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Nicotinic Receptor-Associated 43K Protein and Progressive Stabilization of the Postsynaptic Membrane

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Contents

Abstract

An extrinsic membrane protein of apparent molecular mass 43 kDa is specifically localized in postsynaptic membranes closely associated with the nicotinic acetylcholine receptor (AChR). Since its discovery in 1977, biochemical and morphological studies have combined to provide relatively clear pictures of 43K protein structure and subcellular compartmentalization. Nevertheless, despite these advances, the precise function of this synapse-specific protein remains unclear. Data gathered in recent years indicate that the postsynaptic apparatus develops through the incremental agglomeration of receptor microaggregates; evidence derived from a number of sources points to a role for 43K protein in certain underlying reactions. In this paper, I review 43K protein structural and anatomical data and analyze evidence for its role in the organization and maintenance of the postsynaptic membrane. Finally, I offer a model presenting a view of the role of 43K protein in the ontogeny of the motor endplate.

Index Entries: 43K protein; nicotinic acetylcholine receptor; postsynaptic membrane; motor endplate; *Torpedo* electrocyte.

Introduction

The neuromuscular junction, comprising just 0.1% of the total surface of a muscle fiber, is highly specialized to facilitate efficient neurotransmission. At the adult neuromuscular junction, AChRs are apposed and immobilized in densely packed clusters at the crests of postjunctional folds, close to the sites of acetylcholine (ACh) release. Prominent among nonreceptor synapse-specific postsynaptic elements is a protein termed *43K* for its apparent molecular mass on SDS gels. Accumulated evidence suggests that 43K protein is involved in the development and maintenance of postsynaptic cytoarchitecture accounting for the 100- to 1000-fold gradient in receptor density between junctional and nonjunctional membrane.

First discovered (Sobel et al., 1977) at a model nicotinic cholinergic synapse, the innervated face of the *Torpedo marmorata* electrocyte, 43K protein is present in adult tissue as one molecule per AChR (LaRochelle and Froehner, 1986,1987). The 43K protein colocalizes with AChR in close association with the cytoplasmic face of receptor clusters (Sealock et al., 1984; Kordeli et al., 1986,1989; Bridgman et al., 1987; Flucher and Daniels, 1989) and is thought to couple AChR clusters to the underlying cytoskeleton. Macromolecular clustering, a property shared by several ion channels, is crucial to efficient signal propagation in excitable tissues. Thus, although 43K protein is not required for ligand-gated ion channel function of AChR (Neubig et al., 1979; Mishina et al., 1984), it may be fundamental to AChR-mediated intercellular communication.

Insight into 43K protein function lags behind our understanding of its structure and localization. The 43K protein has been cloned from three species, and preliminary clues regarding regulation of 43K protein expression are emerging. In this paper, I review recent work on 43K protein structure, its posttranslational processing, and regulation of 43K protein expression. I analyze available data concerning the function of 43K protein and conclude that it is likely active in AChR aggregation, immobilization, and stabilization. Finally, I discuss a model of motor endplate morphogenesis that incorporates functional roles for 43K protein in each of these processes.

43K Protein Structure

Sequence Data and Posttranslational Modifications

Studies on 43K protein structure have combined to reveal a phosphorylated, acylated protein whose amino acid sequence is highly conserved across species lines. At the same time, information concerning 43K protein structure has not elucidated its function in the postsynaptic membrane.

Complementary DNA cloning and chemical methods have been used to deduce 43K protein sequences from *T californica* electric organ (Frail et al., 1987; Carr et al., 1987), mouse skeletal muscle (Frail et al., 1988; Froehner, 1989), and *Xenopus* (Baldwin et al., 1988). Overall, these sequences share 70–80% amino acid sequence identity evenly distributed over the length of the protein. Further, two highly conserved regions are apparent: the N-terminus (the first 10 amino acids are identical) and a region near the C-terminus particularly rich in cysteine that shares sequence similarity with a regulatory domain of the protein kinase C (PKC) family and the active site of phospholipase A_2 (Carr et al., 1987; Froehner, 1989). The sulfhydryl content of 43K protein (5.7 mol% cysteine; Carr et al., 1987) is unusually high for a cytoplasmic protein. To date, no significant homology has been found between the primary structure of 43K protein and that of other known proteins.

The 43K protein contains covalently bound myristate in myotubes and in undifferentiated myoblasts (Musil et al., 1988; Frail et al., 1989). Fatty acylation is a property that 43K protein shares with ankyrin and vinculin (Staufenbiel and Lazarides, 1986; Burn and Burger, 1987; Kellie and Wigglesworth, 1987), which fall within a new class of amphitrophic proteins (Burn, 1988). Pos-

tulated to be involved in 43K protein–phospholipid interactions, the functional significance of 43K protein N-myristoylation is unknown.

