

Gap Junctions for Engineers: *A review*

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I. BASIC CONCEPTS

A. Cellular Elements

Cells, embedded in the extracellular matrix, are the basic units of living tissues. Although each particular tissue has its characteristic cells it is possible to identify a basic set of elements that are common to every cell.

A generalized animal cell can be divided in the following main parts:

Plasma (cell) membrane: it is the outer limiting membrane that separates the intracellular space of the extracellular material and external environment.

Cytosol: by cytoplasm we designate all the material enclosed between the plasma membrane and the nucleus. The cytosol is the fluid portion of the cytoplasm, i.e., the intracellular fluid. The cytosol contains ions, small molecules, soluble enzymes and proteins and nutrients. Organelles and inclusions are also suspended in the cytosol.

Organelles: highly organized structures with characteristic shapes that are highly specialized for specific cellular activities.

Inclusions: temporary structure that contain secretions and storage products of the cell.

One important cellular element from the structural point of view is the **cytoskeleton**. It is a complex network of protein filaments allowing the cell to maintain its shape and to perform a variety of coordinated cellular movements. There are three types of filaments that are specialized in particular structures of the cytoskeleton: *microfilaments*, *microtubules* and *intermediate filaments*.

B. The Intracellular and Extracellular Spaces

Water is one of the main components of body. This water is located in two compartments: intracellular and extracellular spaces. The intracellular space comprises approximately 55% of the total water.

The intracellular liquid (ICL) and extracellular liquid (ECL) have different composition as shown in the table below reproduced from [Matthews, 1994]:

	ICL (mM)	ECL (mM)
K⁺	125	5
Na⁺	12	120
Cl⁻	5	125
A^{-1.223 (1)}	108	0
H₂O	55000	55000

Table 1: ICL and ECL composition after [Matthews, 1994]

The extracellular space contains the *extracellular matrix* (EM). The EM is made up of two differentiated parts:

- **Fundamental substance** (or *amorphous substance*): the main constituents are proteins and polysaccharides secreted locally by the cells. Dissolved ions are also present. Glycosaminoglycans (GAG) are a family of non branched polysaccharides that give the EM its characteristic jelly consistence. The specific GAG involved in the extracellular space of a given tissue depends on the tissue itself.
- **Protein Fibers**: which can be of two types depending whether they are *filamentous* (collagen, elastine filaments) or *adhesive* (fibronectine, laminine).

The jelly consistence of the amorphous substance allows easy diffusion of nutrients, metabolites and hormones arriving from blood to the cells present in tissues. The filaments give resistance and elasticity to the tissue while adherent proteins have a cohesive function.

C. Cell Junctions in Tissues

Most of the cells in multicellular organisms are grouped together in cooperative assemblies called tissues. The mechanisms that allow this assemblies to work properly as a functional unit or regulate the interaction between the ICL and ECL are called *junctions*. On the other hand EM surrounds each cell giving structural cohesion to the tissue.

We can differentiate two types of junctions: *cell-cell junctions* (CC) and *cell-matrix junctions* (CM) depending on the two elements being involved. Having this ideas in mind, it is possible to establish the following functional classification of cell junctions [Alberts *et al*, 1993]:

¹ Organic anions.

1. Occluding Junctions (tight junctions)

They are typical of intestinal epithelium and clearly (hermetically) divide the tissue in two functional domains (the apical and basolateral surfaces).

2. Anchoring Junctions**2.a. actin filament attachment sites**

- adhesion belts (CC)
- focal contacts (CM)

2.b. intermediate filament attachment sites

- desmosomes (CC)
- hemidesmosomes (CM)

3. Communicating Junctions

- gap junctions
 - chemical synapses (no physical contact)
 - plasmodesmata (plants only)
-

From the above classification one is acquainted with the diversity of possible cell junctions. On the other hand, it is clear that only gap junctions provide a mean for intercellular communication (physical contact) in animal cells. This special communicating mechanism is the one that we are to review in this report.

II. GAP JUNCTIONS AND INTERCELLULAR COMMUNICATION

It is worthy to devote our attention to gap junctions because it is one of the most widespread communicating mechanism and can be found in large number in almost all animal tissues and practically all animal species [Alberts *et al*, 1993]

A. Brief Historical Background

The first demonstration of this type of cell-cell communication comes from the field of physiology and was carried out in 1958. However, it took almost 10 years before this physiological coupling was correlated to the presence of gap junctions seen in electron microscope.

