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Molecular mechanisms underlying neural circuit formation

Bai Lu,

Section on Neural Development & Plasticity, NICHD, National Institutes of Health, Bethesda, MD 20892-3714

Kuan Hong Wang, and

Unit on Neural Circuits and Adaptive Behaviors, NIMH, National Institutes of Health, Bethesda, MD 20892-3714

Akinao Nose

Department of Complexity Science and Engineering, Graduate School of Frontier Sciences, The University of Tokyo, Chiba 277-8561, Japan

Abstract

The functions of the nervous system are mediated by neural circuits that are formed during development and modulated by experiences. Central to the assembly of neural circuits is the regulation of synaptic connectivity by synaptic molecules and neuronal activity. Extensive studies have focused on identifying molecules involved in synapse formation. Besides factors promoting synaptogenesis, several anti-synaptogenic factors have been discovered. These factors act in concert to ensure the timing and specificity of circuit formation. Moreover, progress has been made in revealing how neuronal activity governs the balance of excitatory and inhibitory synapses. Intriguingly, several transcription factors, as well as activity-dependent transcription of BDNF through promoter IV, have been shown to selectively regulate cortical inhibitory circuits by promoting GABAergic synapse formation.

Introduction

How does an axon (or dendrite) choose among numerous available dendrites (or axons) to form a synapse that not only functions but also lasts for a long time? It is well known that mammalian synapses rarely form at their first encounter. Instead, long-lasting marriage of the pre- and postsynaptic partners is a consequence of a lengthy “dating” process that involves a series of meetings, dancing and touching. Only the right partners eventually wed. The cellular and molecular mechanisms underlying the formation of specific synaptic connections have long been a mystery. Regardless of its complexity, it is generally agreed that the dating of synaptic partners involves two sequential processes. First, the synaptic partners have to click with each other; the presynaptic axon and postsynaptic dendrites need to have the right “physical chemistry”. Second, after initial molecular interactions that ensure the parties have the right matches, an activity-dependent process kicks in. The synaptic partners engage in serious talks. However, the synaptic dating does not need to be one-to-one. In fact, there are often competitions. The relationship gets refined and strengthened if the pre- and postsynaptic partners talk (fire) in synchrony, whereas those who can't speak harmoniously (fire asynchronously) are eliminated. Armed with knowledge cumulated over the last decade in

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related fields as well as advances in tools and technologies, recent studies have begun to reveal the secret of how stable and specific synaptic connections are formed. In this review, we will first discuss a set of new studies that reveal how early axon-dendritic interactions help to find synaptic partners in the right categories. We will then highlight recent progress in identifying synaptogenic and anti-synaptogenic factors that strengthen the appropriate connections, as well as how neuronal activity controls the expression of genes that shape and coordinate the formation and stability of neuronal circuits.

Initial selection of synaptic partners – role of local filopodial Ca^{2+}

Using two-photon time-lapse imaging of fluorescence-labeled pre- and postsynaptic partners, motion pictures have been made to describe the initial “dating” process [1,2]. Surprisingly, dendritic filopodia, the precursor of dendritic spines, seem to know which axonal targets are suitable for a long-term relationship after initial contacts. In slice cultures of hippocampus in which postsynaptic CA3 pyramidal cells were labeled by a red dye, Lohmann and Bonhoeffer observed that filopodia grow, retract, and search for appropriate positions along the axons to make contacts. The lifetimes for most of the contacts are very short (a little more than a minute), and only a few contacts eventually become stable synapses on glutamatergic axons. Remarkably, although dendritic filopodia frequently contacted GABAergic axons (labeled with the GABAergic marker, GAD65-GFP), such contacts never form stable synapses [1]. Conversely, protrusions from labeled GABAergic axons also make transient contacts with dendritic shaft, but they are never transformed into long-lasting, stable contacts. Unlike glutamatergic synapses, new GABAergic synapses were formed exclusively by the creation of new boutons at pre-existing axon-dendrite crossings without the involvement of any dendritic or axonal protrusions [2]. While specific molecules that prohibit the bonding between GABAergic axons and dendritic spines remain to be identified, several lines of evidence suggest that local Ca^{2+} transients in dendritic filopodia play a critical role: 1) the frequency of Ca^{2+} transients increases after filopodia-axon contacts are made; 2) the increase in local Ca^{2+} is more pronounced at stable contacts than at short-lived contacts; 3) the Ca^{2+} change is not observed in filopodia contacting GABAergic axons [1]. Since this Ca^{2+} transient was independent of glutamatergic transmission [1], contact initiated nonsynaptic signaling, perhaps through cell adhesion molecules (CAMs), might contribute to the initial selection of appropriate synaptic partners.

