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TOPICAL REVIEW

THE POSTSYNAPTIC DENSITY: A SUBCELLULAR ANCHOR FOR SIGNAL TRANSDUCTION ENZYMES

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INTRODUCTION

It has been assumed for over a century that information is stored in the brain as changes in synaptic efficiency. In more molecular terms, this means that signals are transmitted from one neuron to the next by the influx of ions through channel proteins. Early on it was discovered that transmission between neurons occurred across specialized junctions called "synapses" (Fig. 1). A synapse consists of two cellular components: a presynaptic terminal on the "upstream neuron" that is adapted for the secretion of excitatory neurotransmitters such as L-glutamate, and a postsynaptic terminal on the "downstream neuron" that contains the glutamate receptor ion-channels. There are three pharmacological classes of glutamate receptor channels activated by synaptic stimulation: the NMDA (*N*-methyl-D-aspartate), AMPA (alpha-amino-3-hydroxyl-5-methyl-4-isoxazole propanoic acid) and kainate receptors [1–3], which have been named for the glutamate analogues that selectively activate them [2, 3]. Despite this pharmacological classification, two functional classes of glutamate receptors predominate: the AMPA/kainate receptors, which

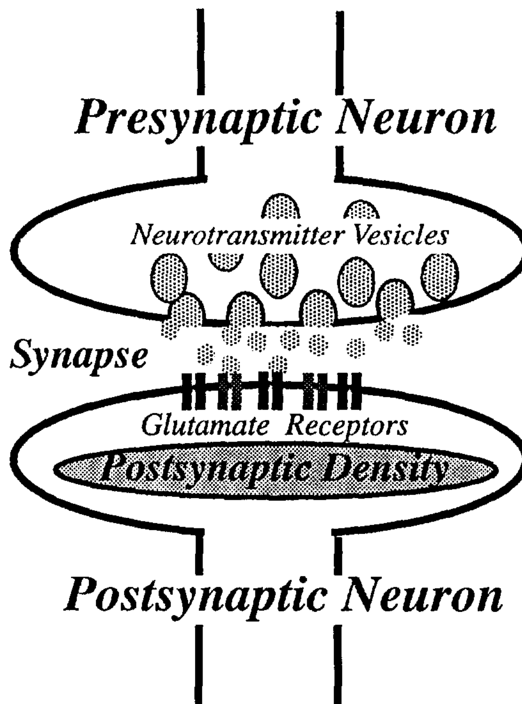

Fig. 1. A schematic diagram of the postsynaptic densities. The structure of a synapse depicting the location of presynaptic neurotransmitter vesicles, glutamate receptors in the postsynaptic membrane and the postsynaptic densities.

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have a preference for monovalent cations, and the NMDA receptors, which are calcium permeable [1]. Occupancy of either class of glutamate receptor on the postsynaptic terminal induces an inward flow of ions that triggers a variety of signal transduction events.

Lying just below the surface of the postsynaptic membrane is a proteinaceous "subsynaptic organelle" termed the "postsynaptic density" (PSD) [4, 5]. Increasing evidence suggests that the PSD orchestrates the regulation of synaptic transmission as proteins associated with the PSD are optimally positioned to respond to signals transduced across the synaptic membrane. Of particular interest has been the identification of several PSD associated proteins which are modulated by the second messengers cAMP and Ca^{2+} [5]. These include the cAMP-dependent protein kinase (PKA), the cAMP-stimulated phosphodiesterase, protein kinase C (PKC), the Ca^{2+}/calmodulin-dependent protein kinase-II (CaM-KII) and the Ca^{2+}/calmodulin-dependent phosphatase, calcineurin (CaN) [6, 7]. The scope of the mini-review is to discuss recent evidence which suggests that many of these signal transduction enzymes are anchored through protein-protein interactions at the PSD.

