Features

Talking Points

Deciphering the language of cells

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Long distance cell-to-cell or organism-to-organism communications may be accomplished by transmission and reception of electromagnetic signals through membrane receptors or enzymes. Consistent with this idea is the observation that membrane ATPases are capable of absorbing energy from oscillating electric fields of defined frequency and amplitude and using it to perform chemical work. The concept of the 'electroconformational coupling' is used to explain how an electric signal can modulate the activity of a membrane protein, and conversely, how an energy-dissipating reaction can produce an electric signal.

Most biochemists agree that cells communicate with each other either by direct exchange of metabolites through gap junctions, or by transfer of messenger molecules or ions over distance. Slime mold and yeast, for example, secrete cAMP to coordinate movement of the cell population towards a center of aggregation at a certain stage of development. In eukaryotic cells neurotransmitters, hormones, growth factors, etc. regulate metabolism, growth, differentiation, biosynthesis and locomotion. Thus, multicellular signals originate from the interaction of ligands with membrane receptors of target cells, which then activate a cascade of biochemical reactions[1–3]. Communication by transmitting molecules or ions over space, however, is a slow process and not effective over long distances. Cells and organisms often need rapid communications, and so they have adopted other methods of transcellular signaling. The most familiar of these is the use of sound waves: birds sing to attract the opposite sex; we speak to convey our thought. The sound waves propagate through the space. No messenger molecules or ions are sent but the messages reach their audience and are perceived. Another familiar example is the electrocommunication of fishes[4,5]. Certain species of electric fishes are able to send EOD (electric organ discharge) signals to warn or to inform other fishes. Multicellular signals and organ-to-organ communications also use electric impulses (see for example Ref. 6). Again, direct transmittal of ligands are not essential. Birds, bats and microorganisms use radiowaves and magnetic fields for orientation or to identify or determine the position of objects. All these activities are crucial to life and are fascinating from a scientific viewpoint, but until recently their molecular mechanisms have received little attention from the biochemistry community. Two popular textbooks of cellular biochemistry, for instance, do not include these essential activities in their lists of methods of transcellular signaling. A prominent biochemist, in a recent conversation with the author, even labeled study of this type of cell-to-cell communication as 'astrology' and maintained that signals could only be carried by 'the substance of chemistry', such as molecules or ions. Although any activity of a cell or an organism must ultimately be accountable or linked to reactions of molecules, these reactions can be and most likely are driven by physical forces. Here we will consider how communications through space by force fields (electrical, magnetic, pressure, etc., i.e. the substance of physics) may also accomplish similar tasks and are universally used by cells and organisms. The study of the molecular mechanisms of these modes of communication might enable us to decipher the language of cells.

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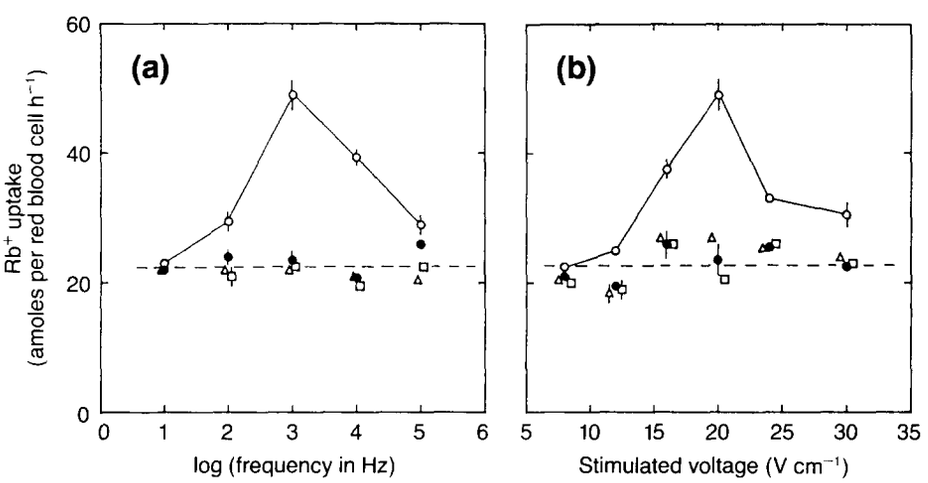

