Using Gap Junctions as Basic Building Blocks

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Abstract

Intercellular communication occurs through exchange of small molecules such as ions, second messengers and small metabolites. This exchange is carried out by clusters of intercellular membrane channels called gap junctions. Structurally, these channels are composed of two hemichannels, or connexons, which span the plasma membranes of two adjacent cells and interact to form a complete gap junctional channel, thus linking the cytoplasm of one cell to another. We discuss some of the relevant properties of connexins and suggest the use of gap junction channels in nanotechnology and nanoscale circuits.

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1 Introduction

Cells communicate with each other in a variety of ways. One such method of communication involves the inter–cellular transmission of molecules through channels in a specialized structure in the cell membrane, the gap junction. Gap junctions provide a regulated pathway linking the cytoplasms of attached cells, and contribute towards ensuring the integration of metabolic activities by setting up networks of directly communicating cell assemblies.

Gap junctions exist between two channels. They allow the movement of small molecules such as Ca^{2+} , cyclic AMP and inositol triphosphate between cells by passive diffusion. but preventing the movement of molecules larger than 1000 Da such as proteins and nucleic acids [11]. Although not showing the selectivity of other ion channels, gap junctions composed of different connexins have been shown to have significant differences in permeability [5].

Co–ordination of cellular activity by gap junctions occurs through a variety of mechanisms which can be classified as follows: speed, synchrony, switching, symbiosis and stimulus/suppression. In the heart, for example, gap junctions permit the rapid cell–to–cell transfer of action potentials, ensuring coordinated contraction of cardiac muscle cells [1]. In the nervous system, gap junctions allow changes in electric potential to be transmitted from one cell to its neighbors [14]. This is significant because electrical synapses function in neuronal pathways requiring maximum speed, synchronous firing of neurons and rapid switching between neuronal pathways. In non–excitable cells, such as those of the eye, gap junctions allow symbiotic exchanges between highly differentiated, functionally compromised cells and more active, renewable cells. Gap junctions can also act to suppress mutations in key metabolic and signaling enzymes by delivering junction–permeable intermediaries which will allow for the survival of mutant cells with blocked enzymes. This may be the basis of the role of gap junctions as tumor suppressors.

1.1 Nomenclature of gap junctions

Connexins are a multi-gene family of proteins, currently believed to have over 20 isoforms. The isoforms have approximately 40% overall sequence identity, with much greater identity in the transmembrane and extracellular domains. Some isoforms are restricted to specific species and others exhibit tissue-specificity. Most cells express more than one isoform. These different isoforms comprise channels with widely varying unitary conductances, permeabilities, voltage sensitivities and sensitivity to modulatory agents [8].

In invertebrates, gap junctions are formed by a family of proteins known as innexins [12]. Although their gene structure is very different from that of connexins, they remarkably exhibit similar channel properties in terms of regulation by pH, voltage and lipophiles and in terms of permeability.

Two major alternative nomenclatures have been proposed for connexin proteins. The most widely used is based on the molecular mass of the polypeptide

Greek N.	Molecular N.	Mass (kDa)	Organs with Expression
$\alpha 1$	Cx43	43.0	Heart
$\alpha 2$	Cx38	37.8	Embryo
$\alpha 3$	Cx46	46.0	Lens
$\alpha 4$	Cx37	37.6	Lung
$\alpha 5$	Cx40	40.4	Lung
$\alpha 6$	Cx45	45.7	Heart
$\alpha 7$	Cx33	32.9	Testis
$\alpha 8$	Cx50	49.6	Lens
$\beta 1$	Cx32	32.0	Liver
$\beta 2$	Cx26	26.5	Liver
$\beta 3$	Cx31	31.0	Skin
$\beta 4$	Cx31.1	31.1	Skin
$\beta 5$	Cx30.3	30.3	Skin

Table 1: Connexin Multigene Family [11]

