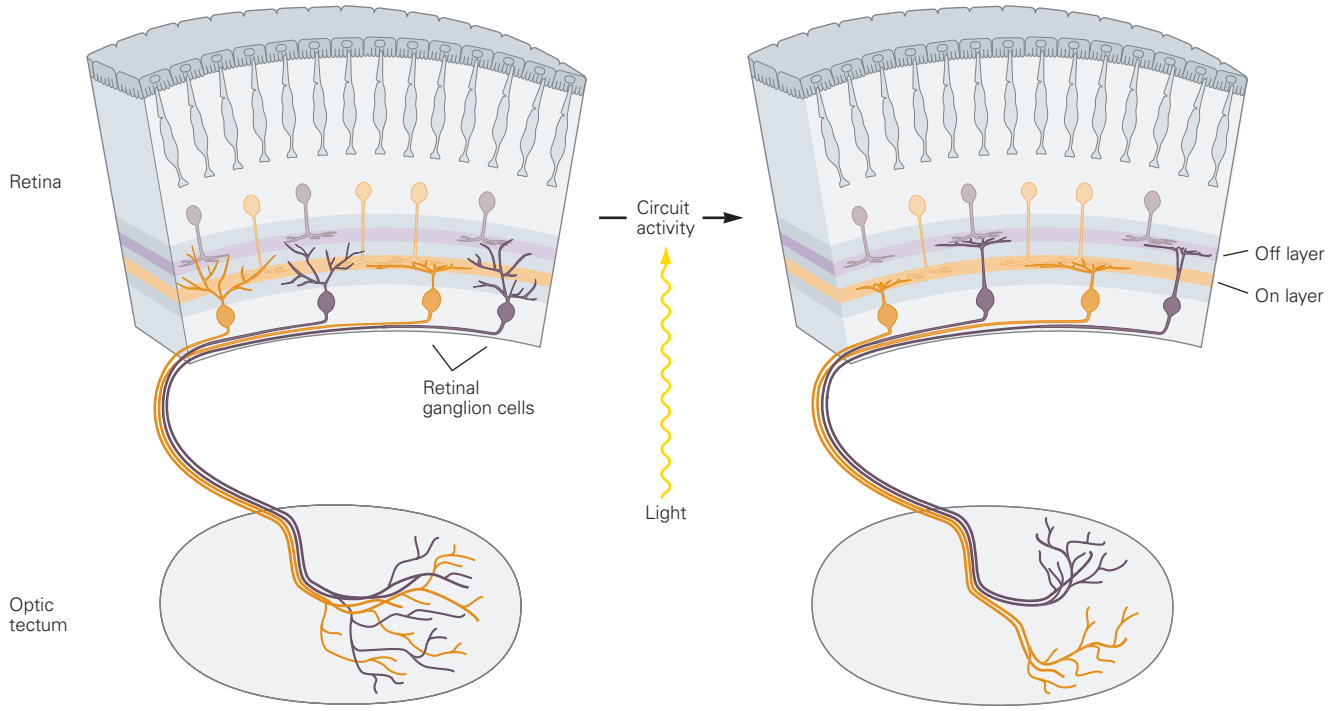

Figure 48–6 Electrical activity refines the specificity of synaptic connections of retinal ganglion cells. Some retinal ganglion cells initially form dendritic arbors that are limited to specific sublaminae in the inner plexiform layer of the retina, whereas others initially form diffuse arbors that are later pruned to form large specific patterns. Similarly, the axonal arbors of retinal ganglion cells initially innervate a large region of their target fields in the superior colliculus. This expansive axonal arbor is then refined so as to concentrate many branches in a small region. Abolishing electrical activity in retinal ganglion cells decreases the remodeling of dendritic and axonal arbors.

through neural activity. In the visual system, sharpening involves loss of synapses. We will return to this process of synapse elimination at the end of this chapter and consider its consequences for behavior in the next chapter.

In a few cases, neural activity promotes specificity in a different way, by turning an inappropriate target into an appropriate one. This mechanism has been most clearly demonstrated in skeletal muscle, where mammalian muscle fibers can be divided into several categories according to their contractile characteristics (Chapter 31). Muscle fibers of particular types express genes for distinctive isoforms of the main contractile proteins, such as myosins and troponins.

Few muscles are composed exclusively of a single type of fiber; most have fibers of all types. Yet the branches of an individual motor axon innervate muscle fibers of a single type, even in "mixed" muscles in which fibers of different types are intermingled (Figure 48–7A). This pattern implies a remarkable degree of synaptic specificity. However, matching does not always come about through recognition in the motor axon of the appropriate type of muscle fiber. The motor axon can also convert the target muscle fiber to an appropriate type. When a muscle is denervated at birth, before the properties of its fibers are fixed, a nerve that normally innervates a slow muscle can be redirected to innervate a muscle destined to become fast, and vice versa. Under these conditions, the contractile properties of the muscle are partially transformed in a direction imposed by the firing properties of the motor nerve (Figure 48–7B,C).

