Meeting Report

Ion transport across biological membranes and its control

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It was the aim of this workshop to bring together scientists from different fields of membrane research to discuss the control of ion transport across biological membranes under a variety of aspects. About 25 speakers presented recent results in an attempt to illustrate the various levels at which such control processes can be defined and described. Major topics were:

Control in terms of the molecular structure of transport and channel proteins;

Control by ion- or potential-mediated gating processes;

Sensory control.

Information about the molecular structure of membrane proteins is of great importance for understanding their functional properties. A preceding workshop of the Sonderforschungsbereich was therefore mainly devoted to the structural analysis of membrane proteins. A selection of particularly well-studied transport proteins served to demonstrate the state of the art in establishing the relation between structure and function [1].

A channel system which has recently attracted considerable interest is the junction channel that controls the exchange of molecules and ions between adjacent animal cells. This kind of channel is important in coordinating cell metabolism and growth and, by virtue of its low resistance, in facilitating transmission of electrical signals, e.g., in synchronously acting cardiac muscle cells. The 3-dimensional structure and transitional states of the gap junction channel protein were presented by N. Unwin (Stanford). An oligomer of 6 identical subunits, each having a molecular mass of about 28 kDa, forms a cylinder which protrudes into the aqueous phase on either side of the lipid bilayer. The complete channel through the membranes of two adjacent cells is constituted by two such oligomers. Three-dimensional analysis of the gap junction by electron microscopy after negative staining [2] has suggested that the subunits can rearrange around the channel lumen by tilting and sliding against each other. When the subunits are strongly tilted tangentially to the channel, the channel appears open (15–20 Å in diameter); when the subunits are only slightly tilted, the channel appears closed. X-ray diffraction studies show the transition to be sensitive to Ca^{2+} [3]. In accordance with physiological studies the 'closed' configuration is observed at high Ca^{2+} concentration. Three-dimensional maps obtained in rapidly frozen aqueous solution, with or without Ca^{2+}, show that the change in tilt of the subunits accomplishes a large change in channel dimensions without the in-

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dividual polypeptide chains becoming grossly distorted.

Ion transport mediated in asymmetric artificial membranes by neutral ionophores was discussed by W. Simon, W.E. Morf, H. Ruprecht and P. Oggenfuss (Zürich). Membranes with asymmetric permselectivity for cations can be realized by combining two half membranes containing the same cation-selective ionophore in different environments [4]. Ideally, such an asymmetric membrane may selectively transport one sort of cation (e.g., Ca^{2+}) in one direction and another (e.g., Na^+) in the opposite direction. The ion selectivity of neutral carrier membranes not only reflects the complexation behaviour of the ionophores but also depends on the dielectric properties of the membrane. According to theory, the selectivity coefficients can be identified with the equilibrium constants of the basic ion-exchange reactions (including complex formation).

Attempts to apply structural principles of biological membranes to the improvement of technical membranes for separation purposes were reported by K. Heckmann and K. Ring (Regensburg, Frankfurt) [5]. In contrast to the highly anisotropic biomembranes, technical membranes still contain an amorphous matrix which causes a number of disadvantages in practical application. It was therefore attempted to construct technical membranes by crosslinking biological membrane constituents or suitable detergents. The most appropriate material so far proved to be a glycosyl-phospholipid with a di-isopranol-tetraether moiety from an archaebacterium, *Thermoplasma acidophilum* [6]; as well as analogous synthetic detergents substituted with saccharides. The lipids or detergents were crosslinked between the saccharide residues without perturbation of the liquid-crystalline structure of the hydrophobic interior. Such membrane systems, stabilized by adequate supporting material, have successfully been doped with ionophorous antibiotics. This may be the prototype of a new class of artificial technical membranes.

G. Boheim and W. Hanke (Bochum) reported on reconstitution experiments with channel-forming proteins in planar lipid bilayers. Highly purified channel proteins from different types of cells were incorporated into solvent-free planar lipid bilayer membranes. The gating activity of the reconstituted channel proteins was then analysed by use of the patch-clamp technique. The condition of having solvent-free lipid bilayers turns out to be essential in order to preserve the channel function of the proteins. On the basis of the complex gating behaviour of the Ca^{2+}-activated and the voltage-dependent K^+ channel from rat embryonic muscle cells [7], a generalized picture of ion channel kinetics was proposed. A variety of further examples of highly purified channel-forming proteins, e.g., phallolysin, TTX-binding Na^+ channels, ACh-gated channels, was presented.

