affect the hydrodynamics of flagellar function. For example (Bouck, 1972), longer and rigid hairs in certain algae called mastigonemes. reverse the direction of flow of water and thus the direction of swimming; cells move with the flagellum directed forward.

Many ciliated protists have such an extensive and dense covering of highly coordinated flagella. These cells often swim very fast and respond instantly to stimuli by reversing the beating patterns of their flagella. The cytoskeleton underlying the cell membrane is extraordinarily complex, utilising a range of fibrous and microtubular components .

Sequences shown, in order

- unidentified flagellate; RT
 - Gonium sociale, RT
- Chlamydomonas; four sequences; all RT
- sperm, newt cells; RT
- newt lung epithelial cells; four sequences; all RT
- Apiocystis (green alga); RT
- Phacus (euglenoid flagellate); three sequences; all RT
- Eutreptiella (euglenoid flagellate); two sequences; RT
- Scripsiella (dinoflagellate); two sequences; RT
- Ceratium maceros (?) (dinoflagellate); RT
- Dinobryon (colonial chrysophyte); RT
- Pyramimonas propulsa (green alga); two sequences; RT
- Paramecium; two sequences; RT

Bouck, G.B. (1972). Architecture and assembly of mastigonemes. Adv. Cell Mol. Biol. 2: 237-271.
Bouck, G.B. & Rogalski, A.A. (1982). Surface properties of the euglenoid flagellum. Symp. Soc,. Exp. Biol. 35: 381-398.
Gibbons, I.R. (1981). Cilia and flagella of eucaryotes. J. Cell Biol. 91: 1075-1245.
Holmes, J.A. & Dutcher, S.K. (1989). Cellular asymmetry in Chlamydomonas reinhardtii. J. Cell Sci. 94: 273-285.

Holwill, M.E.J. (1982). Dynamics of eucaryotic flagellar movement. In Procaryotic and Eucaryotic Flagella (Amos, W,B., Duckett, J.G., eds.), Camb. Univ. Press: pp289-312.

Kamiya. R. & Witman, G.B. (1984). Submicromolar levels of calcium control the balance of beating between the two flagella in demembranated models of Chlamydomonas. .J. Cell Biol. 98: 97-107.

Rieder, C.L. & Borisy, G.G. (1982). The centrosome cycle in PtK cells: asymmetric distribution and structural changes in the pericentriolar material. Biol. Cell. 44: 117-132.

Summers, K.E. & Gibbons, I.R. (1971). Adenosine triphosphate-induced sliding of tubules in trypsin-treated flagella of sea-urchin sperm. Proc. Nat. Acad. Sci. U.S. 68: 3092-3096.

Wetherbee, R., Platt, S.J., Beech, P.L. & Pickett-Heaps, J.D. (1988). Flagellar transformation in the heterokont Epipyxis pulchra (Chrysophyceae): Direct observations using image enhanced light microscopy. Protoplasma 145: 47-54.

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CHAP. 16   FLAGELLA and PREY CAPTURE

Heterotrophic cells must contact their prey to capture it and the chances of encounters are increased greatly if the predator is swimming. Alternatively, stationary cells can use their flagellar/ciliary apparatus to create water currents that bring food organisms to them (reviewed by Fenchel, 1986). The entrapment of prey by this means also attracts larger organisms, even predators, so filter feeders have rapid avoidance reactions, which also rid the cell of unsuitable prey or detritus. The stalk of Vorticella, for example, can contract extremely rapidly.

Fenchel, T. (1986). Protozoan filter feeding. Prog. Protistology 1: 65-113.

Sequences in order
- Paramecium, RT
- Euplotes; five sequences; TLR = 2x
 - Charcesium (?); RT
- Vorticella ; all RT, except repeated sequence

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CHAP. 17   SEX in CHLAMYDOMONAS

While flagella normally are organelles of motility, in higher organisms modified flagella also serve as sensory organelles sensitive to stimuli such as chemicals (for taste and smell), light (in photoreceptors) and mechanical vibrations (e.g., the extremely sensitive sensilla of cockroachs). Even in animals like Hydra, modified cilia in `cnidocils' control discharge of nematocysts. Chapters 17 and 18 illustrate how unicellar organisms also can use flagella to convey external information into the cell.

