Zeitschrift: Archives des sciences et compte rendu des séances de la Société
Herausgeber: Société de Physique et d'Histoire Naturelle de Genève
Band: 42 (1989)
Heft: 1: Archives des Sciences

Artikel: Polarity : from dipoles to biopolarizations. IV. Addenda
Autor: Turian, Gilbert
Kapitel: IV: Subcellular polarizations
DOI: https://doi.org/10.5169/seals-740083

IV. SUBCELLULAR POLARIZATIONS

B. Surface membranes

1. Primitive membranes

The self-assembly process of bilayer membranes involves complex interactions between amphiphilic molecules which have been mimicked by computer simulations. The two terms of the interactive model which involves hard-core *repulsion* between hydrophilic-hydrophobic spheres and anisotropic *attraction* between these particles can lead to assembly of a two-dimensional membrane (Drouffe *et al.*, 1991).

2. Plasma membrane

a) *Biochemical properties*

Water-soluble proteins often expose their polar and charged residues on the membrane surface, whereas apolar residues tend to occur in the interior (Perutz *et al.*, 1965 in **I**; Chothia, 1976 and others in Rees *et al.*, 1989). By contrast, the hydrophobic organization of membrane proteins is explained by models which use the hydrophobic transmembrane α-helix and an "inside out" pattern of more polar interior residues (Engelman and Zaccai, 1980 in Rees *et al.*, 1989).

The lipid bilayer of the plasma membrane is known to be asymmetrical (see Bretscher, 1973; Rothman and Lenard, 1977 in **III**) and this asymmetry might help to keep membrane proteins properly oriented in the bilayer. As for the highly asymmetric association of proteins with the membrane (Alberts *et al.*, 1983 in **I**; Singer, 1990 in **III**) it is of functional significance for the redistribution of mobile membrane proteins and cytoskeletal elements in biopolarizing processes (Poo, 1981 and Luther *et al.*, 1983 in Nuccitelli, 1988).

The anchoring of proteins to the lipid bilayer would be mediated by a phosphatidylinositol-glycan (PI-G)-specific phospholipase D (Scallon *et al.*, 1991). As recently found by many researchers (Hoffman, 1991a), the activity of many membrane-bound proteins needs prenylated lipid tags possibly to help directing them to the right cellular locations. This involves isoprenoid addition which thereby helps a protein such as Ras insert in membrane. Iterations of the positively charged lysine adjacent to the CAAX box would be essential to lead the protein to the plasma membrane.

The surface of the integral, outer membrane channel-forming, trimeric protein porin from Gram-negative bacteria displays polar and nonpolar aromatic side chains at the borderline between the polar and nonpolar parts of the membrane. Ca^{2+} sites can be predicted from electrostatic potential calculation (Weiss *et al.*, 1991).

b) *Structural polarity and recognition systems*

Dielectrophoresis denotes the motion of polarizable particles under the influence of a non uniform electric field (Pohl, 1978 and Zimmermann, 1982 in **I**; Schwan, 1989). It has allowed the determination of the critical frequency F_0 (the polarizability of a shell sphere minus that of the medium) which contains quantitative information on the electric parameters of the membrane of murine myeloma and *Neurospora crassa* slime cells (Marszalek *et al.*, 1991). Non-linear dielectric spectroscopy is a convenient mean to monitor the ability of living cells to transduce exogenous electric field energy (Woodward and Kell, 1990). Low-frequency fields can cause lateral movement of proteins in the plane of the membrane: appropriately charged proteins accumulate at a single pole under the influence of dc fields (Sowers and Hackenbrock, 1981) whilst ac fields cause their accumulation at both poles of spherical cells (Zimmermann and Vienken, 1982). There are many other reports in which very weak electromagnetic fields have been shown to elicit biological or biochemical responses (see Lin, 1989). Applied electric fields can cause a large asymmetry in the distribution of membrane particles on the cathode-facing sides of *Micrasterias* lobes (also see VI.C.c) oriented perpendicular to the fields (Brower and Giddings, 1980). Membrane permeability can be increased by electroporation of cells as reviewed by Lindsey and Jones (1990).