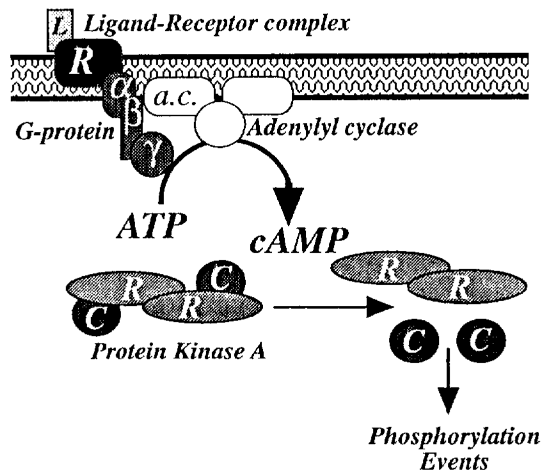

Fig. 2. The cAMP signalling pathway. This diagram indicates the subcellular location of protein components of the cAMP signalling pathway. Individual components are indicated: L, ligand; R, transmembrane receptor; α, β, γ G-protein subunits; a.c. adenylyl cyclase; R regulatory subunits of protein kinase A; C, catalytic subunits of protein kinase A.

I. Localization of the cAMP signalling pathway

The classical view of the cAMP-signaling pathway is relatively straightforward (Fig. 2). A neuropeptide or biogenic amine binds its receptor, which activates adenylyl cyclase via an intermediary G protein, and cAMP is liberated from the intracellular face of the plasma membrane [8, 9]. However, the next stages in the process require a more sophisticated level of molecular organization. First, cAMP activates the cAMP-dependent protein kinase (PKA) by binding to the regulatory subunits (R) of the dormant PKA holoenzyme to release the active catalytic subunit (C). Secondly, although PKA is a multifunctional enzyme with a broad substrate specificity, activation of the kinase somehow permits preferential phosphorylation of specific target substrates (to produce a specific response). For example, phosphorylation of the AMPA/kainate receptors modulates the flow of ions into the neurons, while phosophorylation of nuclear transcription factors (e.g., CREB) alters the expression pattern of certain genes [10–12]. One hypothesis which accounts for the selectivity of PKA action is that individual effectors preferentially activate pools of kinase anchored to neuronal structures [13]. However, enzyme anchoring may be a more widespread process as increasing evidence indicates that G proteins, adenylyl cyclase, and phosphodiesterases are also compartmentalized close to the postsynaptic densities [14–16]. Presumably, the targeting of these enzymes permits more efficient transduction of neural-specific signals.

1.1 G Proteins

Heterotrimeric guanine nucleotide binding proteins (G-proteins) are critical components in

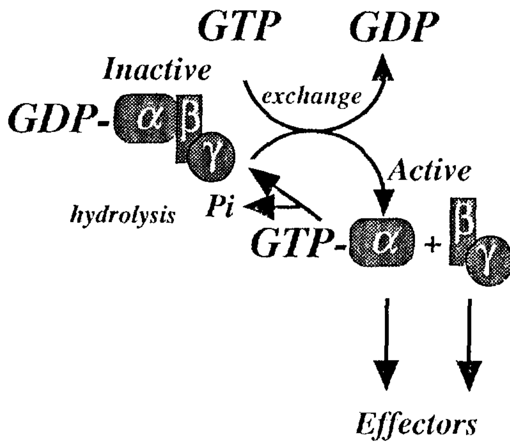

Fig. 3. The G-protein activation cycle. This figure depicts the GTP-dependent activation cycle for the heterotrimeric G-proteins.

almost all cellular signalling pathways as they transduce signals from transmembrane receptors to effector molecules located on the intracellular face of the plasma membrane. Two functional classes of G-protein exist, Gs (stimulatory) and Gi (inhibitory), which are composed of three distinct subunits, α, β and γ. So far, 21 α subunits, 4 β subunits and 6 γ subunits have been identified. These subunits combine to form a multitude of subtly different heterotrimeric forms [14, 16–19]. The currently accepted mechanism for G-protein activation involves a GTP-dependent cycle (Fig. 3). Upon receptor occupancy, GTP binds to the α subunit causing dissociation of the heterotrimeric complex. This allows the α subunit or the βγ complex to activate effector molecules such as adenylyl cyclase or ion channels [17–19]. Deactivation occurs when the GTPase activity residing in the α subunit hydrolyses its bound GTP to favour reassociation of the heterotrimeric α-GDP/βγ complex.