Different patterns of neural activity in fast and slow motor neurons are responsible for the switch in muscle properties. Most strikingly, direct electrical stimulation of a muscle with patterns normally evoked by slow or fast nerves leads to changes that are nearly as dramatic as those produced by cross-innervation (Figure 48–7D). Although activity-based conversion of the type observed at the neuromuscular junction is unlikely to be a major contributor to synaptic specificity in the central nervous system, it is likely that central axons modify the properties of their synaptic targets, contributing to the diversification of neuronal subtypes and refining connectivity imposed by recognition molecules.

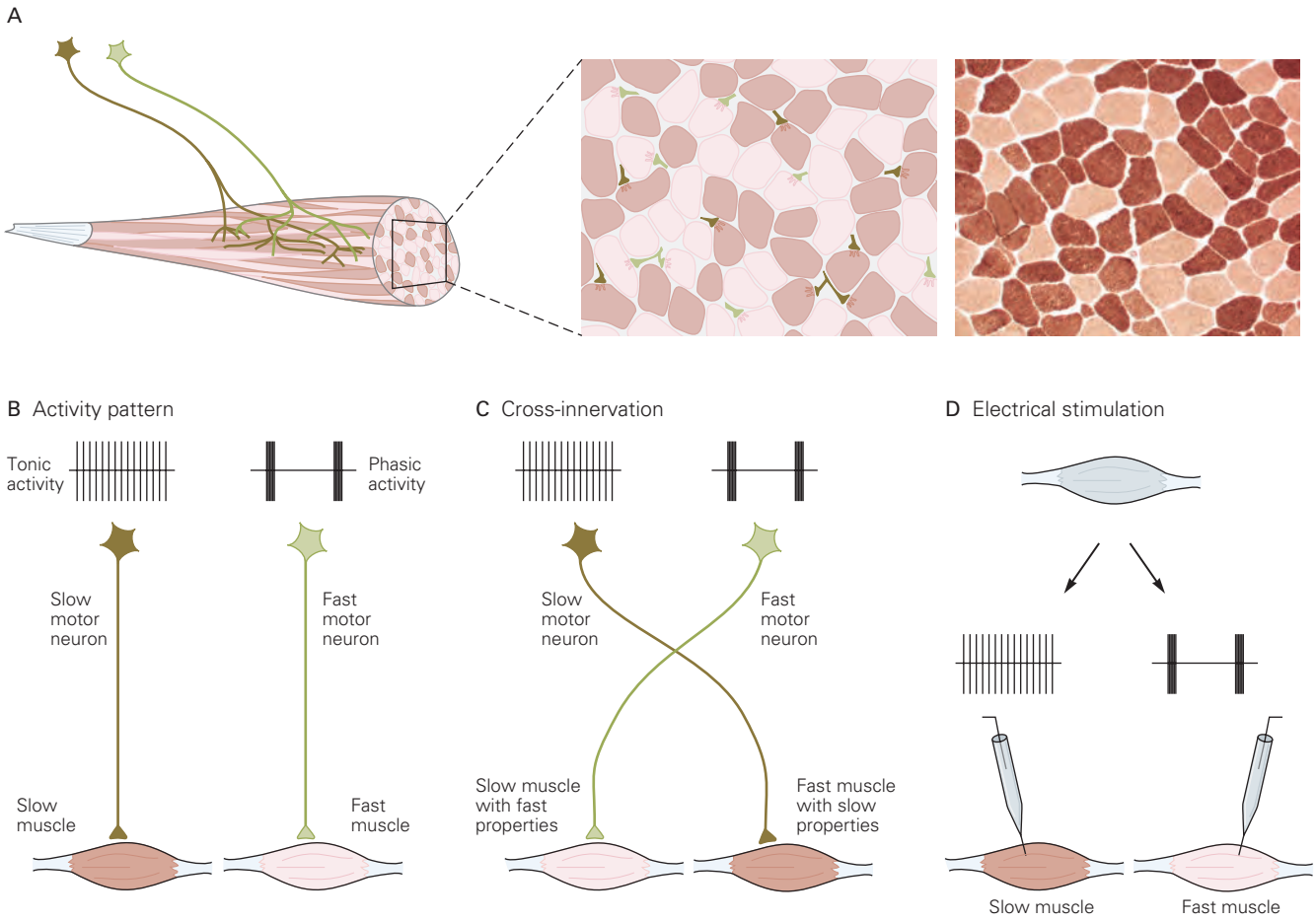

Figure 48–7 The pattern of motor neuron activity can change the biochemical and functional properties of skeletal muscle cells.

A. Muscle fibers have characteristic metabolic, molecular, and electrical properties that identify them as "slow" (tonic) or "fast" (phasic) types. The micrograph on the right shows a section of muscle tissue with histochemical staining for myosin ATPase. The middle sketch shows a section through the muscle, in which motor neurons (**green** and **brown**) form synapses on a single type of muscle fiber. (Photo on right reproduced, with permission, from Arthur P. Hays.)

B. Motor neurons that connect with fast and slow muscle fibers (fast and slow motor neurons) exhibit distinct patterns of electrical activity: steady low-frequency (tonic) firing for slow fibers and intermittent high-frequency bursts (phasic) for fast fibers.

