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. II. Addenda and indexes
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Kapitel: IV: Subcellular polarizations
DOI: https://doi.org/10.5169/seals-740081

IV. SUBCELLULAR POLARIZATIONS

B. Surface membranes

2. a) *Biochemical properties*

The bilayer membrane can be modelled electrically as a thin slab of non conducting material separating two aqueous solutions and thereby acts as a simple parallel-plate capacitor (Gennis, 1989). "Its dielectric constant is a measure of the polarizability of the material and the degree to which any permanent electric dipoles which may be present in the material respond to an electric field (voltage difference)".

The amphiphilic phospholipids form spontaneously well-organized bilayer structures in water which are the basic architecture of biomembrane. Evidence has been obtained with membrane model systems, which support the view that lateral proton conduction occurs at water/lipid interfaces (Tocanne and Teissié, 1990). The polarity at these interfaces in terms of dielectric constant is different of that of bulk water. This means that, "in terms of micropolarity or water molecular dipole moment, the lipid/water interface region is more than likely anisotropic both in terms of structural organization and electrical properties" (see B.2b).

As for the very low permeability of the lipid bilayer to cations as compared to anions (see Tocanne and Tessié, 1990) it is ascribed to the positive polarization potential of the surface membrane (see B.2.d) which would constitute an energy barrier against the transport of positively charged compounds across membranes.

Permeability coefficients have been determined for several kinds of small molecules. Among them, water can relatively easily penetrate the membrane bilayer. As commented by Gennis (1989) "It may seem surprising at first to learn that water can so readily penetrate the phospholipid bilayer". However, "there is no substantial water to be found inside the membrane beneath the carbonyl groups".

2. c) *Energy transduction*

In 1961, two proposals were made as to the way in which electron-transfer reactions of the cytochrome chain — the chain used in the oxidation of NADH by molecular dioxygen — could be connected to ATP formation without the intervention of chemical intermediates (Williams, 1989). Both mechanisms invoked the transduction of the energy of the oxidation/reduction reaction to a proton gradient before the gradient generates ATP. The two mechanisms, sometimes termed the delocalized (Mitchell) hypothesis and the localized (Williams) hypothesis, are very different: in the first, protons generated by oxidation appear only in aqueous phases;

even ATP is generated by an electric field acting on the ATP synthetase and not by proton flow; in the second, protons move in proteins within matrices and aqueous phase equilibrations are ignored in the development of proton gradients, in proton diffusion and in the ATP-synthesis step. To distinguish between these mechanistic possibilities, long series of experiments (Wikström, 1989) have been carried out on separate parts of the cytochrome chain, especially on the last stages of the electron-transfer reactions, those of cytochrome oxidase.

Electric currents produced by oxido-reduction reactions, also called Faraday currents, can be assayed by electrochemical methods such as those of polarography. The polarograph apparatus works with three electrodes (see Monnier *et al.*, 1979): an indicator capillary electrode on which oxido-reduction reactions occur at the surface of mercury drops, a reference electrode allowing to impose to the first one a constant potential while varying the voltage, and an auxiliary electrode insuring passage of current. Registered curves of intensity-potential of chemicals such as metal ions allow their quantitative assay. Dissolved O_2 can also be measured by the polarographic technique (Fork, 1972).

In artificial fuel cells, gases are combined electrochemically such that the exothermicity is converted directly to electrical energy and the only reaction product is water. Dyer (1990) observed gas — electrical energy conversion processes occurring whithin very thin films of gas-permeable, ionically conducting membranes of hydrated aluminium oxide, as a prototypical membrane. Both polarity and the magnitude of the voltage were unexpected. The covered inner platinum electrode was positive and the polarity of the cell could be changed in $H_2 + O_2$ mixtures only when the outer platinum catalyst was changed to a nickel catalyst. This shows the strong dependence of cell polarity on the metals used and their sequence, suggesting that "different electrochemical kinetics might establish the polarity observed" (Dyer, 1990).