Clearly, the subcellular location of G-proteins is important for their action. Tolkovsky and Levitzki first proposed that β-adrenergic receptor activation of adenylyl cyclase proceeds through a collision-coupling mechanism which involves a shuttling of Gαs between the receptor and the effector [20]. However, this simple view has been contradicted by evidence of more highly organized interactions between G-proteins and effectors. Certain neuronal transmembrane receptors appear to interact with functionally distinct pools of (Gαi2). Microinjection of fluorescent G-proteins demonstrates that the α subunits diffuse relatively freely but the lateral mobility of the βγ complex is highly constrained to localized regions of the plasma membrane [14, 21, 22]. This latter finding is consistent with reports demonstrating the physical association of the βγ complex with adenylyl cyclase and the β-adrenergic receptor kinase [18, 23]. Collectively, these findings have led Rodbell and others to propose that the βγ subunits associate with the cytoskeleton through a process that involves multimers of G-proteins in a manner similar to actin polymers [16].

1.2 Adenylyl cyclase

Adenylyl cyclases (AC) catalyze the formation of cAMP from ATP and are coupled to transmembrane receptors through the heterotrimeric G-proteins. So far, cloning studies have identified ten members of the adenylyl cyclase family [24, 25]. Although it is generally considered that the Gα subunits regulate AC, certain subtypes are more efficiently regulated by the βγ complex, Ca^{2+}, PKC or through a feedback mechanism that involves PKA [15, 26–28]. The modes of regulation of various adenylyl cyclase subtypes is indicated in Table 1. The calcium-dependent ACs are predominantly expressed in the brain where the Ca^{2+}/calmodulin-stimulated type I enzyme is present at the PSD in hippocampal neurons [26–31]. Activation of the type I AC represents a critical point of crosstalk between the cAMP and Ca^{2+} signalling pathways and is believed to play a role in long-term potentiation (LTP), a mammalian model for learning and memory acquisition [32–34]. This notion is supported by gene knockout experiments in mice which demonstrate that ablation

Table 1. Regulation of adenylyl cyclase subtypes

Adenylyl cyclase sub-type	Modulators	Expression pattern in the brain
Type I	Gs-α, Ca^{2+}/calmodulin stimulation	Forebrain & Hippocampus
Type II	Gs-α, βγ, PKC stimulation	Cerebellum
Type III	Ca^{2+}/calmodulin, PKC, stimulation	Olfactory neuroepithelium
Type IV	Gs-α, stimulation Ca^{2+} inhibition	None
Type V	Gs-α, stimulation Ca^{2+} inhibition	Caudate nucleus
Type VI	Gs-α, PKA stimulation	Caudate nucleus
Type VII	Gs-α, PKC, stimulation	Cerebellum
Type VIII	Ca^{2+} stimulation	Hippocampus

Note: The regulatory properties of the type XI and X adenylyl cyclases are unknown at this present time. This table is adapted from references [15] and [26].

of the type I AC impairs learning and memory acquisition [33] and is consistent with earlier genetic evidence indicating that the *Drosophila* learning and memory gene, *rutabaga*, encodes a Ca^{2+}/calmodulin-dependent adenylyl cyclase [33, 35].

1.3 Phosphodiesterases

Cyclic nucleotide phosphodiesterases catalyze the degradation of cAMP and cGMP (reviewed by [36, 37]). So far, 30 or so distinct phosphodiesterase enzymes have been identified, which have recently been classified into seven groups on the basis of their regulation and substrate preference [37, 38] (Table 2). A functional role for PDE subtypes in neuronal processes was originally provided by studies on *Drosophila* showing that flies carrying lesions in the *dunce* gene [35], which encodes a cAMP specific type IV PDE, are sterile and exhibit aberrant learning or memory acquisition (reviewed by [39]). Several mammalian homologues of *dunce* have been isolated, and include a brain specific enzyme called RD1, which is anchored to membranes through an N-terminal 25 residue targeting domain [40–42]. However, despite a putative role for type IV enzyme in LTP, the predominant PDE in the brain is the type I enzyme which is selectively expressed in the soma and dendrites of hippocampal neurons and is attached to the PSD [7, 43, 44]. The type I PDE may represent another point of crosstalk between the cAMP and Ca^{2+} signalling pathways at the PSD as the cAMP degrading activity of the enzyme is stimulated tenfold by Ca^{2+}/calmodulin [44, 45].

