Calcium Signaling

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Ionized calcium (Ca\(^{2+}\)) is the most common signal transduction element in cells ranging from bacteria to specialized neurons. Unlike many other second-messenger molecules, Ca\(^{2+}\) is required for life, yet prolonged high intracellular Ca\(^{2+}\) levels lead to cell death. Ca\(^{2+}\) cannot be metabolized like other second-messenger molecules, so cells tightly regulate intracellular levels through numerous binding and specialized extrusion proteins. Normal intracellular Ca\(^{2+}\) levels at ~100 nM are 20,000-fold lower than the 2 mM concentration found extracellularly. Scores of cellular proteins have been adapted to bind Ca\(^{2+}\) tightly, in some cases simply to buffer or lower free Ca\(^{2+}\) levels, and in others to trigger second-messenger pathways. The well-known basic elements in Ca\(^{2+}\) signal transduction have been extensively reviewed (Berridge, 1993; Carafoli, 1992; Clapham and Neer, 1993; Pozzan et al., 1994; Rhee, 1991; Tsien and Tsien, 1990). The aim of this review is to highlight recent findings in Ca\(^{2+}\) signaling and to discuss their implications.

Ca\(^{2+}\) Binding

Why is Ca\(^{2+}\) so important? Seawater magnesium (Mg\(^{2+}\)) is higher in concentration than Ca\(^{2+}\), but unlike Ca\(^{2+}\) is not excluded from the cytosol. One likely argument for the uniqueness of Ca\(^{2+}\) is that it must be maintained at low levels, since it precipitates phosphate, the established energy currency of cells. Ca\(^{2+}\) has a lower affinity for water than Mg\(^{2+}\). Cells evolved strategies for binding Ca\(^{2+}\), perhaps at first simply to reduce its cytosolic levels, but later for signal transduction.

Ca\(^{2+}\) ions are able to accommodate 4–12 oxygen atoms in their primary coordination sphere, but coordination numbers of 6–8 are most common (McPhalen et al., 1991). The artificial chelator EDTA binds divalents through its charged at most biologically relevant pH (Fasman, 1989). Coordinating oxygen atoms of the inner shell, and a third • acts

Sequestration of Ca\(^{2+}\) in the Endoplasmic Reticulum

Since mammalian cells are several micrometers thick and usually >10 \(\mu\)m in length, other Ca\(^{2+}\)-sequestering mechanisms are needed. The endoplasmic reticulum (ER) spreads like a vast three-dimensional spider web within cells, acting as a framework for Ca\(^{2+}\)-binding proteins and actively sequestering Ca\(^{2+}\) into its intraorganellar space. Ca\(^{2+}\) pumps in the ER membrane (SERCA pumps) use ATP to pump Ca\(^{2+}\) ions into the ER, where they are sequestered by high concentrations of specialized buffer molecules, such as calsequestrin, that have known trigger function but act as dynamic storage molecules for low affinity, high capacity Ca\(^{2+}\) uptake. One important question facing cell biologists is whether the high ER Ca\(^{2+}\) has a function beyond warehousing Ca\(^{2+}\). Is protein sorting conducted in this strange environment, or are there separate and dynamically rearranged ER pools? Do high free and bu-

Ca\(^{2+}\) Action Is Local

As would be expected from the number, affinity, and specificity of Ca\(^{2+}\)-binding proteins, Ca\(^{2+}\) can be an extremely localized second messenger. Ca\(^{2+}\) diffuses much more slowly than predicted simply from its ionic (~1 \(\AA\), diffusion coefficient \([D] = 1000 \mu^2/s\)) or even hydrated (\(D = 800 \mu^2/s\)) radius. It is estimated that a Ca\(^{2+}\) atom migrates no further than 0.1–0.5 \(\mu\)m, lasting only ~50 \(\mu\)S before encountering a binding protein (assuming \(10^9 M^{-1} s^{-1} K_p\) and 300 \(\mu\)M binding protein concentration [Allbritton et al., 1992]). Ca\(^{2+}\) diffusion also depends on the degree of saturation of existing Ca\(^{2+}\)-buffering proteins and varies, for example, between 15 and 65 \(\mu\)M/s (Allbritton et al., 1992). However, the image of a cell as a uniform volume for diffusion is a gross simplification, since cellular buffers are undoubtedly distributed in a functional and nonuniform manner. Furthermore, Ca\(^{2+}\) buffers may be either mobile or immobile. Around the mouth of Ca\(^{2+}\) permeant channels, Ca\(^{2+}\) ions exit the 5 \(\AA\) pore at rates exceeding 10\(^6\) ions per second and rapidly reach high concentrations in the immediate surrounding volume.
Table 1. Examples of Mammalian Proteins Triggered by Ca^{2+}

