CA^{2+} DYNAMICS, CA^{2+} WAVES AND THE TOPOGRAPHY OF THE CA^{2+} CONTROL SYSTEM

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Summary

All of lifes responses to the environment are ultimately determined by cellular responses to external signals. These external signals, whether they be light, chemicals, or touch do not directly affect the cells internal biochemical machinery but instead generate an intermediary signal —a second messenger —that carries the information into the cell. Perhaps the most widely used second messenger is the calcium ion. This article traces the information flow from the external signal through the calcium second messenger system and onto the receiver of the calcium signal. The information contained in the external signal is encoded into rapid changes in the calcium concentration. The information, now encoded in the fluctuating calcium concentration, propagates by diffusion into the cell. However, the physical constraints of diffusion preclude sending the signal more than about a micrometer so a number of mechanisms have evolved to permit calcium

signaling in large cells. One mechanism is to change the shape of the cell, another is to use calcium waves. Both mechanisms are discussed here. The mechanism that can generate calcium waves can also generate calcium oscillations. Information in the external signal can be encoded in the frequency of the calcium oscillations. A mathematical model of a calcium oscillator that alters its frequency in response to the magnitude of the external signal is presented. Finally, we present two examples of biochemical systems that can decode the information encoded in the frequency of the calcium oscillations.

1. Introduction

Cells respond to their environment. The external signals cells respond to are diverse. They include foods, toxins, touch, pressure, gravity, light, electric fields, salinity, hormones, pheromones, and much more. Many external signals do not directly enter the cell. Instead the external signal, or primary message, is received by a sensor residing at the cell membrane, which is made of lipids that encloses the cell as a protective pocket. Figure 1 shows a schematic of the cellular signaling system.

Figure 1. Schematic of second messenger signaling system.

When the sensor receives the primary message it *transduces* this information, that is, it encodes the information in a different physical form. This new form is called the *second messenger*. It is the second messenger that carries the information into the cell. We are all familiar with signal transduction. Consider the telephone. When we speak, the speech information is carried through the air as pressure variations (the primary signal). The microphone in the mouthpiece transduces the pressure variation into a corresponding voltage signal. It is the voltage signal that sent through the phone line to the receiver, the person on the other end of the line.

In contrast to the wide diversity of primary signals, the number of second messengers is small. Currently, the known second messengers include nitric oxide (NO), inositol 1,4,5-trisphosphate (IP_3), cyclic AMP (cAMP), diacylglycerol, and the calcium ion (Ca^{2+}). The use of a small number of second messengers is not unique to cells. We see the same limited number of second messengers in electronic systems. Although there are an enormous variety of sensors (motion sensors for automatic switch, light sensors for dawn-and dusk lighting, voltage sensors in cardiac defibrillators, magnetic sensors, pH sensors, touch sensors, the sensor outputs are usually limited to changes in electric current, voltage, resistance, capacitance, and inductance.

Returning to our telephone analogy, you can send virtually an infinite number of different messages and how the listener responds to your message depends on what the message is and who the listener is. Likewise, the cell type and the primary message determine the cellular response to the external information. The

response element (RE) is the molecule or molecules that receive the second messenger and somehow decodes the information carried by the second messenger. Some cellular responses include contraction of a muscle cell, change in the direction of ciliary beating to effect escape or approach in *Paramecium*, release of neurotransmitters in neurons, and release of saliva from salivary glands.

In this article we will focus on one important and ubiquitous second messenger, the Ca^{2+} ion. Our task here is somewhat like that of a telephone company. We wont care about the content of the conversation but we are interested in how the conversation is encoded, transmitted, and decoded. We will explore the following questions: How is the Ca^{2+} signal generated? How is information in the primary signal encoded in the Ca^{2+} signal? How is this information transmitted within the cell? What are the physical constraints on the transmission and how do cells circumvent these constraints? And, finally, how is the Ca^{2+} signal decoded?

2. The Minimal Ca^{2+} Signal Generating System

Cells contain certain concentration of free ions (not bound to proteins). Table 1 lists the concentrations of important free ions inside (intracellular) and outside (extracellular) of the cell, as well as the ratio of the extracellular to intracellular concentration.

Table 1 Intracellular and extracellular concentration of major ions and their ratios (extracellular/intracellular). Data from Alberts, et al., 2002.

Ca^{2+} stands out as exceptional in having such a low intracellular concentration and high concentration ratio. The cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) is between 50 —150 nM while the extracellular Ca^{2+} concentration is around 1 —2 mM in mammalian blood and about 10 mM in squid. Why is $[Ca^{2+}]_i$ so low? Kretsinger (1977) suggests that $[Ca^{2+}]_i$ must be low enough to prevent Ca^{2+} from reacting with phosphates, such as ATP, and forming calcium phosphate precipitate. Rasmussen (1981) presents another possible explanation for why Ca^{2+} is so low. He proposed that early life evolved in an ocean that was low in Ca^{2+} and as Ca^{2+} rose due to weathering of rocks, Nature could have either replaced the metabolic machinery accustomed to the low Ca^{2+} or evolve machinery to keep the cytoplasmic Ca^{2+} low. Whatever the reason, all modern organisms have the machinery to maintain a resting intracellular Ca^{2+} concentration of about 100 nM.

The 10,000-fold concentration gradient (1 mM/100 nM) between the extracellular and intracellular Ca^{2+} concentrations and the electrical potential gradient (-80 mV inside the cell) provide a large electrochemical driving force for Ca^{2+} entry into the cell. Because of this large driving force, Ca^{2+} leakage into the cell is inevitable and if left unchecked will eventually cause the intracellular Ca^{2+} concentration to rise to a level that renders the metabolic machinery dysfunctional and ultimately kill the cell.

Compensating for this leakage and to maintain the Ca^{2+} concentration, $[Ca^{2+}]_i$, at about 100 nM are ATP-dependent Ca^{2+} pumps, Ca^{2+} exchangers, and Ca^{2+} buffers. The operation of the pumps and exchangers require the expenditure of

free energy to move the Ca^{2+} out of the cell against its electrochemical gradient. The cell must also expend energy in making these pumps, exchangers, and buffers, which are large proteins. Having invested into the making of these proteins and expending energy in moving Ca^{2+} out of the cell, Nature has turned this leakage problem into the most diverse and flexible signaling system by adding one additional component: the Ca^{2+} channel.

The Ca^{2+} channel provides a controlled conduit for Ca^{2+} entry into the cell. There are different types of Ca^{2+} channels. The Ca^{2+} channels are usually closed in resting cells but open in response to electric fields, mechanical stretch, or ligands. Upon opening of the channel, extracellular Ca^{2+} flows down its electrochemical gradient and enters the cell. In this way, the Ca^{2+} channel transduces the external signal —the electric field, mechanical stretch, or ligand —into an intracellular Ca^{2+} signal. Figure 2 shows the *minimal Ca^{2+} signal generating system* consisting of the Ca^{2+} channel, pumps and exchangers.

Figure 2. Minimal Ca^{2+} signal generating system. Gray represents region of high Ca^{2+} concentration while white represents low Ca^{2+} concentration. The horizontal line is the membrane, the pair of parallel vertical lines is the Ca^{2+} channel, and the circle and arrow pair represents the Ca^{2+} pump and Ca^{2+} exchanger.

3. Ca^{2+} Signals are Transient

Figure 3 shows the Ca^{2+} concentration in the cytosol of tobacco plant seedlings in response to puffs of air of increasing force delivered at the times indicated by the arrowheads. When the force of the puff is very small (first arrow), there is no detectable change in the cytosolic Ca^{2+} concentration indicated by the aequorin luminescence (a Ca^{2+} indicator). When the force becomes sufficiently strong, the Ca^{2+} concentration rapidly rises. However, the rise in Ca^{2+} is not sustained but decays back to the basal level. The transient Ca^{2+} rise in Figure 3 is typical of Ca^{2+} signals. The Ca^{2+} concentration rises only transiently in response to an external signal. Similar transient Ca^{2+} signals are observed in plant cells in response to a change in direction of gravitational field, in root hairs responding to rhizobium nodulation signals, in eggs activated by sperm, in endothelial cells responding to histamine, and in skeletal and cardiac muscle cells in response to electrical stimulus.

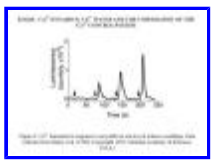

Figure 3. Ca^{2+} transients in response to air puffs (at arrows) in tobacco seedlings. Data redrawn from Haley et al. (1992) (Copyright 1992, National Academy of Sciences, U.S.A.)

The Ca^{2+} increase is transient because the Ca^{2+} channel will close within milliseconds even though the external signal remains present. When the channel closes the flow of Ca^{2+} is staunched and the Ca^{2+} that had entered the cell binds to the Ca^{2+} buffers and is soon removed from the cell by the pumps and exchangers thereby bringing $[Ca^{2+}]_i$ back to the resting level of ~100 nM.

4. Ca^{2+} Carries Information via Diffusion

When the Ca^{2+} channel opens the Ca^{2+} rushes in causing the Ca^{2+} concentration around the mouth of the channel to rapidly increase to 10 —100 μM, much higher than the concentration in surrounding regions that are at the resting level of ~100 nM. Because of this concentration gradient, Ca^{2+} diffuses away from the Ca^{2+} channel. Information about the external signal is carried away from the membrane into the cell with this diffusional flow of Ca^{2+}.

