The Postsynaptic Architecture of Excitatory Synapses: A More Quantitative View

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Annu. Rev. Biochem. 2007. 76:823–47

First published online as a Review in Advance on December 19, 2006

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

This article's doi: 10.1146/annurev.biochem.76.060805.160029

0066-4154/07/0707-0823$20.00

Key Words

dendritic spine, glutamate receptor, mass spectrometry, postsynaptic density, PSD-95

Abstract

Excitatory (glutamatergic) synapses in the mammalian brain are usually situated on dendritic spines, a postsynaptic microcompartment that also harbors organelles involved in protein synthesis, membrane trafficking, and calcium metabolism. The postsynaptic membrane contains a high concentration of glutamate receptors, associated signaling proteins, and cytoskeletal elements, all assembled by a variety of scaffold proteins into an organized structure called the postsynaptic density (PSD). A complex machine made of hundreds of distinct proteins, the PSD dynamically changes its structure and composition during development and in response to synaptic activity. The molecular size of the PSD and the stoichiometry of many major constituents have been recently measured. The structures of some intact PSD proteins, as well as the spatial arrangement of several proteins within the PSD, have been determined at low resolution by electron microscopy. On the basis of such studies, a more quantitative and geometrically realistic view of PSD architecture is emerging.

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INTRODUCTION

Dendrite: the branched projections of a neuron that receive synaptic inputs conveyed by axons from other neurons

AMPA receptor (AMPAR): glutamate receptor channel that mediates most of the fast synaptic transmission in central excitatory synapses

The billions of neurons in the mammalian brain communicate with each other via specialized junctions called synapses. The vast majority of these synapses occur at contacts between presynaptic axons and postsynaptic dendrites, and they use glutamate as the excitatory neurotransmitter. Glutamate released from the presynaptic terminal acts upon postsynaptic glutamate receptor channels [primarily AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (AMPARs) and NMDA (N-methyl-D-aspartic acid) receptors (NMDARs)], which open to allow influx of Na^+ ions (and in the case of NMDARs, also Ca^{2+} ions), thereby propagating electrical depolarization to the postsynaptic neuron (excitatory synaptic transmission).

The strength of synaptic transmission can be modified (synaptic plasticity) by various factors, including specific patterns of synaptic activity (1). By altering synaptic strength in an activity-dependent manner, synaptic plasticity allows synapses and neural networks to store information in response to prior experience, such as occurs during development and in learning and memory. In parallel with adjusting the strength of synaptic transmission, neurons can change the morphology of synaptic connections and even their physical pattern of connectivity (2). Thus information can be stored long-term in the brain not only by modulation of synaptic strength but also by the formation of new synapses or the elimination of existing synapses (2, 3).

Synaptic transmission and plasticity are crucial for all aspects of nervous system function and critical for proper development of the central nervous system. Normal synaptic transmission depends on the proper localization and arrangement of specific proteins on both sides of the synapse. Synaptic plasticity is mediated by changes in the molecular composition of synapses and in chemical modification of synaptic proteins. Pathological synapse development and/or function almost certainly contribute to many neuropsychiatric disorders (e.g., schizophrenia, autism, mental retardation), common neurodegenerative diseases (e.g., Alzheimer's disease), and stroke (4, 5). Therefore, it is of fundamental and clinical importance to understand the biochemical and cell biological basis of synaptic function and plasticity.

We review the molecular and cellular architecture of the postsynaptic specialization of glutamatergic (glutamate-releasing) synapses, which make up the vast majority of central synapses in mammalian brain. Not covered are other types of excitatory synapses (e.g., those using acetylcholine) and inhibitory synapses, which differ in their molecular organization (6, 7). The presynaptic specialization has been recently reviewed (8). The

postsynaptic compartment is where most of the molecular diversity of synapses lies and where the initial signal transduction events take place that lead to long-term synaptic plasticity. On the basis of recent progress in this area, we aim to give a more quantitative description of the postsynaptic specialization, focusing on the postsynaptic density (PSD) and emphasizing three-dimensional (3D) structure, molecular stoichiometry, and spatial arrangement of postsynaptic components.

