

Kinase domain of the muscle-specific receptor tyrosine kinase (MuSK) is sufficient for phosphorylation but not clustering of acetylcholine receptors: Required role for the MuSK ectodomain?

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ABSTRACT Formation of the neuromuscular junction (NMJ) depends upon a nerve-derived protein, agrin, acting by means of a muscle-specific receptor tyrosine kinase, MuSK, as well as a required accessory receptor protein known as MASC. We report that MuSK does not merely play a structural role by demonstrating that MuSK kinase activity is required for inducing acetylcholine receptor (AChR) clustering. We also show that MuSK is necessary, and that MuSK kinase domain activation is sufficient, to mediate a key early event in NMJ formation—phosphorylation of the AChR. However, MuSK kinase domain activation and the resulting AChR phosphorylation are not sufficient for AChR clustering; thus we show that the MuSK ectodomain is also required. These results indicate that AChR phosphorylation is not the sole trigger of the clustering process. Moreover, our results suggest that, unlike the ectodomain of all other receptor tyrosine kinases, the MuSK ectodomain plays a required role in addition to simply mediating ligand binding and receptor dimerization, perhaps by helping to recruit NMJ components to a MuSK-based scaffold.

Formation of the neuromuscular junction (NMJ) depends upon agrin, a protein released by the motor nerve terminal, which interacts with a muscle-specific receptor tyrosine kinase, termed MuSK, as well as an accessory receptor component known as MASC (1–3). These interactions are required to induce the clustering of selected proteins on the muscle surface, resulting in the formation of a complex postsynaptic apparatus (4). The most extensively studied of these postsynaptic proteins is the nicotinic acetylcholine receptor (AChR), activation of which leads to muscle contraction.

Agrin was isolated by virtue of its ability to induce clustering of AChRs on the surface of myotubes in culture (5–8), while MuSK was identified in a search for muscle-specific receptor tyrosine kinases (9). On the basis of the realization that agrin utilizes MuSK as its receptor (3), roles of agrin-induced pathways could be verified *in vivo* by examining mice lacking agrin or MuSK (1, 2). Mice lacking either protein displayed similarly severe deficits in NMJ formation, consistent with the idea that agrin acts by means of MuSK to initiate all aspects of NMJ formation. However, the mechanism by which agrin activates MuSK, and the process by which this activation leads to NMJ formation, remain poorly understood. MuSK itself is not sufficient to bind or to be activated by agrin, but rather requires the yet-to-be isolated accessory receptor component termed MASC (3). Furthermore, other than MuSK phosphorylation, the only intracellular signaling event known to be

induced by agrin is the tyrosine phosphorylation of AChR subunits, which is thought to play a critical role in AChR clustering (10–14); it is not known whether MuSK is necessary or sufficient to mediate this aspect of the agrin response.

Transmembrane receptors with intrinsic tyrosine kinase activity (receptor tyrosine kinases or RTKs) play key roles in many biological responses (15). For most RTKs, the ectodomain serves to bind ligand and mediate ligand-dependent receptor dimerization, resulting in activation of the kinase domain, which in turn associates with and activates an assortment of intracellular signaling substrates that ultimately lead to a biological response. MuSK appears unusual among RTKs, both in its requirement for an accessory receptor component and in the extent of molecular organizations which it induces. To elucidate MuSK's mechanism of action, we have utilized a dominant-negative mutant of MuSK, a chimeric MuSK receptor that can be activated by a surrogate ligand, as well as myotubes lacking MuSK. We demonstrate that kinase activity is required for inducing AChR clustering, verifying that MuSK plays a signaling role, and not merely a structural role, at the NMJ. We also show that MuSK is necessary, and that MuSK kinase domain activation is sufficient, to mediate a key early event in NMJ formation—phosphorylation of the AChR. However, MuSK kinase domain activation, and the resulting AChR phosphorylation, is not sufficient for AChR clustering, as we show that the MuSK ectodomain is also required. These results indicate that AChR phosphorylation is not the sole triggering step that initiates clustering. Thus agrin and MuSK continue to distinguish themselves from other growth factor/RTK systems—in which kinase activation is sufficient to reproduce a receptor's biological activity—perhaps because of the complexity of their organizational roles at the NMJ.