The analogy drawn between Ca^{2+} signaling and the phone company begins to break down here. Electromagnetic waves travel at a constant speed (the speed of light, c). Imagine sending a Morse code message to a friend. If your friend is a distance L from you then he will receive the message at time L/c. If your friend moves to a distance $2L$, he will have to wait twice as long $2L/c$ to receive the message.

Information transmission by diffusion is not so simple. Information flow slows down as the distance between the source and receiver increases and the magnitude of the signal also decreases with distance. These are fundamental constraints of diffusion and the Ca^{2+} signaling system must be structured to circumvent these constraints.

4.1 Range and Time Scale of Ca^{2+} Signaling

Diffusion of a substance in an isotropic medium is described by the diffusion equation

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2}\right),$$

(1)

where C is the concentration, t is time, $x, y,$ and z are the spatial coordinates, and D is the diffusion coefficient. We will use Eq. to calculate the concentration of Ca^{2+} at any point in space and time. This Eq. is incomplete as it ignores the chemical reaction with the buffers; this is a more serious problem. There are no analytic solutions to the diffusion problem when chemical reactions are involved, except for the simple, special case of where the reaction is an isomerization.

However, under certain conditions that occur frequently in cells the diffusion Eq. with chemical reactions converges to the form given by (1), albeit with a modified diffusion coefficient D. When the buffer reaction is fast relative to diffusion (in a manner that is made precise in Wagner & Keizer, 1994) then we

can still use Eq. provided D is replaced by the apparent diffusion coefficient D_{app} (Wagner & Keizer, 1994)

$$D_{\text{app}} = D(C) = \frac{D}{1 + \frac{K_{\text{d}} B_{\text{T}}}{(K_{\text{d}} + C)^2}}.$$

(2)

In this equation, K_{d} is the dissociation constant of the buffer and B_{T} is the total concentration of buffer binding sites. Notice that $D_{\text{app}} \le D$ and that as $C \to \infty, D_{\text{app}} \to D$. This inequality and limit has a simple physical meaning. When the Ca^{2+} concentration is low, there are many free Ca^{2+} binding sites so when a diffusing Ca^{2+} ion comes upon the binding site it becomes trapped, slowing the diffusion of Ca^{2+}. Conversely, when the Ca^{2+} concentration becomes very high ($C \gg K_{\text{d}}$) most of the binding sites are already saturated with Ca^{2+} so a diffusing Ca^{2+} ion will not be impeded and it diffuses as if the buffer were absent. (This is not quite true since a buffer molecule fully bound with Ca^{2+} still provides a physical barrier to the diffusing ion.)

Even using approximation Eq. is too hard to solve. We make one more approximation. We will use the value of D_{app} at one particular Ca^{2+} concentration, the resting concentration of 100 nM. This allows us to determine the order of magnitude for the range and speed of Ca^{2+} signaling.

Let a Ca^{2+} channel carry a Ca^{2+} current i and let it be open for time T. For the Ca^{2+} channel known as the L-type Ca^{2+} channel found in many cells including muscle cells, neurons, and bacterial cells, i is about 0.2 pA (a picoamp is 10^{-12} amperes) and T is about 0.5 ms. The flow of Ca^{2+} through this channel, J, is related to i by $J = i/(2F)$, where F is the Faraday constant (96,485 coulombs/mol) and 2 is the charge of Ca^{2+}. The total number of moles of Ca^{2+} passing through the channel during the opening is $M = J \cdot T$. If we imagine that this many moles of Ca^{2+} ions were instantly deposited then the Ca^{2+} concentration at any point in space and time is given by (see Crank, 1975)

$$C(r,t) = \frac{M}{8(\pi D_{\text{app}} t)^{3/2}} \exp\left(-\frac{r^2}{4 D_{\text{app}} t}\right).$$

(3)

This solution assumes isotropic diffusion and absence of diffusion barriers. In this situation Ca^{2+} spreads out spherically with the radius of the sphere given by $r^2 = x^2 + y^2 + z^2$ and Eq. can be simplified to $\partial C / \partial t = D_{\text{app}} \left(\partial^2 C / \partial r^2 + (2/r)\, \partial C / \partial r\right)$.

4.1.1. Time Scale of Diffusion

Unlike an electromagnetic wave, there is no unique speed of diffusion. Nevertheless we can define a characteristic time scale for diffusion as follows. Suppose that an intracellular Ca^{2+} sensor becomes activated when the Ca^{2+} concentration exceeds a threshold $\tilde{c}$. If the sensor is a distance r from the Ca^{2+} channel then this threshold concentration will be reached at time $\tilde{t}$ obtained by solving

$$C(r,\tilde{t})-\tilde{c}=0.$$

(4)

To solve let $z=\tilde{t}^{-1/2}$ and define

$$\beta=\frac{M}{8(\pi D_{\text{app}})^{3/2}} \text{ and } \gamma^2=\frac{r^2}{4D_{\text{app}}}$$

(5)

then becomes

$$\beta z^3 \mathrm{e}^{-\gamma^2 z^2}-\tilde{c}=0.$$

(6)

Equation can be solved for z and from which we get $\tilde{t}$. Actually, there are two solutions $\tilde{t}_0$ and $\tilde{t}_{-1}$

$$\tilde{t}_{0,-1}=\frac{4\gamma^2}{-6W\left[\frac{2}{3}\left(-\frac{\gamma^3\tilde{c}}{\beta}\right)^{2/3}\right]}.$$

(7)

This solution looks complicated but it can give important insights so it is worthwhile to understand its meaning. W is called the Lambert W function, which solves $W(x)\mathrm{e}^{W(x)}=x$. Lambert W makes its appearance in diffusion problems (as we have here), in time delay problems (such as the delay between turning the hot water faucet and getting hot water out of the shower head), and in determining the optimal firing angle of a projectile when air resistance is present. (See (Corless, et al., 1993 for properties and applications of the Lambert W function.) W is real only when $x\geq -1/\mathrm{e}$ and is double valued on $x\in(-1/\mathrm{e},0)$. The solution $\tilde{t}_0$ is on the branch that extends from $[-1,\infty]$ and $\tilde{t}_{-1}$ is on the branch that extends from $(-\infty,-1]$.

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4.1.2 Range o Ca^{2+} Signaling

In order for $\tilde{t}_{-1}$ and $\tilde{t}_0$ to be positive $W(x)$ must be negative. W is negative only on $-1/\mathrm{e} \le x < 0$ so this is the domain that the argument must be restricted. We can use this restriction to determine the maximum signaling range by setting the argument of W in Eq. to $-1/\mathrm{e}$, that is,

$$\frac{2}{3}\left(-\frac{\gamma^3\tilde{c}}{\beta}\right)^{2/3} = -\frac{1}{\mathrm{e}}$$

(8)

from which we get maximum signaling range $r_{\max}$

$$r_{\max} = \sqrt{\frac{1}{\pi e}}\left(\frac{\sqrt{6}\,M}{\tilde{c}}\right)^{1/3}. \qquad (9)$$

Physical meaning of $\tilde{t}_{-1}$, $\tilde{t}_0$, *and* $r_{\max}$. To gain an appreciation for the meaning of these quantities let us return to the L-type Ca^{2+} channel (LTCC) example mentioned earlier. The current through a single LTCC is $i = 0.2$ pA and the mean open time is $T = 0.5$ ms. The number of moles of Ca^{2+} coming through the channel during one opening is $M = iT/2F = 5\times10^{-22}$ mol. A sensor at a distance of $r = 0.36$μm from the LTCC will see the concentration change shown in Figure 4A. The Ca^{2+} concentration exceeds the sensors threshold of $\tilde{c}$ =1 μM between the times $\tilde{t}_{-1} = 3.37$ ms and $\tilde{t}_0 = 5.67$ ms indicated by the arrows. For these values of M and $\tilde{c}$, $r_{\max} = 0.366$ μm. Figure 4B shows the concentration history at $r_{\max}$. A sensor at this distance will see the Ca^{2+} concentration reach threshold for just an instant at $\tilde{t}_{-1} = \tilde{t}_0 = 4.47$ ms. A sensor farther from $r_{\max}$ at $r = 0.4$ μm will never see the Ca^{2+} concentration exceed threshold as shown in Figure 4C.

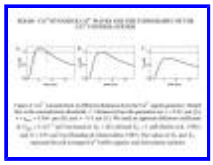

Figure 4. Ca^{2+} concentration at different distances from the Ca^{2+} signal generator. Dotted line is the concentration threshold, $\tilde{c}$. Distances from the generator are $r = 0.36$ μm (A), $r = r_{\max} = 0.366$ μm (B), and $r - 0.4$ μm (C). We used an apparent diffusion coefficient of $D_{app} = 5\times10^{-3}$ μm²/ms based on $B_T = 123$ μM and $K_d = 1$ μM (Berlin et al., 1994) and $D = 0.53$ μm²/ms (Donahue & Abercrombie, 1987). The values of B_T and K_d represent the cell averaged Ca^{2+} buffer capacity and dissociation constant.