Fig. 1. Activation of the Rb^+ (K^+)-pump of Na^+,K^+-ATPase by an oscillating electric field, at 4°C (Ref. 19). (a) Human erythrocytes were exposed to an oscillating electric field of 20 V cm^{-1} at different frequencies for 1 h. Rb^+ uptake was monitored by the radioactive tracer, $^{86}Rb^+$ (see text and Ref. 12). Rb^+ uptake of electric-field stimulated samples (○), stimulated samples treated with 0.2 mM ouabain (□), non-stimulated samples (●), and non-stimulated sample treated with ouabain (△) are plotted against the frequency of the applied field. (b) The same experiment, with an electric field of 1 kHz at different field strengths. Symbols used are the same as in (a).

In similar experiments no ouabain-sensitive Rb^+ efflux was stimulated by the electric fields. The cytoplasmic concentration of Rb^+ was 27 mM, and the external concentration of Rb^+ was 10 mM. Thus, the field induced Rb^+ uptake was an active transport. No consumption of ATP was detected.

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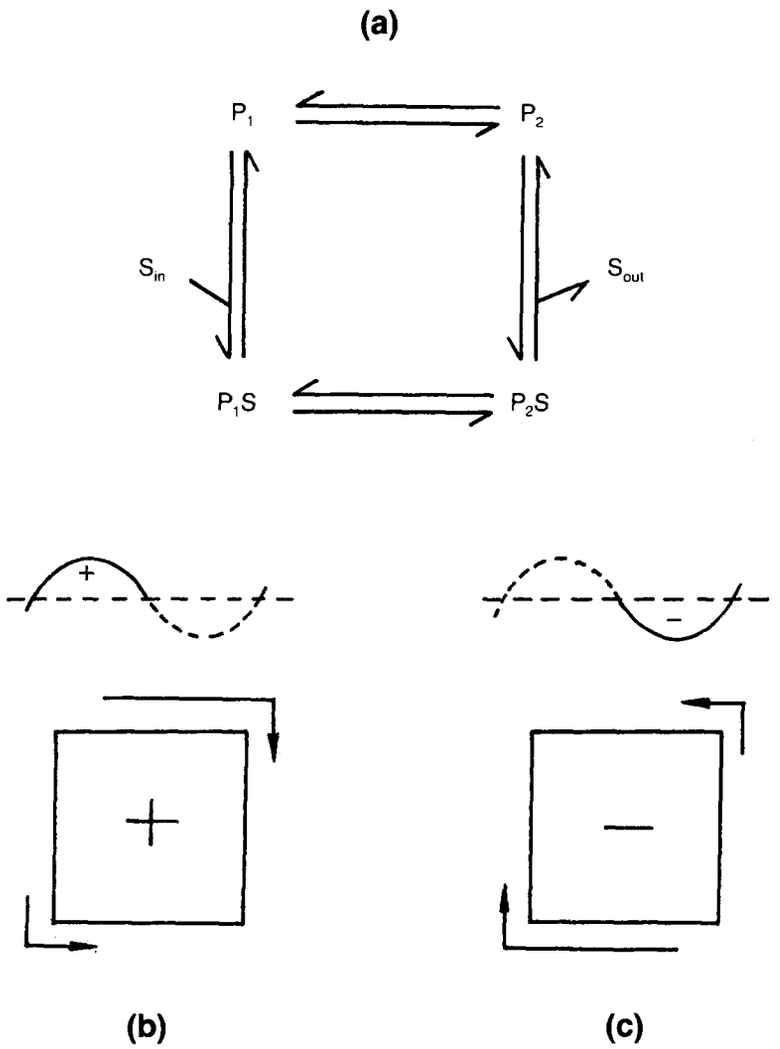

Fig. 2. A cyclic kinetic model which is shown to pump a neutral substrate against its concentration gradient when exposed to an oscillating electric field. (a) *In the scheme, P_2 is assumed to have a greater molar electric moment (ΔM) than P_1. P_2 is also assumed to have higher affinity for S_{out} than P_1 has for S_{in}.* (b) *When the oscillating field (indicated by sinusoidal curves) is in its positive phase, it induces a large flux of P_1 to P_2 transition and a small flux of P_1S to P_2S transition (both given in curved arrows). As the result, there is a net clockwise flux of enzyme conformational transitions, with a concomitant clockwise flux of S.* (c) *When the electric field is in the negative phase, it induces a large flux of P_2S to P_1S transition and a smaller flux of P_2 to P_1 transition. The net result, again, is a clockwise flux of enzyme conformational transitions, with a concomitant clockwise flux of S. The direction of fluxes can be reversed if the affinity of P_1 for S_{in} is greater than the affinity of P_2 for S_{out}.*