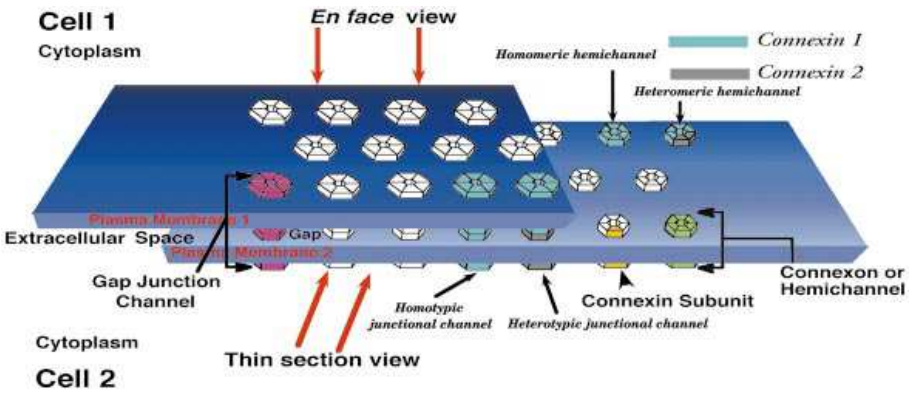

Figure 1: Section view of gap junction channels [8]

while the other used extensively in gene nomenclature aligns the connexins into 3–4 groups (2–8) based on sequence similarity.

1.2 Gap junction structure

Gap junction channels are composed of membrane proteins called connexins. These proteins are organized into hexameric structures known as connexons. An individual connexon on one cell docks with a corresponding connexon on a neighboring cell to form a complete gap junctional channel. Multiple channels then aggregate to form gap junctional plaques. Refer fig 1.

Each connexin has four predominantly hydrophobic, membrane–spanning regions (M1–M4) as shown in fig 2. For Cx43 these domains have been shown to

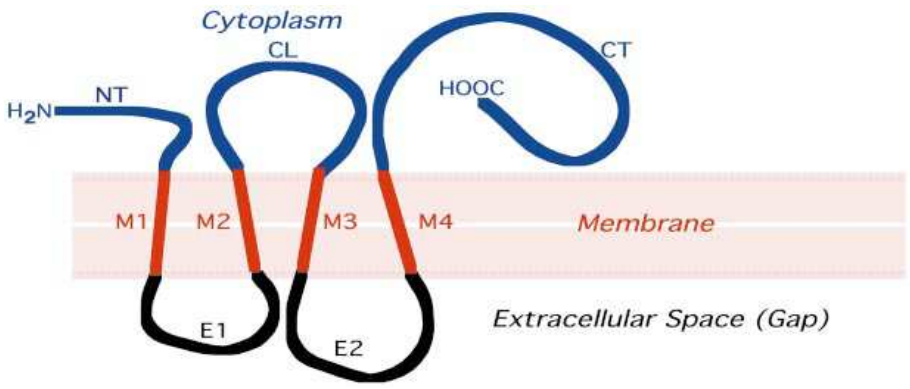

Figure 2: Membrane topology of a connexin monomer [8]

be α–helical. By analogy, the corresponding domains in the other connexins are presumed to be α–helical as well. The carboxy– and amino– terminal domains (CT and NT, respectively) are accessible from the cytoplasm, as is the hydrophilic cytoplasmic loop (CL) domain between M2 and M3. The hydrophilic domains between M1 and M2 and between M3 and M4 form two extracellular loops, E1 and E2. The length of the CT varies among connexins and is responsible for most of the differences in molecular mass [8].

The three–dimensional structure of a recombinant gap junction has been determined at a resolution of 7.5 A° in the membrane plane and 21 A° in the perpendicular direction as in Fig 3. The outer diameter of the connexon is $\sim$70 A° and narrows at the extracellular domain to about 50 A° . The channel pore narrows from an apparent $\sim$40 A° diameter at the cytoplasmic end to $\sim$15 A° at the extracellular side of the bilayer and then widens to $\sim$25 A° in the extracellular region. Since amino acid side chains were not visualized at a resolution of 7.5 A° , the functional diameter of the pore is expected to be only 5 A° .

The α–helical transmembrane domains pack in a left–handed bundle, but a single right–handed packing interaction is observed for one of the two possible helix pairs that line the pore. Two of the transmembrane α–helices are strongly tilted and one of these is positioned directly next to the pore. This tilting causes one of the other three helices to be exposed to the channel, creating a criss–cross arrangement of two of the four helices. In addition, computer modeling studies have shown that the formation of an intercellular channel requires a 30° rotation between hemichannels for proper docking.(See [19] and [4]).