1.4 Protein kinase A

The net effect of increased intracellular cAMP is the activation of a cAMP-dependent protein kinase (PKA). Shortly after the initial purification and characterization of the kinase by Krebs and colleagues, PKA was shown to be equally distributed between the soluble and particulate fractions of certain tissue types [46–50]. More recently it has been shown that the subcellular location of PKA is directed by the

Table 2. The classification of phosphodiesterases

Name	PDE gene family	Number of members
PDE1	CaM-dependent	9+
PDE2	cGMP-stimulated	2
PDE3	cGMP-inhibited	2+
PDE4	cAMP-specific	15+
PDE5	cGMP-specific	2
PDE6	Photoreceptor specific	2
PDE7	HCP-PDE	1

This table is adapted from the Nomenclature guidelines that were proposed by Beavo *et al.* [38] at the recent ASPET meeting on phosphodiesterases in Newport Beach, CA. April 1994.

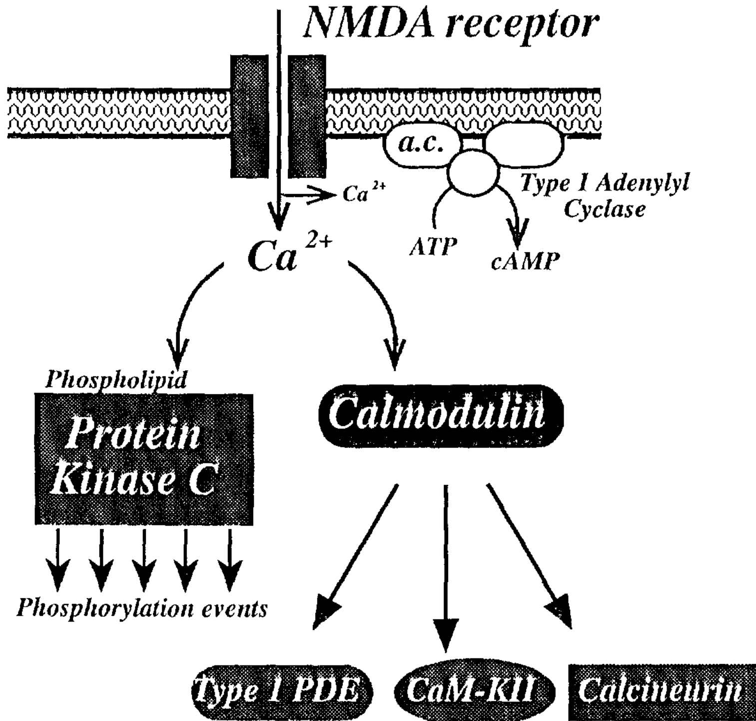

Fig. 4. The Ca^{2+} signalling pathway. An influx of Ca^{2+} through the NMDA receptors could result in the activation of several Ca^{2+} dependent enzymes when combined with other secondary messengers such as calmodulin or phospholipid.

R subunit [43, 51]. Two classes of R subunit exist; RI and RII which form the type I and type II PKA holoenzymes, respectively [47, 52, 53]. The RI isoforms (RIα and RIβ) are predominantly cytoplasmic, whereas up to 75% of the RII isoforms (RIIα and RIIβ) are particulate and associated with either the plasma membrane, cytoskeletal components, secretory granules, the golgi apparatus, centrosomes and the PSD [51, 54–62]. Targeting of PKA to these subcellular structures is mediated by association of the RII dimer with a family of A-kinase anchor proteins (AKAPs) [63–66].

