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Chapter 15
Roles of Neuronal Activity–Induced Gene Products in Hebbian and Homeostatic Synaptic Plasticity, Tagging, and Capture

Yasunori Hayashi, Ken-ichi Okamoto, Miquel Bosch, and Kensuke Futai

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Abstract The efficiency of synaptic transmission undergoes plastic modification in response to changes in input activity. This phenomenon is most commonly referred to as synaptic plasticity and can involve different cellular mechanisms over time. In the short term, typically in the order of minutes to 1 h, synaptic plasticity is mediated by the actions of locally existing proteins. In the longer term, the synthesis of new proteins from existing or newly synthesized mRNAs is required to maintain the changes in synaptic transmission. Many studies have attempted to identify genes induced by neuronal activity and to elucidate the functions of the encoded proteins. In this chapter, we describe our current understanding of how activity can regulate the synthesis of new proteins, how the distribution of the newly synthesized protein is regulated in relation to the synapses undergoing plasticity and the function of these proteins in both Hebbian and homeostatic synaptic plasticity.

Keywords Arc/Arg3.1 • Homeostatic plasticity • Homer/vesl • Synapse tagging and capture • Synaptic plasticity

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15.1 Introduction: Memory, Synaptic Plasticity, and New Protein Synthesis

The synthesis of the right species of protein in the right cells at the right time is crucial for every aspect of cellular function. Therefore, protein synthesis is tightly genetically defined depending on cell type and developmental status. In addition, gene expression is also regulated by external factors. Various cellular environments can also affect the timing, species, and the amount of synthesized proteins.

Protein synthesis in neurons is not an exception from such regulation (for review, see Loebrich and Nedivi 2009; Okuno 2011). The first evidence to link gene expression to the functional modification of neurons comes from the observation that pharmacological stimulation of cultured neuronal cells induces expression of a specific set of genes (Greenberg et al. 1986). Similarly, induction of kindling in animals induces gene transcription (Dragunow and Robertson 1987; Saffen et al. 1988). These observations led to a seminal study by Cole et al. (1989) that linked gene expression to synaptic plasticity for the first time. They induced synaptic plasticity in the hippocampus by delivering a high-frequency stimulation to the input fibers, which leads to long-term potentiation (LTP) of synaptic transmission (Bliss and Lømo 1973; Bliss and Collingridge 1993), and found a correlated increase of specific genes. This induction of gene expression required activation of *N*-methyl-D-aspartate type glutamate receptor (NMDAR), which is also necessary for hippocampal LTP indicating that the electrophysiologically measured LTP and the induction of new gene transcription share at least a common part of cellular signaling (Herron et al. 1986). Arguably, LTP is a cellular counterpart of learning and memory (Bliss and Collingridge 1993), and this work was the first to demonstrate the expression of specific genes in the context of learning and memory. Subsequently, many paradigms of learning such as exposure to a novel environment, fear, and pheromones have been shown to induce expression of genes in specific neurons (Brennan et al. 1999; Guzowski et al. 2001; Hall et al. 2001). Furthermore, because the induction is so robust and reproducible, the induction of those genes could be even used to identify neurons that are activated during learning (Frankland et al. 2004; Reijmers et al. 2007; Kitanishi et al. 2009; Sacco and Sacchetti 2010).

Are gene expression and protein synthesis required for learning and memory? Earlier studies using pharmacological inhibitors of protein synthesis in animals resulted in an inhibition of memory formation (Flexner et al. 1963; Barondes and Cohen 1968). This is followed by multiple studies that tested the effect of protein synthesis inhibitors in various learning paradigms in different animals (Davis and Squire 1984, for review). Overall, the results consistently showed that animals exhibited normal memory performance for a short period of time after the initial training but when tested several days later, their memory was impaired. These observations indicate that the initial formation of memory does not require de novo protein synthesis whereas the retention of the memory over extended periods of time requires newly synthesized proteins. Interestingly, when protein synthesis inhibitors were administered at later time points after the initial memory formation, the memory became resistant to the treatment, indicating that protein synthesis is

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not required for retention or recall once memory becomes consolidated (Davis and Squire 1984).

Consistent with the effect of protein synthesis inhibitors on memory formation, these reagents also blocked LTP consolidation. Inhibition of either protein translation (Krug et al. 1984; Otani et al. 1989) or transcription (Frey et al. 1996) did not affect the early phase of LTP which occurred within 1 h after stimulation (early LTP or E-LTP), suggesting that this initial phase is mediated by existing proteins. However, these treatments did block the maintenance of LTP, especially during the late phase which typically occurs 2 h after the induction (late LTP or L-LTP), which indicates that de novo protein synthesis is required for the retention of LTP. Similarly, if transport of newly synthesized mRNA and protein was prevented by separating dendrites and cell bodies of hippocampal neurons, L-LTP was blocked while E-LTP was not affected, indicating a requirement for transport of newly synthesized molecules from the cell body for maintenance of LTP at the late phase (Frey et al. 1989).