The primary sequence of the 43K protein contains a region compatible with a leucine zipper structure (Fig. 1). This motif is defined as a stretch of leucines (or homologous hydrophobic residues such as isoleucine or valine) in heptad repeats (at least four) within a region compatible with α-helical secondary structure (McKnight, 1991). Leucine zippers stabilize dimerization of some DNA-binding proteins (Landschulz et al., 1988, 1989; Kouzarides and Ziff, 1988) and have been recognized in some membrane-associated proteins (receptors, ion channels, and cytoskeletal elements; McCormack et al., 1989), including recently, 43K protein (Froehner, 1991). Interestingly, the leucine heptad repeat is conserved in the 43K proteins of *Torpedo* electric organ and skeletal muscle from *Xenopus* and mouse (Fig. 1A). This region of the 43K protein sequence may form a hydrophobic face that is involved in 43K protein interaction with AChR, underlying cytoskeletal proteins, or 43K protein itself. This putative hetero- or homodimerization of 43K protein might thus involve coiled–coil interactions (Rasmussen et al., 1991).

Torpedo 43K protein contains covalently bound phosphate (Hill et al., 1991). Extending earlier observations made in AChR-rich membranes (Gordon et al., 1977,1980; Saitoh and Changeux, 1980), we recently have characterized 43K protein as a serine phosphoprotein. Phosphate incorporation in 43K protein occurs exclusively on serine residues at a stoichiometry of 0.65 in a reaction mediated by cAMP-dependent protein kinase A (PKA). Substantial covalent phosphate is detected in 43K protein purified from *T marmorata* electric organ (Hill et al., 1991), suggesting that a physiological role exists for 43K protein phosphorylation. In fact, the c-terminal PKA phosphorylation site, a locus of at least some phosphate incorporation (Hill et al., unpublished observations), is conserved in the primary sequence of 43K protein of mouse muscle (Frail et al., 1988). Protein phosphorylation modulates signal transduction systems at several levels, including neurotransmitter biosynthesis and release, neurotransmitter receptor, and ion channel function (Miles and Huganir, 1988; Huganir and Greengard, 1990). Protein phosphorylation also regulates a number of interactions among components of the cytoskeletal network (Boivin, 1988). Thus, 43K protein phosphorylation may have functional significance to the supramolecular organization of AChR at the neuromuscular junction and the related electromotor synapse of fish electric organ.

43K Protein and AChR Clusters

The 43K protein has been uniformly observed in close association with the cytoplasmic surface of AChR clusters in *Torpedo* electrocyte and mammalian neuromuscular junction, but the exact positioning of 43K protein with respect to the cytoplasmic face of AChR is debated. To date, 43K protein has not been detected in association with isolated nonjunctional AChR molecules, possibly as a result of lack of immunocytochemical sensitivity. Combined morphological and biochemical studies reveal a protein that strictly colocalizes with AChR, with a few notable exceptions; however, the functional significance of colocalization and the mechanisms that underlie it are uncertain.

Early biochemical studies showed 43K protein to be localized on the cytoplasmic face of the innervated electrocyte membrane (Neubig et al., 1979; Wennogle and Changeux, 1980; St.John et al., 1982). Evidence for association of 43K protein with cytoplasmic projections of AChR has been provided using immunogold (Nghiêm et al., 1983; Sealock et al., 1984) and freeze-fracture immunoelectron microscopy (Bridgman et al., 1987). Analysis of electron microscopic images have suggested that the 43K protein molecule may lie beneath the AChR ion channel (Toyoshima and Unwin, 1988) or just to one side, perhaps joining adjacent receptors (Mitra et al., 1989).

These studies, with data gathered using bifunctional crosslinking reagents (Burden et al., 1983),

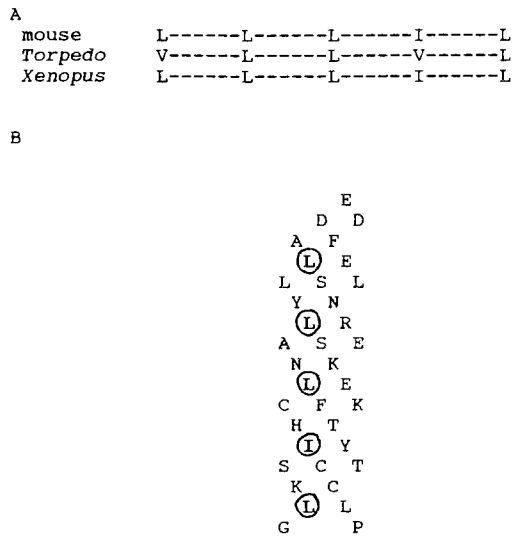

Fig. 1. Aligned 43K protein sequences depicting leucine zipper motif **(A)**. The 43K protein sequences from mouse skeletal muscle (Frail et al., 1988), *T californica* electric organ (Frail et al., 1987), and *Xenopus* muscle (Baldwin et al., 1988) are aligned, extending from amino acids 82 to 110. Leucine residues aligned along a meridian of an alpha helix illustrating the leucine zipper motif (mouse sequence) **(B)**. Calculations indicate that this region of the 43K protein sequence is consistent with predicted α-helical secondary structure (Gibrat et al., 1987), and the occurrence of multiple heptad repeats strongly predicts an α-helical conformation. Leucines (or homologous residues, shown in bold) aligned on one face of an alpha helix are thought to mediate protein–protein oligomerizations via coiled–coiled interactions.

suggest that significant portions of the native 43K protein molecule interact with AChR. Molecules of 43K protein can be crosslinked by disulfide bonds to form homopolymers (Hamilton et al., 1979; Porter and Froehner, 1985), suggesting they lie close to one another in the native postsynaptic membrane. Further, hydrophobic interactions with the bilayer (Porter and Froehner, 1985; Musil et al., 1988) or binding to actin (Walker et al., 1984) may be significant. None of these observations, however, explains the preferential localization of 43K protein with clusters of AChR.