Among the many studies which have shown asymmetric changes in $[Ca^{2+}]$, that of Rooney *et al.* (1990) can be singled out as the first demonstration of oscillatory-Ca^{2+} waves caused by a localized hormone receptor/signal transduction system in a nonexcitable cell. Owing to functional polarization of the cell, the hormone-induced Ca^{2+} signals must originate from a limited membrane domain. The effector-target cell contact probably causes a directional delivery of signals into the effector cell. The transient intracellular polarization thereby established is manifested by the selective redistribution of cytoskeletal proteins and intracellular organelles (Podack and Kupfer, 1991).

c) *Energy transduction*

The plasma membrane H^+-ATPase generates an electric potential and pH gradient across the membrane by extruding protons from the cell; the energy bound in this electrochemical gradient is thought to be the driving force for solute carriers and channels that are responsible for nutrient uptake and maintenance of cell turgor (Palmgren, 1991). An auto-inhibitory domain in the C-terminus of the enzyme might interact with the catalytic site and/or a proton binding site (Palmgren *et al.*, 1990).

d) *Electric potentials*

Ion channels mediate the transmission of electric signals within and between cells of sensory organs. The functioning of several types of voltage-dependent ion channels involves a dual effect of membrane depolarization, first described for the sodium

conductance of the squid axon (see **I**.IV.B.e and also Hodgkin and Huxley, 1952). Such channels open upon depolarization in their activation phase and they spontaneously close, in their inactivation phase, even when the depolarization is maintained. Gating currents express electrically the conformation changes that lead to channel opening. Their measurement in the Shaker potassium channel of the *Drosophila* indicates that the charge on the voltage sensor of the channel is progressively immobilized by prolonged depolarizations (Bezanilla *et al.*, 1991). Neher and Sakmann (1992) have received the Nobel Prize for their patch clamp technique proposed in 1976 (see IV in **III**) to isolate ion channels from cell membranes. Voltage-gated K^+ channels have been reviewed by Miller (1991) and, in higher plant cells, Ca^{2+} channels have been shown to play a major role in such processes as bud formation and polar growth (Schroeder and Thuleau, 1991).

Mechanosensitive channels have been implicated in the regulation of many cell processes including the budding yeast (Gustin *et al.*, 1988). Such a type of channel has been found to transduce the membrane stress into a ionic influx which triggers differentiation of appressoria from the germling apices of *Uromyces* stopped in their polarity growth (Zhou *et al.*, 1991). Electric polarization microelectrode measurements have revealed an apical-basal potential difference in the fungal *Pilobolus* cells (Tarakanova *et al.*, 1991). Membrane potentials which are usually comprised between -60 and -70 mV in animals (see Meissner, 1976; Henquin and Meissner, 1984) exhibit a higher negative membrane potential, of the order of -200 mV (Sanders and Slayman, 1989), in fungi and plant cells.

Phototransduction in retinal rod cells results from a cascade of highly regulated chemical reactions implicating among others the G-protein-coupled receptor rhodopsin (see **I** V.B.5a and Palczewski and Benovic, 1991) that translate the light signal into a hyperpolarization of the cell plasma membrane. Illumination-induced transient hyperpolarization of the plasma membrane is followed by an intracellular acidification in *Trichoderma* (Gresik *et al.*, 1991).

Among the receptors of the central nervous system there are two main classes: ligand-gated ion channels such as the NMDA glutamate receptor recently cloned by Nakanishi's group (Moriyoshi *et al.*, 1991) and metabotropic receptors coupled with G-proteins.

e) *Action potentials*

The electrical properties and the voltage of the nerve cell membrane are determined by ion currents (see Hille, 1984 in **I**). Initiation and conduction of the neuronal action potential is due to voltage-sensitive Na^+ channels (see also d). Modulation of such channels in the brain by protein kinase C phosphorylation might have effects on signal transduction and synaptic transmission in the central nervous system (Numann *et al.*, 1991). Like the nerves, action potentials of algae such as *Chara* were accompanied by a sharp rise in membrane conductance (see Blatt, 1991). In the voltage-gated fluxes measured and simulated in *Acetabularia* the charge balance for the

transient Cl^- efflux, which frequently occurs during an action potential, can be accounted for by the observation of a corresponding release of Na^+ (Mummert and Gradmann, 1991).

Glucose-stimulated insulin secretion provokes B-cell electrical activity which follows an oscillatory pattern in membrane potential on which bursts of action potentials are superimposed (Henquin and Meissner, 1984).

Sensory cells convert different forms of energy to transmembrane potentials. Mechanosensory neurons in the marine mollusk *Aplysia* usually show marked action potential accomodation during prolonged depolarization (Klein *et al.*, 1986, in Walters *et al.*, 1991); axonal injury induces long-term changes in sensory neurons such as: decreased accomodation, decreased hyperpolarization (Walters *et al.*, 1991). Mahowald and Douglas (1991) have exploited the analogy between silicon devices and biological membranes to represent these different ion currents thus providing the voltage-dependent conductances. Complementary metal-oxide-semiconductor circuits have been used to represent the different ion currents thereby forming the silicon analog of a biological neuron.

f) *Synaptic membranes*

Nitric oxide (NO) is polarly transmitted through the neuronal synapse (Hoffman, 1991b). It can thus be considered as a "retrograde messenger" (Barinaga, 1991).