C. Cross-innervation experiments showed that some property of the motor neuron helps to determine whether muscle fibers are fast or slow. Cross-innervation was achieved by surgically rerouting fast axons to slow muscle and vice versa. Although the properties of the motor neurons are little changed, the properties of the muscle change profoundly. For example, fast motor neurons induce fast properties in the slow muscle. (Adapted, with permission, from Salmons and Sreter 1976.)

D. The effects of innervation by fast and slow nerves on muscle are mediated in part by their distinct patterns of activity. Stimulation of a fast muscle in a slow tonic pattern converts the muscle into a slow type. Conversely, fast phasic stimulation of a slow muscle can convert it to a faster type.

Principles of Synaptic Differentiation Are Revealed at the Neuromuscular Junction

The neuromuscular junction comprises three types of cells: a motor neuron, a muscle fiber, and Schwann cells. All three types are highly differentiated in the region of the synapse.

The process of synapse formation is initiated when a motor axon, guided by the multiple factors described in Chapter 47, reaches a developing skeletal muscle and approaches an immature muscle fiber. Contact is made, and the process of synaptic differentiation gets underway. As the growth cone begins its transformation into a nerve terminal, the portion of the muscle

surface opposite the nerve terminal begins to acquire its own specializations. As development proceeds, synaptic components are added and structural signs of synaptic differentiation become apparent in the pre- and postsynaptic cells and in the synaptic cleft. Eventually, the neuromuscular junction acquires its mature and complex form (Figure 48–8).

Three general features of neuromuscular junction development have provided clues about the molecular mechanisms that underlie synapse formation. First, nerve and muscle organize each other's differentiation. In principle, the precise apposition of pre- and postsynaptic specializations might be explained by independent programming of nerve and muscle properties. However, in muscle cells cultured alone, acetylcholine (ACh) receptors are generally distributed uniformly on the surface, although some are clustered as in mature postsynaptic membranes. Yet, when motor neurons are added to the cultures, they extend neurites that contact the muscle cells more or less randomly, instead of seeking out the ACh receptor clusters. New receptor clusters appear precisely at the points of contact with the presynaptic neurites, while preexisting uninnervated clusters eventually disperse (Figure 48–9). Thus, factors on or released by motor axons exert a profound influence on the synaptic organization of the muscle cell.

Likewise, muscles signal retrogradely to motor nerve terminals. When motor neurons in culture extend neurites, they assemble and transport synaptic vesicles, some of which form aggregates similar to those found in nerve terminals. When the neurites contact muscle cells, new vesicle clusters form opposite the muscle membrane, and most of the preexisting clusters disperse.

These studies also revealed a second feature of neuromuscular development: that motor neurons and muscle cells can synthesize and arrange most synaptic components without each other's help. Uninnervated myotubes can synthesize functional ACh receptors and gather them into high-density aggregates. Likewise, motor axons can form synaptic vesicles and cluster them into varicosities in the absence of muscle. In fact, vesicles in growth cones can synthesize and release ACh in response to electrical stimulation, before the growth cone has reached its target cells. Thus, the developmental signals that pass between nerve and muscle do not induce wholesale changes in cell properties; rather, they assure that components of the pre- and postsynaptic machinery are organized at the correct time and in the right places. It is useful therefore to think of the intercellular signals that control synaptogenesis as organizers rather than inducers.

A third key feature of neuromuscular junction development is that new synaptic components are added in several distinct steps. The newly formed synapse is not simply a prototype of a fully developed synapse. Although nerve and muscle membrane form close contacts at early stages of synaptogenesis, only later does the synaptic cleft widen and the basal lamina appear. Similarly, ACh receptors accumulate in the postsynaptic membrane before acetylcholinesterase accumulates in the synaptic cleft, and the postsynaptic membrane acquires junctional folds only after the nerve terminal has matured. Several different axons innervate each myotube around the time of birth, but during early postnatal life, all but one axon withdraws.

This elaborate sequence is not orchestrated by the simple act of contact between nerve and muscle. Instead, multiple signals pass between the cells—the nerve sends a signal to the muscle that triggers the first steps in postsynaptic differentiation, at which point the muscle sends a signal that triggers the initial steps of nerve terminal differentiation. The nerve then sends further signals to the muscle, and this interaction continues.

We now consider retrograde (from muscle to nerve) and anterograde (from nerve to muscle) organizers in more detail.

Differentiation of Motor Nerve Terminals Is Organized by Muscle Fibers

Soon after the growth cone of a motor axon contacts a developing myotube, a rudimentary form of neurotransmission begins. The axon releases ACh in vesicular packets, the transmitter binds to receptors, and the myotube responds with depolarization and weak contraction.

The onset of transmission at the new synapse reflects the intrinsic capabilities of each synaptic partner. Nevertheless, these intrinsic capabilities cannot readily explain the marked increase in the rate of transmitter release that occurs after nerve-muscle contact is made, nor can they explain the accumulation of synaptic vesicles and the assembly of active zones in the small portion of the motor axon that contacts the muscle surface. These developmental steps require signals from muscle to nerve.

