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Local Calcium Signaling in Neurons

Review

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Transient rises in the cytoplasmic concentration of calcium ions serve as second messenger signals that control many neuronal functions. Selective triggering of these functions is achieved through spatial localization of calcium signals. Several qualitatively different forms of local calcium signaling can be distinguished by the location of open calcium channels as well as by the distance between these channels and the calcium binding proteins that serve as the molecular targets of calcium action. Local calcium signaling is especially prominent at presynaptic active zones and postsynaptic densities, structures that are distinguished by highly organized macromolecular arrays that yield precise spatial arrangements of calcium signaling proteins. Similar forms of local calcium signaling may be employed throughout the nervous system, though much remains to be learned about the molecular underpinnings of these events.

Introduction

There is scarcely a reaction within the brain that is not regulated, directly or indirectly, by calcium ions (Ca^{2+}). Phenomena ranging in time scale from the submillisecond triggering of neurotransmitter release at presynaptic terminals to the essentially permanent changes in gene expression that occur during various forms of brain plasticity all require the second messenger actions of Ca^{2+} within neurons. Remarkably, even opposing reactions—such as the growth of neurons during development and the death of these same cells during apoptosis—can be regulated by Ca^{2+}. Given the all-pervasive and sometimes contradictory influences of Ca^{2+}, how are different Ca^{2+}-triggered reactions selectively choreographed within a neuron? The secret lies in localization: neurons have multiple mechanisms for generating intracellular Ca^{2+} signals, and the spatial proximity of these signals to the molecular targets of Ca^{2+} strongly determine the specificity of Ca^{2+} action. In fact, the highly polarized anatomy of neurons (see the review by Horton and Ehlers [2003], this issue of *Neuron*) makes nonlocalized Ca^{2+} signaling rather rare.

The goal of this article is to survey recent progress in understanding the cellular and molecular mechanisms that underlie localized signaling by Ca^{2+} within neurons. Given our own limitations of time and space, we will exclude discussion of local Ca^{2+} signaling in nonneuronal cells. Even within the realm of neuronal Ca^{2+} signaling, we will not discuss the extensive literature on local Ca^{2+} signaling during neural development or the beautiful work being done on Ca^{2+} signaling in the nucleus. We apologize for these and any other omissions and refer interested readers to other recent reviews (West et al., 2001; Spira et al., 2001; Bootman et al., 2001; Verkhratsky, 2002; Delmas and Brown, 2002; Petersen, 2002; Guatimosim et al., 2002; Deisseroth et al., 2003).

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Basic Properties of Calcium Movement Inside Neurons

Understanding of local Ca^{2+} signaling must begin with a consideration of the spatial gradients associated with the movement of Ca^{2+} within neuronal cytoplasm. Such movement arises from the passive entry of Ca^{2+} through Ca^{2+}-permeable membrane channels and the subsequent diffusion of Ca^{2+} away from these sources and into the cytoplasm. This diffusion is limited by homeostatic mechanisms, such as cytoplasmic buffers and membrane pumps and carriers, which affect the time course and magnitude of the Ca^{2+} signal. Diffusing Ca^{2+} ions encounter Ca^{2+} binding effector proteins, which we will call *Ca^{2+} sensors*, and the binding of Ca^{2+} to these sensors ultimately is responsible for the activation of physiological processes.

Given a diffusion-limited Ca^{2+} signal, the geometric relationship between Ca^{2+} channels and Ca^{2+} sensors determines the precise nature of the physiological response to this signal. Several different types of geometries have been considered; these can be distinguished according to the distance between Ca^{2+} channels and between these channels and the Ca^{2+} sensors.

The unitary Ca^{2+} signal is a *nanodomain* caused by the influx of Ca^{2+} though a single Ca^{2+} channel (Figure 1A). In order for this type of signal to encode information, the Ca^{2+} sensor must be located no more than 50 nm from the Ca^{2+} channel. The rapid release of neurotransmitters from certain presynaptic terminals appears to be due to this highly local form of Ca^{2+} signaling. Discrete clusters of Ca^{2+} channels can produce *microdomains* arising from the spatial summation of Ca^{2+} entering from multiple channels (Figure 1B). These microdomains require Ca^{2+} sensors that are placed within a fraction of a micrometer of the Ca^{2+} channel cluster, to allow detection of the summed Ca^{2+} signals. At a slightly less microscopic level, *dendritic spines* generate Ca^{2+} signals that decay over the 1 μm or so length of a spine (Figure 1C). These Ca^{2+} signals arise from the targeting of ligand-gated Ca^{2+} channels to the head of the spine, producing nanodomains or microdomains within the head, as well as the restriction of Ca^{2+} movement by the thin neck of the spine. A longer-range type of local Ca^{2+} signal is found to result from the distributed entry of Ca^{2+} into extended *dendritic segments* (Figure 1D). These signals can spread over distances of 5–500 μm. The degree of heterogeneity in dendritic Ca^{2+} entry and the elaborate branching structure of dendrites determine the magnitude and spatial range of these Ca^{2+} signals, often yielding Ca^{2+} rises with very complex spatial characteristics. The geometrically simplest form of Ca^{2+} signal arises from the radial diffusion of Ca^{2+} from a membrane surface into the cytoplasm (Figure 1E). Such *radial gradients* arise, for example, from bulk diffusion of Ca^{2+} from

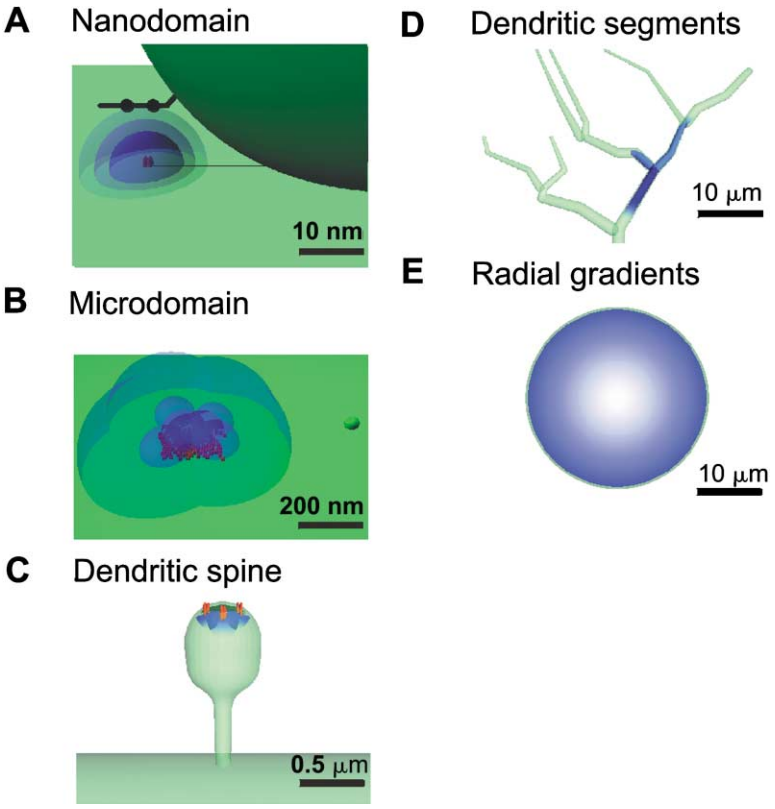

Figure 1. Multiple Spatial Dimensions of Neuronal Ca^{2+} Signaling

(A) Nanodomains arise from Ca^{2+} diffusing from a single open Ca^{2+} channel; in the case illustrated, the nanodomain triggers the fusion of a synaptic vesicle.

(B) Microdomains arise from spatial overlap of Ca^{2+} contributed by multiple Ca^{2+} channels within a fraction of a micrometer of each other. Sphere depicts a nearby synaptic vesicle.

(C) Dendritic spines can compartmentalize Ca^{2+} signals from the rest of the dendrite.

(D) Ca^{2+} signals can be restricted to segments of a dendrite.

(E) Cell-wide radial gradients of Ca^{2+} arise from long-distance diffusion of Ca^{2+} from channels at the plasma membrane.