The first studies were performed in nerve cells of crayfish. It was noted that by applying an electrode in each one of a couple of interacting cells and applying a voltage step, an unexpectedly large current flowed between them. It was a clear demonstration the inorganic ions present in the ICL could freely go from one cytoplasmic region to the other.

Later results showed that small fluorescent dye molecules² injected into one cell could likewise pass to the other cell without leaking into the extracellular space provided that their molecular weight were less than 1kD³.

B. The Structure of Gap Junctions

Gap junctions are formed by a couple of hexameric arrangements of integral proteins⁴ that are known as **connexons**. These arrangements are located in each one of the interacting cells and join each other in the extracellular space. This tubular arrangement leaves an aqueous channel in its interior that connects the cytoplasmic regions of adjacent cells maintaining a relative small separating *gap* that justifies the name of this junction. The width of the gap is 2-4 nm.

Connexons are not disperse within the cell. They are usually grouped in clusters (maculae) with a large number of connexons which are rich in cholesterol [Goodenough *et al*, 1996]. In Fig. 1 we show typical dimensions within a gap junction cluster.

Each connexon is made up of six proteins of a multigene family and they receive the global name of **connexins**. Connexins are responsible for the different properties of gap junctions present in different tissues. However, it is possible to identify a certain common behavior of connexins. In order to understand this point it will be useful to have a look to the constitution of a general connexin.

² The most popular is Lucifer Yellow, molecular weight 443 D [Goodenough *et al*, 1996].

³ Dalton (D). Unit of molecular weight approximately equivalent to the mass of a hydrogen atom.

⁴ *Integral protein*: a protein is said to be integral when it has a transmembrane domain.

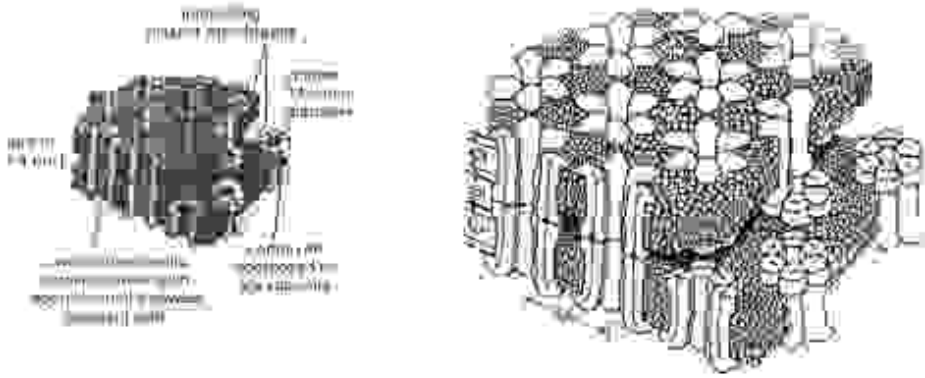


Fig. 1: Two schematic models of gap junctions. A. After [Alberts et al,1993]. B. After [Goodenough et al, 1996].

All connexins have the common structure shown in Fig. 2. They are composed of amino cytoplasmic terminal (NH_2 -), four membrane-spanning regions (M1-M4), a cytoplasmic loop (CL), two extracellular domains (E1-E2) and a carboxyl cytoplasmic terminal (COOH -). The N-terminal, M1-M4 and E1-E2 domains are well conserved between different connexins. On the other hand, the C-terminal and CL domains are quite divergent.

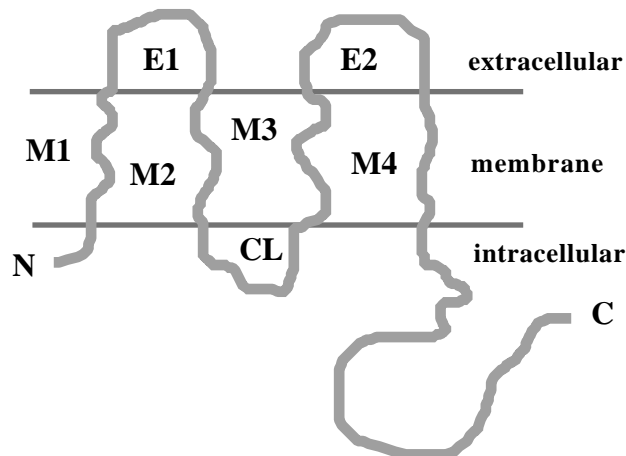


Fig. 2: Connexin structure.