C. Endomembranar and vesicular systems

The secretory pathway targets proteins from the endoplasmic reticulum (ER) via the Golgi apparatus (G) and secretory vesicles to the plasma membrane (Fig. 1 in Reid, 1991). Three hydrophilic, soluble cytoplasmic proteins are required at different stages in vesicle transport (Aalto *et al.*, 1992): Sly1 between ER and Golgi, Sec1 between Golgi and the plasma membrane (Novick and Schekman, 1979), and Slp1 between Golgi and the vacuole. Reverse transport also occurs from Golgi to ER (Dean and Pelham, 1990). Molecular dissection of this pathway related to the Golgi model with a focus on transporting vesicles and their simple coat structure has been recently reviewed by Rothman and Orci (1992). Molecular signals allow the vectorial flow and filter selection of trafficking proteins travelling to the cell surface (Hopkins, 1992).

1. Endoplasmic reticulum

Intracellular trafficking of proteins is concerned with how polypeptides constituents of each cellular organelle and compartment are sorted and polarly delivered to their destinations as recently reviewed in a reference book edited by Steer and Hanover (1991).

When the ribosome becomes engaged with membrane proteins the entity is termed the translocon, and it catalyses the translocation of the nascent protein across the membrane into the lumen of ER. A ribonucleoprotein, signal recognition particle (SRP), functions in this targeting process (Walter and Lingappa, 1986, see **I**). A model for SRP's function has been proposed for yeast ER (Hann and Walter, 1991). Related to the problem of polarized translocation of proteins across membranes, the existence of a protein-conducting channel in the ER has been demonstrated by the use of electrophysiologic techniques (Simon and Blobel, 1991; Lingappa, 1991).

2. Golgi apparatus

Models accounting for donor-acceptor connections in inter-Golgi transport *in vitro* as well as the role of carrier vesicles in that polarized transport have been reviewed by Mellman and Simons (1992).

The fungal metabolite brefeldin A (BFA) can alter the distribution and flow of membrane through an interlocking membrane system (see **II** and Lippincott-Schwartz *et al.*, 1991). Polar transport from ER to the Golgi apparatus (G) has been inhibited by BFA in *Candida albicans* (Arioka *et al.*, 1991). From the G, proteins are involved in vesicular transport and membrane fusion (Waters *et al.*, 1991). In this ER-G transport, the integral membrane protein Sec12p is required for the formation of the transport vesicules generated from the ER (D'Enfert *et al.*, 1991).

Fusion of ER-derived vesicles targeting to G requires calcium and ATP (Rexach and Schekman, 1991). The ER-G traffic is regulated by a GTP-binding SAR1 protein localized to the early compartment of the yeast secretory pathway (Nishikawa and Nakano, 1991).

3. Intracellular vesicles

Receptor mediated endocytosis, well documented in animal cells, has now been reviewed in plants (Robinson and Hillmer, 1990). Evidence has been presented that constitutive exocytosis in yeast is insensitive to changes in cytosolic calcium levels and to voltage and ionic gradients changes across the plasma membrane (Lew and Simon, 1991).

Yeast plasma membrane secretory vesicles have been exploited as "an expression system for site-directed mutants of the [H+]ATPase" (Nakamoto et al., 1991). A GTP-driven mechanochemical enzyme, dynamin, is associated with vesicular traffic (van der Bliek and Meyerowitz, 1991). This nucleotide-sensitive microtubule-binding protein cross-links microtubules into highly ordered bundles, and appears to have a role in intermicrotubule sliding in vitro (Chen *et al.*, 1991).

D. Organelles

1. Lysosomes and microbodies (peroxisomes, etc.)

A peroxisomal targeting tripeptidic signal had been identified in a number of peroxisomal proteins (Gould *et al.*, 1989). The recent detection of the same mechanism

of polar translocation of proteins into glyoxysomes and glycosomes lends support to "a common evolutionary origin for these microbodies" (Keller *et al.*, 1991).

2. Mitochondria

The soluble form of the electron transport enzyme succinate dehydrogenase behaves as a diode gating electron flow in one direction only (Sucheta *et al.*, 1992).

