Cell Intercommunication

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PREFACE

The development of a complex organism is the result of an evolutionary scheme that starts with the synthesis of macromolecules such as DNA, the appearance of unicellular organisms, and the capacity of isolated cells to recognize each other and comunicate in such a way that tissues and organs are formed.

In this long series of events, intercellular channels represent a simple and reliable process of intercellular communication that has been preserved throughout evolution.

The role of gap junctions in electrical synchronization of excitable tissue, growth, differentiation, and cancer is presented in this volume with the purpose of informing the reader and providing the young investigator with clear perspectives for future work.

The molecular organization of gap junctions is also discussed, and this offers an opportunity for close inspection of molecular mechanisms involved in regulation of junctional permeability.

Some Newtonian models of cell communication are presented, and the influence of toxicological factors on junctional communication is reviewed.

I hope this volume will be of help to those interested in the process of cell communication and its implications in cell biology, physiology, pharmacology, and oncology. I want to thank the colleagues who joined me in this project and the staff of CRC Press for their help in the preparation of this book.

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EDITOR

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THE WAY CELLS COMMUNICATE

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I. CELL COMMUNICATION — AN EVOLUTIONARY VIEW

"Whenever we look, we find evolution, diversification, and instabilities."

Jlya Prigogine
Nobel Laureate

Corliss,[1] based on extensive submarine research, suggests that life originated in shallow Archean seas in consequence of the eruption of magma through cracks on the earth's crust. According to this idea submarine hot springs provided the conditions for the generation of life about 3.6 billion years ago.

The biological systems are situated above the physicochemical/matter-energy systems and below the sociocultural systems which together form a theoretical pyramid used by Laszlo[2] to describe the realms of evolution.

It has been suggested that biological evolution occurred by symbiosis of prokaryotic cells into one another to form eukaryotic cells. Margoulis envisages that the first eukaryotic cell was generated when a microbe capable only of anaerobic fermentation of glucose to pyruvate established a symbiotic association with an anaerobic prokaryotic cell which must have had the ability to produce cytochromes and to oxidize all foodstuffs into CO_2.[3]

Although no precise information about when this phenomenon occurred is available, it is probable that the Middle-Late Precambrian Boundary marks the appearance of eukaryotic cells.

The formation of colonies of amoebae is probably one of the oldest examples of intercellular communication. It is known, for instance, that isolated cells can keep growing and dividing as long as bacteria present in the extracellular medium are available as food. Under these conditions the self-sufficient cell seems to be an independent unit of life with no apparent need for interacting with other cells.

A substantial decline in the number of bacteria triggers a process characterized by the generation of pulses of cAMP by some cells followed by interaction with cAMP receptors in cells located far away. The wave of cAMP released into the extracellular space leads to activation of cAMP receptors and consequent movement of these cells towards the cAMP pulsing units.[4-6] In this way an aggregation territory is established. This is an example of how fluctuations in the systems elicited by instability in food supply are able to generate organization.[7]

This primitive example of intercellular communication seems to be exclusively related to the exchange of chemical messages through the extracellular medium, since no intercellular junctions have been found in *Dictyostelium discoideum*.[8]

How did the evolution of biological systems generate eukaryotic cells? According to the theory of punctuated equilibria,[9] evolution occurs when the dominant population within a group of species presenting a similar adaptative plan reaches an unstable state. At this juncture, if instability is strong enough, the dominant species is replaced by a peripheral species.

This theory proposes that evolution concerns the survival of an entire species rather than individuals, as originally proposed by Darwin. The destabilization of a previously dominant community of algae (prokaryotic cells) by the appearance of eukaryotic cells created the opportunity for the generation of more prokaryotes and the consequent

generation of more specialized eukaryotes. This process is relatively rapid and leads to the phenomenon of *speciation*, or the emergence of new species.[2] The aggregation of eukaryotic cells seems to create the necessity of a more elaborated process of intercellular communication. Here is probably when the intimate contact between cells led to the appearance of intercellular junctions.

