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Autor: Turian, Gilbert
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V. POLAR CELL MOVEMENTS

Polarity appears to regulate the direction of cytoplasmic and cell movements. These movements can be "internal", such as cyclosis in plant cells, or result in a major change in shape or position of the cell. In both aspects, microfilaments and/or microtubules are fundamental to these activities. In most cases, an interaction between proteins such as actin-myosin or dynein-tubulin systems, with the simultaneous involvement of an ATPase, would be the underlying biochemical phenomenon (Condeelis, 1983).

Polarized growth of eukaryotic microorganisms such as fungi has the expected attributes of growth (biosynthesis, especially of new hyphal wall material) but also some of the attributes of motility, i.e. some protoplasm, and in extreme cases, all viable material, is moved from its site of synthesis (Carlile, 1980). This effect has something in common with the movement of an amoeba or a myxomycete plasmodium: in one instance empty hyphal walls are left behind, in the other slime from the glycocalyx, and in both protoplasmic streaming is involved.

A. Cytoplasmic movements

Cytoplasmic streaming or *cyclosis* is easily observed in plant cells, in which the cytoplasm is generally reduced to a layer next to the cellulose wall and to fine trabeculae crossing the large central vacuole. Continuous currents can be seen that displace chloroplasts and other cytoplasmic granules. In some plant cells the protoplasmic current can be initiated by chemicals (*chemodynesis*) or by light (*photodynesis*). Cyclosis is modified by environmental factors such as temperature, pH, etc.

Eukaryotic microorganisms can achieve far higher localized growth rates than prokaryotes. A hypha, for example, can increase in length far more rapidly than can a bacterial cell; the hyphae of *Neurospora crassa* can grow at 100 $\mu m\ min^{-1}$ and the sporangiophores of *Phycomyces blakesleeanus* at 60 $\mu m\ min^{-1}$. Materials are transported by protoplasmic streaming to the hyphal apex where extension occurs from a growth zone which may extend for several millimeters behind the apex (Trinci, 1978*a*, *b*).

In microplasmodia of the acellular slime mold *Physarum,* the motive force for protoplasmic streaming is generated in conjunction with the associated contractile apparatus (Wohlfarth-Bottermann, 1975). The actual motive force is provided by peripheral microfilaments situated in the region between the stationary ectoplasm and the moving endoplasm. Cortical microfilaments have mainly been involved in

motive force generation for cytoplasmic streaming activity rather than the fibrillar system extending through the matrix (Brix *et al.,* 1987). Bundles of actin microfilaments all with the same polarity, i.e. with the barbed end anchored in the membrane, form cables. The polarity of their actin filaments is such that the movement of myosin filaments along them could produce the observed cytoplasmic streaming (Fulton, 1984).

The cylindrical cells of the green algae *Nitella* and *Chara* are enormous, being 2 cm to 5 cm in length. These giant, multinucleated cells provide the best examples of cytoplasmic streaming: a continuous ribbon of cytoplasm streams along a gentle helical path down one side of each cell and back across the other side in an endless belt. The streaming cytoplasm moves in only one direction, at speeds of up to 75 μm per second, sweeping internal membranes, mitochondria, nuclei, and cytosol around and around the cell.

Cytoplasmic streaming has been observed to occur at two different velocities: chloroplasts are transported at 1-2 μm s^{-1} and nuclei move in "head-streamed bands" at 3-11 μm s^{-1}. The streamings also differ in the mechanisms of force generation (Koop and Kiermayer, 1980). The cytoplasmic streaming in *Nitella* has been shown by Kamiya (1981) to be driven by an actin and myosin system. The actin cables which exist on the cytoplasmic surface of the *Nitella* membrane are oriented as expected for myosin-driven movements, although there is yet no demonstration that a *Nitella* myosin drives cytoplasmic streaming (Warrick and Spudich, 1987). In Characean algae, direction of streaming has been ascribed to a shearing force generated between the organelle-bound myosin and the actin cable according to the model proposed by Williamson (1975).

In lower animals such as *Paramecium,* similar but slower movements are seen that displace the digestive vacuoles from the site of ingestion to the site of excretion. In many cells of higher animals, particularly in tissue cultures, intracellular movements can also be seen.

B. Cell movements

1. *Cilia-flagella*

Microorganisms may possess cilia or flagella which act as locomotion organelles. The distinction between ciliary and flagellar movement is not always clear-cut, cilia typically causing movement of liquid by beating at right angles to their long axis, while flagella move along the whole length of their axis. Flagella that are located only at one or both poles of the bacterial cell are called polar and bacteria so-called *mono*polar or *bi*polar flagellates. An organism carrying a single polar flagellum would be monopolar monotrichous, while one with a tuft of such flagella would be

monopolar polytrichous or of the lophotrichous type. By contrast, *Spirilla* are bipolar polytrichous or of the amphitrichous type.

The bacterial flagellum consists of three morphological parts: the filament (fibers of the protein flagellin), the hook and the basal body which binds the hook and filament to the cytoplasmic membrane. Some non-flagellated bacteria of the genus *Spirillum* have a polar plate internal to the cytoplasmic membrane.

Reconstitution experiments *in vitro* with *Salmonella* flagella have shown that the flagellar filaments have structural polarity and that they grow by a sequential orderly polymerization of flagellin molecules at a structurally defined end, called the T-end, of each flagellar filament (Asakura *et al.*, 1968). The T-end corresponds to the distal end of a flagellum attached to the cell. Therefore, if flagellar growth *in vivo* is homologous with that *in vitro,* it must take place at the tip.

Bacterial flagella have been found to be assembled by the addition of newly synthesized flagellin monomers onto growing flagella at the tips of the organelles. The assembling process is inverse in non-flagellar, filamentous appendages called fimbriae or pili which are present on every species of Gram$^-$ bacteria and are responsible for the adherence of bacteria to a variety of eukaryotic cell surfaces. According to Lowe *et al.* (1987), the pattern of labelling with gold particles on individual fimbrial organelles indicated morphologically that newly synthesized subunits are added to a growing organelle at its base (Emerson *et al.*, 1970).

