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Electromagnetic effects – From cell biology to medicine

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Abstract

In this review we compile and discuss the published plethora of cell biological effects which are ascribed to electric fields (EF), magnetic fields (MF) and electromagnetic fields (EMF). In recent years, a change in paradigm took place concerning the endogenously produced static EF of cells and tissues. Here, modern molecular biology could link the action of ion transporters and ion channels to the "electric" action of cells and tissues. Also, sensing of these mainly EF could be demonstrated in studies of cell migration and wound healing. The triggers exerted by ion concentrations and concomitant electric field gradients have been traced along signaling cascades till gene expression changes in the nucleus.

Far more enigmatic is the way of action of static MF which come in most cases from outside (e.g. earth magnetic field).

All systems in an organism from the molecular to the organ level are more or less in motion. Thus, in living tissue we mostly find alternating fields as well as combination of EF and MF normally in the range of extremely low-frequency EMF. Because a bewildering array of model systems and clinical devices exits in the EMF field we concentrate on cell biological findings and look for basic principles in the EF, MF and EMF action.

Abbreviations: DC EF, direct current electric fields; EMF, electromagnetic fields; EF, electric fields; MF, magnetic fields; PEMF, pulsed electromagnetic fields; PLMF, power line magnetic field; SMF, static magnetic fields.

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As an outlook for future research topics, this review tries to link areas of EF, MF and EMF research to thermodynamics and quantum physics, approaches that will produce novel insights into cell biology.
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1. Introduction

Here we focus on how cells are influenced by electrical fields (EF), magnetic fields (MF) and alternating electromagnetic fields (EMF) (the term ''EMF'' is also used to summarize the whole field of ''electric'', ''magnetic'' and combined ''electromagnetic'' effects). We begin by reviewing the response of cells to direct current electrical fields (DC EF), which are static electric fields that are generated from the cell mostly by ion transporters. Our focus first turns to mechanisms coupling DC EF to known cell biological phenomena, such as cell adhesion and migration, embryonic and tissue development, and wound healing. We consider these DC EF-induced reaction cascades in the context of known physical principles that link via molecular mechanisms to cell behavior. Later in the review we compare these reactions to those described in response to static magnetic fields (SMF), which come in most cases from outside (e.g. earth magnetic field).

Nothing in a living organism is static, however, because movements of cells, tissues and organs are ever-present phenomenon. Thus in living tissue we mostly find alternating fields as well as a combination of EF and MF. This point is addressed in the last chapters.

EMF frequencies (Table 1) in the body are normally in the range of extremely low frequencies (ELF). These EMF include the action potentials of nerves and heart tissue, skeletal muscle vibrations and frequencies elicited by rhythmic activities within other body tissues. Thus, we concentrate on these frequencies in the present review.

Confusion still persists in the field as to the mechanisms by which high and very high (till microwave) frequencies – encompassing also the mobile radio

Table 1

Frequency zone	Frequency	Wavelength in the air	
Extreme low	0–300 Hz	>1000 km	Many biological processes
Low, middle, high	30 kHz–30 MHz	10 km–10 m	Amateur radio, remote controls
Very, ultra high	30–300 MHz	10–1 m	Radio/TV
Super high	300 MHz–30 GHz	1 m–10 cm	Satellite communication
Extreme high	30–300 GHz	10–1 cm	RADAR
Infrared	300 GHz–300 THz	1 cm–100 μm	
Visible light	429–750 THz	700–400 nm	Light

communication (main area of 900–1800 MHz) – EMF act at the cell and molecular biological level. This is largely due to the numerous "frequency windows" for the biological action (frequencies where a biological respond is found) and by the mixtures of modulated frequencies and carrier frequencies. Researchers have thus concentrated on the thermal effects of radiation at a tissue-specific absorption rate (SAR).

However, as is the case with ELF EMF, the energetic threshold to produce cell-specific information at defined frequencies can be much lower than that required for unspecific heating of the tissue. Interestingly, for therapeutic purposes (e.g. repair of bone fractures, wound healing, etc.) ELF EMF are used as directly applied frequencies or modulated as pulsed magnetic fields. For compiling "hard" data the situation is not very easy: the literature dealing with electric and magnetic field stimuli is full of a bewildering array of model systems, clinical situations, signal configurations and stimulation devices. Thus, we concentrate mainly on cell biological findings in renowned journals and look for basic principles in the mechanisms of EMF influence on biological systems.

The most complex, and currently the most speculative, component of research on EMF is relating the molecular and cell biological findings to observations in multicellular systems and organisms. It is extremely difficult, even in tissues or cells studied in vitro, to determine which responses directly result from EMF. In the body, the wide range of interactions that are likely to occur are too manifold to be defined by clear causal relationships and are thus not discussed here.

Finally, as an outlook to future research topics, we will link areas of EMF research to thermodynamics and quantum physics approaches that are sure to produce novel insight into cell biology.

2. History and literature

2.1. History of electromagnetic field research

The idea of bioelectricity, which refers to the EMF produced by living matter, has long provoked scientific research. Most famously, Galvani elicited muscle contractions from preparations of frog nerves and muscle in the late 1700s using electricity from lightning storms and static electricity generators. He later demonstrated that a frog leg can be made to twitch merely by touching it with the cut end of the sciatic nerve from the opposite leg (1794). Although Galvani believed this experiment demonstrated the existence of bioelectricity, or a mystical "vis vitalis", he had actually revealed what is known today as injury potential (reviewed in McCaig et al., 2005). Using sensitive voltmeters, the anatomist H.S. Burr began experimenting (1930–1950) on the "life field" by measuring differences in electrical potentials of plants, animals and patients (Burr, 1972). In humans he measured a static EF between the left and the right forefinger of 2–10 mV. Individual fields were relatively constant and quite consistent over time for males, however voltage gradients in

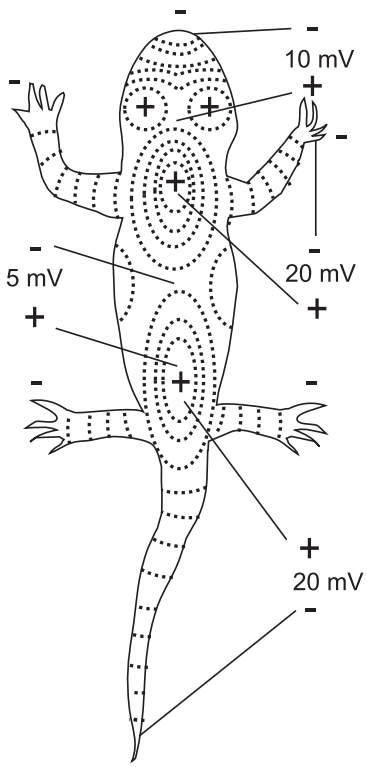


Fig. 1. DC EF field lines measured in a "salamander" by the two electrode method (after Becker, 1985).

fertile females showed a remarkable increase over a period of 24 h during the middle of the menstrual cycle when ovulation occurs.

Lund (1926), Becker (1961) (Fig. 1), Burr (1972) and others importantly revealed that changes in voltage gradients often correlate with morphogenetic events during growth and patterning of both plants and animals (Levin, 2003; McCaig et al., 2005). Later research into this phenomenon was aided by the development of non-invasive extracellular probes to measure ion currents (Bluh and Scott, 1950; Jaffe and Nuccitelli, 1974; Smith et al., 1999). Hotary and Robinson (1990, 1991, 1992, 1994) were among the first to find that these currents were not just physiological signs of standard metabolism or classical membrane potential, but specific and instructive signals for key processes during a range of events, from embryonic development to adult wound healing (Marsh and Beams, 1952; Smith, 1970; Rose, 1974; Stern, 1982; Cooper and Keller, 1984; Hotary and Robinson, 1992; Nishimura et al., 1996; Uzman et al., 1998; Nuccitelli, 2003; Woodruff, 2005; Hildebrandt et al., 2006; see Levin, 2007).

Possibly the biggest revolution in bioelectricity research came with the introduction of functional dyes enabling such things as pH, ions and membrane potential to be visualized at submicrometer levels using fluorescence microscopy methods. Combining these dyes with our current vast array of molecular biology and genetic tools has opened the door to fascinating new insights.

Figs. 2 and 3 (from our recent projects) show the usefulness of these dyes for microscopy at scales from whole axolotl embryos (Fig. 2) to single cells (Fig. 3).

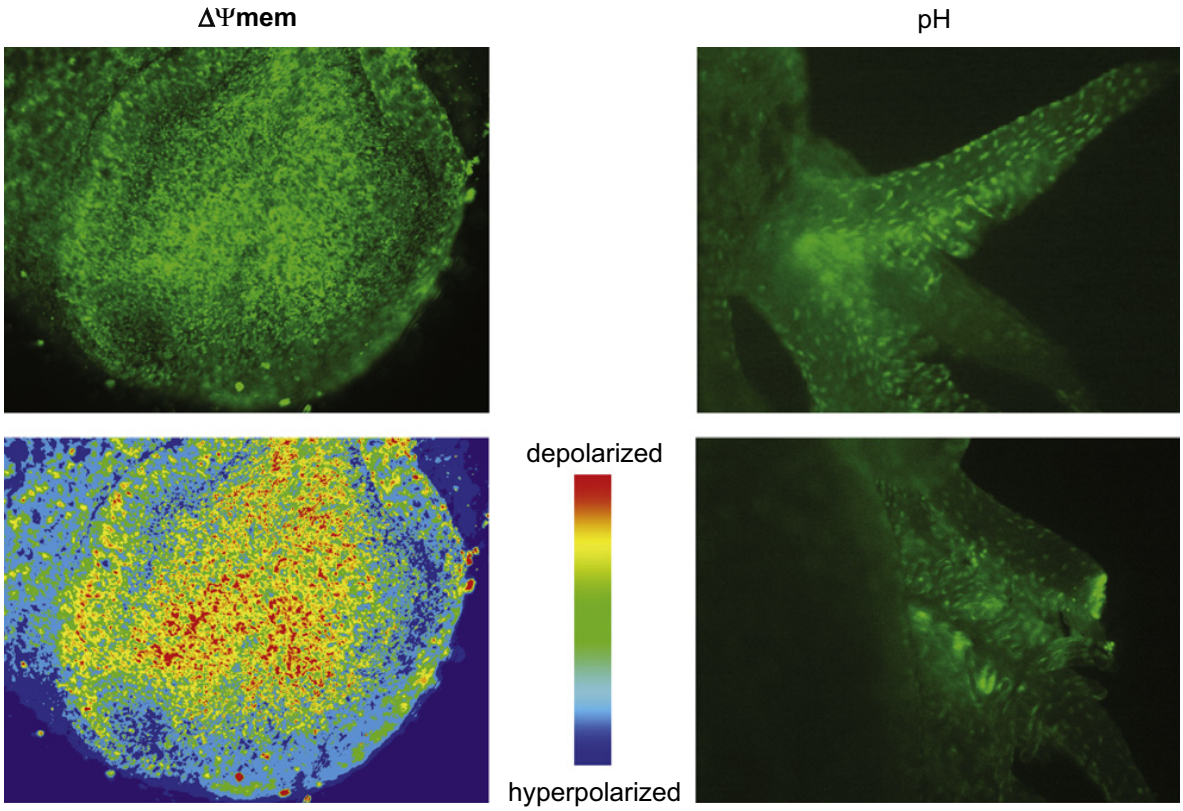


Fig. 2. Screening method of gradients in membrane potential (left, p. 16) and pH (right, p. 44) of a developing axolotl. Note the gradient in pH at injured sites of the gill.

2.2. Studying EMF from a cell migration perspective

After our earlier work on the effects of power line EMF on astrocytes (Golfert et al., 2001), we somewhat circuitously re-initiated our investigation into how EMF impact cell biology during a study on biocompatibility of implant surfaces. Our initial aim was to determine which structural features are involved in directing desired cell function. It was known that cells adhering to an implant surface bearing micrometer-sized groove and ridge patterns often migrate and align in the direction of the surface structure. This process is known as contact guidance (Brunette, 1986). Depending on surface topography, cells in these situations eventually form a well-organized structure. In our study (Monsees et al., 2005), we used patterns of 12 nm deep parallel titanium (Ti) oxide lines with different widths (0.2–10 μm) and distances (2–20 and 1.000 μm). We observed that a significant portion of osteosarcoma cells stretched their cytoskeleton as they aligned along the oxide lines. Concordant with this, small filopodia were extended to contact the lines (Fig. 4) and the majority of focal contacts were placed on the lines. Thus, a nanosized difference in height between the Ti surface and Ti oxide lines was sufficient to provide contact guidance to the osteosarcoma cells. Thus, we hypothesized that gradients in electrostatic potential or surface charge density might be responsible for this phenomenon.

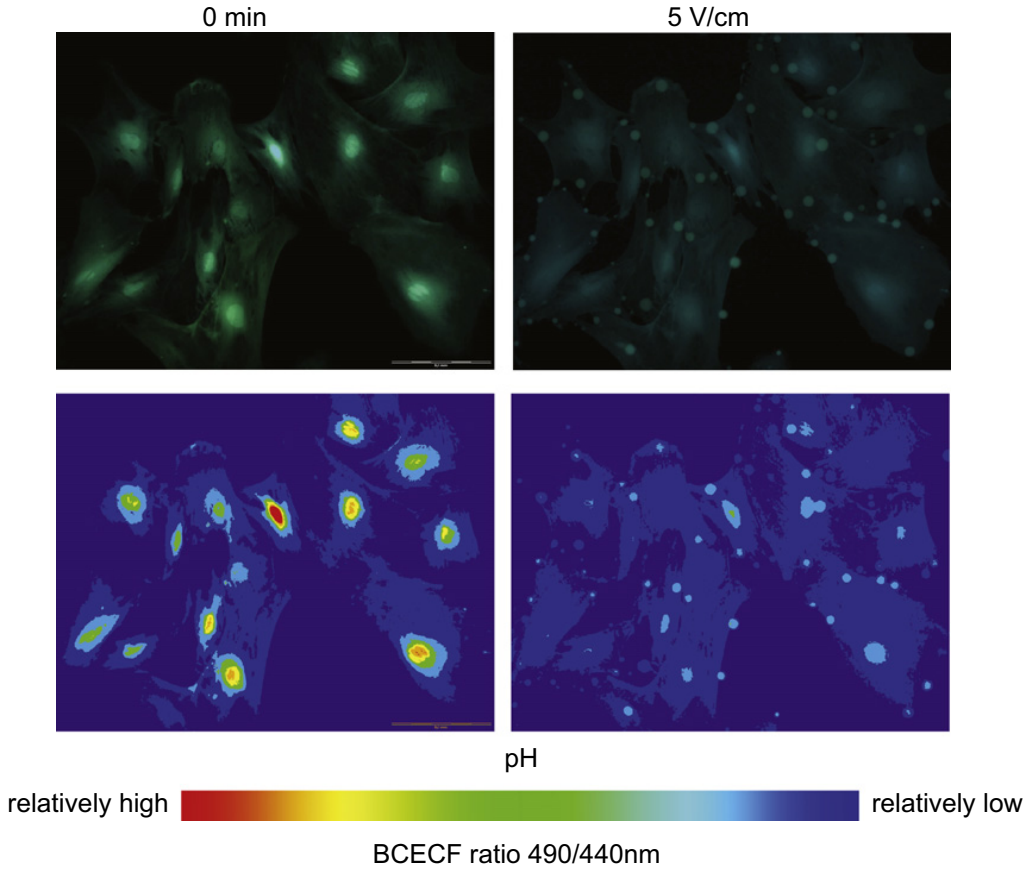


Fig. 3. pH-sensitive dyes in cell biology: calvaria primary culture cells before and after application of DC EF (5 V/cm). Note the localized extrusion of protons (arrows) possibly at sites of proton exchangers. Top: intensity picture and bottom: false color display.

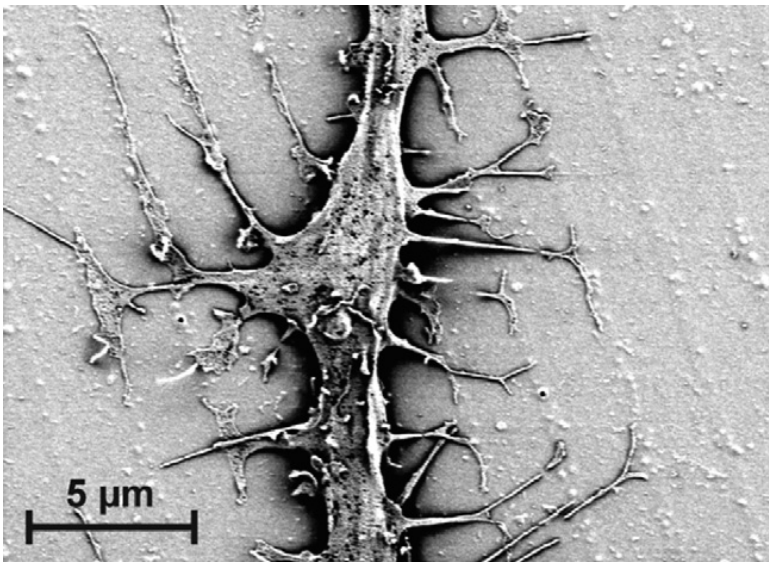


Fig. 4. Scanning electron micrograph of a cell process in a SAOS cell. Note the filopodia that try to come in contact with the oxide lines, 72 h after seeding on titanium/titanium oxide. Oxide lines 2 µm wide, 0.2 µm broad and 20 nm high.

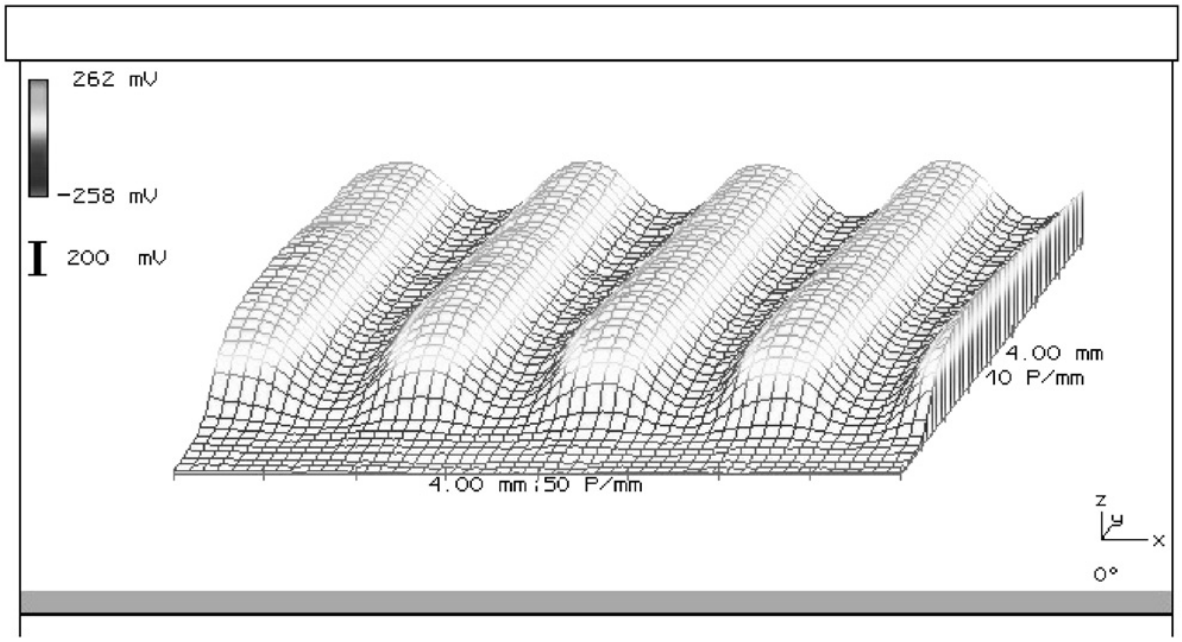


Fig. 5. Scanning Kelvin probe of the surface potential (in mV) at the transition of gold and titanium.

Due to differences in physicochemical properties across Ti/Ti oxide surfaces, it is possible that other factors participated in directing focal contact formation and cell guidance in our experiments. Such cues could include concentration gradients of molecules that facilitate chemotaxis, e.g. growth hormones or extracellular matrix (ECM) components, or gradients of electrostatic potential or surface charge density at the Ti/Ti oxide interface. Our recent studies indicate that osteoblasts 'sense' transitions between two materials, such as Ti/Ti oxide (Monsees et al., 2005) and Ti/glass interfaces (Breme et al., 2007), as cells are significantly more attracted by these transitions than by an individual material alone. In experiments using a raster Kelvin probe (Fig. 5), we showed that approximately 150 mV differences in electrical potential occur at Ti/Au interfaces. This observation not only has implications for coating and structuring implants, but also might lead to more general insights into cell biology.

In further in vitro experiments, we found that application of physiological DC EF-directed movement of bone cells and other cell types. This phenomenon is called electrotaxis or galvanotaxis (Zhao et al., 2002). It has been reported that in vitro many cell types often migrate to the cathode at field strengths of 0.1–10 V/cm (neural crest cells, fibroblasts, keratinocytes, chondrocytes, rat prostate cancer cells and many epithelial cell types) (Robinson, 1985; Nishimura et al., 1996; McCaig and Zhao, 1997; Zhao et al., 1997; Pullar et al., 2006b). In contrast, fewer cell types move to the anode (corneal endothelial cells, bovine lens epithelium, human granulocytes and human vascular endothelial cells). Both speed and movement direction in this case is voltage dependent. As described in our recent review (Funk and Monsees, 2006), current data suggest that species and cell subtype differences affect electrotaxis. For example, human vascular endothelial cells migrate towards the

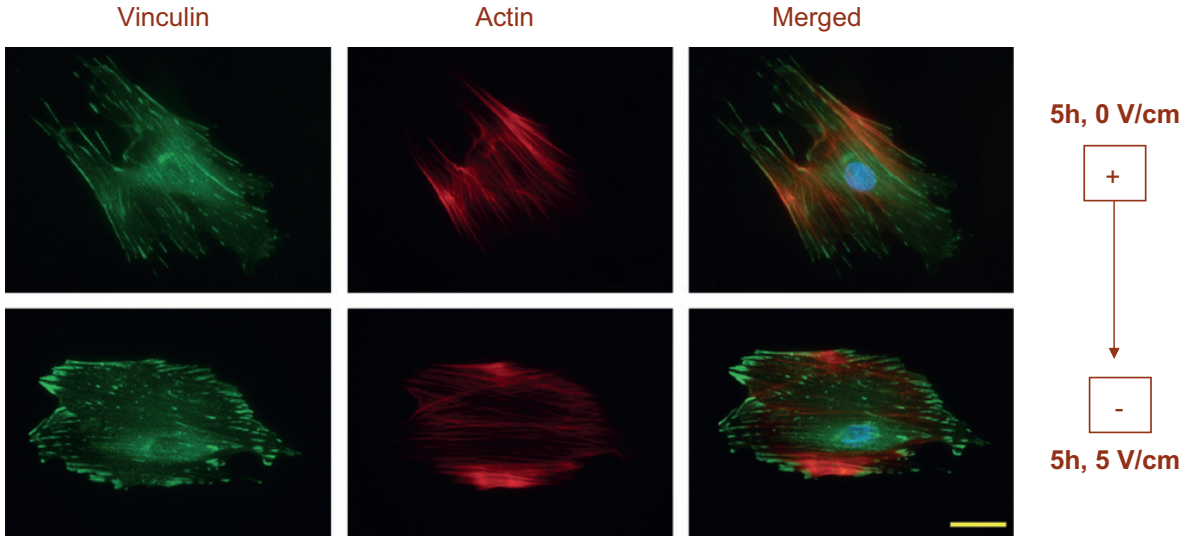


Fig. 6. Fibroblasts in a DC EF field. Note the orientation of the migration direction, the elongation perpendicular to the EF vector and the parallel reorganized cytoskeleton (actin) and the polarized distribution of the focal contacts (vinculin).

anode, whereas bovine aortic endothelial cells move towards the cathode. In SAOS – cells, rat calvaria osteoblasts and fibroblasts we found that during movement, ruffled membranes, lamellipodia and filopodia are formed preferentially in the direction of the anticipated electrotaxis migration and the cells oriented and elongated perpendicular to the electric field lines (Fig. 6) (see also Sulik et al., 1992; Zhao et al., 2002). Several cell types were even reported to change their initial movement direction by 180° when current polarity was reversed (Harris et al., 1990; Soong et al., 1990; Brown and Loew, 1994; Chao et al., 2000; Wang et al., 2000). Higher cathodal migration was noticed with keratinocytes plated on collagen and plastic, whereas lower locomotion occurred on laminin. The cell response on fibronectin was in between the two (Sheridan et al., 1996). Comparable reactions were observed for epithelial cells on laminin or fibronectin coatings (Zhao et al., 1999a). This shows that also the substrate underneath the cells modifies the response of the cells to DC EF.

Interestingly, Sun et al. (2004) noticed directed fibroblast migration at field strengths as low as 0.1 V/cm in three-dimensional collagen gels, but not in conventional two-dimensional cultures. Thus, three-dimensional conditions have the potential to reflect in vivo situations in which DC EF of 0.1–0.2 V/cm are known to be present during many events, including embryonic development (Nuccitelli, 2003), see Section 3.2.1.

Several steps are involved in cell migration: (1) via connection to several adaptor proteins, actin filament growth pushes the cell membrane in the direction of movement; (2) formation of focal contacts at the leading edge, i.e. specific binding via membrane-bound integrin receptors and ECM proteins, which also influences several signaling pathways and adhesion structural elements; (3) focalized proteolysis by recruitment of surface proteases to ECM contacts; (4) cell contraction driven by

myosin II binding to actin filaments and (5) disassembly of focal contacts and detachment of the trailing edge (Lauffenburger and Horwitz, 1996; Friedl and Wolf, 2003; Funk and Monsees, 2006). Many of these events have also been observed in EF-induced cell movement.

These examples show that different events during cell migration are initiated by "electrical" phenomena. In the following sections we will address: How does the cell sense such electrical phenomena? How are they coupled to canonical signaling pathways? And is this relevant to in vivo processes at cellular and organismal levels?

2.3. Literature survey

To date there have been a plethora of studies on electromagnetism, ranging from chemical reaction models and cell biological studies to human epidemiological and clinical investigations. Despite this abundance, it was not until the development of appropriate molecular and cellular tools in the 1990s that a comprehensive link between cell biology and the phenomena of bioelectricity became possible. Before this time, cellular events in response to EMF, such as those used during physical therapy, remained clouded in mystery. This lack of a causal explanation gave rise to a general disregard for EMF studies that reported observations from patients or model organisms. Even a few years ago, as we were preparing a review about surface charges on implant materials and the implications of EMF on cells in this context (Funk and Monsees, 2006), we noticed that the field was only just poised to connect with cutting-edge cell biology.

Surprisingly, a current survey of the literature reveals many recent advances in our understanding of EMF, especially DC EF. Today we are experiencing a revolution because many cell biological findings are now explainable by ion dynamics (Ca^{2+}, H^+ and related ion pumps and voltage sensors) and their action on small signaling molecules. This clearly is a novel link to classical findings in molecular and cell biology. A number of insightful studies by groups including those of McCaig (Zhao et al., 1999a, 2002) and M. Levin (Adams et al., 2007) directly couple EMF to cell biology. Levin (2007) demonstrated that DC EF produced by ion channels (especially for H^+, K^+ and Ca^{2+}) provide specific signals that regulate cell behavior during embryonic development, normal tissue turnover and regenerative repair (Jaffe, 1982; Borgens et al., 1989; Levin, 2007). Thus far, roles have been proposed for endogenous currents (EF, ion currents and secondary distribution of small molecules) in polarizing cells (Jaffe, 1982); patterning embryos during gastrulation, neurulation and organogenesis (Shi and Borgens, 1995) (see also Stern, 1982; Robinson and Messerlie, 2003; Hotary and Robinson, 1992); directing transport of maternal components in insect oocytes (Woodruff, 2005); neural differentiation (Uzman et al., 1998); guiding migration of many vertebrate cell types (Cooper and Keller, 1984; Nishimura et al., 1996); specifying anteroposterior identity during regeneration in flatworms (Marsh and Beams, 1952); Xenopus tail regeneration (Adams et al., 2007); and, general directional signaling and patterning (Marsh and Beams, 1952; Rose, 1974).