Mesozoa, which are more primitive than sponges, Cnidaria, and flatworms, are one of the oldest organisms in which gap junctions can be indentified.[10] In *Ascaris lumbricoides* the primitive giant somatic muscle cells are electrically coupled and healing-over has been described.[11,12]

Of particular interest in the evaluation of evolutionary trends of cell communication is membrane excitability, an essential acquisition which permitted the cell to respond to adequate environmental stimuli with appropriate changes. This basic property was certainly developed before cell communication. Indeed, isolated cells already present this important characteristic. Even in epithelia electrical excitability is observed. In hydromedusae epithelia, for instance, electrical responses that propagate at a velocity of 30 cm/sec can be identified.[13] In siphonores the conduction velocity of electrical pulses in epithelia (50 cm/sec) is similar to that recorded in nerve rings of medusae and the refractory period is identical to that described in vertebrate nerve fibers.[13]

The two basic processes that are essential requirements for intercellular communication — membrane excitability and the ability of cells to generate and receive chemical messages — are preserved throughout the evolution of biological systems.

It is interesting to note that primitive neurons were initially neurosecretory or growth regulatory cells, and only later did their elongated axons become effective cables with the ability to propagate electrical impulses with a high conduction velocity.[14] This stage of the evolutionary process indicates that chemical responsiveness was not totally differentiated. As epithelia are able to respond to specific stimuli with the generation of propagated electrical impulses, what is the reason for the development of nerve cells?

A clear advantage of nerve cells with long axons is that the electrical impulse can be propagated in one specific direction, whereas in epithelia the spread of electrical impulses through gap junctions occurs in all directions simultaneously.

Moreover, it is important to recognize that the further establishment of chemical synapses between the nerve cells made possible the establishment of pathways through which the electrical impulse can be propagated in just one direction.

On analyzing the whole process of evolution it became clear that life is continually searching for novel structures and functions. In the case of intercellular communication, the appearance of specific pathways of communication between cells provided the conditions for the development of complex behavior.

Biological systems are open systems, exchanging energy or matter with their environment; therefore, generation of change is an important characteristic of these systems. The unidirectionality of chemical synapses as well as the synthesis of inhibitory neurotransmitters at nerve endings represented an additional step in the evolutionary process of intercellular pathways. In the human brain, for instance, millions of neurons are activated through specific pathways in a fifth of a second. Here chemical and electrical synapses contribute to brain function, establishing the most complex system of intercellular communication known.

Although the conduction of electrical messages through gap junctions is certainly

more economical because it does not require the complex machinery involved in the synthesis and storage of neurotransmitters, the generation of chemical synapses represents an example of how evolution risks the stability of simple components in order to get more highly developed control systems.[2]

It is recognized today that junctional permeability can be modulated by intracellular factors.[15,16] Therefore, it is reasonable to think that preferential pathways of communication can be achieved in systems containing only gap junctions if the concentration of intracellular regulators or the intensity of extracellular influences, as well as the density of receptors, varies in different areas of the tissue.

Although gap junctions were retained in the evolutionary process as a reliable mechanism of cell communication, evolution generates diversity and it is not surprising that significant variations in the molecular organization of the junctions can be detected among different species. Evidence is indeed available that gap junctional proteins are not the same in different tissues,[17,18] emphasizing again the plasticity of the evolutionary process.

During embryologic development gap junctions are widely distributed. Sheridan[19] demonstrated that the injection of dyes into cells of chick embryos is followed by spread of the dye over large areas. Although neural tube cells are communicated, glial cells and neurons are not coupled at the adult stage.

These findings indicate that embryology occasionally uses some simple and reliable processes of cell communication achieved by evolution, but that more sophisticated control systems are added or preferred when the adult stage is reached.

Studies on the proliferation of neural retinal cells of chick embryo, for instance,[20] showed that the number of gap junctions reaches a maximum just before cessation of cell proliferation. This has been interpreted as evidence that a "factor" diffuses from cell to cell, stopping cell proliferation.[20]

In the embryo of the locust, development is characterized by the appearance of intercellular junctions. The gap junctions are expressed transiently between undifferentiated cells, disappearing later on when the cells begin to cluster and establishing the visual units called ommatidia.[21]

In amphibians the gap junctions are probably important during myogenesis because they make possible the spread of electrical current and consequent muscle activation even before innervation is accomplished.[22,23] One must recognize, however, that more studies are required to clarify the role of gap junctions on differentiation. Lawrence and Green[24] showed, for instance, that insect epidermic cells located in adjacent segments involved in different developmental pathways are coupled to one another and that cells inside the segments are also electrically communicated. These findings are not expected if gap junctions are believed to be involved in differentiation.