The different connexin isoforms may associate with each other in many different combinations. The simplest type of hemichannel is *homotypic*, where the entire connexon is composed of a single connexin. Two such connexons will form a *homotypic* channel. A *heteromeric* connexon is composed of more than one connexin isoform, and two such connexons will dock to form a *heterotypic* channel. Also, two homomeric channels of different connexins form heterotypic channels. See table 1.2 and table 3.

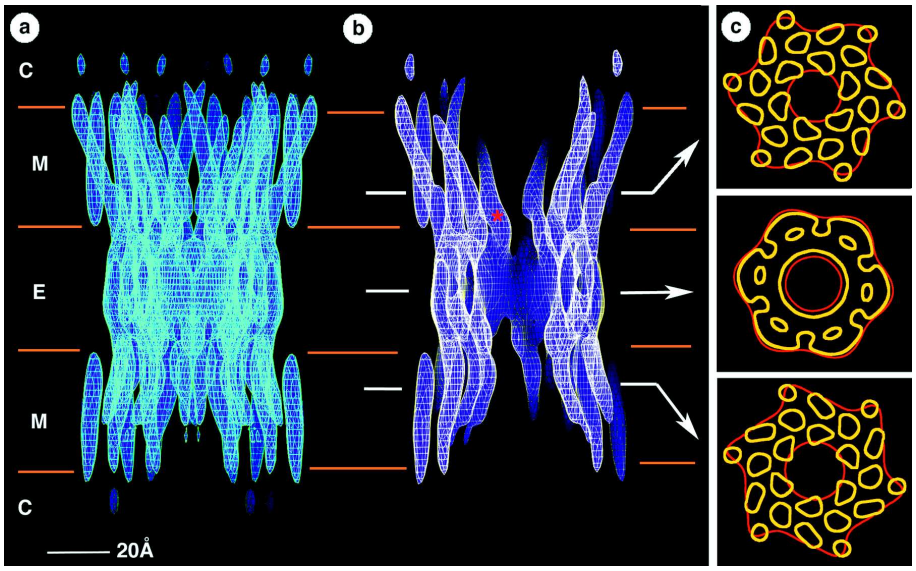

Figure 3: 3 dimensional structure of gap junctions [19]

Table 2: Heterotypic functional compatibilities in mammalian connexins [8]

26	30	30.3	31	31.1	32	33	36	37	40	43	45	46	50	57	
+	+	-	-	-	+		-	-	-	-	-	+	+	-	26
	+	+			+		-	+	+	+	+			-	30
		+	-	-	-		-	+	+	+	-			+	30.3
			+	-	-			-	-	-	-			-	31
				-	-			-	-	-	-			-	31.1
					+	-	-	-	-	-	-	+	+	-	32
						-		-		-					33
							+	-	-	-	-		-		36
								+	+	+	+			+	37
									+	+	+	-	-	-	40
										+	+	+	-	+	43
											+			-	45
												+	+	-	46
													+	-	50
														+	57

Legend: +: forms heterotypic channels, -: does not form heterotypic channels, and blank: not reported.

Group A	Group B
Cx30.3	Cx26
Cx37	Cx32
Cx40	Cx46
Cx43	Cx50
Cx45	
Cx57	

Table 3: Heterotypic functional compatibility groups [8]

2 Properties

Gap junctions exhibit a variety of properties. We will discuss those which might allow the use of gap junctions as basic building blocks for communications and circuits.

2.1 Calcium Wave Propagation and Intercellular Communication

Calcium signals provide a mechanism for the integration and transmission of of information. For example, the astrocytes, neurons and glia of the central nervous system exhibit Ca^{2+} responses in response to neurotransmitters such as acetylcholine, noradrenaline, glutamate and adenosine nucleotides. These can travel as intercellular Ca^{2+} waves over several hundred micrometers and may thus provide a basis for a long–range signaling pathway [17]. In many systems, these waves propagate through gap–junctional channels although propagation through extracellular routes have also been reported.

