adaptor proteins Dok-7, Crk, and CrkL; and strengthening of an interaction between a cytoplasmic protein rapsyn and the ACh receptors. Rapsyn may be the final element in the sequence: It binds directly to the ACh receptors and can induce their aggregation in vitro. In mice lacking rapsyn, muscles form normally and ACh receptors accumulate in normal numbers but fail to aggregate at the synaptic sites on the membrane. Accordingly, muscles of mutant mice lacking Dok7 or rapsyn resemble those lacking MuSK or LRP4: They synthesize ACh receptors but do not have ACh receptor clusters.

Thus, an extracellular protein (agrin), transmembrane proteins (MuSK and LRP4), adaptor proteins (Dok-7, Crk, and CrkL), and a cytoskeletal protein (rapsyn) form a chain that links commands from the motor axon to ACh receptor clustering in the muscle membrane.

Nevertheless, postsynaptic differentiation can occur in the absence of agrin signaling. This capacity was apparent in early studies on cultured muscle (see Figure 48–9) and is also seen in vivo: ACh receptor clusters form initially but then disperse in agrin mutants (Figure 48–12C). Clustering also occurs in muscles that lack innervation entirely. Thus, the signaling pathway that initiates postsynaptic differentiation can be activated without agrin, but agrin is required to maintain clustering of ACh receptors.

The role of agrin is perhaps best understood in terms of the requirement that pre- and postsynaptic specializations be perfectly aligned. ACh receptor aggregates persist in uninnervated muscles but disappear in agrin mutant muscles, suggesting that axons sculpt the postsynaptic membrane through the combined action of agrin and a dispersal factor. One major dispersal factor is ACh itself; clustering persists in mutants that lack both agrin and ACh (Figure 48–12C). Thus, agrin may render ACh receptors immune to the declustering effects of ACh. Through a combination of positive and negative factors, the motor neuron ensures that the patches of postsynaptic membrane contacted by axon branches are rich in ACh receptors.

The Nerve Regulates Transcription of Acetylcholine Receptor Genes

Along with redistribution of ACh receptors in the plane of the membrane, the motor nerve orchestrates the transcriptional program responsible for expression of ACh receptor genes in muscle. To understand this aspect of transcriptional control, it is important to appreciate the geometry of the muscle.

Individual muscle fibers are often more than a centimeter long and contain hundreds of nuclei along their length. Most nuclei are far from the synapse, but a few are clustered beneath the synaptic membrane, so that their transcribed and translated products do not have far to go to reach the synapse. In newly formed myotubes, most nuclei express genes encoding ACh receptor subunits. In adult muscles, however, only synaptic nuclei express ACh receptor genes; nonsynaptic nuclei do not. Two processes contribute to this transformation.

First, as synapses begin to form, expression of the ACh receptor subunit genes is increased in synaptic nuclei (Figure 48–13). Signals acting through MuSK are needed for this specialization. Second, around the time of birth, ACh receptor gene expression shuts down in nonsynaptic nuclei. This change reflects a repressive effect of the nerve, as originally shown by studies of denervated muscle. When muscle fibers are denervated, as happens when the motor nerve is damaged, the density of ACh receptors in the postsynaptic membrane increases markedly, a phenomenon termed *denervation supersensitivity.*

This repressive effect of the nerve is mediated by electrical activation of the muscle. Under normal conditions, the nerve keeps the muscle electrically active, and fewer ACh receptors are synthesized in active muscle than in inactive muscle. Indeed, direct stimulation of denervated muscle through implanted electrodes decreases ACh receptor expression, preventing or reversing the effect of denervation (Figure 48–13B). Conversely, when nerve activity is blocked by application of a local anesthetic, the number of ACh receptors throughout the muscle fiber increases, even though the synapse is intact.

In essence, then, the nerve uses ACh to repress expression of ACh receptor genes extrasynaptically. Current that passes through the channel of the receptor leads to an action potential that propagates along the entire muscle fiber. This depolarization opens voltage-dependent Ca^{2+} channels, leading to an influx of Ca^{2+}, which activates a signal transduction cascade that reaches nonsynaptic nuclei and regulates transcription of ACh receptor genes. Thus, the same voltage changes that produce muscle contraction over a period of milliseconds also regulate transcription of ACh receptor genes over a period of days.

The increase in transcription of ACh receptor genes in nuclei beneath the synapse, along with the decrease in nuclei distant from synapses, leads to localization of ACh receptor mRNA and thus preferential synthesis and insertion of ACh receptors near synaptic sites. This local synthesis is reminiscent of that seen