The development of gap junctions, which seems related to the necessity of cells to share metabolites and electrical information, is completely suppressed in some cells. One reasonable explanation is that cell differentiation provides in some cases a certain degree of metabolic autosufficiency that enables the cells to survive isolated from their neighbors. In skeletal muscle, for instance, the fibers are completely isolated from each other, making possible the establishment of functional units composed of a few fibers and their respective axons. The number of functional units activated varies with the needs of the organism. This particular arrangement is required for the maintenance of posture that requires a constant state of contraction of extensor muscle of the legs, a

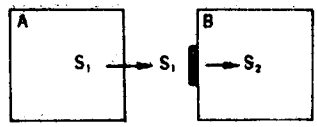

FIGURE 1. Model of cell communication in which cell A releases a chemical signal that produces a change in cell B through receptor (dotted area in B) interaction.

process that is achieved by rotating the number of functional units and consequently avoiding fatigue.

In this particular situation the presence of intercellular junctions between skeletal muscle cells would be incompatible with the organization of isolated functional units as mentioned above. Indeed, the spread of electrical current between the muscle cells would force the whole muscle to respond to a stimulus provided by just one of the axons involved in the innervation of the muscle. This organization contrasts with heart muscle in which intercellular communication is essential for electrical synchronization and development of strong contractions of the ventricular muscle.

Current knowledge of the intricacies of the evolutionary trends of cell communication is limited. Future studies on the comparative biochemistry and physiology of intercellular junctions will certainly provide a better understanding of this important topic.

II. MODELS OF INTERCELLULAR COMMUNICATION

A simple model of cell-to-cell communication is represented by two adjacent cells separated by extracellular fluid. A chemical signal released by cell A can induce changes in cell B if this cell is provided with specific receptors at the level of the surface cell membrane (Figure 1). This is precisely the situation that prevails when colonies of amoebae *(Dictyostelium discoideum)* are organized.[6]

It is quite possible that the establishment of chemical synapses between neurons represents a highly elaborated scheme of the simple process of cell communication of amoebae described above. Indeed, the addition of "autopoietic" function (from the Greek for "self-creating"[28]) to the system make possible the continuous renewal of chemical transmitters and synaptic vesicles that are essential for this type of intercellular communication.

Cross-regulation release of two neurotransmitters such as acetylcholine and vasointestinal polypeptide (VIP) has been described in cerebral cortex and rat submandibular gland[26] and represents another example of how evolution utilizes self-regulatory or autocatalytic processes.[7] As discussed above, this type of mechanism of cell communication was necessary for the establishment of pathways of communication between millions of neurons in the central nervous system of vertebrates.

The price for this complex mechanism is, as expected, high. A major dysfunction in any of the stages of the process leads to severe consequences such as Parkinson's disease or myasthenia gravis. On the other hand, the establishment of gap junctions between apposing cells makes possible the quick spread of electrical current in heart

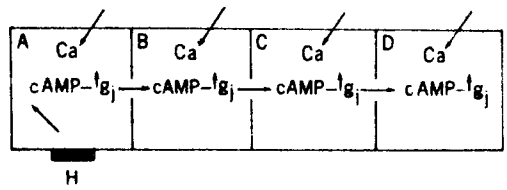

FIGURE 2. In this model of cell communication only cell A presents receptor (dotted area) to a hormone (H). The synthesis of a second messenger (cAMP) in A is followed by increase in junctional conductance (g_j) and diffusion of the nucleotide to cell B in which g_j is also increased. Diffusion of cAMP to cells C and D extends the cascade reaction and makes it possible for cells without receptor to respond to hormonal action. In all cells inward Ca current through surface cell membrane is produced by cAMP.

muscle, dentritic trees, septate axons, epithelia, uterine muscle, and other types of smooth muscle.

Adaptative changes in junctional conductance are probably involved in the maintenance of different values of membrane potential inside the same cell population. In heart, for instance, the membrane potential in pacemaker cells of the sinoatrial node is about 60 mV, whereas in the right atrium values of 75 to 80 mV are found. Although there is a gradation between these values at the border zone, the cells located at the boundary between the two tissues still present a clear difference in their membrane potential. In this particular case, if the junctional conductance prevailing in the pacemaker cells is similar to that found in large Purkinje fibers of the ventricle, for instance, the spread of electrotonic current between pacemaker and atrial cells would increase the membrane potential of pacemaker cells, impairing the effectiveness of these cells to pace the heart.

Evidence exists, however, that the gap junctions between pacemaker cells in the mammalian heart are small in diameter and their number appreciably smaller than that found in atrium or ventricle.[27] These findings might indicate that the maintenance of different values of membrane potential at the boundary between sinoatrial node and right atrium is in part explained by a control of junctional communication in this area.