Color scheme: light green, plasma membrane; dark green, synaptic vesicles; blue, Ca^{2+} signals; red, Ca^{2+} channels.

plasma membrane channels into the interior of a neuron's cell body or into the interior of a cylindrical dendrite. These are among the most diffuse Ca^{2+} signals found in neurons and spread over distances of micrometers.

These different geometric relationships have great impact upon the magnitude and time course of the Ca^{2+} signals that are present at a Ca^{2+} sensor. While the mathematics of Ca^{2+} diffusion can be complex (Crank, 1975), the basic rule is that the distance between Ca^{2+} sources and Ca^{2+} sensors is all important: the speed and magnitude of a Ca^{2+} signal is inversely related to this distance. As illustrated by Figure 1 of Neher (1998a), diffusion causes the tiny difference in distance between a nanodomain signal (20 nm separation between source and sensor) and a microdomain signal (200 nm separation) to generate local Ca^{2+} signals that differ approximately 10-fold in magnitude (100 μM versus 5–10 μM) and approximately 1000-fold in speed (microsecond versus millisecond time scale). These diffusional constraints impose important limitations on the speed of the physiological response of the neuron and also require Ca^{2+} sensors with binding properties that are appropriate for the local Ca^{2+} signal that are to be detected.

Experimental Evidence for Local Ca^{2+} Signaling

In practice, it is not a simple matter to establish that local Ca^{2+} signals exist or to demonstrate that such signals selectively trigger physiological reactions. We will next survey some of the experimental approaches that have been used to demonstrate these various forms of local Ca^{2+} signals.

The more macroscopic forms of local Ca^{2+} signals are sufficiently large to be imaged in living neurons via fluorescence microscopy. Radial gradients in neuronal cell bodies were among the first local Ca^{2+} signals detected (Lipscombe et al., 1988; Hernandez-Cruz et al., 1990; Eilers et al., 1995b), and, as will be described below, many types of Ca^{2+} signals in dendritic segments have now been imaged. Imaging Ca^{2+} signals in individual dendritic spines challenges the limits of many light microscopes. However, the pioneering imaging of Ca^{2+} signals in the spines of hippocampal pyramidal neurons by Muller and Connor (1991) and Guthrie et al. (1991) has motivated many subsequent studies of Ca^{2+} signals in spines (see below). One important caveat to all such imaging studies is that the fluorescent indicators used to image Ca^{2+} signals also serve as Ca^{2+} buffers and, thereby, distort the magnitude and time course of the measured signals (Neher and Augustine, 1992; Schneggenburger et al., 1993). Further, if a Ca^{2+} indicator is more mobile than the endogenous Ca^{2+} buffers of the cell, the indicator will also blur the spatial extent of the signal (Sabatini et al., 2002). Finally, use of indicators with affinities that are high relative to the magnitude of the Ca^{2+} signal under observation will lead to saturation of the indicator and cause the magnitude of the signal to be underestimated (e.g., Regehr and Atluri, 1995; Sinha et al., 1997). Thus, while it now is quite practical to use fluorescence imaging techniques to determine the presence of these macroscopic types of Ca^{2+} signals, deducing the correct spatiotemporal dynamics of these signals requires accounting for distortions that may be introduced by the indicators.

The more microscopic Ca^{2+} signals, the microdomains and nanodomains, were first postulated in the 1980s on the basis of mathematical models of Ca^{2+}

diffusion in the vicinity of open Ca^{2+} channels (Chad and Eckert, 1984; Simon and Llinas, 1985; Zucker and Fogelson, 1986). Although some success has been reported in imaging such local Ca^{2+} signals (Llinas et al., 1992; DiGregorio et al., 1999), they are largely beyond the reach of light microscopy. The spatial dimensions of these signals are a micrometer or less, which tests the resolution of light microscopes. Further, these signals can be quite rapid (microsecond to millisecond), which can outpace the temporal resolution of the cameras typically used on fluorescence microscopes as well as the response time of Ca^{2+} indicator dyes. Finally, while the concentration of Ca^{2+} can be quite high in these cases—tens or even hundreds of micromolar—the minute volumes involved mean that the total number of Ca^{2+} ions is quite small. For example, a nanodomain is associated with the flux of approximately 1000 Ca^{2+} ions through a single open Ca^{2+} channel. Such small quantities severely challenge the sensitivity of any microscope.

Given these limitations, the most localized Ca^{2+} signals are inferred from approaches less direct than imaging. One particularly useful approach has been to compare the ability of BAPTA and EGTA to block Ca^{2+}-triggered physiological responses. Although these two buffers have similar equilibrium affinities for binding Ca^{2+}, they differ in their rate of Ca^{2+} binding, with BAPTA being hundreds of times faster than EGTA. Thus, while BAPTA can bind Ca^{2+} so rapidly that it is relatively effective in buffering all sorts of Ca^{2+} signals, EGTA acts as a high-pass spatial filter that selectively spares signals that occur very locally around Ca^{2+} channels (Augustine et al., 1991; Rios and Stern, 1997; Neher, 1998b). The prototypic use of such criteria was at squid giant presynaptic terminals, where neurotransmitter release was found to be potently blocked by BAPTA but completely insensitive to EGTA (Adler et al., 1991). This indicates that nanodomain-type Ca^{2+} signals trigger transmitter release from these terminals (Neher, 1998a). This criterion has now been used to probe for local Ca^{2+} signaling in many different systems, with the usual conclusions being that high BAPTA/EGTA efficacy points toward nanodomains, and equal BAPTA/EGTA efficacy implicates microdomains or even more spatially diffuse Ca^{2+} signals. Another approach that has been used successfully to probe local Ca^{2+} signals is based on the use of Ca^{2+}-activated potassium (K^+) channels at the plasma membrane to report local Ca^{2+} levels. This technique, originally applied to hair cells by Roberts et al. (1990), has now been used in presynaptic terminals (Yazejian et al., 2000). While use of the technique is complicated by the variable sensitivity of the K^+ channels to intracellular Ca^{2+} concentration and to membrane potential, appropriate calibrations allow the estimation of local Ca^{2+} concentrations. Such efforts have yielded estimates of free Ca^{2+} concentration as high as several hundred micromolar, which are most consistent with nanodomain-type Ca^{2+} signals. A related type of bioassay has been to use rates of Ca^{2+}-dependent exocytosis to estimate the presynaptic Ca^{2+} concentrations associated with transmitter release. For example, presynaptic action potentials produce approximately 10 μM free Ca^{2+} at the calyx of Held synapse (Schneggenburger and Neher, 2000; Bollmann et al., 2000). Finally, given the rapid speed of the most highly localized Ca^{2+} signals, fast kinetics of a Ca^{2+}-dependent physiological response is a good indication of local Ca^{2+} signaling. For example, the submillisecond triggering of transmitter release from presynaptic terminals is taken as good evidence for local Ca^{2+} signaling during this process (Llinas et al., 1981; Augustine et al., 1985; Meinrenken et al. 2002).

In summary, measurement of local Ca^{2+} signals depends upon the degree of localization. The more macroscopic Ca^{2+} signals can be imaged directly, while nanodomains and microdomains are resistant to direct experimental measurement. For these highly localized signals, bioassays are usually employed, with the relative sensitivity to EGTA versus BAPTA being the most widely used diagnostic tool for local Ca^{2+} signaling.

Armed with this general appreciation of local Ca^{2+} signaling, we now consider a few examples among the stunning array of Ca^{2+} signaling events that take place within neurons. Our discussion will focus on Ca^{2+} signaling associated with synaptic transmission, a spatially discrete process that lends itself to the generation of local Ca^{2+} signals. We will first consider the Ca^{2+} signals associated with neurotransmitter release, the best-studied example of Ca^{2+} signaling in neurons (Katz, 1969), and then consider postsynaptic Ca^{2+} signaling.