C. Connexins

As we told before, a *connexin*, is the constitutive element of a connexon. Different connexins are observed in different tissues and confer particular characteristics to them.

Table 2 summarizes the most important connexins and the tissues or cells in which they were observed. The table also shows the relationship between different connexins in the form of a dendrogram that represents the primary sequence identity⁵.

⁵ A percentage related to the number of equal aminoacids in the protein chain.

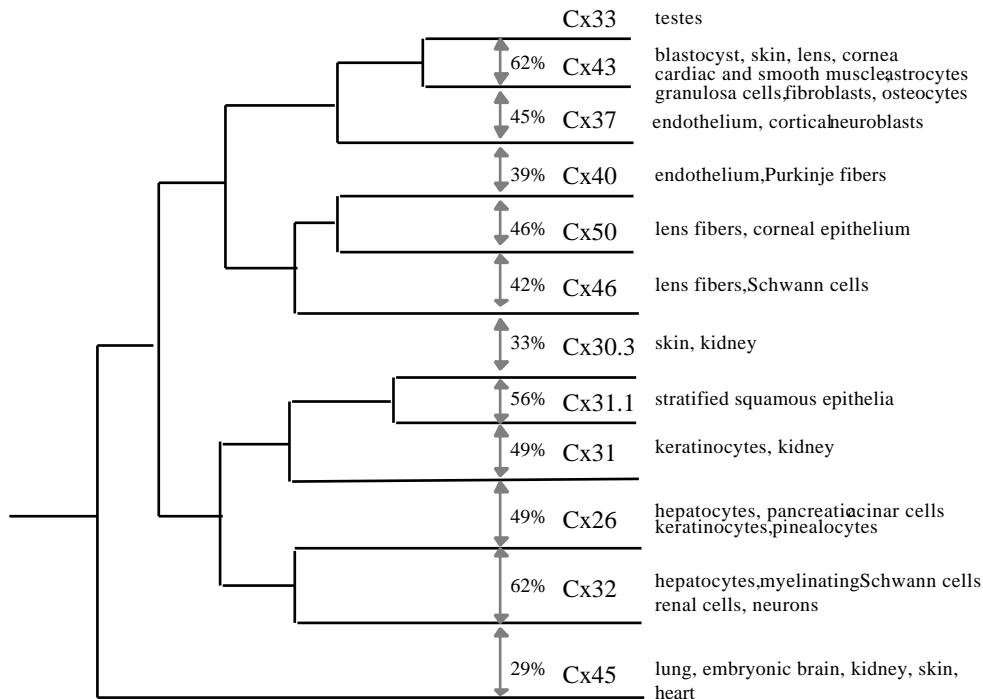


Table 2: Relationship between connexin family members and their location in tissues and cells. After [Goodenough *et al*, 1996]

In order to name different connexins a systematic rule is applied. The connexins are named by the species in which they occur and the predicted formula weight to the nearest kilodalton [Bennett *et al*, 1991].

D. Physiology of Gap Junctions

Gap junctions play an important role in cell communication and signaling. We can classify their functions depending on the excitability of the cells involved in the junction.

Excitable Cells

- Provide a faster mechanism for propagating action potentials than the chemical synapses. Chemical synapses have a typical delay of 0.5 ms while electrical synapses are considered to be instantaneous.
- Facilitates synchronous contraction of cells in muscular tissue by making up a functional syncytium.

Non Excitable Cells

- The intercellular connection provides a means of buffering system that smoothes fluctuations in ions and metabolites concentration in the ICL.
- Facilitates coordinated movements, e.g., cilia movement in epithelial tissue.
- In embryonic development provides positional information depending on the concentration of certain substances.

E. Gating of Gap Junctions

Once that we have summarize the functions of gap junction in intercellular communication we briefly review the gating mechanism that leads to the opening and closing states.

In Fig. 3 and Fig. 4 two models of the gating mechanism of gap junctions are presented. The basic idea is that the connexins that make up the connexon can undergo conformational changes leading to the opening or closing of the gap junction channel. A conformational change is a change in the stereochemistry of a molecule. In the model of Fig. 3 it is shown that the difference between open and closed states is a rotation of the connexins that reduces the effective channel width. In Fig. 4 a more complete view of the mechanism is presented. From this figure it can be seen that the M3 domains of each connexin line the interior part of the channel. By tilting of the connexin the channel becomes narrower and the channel closes.



Fig. 3: Simple gating model. After [Alberts *et al*, 1993].