15.2 Search for Activity-Regulated Genes

These observations triggered many investigations into the involvement of transcription and translation in synaptic plasticity and learning. A number of laboratories set forth to systematically identify genes induced by neuronal activity in an unbiased way. One of the earlier screening efforts was performed by Nedivi and colleagues (1993). They carried out differential cDNA screening between mRNAs from hippocampal dentate gyrus treated with or without an excitatory amino acid analog kainate. They isolated 52 activity-induced genes, of which 35 were novel at the time of report and 17 were known genes such as c-*fos*, c-*jun*, and *zif/268*, which were already reported to be neuronal activity–induced genes, indicating the validity of their approach.

Similar approaches were also undertaken independently around the same time by several other laboratories including those of Inokuchi (e.g., Kato et al. 1997; Matsuo et al. 1998), Worley (Yamagata et al. 1993; Lanahan and Worley 1998), Bliss (Fazeli et al. 1993), Kuhl (Link et al. 1995), and Kandel (Qian et al. 1993). Over the last decade, further analyses furnished with new information and technologies such as entire genome sequence and microarray have led to the identification of many other neuronal activity–induced genes (French et al. 2001; Elliott et al. 2003; Newton et al. 2003; Altar et al. 2004; Hong et al. 2004; Park et al. 2006; Kitamura et al. 2007; Xiang et al. 2007). Currently, it is estimated that around 500–1,000 different neuronal activity–induced genes exist (Nedivi et al. 1993; Loebrich and Nedivi 2009). The identified genes encode proteins ranging from transcription factors, enzymes involved in metabolism, intracellular and extracellular signaling molecules, to cytoskeletal proteins (for review, see Loebrich and Nedivi 2009; Okuno 2011).

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104 Among the various mRNA species detected in such studies, those which
105 increased their amount in the presence of protein synthesis inhibitors were named
106 *immediate early genes* (Cochran et al. 1983). These mRNA species increase their
107 content utilizing only the transcription machineries already present under basal
108 cellular activity and therefore are the primary responder to the external stimulation.
109 Interestingly, some of them encode proto-oncogene transcription factors such as
110 *c-fos, c-jun, jun-B, c-myc,* and *zif/268* (also named *Egr1, NGFI-A, Krox 24*), which
111 have been identified in multiple studies to isolate activity-induced genes both from
112 neuronal and nonneuronal cells (Greenberg and Ziff 1984; Greenberg et al. 1986; Franza
113 et al. 1988; Rauscher et al. 1988; Ryder et al. 1988). Once they are induced, they in turn
114 induce a second surge of gene expression. For example, *c-fos* and *c-jun* gene products
115 associate with each other to form activator protein 1 (AP-1) transcription factor (Raivich
116 and Behrens 2006). The AP-1-responsive consensus sequence is found in genes
117 implicated in the synaptic functions such as α-amino-3-hydroxyl-5-methyl-4-isoxazole-
118 propionate-type glutamate receptor (AMPAR) subunit GluR2, growth-associated protein
119 43 (GAP-43), and the cyclin-dependent protein kinase Cdk5 (Rylski and Kaczmarek
120 2004; Raivich and Behrens 2006).

121 In addition to regulation at the transcriptional level, neuronal activity can trigger
122 translation of new proteins from existing mRNAs. Such translation is regulated in
123 several phases (see Wang et al. 2010 for review). While neurons are at basal activity,
124 the translation from these mRNA must be repressed. When the activity level goes up
125 beyond a certain level, this repression must be removed and the translational machin-
126 ery recruited. Several proteins and noncoding RNAs are involved in the regulation
127 (Feng et al. 1995; Costa-Mattioli et al. 2009; Wang et al. 2010). Of note, abnormality
128 in the translational regulation is suspected to be a major cause of a hereditary mental
129 retardation syndrome, fragile X disease. The disease is caused by a mutation in fragile
130 X mental retardation protein (FMRP), a mRNA binding protein that represses the
131 translation of its target (Feng et al. 1995; Kindler and Monshausen 2002; Bassell and
132 Warren 2008; De Rubeis and Bagni 2010). Autopsy examination of fragile X patients
133 revealed abnormal dendritic spine morphology (Irwin et al. 2001). This is
134 recapitulated in mice lacking FMRP, which show impairment in both synaptic
135 function and morphology, highlighting the importance of the translational regulation
136 for proper synaptic functions (Comery et al. 1997; Nimchinsky et al. 2001; Huber et al.
137 2002; Li et al. 2002; Schütt et al. 2009). There is also evidence that FMRP can regulate
138 the translation of NMDA receptor subunit NR2A through a specific micro RNA
139 (miRNA), miR-125b (Edbauer et al. 2010).