Electric activation of membrane proteins

Recent experiments from several laboratories have shown that many cellular functions are stimulated or suppressed by weak oscillating electromagnetic fields. Pulsed electromagnetic fields have been found to promote bond cell regeneration, DNA-, RNA- and protein biosyntheses[7–10]. The activity of many membrane enzymes are also affected by weak electromagnetic signals[10,11]. From a thermodynamic point of view, a weak electromagnetic field may influence a cellular function only if mechanisms exist which would allow amplification of the signal. The interaction energy between the amplified, effective field and certain cellular components must be large enough to alter the activity of these molecules.

The use of high-intensity electric fields that would produce a transmembrane potential comparable to the endogenous potential of cells or organelles provides another approach. Here membrane enzymes are shown to absorb energy from electric fields for doing chemical work (for reviews see Refs 12 and 13). ATPases of chloroplasts, thermophilic bacteria and mitochondria have been induced to synthesize ATP from ADP and P_i with pulsed electric fields[14–18]. ATP synthesis requires input of approximately 10 kcal (42 kJ) per mole of free energy; an electric field must induce a transmembrane potential of approximately 200 mV to provide such energy. Experimentally, high fields (in the order of kV cm^{-1}) have been used. In these experiments either chloroplasts were deprived of light energy or the electron transport chain was inhibited. The only possible source of energy was that contained in the applied electric field. These enzymes appear to be able to absorb free energy from the external electric fields to perform chemical work. Signal transductions are by nature similar to energy transductions. Examples of corresponding processes include energy absorption for signal reception, energy conversion for signal processing and energy transmission for signal transmission.

Another type of experiment carried out with Na^+,K^+-ATPase is potentially more informative[19–22](D-S. Liu, R. D. Astumian and T. Y. Tsong, submitted). Here the enzyme is shown to utilize free energy transmitted through an oscillating electric field to pump Na^+ and K^+ against their respective concentration gradients. Experiments with red blood cells revealed three important factors that determine the efficiency of the transduction of electric energy. (1) The applied weak electric field (20 V cm^{-1}) must be amplified at the site of the cell membrane to a strength sufficient to shift the equilibrium of a chemical reaction. In this case the field must be amplified by 1000 times to reach a transmembrane electric field of 20 kV cm^{-1}. A cell is like a spherical shell made of insulating material (lipid membrane) and according to the Maxwell relation, an external electric field is amplified by approximately R_0/d times across the membrane (R_0 and d being, respectively, the outer radius of the cell and the thickness of the membrane). Thus, the cell membrane is a site of the field amplification[12,13,23]. (2) There is one optimum frequency for activating the K^+-pump (1 kHz) and another for activating the Na^+-pump (1 MHz)[19,21] (Liu, Astumian and Tsong, submitted). (3) There is also an optimum field strength (20 kV cm^{-1} transmembrane electric field) for activating both pumps[19,21](Liu, Astumian and Tsong, submitted). Some results which show the frequency- and the field strength-dependence of the Rb^+-pumps are given in Fig. 1.

Electroconformational coupling model (ECC)

The above results on Na^+;K^+-ATPase imply that certain membrane

enzymes or receptors possess the ability to decipher electric signals of well defined frequency and amplitude and react accordingly. In the case of Na^+,K^+-ATPase and ATP synthetases, free energy contained in the electric fields is absorbed and coupled directly to drive an endergonic reaction. This need not be always the case, however. If a channel responds to an electric signal, for example, it can either open or close, thus modulating the local ion concentration (e.g. Ca^{2+}) and electric potential. As a result, an enzyme (or a number of enzymes) may be phosphorylated or dephosphorylated, causing changes in activity. Many other mechanisms involving biochemical reactions are possible, as with chemical signaling, but the primary process of recognition here is electric signaling.