Iino's (1969) experiments showed that *Salmonella* flagella grow at their free ends. This agreed with the polarity of flagellar growth *in vitro* (Asakura *et al.*, 1968). Thus, the basic molecular mechanism of flagellar growth *in vivo* may be homologous with that of polymerization of flagellin molecules into flagella *in vitro.*

As for the proximal part of a flagellum which ends as a structure called a "hook", it is connected to a spherical granule, called a "basal granule" (Iterson *et al.*, 1967). These basal structures are presumed to be responsible for the synthesis of flagellin and initiation of flagellar formation as well as flagellar movement.

Movements of flagella are best explained, at least for polar flagellates, according to the theory put forward in 1969 by Vaituzis and Doetsch that the filament of a flagellum is an inert, relatively rigid structure that owes its wavelike appearance to the tertiary structure of the protein; the motion would be initiated by a wobbling action of the basal disc that is embedded in the cytoplasmic membrane (see p. 140).

Among *non*-flagellated bacteria, *Spirochaeta* move by producing helical waves along their length as well as bending, curling and lashing; the motor organelles consist of a single or bundle of fibril(s) attached to both ends and lying under the surface. Possibly related to that type of movement, cell surface of *Spirochaeta* is electrically polarized (James, 1957), contrarily to most other individual organisms in which electric negative charges are distributed evenly and produce there the so-called zeta potential.

Cilia and flagella of eukaryotes are well suited systems for studies of synthesis and assembly of specific proteins. One of the most extensively studied systems is that of *Chlamydomonas* with its model of monopolar flagellar regeneration and its numerous mutants showing aberrant control of flagellar length, assembly, motion, stability and regeneration (Lefebvre and Rosenbaum, 1986). The two ends of flagellar microtubules demonstrate different assembly in vitro at different rates (Allen and Borisy, 1974; Binder *et al.*, 1975). This polarity is unravelled with a method in which partial microtubule sheets are assembled onto preformed microtubules to determine the intrinsic polarity (Euteneuer and McIntosh, 1981*a*). The partial microtubules form hooks whose orientation indicates the polarity of the template microtubules (Lefebvre and Rosenbaum, 1986).

2. *Gliding movements*

There are active even though slow movements in contact with a solid substratum which are carried out by microorganisms such as the primitive Mycoplasmas, and a number of bacteria from Cyanobacteria (blue-green "Algae") to Myxobacteria as well as by certain Algae (diatoms) and Protozoa. The mechanism of this gliding is not yet clearly understood. In Cyanobacteria several alternative models have been proposed (Häder, 1987) to the early hypothesis of an asymmetric secretion of slime.

Mycoplasmas are considered as the smallest prokaryotes possessing very limited genetic information even though endowed with gliding motility. In contrast to the gliding bacteria, the gliding mycoplasmas have a distinct antero-posterior axis; their leading anterior end is distinguishable by a specialized cytoskeletal structure of actin-like proteins (Bredt, 1979; Göbel *et al.*, 1981) as possible locomotor apparatus fueled by cell derived energy. Mycoplasma gliding would therefore insure unidirectional cell movement by an active form of surface translocation rather than a biased Brownian motion (Rosengarten and Kirchhoff, 1988).

Bacterial gliding motility appears to be dependent on the establishment of a transmembrane potential and possible metabolic pathway (cyanide-insensitive) for maintaining it and motility in *Flexibacter* have been proposed (Duxbury *et al.*, 1980). Any depolymerization of the membrane (by 2,4-DNP, CCCP, etc.) results in a cessation of motility. For gliding movements, the energy source for the motor is not ATP but protons circulation (Harold, 1977). Interestingly, the presence of this proton force near one end of the cell would create a potential difference along the long axis of the cell additional to that existing across the plasma membrane and the ensuing polarization may provide a triggering mechanism generating rhythmical oscillations of the bacterium. This requirement for a suitable transmembrane potential in gliding bacteria is consistent with a translation of the electrical potential into some form of propulsion mechanical work (Duxbury *et al.*, 1980).

Gliding filaments of the cyanobacterium *Phormidium uncinatum* rotate during forward locomotion. They respond to a sudden decrease in light intensity by a phobic

reversal of movement. The photophobic response leads to a gliding reversal directed by transductional sensory chain which has been biochemically modelled (see V.B.5).

Temporal polarity of motile filaments of blue-green algae might be due to an electrical potential difference between front and rear end (Häder, 1980). Since this potential is a prerequisite for the phobic reversal of movement, it is possible to inhibit photophobic responses by the application of external electrical potentials. Moreover, as gramicidin inhibits photophobic responses in *Phormidium,* "it can be concluded that an ionic gradient exists across the membrane which is cancelled by the ionophore". The physiological polarity of a trichome determines the direction of movement and is manifested in a potential difference in each cell (Fig. 34 in Häder, 1980).

3. *Amoeboid motion (transient polarity)*

In this form of locomotion, the cell changes shape actively, sending forth cytoplasmic projections called *pseudopodia,* into which the protoplasm flows. Although this special form of locomotion can be observed easily in true protozoan amoebae (amebae), it also occurs in numerous other types of cells from the prokaryotic "amoebae" of *Mycoplasma* and the myxamoebae of slime molds to the human leukocytes (diapedesis).

Some amoebae are predominantly *monopodial* (one pseudopodium), therefore presenting transient *mono*polarity. Others may be temporarily or permanently *polypodial* or in a shape of *poly*polarity. In an unconstrained situation, interphase myxamoebae of *Dictyostelium discoideum* moved around randomly in a polypodial or monopodial fashion. There is good evidence that pseudopodial activity and locomotion are two dissociated events (Roos *et al.,* 1986).