2. d) *Electric potentials*

Many possible factors can contribute to the amount of electrical work to move a charge through a membrane (Gennis, 1989): a) associated work with dielectric constant; b) internal dipole potential by orientation of the dipoles at the membrane surface resulting in a positive potential in the center of the phosphatidylcholine bilayer; c) surface potential which, in most biomembranes, is negatively charged, usually due to the presence of acidic, anionic phospholipids; the electric potential at the shear plane which is the plane defining what migrates in the electric field is called the zeta potential (McLaughlin, 1977); it someway controls the electrophoretic mobility of charged vesicles (electrokinetic effects); d) transmembrane potential which is defined as the difference in the electric potentials of the two bulk aqueous phases separated by the membrane. The asymmetric charge distribution generates

transmembrane potentials which are usually negative inside and can be measured with fluorescence polarity methods using probes such as merocyanine or anilinonaphthalene.

The membrane surface potential (ΔV) is the sum of an electrical term (Ψo) and a dipolar or "polarization" term (ΔVp) which exhibits high positive values (about 300 to 500 mV). The variously oriented and rotating strong dipoles of lipid polar heads would contribute to the surface polarization potential and this view (Tocanne and Teissié, 1990) has been correlated with the concept of "molecular electrometer" as developed by Seelig *et al.* (1987) on the ground of ^{2}H-NMR experiments using parameters such as the deuterium quadrupole splitting.

In fungi, marked changes in the membrane potential detected by [^{3}H]tetraphenylphosphonium (TPP^{+}) uptake rate have been caused by illumination of dark-grown mycelium of *Trichoderma viride.* An initial hyperpolarization of the plasma membrane was found to be accompanied by a rise in the intracellular ATP concentration and by changes in the intracellular level of cyclic AMP (Gresik *et al.*, 1988).

In higher plants, blue light is known to activate the electrogenic proton pump to hyperpolarize the plasmalemma (Assmann *et al.*, 1985 and Shimazaki *et al.*, 1986). Plasma membrane hyperpolarization caused by auxin (IAA), accompanied by short time oscillations in the electric potential of corn coleoptile cells, is paralleled by cytosolic pH drops as well as changes in Ca^{2+} activity (Felle, 1989). Moreover, the activity of the plant plasma membrane enzyme NADH oxidase which transfers the electrons from NADH to oxygen in the absence of added electron acceptors has been linked to membrane polarization (Novak and Ivankina, 1983). In Conjugatophycean green algae photoreception, a tetrapolar gradient of phytochrome created by light perception is achieved by the dichroitic orientation of plasma membrane-bound phytochrome molecules; blue-light also appears to mediate a tetrapolar gradient of the sensor pigment proper mediating tetrapolar actin anchorage sites on the plasmalemma (Grolig and Wagner, 1988).

Gating and ion selectivity of calcium channels have been further studied by electrophysiological experiments. Subtypes of calcium channels have been classified according to their voltage threshold for activation and by their inactivation characteristics (Wray *et al.*, 1989). Current dependence of channel gating has been tentatively ascribed to the formation of dipoles along the trajectories of ion movement that exist during dipole relaxation time (Kostyuk *et al.*, 1989). This new approach would assume that "ion transition through the open channel produces local displacements of charged molecular groups lining the wall of its steric region". During the process, the frequency of ion transitions would increase drastically and become comparable with frequency of dipole relaxation (Kostyuk *et al.*, 1989).

