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IV. SUBCELLULAR POLARIZATIONS

B. Surface membranes

1. Primitive membranes

Polarization of synthetic membranes has been studied by Wijmans (1984).

2. Plasma membrane

a) *Biochemical properties*

The Singer's (1971) ''mosaic'' model has long been the main representation of membrane structure (see **I**, pp. 75-77). The observation that surface proteins and lipids tend to remain on the same membranar surface but are able to move laterally, has provided ground for the more recent ''fluid mosaic'' model of membrane structure in which proteins ''float upon a sea of lipids'' (Rothman and Lenard, 1977). The structure and insertion of integral proteins in membranes have recently been reviewed by Singer (1990).

b) *Structural polarity and recognition systems*

The electrochemical gradient across the membrane is negative inside (see d); it should prove favorable for hydrophobic sequences to insert with their positively charged ends inside (Yamane and Mizushima, 1988). However, according to the ''positive-inside rule'' of von Heijne (1986) the electrical polarization of membranes requires that hydrophobic sequences of proteins to orient themselves with the most positively charged end in the cytoplasm. According to Boyd and Beckwith (1990) ''membrane spanning stretches generally have a positive charge on the cytoplasmic side and few arginine or lysine residues in short extracellular domains''. Thus, charged amino acids play a role in the localization of secreted and membrane proteins. For instance, in bacteria a net dipole around the hydrophobic segment of the signal sequence may be important for this function. Topological models of membrane proteins could be derived from these new facts to explain why improper charge distribution around the signal sequence blocks secretion (Fig. 1 in Boyd and Beckwith, 1990).

Electromagnetic fields (EMF) have been incriminated in growth regulation for ''changing the pattern of charges on the surface of cell membrane so that the receptor is not in the best configuration to transmit its signal'' (Lubin cited by Pool, 1990). EMF exposure has also been reported to alter DNA synthesis (see also Pool, 1990) as well as calcium flow through the cell membrane which is important for the signaling process.

Second messenger waves implicated in cell signaling have many similarities to action potentials (IV.B.e) and to chemical waves in excitable media. According to

Meyer (1991), ''several types of chemical waves have been studied in systems that exhibit chemical reactions far from thermodynamic equilibrium''. This reviewer also questions ''How are diffusion and amplification related to the shape and the velocity of the wave ?'' A model amplifier is then described and mechanisms are postulated for both cAMP and calcium waves as being generated by diffusion and amplification of second messenger molecules in myocytes and oocytes.

Cadherins are newly identified Ca^{2+}-dependent cell-cell adhesion receptors. Their regulated expression controls cell polarity and tissue morphogenesis (Takeichi, 1991). The problem remains of how cadherin-bearing cell-cell junctions achieve a polarized distribution in cells (see VII.C.6b).

c) *Energy transduction and transport*

Mitchell's chemiosmotic hypothesis (see **I** and **II**) is still a challenge in bioenergetics (see in Anthony, 1988) and the important question arises from Kell (1988): ''Is the protonmotive force an energetically-significant intermediate in electron transport-driven phosphorylation?'' Kell's overview differs from many in bioenergetics by its conclusion that ''the proton motive force across energy coupling membranes catalysing electron transport-linked phosphorylation is energetically insignificant''. Such a conclusion is based on many arguments developed in Kell and Westerhoff (1985).

Intracellular pH measurements are important to assay transmembrane pH differences, proton exchanges catalyzed by the H^+ pump or by H^+ cotransport and, more generally, to appreciate cell activity (Kurkdjian and Guern, 1989). Measurements of redox activity at the plant plasmalemma leading to an estimation of proton fluxes have been reviewed by Rubinstein *et al.* (1990).