MICROANATOMY OF THE EXCITATORY SYNAPSE

Ultrastructural features enable unambiguous identification of the pre- and postsynaptic sides of the synapse by electron microscopy (EM). Defining the presynaptic specialization is a cluster of synaptic vesicles (~40 nm diameter), some of which are closely associated ("docked") with a thickening of the presynaptic plasma membrane (the active zone), where vesicle exocytosis occurs (8) (**Figure 1*a*,*b***). Directly apposed to the active zone (and perfectly matched with it in size and shape) is the PSD, an electron-dense thickening of the postsynaptic membrane, where glutamate receptor channels and their associated signaling proteins are highly concentrated (**Figure 1*a*-*c***). The presence of a prominent PSD is characteristic of glutamatergic synapses (hence they are termed *asymmetric*); in contrast, inhibitory (*symmetric*) synapses lack a prominent postsynaptic thickening (9, 10).

The presynaptic active zone and the PSD, which define the extent of the true synapse morphologically, are separated by a gap of 20–25 nm (**Figure 1*a*,*b***). A wide variety of cell adhesion molecules hold pre- and postsynaptic membranes together in register and at the appropriate separation (11, 12). *Puncta adherens* junctions and/or cadherin clusters lie adjacent to the synapse proper or may occur within the PSD-active zone apposition (13).

The postsynaptic membrane can be divided into the PSD itself, as well as perisynaptic and extrasynaptic regions. The latter two terms are often used interchangeably, although it should be borne in mind that the perisynaptic membrane within 100 nm of the PSD probably differs in molecular content and function from the extrasynaptic membrane at greater distances from the PSD. Extrasynaptic regions have specialized postsynaptic functions and are enriched for a distinctive set of proteins, such as metabotropic glutamate receptors (mGluRs) (14) and proteins involved in endocytosis (15) (**Figure 1*d***). On most principal neurons in the mammalian brain (e.g., pyramidal neurons of cortex and hippocampus, Purkinje cells of cerebellum, medium spiny neurons of striatum), the postsynaptic specialization is housed on tiny actin-rich protrusions called dendritic spines. In contrast, inhibitory synapses are made on the shaft of the dendrite (**Figure 1*c***). In inhibitory local circuit interneurons, which usually lack spines, the excitatory synapses are also made on the dendritic shaft. The PSD (and hence the synaptic contact) is typically located on the dilated tip ("head") of the spine. The dimensions of the spine head are highly correlated with the size of the PSD and associated active zone, as well as synaptic strength (16).

Synaptic Cleft

Separating the PSD and the active zone is the synaptic cleft, which measures ~20 nm wide in conventional EM and ~24 nm wide in cryo-EM (17, 18) (**Figure 1*b***). Faint lines bridging the synaptic cleft were noted by early anatomists (19). Recent cryo-EM and tomography studies suggest that the cleft contains more electron-dense material even than the cytoplasm; the density peaks midway between the pre- and postsynaptic membranes (17, 18). The intracleft material appears to consist of transsynaptic complexes that form extensive lateral connections within the cleft (17, 18). The nature of these cleft complexes are unknown, but cell adhesion molecules such as N-cadherin (12) and glutamate receptors may contribute (20).

NMDA receptor (NMDAR): calcium-permeable glutamate receptor channel that contributes to fast synaptic transmission and that regulates synaptic plasticity