We must be aware that $r_{\max}$ is the *absolute upper bound* for Ca^{2+} signaling. The actual range is likely to be smaller because Ca^{2+} buffers will reduce the magnitude of changes in Ca^{2+} concentration.

5. The Relationship between Molecular Geometry and Ca^{2+} Sensitivity

The expression for $r_{\max}$ in Eq. provides us with insights into the relationship between the sensors Ca^{2+} sensitivity and the distance between the Ca^{2+} channel (the signal generator) and the sensor (the signal receiver). We have called $\tilde{c}$ the threshold concentration, which connotes a sharp transition between the sensor being activated or not. This is rarely the case. Sensor activity changes smoothly with the Ca^{2+} concentration. A measure of the sensors Ca^{2+} sensitivity is the dissociation constant K_d. For the bimolecular reaction between Ca^{2+} and sensor

(S), $Ca^{2+} + S \rightleftharpoons S\text{-}C$, half of the sensor molecules are in the Ca^{2+}-bound form, S-C, when $[Ca^{2+}]_i = K_d$. Values of K_d for some Ca^{2+} sensors are given in Table 2.

Table 2. Some cellular Ca^{2+} sensors and their dissociation constants (K_d)

The K_d values differ by a factor of 100 between the highest and lowest in the list above. Let us compare the spatial relationship between the Ca^{2+} channel to two sensors: the ryanodine receptor (RyR) and troponin C. RyR is itself a Ca^{2+} channel; we will talk much more about the function of RyRs later but for now our sole interest is in RyR being a receiver for the signal generated by the LTCC. RyRs are located close to L-type Ca^{2+} channels. The distance between the LTCC and the RyRs is only about 40 nm (0.04 μm). Based on the current through the LTCC and its open time we used before we get $M = 5 \times 10^{-22}$ mol, and using K_d =15 μM for $\tilde{c}$ we get [using Eq.] r_{max} =0.15 μm. Thus the RyRs are well within the signaling range of the Ca^{2+} coming through the LTCCs.

Troponin C (TnC) is part of the contractile machinery. When TnC binds Ca^{2+}, conformational changes occur that allow actin to bind to myosin to cause muscle contraction. There are many TnC molecules within a sarcomere and the distance between the LTCCs and the farthest TnC in a sarcomere is about 1 μm. This distance is far beyond the maximum signaling distance of 0.15 μm so the LTCCs cannot be the signal generator for TnC. It turns out that the RyRs are the Ca^{2+} signal generators for TnC. We will turn to this point in greater detail below.

6. Ca^{2+} Signaling i Small Cells and Small Structures

In small cells and small structures that have a high surface-to-volume ratio, the minimal Ca^{2+} signaling system in Figure 1 is often used. For example, the presynaptic terminal at the neuromuscular junction is a roughly spherical structure about 1 μm across. The membrane of the terminal is studded with voltage activated Ca^{2+} channels (N-type or P-type), schematically shown in Figure 5. When the terminal is depolarized by an action potential, Ca^{2+} enters the terminal and the rise in Ca^{2+} triggers the fusion of acetylcholine containing vesicles with the plasma membrane and releasing the neurotransmitter.

Figure 5. Schematic of a synaptic terminal. Radius (arrow) is ≈0.5 μm. Blue circles are vesicles.

Let us calculate r_{max} for the N-type channel using $i = 2$ pA and $T = 0.2$ ms (Bennett, et al., 2000). Synaptotagmin, the Ca^{2+} binding protein on the vesicles, has K_d of about 25 μM. From these we get r_{max} =0.2 μm. This seemingly small signaling distance, in fact, encompasses 78% of the synapse volume. We arrive at this value by assuming that the synaptic terminal is spherical of radius R =0.5 μm

then the fraction of the synaptic terminal volume within the shell $R - r_{\max} < r < R$ is

$$\frac{\frac{4\pi}{3}\left(R^3 - (R - r_{\max})^3\right)}{\frac{4\pi}{3}R^3} = \frac{3r_{\max}}{R} - \frac{3r_{\max}^2}{R^2} + \frac{r_{\max}^3}{R^3} \tag{10}$$

If the synaptic terminal were 2 μm in diameter then volume of the terminal within the signaling range would fall to 49% of the total terminal volume.

Using Eq. we see that for small cells (such as bacteria) or small structures (such as the synaptic terminal), Ca^{2+} entering through Ca^{2+} channels on the plasma membrane is largely sufficient to activate most of the Ca^{2+} sensors throughout the cell. However, as the cells become larger (bigger R) Ca^{2+} sensors located deep within the cell would be too far to be activated if the Ca^{2+} channels were located only on the plasma membrane. Large cells have developed different strategies for Ca^{2+} signaling and we examine these strategies next.

7. Ca^{2+} Signaling i Large Cells

Large cells have lower surface-to-volume ratios than small cells. This means that a smaller fraction of the total volume of the cell is close (that is, within $r_{\max}$) to the surface; this is the meaning of . Large cells, such as muscle cells, use a number of strategies to circumvent the problem of small surface-to-volume ratios and the constraints on the signaling distance imposed by diffusion.

These strategies all rely on a modification of the minimal Ca^{2+} signaling system shown in Figure 2. Figure 6 illustrates such a system in which the high Ca^{2+} concentration domain is brought *into* the cell. The oval structure is the intracellular Ca^{2+} storage organelle called the endoplasmic reticulum (ER) or (in muscle) the sarcoplasmic reticulum (SR). The Ca^{2+} concentration within the ER/SR is about 1 mM, similar to the extracellular Ca^{2+} concentration. The ER and SR have a high density of ATP-dependent Ca^{2+} pumps called SERCA (Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} ATPase), which moves Ca^{2+} from the cytoplasm into the ER/SR. The mushroom-shaped object represents Ca^{2+} channels, which when open, allow Ca^{2+} to flow down its concentration gradient from the ER/SR into the cytoplasm. The Ca^{2+} channels are either ryanodine receptors (RyRs) or IP_3 receptors (IP_3Rs). These Ca^{2+} channels are named ryanodine or IP_3 receptors because the channels bind the respective compounds that modulate the channel gating properties. In both cases the channels are organized in clusters which open and close in a concerted manner. We will call a cluster a Ca^{2+} release unit or CRU.

Figure 6. Modified Ca^{2+} signal generator.

7.1 Quantal Ca^{2+} Release from CRUs: Ca^{2+} Sparks and Ca^{2+} Puffs

When the Ca^{2+} channels in the CRU receive a signal, the channels open briefly allowing Ca^{2+} to flow from the ER/SR into the cytoplasm. Ca^{2+} released from the CRU can be visualized using fluorescent Ca^{2+} indicators. The indicator fluoresces when it binds Ca^{2+} so the increase in Ca^{2+} concentration is seen as a small flash of fluorescence when imaged with a confocal microscope. These flashes of fluorescence have been imaginatively christened by their discovers Ca^{2+} puffs (when the channels are IP_3Rs, Parker and Yao, Parker & Yao, 1991) and Ca^{2+} sparks (when the channels are RyRs, Cheng, Lederer, and Cannell, Cheng, et al., 1993) . Figure 7A shows examples of Ca^{2+} sparks in a rat ventricular cell.

Figure 7. Ca^{2+} sparks. A, confocal images of a ventricular cell loaded with a fluorescent Ca^{2+} indicator. Top image shows a quiescent cell. Middle image taken 1.2 ms later show two sparks (bright objects). Bottom image taken 25 ms later show the decline of the two sparks. B, 3-dimensional representation of one of the sparks in A. Height represents Ca^{2+} concentration.

The Ca^{2+} that is released in a spark or puff is still subject to the constraints imposed by diffusion. The signaling range of a puff or spark is no more than 1-2 μm as can be seen in Figure 7B. Thus it would at first appear that the strategy of bringing the high Ca^{2+} domain into the cell does not solve the problem of getting Ca^{2+} signals deep within the cell. The problem of getting signals deep into the cell is solved by replicating the CRU, shown in Figure 6Aa, throughout the cell as depicted schematically in Figure 8A. Attaching a fluorescent tag to an antibody that binds to RyRs allows us to determine the positions of the CRUs in the cell. Figure 8B shows the antibody labeled RyR in a ventricular cell. Each bright spot, more easily seen in the magnified section, is a CRU. The CRUs are arranged in a striped pattern. Each stripe marks a structure called a z-line. The mean distance between the CRUs within a z-line is about 1 μm (Chen-Izu et al., 2006). We will see the significance of this distance later on.

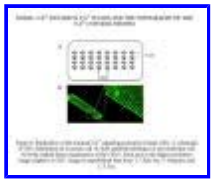

Figure 8. Replication of the minimal Ca^{2+} signaling generator in large cells. A,

schematic of CRU distribution in a muscle cell. B, RyR antibody labeling in a rat ventricular cell. Note the striped linear organization of the CRUs. Each spot in the higher resolution image (right) is a CRU. Image is unpublished data from Y. Chen-Izu, T. Bnysz, and L.T. Izu.