Local Ca^{2+} Signaling in Presynaptic Terminals

Although there is evidence for the regulation of neurotransmitter release by Ca^{2+} released from intracellular stores (Narita et al., 1998; Cao and Peng, 1999; Emptage et al., 2001; Liang et al., 2002), the primary source of Ca^{2+} signals in presynaptic terminals is influx though voltage-gated Ca^{2+} channels. Because these channels are highly enriched in presynaptic terminals relative to the other subcellular compartments of a neuron (Stanley, 1997; Westenbroek et al., 1998; Meir et al., 1999), presynaptic Ca^{2+} signaling must be local to at least some degree. However, there appears to be varying degrees of localization across different presynaptic terminals. In this section, we will compare local Ca^{2+} signaling in various presynaptic terminals and will base this comparison on the types of Ca^{2+} signals employed.

Fast Synapses Where Nanodomains Regulate Exocytosis

As mentioned above, one of the first and most complete cases for nanodomain involvement in neuronal function has come from studies of transmitter release at the squid giant synapse. At this synapse, intracellular EGTA is completely ineffective in reducing transmitter release, even at concentrations up to 80 mM, while BAPTA completely blocks transmitter release at relatively low concentrations (Adler et al., 1991). EGTA is effective in blocking synaptic augmentation, a form of plasticity that apparently relies on delocalized presynaptic Ca^{2+} signals (Swandulla et al., 1991). Experiments using a low-affinity form of aequorin, a Ca^{2+} indicator protein, suggest that presynaptic Ca^{2+} concentrations approach hundreds of micromolar (Llinas et al., 1992), and comparison of the efficacy of Ca^{2+} buffers with differing Ca^{2+} binding affinities indicates a similar conclusion (Adler et al., 1991; Augustine et al., 1991). Elevation of presynaptic Ca^{2+} concentration via photolysis of "caged Ca^{2+}" com-

pounds reveals that a Ca^{2+} concentration of 50 μM produces rates of transmitter release much slower than those produced by a presynaptic action potential (Hsu et al., 1996). Taken together, these results indicate that transmitter release from this terminal is caused by a local Ca^{2+} signal that is 100 μM or greater in amplitude. This is consistent with nanometer-sized distances between presynaptic Ca^{2+} channels and the Ca^{2+} sensors for transmitter release, as predicted for a nanodomain form of Ca^{2+} signal. Finally, broadening presynaptic action potentials, to increase the number of open Ca^{2+} channels, causes a linear increase in transmitter release that is most easily explained by the recruitment of additional independent nanodomains (Augustine, 1990).

Similar lines of experimental data support a similar form of nanodomain Ca^{2+} signaling at presynaptic terminals of bipolar cells from goldfish retina. Here, EGTA seems to be much less effective than BAPTA in blocking exocytosis (von Gersdorff and Matthews, 1994; Burrone et al., 2002), and large (100 μM or greater) concentrations of Ca^{2+} are required to produce high rates of exocytosis (Heidelberger et al., 1994).

What these two presynaptic terminals share in common, beyond their aquatic habitats, is that both are capable of releasing transmitter at prodigious rates. At the squid synapse, approximately 50,000 quanta are released within a millisecond in response to a presynaptic action potential (Hsu et al., 1996), while bipolar terminals have release rate constants on the order of 3000 s^{-1} following a maximal rise in presynaptic Ca^{2+} concentration (Heidelberger et al., 1994; von Gersdorff et al., 1998). It makes sense to use nanodomains to trigger such rapid transmitter release: given the finite rate of Ca^{2+} diffusion within cytoplasm, only such highly localized geometry can generate Ca^{2+} signals within microseconds. Estimates of local Ca^{2+} concentration in motor nerve terminals, another presynaptic terminal with high rates of transmitter release, are consistent with the triggering of exocytosis via nanodomains (Yazejian et al., 2000). The close localization of Ca^{2+} channels with sites of transmitter release at a chick ciliary ganglion synapse also argues for nanodomain triggering of release at this synapse (Stanley, 1997).

Fast Synapses Where Microdomains Regulate Exocytosis

At other presynaptic terminals, it appears that rapid transmitter release arises from microdomain-type Ca^{2+} signals. The first such example was auditory hair cells, mechanosensory cells that release neurotransmitters. The use of Ca^{2+}-activated K^+ channels indicates Ca^{2+} concentration rises as much as several hundred micromolar when these cells are depolarized (Roberts et al., 1990), a result confirmed and extended by analysis of the actions of intracellular Ca^{2+} buffers (Roberts, 1993, 1994; Edmonds et al., 2000). Freeze-fracture electron microscopy and loose patch-clamp measurements of ion currents indicate discrete clusters of Ca^{2+} channels, with sites of exocytosis approximately 100–200 nm outside of this cluster (Roberts et al., 1990). Finally, measurements of exocytosis estimate that $[Ca^{2+}]_i$ rises to 20 μM at release sites during depolarization (Beutner et al., 2001) Taken together, these results point toward a microdomain organization for Ca^{2+} signaling of exocytosis in these cells.

Perhaps the strongest case for microdomain triggering of neurotransmitter release comes from the calyx of Held synapse in the auditory system. Here, EGTA is nearly as effective as BAPTA in blocking transmitter release, with mathematical models suggesting that dozens of Ca^{2+} channels contribute to the Ca^{2+} signal that triggers fusion of a synaptic vesicle (Borst and Sakmann, 1996; Meinrenken et al., 2002). Further, presynaptic action potentials generate local Ca^{2+} signals on the order of 10 μM, consistent with a loose spatial coupling between individual Ca^{2+} channels and the Ca^{2+} sensor for transmitter release (Schneggenburger and Neher, 2000; Bollmann et al., 2000).

Similar but more restricted experimental evidence supports microdomains being involved in triggering release from other rapid central synapses, such as those of the cerebellar parallel fiber-Purkinje cell synapse (Mintz et al., 1995) and cortical synapses (Rozov et al., 2001). For the presynaptic terminals of hippocampal Schaffer collateral synapses, the picture is less clear. Multiple types of Ca^{2+} channels appear to contribute to transmitter release, arguing for a microdomain Ca^{2+} signal (Wu and Saggau, 1994; reviewed in Dunlap et al., 1995). However, other results are more consistent with nanodomain Ca^{2+} signaling at these terminals: transmitter release increases linearly following the broadening of presynaptic action potentials (Qian and Saggau, 1999), and EGTA reportedly does not inhibit transmitter release from these synapses (Ouanounou et al., 1996). Thus, the dimensions of the presynaptic Ca^{2+} signal at these terminals are not yet clear. It is possible that there may be a range of distances between Ca^{2+} channels and sensors, as has been argued for other CNS synapses (Rozov et al., 2001; Meinrenken et al., 2002).

Thus, it appears that rapid triggering transmitter release can also arise from microdomain Ca^{2+} signals. Why use nanodomains at some synapses and microdomains at others? Presumably the contributions of a greater number of Ca^{2+} channels allows for a greater degree of regulation of microdomain Ca^{2+} signals, although this possibility has not yet received experimental scrutiny.

Presynaptic Calcium Signaling at Slow Synapses Is Likely to Be Diffuse

While study of neuronal release of slower-acting transmitters, such as catecholamines and neuropeptides, has lagged behind studies of the release of the more rapidly acting transmitters, evidence available to date indicates that release of these transmitters occurs via diffuse Ca^{2+} signals. The main evidence supporting this conclusion comes from observations that relatively low levels of cytoplasmic Ca^{2+} are required to trigger peptide release from hippocampal synaptosomes (Verhage et al., 1991) and *Aplysia* neurons (Ohnuma et al., 2001). Action potential broadening has larger effects on peptide release than on Ca^{2+} influx, as expected for the triggering of release by diffuse Ca^{2+} signals (Jackson et al., 1991). Further, peptide release seems to track volume-averaged internal Ca^{2+} concentration measurements, while release of fast-acting transmitters does not. Catecholamine release from synaptosomes exhibits intermediate behavior (Verhage et al., 1991), and similar results have been reported for catecholamine release from neuron-like PC12 cells (Kasai et al., 1996; Kishimoto et al., 2001)

and adrenal chromaffin cells (Chow et al., 1994). These results have been interpreted to indicate a looser spatial coupling between Ca^{2+} channels and Ca^{2+} sensors for vesicles containing peptides or catecholamines in comparison to vesicles containing fast-acting transmitters. Thus, it is likely that radial gradients, or microdomains positioned farther from Ca^{2+} sensors, are involved in triggering the release of these slow-acting transmitters.