140 15.3 Synaptic Tagging and Capture Hypothesis

141 In the aforementioned screening studies, multiple molecules implicated in synaptic
142 function have been isolated. This includes synaptic scaffolding proteins, e.g.,
143 Homer1a/vesl-1s (Brakeman et al. 1997; Kato et al. 1998), Arc/Arg3.1 (Link et al.
144 1995; Lyford et al. 1995), and candidate plasticity gene (CPG) 2 (Cottrell et al. 2004);

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intracellular signaling molecules such as kinases, e.g., SNK/polo-like kinase 2 (Kauselmann et al. 1999), protein kinase Mζ (Osten et al. 1996), and GTP-binding proteins or their associated proteins, e.g., RGS2 (Ingi et al. 1998) and Rheb (Yamagata et al. 1994); extracellular signaling molecules, e.g., brain-derived neurotrophic factor (BDNF) (Hughes et al. 1993) and CPG15 (Nedivi et al. 1998); and cell surface adhesion molecules involved in mediating cell–cell communication, e.g., Arcadin (Yamagata et al. 1999) (for review, see Loebrich and Nedivi 2009; Okuno 2011).

One basic feature of LTP is "input specificity," where only stimulated synapses become potentiated and unstimulated synapses are not affected (Bliss and Collingridge 1993). This feature applies not only to the early phase of LTP, which is independent of protein synthesis, but also to the protein synthesis–dependent late phase of LTP. So there may be a mechanism that ensures newly synthesized proteins to function only at potentiated synapses. The presence of such a mechanism was experimentally demonstrated by Frey and Morris (1997). In two-pathway hippocampal recordings, when a high-frequency stimulation was given to one pathway in the presence of a protein synthesis inhibitor, the slice exhibited E-LTP but not L-LTP, consistent with the requirement of protein synthesis in L-LTP. But if a high-frequency stimulation was given to the second pathway before the application of a protein synthesis inhibitor, both pathways could elicit L-LTP. This is interpreted as follows: when the tetanus is given, it generates a "tag" specifically at the stimulated synapse independent of protein synthesis. This will serve as a synapse-specific binding site where newly synthesized proteins required for L-LTP are "captured." The L-LTP induced in the second pathway supplied the necessary protein not only for itself but also for the first pathway stimulated in the presence of protein synthesis inhibitors. The first pathway by itself could not induce protein synthesis because it received tetanic stimulation in the presence of the inhibitor but could still form a "tag," which can "capture" the newly synthesized protein induced by the second pathway and therefore, can induce L-LTP if the second pathway is stimulated in the absence of protein synthesis inhibitor.

This "synapse tag and capture" hypothesis was an attractive model to explain the input specificity of synaptic plasticity and multiple studies have been conducted ever since the proposal (Redondo and Morris 2011 for review). In particular, the identity of the tag has been the source of major research interest. The tag has to fulfill at least four criteria: (1) The tag is formed specifically at potentiated synapses, (2) it does not require synthesis of new proteins for formation, (3) it must stay at the synapse for at least 1–2 h, and (4) it should have a structure that is capable of recruiting newly synthesized plasticity-related proteins (Okamoto et al. 2009).

Okamoto et al. (2004) found that LTP induction induced a rapid formation of filamentous actin (F-actin) in dendritic spines (Fig. 15.1a). F-actin is the major cytoskeletal protein in dendritic spines that serves both as a structural framework of the synapse and as a binding site for other postsynaptic proteins. Consistent with the increase in F-actin, LTP induction also caused an enlargement of dendritic spines that persisted for up to 1 h (Matsuzaki et al. 2004; Okamoto et al. 2004; Honkura et al. 2008). A pharmacological formation of more F-actin at the dendritic spine was sufficient to deliver postsynaptic proteins to the synapse (Okamoto et al. 2004)

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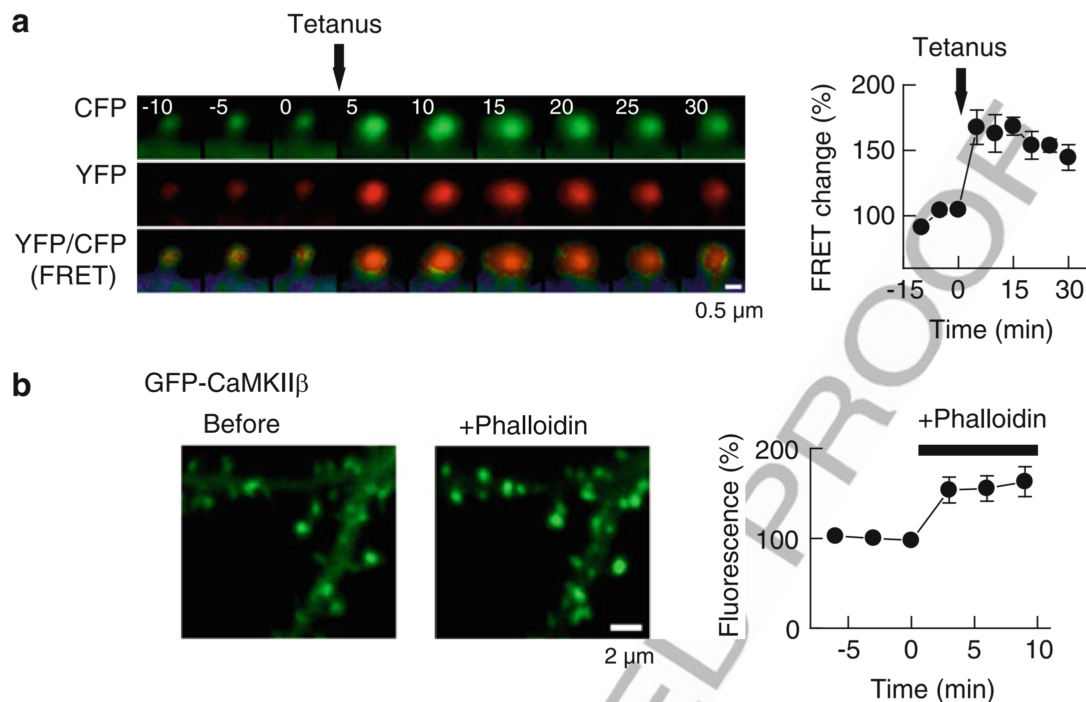