Synptogenic and anti-synptogenic factors

After initial contacts, further development of synaptic relationships depends largely on the right molecular chemistry. Neuroligins and Neurexins are possibly the best known trans-synaptic CAMs that connect pre- and postsynaptic cells [3]. Previous *in vitro* analyses demonstrated that neuroligins and neurexins can reciprocally instruct pre- and postsynaptic specializations, suggesting that these molecules function as bidirectional inducers for synaptogenesis [3]. Surprisingly, however, recent analyses *in vivo* suggest that neuroligins are not required for initial establishment of synapses, but instead play critical roles in the regulation of functional maturation of excitatory and inhibitory synapses. In wild-type animals, neuroligin 1 (NLGN1) is preferentially localized at excitatory synapses whereas neuroligin 2 (NLGN2) is enriched at inhibitory synapses [4,5]. In *Nlgn-1* knockout mice, excitatory synapses are still formed but specifically impaired in NMDA receptor signaling, whereas the *Nlgn-2* knock-out mice exhibit selective defects in inhibitory synaptic transmission [6]. Conversely, over-expression of *Nlgn-1* or *Nlgn-2* in neuronal cultures increases excitatory or inhibitory synaptic responses, respectively [6]. Moreover, a point mutation of *Nlgn-3* specifically increases inhibitory synaptic transmission, possibly due to a gain-of-function effect [7]. These results together raise the possibility that the balance between NLGN1, 2 and 3 signaling may regulate

the excitatory/inhibitory (E/I) ratio and the proper maturation of circuit-specific synaptic junctions, but not the initial formation of synapses.

What molecules may play a more direct role in synaptogenesis? Another well known synaptic receptor-ligand pair is Eph/ephrin, which mediates trans-synaptic signaling in a bidirectional manner [8]. Previous studies on EphB1-3 triple knockout mice showed that the forward signaling, mediated by the binding of presynaptic ephrins to, and the activation of, postsynaptic Eph receptors, is required for spine morphogenesis and postsynaptic differentiation [8]. Recent works in several experimental systems have begun to reveal the importance of ephrin reverse signaling in spine and synapse formation. In *Xenopus* retinotectal system, the activation of presynaptic ephrinB1 by postsynaptic EphB2, as probed through the infusion of EphB2-Fc fusion protein, enhanced presynaptic glutamate release and the number of retinotectal synapses [9]. EphrinBs are also present in the dendrites of hippocampal neurons; and ephrinBs mediated reverse signaling can trigger spine and synapse formation in postsynaptic neurons [10,11].

Other classes of CAMs that contribute to the specificity in synaptic connectivity have also been identified. Previous studies in *Drosophila* and *C. elegans* have shown that Capricious and Syg-1/2, which are expressed in specific synaptic partner cells, mediate synaptic interactions between specific cell pairs [12,13]. While it has been difficult to identify similar molecules in the vertebrate nervous system, significant progress has been made recently towards the understanding of the molecular mechanisms underlying target recognition in complex neuronal circuits (Figure 1A). Sidekicks and Dscams, four closely related CAMs belonging to a subfamily of immunoglobulin superfamily, are expressed in different subsets of pre- and postsynaptic partners in chick retina and direct lamina-specific synaptic connectivity [14]. Another immunoglobulin-like protein, Close Homologue of L1 (CHL1), is involved in the guidance of stellate axons towards a specific subcellular region of the target Purkinje cell [15].