<table>
<thead>
<tr>
<th>Protein</th>
<th>Ca^{2+}-Binding Site</th>
<th>Protein Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin C</td>
<td>EF hand</td>
<td>Modulator of muscle contraction</td>
</tr>
<tr>
<td>Calmodulin</td>
<td>EF hand</td>
<td>Ubiquitous modulator of protein kinases and other enzymes (MLCK, CaM kinase II, adenyl cyclase)</td>
</tr>
<tr>
<td>Calretinin, retinin, visinin</td>
<td>EF hand</td>
<td>Activator of guanylyl cyclase</td>
</tr>
<tr>
<td>Calcineurin B</td>
<td>EF hand</td>
<td>Phosphatase</td>
</tr>
<tr>
<td>Calpain</td>
<td>EF hand</td>
<td>Protease</td>
</tr>
<tr>
<td>Inositol phospholipid-specific PLC</td>
<td>EF hand</td>
<td>Generator of InsPs and diacylglycerol</td>
</tr>
<tr>
<td>α-Actinin</td>
<td>EF hand</td>
<td>Actin-bundling protein</td>
</tr>
<tr>
<td>Annexin</td>
<td></td>
<td>Implicated in endo- and exocytosis, inhibition of PLA_2 ion channel?</td>
</tr>
<tr>
<td>Phospholipase A2</td>
<td></td>
<td>Producer of arachidonic acid</td>
</tr>
<tr>
<td>Protein kinase C</td>
<td></td>
<td>Ubiquitous protein kinase</td>
</tr>
<tr>
<td>Gelsolin</td>
<td></td>
<td>Actin-severing protein</td>
</tr>
<tr>
<td>Ca^{2+}-activated K^+ channel</td>
<td></td>
<td>Effector of membrane hyperpolarization</td>
</tr>
<tr>
<td>InsP₃ Receptor</td>
<td></td>
<td>Effector of intracellular Ca^{2+} release</td>
</tr>
<tr>
<td>Ryamodine receptor</td>
<td></td>
<td>Effector of intracellular Ca^{2+} release</td>
</tr>
<tr>
<td>Na^+ /Ca^{2+} exchanger</td>
<td></td>
<td>Effector of the exchange of Ca^{2+} for Na^+ across the plasma membrane</td>
</tr>
<tr>
<td>Ca^{2+} ATPase</td>
<td></td>
<td>Pump of Ca^{2+} across membranes</td>
</tr>
<tr>
<td>Ca^{2+} antiporters</td>
<td></td>
<td>Exchanger of Ca^{2+} for monovalent ions</td>
</tr>
<tr>
<td>BoPCAR</td>
<td></td>
<td>G protein–linked Ca^{2+}-sensing receptor</td>
</tr>
<tr>
<td>Caldesmon</td>
<td></td>
<td>Regulator of muscle contraction</td>
</tr>
<tr>
<td>Villin</td>
<td></td>
<td>Actin organizer</td>
</tr>
<tr>
<td>Arrestin</td>
<td></td>
<td>Termination of photoreceptor response</td>
</tr>
<tr>
<td>S1000</td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>Calreticulin</td>
<td></td>
<td>Ca^{2+} buffer/modulator of nuclear hormone receptor</td>
</tr>
<tr>
<td>Parvalbumin</td>
<td>EF hand</td>
<td>Ca^{2+} buffer</td>
</tr>
<tr>
<td>Calbindin</td>
<td>EF hand</td>
<td>Ca^{2+} buffer</td>
</tr>
<tr>
<td>Calsequestrin</td>
<td></td>
<td>Ca^{2+} buffer</td>
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fered concentrations of Ca^{2+} in the ER and Golgi network contribute to condensation of proteins observed in protein sorting, and if so, what happens to protein processing when stores are emptied?