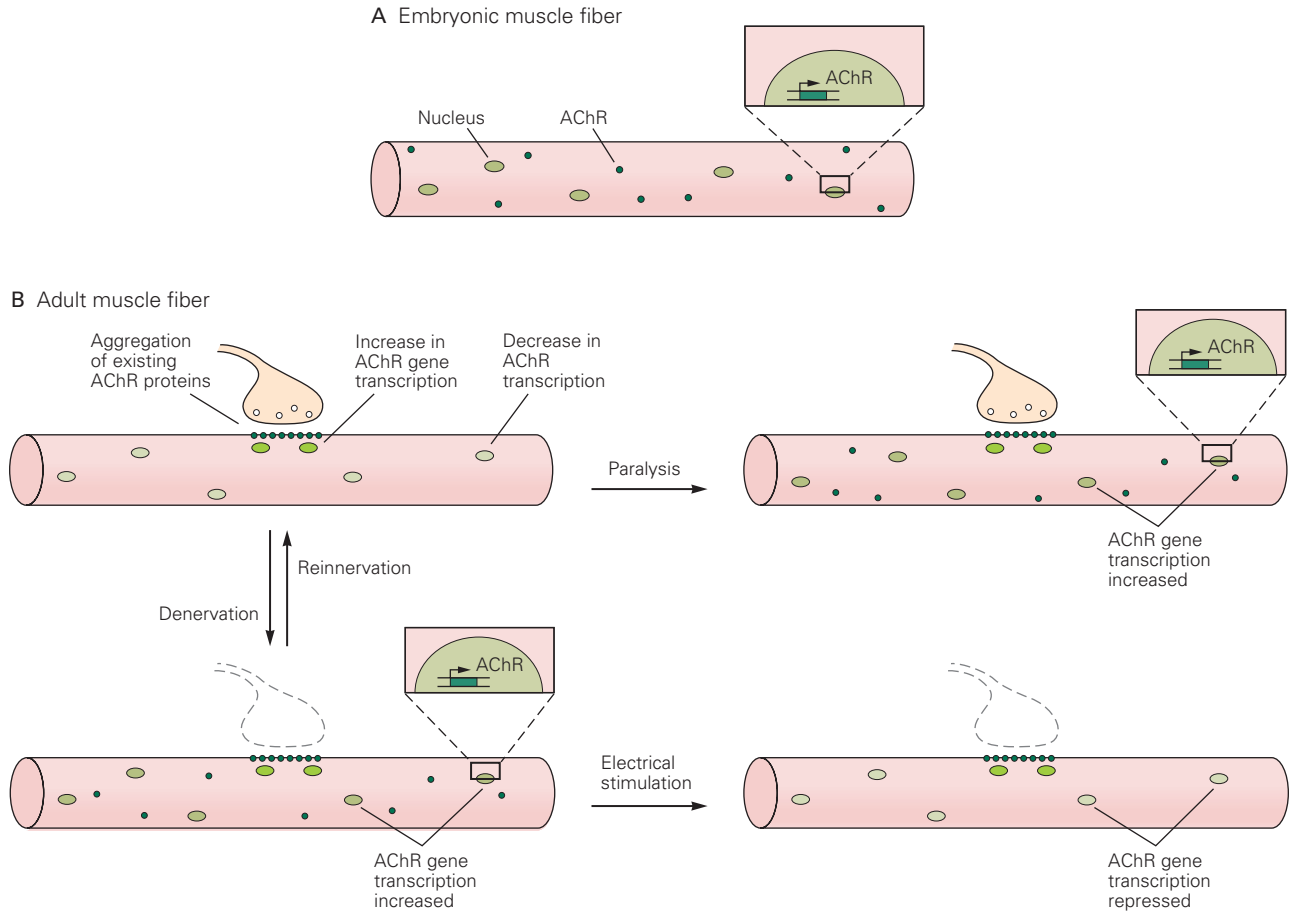

Figure 48–13 Clustering of acetylcholine (ACh) receptors at the neuromuscular junction results from transcriptional regulation and local protein trafficking.

A. ACh receptors (**AChR**) are distributed diffusely on the surface of embryonic myotubes.

B. After the muscle is innervated by a motor axon, the number of receptors in extrasynaptic regions decreases, whereas receptor density at the synapse increases. This reflects the aggregation of preexisting receptors and enhanced expression of ACh receptor genes in nuclei that lie directly beneath the nerve terminal. In addition, the transcription of receptor genes is repressed in nuclei in extrasynaptic regions. Electrical activity in muscle represses ACh gene expression in nonsynaptic nuclei, leading to a lower density of ACh receptors in these regions. The nuclei at synaptic sites are immune to this repressive effect. Following denervation, ACh receptor gene expression is upregulated in extrasynaptic nuclei, although not to the high level attained by synaptic nuclei. Paralysis mimics the effect of denervation, whereas electrical stimulation of denervated muscle mimics the influence of the nerve and decreases the density of ACh receptors in the extrasynaptic membrane.

at postsynaptic sites on dendritic spines in the brain. Local synthesis in muscle is advantageous since ACh receptors synthesized near the ends of fibers would never reach the synapse without degradation.

Many components of the postsynaptic apparatus are regulated in ways similar to those we have described for ACh receptors—their aggregation depends on agrin and MuSK, and their transcription is enhanced in synaptic nuclei and repressed in extrasynaptic nuclei by electrical activity. Thus, synaptic components have tailor-made regulatory mechanisms, but many of these components are regulated in parallel.

The Neuromuscular Junction Matures in a Series of Steps

The adult neuromuscular junction is dramatically different in its molecular architecture, shape, size, and functional properties from the simple nerve-muscle contact that initiates neurotransmission in the embryo. Maturation of the nerve terminal, the postsynaptic membrane, and the intervening synaptic cleft occurs in a complex series of steps. We illustrate this stepwise synaptic construction with a continued focus on the development of ACh receptors.

As we have seen, ACh receptors aggregate in the plane of the membrane as the neuromuscular junction begins to form, and receptor gene transcription is enhanced in postsynaptic nuclei. A few days later, activity begins to decrease the level of extrasynaptic receptors. These transcriptional changes are soon followed by changes in the stability of the receptors. In embryonic muscle, ACh receptors are turned over rapidly (with a half-life of approximately 1 day) in both synaptic and extrasynaptic regions. In contrast, in adult muscle, the receptors are relatively stable (with a half-life of approximately 2 weeks). The metabolic stabilization of ACh receptors helps concentrate them at synaptic sites and stabilize the postsynaptic apparatus.