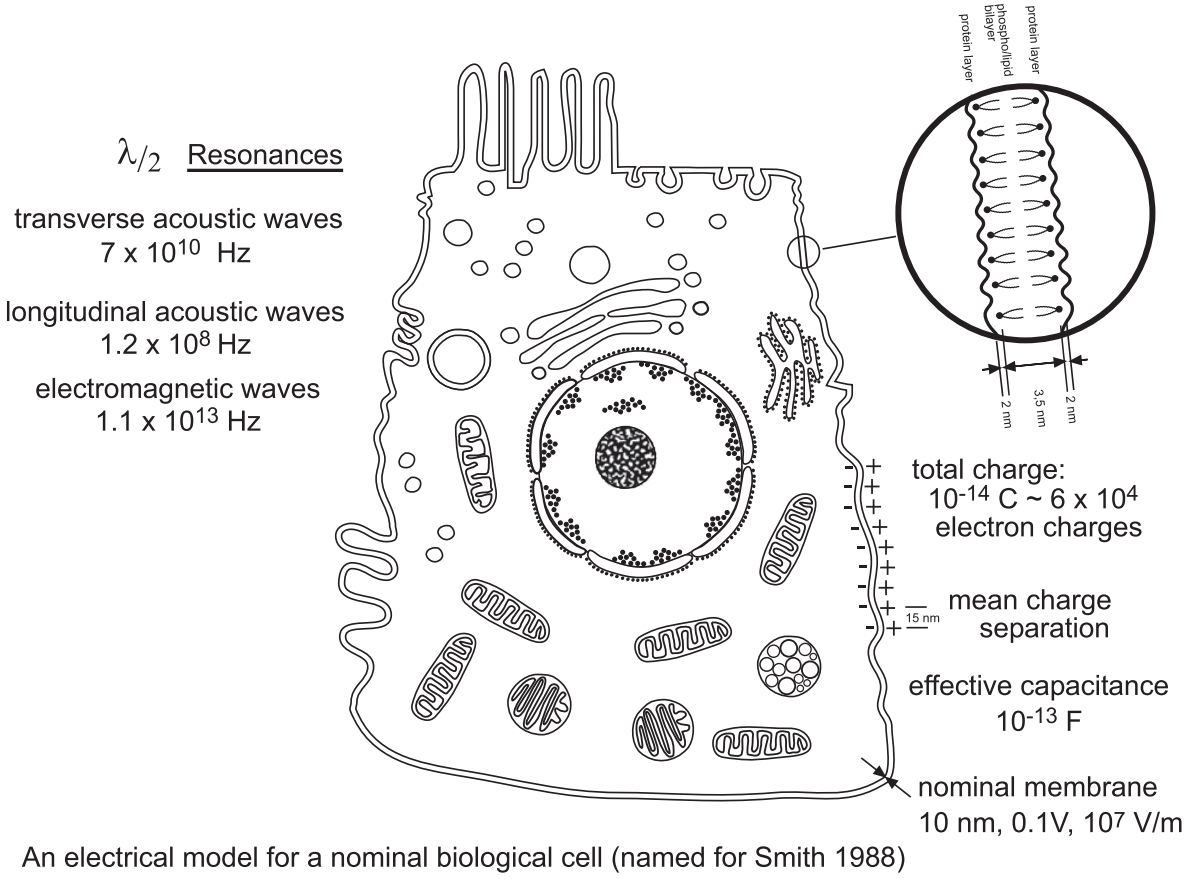


Fig. 7. Physical parameters found in an idealized model of the cell and plasma membrane (after Smith, 1988).

In some ways it is not surprising that nature uses endogenously (by ion channels) produced DC EF as fast and far reaching information carriers because these fields are ideally suited to bridge the informational gap between the short-range action of molecules and far reaching external influences (e.g. temperature and radiation). As we see in the chapters below (see Rohon-Beard ''neurons''; Spitzer, 1982), ''bioelectric fields'' of small magnitude are possibly a general cellular phenomenon and required for intracellular short-range communication. On the other hand, cells must be sheltered from neighboring stochastic EMF and must be able to discern cell biologically relevant information. The cell membrane, acting as a ''Faraday cage'', serves as a very good shield against irrelevant DC EF. As discussed later, EF rhythms can be detected via receptors, charged molecules or other mechanisms (Fig. 7). However, the magnetic component of EMF can also induce EF within cells (Faradays law, see section EMF).

We first must define the physical phenomena of electricity, magnetism and electromagnetisms that can influence cells, tissues and organisms in order to introduce the biologist or physician to this topic. Thus, we address the following questions: is there a relevant appearance in the natural environment of biological systems, how are the mechanisms to couple these fields into biological signaling or

metabolic cascades, are there examples for this in cell biological studies (we only pick out relevant typical studies to show the principal mechanisms, there are many reviews – which we refer – showing the vast array of existing studies in this field) and finally, can we form new hypotheses regarding physiological functions of these fields in cell biology?

3. Static electric fields (DC EF)

3.1. DC EF strength in the environment

Because of the high conductivity of body tissues relative to air, and because EF are conducted around the body, exposure to DC EF via air does not produce an internal field but instead builds up surface charge on the body (Tenforde, 1991; see Bracken et al., 2004). At sufficiently high levels, the induced surface charge can lead to hair deflection and cutaneous perception of strong static electric fields. The mean threshold for field perception by human volunteers is about 22 kV/m, when standing on an insulated mat (Hill et al., 1976). Under dry conditions, perception of the field through hair stimulation occurs at field levels of approximately 30 kV/m (Hill et al., 1976). Others reported the threshold for human perception of DC EF to be 40–45 kV/m (Blondin et al., 1996). The surface charge induced by a high-intensity static EF can also lead to spark discharges between a person and electrically conductive objects. The threshold for perceiving these shocks is 0.3–0.4 mJ in a dry environment (Zaffanella and Deno, 1978).

If one considers the dimensions of a cell, then the thickness of a cell membrane (10 nm) with a 0.1 V difference corresponds to field strength of 10^6–10^7 V/m. This halts weaker DC EF from entering the cell. The magnetic component of EMF, however, can penetrate the cell membrane. The EF for electroporation of drugs or genetic material into cells lies in the range of 1000–5000 V/cm (Mir et al., 1999). Despite these high-field strengths required to penetrate cell membranes, it is important to note that DC EF couple to internal signaling pathways via interactions with sensor mechanisms located at the cell membrane.

3.2. DC EF under physiological conditions

3.2.1. Embryonic development

Under which circumstances are endogenous DC EF found? In the embryo, cells move and grow in specific directions to form tissues and organs. EF normally arise during various stages of embryo development. During early development of amphibian and chicken embryos, endogenous ionic currents can be measured. The currents and related fields are actively generated by passive Na^+ uptake from the environment that leads to an internally positive transepithelial potential difference (TEP) (see Fig. 8). Differences in TEP between various regions form an intraembryonic voltage gradient (see Fig. 9). The magnitude of these endogenous

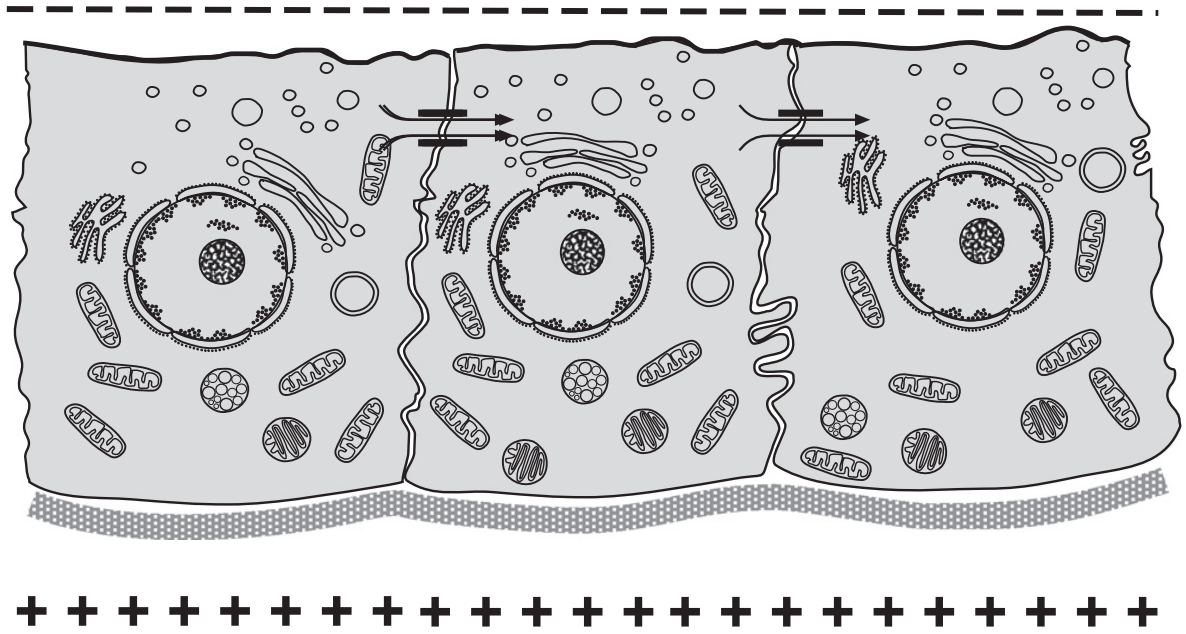


Fig. 8. DC EF potential generated by an epithelial layer (basement membrane at the bottom of the cells), transepithelial potential (TEP). Arrows between the neighboring cell membranes = ionic flow through gap junctions.

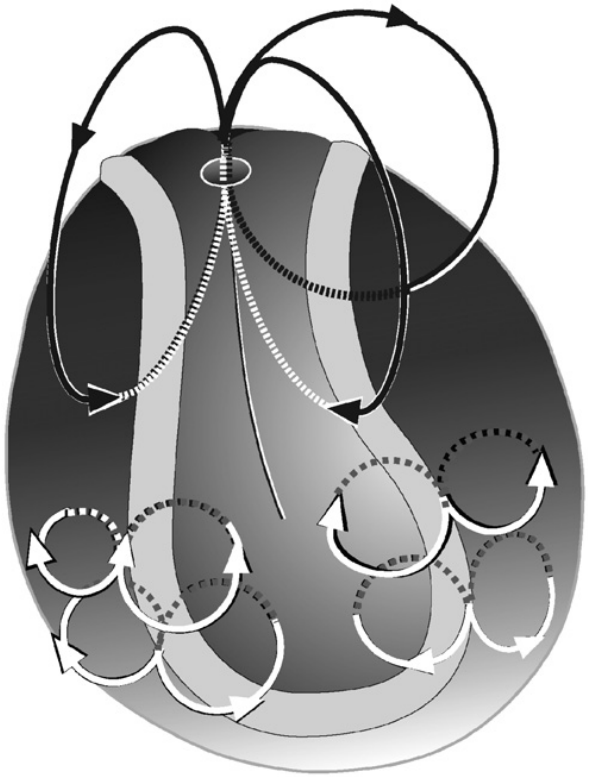


Fig. 9. Transepithelial potential differences are the source of current loops detected using non-invasive vibrating electrodes. The electrode vibrates rapidly near an axolotl embryo over a distance of about 20 μm. Outward currents are found at the lateral edges of the neural ridges and at the blastopore, whereas inward currents are found at the center of the neural groove and at the lateral skin (after Shi and Borgens, 1995).

static EF is in the order of 1–5 V/cm and therefore well above the minimum level needed to affect morphology and migration of embryonic cells in vitro (Hotary and Robinson, 1990; Metcalf et al., 1994; Levin, 2007). It has also been stressed by McCaig et al. (2005) that "it is important to put the dimensions of the electric fields into the right context": a depolarization of a neuron by surface electrodes requires a field of 10–20 V/cm. Nerve cell action potentials are confined to cell membranes and are propagated along the cell membrane. The DC EF mentioned in the present review last much longer and are present across hundreds of micrometer. Furthermore, the field gradients spread in the extracellular space as well as within the cytoplasm of one or more cells, coupled by e.g. gap junctions.

In contrast to short-lived action potentials, small endogenous DC EF last very long and build up gradients that persist from days to weeks. It has been difficult to measure such gradients, a factor that contributed to the past lack of regard for bioelectricity in cell biology and medicine. Furthermore, the term "bioelectricity", which has been long used by the "vitalistic" fraction in biology and philosophy, was used to define the target of dubious therapeutic instruments. However, present work is rehabilitating this term by linking the historic knowledge of EMF action to modern molecular and cell biology. For example, Levin et al. (2002) and Adams et al. (2006) found that development of left-right asymmetry utilizes an EF between the blastomeres, which is generated by an asymmetrical flow of H^+ ions generated by K^+/H^+-ATPases (Fig. 10). This special DC EF in the early embryo appears to be a driving force to dislocate small molecules such as serotonin (electrophoresis, see section "physical principles behind DC EF", Fig. 10).

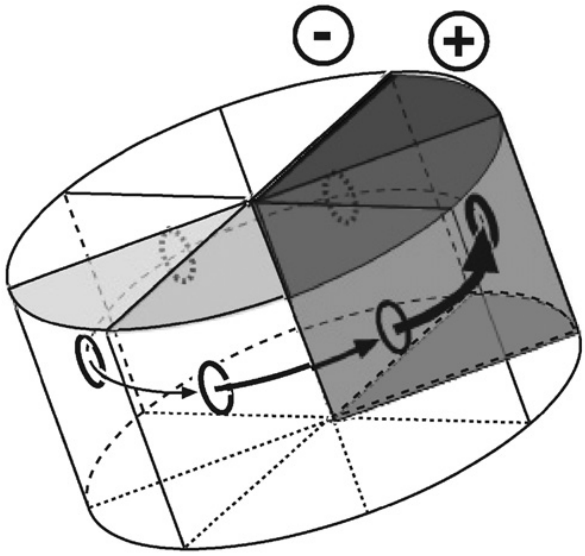


Fig. 10. DC EF potential-driven gradient in the concentration of a small molecule (e.g. serotonin) through an array of cells via gap junctions (arrows through rings). Maximal accumulation at + and minimal concentration at −. By this ± gradient, e.g, laterality is determined in early development (after Adams et al., 2006).

3.2.2. Ion flux dependent gradients of signaling molecules

With respect to laterality in Xenopus, Levin et al. (2002) found that proteins forming gap junctions, structures that allow bidirectional flow of ions and of small molecules, can also form one-way junctions. Additionally, they showed that small molecules including serotonin could be electrophoretically forced to form long-range gradients across paths of gap junction-coupled cells. These results shed light on how gradient formation in single cells leads to large-scale morphogenetic gradients (Fig. 10).

Regarding coupling of cells, an interesting phenomenon is seen in the developing nervous system of the tadpole. Electrical coupling of Rohon–Beard neurons via Ca^{2+} takes place at early stages. After 28 days, this coupling disappears as the Na^{+} component of the action potential appears (Spitzer, 1982). This potentially shows that bioelectricity diverged during evolution: small currents steadily produce relatively far reaching EF to convey basic cellular information, whereas typical action potentials of neural cells are guided and confined to the related cell membrane.

Small DC EF are also present during gastrulation and neurulation. Hotary and Robinson (1994) measured currents of $100\,\mu A/cm^2$ in the blastopore of Xenopus embryos (voltage gradients of about 100 mV/cm) during neural tube formation (reviewed in (McCaig et al., 2005). The anterior neural folds are also reported to be sites of current exit, where current densities of $2\,\mu A/cm^2$ have been measured. Notably, regions of major tissue movements are generally sites of current leakage (Robinson and Messerlie, 1996) due to the transient disruption tight junction seals as cells move apart (Decker, 1981). Current flows parallel to tightly sealed epithelium in areas of high resistivity (intact tight junctions) and exits the embryo in regions of low resistivity (severed tight junctions) (see Fig. 9). EF gradients that develop in the embryo by such a mechanism can direct cell migration. At different developmental stages, localized DC EF are switched on and off (reviewed in McCaig et al., 2005).

The existence of a voltage gradient across the neural tube gives rise to speculation that it may function in directing neuroblasts to migrate and differentiate (e.g. via voltage-sensitive PTEN phosphatase) (reviewed in McCaig et al., 2005). Observations of nerves sprouting in response to wounds in the skin (Fitzgerald et al., 1975; Matsuda et al., 1998) and cornea (Rozsa et al., 1983; Beuerman and Rozsa, 1984), presumably along an electric gradient, hint that DC EF also deliver guidance cues during normal neuronal development.

3.2.3. Ion flows during differentiation of tissues and organs

A strong electrical gradient has also been detected across the wall of the neural tube (McCaig et al., 2005). Additionally, voltage drop across the neural tube wall, division and differentiation of neurons in the presumptive CNS lumen, and axis of cell division can be influenced by applied and endogenous EF (Zhao et al., 1999b; Song et al., 2002). Ion flows are also involved in differentiation control. Recent findings have implicated the calcineurin pathway, which upregulates myogenin and MEF2 activity, as linking K^{+} channel (Kir2.1)-mediated hyperpolarization with differentiation in human myoblasts (Konig et al., 2006). Thus, it is clear that

vertebrate embryos possess steady voltage gradients, particularly in areas where major developmental events related to cell movement and cell division occur. Disrupting these electrical fields disrupts normal development (Zhao et al., 2006).

3.2.4. Differentiation of single organs

The activities of EMF observed in embryos may also apply to differentiation of single organs, e.g. in the vertebrate lens, basolateral membranes of anterior epithelial cells produce a DC EF by Na^+/K^+ pumps (Fig. 11) (Wang et al., 2003a). Using published values for equatorial and polar lens resistivity (0.5 and 500 kΩ/cm) (McCaig et al., 2005) has calculated that lens currents give rise to steady DC EF of between 0.02 and 6 V/cm, a normal physiological range. Current flow draws associated water through the avascular lens, and this may flush out metabolites (Mathias et al., 1997). The main current efflux is concentrated at the lens equator, where important aspects of lens physiology, such as growth of new cells, take place. During adult life, lens epithelial cells move towards the equator, probably by active migration, proliferate and transdifferentiate into lens fiber cells (see also Funk et al., 2002).

3.3. Coupling physical forces to cell biology

What are the physical mechanisms behind the observed biological phenomena elicited by DC EF during embryonic and tissue differentiation? The effects of DC EF

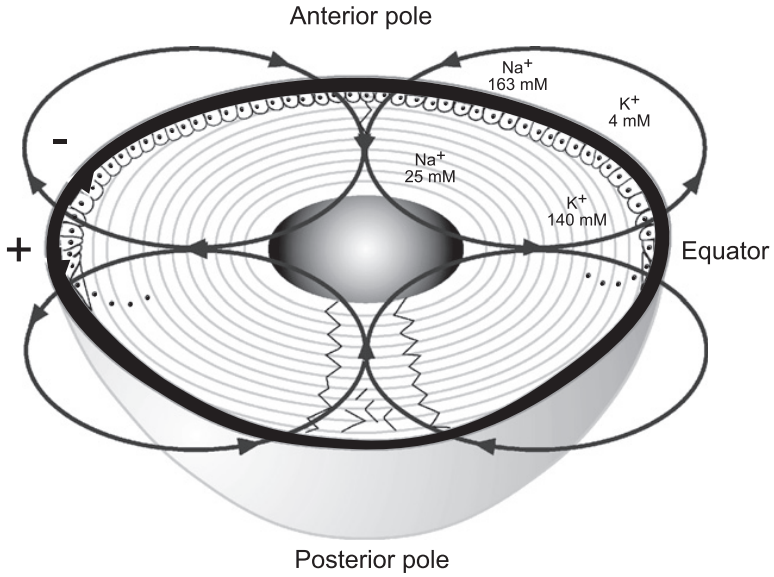


Fig. 11. Diagram illustrating endogenous EFs in the lens. At the equator a large outward current is present which returns inward at both poles. The currents are generated by Na-K-ATPase pumps in the basolateral membranes of the anterior epithelium. This leads to K+ accumulation in the lens interior, and Na+ accumulates outside at the anterior pole. The lens epithelial cells proliferate and begin to migrate at a germinative zone near the lens equator. The cells migrate towards the equator, elongate, and differentiate into lens fiber cells (see Funk et al., 2002; McCaig et al., 2005).

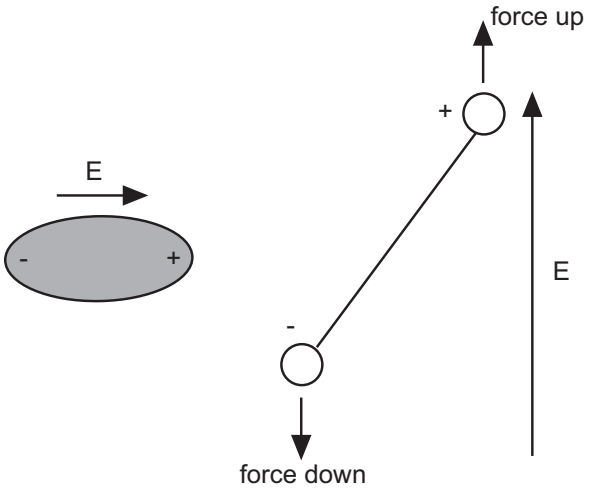


Fig. 12. Left: DC EF-induced polarization of bond charges; right DC EF-induced orientation of permanent dipoles (after Hurt, 2000).

that we have seen in the chapter before can be attributed to a few principle mechanisms:

- *Polarization of bound charges*: Bound charges are so tightly controlled by restoring forces that they can move only very slightly. Without an applied E-field, positive and negative bound charges in an atom or molecule are essentially superimposed upon each other and effectively cancel out. When an E-field is applied, the forces on the positive and negative charges are in opposite directions and the charges separate, resulting in an induced field (Pilla, 2003, Fig. 12).
  The arrangement of charges in some molecules produces permanent dipoles which exist regardless of whether an E-field is applied to the material. With no E-field applied, permanent dipoles are randomly oriented because of thermal excitation. With an E-field applied, the resulting forces on the permanent dipoles tend to align the dipole with the applied E-field. This orientation effect is a net alignment of dipoles over the (thermal) randomness that existed without an applied E-field (Fig. 12). Like it is found in induced dipoles, the net alignment of permanent dipoles produces new fields.
  The two effects, creation of polarization charges by an applied field and creation of new fields by these new charge distributions are called dielectric value (Pilla, 2003). This is a measure of how easily the polarization in a material occurs. If an applied E-field results in many induced dipoles per unit volume or a high net alignment of permanent dipoles per unit volume, the dielectric value is high.
- *Significant orientation of permanent dipoles resulting in topographical changes in molecules*: This force can also drive the electroconformational coupling (energies needed for changes in conformation are much lower than those of chemical bonds).
- *Drift and diffusion of conduction charges*: The drift of conduction charges in an applied E-field occurs because these charges are free enough to move in response to forces of the applied field. Electrons, protons and ions can be conduction

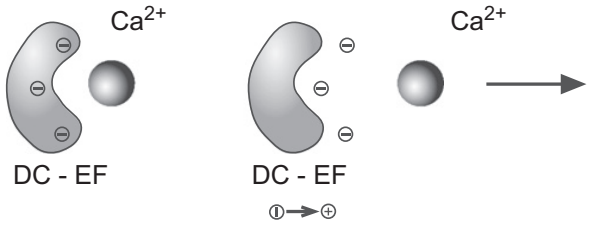


Fig. 13. Separation of an ion from a prebound state in relation to a protein (see Section 3.4.2.3) to a "free" state, induced by a DC EF.

charges. Movement of the conduction charges is called drift because thermal excitation causes random motion of the conduction charges, and the force due to the applied fields superimposes a slight movement in the direction of the force on this random movement. The drift of conduction charges builds up a current, and this current produces new fields that did not exist before the E-field was applied (Pilla et al., 1987).

The drift of conduction charges is called conductivity – a measure of how much drift occurs for a given applied E-field. A large drift means high conductivity.

- *Ions can be bound to or released from proteins* (bound ion model by Liboff (Liboff, 2003); Fig. 13).
- *Ion channel- or receptor clustering can occur in the cell membrane*: (Kindzelskii and Petty, 2005) (see also "EF-induced migration", Fig. 14); this is also a special form of micro-iontophoresis (see paragraph "Drift of charges" in the chapter "In vitro response of single cells and cell organelles to externally applied EMF").
- *Complex voltage-sensitive enzymes*: like the voltage-sensitive phosphatase (VSP) were found as direct EF sensors (see Section 5.5). This is again a special case of electroconformational coupling of channel proteins (because here, e.g. a molecular lever is moved by the charges leading to conformational changes) which leads to an ion flux (see below: VSP) and hence produces new currents and fields by this ion flux.

3.4. Externally applied DC EF

3.4.1. EF- triggered cell shape and cytoskeletal deformation

Our understanding of the cell reactions to DC EF comes from the migration studies of cells in an electric field and the related phenomenon of galvanotaxis (movement of cells to the cathode or anode) which is known since the beginning of the 20th century.

Cells in an externally applied DC EF always try to orient themselves perpendicular to the direction of the field lines. DC EF are also able to change the geometry of the cell: round-shaped cells elongate and stretch to experience minimal field gradients (Hinkle et al., 1981) (Fig. 15). The mechanisms behind this phenomenon are complex, possibly involving micro-iontophoresis of receptors, channels or other molecules. As stated here and elsewhere (Jaffe, 1977; Poo and Robinson, 1977; Poo,

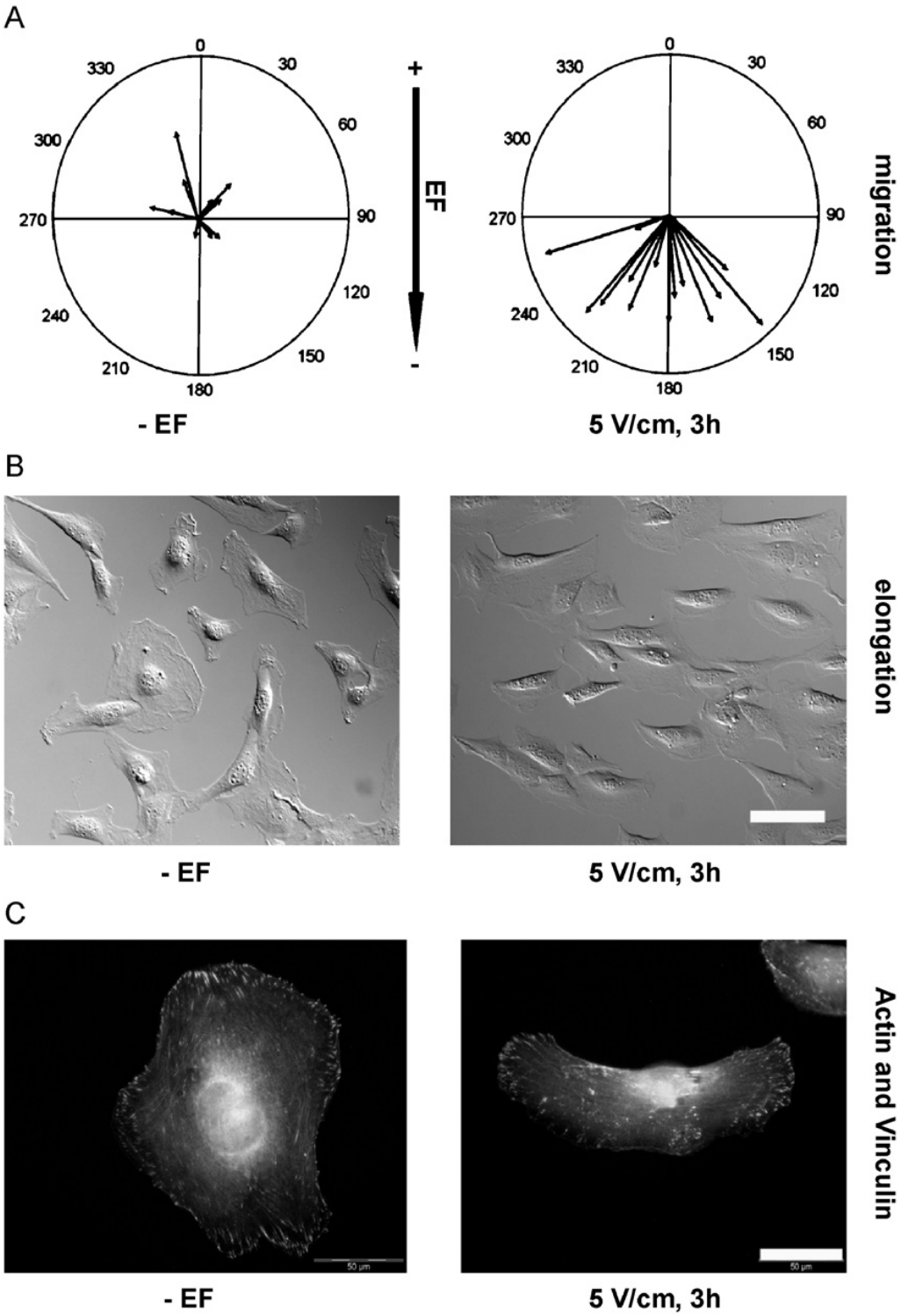


Fig. 14. EF-induced migration in 3T3 mouse fibroblast cells: (A) Centered trajectories showing the direction and relative distances of 3T3 cell migration after DC EF application of 5 V/cm for 3 h. (B) DIC images of elongated and perpendicular oriented cells to EF vector after 3 h of 5 V/cm DC EF. Bar: 100 μm. (C) Parallel reorganized actin cytoskeleton (filaments, phalloidin-TRITC) and polarized distribution of focal contacts (dots, Vinculin-FITC) after 3 h of 5 V/cm DC EF. Bar: 50 μm.