Several neuronal AKAPs have been characterized [67]. Dendritic attachment of type II PKA occurs through interactions between RII and microtubule associated protein 2 [68]. RII also associates with a human calmodulin-binding protein designated AKAP79 [57]. Immunological studies and subcellular fractionation experiments have demonstrated that AKAP79 is predominantly expressed in the hippocampus and cerebral cortex where it is specifically localized to PSD [57]. Based upon these observations, we have proposed that AKAP79 functions to tether PKA to the PSD where the kinase is optimally positioned to phosphorylate transmembrane proteins such as the NMDA and AMPA/kainate receptors [13]. This hypothesis is strengthened by evidence that perfusion of cultured hippocampal neurons with peptides that block RII/AKAP interaction caused a time dependent rundown in AMPA/kainate responsive currents [69]. Presumably, PKA phosphorylation of the channel was interrupted by the displacement of the kinase from anchoring sites at the PSD. More recently, we have demonstrated that AKAP79 associates with the Ca^{2+}/calmodulin-dependent phosphatase 2B, calcineurin (CaN) and have shown that a ternary complex between PKA,

AKAP79, and CaN exists in brain and hippocampal neurons [70, 71]. These results suggest a novel model for reversible phosphorylation of proteins in the postsynaptic membrane in which the opposing effects of kinase and phosphatase action are co-localized in a signal transduction complex by association with AKAP79 [70].

2. Localization of the Ca2+ signalling pathway

Calcium serves as a key second messenger at the PSD and regulates enzymes that mediate several excitatory synaptic events such as transcription, and LTP [12, 72]. The NMDA receptors, which control neuronal calcium entry, are sequestered at the postsynaptic membrane [73, 74]. Calcium influx regulates two classes of signalling events at the PSD (Fig. 4): Ca^{2+}/phospholipid-dependent processes such as the activation of protein kinase PKC and the activation of calmodulin-dependent enzymes such as Ca^{2+}/calmodulin-dependent kinase II (CaM KII) and the Ca^{2+}/calmodulin-dependent phosphatase 2B, calcineurin (CaN) [75]. Neuronal calcium entry also influences the cAMP signalling pathway through activation of the Ca^{2+}/calmodulin-dependent type I AC and type I PDE [44, 45, 76].

2.1 Protein kinase C

The PKCs are phospholipid-dependent serine/threonine kinases that play a major role in a variety of postsynaptic events [75]. At least nine members of the PKC family have been identified that differ with respect to their tissue distribution, subcellular localization, molecular weight, substrate specificity and activation requirement [77–79]. Within the central nervous system several PKC isoforms are regionally expressed. For example, the α, β and γ isoforms are differentially compartmentalized in retinal neurons, with γ-PKC being specifically localized to the rod bipolar and amacrine cells [77]. Originally, PKC targeting was thought to occur exclusively through association with lipids and involved translocation from the soluble to the particulate fraction upon activation [78, 80]. However, it has now been shown that the active kinase is also localized through interaction with specific anchoring proteins in a phospholipid dependent manner [81–87]. Protein overlay methods and interaction cloning strategies have shown that PKC binds cytoskeletal proteins, including cytokeratins 8 and 18, β-adducin, MARCKS and PSD proteins of 110,000 M_r and 115,000 M_r [81–83, 86, 88–90]. Targeting of PKC to the PSD is likely to adapt the kinase for a specific role in the phosphorylation of postsynaptic substrates such as the NMDA receptors. This is supported by physiological evidence that treatment of hippocampal neurons with phorbol esters or microinjection of PKC induces LTP [32, 91].

2.2 CaM-Kinase II

Upon activation by the synergistic actions of Ca^{2+} and calmodulin, the multifunctional kinase, CaM-KII, mediates several neuronal events including induction of LTP and stimulation of Ca^{2+}-responsive gene transcription [92, 93]. CaM-KII phosphorylation events have long been considered a critical component of LTP and mice lacking the major neural form of CaM-KII exhibit defects in learning and memory [94, 95]. CaM-KII is a significant component of the PSD where it constitutes up to 20% of the total protein and is optimally positioned to respond to fluctuations in postsynaptic Ca^{2+} concentrations [74, 96]. The α-subunit of CaM-KII is believed to be the principal isoform present in the PSD [92, 97]. However, recent reports imply that a novel CaM-KII isoform may also be present [34]. Unlike PKA and PKC which appear to be attached to the PSD through association with anchoring proteins, CaM-KII may be a structural component of the PSD. The precise mechanism of CaM-KII attachment to the PSD is unclear. Recently, a 190,000 M_r PSD protein called p190, which binds to the autophosphorylated Cam-KII with sub micromolar affinity, has been identified by overlay techniques. This