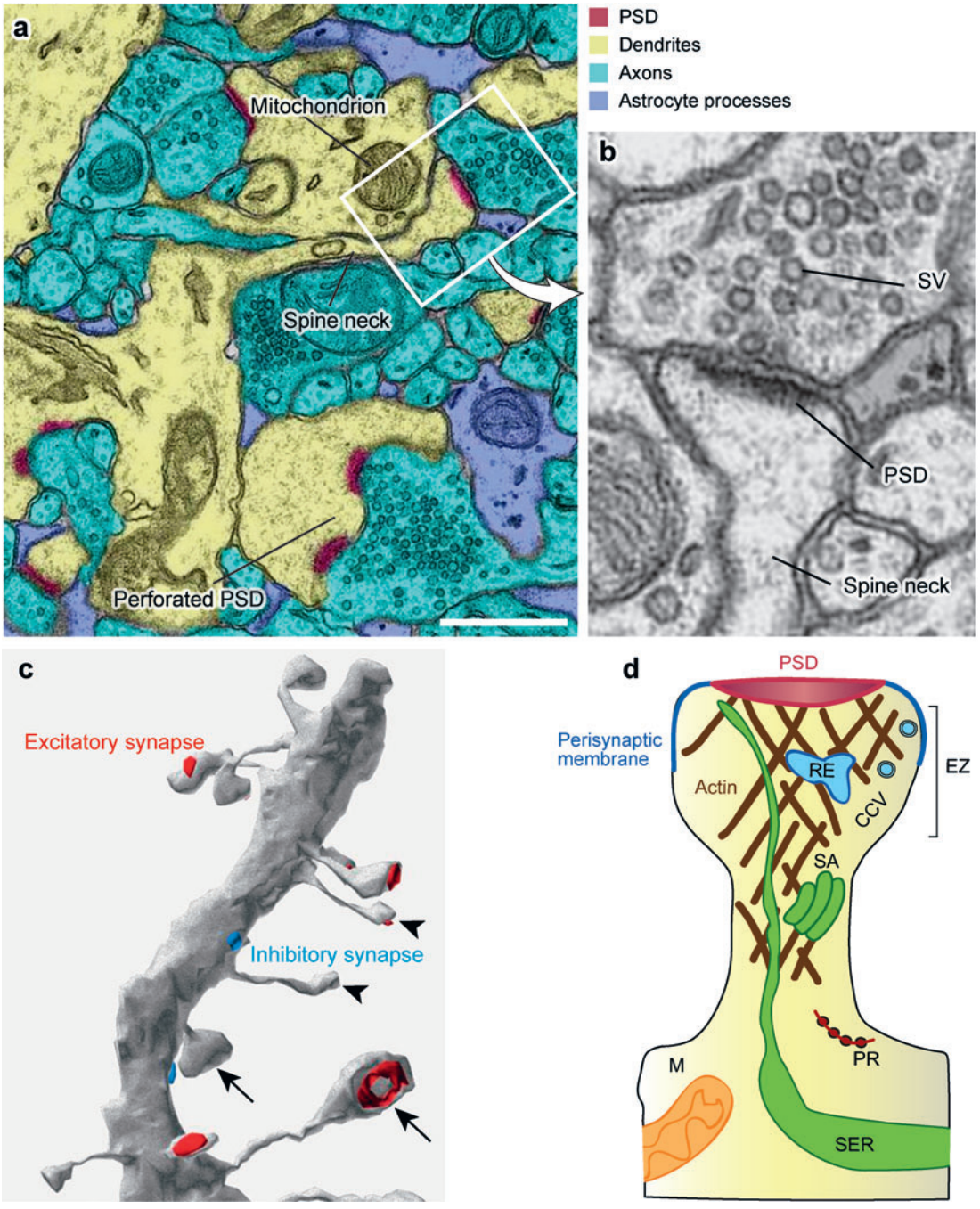
Synaptic plasticity: the ability of the synapse to alter its strength and structure

Excitatory synapse: contact site where synaptic transmission occurs by secretion of neurotransmitter (glutamate) from presynaptic neuron to postsynaptic neuron

Postsynaptic density (PSD): an electron-dense structure at the postsynaptic membrane containing many glutamate receptors, scaffold proteins, and signal transduction molecules

EM: electron microscopy

Active zone: region of the presynaptic membrane directly apposed to the PSD, where synaptic vesicles fuse with the presynaptic membrane



The Dendritic Spine

Dendritic spines are small (typically 0.5–2 μm in length) membranous protrusions that house the essential postsynaptic components, including the PSD, actin cytoskeleton, and a variety of "supporting" organelles (**Figure 1*d***). Spines occur at a density of 1–10 spines per μm of dendrite length on principal neurons (21), and they receive most of the excitatory synapses in the mature mammalian brain. Typical spines have a bulbous head (receiving a single synapse) connected to the parent dendrite through a thin spine neck (**Figure 1*c***). Because the neck hinders diffusion of molecules to and from the parent dendritic shaft, spines serve as microcompartments in which biochemical changes in one individual synapse can be isolated from other synapses on the same neuron (22). The geometry of the spine neck determines calcium efflux into the dendrite shaft and hence the degree of calcium elevation in the spine head following NMDAR activation (23). Recent evidence suggests that the spine neck diffusion barrier can be controlled by neuronal activity (24).