Gap junction channels allow the diffusion of signaling molecules from cell to cell, thus sustaining the propagating event. These molecules are now generally believed to be inositol triphosphate (IP_3) or Ca^{2+} , although cyclic ADP ribose and other intercellular molecules are within the size limits for passage through gap junctionas and might also trigger regenerative Ca^{2+} mobilization [2]. The neurotransmitters (or agonists) would bind to receptors on the cell membrane, causing activation of the enzyme phospholipase C β. The subsequent rise in IP_3 levels within the cell causes release of Ca^{2+} from intracellular stores (Refer Fig 4). IP_3 can also be generated in downstream cells by PLC activation, thus forming a regenerative step in the propagation mechanism.

Thus intercellular communication can be achieved by controlling the levels of the agonist (activator) which ultimately influences the concentration of Ca^{2+} in the neighboring cells. This behavior is also dependent upon the relative receptor density. It is seen that saturating doses of varying agonists evoke responses of different magnitudes, which may be attributed to differences in overall density and distribution of the receptors for these agonists. In the single–cell model of Hofer et al [17], the Ca^{2+} amplitudes computed for different relative receptor densities

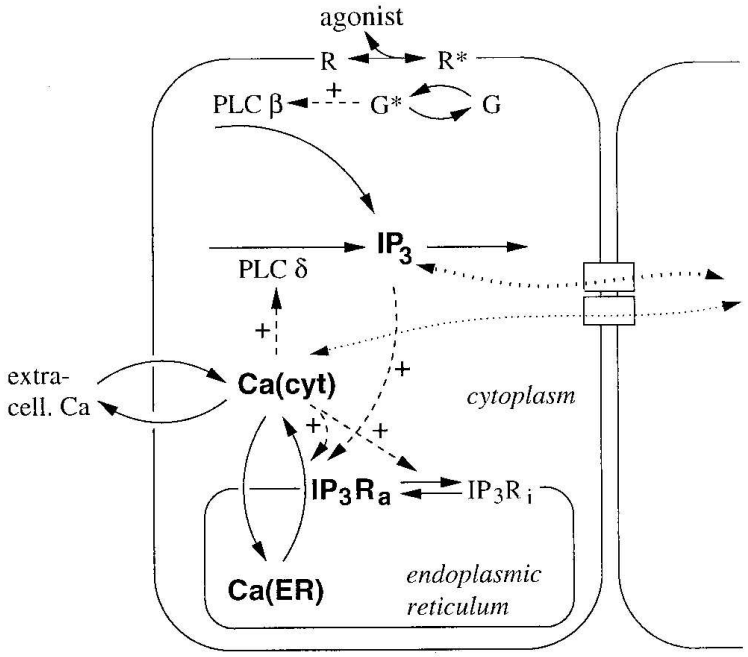

Figure 4: Scheme of the Ca^{2+} and IP_3 dynamics [17]

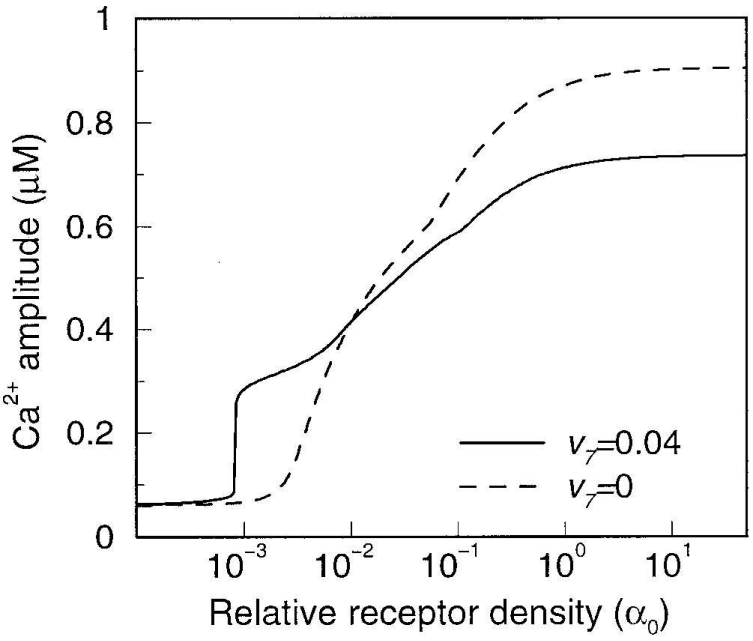

Figure 5: Agonist response in the model of a single cell [17]

reproduce this behavior, as in Fig 5. Similar ideas are also discussed in [13].