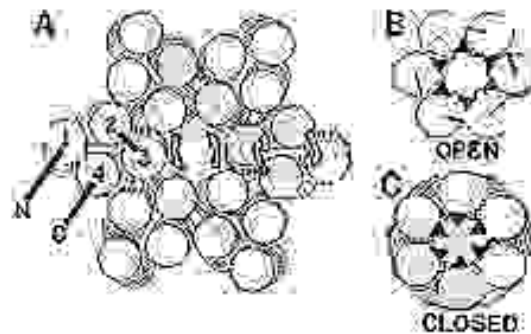


Fig. 4: More complex model indicating transmembrane domains. After [Bennet *et al*, 1991].

F. Agents that Regulate Permeation of Gap Junctions

The permeability of gap junctions does not remain constant but, like voltage-gated ion channels, it can be modulated according to certain variables. On the other hand, this process is, in general, reversible.

As a general rule, it can be stated that the molecular weight of substances being allowed to cross the junction should be less than 1kD as suggested by certain experiments with dye molecules [Alberts *et al*, 1993].

The permeability of gap junctions is widely non selective allowing the pass of small molecules (charged or not), second messengers and small metabolites. On the other hand gap junctions are impermeable to nucleic acids and proteins [Bennett *et al*, 1991]. In summary, their behavior is quite less selective than non junctional channels of the plasma membrane (ionic channels) which makes that the coupled cells form a functional syncytium.

The main agents that regulate the permeability of gap junctions are the following:

- **Voltage.** Both transjunctional (V_j) and transmembrane (V_{i-o}) potential influence the channel conductance. Gap junctions are not linear (they do not follow Ohm's law). If a channel (molecule) can exist in two states, say closed and open, and the energy difference between these states is a linear function of voltage, then the equilibrium distribution between these two states will be given by a Boltzmann relation of the form:

$$p_c / p_o = \exp(A(V - V_o))$$

where p_c and p_o are closed and open probabilities, V is the applied voltage, V_o is the voltage at which $p_c = p_o$, $A = nq / (kT)^{-1}$ is a constant expressing gating charge, and n is the equivalent number of unitary positive charges, q , moving through the entire applied voltage. A more useful form of the relation is

$$p_o = 1 / (1 + \exp(A(V - V_o)))$$

If the closed state conductance is zero, $g_j = N p_o$ where N is the number of channels. Bennett and Verselis [Bennett & Verselis, 1992] also comment that in most cases of voltage dependence g_j does not go to zero but, instead approaches a minimum value. By accepting this latter idea, they propose the following expression:

$$g_j = (g_{j\max} - g_{j\min}) / (1 + \exp(A(V - V_o))) + g_{j\min}$$

Up to this point it has been supposed that conductance has a symmetrical voltage dependence. However, in heterotypic junctions⁶ a rectifying behavior is observed.

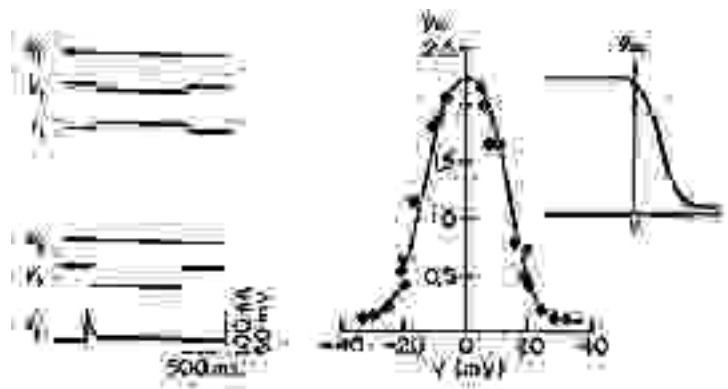


Fig. 5: Voltage sensitivity of gap junctions between pairs of amphibian blastomeres. After [Bennet & Verselis, 1992].

In Fig. 5, taken from [Bennet & Verselis, 1992], we show the voltage sensitivity of special type of cell (blastomeres) in amphibian. A Boltzmann distribution was fitted for each voltage polarity. For this case $V_o = 15$ mV and the effective charge was about 6 which is comparable to that of channels of electrically excitable membranes.

- **Lipophiles.** Several lipophilic⁷ compounds are among the pharmacological agents that block electrical communication. The main compounds investigated were⁸:

⁶ Junctions with two connexons made up of different connexins.