The shuttle streaming of endoplasm in plasmodial veins of the slime mold *Physarum polycephalum* follows local hydrostatic pressure alterations in different regions of the plasmodium ("pressure flow"). Hydrostatic pressure gradients result from intrinsic contraction automaticity of cytoplasmic actomyosin in the ectoplasmic wall of the veins. The flowing (i.e. relaxed) endoplasm contains depolymerized actin. In the ectoplasmic wall of the strands, the actomyosin undergoes rhythmic contraction-relaxation cycles accompanied by $G \rightleftharpoons F$ actin transformation and actomyosin fibrillogenesis (during isometric contraction). According to Wohlfarth-Bottermann (1983), ecto-endoplasm transformations involved in plasmodial locomotion can be regarded as a part of the contraction-relaxation cycle of cytoplasmic actomyosin.

The protoplasm of the amoeba has a clear ectoplasm, which expands considerably toward the end of the pseudopodium. The axial endoplasm is surrounded by a "shear zone" where particles move freely. At the advancing end is the hyaline cap and just posterior to it, the "fountain zone", where the axial endoplasm appears

to contract actively and flows below the ectoplasmic tube. At the opposite end is the tail process, also called the *uroid,* and near it the *recruitement zone,* where the endoplasm is recruited from the walls of the ectoplasm in the posterior third of the cell (Giese, 1979; Taylor *et al.,* 1982; Fulton, 1984).

Motile activity in amoeba of cellular slime molds has also been biochemically characterized (Taylor and Condeelis, 1979; Taylor *et al.,* 1982). Actin and myosin were shown to interact to produce amoeboid movement. In aggregating, polarized amoeba, actin was found primarily in the anterior pseudopodium. However, myosin was not always codistributed with actin (Rubino *et al.,* 1984), a puzzling fact with respect to the generally accepted actomyosin theory of amoeboid movement. Indeed, myosin filaments in myxamoebae were found to be primarily localized at the posterior cortex which would thus be the site of motive force production to move the polarized cell (Fukui and Yumura, 1986). Changes in myosin heavy-chain, as well as light-chain phosphorylation occur in *Dictyostelium* cells responding to the chemoattractant cAMP (Berlot *et al.,* 1985). Surprisingly, this myosin has been shown by genetic manipulation experiments to be nonessential for chemotaxis (De Lozanne and Spudich, 1987; Knecht and Loomis, 1988). Pseudopods extension is correlated with the appearance of dense microfilament networks at sites of pseudopod growth. Actin polymerization may constitute one of the driving forces for extension of pseudopods, and nucleation sites regulating polymerization are under the control of chemotaxis receptors (Condeelis, *et al.,* 1988).

In migrating giant amoebae of the *Amoeba proteus* type, the dense filamentous actin-containing cortical layer is always continuous over the entire surface and varies in thickness depending on the cell polarity. At the front of the cell it is thin while in the trailing uroid regions it increases in thickness. Thin actin filaments are in all parts of the cortex, contrarily to thick filaments, probably myosin, which are present mainly in the uroid region. In intermediate regions, "a flow of cortex from the front to the back of the cell is coupled to the plasma membrane and through it to the substratum, where it drives the forward migration of the cell" (Bray and White, 1988).

An important factor in amoeboid motion is adhesion to a solid *support.* An amoeba that floats freely in the liquid medium can emit pseudopodia, but does not progress; only when it adheres to a solid surface does it commence this type of locomotion. This is also known in aquatic Fungi where the zoospores are creeping on solid surface.

In their natural environment, cells crawl over a complex network of molecules known as the extracellular matrix. The matrix consists largely of the protein collagen and various long chain polysaccharides. Included in the network are some specialized large proteins — like fibronectin — that the cell recognizes as it moves along. The cell's attachment is mediated by a receptor for fibronectin in the plasma membrane.

The receptor recognizes fibronectin and binds to it, forming a "foot" that provides the thrust for forward motion.

According to Abercrombie *et al.* (1970), cell movement is achieved by a continuous backward flow of membrane from the leading edge of the cell to its trailing edge. They saw that such a membrane-transfer cycle would thus enable the cell to extend itself forward over the substratum. To explain the mechanism of amoeboid movement, Bignold (1987) has proposed a "membrane ratchet" model which invokes the existence of specific, laterally mobile, transmembranous structures in the cell membrane; such structures are reversibly adhesive for both the contractile apparatus of the cell internally, and the substratum externally. A more recent model for general cell locomotion has been proposed (Fig. 17). The driving force involves a concerted flow of actin filaments associated with the inner face of the plasma membrane: "the movements originate at a specific region at the cell surface — the leading edge of migrating cells or the polar region of a dividing cell — and carry material back over the cell surface to a more proximal position" (Bray and White, 1988).

Receptors are concentrated at the leading edge, as shown by the high concentration of ferritin receptors on the ruffling edge of the cells tested (Bretscher, 1987). A similar distribution was found for transferrin receptors on giant HeLa cells. Non circulating proteins, on the other hand, were found to be distributed more or less at random on the surface. Such experiments imply that exocytosis occurs at the front edge of a motile cell and that this process, directed toward the leading edge, is sufficient to account for the actual forward movement seen in fibroblasts (Bretscher, 1987). Nevertheless, remains the challenging question of what distinguishes the leading edge of a motile cell so that exocytosis takes place here and not elsewhere.