Fig. 15.1 *Polymerization of F-actin induced by local tetanic stimulation and its effect on synaptic protein distribution*. (**a**) F-actin formation was visualized by Förester resonance energy transfer (FRET) between donor- and acceptor-tagged actin molecules. Warmer hue indicates higher FRET. Time stamp in minutes. (**b**) Pharmacological polymerization of actin is sufficient to deliver CaMKII into dendritic spine. A neuron was transfected with GFP-tagged CaMKIIβ and injected with phalloidin, which stabilizes F-actin. The synaptic distribution of GFP-CaMKIIβ was monitored before and after the injection (Modified from Okamoto et al. 2004)

(Fig. 15.1b). This increase in F-actin levels along with the resultant increase in spine volume and the enhanced capacity of binding of the synapse perfectly fulfill all four criteria listed above. In fact, it has been recently shown that a pharmacological disruption of F-actin prevented synapse tagging (Ramachandran and Frey 2009). Therefore, formation of new F-actin together with the resulting structural changes is a prime candidate for the synapse tag.

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15.4 Synaptic Capture of Newly Synthesized Protein

Another feature of the synapse tag and capture hypothesis is the selective capture of newly synthesized proteins at potentiated synapses. There can be several ways to deliver proteins to the tagged synapses. One is to deliver mRNA to the tagged synapse and have the proteins locally translated. The dendrite contains hundreds of mRNA species, ribosomes and intracellular organelles such as endoplasmic reticulum or Golgi apparatus required for protein synthesis (Steward and Levy 1982; Eberwine et al. 2002; Kindler and Monshausen 2002; Ostroff et al. 2002; Horton and Ehlers 2003; Moccia et al. 2003; Poon et al. 2006).

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One representative mRNA of a dendritically localized immediate early gene is Arc/Arg3.1. It was initially identified as an immediate early gene induced after neuronal activation (Link et al. 1995; Lyford et al. 1995; Hevroni et al. 1998; Steward et al. 1998; Steward and Worley 2001). In dendrites, the Arc/Arg3.1 mRNA is specifically localized to the activated dendritic region thereby confining the protein product within the vicinity of the activated synapse (Steward et al. 1998; Steward and Worley 2001).

The mRNA of Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) is another abundant dendritic mRNA. It has a dendritic localization element in the 3′-untranslated region (UTR) (Mayford et al. 1996). LTP induction induced a rapid increase in local translation of CaMKIIα (Ouyang et al. 1999; Bagni et al. 2000). Genetic elimination of the 3′-UTR in mice not only disrupted the dendritic targeting of CaMKIIα but also impaired the stabilization of synaptic plasticity and memory consolidation (Miller et al. 2002).

However, these studies still have not demonstrated whether the newly synthesized proteins are specifically captured specifically at the potentiated synapse or not. It has been difficult to address this issue in the mammalian central nervous system, primarily due to the small size of individual synapses, the high density of synapses, and the lack of appropriate methods to induce synaptic plasticity specifically in the synapse under observation. To overcome these problems, Wang et al. (2009) used the *Aplysia* sensory-motor neuron co-culture system that mimics a simple neuronal circuit which underlies sensitization and habituation of gill-withdrawal reflex. In this preparation, focal application of neurotransmitter serotonin can trigger protein synthesis–dependent synapse-specific plasticity (Martin et al. 1997). By time-lapse imaging of photoconvertible translational reporters introduced to neurons in this system, they demonstrated that the translation was spatially restricted to the activated synapse.