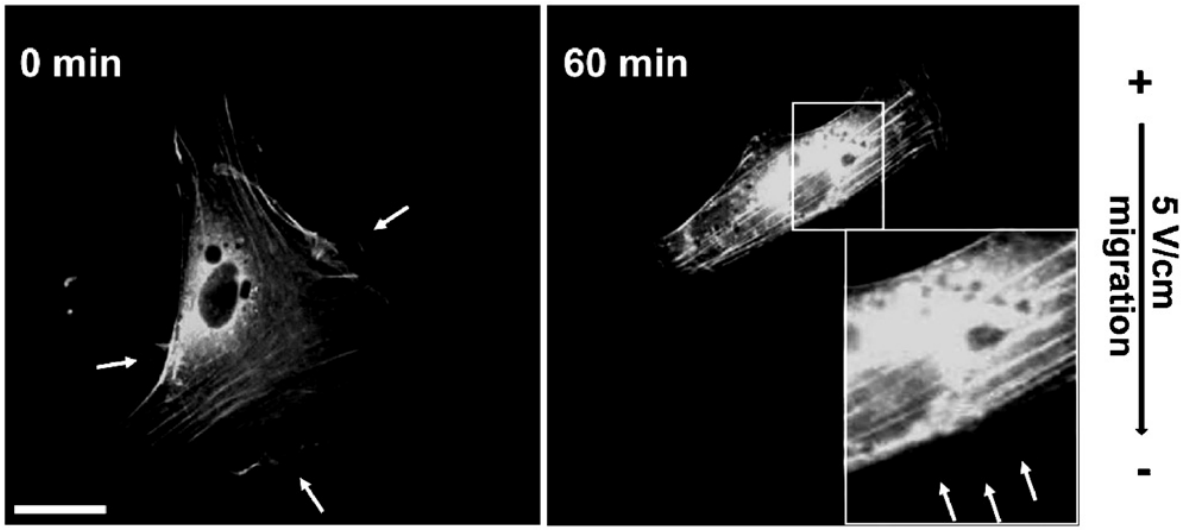


Fig. 15. Time lapse frames (0.60 min) from GFP-actin transfected mouse fibroblast (3T3) cells showing parallel organized actin filaments and cell elongation after 1 h of 5 V/cm DC EF. Arrows indicate the lamellipodia formed only towards the direction of migration, cathode.

1981), DC EF trigger the separation of charged membrane components. In order to achieve perpendicular elongation, a certain self-induced cytoskeletal tension is required along the axis. Assuming that all free integrin receptors drift towards an electrode and accumulate there, the cell is only able to connect to two focal adhesions and increase tension between them if they are on an axis perpendicular to the drift. Cell adhesion is a dynamic process, thus after some time, disassembly of focal contacts on the opposite side of the drift destination leads to another drift. Consequently, the cell is no longer able to maintain protrusions with the same orientation as the EF (Curtze et al., 2004).

It has been reported that membrane extensions from the trailing edge of chondrocytes concomitantly retract (Chao et al., 2000). Additionally, osteoblasts and osteoblast-like cells undergo processes of retraction and elongation that ultimately realign their long axis perpendicular to the EF (Curtze et al., 2004). One theory to explain EF-induced perpendicular orientation of cells is that cell attachment is made by the adhesive interaction of ECM proteins with a group of transmembrane adhesion receptors, the integrins (Dzamba et al., 2001; Geiger et al., 2001). After binding to their specific ligands, integrins cluster within the membrane and recruit several adaptor and signaling proteins to form focal contacts (focal adhesions) that anchor the ends of intracellular actin filaments (Fig. 6). Vinculin is one of these adaptor proteins and is frequently used as a marker for integrin-based focal contacts. As DC EF trigger migration and orientation, cells must orchestrate the response of cytoskeletal elements and shape to this trigger. In fibroblasts, EF exposure results in the orientation of actin stress fibers and microtubules perpendicular to the EF (Harris et al., 1990).

Our studies revealed that mouse fibroblasts (3T3) show time- (0–5 h) and voltage (0–5 V/cm)-dependent perpendicular orientation to an EF vector. Using immunohistochemistry, we could demonstrate that focal contact distribution in these cells

was polarized, accumulating on the left and right sides as well as on the leading edges of perpendicularly oriented cells after exposure to a 5 V/cm DC EF for 3 h. However, more actin was found at the leading edges of migrating cells (Fig. 15). Migration followed elongation, and formation of new filopodia and lamellipodia was also observed at the leading edge. Mouse fibroblasts exhibited directional migration towards the cathode under physiological DC EF (5 V/cm). Additionally, fibroblast cells migrated faster when compared to other cell types, e.g. osteoblast-like cells. In agreement with other reports, an accumulation of actin stress fibers was observed at the cathodal (leading) edge of several cell types (Sulik et al., 1992; Zhao et al., 2002). Often, the total amount of filamentous actin transiently increased and became selectively enriched in the leading lamellipodia (Li and Kolega, 2002b) along with focal contacts (Chang et al., 1996). Recent experiments showed that EF-mediated motility in fibroblasts can be halted by inhibition of microfilament dynamics, whereas inhibition of microtubules only reduces migration speed (Finkelstein et al., 2004).

Calcium: is an essential ion that is involved in many biological/physiological cascades. Our time-lapse observations with fibroblasts reproducibly showed that cellular Ca level increases shortly (~17 s) after application of a DC EF (14 V/cm) (Fig. 16). Additionally, the initial area of elevation seems to be dependent on, or at least affected by, the direction of the migrating cell in the EF. We initially observed an elevation in calcium at the anode side, which then propagated through the cell body towards the cathode as the fibroblasts migrated towards it. However, it is still unclear as to the type of Ca channels involved and the correlation between intracellular calcium and cell migration. Since we only detected calcium elevation above 14 V/cm, it is possible that these channels are not activated below this voltage and that other ion exchangers/pumps, e.g. H^+, K^+, could be involved in migration.

In contrast to calcium, clear changes in cell membrane potential and H^+ potential (pHi) in osteoblast-like cells could be detected after exposure to both high (14 V/cm) and low (5 V/cm) strength DC EF (Fig. 17). Together these findings lead to the conclusion that cellular migratory events may be affected more by K^+ and H^+ ions, which regulate membrane potential and pHi, than by calcium during physiological DC EF-directed cell migration in vitro. As mentioned, ''charge drift'' can be responsible for receptor redistribution on the cell membrane, thus redistribution of charged molecules in the medium may also be involved.

Chernyavsky et al. (2005) showed that pharmacologic and molecular modifiers of the Ras/Raf-1/MEK1/ERK signaling pathway altered both chemotaxis toward choline and galvanotropism toward a cathode in similar ways, indicating the involvement of the same signaling steps. Galvanotropism could be abrogated by inhibiting acetycholin (ACh) production by hemicholinium-3 and restored by adding exogenous carbachol. The concentration gradients of ACh and choline toward the cathode in a DC field were determined using high-performance liquid chromatographic measurements. Their results suggest that keratinocyte galvanotaxis is chemotaxis toward the concentration gradient of ACh, which is created in a DC field due to its highly positive charge. A time-course immunofluorescence study of ACh receptor redistribution on the membrane of keratinocytes exposed to a DC field

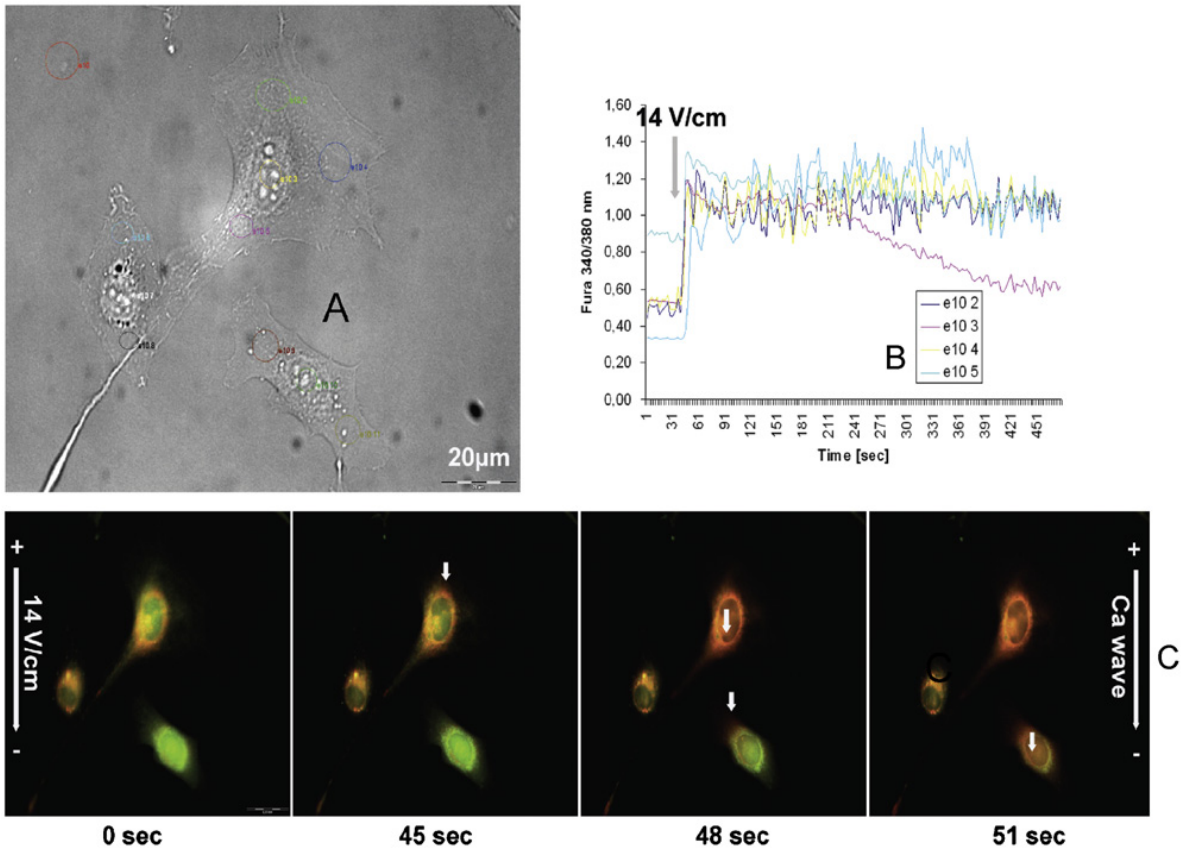


Fig. 16. (A) Phase contrast image of 3T3 cells showing the regions of interest (colors). (B) Ratio kinetics of Fura-2AM representing the $[Ca^{2+}]_i$ elevation under 14 V/cm of DC EF (applied at 30 s; arrow). Cells responded to applied DC EF in 10 s. (C) Fura 2-loaded cells showing the elevation of $[Ca^{2+}]_i$ initiated at rear-end (anode side) and propagating through entire cell body as a wave (small arrows).

revealed that α7 nicotinic and M_1 muscarinic receptors rapidly relocate to and cluster at the leading edge.

Mechanical load from outside is also discussed as a possible transducer of EF. EF-induced effects such as dynamic changes in intracellular calcium concentration, cell traction and orientation are similar to cellular responses after mechanical stimulation (Oliver et al., 1999; Munevar et al., 2001; Curtze et al., 2004). Converse flexoelectricity (Fig. 18) may therefore be a way for cells to sense small DC EF. Charged proteins in the lipid bilayer of the cell membrane repel each other, influencing membrane tension (Petrov et al., 1993). If the charge on one side of the membrane is changed by a DC EF, the membrane tension also changes, resulting in a modified curvature of the membrane. Such flexoelectric effects have already been demonstrated on voltage-clamped cells (Zhang et al., 2001).

Using traction force microscopy, Curtze et al. (2004) noticed that the first detectable reaction of osteoblasts after DC EF exposure (10–30 s) was a 5–30% increase in the average traction force magnitude. The visible retraction phase started after 5–10 min, then cells subsequently elongated and oriented perpendicular to the EF lines. Traction forces at the margins tangential to the EF decreased below their

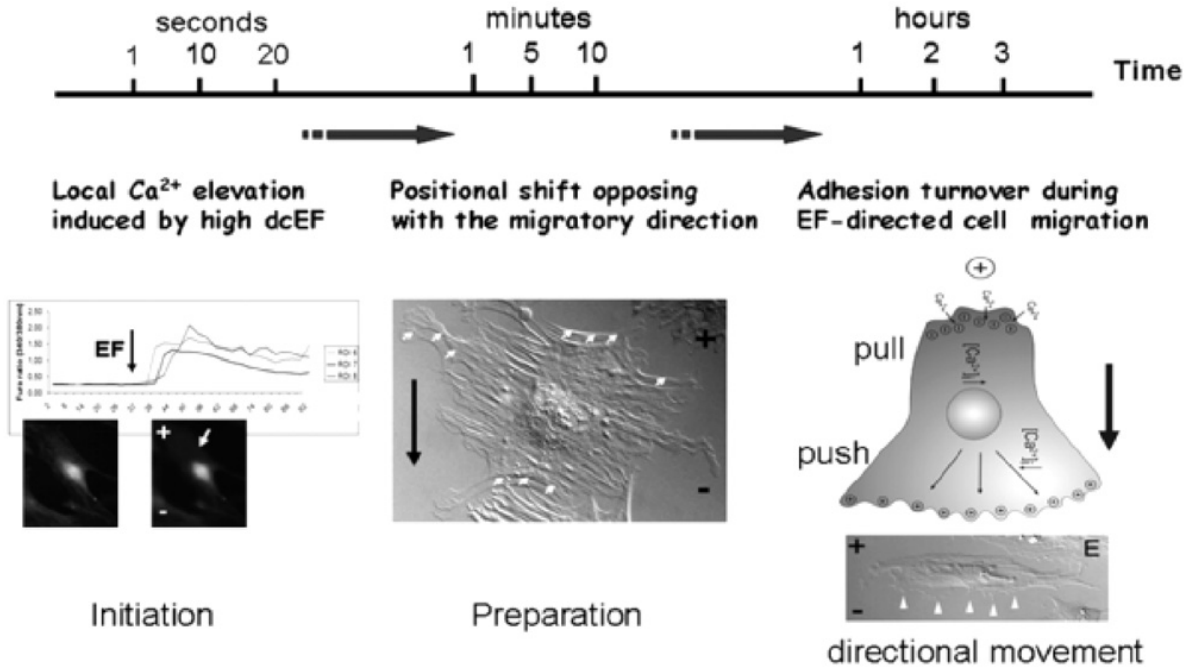


Fig. 17. Flow diagram is showing DC EF-induced early and late events in a single calvaria osteoblast-like cell as a model. DC EF of 14 V/cm triggers a local elevation in intracellular calcium (in seconds) on anode-facing cell side followed by a rapid positional shift (in minutes) towards anode and finally directed migration (in h) towards cathode.

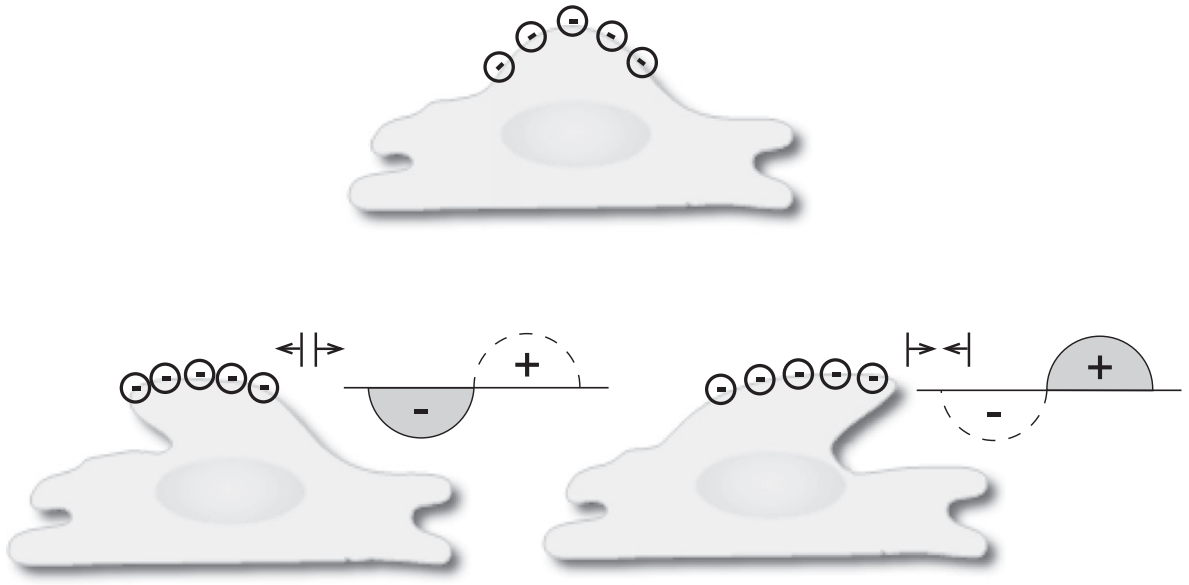


Fig. 18. Rhythmic traction forces by sinusoidal EMF onto a surface charges bearing part of a cell.

initial values 2–15 min after the start of EF exposure. A mean delay of 85 s between EF application and the first observable changes in Ca^{2+} levels occurred, suggesting that stretch-activated Ca^{2+} channels (Glogauer et al., 1997) may be responsible for

the Ca^{2+} influx. Appropriate strain sensors that produce biochemical signals to modulate cell reactions were proposed by Sheetz et al. (1998).

Sun et al. (2007) showed that a potential electrocoupling mechanism involves activation of cell surface receptors (e.g., G-proteins receptors) in the cell membrane by electrical stimulation. This can lead to the PLC enzyme-mediated signaling through the IP3 pathway. It is known that this pathway is able to generate $[Ca^{2+}]_i$ oscillations (see Section 3.7.2). Other coupling mechanisms could include multiple types of calcium channels and signal-transducing molecules such as integrins.

3.4.2. Coupling the DC EF effect to common signaling pathways

The most important candidate is ion movement (e.g. by voltage-sensitive Ca^{2+} channels) (Sasaki et al., 2000). However, in the case of K^+-dependent signaling, Ca^{2+} fluxes were not affected by K^+ channel activity. Levin (2007) reports that in some studies, cell biological effects seem to depend on the particular transporter involved, indicating that the type of ion and perhaps its movement dynamics also act as signals (Ng and McAinsh, 2003).

3.4.2.1. Pattern of ion pumps and DC EF. A typical eukaryotic cell possesses multiple types of ion pumps, which are each present to different degrees. This means that there are two degrees in freedom to form cell-type (and possibly cell individual) characteristic patterns of DC EF.

Microdomains of ion channels and transporters distributed in a pattern across the entire two-dimensional surface of a cell can encode an enormous amount of information (Levin, 2007). In addition, these ion pumps normally do not maintain the same level of work over time. A characteristic pattern of fluctuation in activity, often metabolically triggered (Kindzelskii and Petty, 2005), can add specific rhythms to the spatial patterns. Thus, a single cell can generate different EMF patterns over time that can be as manifold as the morphology of, e.g., a cell membrane with its thousands of types of proteins, glycolipids (glycocalix) and other characteristic features. In the context of a specific tissue, these EMF patterns can form a kind of ''hologram'' that renders information for the whole set or subset of cells.

In parenchymal tissues with many cells lying close together, the extracellular space near the cell membrane is normally the area in which biomolecules bind to membrane receptors. Its lower electrical impedance also makes it the preferred site over transmembrane regions for induced currents of intrinsic and environmental EF (Adey, 1992a). Thus, in parenchymal tissues, extracellular space transfers at least 90% of the currents, directing them along cell membrane surfaces.

3.4.2.2. Gap junctions. In many organs, cells are directly coupled by gap junctions, which form channels that enable their capacitative coupling. A model, namely the transmission line model, has been proposed by Pilla (2003) to account for tissue sensitivity to weak electric currents commonly found in developing and regenerating tissues (Cooper, 1984). This is a ladder network that provides a pathway for internal currents (Pilla et al., 1994b). This network and the effective shape of most cells leads to tissues with higher sensitivity than that predicted by mathematical models: the

cells in a living tissue are often more elongated and thus grasp a larger field gradient than that projected by standard models using round cells in mathematical calculations of signal-to-noise ratios. Furthermore, there can be enough anisotropy in one cell and more in such coupled arrays (McLeod et al., 1987). Gap junctions also increase the effective electrical "size" of the cell/tissue target. This means that the gain of an array of cells in gap junction contact is significantly larger than that of a single cell. This also increases the EMF sensitivity beyond of a single cell, with a further resultant increase in signal-to-noise ratio. Beyond a possible initial role in weak EMF transduction, gap junctions may be involved in cell surface signal amplification through the highly cooperative binding or release of calcium ions (Bawin et al., 1975; Bawin and Adey, 1976; Blackman et al., 1979, 1985a).

Small molecules, such as serotonin (Fig. 10), can be driven through connexin gap junction channels by DC EF. They enable intercellular, bidirectional transport of ions, metabolites, second messengers and other molecules smaller than 1 kD. In this context, Levin et al. (2006) and Adams et al. (2007) have identified serotonin as a "second" messenger. Serotonin accumulation produces a chemical gradient that can drive further signaling cascades to transduce and enhance primary DC EF signals meant to cause biologically relevant reactions. As an active transport event, Levin (2007) lists the activity modulation of voltage-sensitive small-molecule transporters (Levin et al., 2006), including voltage-sensitive serotonin transporters, which could convert membrane voltage into the influx of specific chemical signals. Charged morphogens or other signaling molecules can be moved inside cells to build up a gradient inside one or – via gap junctions – more cells, thus inducing further events (Levin, 2007) (Fig. 10).

3.4.2.3. Bound water. Along with the topic of electric gradients and currents acting near the cell membrane, we must consider the most important biochemical agent on our planet: water. All surfaces of the extracellular space, which transfer the majority of DC EF and direct them along the cell membrane, and all surfaces within the cell are characterized by water associated with charged molecules (bound water). Water itself is also a charged molecule and exists in a dipole (Modig et al., 2003; Despa, 2005). Forrester et al. (2007) state such that application of DC EF to cells in an aqueous environment generates forces acting on the surface of the cell by a form of hydrodynamic drag, termed electro-osmosis through various membrane channels and metabolic pumps. This is because of taking part of larger Na^+ ions externally and smaller K^+ ions internally. External sodium thus attracts to it a larger aqueous shell of water molecules than internal sodium, and thus creates a stronger external dragging force in the presence of an electric field (McLaughlin and Poo, 1981).

Around ions, bound water molecules (Fig. 19) can cause hydrodynamic friction in the presence of moderate EF whereas "electrofriction" occurs at highly charged surfaces (Kim and Netz, 2006). Similar to ions (Nordenström, 1994; Halle and Davidovic, 2003), proteins and sugars are also normally surrounded by structured water (Pollack and Reitz, 2001; Pollack, 2002, 2003; Hildebrandt et al., 2006) (Fig. 20). Water molecules, due to their polarity, form a shell around all charged molecules. Outside the cell, bound water in the vicinity of charged surfaces is

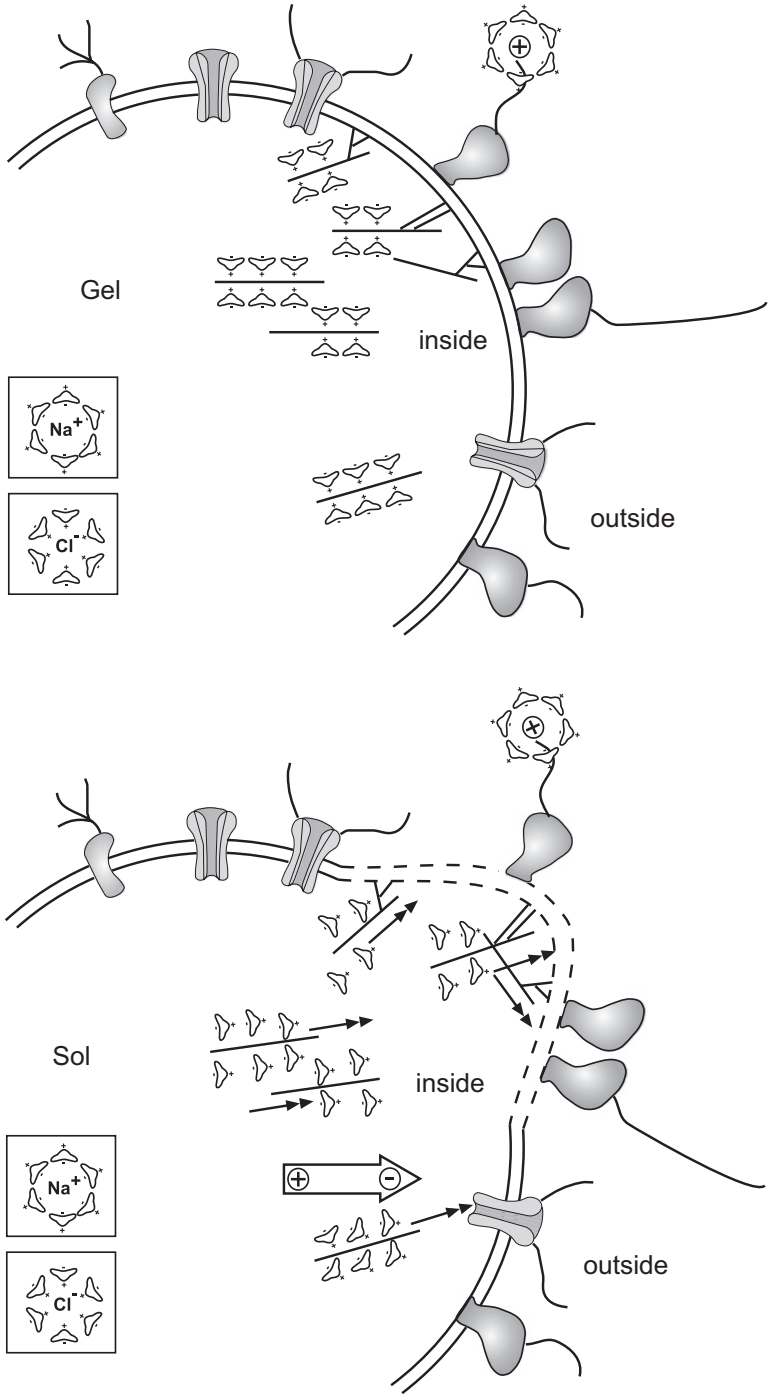


Fig. 19. Ions (+, above and in the insert) as well as filamentous proteins (here, negatively charged) are surrounded by layers of ordered water molecules (bound water). According to Forrester et al. (2007), water exists as a dipole and may adopt a structured network of water dipoles around the polymer (interfacial water). Biopolymers together with the network of 'structured water' should constitute a gel, e.g. in the extracellular matrix and the cytoplasm of cells. Application of a DC EF may lead to disruption of this gel. A subsequent entry of Na^+ ions and escape of K^+ ions may then occur. Furthermore, a depolymerization of the gel near the membrane inside the cell, together with changes in the cytoskeleton, may allow an extension of the plasma membrane (e.g. as a lamellipodium).

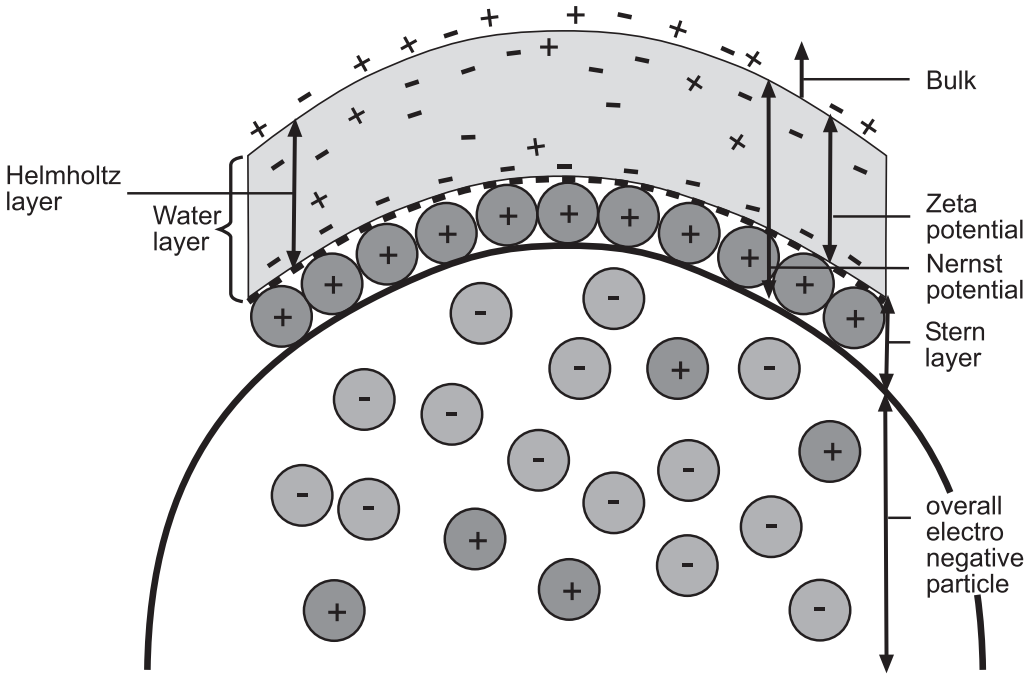


Fig. 20. Water layers (“bound water”) around an overall negatively charged particle.

somewhat different to “free” bulk water, which has no bonding or relation to molecules. Bound water can influence molecules, especially protein charges. For example, in proteins the positive charges of water molecules line along the negative surface charges of the proteins with further layers of water molecules (Fig. 19).