The system of *myo*-inositol transport in the bacterium *Klebsiella aerogenes* is by H^+-symport. The concentrative system of this molecule is bidirectional but is highly asymmetric. Energization of the membrane is essential to render the system asymmetrical as demonstrated by the abolition of the proton gradient in the presence of uncouplers (Deshusses and Reber, 1977). A periplasmic binding protein is involved in the process as also reported for mannitol transport in *Pseudomonas aeruginosa* (Eisenberg and Phibbs, 1982).

d) *Electric potentials*

The plasmamembrane Ca^{2+} pump, discovered 25 years ago by Schatzmann, is a model membrane protein for the study of ATP-driven cation transport as recently emphasized by Strehler (1991).

A special class of channel proteins is reponsible for the electrical activity of neurons. The structure of these voltage-gated channels spans 6 transmembrane helices from the NH_2 (+) to COOH (-) poles. Recent experiments (Yool and Schwarz, 1991; Hartmann *et al.*, 1991) involving site-directed mutagenesis identify the pore region (P)

as the pore-forming sequence. They demonstrate that P is critically involved in ions permeation and selectivity (potassium ions can pass through while sodium ions are excluded). The recording of ions currents from biological membranes has been revolutionized by the highly sensitive electrophysiological, patch-clamp technique (Neher and Sakmann, 1976). In mature plant cells, the two membranes - plasmic and vacuolar - are positioned in series. Their current rectifications are in opposition upon depolarization (ref. in Hedrich and Schroeder, 1989). Therefore as reported for gap junctions (see p. 414), ''bipolar gating phenomena may be found when ion currents through both membrane are recorded in series''.

Depolarization of membrane potential is not only provoked by rise in Ca^{2+} concentration (ref. in **I** and, Tsien and Tsien, 1990). Uptake of K^+ also depolarizes the cells, suggesting that it is accompanied by the influx of a positive charge (Bakker and Harold, 1980).

Hyperpolarization of the cell membrane of *Saccharomyces cerevisiae* can be caused by metabolic substrates such as glucose or ethanol (van de Mortel et al., 1988). The glucose-induced hyperpolarization activates Ca^{2+} influx by reducing the negative membrane potential below a threshold value (Eilam and Othman, 1990). There are also indications that the transient hyperpolarization of the yeast cell membrane by glucose is caused by opening of specific K^+ channels (Borst-Pauwels *et al.*, 1988). In hyperpolarized algal cells, inward K^+ current rectifies the membrane polarization as found (Sokolic and Yurin, 1986).

Among the channels contributing to ion (I) transport through the plasma membrane, the I_K+ out channels allowing K^+ efflux become activated by depolarization of the membrane potential. The observed depolarization can be provoked by the addition of K^+ to the extracellular medium of *Neurospora* (Rodriguez-Navarro *et al.*, 1986).

Changes in circular dichroism suggest ''a decrease in α-helicity and increase in β-structure with the membrane potential positive inside and vice versa when the potential was positive on the outerside of membrane vesicles from where alamethicin was inserted into the membrane''. These alamethicin channels therefore depend upon electric field (Brumfeld and Miller, 1990).

Channel-like analogues in icosahedral viruses, called ''channelogs'', encompass structures with narrow regions analogous to selectivity filters which can be lined by negative or positive dipoles. The source of the impermeable barrier is the repulsive ion-charge interactions, largely due to the parallel orientation of the helix pentamers. This ''helix dipole'' effect (Hol *et al.*, 1978) was verified by Langevin energy profiles calculations (Eisenman and Alvarez, 1991). The concentrations of permeant species can be modified by interactions with ionizable, dipolar, and hydrophobic groups. Helix dipoles can thus cause a sterically open structure to be impermeable to ions (Eisenman and Alvarez, 1991).

The single channel conductance and ion selectivity of the membrane channels are controlled by pH. Thus, the large voltage-dependent channels formed in planar lipid

layers by botulinum, tetanus and diphtheria toxins are pH-gated. Channel formation was shown to be optimal when the protein-containing *cis*-side of the plasma bilayer is at low pH (4.5) and the opposite, *trans*-side is at physiological pH (7.0). A pH gradient would therefore enhance formation of channels which could function as tunnel proteins (Hoch and Finkelstein, 1985).