Dendritic spines are highly heterogeneous structures that show dynamic motility, especially during development (25, 26). Their number, size, and shape undergo plastic changes correlated with long-term modifications of synaptic strength and interneuronal connectivity (2, 27). Spine shape has been categorized as "mushroom," "thin," or "stubby," but EM studies show a continuum between these categories. There is growing evidence that different spine shapes and sizes reflect different developmental stages and/or altered strength of synapses (2, 16, 27). Sophisticated imaging experiments indicate that the volume of spine heads can increase with stimuli that strengthen synapses and can decrease with stimuli that weaken synapses (16, 27). The molecular mechanisms that coordinate synaptic strength with spine morphogenesis is a subject of current interest (25).

Spines with large heads are generally stable, express large numbers of AMPARs, and contribute to strong synaptic connections. By contrast, spines with small heads are more motile, less stable, and contribute to weak synaptic connections (28, 29). In vivo time-lapse studies show that spines turn over at various rates in the mouse brain; a large fraction of mushroom spines are persistent, with lifetimes up to many months (30, 31).

Dendritic spines: small membranous compartments protruding from dendrites that receive synaptic contacts from glutamate-releasing axons

Figure 1

Microanatomy of the excitatory synapse and dendritic spine. (*a*) Thin section electron microscopy (EM) image of the CA1 region of the hippocampus. Morphological features are highlighted in color for illustrative purposes: postsynaptic density (PSD); dendrites, including spines; axons, including presynaptic terminals containing synaptic vesicles; astrocyte processes, which are found frequently at the edge of synapse. The scale bar is 1 μm. (*b*) EM morphology of an excitatory synapse. The presynaptic terminal contains synaptic vesicles loaded with glutamate, facing the PSD located on the tip of the dendritic spine. The synaptic cleft separating pre- and postsynaptic membranes is 20–25 nm wide. Abbreviation: SV, synaptic vesicle. (*c*) Three-dimensional EM reconstruction of a segment of dendrite from the CA1 region of the hippocampus. Red indicates the PSD of an excitatory synapse. Blue indicates inhibitory synapse. Some PSDs are not visible because they face away from the viewer. Note a central perforation in the PSD of the large mushroom spine at bottom right. Examples of thin spines (*arrowheads*) and mushroom spines (*arrows*) are indicated. (*d*) Schematic diagram of mature mushroom-shaped spine, showing the PSD, the perisynaptic membrane, and other organelles. The endocytic zone (EZ) is located lateral of the PSD in extrasynaptic regions of the spine, where it may be associated with clathrin-coated vesicles (CCV) and recycling endosomes (RE). Smooth endoplasmic reticulum (SER), polyribosomes (PR) and mitochondria (M) are found mainly in the dendritic shaft near the base of spines but may extend into the spine. The abundant actin cytoskeleton (*brown lines*) is connected to the PSD and determines spine structure and motility. Other abbreviation: SA, spine apparatus. Images for panels *a-c* of this figure were kindly provided by Kristen Harris (Medical College of Georgia, USA).