Yet another alteration is in the composition of the ACh receptors. In the embryo, ACh receptors are composed of α-, β-, δ-, and γ-subunits. During the first few postnatal days, the γ gene is turned off and a closely related gene called ε is activated. As a result, new ACh receptors inserted in the membrane are composed of α-, β-, δ-, and ε-subunits. This altered subunit composition tunes the receptor in a way that is suited to its mature function. However, although it occurs at the same time as the metabolic stabilization, the two changes are not causally linked.

These molecular changes in the ACh receptors are accompanied by changes in their distribution (Figure 48–14). Soon after birth, junctional folds begin to form in the postsynaptic membrane and ACh receptors become concentrated at the crests of the folds, along with rapsyn, whereas other membrane and cytoskeletal proteins are localized in the depths of the folds. The initial aggregate of ACh receptors appears to have a plaque-like appearance. Perforations that undergo fusion and fission eventually transform the dense plaque into a pretzel shape that follows the branches of the nerve ending. New receptor-associated cytoskeletal proteins are added to the aggregate, presumably to drive the geometric changes. Finally, the postsynaptic membrane enlarges and eventually contains many more ACh receptors than were present in the initial cluster. Each of these changes occurs while the synapse is functional, suggesting that ongoing activity plays an important role in synaptic maturation.

Central Synapses and Neuromuscular Junctions Develop in Similar Ways

Synapses in the central nervous system are structurally and functionally similar to neuromuscular junctions in many ways. Presynaptically, most of the major protein components of synaptic vesicles are identical at both types of synapses. Likewise, the mechanisms of transmitter release differ only quantitatively, not qualitatively. Postsynaptically, neurotransmitter receptors are concentrated beneath the nerve terminal and associated with "clustering" proteins.

These parallels extend to synaptic development. Studies of cultured neurons have shown that the cellular logic of synapse formation is conserved between neuromuscular junctions and central synapses. At both synaptic types, pre- and postsynaptic elements regulate each other's differentiation by organizing synaptic components rather than by inducing their expression, and synapses develop in a progressive series of steps (Figure 48–15). The molecular details differ, however. Neuromuscular organizing molecules such as agrin and laminins do not play key roles at central synapses, suggesting that other synaptic organizers are involved. Recently, some of these organizing molecules have been identified.

Neurotransmitter Receptors Become Localized at Central Synapses

The concentration of neurotransmitter receptors in the postsynaptic membrane is a feature shared by many synapses. In the brain, receptors for glutamate, glycine, γ-aminobutyric acid (GABA), and other neurotransmitters are concentrated in patches of membrane aligned with nerve terminals that contain the corresponding transmitter.

The processes by which these receptors become localized may be similar to those at the neuromuscular junction. In cultures of dissociated hippocampal neurons, for example, both glutamatergic and GABAergic nerve terminals appear to stimulate clustering of appropriate receptors in the postsynaptic membrane. Moreover, nerves can induce expression of genes encoding glutamate receptors in central neurons, much as occurs for ACh receptors in muscle. Finally, electrical activity also regulates expression of neurotransmitter receptors in neurons.

In forming receptor clusters, central neurons face an obvious challenge that myotubes do not: They are contacted by axon terminals from distinct classes of neurons that use different neurotransmitters (Figure 48–16A). Thus, the nerve terminal probably has an instructive role in the clustering of receptors. In cultures of hippocampal neurons, glutamatergic and GABAergic axons terminate on adjacent regions of the same dendrite. Initially, glutamate and GABA receptors are dispersed, but soon, each type becomes selectively clustered beneath terminals that release that neurotransmitter. This observation implies the existence of multiple clustering signals with parallel pathways of signal transduction.