Increases in Cytosolic Ca^{2+} for Signal Transduction

There are several mechanisms to introduce small bursts of Ca^{2+} into the cytosol for signal transduction. Ca^{2+} ions from the two largest Ca^{2+} sinks, the extracellular space and the ER, are injected into the cytosol either across the plasma membrane or from the ER through ion channels. There are two common motifs for Ca^{2+} signaling, illustrated in Figure 1.

Nonexcitable Cells: Overview

In nonexcitable cells such as blood cells, hepatocytes, and endothelia, the slow inositol (1,4,5)-trisphosphate (InsP₃)-mediated pathway predominates. Two receptor classes, the G protein–coupled receptor class of seven transmembrane–spanning receptors (GCRs) and the receptor tyrosine kinases (RTKs), release InsP₃ via the pathways shown in Figure 1a. GCRs activate phospholipase Cβ (PLCβ), while RTKs stimulate phospholipase Cγ (PLCγ) to convert phosphatidylinositol (4,5)-bisphosphate (PtdInsP₂) into InsP₃ and diacylglycerol (Berridge and Irvine, 1989). InsP₃ acts as an intracellular second messenger by binding to the specialized tetrameric InsP₃ receptor that spans the endoplasmic reticular membrane and triggering release of Ca^{2+} from the ER. Table 2 lists common G protein–linked and tyrosine kinase–linked receptors that stimulate intracellular increases in Ca^{2+} levels. Either of these InsP₃-mediated signal transduction pathways can increase intracellular [Ca^{2+}] from ~100 nM to ~1 μM.

Ca^{2+} can also enter nonexcitable cells by crossing the plasma membrane. Nonexcitable cells enhance Ca^{2+} entry by hyperpolarization. Open potassium (K) channels force the membrane potential to more negative potentials, drawing Ca^{2+} more rapidly across the plasma membrane. Ca^{2+} selectivity is ensured by the structure of the channel pores, which strain out all other ions.

Excitable Cells: Overview

In addition to the system described for nonexcitable cells, excitable cells contain voltage-dependent Ca^{2+} channels that enable these cells to increase cytosolic Ca^{2+} levels dramatically. Specialized Ca^{2+} trigger proteins near the plasma membrane inner surface initiate functions as diverse as exocytosis in neurons and contraction in muscle. In excitable cells, depolarization from the resting membrane potential (~−70 mV) initiates conformational changes in Ca^{2+}-selective ion channels (voltage-dependent Ca^{2+} channels) via special voltage-sensing regions (S4) of these molecules, catalyzing the flood of Ca^{2+} across the membrane. Since the forces between chemical and electrical balance for Ca^{2+} are equal at ~+150 mV, Ca^{2+} flows into the cell at all physiological membrane potentials (~90 to +60 mV). Voltage-dependent Ca^{2+} channel activity is self-limiting—the Ca^{2+} channel itself closes in a time-dependent fashion, while further depolarization only decreases the electrochemical driving force for Ca^{2+} entry.
In excitable cells (e.g., neurons), Ca\(^{2+}\) entering through voltage-dependent Ca\(^{2+}\) channels may directly activate ryano dine receptors (RYR), the excitable cell counterparts to the InsP\(_3\) receptor, to release Ca\(^{2+}\) from intracellular stores (Figure 1b). Skeletal muscle is a specialized case of this theme, in which dihydropyridine receptors on the surface of the plasma membrane and in T tubules abut the ER tetrameric RyR. Conformational changes induced by voltage in the dihydropyridine receptor result in Ca\(^{2+}\) influx and perhaps directly modulate the RyR to release Ca\(^{2+}\) from intracellular stores (McPherson and Campbell, 1993).