When a positively charged polymer such as polylysine is added to the medium, it reduces the net charge on the surface of the membrane and the membrane contracts. This contraction can be neutralized by washing or by adding a negatively charged polymer such as polyglutamic acid or heparin. All these experiments are carried out in the absence of bivalent cations such as calcium or magnesium; but if calcium or magnesium are present it is possible to produce contraction also with negatively charged substances. For example, polyglutamic acid or heparin in the presence of calcium or magnesium will cause contraction and more important still, ATP in the presence of magnesium will also cause contraction. In the case when the net charge is reduced by a negatively charged substance associated through calcium or magnesium with the cell surface, we are presumably dealing with a bridge between the two, formed by the bivalent calcium or magnesium ion. The important point here is that the net charge on the surface of the isolated nuclear membranes is acting in very much the same way as the net charge acts between cells: when there is a high net charge, the charges repel each other strongly and cause the membrane to expand; when the charge is reduced the repulsive force at the surface (that is, tangentially along the surface) is reduced and the membrane contracts. This effect has been

demonstrated to be related to actual cellular locomotion and to transmembrane potentials. In *Amoeba proteus* a local injection of polylysine with a micropipette near the leading pseudopodium causes immediate contraction and that region of the cytoplasm begins to flow in the reverse direction.

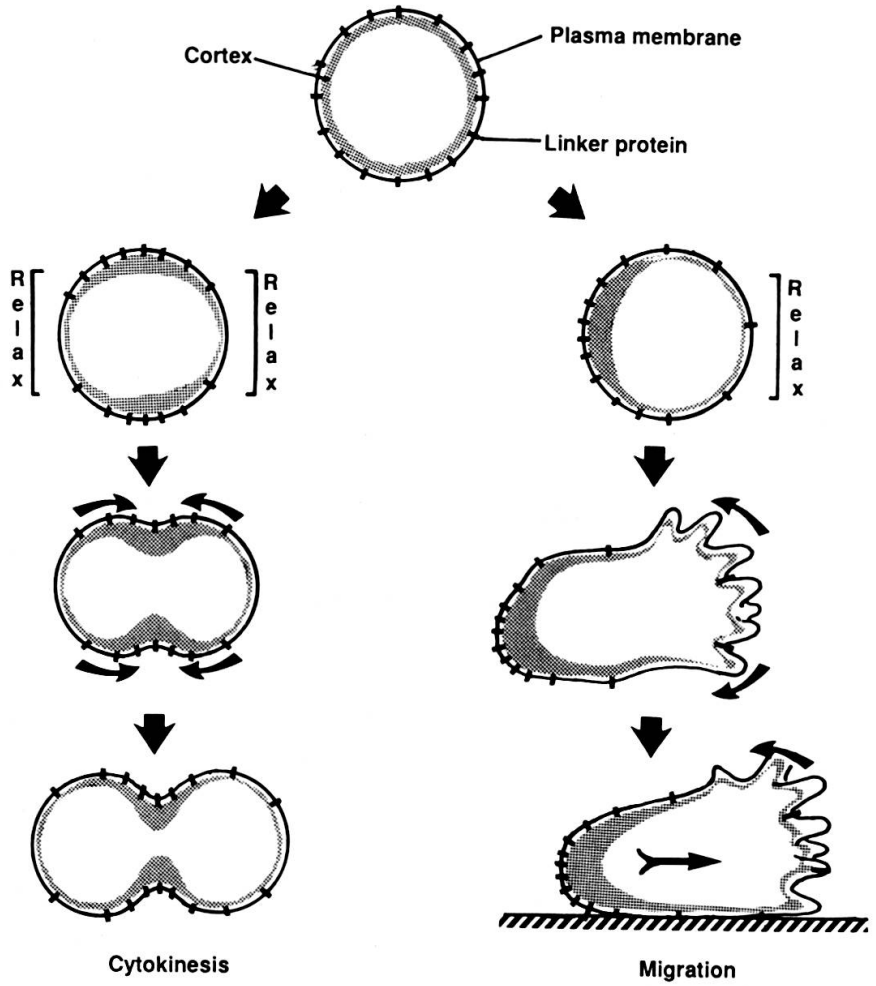

FIG. 17.

Analogy between cortical movements during cytokinesis and cell migration. In this diagram the cortex is represented in cross section, lying close to the inner face of the plasma membrane to which it is attached through special linker proteins. The linker proteins are connected with molecules on the external surface of the cell, such as those concerned with adhesion to a substratum. In *cytokinetic* cell, relaxation at the polar regions (or, what is equivalent for the present purposes, contraction at the equator) leads to the accumulation of actin and associated protein in a band around the equator of the cell. In *migrating* cell, only one region is relaxed and cortical components are pulled in a unidirectional flow toward the region of greatest tension. Here components of the cortex are dispersed and carried forward through the cytoplasm to complete the cycle. Although the flow continues in a cell suspended in fluid medium, contact with a solid substratum permits attachments to be formed with the cortical linker proteins; the cortical flow will consequently drive the cell forward, as shown. Bray and White, Fig. 2, *Science,* 239: 883 (1987), with permission.

Electrical polarity can also be demonstrated with amoebae, the regions of advancing pseudopodia having high negative charge and the tail having a lower

charge. There is a striking correlation between amoeba's morphology changes produced by spontaneous reversals of cytoplasmic streaming polarity and changes in transcellular current patterns. The giant amoeba *Chaos chaos* drives a steady current (0.1 $\mu m/cm^2$), probably carried by Ca^{2+}, into its tail and out its pseudopods (Nuccitelli *et al.,* 1977). The reversals of polarity are always *preceded* by a change in the transcellular current such that the region of largest inward current becomes the new tail (Nuccitelli, 1983).

In an oriented process known as diapedesis, leukocytes can wander out of the blood vessels by active amoeboid motion and progress toward the focus of infection (Schiffmann, 1982). With their dynamic elastic behavior, erythrocytes provide a simple viscoelastic model of cellular elongation which could microscopically be recorded. When an erythrocyte cell suspension was placed between two electrodes with sharp edges, it led to the accumulation of opposite charges at the two ends of the cell opposing the electrodes (Engelhardt *et al.,* 1984). Due to this so-called Maxwell-Wagner polarization, each cell behaved as an electric dipole which could be coupled to the electric field (Pohl, 1978).