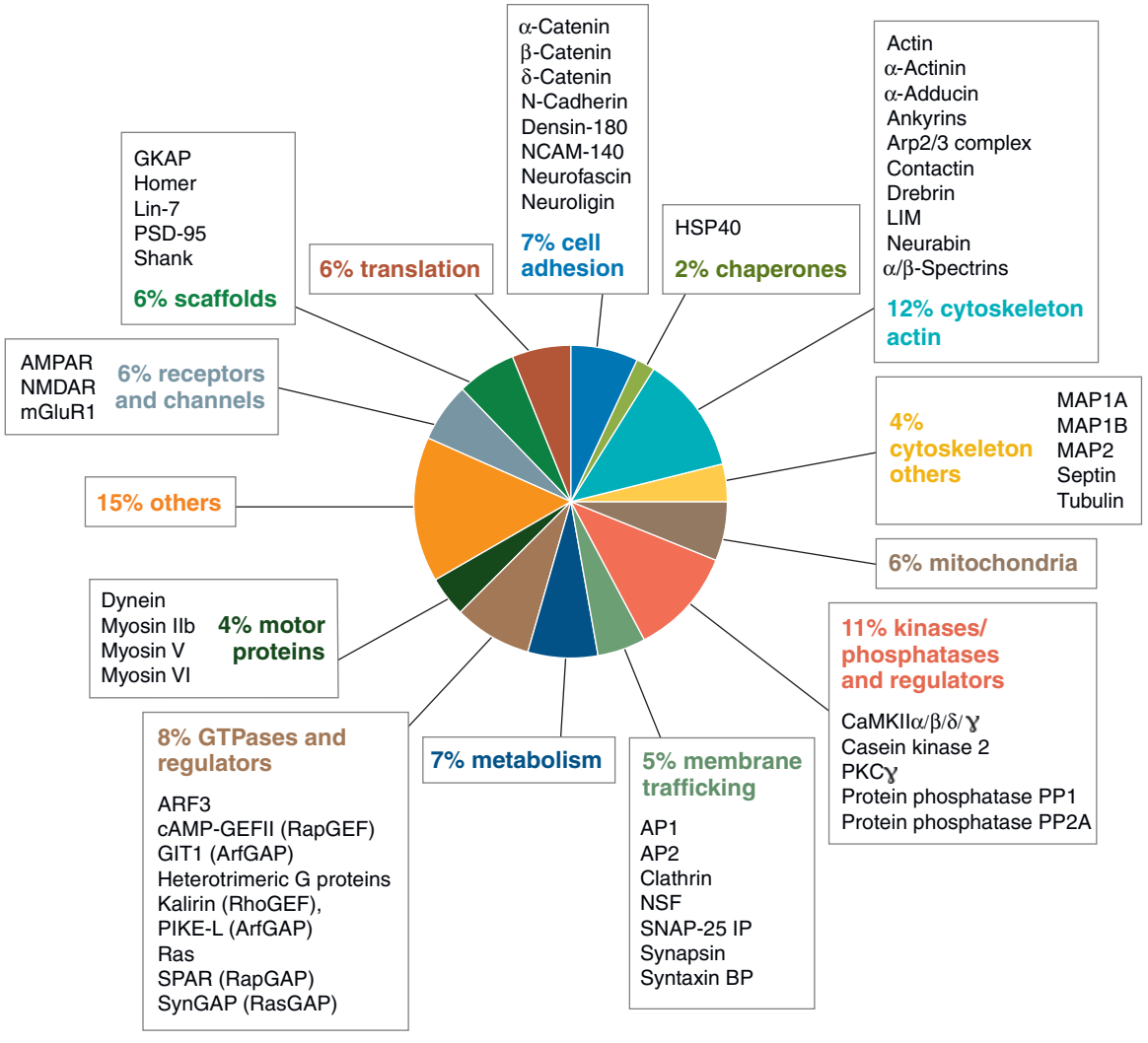


Figure 2

The variety of proteins in the PSD fraction. Pie chart showing the wide variety of proteins identified in the PSD fraction of the forebrain, categorized according to cellular function. Only small subsets of identified proteins are shown as examples. PSD proteins are involved in cellular communication and signal transduction (adhesion, GTPases, kinase/phosphates, receptors, and channels), cellular organization (cytoskeleton, membrane traffic, motors, and scaffolds), energy (mitochondria and metabolism), protein synthesis and processing (translation and chaperones), and others. The percentage of PSD proteins per category was obtained from the mass spectrometry data by Peng and colleagues (70).

proteome (63). Current estimates based on MS analysis range from a few hundred (~100–400) to as many as one thousand proteins in the PSD. However, this number includes many potential contaminants of the PSD fraction (false positives), such as mitochondrial and glial proteins, abundant metabolic enzymes, and cytoskeletal elements. Proteomic analysis of the PSD is also prone to miss bona fide components that are in low abundance or only transiently associated with PSDs (false negatives).