Fig 6 depicts the intercellular Ca^{2+} waves. These Ca^{2+} waves could be used to form patterns as in [9] and in Amorphous Computing [6], the goal of the project being able to engineer prespecified, coherent behavior from the co–operation of vast numbers of unreliable parts that are interconnected in unknown, irregular, and time–varying ways.

In [15], intra–cellular genetic circuits are used for cellular communications. Genetic circuits were engineered with the ability to send a controlled signal from one cell, diffuse that signal through the intercellular medium, receive that signal within a second cell, and activate a remote transcriptional response. In contrast to this work, Ca^{2+} wave propagation through gap junctions can be used, which uses non–genetic cellular processes as those described earlier to achieve similar communication. A possible benefit of this method could be that Ca^{2+} wave propagation happens within an order of seconds, whereas genetic circuits might take an order of minutes to react.

2.2 Gating Mechanisms

Gating of a gap junction channel refers to the phenomenon of the opening or closing of the channel due to some stimulus. There are at least three different gating mechanisms associated with gap junction channels—a docking gate, a chemical gate, and a voltage–sensitive gate. [7]

Although the characteristics of voltage–dependent closure of gap junctions have been well studied, the molecular mechanisms involved and even the precise physiological role remain unclear. Channel closure in response to V_j (a voltage difference between neighbouring cells) may uncouple a dying cell from its neighbors. Furthermore, mutations that affect voltage gating of Cx32 are linked to a neurodegenerative disease and a form of congenital deafness. Knowledge of gating mechanisms is important for identification and classification of connexin proteins as well as in the study of connexin structure and interactions [7].

Connexin channels have several voltage–sensitive processes. The most prominent and well–characterized is the sensitivity to transjunctional voltage (V_j) as shown in fig 7. The conductance is typically maximal at $V_j = 0$ and relaxes symmetrically and slowly (over hundreds of milliseconds to seconds) for hyperpolarizations and depolarizations of either cell [8]. This symmetry is the result of gap junctional channels being composed of two mirror–image hemichannels that are identical in homotypic channels (upper Z traces in fig 7 but different in heterotypic channels (lower trace in fig 7).

In [3], it was found that the Cx43 cell–cell channel when closed to the residual state, while still showing conductance, were not permeable to monovalent positively and negatively charged dyes, which are readily permeable through the fully open channel. These data indicate that a narrowing of the channel pore accompanies gating to the residual state. Consequently, the fast V_j–sensitive gating mechanism can serve as a selectivity filter, which allows electrical coupling but limits