- *Octanol and heptanol*: these substances reversibly block conductance in a dose-dependent manner⁹.
- *Volatile Anesthetics (halothane and ethrane)*: applied in mM concentrations reversibly block cardiac gap junctions.
- *Arachnidonic acid, unsaturated fatty acids and doxyl stearic acids*: block junctional conductance at relatively low (μM) concentrations.

All of these lipophilic agents are likely to act by incorporation into the lipid bilayer.

- **Cytoplasmic pH and Ca^{2+} concentration.** Cytoplasmic acidification or increase of $[\text{Ca}^{2+}]$ lead to a reduction in junctional conductance in many systems. Although the influence of pH in junctional conductance is important under physiological conditions, the influence of variations in channel conductance due to Ca^{2+} is only important under pathological circumstances.

An interesting interpretation for Ca^{2+} dependence is as mechanism of cell defense. Suppose that a diseased cell begins leaking out its cytoplasmic material because a rupture in its plasma membrane. As a consequence of this, Ca^{2+} begins entering the cell because it is more concentrated in the ECL and valuable metabolites of the ICL are lost. Gap junctions close avoiding in this way that this unbalance in the chemistry of the intracellular space may affect the healthy neighboring cells.

- **Phosphorylation.** [Goodenough *et al*, 1996; Bennett & Verselis, 1992] The action of kinases to phosphorylate connexins and that of phosphatases to desphosphorylate them are mechanisms that seemly influence the energy barrier between open and closed states of gap junctions. In this way, they can modify the effective conductance of channels. The main problem is that different kinase affect junctional conductance differently in different tissues expressing the same or different connexins.

Table 3 summarizes different kinase action following the work of Bennett *et al* [Bennett *et al*, 1991]:

⁷ Substances which can be dissolved in non polar solvents.

⁸ Cf. [Beyer, 1993] for original references on each substance group.

⁹ Takens-Kwak [Takens-Kwak *et al*, 1992] found that heptanol reduced all non-junctional membrane ionic currents, in addition to gap junction conductance. These authors propose that heptanol affects the membrane lipid structure rather than interacting directly with gap junctional channels.

Kinase	Cell Type	Junctional Conductance	Connexin	Reference
cAMP-dependent protein kinase (A kinase)	Hepatocytes		32 and 26	Sáez et al., 1986, 1990
	Cardiac Myocytes		43	De Mello, 1988, Burt & Spray, 1988
	Myometrium		43	Cole & Garfield, 1986
	Sertoli cells		43	Grassi et al., 1986
Protein kinase C	Horizontal cells		?	Piccoline et al., 1984, DeVries & Schwartz, 1989, McMahon et al., 1989
	Sertoli cells		43	Grassi et al., 1986
	Cardiac myocytes		43	Spray & Burt, 1990
	Hepatocytes	?	32 and 26	Sáez et al., 1990
cGMP-dependent protein kinase (G kinase)	Cell lines		?, 43+?	Trosko et al., 1988, Chanson et al., 1988
	Cardiac myocytes		43	Burt & Spray, 1988
Tyrosine kinase	Horizontal cells		?	DeVries and Schwartz, 1989
	Fibroblast		43	Crow et al., 1990
Unknown	Cardiocytes, leptomenigeal cells	?	43	Hertzberg et al., 1989
	Lens	?	43	Musil et al., 1990
	Cell lines		43	Musil et al., 1990

Table 3: Kinase action on junctional conductance.

G. Conductance of Gap Junctions

Macroscopic Junctional Conductance (voltage clamp): a method for assessing macroscopic junctional conductance is by voltage clamping two interconnected cells as shown in Fig. 6 where an electric model for two cells is presented. In this figure, g_j , g_{nj1} and g_{nj2} stand for the junctional and non junctional conductances involved.

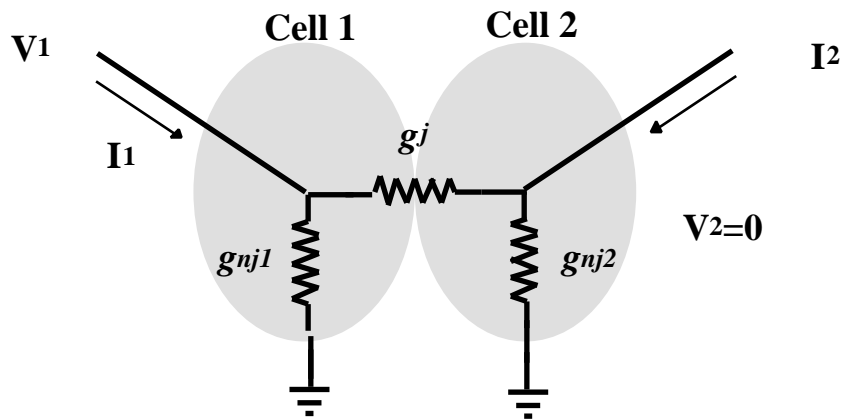


Fig. 6: Simplified model of voltage clamp method.