While each Ca^{2+} spark or puff has only a limited signaling range, the collective response of all CRUs can cause a rapid Ca^{2+} increase throughout the cell. We next turn to discuss the collective behavior of CRUs.

7.2 The Collective Behavior of CRUs

How does the primary signal on the plasma membrane trigger Ca^{2+} release deep within the cell? We will examine two paths that large cells have taken to solve this problem. The first path, used by skeletal muscle cells and mammalian ventricular cells, cleverly brings the plasma membrane into the cell, effectively making all points in the cell close to the surface. The second path uses propagating Ca^{2+} waves.

7.2.1 Bringing the Surface into the Cell.

Figure 9 is a schematic of a skeletal muscle or mammalian ventricular muscle cell. At periodic intervals of about 2 μm, the plasma membrane invaginates into the cell. These invaginations are called transverse tubules or t-tubules. These t-tubules are analogous to the depression made when you carefully press your finger into a balloon without rupturing the rubber. The extracellular fluid (*gray color*) fills the t-tubules. L-type Ca^{2+} channels (LTCCs) are on the cell membrane that form the t-tubules and the RyRs of the CRUs inside the cell lie in close opposition to the LTCCs. The complex of LTCC and CRU is called a *dyad* and the separation distance is only between 20 and 40 nm. The action potential travels rapidly along the surface membrane and down the t-tubules. The action potential causes the LTCCs to open and admit a small amount of Ca^{2+} into the cell. This Ca^{2+} then binds to the RyRs causing the CRU to open and release a much larger amount of Ca^{2+} into the cytoplasm from the SR. Because the action potential travels rapidly down the t-tubules, the LTCC both near the surface and deep within the cell open nearly synchronously. Each CRU that releases Ca^{2+} produces a spark with a signaling range limited to about 1 —2 μm, but the collective result of the synchronous triggering of CRUs by the L-type Ca^{2+} channels results in synchronous and rapid Ca^{2+} release throughout the cell.

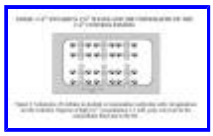

Figure 9. Schematic of t-tubules in skeletal or mammalian ventricular cells. Invaginations are the t-tubules. Regions of high Ca^{2+} concentration (~1 mM, gray color) are in the extracellular fluid and in the SR.

1. Introduction

7.2.2 Ca^{2+} Signals

Its Own
Release.

CA^{2+} DYNAMICS, CA^{2+} WAVES AND THE TOPOGRAPHY OF THE CA^{2+} CONTROL SYSTEM

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Keywords: calcium control, heart, calcium sparks, second messenger, arrhythmia, oscillation, information coding, information decoding, calcium signaling.

Contents

7.2.2 Ca^{2+} Signals Its Own Release.

The RyR has the important property that the probability of its opening increases as the ambient Ca^{2+} concentration increases. This property is known as *Ca^{2+}-induced Ca^{2+} release* or CICR. CICR is a very important property of RyRs so let us reexamine what happens in the ventricular cell in light of it. The opening of the LTCC brings a small amount of Ca^{2+} into the small dyadic space between the LTCC and the RyRs of a CRU. Because of CICR, the rise in Ca^{2+} concentration in this small space increases the probability that the RyRs will open. When an RyR does open, more Ca^{2+} flows into the dyadic space increasing the Ca^{2+} concentration that further increases the probability that other RyRs will open. This is a positive feedback loop. There are about $N \approx 100$ RyRs in a CRU. There are good reasons why it is important for RyRs to be arranged in clusters.

First, clustering increases *reliability.* Suppose that the increase of Ca^{2+} due to the opening of the L-type Ca^{2+} channel increases the probability of opening of any RyR in the cluster to increase to some value *p.* Then the probability that *none* of the *N* RyRs will open is $(1-p)^N$, or equivalently, the probability that *at least one* RyR will open is $\overline{P} \equiv 1-(1-p)^N$. From this Eq. we can calculate the number *N* needed to achieve the value of $\overline{P}$, which we call the reliability. This value of N is $N = \log(1-\overline{P})/\log(1-p)$. If $p = 0.05$, then for 90% reliability ($\overline{P} = 0.9$), $N = 45$; for 99% reliability, $N = 90$. When one RyR opens, more Ca^{2+} flows into the small dyadic space thereby increasing the probability that other RyRs in the cluster opens. Therefore, once one RyR opens then, because of CICR, more RyRs are likely to open. Even though a single RyR may have a low reliability of responding to the L-type Ca^{2+} channel opening (5%), the reliability of a large cluster containing more than 100 RyRs can be very high (>99%).

Second, clustering provides signal *amplification.* The Ca^{2+} current through a single RyR is about 0.4 pA, about twice as large as the current through the L-type Ca^{2+} channel. The total Ca^{2+} current through the 10 —30 RyRs is between 4 and 12 pA so the Ca^{2+} signal that comes through the L-type Ca^{2+} channel (0.2 pA) is amplified between 20 and 60 times. Without this large amplification, muscle contraction would not occur with the speed and magnitude necessary for pumping blood or lifting objects.

7.2.3 Ca^{2+} Waves.

Skeletal muscle and mammalian ventricular muscle cells are unusual in the extent and organization of the t-tubular system. Other cells do not have t-tubules or invaginations seen in these two muscle types. For example, mammalian atrial cells have only a sparse and poorly organized t-tubular-like system and avian ventricular cells have none. How do large cells without t-tubules or extensive invaginations get the Ca^{2+} signal into the interior of the cell far from the surface? The use of Ca^{2+} waves is widespread for transmitting Ca^{2+} signals in the cell far from the surface. Ca^{2+} waves are found in mammalian atrial cells, medaka eggs, astrocytes, as well as in mammalian ventricular cells under pathological conditions such as heart failure.

What are Ca^{2+} waves? The sequence of images in Figure 10 shows the development and propagation of two Ca^{2+} waves starting at both ends of a rat ventricular cell. In these images, the height represents the Ca^{2+} concentration. The Ca^{2+} waves look like water waves confined to a channel. Like a water wave, the Ca^{2+} wave amplitude does not change appreciably and the wave propagates at a constant speed. However, unlike a water wave, when the Ca^{2+} waves collide they annihilate each other; water waves will pass through each other unperturbed.

Figure 10. Confocal images of Ca^{2+} waves in a rat ventricular cell. Images are sequentially arranged (left to right, top to bottom). The cell is initially quiescent (1st row, left). A Ca^{2+} wave starts at the left end of the cell (1st row, right). Another Ca^{2+} wave starts at the right end (2nd row, left). The waves travel toward each other then collide (3rd row, left) and annihilate each other (3rd row, right). Unpublished data from Y. Chen-Izu, T. Bnysz, and L.T. Izu.

CICR underlies Ca^{2+} waves. Ca^{2+} released from a CRU in a muscle cell diffuses to its target protein, troponin C. When Ca^{2+} is bound to troponin C, it triggers the interaction between actin and myosin causing muscle contraction. This is the job that Ca^{2+} is doing in muscle cells. However, the Ca^{2+} that diffuses to troponin C will also diffuse to an adjacent CRU. If the Ca^{2+} concentration is high enough then Ca^{2+} release from the RyRs in this neighboring CRU will also occur. The released Ca^{2+} will diffuse yet again to another CRU and trigger further Ca^{2+} release. This chain reaction of Ca^{2+} release is the mechanism underlying Ca^{2+} waves in muscle cells. In astrocytes and eggs, the mechanism is slightly more complicated as Ca^{2+} wave generation involves both IP_3 and Ca^{2+} (see Scemes & Giaume, 2006 for review). We will limit our discussion to Ca^{2+} waves in muscle cells.

To understand the initiation and propagation of Ca^{2+} waves it is useful to use a gunpowder analogy. CRUs are analogous to piles of gunpowder. Lighting one gunpowder pile may ignite a neighboring pile if the piles are close enough ($r_{\max}$), if the thermal energy released is large enough (M), and the gunpowder is sufficiently unstable ($\tilde{c}$). The expressions in parentheses are the analogous quantities in Ca^{2+} signaling that are in Eq. . The gunpowder analogy helps to explain why colliding Ca^{2+} waves annihilate themselves. Imagine lighting a row of gunpowder piles at each end. As the burning wavefronts race toward each other, the fuel behind each front is depleted. When the wavefronts collide the waves stop since there is no more fuel. The annihilation of colliding Ca^{2+} waves occurs for similar reasons. Behind the wavefront, the SR is depleted of Ca^{2+}, which, like the depletion of fuel, precludes colliding wavefronts from progressing further. The Ca^{2+} pumps will eventually refill the SR with Ca^{2+} but this does not occur quickly enough to allow Ca^{2+} waves to pass through each other like water waves.

7.3 A Simple Model for Ca^{2+} Waves

Mathematical models of Ca^{2+} waves, which emerge from the chain reaction of Ca^{2+} release described above, are all variations of fire-diffuse-fire models (Keizer et al., 1998; Izu, et al., 2006). The firing refers to the opening of the RyRs in the CRU. We can construct a very simple model of a Ca^{2+} wave using the machinery we have already developed. This model is adapted from the model of Keizer et al, (1998).