Different bond strengths cause conformational “mechanics”, internal “electronics” and quantum effects (see below, “flickering protons”).

In a situation where many proteins are tightly packed and thus “polymer-like”, the structured water molecules allow an overall supramolecular structure with increasing intramolecular forces, leading to the formation of a polymeric gel (Forrester et al., 2007).

This gel clearly can interact with ion channels, receptors, etc. in cell membranes, which are tightly packed with proteins, lipids and sugars (glycocalix). According to Forrester et al. (2007), intracellular cytoskeletal proteins, in particular near the cell membrane (cortex) are packed together with structured water molecules in a gel formation, held together with Ca^{2+} ions. Under the influence of DC EF, this water/protein polymer can be instantaneously altered in its structure by changing the orientation of the water dipoles (see also the hypothesis of Lange and Gartzke, 2006, below). The gel becomes more a “sol” and releases large amounts of trapped Ca^{2+} ions (see calcium wave in section “migration”, Fig. 16). During migration, loss of gel structure can lead to lamellipodial protrusion and other concomittant processes (Forrester et al., 2007).

Regarding this gelatinous state of water (found as bound water) from a quantum physics perspective, many new aspects arise: the gelatinous state favors a collective effect of coherent nonlinear (quantized) states that allow readout by other biomolecules (Watterson, 1996). Concerning subtle forces, such as London forces,

that can act on e.g. microtubules, Hameroff and Penrose (1996a, b) formulated a hypothesis as to the ability of biomolecules such as microtubules to act as quantum computing devices (Hameroff, 2004). Here, the gel–sol transitions of bound water have also been in conjunction with shielding of microtubules in neuronal axons during rhythmic firing (see the last section).

3.5. Consequences of DC EF on signaling between cells and tissues

Now, we will present an outline for a hypothesis regarding the weak DC EF effects of ''bioelectricity'' and for the combined effects of electric and magnetic fields. Such a hypothesis may be preliminary and rudimentary in this new and rapidly expanding branch of molecular cell biology. However, it has helped us to search more systematically for data linking EF, MF and EMF (we use the term ''EMF'' to summarize all three entities) and molecular cell biology. Only those EMF effects will be regarded which have direct connections to physiological processes in (A) development, (B) adult states, (C) wound healing and (D) regeneration. We will also refer to pathological states if they have a connection to the coupling of EMF and cell biology during these processes. Studies are not covered that show damaging actions and safety hazards of special EMF strengths and frequencies.

In the previous chapter we have seen that cells can generate EF on their own. Additionally, cells are capable of sensing EF, or at least EF gradients. We now present the following hypothesis: (a) cells send EF into their surroundings to ''see'' or ''touch'' their neighbors with EF gradients; (b) there may be a feedback pattern by this interference with related EF gradients from other cells and other field lines reflected from the surroundings; and (c) cells use this mechanism to subtly ''feel and see'' the position, movement and state of other cells and tissues. In the first instance this might sound strange, but there are many advantages to this mechanism of ''going down from the more preliminary and more subtle'' (like fields) to biochemical signaling: many factors can be ''calculated and integrated'' before ''things go real''.

With ion and EF gradients, changes and movements in the neighborhood could also be sensed with greater temporal resolution (milliseconds) than with slow diffusion processes, e.g. the diffusion time for an active component in 1 nano molar solution of 100 mm^3 is 10 s. Diffusion of molecules, such as morphogens, growth factors, etc. can follow these EF processes and will yield a more stable fixation of successive emerging patterns, until they are morphologically stable (see the above example of serotonin, Fig. 10). Because all molecules in the body are in a steady motion, not only static (DC) EF should be considered. But also induced electric currents arise (by Faradays law) and by them magnetic components arise. Vice versa, the magnetic components can induce currents and create again EF.

It is clear, that our hypothesis of the physiological role of bioelectricity will only be one player within a concert of other factors leading to the more durable ''hardware'' of cells, tissues and larger morphological entities. However, it must be investigated: Are there actors that can produce a field according to the needs of the cell and its surrounding? Could it be that EMF are a driving force for morphogens (like serotonin, see above) in general?

3.6. Actors and sensors of DC EF

3.6.1. Actors of DC EF on the cell membrane?

Are EF really present in physiological situations? In development, we have seen that small DC EF are present during gastrulation and neurulation. In the immature oocytes of both fish and frogs, a transcellular current is already preset that enters the animal pole and exits in the vegetal pole, preceding the development of pigment asymmetry (Nuccitelli, 1988). A further hint that EF are involved in early processes is the fact that the cleavage planes in frog eggs are altered by strong magnetic fields (Denegre et al., 1998, PNAS). In a biophysical model, Valles (2002) showed that the altered cleavage plane geometry results from magnetic field-induced realignment of mitotic structures, which causes a rearrangement of the centrosome replication and spreading processes.

Zhao et al. (1999a) report that in a small physiological DC EF cells divided while attached to the culture dish, and most did so with a cleavage plane perpendicular to the EF vector. Cell divisions in vivo are enhanced in the presence of physiological DC EF: thus, endogenous physiological EF may play important roles in the processes of rapid cell division (morphogenesis, wound healing, etc.) by regulating the axis of cell division and via this mechanism, the positioning of daughter cells.

Furthermore, mitotic spindles orient according to EF generated during the disruption of epithelium and wound healing (see McCaig et al., 2005, see below).

Major steps such as gastrulation and neurulation are also accompanied by changes in EF gradients: the epithelium of the embryo is characterized by a transepithelial potential (Fig. 8) (McCaig et al., 2005). This results from a principal mode of cell polarity: the apical cell membrane has specialized amiloride sensitive sodium channels (Na^+ can enter the cell) and the basolateral membranes contain ouabaine-sensitive Na^+-K^+-ATPase (pumping 3 Na^+ out, while 2 K^+ can enter, Fig. 21). Thus, ion transport is directed through the epithelium into the embryo. Sealing tight junctions leads to high electrical resistance between neighboring cells by reducing the electrical conductivity of the paracellular space. During neurulation Shi and Borgens (1995) have measured stable EF gradients with concomitant electric currents around the neural plate, detecting 0.75–1 V/cm underneath the epithelium in the extracellular space in the head region. In the region of the developing blastopore, EF of only 0.3 V/cm are found (Robinson and Messerlie, 1996). McCaig et al. (2005) points out that the sites of current leaks are regions of major tissue movements with transient disruptions of tight junction seals. This group further showed that a voltage gradient exists across the neural tube and that neuroblasts differentiate in this gradient (see McCaig et al., 2005). If in axolotl this trans-neural tube potential is eliminated during stages 34–36, major abnormalities in development of the cranial and central nervous system occur (McCaig et al., 2005). Simply by collapsing the trans-neural tube potential, the internal structure of most of these embryos reduces to a formless mass of dedifferentiated cells. Remarkably, the external form of some embryos with collapsed trans-neural tube potential continues to develop, despite the complete absence of concomitant internal histogenesis (Borgens and Shi, 1995).

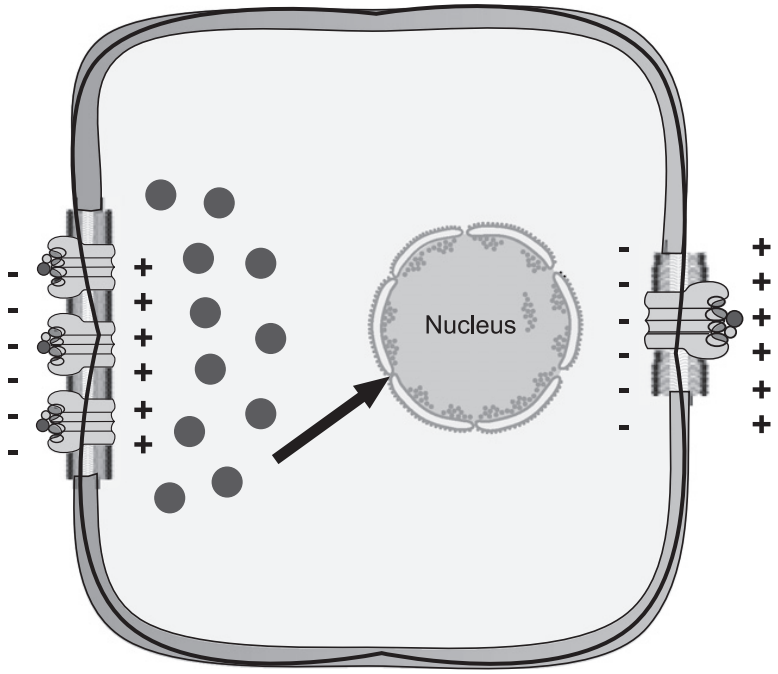


Fig. 21. Directed transport of small signaling molecules (e.g. serotonin) entering on one side of the cell via the membrane-voltage-gated transporter. This event can change the functional state of the cell in a coordinated manner (cf. axial patterning with a DC EF potential-driven gradient in the concentration of a small molecule, Fig. 10).

Is the cell able to actively direct actors, the producers of field gradients, to certain places? This is a question that leads us to the localization and activity of distinct ion pumps. We have seen above that sol–gel transitions are discussed in conjunction with "bound water". Gel–sol transitions are important for the translocation of proton pumps such as the H^+-ATPases (see below). Regarding the gel–sol transition, Beaulieu et al. (2005) showed that in the epididymis, in which luminal acidification is crucial for sperm maturation and storage, modulation of the actin cytoskeleton by the calcium-activated actin-capping and severing protein gelsolin plays a key role in regulating vacuolar H^+-ATPase (V-ATPase) recycling. Maintenance of the actin cytoskeleton in a depolymerized state by gelsolin facilitates calcium-dependent apical accumulation of V-ATPase in response to luminal pH alkalinization. Gelsolin is also present in other cell types that express V-ATPase in their plasma membrane and in recycling vesicles, including renal intercalated cells and osteoclasts. Therefore, modulation of the actin cortex by this severing and capping protein may represent a common mechanism by which these cells regulate their rate of proton secretion. Furthermore, a subunit of the H^+-V-ATPase has a function in mediating the direct binding of the enzyme to the actin cytoskeleton (Vitavska et al., 2003).

V-ATPases, in general, serve various functions in normal physiology at a number of intracellular sites. Mainly, they are used to generate an acidic pH. In secretory vesicles, the proton and membrane potential gradients established by V-ATPases are used to drive uptake of small molecules, such as neurotransmitters (Forgac, 2007).

If we look at general *mechanisms for placing and aligning ion transporters*, we find that investigations have mainly been performed using the models of (a) wound healing, (b) migration of cells in DC EF and (c) cell adhesion. The models of regeneration (e.g. limb regeneration in salamanders and newts (Borgens et al., 1977, 1984; Altizer et al., 2002) encompass so many facets that it is at the moment very difficult to find out all molecular aspects of ion transporter location. It is known from a recent paper by Adams et al. (2007) that H^+ pump (V-ATPases)-dependent changes in membrane voltage are an early mechanism, which is necessary and sufficient (!), to induce tail (spinal cord, muscle and vasculature) regeneration in Xenopus. After amputation, the normal regeneration bud depolarizes, but after 24 h it repolarizes due to V-ATPase activity (Fig. 22). It is indeed the specific ion flux per se that is necessary and sufficient for regeneration. This paper of Adams et al. (2007) reveals active upregulation of a pump mechanism specifically during regeneration, in contrast to passive injury currents that result from breaks in ubiquitously polarized epithelia during limb regeneration (Borgens, 1984). Here, the cell-surface V-ATPase is upregulated at the mRNA and protein levels within 6 h of amputation (!) and V-ATPase-dependent expression of downstream gene expression indicates that this is an extremely early step in the regeneration process. This is important, indeed: there are two physiological events (1) a DC EF that attracts innervation to the bud (Britland and McCaig, 1996) and (2) a depolarization of more rostral cells that perhaps upregulates proliferation (Cone and Tongier, 1971) and can induce a degree

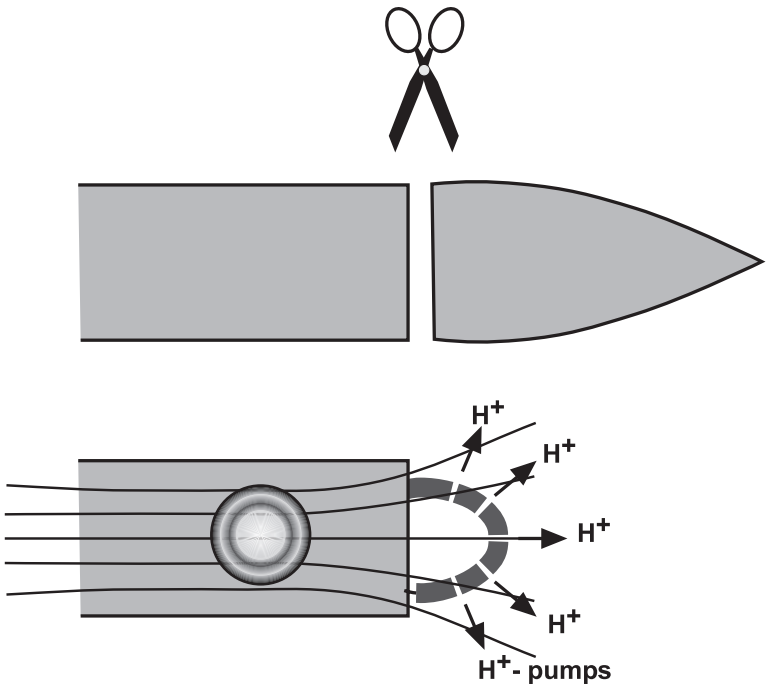


Fig. 22. After cutting the tail of Xenopus laevis larva, a depolarized "blastema" zone forms (spheroid area). At the regenerating edge proton extruders are located within the cell membrane (arrows, H^+) (see Adams et al., 2007). The transepithelial potential possibly results in an electric field (curved lines) that may guide axons into the bud.

of plasticity necessary for mature somatic cells to rebuild the tail (Binggeli and Weinstein, 1986; see Levin, 2007).

Levin (2007) further cites that this finding shows that several ion channels and pumps have roles as information cue for different cell biological processes unrelated to their ion transport. Thus, artificial modulation of wound physiology by gene therapy, with a judicious choice of ion transporters, would be a biomedically promising direction for augmenting and inducing regeneration in otherwise non-regenerating tissues.

DC EF are, indeed, present in *wound healing*: An EF is generated immediately upon wounding, with the cathode at the wound center. It is possible that a DC EF is the earliest signal an epithelial cell receives to initiate directional migration into the dermal wound bed (Nuccitelli, 2003; Ojingwa and Isseroff, 2003). Even transient breaches in an epithelium, also during natural turnover, induce short-lived, local electrical signals that influence cell regulation (see McCaig et al., 2005) (Fig. 23). During disruption of an epithelium, the potential difference becomes short-circuited, either across the whole epithelial sheet, or across a single cell membrane (see McCaig et al., 2005). A wound-induced electrical signal lasts for many hours (see McCaig et al., 2005) and regulates different cell behaviors within 500 μm to 1 mm from the wound edge. After complete reepithelialization, the signal fades. A similar phenomenon is referred to by McCaig et al. (2005) for a single neuron: using a vibrating probe they showed that a drop in injury current at the cut end of an axon indicated that an ionic seal forms in 1 h (Eddleman et al., 2000; Fishman and Bittner, 2003).

In his review McCaig et al. (2005) states: "in evolutionary terms, membrane resealing to close an electrical leak is among the most primitive activities that cells

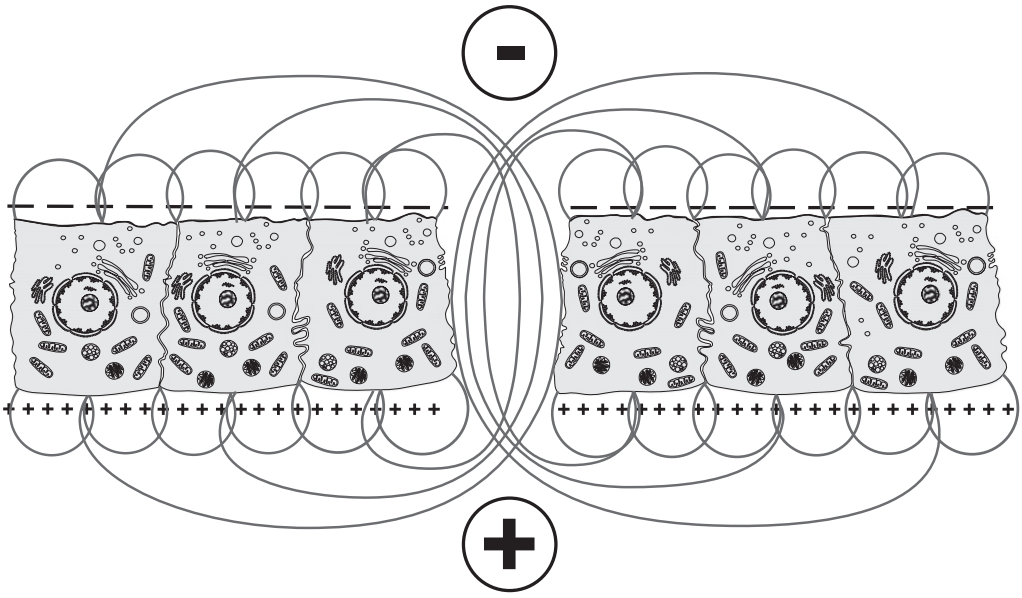


Fig. 23. At the site of the wound the transepithelial potential breaks down due to a current leak. In the adjoining regions where the epithelium and the ion transport properties remain intact a voltage gradient returns back and reaches normal values at greater distances.

undertake. Perhaps both single cells and sheets of cells use the instantaneous electrical signal induced by injury to seal a membrane and to close a wound, respectively''

Chifflet et al. (2005) demonstrated that non-specific depolarization of the plasma membrane potential of epithelial cells during wounding can promote characteristic *cytoskeletal rearrangements*. As to the role of ion channels, Chifflet et al. (2005) showed a connection between membrane depolarization and the cytoskeleton of wound healing epithelia. Spontaneous depolarization of the plasma membrane, potentially by a rise in epithelial Na^+ channel activity, constitutes an additional factor in the intermediate cellular processes leading to wound healing. By this, membrane potential depolarization occurs at the leading edge of wounds and gradually extends inward toward the neighboring cells. Chifflet et al. (2005) exchanged the type of ion to show that membrane depolarization per se, not the increase in intracellular Na^+ concentration, is responsible for the formation of actin cables.

The respective EF may elicit small direct currents that participate in the orientation and actin reorganization of migrating cells and may act via a complex interaction with enzymatic reactions (see Grasso et al., 2007).

Integrin receptors within focal adhesions stabilize lamellipodia of migrating keratinocytes (Frank and Carter, 2004), allowing these cells to interact with a variety of extracellular matrices found at the wound site, including fibronectin, vitronectin, stromal type I collagen and fibrin (Larjava et al., 1993). Regarding proton pumps, EF and integrin-mediated anchoring of the cytoskeleton, Schwab et al. (2007) report that controlling cell adhesion depends on the extracellular pH and involves activity of the Na^+/H^+ exchanger NHE1 (Stock et al., 2005). They further state that colocalization of NHE1 and integrins at the leading edge of lamellipodia (Grinstein et al., 1993; Plopper et al., 1995; Denker et al., 2000; Klein et al., 2000) creates a proton-enriched nanoenvironment in the immediate vicinity of the focal adhesion complexes. Here, the local extracellular pH at focal adhesion sites modulates the strength of cell adhesion and thereby migration on a collagen I matrix (Stock et al., 2005) an increase in NHE1 activity (more protons) leads to tighter adhesion and decreased cell migration, whereas a lack of protons due to low NHE1 activity prevents adhesion and migration (see Stock and Schwab (2006) for a review NHE1 function in cell migration).

At this point in our review, we aim to more precisely define the *coupling of DC EF signals to intracellular signaling cascades*. Sun et al. (2004) found that the primary event required to induce migration in fibroblasts is the activation of a cell surface receptor-coupled phospholipase C by an external electrical stimulus. However, the sustained cell movement was mediated by mechanically operated stretch-activated cation channels and subsequent Ca^{2+} influx. For keratinocyte directional migration in response to a DC EF stimulus, Pullar et al. (2006a) showed that both integrin ligand-binding and cytoplasmic domains together with EGF were required for the synergistic activation of a Rac-dependent signaling pathway.

Finkelstein et al., 2004 tested the hypothesis if microtubules enhance adhesion/de-adhesion remodeling during galvanotaxis and if EF mediate motility of cells poorly

or dynamically attached to substrata. They found that incompletely spread cells migrated more rapidly than fully spread cells. Also, overexpression of PAK4, a Cdc42-activated kinase that decreases adhesion, enhanced galvanotaxis speed, whereas its loss decreased speed. They concluded that EF mediate fibroblast migration via participation of microtubules and adhesive components, but their participation differs from that during spontaneous motility.

Concerning the directionality for cell migration, DC EF are also responsible that ion channels are moved and clustered in the lamellipodia in the direction of the migration (Rosenspire et al., 2001) (Fig. 24).

Exposure of both neutrophils and keratinocytes to EF in serum-free medium induces rapid, specific and long-term phosphorylation of protein kinases including mitogen-activated protein kinase (MAPK), ERK, Src and Akt (Wang et al., 2003a). Recent studies in yeast, mammalian cells, and Dictyostelium discoideum revealed another critical role of Ras, PI3K and TOR in regulating the actin cytoskeleton, cell polarity, and cellular movement, and also demonstrate that multiple steps in the signal transduction pathway coordinately regulate cell motility in the response to DC EF. Similar to a chemotactic response, phosphorylated Src and PIP 3 are polarized in the direction of migration. However, Zhao et al. (2006) could show in chemotactic

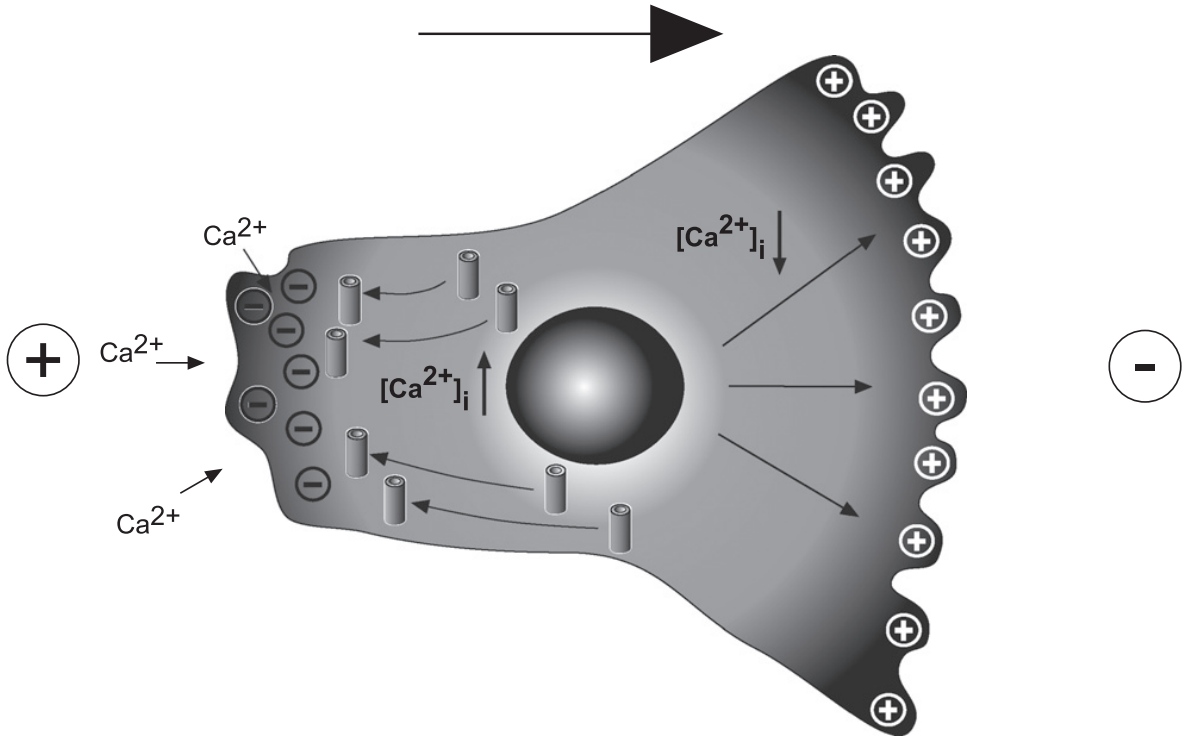


Fig. 24. DC EF can lead to clustering of ion channels in the lamellipodia toward direction of migration (arrows at the cathodal side). The initial area of Ca^{2+} elevation seems to be dependent to or affected by the direction of the migrating cell in the EF. Calcium elevation at anode side and then propagation through the cell body towards cathode is present as (e.g.) fibroblasts migrate to cathode. Arrows at the anodal side: presumed drift of Ca^{2+} channels to the anodal pole.

relevant protein knockouts that Dictyostelium discoideum can detect DC EF independently of chemotactic cues (Zhao et al., 2006; see Levin, 2007).

During wound healing DC EF stimulation as a prime directional cue triggers activation of Src and inositol-phospholipid signaling, which polarizes in the direction of cell migration (Zhao et al., 2006). Genetic disruption of phosphatidylinositol-3-OH kinase (PI(3)K) decreases EF-induced signaling and abolishes directed movement of healing epithelium in response to electric signals. With this finding, a specific gene involved in electric-field-induced cell migration was identified for the first time (Levin, 2007). Following the PIP3 mechanism further, Zhao et al. (2006) looked at a tissue-specific deletion of the PTEN gene in keratinocytes. The lipid phosphatase PTEN negatively regulates the PI3K and Akt pathway by reducing the available amount of PtdIns(3,4,5)P3 (Li et al., 2002a). The genetic abrogation of PTEN enhanced ERK and Akt phosphorylation, and potentiated field-induced keratinocyte migration. These results implicate function of the tumor suppressor PTEN in the response of cells to electrical signals (see Levin, 2007).

In skeletal muscle cells, NF-κB activation is linked to membrane depolarization and depends on the duration of elevated intracellular calcium. Valdes et al. (2008) showed that this can be regulated by sequential activation of calcium release mediated by the ryanodine and IP(3) receptor. Furthermore, both K^+-induced depolarization and electrical stimulation mediated by calcineurin activity increase mRNA levels of type 1 IP3 receptor, which suggests that depolarization may regulate IP3 receptor transcription. The results of Valdes et al. (2008) confirm the presence of at least two independent pathways for excitation–transcription coupling in skeletal muscle cells, both dependent on calcium release and triggered by the same voltage sensor but using different intracellular release channels.

3.6.2. What are the specific sensors of DC EF at the cell membrane?

As early as 1996, Olivotto et al. (1996) wrote: ''Membrane proteins possess certain features that make them susceptible to the electric fields generated at the level of the plasma membrane''. A reappraisal of cell signaling, taking into account protein interactions with the membrane electrostatic profile, suggests that an electrical dimension is deeply involved in this fundamental aspect of cell biology. At least three types of potentials can contribute to this dimension: (1) the potential across the compact layer of water adherent to membrane surfaces, (2) the potential across the Gouy-Chapman double layer (see water layers Fig. 20), which accounts for the effects of extracellular cations in the modulation of differentiation and (3) the resting potential'' (see Section 3.4.2.3).