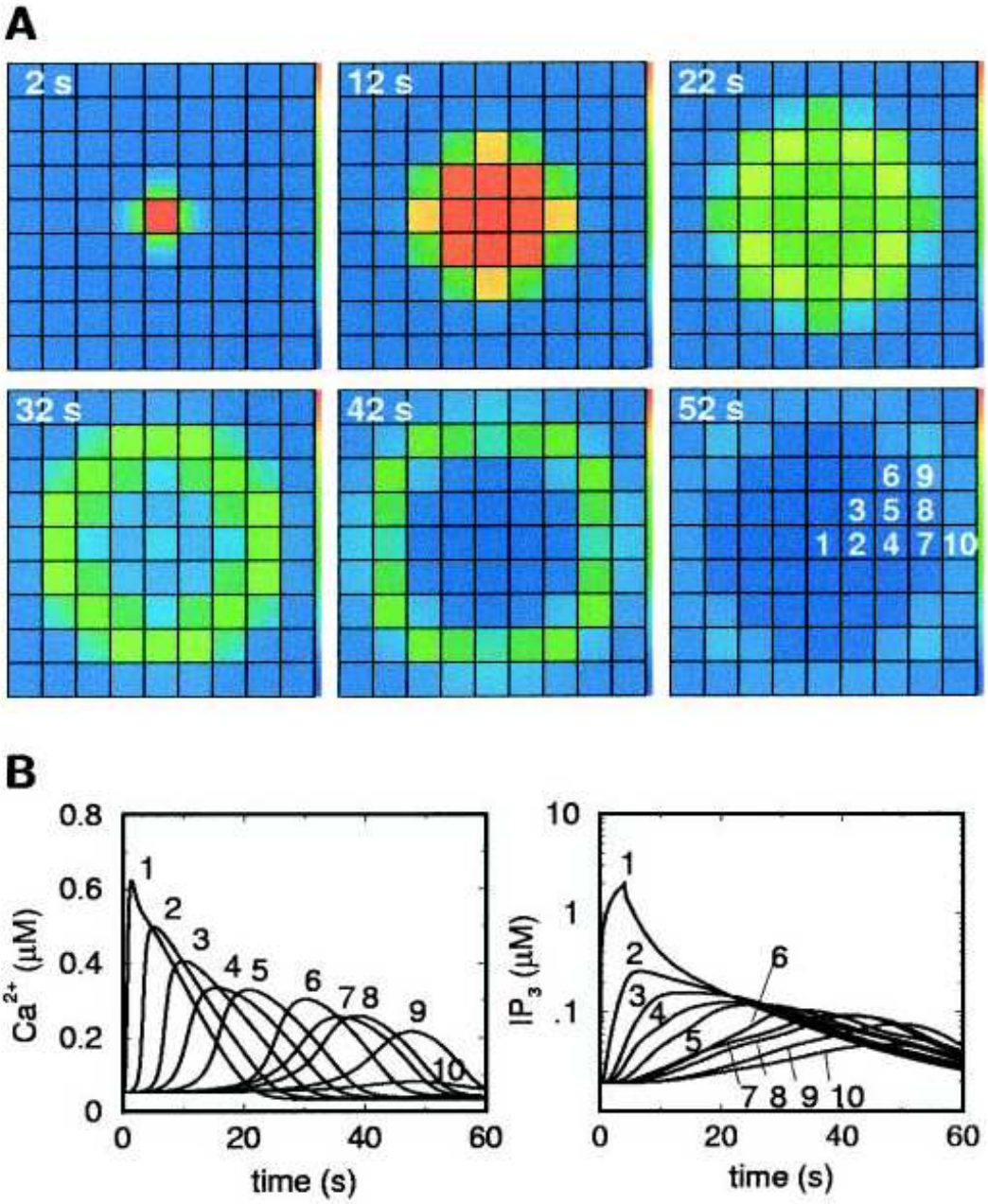

Figure 6: A: Model for agonist evoked intercellular Ca^{2+} waves. The average wave speed in the model for signal propagation through the first three rows of cells is in the same range as the speed computed from experimental data. B: Time courses of $[Ca^{2+}]$ and IP_3 in the cells as labelled in the last frame [17]

metabolic communication.

V_j–gating (fig 8 (A,B,C,D))involves rapid voltage–sensitive transitions between a primary open state and one or more subconductance states, but not to a fully closed state hence explaining the residual condition seen in each of the traces in fig 7 and fig 8. In addition to V_j–gating, a second gating process dependent on junctional voltage is seen in single–channel currents. Unlike V_j–gating, it involves slow transitions into and out of a fully closed state. This has been termed *loop gating* [8] as it is thought to represent the same gating that occurs when the extracellular loops of apposed connexons interact when the Z hemichannels dock to form the complete channel.

Several connexin mutants with altered voltage sensitivity show a "reversed" response to V_j in that junctional currents activate rather than inactivate when V_j is applied. This "reverse gating" phenomenon was first reported for Cx26 mutants lacking a conserved proline in M2 [7]. The proline is thought to play an essential role in the transduction of a conformational change essential for voltage gating. Amino acid substitutions at a number of sites in M1 of Cx32 influence gating in a similar way and some of these have been associated with disease phenotypes.

2.3 Rectification

The functional diversity of gap junction intercellular channels arising from the large number of connexin isoforms is significantly increased by heterotypic and heteromeric interactions between members of this family. In [18], a model of electrodiffusion of ions is developed, which predicts that asymmetries in the conductance/permeability properties of hemichannels (refer to fig 7) will produce either an accumulation or a depletion of ions within a channel, depending on voltage polarity, that will result in rectification. Such a property could be exploited while constructing nanoscale circuits.