Recently,Inokuchi's group tested whether the somatically synthesized immediate early gene product, Homer1a, can be trapped at the activated synapse in cultured hippocampal neurons (Okada et al. 2009). Homer (also called cupidin/Vesl/PSD-Zip45/Ania3) is a family of synaptic scaffolding protein identified in various studies as a gene product induced by neuronal activity (Nedivi et al. 1993; Brakeman et al. 1997; Kato et al. 1997; Xiao et al. 2000; Shiraishi-Yamaguchi and Furuichi 2007). Okada et al. (2009) expressed one of the subtypes, Homer1a fused with green fluorescent protein (GFP) and monitored its translocation in neurons. When synapses were locally stimulated with NMDA and glycine, more GFP-tagged Homer1a became localized at the stimulated synapse. To test if the somatically synthesized Homer1a could be captured by the stimulated synapse, they used photoactivatable GFP (PA-GFP)-tagged Homer1a and selectively photoactivated PA-GFP-Homer1a in the cell body. The authors demonstrated that the soma-derived PA-GFP-tagged Homer1a protein was captured by activated synapses.

Is the capture of protein to activated synapses a unique property of Homer1a, or is it a more general property shared by various synaptic proteins (Okabe 2007; Sheng and Hoogenraad 2007)? To address this question, Bosch et al. (2009) investigated the time course of translocation of multiple postsynaptic proteins including receptors, enzymes, and scaffolding components of the postsynaptic

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density (PSD), during the selective induction of LTP onto single dendritic spines using two-photon glutamate uncaging. They cotransfected the GFP-fused protein of interest with a red fluorescent protein (RFP) to simultaneously observe the localization of the protein and spine volume changes upon synaptic stimulation. They found that most of the proteins were translocated to the synapse following LTP induction. For the majority of them, including Homer1a, AMPAR GluR1, actin, and CaMKIIα, the total amount of protein that accumulated at the spine was comparable to the changes in spine volume, making their concentration before and after LTP induction equivalent (Okamoto et al. 2004; Kuriu et al. 2006; Bosch et al. 2009). This supports the idea that the change in the volume of the spines induced by F-actin polymerization provides an enhanced binding capacity to that synapse and acts as the tag that can quantitatively capture the new proteins needed to maintain the potentiated state (Okamoto et al. 2009; Redondo and Morris 2011).

15.5 The Functions of the Neuronal Activity–Induced Gene Products in Hebbian and Homeostatic Plasticity

As we have seen above, synaptic activity induces the expression of many neuronal genes. How then do these neuronal activity–induced gene products affect synaptic transmission in turn? We overexpressed Homer1a in hippocampal pyramidal neurons and compared the response amplitude of glutamatergic synaptic transmission between control and overexpressing neurons (Sala et al. 2003) (Fig. 15.2a). Surprisingly, overexpression of Homer1a reduced the postsynaptic current amplitude instead of increasing it. The reduction in transmission occurred in both AMPARs and NMDAR-mediated synaptic currents. Sala et al. (2003) carried out morphological analyses of Homer1a overexpressing neurons and found that the neurons not only had a reduced surface glutamate receptors but also a decrease in various postsynaptic proteins as well as the density and size of dendritic spines (Fig. 15.2b). So the effect of Homer1a is the global suppression of both synaptic structure and function, which is seemingly counterintuitive considering that Homer1a is induced by neuronal activity.

To elucidate the molecular mechanisms of this effect, a better understanding of the role of Homer1b, the long form of Homer is critical (Shiraishi-Yamaguchi and Furuichi 2007). Homer1b is generated from the same gene as Homer1a but from a longer transcript (Fig. 15.3a). Homer1a is a short, monomeric form that contains only an EVH1 domain and is expressed in an activity-dependent manner. In contrast, the long forms, Homer1b and c, have both the EVH1 and the coiled-coil domains that form a tetramer and are constitutively expressed (Hayashi et al. 2006, 2009). Through the EVH1 domain, both short and long Homers can bind to various other scaffolding and signal transduction molecules, which include group I metabotropic glutamate receptors (mGluR) (Brakeman et al. 1997), inositol-1,4,5-trisphosphate receptors