Blue light and intracellular pH induce changes in the electric membrane potential of hyphae of the fungus *Phycomyces blakesleeanus*. The induced hyperpolarization is mediated by the parallely induced rate of acidification in the medium (Weiss and Weisenseel, 1990).

Movement of nutrients across the bacterial plasma membrane is by no means *uni*directional. Bacteria are known to export and secrete antibiotics, iron-sequestering siderophores, toxic byproducts. For instance, ''protein must ''know'' where it is destined to go''. Such a polarized process ''implies the existence of targeting and localization (stop transfer) signals within the protein structure'' (Pugsley and Schwartz, 1985).

In symbiotic associations, such as in vesicular-arbuscular endomycorrhizas, the host plasmalemma apparently retains its normal function of *bi*-directional nutrient transfer whereas in pathogenic relationships this transfer is *mono*-directional. There should occur some type of depolarization of the electrical potential difference associated with H^+-ATPase activity across the host-fungus membrane interface (Gianinazzi-Pearson *et al.*, 1991). It remains to understand how changes of the membrane potential ($\Delta\Psi$) decide of the monopolar or bipolar directionality of the nutrient transport (Smith and Smith, 1989).

e) *Action potentials*

The organization of the top three outer layers of the retina - photoreceptors, horizontal cells and bipolar cells - has been modellized to build a silicon chip (Mahowald and Mead, 1991). However, ''biological computation must be very different from its digital counterpart'': in the human retina, bipolar cells transmit a signal corresponding to the ratio of the signals from rods and horizontal cells through the ganglion cells while, in the silicon retina bipolar cell, circuitry amplifies the difference between the signal from the photoreceptor and the local average.

Depolarizing bipolar retinal neurons can be hyperpolarized by the neurotransmitter glutamate which supresses their cGMP-activated conductance (Nawy and Jahr, 1990). Bipolar cells isolated from the salamander retina are thought to be the hyperpolarizing or off-center bipolar cells. In such cells permeation of calcium ions occurs through non-*N*-methyl-D-aspartate - glutamate channels (Gilbertson *et al.*, 1991).

Stimulation of olfactory neurons developed an inward current when stimulated with odorants. The resulting depolarization can be sufficient to induce action potentials, as recently achieved by tight-seal patch-clamp recordings of frog olfactory cilia (Kleene and Gesteland, 1991).

f) *Synaptic membranes*

Synapsins, proteins interacting with synaptic vesicles and targeted in nerve terminals (Huttner *et al.*, 1983, see **I**; De Camilli *et al.*, 1990) appear in development during synaptogenesis. Synapsin is a filamentous protein which is thought to link synaptic vesicles and actin as shown in quick-freeze electron micrographs of nerve terminals. From these recent results (Han *et al.*, 1991), a model of synapse formation is proposed.

C. Endomembranar and vesicular systems

1. Endoplasmic reticulum

Intrinsic membrane proteins are inserted vectorially into the endoplasmic reticular membrane during or immediately after biosynthesis. Such asymmetric insertion provides a functional model for the small-intestinal Na^+, D-glucose cotransporter. In that gated transport agency, the substrate binding sites are more easily accessible from the cytosolic pole, the negatively charged mobile pole being a part of the Na^+ binding site (Semenza *et al.*, 1985).

The biphasic process of protein transport across the endoplasmic reticulum (ER) membrane comprises (1) an initiation of targeting cycle, and (2) the actual transfer of the polypeptide chain through the membrane. The question remains to know if this last process occurs through a translocation tunnel. According to one model (Rapoport, 1990 and **I** pp. 95-97), polypeptides are transported through a hydrophilic or amphiphilic tunnel that is assembled from transmembrane proteins.