In summary, local Ca^{2+} signaling is an integral part of the triggering of neurotransmitter release from presynaptic terminals. These signals vary from one synapse to another, with the speed of release being an important—but not the sole—determinant of the spatial organization of Ca^{2+} signaling.

Local Calcium Signaling in Postsynaptic Processes

Ca^{2+} plays fundamental roles in local signaling in postsynaptic processes. Changes in internal Ca^{2+} concentration in dendrites also can be localized, though postsynaptic Ca^{2+} signals have a wider spatial repertoire than those of presynaptic terminals and range from dendritic segments extending over hundreds of micrometers down to submicrometer microdomains. Dendritic Ca^{2+} signals also have more numerous sources, including entry through voltage-gated Ca^{2+} channels, entry through transmitter-gated channels, and release from intracellular stores. This section will explore several examples of local Ca^{2+} signaling in postsynaptic structures, again grouped according to the types of Ca^{2+} signal involved, and then consider their physiological functions.

Ca^{2+} Signaling in Dendritic Segments

Several different types of postsynaptic Ca^{2+} signals have been detected in dendrites and range in localization from spines to large portions of the dendritic tree.

Postsynaptic Ca^{2+} Action Potentials. One of the most-studied types arises from action potentials caused by regenerative activation of voltage-gated Ca^{2+} channels. These Ca^{2+} signals can be generated directly from synaptic excitation of dendrites or can arise from action potentials backpropagating from the soma. Except for a few unusual cases, such as climbing fiber activation of cerebellar Purkinje cells (Ross and Werman, 1987; Knopfel et al., 1991; Konnerth et al., 1992; Miyakawa et al., 1992a), generation of these *postsynaptic Ca^{2+} action potentials* (Figure 2A) typically requires the activation of multiple synapses to reach a threshold. Below this threshold, Ca^{2+} signals are graded and depend on the strength of synaptic activity (Eilers et al., 1995a; Schiller et al., 1997; Wei et al., 2001). These subthreshold signals are due to glutamate directly activating Ca^{2+} influx through NMDA-type glutamate receptors (NMDA receptors; Schiller et al., 2000; Wei et al., 2001) or due to glutamate activating AMPA-type glutamate receptors (AMPA receptors) and producing subthreshold depolarizations that activate voltage-gated Ca^{2+} channels (Eilers et al., 1995a; Miyakawa et al., 1992b). Dendritic Ca^{2+} action potentials are localized primarily to dendrites but can spread over greater distances (Schiller et al., 2000; Golding et al., 2002), including invasion of the cell body (Golding et al., 1999). Recently, it has been shown that dendritic Ca^{2+} action potentials propagate actively but

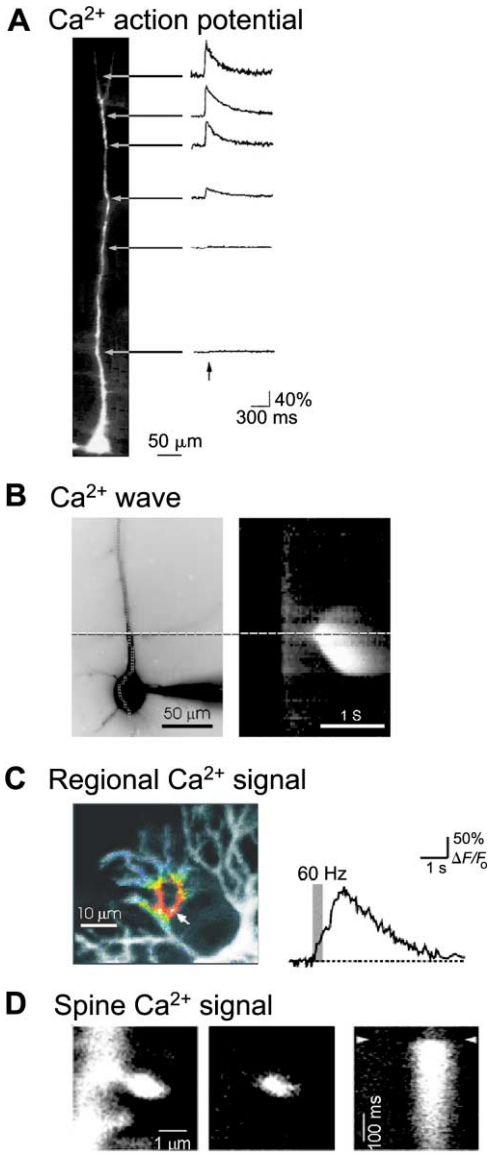

Figure 2. Examples of Postsynaptic Ca^{2+} Signaling over Different Spatial Ranges

In all cases, fluorescent Ca^{2+} indicator dyes were introduced into postsynaptic neurons and used to measure Ca^{2+} concentration dynamically over time. (A) Ca^{2+} spikes propagating along the apical axon of a cortical neuron after synaptic stimulation (from Schiller et al., 1997). Traces indicate the time course of Ca^{2+} signals recorded at different locations. (B) Ca^{2+} waves propagate for several microns in CA1 pyramidal cells. Left, image of a pyramidal cell filled with a Ca^{2+} indicator dye. Right, a line scan of Ca^{2+} concentration over time, measured along the apical dendrite, showing that a Ca^{2+} wave elicited by synaptic activity starts at a dendritic branching point (dashed line) and spreads some distance along the dendrite (from Nakamura et al., 2002). (C) Localized Ca^{2+} signaling in Purkinje cells. Left, image of a Ca^{2+} signal (amplitude encoded in pseudocolor) produced by a train of activity of presynaptic parallel fibers. Right, the synaptic Ca^{2+} response shows two temporal components (from Finch and Augustine, 1998). (D) Ca^{2+} signals evoked in a pyramidal neuron spine in response to synaptic activity. Left, two-photon image of the resting spine, with the parent dendrite on the left side of the image. Center, change in dye fluorescence produced by synaptic activity is restricted to the spine. Right, line scan across the length of the spine and across the width of the parent dendrite shows the restricted spatiotemporal dimensions of the Ca^{2+} signal (from Sabatini et al., 2002).

can stop abruptly at branching points (Wei et al., 2001). The mechanisms responsible for this abrupt termination have not yet been determined but could require a combination of changes in dendritic surface-to-volume ratio, density of voltage-gated channels, and/or an impedance mismatch due to changes in dendritic diameter. Backpropagating action potentials have been extensively studied (Jaffe et al., 1992; Yuste et al., 1994; Schiller et al., 1995; Yuste and Denk, 1995; Svoboda et al., 1997, 1999) and arise largely from the activation of voltage-gated Ca^{2+} channels (Markram et al., 1995; Larkum et al., 1999).

Ca^{2+} Waves. A second type of dendritic Ca^{2+} signal consists of *Ca^{2+} waves*. These waves arise from the propagation of intracellular Ca^{2+} release in dendrites following synaptic activity or by synergistic activity of metabotropic glutamate receptors (mGluRs) and backpropagating action potentials (Figure 2B). This type of Ca^{2+} signal has been characterized most extensively in hippocampal CA1 pyramidal neurons (Nakamura et al., 1999, 2000). In general, dendritic Ca^{2+} waves have a single site of origin and can propagate bidirectionally. When induced by synaptic activity, a Ca^{2+} wave typically spreads approximately 50 μm (Nakamura et al., 2002), a range somewhat smaller than that produced by Ca^{2+} action potentials (Schiller et al., 1997). The probability of producing a Ca^{2+} wave following synaptic activity depends upon the distance between the active synapses and the cell body: Ca^{2+} waves are reliably generated by synapses innervating proximal segments of apical dendrites ($<$100 μm from the cell body) of CA1 pyramidal cells and do not travel into the oblique dendrites (Nakamura et al., 2002). At more distal locations, synaptic activity is effective in generating Ca^{2+} waves only when backpropagating action potentials occur simultaneously (Nakamura et al., 1999). Although these waves may be confined to dendrites, in some cases they can propagate through the cell body and spread to dendrites on the other side of the cell body. The rate of wave propagation is approximately 70 μm/s (Nakamura et al., 1999), which is in line with IP3-induced Ca^{2+} waves observed in many other types of cells (for a review, see Jaffe, 1993).