A clue to the source of these signals came from studies on the reinnervation of adult muscle. Although axotomy leaves muscle fibers denervated and leads to insertion of ACh receptors in nonsynaptic regions, the postsynaptic apparatus remains largely intact. It is still recognizable by its synaptic nuclei, junctional folds, and the ACh receptors, which remain far more densely

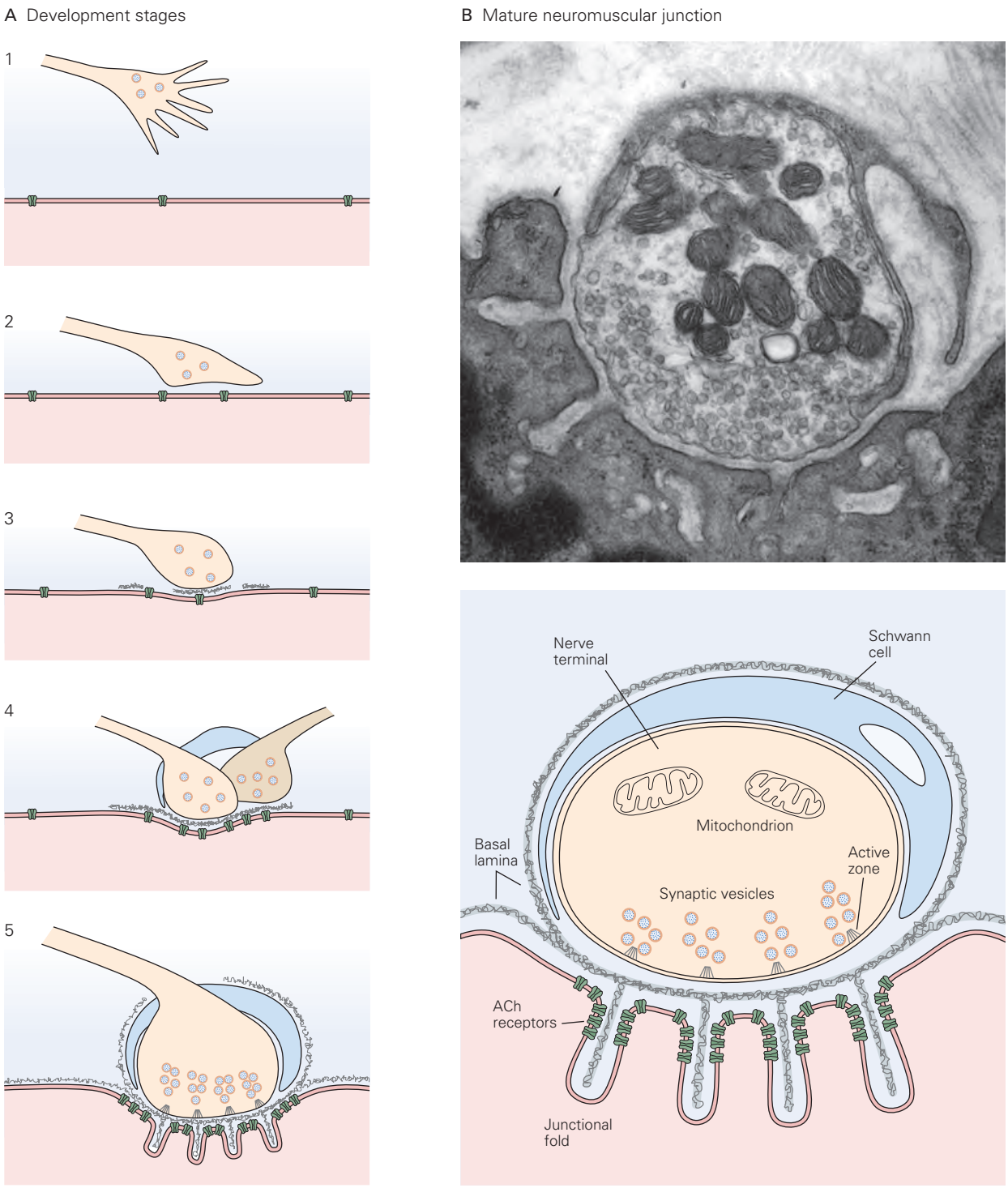

Figure 48–8 The neuromuscular junction develops in sequential stages.

A. A growth cone approaches a newly fused myotube (**1**) and forms a morphologically unspecialized but functional contact (**2**). The nerve terminal accumulates synaptic vesicles and a basal lamina forms in the synaptic cleft (**3**). As the muscle matures, multiple axons converge on a single site (**4**). Finally, all axons but one are eliminated and the surviving terminal matures (**5**). As the synapse matures, acetylcholine (**ACh**) receptors become concentrated in the postsynaptic membrane and depleted from the extrasynaptic membrane. (Adapted, with permission, from Hall and Sanes 1993.)

B. At the mature neuromuscular junction, pre- and postsynaptic membranes are separated by a synaptic cleft that contains basal lamina and extracellular matrix proteins. Vesicles are clustered at presynaptic release sites, transmitter receptors are clustered in the postsynaptic membrane, and nerve terminals are coated by Schwann cell processes. (Micrograph reproduced, with permission, from T. Gillingwater.)