The results of Na^+,K^+-ATPase also allowed us to formulate a mechanism based on the concept of 'electroconformational coupling' (ECC) for cellular signal and energy transductions[13,24,25]. This model postulates that a protein can undergo conformational changes by a coulombic interaction with an oscillating electric field (or any oscillating force field with which the protein can interact). When the frequency of the electric field matches the kinetic characteristic of the conformational transformation reaction, a phenomenological oscillation among different conformers of the enzyme is induced. At the optimum field strength the conformers formed are functional. The oscillation of these productive conformers when coupled to the binding of ligands, such as K^+ and Na^+, can lead to active pumping of the ligands (see Fig. 2). Likewise, when the reaction is coupled to the binding of ADP and P_i, ATP can be synthesized, as in the case of mitochondrial ATPase. The maximum energy transferred is $\Delta M \cdot E$, in which ΔM is the difference in the molar electric moment of the two conformers and E is the field strength (ΔM is the sum of changes in permanent and induced dipole moments). For example, the interaction energy for a conformational change involving a ΔM of 200 Debye under an electric field of 400 kV cm^{-1} ($\Delta\Psi$ of 200 mV) would be ~ 4 kcal (16.8 kJ) mol^{-1}.

In principle, each class of proteins is adapted to respond to an oscillating force field (electrical, sonic or chemical potential) of defined frequency and strength. Conversely, chemical reactions of the reverse order would transmit signals via the same enzymes or receptors. The transmembrane electric potential of a cell does play a role in transcellular communication. The most familiar one is the generation and propagation of the action potential in a neuron. According to the analysis based on the ECC, model signals are expressed only in oscillating, or fluctuating force fields[13,24–26]. Figure 3 gives several types of waveforms which have been shown to work with the ECC model by the computer analyses[26]. Electric fields of these waveforms have been shown to drive the enzyme modeled in Fig. 2 to perform chemical work. Most electrophysiological measurements reported in the literature, however, give only constant values for the transmembrane potential of, cells, including the resting potential of neurons.

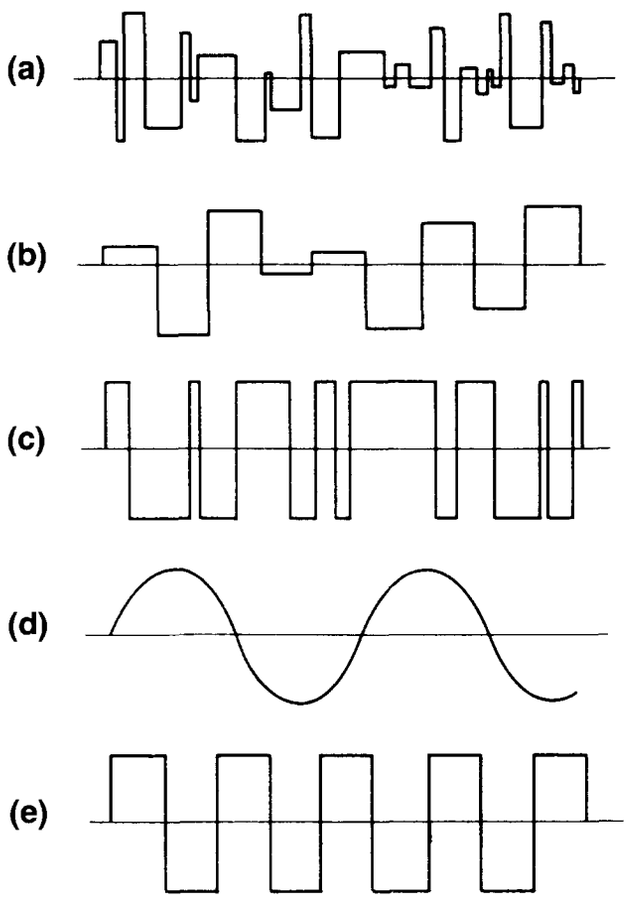

Fig. 3. Various waveforms of stochastic noise (**a**)–(**c**) *and regularly oscillating* (**d**) *and* (**e**) *electric fields shown to drive the kinetic scheme of Fig. 2 for doing chemical work. The electric field must be sustained by an energy-dissipating process, such as the metabolite-supported electron transport or the light driven photo-oxidative reaction. In* (**a**) *both amplitude and lifetime are random; in* (**b**) *lifetime is constant but amplitude random; in* (**c**) *amplitude is constant but lifetime random; in* (**d**) *a cosine wave; and in* (**e**) *a square wave or meander function signal is shown. (See text and references for details.)*