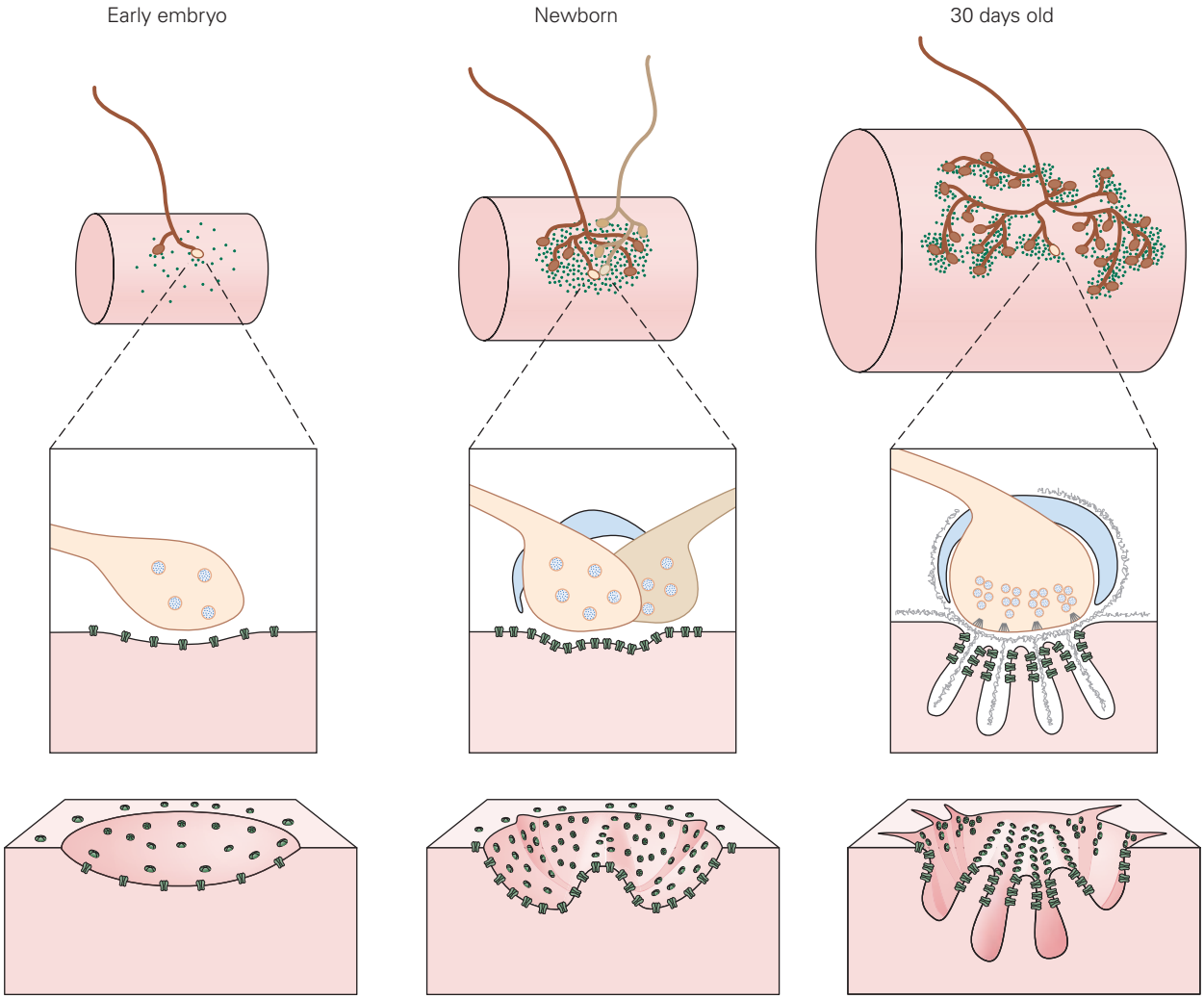

Figure 48–14 **The postsynaptic membrane at the neuromuscular junction matures in stages.** During early embryogenesis, ACh receptors exist as loose aggregates. Later, these aggregates condense into a plaque-like structure. After birth, the dense cluster opens up as the nerve develops multiple terminals. These axon branches expand in an intercalary fashion as the muscle grows, and the plaque indents to form a gutter, which then invaginates to form folds. Receptors are concentrated at the crests of the folds. (Adapted, with permission, from Sanes and Lichtman 2001.)

At the neuromuscular junction, rapsyn binds to the intracellular domain of ACh receptors and clusters them. Several proteins have been found to play similar roles at central synapses. One, gephyrin, is highly concentrated in the synaptic densities at glycinergic and some GABAergic synapses (Figure 48–16A). Gephyrin is not structurally related to rapsyn but has the same function: It links the receptors to the underlying cytoskeleton. In nonneural cells, glycine receptors cluster when gephyrin is co-expressed; conversely, clusters fail to form at inhibitory synapses in gephyrin-deficient mutant mice (Figure 48–16B). Similarly, a class of proteins that share conserved segments called PDZ domains—the prototypes being PSD-95 or SAP-90—facilitate clustering of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors and their associated proteins. Other PDZ-containing proteins interact with α-amino-3-hydroxy-5-methylisoxazole-4-propionate acid (AMPA), kainate, and metabotropic types of glutamate receptors.

Synaptic Organizing Molecules Pattern Central Nerve Terminals

Although central synapses and neuromuscular junctions share many features, their synaptic clefts differ