In addition, 43K protein-like immunoreactivity has been detected at the mammalian neuromuscular junction (Froehner et al., 1981; Froehner, 1984) and at aggregates of AChR in aneural myotubes in vitro (Burden, 1985; Peng and Froehner, 1985; Bloch and Froehner, 1987). Peng and Froehner (1985) and Burden (1985) have shown that 43K protein appears at the developing neuromuscular synapse in *Xenopus* nerve-muscle cocultures as early as AChRs accumulate at synaptic sites.

43K Protein may interact with the cyto-skeletal matrix underlying the postsynaptic membrane. A number of proteins are concentrated near the cytoplasmic face of AChR clusters including actin (and associated proteins such as vinculin, talin, α-actinin, and filamin), spectrin, and clathrin (Bloch and Pumplin, 1988; Froehner, 1991). Also, dystrophin (Jasmin et al., 1990), dystrophin-related protein (Love et al.,1989; Khurana et al., 1990; Froehner, 1991), and polypeptides of apparent molecular mass 54 (Cartaud et al., 1989), 58 (Froehner et al., 1987), and 87 kDa (Carr et al., 1989) have been detected in *Torpedo* innervated membranes and/or at motor endplates. In muscle, β-spectrin is associated with clustered AChR (Bloch and Morrow, 1989), and treatments that dissociate spectrin and actin from clusters cause AChR to diffuse into surrounding membrane domains (Bloch, 1986). Some data suggest that the actin-spectrin network, which appears early in cluster development (Daniels, 1990), can be differentially extracted from the AChR-43K protein complex (Bloch, 1986; Bloch and Froehner, 1987).

Recent work has revealed a shift of 43K protein from a predominantly cytoplasmic pool to a membrane-associated pool during the course of *T marmorata* electrocyte development (Kordeli et al., 1989, LaRochelle et al., 1990; Nghiêm et al., 1991). In contrast, in the case of C2i muscle cells in culture, approx 80% of immunoprecipitable 43K protein is tightly associated with the membrane in both differentiated and undifferentiated cells (Frail et al., 1989). Musil and colleagues (1989) have reported that 43K protein is tightly associated with the membrane in nonmuscle cell types that express 43K protein. 43K Protein spontaneously aggregates in small clusters associated with the membrane when expressed in *Xenopus*

oocytes (Froehner et al., 1990) or in fibroblasts (Phillips et al., 1991). The reasons underlying these discrepancies are unclear but might stem from species or developmental differences, e.g., 43K protein myristoylation or phosphorylation.

Regulation of 43K Protein Gene Expression

During *Torpedo* electrocyte ontogeny, AChR and 43K protein are detectable prior to development of synaptic contacts (less than 55 mm in body length; Witzemann et al., 1983; Kordeli et al., 1989; LaRochelle et al., 1990; Nghiêm et al., 1991). Prior to synaptogenesis, however, AChRs are organized at the ventral cell surface, but little membrane-associated 43K protein is observed (Kordeli et al., 1989); rather, 43K protein immunoreactivity is diffusely distributed over the electrocyte cytoplasm (Kordeli et al., 1989; LaRochelle et al., 1990). Upon electrocyte innervation, AChR and 43K protein concentrations rise markedly and in concert, eventually attaining the approx equimolar ratio characteristic of the adult (LaRochelle et al., 1990; Nghiêm et al., 1991).

Similarly, in *Xenopus* embryos, 43K protein transcript can be detected before synapses form and before AChRs cluster at synapses (Baldwin et al., 1988); after synaptogenesis, 43K protein expression increases markedly. Working with C2 muscle cells in culture, Frail et al. (1989) observed a twofold increase in 43K protein mRNA upon differentiation but similar amounts of immunoprecipitable 43K protein. In variants of the C2 mouse muscle cell line with reduced AChR expression, the amount of 43K protein is also reduced (LaRochelle et al., 1989). As a marked exception to the equimolar AChR-43K protein rule, 43K protein is expressed in some cells in the absence of AChR (Frail et al., 1989; Musil et al., 1989).

Expression of 43K protein in skeletal muscle is down-regulated by electrical activity (Baldwin et al., 1988; Frail et al., 1989; Froehner, 1989). Upon denervation, 43K protein mRNA levels in muscle increase two- to threefold (Frail et al., 1989); under similar conditions, AChR α-subunit mRNA increases dramatically (Merlie et al., 1984; Goldman et al., 1985; Klarsfeld and Changeux, 1985). Similarly discoordinate regulation of gene expression was observed in rat α-subunit and 43K protein (Froehner, 1989). In *Xenopus* muscle, however, comparable increases upon denervation (30-fold) were observed for AChR α-subunit and 43K protein mRNAs (Baldwin et al., 1988). It is unclear whether these inconsistent observations reflect species, developmental, or experimental dissimilarities.