MATERIALS AND METHODS

Production of the Rat TrkC/Human MuSK Chimera. An expression construct encoding the TrkC/MuSK chimeric receptor was assembled as follows. A three-way ligation was performed using the following three fragments: (i) An expression construct for rat TrkC, designated pMT21-rTrkC, was cut with *SalI* and *NotI* to cut out the signaling region of TrkC. (ii) An expression construct for human MuSK, designated pMT21-hMuSK, was cut with *ApaI* and *NotI* to yield the cytoplasmic domain of hMuSK, downstream of the *ApaI* site. (iii) An adjoining hinge region (*SalI* to *ApaI*) was created by amplification from hMuSK so as to add in a *SalI* site compatible with that at the end of the transmembrane domain of TrkC (using

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Abbreviations: NMJ, neuromuscular junction; MuSK, muscle-specific receptor tyrosine kinase; hMuSK, human MuSK; MASC, MuSK accessory specificity component; AChR, acetylcholine receptor; RTK, receptor tyrosine kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NT3, neurotrophin-3.

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the sequence GCA AAG CTG CTC AGC ACA GGA GAG GGG TGG GGG, which is just downstream of the unique *ApaI* site, as the 3' primer, and the sequence CAA AAA GGT CGA CGG AGA GAA TCA GCA GCA GTA ACC CTC ACC ACA CTG CC as the 5' primer, which adds a *SalI* site just at the second stop-transfer sequence). The resulting protein is rat TrkC through the sequence MINKYG, which is just COOH-terminal to the transmembrane domain, and then switches into the hMuSK stop-transfer sequence, RRRRK, and continues on with the hMuSK sequence.

Stable Transfections of TrkC/hMuSK, TrkC, and MuSK-Encoding Plasmids into MG 3T3 and C2C12 Cell Lines, and Growth Assays. Stable transfections of expression constructs for rat TrkC, hMuSK, and the TrkC/hMuSK chimera into MG 3T3 fibroblasts and C2C12 myoblasts were performed as described (16, 17). Growth assays on the MG 3T3 fibroblasts were performed as described (18), using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay to quantitate viable cell number.

Growth Factors. Purified human c-agrin_{4,8} was made by the Protein Sciences Division of Regeneron; it corresponds to the 50-kDa COOH terminus of the full-length protein. CHO cell-expressed 95-kDa COOH-terminal rat agrin was used for the Sol8 dominant-negative experiment (18).

Sol8 Dominant-Negative Experiment. Sol8 cells, a fusion-competent mouse muscle cell line (19), were maintained and transfected as described previously (20). Cells were colabeled with an anti β -galactosidase (LacZ) antibody and α -bungarotoxin to visualize AChR clusters, or with an anti-MuSK antibody (3) and α -bungarotoxin. For quantitation, transfected myotubes expressing introduced MuSK were first identified by looking for myotubes that exhibited bright MuSK staining well above the signal from nontransfected myotubes, and these myotubes were then scored for whether or not they contained AChR clusters. Quantitative results from three independent experiments for each condition were obtained, and at least 50 transfected myotubes were scored in each case.

Muscle Cell Culture. C2C12 myoblasts and primary myoblast cultures were maintained, differentiated into myotubes, and used in AChR clustering assays as previously described (3).

Receptor Tyrosine Phosphorylation Assays. The ability of agrin and neurotrophin 3 (NT3) to induce tyrosine phosphorylation of MuSK, TrkC, or the TrkC/MuSK chimera was evaluated as previously described (3).

RESULTS

MuSK Is Necessary for Agrin-Induced AChR Phosphorylation. Other than the induction of MuSK phosphorylation, the only defined intracellular signaling event downstream of agrin is the tyrosine phosphorylation of AChR subunits (10–14). To ask whether MuSK is a necessary intermediary in this aspect of the agrin response, as it is for AChR clustering (3), we derived myotubes from normal mice or mice lacking MuSK (3) and examined them for AChR phosphorylation in response to agrin. AChR levels were equivalent in wild-type and MuSK^{-/-} myotubes. However, while agrin induced both MuSK and AChR phosphorylation in normal myotubes, it could not induce either MuSK or AChR phosphorylation in myotubes lacking MuSK (Fig. 1). Thus, MuSK is required not only for agrin-induced AChR clustering but also for AChR phosphorylation, which precedes clustering by several hours.