Recently, Hegle et al. (2006) demonstrated in Drosophila neurons that a voltage-driven switch for ion independent signaling exists by ''ether-à-go-go K^+'' channels. These channels increase p38 and MAP kinase activity, a role that previously has been ascribed only to channels regulating calcium influx. Furthermore, a voltage-sensitive phosphatase (VSP) was found to be a direct EF sensor and link to the relevant signaling cascades (Murata et al., 2005). VSP is a phosphoinositide phosphatase that converts PtdIns(3,4,5)P3 to PtdIns(4,5)P2, under regulation of a voltage sensor

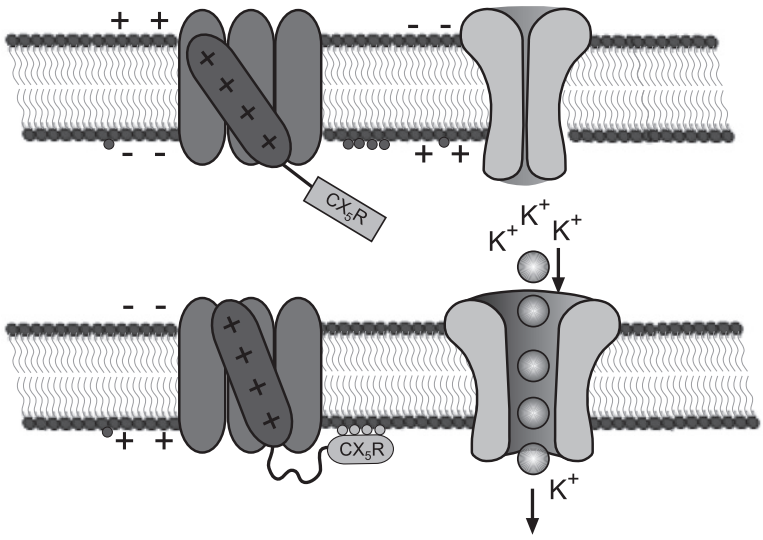


Fig. 25. Voltage-sensitive phosphatase (VSP, left side): a phosphoinositide phosphatase that converts PtdIns(3,4,5)P3 to PtdIns(4,5)P2, regulated by a voltage sensor domain (CX5R). Right side: voltage-gated K^+ channel.

domain (Iijima et al., 2004) (see Fig. 25). This system is therefore able to be effective in all the mentioned phenomena elicited by DC EF.

Levin (2007) reports that the lipid phosphatase PTEN was found to be a component of an intrinsic voltage sensor (Murata et al., 2005). PTEN negatively regulates the PI3K and Akt pathway by reducing the available amount of PtdIns(3,4,5)P3. Furthermore, genetic abrogation of pten enhanced ERK and Akt phosphorylation, and potentiated field-induced keratinocyte migration (Zhao et al., 2006; see Levin, 2007).

In neural precursor cells, Li et al. (2002a) showed that PTEN keeps PIP3 levels relatively low and thereby maintains the proper number of cells. In the cascade of an intrinsic voltage sensor the PI3K/PTEN balance is then shifted by an unknown signal, the PIP3 levels increase, and, the migration is initiated (Li et al., 2002a).

In the chapter on development (Section 3.2.1) we have seen that DC EF are also involved in the development of laterality. In this case, the Notch pathway is involved as an important genetic 'sensor' for bioelectrical events (Raya et al., 2004).

3.7. DC EF, proliferation and stem cells

3.7.1. DC EF and proliferation

DC EF (2 V/cm) inhibit proliferation of vascular endothelial cells or lens epithelial cells by inducing a cell cycle arrest at the G1/S phase (Wang et al., 2003b). In both cell types, DC EF significantly decreased the expression of cyclin E, whereas levels of the inhibitor of the cyclin E/Cdk2 complex, $p27^{kip1}$, increased. Also the healing of lens epithelial monolayer wounds was inhibited at the cathodal side after exposure to DC EF. Extracellular signal-regulated kinase (ERK 1/2) activity was increased in this case, but became asymmetrically distributed with much weaker activity on the cathodal side than on the anodal side (Song et al., 2002; Wang et al., 2003b). Konig

et al. (2006) found in human myoblasts triggered to differentiate, that hyperpolarization resulting from K^+ channel (Kir2.1) activation generates an intracellular Ca^{2+} signal. The authors found that out of the calcineurin, p38-MAPK, PI3K and CaMK pathways, only the calcineurin pathway was inhibited when Kir2.1-linked hyperpolarization was blocked. The CaMK pathway, although Ca^{2+} dependent, is unaffected by changes in membrane potential or blockage of Kir2.1 channels. They concluded that Kir2.1-induced hyperpolarization triggers human myoblast differentiation via activation of the calcineurin pathway, which, in turn, induces expression/activity of myogenin and MEF2.

3.7.2. DC EF and stem cells

A new field has opened to study of the relationship between DC EF and stem cells. In general, regeneration events are accompanied by strong currents, and inhibition of endogenous currents specifically prevents regeneration. Artificial induction of currents can induce a significant degree of regeneration in normally non-regenerating species (Borgens et al., 1989; Nuccitelli, 2003; see also Becker, 2002) (Fig. 1). If one considers the blastema, or ''bud'', that forms during regeneration of an amputated amphibian tail (Fig. 22), questions arise about the general role of stem cells. These questions also hold important relevance for the adult organism.

During *limb regeneration*, e.g. in the salamander, the major source of blastemal cells is the reversal of the differentiated state of mature cells at the amputation site (Stocum, 1997; see Becker, 2002). These dedifferentiated cells redifferentiate into the cells needed and regenerate missing structures. At this time point stem cells become established and are dispersed in the more differentiated tissue, like it is in the mature tissues of higher vertebrates (Brockes, 1997; Lowenstein and Parent, 1999). It has been known since 1961 (Becker, 1961, 2002; Becker and Murray, 1970) that neurally generated direct current factors at an injury site can lead to dedifferentiation of blastema cells. Stimulation of these factors by DC EF leads to regrowth of a complete limb at the amputation site in mature amphibians species that are normally incapable of limb regeneration (Smith, 1967). Even human children have the capability to regenerate traumatically amputated finger tips (Illingworth, 1974), a process which is associated with the appearance of a blastema-like structure and measurable electrical potentials similar to those demonstrated in regenerating salamanders. Importantly, such amputated finger tips heal much better (till the age of about 10 years) and can only regenerate completely if the wound is covered by a moist plaster (see Illingworth, 1974). This is possibly due to electric currents which can spread at moist tissue, however, if the surface of the skin or wound is dry, this spreading of currents is prevented (Becker, 1961; Becker and Murray, 1970). However, at present it cannot be determined if these examples of regeneration are caused by proliferation of a small, pre-existing stem cell population or the dedifferentiation of mature mammalian cells (Becker, 2002).

Yamada et al. (2007) showed that mild electrical stimulation strongly influenced embryonic stem (ES) cells to assume a neuronal fate. They proved that induction of calcium ion influx is significant in embryoid bodies forming ES cells. Because Ca^{2+} is one of the most important signaling ions, many downstream pathways may be

involved. For example, Ca^{2+} is known to be involved in the non-canonical Wnt signaling pathway (see Section 5.5). Yamada et al. (2007) further point out that physical alteration of cell surface membranes may initiate signaling, even though normal signaling molecules take over later (see Fig. 18). Again we see that ionic flux constitutes a novel category of differentiation signals.

This primitive signaling pathway for early development and neural regeneration may be a prototype that mediates environmental effects on cells. Receptor-ligand signaling systems may have evolved to stabilize and refine environmental cues imposed on cells. In neuronal tissues and other cell types (Levin, 2007), ionic currents are continuously flowing, and these currents may instruct and ensure that undifferentiated cells assume a neuronal progenitor fate.

Again, recent findings show that excitatory neural activity induces adult neural stem cells to adopt a neural fate. Chun et al. (2006) showed that tetanic stimulation of long-term potentiation (LTP) increases *neural progenitor proliferation* in the adult dentate gyrus (DG) in an NMDA receptor-dependent manner. Several lines of evidence suggest a role for DG neurogenesis in learning and memory by LTP. The DG is also one of the few areas in the mammalian brain where production of new neurons continues into adulthood. Thus, they conclude that electric stimulation of LTP and neurogenesis are closely related.

D'Ascenzo et al. (2006) could show that neural stem/progenitor cells differentiation is strongly correlated with the expression of voltage-gated Ca^{2+} channels, especially the Ca(v)1, and that Ca^{2+} influx through these channels plays a key role in promoting neuronal differentiation.

Finally, in rat bone marrow-derived mesenchymal stem cells, Deng et al. (2007) demonstrate that membrane potential, IK(DR) and I(KCa) channels change with cell cycle progression.

Human *mesenchymal stem cells* (hMSC) (Sun et al., 2007), as do many other cell types, possess characteristic Ca^{2+} waves that are involved in intracellular signaling. These waves come in short and long periods – the longer also operate during transcellular signaling. Sun et al. (2007) showed that a DC 0.1 V/cm stimulus (30 min/day for 10 days) facilitated, synergistically with osteoinductive factors, hMSC differentiation into the osteogenic cell lineage by reducing the Ca^{2+} wave frequency, which is typically found in differentiation processes. These naturally occurring fluctuations in Ca^{2+}, or other metabolic or signaling waves (rhythms of the cell), can be accessible to appropriate EMF impulses. However, the coupling devices and mechanisms channeling EMF information into these pathways are now the subjects of detailed investigation.

Regarding stem cells and EMF, significantly more studies have been performed using pulsed (E or M) fields, or by varying electromagnetic field stimulation than by DC EF (see EMF and stem cells discussed below).

Interestingly, another hint for the importance of correct timing comes from genetic network oscillators in which now are found, e.g., in the tissue organization during zebrafish somitogenesis (Mara and Holley, 2007; see section EMF). However, the research field "connection between genetic network oscillators and electric- or EM-fields" is still untouched.

Cell membrane surface charges can also change with pathophysiological state. Malignant cancer cells showed indeed increased negative surface charges (although low overall membrane potential, see below) and this could affect field-induced effects such as electrotaxis (Mycielska and Djamgoz, 2004).

It is very intriguing that, in general, malignant cancer cells (<-10 mV) as well as proliferating cells (CHO, 3T3, etc., -12 to -25 mV) have low cell membrane potentials whereas quiescent and differentiated cells possess a high membrane potential (>-50 mV) with the highest for neurons (-75 mV), glia (-90 mV) and skeletal muscle >-90 mV) (Binggeli and Weinstein, 1986).

3.8. Resumé: actors/sensors

The literature points to the existence of DC EF actors, such as ion channels, and the controlled placement of these actors by the cell and its cytoskeleton, proteins, bound water and other structures. The same is true for specific sensors and their coupling to canonical signaling pathways which reach till the genome and go back into translation. Thus, we are now literally witnesses to this growing novel aspect of molecular biology, which has been introduced mainly by the groups cited initially in this review. Many questions are still open but can now be addressed. One of these, possibly the most complicated, is how this complex information processed between the many actors and sensors (''tertium comparationis'' of our hypothesis). Further aspects not yet addressed in this review and nearly untouched by cutting-edge cell biology are the influence of MF components and the topic of rhythm. Do intrinsic EMF interfere with actor and sensor systems? This will be discussed in the following chapters ''SMF'' and ''EMF''.

4. Static magnetic fields (SMF)

Compared to DC EF, endogenous sources of static magnetic fields are presumably negligible because in living systems everything from molecule to organelle is in motion. Even SMF can induce EMF if the change in movement is rhythmic. Thus one can apply Faraday's law that ''a changing magnetic field is associated with a changing electric field''. In most cases of endogenous MF generation by muscle, nerve, piezo, streaming and other E potentials, the concomitantly generated magnetic (H) component is short, thus generating a pulsed MF (= PEMF, EMF because of the additional EF). On the other hand, EF in the body and all the effects seen above, including nerve potential and ECG, can be influenced by SMF from outside. Here, one has to discern natural SMF, e.g. the earth's magnetic field (geomagnetism) from artificial ones in our technical environment.

In principle, coupling MF effects to cell biology is still for more enigmatic, as it is with DC EF. Whereas EF represent forces at the surface of molecules, cell membranes and even the whole body, MF penetrate deeper. Since the body is ''semitransparent'' to MF, MF can go inside the cell and influence even chemical and

biochemical reactions. Magnetic field gradients can intrude into cells and even deeper into layers of living tissue, unlike electric fields which are shielded by the high dielectric property of the cell membrane (Markov, 2007). Through this mechanism, every signaling pathway or cell biological event can be altered in a subtle way.

First let us look at, what MF strengths are present in our surroundings, and then we will ask: Is this relevant for biology? In the following sections we will outline the concepts currently used to explain MF coupling.

4.1. Natural MF

The MF of the earth has a strength of 0.5 G, which is equivalent to about 5×10^{-5} T. Magnetic fields can be measured either as magnetic flux density or as magnetic field strength. In the US and Western Europe field strengths are usually specified in units of magnetic flux density (Tesla or Gauss): exact conversion: $1\,T = 100\,V\,s/cm^2 = 100\,G = V\,s/m^2$ 10,000 G equals 1 T, 1 G = 100 μT. The geomagnetic field shows large topographical variations that can be measured exactly along, e.g. geologically relevant structures (Kertz, 1971). Geomagnetic field changes occur continually at periods ranging from a few milliseconds up to 1012 years (Kertz, 1971). The vertical component of the dipole field of the earth reaches a maximum of about 70 μT at the magnetic poles, and approaches zero at the magnetic equator; conversely, the horizontal component is close to zero at the poles and has a maximum just over 30 μT at the magnetic equator. Changes in the dipole field with periods on the order of about 100 years are explained by eddy currents located near the core boundary (Bullard, 1949). Variations in period shorter than this have their primary cause outside the Earth, and are associated with processes in the ionosphere and magnetosphere (Kertz, 1971; Garland, 1979). The typical solar diurnal cycle shows variations of no more than a few tens of nanoteslas, whereas large magnetic "storms" may reach variations of 0.5–1 μT over 72 h. For further rhythmical (EMF) phenomena in the atmosphere and in the natural environment see the chapter on EMF.

Geomagnetic rhythms may serve as time cues to organize physiological rhythms, e.g. Gauquelin and Gauquelin (1967), Wever (1968) and Cremer-Bartels et al. (1984). A variety of behavioral changes in humans are statistically related to disturbances in the earth's electromagnetic field. There is a documented relationship between increased geomagnetic activity and the rate of seizures as well as of admission of patients to 35 psychiatric facilities (Friedman et al., 1963; Venkatraman, 1976; Rajaram and Mitra, 1981). Hypo-geomagnetic periods may also affect patients with psychic disorders (Kay, 2004).

Shielding humans from ambient MF significantly desynchronizes circadian rhythms, which can be gradually resynchronized after application of MF. Hundreds of subjects were observed over many years in an underground bunker built to study human circadian rhythms. One of the two experimental rooms was shielded against natural magnetic and electric fields (Wever, 1970), and the other had an electromagnetic shield around it, consisting of a mesh of steel rods and plates that reduced the influence of geomagnetic rhythms by 99%. The rhythms of body

temperature, sleep, waking, urinary excretion and other physiological activities were monitored. All subjects developed longer and irregular, desynchronized or chaotic physiological rhythms. Those in the magnetically shielded room developed significantly longer and more irregular physiological rhythms. In some experiments, artificial electric and magnetic rhythms were pulsed into the shielding. A very weak 10 Hz electric field dramatically restored normal biorhythm patterns. Thus, the geomagnetic field and its normal fluctuations seem to be important for the "vitality" of the organism. Many papers report a decrease in "vitality" in a hypo-geomagnetic field. In such a reduced field (reduction by factory of 10 s), Nepomnyashchiida et al. (1997) found impaired regeneration in the mouse myocardium. Regarding stress-induced analgesia (SIA) in mice, Del Seppia et al. (2000) and Choleris et al. (2002) observed that SIA decreased in a hypo-geomagnetic environment (see also Del Seppia et al., 2007).

4.2. MF in technical environment

Considering high MF in the technical environment, workers in the aluminum industry and at particle accelerators experience flux densities of 1–4 T for long time periods. Patients are briefly exposed to fields reaching 3 T in new MRI machines. In a review of the physiological effects of exposure of humans to high-field [1.5–8 T] MRI units, Chakeres and de Vocht (2005) concluded: "There were no clinically significant changes in the subjects' physiologic measurements at 8 T. There was a slight increase in the systolic blood pressure with increasing magnetic field strength. There did not appear to be any adverse effect on the cognitive performance of the subjects at 8 T. A few subjects commented at the time of initial exposure on dizziness, metallic taste in the mouth (think on metal containing enzymes and on iron in hemoglobin), or discomfort related to the measurement instruments or the head coil. There were no adverse comments at 3 months". Changes in the ECG (increase in the amplitude of the *T* wave) can be found in humans at flux densities >1 T. These changes originate from electric potentials that are induced in blood flowing through the aorta, and by this "superimpose additional voltage on the normal electrocardiogram" (Tenforde et al., 1983).

For high SMF, relatively few reports exist regarding experimental cell biological effects. Very strong fields (10–16.7 T) can directly alter the cleavage plane in frog eggs (Denegre et al., 1998). MF greater than 0.5 T applied to a frog embryo during its first three cleavages can lead to severe gastrulation abnormalities much later in development. (Denegre et al., 1998, see above). As mentioned above, the present review focuses on physiological effects and the relevance to cell biology and medicine. Thus, it is more important to look at MF effects low on the Tesla scale.

4.3. Coupling MF into cell biology

There are two sides in this discussion: (1) The physical side, which has a background in solid state physics, testing hypotheses with "big machines". Here

nanotechnology and microelectronics findings at the level of quantum physics will help to better understand biology. (2) The biological side with its immense number of charged particles and surfaces – from single molecules to large macromolecules and cell organelles. In addition, numerous recently discovered phenomena exist in the physics of biology, e.g. open dynamic systems with special compartmentation related to thermodynamic needs, coupling by solitons, coherence in general at different levels, and diverse effects of quantum physics including tunneling, entanglement, "flickering" protons and electrons, etc. However, solid state physics has until now mainly focused on mechanisms by which MF affect living systems in the context of variants of Faraday's Law, which states that a "changing magnetic field is associated with a changing electric field". The majority of these mechanisms require flux densities in at least the mT–T range (see below).

Besides Faradays Law, the main principle behind magnetic "influence" is the direct action on molecules by magnetomechanical interactions by (a) rotational motion of a substance in a uniform field until it achieves a minimum energy state or (b) translational force exerted on a paramagnetic or ferromagnetic substance placed in a MF gradient. Biological tissues are diamagnetic with few exceptions, thus their magnetic susceptibilities are close to vacuum. Only under special circumstances, such as if tissue contains aligned magnetite (ferromagnetic) particles, do MF exert torque on the magnetic dipole. Bacteria containing chains of magnetite particles are able to sense the earth's MF. Several authors have discussed a role for iron-containing molecules such as transferrins, which have specific receptors at the cell surface, and heme-containing proteins that couple receptors to enzymes at cellular membranes (Phillips, 1986; Adey, 1993, see Markov, 2007). Amara et al. (2007) describe the effects of a static MF (250 mT for 3 h) on a labile zinc fraction as causing slightly lower viability in a monocyte line.

Furthermore, the classic concern against weak ($<1\,\mu T$) MF is that the energies involved are not sufficient to compensate for random thermal ("Brownian") agitation of the molecules (the Boltzmann "boundary") (see Whissell and Persinger, 2007b). However, there are realistic ways to influence biological systems by MF: Larmor precession (discussed below) is a strong candidate in rhythmic processes – if a SMF hits biological system in motion, like blood or moving cell organelles or macromolecules.

In moving systems, which includes the entire organism, the *Hall effect* (exerted by the Lorentz force) comes into play, which is in connection to orbital electrons and electron spins (at very high flux densities: nuclear spins) and magnetic force (Fig. 26).

Because electrons are in motion in an orbit and have spin, the torque exerted produces a change in angular momentum perpendicular to that angular momentum, causing the magnetic moment to precess around the direction of the magnetic field rather than settle down in the direction of the magnetic field. This is called *Larmor precession* (Fig. 27). A bound ionic oscillator (e.g., ion bound to a protein) in a static magnetic field will precess at the Larmor frequency in the plane perpendicular to the applied field. This motion will persist in superposition with thermal forces, until thermal forces eventually separate the oscillator from a binding site. Interestingly,

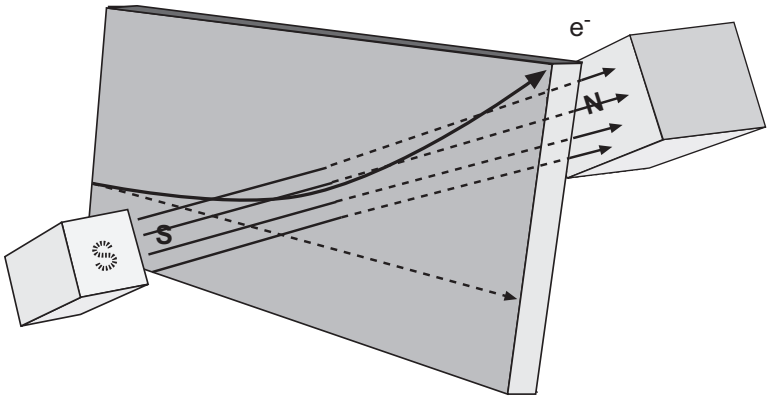


Fig. 26. Hall effect: deflection of charged particles of a current flow in a conductor by the Lorentz force (= entire electromagnetic force on a charged particle with charge q and velocity v) due to a perpendicular oriented magnetic field. Moving charges accumulate on one face of the conductor. This results in an asymmetric distribution of charge density across the "hall element".

the high sensitivity to (alternating) EMF of only 10–100 nT, possibly is only given, if the EMF is overlaid to a static magnetic field at most in the order of the geomagnetism ($\leqslant 100\,\mu T$) (see Pazur et al., 2006).

4.4. Signal transduction

In general, there are excellent reviews about the various effects of MF on cell biology which show a plethora of all possible effects (Adey, 1992a; Barnes and Greenebaum, 2007; Markov, 2007).

In the short history of EMF coupling to biological sytems (over the last 30 years, see Pilla, 2003) the first explanations focussed on ions near ion channels of the cell membrane. Here, researchers recognized that the greatest problem for EMF sensing is the random motion of ions by the thermal stochastic (Brownian) molecular movement in all 3 directions (Boltzmann "boundary" discussed above). A force exerted by an EMF field should modify these 3 D vectors until finally an ion channel is triggered by this effect.

However, ions in the body are not in a free vacuum, thus it is not trivial to apply the "solid state physics" approach to these problems. On has to keep in mind the "bound water" situation (see Section 3.4.2.3) and the ligation of ions to the ubiquitously existing proteins or other macromolecules (including also glycoproteins and lipids).

The cell membrane is often considered to be the main target for MF signals and most results point to an MF effect on the rate of ion or ligand binding to, e.g., a receptor site acting as a modulator of signaling cascades often involving calcium/calmodulin-dependent processes, cAMP, and growth factors (see Markov, 2007). In most cases the magnetic effect is ascribed to Ca^{2+} ions. The biochemical reactivity of ions bound in the molecular clefts of macromolecules may be affected by SMF via

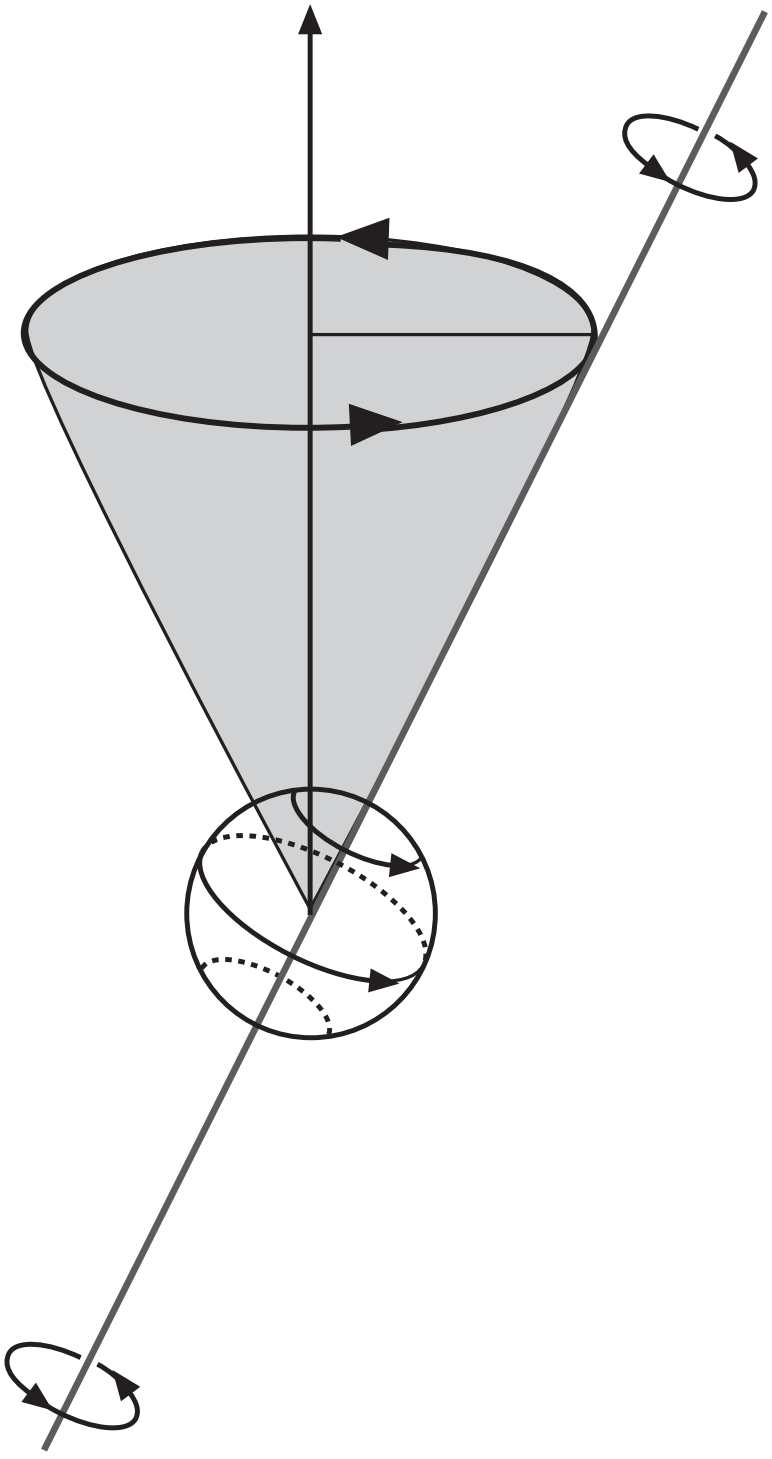


Fig. 27. Larmor precession: precessional motion (thick circle with arrowheads) of the axis (thick dark gray line) of a charged particle (sphere). One circulation around the axis (small arrow) of the precessional circle determines the Larmor frequency.

changes in the spatial orientation of movement or by changing Larmor precession frequencies. For fields in the mT range, the bound lifetime must be sufficiently longer than 1 ms (Muehsam and Pilla, 1996).

For biological systems, the Larmor precession model (Fig. 27) postulates that precessional motion at the Larmor frequency of charged particles bound inside a binding site or molecular cleft can be modulated by applied MF to affect binding kinetics. This coherence modulates the fluctuations of the dielectric constant at the binding site and by this modulates the binding situation. A threshold in the 0.1–1 μT range is predicted by this model in the presence of thermal noise, with bound times of 0.5–1 s (see Pilla, 2003). The charm of this system is that no substantial input of energy or angular momentum into the system is required (Edmonds, 1993). Coherence is brought into thermal fluctuation and thus, Larmor precession may be a transduction mechanism for weak magnetic field effects on the physicochemical properties of aqueous solutions, which in turn may modulate biological responses (Edmonds, 1993; Muehsam and Pilla, 1996; Pilla et al., 1999). Furthermore, Larmor precession allows the system to be a highly sensitive detector of the MF environment in the presence of thermal noise (Pilla et al., 1997). Many studies of the Markov–Pilla group show that Ca^{2+}–Calmodulin-dependent myosin light chain kinase and protein kinase C-dependent processes speed up to twofold (Shuvalova et al., 1991; Markov et al., 1992, 1993, 1994; Pilla and Markov, 1994; Markov and Pilla, 1994a, b, 1996, 1997; Engstrom et al., 2002; Liboff et al., 2003; see Pilla, 2007). Also the rate of Ca^{2+} binding to Calmodulin was increased twofold in a 2 G SMF (Markov and Pilla, 1997; Liboff et al., 2003).

Larmor precession of water molecules may also be a generalized transduction mechanism for weak MF effects on all aqueous interactions by altering the physicochemical properties of aqueous solutions, which in turn may modulate biological responses (see Pilla, 2003 and below).