By applying a voltage step V_1 to one of the cells (to cell no 1 in the figure) and a current I_2 to the other cell (cell no 2) that equals the junctional current, it is possible to maintain the last cell to a constant potential. In this way, it is possible to obtain the value of both junctional and non junctional currents as:

$$g_j = - I_2 / V_1$$

$$g_{nj1} = (I_1 + I_2) / V_1$$

In a similar way can be estimated the non junctional conductance of the second cell.

Single Channel Conductance of Gap Junctions (patch clamp): recording of single gap junction channels is possible by using cells with non-junctional membranes of very low conductance. By *patch clamp techniques* [Sakmann & Neher, 1984] it was possible to show that many cells have non junctional conductances in de nanosiemens range and single gap junctions have unitary conductance () readily detectable against that background [Bennet & Verselis, 1992]. A simple procedure for gap junctions is to hold two coupled cells at different potentials; under these conditions opening of a junctional channel is signaled by an increase in current that is equal and opposite in the two cells. A single channel event in non junctional membrane is signaled by a current step in that cell alone. In Table 4, adapted from [Bennet & Verselis, 1992], some typical values for single channel conductance are shown.

Tissue:	Unitary conductance (pS)	Connexin type
Rat lacrimal gland	110-130	Cx32?, Cx26?
Rat neonatal heart	50, 45	Cx43
Chicken embryonic heart	60-80, 165	Cx42, Cx43, Cx45
Guinea-pig heart	30-40	Cx43?
Hamster ovarian cells	22-120	Cx43?
Pancreatic acinar cells	27, 130	Cx32
Earthworm MGA septum	100	?
Astrocytes	50-60	Cx43
Horizontal cells	50	?
<i>Xenopus</i> embryonic muscle cells	100	<i>Xen</i> 43
Rat leptomeningeal cells	40-90	Cx43, 34kD protein
WB cells	80-90	
Exogenous expression		
SKHep1 transfected cells		
Cx32	130,150	
Cx43	60, 90	
Artificial bilayers		
Isolated rat liver junctional membranes (Cx32, Cx26)	150	
Isolated junctional membranes	130-160	
Solubilized Cx32	50, 130	

Table 4: Single-channel conductance of different tissues. Adapted from [Bennett & Verselis, 1992]

III. GAP JUNCTIONS AND FAILING HEART

A. Cardiac Muscle Tissue

Animal body is organized at different levels. At tissue level, four different kind of tissues can be distinguished: *epithelial*, *nervous*, *connective* (proper, vascular or blood, osseous or bone and cartilage) and *muscle*. There are three types of muscular tissue: skeletal muscle, smooth muscle and **cardiac muscle**. In fact cardiac muscle tissue has some characteristics in common with both skeletal (both are striated muscles) and smooth muscles (both are involuntary).

Cardiac Muscle Tissue *Description:* branched cylindrical, striated fibers with one or two centrally located nuclei; contains intercalated discs; mainly involuntary control.
Location: heart wall.
Function: pumps blood to all parts of the body.

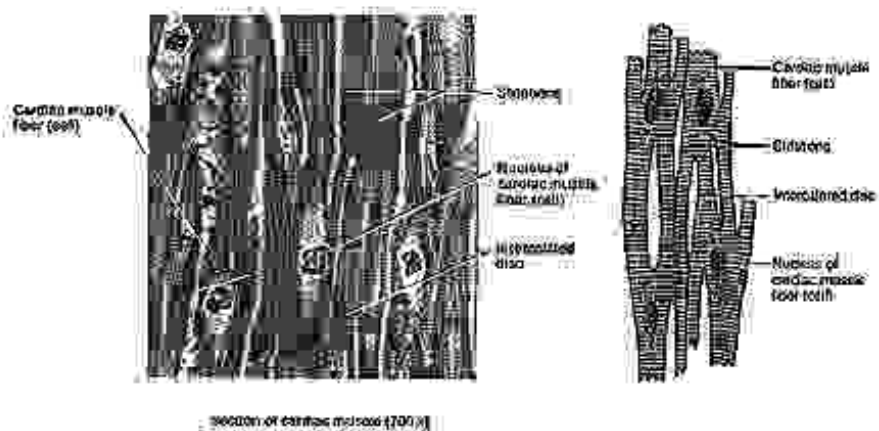


Fig. 7: Cardiac muscle tissue. From [Tortora & Grabowsky, 1993].