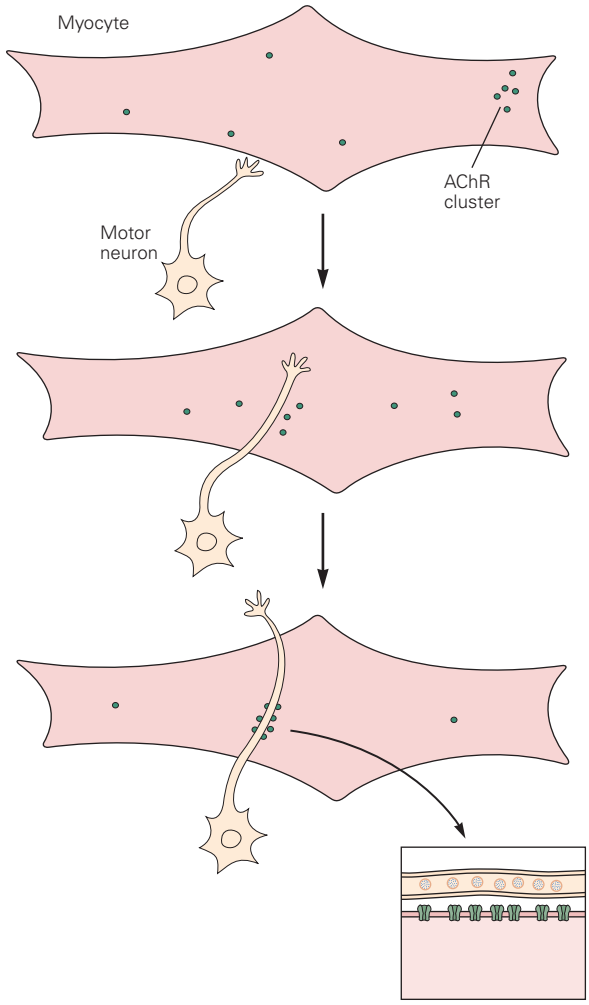

Figure 48–9 Nerve and muscle cells express synaptic components, but synaptic organization requires cell interactions. Acetylcholine receptors (AChR) are synthesized by muscle cells cultured without neurons. Many receptors are diffusely distributed, but some form high-density aggregates similar to those found in the postsynaptic membrane of the neuromuscular junction. When neurons first contact muscle, they do not restrict themselves to the receptor-rich aggregates. Instead, new receptor aggregates form at sites of neurite-muscle contact, and many of the preexisting clusters disperse. Similarly, motor axons contain synaptic vesicles that cluster at sites of neurite contact with muscle cells. (Adapted, with permission, from Anderson and Cohen 1977; Lupa, Gordon, and Hall 1990.)

packed in synaptic areas than in extrasynaptic areas of the cell. Damaged peripheral axons regenerate readily (unlike those in the central nervous system) and form new neuromuscular junctions that look and perform much like the original ones.

A century ago, Fernando Tello-Muñóz, a student of Santiago Ramón y Cajal, noted that the new junctions form at preexisting synaptic sites on the denervated muscle fibers even though the postsynaptic specializations occupy only 0.1% of the muscle fiber surface (Figure 48–10A). Later, electron microscopy showed that specialization in the axon occurs only in the terminals that contact the muscle. For example, active zones form directly opposite the mouths of the postsynaptic junctional folds. This striking example of subcellular specificity implies that motor axons recognize signals associated with the postsynaptic apparatus.

When regenerating axons reach a muscle fiber, they encounter the basal lamina of the synaptic cleft. To explore the significance of this association, muscles were damaged in vivo in a way that killed the muscle fibers but left their basal lamina intact. The necrotic fibers were phagocytized, leaving behind basal lamina sheaths on which synaptic sites were readily recognizable. At the same time that the muscle was damaged, the nerve was cut and allowed to regenerate. Under these conditions, motor axons reinnervated the empty basal lamina sheaths, contacting synaptic sites as precisely as they would have if muscle fibers were present. Moreover, nerve terminals developed at these sites and active zones even formed opposite struts of basal lamina that once lined junctional folds. These observations implied that components of the basal lamina organize presynaptic specialization (Figure 48–10B).

Several such molecular organizers have now been identified. Among the best studied are isoforms of the protein laminin. Laminins are major components of all basal laminae and promote axon outgrowth in many neuronal types. They are heterotrimers of α, β, and γ chains, comprising a family of five α, four β, and three γ chains (Chapter 47). Muscle fibers synthesize multiple laminin isoforms and incorporate them into the basal lamina. Laminin-211, a heterotrimer containing the α2, β1, and γ1 chains, is the major laminin in the basal lamina, and its absence leads to severe muscular dystrophy. In the synaptic cleft, however, isoforms bearing the β2 chain predominate (Figure 48–11A), and nerve terminals fail to differentiate fully in mutant mice that lack the β2 laminin (Figure 48–11B). The β2 laminins appear to act by binding to voltage-sensitive calcium channels that reside in the axon terminal membrane, where they couple activity to transmitter release. Laminins act on the extracellular domain of the channels, whereas the intracellular segment recruits or stabilizes other components of the release apparatus.