Consider a line of CRUs spaced λ microns apart. Assume that when the *j-th* CRU fires, *M* moles of Ca^{2+} are instantaneously deposited at $x = j\lambda$. If the Ca^{2+} concentration at the adjacent CRU at $x = (j+1)\lambda$ exceeds $\tilde{c}$ then the CRU fires. The velocity of the wave equals the distance between CRUs divided by the time it

takes for the Ca^{2+} concentration at $(j+1)\lambda$ to exceed $\tilde{c}$. This time is $\tilde{t}_{-1}$ given by Eq.(We choose the time on the -1 branch because it is the smaller of the two times.) Thus the wave velocity, if a wave exists, is

$$v = \frac{\lambda}{\tilde{t}_{-1}(M, D, \lambda, \tilde{c})}$$

(11)

The argument list is included in $\tilde{t}_{-1}$ as a reminder that $\tilde{t}_{-1}$ depends on all of these parameters. The wave velocity as a function of the CRU spacing is shown in Figure 11; for $0.4\text{mm} < \lambda <$ 1μm, v behaves as $1/\lambda$. The values of M and $\tilde{c}$ used in generating this graph were based on measured parameters for mammalian ventricular CRUs. The mean CRU spacing in the plane of the z-line is between 0.6 and 1.05 μm (Chen-Izu et al., 2006, Soeller, et al., 2007). The calculated wave velocity is 182 μm/s when $\lambda = 0.6$ μm and is 62 μm/s for $\lambda = 1$ μm. The inset in Figure 11 shows that the wave velocity rapidly declines as λ approaches r_{max} = 1.17 μm. A Ca^{2+} wave will not exist for CRU spacing > r_{max} because the Ca^{2+} concentration will not exceed the threshold concentration $\tilde{c}$ at these distances.

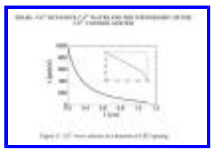

Figure 11. Ca^{2+} wave velocity as a function of CRU spacing.

7.4 Physiological Role of Ca^{2+} Waves in Cardiac Muscle

Mammalian atrial cells do not have the well-organized and extensive t-tubular system of mammalian ventricular cells. Therefore, the L-type Ca^{2+} channels on the surface membrane interact only with the CRUs that are on the periphery of the cell as shown in Figure 12. Ca^{2+} entry through the L-type Ca^{2+} channel (straight arrows in second column of CRUs) triggers Ca^{2+} release just from the CRU that lies in close apposition. The Ca^{2+} that is released diffuses inward and triggers Ca^{2+} release from the next CRU. This chain reaction continues and the Ca^{2+} wave hops from CRU to CRU. The net result is a wave of Ca^{2+} release that starts at the periphery and progresses to the center of the cell. This kind of Ca^{2+} wave has been seen in atrial cells of rats (Berlin, 1995), cats, and humans. The wave velocity ranges from about 115 to 230 μm/s. The good agreement between the calculated and the experimentally measured velocities is satisfying given the simplicity of our model for Ca^{2+} wave propagation. If the spacing of the CRUs were much larger than ~1 μm, Ca^{2+} waves would probably not exist.

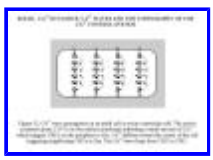

Figure 12. Ca^{2+} wave propagation in an atrial cell or avian ventricular cell. The

action potential opens LTCCs on the surface membrane admitting a small amount of Ca^{2+}, which triggers CRUs on the periphery to fire. Ca^{2+} diffuses toward the center of the cell triggering neighboring CRUs to fire. The Ca^{2+} wave hops from CRU to CRU.

Finch and pigeon ventricular myocytes, unlike their mammalian counterparts, do not have t-tubules yet their fast heart rate (5-10/s) demands that Ca^{2+} rapidly reach the center of the cell. The avian myocyte ultrastructure ensures that Ca^{2+} waves could rapidly increase the Ca^{2+} concentration in the center of the cell. The myocytes are thin (~10 μm vs. ~20 μm in mammalian heart) so the distance the Ca^{2+} wave needs to travel is small. Whereas the CRU spacing is 0.6 —1 μm in mammalian atrial and ventricular cells, the spacing in avian myocytes is ~0.2 μm. The small CRU spacing will allow the Ca^{2+} wave to propagate very rapidly as seen in Figure 11.

7.5 Pathological Spontaneous Ca^{2+} Waves in Cardiac Muscle

Ironically, Ca^{2+} waves are most extensively studied in mammalian ventricular cells that do not use Ca^{2+} waves for signal propagation. Part of the reason is technical practicality: ventricular cells are more plentiful and more robust than atrial cells for getting experimental data. (In this section all references to ventricular cells refer to mammalian ventricular cells.) The other reason is that Ca^{2+} waves do occur in ventricular cells with possibly dire consequences. There is now great interest in defining the relationship between spontaneous Ca^{2+} waves and life threatening cardiac arrhythmias. In ventricular cells Ca^{2+} waves can emerge spontaneously without being triggered by an action potential. How do Ca^{2+} waves arise spontaneously? It is useful again to return to the gunpowder analogy. Imagine now a two-dimensional array of gunpowder piles. If the piles are far enough apart, if the energy released is not sufficiently large, or the gunpowder is stable enough then one burning pile will not trigger a chain reaction. However, if several gunpowder piles in small neighborhood are lit at once then their combined energy may be enough to initiate the chain reaction.

Evidently muscle cells are stable. CRUs fire spontaneously and randomly at a low rate. These normal random firings are manifest as occasional sparks in the cell shown in Figure 7. These occasional sparks do not trigger Ca^{2+} waves. But under conditions where the sensitivity of the CRU becomes greater ($\tilde{c}$ becomes smaller), or the amount of Ca^{2+} released becomes greater (M increases), or the rate of spontaneous firing becomes greater, then there is a greater chance that several CRUs in a small neighborhood could fire almost simultaneously and cause a large enough increase in the cytoplasmic Ca^{2+} concentration in this neighborhood to initiate a Ca^{2+} wave.

Defining the kinds of pathophysiological changes that alter $\tilde{c}$, M, or spontaneous firing rates are the subject of current research. Proximate factors include altering RyR Ca^{2+} sensitivity by pH, phosphorylation, or substances like caffeine (which decreases $\tilde{c}$) and increasing the amount of Ca^{2+} in the SR (which increases M, decreases $\tilde{c}$, and increases spontaneous firing rate). Distal causes include increase in catecholamine levels due to stress, exercise, or disease (such as heart failure) and acidosis due to ischemia.

The mechanism linking spontaneous Ca^{2+} waves and arrhythmias is currently believed to involve the sodium-calcium (Na^{+}-Ca^{2+}) exchanger. Part of the Ca^{2+} released from the SR during a spontaneous Ca^{2+} wave is taken back up into the SR. The remaining part is removed from the cell largely through the Na^{+}-Ca^{2+} exchanger. The Na^{+}-Ca^{2+} exchanger exchanges 3 Na^{+} ions into the cell for every Ca^{2+} ion moved out of the cell. The net positive charge gained depolarizes the cell. If the depolarization is sufficiently great then the cell will fire an action potential; this action potential is aberrant because it is not started by the pacemaker cells in the sino-atrial node. If there are enough ventricular cells having aberrant action potentials these could trigger an ectopic firing (meaning starting from the wrong part of the heart). Ectopic heartbeats are a form of arrhythmia and can lead to life-threatening ventricular fibrillation.

4.1.2 Range o Ca^{2+} Signaling

8. Ca^{2+} Oscillations

CA^{2+} DYNAMICS, CA^{2+} WAVES AND THE TOPOGRAPHY OF THE CA^{2+} CONTROL SYSTEM

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Keywords: calcium control, heart, calcium sparks, second messenger, arrhythmia, oscillation, information coding, information decoding, calcium signaling.

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8. Ca^{2+} Oscillations

Ca^{2+} oscillations are ubiquitous. They were first described in skeletal muscle cells in 1970 (Endo et al., 1970) and subsequently found in cardiac muscle, eggs (Ridgeway et al., 1977), astrocytes (see Scemes and Giaume, 2006 for review), plant guard cells, plant root hairs, green alga, and *Paramecium*.

The Ca^{2+} oscillator, like any oscillator, must have a mechanism to produce positive feedback. Positive feedback is usually provided by Ca^{2+}-induced Ca^{2+} release (CICR) from IP_3- or RyR-sensitive Ca^{2+} stores. Therefore, the same mechanism that generates Ca^{2+} waves can generate Ca^{2+} oscillations.