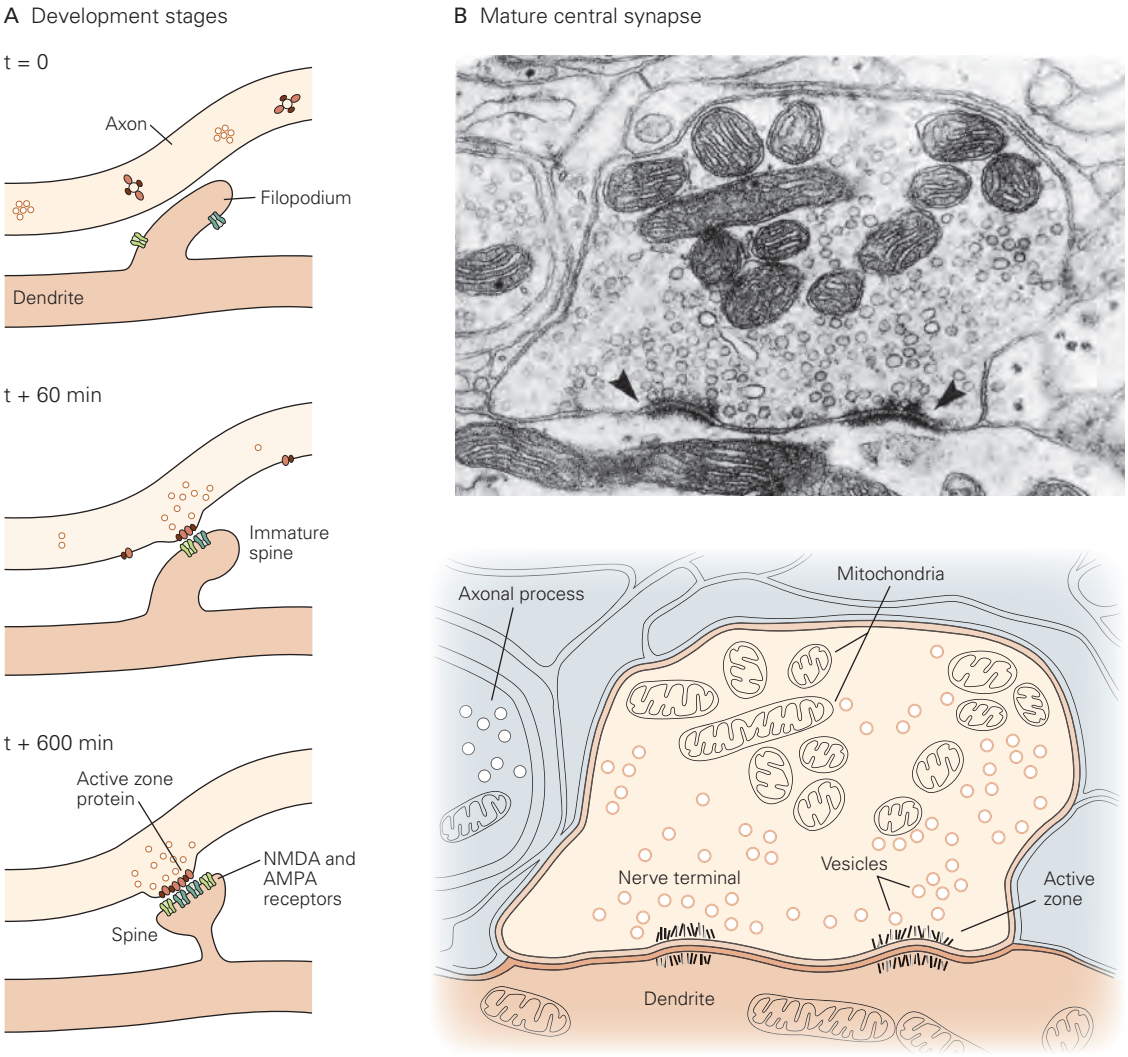

Figure 48–15 Ultrastructure of a synapse in the mammalian central nervous system.

A. Initial contact between an axon and a filopodium on a developing dendrite leads to a stable dendritic spine and an axodendritic synapse. This entire process can take as little as 60 minutes. (Abbreviations: **AMPA**, α-amino-3-hydroxy-5-methylisoxazole-4-propionate acid; **NMDA**, *N*-methyl-D-aspartate.)

B. In a mature interneuron synapse in the cerebellum, synaptic vesicles in the nerve terminal are clustered at active zones (**arrows**) directly opposite receptor-rich patches of postsynaptic membrane. (Reproduced, with permission, from J.E. Heuser and T.S. Reese.)

dramatically. Whereas muscle fibers are ensheathed by a basal lamina that has a distinctive molecular structure at the neuromuscular junction, central neurons do not have a prominent basal lamina. Instead, formation of central synapses is regulated in large part by molecules embedded in the pre- and postsynaptic membranes.

Several interacting pairs of membrane proteins have now been found that link the pre- and postsynaptic membranes and also organize synaptic differentiation as synapses form. Perhaps the best studied are a set of proteins called neurexins, which are enriched in presynaptic membranes, and their partners, the neuroligins, which are concentrated in postsynaptic membranes (Figure 48–17A). There are three neurexin and four neuroligin genes in the mammalian genome. The ability of neurexins and neuroligins to promote synaptic differentiation was first revealed by culturing neurons