Molecular models of ion transport systems in the inner mitochondrial membrane and the erythrocyte membrane were the subject of a series of contributions. Control of transport by substrate-, ligand- or environment-induced conformational transitions and functional as well as structural asymmetries become evident in an increasing number of ion-translocating proteins. M. Klingenberg (Munich) reported studies suggesting that the uncoupling protein from brown adipose tissue mitochondria, likely to be involved in thermogenesis by inducing a short-circuit of H^+, shares structural (M_r, dimeric structure, relative hydrophobicity) and functional properties (half site reactivity, nucleotide specific binding sites) with the ADP/ATP exchange carrier of the mitochondrial membrane. In contrast to that system, however, the function of the uncoupling protein is inhibited by ATP, presumably due to a conformational change. Evidence for an allosteric relationship between nucleotide and H^+ binding comes from a pronounced pH dependency of pK_{ATP} in the range pH > 7 [38].

The problems met in defining whether a transport system behaves symmetrically were illustrated by R. Krämer (Munich) for the mitochondrial ADP/ATP exchange carrier. Using a reconstituted system it was demonstrated that with respect to substrate affinity, kinetics, and dependence on membrane potential, the system behaves symmetrically [8,9]. In other respects, however, such as inhibitor binding, activation by anions, and dependence on surface potential, it is remarkably asymmetric. These findings imply that in ion translocators a pronounced structural asymmetry may be accompanied by symmetric transport kinetics.

A particular aspect of these studies concerned the effect of charged lipids on transport parameters. These effects were also strongly asymmetric and suggest that the substrate binding sites at the opposite faces of the membrane sense the surface potential to a very different extent.

The relevance of the composition of the lipid domain of the membrane for the properties of transport proteins was further underlined in a communication by W. Köhne and B. Deuticke (Aachen). Using band 3 protein from the erythrocyte membrane combined with the 4 major phospholipid species of the membrane, they could demonstrate that phosphatidylcholine and phosphatidylethanolamine maintain a high activity of the system, whereas sphingomyelin and phosphatidylserine suppress transport activity. These findings raise some interesting problems with respect to the control of anion exchange in the erythrocyte membrane, well-known for its highly asymmetric distribution of phospholipids [10].

Possible molecular mechanisms of anion movement via band 3 protein of the erythrocyte were reviewed by H. Passow and M. Glibowicka (Frankfurt) [11]. Information on such mechanisms has recently been obtained by comparing the different modes of operation of the transporter, namely, (1) electrically silent, obligatory anion exchange, (2) conductance by 'slippage', defined as the occasional return of the empty carrier (i.e., net transport of classic carrier transport), and (3) electro-diffusion (or transit) of anions through the 'open' system without liganding to the reorientation of a binding site. Substrate binding is crucially dependent on the integrity of lysyl and arginyl residues. Transport rates and binding of modifiers are influenced by anions and inhibitors in a mode indicating allosteric interactions between various domains of the protein. Two different models of operation of the anion exchange protein have been proposed. In the 'knock-on' (or 'zipper') mechanism [12] substrate anions are translocated by breaking sequentially a chain of salt bridges between arginyl and carboxyl groups. The alternative 'lock in' model ascribes anion exchange to a one-step conformation change of two arginyl groups which entrap the arriving anion, thereby closing the originally open gate near the mouth of the anion channel [13].

A further example of the relationship between structure and function in an H^+ transport protein was put forward by G. Buse and G.C.M. Steffens (Aachen). Comparative analyses of the primary structure of cytochrome *c* oxidase from mammalian tissues and from *Paracoccus denitrificans* suggest a structural domain, invariant from bacteria to man, which may function as an electron-conducting bridge between the metal centers (copper, heme *a*) of the enzyme (subunit II, I) [14–16]. Three-dimensional models show that this bridge might consist of aromatic residues (Tyr, Trp) forming a stacked π-bonding system of 3–6 members which span a distance of 10–20 Å. Electron conduction within this system might force a tyrosine residue to shuttle between a benzenoid and a quinoid structure and thus to act alternately as an H^+ donor or acceptor site. Quantum-mechanical calculations (in collaboration with J. Fleischhauer, Aachen) suggest that electron conduction within such a system might directly promote H^+ transport.