3. Chloroplasts and phototransducing membranes

The hydrophobic organization of transmembrane regions of the photosynthetic reaction center from *Rhodobacter* (ex *Rhodopseudomonas*) *sphaeroides* has been studied by Rees *et al.*, (1989, see also IV.B.2). The polarity of the interior of the reaction center structure resembles that of soluble proteins and is "intermediate between protein surfaces exposed to aqueous solution and those exposed to hydrophobic cores of membranes" (Deisenhofer and Michel, 1991).

Long-lived photoinduced charge separation has been achieved and maintained in a redox, artificial photosynthetic system (Slama-Schwok *et al.*, 1992).

E. Cytoskeletal components

For a helpful book on these macromolecules, see Amos and Amos (1991).

1-2. Microfilaments (actin-myosin)

The dynamic filamentous structure of F-actin and the consensus reached on its three-dimensional atomic model (see **I**) have been reviewed (Bremer and Aebi, 1992). Phalloidin reduces the dissociation rate constants at both ends of actin filaments to near zero and also reduces the association rate constant at the barbed end by about 50% (Sampath and Pollard, 1991).

Dynamics and density of actin microfilaments and their turnover process in lamellipodia of locomoting cells have been accounted by a few models (Heath and Holifield, 1991) among which a first embodies a polarized array of actin filaments and another one of nucleation-release (Theriot and Mitchison, 1991). The force fluctuations in a single actin filament and the quantization of actin filament velocities have been described by Ishijima *et al.* (1991) and Uyeda *et al.* (1991).

"Myosin rods" have been studied in amoebae of *Dictyostelium discoideum* by immunoelectron microscopy (Yumura and Kitanishi-Yumura, 1990). The yeast cell type specific budding pattern is maintained by a myosin heavy chain gene (Rodriguez and Paterson, 1990). A myosin organization center would play a role in the determination of cell polarity in *Dictyostelium* (Fukui *et al.*, 1991) while a 110-kDa calmodulin complex identified in kidney microvilli shares with myosin the ability to associate with actin filaments to give them a defined polarity (Coluccio, 1991).

3. Microtubules-tubulins

Viewed from the outside, all protofilamentous subunits have the same orientation ("outside-out") and the same polarity ("polarity up", with two distinct ends, "plus" and "minus"). The intrinsic polarity of microtubules is the consequence of their formation from aligned asymmetric subunits. As a component of the spindle pole body, the newly discovered γ-tubulin first isolated from *Aspergillus nidulans* (Oakley *et al.*, 1990, see **III**) has been proposed to establish microtubule polarity *in vivo*. γ-tubulin genes have now been cloned and characterized from *Schizosaccharomyces pombe* and *Xenopus laevis* (Stearns *et al.*, 1991) as well as *Drosophila melanogaster* and *Homo sapiens* (Zheng *et al.*, 1991). Posttranslationally modified tubulins exhibit restricted subcellular distribution in polarized cells of developing *Artemia* (MacRae *et al.*, 1991).

When microtubules depolymerize *in vivo* up to a specific point, they either regrow along exactly the same path or abruptly reverse their directional growth (Schulze and Kirschner, 1988). Cytoplasmic components such as mitochondria might influence this directional process involving intramitochondrial chaperone proteins accounted by a new model for *in vivo* microtubule assembly (Gupta, 1990).

The dynamic instability of microtubules accounted by the GTP-cap model has been reviewed by Caplow (1992) and the role of microtubules in the orientation of plant cell wall cellulose microfibrils has been modellized by Emons *et al.* (1992).

4. Microtubule-associated "motor" proteins

This year has seen an explosion of new data about additional cytoplasmic microtubules motors. A novel kinesin, *unc-104* encoded, is a neuron-specific motor used for anterograde translocation *in vivo* of synaptic vesicles along axonal microtubules in the worm *C. elegans* (Hall and Hedgecock, 1991; see also VIII.2.a). The microtubule-based dyneins and kinesins were first thought to move in opposite directions along the asymmetric microtubules. The fact that the newly found kinesin-like motor, *ncd*, can move in the same directions as dynein raises the possibility that dyneins and kinesins have similar motor domains (McDonald *et al.*, 1990 and Walker *et al.*, 1990 in Goldstein and Vale, 1991). Using the fission-yeast with its bipolar intranuclear spindle, Hagan and Yanagida (1992) have proposed two models in which a spindle pole-body-associated, plus-end directing cut7 reorientates the microtubule arrays during spindle formation. Evidence has recently been obtained of another member of the kinesin family generating force toward the microtubule minus end, and that dynein may be either a bidirectional protein, or composed of closely related retrograde and anterograde isoforms (Bloom, 1992). However, motor proteins are not only microtubule-based as recently evidenced for unidirectional organelle movement in squid axoplasm shown to be ATP- and actin-dependent, and probably generated by a myosin-like motor (Kuznetsov *et al.*, 1992). This actin-based myosin motor uses the free energy of ATP hydrolysis to produce force or motion on a track which moves unidirectionally actin filaments. The polarity of the velocities was assayed *in vitro*

(Uyeda *et al.*, 1991) and determined such that the direction of the active movement is positive.