The finding that presynaptic differentiation is only partially compromised in the absence of laminins indicated that additional muscle-derived organizers of axonal specialization must exist. Several have now been identified, including members of the fibroblast

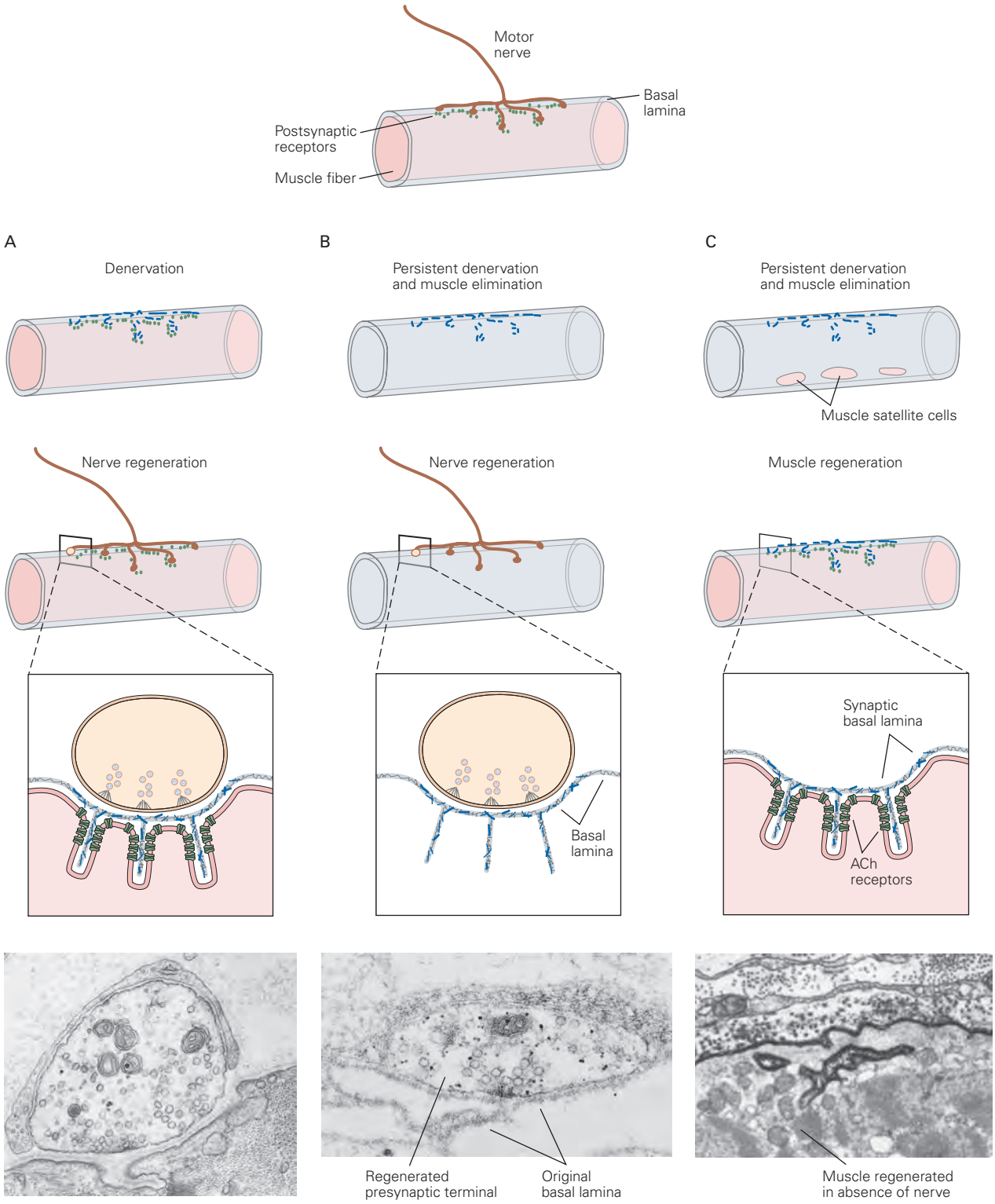

Figure 48–10 Synaptic portions of basal lamina contain proteins that organize developing and regenerating nerve terminals.

A. Damaged motor axons regenerate and form new neuromuscular junctions. Nearly all of the new synapses form at the original synaptic sites. (Micrograph reproduced, with permission, from Glicksman and Sanes 1983.)

B. A strong preference for innervation at original synaptic sites persists even after the muscle fibers have been removed, leaving behind basal lamina "ghosts." Regenerated axons develop synaptic specialization on contact with the original synaptic sites on the basal lamina. (Micrograph reproduced, with permission, from Glicksman and Sanes 1983.)

C. Following denervation of a skeletal muscle fiber and elimination of mature muscle fibers, muscle satellite cells proliferate and differentiate to form new myofibers. The expression of acetylcholine (ACh) receptors on the regenerated myofiber surface is concentrated in the synaptic areas of basal lamina, even when reinnervation is prevented. (Micrograph reproduced, with permission, from Burden, Sargent, and McMahan 1979.)