Certainly other factors, such as the effectiveness of Na/K pump and the different ionic permeability of the nonjunctional cell membrane in pacemaker and atrial cells, contribute to the preservation of the difference between their membrane potentials.

An important model of cell communication is represented by cells coupled through gap junctions, but only a few contain specific receptors in the nonjunctional cell membrane to neurotransmitters (Figure 2). The interaction of the transmitter molecules with the receptor (epinephrine, for instance) increases the intracellular concentration of cAMP through the activation of adenylate cyclase. In some systems the increase of the intracellular levels of cAMP leads to phosphorylation of junctional proteins and a quick increase in junctional conductance (g_j).[16,28] Cyclic AMP not only increases g_j but diffuses easily to nearby cells through gap junctions.[29] If the concentration of cAMP diffusing into the nearby cell devoid of adrenergic receptor is enough to counterbalance the effect of protein kinase inhibitor[16] then the level of phosphorylation of junctional proteins will be increased and g_j enhanced in cell B. The area involved in this cascade

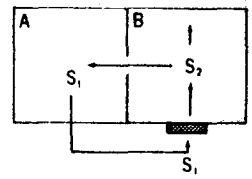

FIGURE 3. Model of intercellular communication showing two cells coupled through a gap junction. Cell A releases a signal (S_1) that activates a receptor in cell B causing the synthesis of an essential messenger (S_2) in this cell. The diffusion of S_2 to cell A might release or inhibit the synthesis of S_1.

reaction will depend on the amount of cAMP that diffuses into cell C or D. According to this model a group of cells without adrenergic receptors on their surfaces will respond to the neurotransmitter indirectly.

The consequences of this model are not limited to the diffusion of cAMP and increase in g_j but extend to the nonjunctional cell membrane of the cells devoid of receptors. In these cells the nucleotide will also increase the current that flows through the voltage-dependent Ca channels in nonjunctional membranes and will influence their excitability or contractility.

This model might be of importance under certain conditions in which the establishment of membrane receptors is suppressed by disease or downregulation. Experimental evidence that cAMP modulates g_j is presented below.

Figure 3 shows a variant of the previous model. Here two cells coupled through gap junctions are interacting in a different way. Cell A releases a signal that activates a receptor located at the surface of cell B and causes the formation of a second messenger inside cell B. This messenger, or some molecules generated by its presence, will diffuse to cell A and will be used appropriately. The molecule S2 might enhance the release of S1 or inhibit its synthesis. If the formation of S1 is increased, the intercellular communication will be greatly increased through an autocatalytic process. If S2 inhibits the synthesis or release of S1 the process will be interrupted and the system will return to its previous steady state. This model will be of value when cell A needs S2 and is not able to synthesize it.

According to this model a biochemical heterogeneity is generated within the system in which some cells play the role of suppliers and others of receivers. In such a system the interruption of junctional communication might represent serious physiological consequences for cell A due to lack of S2, as well as for cell B that is also dependent on S1 for the activation of the receptor and the synthesis of S2.

Electrotonic junctions are frequently observed to occur immediately adjacent to chemical synapses. The meaning of this combination of electrical and chemical junctions is not known.

A possible consequence of this occurrence is presented in a model (Figure 4). The depolarization of the postsynaptic membrane caused by the release of neurotransmitter spreads out back into the presynaptic cell modulating or enhancing the release of the transmitter. A change of less than 1 mV in membrane potential of the presynaptic neuron is enough to modify the synaptic transmission.[40]

This type of feedback between pre- and postsynaptic cells might represent a self-

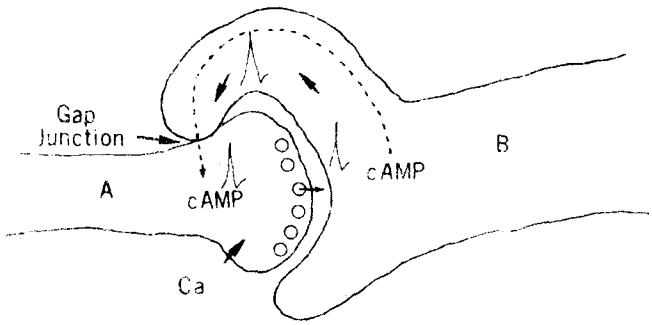

FIGURE 4. Model of cell communication showing modulation of transmitter release (cell A) caused by spread of depolarizing current or cAMP diffusion back into the presynaptic ending through gap junction.

sustaining mechanism of neurotransmitter release — a kind of electric clock.