**G Proteins**

At least 30 seven transmembrane–spanning receptors initiate Ca\(^{2+}\) release through the activation of PLC\(\beta\) (Table 2). Experiments using pertussis toxin (which disrupts coupling between receptors and G\(_{\text{a11}}\), G\(_{\text{a14}}\), and G\(_{\text{a16}}\)) demonstrate that both pertussis toxin–sensitive and –insensitive G proteins transduce signals between receptors and PLC\(\beta\) (Sternweis and Smrcka, 1992). The most well-established path for activation of PLC\(\beta\) is through the pertussis toxin–insensitive G\(_{\text{a11}}\) (and presumably others of the family, G\(_{\text{a14}}\), G\(_{\text{a16}}\); Lee et al., 1992; Smrcka et al., 1991; Taylor and Marshall, 1992). G\(_{\text{a11}}\)-linked receptors, such as the muscarinic type 3 and serotonin 5HT1C, rapidly increase intracellular Ca\(^{2+}\). The pertussis toxin–sensitive G proteins that mediate Ca\(^{2+}\) release are less well established, but there is evidence that both G\(_{\text{a11}}\) and G\(_{\text{a14}}\) subunits are involved (Moriarty et al., 1990).

It is now firmly established that G\(_{\text{a11}}\) subunits also activate PLC\(\beta\) (Clapham and Neer, 1993; Neer, 1995 [this issue of Cell]), giving rise to speculation that pertussis toxin–insensitive PLC\(\beta\) activation may result from G\(_{\text{a11}}\) rather than G\(_{\text{a14}}\) and G\(_{\text{a16}}\). G\(_{\text{a11}}\) and G\(_{\text{a14}}\) appear to interact with separate domains of the PLC\(\beta\) molecule, implicating independent regulation by both effector arms of the heterotrimeric G protein. As yet, very little is known about the specificity of G\(_{\text{a11}}\) subunits in activating PLC\(\beta\) subtypes, except...

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**Table 2. Plasma Membrane Receptors Increasing Intracellular Ca\(^{2+}\)**

<table>
<thead>
<tr>
<th>Via PLC(\beta)</th>
<th>Via PLC(\gamma)</th>
<th>Directly</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-Adrenergic</td>
<td>Epidermal growth factor receptor</td>
<td>Nicotinic ACh channels</td>
</tr>
<tr>
<td>Muscarinic m1, m3, m5</td>
<td>Platelet-derived growth factor receptor</td>
<td>Glutamate receptor family of ion channels</td>
</tr>
<tr>
<td>Purinergic P2y P2u, P2t</td>
<td>Fibroblast growth factor receptor</td>
<td></td>
</tr>
<tr>
<td>Serotonin 5HT1C</td>
<td>ErbB2</td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>T cell receptor</td>
<td></td>
</tr>
<tr>
<td>GnRH</td>
<td>TRH</td>
<td></td>
</tr>
<tr>
<td>Glucagon</td>
<td>Cholecystokinin</td>
<td></td>
</tr>
<tr>
<td>Vasopressin V-1a, V-1b</td>
<td>Angiotensin II</td>
<td></td>
</tr>
<tr>
<td>Oxytocin</td>
<td>Thrombin</td>
<td></td>
</tr>
<tr>
<td>ANP</td>
<td>Bombesin</td>
<td></td>
</tr>
<tr>
<td>Vasoactive intestinal peptide</td>
<td>Bradykinin</td>
<td></td>
</tr>
<tr>
<td>Tachykinin</td>
<td>Thromboxanes</td>
<td></td>
</tr>
<tr>
<td>Platelet-activating factor</td>
<td>F-Met-Leu-Phe</td>
<td></td>
</tr>
<tr>
<td>Endothelin octapeptide</td>
<td>BoPACAR</td>
<td></td>
</tr>
</tbody>
</table>
specific tol (1,3,4,5)tetrakisphosphate (Ins(1,3,4,5)P4) enhances a set of enzymes mediates the generation of multiple inositol splits PtdlnsP2 into InsP3 and diacylglycerol, a complex dylinositol 4-phosphate (PtdlnsP), and PtdlnsP2. Once PLC catalyzes the hydrolysis of PtdlnsP2 to produce InsP3 and diacylglycerol. Tyrosine kinase-activated PLC increases Ca2+ more slowly and for longer durations than do G-mediated PLCs. Tyrosine kinase receptors activate PLCγ. In brief, single (and papers).