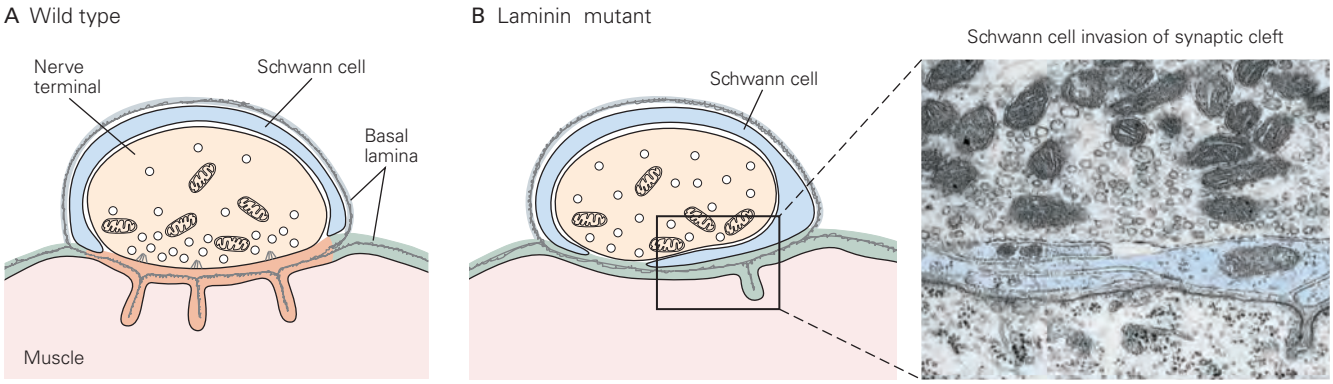

Figure 48–11 Different laminin isoforms are localized at synaptic and extrasynaptic areas of the basal lamina.

A. Different laminin isoforms are found in synaptic (**brown**) and extrasynaptic (**green**) areas of basal lamina. Isoforms, containing the β2 chain, are concentrated in the synaptic areas.

B. Maturation of neuromuscular junctions is impaired in mice lacking β2 laminins. These mutants have few active zones, and the synaptic cleft is invaded by Schwann cell processes (**blue**). (Micrograph reproduced, with permission, from Noakes et al. 1995.)

growth factor and collagen IV families, as well as a muscle membrane-associated protein, LRP4, that we will soon encounter again in the context of postsynaptic differentiation. Thus target-derived proteins from multiple families collaborate to organize the presynaptic nerve terminal.

Differentiation of the Postsynaptic Muscle Membrane Is Organized by the Motor Nerve

Soon after myoblasts fuse to form myotubes, the genes that encode ACh receptor subunits are activated. Receptor subunits are synthesized, assembled into pentamers in the endoplasmic reticulum, and inserted into the plasma membrane. As noted above, some receptors spontaneously form aggregates, but the majority are distributed throughout the membrane at a low density, approximately 1,000 per μm^2.

Once synapse formation is complete, however, the distribution of the receptors changes drastically. The receptors become concentrated at the synaptic sites of the membrane (to a density up to 10,000 per μm^2) and depleted in the nonsynaptic membrane (reduced to 10 per μm^2 or less). This thousand-fold difference in ACh receptor density occurs within a few tens of micrometers from the edge of the nerve terminal.

Appreciation of the critical role of the nerve in the redistribution of ACh receptors inspired a search for factors that might promote their clustering. This quest led to the discovery of a proteoglycan, agrin. Agrin is synthesized by motor neurons, transported down the axon, released from nerve terminals, and incorporated into the synaptic cleft (Figure 48–12A,B). Some agrin isoforms are also made by muscle cells, but the neuronal isoforms are about a thousand-fold more active in aggregating ACh receptors.

The phenotype of mutant mice lacking agrin shows that agrin has a central role in the organization of ACh receptors. Agrin mutants have grossly perturbed neuromuscular junctions and die at birth. The number, size, and density of ACh receptor aggregates are severely reduced in these mice (Figure 48–12C). Other components of the postsynaptic apparatus—including cytoskeletal, membrane, and basal lamina proteins—are also reduced. Interestingly, the differentiation of presynaptic elements is also perturbed. However, the defects in the presynaptic element do not result directly from lack of agrin in the motor neuron, but rather indirectly from the failure of the disorganized postsynaptic apparatus to generate signals for presynaptic specialization.

How does agrin work? Agrin's major receptor is a complex of a muscle-specific tyrosine kinase called MuSK (muscle-specific trk-related receptor with a kringle domain) and a coreceptor subunit called LRP4 (Figure 48–12A). MuSK and LRP4 are normally concentrated at synaptic sites in the muscle membrane, and muscles of mutant mice lacking MuSK or LRP4 do not have ACh receptor clusters (Figure 48–12C). Myotubes generated in vitro from these mutants express normal levels of ACh receptors, but these receptors cannot be clustered by agrin. Binding of agrin to the MuSK/LRP4 complex initiates a chain of events that ends in receptor clustering. Key events are agrin-induced activation of MuSK's kinase activity; autophosphorylation of the MuSK intracellular domain; recruitment of