Locally fluctuating transmembrane electric fields

This brings us to another question critical for the understanding of transcellular signaling. For an electroconformational change to occur, one need merely consider the short-range interaction between a protein and an electric field[13,24–26]. That is to say, the time-average and space-average value of transmembrane electric field is irrelevant in the analysis of the ECC model. It is the local electric field that is pertinent. A coulombic interaction between two charges is inversely proportional to the square of the distance separating the two charges. The interaction energy fades away rather quickly. It is conceivable that the transmembrane potential of a cell does indeed display large amplitude fluctuation or oscillation when it is time-resolved to microseconds or when it is recorded at a small region, say at the vicinity of an energy-transducing protein. A constant potential can easily be modulated either by electron transport to become locally oscillatory or fluctuating (as might be the case for the inner membrane in mitochondria), or by the opening and closing of an ion channel with a characteristic frequency (as in neurons).

There are other possible interpretations of the above results on electric field activation of membrane ATPases. In particular, the surface compartmental model of M. Blank[27], which is based on field-induced ion accumulation near the cell membrane surface, has been shown to reproduce some results obtained with the Na^+,K^+-ATPase. However, the ECC model is consistent with the electric property of most membrane proteins[13,24–26], and acknowledges the active role of some proteins. Another advantage of the present model is that it can bridge the gap between the chemiosmotic hypothesis and the conformational coupling hypothesis of the bioenergetics[28–31]. Here

the electric potential is assumed to be responsible for the conformational change of ATPase. The activated state of the ATPase is then coupled to drive an endergonic reaction. Localized proton movement is given the role of modulating the electric potential to become 'locally oscillatory', thus, allowing the ATPase to turnover[13,24–26,32]. An experimental challenge posed by the ECC model is to investigate the pattern of the transmembrane electric potential of a cell or an organelle in a limited region. Is it stationary or locally oscillatory? This requires a time resolution of many chemical and physical parameters of a cell membrane to microseconds and a space resolution to nanometers. These parameters may include concentrations of Ca^{2+}, Na^{+}, K^{+}, cAMP and hormones, as well as physical properties such as transmembrane electric field, osmotic pressure, local thermal energy and mechanical force.

Techniques for fluorescence imaging of transmembrane electric fields using sensitive fluorescent dyes have recently been developed[33–35]. The microsecond resolution of a membrane potential induced by a pulsed electric field is of special interest to the present discussion[35]. Although space resolution of optical microscopy is limited, future developments using more advanced technologies, might be able to improve upon it. If large amplitude local fluctuations of a transmembrane electric field is indeed an inherent feature of a cell membrane, and if they can be faithfully recorded, we might be able to analyse the messages contained in these signals. Even at the current state of instrumentation, the time may be ripe for us to learn the language of the cell at the single cell level. Hopefully, we will then be able to control and regulate cell functions, such as metabolism, differentiation and growth, by 'speaking' to them using the language they best understand.

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To transform or not to transform

To transform, or not to transform, that is the question.
Whether 'tis nobler in the cell to suffer
The ons and offs of multiple phosphorylations
Or to take up oncogenes against a sea of factors
And by mutating, change them. To mutate, to change,
No more, and by a change to say we end
The control, and the thousand second signals
The cell is heir to; 'tis a consummation
Devoutly to be wish'd. To mutate, to change
To change, perhaps to die; ay, there's the rub,
For in that transformation to malignancy what cancer may come
When we have altered genetic control
Must give us pause; there's the respect
That makes calamity of too much UV
For who would bear the protein kinase C,
The receptor's signal, the membrane channels or
Transcriptional control or AMP
The DAG and then the calcium flux
Which PIP does give occasion to,
When he himself might his mutation make
With a retrovirus? who would regulation bear,
To be quiescent – such a weary life –
But that the dread of something after change,
The unexplored mutation from who's altered state
No normal cell returns, puzzles the will
And makes us rather bear the controls we have
Than change to others that we know not of.
Thus regulation doth make cowards of us all,
And thus the native hue of cell division
Is sicklied o'er with the pale cast of G_0,
And cell divisions of great pitch and moment
With this regard their currents turn awry
And lose the name of mitosis.

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