Figure 2. Potential G Protein Subunit Signaling Pathways Stimulating PLCβ
Abbreviation: PTX, pertussis toxin.

that transducin Gβγ (Gβγ) is less effective than other dimer combinations.

The fact that both αand Gβγ can modulate PLCβ greatly increases the potential complexity of signal transduction (Figure 2). Ca2+ is an important modulator of PLCβ, adenylyl cyclase, nitric oxide synthase, phospholipases, and calmodulin (CaM) kinases. Several CaM kinases are activated by Ca2+ in response to neurotransmitters and hormones. CaM kinases are characterized by the presence of a Ca2+-calmodulin binding domain and a catalytically active domain. CaM kinases are involved in a variety of cellular processes, including gene expression, cell growth, and signal transduction.

Receptor Tyrosine Kinases
Tyrosine kinase receptors activate PLCγ. In brief, single transmembrane–spanning receptor molecules, such as the platelet-derived growth factor receptor, dimerize and autophosphorylate on tyrosines upon ligand binding. The autophosphorylated tyrosines form docking sites for the PLCγ, which is a complex enzyme activated by InsP3 and diacylglycerol. Formation of docking sites is mediated by SH2 domains, bringing PLCγ into proximity with PtdlnsP2. As a result of docking, PLCγ hydrolyzes phosphatidylinositol (Ptdlns) phosphates (Berridge and Irvine, 1989). It is clear that PLCγ's unique autophosphorylation properties as cytoplasmic [Ca2+] or InsP3-induced desensitization, differing receptor isoform affinity for InsP3, InsP3 receptor distribution, and intraluminal Ca2+ regulation of the InsP3 receptor need further quantitation. There is evidence both for and against significant intraluminal control of InsP3 receptor gating (Missiaen et al., 1991; Shuttleworth, 1992). Another area of controversy has been the significance of InsP3 receptors found in the plasma membrane. Although the ER contains by far the highest number of InsP3 receptors, InsP3 receptors have also been identified in the plasma membrane (Khan et al., 1992).

The RyR (the excitable cell counterpart of the InsP3 receptor) is composed of a tetramer of four ~560 kDa subunits and is gated either by electromechanical coupling to the plasma membrane dihydropyridine receptor in skeletal muscle (see Figure 1b), by Ca2+, or by cADP-ribose in some cell types (Coronado et al., 1994; Ehrlich et al., 1994). Like the InsP3 receptor, it is modulated by Mg2+, ATP, and Ca2+, although Ca2+ and Mg2+ inhibition occurs in the mM range. Similarly, the RyR is relatively nonselective for cations, although it excludes all anions. Three separate genes (gry-1, gry-2, and gry-3) encode RyR proteins expressed predominantly in skeletal muscle, cardiac muscle, and brain or smooth muscle, respectively. FKB12, a cie-trans peptidehydrolylserase that binds the immunosuppressants FK506 and rapamycin, copurifies with RyR and modulates RyR channel opening in lipid bilayers (Brilantes et al., 1994). A newly discovered second messenger, CADP-ribose, releases Ca2+ in sea urchin eggs and may be a physiologically relevant RyR agonist in cardiac and pancreatic cells (gry-2, gry-3; Mészáros et al., 1993; Thorn et al., 1994). CADP-ribose is synthesized from nicotinamide adenine dinucleotide (NAD+) by ADP-ribose cyclase, an enzyme found in both invertebrate and mammalian cells. Interestingly, the second-messenger cyclic GMP regulates CADP-ribose levels in some cells (Galione, 1993). One mystery of neuronal Ca2+ signaling is the func-
tion of the large numbers of low affinity InsP$_3$ receptors in Purkinje cells of the cerebellum. The receptors seem to be too dense and too ineffective at Ca$^{2+}$ release to play the usual role of receptor-mediated intracellular Ca$^{2+}$ release. InsP$_3$ receptors are dense but RyRs sparse in cerebellar Purkinje and hippocampal CA1 pyramidal cells. In contrast, RyRs are dense and InsP$_3$ receptors sparse in the dentate gyrus and CA3/4 areas of the hippocampus (Sharp et al., 1993). A second area ripe for exploration is the physiological role of unique distributions of RyRs and InsP$_3$ receptors in axons, dendritic spines and shafts, and cell bodies. Electron microscopy of hippocampal showed RyR in axons, dendritic spines, and dendritic shafts near the spines, while the InsP$_3$ receptor was more prominent in dendritic shafts and cell bodies (Sharp et al., 1993).