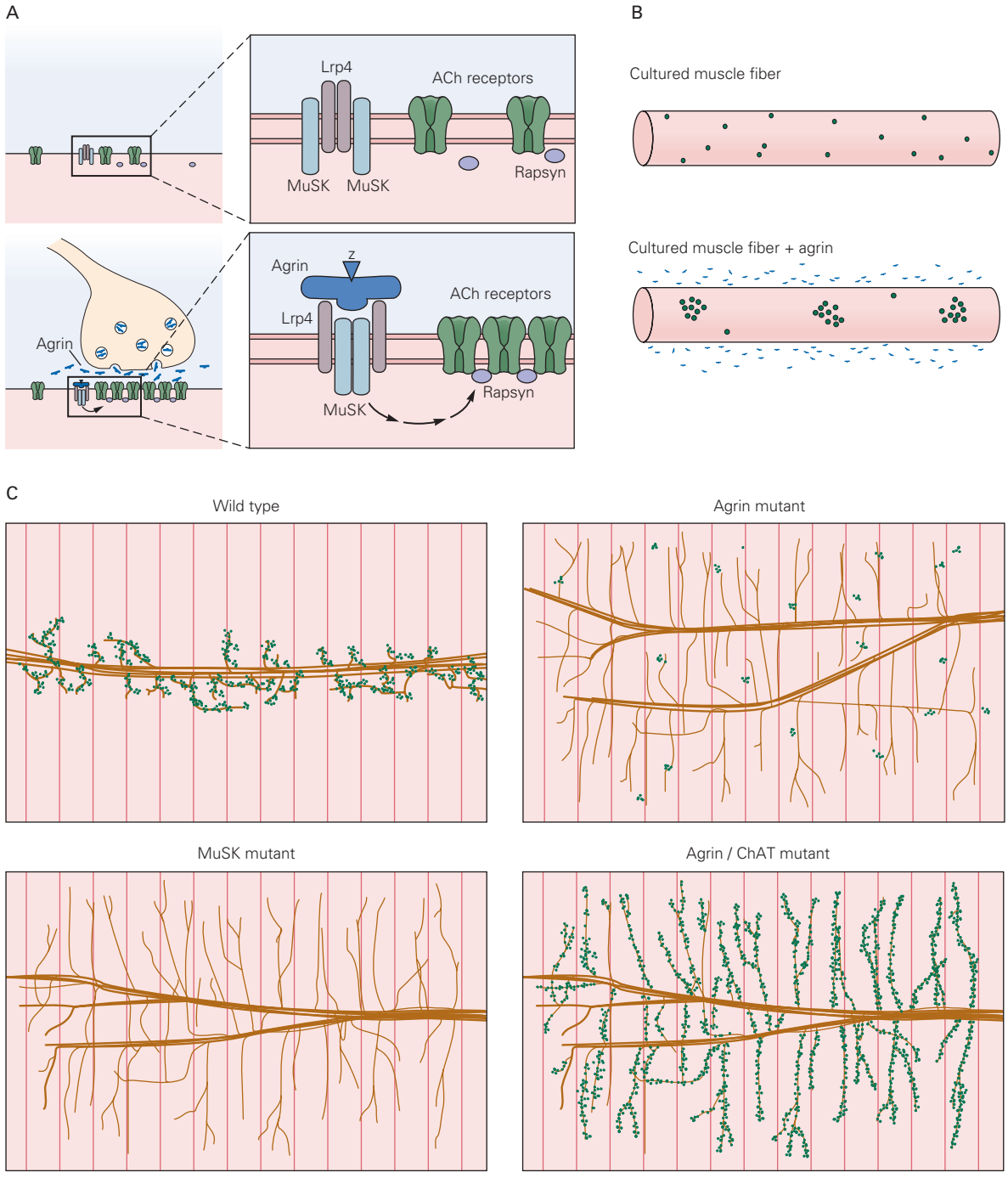

Figure 48–12 Agrin induces aggregation of acetylcholine (ACh) receptors at synaptic sites.

A. Agrin is a large (~400 kDa) extracellular matrix proteoglycan. Alternative splicing includes a "z" exon that confers the ability to cluster ACh receptors. When released by a nerve terminal, agrin binds Lrp4 on the muscle membrane, activating the membrane-associated receptor tyrosine kinase MuSK and triggering an intracellular cascade that results in ACh receptor clustering. Key intracellular signaling molecules are Dok7, Crk, and CrkL. These signal to rapsyn, a cytoplasmic ACh receptor–associated protein, which physically interacts with and clusters the ACh receptors. (Adapted, with permission, from DeChiara et al. 1996.)

B. Few ACh receptor clusters form on myofibers grown in culture under control conditions, but addition of agrin induces ACh receptor clustering. (Adapted, with permission, from Misgeld et al. 2005.)

C. Muscles from wild-type neonatal mice and from three mutant types. Muscles were labeled for ACh receptors (**green**) and motor axons (**brown**). In wild-type mice, ACh receptor clusters have formed under each nerve terminal by birth, whereas in agrin mutants, most clusters have dispersed. ACh receptor clusters are also absent in MuSK, Dok7, and rapsyn mutant mice. When the genes for agrin and **ChAT** (choline acetyltransferase) are mutated, clusters of ACh receptors remain, indicating that agrin works by counteracting receptor dispersion mediated by ACh. All mutant conditions also show axonal abnormalities, reflecting defects in retrograde signaling to the motor axon. (Abbreviation: **MuSK**, muscle-specific trk-related receptor with a kringle domain.) (Adapted, with permission, from Gautam et al. 1996.)

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