Mathematical models of Ca^{2+} oscillations vary in their degree of complexity but

all have a common generic basis of two components: the cytoplasmic Ca^{2+} concentration, designated x, and the Ca^{2+} concentration in the intracellular Ca^{2+} store, y. These models are sometimes called tank models wherein Ca^{2+} flows from one tank (Ca^{2+} store) to another (cytoplasm). The differential equations describing these flows are of the form

$$\frac{dx}{dt} = J_{\text{release}}(x, y, \vec{\gamma}) - J_{\text{pump}}(x, y, \vec{\gamma}) + \sigma - kx$$
$$\frac{dy}{dt} = -\alpha\left(J_{\text{release}}(x, y, \vec{\gamma}) - J_{\text{pump}}(x, y, \vec{\gamma})\right)$$

(12)

A water tank analog is shown in Figure 13. Think of the tank on the left as the intracellular store and the tank on the right as the cytoplasm. $J_{\text{release}}(x, y)$ is the outflow of Ca^{2+} from the intracellular store to the cytoplasm; $J_{\text{pump}}(x, y)$ represents the pumping of Ca^{2+} from the cytoplasm back into the intracellular store. The symmetry in the equations for x and y reflects the conservation condition for Ca^{2+}: Ca^{2+} lost by the store is gained by the cytoplasm and vice versa. The factor α is the ratio of the volume of the cytoplasm to the volume of the intracellular store. The vector $\vec{\gamma}$ is simply a collection of model parameters. σ is some kind of flow of Ca^{2+} into the cytoplasm distinct from the Ca^{2+} store. If $\sigma = 0$ then Ca^{2+} oscillations would be impossible. σ could be Ca^{2+} coming in from the plasma membrane or from IP_3-sensitive stores (see the Goldbeter model below). The term $-kx$ represents the removal of Ca^{2+} from the cytoplasm by the Na^{+}-Ca^{2+} exchanger or a plasma Ca^{2+} pump, for example.

In Figure 13 the gate (*black vertical rectangle*) controls the flow between the tanks. Note the position of the float (*black horizontal rectangle*) relative to pivot point (*circle with arrow*). In this configuration the following scenario is possible. Suppose that the water levels in the left and right tanks are steady at (x_0, y_0). Suppose a small amount of water is added to the right tank raising the level to $x_0 + \delta x$. The float rises by δx and pushes up the gate by the amount $(L_2 / L_1)\delta x$. The ratio L_2 / L_1 is called the feedback gain. As the gate is raised, more water flows into the right tank, which further raises the float (increasing δx), which further raises the gate. This is a positive feedback system. In this system it is possible that the water level in the right tank will continue to rise. Whether this will in fact occur depends on how fast the pump operates, the value of σ, and on the magnitude of the feedback gain. If the feedback gain is large enough, then the small disturbance δx will be amplified as the water level continues to rise in the right tank. In this situation the steady state (x_0, y_0) is *unstable.*

Figure 13. Water tank analog for Ca^{2+}oscillators. The flow of water from the left to right hand tanks is controlled by the gate (black vertical rectangle). As the water level in the right hand tank rises, the float (black horizontal rectangle) pushes the gate upward allowing even more water to flow setting up a positive feedback loop.

If, on the other hand, the feedback gain is not sufficiently large relative to the speed of the pump and σ, then the small disturbance δx will dissipate and the water level will return to the original value of (x_0, y_0). In this case the steady state is *stable.*

RyRs and IP_3Rs increase their probability of opening as the cytoplasmic Ca^{2+} concentration increases in a low to moderate range (0.1 to 10s of μM) but decrease at higher (~100 μM) concentrations. Within low to moderate concentration ranges then the feedback gain, $(\partial J_{\text{release}} / \partial x)$, is positive. However, if its magnitude is not sufficiently large then small disturbances (such as those produced by random Ca^{2+} sparks) will be damped out and the system is stable.

As the values of the parameters change, the steady state can become unstable. In models of the type in Eq. the system often undergoes what is called a Hopf bifurcation. Bifurcation means splitting and in a Hopf bifurcation the once stable steady state splits into two solutions: an unstable steady state and a stable oscillatory solution.

Goldbeter et al (1990) have proposed a specific example of a model Ca^{2+} control system of the type given in . In their model,

$$J_{\text{release}}(x,y) = V_{\text{m3}} \frac{y^m}{K_2^m + y^m} \cdot \frac{x^p}{K_A^p + x^p} - k_{\text{f}} y \ , \ J_{\text{pump}}(x,y) = V_{\text{m2}} \frac{x^n}{K_2^n + x^n}$$

$$\sigma = v_0 + v_1 \beta$$

(13)

J_{release} is the flow of Ca^{2+} from the store to the cytoplasm. V_{m3} is the maximal release rate (μM/s) of Ca^{2+} through, say, RyRs. K_{A} and K_2 (having units of μM) measure how sensitive the release is to the store Ca^{2+} concentration (y) and cytoplasmic Ca^{2+} concentration (x) respectively; and $k_{\text{f}} y$ represents leakage from the store not via RyRs. The exponents $m, p,$ and n (for the pump) are Hill coefficients, which measure the degree of cooperativity. The positive feedback gain, $(\partial J_{\text{release}} / \partial x)$, is critically dependent on the value of p and K_{A}. σ is the rate of flow of Ca^{2+} from IP_3-sensitive stores. In this model, $\alpha = 1$ and For RyRs, an increase in catecholamine levels (due to stress or exercise, for example) can decrease K_{A}, rendering the RyRs more sensitive to Ca^{2+}. This sensitization translates to an increase in $(\partial J_{\text{release}} / \partial x)$. Figure 14A shows what happens when K_{A} changes from 0.95 to 0.90 at $t = 5s$. When $K_{\text{A}} = 0.95$, the system is stable. However, when K_{A} changed to 0.90, the positive feedback gain became large

enough to destabilize the system and the Ca^{2+} concentration began to oscillate.

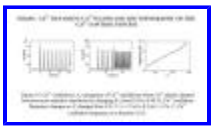

Figure 14. Ca^{2+} oscillations. A, emergence of Ca^{2+} oscillations when Ca^{2+} release channel becomes more sensitive sensitivity by changing K_A from 0.95 to 0.90. B, Ca^{2+} oscillation frequency changes as β changes from 0.3 ($5 < t < 15$ s) to 0.5 at $t > 15$ s. C, Ca^{2+} oscillation frequency as a function of β.

7.2.2 Ca^{2+} Signals Its Own Release.

8.1. Frequency Coding of External Signals

CA^{2+} DYNAMICS, CA^{2+} WAVES AND THE TOPOGRAPHY OF THE CA^{2+} CONTROL SYSTEM

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8.1. Frequency Coding of External Signals

In responding to its environment, cell must be able to sense not only whether a signal is present but also the magnitude of the signal. How is the magnitude of the external signal encoded and transmitted into the cell? One possibility that has received much attention is the use of frequency coding. In a number of cell types, the frequency of Ca^{2+} oscillations changes with the magnitude of the external signal. A classic example of this is found in the parallel increase of Ca^{2+} oscillation frequency in hepatocytes with the increasing concentration of the peptide hormone vasopressin (Woods, et al., 1986).

Vasopressin activates IP_3Rs. In Eq. σ is the flow of Ca^{2+} from IP_3-sensitive stores and β is the fraction of IP_3Rs that is activated. The model given by and was run

with increasing values of β to simulate increasing vasopressin concentration. The results are shown in Figure 14B. Between $0 < t < 5$ s, β was equal to 0.25 meaning that 25% of the IP3Rs were activated. For this β, the steady state was stable and no oscillations occurred. Between $5 < t < 10$ s, β was increased to 0.3. The steady state was no longer stable and oscillations of ~0.47 Hz are seen. From $t > 10$ s, β was increased to 0.5 and the oscillation frequency tripled to 1.43 Hz.

The relationship between β and the Ca^{2+} oscillation frequency is shown in Figure 14C. Because the relationship is monotonic, every frequency is associated with a unique β. Therefore, if the cell could somehow detect the Ca^{2+} oscillation frequency, then it would know the magnitude of the external signal. If Ca^{2+} oscillations are to have any meaning to the cell, then there must be some mechanism for decoding the information that is encoded in the oscillating signal. We now turn to the problem of decoding the Ca^{2+} signal.

8.2. Decoding Ca^{2+} Signals

Figure 15 shows a cell that has two transducers A and B that can sense external signals a and b. Activation of transducer A produces a Ca^{2+} pulse and B produces Ca^{2+} oscillations. Response elements RE_a and RE_b are both Ca^{2+} binding proteins and are supposed to respond to signals a and b, respectively. There are two aspects to the decoding problem that we might call *selectivity* and *sensitivity*. *Selectivity* refers to the ability of RE_a to respond to the Ca^{2+} signal generated by A but not to Ca^{2+} signal generated by B. *Sensitivity* refers to the ability of RE_a to respond to the Ca^{2+} signal generated by *A,* which depends on the amplitude of the Ca^{2+} signal (C_{max}), the threshold of Ca^{2+} (ε) for the *RE* response, the relative time scales of the Ca^{2+} signal (τ_{Ca}) and the *RE* response time (τ_{RE}). Let us consider the signal amplitude and sensitivity first.

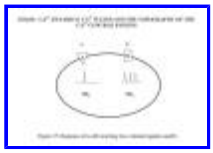

Figure 15. Response of a cell receiving two external signals a and b.