Protein	Stoichiometry	Domain structure	Description—Function
α-Actinin		CH, Spectrin, EFh	Actin-binding protein that interacts with several PSD proteins, including NR2B and CaMKII—regulates spine morphogenesis (145)
AKAP79/150	20	PA	PSD-95/SAP97-associated A-kinase-anchoring protein that binds to protein kinase A and PP1 (151)
CaMKIIα/β	5600	CK	Abundant postsynaptic Ca^{2+}/calmodulin-dependent serine/threonine kinase important for synaptic plasticity (79)
N-cadherin		CA	Calcium-dependent homophilic adhesion molecule—involved in synapse and dendrite development (150)
β–Catenin		Arm	Signaling protein that links N-cadherin to the actin cytoskeleton (150)
Cortactin		SH3	Actin-binding protein and activator of the Arp2/3 actin nucleation machinery—interacts with Shank, regulates spine morphogenesis (144)
Densin-180		Leucine-rich repeat	Leucine-rich repeat and PDZ domain–containing protein—interacts with δ-catenin, Shank, CaMKIIα, α-actinin (145)
GluR1/2/3	60		Subunits of AMPA-type glutamate receptor channels
Homer1/2/3	60	EVH1	Adaptor proteins that bind to Shank and several other postsynaptic proteins, including mGluR1/5 (98)
IRSp53	80	SH3	Adaptor protein that binds to PSD-95 and Shank—links activated Rac1/Cdc42 to actin regulation, involved in spine morphogenesis (149)
Kalirin7		Sec14, Spectrin, RhoGEF, PH	PSD-95-associated RhoGEF—regulates actin cytoskeleton and dendritic spine morphogenesis (143)
mGluR1/5	20		Metabotropic glutamate receptors, G protein–coupled receptors for glutamate
Neurabin-I/ spinophilin		SAM	Scaffold proteins that bind to PP1 and actin—regulate synaptic function, plasticity, and spine morphology (146)
Neuroligin		ELHD	Postsynaptic adhesion molecule that binds to presynaptic neurexin—involved in formation and development of synapses (142)
NR1/NR2A/ NR2B	20		Subunits of NMDA-type glutamate receptor channels
PSD-95	300	SH3, GK	PDZ scaffold protein that regulates synaptic plasticity (58, 59)
SAP97	10	L27, SH3, GK	Scaffold protein of the PSD95 family that regulates synaptic function (58, 93)
SAPAP1–4/ GKAP	150	GKAP	Abundant multidomain scaffold that links PSD-95 with Shank (58)
Septin7		GTP-CDC	GTP-binding protein that forms polymers (148)
Shank1/2/3	150	Ank, SH3, SAM	Scaffold proteins that bind to GKAP and Homer—promote morphological and functional maturation of synapse and dendritic spine (58, 80)
SPAR		RapGAP	RapGAP that binds to PSD-95—regulates actin and spine morphogenesis (102)
SynGAP	360	RasGAP	Abundant RasGAP that binds to PSD-95—regulates MAP kinases and synaptic function and development (147)