The import of secretory protein precursors into the ER of *Saccharomyces cerevisiae* requires components such as a polypeptide (p) coded by the gene *SEC62*. This *SEC62p* spans ''a ER membrane twice, displaying hydrophilic amino- and carboxy-terminal domains towards the cytosol'' (Deshaies and Schekman, 1990).

2. Golgi apparatus

Proteins imported from the endoplasmic reticulum are polarly transported across the Golgi stack before secretion or storage in vacuoles. According to the endomembrane flow theory, in their polar progression, the cisternae of the dictyosomes would be formed consecutively at the *cis*-face and, after maturation steps, would be lost at the *trans*-face during the formation of secretory vesicles (see Steer, 1991). The alternative ''Rothman model'' suggests that individual cisternae are static and that transported molecules are successively passed from one cisterna to the next in vesicle shuttles (Rothman, 1985).

Monovalent cations are involved in the mechanism of Golgi secretion by animal cells as shown by blocking export of cell wall matrix polysaccharides from pea cells by the H^+- and K^+ exchanging ionophore, nigericin. The associated requirement of an

acidic internal pH in the cisternae might be explained by an osmotic model (Griffing and Ray, 1985). Another ionophore, monensin, is known to disturb the polarized traffic from the Golgi (see **I**, p. 99 and Tartakoff, 1983). Lipid traffic has further been studied by Pagano (1990) using his fluorescent marker microscopic technique.

3. Intracellular vesicles

Endocrine and exocrine secretory processes mediated by endocrine and exocrine vesicles respectively are directionally inverse shuttling operations. These shuttles involve a special class of lipid-rich, proton-poor membranes that appear to use an inwardly directed H^+-translocase ATPase activity operative for pH-dependent sorting of transported molecules (Castle *et al.*, 1987).

Within polarized epithelial cells (see VII.C.6b), the export traffic can be directed to a distinct plasmalemmal domain which distinguishes exocrine from endocrine secretion and import traffic can be directed transcellularly.

Exocytosis is a membrane process, which requires calcium and ATP as shown by membrane-bound chromaffin granules which store secretory products of the adrenal medulla cells and release their contents (catecholamines) to the cell exterior. Such release is triggered on cell depolarization by acetylcholine or by certain concentrations of K^+-ions; Ca^{2+} channels then open up and an increase in Ca^{2+} influx follows to reach its critical level of intracellular concentration. If exocytosis is a true contractile event involving cytoskeleton proteins (actin, α-actinin, myosin) and their regulatory ones, ''a sliding filament mechanism similar to that found in muscle would operate in chromaffin cells'' (Trifaró and Fournier, 1987). The intracellular Ca^{2+}-concentration reached during stimulation would activate calmodulin-dependent processes and phosphorylation of myosin light chains, a condition required for chromaffin cell myosin-ATPase actin activation and formation of bipolar myosin filaments (Trifaró *et al.*, 1985).

In an attempt to explain the exocytotic fusion of membranes of secretory vesicles with plasma membranes, Pollard *et al.* (1987) have proposed a novel ''hydrophobic bridge'' hypothesis for how synexin, a new calcium-binding protein, both drives and directs the fusion process. Conformational changes of the protein in the presence of calcium would expose its hydrophobic domains and shield its charged and neutral domains.

The cells make use of ionic gradients across the plasma membrane for signaling purposes, as in the propagation of the action potentials in excitable cells (see IV.B.2e), and for doing osmotic work, as in Na^+-dependent transport systems in epithelial cells (see VII.C.6b) and in the process of acidification of vesicles and other intracellular organelles. Moving of H^+ ions from the cytoplasm to the vesicle lumen by the ATP-driven H^+ pump has two consequences (Rudnick, 1987): the vesicle lumen becomes acidic and also positively charged with respect to the cytoplasm. For net H^+-pumping to occur, counter ion movement (anion influx or cation efflux) must dissipate the transmembrane potential.