When a fibroblast from a tissue culture is in motion, it is often roughly triangular in top view: one side of the triangle forms the front, or leading edge, and the cell tapers off behind to a narrow trailing edge. This activity does not follow a predictable pattern. One region may briefly extend forward over the substratum; then, when the activity subsides, another region along the leading edge may spread out. The sequence is repeated until the cell has advanced along its entire front (Fulton, 1984).

When single kidney cells are seeded out in a blood plasma medium, each cell secretes an as yet unidentified liquid product — perhaps primitive urine — a large vacuole which indents the nucleus and eventually is extruded from the cell. Each isolated cell thus forms a liquid puddle around it in the plasma clot; however, the point of extrusion is in each cell quite haphazard. As soon as the dispersed cells combine into groups, as a result of accidental collision and homotypic aggregation, they begin to polarize each other, each acquiring a basal apical axis, with secretion no longer being arbitrary, but all cells now extruding at their new apical surface — that is, towards the inside — which leads to the physical result of, at first, a vesicle. As soon as a certain critical mass of cells has developed, the lumen is closed off. This deforms the structure further, the nucleus moves towards the polar axis and elongates, and if mesenchymal (stromal) cells are in the vicinity, a basement membrane is formed, the mechanical properties of which confine the total diameter of the structure to a standard size. That leads to a cylindrical extension, which may branch whenever the growing mass exceeds a certain threshold value.

4. *Amoeba-flagellate reversible transformation*

In the acellular slime mold *Physarum polycephalum,* haploid cells transform reversibly between biflagellated swarm cells and amoeboid cells depending on

environmental conditions. Events which take place during these changes include "de novo" formation or disintegration of flagella, redistribution of organelles and morphological changes between a non-polarized amoeba and an elongated, polarized cell with a distinct shape (Goodman, 1982). This strongly suggested the involvement of changes in cytoskeletal structures, as cytochemically unravelled by Uyeda and Furuya (1985). The microtubular cytoskeleton of *Physarum* flagellates was found to be formed by some modification into a cone structure of microtubules already present in amoeba. Microfilaments were detected in both stages of the slime mold, more peripherally with extrusions toward pseudopods in the amoebae, as a dorsal ridge in the flagellates. This last structure apparently coincided spatially with one of the bundles of microtubules (Uyeda and Furuya, 1985). According to Schuster's model (1965) for amoeba-to-flagellate transformation, "once the cell acquired an elongated shape, this was maintained thereafter". In their contrasting model, Uyeda and Furuya (1985) have proposed that "after a cell has become elongated and polarized, accompanying extrusion of the flagellar cone, it temporarily rounds up into a disk-like shape, while retaining its polarity".

Free-living amoebae of the protozoan genus *Naegleria* display a predominantly monopodial "limax" pattern of locomotion. Enflagellation normally occurs when amoebae are subjected to nutritional deprivation (Marciano-Cabral, 1988). Oppositely to the myxamoebae of *Physarum,* interphase *Naegleria* amoebae were found to lack of microtubular structures (Fulton, 1977) and, thus, cytoskeletal microtubules of its flagellates were suggested to be formed completely "de novo" together with flagella and basal bodies (Walsch, 1984).

As an amoeba, *N. gruberi* possesses no fixed polarity, forming pseudopodia in all directions. Within minutes after exposure to an inducing signal (low ionic strength) pseudopodial outgrowth becomes polarized; only one pseudopodium appears at one end and exhibits directed movement. Shortly thereafter fine projections appear at the posterior end and transform into a flagellum. This latter region now becomes the anterior, leading end, reversing the polarity of the cell.

Polarity of the cell is probably determined by factors controlling the position of the basal granules. In most cells these granules preexist or are derived from centrioles, but in *Naegleria* no preexisting centrioles or basal granules have been seen. This implies that the basal granule arises de novo by mechanisms still unknown and becomes fixed within the cortex at the posterior end. It is not clear whether the granule forms randomly in any region of the cell and migrates to the posterior end, or whether it is assembled in its final position. It does become fixed in the region of an amoeboid cell that is most rigid and under a state of contraction.

The change between the two motility forms of the amoeba *Naegleria gruberi* has been further studied by Lai *et al.* (1984) as a Yin-Yang, in Chinese thought the two complementary forces or principles that make up all aspects and phenomena of life (Encyclopedia Britannica, 1980). As explained by Lai *et al.* (1984). "This is

consistent with the current understanding of cell shape and motility as it applies to this differentiation, but it is also simplistic. Although no cytoplasmic microtubules have been observed in *Naegleria* amoebae, the amoebae use tubulin for mitosis. Therefore the capacity for amoeboid motility remains, albeit latent, in flagellates."

The cells change from amoebae with an actin-based motility system to flagellates with a tubulin-based system. Thus, they alternate between two fundamental motility forms. The actin-based motility system remains latent in the flagellates, but can be reactivated almost instantly by suitable stimuli (Fulton, 1977). In addition to the microtubules of the two flagella and basal bodies, the flagellates have a cage of microtubules just beneath the cell surface (Fulton, 1977; Walsh, 1984). The tubulin-based system is more impressive than the actin-based system in the sense that flagellates swim about 100 times as fast as amoebae walk. According to Lai *et al.* (1984), during the time that the cells are changing from an actin based to a tubulin-based motility, actin, the major protein of both amoebae and flagellates, is neither synthesized nor degraded, whereas tubulin for the flagella is synthesized de novo. The abundance of translatable mRNAs for tubulin and actin rise and fall in parallel with these changes.

The amoeba-to-flagellate transformation appears to be both directly and indirectly controlled by calcium (Marciano-Cabral, 1988). Its inhibition by the ionophore A23187 reveals a direct role of calcium while its inhibition by trifluoperazine is indirectly mediated by the calcium-regulatory protein calmodulin, itself controlled by cyclic nucleotide levels (Schuster and Twomey, 1983).