4.5. MF effects on physicochemical properties of aqueous solutions

Many papers describe EMF-induced changes of the physicochemical properties of aqueous solutions, e.g. of different salt solutions (Berton et al., 1993), and crystal growth rates of diamagnetic inorganic salts (Lundager Madson, 1995). These effects often persist up to several days after removing the applied EMF. Magnetic pretreatment of water has been reported to alter cell density, size and nuclear diameter in catfish hepatocytes (Garg et al., 1995), suggesting that physicochemical changes in the aqueous solution itself may mediate some EMF bioeffects (see Pilla, 2003). Furthermore, magnets are commonly used to inhibit scaling in heat exchangers and water pipes (Busch et al., 1986; Yoon and Lund, 1994). The cause of this phenomenon is still unclear. Pilla (2003) states that "Larmor precession hypothesis requires that water forms stable complexes at interfaces or in clusters with lifetimes comparable to the period of the Larmor precession frequency, on the order of one second for pT-range (environmental) fields and one millisecond for mT-range (therapeutic) fields". Sufficiently long lifetimes can exist in the case of water molecules at a molecular clefts (Conway, 1981; Otting and Wuthrich, 1988), but it is as yet unclear as to whether sufficiently stable structures participate in solvent–precipitate interactions or in bulk water (see also Smith, 1999).

In general, *water is diamagnetic*. MF (0.2 T) have been shown to increase the number of monomer water molecules (Zhou et al., 2000) but, rather surprisingly, they also simultaneously increase tetrahedrality. Magnetic treatment may also increase the formation of clathrates and hydrogen bond strength (Hosada et al., 2004). An effect of MF on hydrogen bonding has been further supported by the rise in the melting point of H_2O (5.6 mK at 6 T) and the 3 °C lowering of the sol–gel transition (at 0.3 T) in methylcellulose (Wang et al., 2007), indicating a weakening of the van der Waals bonding of the water molecules within a magnetic field. It is probable that these effects are based on the quantum electrodynamic properties of water, which however are still only poorly studied. Del Giudice et al. (2002) propose a two-phase state of water with regions providing quantum coherence, and thereby a decoupling factor against the surrounding thermodynamical equilibrium (see Pazur et al., 2006).

EMF should weakly couple to water-inherent coherent oscillations, measured in water and in human subjects (Smith, 1994). These oscillations are described by wave equations describing their quantum state also using the magnetic vector potential (Smith, 1994; Cardella et al., 2001).

But we should now go back and look further to the action of moderate-intensity SMF on biological systems.

4.6. SMF effects on cells

A special mechanism of action of moderate-intensity SMF on biological systems is described by Rosen (2003). This mechanism is based on the *diamagnetic anisotropic properties of membrane phospholipids*. It is proposed that reorientation of these molecules during moderate SMF exposure deforms imbedded ion channels, thereby altering their activation kinetics. Channel inactivation is not expected to be influenced by these fields because this mechanism is not located within the intramembraneous portion of the channel. Patch-clamp studies of calcium channels have provided support for this hypothesis, as well as demonstrating a temperature dependency that is understandable on the basis of the membrane thermotropic phase transition. Additional studies have demonstrated that sodium channels are similarly affected by SMF, although to a lesser degree. These findings support the view that moderate SMF effects on biological membranes represent a general phenomenon, with some channels being more susceptible than others to membrane deformation.

Most studies about MF effects were driven by the long history of using magnets for *therapy*. Modern and more serious medical applications of electromagnetic fields are to heal non-unions of bone fractures and treat some bone-related diseases, such as osteoporosis and osteoarthritis, although the specific molecular mechanisms are not fully understood. MF, in general, have been successfully applied to therapeutically resistant problems in the musculoskeletal system (Markov, 2007). On the other hand, the use of "magnetic blankets" and other magnetic devices has garnered this kind of therapy a bad reputation and little acceptance in natural science-based medicine. However, like Markov and Ayrapetyan (2006) state: "there are now experimental and clinical data which suggest that exogeneous MF at

surprisingly low levels can have a profound effect on a large variety of biological systems (Todorov, 1982)''.

Furthermore, ample reviews exist about MF and EMF effects on nociception (Del Seppia et al., 2007) and pharmacodynamics (Whissell and Persinger, 2007a).

Reports of SMF effects on *cell growth* do exist. However, different effects have been noted depending on the MF magnitude. Inhibition of human lymphocyte growth (4–6.3 T) was detected by Norimura et al. (1993), while Balyasnikova et al. (1994) observed stimulation of mammalian cell growth at 140 mT. Both stimulation and inhibition of DNA synthesis was seen in fibroblasts at 610 mT (McDonald, 1993). Aldinucci et al. (2003) reported decreased proliferation of human leukemia cells at 4.3 T, but no effect on normal lymphocytes. Stolfa et al. (2007) found significantly enhanced viability (MTT test) of human chondrocytes cultured in medium under SMF (0.6 T).

In a 2004 review of the cellular effects of SMF, Miyakoshi (2005) came to the assumption that: ''Studies have shown that a static magnetic field alone does not have a lethal effect on the basic properties of cell growth and survival under normal culture conditions, regardless of the magnetic density. Most but not all studies have also suggested that a static magnetic field has no effect on changes in cell growth rate [or] cell cycle distribution. Many studies have found a strong magnetic field that can induce orientation phenomena in cell culture.''

4.7. SMF effects on blood flow

SMF (up to 8 T) can evoke changes in cutaneous blood flow and temperature in mice. After exposure, microcirculatory blood flow initially increased for about 5 min followed by a gradual decrease and a return to the control value (Ichioka et al., 1998, 2000). Increased blood flow in skeletal muscle has been reported in response to whole body SMF (0.3, 1 and 10 mT for 10 min) exposure (Xu et al., 2001). The authors revealed a threshold of 1 mT for enhancing muscle microcirculation in mice under pentobarbital-induced hypnosis. Applying SMF of 4 T for 15 min, Mayrovitz and Groseclose (2005) showed that resting finger skin microcirculation decreased in conscious human volunteers. Morris and Skalak (2005) found that SMF (70 mT for 15 min) influence arteriolar diameters in a restorative fashion, acting to normalize the tone to the median tone value following exposure. They argue that SMF application could be efficacious for treating both ischemic and edematous tissue disorders involving compromised microvascular function because this response occurs primarily in the resistance arterioles, which significantly influence tissue perfusion. In a recent study (Morris and Skalak, 2007), the same authors stated that chronic SMF exposure can alter the adaptive microvascular remodeling response to mechanical injury. Furthermore, in another study they revealed that acute exposure to a moderate strength MF reduces edema formation in rats (Morris and Skalak, 2008). Here, application of a 10 or 70 mT, but not a 400 mT SMF for 15 or 30 min immediately following histamine-induced edema resulted in a significant 20–50% reduction in edema formation. Additionally, a 2 h 70 mT field application to lambda-carrageenan-induced edema also resulted in significant (33–37%) edema reduction.

The authors further hint that the potential mechanism of SMF action may be via modulation of vascular tone through effects on L-type Ca^{2+} channels in vascular smooth muscle cells.

Findings on blood flow after pulsed electromagnetic fields (PEMF) are in contrast to the mostly vasoconstrictory effects of SMF. Studies of arteriolar microvessel diameters in the rat cremaster muscle showed that PEMF stimulation produced significant vasodilation compared to prestimulation values (Smith et al., 2004).

Further hypotheses on coupling SMF to cell biology and therapeutic uses will be addressed in the next chapter because it is difficult to completely separate the cell biological effects of SMF from those of PEMF and EMF.

5. EMF/PEMF

5.1. Bone, muscles and nerves

As we already mentioned, everything in living systems is in motion and changing MF are associated with changing EF. Endogenous EMF and PEMF arise from the movement of muscles, tendons, etc. and the actions of the musculoskeletal system itself. Mechanical deformation of dry bone caused piezoelectricity, i.e. bending strain couples to the spatial gradients of permanent dipoles in collagen molecules (Hastings and Mahmud, 1988). However, in the moist surroundings of living bone, small piezoelectric potentials are rapidly shielded (Otter et al., 1992). At physiological conditions, mechanical stress-generated potentials are formed by different mechanisms including: (a) the streaming potential, which is the electric potential difference between a liquid and a capillary, diaphragm, or porous solid through which it is forced to flow, or (b) the electrokinetic processes, i.e. entrainment of ions because of fluid motion through the bone (Otter et al., 1998). In any case, the EMF caused by these reactions are able to penetrate tissue and the MF component can induce electric currents in the bone or muscle tissue by Faraday coupling.

Vibrations of human muscles induce mechanical strains and currents of certain frequencies (5–30 Hz) were found during postural muscle activity (quiet standing) and <10 Hz during walking (Antonsson and Mann, 1985). These muscle contractions also induce EF in the underlying bone tissue and are important in maintaining bone mass. For example, a vertical whole body vibration of 30 Hz with an acceleration of 0.2 G causes tibial strains that in turn significantly stimulate gain in trabecular bone density (Inbar and Noujaim, 1984; McLeod et al., 1997). Interestingly, bone cells have strong frequency selectivity with EMF effectiveness peaking in the range of 15–30 Hz. Here fields as low as 0.01 mV/cm affect remodeling activity (McLeod and Rubin, 1993). Endogeneous EM current densities produced by mechanical loading (e.g. 1 Hz during walking) in bone approximate 0.1–1.0 mA/cm^2 (Lisi et al., 2006).

In addition we have to keep in mind that ligaments, tendons, fasciae and other connective tissue elements also consist of collagen. This collagen accounts for

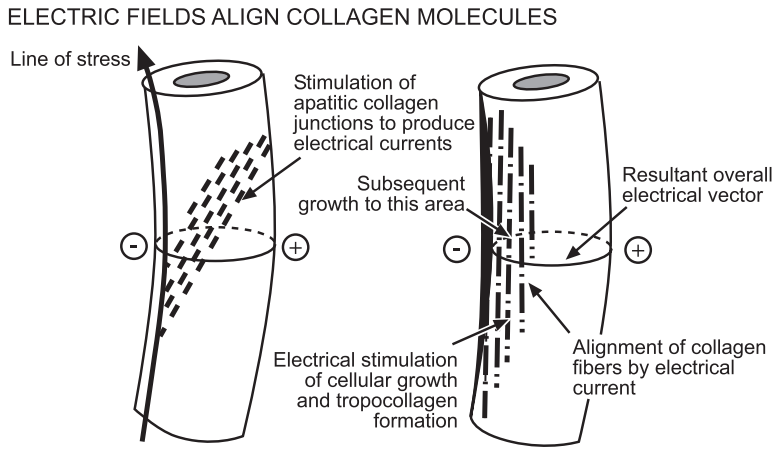


Fig. 28. Alignment of collagen molecules (representing piezoelectric molecules) due to mechanical stress that induces an electrical vector. In addition the electrical current stimulates cell growth and tropocollagen formation (after Becker, 1985).

30–40% of body protein having piezoelectric properties (Becker, 1985; see Fig. 28). The extracellular matrix of hyaline cartilage is also piezoelectric and able to convert mechanical vibrations into EMF and vice versa (Jacobson et al., 2001). Thus, all motion is accompanied by EMF pulses.

The mentioned endogenous rhythms of the musculoskeletal system, as well as the pulse of the circulatory system, create ubiquitous EMF often characterized by spikes (like the propagating action potential of nerves) and thus often resemble pulsed EMF (PEMF). On the other hand, the little higher frequencies of brain activity (although still extremely low-frequency EMF – most of the EEG spectral density is below 100 Hz) are more related to the typical sinusoid waveforms of the EMF. The current densities lie normally in the range from 1 to 10 mA/m^2 (nerve firing around 1–60 Hz), however, current densities of 1000 mA/m^2 can develop during brief periods of activity (e.g. action potentials) on the surface of nerve or muscle cells (Wachtel, 1995).

Furthermore, the enzymatic and metabolic activities of cells are mostly processed rhythmically. Thus, every substrate change and every small metabolic cycle has its own up and down often in a sinus wave with a typical frequency (Bertram et al., 2007; Goyal and Wingreen, 2007). This begins during early embryogenesis, with oscillators for tissue organization (Mara and Holley, 2007), till the very short flickering (picoseconds of molecular bonds) and the influence of EMF on this flickering (see below, Blank, 2005).

There is an interesting correlation between EMF in the natural environment and the endogeneous rythyms of the heart (ECG), brain (EEG) and peripheral nerve activity. It looks as if organisms, including mammalians and humans, have internalized environmental EMF rythyms. For example, the Schumann frequency (see below) is reflected in the EEG as well as in the brain stem (10 Hz). These low frequencies are based mostly on metabolic cycles, as seen in experiments with neutrophils and PEMF (Rosenspire et al., 2005). In neuronal groups of the thalamus

von Krosigk et al. (1993) found gamma amino buturic acid and GABAergic neurons with stable 0.1 Hz oscillations. Despite this progress, there is much to be studied regarding the correlation of metabolic cycles and ELF EMF.

Often, these rhythms seem to be chaotic but then reveal as ''ordered'' (deterministic) chaos. This kind of chaotic dynamics between cells or within cells are easier to control for nature (Glass, 2001): e.g. in the olfactory bulb of the rabbit, spatiotemporal temporal oscillations in the olfactory bulb related to sensing of odor reveal chaotic patterns (Scarda and Freeman, 1987). Complex dynamics in cardiac (Garfinkel et al., 1992), neural (Schiff et al., 1994) systems using chaos-control techniques led to attempts to manipulate such complex rhythms, although the mechanisms are not fully understood (Scarda and Freeman, 1987).

Possibly, these dynamics can be disturbed by EMF: magnetic fields at 1 and 60 Hz destabilize rhythmic oscillations in brain hippocampal slices via yet unidentified *nitric oxide* mechanisms (Bawin et al., 1996). Adey (2003) reports that studies on the role of NO in controlling the regularity of EEG waves in rat brain hippocampal tissue have shown that inhibition of its synthesis is associated with shorter and more stable intervals between successive bursts of rhythmic waves. Conversely, donors of NO and cGMP analogs applied during blockade of NO synthesis lengthen and destabilize intervals between successive rhythmic wave bursts (Bawin et al., 1994). Rhythmic EEG wave bursts in rat brain hippocampal tissue can also change due to exposure to weak (peak amplitudes 0.08 and 0.8 mT) 1 Hz sinusoidal magnetic fields (Bawin et al., 1996). Adey (2003) reports that these field effects depend on synthesis of NO in the tissue. They are consistent with reports of altered EEG patterns in man and laboratory animals by ELF EMF (Bell et al., 1992; Lyskov et al., 1993).

5.2. EMF and PEMF in the environment

Naturally occurring ELF EMF are found in the atmosphere and are known as Schumann resonance (Sentmann, 1985). This phenomenon is the reflection of charges (especially lightning, about 200/s) between the earth's surface and the ionosphere causing EMF in the range of 7.83–250 Hz (ground frequency and additional harmonics). Lightning creates electromagnetic standing waves that travel around the globe. The waves are reflected from the ionosphere, back to the earth, and then back to the ionosphere. The electric component consists of about 0.01 V/m, and the magnetic fields amounts to 1–10 nT (the earth's static geomagnetic field, typically around 50 μT is much larger). As electromagnetic waves, the Schumann resonance can be detected either as electric or magnetic micropulses. This phenomenon has been widely studied because it is the basis for long distance radio communication. When the ionosphere gets higher, the cavity gets larger and the resonant frequency drops. The overlap of Schumann resonances and biological fields may not be accidental, rather it may be part of a close interplay between geomagnetic and biomagnetic fields throughout evolution (Direnfeld, 1983). Thus, organisms may have incorporated these natural frequencies (see above). In the human brain stem an intrinsic ''ground'' frequency of about 10 Hz exists in the sympathetic part of the autonomic nerve system (Gebber et al., 1999). It is not

known if the static geomagnetic field directly influences cellular systems, however, the reactions of some patients to magnetic disturbances points to partial susceptibility (Kay, 2004).

5.3. Therapeutic relevance of EMF

The use of these fields has a long history. In the first century AD, use of an electric fish was described to cure headache and gout. Later, Paracelsus (1493–1542) studied the medical use of lodestone and Sir Kenelm Digby (1603–1665) described the magnetic cure of wounds (Macklis, 1993). Modern and more serious medical applications of electromagnetic fields are to heal non-unions of bone fractures and to treat some bone-related diseases (e.g. osteoporosis, osteoarthritis), although the specific molecular mechanisms are not fully understood. The use of electromagnetic fields to stimulate osteogenesis is based on the idea of stimulating the natural endogenous streaming potentials in bone. At first, currents were applied directly via electrodes or induced by external EMF. Later, magnetic fields were produced by forcing electric currents through wire coil placed over the fracture. Periodic changes in the MF then produced the required EF in bone via Faraday induction. The most effective medical devices today use time varied (pulsed) EMF (1–100 Hz), inducing EF in the μV/cm level at the fracture site (Otter et al., 1998; Pilla, 2002). The physiological frequencies (8–30 Hz) caused by natural muscle contractions and the subsequently induced EF in bony tissue are also used for therapy. Here, these frequencies are applied as mechanical vibrations to the connective tissue and muscle (Randoll and Funk, 2004). It is important to realize that MF or EMF only induce physiological effects within certain parametric windows, i.e. ELF (8–60 Hz) and low amplitudes ($\leqslant 1$ G) (Gartzke and Lange, 2002). It must be pointed out that not all reports pay attention to this topic. Thus one must have healthy skepticism.

Nevertheless, based upon multicenter, randomized and prospective clinical studies, the FDA approved pulsed EMF as safe and effective for treating non-unions and osteoporosis (Otter et al., 1998; Pilla, 2002; Chao and Inoue, 2003; Chao et al., 2004). Bone remodeling is a highly integrated process of resorption (by osteoclasts) and formation (by osteoblasts) of bone tissue that results in precisely balanced skeletal mass with renewal of the mineralized matrix. In bone diseases such as osteoporosis, the balance between bone resorption and bone formation is disturbed. Resorption outstrips formation, eventually leading to reduction in bone mineral density that enhances the risk of fracture. Regarding PEMF, most investigations have been motivated by observations of positive therapeutic results of PEMF treatment. On the other hand PEMF frequencies and application profiles have often been "copied" from the above-mentioned naturally occurring frequencies in order to give "healing signals" to the body. PEMF can enhance osteoblast activity but significantly reduce osteoclast formation (Otter et al., 1998; Hartig et al., 2000; Chang et al., 2004). Thus, treatment with PEMF may shift the balance towards osteogenesis.

5.4. PEMF interaction with biological tissue

This interaction might occur outside the plasma membrane, but could involve interactions with transmembrane proteins (McLeod et al., 1995; Otter et al., 1998). Patterson et al. (2006) are mentioning an example of exposure of NIH3T3 cells to a 50 Hz PEMF signal for greater than 2 h which increased the clustering of intermembrane proteins compared to unexposed cells (Bersani et al., 1997; see also Liu et al., 1996; Heermeier et al., 1998; Ciombor et al., 2002). This signal is then transduced to signaling via tissue factors like TGF-b (Lohmann et al., 2000; Guerkov et al., 2001; Aaron et al., 2002; Lohmann et al., 2003), FGF-2 (Tepper et al., 2004) and prostaglandin E2 (Lohmann et al., 2000, 2003; Guerkov et al., 2001). By these events is triggered later callus formation (Inoue et al., 2002; Midura et al., 2005) and fracture healing (Sharrard, 1990; Garland et al., 1991; Gossling et al., 1992; Scott and King, 1994).

Other in vitro studies have shown that PEMF exerts a reproducible osteogenic effect on rat osteoblasts and induces transcription of bone morphogenetic protein (BMP)-2 and BMP-4 mRNA (Bodamyali et al., 1998). Combined treatment of BMP-2 and PEMF had additive effects on osteoblastic cell proliferation and differentiation (Selvamurugan et al., 2007). Very recently, an ample review appeared regarding microvasculature and SMF, PEMF and EMF (McKay et al., 2007).

Where are the *thresholds for biological effects of EM fields?* In their review "Biomedical Applications of EMF", Blank and Goodman (2003) report, that the threshold of interaction between an EMF and enzyme activity is as follows: for Na-K-ATPase 2–3 mG (Blank and Soo, 1995); for cytochrome oxidase 5–6 mG (Blank and Soo, 1998a); for ornithine decarboxylase <20 mG (Mullins et al., 1999) and, for stress proteins (in HTB124 normal breast cells) the values are <8 mG (Han et al., 1998). In a very instructive review, Adey (1997) reported that at tissue electric gradients in the range of 10^{-7}–10^{-1} V/cm and concomitant ELF MF in the range 1.2–10 μT, a spectrum of physiological and behavioral sensitivities were found in organisms from marine vertebrates to man (Adey, 1981a, b), and in laboratory studies at the cellular level (Adey, 1992a, b, 1997).

Effective EMF-stimuli are coherent (Adey, 1993), presenting a train of regularly recurring signals that must be present for a certain minimum duration (Litovitz et al. 1993). Thus, *windows* were found for certain frequencies at cell and molecular levels in cerebral tissue (Bawin et al., 1975; Blackman et al., 1985a; Kolomytkin et al., 1994) and in non-neural cells (Byus et al., 1987; Walleczek, 1994).

Sontag and Dertinger (1998) investigated the liberation of PGE2 of human granulocytes (HL – 60 g) during application of EMF of different frequencies: they found "windows" at 6 and 16 Hz where PGE was 200% above 0 Hz baseline and beneath these "windows" (e.g. at 10 Hz) PGE was only slightly above the baseline.

This *resonance and coherence* is the secret of inducing large effects with low thresholds. Conservative estimates show that a 1-μV-induced membrane potential can be detected after 10 ms by an ensemble of less than 10^8 ion channels. Thus, strong PEMF are not required. According to Jacobson (1994), Jacobson and Yamanashi (1995), Sandyk (1996), Persinger (2006) and Persinger and Koren (2007)

even pT–nT magnetic fields are effective with appropriate resonance as a function of the charge and mass of the target molecule.

Which resonance phenomena could cause the more dose effective profiles of PEMF compared to SMF? For example, in the Ca^{2+}/CaM-dependent myosin phosphorylation system. Pilla (2003) reports that compared to a static field, a by far smaller pulsed radio frequency field (PRF) is effective. To explain: the PRF signal induced a time-varying electric field and negligible magnetic field vs ambient. The authors (Pilla et al., 1997) compared the effect of a 0.2 G PRF signal having a 500 ps burst of 27.12 MHz sinusoidal waves repeating at 1/s, and a 450 G SMF on Ca^{2+}/CaM-dependent myosin phosphorylation on neurite length from embryonic chick ganglia explants. They found that a MF of approximately 25 G PRF equals by Larmor resonance the effect of a SMF at 450 G.

We already have seen that the Larmor effect is also relevant for SMF effects. This is because of the movement of biological components in a cell or organism. If we use PEMF in therapy, then the pulse (time component) is imposed from outside and an effect can be seen if the Larmor frequency hits the right resonance frequency (coherence) of the desired biological systems. This example shows that it is generally difficult to draw a sharp line between SMF, PEMF and EMF.

Are there other effects of EMF on cell biology? EMF can influence the movement of whole organisms during development. Komazaki and Takano (2007) examined the influence of EMF on early development of the amphibian embryo. When embryos developed under the influence of a low-frequency EMF (50 Hz, 5–30 mT) the rate of early development was accelerated. EMF effects were preferentially exerted at the gastrula stage, as the period of gastrulation was shortened. Histological observations showed that EMF promoted morphogenetic cell movements during gastrulation. EMF specifically increased the intracellular calcium concentration of gastrula cells, thereby accelerating the rate of morphogenetic cell movements.

EMF also act directly on *cell differentiation* when coupled to Ca signaling. Lisi et al. (2006) found that exposure to a 50 Hz ELF EMF (magnetic flux density of 2 mT) promotes differentiation of pituitary corticotrope-derived cells from an AtT20 D16V cell line that responds to nerve growth factor (NGF) by extending neurite-like processes and differentiating into neurosecretory-like cells. During exposure, intracellular calcium ($[Ca^{2+}]_i$) significantly increased and intracellular pH decreased. ELF EMF were also able to stimulate neurogenesis in the subventricular zone of adult rats (Arias-Carrion et al., 2004).

This leads to *studies of EMF on stem cells*. Nikolova et al. (2005) found that EMF affect transcript levels of apoptosis-related genes in mouse ES cell-derived neural progenitors. ES cells were exposed to ELF EMF simulating power line MF (PLMF) at 50 Hz. This significantly affected transcript levels of the apoptosis-related bcl-2, bax and cell cycle regulatory growth arrest DNA damage inducible (GADD45) genes, whereas mRNA levels of neural-specific genes were not affected. No effects were found on mitochondrial function, nuclear apoptosis, cell proliferation, or chromosomal alterations. Czyz et al. (2004) investigated the non-thermal effects of PLMF as (50 Hz) on gene expression levels of pluripotent ES cells and the role of the tumor suppressor p53. In the model of pluripotent mouse ES cells, they found that

5 min ON/30 min OFF intermittent PLMF (50 Hz) exposure is capable of evoking non-thermal responses in ES cells, dependent on the cellular p53 function.

In human mesenchymal stem cells, Cho et al. (2006) characterized calcium oscillation profiles before and after subjecting cells to osteoinductive factors. They found that calcium spikes decreased rapidly with osteodifferentiation to a level observed in terminally differentiated human osteoblasts. In addition, calcium oscillations appeared to serve as a bidirectional signal during hMSC differentiation. While an altered calcium oscillation pattern may indicate hMSC differentiation, it is also likely to be involved in directing hMSC differentiation. Treatment of hMSCs with a non-invasive electrical stimulation, for example, not only altered the calcium oscillations but also facilitated osteodifferentiation. Thus, regulation of calcium oscillation by external physical stimulation could amplify hMSC differentiation for some tissue-specific lineages and may offer an alternate biotechnology to harness the unique properties of stem cells.

Chao et al. (2007) applied pulsing direct current EF to calf anterior cruciate ligament (ACL) fibroblasts. ACL fibroblasts demonstrated enhanced migration speed and perpendicular alignment to the applied EF. The motility of ligament fibroblasts was further modulated on type I collagen. In addition, type I collagen expression increased in ACL fibroblasts after exposure to pulsing EF. In vitro wound healing studies showed inhibitory effects of static EF, which were alleviated with a pulsing EF. These results demonstrate that applied EF augment ACL fibroblast migration and biosynthesis. Chao et al. (2007) conclude that this is a mechanism by which EF may be used to enhance ligament healing and repair.

The influence of ELF EMF on *Ca^{2+} signaling and NMDA receptor* functions in rat hippocampus was investigated by Manikonda et al. (2007). EMF (50 Hz magnetic fields at 50 and 100 μT) were examined for their influence on Ca^{2+} signaling enzymes in the rat hippocampus and related with NMDA receptor functions. Exposure caused increased intracellular Ca^{2+} levels concomitant with increased activities of Ca^{2+}-dependent protein kinase C (PKC), cAMP-dependent protein kinase and calcineurin as well as decreased activity of Ca^{2+}–calmodulin-dependent protein kinase in hippocampal regions. Simultaneous ligand-binding studies revealed decreased binding to *N*-methyl-d-aspartic acid (NMDA) receptors. The combined results suggest that perturbed neuronal functions caused by ELF exposure may involve altered Ca^{2+} signaling events contributing to aberrant NMDA receptor activities.

Regarding EMF effects on tumor promotion, some studies show a cocarcinogenetic effect (Simko et al., 2001), others demonstrate the ability of EMF to significantly inhibit tumor growth in athymic mice and other neoplastic disease models (Tofani et al., 2001, 2002; Athanasiou et al., 2007).

That EMF ultimately affect ions may explain the diffuse and variable nature of cellular reactions linked to EMF, and explain the function of ion channels in DC EF.

Again we turn to the phenomenon of low EMF thresholds found in cell biology. Bone cells for instance respond to extremely small ($\leqslant$0.01 mV/cm) induced sinusoidal EMF (McLeod et al., 1995). These effective EF or MF have very low energies. This prompts two questions: (1) Which cellular mechanism is able to

convert this very low field energy to physiological responses that require much higher energies? and (2) Why does thermal noise not interfere with the conversion and transduction of the much smaller energy EMF? Several mechanisms have been proposed to solve these problems.

5.5. Coupling EMF into cell biology

According to the principles of radio receivers, extraction of ELF modulation information from an amplitude-modulated signal requires a nonlinear element in the detection system (Adey, 2003; see Adey, 2004). What kind of nonlinearities can we find? First, in all tissues there is known to be very sharp border in the form of the cell membrane. An extreme functional nonlinearity within the cell membrane is associated with transmembrane charge tunneling (DeVault and Chance, 1966). Early experimental studies by Chance (1970) have been extended theoretically (Moser et al., 1992; see Adey, 2004). Given the positive effect of very small EMF on cellular and organelle functions, several mechanisms must add or multiply to explain the low threshold and apparent signal–noise detection. In addition, multiplicity in the hypotheses and/or observed ways of coupling to biology show that for time-coded magnetic PEMF or EMF, the situation is far more unclear than that for DC EF (Moser et al., 1992; see Adey, 2004).