Sexual reproduction involves the fusion of two specially differentiated gametes. In Chlamydomonas, sexual reproduction is initiated when vegetative cells divide to form two types of gametes. While superficially identical, TEM shows that these "+" and "-" gametes are different with complementary mating structures. Gametes use their flagella to find compatible gametes (Musgrave & van den Ende, 1987), first by forming clumps adhering by their flagella. Soon, gametes pair off, always "+" with "-". Thus the flagella serve as sensory organelles in the search for a compatible mating gametes.

The flagella adhere due to macromolecules called agglutinins. The flagella usually initiate pairing at their tip and adhesion proceeds lengthwise along the flagella, steadily bringing a specific area of the gametes' membrane between the flagellar bases into close juxtaposition. It also triggers differentiation of a tiny mating papilla from one gamete between the flagellar bases (just visible in the video sequences). The incessant movement of the gametes brings this papilla into repeated contact with an equivalent region of differentiated membrane of the other gamete, whereupon the cell membranes fuse. The papilla contains a system of actin filaments that pull the gametes together (Detmers et al., 1983). Meanwhile, the cell wall is shed (see Chap. 19), allowing the cells to fuse.

Detmers, P.A., Goodenough, U.W. & Condeelis, J. (1983). Elongation of the fertilization tube in Chlamydomonas: new observations on the core microfilaments and the effect of transient intracellular signals on their structural integrity. J. Cell Biol. 97: 522-532.

Musgrave, A. & van den Ende, H. (1987). How Chlamydomonas court their partners. Trends Biochem. Sci. 12: 470-473.

Sequences in order
- All Chlamydomonas; RT

_____

CHAP. 18 FEEDING in EPIPYXIS

The chrysophyte Epipyxis secretes a vase-shaped lorica (cell wall) attached to a substrate. The biflagellate cell attaches to the base of this lorica via a long thin, contractile stalk. The cell will leave the lorica if disturbed.

Like many chrysophytes, Epipyxis is heterotrophic, supplementing photosynthesis by capturing and ingesting small organisms. During feeding, the longer flagellum, covered with hairs, beats rapidly, creating a current which sweeps particles on to the relatively immobile, shorter flagellum. The particles are held on the short flagellum; often the long flagellum briefly stops beating and the particle is held between them. Within a second or two, the cell decides how to respond, a decision presumably based on sensory information from the shorter flagellum. Rejected particles are cast off immediately, but others are rapidly enveloped by a loop of cytoplasm (Wetherbee & Andersen, 1992; Andersen & Wetherbee, 1992)

Andersen, R.A. & Wetherbee, R. (1992). Microtubules of the flagellar apparatus are active during prey capture in the chrysophyte alga Epipyxis pulchra. Protoplasma 166: 8-20.

Wetherbee, R. & Andersen, R.A. (1992). Flagella of a chrysophycean alga play an active role in prey capture and selection. Protoplasma 166: 1-7.

Sequences shown
-        all are of Epipyxis pulchra, RT except that mentioned: TLR = 6x

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CHAP. 19 CELL WALLS

The importance of even thin cell walls can be seen in Chapter 9 on heliozoa. The only part of the Chlamydomonas cell exposed to the external environment is the membrane covering the flagella.   The value of thick gelatinous walls in protecting cells against dessication is obvious. Such coatings also inhibit other cells (e.g., bacteria or fungi) attacking or attaching to them. Many unicellular plants secrete copious amounts of mucilage for such purposes. Spirogyra, for example, is slimy and very few organisms can attach to it.

Sequences in order
- Chlamydomonas; RT
- Haematococcus; two sequences; RT
- Apiocystis (green alga); RT
- Micrasterias hardyii (desmid, green alga); TLR = 25x
- Euglena; TLR = 25x
- Mallomonas splendans (chrysophyte); two sequences; RT
- Choanocystis (Acanthocystis); TLR = 25x
- Surirella, Cymatopleura (diatoms); TLR = 25x
- Odontella; first sequence: RT; cell division: total time 1.5 hrs. approx.