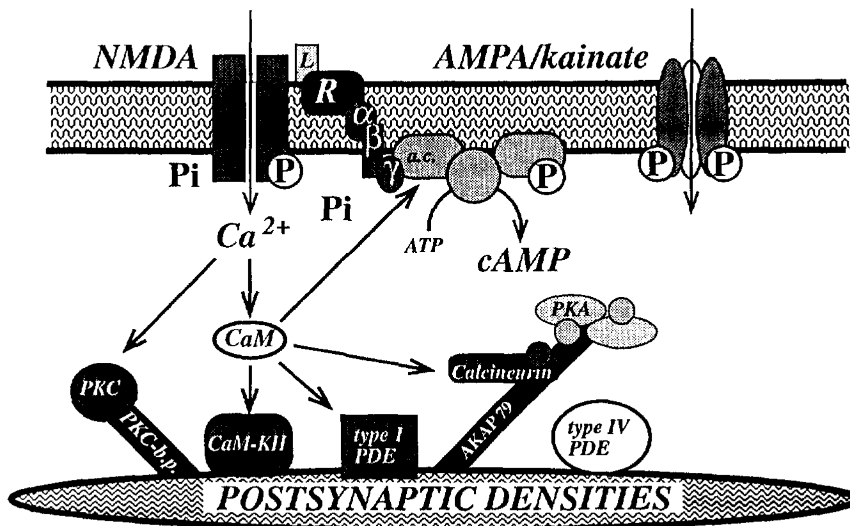

Fig. 5. Enzyme targeting at the postsynaptic density. This diagram condenses the subject matter of this mini-review, showing that several key signalling enzymes that function in postsynaptic events are targeted to the postsynaptic densities. In several cases, such as PKA, PKC, CaM-KII and calcineurin, postsynaptic targeting appears to be maintained through association with anchoring proteins.

later finding suggests that CaM KII may be anchored to the PSD by p190 following its activation by an increase of dendritic Ca^{2+} concentrations [98].

2.3 *Calmodulin*

Calmodulin (CaM) is an ubiquitously expressed multifunctional Ca^{2+} binding protein that mediates the intracellular effect of calcium on numerous enzymes and intracellular processes [99, 100]. Although calmodulin is not thought to be directly attached to the PSD it is evident that significant concentrations of the protein are maintained at, or close to, postsynaptic sites through association with several of the enzymes and anchoring proteins discussed in this article. For example, Ca^{2+}/CaM binds to and activates both the CaM-kinase II, and the phosphatase 2B, calcineurin, enzymes which regulate the phosphorylation state of key neuronal substrates [101, 102]. Calmodulin also indirectly modulates accumulation of cAMP at the PSD by activation of the type I and type III adenylyl cyclases, and metabolism of cAMP through stimulation of the type I phosphodiesterase [31, 44, 45, 101]. Furthermore, AKAP79, the dual function anchoring protein which targets PKA and calcineurin to the PSD, and several of the PKC-binding proteins, such as MARCKS, associate with CaM [70, 71, 83].

3. *Conclusions and perspectives*

Neurons are highly polarized cells that express a variety of cytoplasmic and membrane proteins which are differentially distributed in axons, dendrites and somata. The evidence presented in this mini-review suggests that dendritic signalling events are efficiently controlled because certain signal transduction molecules are clustered together at the postsynaptic densities. Further evidence for the specialized function of these synapses may be indicated by ultrastructural evidence showing that the protein synthesis

machinery is targeted to specific postsynaptic sites. For example, polyribosomes have been localized at postsynaptic sites while mRNA's for PSD proteins such as CaM-KII and various glutamate receptor subtypes respectively have been localized to sub synaptic regions by a combination of in situ hybridization and PCR based strageties [103, 104]. These studies have led to the suggestion that dendrites themselves are compartments for macromolecular synthesis [104]. Future studies will test this hypothesis and establish whether PSD proteins, including those discussed in this article, are synthesized at their site of action.