The generation of a Ca^{2+} wave requires the repetitive activation of mGluRs. Production of these waves following synaptic activity has been shown to arise from a delayed increase in internal Ca^{2+} concentration that then becomes regenerative (Larkum et al., 2003). Activation of mGluRs produces the second messenger IP3, which in turn binds to IP3 receptors in the smooth endoplasmic reticulum and liberates release of Ca^{2+} from these organelles. Ca^{2+} waves of peripheral neurons are believed to arise from Ca^{2+}-induced calcium release via ryanodine receptors, another type of Ca^{2+}-releasing channel found on the smooth endoplasmic reticulum (Friel and Tsien, 1992; Usachev and Thayer, 1997). Although ryanodine receptors and IP3 receptors share a common Ca^{2+} pool in Purkinje cells (Khodakhah and Armstrong, 1997), ryanodine receptors contribute very little to Ca^{2+} waves in pyramidal neurons. In the case of hippocampal pyramidal cells, IP3 is the main intracellular signal for triggering Ca^{2+} waves, with rises in Ca^{2+} from other sources synergistically contributing to the activation of IP3 receptors by IP3 (Nakamura et al., 1999, see also Iino, 1990; Finch et al., 1991; Bezprozvanny et al., 1991).

The mechanisms underlying the localized generation of dendritic Ca^{2+} waves have not yet been determined. Key remaining questions are the regional distribution of IP3 receptors in dendrites and how the density of IP3 receptors affects the generation of Ca^{2+} waves. Propagation of Ca^{2+} waves requires local Ca^{2+} release by generation of IP3, with the diffusion of Ca^{2+} and/or IP3 leading to spreading of the wave (Wang and Thompson, 1995). Ca^{2+} buffers with different binding rates have been used to explore the range of action of the released Ca^{2+} upon downstream IP3 receptors. Early studies using EGTA and BAPTA suggested that the feedback Ca^{2+} activation of IP3 receptors was on distances of 1 μm or less; these experiments were carried out in neuroblastoma cells (Wang and Thompson, 1995). More recently, similar experiments in CA1 pyramidal cells show that these two buffers are comparable in their ability to suppress Ca^{2+} waves (Nakamura et al., 2000), suggesting that local Ca^{2+} release activates IP3 receptors over a greater spatial range than previously thought (Horne and Meyer, 1997; see review by Berridge, 1998).

Regional Ca^{2+} Signals. Synaptic activity can also result in more local, nonpropagating Ca^{2+} signals in dendrites, which we will term *regional Ca^{2+} signals*. Such signals have been measured in terminal spiny dendrites due to the activity of parallel fiber synapses on Purkinje neurons (Figure 2C; Eilers et al., 1995a; Hartell, 1996; Finch and Augustine, 1998; Takechi et al., 1998) and similar signals have been observed in CA1 pyramidal cells (Emptage et al., 1999). Synaptic activity produces regional Ca^{2+} signals both in dendritic spines and in the dendrite to which these spines are attached. What makes these signals regional is that they do not propagate through the dendritic arbor, even when synaptic activity is sufficiently strong to produce an action potential in the cell body of these neurons. These Ca^{2+} signals spread only a few micrometers from the site of stimulation and terminate abruptly, in many cases at dendritic branching points (Finch and Augustine, 1998). The amplitude of regional Ca^{2+} signals is graded and has a higher threshold than for the generation of unitary EPSPs.

Ca^{2+} signals generated by a single round of synaptic activity are rapid, rising within milliseconds and decaying within hundreds of milliseconds. These responses are due to the activation of postsynaptic AMPA receptors, with subsequent activation of an undefined type of voltage-gated Ca^{2+} channel (Eilers et al., 1995a; Hartell, 1996). However, repetitive synaptic activity can generate more Ca^{2+} signals that are more complex, consisting of two discrete temporal components (Finch and Augustine, 1998; Takechi et al., 1998). The initial, more rapid component of these signals is due to Ca^{2+} influx, via the same mechanism elicited by single rounds of synaptic activity, while the slower component is due to Ca^{2+} release from smooth endoplasmic reticulum via IP3 production by activated mGluR (Finch and Augustine, 1998; Takechi et al., 1998). The requirement for multiple rounds of synaptic activity probably is due to the nonlinear activation of IP3 receptors by IP3 (Khodakhah and Ogden, 1993) coupled with the production of a sub-

threshold quantity of IP3 by a single round of activation of mGluR (Finch and Augustine 1998).

The spatial range of regional Ca^{2+} signals in Purkinje cells is limited by the range of activation of voltage-gated Ca^{2+} channels, with IP3-mediated Ca^{2+} release being more restricted in its spatial range (Finch and Augustine, 1998; Takechi et al., 1998). In fact, IP3-mediated Ca^{2+} release can vary in spatial range from individual spines to general Ca^{2+} release in a dendritic branchlet. The pattern of synaptic activity regulates the range of IP3-mediated Ca^{2+} release (Finch and Augustine, 1998), presumably by varying the concentration of IP3 that is produced and/or by saturating dendritic Ca^{2+} buffers (Maeda et al., 1999; Schmidt et al., 2003a, 2003b). It is not yet clear why IP3-mediated Ca^{2+} release causes only regional Ca^{2+} signals in Purkinje cells but yields Ca^{2+} waves in hippocampal pyramidal neurons.

Ca^{2+} Signaling in Single Dendritic Spines

At most excitatory synapses, presynaptic terminals release glutamate onto dendritic spines and generate both electrical signals and Ca^{2+} signals in the spines. Here we will consider how the spine Ca^{2+} signals are generated in and localized to the spine.

Ca^{2+} signals in spines arise from influx through voltage-gated Ca^{2+} channels, influx though neurotransmitter receptors, and/or via release of Ca^{2+} from intracellular stores. At present, the most complete picture of spine Ca^{2+} dynamics has been obtained in pyramidal cells (Sabatini et al., 2001). Ca^{2+} sources in these spines include NMDA receptors (Kovalchuk et al., 2000; Mainen et al., 1999), voltage-gated Ca^{2+} channels (Yuste et al., 1999), and smooth endoplasmic reticulum (Emptage et al., 1999). Ca^{2+} is highly buffered in spines by a number of Ca^{2+} binding proteins, including calmodulin (Grab et al., 1979), parvalbumin, and calbindin (Schmidt et al., 2003b). Ca^{2+} is extruded by ATPase Ca^{2+} pumps in the smooth endoplasmic reticulum (Emptage et al., 1999) and/or the plasma membrane (Stauffer et al., 1995). As in most other cells, this plethora of removal mechanisms causes Ca^{2+} ions to be efficiently and rapidly removed from the spine, so that less than 1% of the entering Ca^{2+} will diffuse into the dendritic shaft (Sabatini et al., 2002). Spine Ca^{2+} signals can have a biexponential decay (Majewska et al., 2000a), perhaps due to the differential contributions of fast and slow buffers to Ca^{2+} regulation (Markram et al., 1998; Schmidt et al., 2003b). In the complex mossy fiber synapses on CA3 hippocampal pyramidal neurons (Reid et al., 2001) and neocortical neurons (Koester and Sakmann, 1998), synaptic activity also produces localized Ca^{2+} signals in spines. The Ca^{2+} sources in CA3 pyramidal neurons and in neocortical neurons are different, being influx through voltage-gated Ca^{2+} channels in the first case and influx through NMDA receptors in the second case.