**Ca$^{2+}$ Pumps**

Although buffers help maintain low cytosolic Ca$^{2+}$ ions, they alone cannot combat the infinite source of high Ca$^{2+}$ surrounding the cell. For this purpose, Ca$^{2+}$ pumps transport Ca$^{2+}$ ions into the ER or extracellular space at the cost of 1 to 2 ATPs per Ca$^{2+}$ ion removed. Both smooth ER (SERCA) and plasma membrane (PMCA) Ca$^{2+}$ pumps are P type ATPases (Pederson and Carafoli, 1987), defined by an obligatory phosphorylated intermediate in the pump cycle. Although both have ten putative transmembrane-spanning domains, homology between the two classes is surprisingly low. The sarcoplasmic reticulum Ca$^{2+}$ ATPase pumps are the products of three different genes, known as SERCA1, SERCA2, and SERCA3. SERCA1 pumps are exclusively expressed in fast-twitch skeletal muscle, while SERCA2 pumps are expressed in cardiac and slow-twitch skeletal muscle, and SERCA3 pumps are expressed in nonmuscle tissues (Pozzan et al., 1994). Although pharmacological tools for studying these putative ion channels have been purified or cloned, none of these putative ion channels have been purified or cloned. The most well-established pathway in this regard is I$_{CRAC-DAC}$ (Ca$^{2+}$ release-activated or depletion-activated current; for review, see Fasolato et al., 1994). I$_{CRAC}$ has an extremely low conductance (~20 fS), approximately 1,000-fold lower than the conductance of most ion channels. The net current passing through the Ca$^{2+}$ entry pathways in an entire cell is ~5 pA; in comparison, the net current activated in neurons by voltage-dependent Ca$^{2+}$ channels is hundreds of pA. I$_{CRAC}$ is activated by several experimental procedures that result in depletion of stores, probably the most reliable of which is thapsigargin, the inhibitor of the SERCA pump. I$_{CRAC}$ is highly Ca$^{2+}$-selective and, like many other Ca$^{2+}$ trigger proteins, is inactivated by high intracellular Ca$^{2+}$ levels.

Several other Ca$^{2+}$ entry pathways have been proposed but are probably of even lower density and more localized in function (Table 3). These include a Ca$^{2+}$-activated entry pathway modulated by Ins$(1,3,4,5)$P$_4$ (Lückhoff and Clapham, 1992), InsP$_3$-modulated Ca$^{2+}$ entry pathways (Kuno and Gardner, 1987; Vaca and Kunze, 1994), and a higher conductance depletion-activated entry channel found in epithelial cells (Lückhoff and Clapham, 1994). Candidate genes for the Ca$^{2+}$ entry pathways include the Drosophila gene products trp and trpl (Hardie and Minke, 1993). In Drosophila, light-induced PtdIns hydrolysis by PLC activates a Ca$^{2+}$-selective channel absent in the trp mutant. However, it is far from established that trp or trpl produce the protein responsible for I$_{CRAC}$, and no mammalian homo-
... Ca\textsuperscript{2+} entry channel. Numerous second messengers have been proposed to initiate I\textsubscript{Ca,AC}, including small G proteins, pertussis toxin–sensitive heterotrimeric G proteins, cGMP, a product of cytochrome P450 activity, various lipids, tyrosine phosphorylation, and InsP\textsubscript{3}, but to date none has been demonstrated to do so convincingly. The most interesting and controversial candidate second messenger is Ca\textsuperscript{2+} influx factor (CIF). CIF was initially isolated from Jurkat T cells stimulated to deplete their Ca\textsuperscript{2+} stores by phytohemagglutinin treatment (Randriamampita and Tsalen, 1993). It has been partially characterized as a <500 kDa phosphorylated protein whose mechanisms and purposes must be defined (Hu et al., 1994).