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CHAP. 20 CONTRACTILE VACUOLES

Contractile vacuoles are essential organelles freshwater cells that are naked or have a non-structural wall. The osmotic potential of cytoplasm is high and water is taken up continuously through the cell membrane; the cell will expand and burst unless water is pumped out of the cell at an equal rate. The contractile vacuole(s) serves this function.

Many algae enclosed in a rigid wall, reproduce by motile swarmers (zoospores, gametes). Once these motile cells emerge from the parental wall, they immediately develop contractile vacuoles to balance osmotic uptake of water . Contractile vacuoles also shrink gametes within the wall, for example, during conjugation in Spirogyra (Chap. 12) and Closterium (Chap. 1).

Sequences in order
  - Mallomonas splendans; TLR = 25x
  - heliozoan (probably Actinophrys)
  - Phacus (two sequences); TLR = 50x
  - Paramecium; four sequences; TLR = 2-5X

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CHAP. 21 VACUOLES: TURGOR PRESSURE

Many plant cells contain a large watery vacuole which may occupy more than 95% of the cell . The vacuole is contained within the vacuolar membrane or tonoplast and it often becomes the repository for secondary metabolites, waste compounds, storage materials, or else pigments like anthocyanins which give many flowers their bright colours. The vacuole also confers protection against herbivores when toxic or evil-tasting compounds are concentrated within it.   Chap. 23 shows a remarkable development of the vacuole for protective purposes.

In cells that make up plant tissues, the ions and metabolites in the vacuole generate turgor pressure (5-20 atmospheres). This pressure, contained within a wall, gives cells and thus tissues their rigidity. When plants wilt through lack of water, turgor pressure drops. Upon watering, wilted plants recover by taking up water through their roots and rapidly transporting it through all tissues.

The experiments illustrated here on Nitella are simple and reliable and they are recommend ed for biology practical classes.   Green & Stanton (1967) have directly measured the internal pressure in Nitella at between 5.1-5.7 atmospheres.

Green, P.B. & Stanton, F.W. (1967). Turgor pressure: direct manometric measurement in single cells of Nitella. Science 155: 1675-1676.

Sequences in order
  - the pot plant is Coleus; recovery took about 45 mins.
  - cut stems are from celery
  - epidermis, red onion; TLR = 25x
  - Nitella; TLR 2 – 4x

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CHAP. 22   TURGOR PRESSURE and GROWTH

Plant cells are able to grow in volume very rapidly (e.g., up to 1uM/min.), much faster than animal cells. Turgor pressure drives cell expansion in cells enclosed within a wall . This wall must be plastic enough to permit stretching and it is called a primary cell wall . Once higher plant cells stop growing, they deposit a rigid, strong secondary cell wall within the primary wall.
Single-celled protists illustrate interesting variations on this theme.   In desmids (green algae), cytokinesis cuts the symmetrical parent cell in half. Then each daughter cell secretes a ballooning complementary half cell enclosed in a primary wall; expansion is driven by turgor pressure. Once the new cells reach full size, the secondary wall is laid down inside the primary wall which is later shed. Expansion of the primary wall is controlled with remarkable precision by unknown mechanisms (Pickett-Heaps,

1983).. The filamentous alga Oedogonium has evolved a unique mechanism for achieving growth. The rigid wall of each cell has a circumferential weakening at the apical end. Prior to division, a dough-nut shaped mass of primary wall material is secreted at exactly this site. After cytokinesis, turgor pressure ruptures the wall at the weakening and then stretches the ring material to enclose one of the two daughter cells. Later, a secondary wall is secreted within this primary wall (Pickett-Heaps (1975).

Diatoms have evolved a completely different approach. Their protective walls or thecae are made of silica which is completely inextensible. So each theca is composed of two overlapping segments which fit tightly together. To allow growth, the theca slide apart slide.

