Comment

Shaping Membrane Architecture: Agrins In and Out of the Synapse

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Effective interactions between cells and their environment often rely on the creation, maintenance, and regulation of specialized membrane domains. Such domains are typically comprised of selected cytoskeletal, signaling, and adhesion molecules. The task confronting the cell is to deploy these elements into functional units in the right places at the right times. Skeletal muscle cells offer some of the best examples of such selective spatiotemporal regulation of cell surface specializations. For example, the postsynaptic apparatus occupies only about 0.1% of the plasma membrane, yet possesses all of the machinery necessary for rapid communication with the nerve terminal. Most notably, the density of acetylcholine receptors (AChRs)[1] in this domain exceeds 10,000/μm^2 (Colledge and Froehner, 1998).

The large expanse of extrasynaptic membrane, which in normal muscle bears only about 10 AChRs/μm^2, is nonetheless specialized in its own right. The dominant features here are costameres: discrete, rectilinear domains arrayed in transverse stripes and aligned with the Z-lines of the sarcomeres (Berthier and Blaineau, 1997). Costameres have at least three subdomains that are characterized by distinct membrane–cytoskeleton associations: integrin–talin/vinculin, Na^+K^+–ATPase-spectrin, and dystroglycan–dystrophin (Fig. 1 A). Each of these in turn interacts with the actin cytoskeleton and the extracellular matrix (Rybakova et al., 2000). This complex is thought to stabilize the plasma membrane and to distribute force from the sarcomere to the extracellular matrix during muscle contraction. Many of the elements enriched in costameres are found at neuromuscular and myotendinous junctions, and also turn up in focal adhesions and membrane ruffles in a variety of nonmuscle cells. Finally, costameres have received considerable attention because one of their core constituents, dystrophin, is the product of the gene mutated in Duchenne muscular dystrophy (Williams and Bloch, 1999).

How are such domain-specific specializations formed and maintained? A large body of work has established that the neuromuscular junction is induced by the secretion of "neural" agrin from the nerve terminal (Sanes and Lichtman, 1999). However, synapse formation is not a single-hit event and much remains to be learned about the ongoing information exchange between nerve and muscle that ultimately shapes the synapse. On the other hand, the factors controlling costamere organization are completely unknown. In two papers in this issue, Lømo and colleagues use a new approach—the direct application of recombinant agrin protein to muscle in vivo—to provide novel insights into both of these questions (Bezakova and Lømo, 2001; Bezakova et al., 2001). One remarkable finding is the demonstration that individual agrin molecules can persist at postsynaptic specializations in vivo for almost two months. This observation has potentially far-reaching implications for understanding how synaptic specializations are maintained in muscle cells and could influence our understanding of this process in the central nervous system (CNS) (Lisman and Fallon, 1999). Further, they show that electrical activity and "muscle" agrin play important and previously unsuspected roles in costamere organization at both junctional and extrajunctional regions of the myofiber.

Agrin is a heparan sulfate proteoglycan that was originally purified from basal lamina based on its ability to induce clustering of AChRs on cultured myotubes. The polypeptide core consists of distinct domains that mediate binding to laminin and α-dystroglycan. A third domain at the COOH-terminal end of the molecule is necessary for agrin-induced activation of the muscle-specific receptor tyrosine kinase (MuSK), which leads to AChR clustering (Hoch, 1999). Alternative splicing yields agrin isoforms that vary slightly in primary sequence but greatly in function. The so-called neural isoforms of agrin are unique to neurons and contain inserts of 4 and 8 (and/or 11) amino acids at two sites in the COOH-terminal fifth of the molecule. Agrin splice forms lacking 8/11 inserts are termed "muscle" agrin, although they are also expressed in many other tissues including the brain. Picomolar concentrations of neural agrin can induce MuSK phosphorylation and AChR clustering on cultured myotubes, whereas muscle agrin is inactive even at 1,000-fold higher levels.

The bulk of information on agrin function has been derived from work in two general systems: tissue culture and genetic manipulations in vivo (Hoch, 1999; Sanes and

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[1]*Abbreviations used in this paper:* AChR, acetylcholine receptor; CNS, central nervous system; DAPC, dystrophin-associated protein complex; MuSK, muscle-specific receptor tyrosine kinase.