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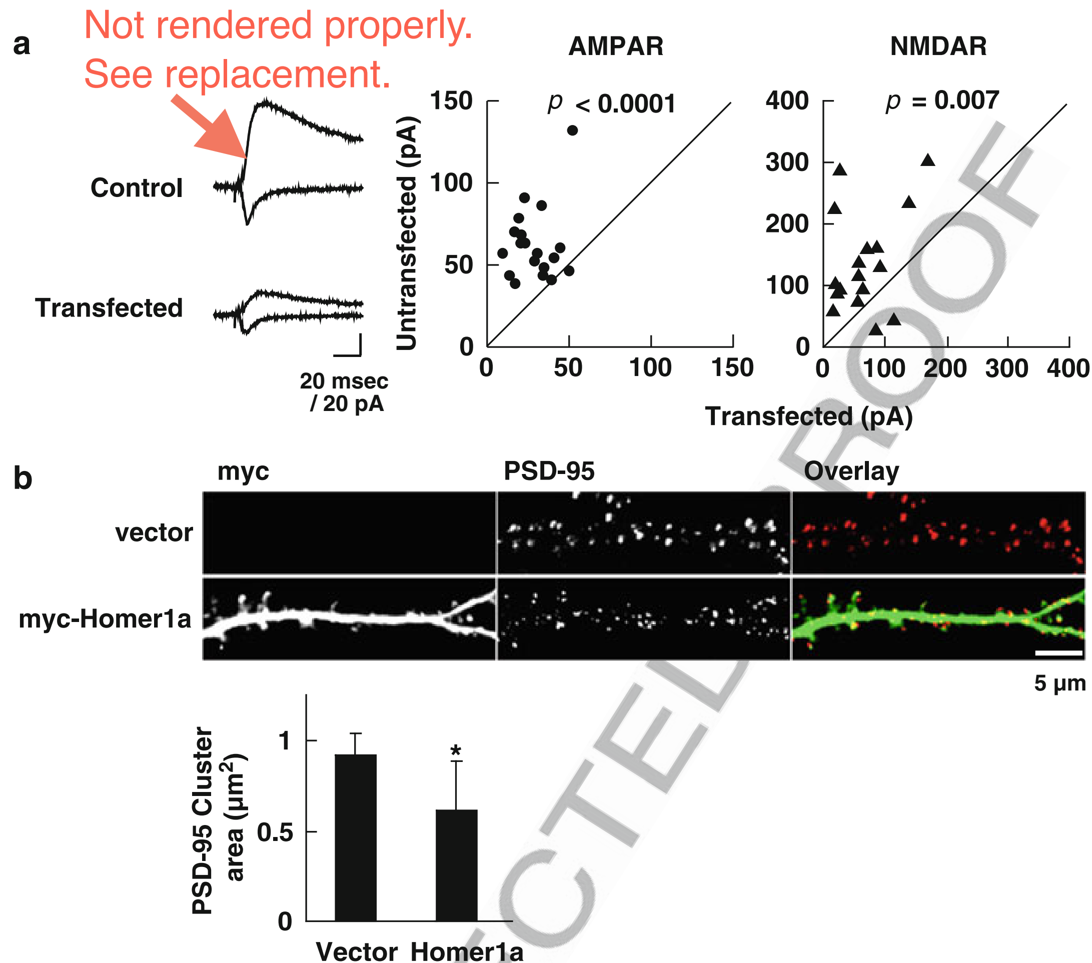

Fig. 15.2 *Reduction of synaptic current and components in neurons expressing Homer1a.* (**a**) A paired recording from a hippocampal pyramidal CA1 pyramidal neuron expressing Homer1a or adjacent untransfected neuron. *Downward trace*: AMPAR EPSC recorded at −60 mV; *upward trace*: NMDAR-EPSC recorded at +40 mV in the presence of AMPAR blocker. Results of recording from multiple cells. Note that both AMPAR and NMDAR components are similarly reduced. ~~Scale bar: 20 pA/20 mV.~~ (**b**) Reduction of PDS-95 synaptic clusters, a representative postsynaptic protein, in neurons overexpressing Homer1a. $^{*}p < 0.01$ (Modified from Sala et al. 2003)

(IP_3R) (Tu et al. 1998), Shank (Tu et al. 1999), TRPC family channels (Yuan et al. 2003), and PI3 kinase enhancer (Rong et al. 2003).

Sala et al. (2001) found that when Homer1b is coexpressed with its binding partner Shank in neurons, an enlargement in the size of dendritic spines together with the recruitment of multiple postsynaptic proteins was observed. Shank itself can also form oligomers through homomeric association (Im et al. 2003; Romorini et al. 2004; Baron et al. 2006). Hayashi et al. (2009) found that when Homer1b and Shank were mixed together, they formed a high-order mesh-like complex (Fig. 15.3b). This complex can carry postsynaptic adapter protein GKAP, which is further linked to synaptic surface glutamate receptor proteins. When tetramer formation of Homer1b or interaction with Shank was prevented by point mutations, Homer and Shank could not form the high order complex. When the mutant that abolish tetramer formation was introduced to a neuron, the number of dendritic spines decreased and the spines