Regarding PEMF the interplay between pulsed electric and magnetic fields requires attention because EF and MF can produce different biologic effects (Haddad et al., 2007). The frequency-dependent synergy was reported by Goodman and Shirley-Henderson (1991) with their in vitro study regarding transcription and translation in cells exposed to an extremely low PEMF where the DC EF change rate was too weak to drive adequate current through the tissue.

What coupling phenomena can occur?

(1) The cell membrane as major nonlinearity: in this case the charge to mass ratio is important: (a) at receptors, (b) in ion–ligand bondings during signal transduction and signal enhancement – problem of ionic signaling, mostly focussed on calcium, (c) in complex topography like molecular levers (in this case the charge to mass ratio and distances are important for resonating in the EMF frequency), (d) in conformational changes, e.g. in ion channels, (e) in sheltering ions in ion channels (ion cyclotron resonance, Larmor precession).
Cell shape (Fig. 18) and clustering of cells, electrically or otherwise coupled by gap junctions, is also important for these effects to take place (Fig. 8).
Additionally, reflections in the water bound phase of the cell membrane (see Pilla et al., 1997) make spatial focusing of the EMF possible, like in curved mirrors.
Time dependence (phase, coherence) and charge to mass ratio are also decisive factors that can lead to linear shifts in the cell membrane, such as electrophoretic mobility in DC EF.
(2) Bonding of ions to "polyelectretes" is important underneath the cell membrane in the cortical cytoskeleton. In this case, the magnetic component can induce, by Faradays Law, small electric currents that act like EF (see Section 3).

(3) Influence on chemical bonds via radical pair mechanism (hyperfine couplings with quantum effects, in general).
(4) Paramagnetic property of metal atoms/ions in enzyme systems.

(Ad 1) Membrane receptors as mediators of signal transduction

The cell membrane represents a significant barrier to charged molecules such as peptide hormones and neurotransmitters that may carry regulatory signals from one cell type to another. Similarly, due to its high resistance, the cell membrane represents a major barrier to electric currents that are flowing in the medium outside the cell. The mechanisms developed by living cells to sense the presence of signaling molecules outside the cell have many of the properties that are required to receive an electrical signal. For example, sensitive detection, amplification, rectification, and transduction can be accomplished by enzyme systems residing in cell membrane. The receptors for hormones, neurotransmitters, and growth factors thus are logical targets to investigate as sites of EMF effects.

In the history of EMF coupling, the first phenomenon which was discussed was the coherent vibration of charged molecules (mostly ions and here Ca^{2+}) with the EMF in phase. This led to the discussion of signal–noise ratio with the thermal (white) noise as background vibration.

(Ad 1a) Then, the *cyclotron resonance* was discussed for ions (see Section 4) (Liboff, 1985a, 1992). However, it could be shown that the force applied by an MF on a charge (which is ''free'', that is outside a binding site) is too week compared to the background thermal noise (see Bianco and Chiabrera (1992) and Pilla (2007), see below).

(Ad 1b) It was first proposed by Pilla (1972) that EMF might affect *ion adsorption/binding* and possibly alter the related cascade of biological processes. This electrochemical information transfer hypothesis postulated that the cell membrane is the site of interaction of low-level EMF. Ligand–receptor interactions play a pivotal role in mediating signal cascade events inside the cell membrane by altering the rate of binding of, e.g. calcium ions to enzymes and/or receptor sites. The ligand–receptor interactions play a pivotal role in mediating the following signal cascade events inside the cell membrane. In the bound water phase of the cell membrane (see Sections 3 and 3.4.2.3)–ion binding or dissociation is the hopping between two states (either bound in the molecular cleft or prebound in the plane of closest approach to the hydrated surface-Helmhotz plane) separated by few kT (see Pilla, 2007, Fig. 20).

(Ad 1c) Also, *Larmor precession* is a very strong candidate for EMF coupling (like in Section 4). Here, the precession frequency is unaffected (!) by thermal noise while the oscillator is bound. According to Pilla (2007) the threshold for the Larmor precession mode is therefore determined only by the bound lifetime of the charged oscillator. Thus, magnetic fields in the 0.1–1 μT can be detected if the oscillator (e.g. protein-bound ion) remains bound in the order of a second. The topography of the binding site can create locally a hydrophobic region from which dipolar molecules (like water) are repelled (Cox, 1988; see Pilla, 2007). Thus, in the binding site the bound ion experiences only few collisions in a surrounding which has a significant

lower viscosity than the bulk water. This fact accounts for the long bound times reported for the Ca^{2+}–Calmodulin system.

Thus, Larmor precession can explain such low intensities as 20 μT in 65 μs bursts of pulsed radio frequencies. Under these circumstances 600 bursts/s were enough to increase the myosin phosphorylation reaction twofold in a cell-free system (see Pilla, 2007).

(Ad 1d) Faraday coupling: EMF should also have the property to induce surface charges on the cell membrane (McLeod et al., 1995). *Coupling of an EF component* was described between *Coulombic forces at the surface of the cell membrane* that are able to distort the shape of the membrane and of the underlying cytoskeleton (Peskin et al., 1993). If a Coulombic force is large enough then an insertion of actin monomer between the cytoskeleton and cell membrane can occur – then, after the polymerization of actin the cell shape is altered more durably. Such manipulations distort transmembrane proteins (ion channels, etc.) and thus lead to intracellular signaling to the cytoskeleton.

(Ad 1e) It has been proposed that charged receptors or other kinds of *'antennae' on the outside of the cell membrane* recognize EMF by their ability to resonate with varying EMF frequencies because of the appropriate lengths of the moving parts that hold a charge on the free end. The resonance frequency thereby depends on the length of this lever (Fig. 29) and induced surface charge movements on the membrane trigger a signaling pathway (Fitzsimmons et al., 1992; Fitzsimmons and Baylink, 1994). This phenomenon is similar to the *electrophoretic mobility of charged molecules* in the cell membrane exposed to a static EF. However, this reaction could be faster than the 2–10 min reaction during electro-osmosis. The induced charge movement would represent at least a modification of Coulombic forces on the outside of the cell (Otter et al., 1996, 1997) or a modification of the charge distribution on the attachment surface.

An EF of 0.01 mV/cm is capable of inducing a sufficient charge density (1–10 C/m^2) to elicit a cell reaction (Otter et al., 1998). Kindzelskii and Petty (2005) found that application of a phase-matched EF in the presence of ion channel clusters caused myeloperoxidase (MPO) to traffic to the cell surface. As MPO participates in

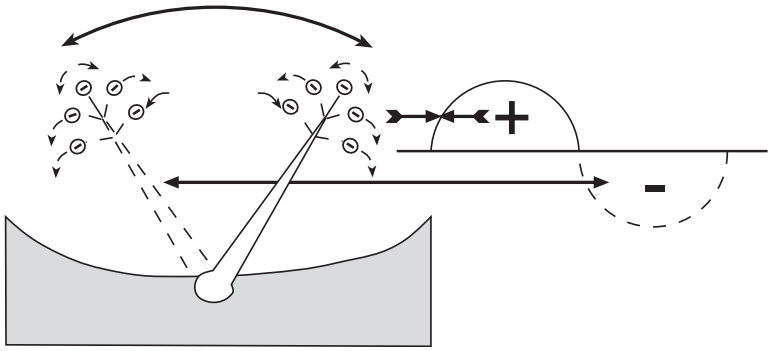


Fig. 29. Resonance of molecules with charges on a movable lever (left). Such levers can be part of a receptor and are addressed 'unspecifically' by, for example, a sinusoidal EMF wave (right).

high amplitude metabolic oscillations, this suggests a link between the signaling apparatus and metabolic changes. Furthermore, EF effects could be blocked by MPO inhibition or removal while certain EF effects were mimicked by the addition of MPO to untreated cells. Therefore, channel clustering plays an important role in EF detection and downstream responses of morphologically polarized neutrophils (Fig. 24).

(Ad 1f) Induced electric currents by MF (Faraday coupling) as active agent are discussed by Schimmelpfeng and Dertinger (1997).

For proliferation of SV40-3T3 mouse fibroblasts and human HL-60 promyelocytes they found after treatment with a sinusoidal 2 mT 50 Hz magnetic field that cell growth was only affected by an induced electric field above a threshold between 4 and 8 mVpeak/m at 2 mT.

Pilla (2007) explained in an instructive review that the ion cyclotron resonance described frequency-specific combinations of DC and AC magnetic fields, which can increase ion mobility near receptor sites and through ion channels. Although the individual influences of both AC and DC magnetic fields to ligand-receptor binding and motions of ions or other charged molecules were respected and calculated by the Lorentz force equation the main objection remained that the Brownian motion (thermal noise) will mask the additional force of a respective magnetic field.

Thus a direct energy transfer via cyclotron resonance on larger biomolecules (Karnaukhov, 1996) is considered unlikely.

(Ad 1g) This is why Liboff (2003) put the hypothesis that *ion channels in the cell membrane* would shelter the ions from the otherwise strong thermal motion outside the membrane. In this compartment external forces like cyclotron resonance would also be able to lower the signal–noise ratio (see Fig. 31). But the large radius required for cyclotron resonance and the high energies needed favor other coupling mechanisms (see below).

(Ad 1h) To overcome the problem of thermal noise, two physical models for a *direct energy coupling between* Ca^{2+} *and MF* are discussed: (1) the *ion parametric resonance model* (Lednev, 1991) and (2) the *ion interference model* (Binhi, 1997). Lednev's theory uses the phenomenon of Zeeman splitting of excited states of bound ions by a static MF (nonlinear effect). We address this model in the outline of the radical pair mechanism (see also Fig. 30). ELF EMF might modulate these states, and thus be a mechanism for MF interaction with Ca^{2+} and other cations bound to biomolecules. Binhi (1997) suggested the ion interference model, which is based on the interference of nonlinear energy states of bound Ca^{2+}. As an example, ELF EMF (10–100 Hz) delivered to human dermal fibroblasts in a collagen matrix demonstrated a 'window' behavior.

Nonlinear quantum interference effects on protein-bound substrate ions are therefore proposed. Due to EF, ions experience electric gradients as small as 0.01 mV/cm produced by polarized binding of the ligand atomic shells. Through this effect, electric gradients interfere with ion quantum states (Binhi and Goldman, 2000).

Both theories reflect the observed optimal amplitude and frequency windows of EMF action. Both models work with the cyclotron frequency, a frequency that is able to accelerate a Ca^{2+} ion moving on a curved pathway. The force, which is based

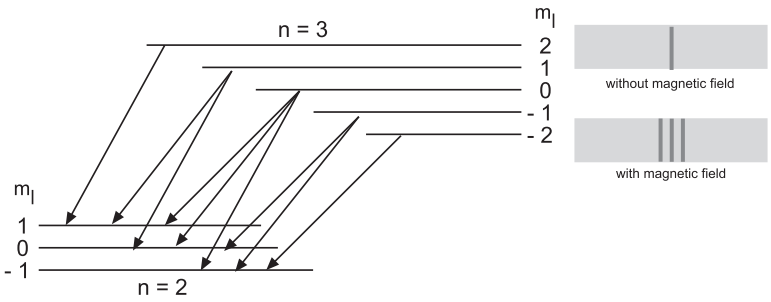


Fig. 30. Zeeman effect (“splitting”) in (e.g.) hydrogen. The spectral lines of the transition of hydrogen from $n = 3$ to 2 split into three lines (at 0) or two lines (at 1 or -1) when an external magnetic field is applied. The transitions are according to the selection rule that does not allow a change of more than one unit in the quantum number m_l.

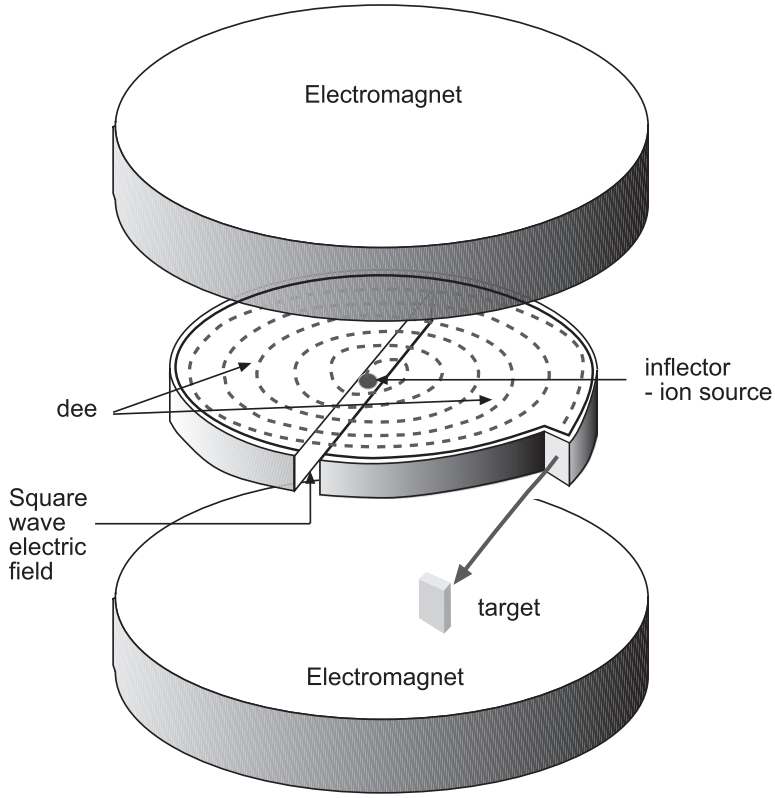


Fig. 31. The cyclotron resonance is named after an early type of particle accelerator (cyclotron, for electrons called “betatron”). The principle is that the magnetic force (induced by the two electromagnets at top and bottom) bends the moving charge between the two halves (dee). A square wave electric field accelerates the charge at each gap crossing and the charge spirals outward increasing in speed. The time to complete one orbit is the cyclotron frequency.

on the Lorentz force, tends to bend the path of a charged ion moving through a constant MF (Liboff, 1985b) (see Fig. 31). Since such charges tend to circulate, they may gain energy from an alternating field at a frequency determined by the charge/mass ratio and MF strength. For ions including Ca^{2+}, resonance occurs at

frequencies of 10–100 Hz. However, in free Ca^{2+} ions the cyclotron radius is too large and the energy needed is too high, and thus this is inadequate to explain the biological findings. Furthermore, the acceleration of the bound ion oscillating at frequencies of 10^{12} (Larmor frequency in the infrared band) cannot be affected by the negligible perturbations of the ion orbit generated by weak magnetic fields at 10^{10} lower frequencies. Therefore, the transition rate to the ground state cannot be affected by ELF EMF and thus, ion parametric resonance cannot occur (see Pilla, 2007).

(Ad 1i) Using cyclotron or quantum resonance conditions, *EMF effects on calcium binding in tissue* have been studied. One important study has shown that nifedipine, a calcium channel blocker, inhibits the EMF effect, indicating a role for calcium channels (Lisi et al., 2006).

As we have seen with DC EF, a DC 0.1 V/cm stimulus reduces hMSC differentiation into the osteogenic cell lineage by reducing the *Ca^{2+} wave frequency* (Sun et al., 2007). SMF (reactivity of ions bound in the molecular clefts of macromolecules may be affected by SMF) and PEMF (e.g. Ca^{2+}/CaM-dependent myosin phosphorylation system), as well as Ca^{2+} ions are the major target for ELF EMF. Many experiments on isolated cell systems point to Ca^{2+} as a signaling ion involved in multiple pathways. Furthermore, a number of authors propose that this ion is a supposed target of EMF during the initial step of coupling (Carson et al., 1990; Walleczek and Liburdy, 1990; Lyle et al., 1991; Walleczek and Budinger, 1992; Fitzsimmons and Baylink, 1994; Barbier et al., 1996; Golfert et al., 2001; Pessina et al., 2001).

EMF cause an increase in Ca^{2+} in many cell types (Carson et al., 1990; Lyle et al., 1991; Lindstrom et al., 1993, 1995; Fitzsimmons and Baylink, 1994; Barbier et al., 1996; Pessina et al., 2001). However, a decrease in Ca^{2+} has also been reported when metabolic activity is retarded and action potentials blocked (Cavopol et al., 1995; Rosen, 1996; Sabo et al., 2002).

EMF-stimulated calcium efflux and insulin release from isolated rabbit pancreatic islet cells was reported (Jolley et al., 1983). Calcium efflux and insulin receptor activity are increased in fibroblasts (Bourguignon et al., 1989), and cytosolic-free Ca^{2+} is increased, after exposure to EMF in HL60 cells (Carson et al., 1990). Qualitatively similar results in lymphocytes and diatom have also been observed (Lyle et al., 1991; Reese et al., 1991). EMF conditions set to the Ca^{2+} resonant frequency increased diatom motility detuning to a resonant frequency for K^+ significantly reduced this effect (McLeod et al., 1987). These studies were extended to mitogenic stimulation of lymphocytes, which showed that tuning to calcium frequencies led to enhancement on mitogenic stimulation (Liboff et al., 1987).

EMF affects calcium efflux (Bawin et al., 1975; Blackman et al., 1982, 1985b; Liburdy, 1992) and influx (Colacicco and Pilla, 1984) in numerous biological systems. In addition, EMF influences various calcium-dependent cellular responses, such as bone resorption (Cain and Luben, 1987) and collagen synthesis (Luben et al., 1982):

- The effects of the ELF magnetic fields on intracellular Ca^{2+} concentration of rat chromaffinoma PC-12 cells were examined by using a digital fluorescence image

microscopy system. The results showed that a 50 Hz, 100 pT sinusoidal ELF magnetic fields increased Ca^{2+} to a marked level. It seemed likely that the Ca^{2+} transport ability of the cell membranes was increased by the 50 Hz magnetic fields (Huang et al., 2000).

- It has already been observed that simple, chronic, ionic changes in the extracellular fluid can cause a modification in cell state function (Pilla, 1974; Becker and Pilla, 1975; Chiabrera et al., 1979, 1980). In fact, simple changes in extracellular ionic microenvironment can influence the rate of cell differentiation and even redirect its development pathway (Barth and Barth, 1969, 1974a, b).
- Transmembrane potentials, generally regarded as stationary in the past, are in fact fluctuating (oscillatory), and this dynamic property of an electric field is important in understanding its interaction with a membrane protein.

(Ad 1j) First experimental hints that *channel clustering in cell protrusions* is very important came from experiments of Kindzelskii and Petty (2005) who showed that in neutrophils this mechanism can significantly lower the signal–noise ratio (see above). At the lamellipodia of these cells, store operated Ca^{2+} channels (SOCs) are clustered and inhibition of these channels abolished the migration response of these cells. The authors argue that it seems likely that SOCs are part of a complex of plasma membrane proteins which can be affected by weak EF. In addition, several SOCs are members of the transient receptor potential-like (TRP) family of gene products. Among these proteins, TRP1 is a lipid raft-associated protein (Lockwich et al., 2000; Trevino et al., 2001) in Kindzelskii and Petty (2005) and the lamellipodia are enriched in these proteins. Blocking SOCs with Gd^{2+}, Trollinger et al. (2002) could inhibit galvanotaxis (see Kindzelskii and Petty, 2005). Kindzelskii and Petty (2005) could confirm predictions in a hypothesis of Galvanovskis and Sandblom (1997), see Kindzelskii and Petty (2005) that clusters of such proteins enhance the sensitivity for EF detection and that a discontinuous cell geometry with clustered ''receptors'' (e.g. in cell protrusions) favors EF detection whereas spherical cells with equal distribution of receptors are relative insensitive. The number of the clustered ''receptors'' can amount to 10^6 in clusters of μm size. Thus, an estimated signal/noise ratio of at least a factor of 30 can result.

An additional effect for enhancing sensitivity (already addressed in the present review) was also discussed by Kindzelskii and Petty (2005): the coherence and cooperative interaction of receptors to receptors or channels to channels (the distance of individual channels being only about 7 nm). The coupling may take place via conformational mechanisms or via other coupling (electron tunneling or other quantum effects, see below). All these mechanisms may further improve the signal amplification (Duke and Bray, 1999; Neumann et al., 2000; see Kindzelskii and Petty, 2005).

(Ad 2) Polyanions, ''electretes''

The binding of calcium or other cations is very important to special sides, as ions are not only herded in vesicles or membrane compartments (e.g. in the endoplasmic reticulum) but are also bound to polyanions, such as polyamines. Adey (2003) states

that in inward rectifier channels, highly hydrophobic negatively charged pores at the inner end of the channels attract complementary positively charged spermine and other polyamine molecules into the cytoplasmic pores. Polyamines have the highest charge/mass ratio of any biomolecule. They "herd and queue" (Adey, 2003) K^+ ions towards the transmembrane exit (Nishida and MacKinnon, 2002; Matsuda et al., 2003). Interestingly, polyamines are synthesized from ornithine in response to ELF exposure (Byus et al., 1987).

The idea of Ca^{2+} storage at the high-affinity polyanionic binding sites of actin filaments will be presented as an alternative hypothesis of Gartzke and Lange (2002) (see below, Figs. 32 and 33). Cellular sites of F-actin-based Ca^{2+} storage are located in the submembrane cytoskeleton, e.g. in the microvilli and lamellipodia, and in all cell protrusions (think to the sharp anisotropy, "nonlinearity" in the very small dimensions of cell prtrosions).

Several specific features of Ca signaling such as store-channel coupling, quantal Ca^{2+} release, spiking and oscillations, biphasic and "phasic" uptake kinetics, and induced Ca^{2+} release, which are not adequately described by the current hypotheses, are inherent properties of the F-actin system and its dynamic state of treadmilling (see Gartzke and Lange, 2002).

Because the magnetic component of EMF can intrude into the cell, *Faraday's induction law* is also applicable, as demonstrated by reorganization of the

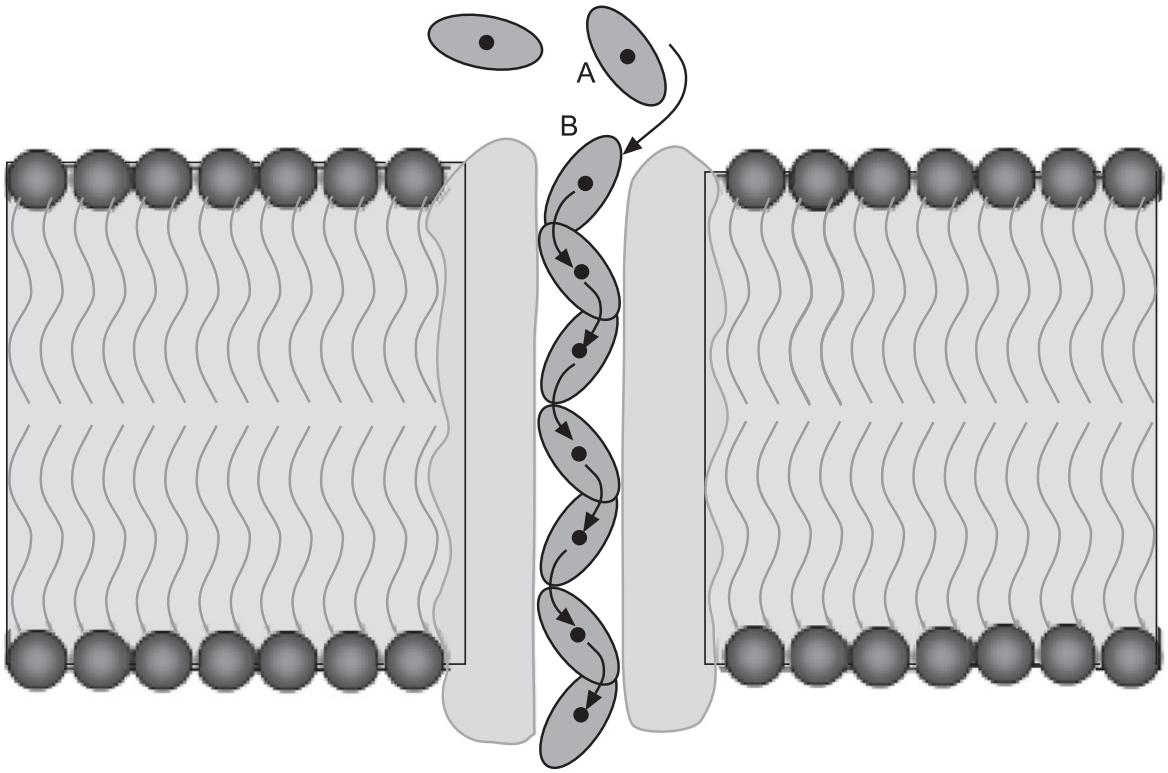


Fig. 32. Transport of ions through an ion channel in the cell membrane. The hydration state of the ions changes at the mouth of the ion channel (from A to B). Without hydration shell the charge to mass ratios will necessarily be reduced from A to B leading to lowered values of ion cyclotron resonance frequencies (after Liboff et al., 2003).

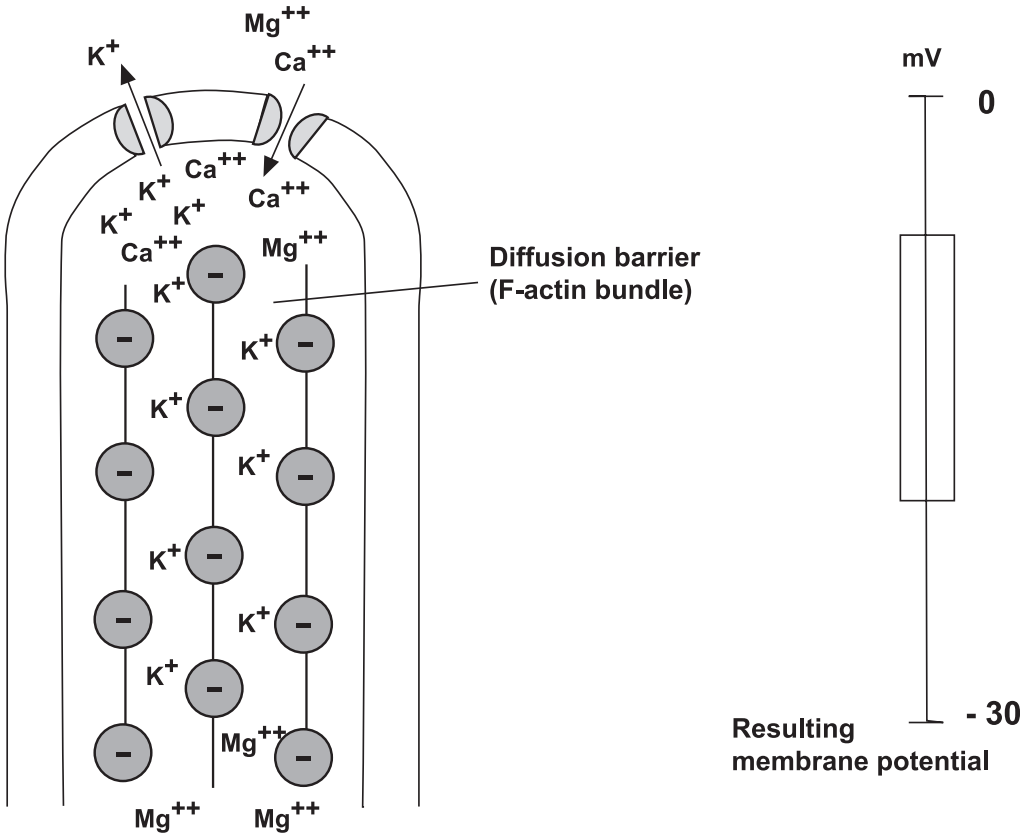


Fig. 33. Microvilli on the cell surface as sealing against Ca^{2+} influx via membrane channels and to establish the intracellular ionic conditions necessary for Ca^{2+} signaling: barrier function of the microvillar actin filament bundle by the dense polyelectrolyte matrix. Cations are in a loosely bound state and can be moved by EMF as "clouds" (modified after Gartzke and Lange, 2002).

electrostatically negative charged actin filaments. Cho et al. (1996) showed that a 1 or 10 Hz field changed microfilament structure from an aligned form to globular patches, whereas higher frequencies (20–120 Hz) had no effect. Possibly, the moment of inertia in the actin fibers could not follow the changing field at higher frequencies, whereas at low frequencies the steady distortion inhibited formation of the typical cable-like structures.