Thus, during development 43K protein levels roughly parallel those of AChR, suggesting that similar mechanisms regulating gene expression are at work. Levels of 43K protein transcript are also modulated by denervation, but the degree to which this occurs is controversial. To date, isolation of the 43K protein gene promoter has not been reported, and definitive analysis of the regulation of 43K protein gene expression must await these data.

Studies to Probe Function

Although a precise role has not been defined, considerable evidence suggests that 43K protein is involved in AChR stabilization, recruitment, and immobilization. 43K protein can be extracted from receptor-rich membranes by exposing them to detergent (Sobel et al., 1977), alkaline pH (Neubig et al., 1979), or chaotropic agents (Elliott et al., 1980). Studies of membranes from which 43K protein has been removed reveal increased lateral (Barrantes et al., 1980; Cartaud et al., 1981, Rousselet et al., 1982; Bloch and Froehner, 1987) and rotational (Rousselet et al., 1982; Lo et al., 1980) mobility of AChR. Increased receptor susceptibility to heat inactivation (Saitoh et al., 1979) and proteolytic attack (Klymkowsky et al., 1980; Bloch and Froehner, 1987) have been described. Several investigators have speculated that 43K protein anchors AChR in the postsynaptic membrane (Cartaud et al., 1981; Rousselet et al., 1982; Burden et al., 1983; Walker et al., 1984;

Froehner, 1986). Indeed, AChR clusters remain with the cytoskeleton after the bulk of the cell has been extracted using nonionic detergents (Prives et al., 1982), although 43K protein has not been directly implicated in this linkage. Despite data suggesting that 43K protein is involved in the processes of AChR stabilization and immobiliztion, direct demonstration of 43K protein–AChR interaction is lacking.

Consistently, 43K protein is observed at AChR clusters during development and under in vitro experimental conditions. In cultured muscle cells, AChR clusters associated with 43K protein develop spontaneously in the absence of exogenous stimuli. Similarly, in denervated muscle, new AChR aggregates appear at sites remote from the original endplate (Ko et al., 1977). Upon innervation, extrajunctional clusters are dispersed (Peng et al., 1981; Moody-Corbett and Cohen, 1982). Nerve-induced AChR aggregation appears to be a property specific to motor nerves, since sensory or sympathetic neurons do not trigger the clustering process (Cohen and Weldon, 1980). Receptor clustering and the appearance of subsynaptic densities can be induced experimentally by assorted stimuli, including exposing tissue in culture to basic polypeptide-coated latex beads (Peng et al., 1981: Peng and Cheng, 1982; Peng and Froehner, 1985), or basic fibroblast growth factor-coated beads (Peng et al., 1991), DC electric field (Orida and Poo, 1978), and a variety of soluble factors derived from nerve cells or tissues (Bloch and Pumplin, 1988). The 43K protein is associated with these newly formed AChR clusters (Peng and Froehner, 1985).

Collagenase digestion of muscle (Bloch et al., 1986) disrupts AChR clusters at the adult neuromuscular junction, suggesting that the entire AChR-43K protein complex is mobile in the plane of the membrane. Thus, disorganization of the extracellular matrix perturbs AChR cluster stability and frees individual AChR-43K protein complexes to disperse in the membrane. Similar degrees of metabolic stability for 43K protein have been observed in differentiated and undifferentiated muscle cells ($t_{1/2} \approx 2.4$–2.8 h), indicating that the presence of AChR does not influence the rate of 43K protein degradation (Frail et al., 1989).

A unifying mechanism to explain the reversible nature of AChR clustering is unavailable. Depletion of ATP in cultured muscle cells leads to rapid dispersal of AChR clusters (Bloch, 1979), indicating that metabolic energy is a factor in maintenance of cluster stability. Activation of PKC or exposure to the cholinergic agonist carbamylcholine leads to cluster dissolution (Bloch, 1986; Ross et al., 1988), accompanied by increased phosphorylation of AChR δ-subunit. Transformation of cultured muscle cells by *Rous sarcoma* virus inhibits the development of AChR clusters, an effect mediated by the *src* gene product (Anthony et al., 1984;1988). Exposure to azide (Bloch, 1986), elevated temperature (Olek et al., 1986), or reduced extracellular calcium (Bloch, 1983; Krikorian and Daniels, 1989) also causes AChR aggregates to break up. Data from experiments in culture show that entire AChR-43K protein complexes migrate in the plane of the membrane (Tsui et al., 1990).