Two contributions were concerned with patch clamp analysis of Ca^{2+}-activated K^+ channels [17]. W. Schwarz and R. Grygorczyk (Frankfurt) reported on Ca^{2+}-activated highly K^+-selective channels in the cell membrane of human erythrocytes [18]. The single channel conductance which decreases with decreasing K^+ concentration does not vary with the Ca^{2+} concentration, but depends on membrane potential and K^+ concentration. It ranges from >40 pS for inward currents to 10 pS for outward currents. Activation (the probability of a channel to be in the open state) is zero for Ca^{2+} concentrations below 0.5 μM and saturates at 5–10 μM free Ca^{2+}. It becomes smaller for more positive membrane potentials. The mean open time increases linearly, the mean closed time decreases nonlinearly with increasing Ca^{2+} concentration. The data are consistent with a model where the binding of two Ca^{2+} is necessary for the activation of a single channel.

The open and closed interval distributions of Ca^{2+}-activated K^+ channels in the cell membrane of cultured rat muscle were studied by K.L. Magleby and B.S. Pallotta (Miami). The distribution frequencies of the two major open channel states and of the 3 closed states all depend differently on $[Ca^{2+}]$ (0.1–1 μM). The long closed interval decreases with a power of about 2 with increasing $[Ca^{2+}]$, indicating that the openings re-

quire the binding of 2 or more Ca^{2+}. A model, analogous to that for the ACh receptor with 3 closed states, 1 inactivated state and 2 or 3 open states, can describe many of the observed kinetic properties [19].

The control of ion transport across epithelial membranes was the subject of two contributions. M. Henrich and B. Lindemann (Homburg) discussed the voltage dependence of currents and densities of apical Na^+ channels in the toad urinary bladder measured by noise analysis in the presence of amiloride or triamterene applied to the mucosal side. Power density spectra of current fluctuations obtained under steady-state voltage clamp conditions showed blocker-induced Lorentzians which were analyzed for blocking rate constants, Na^+ channel currents (i) and area densities of open channels (N_o). When the clamp voltage was increased, i increased nonlinearly and roughly as predicted by the Goldman-Hodgkin-Katz equation. The relative increase of the macroscopic current was less than that of i, indicating that N_o decreases with increasing voltage. At +100 mV about 70% of the N_o active at 0 mV are electrically silent. This effect is observed within 2 min after changing the voltage and occurs irrespective of the degree of transport inhibition by the extrinsic blocker [20,21].

The influence of the ionic microclimate on ion transport across membranes of intestinal and gallbladder epithelia and its consequences for modelling these transport systems were presented by K.U. Petersen and K. Heintze (Aachen). As observed already by several authors, the activities of H^+, Na^+, K^+ and Cl^- in the immediate vicinity of the luminal sides of such membranes may markedly differ from those in the bulk solutions. It is the ion activity of this thin fluid layer, however, which defines the concentration gradients driving passive and secondary active ion flow. Ion activities in this layer should be monitored during ion substitution experiments. In some cases it may be essential to introduce appropriate corrections into transport models. For instance, the feasibility, in energy terms, of Na^+/Ca^{2+} exchange, ascribed to the basolateral membranes of several epithelia, is still a matter of dispute. The ambiguity of the results may be due in part to uncertainties concerning the Na^+ activity in the large submucosal unstirred layer, different from that in the serosal bulk solution [22,23].

Mechano-electric control of ion transport was the subject of several contributions. U. Thurm (Münster) reported on sensory transduction in mechanoreceptor cells of insects. At the site of force impact, the receptor membrane is tightly connected to cuticular structures stabilizing it against being stretched and in some sensilla also against being bent. Within this area of 0.3–5 μm^2, membrane-integrated cones, rather regularly spaced (2000/μm^2) protrude for about 18 nm from the inner membrane surface. They are connected to microtubules arranged in a layer beneath the membrane. The stimulating mechanical force is concentrated on the small cross-section of the membrane cones. The short latency of 50–70 μs of the electric response suggests a mechanical control of ion channels, which probably occurs in that membrane area on which the stimulatory force is concentrated. The data suggest that each cone–membrane complex represents a single sensor-channel unit [24].