Spines are morphologically heterogeneous (see Fiala and Harris, 1999) and contain a variety of intracellular organelles, including actin-based cytoskeletal elements, smooth endoplasmic reticulum, and occasionally mitochondria. In the vicinity of the synaptic membrane is a dense concentration of proteins termed the postsynaptic density (PSD). Collectively, these structures can further compartmentalize the already small volume of a spine. The spine neck serves as a limiting resistance that serves to slow diffusion between the spine and the parent dendrite (Svoboda et al., 1996). Given that spine Ca^{2+} signals are short-lived, it is widely thought that this property of the spine neck allows Ca^{2+} signaling in spines to be uncoupled from that of dendrites (Harris and Kater, 1994; Nimchinsky et al., 2002; Sabatini et al., 2002). Under some experimental conditions, diffusion of Ca^{2+} from spines into the parent dendrite can be observed (Yuste et al., 1999; Majewska et al., 2000a, 2000b), perhaps due to shuttling of Ca^{2+} by indicator dyes.

While the spine neck may limit diffusion of Ca^{2+} from the spine, longer-lived second messengers can diffuse out of the spine over physiologically relevant time scales. For example, IP3 can diffuse between spines and dendritic shafts of cerebellar Purkinje cells. The evidence supporting this conclusion is that uncaging IP3 in shafts can release Ca^{2+} in adjacent spines (Finch and Augustine, 1998) and that genetic deletion of IP3-sensitive stores in spines still allows IP3 generated in spines to release Ca^{2+} in the parent dendrite (Miyata et al., 2000). Thus, diffusional uncoupling of spines and dendrites may be unique to Ca^{2+} and is due to the rapid and virtually complete clearance of Ca^{2+} from the spine.

The tight compartmentalization of Ca^{2+} signals within spines automatically creates a geometry functionally equivalent to microdomains (Figure 1C; see also Blackstone and Sheng, 2002). In fact, spine Ca^{2+} signaling may be even more localized. The PSD is approximately 500 nm in width and contains Ca^{2+} sources, such as NMDA and AMPA receptors, as well as several Ca^{2+}-activated proteins that may serve as Ca^{2+} sensors (see below). Thus, Ca^{2+} may need to diffuse only molecular distances to activate its sensors, which would create nanodomains similar to those seen in presynaptic terminals. While few studies have directly considered this possibility, it is consistent with the results of a recent study of the differential actions of EGTA and BAPTA upon postsynaptic plasticity (Hoffman et al., 2002).

Physiological Consequences of Local Postsynaptic Ca^{2+} Signaling

The functional significance of local postsynaptic Ca^{2+} signaling is not understood as well as presynaptic Ca^{2+} signaling. Ca^{2+} clearly is an important intracellular second messenger that regulates many postsynaptic enzymes, and these, in turn, trigger modifications of synaptic strength or structure. Here we summarize several postsynaptic functions that depend upon local Ca^{2+} signaling.

Modification of Synaptic Strength. Most forms of long-term synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), require postsynaptic Ca^{2+} signals (Zucker, 1999; Bear and Linden, 2001). Given that these changes in synaptic strength arise from changes in local glutamate receptor trafficking within the spine head (Malinow and Malenka, 2002; Barry and Ziff, 2002; Duprat et al., 2003), it is likely that local Ca^{2+} signaling in the spine plays important roles in the modification of synaptic efficacy.

Perhaps the clearest example of a role for local Ca^{2+} signaling in synaptic plasticity occurs at the glutamatergic synapses between the parallel fibers and Purkinje cells of the cerebellum. At these synapses, LTD is induced when both parallel fiber synapses and climbing fiber synapses on the Purkinje cell are simultaneously

active. This form of LTD requires activation of mGluRs on spines of the parallel fiber-Purkinje cell synapse (Ito, 2001; Bear and Linden, 2001). Activation of this synapse leads to IP3 production and release of intracellular Ca^{2+} (Finch and Augustine, 1998; Takechi et al., 1998), which is necessary and sufficient to trigger LTD (Khodakhah and Armstrong, 1997; Inoue et al., 1998; Finch and Augustine, 1998; Wang et al., 2000). Both the dendrites and spines of Purkinje cells are filled with smooth endoplasmic reticulum that is studded with IP3 receptors (Takei et al., 1992; Martone et al., 1993). In mutant mice and rats lacking myosin Va, smooth endoplasmic reticulum is selectively lost from the spines of Purkinje cells and LTD is abolished (Miyata et al., 2000). This defect in LTD is due to the loss of local Ca^{2+} signaling associated with spine smooth endoplasmic reticulum, because LTD can be rescued by photolysis of caged Ca^{2+}. Thus, local release from IP3-sensitive Ca^{2+} stores in spines is required for induction of cerebellar LTD.

In hippocampal CA1 pyramidal neurons, both LTP and LTD can be induced by repetitive synaptic activity. In contrast to Purkinje cells, in these neurons the Ca^{2+} source for both forms of plasticity is mainly influx through NMDA receptors on postsynaptic spines (Sabatini et al., 2001). LTP is induced by conditions that lead to strong activation of NMDA receptors, such as a brief bout of high-frequency synaptic activity, while LTD is induced when NMDA receptors are weakly activated during prolonged low-frequency activity. Photolysis of caged Ca^{2+} indicates that LTP is caused by brief but large increases in postsynaptic Ca^{2+} concentration, while LTD arises from prolonged but smaller Ca^{2+} signals (Yang et al., 1999), indicating that different forms of Ca^{2+} signal are required for induction of these different types of long-term plasticity. Three lines of evidence suggest that local signaling in postsynaptic spines may confer this Ca^{2+} regulation. First, as mentioned above, Ca^{2+} signaling in these spines is uncoupled from that of dendrites (Sabatini et al., 2002). Second, synaptic activity causes changes in spine Ca^{2+} levels that parallel the Ca^{2+} requirements for LTD and LTP. A single weak shock stimulus causes small rises in Ca^{2+} concentration that are almost restricted entirely to individual spines (Yuste and Denk, 1995; Yuste et al., 1999; Kovalchuk et al., 2000). Repetitive weak synaptic stimulation can be used to produce LTD. In contrast, tetanic synaptic stimulation, a condition that can induce LTP, causes larger Ca^{2+} signals that spread to adjacent dendrites but are largest in spines (Petrozzino et al., 1995). Finally, comparison of the differential actions of EGTA and BAPTA indicates that the range of Ca^{2+} action is between 30 to 350 nm during theta-burst potentiation, a form of plasticity believed to share intracellular signal transduction mechanisms with LTP (Hoffman et al., 2002). Taken together, it seems likely that local Ca^{2+} signaling is important for the induction of both LTP and LTD in these neurons.

Ca^{2+} Control of Synaptic Structure. Local Ca^{2+} signaling also may play an important role in regulating postsynaptic structure. Changes in spine shape or formation or loss of entire spines has been reported to occur under a variety of conditions (Maletic-Savatic et al., 1999; Engert and Bonhoeffer, 1999; Toni et al., 1999; Dunaevsky et al., 1999; Fischer et al., 2000; Korkotian and Segal, 2001). These structural changes are thought to contribute to synaptic plasticity, both during development and in mature brain (Harris, 1999; Matus, 2000; Segal, 2001). Changes in spine structure seem to depend upon actin, a cytoskeletal protein that is concentrated in spines (Fischer et al., 1998; Halpain et al., 1998; Rao and Craig, 2000). Time-lapse imaging of GFP-tagged actin reveals that actin is highly dynamic in spines (Fischer et al., 1998), with fluorescence recovery after photobleaching indicating a turnover time of less than a minute for spine actin (Star et al., 2002). Activation of AMPA or NMDA receptors regulates actin dynamics, due to the effects of these receptors on Ca^{2+} influx into spines (Fischer et al., 2000; Star et al., 2002). Conversely, changes in structure influence the kinetics of Ca^{2+} signals within spines (Majewska et al., 2000b; Korkotian and Segal, 2000). Therefore, Ca^{2+}-induced changes in spine structure might be self-regulating. The degree of localization of the relevant Ca^{2+} signals is not yet clear for the case of mature neurons, although local Ca^{2+} signaling clearly affects dendritic structure in developing retinal ganglion cells (Lohmann et al., 2002).