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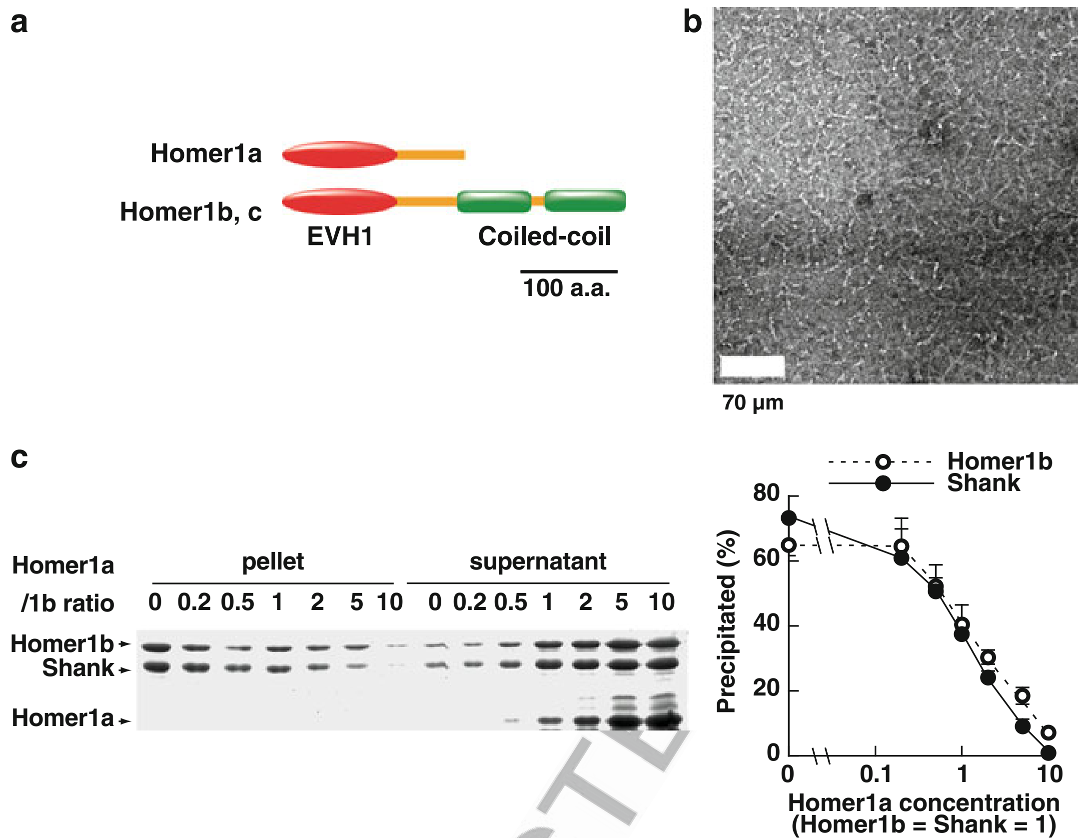

Fig. 15.3 *Interaction between Homer1a and Shank-Homer1b complex*. (**a**) A schematic drawing of Homer1a and Homer1b. (**b**) Electron microscopic picture of complex between Homer1b and Shank. The proteins were expressed separately, reconstituted in vitro, and observed with electron microscopy. Note a mesh-like high-order structure. (**c**) Inhibition of Homer1b-Shank by the addiction of Homer1a. To the fixed amount of Homer1b and Shank, an increasing amount of Homer1a was added. The mixture was centrifuged to precipitate high order complex and the amount of Homer1b and Shank in pellet fraction was measured (Modified from Hayashi et al. 2009)

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~~got~~ became longer and thinner similarly to those which overexpress Homer1a (Sala et al. 2003; Hayashi et al. 2009). These results indicate that the mesh formation between Homer1b and Shank is required for the maturation of synapses, likely by forming a two-dimensional lattice where other postsynaptic proteins rest. Interestingly, addition of Homer1a prevented the Homer1b-Shank mesh formation in a dose-dependent fashion. This observation reasonably explains the general suppressive function of Homer1a on postsynaptic structure and function (Fig. 15.3c).

Hu et al. (2010) also found that Homer1a reduces synaptic AMPAR currents similarly to Sala et al. (2003). But they considered this effect to be mediated by the activation of group I mGluR. Binding of Homer1a with the intracellular carboxyl tail of group I mGluR activates the receptor without glutamate (Ango et al. 2001), which leads to the reduction of tyrosine phosphorylation of AMPAR subunit GluR2 and then to the reduction of surface AMPAR (Hayashi et al. 1999; Hayashi and Huganir 2004). However, this mechanism does not fully explain the reduction of other postsynaptic proteins such as NMDA receptor, Homer1b, actin, and Shank or the shrinkage of the

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overall structure of dendritic spines (Sala et al. 2003). Therefore, it still remains to be determined what is the exact role of Homer1a at the synapse.

Overexpression of Arc/Arg3.1 also reduced the level of AMPAR-mediated synaptic transmission without changing NMDAR-mediated synaptic transmission unlike Homer1a, indicative of different mechanism of action between these two proteins (Chowdhury et al. 2006; Rial Verde et al. 2006; Shepherd et al. 2006). Arc/Arg3.1 interacts with endophilin 2 and 3 and dynamin, which are components of the clathrin-mediated endocytotic machinery. The interaction stimulates the clathrin-mediated endocytosis of synaptic AMPAR, thereby leading to a specific reduction of AMPAR-mediated synaptic transmission. This mechanism works even at a single synapse level (Béïque et al. 2011). Mice with Arc/Arg3.1 gene disruption exhibited an enhanced E-LTP but diminished L-LTP and impaired memory (Plath et al. 2006). However, it remains to be determined whether Arc/Arg3.1 protein is captured by an activated synapse to the same extent as that reported for Homer1a. Further study is needed to clarify the role of Arc/Arg3.1 at specific synapses during memory formation.