Recent data establish that 43K protein can effect AChR clustering. The 43K protein expressed in *Xenopus* oocytes induces the formation of small (less than 1μm diameter) clusters of AChR, which otherwise are diffusely distributed at the oocyte membrane (Froehner et al., 1990). Similarly, both fetal and adult muscle AChRs aggregate in fibroblasts when coexpressed with transfected 43K protein (Phillips et al., 1991). These observations suggest that 43K protein participates in AChR cluster assembly and may function as (one of) the crucial component(s) that drive(s) cluster formation (possibly via 43K protein–43K protein interaction). Loose aggregates of small receptor patches that coalesce into a larger network have been recognized in developing muscle (Steinbach, 1981; Olek et al., 1983; Krikorian and Daniels, 1989). It has not been excluded that AChR-43K protein clusters may require other cytoskeletal or extracellular elements and/or 43K protein modification to achieve the supramacromolecular dimensions and stability observed in vivo.

A Role For 43K Protein at the Postsynaptic Membrane

As discussed earlier, considerable data have accumulated concerning 43K protein chemistry and subcellular compartmentalization. General principles regarding 43K protein expression during development are emerging along with facts concerning transcriptional regulation of the 43K protein gene. Notwithstanding the availability of detailed information on 43K protein biology, however, the function of 43K protein remains elusive.

Consensus in the field holds that 43K protein is involved in the dynamic control of AChR cluster formation and stabilization. Currently, it is not clear where 43K protein is active in the continuum of AChR stabilization, recruitment, and immobilization. Indeed, functional data implicate 43K protein in several roles, not mutually exclusive, during development of the postsynaptic membrane.

43K Protein Stabilizes AChR

Extraction of 43K protein from postsynaptic membranes leads to increased AChR susceptibility to thermal and proteolytic degradation (*see above*). This may reflect a direct stabilizing effect of AChR-43K protein interaction. On the other hand, simple steric considerations may be important: 43K protein augments the size of the receptor complex, rendering it more heat stable and possibly blocking access of cytoplasmic proteases.

43K Protein Recruits AChR

Recent obervations in heterologous expression systems suggest that 43K protein is critical to recruitment of AChR into receptor clusters (Froehner et al., 1990; Phillips et al., 1991). Also, 43K protein is associated with experimentally induced AChR clusters in vitro (Peng and Froehner, 1985); this spatial and temporal correlation hints at a causal role. In other systems, however, 43K protein is not essential to AChR aggregation. In *Torpedo* electroplaque, AChR aggregates are clearly detected in electrocyte precursor cells before 43K protein immunoreactivity is associated with them (Kordeli et al., 1989; LaRochelle et al., 1990; Nghiêm et al., 1991). Approximately one third of AChR aggregates in cultured chick myoblasts are negative for 43K protein-like immunoreactivity (Tsui et al., 1990). Moreover, both BC3H1, a muscle cell line that does not exhibit spontaneous AChR clusters, and C2, a muscle cell line that does, express 43K protein (LaRochelle and Froehner, 1987; Frail et al., 1989). Finally, *Torpedo* AChRs heterologously expressed in fibroblasts aggregate under the influence of extracellular synaptic factors even in the presence of little or no 43K protein (Hartman et al., 1991).

43K Protein Immobilizes AChR

Extraction of 43K protein from postsynaptic membranes releases AChR from receptor clusters (*see above*). Removal of 43K protein, however, involves exposure to chaotropic agents or strongly alkaline pH with unknown coincident modifications of membrane proteins and/or phospholipids. Indeed, pH 11 extracts of electrocyte membranes contain several protein bands in addition to the prominent one at 43 kDa. Also, there are data to suggest that AChR-43K protein complexes are mobile in the membrane (Bloch et al., 1986; Bridgman et al., 1987; Tsui et al., 1990). It remains plausible that other extracted membrane components, even minor constituents, are crucial to AChR immobilization.

Thus, despite the application of diverse experimental approaches to the question of 43K protein function, no convincing demonstration has been reported. With the recent introduction of new information, it is timely to synthesize a model of 43K protein function in postsynaptic membrane ontogeny. The following is a scheme based on temporal and spatial control of cluster assembly through progressive stabilization of partially assembled components, an emerging principle of erythrocyte morphogenesis (Lazarides

and Woods, 1989). Therein, 43K protein serves as an intermediate element stabilizing individual AChR molecules and via interactions with other cellular elements, assisting in AChR recruitment and cluster maturation.

I have chosen to define AChR clusters in strictly physiologic terms; a cluster exists when AChR molecules are immobilized and stabilized in tightly packed masses. Formerly, clusters have been defined in stereologic terms to refer to all AChR aggregates in the continuum between groups of loosely associated receptors and mature clusters. In my view, some controversy in the literature concerning the role of 43K protein in cluster formation stems from the casual usage of the term cluster. In the following scheme, I have used *AChR crowd* to denote immature receptor aggregates distinct from true clusters.

Progressive Stabilization of Partially Assembled Components

I propose that newly synthesized AChR is inserted into the plasma membrane—in analogy with the anion transporter in the developing red blood cell—before association with 43K protein or other cytoskeletal elements (Fig. 2). Consistent with the known stabilizing effects of 43K protein association, these AChRs, which may already be congregated in pools at the site of membrane insertion, degrade rapidly unless coupled with 43K protein. The 43K protein binds to AChR, the former reaching the postsynaptic membrane from a preexisting cytoplasmic pool (Kordeli et al., 1989; Nghiêm et al., 1991).