5. *Taxis*

a) *Chemotaxis*

Chemotaxis can be defined as the property of several substances to influence cell motion by attracting or repelling the cells or, in short, cell motion directed by external chemical gradients. Most relevant among the issues recently raised by Devreotes and Zigmond (1988) about "how do cells accomplish chemotaxis?" there is that of "how is the directional response achieved?" Other unsolved questions are "Do chemoattractant receptors move in the plane of the membrane? What localizes components of the cytoskeleton in a locomoting cell?"

Chemotaxis is not only most clearly displayed by eukaryotic models — slime mold and leukocytes — but also by many prokaryotes. Among mycoplasmas, helical spiroplasmas (Daniels and Longland, 1984; Daniels *et al.*, 1980) display a motion that appears to be a rotation around the helix and a flexing of the cell body (Davis and Worley, 1973). Using the motility track technique Kirchhoff *et al.* (1987) were able to demonstrate chemotactic behavior of a gliding mycoplasma that is moving very fast. Different opinions exist about chemotactic behavior of gliding, flagellated bacteria (Adler, 1975). This behavior has been described by some authors (Ho and

McCurdy, 1979), but negated by others (Dworkin and Eide, 1983) and it should be determined whether the use of the simple motility track technique will end this controversial discussion. In chemotactic bacteria *spatial* concentration differences of attractants can be translated into changes of concentration with time. Their *temporal* response has been found to be based on adaptation due to the methylation of a set of membrane proteins (Goy *et al.*, 1977).

Bacteria have sensory receptors — chemoreceptors — that detect the attractants per se; the first identified at the molecular level, by using deficient mutants, was the one for galactose which turned to be the galactose binding protein (Adler, 1980). Bacterial chemoreceptors serve a *dual* function: transport and chemotaxis; chemoreceptors being located in the cell surface, chemicals can serve as attractants without entering the cell. The control of the frequency of tumbling is the central feature of bacterial chemotaxis; its control applies not only to spatial gradients but also to temporal ones (Koshland's group, 1972-73, in Adler, 1980). Between the receptors of stimuli and the effectors is a system for integrating the sensory information and transmitting a message to the flagella. The system of sensory transduction somehow involves methylation of membrane proteins (Adler, 1980).

Proteins regulated chemotactic responses in *E. coli* are methyl-accepting chemotaxis proteins or MCPs (see Saier and Jacobson, 1984). Attractants stimulate methylation of one of these proteins (by a methyltransferase) while repellents cause demethylation (by a methylesterase). By their autophosphorylation, these proteins initiate a cascade of protein (histidine-) phosphorylation which may be important in the signal transduction pathway of bacterial chemotaxis (Hess *et al.*, 1988). Sensory information for chemotaxis is gathered by a set of transmembrane receptors, integrated and transmitted to the flagellar "switch" which regulates the direction of rotation of flagellar filaments in response to extracellular stimuli. The MCPs therefore play the decisive role in the conversion of sensory stimulus into behavioral, positive or negative polar response modulated by flagellar rotation.

The energy for rotation of bacterial flagella was shown not to be the ATP (unlike eukaryotic flagella), but rather the proton motive force (Adler's group, 1974 and Berg's group, 1977, in Adler, 1980). Prokaryotic flagella rotate at their base where is located the rotary motor comprising rings, thought to act as stators and rotors for the proton motor. Rotation of these ring structures with respect to another could then generate the torque necessary to spin the flagellar filament of flagellin. It is believed that the proton electrochemical gradient provides the energy for flagellar rotation (see Lehninger, 1982; Saier and Jacobson, 1984).

In the cellular slime mold *Dictyostelium discoideum,* amoebae are aggregated by a sophisticated chemotactic system (Bonner, 1974). Assessment of the directional role of a cyclic AMP gradient has provided one of the best understood examples of signal transduction across a biological membrane. Amoebae migrating away from the "source" of cyclic AMP in the spatial gradient extend lateral pseudopods in a

polarized fashion (McRobbie, 1986). Even though a spatial mechanism has been involved in assessing spatial gradient of the chemoattractant, a temporal gradient of cyclic AMP generated by an amoeba moving through the spatial gradient might play a major role in chemotaxis (Varnum-Finney *et al.*, 1987). In a recently proposed model (Klein *et al.*, 1988), the cyclic AMP receptor — rhodopsin-like, hydrophobic residues-rich — crosses the amoebal membrane with a serine-rich cytoplasmic carboxyl terminus, the proposed site of ligand-induced receptor phosphorylation.

Amoebae have two possibilities of sensing a spatial gradient of chemical attractant: they can measure concentration differences over their surfaces, or they can extend pseudopods into different directions using them as sensors. During its extension a pseudopod can translate spatial concentration differences into changes of concentration with time. It is an open question if, in the chemotactic response of amoeboid cells, such a temporal mechanism is involved. To solve this question for amoebae, chemical mediators of the chemotactic response have been searched to study their temporal behaviour after addition of attractant to living cells. This search for intracellular mediators of the chemotactic response has been focussed on changes which occur within the first few seconds after the stimulation of cells by cyclic AMP or other attractants like folic acid (pterins). A strong polarization could prevent the cells of *D. discoideum* from reorienting in a reversed gradient: cells attracted to a pipette-imposed gradient can reverse reorientation when the pipette is repositioned at the back (Loomis, 1982).

Interference of colchicine with chemotaxis (Oliver and Berlin, 1982) has led to consider that arrays of microtubules, organized from the centrosome, might provide direction to cell movement (Schliwa *et al.*, 1983). However, colchicine-treated polymorphonuclear leukocytes viewed microscopically still point in the right direction and, although they show wider angles of turn than untreated cells, they continue to move toward the chemotactic source.