Pickett-Heaps, J.D. (1983). Morphogenesis in Desmids: Our current state of ignorance. IN: Spatial Organization of Eucaryotic Cells, (J.R. McIntosh, ed.), Alan R. Liss, Inc., New York. Vol. II, pp. 241-258. Pickett-Heaps, J.D. (1975). Green Algae: Structure, Reproduction and Evolution in Selected Genera. Sinauer Assoc., Stamford, CT; 606 pages, 1975.

Sequences in order
- Micrasterias thomasiana (desmid, green alga); total time 1.5 hrs. approx.
- Micrasterias sp. (probably M. lux); total time 1.75 hrs. approx.
- Oedogonium cardiacum (green alga): total time 1.5 hrs. approx.
- Odontella: total time = 1.25 hrs. approx.

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CHAP. 23 CELLS in ATTACK and DEFENSE

Many protistal cells possess ejectile organelles (generically called ejectosomes) which discharge explosively when the cell is irritated. These complex organelles are of several different types and their roles are uncertain. They are often said to be involved in prey capture or predator avoidance but direct demonstration of such functions is rare. The single-celled chloromonad Vacuolaria is shown discharging spiral trichocysts. Surprisingly, the cell is pulled in the direction the shaft is discharged. Since water is viscous at the scale of the cell, the spiralling shaft probably "screws" itself forward at the instant of discarge.

Animals and higher plants have evolved some remarkably complex cells for offence or defence. One example of each is given. Many plants deter grazers by concentrating unpleasant or toxic materials such as calcium oxalate in the vacuoles of certain cells (Chap. 23). This strategy has evolved to an extraordinary degree in Diffenbachia (Middendorf, 1982) where oxalate is crystallized into hundreds of needles, the raphides, stored in cells called idioblasts which are also filled with mucilage. When the leaf is chewed and the idioblasts damaged, the mucilage absorbs water. Increasing pressure ejects the needles over 20-30 minutes. Additioally, these cells synthesise and store toxic enzymes related to venoms (Middendorf, 1982) which inflame throat tissue already irritated by the raphides.

The cnidarians (jellyfish, corals, hydroids) are primitive animals that possess tentacles in which are found remarkable stinging cells, the cnidocytes. These differentiate a long, complex barbed shaft called the nematocyst. When the cell's trigger (a modified cilium), is touched, the nematocyst is explosively ejected, penetrating and attaching to a prey organism which is injected with a paralysing venom. Note that the nematocyst is jerked forward, in the direction of discharge, like the trichocysts shown just previously. The cnidocytes of the box jellyfish, shown here, are so toxic that multiple stings have proved fatal to humans.

Middendorf, E. (1982). The remarkable shooting idioblasts. Carolina Tips 45: 5-26.

Sequences in order
- the alga is Vacuolaria viriscens
- the plant is Diffenbachia; sequences of release: TLR = 10-25x
- cnidocytes of Hydra, box jellyfish

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Symbiosis is the association of two different organisms that live in a partnership that benefits both of them, and maintained even if they are capable of independent existence. Lichens, for example, are the result of a symbiosis between fungi and algae which allows them to survive in ecosystems where neither can live by itself. (Technically, this is a 'secondary' symbiosis – see below - since the alga itself arose from a previous symbiosis). A symbiotic association between corals and dinoflagellates is essential to the survival of coral reefs (Trench, 1987).

Symbiotic associations between algae and non-pigmented protists are common. That chloroplasts evolved from such symbiotic algae was proposed in the 1880s (Rothschild & Heywood, 1987). Symbionts have progressively lost or transferred more and more of their genetic information to their host cell nucleus. In Secondary Endosymbiosis, a host cell took up an alga that was itself the result of an earlier symbiosis; S.E. gve ris to the all the golden brwn resulted in the range of extant protists (Margulis, 1981; Cavalier-Smith, 1986; Reisser, 1992). The evidence for such an origin of chloroplasts and mitochondria is various and compelling. For example, they both have their own DNA and a procaryotic physiology and molecular biology. Molecular phylogenies confirm a phyletic origin of host eucaryotic cells separate to that of the symbionts.