MuSK Kinase Activity Is Necessary to Mediate Agrin Responses. The analysis of mice and myotubes lacking MuSK demonstrated that MuSK is required for NMJ formation and for agrin-induced AChR clustering and phosphorylation, but did not specifically test whether the kinase activity of MuSK is required for any of its functions (refs. 2 and 3 and above data). Formally, it remained a possibility that MuSK plays a strictly structural role in agrin responses—for example, by serving as

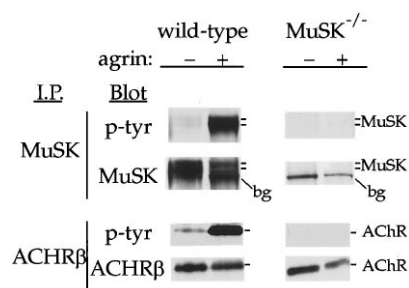


FIG. 1. MuSK is required for phosphorylation of the β subunit of the AChR. Myotubes from either wild-type or MuSK^{-/-} mice were stimulated with agrin and then lysed. Lysates were immunoprecipitated with antibodies specific for the MuSK or AChR β , as indicated, then immunoblotted for phosphotyrosine and receptor levels. The MuSK^{-/-} myotubes lack MuSK protein (the visible band, labeled bg for “background,” is nonspecific). The lower four blots indicate that MuSK^{-/-} myotubes have as much AChR β as in the control, yet AChR β is not phosphorylated upon agrin stimulation in MuSK^{-/-} myotubes.

a scaffolding protein. Indeed, we present evidence for such a scaffolding role in a separate paper (21). To determine whether MuSK kinase activity is also required for its role in AChR clustering, we engineered a construct encoding a mutant rat MuSK in which we replaced a single key lysine residue in the consensus ATP-binding site with an alanine (rMuSK^{K608A}). Such mutations in other RTKs abolish tyrosine kinase activity (15); over-expression of this mutant MuSK in COS cells confirmed that it lacked kinase activity (data not shown). This mutant MuSK, wild-type rat MuSK, or a control plasmid (pSK), was transiently over-expressed in myotubes, which were then examined for agrin-induced AChR clustering. Transfected cells were marked by staining for MuSK or by expression of a cotransfected gene encoding the enzyme LacZ. In cases where wild-type MuSK or the control plasmid was transiently expressed, LacZ-positive myotubes exhibited normal patterns of agrin-induced AChR clustering, detected by staining with α -bungarotoxin (Fig. 2). In contrast, expression of the MuSK K608A mutant abolished agrin-mediated clustering in myotubes marked by the coexpressed *lacZ* gene (Fig. 2). Thus, the mutant MuSK seems to function as a dominant-negative inhibitor of the endogenous MuSK, as described for similar mutants of other RTKs (22, 23), and demonstrates that the kinase activity of MuSK is required for mediating its responses to agrin.

A TrkC/MuSK Chimera Allows for MuSK Kinase Activation in Nonmuscle Cells. On the basis of the above findings that MuSK is required for AChR phosphorylation and that MuSK kinase activity is required for agrin-induced AChR clustering, we asked whether MuSK kinase activation was sufficient for these purposes. To this end, we constructed a chimeric receptor, in which the ectodomain of a different RTK was fused to the kinase domain of MuSK, thus allowing MuSK kinase activity to be regulated by a surrogate ligand which normally does not act on myotubes. We and others have made many similar chimeric receptors, and activation of these chimeric receptors by the appropriate surrogate ligands has invariably been found to mimic the biologic responses normally seen following activation of the RTK donating the kinase domain (e.g., refs. 24–26).

To create a chimeric MuSK receptor, we engineered a construct in which the coding regions for the extracellular and transmembrane (TM) domains of the rat receptor tyrosine kinase TrkC were fused to those encoding the entire cytoplasmic domain of human MuSK (Fig. 3A). TrkC serves as the primary receptor for a neurotrophic factor, NT3 (27, 28); TrkC is not normally expressed in myotubes, nor does NT3 normally have any effects on cultured myotubes (D.J.G., P.J., and