2.4 Cell–Free Synthesis and Assembly of Gap Junctions

One of the reasons why gap junctions could be attractive in nanotechnology is that it is possible to synthesize and assemble them *in vitro*. In [10], synthesis and assembly of a gap junction connexon was done in microsomal membranes. These were reconstituted into synthetic lipid bilayers. Single channel conductances recorded from these lipid bilayers were similar to those measured for these connexons produced *in vivo*. Further results on synthesis and assembly of connexins *in vitro* into homomeric and heteromeric functional gap junction hemichannels can be found in [16]. However, yields of connexin proteins from *in vitro* assembly are currently low, being about 15%. Also, it is important to note that only hemichannels were assembled, and making gap junctional channels would be significantly more complex.

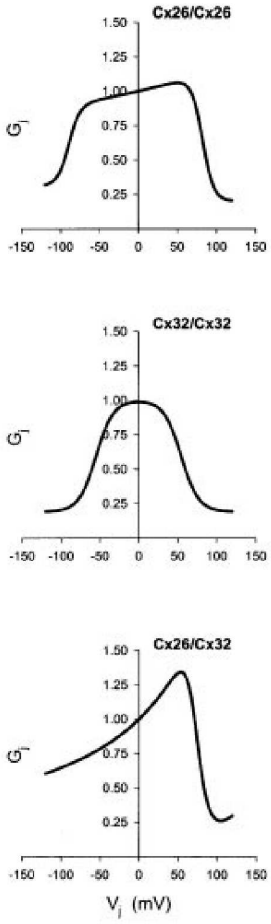

Figure 7: Macroscopic steady state G–V relations for homomeric Cx26, homomeric Cx32 and heterotypic Cx26/Cx32 junctional channels expressed in oocytes [8]

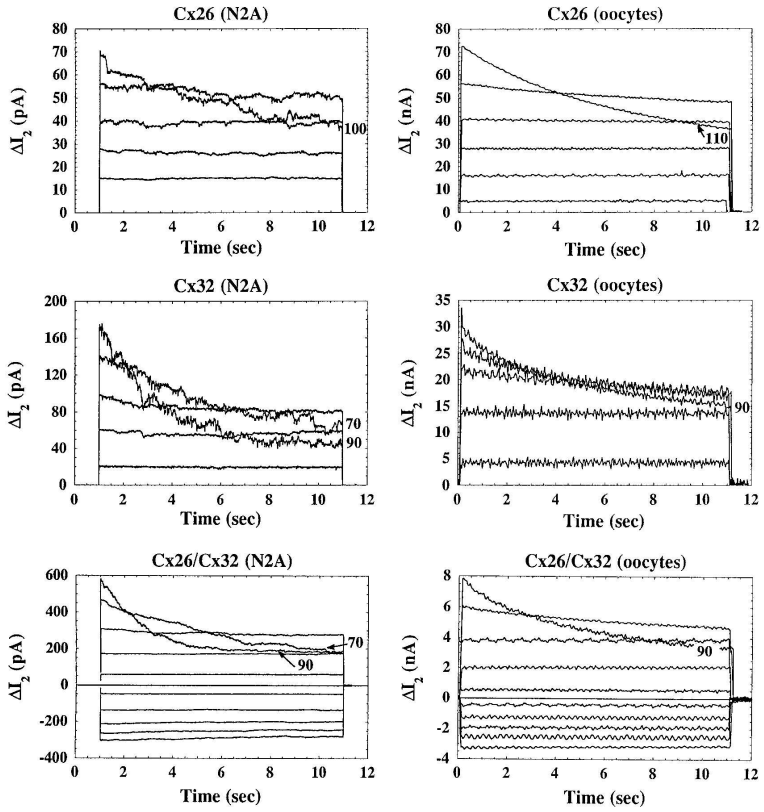

Figure 8: A, B, C and D show voltage gating in homotypic Cx26 and Cx32 in paired N2A cells and Xenopus oocytes. E and F show show voltage gating for the heterotypic channel, and show time– and V_j–dependent decay only for positive V_j steps. [8]