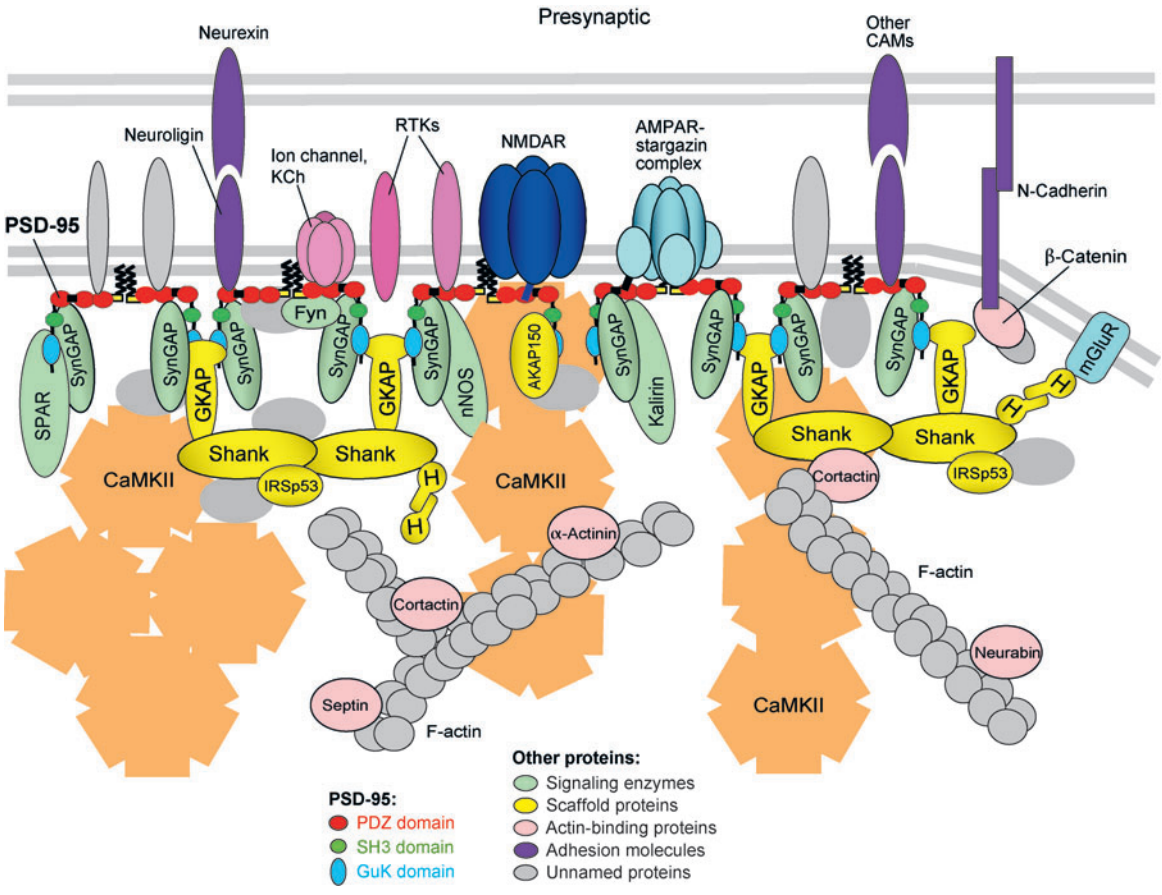


Figure 4

Organization of proteins and protein-protein interactions in the postsynaptic density (PSD). Schematic diagram of the network of proteins in the PSD, with edge of PSD depicted at right. Only major families and certain classes of PSD proteins are shown [in approximate stoichiometric ratio and scaled to molecular size, if known (see text)]. Contacts between proteins indicate an established interaction between them. Domain structure is shown only for PSD-95 (PDZ domain, SH3 domain, GuK domain). Other scaffold proteins are colored yellow; signaling enzymes, green; actin binding proteins, pink. CaMKII (calcium/calmodulin-dependent protein kinase II) is depicted as dodecamer. Unnamed proteins signify the many other PSD proteins that are not illustrated in this diagram. Abbreviations: AKAP150, A-kinase anchoring protein 150 kDa; CAM, cell adhesion molecule; Fyn, a Src family tyrosine kinase; GKAP, guanylate kinase-associated protein; H, Homer; IRSp53, insulin receptor substrate 53 kDa; KCh, K^+ channel; mGluR, metabotropic glutamate receptor; nNOS, neuronal nitric oxide synthase; RTK, receptor tyrosine kinases (e.g., ErbB4, TrkB); SPAR, spine-associated RapGAP.

in addition to differential expression of synaptic proteins in cultured neurons versus adult brain. For instance, the levels of GKAP, as well as Shank and Homer family, proteins are influenced by synaptic activity (81), and different levels of activity prevail in culture versus in vivo. Another possibility is that a fraction of Shank and Homer proteins is lost by Triton X-100 extraction during purification of PSDs for MS analysis. Despite these quantitative discrepancies, it is clear that the PSD-95/GKAP/Shank/Homer scaffold assembly accounts for a substantial proportion of total protein mass of the PSD (76).

also be directed to lysosomes for degradation (132, 133).

It is generally believed that AMPARs are exocytosed at extrasynaptic sites (depending on the AMPAR auxiliary subunit stargazin), followed by lateral movement into synapses, where they become relatively immobile owing to interactions with, or hindrance by, other postsynaptic proteins such as PSD-95 (88, 134–138) (**Figure 5*c***). Compared with endocytosis, the precise location on the neuronal surface where AMPAR insertion occurs is less certain; it could be in extrasynaptic regions of the spine or in the dendritic shaft and soma.

A variety of proteins that directly interact with specific AMPAR subunits play important roles in AMPAR trafficking (37, 129). For example, GluR2 binds to *N*-ethylmaleimide-sensitive factor (NSF) [an ATPase required for dissociation of the SNARE protein complex and recruited to the PSD during brain ischemia (105)], and this interaction is critical for the recycling of GluR2-containing AMPARs to the surface and for their incorporation into the synapse (129, 139–141). It is tempting to speculate that NSF functions by dissociating protein interactions that block GluR2 entry into the PSD.