Next, AChR-43K protein complexes spontaneously gather, forming "speckles," (Steinbach, 1981) or microaggregates (Olek et al., 1983). Under the influence of motor nerve-mediated stimuli, AChR-43K protein complexes redistribute, coalescing as large stable clusters (Krikorian and Daniels, 1989). Finally, macromolecular aggregates are remodeled as mature postsynaptic clusters upon chemical modification (AChR and/or 43K protein phosphorylation?) or association of other cytoskeletal elements (possibly unstably preassembled) of the subsynaptic density.

Discussion

Involvement of 43K protein is proposed at three distinct stages of postsynaptic membrane ontogeny: stabilization of AChR molecules, recruitment of AChR-43K protein complexes into small aggregates, and immobilization of AChR by coupling with the underlying cytoskeletal network. Crucial to the model is the existence of isolated or small groups of AChR-43K protein complexes mobile in the plane of the membrane. The existence of such complexes is suggested in rotary shadowed freeze-etched images of electrocyte membrane vesicles (*see* Fig. 16 in Bridgman et al., 1987) and cultured rat myotubes (Pumplin and Bloch, 1987). These studies reveal tiny AChR clusters (≈0.05-0.1 μm diameter) in the membrane that probably represent a small number of AChR-43K protein complexes. The cytoplasmic surfaces of these miniature aggregates of AChR react with a monoclonal antibody (MAb) against 43K protein (Bridgman et al., 1987), indicating that 43K protein is associated with AChR at this stage of cluster development.

Recent evidence indicates that AChR-43K protein complexes spontaneously form intermediate-sized (≈0.5–2 μm diameter) clusters (Froehner et al., 1990; Phillips et al., 1991). In developing skeletal muscle, "intermediate" clusters have been observed (Steinbach, 1981; Olek et al., 1983) in association with 43K protein (Daniels, 1990); these clusters may be analogous to those observed in reconstitution studies. Finally, experimental evidence exists for the gradual fusion in developing muscle membrane of small AChR clusters into larger, mature-sized aggregates (Krikorian and Daniels, 1989).

In the proposed scheme, the formation of miniature (less than 1 μm) and intermediate (≈0.5–2 μm) AChR clusters is spontaneous, not requiring the external influence of nerve-medi-

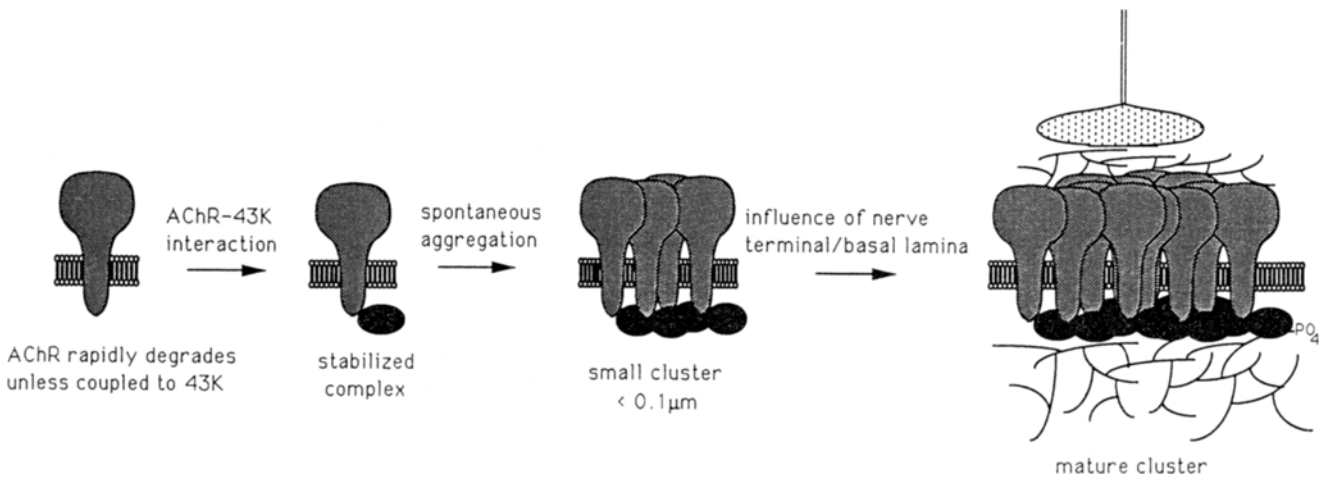

Fig. 2. Model of 43K protein involvement in development of the postsynaptic membrane (*see text for complete explanation*). Newly synthesized AChR is inserted into the membrane where they may reach relatively high densities as AChR crowds before association with 43K protein. AChR-43K protein complexes spontaneously aggregate to form microclusters. Under the influence of motor nerve-mediated stimuli, AChR-43K protein complexes redistribute and coalesce into large, stable clusters.

ated factors. Additional cytoplasmic or cytoskeletal factors may intervene in these steps, but the process remains spontaneous in the sense that assembled components are sufficient to drive the reaction, and external cell–cell interactions are not required. It should be noted that such hypothetical additional elements must be common to muscle and muscle-derived cells, as well as fibroblasts and frog oocytes (in heterologous expression studies). It is only at the transition from small aggregate to mature cluster that the program is driven by external elements. These elements, possibly anterograde factors released from the motor nerve terminal, set off a cascade of cytoskeletal reactions leading to maturation of the AChR cluster.