The surface membrane of ciliated protozoa is a convenient system for analysing mechanosensory transduction and electrically controlled cation fluxes. The behaviour of ciliates upon mechanical stimulation is controlled by at least two different receptor channels. Activation of one type, which is permeable to Ca^{2+} and other divalent cations, leads to membrane depolarization. The other type is exclusively permeable to K^+, its activation leading to hyperpolarization. As reported by H. Machemer (Bochum), the depolarizing channels are distributed along the cell, with the largest receptor currents recorded at the anterior end. In contrast, the conductance for K^+ is maximal at the posterior end. As a consequence of superposition of these two gradients the mechanosensitivity is defined by the site of the stimulus input.

Deciliation experiments have shown that mechanoreceptor channels are restricted to the somatic membrane; voltage-sensitive Ca^{2+} channels are localized in the ciliary membrane [25]. Due to the electrotonic spread of a depolarizing receptor potential, ciliary Ca^{2+} channels open and Ca^{2+} enters the ciliary space, thereby causing ciliary reorientation.

The role of cilia in stimulus transduction has been investigated using the long immobile 'tail cilia' of *Paramecium* [26]. Local mechanical

stimulation of these cilia causes a delay of several milliseconds in receptor current activation as compared to direct somatic stimulation. The observations suggest that tail cilia are passive members during stimulus reception; they may serve to process the mechanical input prior to transduction.

Membrane currents during excitation of the ciliary membrane were analysed by Y. Naitoh (Ibaraki). Based on the classical Hodgkin-Huxley-type kinetic analysis the Ca^{2+} conductance (g_{Ca}) during a membrane depolarization was formulated as $g_{Ca} = \bar{g}_{Ca} \cdot m^5(1 - (1 - h)^5)$. Unlike the Na^+ current, the inactivation of the Ca^{2+} current in *Paramecium* depends on the intraciliary accumulation of Ca^{2+} during entry. The time course of changes of g_K during an action potential can be formulated as $g_K = \bar{g}_K \cdot n$ [27].

Voltage clamp experiments revealed that K^+ and Ca^{2+} in the medium have antagonistic effects on most of the membrane properties. The response was found almost constant when the ratio $[K^+]/[Ca^{2+}]^{1/2}$ was kept constant. The results indicate that Ca^{2+} influx as well as the kinetic properties of Ca^{2+} and K^+ channels depend primarily on membrane-bound calcium [28].

Competition between Ca^{2+} and other divalent cations at the ciliary membrane was investigated by F. Wehner and E. Hildebrand (Jülich). Exchange of Sr^{2+} for Ca^{2+} in the medium does not reduce the voltage-dependent Ca^{2+} inward current, and Ba^{2+} even causes a slight increase. However, both activation and inactivation kinetics of the Ca^{2+} current are slower with Sr^{2+} and by far slower with Ba^{2+}, indicating that the gating mechanism is ion-dependent. Other divalent cations tested inhibit the Ca^{2+} inward current, most probably in a competitive manner. Evaluation of the data in terms of Michaelis-Menten shows the ratio of 'dissociation constants' to be: $K_{Ni}:K_{Co}:K_{Ca}:K_{Mn}:K_{Mg} = 1:3:4.5:5:6.5$. This sequence indicates specific binding of divalent cations and rules out simple screening of surface charges as the only mechanism of Ca^{2+} current inhibition. Rather, competitive binding to anionic sites at the outer surface of the membrane and/or to constituents of the Ca^{2+} current channels itself seem to control the Ca^{2+} conductance.