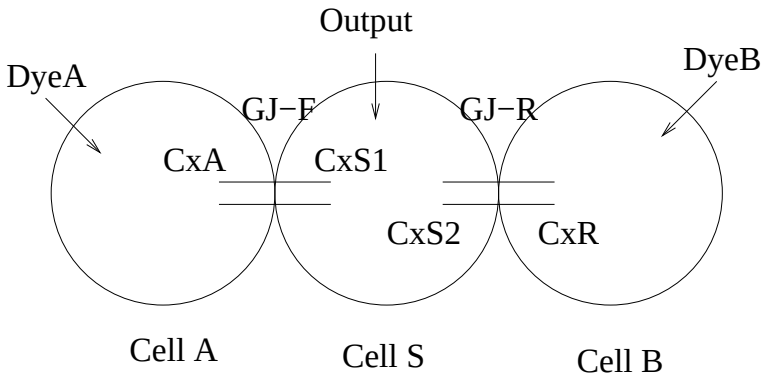

Figure 9: Experimental setup of the 1–bit multiplexer

3 Constructing a 1–bit cellular multiplexer

Different connexins can be made to interact in different ways within the cellular environment to produce a combinatorial connexin library. Table 1.2 gives a connexin interaction matrix.

We will now propose a thought experiment to construct a 1–bit multiplexer using gap junction channels, as shown in fig 9. Our setup consists of 3 cells C_A, C_S and C_B. We will call cell C_S the select cell and the cells C_A and C_B the input cells. The output of the multiplexer will also be in C_S. C_S expresses 2 connexins, one showing normal gating behavior and one showing reverse gating behavior. We will label the connexin with normal gating behaviors as CxA, CxS1, CxS2 and the one with the reverse gating behavior as CxR. C_A expresses a connexin CxA such that it docks only with CxS1 and not with CxS2. C_B expresses a connexin CxR such that it docks only with CxS2 and not with CxS1. A desirable property for CxS1 and CxS2 would be that they form distinct plaques on the cell surface. The gap junction between C_A and C_S is GJ_F and the gap junction between C_S and C_B is GJ_R. The input at C_A is specified by a certain dye dye_A and that at C_B is specified by a dye dye_B.

In the resting state, GJ_F is open, and there is transfer of dye dye_A from C_A to C_S. When C_S is raised to a higher potential as compared to C_A and C_B, GJ_F begins to close. On the other hand GJ_R begins to open and transfer of dye dye_B from C_B to C_S should be observed.

Hence, by setting the state of C_S, we have been able to successively select dye transfer from C_A or C_B, and thereby demonstrated the working of a 1–bit cellular multiplexer.

Selective formation of heterotypic channels in mammalian connexins is documented in Table 1.2. Therefore it is theoretically possible to select a combination of connexins that displays suitable experimental behavior. For example, connexins 26 and 43 do not interact, and are known to form distinct and separate plaques when co–expressed in the same cell. Furthermore, reverse gating behavior has been

observed in proline mutants of Cx26 and Cx32 mutants where the mutation occurs in the M1 domain of the protein. These reverse gaters are closed in a resting state, and open upon the application of current, this behavior being the exact opposite of that displayed by wild–type connexins. In our thought experiment, the select cell C_S would express both Cx43 and Cx26. Cell C_A would express Cx45, which interacts with Cx43 but not 26, while cell C_B would express a reverse–gating mutant of Cx26. These conditions should satisfy the parameters outlined initially. Based on observed heterotypic interactions, connexins can be classified into two groups that preferentially interact among themselves, as in Table 3. Such behavior can be used to generate a combinatorial connexin library as mentioned earlier.

However this design of a 1–bit multiplexer exhibits some limitations. One problem is that the C_S also acts as the output cell, and hence the output has to be measured in C_S. An extra cell could be attached for measuring output, but would make the experiment complicated. Also, when the state is changed more than once, both the dyes from the input cells will be present in C_S, and one needs a mechanism to eliminate this interference.

4 Conclusion

Hence, we have discussed various properties of gap junctions that make them suitable as basic building blocks for circuits and for use in nanotechnology. However many practical and technical issues need to be resolved before experimental realization of the aforementioned ideas is possible. Nonetheless the potential application of gap junctions in these areas remains a novel and interesting idea.

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