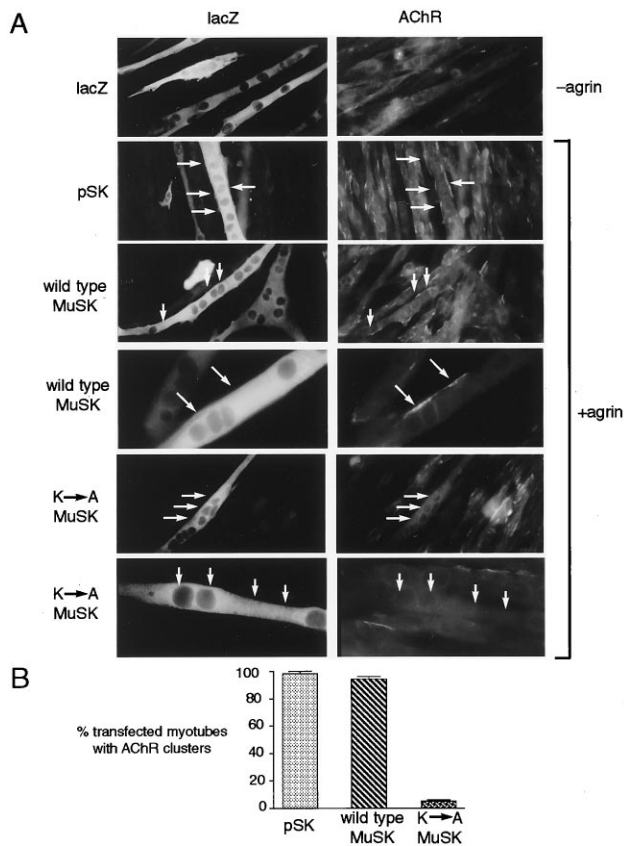


FIG. 2. Kinase activity of MuSK is required for AChR clustering. Sol8 cells were transfected with a rat MuSK dominant-negative kinase mutant, K608A, or with wild-type MuSK or vector. All cultures were cotransfected with a second plasmid encoding LacZ. Three days later, cultures were stained with α -bungarotoxin, to reveal AChR clusters, plus anti-LacZ or anti-MuSK antibodies to mark transfected myotubes (arrows indicate lacZ-transfected myotubes in side-by-side panels). Agrin stimulated AChR clustering in untransfected myotubes and in myotubes transfected with vector or wild-type MuSK, but not in cells harboring the K → A mutant. (Bars are 25 μ m.)

G.D.Y., unpublished data). To verify that this TrkC/MuSK chimeric receptor allows for the surrogate ligand, NT3, to activate the MuSK kinase domain, we stably transfected the construct encoding this chimeric receptor into a fibroblast cell line (the MG 3T3 variant of NIH 3T3 cells). NT3 could indeed regulate the tyrosine phosphorylation of the MuSK kinase domain of this chimeric receptor expressed in fibroblasts, as it does for the parental TrkC receptor ectopically expressed in these cells (Fig. 3B). Moreover, NT3 could mediate growth and survival responses in cells expressing the chimeric TrkC/MuSK receptor (Fig. 3C). Although these growth responses were more modest than those seen in cells expressing the parental TrkC receptor (Fig. 3C), the relative magnitude of the responses may simply reflect the relative phosphorylation inductions noted with these two receptors (Fig. 3B), consistent with the idea that the lower responses seen in cells expressing the chimeric receptor were due to a decreased expression of this receptor as compared with the parental TrkC receptor (D.J.G., S.S. and G.D.Y., unpublished data). Alternatively, the MuSK kinase domain might signal through distinct (or fewer) substrates than the TrkC kinase domain, rendering it less able to mediate proliferation in fibroblasts. Nevertheless, even the modest growth-promoting capability of MuSK raises the possibility that agrin may, in some cases, have actions more similar to conventional growth factors, in addition to its unique organizing role at the NMJ. It should be pointed out that since MuSK normally requires the still-to-be-defined muscle-