In the ciliate Protozoa of the genus *Paramecium,* the "avoidance" reaction involves a quick change in the direction of ciliary beating. This reaction is critically dependent on the presence of Ca^{2+} ions in the medium and dependent on local intracellular concentration of this ion (excess of 10^{-6} M) influx of Ca^{2+} down its electrochemical gradient causes membrane depolarization, thus provoking the avoidance reaction by ciliary reversal and transient opening of Ca^{2+} channels in the plasma membrane covering the cilia (see Saier and Jacobson, 1984). By contrast, hyperpolarization of the cell membrane resulting from K^+ cell outflow provokes an escape behavioral response in *Paramecium.* Mechanically responsive ion channels are therefore differentially localized in the cell membrane and, as further stated by Saier and Jacobson (1984), "since an anterior stimulus elicits a response opposite to that resulting from a posterior stimulus (i.e. depolarization versus hyperpolarization), and channels of different specificities are initially activated in each case (Ca^{2+} versus K^+), there must be spatial localization of the two receptor channels at

opposite ends of the cell''. Both Ca^{2+} and K^+ channels exhibit the classic voltage-dependence (see IV.B.2d) and their functionings have been compared in several behavioral mutants in *Paramecium* (see Saier and Jacobson, 1984). Recently, the deficient Ca^{2+}-dependent K^+ current mutants called pantophobiacs could be corrected by the microinjection of wild type *Paramecium* calmodulin (Hinrichsen *et al.*, 1986).

Leukocytes are also polarized in their response to changes in chemotactic gradients: when the direction of a spatial gradient is reversed, new pseudopods extend from the front of most leukocytes (Zigmond *et al.*, 1981).

b) *Phototaxis*

Phototaxis is an orientation depending on the direction of the incoming light rays. The red *Halobacterium halobium* can use the light as a signal for orienting itself in the environment through a photoreceptor pigment, bacteriorhodopsin. This retinyl pigment (Häder, 1987) is concentrated in the cell membrane at particular regions of this so-called purple membrane. Upon light absorption, bacteriorhodopsin is deprotonized; this exchange of protons is a vectorial process and the resulting electrochemical H^+ gradient can be used by the cell in different ways such as the synthesis of ATP and the proton-driven pumping of Na^+ ions out of the cell. If the light intensity is suddenly increased or decreased, the halobacterial cell can perform a typical photophobic response (reversed swimming direction). Thus, bacteriorhodopsin is responsible for a dual use of light, both as source of energy and signal for behaviour (Haupt, 1980).

Probably Cyanobacteria are the only prokaryotes capable of a true phototactic orientation (Häder, 1987). The oriented movement takes the form of positive phototactism (toward the light source) or negative phototactism (away from the light source). The two families of *Oscillatoriaceae* and *Nostocaceae* differ in the mechanisms of phototactic orientation. A model for the sensory transduction chain powering the motor of the photophobic gliding *Phormidium (Oscillatoriaceae)* has been proposed by Häder (1982). A proton gradient built up in light through the photosynthetic electron transport chain causes a hyperpolarization in the cytoplasm. When the front cells enter a dark field, this proton gradient breaks down which causes depolarization. The small electrical potential generated between the two morphologically equal ends of the filament — front end more negative than rear end — is supposed to open voltage-dependent Ca^{2+} specific channels in the plasmalemma. This initial small potential change causes voltage-dependent Ca^{2+} channels to open and allow a transient massive Ca^{2+} influx. The resulting large electrical potential change reverses the potential difference between the front and rear ends, which eventually reverses the direction of movement (Häder, 1987). A partially different mechanism for the sensory transduction in Cyanobacteria has been proposed by

Glagolev's group (Gabai and Glagolev, 1985) which implies redox sensing through plastoquinone. From these models of photosensory behaviour in prokaryotes it can at least be concluded that motile filaments of blue-green algae show a temporal polarity which might be due to an electrical potential difference between front and rear end. Consequently, the photophobic reversal of their movements can be inhibited by the application of external electrical potential. The following scheme, proposed by Haupt (1980) summarizes such sensory transduction of the photophobic response of a *Phormidium* trichome:

Light STIMULUS dI/dt --► TRANSDUCTIONAL changes in: electron flow rate --►plastoquinone --►proton transport across thylakoid and plasma membranes ---►electrical polarity of cell ---►reversal of anterior-posterior cell polarity --► amplification by cell-to-cell transfer --►cell polarity controls direction of motor activity --►RESPONSE: reversal of gliding movement.

The gliding flagellate *Euglena gracilis* shows positive and negative phototaxis depending on the light intensity and implicating a flavin-like signal. Since phototactic orientation can be induced in some cases by very low light intensities, a signal amplification by gated cation fluxes needs to be incorporated in the sensory transduction chain (Doughty and Diehn, 1982). Ca^{2+} currents generated by light stimulus also influence the motion of flagella and thereby a positive phototaxis in the green algal-flagellate *Chlamydomonas.*

Dictyostelium discoideum myxamoebae show a positive phototaxis in low light intensity. The direction of light was detected by a comparison between the signal strengths at a multitude of receptor sites around the circumferences of the myxamoebae. A low light beam focussed onto the periphery of a cell induced a reorganization of its internal polarity and movement in the direction of the microbeam (Häder and Burkart, 1983).

Phototactic behavior in *Physarum polycephalum* has been discussed by Mori *et al.* (1986) in terms of bifurcation in spatio-temporal organization in a self-organizing system. Twice more ATP was measured in the front than in the rear of a migrating plasmodium. This polar pattern became unstable, and a new wavy pattern appeared by stimulating a local frontal part with blue light.

In amoeba, transient polarity can also be induced by lateral incident light (Lazowski, 1986). The negative phototactic reaction of *Amoeba proteus* is related to a semi-permanent organization of its cell. Posterior part of the illuminated cell is developed on the more irradiated side, anterior part with advancing pseudopods on the shadowed side. In order to minimize the body area direction, exposed to light, amoeba turns its body to a parallel position in respect to light direction, or it produces the new pseudopods on the shadowed cell side. Then, it gradually orients its body along the light direction, as the leading pseudopod advances. The mechanism underlying the light-induced functional polarity of the amoeba appears to involve

intact membranes as suggested by the disturbing effects of different drugs such as DMSO, digitonin and saponin on the light-induced cell polarity (Lazowski, 1986).