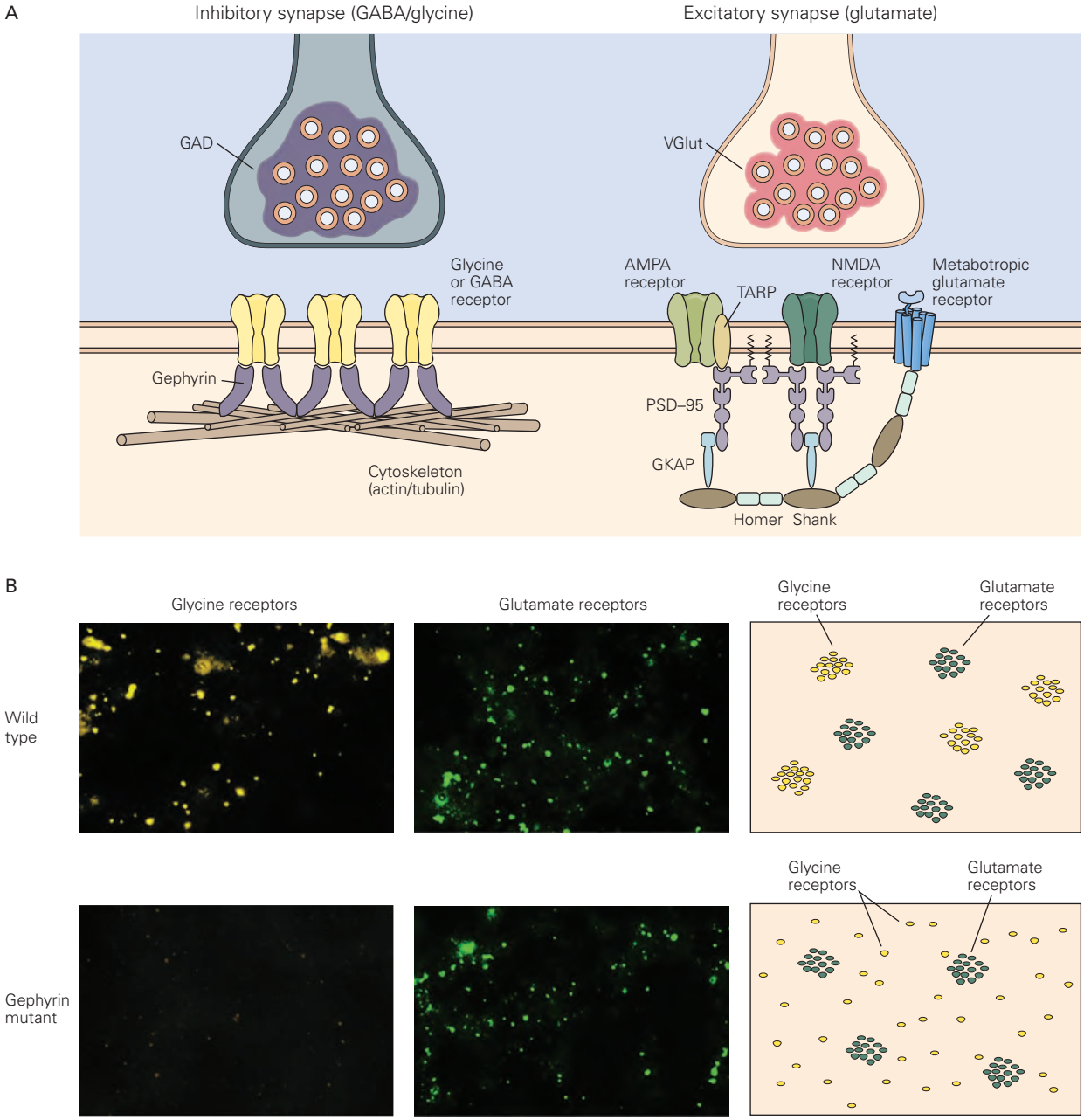

Figure 48–16 Localization of neurotransmitter receptors in central neurons.

A. Glutamate receptors are localized at excitatory synapses, and γ-aminobutyric acid (**GABA**) and glycine receptors are localized at inhibitory synapses. The receptors are linked to the cytoskeleton by adaptor proteins. Glycine receptors are linked to microtubules by gephyrin (*left*), and *N*-methyl-D-aspartate (**NMDA**)-type glutamate receptors are linked to each other and to the cytoskeleton by PSD-95–related molecules (*right*). The PSD family of molecules contains PDZ domains that interact with a variety of synaptic proteins to assemble signaling complexes. Other PDZ-containing proteins interact with α-amino-3-hydroxy-5-methylisoxazole-4-propionate acid (**AMPA**)-type and metabotropic glutamate receptors (see Chapter 13). (Abbreviations: **GAD**, glutamate decarboxylase; **GKAP**, Guanylate-kinase-associated protein; **TARP**, transmembrane AMPA receptor regulatory proteins; **VGlut**, vesicular glutamate transporter.)

B. In gephyrin mutant mice, glycine receptors do not cluster at synaptic sites on spinal motor neurons, and the animals show spasticity and hyperreflexia. In the same neurons, glutamate receptor clusters are unaffected. (Adapted, with permission, from Feng et al. 1998.)