Membranes of neuroendocrine secretory vesicles contain a H^+-translocating ATPase responsible for the generation and maintenance of the electrochemical proton gradient, ΔpH inside acidic, and transmembrane potential, $\Delta\Psi$ inside positive. This electrochemical proton gradient serves as the driving force for the intravesicular accumulation of compounds such as the biogenic amines inside the chromaffin granules (Johnson *et al.*, 1987). Synaptic vesicles and vesicles of the *Torpedo* electric organ also show ATP driven proton pump acidification (Stadler, 1987).

D. Organelles

2. Mitochondria

Direct evidence for an active role of microtubules in moving mitochondria in Spongillid pinocytes has recently been obtained (Weissenfels *et al.*, 1990).

3. Chloroplasts and phototransducing membranes

In photosynthetic systems, carotenoids serve the dual fonctions of light harvesting and photoprotection. Moreover, shifts in their absorption spectra are used to measure transmembrane potentials and the electrogenicity of charge separation steps. Such polyenes are highly polarizable, that is, electric fields can induce substantial dipole moments. Measurements of the seemingly anomalous dependence of carotenoid band shifts on transmembrane potential have revealed the production of large protein-induced dipoles for a symmetric carotenoid in the photosynthetic antenna complex of *Rhodobacter sphaeroides* (Gottfried *et al.*, 1991).

E. Cytoskeletal components

They are the actin microfilaments and the tubulin microtubules, on one hand, and the myosin, kinesin, dynein and dynamin microfilamentous ''motor'' proteins which are mechanochemical nucleotide triphosphates (ATPases), on the other hand (see new § E 4.). Such microtubule motors have not yet been identified in plants. As a cause of microtubule movements within the phragmoplast, tubulin might be incorporated at the interdigitating + ends and could move through microtubules by flux or minus-end assembly might also be involved (Lloyd, 1991). According to Asada et al. (1991) the equatorial region of the phragmoplast would be associated with a mechanochemical enzyme that generates the force for microtubule translocation by hydrolysing GTP.

Microtubules and neurofilaments (intermediate filaments, see new § E 5) are major elements of the neuronal cytoskeleton. However, contrarily to microtubules, neurofilaments are composed of an antiparallel array of subunits which may not be polar filaments and therefore could not support directed movement of organelles.

Cytoskeleton of the *Drosophila* fly has been extensively reviewed in 1990 by Fyrberg and Goldstein.

1. Microfilaments-actin

Actin filaments (F-actin) are important components of the cell cytoskeleton where they are often involved in transport processes. The actin monomer (G-actin) has been originally isolated by Straub (1942). Isolated F-actin filaments are suitable for three-dimensional reconstruction (Aebi *et al.*, 1986b). The atomic structure of their monomer complexed with the enzyme DNase I as well as an atomic model for the structure of the filament, a helical polymer of actin subunits have been recently unraveled by Holmes *et al.* (1990) and Kabsch *et al.* (1990).

The association between types of filaments and plasma membrane specializations first seemed to respond to a general rule. Thus, single actin microfilaments have been described as uniformly polarized at their attachment, with arrowheads (- ends) pointing away from the plasma membrane (Ishikawa, 1979). In other words, ''cables of actin consist of bundles of microfilaments all with the same polarity, i.e. with the barbed end (+) anchored in the membrane'' (Fulton, 1984, see **I**). However, at odds with this directionality there are reports of actin filaments anchored at their pointed end to cell membranes (Stossel *et al.*, 1985, see **I**, pp. 114-115).

According to a recently proposed working model (Schwartz and Luna, 1988), based on geometric considerations and observations from equilibrium binding studies, membrane-bound actin nuclei initially assemble with both barbed and pointed ends free (Shariff and Luna, 1990).

Within a bundle, actin microfilaments are arranged according to two types with respect to the polarity: uni- and bidirectional arrangements. According to such dual directionality, the bundle can be attached to the plasma membrane in side-to-membrane association and only with one end, if any end-to-membrane association exists (Ishikawa, 1979).