Two different mechanisms are proposed whereby 43K protein participates in cluster development: intrinsic cohesion among AChR-43K protein complexes (recruitment) and 43K protein-mediated attachment to the subsynaptic cytoskeleton (immobilization). This implies that 43K protein interacts not only with AChR but also with other elements: itself (43K–43K), other AChR molecules (bridging), and/or cytoskeletal proteins. This idea will require testing in the laboratory using a binding and reconstitution approach, which has heretofore proven difficult (Hill et al., unpublished observations).

What of AChR aggregates that form in myoblasts and developing electrocytes without 43K protein? In both instances, rapid AChR biosynthesis followed by specific triage and targeting to the postsynaptic membrane may account for the apparent paradox. In these tissues, during development, AChR molecules are precisely targeted to a single membrane domain (Witzemann et al., 1983) where they accumulate prior to association with 43K protein (Kordeli et al., 1989). AChR aggregates transiently observed early in development may not be true clusters but rather pools of AChR newly inserted in the membrane; AChRs are merely crowded rather than truly clustered. Consistent with this idea, some evidence indicates that AChR aggregates in cultured myoblasts are less stable than those in myotubes (Tsui et al., 1990).

Some data suggest that preexistent clusters break up before new ones form (Anderson and Cohen, 1977; Anderson et al., 1977; Kuromi and Kidokoro, 1984), implying direct destabilization of clusters rather than depletion of surface AChR

toward the nascent endplate. Thus, in analogy to the regulation of AChR expression, cluster development is mediated by a local signal and accompanied by a more global signal that destabilizes preexisting extrasynaptic clusters. Studies of reinnervated muscle have suggested that AChR loss precedes elimination of redundant nerve terminals (Rich and Lichtman, 1989), again implicating a postsynaptic signal controlling cluster dynamics and synapse remodeling. It is tempting to speculate that electrical activity, an established mechanism of suppression of AChR gene expression (Laufer and Changeux, 1989), may be directly involved in regulating extrasynaptic aggregate formation and dispersal, possibly via Ca^{2+}-dependent mechanisms (Rotzler et al., 1991).

The model is founded on the progressive stabilization of AChR in increasingly large receptor clusters. Accordingly, it should account for measurements of AChR stability in the postsynaptic membrane. At the innervated neuromuscular junction, AChR degrades slowly ($t_{1/2} \approx 10$ d). Upon denervation, degradation rates accelerate for AChR present at the neuromuscular junction prior to denervation; $t_{1/2} \approx 3$ d). This shift in degradation half-life can be antagonized by elevated intracellular Ca^{2+} levels (Rotzler et al., 1991), cAMP (Shyng et al., 1991), and direct electrical stimulation (Fumagalli et al., 1990). New AChRs synthesized after denervation and present at both junctional and extrajunctional regions degrade yet more rapidly ($t_{1/2} \approx 1$ d). Consistent with the model, 43K protein attachment to newly synthesized AChRs stabilizes the otherwise rapidly degraded receptor. Motor innervation then leads to further increases in AChR stability, specific to junctional AChR (Rotzer and Brenner, 1990) and possibly mediated by cAMP-dependent mechanisms (Shyng et al., 1991). It has been proposed that anterograde factors released from the motor neuron regulate gene expression at subsynaptic nuclei (Changeux et al., 1990). Calcitonin gene-related peptide, a factor that coexists with ACh in several vertebrate motor systems (Hökfelt et al., 1986), induces PKA-mediated phosphorylation of AChR (Miles et al., 1989). Agrin, a protein isolated from *Torpedo* electric organ, provokes AChR aggregation in cultured myotubes (Nitkin et al., 1987); agrin induces AChR β-subunit phosphorylation on tyrosine residues and possibly serine residues of γ- and δ-subunits (Wallace et al., 1991). Thus, phosphorylation mechanisms may be fundamental to the development and maintenance of a topologically organized structure at the postsynaptic membrane (Ross et al., 1988).

Implicit to the model is the potential existence of AChR in four different contexts: isolated AChR, isolated AChR-43K complex, aggregate of AChR (crowd), and receptor cluster. The model thus provides several additional testable ideas. One might expect to observe diffusion coefficient values for AChR-43K protein complexes intermediate between those of isolated AChR molecules and mature clusters. The concept of AChR crowds assumes that receptor molecules are inserted in discrete patches of membrane but are not immobilized there. Thus, measurements of AChR mobility and turnover rates should reveal differences between crowds and clusters. One might expect that AChR crowds are packed less densely and break up more rapidly than true clusters (Tsui et al., 1990). There may be a gradient in AChR density away from the locus of insertion as AChR molecules diffuse from the crowd. AChR may be less metabolically stable in the absence of 43K protein, a question addressable using reconstitution systems.