In summary, although Ca^{2+} signaling certainly is important for controlling postsynaptic structure, to date there have been few attempts to define the precise role of signal localization.

Molecular Mechanisms of Local Ca^{2+} Signaling

Given that various types of Ca^{2+} signaling arise from the spatial proximity of Ca^{2+} sensors and Ca^{2+} channels, it next is necessary to define how such spatial relationships are produced. Ultimately, understanding local Ca^{2+} signaling requires definition of the molecular mechanisms that position Ca^{2+} channels and Ca^{2+} sensors at prescribed distances relative to each other. While not enough has been done to define the molecular mechanisms of targeting of Ca^{2+} signaling components, recent studies on presynaptic terminals and postsynaptic spines have shed some initial insights into this fundamental question.

Presynaptic Ca^{2+} Channel/Synaptotagmin Interaction Promotes Local Signaling

In presynaptic terminals, it is clear that voltage-gated Ca^{2+} channels serve as the main sources for Ca^{2+}, and it is likely that the vesicular protein synaptotagmin serves as the main Ca^{2+} sensor for neurotransmitter release (Augustine, 2001; Chapman, 2002; Sudhof, 2002; Koh and Bellen, 2003). How are these two types of proteins kept in close spatial proximity to create nanodomains and microdomains?

The structure of presynaptic terminals is extremely well organized into a junction known as an *active zone* (Burns and Augustine, 1995). This structure includes many of the protein and lipid components involved in exocytosis, as well as cytoskeletal elements, synaptic vesicles, and perhaps other organelles. How the active zone is assembled is not yet clear, although an important clue comes from recent observations that active zone precursors are partially assembled in advance and then transported into presynaptic terminals (Ahmari et al., 2000; Zhai et al., 2001; Shapira et al., 2003). Correct coupling of presynaptic Ca^{2+} channels to active zones apparently involves α-neurexins, integral proteins of the plasma membrane, because genetic deletion of neurex-

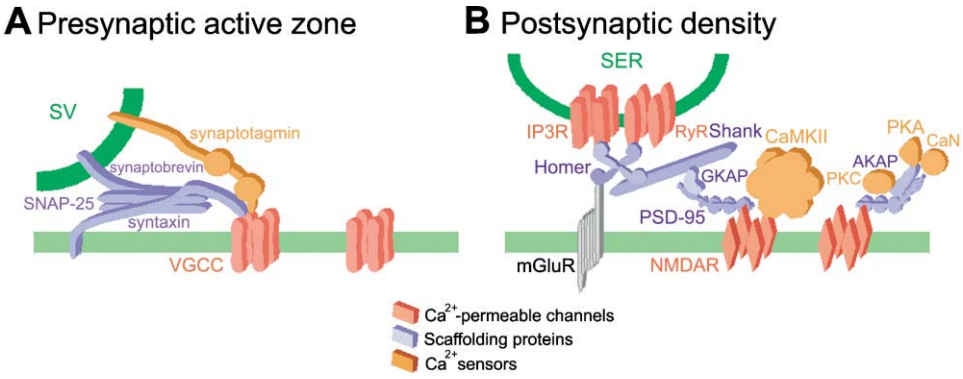

Figure 3. Molecular Mechanisms of Local Ca^{2+} Signaling in Presynaptic and Postsynaptic Compartments

Left: In presynaptic terminals, the close association of synaptotagmin and Ca^{2+} channels arises from direct binding of these two proteins to each other, as well as indirectly via SNARE proteins that bind to both. Right: In postsynaptic PSDs, Homer links mGluRs to IP3 receptors (IP3R) to allow both local IP3 signaling and local activation of Ca^{2+} sensors within the PSD. PSD-95 links NMDA receptors (NMDAR) to Ca^{2+} sensors, such as calmodulin, CaMKII, and others, as well as to Homer via a linkage mediated by Shank. Red, blue, and orange color shows Ca^{2+}-permeable channels, scaffolding proteins, and Ca^{2+} effectors, respectively. The interactions of each protein are indicated by overlapping shapes. Synaptic vesicle (SV) and smooth endoplasmic reticulum (SER) are indicated in dark green, and plasma membrane is in light green. RyR, ryanodine receptor; CaN, calcineurin. See text for other abbreviations.

ins disrupts this coupling (Missler et al., 2003). This coupling also appears to involve the interaction of SNARE proteins with Ca^{2+} channels (Mochida et al., 2003).

While the mechanisms responsible for the spatial organization of synaptotagmin relative to Ca^{2+} channels are not completely clear, interactions between these two proteins seem to play a key role (Figure 3A). Biochemical evidence indicates that Ca^{2+} channels and synaptotagmin interact with each other (reviewed in Seagar et al., 1999; Catterall, 1999; Zamponi, 2003). This interaction can be due to direct binding of Ca^{2+} channels to synaptotagmin (Martin-Moutot et al., 1993; Charvin et al., 1997; Sheng et al., 1997) or due to Ca^{2+} channels binding to SNARE proteins (Bennett et al., 1992; Abe et al., 1993; el Far et al., 1995; Kim and Catterall, 1997), which in turn bind to synaptotagmin (Shao et al., 1997; Schiavo et al., 1997; Davis et al., 1999; Zhang et al., 2002). Such protein-protein interactions should act to maintain Ca^{2+} channels within molecular distances of synaptotagmin.

Physiological evidence suggests that these interactions do serve such a function in neurotransmitter release. Microinjection of a peptide representing the site of interaction between Ca^{2+} channels and SNARE proteins inhibits transmitter release, perhaps by increasing the distance between Ca^{2+} channels and the Ca^{2+} sensor (Mochida et al., 1996; Rettig et al., 1997; Mochida et al., 1998). Conversely, disrupting the binding of synaptotagmin and/or SNAREs alters the gating properties of Ca^{2+} channels (Bezprozvanny et al., 1995; Wiser et al., 1996; Zhong et al., 1999; Bergsman and Tsien, 2000; Arien et al., 2003; Cohen et al., 2003). Thus, it appears that the close spatial relationship between Ca^{2+} channels and synaptotagmin is important for local Ca^{2+} signaling during transmitter release, although it is likely that many other protein-protein interactions are also involved in determining the highly-ordered structure of the active zone.

PSD Proteins Help Compartmentalize Postsynaptic Ca^{2+} Signaling

Local postsynaptic Ca^{2+} signaling requires that spines accumulate many signal transduction components, including Ca^{2+}-permeable channels and Ca^{2+} sensors, as well as Ca^{2+} mobilizing signals and numerous other signaling proteins. Several lines of evidence show that protein-protein interactions within the PSD assemble a complex of signaling molecules that creates conditions that are favorable for local Ca^{2+} signaling (Figure 3B). Although the composition and organization of PSD proteins has been the topic of many comprehensive reviews (see, for example, Kennedy [2000] and Sheng and Kim [2002]), here we will highlight features of PSD organization that are of special relevance for local Ca^{2+} signaling.

PSD Organizes Ca^{2+} Sources. The mechanisms involved in the localization of postsynaptic Ca^{2+} sources have been defined in some detail. The key to localization of IP3 receptors within spines appears to be a protein complex involving IP3 receptors, mGluRs, and a linking protein called Homer. Several Homer isoforms have been identified (Kato et al., 1998; Xiao et al., 1998), and all of these bind to mGluRs and to IP3 receptors (Tu et al., 1998) as well as to ryanodine receptors (Feng et al., 2002). Long isoforms of Homer can dimerize via a C-terminal coiled-coil domain. Dimerization is thought to allow these versions of Homer to serve as a link between mGluR and IP3 receptors. Homer 1a, a short form of Homer, lacks a coiled-coil domain and cannot dimerize. This isoform seems to serve as a dominant-negative by disrupting the linkage between mGluR and IP3 receptors (Xiao et al., 1998) and is capable of slowing the kinetics and reducing the magnitude of mGluR-evoked Ca^{2+} release from IP3 receptors (Tu et al., 1998). These results make clear that Homer is the central player in a protein complex that allows IP3 produced by mGluR activation to rapidly and effectively induce Ca^{2+} release from nearby IP3 receptors. This local release of Ca^{2+}, in turn, should selectively activate Ca^{2+} sensors that are targeted to the PSD (see below). Likewise, the binding of Homer to ryanodine receptors may allow local Ca^{2+}-induced Ca^{2+} release from ryanodine receptors (Lazarewicz et al., 1998; Caillard et al., 2000), although this is still unresolved as some groups have reported this type

of release (Emptage et al., 1999) while others have ruled it out (Kovalchuk et al., 2000).