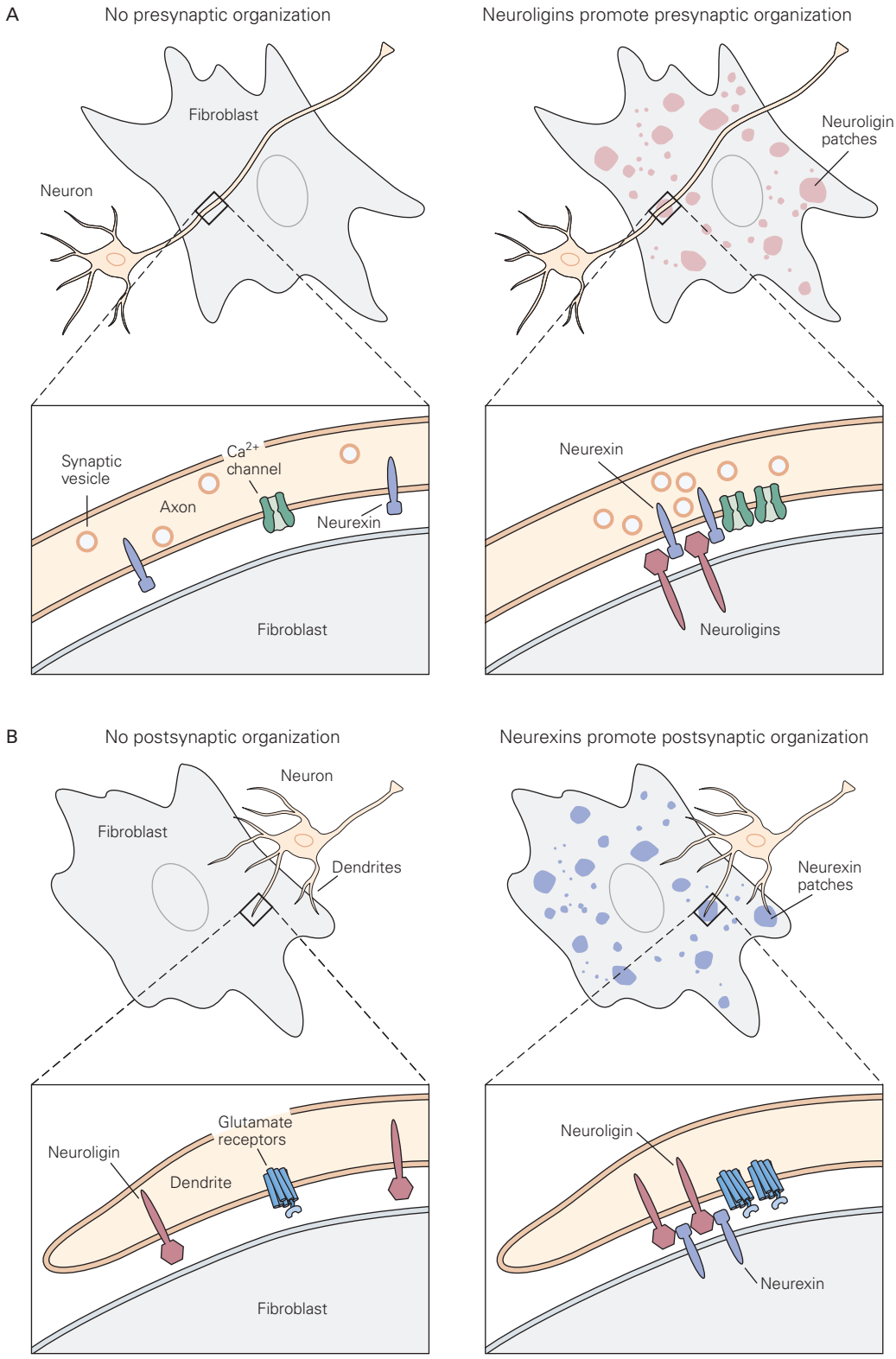

Figure 48–17 Synaptic organizers such as neurexins and neuroligins promote differentiation of central synapses.

A. When brain neurons are cultured with fibroblast cells that express neuroligin, those segments of the axon that contact these cells form presynaptic specializations, marked by clustered neurexin, Ca^{2+} channels, and synaptic vesicles.

B. Similarly, when neurons are cultured with cells that express neurexin, dendrites that contact these cells accumulate aggregates of glutamate receptors, accompanied by scaffolding molecules (not shown) and clustered neuroligins. Neurons grown with control cells fail to form such pre- and postsynaptic specializations.

with nonneural cells engineered to express one or the other. In culture, synaptic vesicles form clusters at sites of contact with the neuroligin-expressing cells, and they are capable of releasing neurotransmitter when stimulated (Figure 48–17A). Conversely, neurotransmitter receptors in dendrites aggregate at sites that contact nonneural cells engineered to express neurexins (Figure 48–17B). Thus, neurexin–neuroligin interactions facilitate precise apposition of pre- and postsynaptic specializations.

How do neurexins and neuroligins work? Part of the answer is that their carboxy terminal tails bind to PDZ domains in proteins such as PSD-95 (Figure 48–16). Indeed, a remarkable number of proteins in both pre- and postsynaptic membranes have PDZ domain-binding motifs, notably adhesion molecules, neurotransmitter receptors, and ion channels. Moreover, many cytoplasmic proteins that possess PDZ domains are present in nerve terminals and beneath the postsynaptic membrane. Thus, PDZ-containing proteins can serve as scaffolding molecules that link key components on both sides of the synapse. Interactions of proteins such as neurexins and neuroligins may provide a means of coupling the intercellular interactions required for synaptic recognition to the intracellular interactions required to cluster synaptic components within the cell membrane.

Although neurexin–neuroligin interactions promote synaptic differentiation in culture, mice lacking neurexins or neuroligins form synapses in vivo. However, the synapses that form in the mutants are defective, with the nature and severity of the defects varying among synaptic types. Thus, the primary role of these synaptic organizers may be to specify the properties of particular synapses. For example, neuroligin1 is concentrated in the postsynaptic membrane of excitatory synapses, and levels of glutamate receptors are reduced at excitatory synapses in neuroligin1 mutants. Conversely, neuroligin2 is concentrated at inhibitory synapses and plays a critical role in patterning the inhibitory postsynaptic membrane.

Additional complexity in the tuning of central synapses by neurexins arises from the fact that they bind to multiple postsynaptic organizing molecules in addition to the neuroligins (Figure 48–18). Moreover, thousands of neurexin isoforms are generated from each neurexin gene as a result of differences in promoter choice (generating α and β forms) and alternative splicing at multiple sites. Different neurexin isoforms are differentially expressed by neurons and have different affinities for the various neurexin ligands. Neuroligins are also alternatively spliced and differentially expressed and thus are likely to have multiple presynaptic partners.

More recently, other synaptic organizing molecules have been found; they include protein tyrosine phosphatases and leucine-rich repeat proteins as well as members of the fibroblast growth factor (FGF) and