Enzymes are associated with an F-actin microfilament, leaving in the interstices of the Porter microtrabecular lattice (see **I**) a solution containing ions and some metabolites, but no proteins. Most of charged residues (more negative than positive) of actin must be in contact with water, so that, according to Clegg's model (1984), the F-actin filament is a highly charged, high-molecular-weight polyelectrolyte. As such it must generate special kinds of water structure involving a counterion adjacent to each charged group. In the electric balance, the ''couple'' H_3O^+ and OH^- compensates the deficit in diffusible inorganic ions (Wiggins, 1990).

2. Microfilaments-myosin

The myosin motors are force-generating enzymes (ATPases) which move toward the barbed (or plus) end of the actin filament (see Fig. 13 in **I**). In their bidirectional movement, myosin filaments can translocate actin filaments both toward and away from

their central bare zone. This illustrates the polarity of such sliding filaments (Sellers and Kachar, 1990).

3. Microtubules-tubulins

In microtubules, tubulin subunits are arranged in tandem composing a complex helical array (Vallee and Shpetner, 1990). Therefore, "the polar properties of microtubules on the supramolecular level are based on the polar properties of the tubulin subunits" (Mandelkow *et al.*, 1987). Evidence for the asymmetric shape of the tubulin molecule came mainly from image processing of electron micrographs of negatively stained microtubules or related polymorphic tubulin assemblies. Axial polarity was observed along protofilaments (up/down) siddedness (left/right) and radial asymmetry (inside/outside). Each subunit, and the microtubule as a whole, has therefore an inherent or intrinsic polarity.

Polarity was first deduced from this highly organized array of microtubules that make up the ciliary and flagellar axoneme (Borisy, 1978). These microtubules all have the same orientation with the end proximal to the cell body designated as "-" and the distal end as "+". This is what occurs in axons with their "anterograde" (away from the cell body) and "retrograde" (toward the cell body) directional transport of organelles. Such movements are therefore toward the + and - ends of the axonal microtubules, respectively. By contrast, neuronal dendrites contain microtubules of *mixed* polarity (Burton, 1988; Baas *et al.*, 1988), which "raises perplexing questions regarding the expected behavior of dendritic organelles" (Vallee and Shpetner, 1990).

As in the actin microfilaments, the inherent or intrinsic polarity (Huxley, 1963, Amos and Klug, 1974 in **I**) is reflected in the asymmetric addition of subunits at the two ends of the polymer (Haimo, 1989; see **I** and **II**).

Many factors regulate microtubule assembly and organization in the cytoplasm among which are the thermodynamic polarity of the microtubules themselves, their number, the presence and number of nucleating sites and the concentration of tubulin (Kirschner and Mitchison, 1986).

For measuring the polarity of cytoplasmic microtubules two methods have been developed (see Linck, 1989): 1) flagellar dynein reassociation with non-flagellar microtubules (Haimo *et al.*, 1979 in **I**); 2) brain tubulin assembly onto existing microtubules, forming curved arcs or hooks in cross section (Heidemann and McIntosh, 1980 in **I**).

The fundamental structural polarity of microtubules derives from the head-to-tail organization of α- and β-tubulin heterodimers in the profilament lattice constituting the wall of the tubule. "This polarity is made apparent on a larger scale by the polarized grouping of such accessory structures as the radial spokes on ciliary and flagellar tubules and by the different rates at which tubulin assembles and disassembles at the two ends of a tubule" (ref. in Gibbons, 1989).

In plants, the orientation of microtubules is transverse across the long axis of the stem, and perhaps might be causely related to the orientation of the cellulose microfibrils that give structural integrity to the cell wall. Shortly after growth begins to slacken, the horizontal orientation of microtubules rapidly changes toward longitudinal positioning in the pea stem (Laskowski, 1990). Cellulose microfibrils are also reoriented during cell elongation to a longitudinal orientation. Such a parallel orientation to the long stem axis provides longitudinal strength to its mature cells.