8.3. Spatial and Temporal Decoding

We know that the threshold ε and the total amount of available Ca^{2+} *M* determine the transducer signaling distance r_{max} (Eq.). If the *RE* is located within r_{max} of the transducer then it can hear the message; outside of this distance, *RE* is deaf. This simple strategy of moving the *RE* closer or farther from the transducer can confer both sensitivity and specificity. This idea is demonstrated by two important Ca^{2+} response elements: RyR and troponin C. As already noted, the RyRs are located very close (20-40 nm) to the LTCCs. Because of their close proximity, the opening of the LTCC generates a huge Ca^{2+} concentration surge (~100 μM) in the vicinity of the RyRs. Therefore, despite the low Ca^{2+} sensitivity of the RyRs, ~15 μM, they still can respond to the opening of the LTCC. Troponin C, on the other

hand, can be as far away as 1 μm (1000 nanometers) from the LTCCs. This distance is far beyond the signaling range so despite its higher Ca^{2+} sensitivity, ~1 μM, troponin C is deaf to the opening of the LTCC. RyRs, but not troponin C, respond to Ca^{2+} signals generated by the LTCCs.

Let us ask why Nature has chosen for RyRs the combination of relative insensitivity and close proximity to the LTCCs. The answer probably lies in that this combination confers stability to the Ca^{2+} signaling system. Let us see what would be the consequence if RyRs opened when the Ca^{2+} concentration reached $\tilde{c}$ =1 μM instead of 15 μM. Recall that the CRUs (clusters of RyRs) act as signal amplifiers for the LTCCs and release a much larger amount of Ca^{2+} into the cytoplasm from the ER or SR. The Ca^{2+} current through the CRU is ~10 pA compared to ~0.2 pA through the L-type Ca^{2+} channel. The time the CRUs remain open is ~5 ms so the amount of Ca^{2+} flowing through the CRU is M=2.510^{-19} mol. From Eq. we find that the signaling distance, r_{max}, is 1.2 μm when $\tilde{c}$ =15 μM but becomes 2.9 μm when $\tilde{c}$ =1 μM. Recall from Figure 8 that the spacing between the CRUs in the plane of the z-line is ~1 μm in both atrial and ventricular cells (Chen-Izu et al., 2006). This distance is close to r_{max} for $\tilde{c}$ =15 μM. Remember that r_{max} is the upper bound for the signaling distance and the actual signaling distance is somewhat shorter because of Ca^{2+} buffers. This means that when $\tilde{c}$ =15 μM, the CRUs are largely isolated from each other and incommunicado. Being incommunicado, the spontaneous opening of a CRU (generating a spark) will not activate neighboring CRUs to trigger a Ca^{2+} wave. On the other hand, if $\tilde{c}$ were 1 μM, then a spark would most likely activate a number of neighboring CRUs and trigger a Ca^{2+} wave; the system now becomes unstable. Therefore, the combination of low RyR Ca^{2+} sensitivity and close proximity to L-type Ca^{2+} channels ensures that the CRUs listen to the L-type Ca^{2+} channels but not to each other.

8.4. Temporal Decoding

Ca^{2+} signals always have a temporal component. The Ca^{2+} signal may be a single brief spike or a sustained plateau; it may be a series of spikes with varying frequency and duration. Whether meaningful information can be encoded in the temporal properties of the Ca^{2+} signal depends on whether the Ca^{2+} sensor is sensitive to temporal changes of Ca^{2+}.

Ca^{2+} sensors that respond to the temporal properties of the Ca^{2+} signal can be divided into two classes. One class is called *integrators* because the activity of the Ca^{2+} sensor is a function of the integral of the Ca^{2+} signal. An example model system of this class involves a Ca^{2+}-dependent kinase (Y) and some target protein (X) that becomes active upon phosphorylation by the kinase. We follow the analysis of Salazar et al (2008), who use a biochemical system that is representative of this class of temporal detectors. Upon binding n Ca^{2+} ions the kinase goes from the inactive (Y') to the active (Y) form

$$Y' + n\,\mathrm{Ca} \underset{\beta_Y}{\overset{\alpha_Y}{\rightleftharpoons}} Y. \qquad (14)$$

Y then changes the inactive form of the target protein (X') to the active form X

$$X' + Y \underset{\beta_x}{\overset{\alpha_x}{\rightleftharpoons}} X.$$

(15)

α_y and α_x are the forward rate constants; β_x and β_y are the reverse rate constants. Except for α_y, the rate constants have units of s^{-1}; α_y has units of $\mu M^{-n} s^{-1}$. Let $\overline{X}$ and $\overline{Y}$ be the total concentrations of target protein and kinase (active and inactive forms) and define dimensionless variables $x = X / \overline{X}$ and $y = Y / \overline{Y}$ then the time evolution of X and Y are given by

$$\frac{dy}{dt} = \alpha_Y C(t)^n (1 - y) - \beta_Y y$$
$$\frac{dx}{dt} = \alpha_x \overline{Y} y (1 - x) - \beta_x x$$

(16)

where $C(t)$ is the time-dependent Ca^{2+} concentration. Figures 16 Aa and Ab show the fluctuating Ca^{2+} concentration and the concentration of the active target protein. The temporal features of the Ca^{2+} signal are defined by the oscillation period (T), the time that the Ca^{2+} concentration is above zero (Δ), and the amplitude (S_0). The gradual rise and saw-tooth shape of $x(t)$ reflects the integration of the Ca^{2+} signal. Taking the mean value of x ($\langle x \rangle$) as a measure of sensor output, Salazar et al show that frequency discrimination occurs only when $n > 1$. In other words, a biochemical system capable of decoding information contained in the Ca^{2+} oscillation frequency must involve binding of multiple Ca^{2+} ions to the kinase Y.

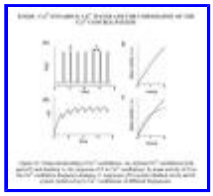

Figure 16. Temporal decoding of Ca^{2+} oscillations. Aa) stylized Ca^{2+} oscillations with period T and duration Δ. Ab) response of X to Ca^{2+} oscillations. B) mean activity of X as the Ca^{2+} oscillation frequency changes. C) responses of Fs system (dashed curve) and Si system (solid curve) to Ca^{2+} oscillations of different frequencies.

Figure 16B shows how the system decodes the information encoded in the frequency of the Ca^{2+} oscillations. The mean activity of the sensor $\langle x \rangle$ increases monotonically with the Ca^{2+} oscillation period so every $\langle x \rangle$ is uniquely associated with an oscillation frequency. We have also seen in Figure 14C that each Ca^{2+} oscillation frequency is uniquely determined by β. Therefore, there is a one-to-

one correspondence between the external signal and the sensor activity. This one-to-one correspondence means the external signal has been decoded.

8.5. Frequency Selectivity

Consider two systems $(X_{\mathrm{Si}}, Y_{\mathrm{Si}})$ and $(X_{\mathrm{Fs}}, Y_{\mathrm{Fs}})$, which participate in reactions of form and . These two systems can show frequency selectivity by a suitable choice of parameters. The subscripts indicate that the system is either ***s***low and ***i***nsensitive (*Si*) or ***f***ast and ***s***ensitive (*Fs*). The Si system is made slow relative to the Fs system by choosing $\alpha_{X,\mathrm{Si}} = \alpha_{X,\mathrm{Fs}}/10$, and $\beta_{X,\mathrm{Si}} = \beta_{X,\mathrm{Fs}}/10$ and insensitive because $K_{\mathrm{Si}} = 3\mu\mathrm{M}$ while $K_{\mathrm{Fs}} = 1$ μM. (*K* is the dissociation constant $K = (\beta_Y/\alpha_Y)^{1/n}$.)

Figure 16C shows the response of two systems to Ca^{2+} signals of varying frequencies. When the Ca^{2+} oscillation frequency is high (small oscillation period) the *Fs* system (*dashed curve*) has a higher mean activity than the *Si* system (*solid curve*). However, the opposite holds when the Ca^{2+} oscillation frequency is low. Consider two external signals σ_1 and σ_2 that induce Ca^{2+} oscillations of frequencies 2 and 0.5, respectively. When the cell is presented with σ_1, the *Fs* system is almost fully activated while the *Si* system is largely inactive. When σ_2 is present, both systems become fully active.

8.6. Parametric Resonators

Another class of biochemical Ca^{2+} oscillation decoders involves *parametric resonance* of an excitable system (described in Izu & Spangler, 1995). An excitable system is one that is stable to small disturbances but fires when the disturbance exceeds some threshold. The biological world is replete with excitable systems. The cardiac action potential is a good example. A small voltage fluctuation during diastole causes only small current flow and the membrane potential is kept close to the resting potential of -80 mV. If a perturbation, for instance a spontaneous Ca^{2+} wave induced Na^+/Ca^{2+} exchange current, depolarizes the membrane to exceed a certain threshold value (about -60 mV), however, it would open the voltage-sensitive Na^+ channels, which further depolarizes the membrane and opens even more Na^+ channels. Thus the membrane potential shoots up rapidly (within several ms) to about +60 mV; the cardiac cell has fired an action potential in response to a spontaneous Ca^{2+} wave (which is known to cause arrhythmias).

In these parametric resonators, Ca^{2+} is not a direct participant in the chemical reactions (unlike in the integrators above) but modulates parameters, such as the reaction rates. By modulating the parameters, Ca^{2+} oscillations rock the position of the steady state. The action is similar to rocking a car stuck in a small rut. By gently rocking the car back and forth at the resonant frequency the car will soon pop out of the rut. Rock the car at the wrong frequency, however, and the car will stay in the rut. Similarly, when the Ca^{2+} oscillations are at the correct frequency, the system fires.