Release of Ca^{2+} from the sarcoplasmic reticulum (SR) following depolarization of transverse tubules (T-tubules) triggers contraction of the skeletal muscle. The foot

structure of the SR is part of a molecular bridge which spans a short gap between the T-tubules and the terminal cisternae of the SR. Large cytoplasmic extensions of the molecule evidently attach to the dihydropyridine receptor complex in the T-tubules (Agnew, 1989). There is also evidence that the dihydropyridine receptor in the T-tubule membrane of skeletal muscle functions not only as slow calcium channel but also as an essential component of coupling, probably as the voltage sensor (Takeshima *et al.*, 1989). A model of the structure of the dihydropyridine-sensitive calcium channel has been proposed (Catterall *et al.*, 1989) in analogy with current models of the structure of voltage sensitive sodium channels.

Chloride (Cl^-) channels (normal and pathological) were activated by patch excision which caused large membrane depolarization. This allowed Welsch *et al.* (1989) "to use depolarization as a "tool" to determine if a Cl^- channel was present in a patch". Active chloride transport can be light-driven by retinal proteins. These bacterio- or halorhodopsins function as inward-directed electrogenic pumps for Cl^- ions (Zimányi and Lanyi, 1989). Parallely, these pumps transport protons out of the cell interior, thereby generating an inside-negative membrane potential.

Opening and closing of chloride channels studied in the electric ray *Torpedo californica* are unequally timed. This asymmetric electric conduction increases with transmembrane electrochemical gradient for the chloride ion thus demonstrating that the channel-gating process is not at thermodynamic equilibrium (Richard and Miller, 1990).

2. e) *Action potentials*

They are not only generated in animals (see **I**) but also in fungi, algae and higher plants in response to light, heat, cold, chemicals, electrical stimulus, and wounding as reviewed by Pickard (1973) and Simons (1981). Davies (1987) considered action potentials as multifunctional signals in plants and proposed a unifying hypothesis to explain apparently disparate wound responses. Action potentials could also be a unifying factor to explain the involvement of an interaction between Ca^{2+} flux and auxin transport in the role of gravity in geotropisms (De la Fuente, 1984, also VIII.A.2.c[4]).

Cell electrophysiology and membrane transport in plants have been recently reviewed by Bentrup (1989) who stated that "the evergreen question of the role of Ca^{2+} during the characean action potential will remain elusive as long as the characean plasmalemma is not routinely accessible to patch clamp technics". In the *Characeae,* depolarization occurs by diffusive Cl^--efflux and repolarization by diffusive K^+-efflux (Köhler *et al.*, 1986; Gradmann, 1989).

The role of K^+ in the mechanisms of action potentials has been further analyzed in the green alga *Eremosphaera viridis* by Köhler *et al.* (1985, 1986) who showed that it is caused by a transient opening of a K^+ channel which is not gated by the membrane potential.

In animals, action potentials experimentally evoked by electrical activity can suppress neurite elongation and growth cone motility (Cohan and Katter, 1986) and thereby may influence structure and connectivity within the nervous system (see also VI.A.2.i).

Following electrical activity in excitable cells, there is an increase in intracellular Ca^{2+} concentration. Silver *et al.* (1990) also report that clustering of L-type Ca^{2+} channels causes intracellular Ca^{2+} hotspots at the neural growth cone. Enzymes with a micromolar requirement for Ca^{2+} at the hotspots are therefore activated by the ensuing depolarization. The role of voltage-dependent calcium influx in controlling nerve cell outgrowth remains puzzling because "also raised intracellular Ca^{2+} concentration triggers outgrowth of the growth cone margin, neurite elongation requires low intracellular Ca^{2+} concentration". According to Silver *et al.* (1990), the fact that "electrical activity can selectively raise intracellular Ca^{2+} concentration in the growth cone, leaving neurite calcium concentration low would resolve this paradox".