We will briefly review the organization of cardiac muscle tissue in order to correctly locate gap junctions. Cardiac muscle is composed of an arrangements of cells called **myocytes**. They are branched cylindrical cells with striated fibers responsible of the contractile properties of the tissue. Each myocyte may contain one or two centrally located nucleus. Mitochondria may be quite large in cardiac muscle. They are typically 2.5 μm long but may occasionally arrive to 7 or 8 μm . Another important aspect is the existence of the transverse tubular system which is composed of invaginations of the sarcoplasmic membrane. This system allows easy propagation of action potential through the interior of the myocyte in order to facilitate the synchronous contraction. It is said that every interior point is within a range of 2 to 3 μm of the extracellular space [Fawcett, 1986]. Fig. 7 shows a simplified diagram of the structure of a myocytes' network.. Fig 8 shows a diagram of the interior of a myocyte based on an electron micrograph.

Cardiac muscle fibers branch and interconnect with each other, but form two separate networks. The muscular walls and partition of the upper chambers of the heart (atria) compose one network. The muscular walls and partition of the lower chambers (ventricles) compose the other network. Each fiber in a network is connected to its

neighbors by irregular, transverse thickenings of the sarcolemma¹⁰ called *intercalated discs* (Fig. 9).

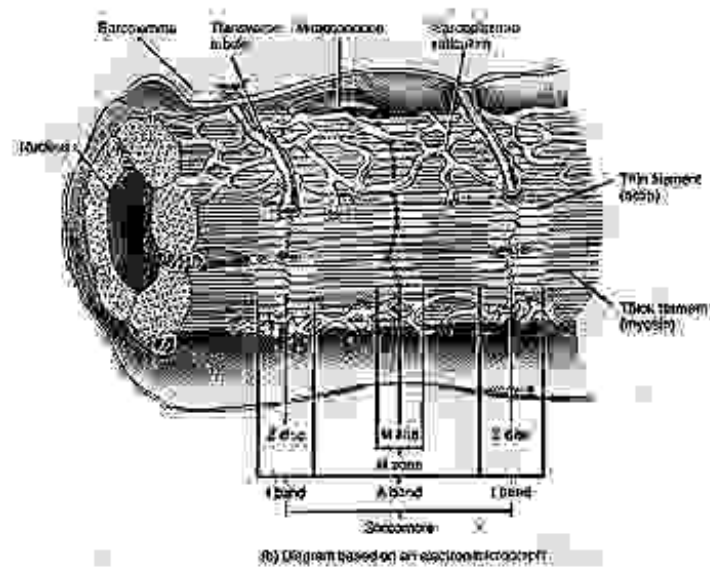


Fig. 8: Diagram of the interior of a myocyte based on an electron micrograph. After [Tortora & Grabowsky, 1993]

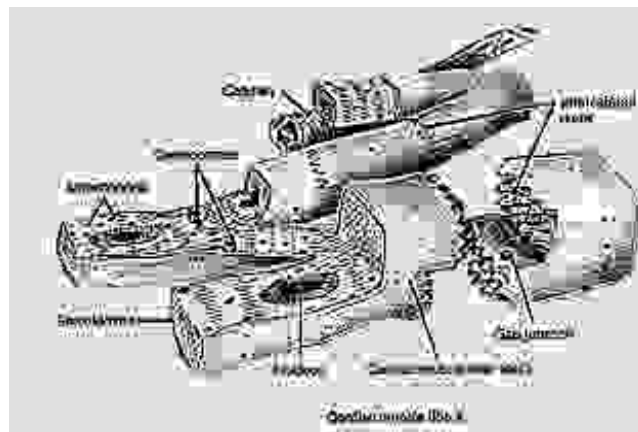


Fig. 9: Detail of the junctional portion of myocytes.

The intercalated discs contain three types of junctions: *desmosome* and *fascia adherens*¹¹ which are responsible of myocytes' adhesion -anchoring junctions- and *gap junctions* which allow easy spread of action potentials through the network that works as

¹⁰ Sarcolemma and sarcoplasm are the names of the cytoplasmic region and plasma membrane of muscle cells.