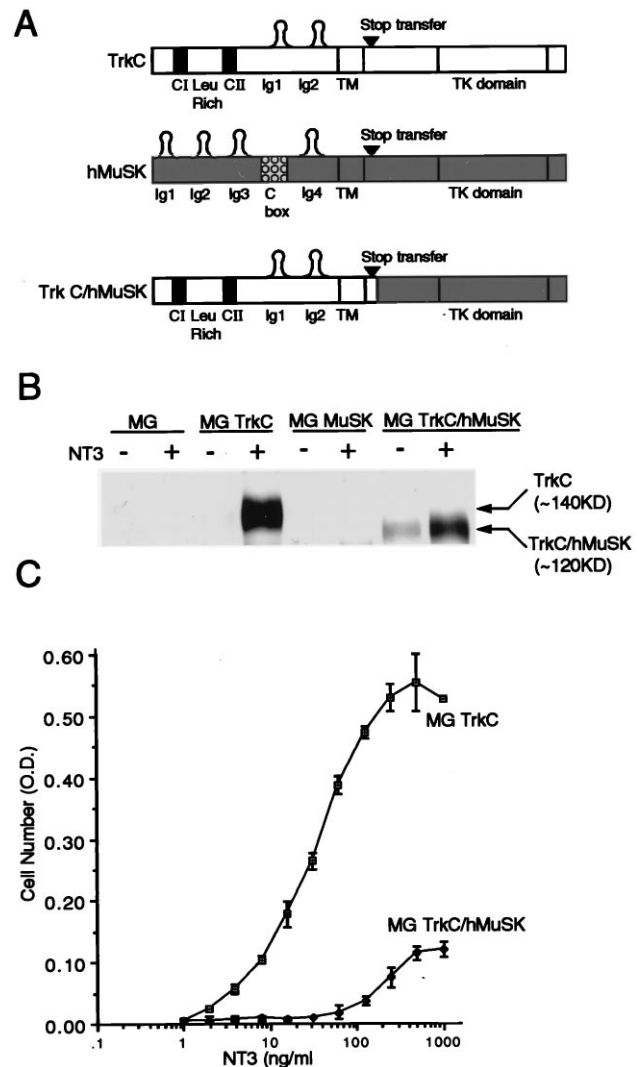


FIG. 3. A TrkC/MuSK chimera introduced into fibroblasts can be regulated by the TrkC ligand, NT-3, and allows for conventional growth responses. (A) Schematic depiction of chimeric TrkC/MuSK receptor. The cysteine-containing regions (CI and CII), the leucine-rich region (Leu Rich), and Ig domains (Ig1 and Ig2) of TrkC are indicated, as are the Ig domains (Ig1, Ig2, Ig3, and Ig4) and cysteine-rich domain (C box) of MuSK; TM, transmembrane region; TK domain, tyrosine kinase domain. (B) NT3-regulated phosphorylation of TrkC and the chimeric TrkC/MuSK receptor, but not of MuSK, expressed in MG 3T3 fibroblasts, as determined after phosphotyrosine immunoblotting of receptor immunoprecipitation. (C) NT3-regulated growth responses in MG 3T3 fibroblasts expressing TrkC and the chimeric TrkC/MuSK receptor; NT3 had no growth effects on parental MG 3T3 fibroblasts lacking these receptors (not shown). Cell number is reflected in arbitrary optical density units (O.D.) at 570–650 nm resulting from the MTT assay used to quantitate viable cell number.

specific MASC to be activated by agrin, parental MuSK could not be evaluated for its capabilities in nonmuscle cells.

The TrkC/MuSK Chimera Mediates the Phosphorylation, but Not the Clustering, of AChR β . Having verified that the chimeric TrkC/MuSK receptor could be regulated by a surrogate ligand, we introduced it or TrkC itself into C2C12 myoblasts, an immortalized myoblast cell line which can be induced to differentiate into myotubes. Clonal transfectants expressing the introduced chimeric receptor, which were differentiated into myotubes, exhibited NT3-mediated activation of the chimeric receptor equivalent to that of an introduced TrkC receptor (compare C2C12-TrkC with C2C12-TrkC/MuSK subclones in Fig. 4A). Moreover, NT3-mediated phos-