C. Endomembranar and vesicular systems

1. Endoplasmic reticulum

In the endomembranar sorting process, proteins destined for transfer are sequestered within membrane vesicles that bud off from a donor organelle and then fuse with the appropriate acceptor organelle. Vesicle fusion in several distinct branches of this complex distribution network as well as transfer of vesicles between the rough endoplasmic reticulum (ER) and the Golgi complex require the same cytosolic protein, a tetrameric, *N*-ethylmaleimide-sensitive protein (NEM) called NSF (Beckers *et al.*, 1989). Such transfer requires ATP and is inhibited by NEM or the monoclonal antibody against NSF. NSF is required in a late, calcium-dependent transfer step; this step is most likely the fusion step. Surprisingly, the deduced protein of cloned and sequenced NSF product showed sequence similarity with the product of a yeast gene *(SEC18)* previously shown by Schekman and Novick (1982) to control the transfer of vesicles between the rough endoplasmic reticulum and the Golgi complex; more recent studies suggested it has a function in endocytosis (Riezman, 1985). These results raise the possibility that "fusions between different organelles derived from the rough endoplasmic reticulum may all be catalyzed by the same set of proteins" (Schatz, 1989).

2. Golgi apparatus

This compact structure colocalizes with the microtubule organizing center (MTOC) in a perinuclear region of fibroblasts. Intact interphase microtubules but

not microfilaments appear to be required for this specific location of the Golgi apparatus. This has been demonstrated by the scattering of Golgi elements after treatment with the microtubule depolymerizing drug nocodazole, and by the subsequent reclustering of the Golgi elements when nocodazole is removed (Ho *et al.*, 1989). A protein may be involved in linking the Golgi apparatus to the microtubule network and the MTOC in vivo (Allan and Kreis, 1986). A fungal antibiotic, brefeldin A, produces a reversal of traffic polarity i.e. a rearrangement of Golgi elements into the ER, thereby inducing a secretion block (Bosshart *et al.*, 1990). Such "violation of the one-way system" has been further discussed by Armstrong and Warren (1990).

D. Organelles

3. Chloroplasts and phototransducing membranes

Most of the chloroplast proteins are imported from the cytosol and polarly directed into six different compartments (Smeekens *et al.*, 1990). Two sorting systems are involved in this import and intraorganellar transport of nuclear-encoded protoplast proteins. Additional sorting informations located at N- termini are contained in thylakoid lumen proteins. The information present in transit peptides, decoded by the chloroplast import machinery, is not yet known.

The electron transfer reactions in photosystem II take place within the so-called reaction center grouping numerous antenna pigment molecules (chlorophyll, etc.) as well as organic ions and charged atoms (manganese, calcium, etc.). The stepwise transfer of electrons through this reaction center succeeds in pulling far apart the mutually attractive positive and negative charges. The task of the photosystem II is thus to act as a tiny capacitor, storing energy by separating and stabilizing positive and negative charges on either side of the thylakoid membrane (Rutherford, 1989). The water-splitting reaction produces four protons and four electrons released simultaneously with O_2 in that water-oxidizing clock which is a cyclic mechanism of four states (Gowindjee and Coleman, 1990).

E. Cytoskeletal components

That the cytoskeleton is someway involved in plants intracellular movements, perception mechanism and transmission effects has again been emphasized by Hensel (1989b) who concluded that "the function of the cytoskeleton is to generate and maintain cell polarity".

As for fungal cells, they have been comprehensively surveyed in 1987 and 1989 by Hohl.

1-2. Microfilaments (actin-myosin)

Both actin and myosin filaments have definite polarities and well-ordered structures (see **I**). Actin filaments can move in opposite directions on tracks of myosin heads. They always move foreward but never backward reversing the polarity of the movement. According to Toyoshima *et al.* (1989) "The direction of movement is therefore determined by the polarity of the actin filament".

Myosin heads can form reverse chevrons and, when tethered in a single thick filament of a mutated *Drosophila* flight-muscle sarcomere, can bind with opposite rigor crossbridge angles to flanking thin filaments, which are apparently of opposite polarities (Reedy *et al.,* 1989).