The second major question in the field of capacitative entry is the mechanism by which depleted stores signal the Ca\textsuperscript{2+} entry channel. Numerous second messengers have been proposed to initiate I\textsubscript{Ca,AC}, including small G proteins, pertussis toxin–sensitive heterotrimeric G proteins, cGMP, a product of cytochrome P450 activity, various lipids, tyrosine phosphorylation, and InsP\textsubscript{3}, but to date none has been demonstrated to do so convincingly. The most interesting and controversial candidate second messenger is Ca\textsuperscript{2+} influx factor (CIF). CIF was initially isolated from Jurkat T cells stimulated to deplete their Ca\textsuperscript{2+} stores by phytohemagglutinin treatment (Randriamampita and Tsalen, 1993). It has been partially characterized as a <500 kDa phosphorylated protein whose mechanisms and purposes must be defined (Hu et al., 1994).

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Calcium Signaling

Review:

Travel 15-30 μm/s. Many other complex patterns, as well as simple fluorescent dye Fluo3 (15 μM) prior to confocal imaging. The distance with InsP3 (10 μM) to evoke Ca2+ release and with the Ca2+-sensitive Figure 3. Ca2+ Waves signal transduction cascade to produce InsP3, which diffuses rapidly throughout the cell. Where [InsP3] is very high, ER Ca2+ stores release (A) Ca2+ wave observed in an intact Xenopus oocyte. Green indicates high [Ca2+] (peak level, approximately 0.5 μM). An oocyte was loaded with InsP3 (10 μM) to evoke Ca2+ release and with the Ca2+-sensitive fluorescent dye Fluo3 (15 μM) prior to confocal imaging. The distance between expanding wavefronts, or wavelength, is 250 μm, and waves travel 15-30 μm/s. Many other complex patterns, as well as simple planar waves, can be observed within the same oocyte. (B) How Ca2+ waves are generated. The bell-shaped dependence of the InsP3 receptor on [Ca2+], shown at the left side of the figure, is the key to understanding the model. The activated receptor initiates a signal transduction cascade to produce InsP3, which diffuses rapidly throughout the cell. Where [InsP3] is very high, ER Ca2+ stores release Ca2+. High [Ca2+] increases the sensitivity of InsP3 receptor to InsP3, rapidly releasing Ca2+ from stores and forming the wavefront (middle row). The very high [Ca2+] generated by Ca2+ pouring out of the InsP3 channel decreases the sensitivity of the channel to InsP3 and terminates Ca2+ release (top row, behind the Ca2+ wavefront). Full stores ahead of the diffusing Ca2+ wave (bottom row) have not yet released and are still relatively insensitive to InsP3. Ca2+ pumps replete stores and lower cytoplasmic Ca2+ levels. The model in sympathetic neurons is similar, except that RyRs replace the InsP3 receptors, and only the rising phase of the bell-shaped dependence on Ca2+ has significance. Pump activity and store depletion account for return of Ca2+ levels to normal in the neuronal model.

Ca2+ gradients within cells have been proposed to initiate cell migration, excocytosis, lymphocyte killer cell activity, acid secretion, transcellular ion transport, neurotransmitter release, gap junction regulation, and numerous other functions (Tsien and Tsien, 1990). The most complex wave patterns, exhibiting hotspots and spherical, spiral, and planar waves, were demonstrated in Xenopus oocytes (Lechleiter et al., 1991b). There appears to be insufficient space within a single 10-20 μm mammalian cell for such complex patterns, but similar patterns have been observed in larger cardiac cells and in networks of astrocytes and glia. Ca2+ signals between cells have also been identified in brain and epithelial function. Our understanding of the brain may be radically changed by observations of Ca2+ waves spreading across astrocytes and exciting overlying neuronal cells, either through gap junctions (Nedergaard, 1994) or via glutamate neurotransmission between astrocytes and neurons (Parpura et al., 1994).