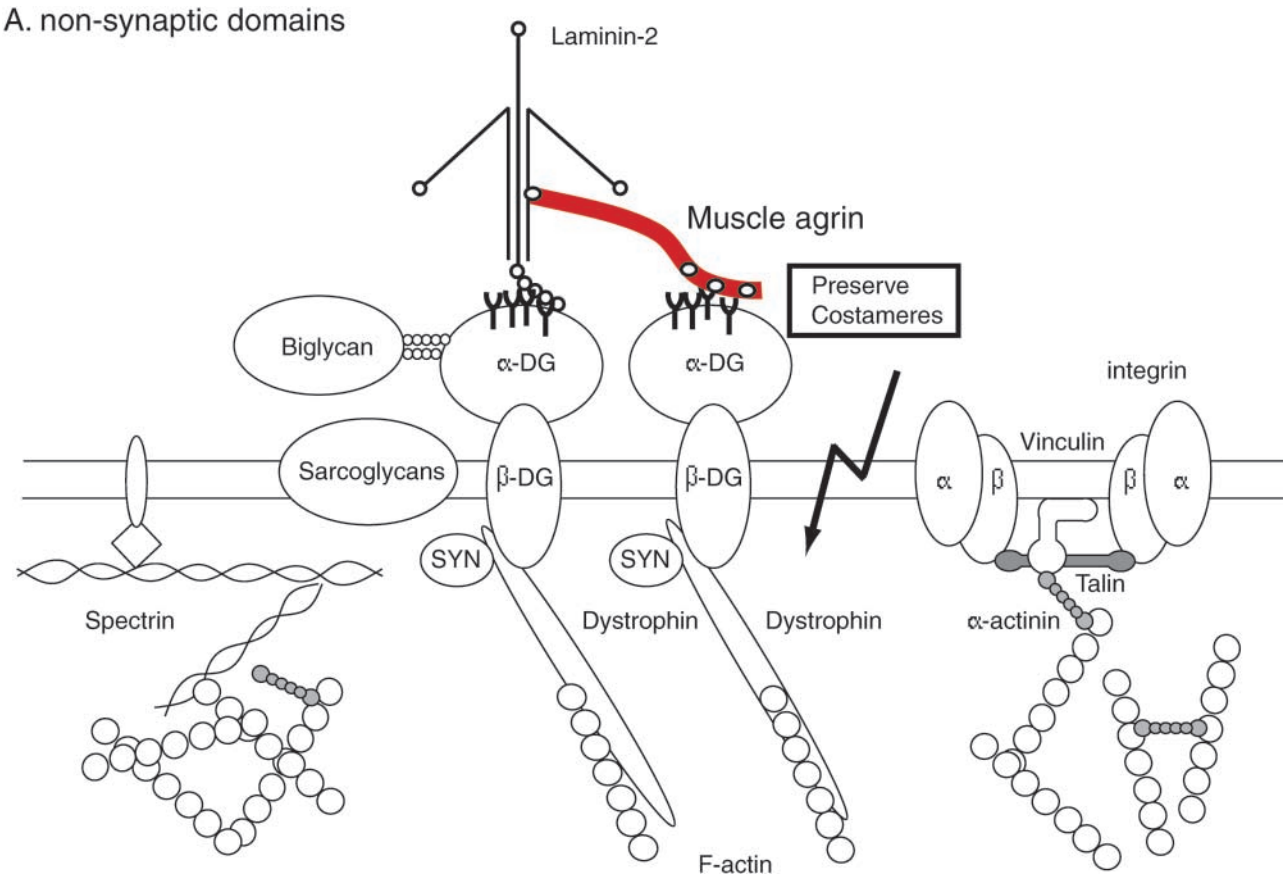

Figure 1. Model for role of agrin isoforms in nonsynaptic (A) and synaptic (B) domains on the muscle cell surface. (A) At the nonsynaptic domains, muscle agrin and electrical activity (jagged arrow) can regulate costamere organization. α- and β-DG, α- and β-dystroglycan; SYN, syntrophin. (B) At the neuromuscular junctions, neural agrin activates MuSK phosphorylation and triggers a local signal to induce phosphorylation of β-subunit of nearby AChRs. This phosphorylation is accompanied by AChR clustering and linkage of the AChR to the cytoskeleton, which is dependent upon rapsyn. Neural agrin signaling also activates Rac, Cdc42, and Src-related kinases. Muscle agrin is likely to play a role in stabilizing AChRs. DB, dystrobrevin; MASC, myotube-associated specificity component; RATL, rapsyn-associated transmembrane linker.