A couple of reports were concerned with photoreception, especially with the origin of elementary excitatory events, the so-called quantum bumps. Based on experimental data from the fly photoreceptor, K. Hamdorf (Bochum) described his hypothesis of the microvillus as the unit of excitation and adaptation (microvillus hypothesis) [29]. This hypothesis includes: Every microvillus behaves as a physiological unit. Excitation by adsorption of a photon leads to local production of an excitatory substance E. After reaching a threshold concentration, substance E induces the opening of ion channels in the membrane of the 6 surrounding microvilli and thus elicits a quantum bump. Simultaneous photoactivation of neighbouring microvilli accelerates the time to threshold concentration of E, thus shortening the bump latency. After excitation the microvillus enters a refractory phase. The state of adaptation is determined by a simple equilibrium between the excitable and refractory microvilli. As H. Stieve (Jülich) reported, a quantum bump in the *Limulus* ventral photoreceptor involves the transient opening of an estimated number of up to 10^4 ion channels located on 10^3–10^4 microvilli. Bumps follow photon absorption after considerable, greatly variable delays (on average 310 ms). Weak light adaptation shortens the bump latency to about 250 ms and reduces both bump size and width. Very weak conditioning flashes cause facilitation, namely, an increase in the number and size of bumps. Spontaneous bumps have a smaller average size than light-evoked bumps [30,31].

A statistical evaluation of the experimental data of single photon responses in the ventral photoreceptor of *Limulus* as demonstrated by H. Stieve was presented by J. Schnakenberg and W. Keiper (Aachen) [32]. On the basis of this evaluation, the following assumptions for the mechanism involved were proposed: (1) The latency is due to a time-consuming process without amplification which follows the absorption of a photon but precedes the opening of channels. (2) A 'transmitter' which is released in the region of the microvillus hit by the photon transfers the information to the ionic channels. This release starts at the end of the latency period and includes amplification. (3) The transmitter molecules diffuse to the light-dependent channels and cause their opening. The open channels close at random after some average open time. These assumptions have been put together into a quantitative model which allows a comparison of the calculated

bumps and of the distributions of their parameters with the experimental data. In agreement with the experimental data, the model exhibits 3 uncorrelated sources of fluctuations: the duration of the latency period, the extent of amplification and the local density of channels and their conductances.

The electrical properties of the disc membrane in vertebrate photoreceptors are almost unknown. Light-induced changes of the electric field near the disc surface were investigated by P.J. Bauer, E. Bamberg and A. Fahr (Jülich, Frankfurt, Berlin) with bovine discs attached to black lipid bilayer membranes [33]. At physiological ionic strength the polarity of the light-induced voltage transient was negative at the rhodopsin-containing side of the bilayer, while at low KCl it was positive. The action spectrum of the signal height matched the adsorption spectrum of rhodopsin. The observations cannot be explained simply by interfacial charge displacement in the disc membrane, as assumed for the early receptor potential. Most probably, the photoresponse is generated by ionic fluxes across the disc membrane.

Direct measurements of the Ca^{2+} content and Ca^{2+} exchange in rod outer segments of the toad by means of laser-micro-mass-analysis (LAMMA) were done by W.H. Schröder and G.L. Fain (Jülich, Los Angeles). It was shown that rod outer segments contain large amounts (~4 mM) of Ca^{2+}. Physiological levels of illumination produce a Ca^{2+} efflux up to about 10^4 ions per rhodopsin bleached. Since light does not change the rate of Ca^{2+} influx, the total Ca^{2+} content of the rod decreases upon illumination. In bright light, about half of the total Ca^{2+} leaves the rod within only 1 min of illumination [34].

Another kind of control of membrane function was reported by S. Wendler and U. Zimmermann (Jülich). Algal cells respond to osmotic changes in their environment by changing the rates of ion transport or metabolic reactions to maintain constant turgor pressure. In contrast to other algal cells, a continuous turgor increase was observed in *Acetabularia*, even at constant osmotic pressure of the medium. At a critical point, the pressure is down-regulated by a burst-like chloride salt efflux. The results are consistent with the idea that the plasmalemma and/or tonoplast are able to sense the turgor and that the permeability to Cl^- is pressure-controlled. Charge-pulse experiments on *Valonia* cells indicate that changes in thickness of the cell membrane could be the primary step in the conversion of pressure signals to metabolic processes [35]. Relaxation measurements after application of current pulses suggest a redistribution of charges within one (plasmalemma) or both (plasmalemma and tonoplast) membranes, presumably as part of a transport system [36]. The results are consistent with an electromechanical model recently proposed [37].

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