F. Nuclei and mitotic figures

Structural bipolarity is the fundamental property of mitosis. This polarity is not yet evident during the transition from prophase to metaphase. The movements of the centrioles at prophase, similar to those of the "polar plaques" in simpler organisms, are controlled by microtubules which are oriented in the spindle with their minus ends at the pole.

1. Interphasic and mitotic structures

Information complementary to that of Gerace and Burke (1988, see **I**) about the nuclear pore complex and its aqueous channels has been provided by Silver (1991) and cytosolic proteins which specifically bind nuclear location signals have been described as receptors for nuclear import (Adam and Gerace, 1991). Nuclear targeting signals allow selective entry of the acidic protein nucleoplasmin into the nuclear pore complex. Two interdependent basic domains have been identified in the nuclear targeting sequence (Robbins *et al.*, 1991). The bidirectional nature of this import-export exchange at the nuclear envelope is distinct from transport across the membranes of other organelles as reported in a recent meeting review (Nigg *et al.*, 1991).

The symmetry of the mitotic figure may exist without centrioles (Molè-Bajer, 1975, in Dustin, 1978) but these organites are "indicators of poorly understood factors which lead the cell to the bipolarity found at metaphase". As further stated by Dustin (1978) "mitosis is polarized, either by MTOC, by centrioles, or by unknown polar forces (as in higher plants)". The classical separation and migration of centrosomes to opposite poles on (or within) the nuclear envelope can be deviated to *multi*polar mitosis (Baltzer, 1908, in Czihak *et al.*, 1991). Such a deviation can be produced by mitotic inhibitors such as carbamates (urethane, etc., ref. in Dustin, 1978). Conversely, some special conditions such as colchicine poisoning (C-mitosis, see Dustin, 1978) provoke uni(mono)polar mitoses. The forces which must normally move bipolarly to position centrosomes, involving actin and microtubules (Euteneuer and Schliwa, 1985 see **I**; Schatten *et al.*, 1988), are someway disturbed. Microsurgical experiments designed by Maniotis and Schliwa (1991) have shown that animal cells never enter mitosis following removal of the centrosome. Centriole-free regenerated microtubule-organizing center (MTOC) regenerated in karyoplasts cannot partition and thus cannot generate the *intrinsic* bipolarity of the mitotic spindle. Intra-astral bidirectional motility – retrograde-anterograde – known to occur in animal cells (Rebhun, 1972; Bajer and Molè-Bajer, 1975 in **I**) has also been observed in fungal mitotic asters (Aist and Bayles, 1991). Models predict that a sliding mechanism operates between microtubules of opposite polarity to produce spindle elongation as observed by the occurrence of spaced bridges in amoebae of *Dictyostelium discoideum* (Jensen *et al.*, 1991).

At mitosis, spindle microtubules are assembled with a common polarity, the minus ends being embedded in pericentriolar material, the plus ends being free to grow into the cytoplasm. The way that this polarity is achieved starts only now to be understood. γ-tubulin has just been localized in the animal centrosome (Stearns *et al.*, 1991) and Zheng *et al.* (1991) also point to the role of this new tubulin on the side of α- β-tubulins in the establishment of this polarity in the microtubule organizing center.

2. Polewards chromosome movements

Chromosome-to-pole spindle forces involve complex molecular mechanisms (Koshland, 1991) and, in answer to the major question of the basis of their generation, it has recently been found that one of the best candidates for a "motor" for mitosis, cytoplasmic dynein (Salmon, 1989), is present at the kinetochore at least to meet the energy requirements for chromosome motion in prometaphase (Farr *et al.*, 1990; Steuer *et al.*, 1990). This finding shifts the emphasis on force generation within the chromosome from that of microtubule polymerization and depolymerization to a motor-dependent mechanism itself dependent upon the existence of a microtubule continuum (Snyder *et al.*, 1991a).