Lichtman, 1999). The culture models have been valuable because they have allowed detailed dissection of the early events in agrin-induced AChR clustering. Knockout animals have provided convincing evidence for the essential role of neural agrin in triggering post- and presynaptic differentiation in development. Unfortunately, the early lethality of these mutants has precluded studies of agrin's role in synapse maturation, maintenance, and stability. Another useful approach has been the injection of cDNA into muscles in vivo. These results have shown that both agrin and MuSK can induce ectopic postsynaptic specializations on innervated or denervated muscle fibers. However, this system affords relatively little control over the level and timing of agrin expression.

In this issue, Lømo and colleagues introduce a new and quite powerful approach that overcomes many of these limitations: the direct application of recombinant agrin proteins to muscle cells in intact animals (Bezakova and Lømo, 2001; Bezakova et al., 2001). The most striking finding with regard to synaptic differentiation is the observation that a single dose of agrin results in the formation of postsynaptic specializations that are maintained for at least seven weeks. Remarkably, tagging studies show that the original agrin molecules persist at these AChR clusters for this entire time. In addition, this work provides the first quantitative demonstration that denervation increases the sensitivity of the extrajunctional membrane to neural agrin. This finding provides a neat explanation for the classic experiments of Lømo, showing that innervated, but not denervated, muscle is refractory to innervation by foreign nerves (Lømo and Slater, 1978). Finally, this work provides strong support for muscle agrin's role in the stabilization of AChR clusters (Fig. 1 B).

Bezakova and Lømo (2001) opens up an entirely new line of investigation into the structure and regulation of costameres (Rybakova et al., 2000). The first novel and unexpected observation is that denervation leads to wholesale reorganization of costameres. In innervated muscle these dystrophin-containing structures appear primarily as transverse stripes with a minor longitudinal organization. However, following denervation the dystrophin-based costameric constituents are localized in longitudinal arrays. Intriguingly, direct electrical stimulation of denervated muscle prevents this reorganization. Thus, costameres show activity-dependent plasticity. The second major finding provides a molecular basis for this regula-