In addition to synaptogenic factors, anti-synaptogenic factors are important for the specificity of synapse formation (Figure 1B). For example, Wnt4 could act as an anti-synaptogenic signal, preventing the development of the neuromuscular synapse between a motor axon and one of the two target muscle cells in *Drosophila* [16]. Wnt/Lin-44 in *C. elegans* determines the subcellular location of synapses by preventing synapse formation in a specific domain of DA9 axons [17]. Unc-6/Netrin regulates DA9 synapse formation in a similar manner [18]. Taken together, Wnt/Lin-44 and Unc-6/Netrin are expressed in different parts of the body and act in concert to restrict synapse formation to a discrete domain of the axon where both molecules are absent. These results indicate that spatial specificity of synapses is regulated not only by attraction from the target but also by exclusion from neighboring cells or local environments. Timing of synaptogenesis can also be controlled by anti-synaptogenic factors. Slit1a, through Robo2 receptor, inhibits arborization and synaptogenesis of retinal ganglion cells in zebrafish. In the absence of Slit-Robo signaling, the arbors form earlier, suggesting that Slit-Robo prevents premature synapse formation [19]. Similarly, polysialic acid (PSA), which is attached to NCAM, prevents precocious maturation of GABAergic synapses in the visual cortex of mammals [20]. Interestingly, some of the aforementioned anti-synaptogenic factors, Wnts and Netrins, are also known to act as pro-synaptogenic factors [21,22]. Thus, these molecules might exert both pro- and anti-synaptogenic effects depending on the context. In the case of Unc-6/Netrin, the difference appears to be regulated by different receptors: Unc-40/DCC mediates synaptogenesis and Unc-5 anti-synaptogenesis [18,22]. An important future challenge is to elucidate the context-dependent signaling pathways that differentiate pro- and anti-synaptogenic response in the cells.

Activity-regulated gene expression and coordinated development of glutamatergic and GABAergic synapses

While genetically pre-specified molecular recognition mechanisms may be important in connecting specific synaptic partners, neural activity-regulated gene expression programs appear to play a key role in orchestrating the assembly of neural circuits, which contain synaptic connections among diverse types of neurons. Genome-wide gene expression analyses have revealed several hundreds of genes whose expressions are acutely regulated by membrane depolarization or neural transmitter release [23]. These genes encode both transcription factors and downstream effectors and regulators of synaptogenesis. Recent studies have highlighted the specific roles of several activity-regulated transcription factors in controlling synapse development. They can regulate the numbers of glutamatergic or GABAergic synapses, and affect E/I balance through activity-dependent positive or negative feedback loops in individual cells or across neuronal circuits (Figure 2).

For glutamatergic synapses, MeCP2, a transcriptional regulator that binds to methylated DNA, has been shown to promote the number and strength of excitatory synaptic connections. Loss of MeCP2 in mice led to decreased excitatory synaptic responses and developmentally reduced excitatory synapse numbers, while inhibitory synaptic responses were normal [24,25]. Conversely, doubling of MeCP2 expression enhanced excitatory synaptic responses and synapse formation [24]. Since neuronal activity triggers the phosphorylation of MeCP2, which is necessary for MeCP2-mediated induction of target genes and modulation of dendritic growth and spine maturation [26,27], MeCP2 may participate in an activity-dependent positive feedback loop to promote excitatory synaptogenesis. In contrast, the activity-dependent transcription factor MEF2 has been shown to restrict the number of excitatory synapses. Disrupting MEF2 function *in vitro* or *in vivo* resulted in an increase, whereas overexpression of MEF2 resulted in a decrease, of excitatory synapses [28,29]. Since increased neuronal activity dephosphorylates MEF2, activates MEF2 mediated transcription of target genes, and suppresses excitatory synapse numbers [29], MEF2 seems to mediate an activity-dependent negative feedback loop to maintain E/I balance by restricting excitatory synapses.