9. Conclusion

Ca^{2+} is a universal second messenger in biological cells. Receptors on the cell membrane transduce the information carried by the external signals to cytoplasmic Ca^{2+} concentration changes. The Ca^{2+} that flows through the channels may come from the extracellular environment or from intracellular stores. The transmission of the Ca^{2+} signal in the cytosol is by diffusion and is therefore subject to the constraints imposed by the diffusion process. Large cells, such as cardiac muscle cells, overcome the limited signaling range by replicating Ca^{2+} release units throughout the cell just as telephone companies use repeaters. These repeaters allow a synchronous Ca^{2+} release throughout the mammalian ventricular myocyte in response to the opening of L-type Ca channel during action potential, and allow propagation of Ca^{2+} waves to rapidly spread the Ca^{2+} signal throughout the atrial myocyte. The Ca^{2+} induced Ca^{2+} release property also enable the cell to generate Ca^{2+} oscillations. Information can be encoded in the temporal properties of these oscillations. Biochemical sensors can decode the information carried by the Ca^{2+} oscillations and waves. Thus Ca^{2+} serves as a messenger to transmit the information carried by the external signal to the biochemical systems inside the cell to trigger cellular responses.

Acknowledgements

This work was partially funded by grants from the National Institutes of Health (K25 HL068704 and R01HL090880 to LTI, R03 AG031944 to YC) and from the American Heart Association (SDG 0335250N to YC).

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Glossary and Abbreviations

Ca^{2+} : Calcium ion. Typical resting concentration in cells is about 0.1 μM (10^{-7} M).

Ca^{2+} spark : The term Ca^{2+} spark is commonly used in two ways. It refers to the Ca^{2+} that is released from the sarcoplasmic reticulum by cluster of ryanodine receptors (RyRs). It also refers to the fluorescent image of this released Ca2+, as shown in Figure 7. When Ca2+ sparks are imaged with a high-speed confocal microscope they appear as brief, small, and bright points of increased fluorescence —like sparks! Cheng, et al. discovered Ca2+ sparks in 1993.

Ca^{2+} puffs : These are similar to Ca2+ sparks but refer to the Ca2+ released from IP3 receptors. Ca2+ puffs are broader in spatial extent and last longer than Ca2+ sparks. Parker

and Yao discovered Ca2+ puffs in 1991.

Ca^{2+}release unit (CRU): Cluster of ryanodine receptors that act in concert to release Ca^{2+} from the sarcoplasmic reticulum. The opening of this cluster of ryanodine receptors generates a Ca^{2+} spark.

Ca^{2+}-inducedCa^{2+}release (CICR): This refers to the important property possessed by RyRs, in which the probability that they will open and release Ca^{2+} from the sarcoplasmic reticulum *increases* with the Ca^{2+} concentration. Therefore, Ca2+ released by one RyR will increase the probability that a neighboring RyR will release Ca2+, thus further increasing the Ca2+ concentration and the likelihood that yet another RyR will open. This regenerative feedback is necessary to generate Ca2+ waves and Ca2+ oscillations.

Ca^{2+} wave: Propagating wave of regenerative Ca2+ release from sarcoplasmic or endoplasmic reticulum. Ca2+ waves in cardiac muscle cells travel at about 100 μm/s. An example is shown in Figure 10.

cyclicAdenine MonoPhosphate (cAMP): Discovered by Earl W. Sutherland in 1958 he later showed that the formation of cAMP is catalyzed by adenylyl cyclase when epinephrine binds to a surface receptor, which stimulates adenylyl cyclase. Based on this discovery Sutherland proposed the concept of the second messenger. Sutherland won the Nobel Prize in 1971 for his discovery of cAMP and the second messenger concept.

IP_3: Inositol 1,4,5-trisphosphate. IP_3 is a widely used second messenger derived from the hydrolysis of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate.

IP_3 receptor: The IP3 receptor is a Ca2+ channel that, like the ryanodine receptor, is located on the sarcoplasmic or endoplasmic reticulum (see below). The opening of the IP3 receptor is regulated by binding to IP3 and, in some isoforms, by Ca2+.

L-typeCa^{2+}channel (LTCC): There are many types of voltage-dependent Ca2+ channels (channels that selectively admits Ca2+ over other ions). The LTCC is the most prominent one in ventricular myocytes while T-type Ca2+ channels play an important role in atrial myocytes and atrial pacemaker cells. The L designation comes from the long time course of the channel opening while the T designation comes from the more transient time course of channel opening. Other voltage-gated Ca2+ channels are P-type, Q-type, and N-type.

Ryanodine receptor (RyR): Channel that controls Ca^{2+} release from the

sarcoplasmic reticulum membrane. It derives its name from its ability to bind the toxin ryanodine from the plant *Ryania speciosa.*

Sarcoplasmicreticulum (SR): The intracellular Ca2+ storage organelle is called the SR in muscle cells but in other cell types it is called the endoplasmic reticulum or ER. Ca2+ is released into the cytoplasm from the SR through the RyRs and Ca2+ is taken back in the SR via the ATP-dependent Ca2+ pump called SERCA.

SERCA: This is the abbreviation for Sarcoplasmic-Endoplasmic Reticulum Ca^{2+} ATPase. SERCA pumps Ca^{2+} from the cytoplasm back into the SR or ER. The energy needed to move the Ca^{2+} against the concentration gradient is provided by ATP hydrolysis.

Sodium-calciumexchanger (Na^{+}-Ca^{2+} exchanger):Ca2+ that enters the cell through Ca2+ channels such as the LTCCs must be eventually extruded from the cell lest there be a continual buildup of Ca2+ to toxic levels. The Na+-Ca2+ exchanger is one important pathway for Ca2+ extrusion. The Na+-Ca2+ exchanger moves Ca2+ from the low concentration in the cell (about 0.1 to 1 μM) to the high concentration outside the cell (about 1000 μM). The energy needed for this uphill transport is provided by coupling this flow to the downhill transport of sodium from a high concentration outside the cell (about 140 mM) to the low concentration in the cell (about 10-20 mM). Three Na+ ions move into the cell for each Ca2+ ion that moves out. This 3 1 exchange means that there is net positive flow of current into the cell, which can depolarize the cell unless countered by an opposite current flow.

Troponin-C (TnC): The troponins are a class of proteins involved in regulating muscle contraction by modulating the interaction of actin and myosin. Ca^{2+} sensitivity of muscle contraction is conferred by Ca^{2+} binding to troponin-C.

Transversetubules(t-tubules): These are invaginations of the plasma membrane in mammalian ventricular myocytes. They plunge from the cell surface to about the center of the cell. The t-tubules are studded with ion channels (sodium channels, LTCC) that allow the action potential to rapidly travel down the t-tubules and rapidly trigger Ca2+ entry through the LTCCs thereby causing synchronous Ca2+ release throughout the large myocyte. Atrial myocytes and avian ventricular myocytes do not have the dense, well-organized t-tubular system possessed by mammalian ventricular myocytes.

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Biographical Sketches

Leighton T. Izu received his BA in mathematics and psychology at the University of Hawaii at Manoa in 1979 and his PhD in biophysics from the State University of New York at Buffalo in 1990. His dissertation under the guidance of Professor Robert A. Spangler was on bifurcations in reaction-diffusion equations with nonlinear boundary conditions, which include models for Ca^{2+} oscillations in heart cells.
He is currently associate professor in Pharmacology at the University of California at Davis. His long-term interest is to understand how systems suddenly change their behavior. Of particular interest is in understanding the origin of cardiac arrhythmias. To this end, he and his collaborators combine large-scale simulations, high-speed confocal microscopy to measure Ca^{2+} dynamics, and electrophysiological methods to measure cell membrane currents and voltages.
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Ye Chen-Izu was born in 1963 at Nanjing, China. Her educational background includes Bachelor of Science degree in physics (B.S., 1985) from the National University of Defense Sciences and Technology, Changsha, China; Master of Science degree in bioengineering (M.S., 1988) from the Tsinghua University, Beijing, China; and Doctor of Philosophy degree in biophysics (Ph.D., 1994) from the State University of New York at Buffalo, New York, United States of America.
During 1995-1999, she conducted scientific research as Staff Fellow in the Laboratory of Cardiovascular Sciences, the National Institute on Aging, USA. During 1999-2004, she further

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Dr. Ye Chen-Izu is a member of professional scientific societies including Biophysical Society, American Heart Association/American Stroke Association, International Society for Heart Research, Federation of American Societies for Experimental Biology, and American Association for the Advancement of Science.

To cite this chapter

Leighton T. Izu,Tam□s B□ny□sz ,Ye Chen-Izu, (2011),CA2+ DYNAMICS, CA2+ WAVES AND THE TOPOGRAPHY OF THE CA2+ CONTROL SYSTEM, in *Mathematical Physiology*, [Eds.Andrea de Gaetano, Pasquale Palumbo],in *Encyclopedia of Life Support Systems(EOLSS),* Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford ,UK, [http://www.eolss.net] [Retrieved May 31, 2011]

8. Ca^{2+} Oscillations