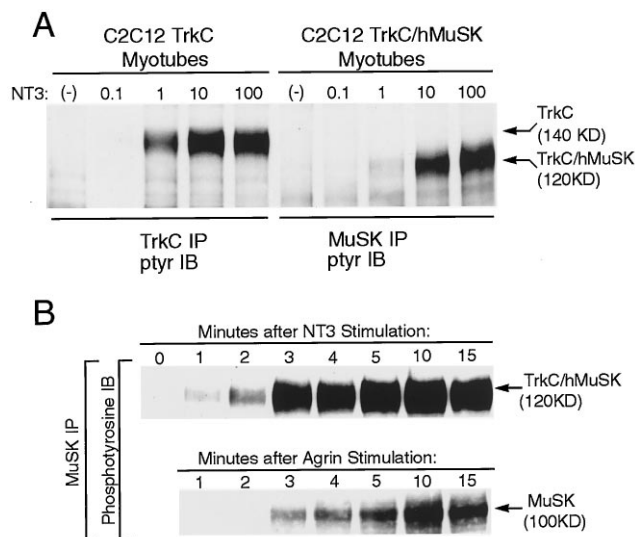


FIG. 4. TrkC/MuSK chimera expressed in differentiated C2C12 myotubes is inducibly regulated by NT3. Phosphotyrosine immunoblots (IBs) of receptor immunoprecipitations (IPs) are depicted. (A) TrkC and TrkC/MuSK receptors display similar NT3-dependent activation when introduced into C2C12 cells; clonal transfectants induced to differentiate into myotubes are depicted. (B) NT3-mediated phosphorylation of the introduced TrkC/MuSK chimera was similar in timing and even more robust than that of endogenous MuSK induced by added agrin.

phorylation of the introduced TrkC/MuSK chimera was similar in timing and even more robust than that of endogenous MuSK induced by added agrin (Fig. 4B Upper); longer time courses were also performed, with phosphorylation of both receptors exhibiting similar regulation as long as 2 hr after stimulation (data not shown).

Having shown that MuSK is required for mediating both phosphorylation and clustering of AChRs, we next used the TrkC/MuSK chimera to test whether MuSK kinase activation is sufficient for either of these purposes. Myotubes expressing the TrkC/MuSK chimeric receptor were stimulated either with NT3 to activate the chimeric receptor or with agrin to activate endogenous MuSK receptors (Fig. 5A Upper). Similar levels of AChR β phosphorylation were induced in both cases (Fig. 5A Lower), indicating that MuSK kinase activation is sufficient for mediating this aspect of the agrin response.

We also asked whether activation of the kinase domain of MuSK was sufficient for mediating AChR clustering. Surprisingly, the addition of NT3, even at concentrations well above those required for phosphorylation, did not lead to AChR clustering in these myotubes, although the myotubes responded normally to agrin (Fig. 5B). Thus, while activation of the kinase domain of MuSK is sufficient to induce early events in the response, such as the phosphorylation of AChRs, it is not sufficient to mediate all aspects of the agrin response, primarily as measured by AChR clustering.

DISCUSSION

We have previously demonstrated that MuSK is required to mediate NMJ formation *in vivo* and agrin-induced AChR clustering *in vitro* (2, 3). Here we report that MuSK is also required for the earliest known agrin-induced signaling event after MuSK activation—AChR phosphorylation. We further demonstrate that the kinase activity of MuSK is required for agrin-induced responses, ruling out the possibility that MuSK plays only a structural or scaffolding role in agrin responses. While we show that MuSK kinase activation is sufficient to mediate phosphorylation of AChRs, we unexpectedly find that