For GABAergic synapses, a recent microarray screen of membrane depolarization activated genes identified a transcription factor, Npas4, whose transcription was rapidly and transiently induced in excitatory neurons following calcium influx [30]. The inhibition of Npas4 specifically downregulated, whereas overexpression of Npas4 upregulated, the number of GABAergic synapses formed on developing excitatory neurons. Therefore, Npas4 may mediate an activity-dependent negative feedback loop to maintain E/I balance by enhancing inhibitory synapses. Another transcription factor, the homeoprotein Otx2, regulates the activity-dependent maturation of inhibitory neurons [31]. Remarkably, the effect of Otx2 is non-cell autonomous. Cortical infusion of Otx2 accelerated the maturation of inhibitory cells and the onset of ocular dominance plasticity, whereas conditional knockout of Otx2 in non-inhibitory cells or from the subcortical visual pathways blocked these processes. Otx2 appeared to be synthesized in subcortical sites such as retina and thalamus, then transported into cortical inhibitory neurons in response to visual experience, suggesting a novel circuit-level mechanism by which neural activity can promote the maturation of inhibitory synapses.

The second group of activity-regulated genes encodes molecules that are effectors or regulators of synapses such as Arc, Homer, Cpg15, MHC Class I [32-36]. Of particular interest is the activity-dependent transcription of BDNF through promoter IV. Disruption of *Bdnf* promoter IV function appears to selectively affect the development of cortical inhibition [37] [38]. A subtle mutation that disrupts the ability of CREB to bind *Bdnf* promoter IV results in fewer inhibitory synapses in cultured cortical neurons, deficits in miniature IPSCs in cortical slices, and reduced expression of GABAergic markers, but not glutamatergic markers, in the visual

cortex [37]. Mice that completely lack promoter IV-driven *Bdnf* transcription exhibit significant deficits in GABAergic, but not glutamatergic, synaptic transmission, leading to an aberrant spike-timing dependent synaptic potentiation (STDP) in the prefrontal cortex [38]. These results demonstrate the importance of activity-dependent BDNF transcription in the formation of cortical inhibitory circuits, and reveal a selective modulation of GABAergic function by promoter IV-derived BDNF.

Conclusion

Contrary to previous belief, developing neurons exhibit preference to their future synaptic partners, at least grouped by large categories (GABAergic or glutamatergic), during early stages of synaptogenesis. It has become increasingly clear that the formation of appropriate synaptic networks involves both synaptogenic and anti-synaptogenic factors, which control the specificity as well as timing of synaptogenesis. Cumulating evidence also suggests that neuronal activity orchestrates the development of neuronal circuits through activity-regulated genes, particularly transcriptional factors and their downstream effectors, such as BDNF. Distinct factors seem to regulate excitatory and inhibitory synaptic connections. For example, activity-dependent transcription of BDNF appears to selectively promote the development of cortical GABAergic synapses. An important question for the future is to identify molecular mechanisms underlying the formation of specific synapses within a sub-region of the brain (e.g. CA3 of hippocampus) or within the same class of neurons (e.g. synapses formed in CA3 pyramidal neurons by recurrent collaterals versus those by mossy fibers from dentate gyrus). Another major challenge is to elucidate the distinct yet overlapping sets of genes that are regulated under different activity-dependent programs, and unravel the molecular logic underlying the spatial selectivity and temporal coordination of synapse development in neural circuits.

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A synaptogenic

B anti-synaptogenic

Figure 1. Regulation of synaptic specificity by synaptogenic and anti-synaptogenic factors
 (A) Generation of synaptic specificity by cell adhesion molecules. left: Dscams mediate lamina specificity, right: CHL1 mediates subcellular targeting.
 (B) Generation of synaptic specificity by anti-synaptogenic factors. left: Wnt4 mediates cellular targeting, right: Lin-44/Wnt and Unc-6/Netrin determines subcellular specificity.

Figure 2. Activity-regulated gene expression and coordinated development of glutamatergic and GABAergic synapses

Excitatory synapses (shown in green) are mostly formed on the dendritic spines of excitatory neurons, whereas inhibitory synapses (shown in red) are mostly formed on the dendritic shafts and cell soma. Activity-regulated transcription factors MeCP2 and MEF2 (in green) modulate excitatory synapses (in green) to enhance or decrease excitatory/inhibitory (E/I) ratio.

Activity-regulated transcription factor Npas4, and secreted molecule BDNF (in red) modulate inhibitory synapses (in red) to decrease E/I ratio. The homeoprotein Otx2 promotes inhibitory neuron maturation and decreases E/I ratio through activity-dependent transfer into inhibitory neurons.