CONCLUDING REMARKS

In recent years, a more comprehensive and increasingly quantitative view has been emerging of the protein composition of the PSD, but numerous details remain to be elucidated, much of it descriptive in nature. False negatives, which inevitably contaminate the PSD fraction, need to be weeded out, and the biochemistry and functional significance of the real constituents of the PSD must be determined. We have obtained a low-resolution picture of the 3D shape of the PSD, but it was in an unnatural state, divorced from the plasma membrane and actin cytoskeleton with which the PSD usually associates. Further work combining modern EM and molecular genetics in more intact preparations is essential to reveal the molecular architecture of postsynaptic specialization and inform its structure-function relationships.

Even as we struggle to reach a stoichiometric and geometric description of the PSD and its constituent proteins, it is clear that we are chasing a moving target that changes rapidly and substantially in response to neural activity and developmental experience (akin to taking a census of a fluid society). Both the PSD and the dendritic spine, on which it sits, are dynamic structures made up of parts that move and turn over constantly; components of the PSD are in constant flux with the extrasynaptic membrane and cytoplasm, and contents of the spine with the parent dendrite. Regulated modifications of these fluxes (e.g., altered AMPAR trafficking) result in profound changes in synaptic strength in response to specific patterns of activity. And yet, despite being mutable on a timescale of seconds to minutes, and in the face of constant protein turnover, the PSD and dendritic spine remain coordinated with each other and with the presynaptic specialization, and they are able to maintain over days to months the synaptic weights that presumably encode memories in the brain. Perhaps more than anything else, these remarkable capacities for dynamic plasticity and long-term stability of synapses intrigue neuroscientists. Sorting out the underlying mechanisms will fuel research for years to come.

SUMMARY POINTS

1. Excitatory synapses in the mammalian brain mainly use glutamate as the neurotransmitter and are usually situated on the tips of specialized postsynaptic compartments called dendritic spines.

2. Dendritic spines provide synapse-specific biochemical microcompartments; they contain not only the synapse proper, but also other organelles involved in protein synthesis, membrane trafficking, and ATP and calcium metabolism.
3. The postsynaptic density (PSD) contains the glutamate receptors that detect the release of glutamate from the presynaptic terminal and a host of associated signaling molecules that transduce glutamate binding into postsynaptic biochemical responses.
4. A variety of abundant scaffold proteins (e.g., PSD-95, Shank) assemble the PSD by binding to glutamate receptors, other postsynaptic membrane proteins, cytoplasmic signaling enzymes, and cytoskeletal elements.
5. The molecular size of the PSD and the relative stoichiometry of many major constituents have been measured by MS, EM, and other imaging approaches, leading to a more quantitative understanding of protein composition of the PSD.
6. The 3D structures of glutamate receptors and other PSD proteins and their highly organized spatial arrangement within the PSD are emerging through EM and X-ray crystallography.
7. The PSD is a dynamic and heterogeneous structure whose shape, size, and composition changes during development and in response to synaptic activity.
8. The extrasynaptic membrane contains glutamate receptors that are in dynamic flux with those in the PSD, but it is also specialized for different functions, such as endocytosis.

FUTURE ISSUES

1. Hundreds of different proteins have been identified in the PSD, but for most of them, their specific roles in synaptic structure and function are unknown.
2. Quantitative stoichiometry, protein-protein interactions, and 3D structures remain to be determined for the vast majority of PSD constituent proteins.
3. The detailed molecular architecture of the PSD, and its relationship with the actin cytoskeleton and other postsynaptic organelles, should be studied in depth with EM tomography combined with molecular manipulations.
4. The dynamics and mechanisms of ontogeny and turnover of PSD proteins are only beginning to be uncovered. The functional significance of the regulated change of PSD proteins needs to be investigated at the cell biology and neural systems levels.

ACKNOWLEDGMENTS

We are grateful to Kristen M. Harris for providing EM sections of brain and 3D reconstructions of spines for **Figure 1** and to Tom Reese and Xiaobing Chen for providing EM images of purified PSDs for **Figure 5**. We thank the following for critical comments on the manuscript: Junmin Peng, Yasunori Hayashi, Richard Weinberg, Li-Huei Tsai, Baris Bingol, Honor Hsin, and members of the Hoogenraad lab. M.S. is an Investigator of the Howard Hughes Medical