There is a special isoform of atypical protein kinase C (PKC) called PKMζ. Unlike full-length PKC, which requires diacylglycerol and Ca^{2+} for activation, PKMζ is constitutively active. Instead, the activity of PKMζ is regulated through a unique translational machanism. Under basal neuronal activity, translation of PKMζ is repressed. But by LTP induction and resultant activation of intracellular signaling cascade, this repression is unmasked and translation of PKMζ protein is initiated (Hernandez et al. 2003). Once active PKMζ is formed, it induces its own translation, thereby maintaining its own protein levels (Westmark et al. 2010). The activated PKMζ reduces internalization of AMPAR subunit GluR2 by increasing the interaction between GluR2 and NSF, thereby increasing the surface amount of AMPAR (Yao et al. 2008). Interestingly, once PKMζ increases the synaptic GluR2, it is kept within the vicinity of GluR2 through a PDZ containing protein Pick1 and this is proposed to be a self-perpetuating mechanism to maintain the increased transmission seen in LTP (Yao et al. 2008). Consistently, a peptide inhibitor of PKMζ blocks the maintenance of LTP as well as retention of memory (Sajikumar et al. 2005; Shema et al. 2007). However, again, a detailed intracellular distribution of PKMζ has not been visualized at the resolution carried out for Homer1a. Therefore, it is still an open question whether the proposed mechanism works specifically at the potentiated synapse and, if it is the case, what is the mechanism for the selective action of the protein. Also, the fact that L-LTP can be still induced in GluR2 knockout animals contradicts the proposed self-perpetuating mechanism (Asrar et al. 2009).

CPG2 was isolated and functionally characterized by Nedivi and colleagues (Nedivi et al. 1993; Hevroni et al. 1998; Cottrell et al. 2004; Loebrich and Nedivi 2009). It encodes a protein with homology to dystrophin and also contains several structural domains, such as spectrin repeats and coiled-coil domain. Interestingly, this protein is localized to the postsynaptic endocytotic zone and is also involved in AMPAR internalization (Cottrell et al. 2004). RNAi-mediated suppression of CPG2 resulted in a decreased internalization of AMPAR and an increase in the size of dendritic spines. Overexpression of CPG2 reduces the size of dendritic spines.

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Another activity-induced gene product with a known synaptic role is SNK/polo-like kinase 2, isolated by Kuhl's group (Kauselmann et al. 1999). Seeburg et al. found that it phosphorylates spine-associated RapGAP (SPAR) and destines it to the degradation pathway, which ultimately leads to a reduction of synaptic transmission (Seeburg et al. 2008; Seeburg and Sheng 2008). Arcadin is an activity-induced cadherin-like transmembrane molecule (Yamagata et al. 1999). Overexpression of this molecule in hippocampal neuron makes dendritic spines smaller (Yasuda et al. 2007).

From these studies, a feature shared by many, though not all, of the neuronal activity–induced gene products emerges. They are consistent in reducing excitatory synaptic transmission, rather than potentiating the transmission. Considering how most of these genes were isolated, it is actually a logical consequence. Most of the studies used massive neuronal stimulation typically by pharmacological reagents or electrical stimulation above the physiological range of neuronal activity, such as seizure and kindling. This is an understandable experimental choice to obtain sufficient sample materials for biochemical or molecular biological identification of the genes. But as a result, most of the identified activity-induced gene products, instead of potentiating the excitatory synaptic response, downregulate the synaptic response. These genes are most likely involved in homeostatic plasticity, the neurons ability to reduce the input activity when their excitability is too high (Turrigiano et al. 1998; Turrigiano 1999). This indicates that while these studies show that neuronal activity–induced genes shapes our initial understanding of the biology of synapse, there is still a lot to be investigated in order to fully comprehend the roles of neuronal activity–induced gene in regulating synaptic plasticity.

15.6 Concluding Remarks

Experimental efforts from a number of laboratories over the last two decades have elucidated the roles of various neuronal activity–induced genes. Among the estimated 500–1,000 neuronal activity–induced genes (Loebrich and Nedivi 2009), only a handful of them have been characterized in any great detail. Nevertheless, these studies have already illustrated the diverse mechanisms by which neuronal activity–induced gene products regulate synaptic transmission. Interestingly, most of these genes have been found to be negative homeostatic regulators of neuronal function. Critical information still largely lacking is the precise intracellular distribution of the neuronal activity–induced gene products in relation to the synapse underwent plasticity. It is not clearly known whether these proteins specifically act on potentiated synapses or they act nonspecifically on all synapses. Proteins in neurons are diffusible and can even be shared between neighboring synapses (Gray et al. 2006; Kuriu et al. 2006; Dieterich et al. 2010). For neuronal activity–induced genes to function at a synapse which has undergone Hebbian-fashion potentiation, it has to be captured specifically at the activated synapse but not at others. Further examination of the precise intracellular dynamics of these

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activity induced gene products using more advanced imaging techniques will be necessary to fully understand the role of neuronal activity–induced genes. 402 403

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