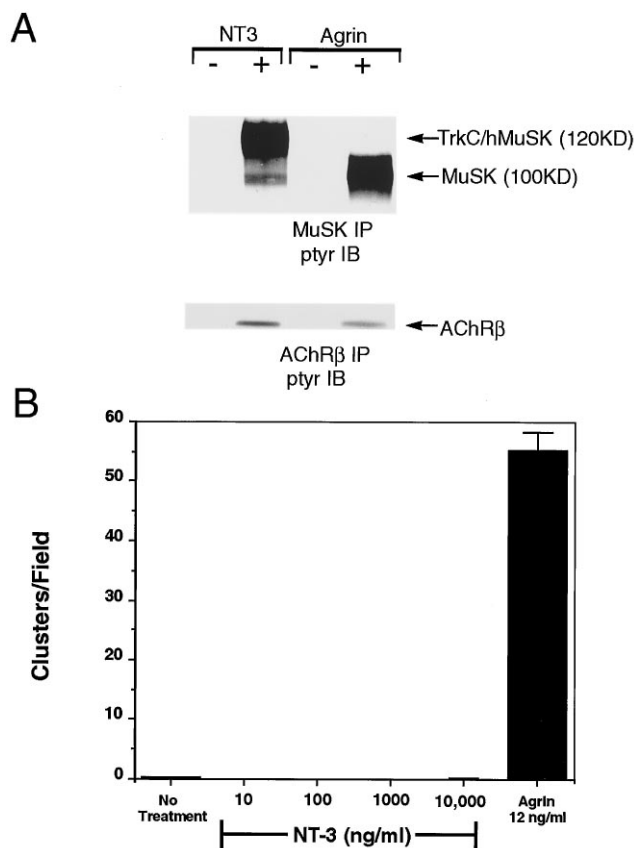


FIG. 5. The TrkC/MuSK chimera mediates the phosphorylation, but not the clustering, of AChRs in C2C12 myotubes. (A) AChR β tyrosine phosphorylation is similarly induced by either NT3 or agrin in myotubes derived from C2C12 clones expressing the TrkC/MuSK chimera. Phosphotyrosine immunoblots of MuSK immunoprecipitations (Upper) or AChR β immunoblots (Lower) are depicted. (B) The addition of NT3, even at concentrations well above those required for phosphorylation, did not lead to AChR clustering in these myotubes, although the myotubes responded normally to agrin.

it is not sufficient to mediate all aspects of the agrin response—in particular, it does not induce AChR clustering. Finally, we show that activation of MuSK kinase activity in a nonmuscle background can lead to modest growth responses, raising the possibility that in some cases agrin may share signaling pathways and actions with other growth factors in addition to playing its unique organizing role at the NMJ.

The failure of the TrkC/MuSK chimera to reproduce the organizing phenotype of native MuSK indicates that AChR phosphorylation is not the sole triggering event which leads to AChR clustering. Moreover, it suggests that the simple model, in which agrin merely activates MuSK kinase activity, which in turn leads to AChR phosphorylation and then AChR clustering (Fig. 6A), is incomplete. One explanation for the inability of the chimeric MuSK receptor to mediate clustering is that agrin might need to activate a receptor other than MuSK (Fig. 6B and C). We have previously shown that MuSK requires an accessory receptor component, termed MASC, to bind and respond to agrin stimulation (3). However, the TrkC/MuSK chimera bypasses the need for MASC, as it substitutes NT3 for agrin stimulation. Thus, the inability of the TrkC/MuSK chimera to induce AChR clusters might indicate that MASC serves another required function as well; for example, it may be necessary to activate a parallel signaling pathway that MuSK cannot (Fig. 6C) or to recruit other critical organizing components of the NMJ to a developing scaffold (Fig. 6C). Alternatively, the extracellular domain of MuSK, which is missing from the chimeric receptor, might itself recruit these

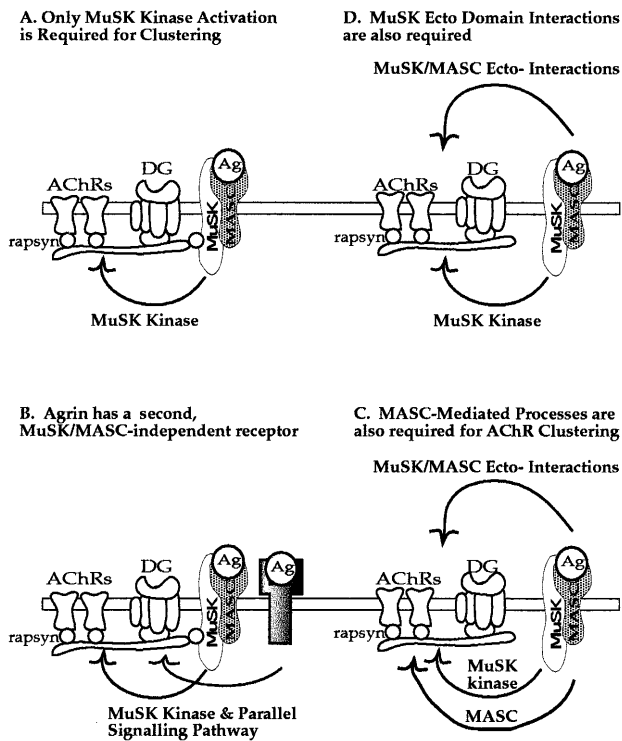


FIG. 6. Models for Agrin/MuSK-mediated NMJ formation: Four distinct models for how agrin and MuSK orchestrate the organization of the motor endplate. Our data argue strongly against the first, simple, model (*A*), in which agrin-mediated MuSK kinase activation is sufficient to lead to AChR clustering. In the second model (*B*), MuSK kinase activation is not sufficient because agrin independently activates a second, distinct, receptor system that is also required for AChR clustering. A variety of data, discussed in the text, argue against this model. Although it remains possible that MASC provides its own signaling pathway (*C*), we have no data to support this. The model (*D*) most strongly supported by our data and that of Apel *et al.* (21), indicates that in addition to functioning through intracellular pathways activated by the MuSK kinase domain, the agrin/MASC/MuSK complex acts extracellularly to help recruit and assemble components of the NMJ.