Moreover, the presence of intercellular channels between the two cells makes possible the flow of chemical information between them. A reasonable possibility is that the generation of cAMP inside the postsynaptic cell diffuses into the presynaptic terminal enhancing the inward Ca current through the surface membrane and consequently increasing the transmitter release.

The coexistence of gap junctions and chemical synapses between neurons might be also involved in a type of negative feedback as presented in Figure 5. According to this model the postsynaptic cell is connected to an intermediary neuron through gap junctions. The establishment of an inhibitory chemical synapse between the presynaptic cell and the intermediary neuron leads to hyperpolarization of the presynaptic neuron every time the postsynaptic cell is excited.

III. JUNCTIONAL PERMEABILITY REGULATION

A central question in gap junction physiology is the meaning of junctional permeability regulation.

The answer to this question is certainly related to the physiological characteristics prevailing in each tissue. The regulation of junctional conductance in cardiac muscle or other excitable tissues, for instance, is supposed to have a different meaning than that of salivary glands or pancreatic cells.

This focuses our attention on a strategic point: the understanding of the physiological meaning of intercellular communication depends on the good use of an intercellular approach and not exclusively on the junction ultrastructure or molecular properties of junctional proteins.

Let us take the cardiac muscle as an example. This is an excitable tissue in which the electrical coupling between the cells is fundamental for the spread of electrical impulses and consequent synchronization of the electrical firing of many cardiac cells. As each cell represents a contractile unit it is easy to visualize that electrical synchronization is required for mechanical synchronization also.

This means that myocardial cells must be always electrically connected in order to

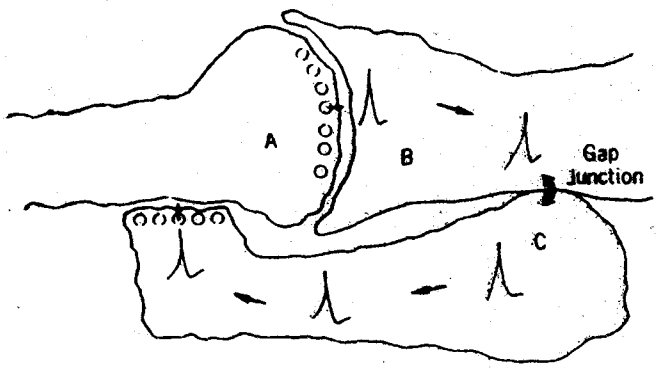

FIGURE 5 In this model of cell communication the postsynaptic cell (B) is connected to an intermediary neuron (C) through gap junctions. The establishment of an inhibitory chemical synapse between the presynaptic cell (A) and the interneuron leads to hyperpolarization of the presynaptic ending any time the postsynaptic cell is excited.

provide the physiological conditions for the heart muscle. In other words, cell decoupling in cardiac muscle is not related to physiological regulation of heart function; on the contrary, cell decoupling is synonymous with heart pathology.

The finding that the intracellular Ca injection leads to cell decoupling in heart[31] as well as in Chironomus salivary gland[32] represents in itself evidence that junctional conductance can be suppressed by an important intracellular factor. Again when the physiological meaning of this finding in heart, for instance, is analyzed, the conclusion is that such an event is not expected to occur under physiological conditions. However, the finding that Ca is a good cell decoupler has pathological significance because during myocardial damage the inward movement of Ca markedly enhances the junctional resistance isolating the normal from the lesioned cells (healing-over) and consequently avoids depolarization of normal tissue.[33,34]

The free $(Ca^{2+})_i$ required to suppress the electrical coupling has been estimated in Chironomus salivary gland and found to be 5 to 8×10^{-5} *M*.[35]

In cardiac muscle the intracellular free $(Ca^{2+})_i$ needed to supress the electrical coupling was reported to be the same or higher than that required to activate contraction.[36] More recently elegant studies made in isolated cell pairs indicated, however, that 0.2 μM of Ca is enough to abolish the cell-to-cell coupling in heart.[37] As stressed before,[38] the hypothesis that Ca is a physiological modulator of g_j cannot be tested on the basis of Ca concentration necessary to abolish the electrical coupling. It is reasonable to think that cell decoupling in many tissues has no physiological significance at all.

The answer to this question can only be achieved if the minimum Ca concentration needed to change g_j over a physiological range is determined, and that this is indeed a normal occurrence is confirmed.

This task is particularly difficult because (1) the compartmentalization of the cytosol imposes uncertainty on the interpretation of measurements of free $(Ca^{2+})_i$, since