Life, Death, and Ca2+

Ca2+ is essential for cell growth and survival, although its effects are so widespread that it has been difficult to pin down specific mechanisms. Ca2+ affects the cell cycle in more than one way: depletion of the InsP3 receptor–gated Ca2+ pool results in cell cycle arrest at G0/G1 and S phases, and Ca2+ is necessary and sufficient for resumption of meiosis in marine eggs; a spike of Ca2+ triggers completion of meiosis and initiation of mitosis (Means, 1994). However, our understanding of the specific mechanisms for these effects is still at an early stage. In Xenopus oocytes, resumption of meiosis is mediated by Ca2+/calmodulin stimulation of CaM kinase IIa to phosphorylate as yet unidentified targets (Lorca et al., 1993). G0 to G1 transitions in yeast and mammalian cells appear to require calcineurin, the Ca2+-dependent Ser/Thr phosphatase 2B (Means, 1994).

A more defined endpoint than cell division is activation of a specific transcription factor. For example, Ca2+ stimulates CaM kinase–dependent phosphorylation of the cAMP response element–binding protein (CREB) (Sheng et al., 1991). The local nature of Ca2+ action is again demonstrated by the finding that gene transcription depends on how Ca2+ enters the cell. Ca2+ entry through voltage-dependent L type Ca2+ channels and N-methyl-D-aspartic acid (NMDA) receptors initiates gene transcription through distinct DNA-regulatory elements (Bading et al., 1993). Not only does the route of Ca2+ entry affect which genes are transcribed, but cellular [Ca2+] levels quantitatively correlate with transcription factor expression in single cells (Negulescu et al., 1994). More surprises on the far-reaching effects of Ca2+ on gene transcription are undoubtedly in store. For example, calreticulin, a molecule previously thought to act only as a Ca2+ buffer, appears to regulate the glucocorticoid nuclear hormone receptor (Burns et al., 1994).

Intracellular Ca2+ increases initiate gene expression and cell cycle progression, but also can activate degradative processes in programmed cell death, or apoptosis. Prolonged high [Ca2+] activates nucleases that cleave DNA and degrade cell chromatin. Ca2+ promotes DNA digestion by direct stimulation of endonucleases, or indirectly by its activation of Ca2+-dependent proteases, phosphatases, and phospholipases, resulting in a loss of chromatin structural integrity (Nicotera et al., 1994). Although many loose correlations can be drawn be-
between Ca\(^{2+}\), transcriptional control, and cell division, the intervening details beg further study. In particular, a quantitative correlation between Ca\(^{2+}\) wave oscillation frequency and amplitude and transcription of a particular element would be an exciting development. Such a correlation would imply that receptors encode specific information in the frequency and amplitude of induced Ca\(^{2+}\) oscillations.

Conclusion
Evolution of molecular strategies to buffer cytosolic Ca\(^{2+}\) levels resulted in specialized Ca\(^{2+}\)-binding regions in proteins. These Ca\(^{2+}\)-binding motifs have been incorporated in many proteins, enabling Ca\(^{2+}\) to act as a triggering second-messenger element that induces conformational changes in effector molecules. Scores of receptors and ion channels use the Ca\(^{2+}\) signal to initiate events as basic as cell motility, contraction, secretion, and division. Ca\(^{2+}\) as a signal transduction element in excitable cells is controlled by depolarization of membrane potential, inducing conformational changes in Ca\(^{2+}\)-selective voltage-dependent channels. Ion channels raise cytosolic Ca\(^{2+}\) levels just under the plasma membrane within milliseconds, rapidly initiating events such as neurotransmission and muscle contraction. In nonexcitable cells, G-protein-linked receptors and receptor tyrosine kinases initiate slower Ca\(^{2+}\) release at the endoplasmic reticulum/cytosolic interface. In nonexcitable cells, these events are potentiated by hyperpolarization rather than depolarization. Complex mechanisms for Ca\(^{2+}\) release from the ER and for Ca\(^{2+}\) entry across the plasma membrane, and for sequestering released Ca\(^{2+}\), lead to an inherently oscillatory system. Whether these oscillations specify receptor or cell-specific information is one of the important questions yet to be answered in biology.

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