4. Microtubule-associated "motor" proteins

There are three classes of microtubule-associated motor proteins to power intracellular movements. Relative to the intrinsic structural polarity of the microtubule, dynein produces movements toward the minus end (Vallee and Shpetner, 1990), while kinesin generates movements toward the plus end (Vale *et al.*, 1985, in **I**), and dynamin bundles microtubules and causes them to slide relative to one another (Shpetner and Vallee, 1989, in **II**). The polarity of movement of the recently found kinesin-like *ndc* protein of *Drosophila* is also minus end directed (McDonald *et al.*, 1990).

The microtubule-associated mechanochemical enzymes or "motor" proteins kinesin and dynein contain a specialized enzymatic domain that hydrolyzes ATP and uses the derived chemical energy to produce force and movement along a cytoskeletal polymer. With kinesin and myosin adenosine triphosphatases (ATPases), dynein represents one of the three general classes of ATPase that couple energy derived from the hydrolysis of ATP to the movement of cellular components along stable tracks of either microtubules (dynein, kinesin) or actin filaments (myosin) (see Warner, 1989). The recently discovered dynamin (Shpetner and Vallee, 1989) has a microtubule-activated ATPase activity. This mechanochemical ATPase mediates sliding between microtubules and its properties are distinct from those of the other two proteins. Indeed, its molecular cloning has recently revealed homology with a new family of GTP-binding proteins (Obar *et al.*, 1990). By contrast, dynein and kinesin provide locomotive forces along cytoplasmic microtubules, but by analogy to a railroad, the microtubules themselves are far more than simple, inert tracks (Linck, 1989). Dynein can bind to axonemes or to nonaxonemal microtubules thereby revealing their intrinsic structural polarity.

Movement of the motor along the polymer is unidirectional, which is "a consequence of the inherent asymmetry of the polymer and the motor" (Vale and Goldstein, 1990). These microtubule motors are either plus-end directed (kinesin) or minus-end directed (dynein). Dynein (22S and 14S) extracted from axonemes of *Tetrahymena* produces force in the direction of the minus-end predicted from the outer doublet sliding experiments of Sale and Satir (1977). This direction is opposite to that of kinesin-induced movement along a microtubule (see Vale and Toyoshima, 1989).

Microtubles and associated molecular protein motors are also known to mediate the movement of pigment granules (chromatophores) in cytomatrix protein (Weissenfels

et al., 1990) and other-surrounded cell compartments (Couchman and Rees, 1982; Schroer *et al.*, 1988).

5. Intermediate filaments

There is ample evidence that microtubules and microfilaments are of importance for the onset of cell polarity and generation of cell movements, while the function of intermediate filaments remains elusive (Traub, 1985). Some of their subunit proteins have a specificity for binding to DNA rather than to RNA intermediate filaments and could thus be involved in signal transduction from the plasma membrane or cytoplasm to the nucleus. In fact the nuclear lamina is a meshwork of intermediate-type filaments lining the nucleoplasmic surface of the inner nuclear membrane (see Aebi *et al.*, 1986a).

F. Nuclei and mitotic figures

1. Interphasic and mitotic structures

The development of a bipolar spindle is an essential prerequisite for the segregation of chromosomes during mitosis (Mazia, 1961 and Nicklas, 1971 in Borisy, 1978) and the polar functions of the spindle reflect the polarities of the microtubules originating from the centrosomes and the chromosomes (Subirana, 1968 and McIntosh *et al.*, 1969 in Borisy, 1978). "Given that microtubules possess intrinsic molecular polarity (see IV.E.3), there are two ways in which they might be oriented relative to a nucleation site and therefore four possible polarity relations for the microtubules of opposite centrosomes and chromosomes" (McIntosh, 1977 in Borisy, 1978). There is not yet a readily interpretable indicator (morphological marker) for microtubules polarity such as heavy meromyosin in the case of actin filaments (Huxley, 1963, see **I**; Ishikawa *et al.*, 1969). For cytoplasmic microtubules, their intrinsic polarity is therefore reflected in their direction of growth. Their elongation *in vitro* is known to be a biased-polar process (Allen and Borisy, 1974, see **I**; other ref. in Borisy, 1978) in which the tubulin dimers are added at the ends of the tubule (see IV.E.3).