The driving force for the rearrangements of the actin cytoskeleton in cell motility, division and differentiation is provided by actin-binding proteins. The addition of actin subunits to the barbed end of actin filaments and the nucleation of polymerizing actin *in vitro* are controlled by capping protein. Recent experiments suggest that capping protein regulates polar distribution *in vivo* of actin filaments. The actin cytoskeleton is disrupted in yeast capping protein mutants, indicating that "the asymmetric distribution of actin in budding yeast (see VI.A.1.a[2] in **I**) depends on the proper functioning of several actin-binding proteins with apparently different functions" (Amatruda *et al.,* 1990).

The uniform angle and conformation of myosin subfragment 1 (S1) bound to actin filaments (F-actin) "attest to the precise alignment and stereospecificity of the binding of these two contractile proteins. Because actin filaments are polar, myosin heads must swing or rotate about the head-tail junction in order to bind" (Reedy *et al.,* 1989). Adams and Pollard (1989) have shown for the first time that the single-headed myosins called myosin-I can bind directly to NaOH-extracted membranes isolated from *Acanthamoeba* and to vesicles of pure lipids with an affinity sufficient for extensive binding in the cell. Membrane-bound myosin-I may provide a mechanism for many cellular movements previously thought to involve filamentous myosin-II (see V, in **I**) and for the specification of sites of cell surface growth (Drubin *et al.,* 1990).

For a general review about cytoskeleton microfilaments, see Kristen (1987).

1-3. Microfilaments-microtubules (actin-tubulin)

In the cortex of the giant coenocytic green alga *Caulerpa,* amyloplasts are transported along microtubular strands as shown by the fact that both microtubule- and dynein-specific inhibitors block movements of these organelles. In contrast, chloroplast movement is blocked by cytochalasin but not by colchicine thereby showing that immobilization and movement of chloroplasts are dependent on intact microfilaments of actin but not on microtubules (Menzel and Elsner-Menzel, 1989).

F. Nuclei and mitotic figures

2. Polewards chromosome movement

The bipolar attachment of chromosomes to the spindle occurs well before all the chromosomes congregate metaphasically. In the normal functioning of the mitotic spindle most of its growth and disassembly take place at the end of the microtubule away from the pole. All microtubules have the same polarity and the fibers behave differently depending on the structure in the spindle to which they bind. Most important as microtubule-organizing center is the centrosome which serves as a seed to start microtubule polymerization; thereby it defines their polarity. That polarity, or asymmetry, is crucial to the functioning of microtubules (see I) by at least two of its functional consequences: at the ends it causes the (+) end to add and lose subunits faster than the (−) end; along the surface it influences the orientation with which proteins will bind to the microtubule surface (McIntosh and McDonald, 1989).

The molecules involved in the mechanical forces moving polewards chromosomes begin to be unraveled (Vale and Goldstein, 1990). Among such mitotic motors there are kinesin motors and perhaps the newly discovered dynamin motor (Shpetner and Vallee, 1989) which forms cross-bridges and induces ATP-dependent sliding between antiparallel microtubules *in vitro* (McIntosh and Koonce, 1989). Kinesin is a microtubule-interactive, force-generating ATPase acting as a plus-end motor in intracellular transport of vesicles along microtubules (Vale, 1987, and others, see in **I**). The inherent asymmetry of the polymer (actin or tubulin) and the motor is necessary for the unidirectional movement of the motor along the polymer. It is toward the barbed (or +) end of the actin filament that myosin motors such as myosin I (single ellipsoidal head) move.

A superfamily of kinesin motors acting in fungal nuclear fusion and division has now been described in *Saccharomyces cerevisiae* (Meluh and Rose, 1990) and in *Aspergillus nidulans* (Enos and Morris, 1990). Such kinesin motors bear either round or rectangle heads at the end of the α-helical coiled coils. Short single-headed kinesins analogous to myosin I, kinetochore-specific kinesins, and perhaps kinesins may also be expected to be involved in morphogen or RNA transport as force-producing proteins (Vale and Goldstein, 1990).