¹¹ *Fascia adherens* (sometimes also called *zonula adherens*) is a special type of adhesion belt junction.

a functional unit (syncytium). The main connexin type found in normal human myocardium is Cx43. Each myocyte interconnects with about 11 other myocytes through its branches [Severs, 1994a]. Desmosomes and fascia adherens are mainly located in the transverse regions of the intercalated discs while gap junctions' macula are more concentrated in the longitudinal faces between myocytes. Both desmosomes and fascia adherens have a much wider intermembrane space (20-25 nm) than gap junctions (2-3 nm) [Severs, 1994a]. Myocytes are roughly quadrangular when viewed in a transverse cut, and about 14µm diameter [Tortora & Grabowsky, 1993].

The quantity and distribution of the gaps in the heart follows the functional needs. While in the atrioventricular node there are few and sparse gaps in order to increase the delay in the action potential propagation, Purkinje fibers and active myocardium are rich in gaps.

A final observation about action potential propagation is its anisotropy. In normal myocardium conduction parallel to the longitudinal axis of the myocytes is three times faster than in the transverse direction [Severs, 1994a]. This may be because gap junctions are predominantly distributed in the longitudinal pathway joining myocytes. Moreover the ratio between the cytoplasmic membrane resistance and the resistance due to gap junctions may be as large as 400 [Guyton & Hall, 1996].

B. Ischemia and Myocardial Infarct

In next section we will summarize some experimental results about gap junctions in normal and failing heart. By failing heart we will consider ischemic heart. **Ischemia** is a heart fault arising from a *reduction of blood flow* in a certain region of the heart. Ischemia usually causes **hypoxia** which is a reduction in oxygen supply that may weaken cells without killing them. The main consequence of a poor blood supply is a reduction in glucose and oxygen concentrations in the extracellular fluid.

More serious than ischemia is **myocardial infarction**, commonly called heart attack. Infarction means the death of an area of tissue because an interrupted blood supply. Myocardial infarction may result from a *thrombus* (stationary blood clot) or *embolus* (blood clot transported by blood) in one of the coronary arteries. The tissue distal to the obstruction dies and is replaced by non contractile scar tissue. Thus the heart muscle loses at least some of its strength being the aftereffects dependent upon the size and location of the infarcted or dead area.

C. Gap Junction Alterations in Failing Heart

Severs [Severs, 1994a; 1994b] reported two main alterations in gap distribution and quantity in ischemic myocardial tissue:

- Disturbance in the spatial distribution of gap junctions at the border zone of healed infarcts.
- Reduction in the quantity of immunodetectable Cx43 in regions of normal gap junction distribution distant from infarct scar.

Gap junctions in ischemic tissue are not confined to the intercalated discs but may be sparsely distributed in the cellular surface. The myocytes presenting this alteration may be several hundred micrometers from the border of the infarct scar. They may be even mixed among apparently normal myocytes.

There is also a reduction in the number of gaps per unit length and in their size selectively affecting long gap junctions detected in transverse planes of section. The number of intercalated disc contacts per myocyte is markedly reduced, a change largely confined to the discs making side-to-side connections between cells (those in branches rather than at the ends of the main body of the cell).

All these effects may contribute to the anisotropic propagation of the wavefront by reducing the coupling between myocytes and stressing the difference in propagation speed of the action potential in the longitudinal and transverse directions.

Although myocardium distant from infarct scars has seemingly normal arrangement of gap junctions, the occurrence of ventricular arrhythmias in other clinical settings, such as hypertrophy, raises the question as to whether the diseased heart is afflicted with a more wide-spread gap-junctional abnormality.

It seems that reduced expression of connexin 43 may be a key factor. It has been shown that this reduction in expression is not only confined to ischemic heart disease. This abnormality may be also found in non ischemic hypertrophied myocardium.

It has also been reported that cultured myocytes infected with *Trypanosoma cruzi*, the unicellular parasite responsible for Chagas' disease (the most common cause of heart disease in South America), show reductions in gap junctional conductance and immunocytochemically detectable connexin 43 levels. The occurrence of a common gap-junctional abnormality in the form of reduced connexin 43 in these diverse disease settings, all of which are associated with an arrhythmic tendency, is highly suggestive of a direct link between connexin 43 levels and conduction disturbances.

IV. ACKNOWLEDGMENT

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