In the model for cytokinesis proposed by Pollard *et al.* (1990), actin filaments of the cellular isotropic gel are attached by their barbed ends to the plasma membrane (see also E.1) and cross-linked by alpha-actinin and other proteins preexisting in the cortex. Myosin II assembled into bipolar filaments also interacts with actin filaments thereby activating the cleavage process. Actomyosin is also involved in the organization of mitosis. In *Dictyostelium amoebae*, specific accumulation of myosin I

might occur in the polar amoebal lamellipodia and be related to apparent ''axial relaxation'' (Fukui, 1990).

The mitotic spindle in higher plants is typically anastral (without polar bodies) but some cell types possess the potential to develop asters. With the exception of certain algae, plant spindles are also acentriolar. The preprophase band is a unique feature of cell division in such non-algal plants (Pickett-Heaps and Northcote, 1966). By late preprophase, microtubules appear around the nucleus of the fern *Athyrium filix-femina* in a preferred orientation establishing division polarity before the onset of prophase. Thus ''the polar ''caps'' of microtubules concomitantly appear as an additional element re-enforcing the axial polarity'' (Jenni *et al.*, 1990).

The organization of microtubules into arrays such as the mitotic spindle is choreographed by structures known as microtubule organizing centres (see **I**, pp. 126-128). A particularly well-studied centre, the spindle pole body of the budding yeast *S. cerevisiae* has now been isolated by Rout and Kilmartin (1990). This should provide an attractive model to study the ability of the pole body to assemble microtubules and a tool for its still unknown biochemistry.

It has been proposed by Oakley *et al.* (1990) that γ-tubulin attaches microtubules to the spindle pole body, nucleates microtubule assembly, and establishes microtubule polarity *in vivo*. A microtubule-severing protein has now been identified in *Xenopus* egg extracts (Vale, 1991). This factor may be involved in disassembling the interphase microtubule network prior to constructing the mitotic spindle.

2. Polewards chromosome movement

There are three types of spindle microtubules, kinetochore microtubules, central spindle microtubules, and astral microtubules. They originate from a spindle pole, the centrosome, and are oriented with their minus ends at the poles, plus ends out. There are two known motive forces on chromosomes: polewards movements transmitted via the kinetochores, and forces away from the poles, the polar ejection forces transmitted to chromatin. Carpenter (1991) concludes that the balance between the polar wind, pole-directed kinetochore forces, and bonding by interchromosomal microtubules can explain all known interactions within the process of distributive segregation involved in mitosis.

A model for the dynamics of chromosome movement has been proposed by Palmer *et al.* (1989) founded on the analysis by digital imaging microscopy of DNA nuclear movement in live cells of the budding yeast *Saccharomyces cerevisiae*. The mechanism of these movements and their induction have been studied in certain cell deficient cycle mutants (cdc), and the axis defined by the segregating genomes found to rotate relative to the cell surface. Quite recently, two different microtubule-based motor activities with opposite polarities have been unraveled in kinetochores by video microscopy (Hyman and Mitchison, 1991).

The radial microtubule array emanating from the centrosome is one of the important controlling elements in cell form and polarity. "Since kinesin would be expected to move objects to the periphery of the cell on those microtubules, it could be very useful in building and maintaining polar distributions of materials within cells" (Sheetz *et al.*, 1987). For further information about kinesin and dynein implications as motor molecules in chromosome movements, see Carpenter (1991).