According to our model for postsynaptic targeting, which is presented in Fig. 5, we propose that anchoring at the PSD increases synaptic efficiency by permitting the preferential activation of certain signalling enzymes. However, it must be stressed that enzyme targeting only represents one part of story. It is evident that the diffusion of the second messengers Ca^{2+} and cAMP are essential steps in the activation of these enzymes [96, 105]. For example, activation of adenylyl cyclase in the postsynaptic membrane generates soluble cAMP which freely diffuses to the PSD. There it is likely to activate PKA associated with AKAP79 as opposed to other pools of the kinase which are tethered at other dendritic sites through association with MAP2 or at the endoplasmic reticulum through AKAP100 [106]. Another important component of the targeting model is that phosphodiesterases regulate the accumulation of cAMP. It is evident that phosphodiesterases tethered to the PSD regulate accumulation of cAMP and restrict activation of PKA by preventing diffusion of the second messenger to adjacent sites [7].

Another important theme that seems to be emerging from the study of anchoring proteins is that pools of kinase and phosphatase can be targeted at the same site. For example, our own findings suggest that AKAP79 represents a novel anchoring protein as it serves a bifunctional role in localizing both PKA and calcineurin [70]. This suggests a model for reversible phosphorylation of proteins in the postsynaptic membrane in which the opposing effects of kinase and phosphatase action are co-localized in a signal transduction complex by association with a common anchor protein. This latter statement makes one important assumption that has yet to be completely proven, namely, that the multifunctional kinases and phosphatases are co-localized with their substrates.

Although PKA is targeted to the PSD it is likely that the Ca^{2+} activated enzymes, PKC and Cam-KII, are the predominant kinases at this dendritic site. This process places either enzyme close to receptors, ion-channels or structural proteins which are known substrates for both kinases, and permits their efficient activation by the influx of Ca^{2+} through the NMDA receptor [79. 107]. Both enzymes play a critical role in LTP as microinjection of pseudosubstrate inhibitors for either kinase into cultured neurons resulted in a loss of tetantic stimulation [108]. Furthermore, selective activation of either kinase is achieved through the requirement of a second activator: phospholipid for PKC and calmodulin for CaM-KII. It would appear that the Ca^{2+} activated kinases are associated with the PSD. For example, PKC may be anchored to several proteins including MARCKS, a protein that was identified as the major PKC substrate; while a 190,000 M_r protein has recently been identified for CaM-KII [83, 98].

Finally, the emphasis of this article has focused predominantly on the phosphorylation of proteins at the PSD. However, it would be remiss not to mention the reverse process, namely the dephosphorylation of proteins at the PSD. Over the past year, biochemical and physiological evidence has emerged which suggests that targeting of phosphatase at the PSD is an important process. The activity of NMDA currents are blocked in cultured hippocampal neurons by the phosphatase 1 and 2A inhibitors okadaic acid, microcystin and the calcineurin inhibitor FK-506 [109–111]. In addition, Jahr and colleagues have recently demonstrated that application of CaN into these neurons attenuates the NMDA receptor current [112]. This finding is of particular interest to us as we have recently

demonstrated that CaN is targeted to the PSD through association with AKAP79 [78]. Calcineurin activity has also recently been implicated in synaptic long-term depression (LTD). This involves a phosphatase cascade whereby neuronal Ca^{2+} influx stimulates CaN which, in turn, dephosphorylates the type I phosphatase inhibitor proteins, inhibitor 1 or DARP-32. This leads to the activation of PP-1 which is believed to promote dephosphorylation of those proteins involved in LTD [113, 114]. Future studies are planned to establish if PP-1 and PP-2A are anchored to the PSD and their roles in the dephosphorylation of PSD proteins.

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