(Ad 3) Radical pair mechanism

Because of the fine architecture of molecules and charges, and their manifold interactions, Adey (1997) suggested the occurrence of far finer physical processes at the atomic level rather than chemical reactions between a "soup" of random colliding biomolecules controlled only by the laws of gross thermodynamics formulated over 100 years ago for closed systems. Physical actions of EMF may regulate the rate and the amount of products from biochemical reactions, possibly through free radical mechanisms (McLauchlan and Steiner, 1991; Till et al., 1998;

Timmel et al., 1998), including direct influences on enzyme action (Grissom, 1995). Chemical bonds are magnetic bonds formed between adjacent atoms through paired electrons having opposite spins and thus attracted magnetically. When bonds are broken in chemical reactions, each atomic partner reclaims its electron and moves away as a free radical to seek another partner with an opposite electron spin.

The pair can either recombine to form the original molecule or separate into free radicals. However, if the relative orientation of the spins is altered (singlet to triplet) the kinetics of recombination is modified. Three types of processes can alter spin orientation: (1) hyperfine coupling (linked to the magnetic environment of the pair); (2) differences in Larmor precession rates ('Δg' mechanism); and (3) crossing from one energy level to another (Zeeman effect, Fig. 30).

In the cell, the most prevailing species of free radicals are radical oxygen species (ROS and hydroxyl radicals) and radicals formed by nitric oxide (NO) (Simko, 2007). The lifetime of a free radical varies grossly: highly unstable radicals such as hydroxyl radicals are present for less than a nanosecond whereas more stable ones such as NO can have lifetimes of around 3–5 s (Simko, 2007).

Adey (2003) cites a statement of McLauchlan who points out that radical pair model predicts a potentially "enormous effect" on the rate and amount of product of chemical reactions for EMF bioeffects. The highest levels of free radical sensitivities to imposed MF may reside in spin-mixing of orbital electron spins with nuclear spins in adjacent nuclei, where potential sensitivities may exist down to zero MF levels (reviewed in Adey, 2003).

In the mentioned review of Adey (2003), Lander (1997) is cited: that "we are at an early stage of understanding free radical signal transduction. Future work may place free radical signaling beside classical intra- and intercellular messengers and uncover a woven fabric of communication that has evolved to yield exquisite specificity, but not necessarily through "lock and key" mechanisms".

The radical pair phenomenon (NO, ROS) can thus interfere with endogeneous oscillations in cell or metabolic systems. Resonance effects that greatly enhance (in phase), or diminish or extinguish (counter phase) characterize this interplay:

Rosenspire et al. (2005) have shown in experiments with neutrophils and weak magnetic pulses that it is possible to trigger, enhance and dampen endogenous metabolic oscillations, including NAD (P)H- and flavoprotein oscillations, which influence the *production rate of reactive oxygen species and nitric oxide*. They propose an electrically sensitive membrane-embedded receptor complex, such as VSP, which transduces the signal to 1–25 Hz Ca^{2+} pulses. The frequency of the calcium pulses must be compared with the fundamental 0.05 Hz metabolic oscillations. Rosenspire et al. (2005) argue that: "the intermediate metabolism of the cell functions as a biochemical bandwidth filter centered at 0.05 Hz. In this way, the 0.05-Hz electrical-pulse-frequency domain of interest is seen to arise quite naturally. Moreover, the physiological response of the cells to the pulsed fields must also depend upon the phase relationship of the pulses with the metabolic oscillation."

(Ad 4) The paramagnetic property of metal atoms/ions

Suggestive of EMF sensing, a significant increase in norepinephrine and glutamine levels was found in chicken embryos after ELF EMF exposure (Rajendra et al.,

2004). The authors propose that the enzyme dopamine beta hydroxylase (DBH) is the coupling mediator because of its two copper atoms. Copper is paramagnetic and its role in the activity of DBH may be altered by external magnetic fields. The same may be true for glutamine synthetase, which requires paramagnetic manganese for activity. The finding that ion–protein complexes can rotate under static MF supports this theory (Binhi et al., 2001).

5.6. Problem of signal-to-noise ratio

Is it too early for a hypothesis proposing general cell–cell interactions based on weak low-frequency EMF? Adey, one of the leading scientists in the EMF field, was the first to show in reviews that sensitivity to weak low-frequency EMF may be a general property of cells and tissues (Adey, 1992a, b, 1999). He pointed out that ELF EMF might be a private language of intrinsic communication by which cells may "whisper together" in activities such as metabolic cooperation and growth regulation (Adey, 2003).

This is similar to the hypothesis that we presented in the DC EF chapter. For some EF, however, the problem of timing is essential along with the magnetic components that directly influence molecular reactions. Furthermore, there is the problem of signal detection in the presence of ambient thermal noise. Given that modern cell biology has revealed intriguing topographies of molecular and charge patterns, combining this work with quantum physics should soon lead to the an explanation of low signal detection thresholds. Some theories of EMF detection (with experimental hints) arised in the last decade:

A 0.01 mV/cm extracellular EF will only generate a 10 nV perturbation in membrane potential and needs at least 100 μV to ensure a cellular response (Otter et al., 1998). In addition, a channel "noise" of 100 μV will mask the 10 nV signal. Even cellular coupling by gap junctions does not sufficiently reduce the noise to solve this problem (Spach and Heidlage, 1992) (Fig. 8).

On the other hand, tissue electric gradients in the range of 10^{-7}–10^{-1} V/cm in combination with ELF MF in the range of 1.2–10 μT, produce a spectrum of physiological and behavioral sensitivities that have been reported in a number of species (Adey, 2003). Evolution of heightened sensitivity by increasing the number coupled cells has given rise to the ampullae of Lorenzini, an extremely sensitive organ in certain fish, especially sharks. In sharks these organs can detect field gradients up to 10^{-8} V (Fields, 2007). Generally, in complex arrays of receptors the thresholds are 100 times below measurable thresholds of individual electroreceptor organs (Valberg et al., 1997). Furthermore, physiological responses are only observed within certain windows of MF parameters, i.e. at very low amplitudes (<1 G) and frequencies (8–60 Hz) (Gartzke and Lange, 2002). This frequency dependency suggests a nonlinear, i.e. discrete or quantized, physical mechanism of energy transfer.

As we have seen in the chapter DC EF an *increase in sensitivity* can be found due to a *coupling of several cells via gap junctions* and via other cell biological phenomena. A cell array model was proposed which takes these mechanisms into

account (Pilla et al., 1994a). Pilla (2007) calculated that the induced induced transmembrane voltage can be 100 times higher for a cell array model of 1 mm length (at a frequency below 100 Hz). At longer distances (at least till 1 cm) this increases further, thus the signal/noise ratio increases further, too.

The problem of thermal molecular motion, i.e. thermal noise, might generally be overcome by stochastic resonance (Kruglikov and Dertinger, 1994) or molecular 'Brownian' ratchets (Astumian, 1994, 1997). This phenomenon can amplify weak signals more than 1.000-fold by using system-inherent noise. Stochastic resonance can actually enhance the information and thus improve sensing and processing of otherwise undetectable signals – also for example in oscillations between different quantum energy levels (Gartzke and Lange, 2002; Badzey and Mohanty, 2005; see Funk and Monsees, 2006).

Brownian or thermal ratchets can bias thermal noise in one direction (rectification). This is achieved through small periodic forces that bias cytoskeletal Brownian ratchets or receptors, or other parts of the cell membrane. The main direction of the rectified sum movement will be provided by the external EMF. For example, the effect of an induced EF is reversed in the second half of the sine wave. Such rectifications can occur with the shape of the cell as a whole (Fig. 18) and with the cytoskeleton as mediators (e.g. by inserting actin fibers in one direction or by motor proteins like prestin which can give a coherent swing).

Audioreceptor cells in the ear or photoreceptor cells in the retina are examples of such swinging elements. In the ear, bundles of microvilli from hair cells can be laterally moved by applied acoustic fields. This swinging movement generates a synchronously oscillating membrane potential that changes about 10 mV around its normal value. In addition to this mechano-electrical transduction, hair cells display high sensitivity and frequency selectivity by adding self-generated mechanical energy to low-level signals. Thus, detection of signals that are much smaller than thermal molecular motion is possible (Gartzke and Lange, 2002; Chang et al., 2003). In the membranes of the hair cells also a special situation is found regarding "flexoelectricity" (see Section 3): This flexoelectricity is combined with electro-motility by a special arrangement of the connection between cytoskeleton and cell membrane (Raphael et al., 2000; Petrov, 2006). Here, the piezocoefficient (20 μm/V) is 10^7 higher that in quartz or 10^9 higher than in the cow femur (El Messiery et al., 1979; Ferrier et al., 1986; Petrov, 2006).

Coupling EMF to a biological system is a multistep procedure: (1) EMF energy is coupled to a bound cation; (2) interaction with larger biomolecules occurs; and (3) a classical signaling cascade is triggered (see below and Fig. 34).

5.7. Alternative hypothesis for EMF coupling

To close the gap between EMF coupling steps, Lange and Gartzke (2006) proposed an alternative hypothesis for calcium storage and Ca^{2+} signaling by EMF induction. This hypothesis differs mainly in the mechanism of Ca storage. Current theory rests on the assumption that Ca-accumulating endoplasmic/sarcoplasmic reticulum-derived vesicles are equipped with an ATP-dependent Ca pump and

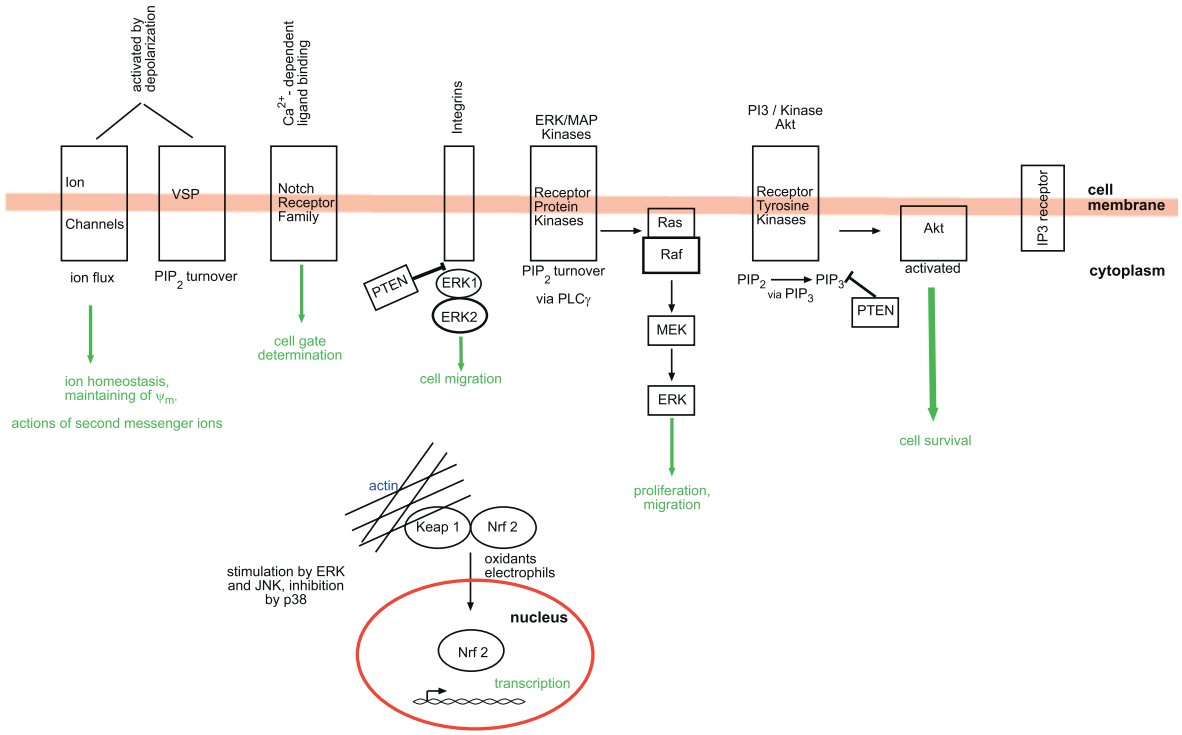


Fig. 34. Overview showing the different "receptors" of EF at the cell membrane and associated signaling pathways leading to nuclear activation (transcription) (details mentioned in the text).

IP3- or ryanodine-sensitive channel receptors for Ca release. The Gartzke and Lange hypothesis proceeds from the idea of *Ca storage at high-affinity binding sites on actin filaments*. Cellular sites of F-actin-based Ca storage are known to be microvilli and the submembrane cytoskeleton.

It is very important to keep in mind that the *dissociation energy of single bound* Ca^{2+} *in organic molecules* exceeds, by orders of magnitude, the energy level of ELF EMF (see table for binding energies). Thus, coupling with larger biomolecules cannot occur only by changing single bonds. With regard to the influence of EMF on bonding, Gartzke and Lange (2002) proposed ionic conduction along actin fibers in charged clusters or vortices formed by Ca^{2+} ion clouds (Fig. 33). Ideally, this should happen in the microvilli and cortical cytoskeleton. Each of the negatively charged actin bundles has 5–6 binding sites for cations, preferentially for divalent cations such as Ca^{2+} or Mg^{2+}. The proposed concept is appealing because actin has a central role in Ca^{2+} signaling and its polyelectrolyte nature has specific ion conduction properties.

As we cited Gartzke and Lange (2002) in our review Funk and Monsees (2006): Ca^{2+} entry from the actin core of the microvilli into the cytoplasm would be via a nonlinear cable, like cation conduction through arrays of condensed ion clouds. Consequently, free ionic movement along an EF is restricted. The transport of cations along the linear matrix of fixed negative charges requires a simultaneous (coherent) jump of counterions between all centers at the same time and in the same direction (Fig. 35). Thus, in contrast to stochastic activation of ion transfer by

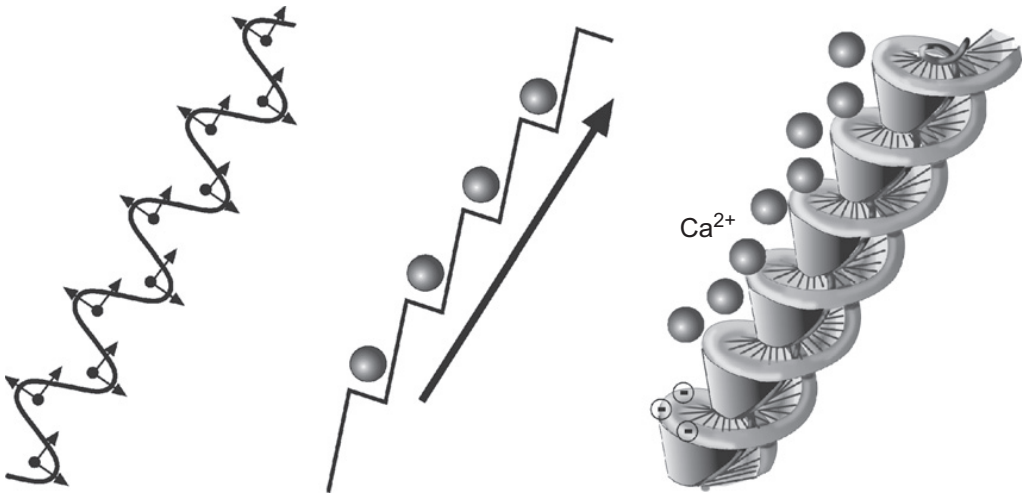


Fig. 35. Left: sinusoidal EMF wave (with vector components). Middle: principle of a molecular ratchet which is able to direct the random 'Brownian' molecular movement if a triggering vector is present. Right: model of ion clouds (Ca^{2+} ions) moving along a ratchet-like molecule (e.g., actin) and thus, leading to a polarization of charges (modified after Gartzke and Lange, 2002).

thermal effects, energy transfer from the applied EMF may result in time-, space- and vector-coherent excitation of ions within the whole conducting path. Thus, an array of coupled low-potential barriers between the charge centers efficiently discriminates thermal activation of ion conduction along the polyelectrolyte.

It is very important that the activation energy for an ion transfer between charge centers of the ion cloud is lower than or similar to the thermal energy level. Therefore, MF of similar low energy can move ions between the centers (Gartzke and Lange, 2002). The resulting influx of cations (Ca^{2+}) into the cell induces the third step, the triggering of classical signaling cascades.

Microvilli with actin bundles shielded by a lipid membrane can function like electronic integration devices for signal–noise enhancement; the influence of EMF on cation transduction is amplified, whereas that of random noise is reduced. Thus, microvilli might be some kind of antennae for EMF. In this respect, it is interesting to note that in macrophages, the formation of microvilli-like structures (podosomes) is induced at 1 Hz and a 2-V/cm field (Cho et al., 2000). Thus, one should apply this concept of coupling not only to microvilli but also to microvilli-like structures including podosomes, filopodia and nonlinearities in the cell border. On the other hand, microvilli-like structures can be damaged by EMF of the wrong frequency. PEMF at frequencies between 50 and 70 Hz and a 0.6-V/cm field cause loss of microvilli-like structures and a collapse of apical membrane in endoderm cells of the embryonic yolk sack (Zhang et al., 1997).

The charm of this system is that several specific features of Ca signaling, such as store–channel coupling, quantal Ca release, spiking and oscillations, biphasic and "phasic" uptake kinetics, and Ca-induced Ca release, are inherent properties of the F-actin system and its dynamic state of treadmilling (Lange and Gartzke, 2006). However, the general model proposed by Gartzke and Lange (2002), which states

that ionic conduction along actin fibers in charged clusters or vortices formed by Ca^{2+} ion clouds remain to be proven in living cells.

5.8. What other novel hypotheses/findings are now in discussion?

If we go down to EMF and molecular reactions (like we have seen with the radical pair mechanism), we again encounter proton- and electron-driven mechanisms:

EMF can accelerate certain dynamic chemical systems (Blank and Soo, 2001) or activities of enzymes such as cytochrome oxidase (Blank and Soo, 1998a, b, 2003) and Na^+ /K^+-ATPase (Yoda et al., 1984; Blank, 2005). For both enzymes, the frequency optimum in the response to EMF is very close to the enzyme turnover number indicating that the EMF interacts with components critical for determining reaction rates.

Recent work has pointed to coupling mechanisms via transient electrons, where measurements flickering in H-bonded molecule networks (Fecko et al., 2003) indicate that protons regularly move between oxygens, suggesting that electrons (the de Broglie wavelength of electrons is much greater and thus they can tunnel over greater distances) would do the same. Like in water, flickering protons and electrons would also be expected in hydrated and internally H-bonded proteins at a similar flicker rate (nanometers/picosecond). Furthermore, covalent bonds have been shown to be preferred paths for quantum tunneling (Wenger et al., 2005). In the model of Blank (2005), the speed of the moving charges (1000 m/s) is comparable with electron speeds in DNA (Wan et al., 1999), proposing that electrons are the moving charges affecting the rate of the enzymatic reaction.

Regarding our reflections regarding coherence and rhythm, this model also offers a rationale for why large static MF do not have an effect on enzymes like the Na^+ / K^+-ATPase, even though weak ELF EMF do. In weak ELF EMF, electrons moving at 1000 m/s could be displaced approximately 1 nm/ps (Fig. 36). This distance is smaller than the membrane thickness (approximately 10 nm) and well within a protein. In contrast, MF penetrate the protein and interact with transient electrons throughout the membrane. EF do not penetrate the protein but can change the charge distribution at the interface. Therefore, effects can only be propagated indirectly, and thus, differences between responses of membrane enzymes to MF and EF can be explained.

Thus, EMF are able to accelerate such chemical or enzymatic reactions, also via a ratchet mechanism, where the effect of an induced EF is reversed in the second half of the sine wave. This ratchet mechanism is especially important in a more complex molecular topography: an MF generates a force orthogonal to the direction of movement of the electron, and in the second half of the sine wave, the force is orthogonal in the opposite direction. However, in both halves of the sine wave, the electron has a component in the original direction. Therefore, interaction with an MF provides a ratchet mechanism that allows a process to proceed essentially in one direction only (Blank and Soo, 2001).

In these nanometer scale processes also quantum physics is very real: according to Matsuno (2001), EMF coupling may occur via interference with quantum coherence,

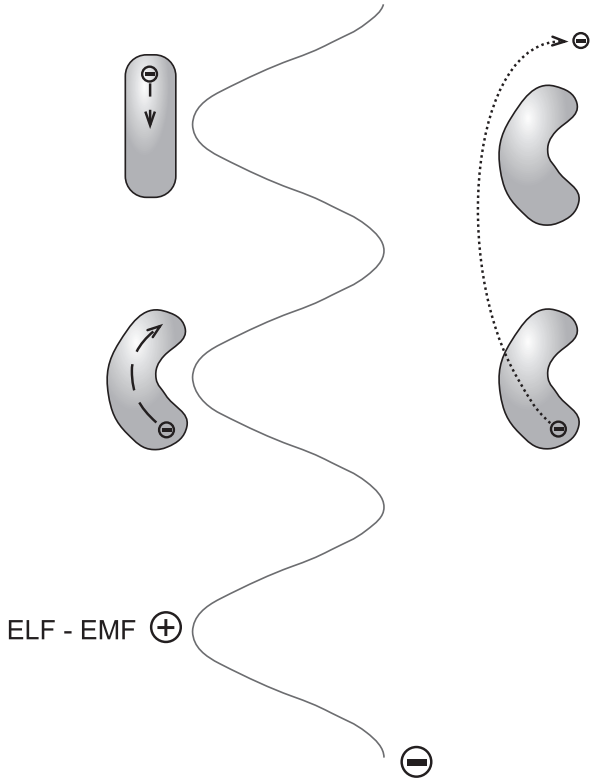


Fig. 36. In weak ELF EMF, electrons could be displaced in a distance that allows a charge shift within a protein leading to a subsequent conformational change (left). In contrast, MF would penetrate the protein and move the charge apart failing to change the conformation (right).

as an actin filament sliding on myosin molecules in the presence of ATP always exhibits magnetization as a marker of quantum processes. These processes can get a coherent "swing" from EMF applied from the outside.

5.9. Resumé of our initial hypothesis on EMF as information- and interaction medium

In the chapters above, we have seen that it is, indeed, possible that cells can inform and interact with each other via EMF. A main hint for this intrinsic property of the cell is the existence of a membrane potential per se (maintained despite this potential is a high energy demand for the cell) and that the cells can also use EMF signaling in addition to DC EF signaling – the new finding of metabolic oscillations and related alternating fields. Here, it has to be kept in mind the existence of the great differences in the membrane potential in different cell types and the astonishing low potentials in malignant cells (see above).[1]

[1]Here, also new possibilities arise for cancer research and therapies. Nordenström (1994) possibly came out too early with his electrotherapy of tumours because with the limited methods of his times a real coupling of his hypotheses to cell biology was not possible. The same was true for the early projects of Becker (1961). However, later he could tie his projects regarding bone fracture healing to the

Which EMF coupling mechanisms are realistic for physiological processes? In DC EF and in EMF the list of the coupling mechanisms can be elegantly completed by the newly discovered EF-sensitive receptors (e.g. VSP and SOCs, see above), however, the mechanisms and thresholds for the detection of the magnetic field component are less cear.

Among all the hypotheses of "magnetic interaction" the following are still in debate: (a) the Larmor precession, where the axis of vibration in bound ligands can be affected and some reorientations may cause biological effects, (b) some of the quantum processes including radical pair mechanism, (c) the hypotheses of Gartzke and Lange (2002), (d) of Blank (2005), (e) all the cooperative phenomena of modern solid state physics to be revisited by cell biology, see below) and (f) the Faraday coupling which leads back to the exciting finding of the EF-sensitive receptors.

If we look to the next step in biological response, many papers refer to EMF effects on the expression of early-induced genes such as c-myc, c-fos, c-jun and to the effects on the synthesis of various proteins: among them the tumor suppressor gene p53 (Tofani et al., 2002).

We do not want to list again all the EMF effects on cell, tissues and organs (adverse, favorable or deleterious) because there are excellent other already mentioned reviews about this topic.

To sum up the quintessence: recent developments show that electro sensitive receptors (VSP and SOCs, be it for DC EF as well as for ELF EMF, see above) can couple directly to many important common signaling pathways. However, the development of the field is still at its very beginning – so we can only show the first citations in literature. An additional drawing summarizes first results in coupling EMF to signaling cascades (Fig. 34).

However, it will be fascinating to track these hints further using the methods of modern cell and molecular biology and genetics.

6. Aspects for future research

Metabolic oscillations and concommittant, ELF EMF signals are generated by distinct molecule arrays (see above). Possibly, this is also important for *information processing* of the cell. The point is that these information "routes" would add to all other hitherto known cell biological signaling and information pathways and this would not be an alternative explanation of cell physiology.

We are still far away to understand all these endogenous processes. Not until we really know more about their spatiotemporal patterns, we can trigger deliberately these signals from outside for therapy.

However, the *translation of these molecular and cell biological findings into a multicellular system*, into tissues as well as into a whole organism is very complicated

(*footnote continued*)
piezoelectricity of bone (although it is now clear that in wet tissues also the streaming potential of ions is also important, see above).

and thus most speculative. From the clinical point of view, the problem with EMF-related complaints, e.g. is that patients report weariness, exhaustion, weakness of the immune system, etc. after a possible encounter with ''EMF''. Hopefully, the present review has shown, how complicated is the interaction of the different forms of EMF with biological tissues.

Thus, the direct assignment of actual and manifest changes possibly caused by EMF is extremely difficult – as we have seen in tissues or cells looked upon under in vitro experimental conditions. In the living body, interactions between the different levels of complexity are likely to occur and they are too manifold to be defined by simple–causal relationships. Here, we should think, e.g. to the relationship of electrostimulation of acupuncture points which showed in a controlled and randomized study significant changes in the pattern of cytokines in the peripheral blood (Jong et al., 2006).

In looking to the whole organism, a vast array of additional factors have to be considered: to name only some well-described factors like oxidative state and the state of cellular and other defence mechanisms (e.g. ELF EMF induce rapid transitory intracellular expression of heat-shock proteins that mediate a wide range of cellular stress responses, (Lin et al., 1997, 1998; Junkersdorf et al., 2000; Chater et al., 2006; Gottwald et al., 2007). Furthermore, cells and organs are normally robust against triggering by false 'technical' EMF from outside. The individual organism, however, can react differently. Thus, in rare cases, enigmatic processes can be triggered, as is seen in the 'electrosensitivity' (as ''allergy to EMF'') of patients.

Another important general aspect what we have learned is that *molecules in the cell not only represent mechanical molecular machines* working alone by their topography but are also nanodevices working by their charges (electric properties, and while moving also magnetic properties). Material science has recently recognized this potential in organic molecules and found that these molecules are comparable, e.g. to transistors in a microchip, the fields is called now 'molecular electronics' (Piva et al., 2005; Kushmerick et al., 2006). To bring only one example: a molecular memory device was found in porphyrin-based molecules (Aswal et al., 2006). This molecule works with the principle of charging and discharging into different chemically oxidized (writing) or reduced (reading out) states. The example above also minds us that we always should look for analogies found in modern nanotechnology, electronics and quantum physics and that we should revisit all our molecular and cell biological facts with this novel approach. Another example for additional properties of biomolecules is found in the interior of microtubules: they can work as electromagnetic wave guides, full of water in an organized collective state and are able to transmit information (Rosa and Faber, 2004). Other recent findings in (ELF) EMF processing remind to new findings in quantum photosynthesis (''light harvesting'') in plants. Here, an ensemble of pigments and proteins absorbs light and channels its energy and information into chemicals. Components oscillate after they get excited and the excitations are kept synchronized by specific vibrations of the protein connecting these components. By these coherent swings, the ensemble functions together as a ''supermolecule'' (Lee et al., 2007).

The detection of the ''coherent supermolecules'' (discussed above) shows that especially in the neighborhood of membranes energetic pumping (like in other *coherent systems* as in lasers) seems very probable. The electric membrane potential is one aspect of this phenomenon. Especially, Fröhlich (1968, 1969) and Smith (1988) (see Fig. 7) and others have given interesting quantum field examples of this type which appear to be suitable for an interpretation of living systems. Here, the electric dipole properties of biological molecules play the role of the quantum mechanical ordering parameter (Del Giudice et al., 1985; Del Giudice, 1986, 1988). Due to this, the arrangement of many biological important molecules can be regarded as liquid crystals with coherent polarizations ''dancing'' in the rhythm of signaling cascades or metabolic oscillations.

This quantum field approach also comes to mind if we regard the EF which are generated in an oocyte and in the subsequent cell divisions: It has been a well-known fact for a long time that quantum theory has spatially extended individuals as objects of the theory, ''in case of their division essentials are lost or changed''. Therefore, ''also the fundamental properties of biological units or even individuals can indeed understood through this theory'' (Görnitz et al., 1992; Görnitz, 1999).

Cell biology has to learn still a lot from other scientific disciplines like quantum physics and we have to look for analogies of respective phenomena and then try to find them again in the wet environment of the cell. Surely, the rapidly developing devices of nanotechnology will help us.

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