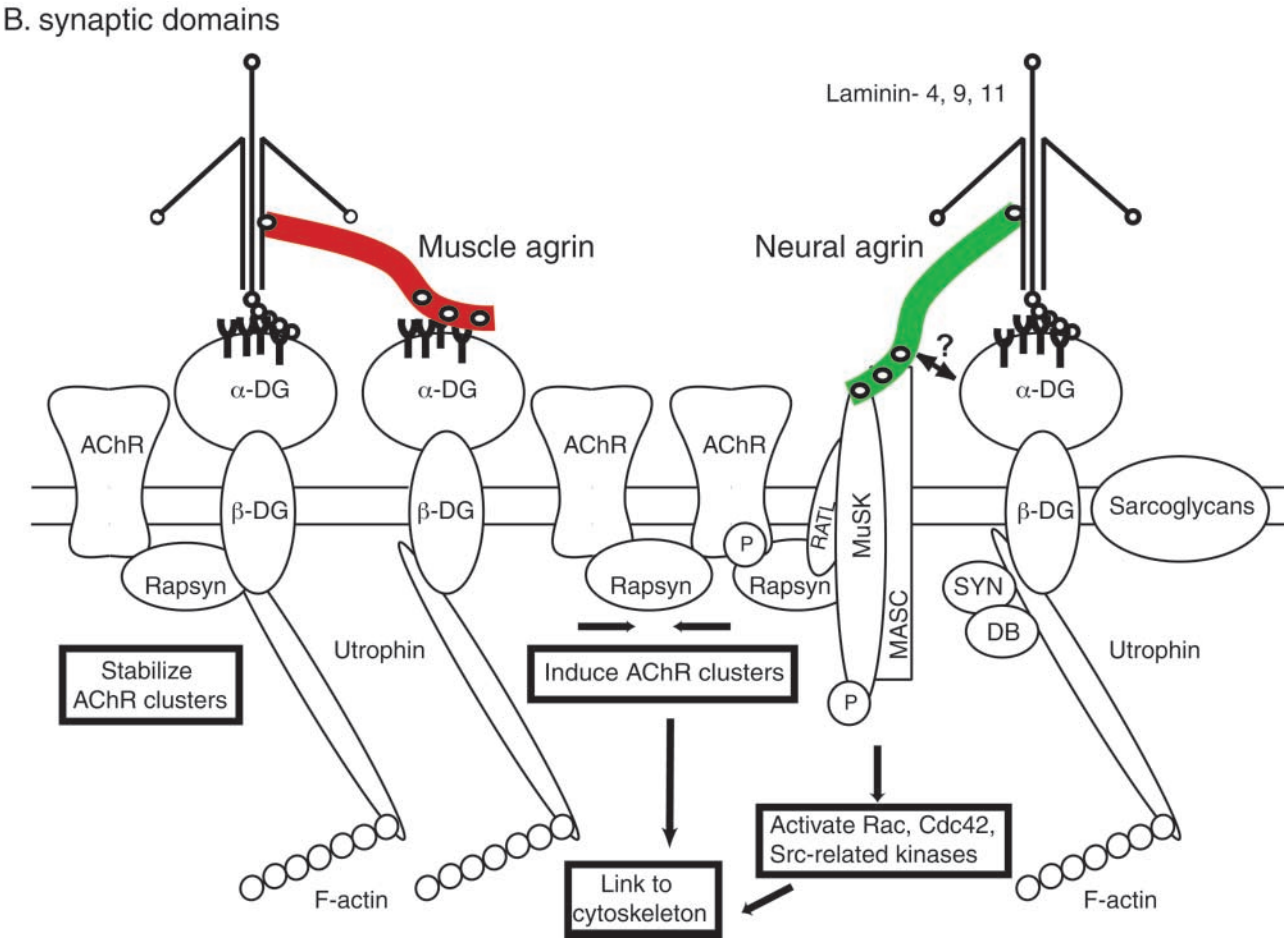

Figure 1 (continued)

tion of costamere organization: muscle agrin. Application of muscle agrin at nanomolar concentrations preserves normal (transverse) costamere orientation in denervated muscle. This result provides the first clear-cut function for muscle agrin.

Much remains to be learned about how agrin brings about these changes in membrane architecture. For example, although MuSK is clearly essential for the induction of neural agrin–induced postsynaptic specializations, it is likely that integrins and dystroglycan also contribute to later events in neuromuscular junction differentiation. Biglycan, a newly discovered binding partner of α-dystroglycan, may also play a role in this process (Bowe et al., 2000). Further, MuSK can be activated even in the absence of agrin (Lin et al., 2001). The intracellular signaling pathways triggered by agrin include activation of Src-class kinases as well as Rac and Cdc 42 (Weston et al., 2000; Mohamed et al., 2001). Muscle agrin binds to α-dystroglycan, but the role of this interaction and its signaling is unknown. In addition, we need to know whether activity or muscle agrin regulate the spectrin- and integrin-based costamere domains. Finally, the link between electrical activity and agrin-dependent membrane organization is also obscure; however, it is noteworthy that the dystrophin-associated protein complex (DAPC) harbors two other activity-regulated molecules: voltage-gated sodium channels and nitric oxide synthase (Colledge and Froehner, 1998).

The observation that a given molecule of agrin can persist at synaptic specialization for at least seven weeks invites speculation about whether agrin might be used for long-term information storage. Although there has been much progress in elucidating the way in which synapses are formed, little is known about how they are stabilized. Similarly, while the mechanisms underlying the induction of synaptic modifications in the CNS (e.g., long-term potentiation) are coming into focus, how such changes in efficacy are maintained remains mysterious (Lisman and Fallon, 1999). Although the function of agrin in the brain has not been established, some evidence suggests that it plays a role in the differentiation of neuron–neuron synapses (Bose et al., 2000). Further, the brain is rich not only in transcripts with the 8/11 splice inserts, but also in a newly discovered agrin isoform that is retained on the cell surface (Burgess et al., 2000). It is tempting to speculate that these different agrins may play distinct roles at neuronal synapses, perhaps including synaptic maintenance.

The results presented in Bezakova and Lømo (2001) and Bezakova et al. (2001) could have important implications for understanding muscular dystrophies. The vast majority of dystrophies are caused by derangements in the DAPC, which is an integral component of costameres. Agrin can induce the organization of several DAPC elements on the muscle cell surface including utrophin, the autosomal homologue of dystrophin. Understanding how these struc-

tures are formed and regulated will deepen our insight into these diseases and could reveal strategies for the restoration of DAPC structure and function on the cell surface. Such rescue of DAPC function could form the basis for therapies for muscular dystrophies.

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