The green alga Chlorella commonly establishes symbiotic relationships with protists such as Vorticella (Graham & Graham, 1980) and small animals like Hydra. Paramecium bursaria and its Chlorella symbiont can grow separately, but immediately establish a symbiosis if they encounter each other (Reisser, 1986). Chlorella and relatives such as the Crucigenia shown ingested in the amoeba in this chapter, are enclosed by an extraordinarily resistant wall (Atkinson t al., 1972), perhaps a useful preliminary step toward symbiosis.

Cyanophora paradoxa represents a later stage in the evolution of chloroplasts, in this case from a blue-green symbiont. Its cyanelle strongly resembles a blue-green alga but neither host nor cyanelle can survive independently. The cyanelle has lost about 90% of its genome while acquiring a few genes from its host. Thus, the cyanelle has become a true organelle. The chloroplasts of euglenoid algae were derived from a green alga (Gibbs, 1978). The colorless euglenoids have lost their chloroplasts and must survive heterotrophically. The chloroplasts of Chlorarachnion were derived from a green alga (Hibberd & Norris, 1984). Each is still contained within its original cell membrane which also includes some cytoplasm and a nucleomorph with three small chromosomes ((Eschbach et al., 1991). The nucleomorph was first recognised in cryptophytes (Greenwood et al., 1977; Gillot & Gibbs, 1980).

A binucleate dinoflagellate was first described by Dodge (1970) and its second nucleus turned out to belong to a chrysophyte or diatom symbiont (Tomas & Cox, 1973; Jeffrey & Vesk, 1976). More recently, a green, chlorophyll b-containing dinoflagellate has been discovered (Watanabe et al., 1987, 1990); chlorophyll b is characteristic of green algae.

Atkinson, A.W., Gunning, B.E.S., & John, P.L.C. (1972). Sporopollenin in the cell wall of Chlorella and other algae: ultrastructure, chemistry, and incorporation of 14C-acetate, studied in synchronous cultures. Planta 107: 1-32.
Cavalier-Smith, T. (1986). The kingdom Chromista: origin and systematics. Prog. Phycol. Res. 4: 309-347.
Dodge, J.D. (1970). A dinoflagellate with both a mesocaryotic and a eucaryotic nucleus. Protoplasma 73: 145-157.
Douglas, S.E. (1992). Eukaryote-eukaryote endosymbioses: insights from studies of a cryptomonad alga. Bio Systems 28: 57-68.
Eschbach, S., Hoffmann, C.J.B., Maier, U.-G. Sitte, P. & Hansmann, P. (1991). A eukaryotic genome of 660kb: electrophoretic karyotype of nucleomorph and cell nucleus of the cryptomonad alga, Pyrenomonas salina. Nucleic Acids. Res. 19: 1779-1781.
Graham, L.E. & Graham, J.U. (1980). Endosymbiotic Chlorella (Chlorophyceae) in a species of Vorticella

(Ciliophora). Trans. Am. Microscop. Soc. 99:160-166.

Greenwood, A.D., Griffiths, H.B. & Santore, U.J. (1977). Chloroplasts and cell compartments in Cryptophyceae. Br. Phycol. J. 12: 119.

Gibbs, S.P. (1978). The chloroplasts of Euglena may have evolved from symbiotic green algae. Can. J. Bot. 56: 2883-2889.

Gillot, M.A. & Gibbs, S.P. (1980). The cryptomonad nucleomorph: its ultrastructure and evolutionary significance. J. Phycol. 16: 558-568.

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Sequences shown, in order
- Paramecium
- Unidentified amoeboid protozoan and blue-green algae
- Amoeba; TLR = 25x
- Paramecium bursaria; TLR = 10x
- Vorticella; RT
- Cyanophora paradoxa; two sequences; RT
- Chlorarachnion reptans; several sequences; TLR = 10 – 25x
- Lepidodinium
- Hydra; two sequences; TLR = 2x