other critical organizing components, either alone or in concert with MASC (Fig. 6*D*). Results in a separate manuscript (21), in which we have performed a detailed examination of the role of a cytoplasmic protein called rapsyn (also known as the 43K protein) in the agrin/MuSK pathway, strongly supports the latter model, in which the MuSK ectodomain acts to help recruit other key organizing components (Fig. 6*D*). Earlier work had found that rapsyn colocalizes with AChRs at developing and adult NMJs (29, 30), and it may anchor AChRs to a subsynaptic cytoskeleton complex (31). Further, rapsyn has been shown to be sufficient to induce clustering of the AChR when the two proteins are coexpressed in nonmuscle cells (32, 33), and more recently rapsyn was shown to also recruit MuSK into such clusters along with AChRs (34). In a separate paper (21), we report that a primitive NMJ scaffold that includes MuSK but not AChRs is seen in mice lacking rapsyn, suggesting that MuSK uses rapsyn to recruit AChRs to the synapse (21). We also report that the ability of rapsyn to cluster MuSK in nonmuscle cells depends purely on the ectodomain of MuSK, presumably due to association with a transmembrane linker protein that also interacts with rapsyn on its cytoplasmic side (21). This indirect association between the MuSK ectodomain and rapsyn supports the idea of a required role for the MuSK ectodomain (Fig. 6*D*) and thus why simply activating the MuSK kinase domain within a chimeric receptor may not suffice for AChR clustering.

Although we favor the possibility that the ectodomain of MuSK is required to recruit other components to a developing MuSK-based scaffold (Fig. 6*D*), explaining the inability of the chimeric receptor to induce AChR clustering, it is worth returning to the possibility that agrin might activate another required receptor in addition to the MuSK/MASC system (Fig. 6*B*). It should be pointed out that no agrin-induced signaling event has yet been described that is independent of MuSK, and that mutational analysis reveals that portions of agrin required for clustering are identical to those required for binding and activating the MASC/MuSK (D.J.G., D.B., and G.D.Y., unpublished work). Nevertheless, one extracellular protein which needs to be considered for a role in agrin-mediated clustering of the AChR is α -dystroglycan, an extrinsic peripheral membrane protein that has been shown to directly bind agrin (35–40). α -Dystroglycan is attached to the cell surface by linkage to β -dystroglycan, which in turn couples to the intracellular cytoskeletal scaffold through an associated protein complex. The dystroglycan complex coclusters with AChRs in response to agrin *in vitro*, and components of this complex are concentrated at the endplate *in vivo*. Thus, the dystroglycan complex may act as an independent required receptor system for agrin. Alternatively, agrin may be involved in bridging the dystroglycan complex to the extracellular face of the MuSK/MASC complex, and this coupling may be required to recruit AChRs to a developing motor endplate.

Regardless of whether agrin requires another receptor system in addition to MuSK and MASC, or whether the ectodomains of MuSK and MASC play a unique scaffolding/recruiting function, our findings suggest agrin and MuSK display unprecedented complexity for a growth factor/receptor system. The effects of all other growth factors that use RTKs appear to be entirely attributable to activation of the catalytic activity of the receptor's cytodomain. This central paradigm has been reinforced by the construction of receptor chimeras in several different systems. An early example of this use of chimeras involved the construction of an epidermal growth factor receptor-insulin receptor (EGFR-IR) chimera, in which the extracellular domain of the EGFR was fused to the cytoplasmic domain of the IR, allowing epidermal growth factor to elicit all responses characteristic of insulin (24, 25). More recent examples of chimeric receptors include those involving the cytoplasmic domain of TrkA, a receptor for nerve growth factor (NGF), which can be activated by surrogate ligands and still mimic all the complex neurotrophic actions of NGF (41). Thus, for all other RTKs examined, surrogate activation of the kinase domain within the context of a chimeric receptor results in biological responses mimicking those of the native ligand. Unlike other ligands for RTKs, agrin has a macromolecular organizing function that may require a very complex receptor system(s).

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