NMDA receptors are very important Ca^{2+}-permeable channels and are localized to postsynaptic spines (Kennedy, 2000). Clustering of NMDA receptors in spine heads both optimizes the binding of glutamate that is released from presynaptic terminals and also yields high and localized rises in postsynaptic Ca^{2+} concentration. Several protein interactions that are involved in the clustering of NMDA receptors within the spine have been defined. Many prominent PSD proteins bind directly or indirectly to NMDA receptors and contribute to the formation of signaling complexes within the PSD (Kennedy, 2000). Among those proteins is PSD-95, which binds directly to NMDA receptors (Sheng and Pak, 2000; Garner et al., 2000; Scannevin and Huganir, 2000). PSD-95 also binds to other PSD proteins, including guanylate kinase-associated protein (GKAP), which in turn binds to Shank (Naisbitt et al., 1999). Shank interacts with Homer (Tu et al., 1999) and, like Homer, can multimerize (Naisbitt et al., 1999). Such multimerization appears to be involved in the clustering of NMDA receptors, because overexpression of Shank and Homer can recruit NMDA receptors to synapses (Sala et al., 2001). Therefore, it appears that a complex involving PSD-95, GKAP, Shank, and Homer contributes to localized Ca^{2+} influx into spines through NMDA receptors. Ca^{2+}-permeable nicotinic receptors also bind to PSD-95, and this interaction is similarly thought to localize Ca^{2+} signaling pathways (Conroy et al., 2003).

Related targeting mechanisms may contribute to local Ca^{2+} signaling at synapses that do not have PSDs. Sympathetic neurons express both M1 muscarinic and B2 bradykinin receptors, each of which are coupled to phospholipase Cβ and thereby generates IP3 (del Rio et al., 1999; Bofill-Cardona et al., 2000). However, only IP3 produced by bradykinin receptors can activate Ca^{2+} release from IP3 receptors (Cruzblanca et al., 1998; del Rio et al., 1999; Delmas et al., 2002). This privileged coupling is due to spatially restricted complexes that link bradykinin receptors to IP3 receptors (Delmas et al., 2002). While the identity of the protein that links together these components is unclear, the resulting complex forms a postsynaptic structure for Ca^{2+} mobilization (Delmas et al., 2002).

PSD Organizes Ca^{2+} Sensors and Effectors. The restricted targeting of Ca^{2+} sources to the PSD can only encode local information if Ca^{2+} sensors are positioned nearby. Several Ca^{2+} binding proteins and Ca^{2+}-activated enzymes are found in the PSD. Calmodulin, a ubiquitous Ca^{2+} sensor protein, is localized to PSDs (Grab et al., 1979). The Ca^{2+} bound form of calmodulin is necessary for the activation of many important postsynaptic enzymes, including the type II Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) and calcineurin, a protein phosphatase. Further, Ca^{2+} bound calmodulin binds to and inhibits NMDA receptor function (Ehlers et al., 1996) and IP3 receptors (Taylor and Laude, 2002).

CaMKII is the most prominent signaling protein of the PSDs. CaMKII is a target for Ca^{2+} entering through NMDA receptors and is required for induction of LTP in CA1 pyramidal neurons (Silva et al., 1992). Activation of CaMKII, by Ca^{2+}/calmodulin or via autophosphorylation, stimulates the binding of CaMKII to NMDA receptors (Strack and Colbran, 1998; Leonard et al., 2002). This binding interaction prevents autoinhibitory interactions within CaMKII and thereby locks CaMKII in an active state (Bayer et al., 2001). Such prolonged activation of CaMKII may be important for LTP induction. The location of nonactivated CaMKII is not yet clear. Rises in cytoplasmic Ca^{2+} cause CaMKII to translocate from the vicinity of the PSD to the PSD (Shen and Meyer, 1999; Dosemeci et al. 2001). However, inactive CaMKII can bind to α-actinin, an actin binding protein enriched in PSDs (Walikonis et al., 2001). Thus, some inactive CaMKII might pre-exist in PSDs by linking to the actin cytoskeleton via α-actinin binding. How CaMKII is regulated by local Ca^{2+} signaling has not yet been clarified.

Several other Ca^{2+}-dependent enzymes that are known to participate in the long-term regulation of postsynaptic function are concentrated in PSDs (Kennedy, 2000; Sheng and Kim, 2002). These enzymes include protein kinase C (PKC), protein kinase A (PKA), and calcineurin (Yamauchi, 2002). PKC, PKA, and calcineurin are localized to the PSD by associating with an anchoring protein, A-kinase anchoring protein 79 (AKAP79), that is enriched at the PSD (Coghlan et al., 1995; Klauck et al., 1996). AKAP79, in turn, associates indirectly with both AMPA and NMDA receptors (Colledge et al., 2000). Neuronal NO synthase (nNOS), which is activated by Ca^{2+}/calmodulin, interacts with PSD-95 (Hillier et al., 1999). This interaction is thought to keep nNOS close to NMDA receptors, so that Ca^{2+} entering through NMDA receptors can activate this enzyme to produce NO in certain types of postsynaptic neurons. Finally, the MAP kinase, as well as many proteins related to the MAP kinase signaling cascade, are located at the PSD (Sheng and Kim, 2002). Although the mechanisms involved in their targeting to the PSD are not yet clear, these proteins are also thought to participate in local postsynaptic Ca^{2+} signaling.

In summary, many proteins that are important for Ca^{2+} signaling are exquisitely targeted within the PSD. This arrangement suggests that spine Ca^{2+} signaling has a high degree of spatial organization. Unfortunately, we do not yet know the functional consequences of such spatial organization for postsynaptic information transfer.

Conclusions

Local Ca^{2+} signaling is an essential part of information processing in the brain. There are many forms of Ca^{2+} signaling, and the spatiotemporal dynamics of these signals encode qualitatively different types of information. While our focus has been on local Ca^{2+} signaling during synaptic transmission, it is likely that similar principles apply to other neuronal processes and to those of nonneuronal cells as well.

Within the synapse, local Ca^{2+} signals trigger numerous reactions in both presynaptic and postsynaptic elements. One common design principle that is shared by both presynaptic and postsynaptic elements is that Ca^{2+} signaling components are concentrated in multimolecular complexes that spatially link Ca^{2+} sources and Ca^{2+} sensors (Figure 3). For the case of the presynaptic terminal, this structure is the active zone, while the postsynaptic homolog is the PSD. In the case of the presynaptic terminal, the spatial organization of local Ca^{2+} signals

is clear, but our understanding of the molecular mechanisms that are responsible for the localization of Ca^{2+} signaling components within the active zone is incomplete. In postsynaptic spines, on the other hand, the binding reactions involved in the localization of Ca^{2+} signaling proteins to the PSD have been defined, but the physiological significance of these spatial relationships is not yet defined. Completing our understanding of the organization of local Ca^{2+} within these two synaptic structures is a clear goal for the immediate future.

Appreciation of the importance of active zones and PSDs as destinations for Ca^{2+} signal transduction proteins emphasizes that Ca^{2+} signaling events can occur over molecular dimensions. Because these scales are beyond the resolution of current Ca^{2+} imaging methods, it will be important to develop improved methods for probing Ca^{2+} signaling over molecular dimensions. The insights that have emerged from studies of synaptic Ca^{2+} signaling highlight the need to focus more attention on information processing at similar macromolecular signaling complexes that must occur elsewhere within neurons.

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