6. *Structural basis of directionality*

The apparent relationship between cell polarity and the position of the centrosome has been postulated a century ago by van Beneden (1883). It received support from recent experimental studies that correlate shifts in centrosome position with changes in the cell axis and/or changes in the direction of cell locomotion. Even though such a correlation between cell polarity and centrosome position could not be established in all instances (Oliver and Berlin, 1982). These and other observations have led to the widely held belief that cell polarity and the direction of cell locomotion are determined by the centrosome, which transmits the necessary information to the rest of the cytoskeleton by way of the microtubules associated with it. It needs to be emphasized, however, that, whereas these studies present evidence for a positive correlation between centrosome position and the establishment of a cell axis or the direction of cell locomotion, it is difficult to determine cause and effect. This question is central to the issue of cell polarity during directional locomotion, but it cannot be resolved on the basis of the available evidence.

Mechanisms required for cell locomotion to occur are: a) intracellular force-generation involving the cytoskeleton; b) polarity-determination involving the insertion of new membrane mass into the leading edge; c) adhesive interactions of the cell surface with the substratum. The insertion of new membrane mass into the leading edge implies an equivalent rate of retrieval of old membrane mass towards the rear of the motile cell. Required for this directed locomotory process is that "ordinary membrane recycling be polarized, i.e. that at least some of the processes of membrane insertion and retrieval (recycling) that normally occur *uniformly* over the surface of a nonmotile cell, be *polarized* so that insertion always occurs at the leading edge and retrieval towards the rear" (Singer and Kupfer, 1986).

Focal adhesions usually form within or just behind the ruffling leading edge of motile cells. A microspike or bundle of radially-oriented filaments of actin precedes the formation of these adhesions. According to Burridge *et al.* (1988) "Talin, but not vinculin, has been detected at the leading edge and is concentrated at the tips of the distal portion of the precursor (Izzard, 1988) where microfilaments terminate in an electron dense patch of material associated with the plasmalemma (Small, 1981)".

Euteneuer and Schliwa (1985*a*) have proposed that the cell cortex influences the position of the centrosome/microtubule complex. This interaction, whose molecular basis remains to be determined, also takes place in polar migratory cells, where the cell cortex, notably extending anterior lamellipod, plays a dominant role. As commented by these authors, the proposed dominance of the cortex, however, does not

mean that microtubules are dispensable and do not take part in directional locomotion of many cell types, perhaps except fish epidermal keratocytes; these last cells appear to be "permanently polarized and therefore may circumvent the need to achieve the polarization that is required by an amorphous cell in order for it to migrate" (Singer and Kupfer, 1986). In keratocytes, the emphasis thus appears to be shifted from a cell component — microtubules — that sets up polarity to an element that helps reinforce polar organization, set up by the cell cortex. Trinkaus (1984) discusses a scenario where microtubules become passively aligned by the cortex, help stabilize cortically determined asymmetry, and thus, in a process of mutual reinforcement, establish cell polarity. In this way the centrosome-aster complex tends to exert a constraining influence and serves to stabilize the bulk of the cell.

The polarizing influence from basal granules is also expressed as a pattern of oriented cilia beating synchronously in waves that direct movement of a ciliated cell. In this way polarity at the molecular level is transformed into organismic asymmetries.

Activation of the cell cortex by chemoattractants (Schliwa *et al.,* 1982) induces cell spreading that is likely to exert a force on the microtubules extending into the peripheral filament network, thus pulling the microtubule aster apart and leading to two, occasionally three, independent asters. As long as the cortex remains active, this condition is maintained. It is reversed upon return to a normal migratory morphology (Schliwa *et al.,* 1982) or experimental disruption of the actin network.

Many cell types are capable of persistent, directional locomotion in the absence of known external stimuli such as chemical signals, substrate properties, or electric fields. Essentially all these locomoting cells possess an external polar organization characterized by the presence of an anterior lamellipod of variable shape and complexity, and a posterior uropod or tail from which retraction fibers may extend. This external asymmetry seems to be complemented in many instances by an internal axis defined by the position of two prominent cell organelles, namely, the nucleus and the centrosome/microtubule complex. The finding of an apparent relationship between cell polarity and the position of the centrosome, postulated a century ago, received support from recent experimental studies that correlate shifts in centrosome position with changes in the cell axis and/or changes in the direction of cell locomotion. Even though such a correlation between cell polarity and centrosome position could not be established in all instances, these and other observations have led to the widely held belief that cell polarity and direction of cell locomotion are determined by the centrosome which transmits the necessary information to the rest of the cytoskeleton by way of the microtubules associated with it.

The leading edge of migrating cells is internally propelled by force-generating systems that are components of the cytoskeleton: cytochalasin B inhibits not only directional migration but all protrusive activity (blebbing). As the primary effect of this drug is to "cap" the barbed ends of F-actin filaments (see IV.E.1), a role for

actin in cell migration is strongly implied by these findings (Singer and Kupfer, 1986). The possible relationship between cell motility and mitochondrial membrane potential has been explored in epithelial cells in culture and mitochondria of the leading edge found to have a higher potential than those located elsewhere (Johnson *et al.,* 1981; Chen, 1988).

In migrating cells, the cytoplasmic vesicular structures and their associated microtubules are more or less oriented in front of the nucleus and face the average direction of motion. Serial sectioning was required to demonstrate such cell polarization for polymorphonuclear leukocytes migrating along a chemotactic gradient (Malech *et al.,* 1977). In fibroblasts migrating out of chick heart explants, mitochondria were highly clustering near the nucleus while they were dispersed more evenly when the cells came to rest in confluent growth (Parsons, 1986). This structural polarization of cells in motion was confirmed in a computer-aided three-dimensional graphics reconstruction of serial thin sections of migrating fetal monkey neurons (Parsons, 1986) and further work is now being directed at quantitating the polarization of organelles in moving cells.