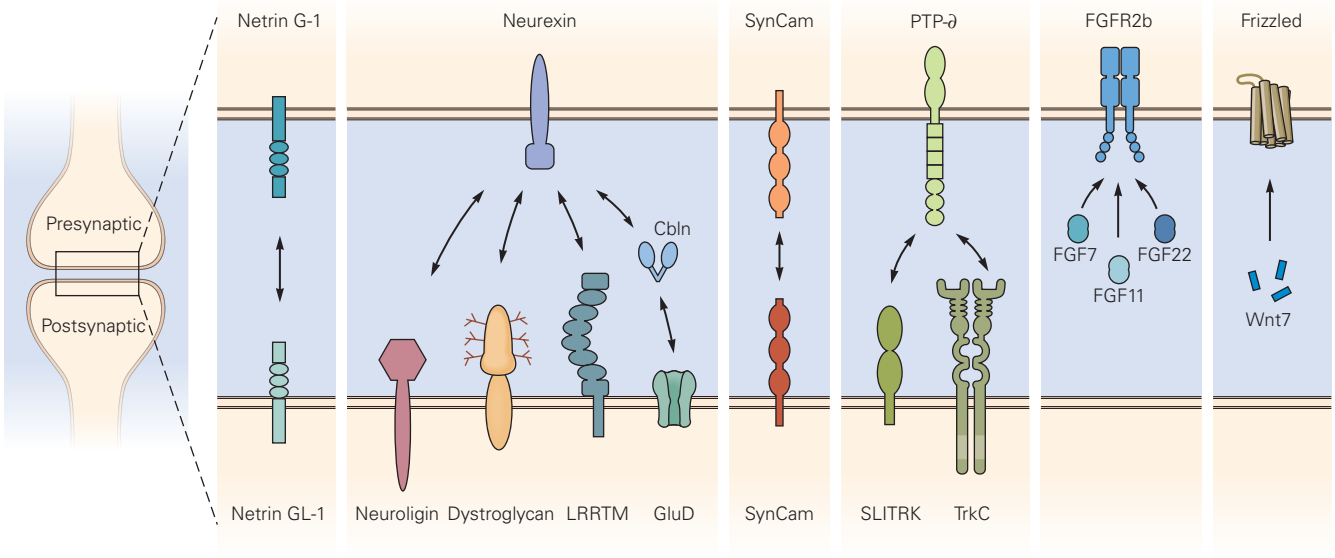

Figure 48–18 **Numerous macromolecular complexes link pre- and postsynaptic membranes at central synapses.** The figure shows some of the many transsynaptic proteins that interact at synaptic sites. Some bias synapse formation in favor of appropriate partners, whereas others act to regulate the properties of the synapse; some may do both.

Wnt families of secreted morphogens and their receptors (Figure 48–18). They are present at specific subsets of synapses and play distinct roles. For example, similar to neuroligin1 and neuroligin2, FGF22 and FGF7 are localized to and promote differentiation of excitatory and inhibitory synapses, respectively. Some of these organizing proteins may act in parallel with neurexins, while others may act as initial organizers, with neuroligins and neurexins consolidating the synapses at a later time and specifying their particular properties.

Together, these results suggest that central synapses are not patterned by master organizers akin to agrin, MuSK, LRP4, and laminins. Indeed, loss of no single central organizer studied to date is lethal in the manner observed for agrin, MuSK, LRP4, and laminin mutants. Instead, the enormous variety of neuronal and synaptic types in the central nervous system and their wide range of functional properties arise from a multitude of organizers that act combinatorially and in cell type–specific ways. Consistent with this view, genetic variation in many central organizers and synaptic recognition molecules, including neurexins, neuroligins, cadherins, and contactins, has been associated with behavioral perturbations in experimental animals and with behavioral disorders, including autism, in humans (Chapter 62).

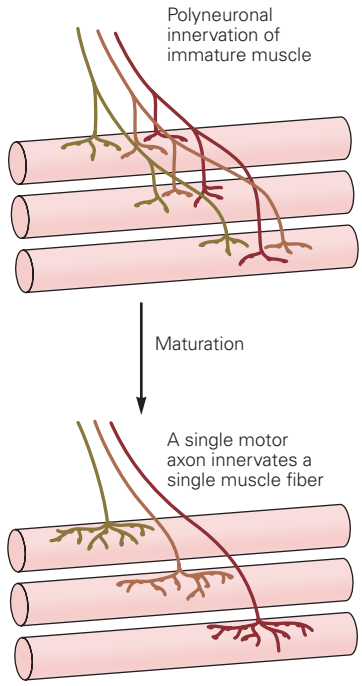

Figure 48–19 Some neuromuscular synapses are eliminated after birth. Early in the development of the neuromuscular junction, each muscle fiber is innervated by several motor axons. After birth, all motor axons but one withdraw from each fiber, and the surviving axon becomes more elaborate. Synapse elimination occurs without any overall loss of axons—axons that "lose" at some muscle fibers "win" at others. Central synapses are also subject to elimination.

Some Synapses Are Eliminated After Birth

In adult mammals, each muscle fiber bears only a single synapse. However, this is not the case in the embryo. At intermediate stages of development, several axons converge on each myotube and form synapses at a common site. Soon after birth, all inputs but one are eliminated.

The process of synapse elimination is not a manifestation of neuronal death. Indeed, it generally occurs long after the period of naturally occurring cell death (Chapter 46). Each motor axon withdraws branches from some muscle fibers but strengthens its connections with others, thus focusing its increasing capacity for transmitter release on a decreasing number of targets. Moreover, axonal elimination is not targeted to defective synapses; all inputs to a neonatal myotube are morphologically and electrically similar, and each can activate the postsynaptic cell (Figure 48–19).

What is the purpose of the transient stage of polyneuronal innervation? One possibility is that it ensures that each muscle fiber is innervated. A second is that it allows all axons to capture an appropriate set of target cells. A third, intriguing idea is that synapse elimination provides a means by which activity can change the strength of specific synaptic connections. We will explore this idea in Chapter 49.

Like synapse formation, synapse elimination results from intercellular interactions. Every muscle fiber ends up with exactly one input: None have zero, and very few have more than one. It is difficult to imagine how this could occur without feedback from the muscle cell. Moreover, the axons that remain after partial denervation at birth have a larger number of synapses than they did initially. Thus, synapse elimination appears to be a competitive process.

What drives the competition, and what is the reward? There is good evidence that neural activity plays a role: Paralysis of muscle reduces synapse elimination, whereas direct stimulation enhances it. These findings showed that activity was involved but did not reveal how the outcome was determined, because all axons were stimulated or paralyzed together. Because the essence of the competitive process is that some synapses gain territory at the expense of others, differential activity among axons may be a determinant