Any final conception of 43K protein function must take into account the fact that 43K protein is present in undifferentiated myoblasts (Frail et al., 1989) as well as in certain nonmuscle cell types that do not express AChR (Musil et al., 1989). This 43K protein, biochemically indistinguishable from that isolated from mature myocytes, is tightly associated with the membrane even in the absence of AChR. It has been suggested that 43K protein may be involved in membrane events other than AChR clustering (Musil et al., 1989), although evidence for this is currently unavailable. In any event, we cannot exclude a heretofore undiscovered enzymatic or signal transductional role for 43K protein.

Summary

The specialized molecular architecture of the chemical synapse is critical to efficient signal transmission at the neuromuscular junction. Release and subsequent degradation of neurotransmitter occur close to dense clusters of nicotinic receptor in the postsynaptic membrane of the motor endplate. AChRs aggregates, reaching a density of approx 10,000 AChR per μm^2 in the adult neuromuscular junction (Fertuck and Salpeter, 1976) are stable and cannot easily be dispersed (Bloch and Pumplin, 1988). Mechanisms underlying the development, maintenance, and regeneration after injury of this domain are the subject of intensive research (Peng and Poo, 1986; Schuetze and Role, 1987; Bloch and Pumplin, 1988; Changeux et al., 1990).

During muscle embryogenesis, diffusely dispersed receptors aggregate into densely packed clusters close to motor nerve terminals. The concerted mechanisms of lateral AChR diffusion and capture underneath the motor nerve ending and compartmentalized gene expression at "fundamental" nuclei underlie the morphogenesis and maturation of the postsynaptic domain. In association with this morphogenetic rearrangement, AChR becomes immobilized (Bourgeois et al., 1978; Rousselet et al., 1982) and metabolically stabilized (Berg and Hall, 1975; Chang and Huang, 1975; Devreotes and Fambrough, 1975). Due to the combined efforts of several laboratories, evidence is available suggesting that 43K protein is important throughout the cascade of cluster elaboration.

Insights derived from 43K protein studies may be extrapolated to other systems since macromolecular clustering of ion channels is a general property of excitable membranes. Glycine receptors are disposed in patches (Triller et al., 1985; Seitanidou et al., 1988), the size and number of which vary according to subcellular localization (Triller et al., 1990). Voltage-sensitive sodium channels congregate as clusters in the troughs of postjunctional folds (Beam et al., 1985; Angelides, 1986; Haimovich et al., 1987; Flucher and Daniels, 1989), a configuration that may facilitate initiation of the muscle action potential. In an analogous fashion, Ca^{2+} channels cluster at presynaptic active zones (Robitaille et al., 1990). The mechanisms governing molecular assembly in these systems are not known. However, a 33-kDa protein that binds with high affinity to the sodium channel has been described (Edelstein et al., 1988).

Ankyrin and spectrin have been shown to associate with sodium channels in brain (Srinivasan et al., 1988) and to codistribute with sodium channels at the neuromuscular junction (Flucher and Daniels, 1989). An intracellularly disposed phosphoprotein (β-subunit) is associated with the skeletal muscle voltage-sensitive calcium channel (Catterall, 1991); β-subunit coexpression with α1 normalizes the activation kinetics of dihydropyridine-sensitive calcium currents (Lacerda et al., 1991), a modulatory effect not reported for 43K protein and AChR. A peripheral 93-kDa protein (Schmitt et al., 1987) associated with the glycine receptor in fixed stoichiometry (Becker et al., 1989) may function in an analogous way to the AChR-associated 43K protein.

Mechanisms underlying assembly of the postsynaptic domain in the central nervous system are poorly characterized. In analogy with the experimentally accessible fish electric organ or mammalian neuromuscular junction, gradual stabilization of the developing postsynaptic apparatus may be fundamental. Already, dystrophin has been localized to postsynaptic regions in cortical neurons (Lidov et al., 1990). It will be interesting to investigate the existence of an isoform of 43K protein in nervous tissue. It is likely that progressive stabilization of focal aggregates is critical in the elaboration of an anisotropic distribution of receptors at the surface of central neurons (Changeux, 1986).

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References

Anderson M. J. and Cohen M. W. (1977) Nerve-induced and spontaneous redistribution of acetylcholine receptors on cultured muscle cells. *J. Physiol.* **268,** 757–773.

Anderson M. J., Cohen M. W., and Zorychta E. (1977) Effects of innervation on the distribution of acetylcholine receptors on cultured muscle cells. *J. Physiol.* **268,** 731–756.

Angelides K. J. (1986) Fluorescently labeled Na^+ channels are localized and immobilized to synapses of innervated muscle fibers. *Nature* **321,** 63–66.

Anthony D. T., Schuetze S. M., and Rubin L. L. (1984) Transformation by Rous sarcoma virus prevents acetylcholine receptor clustering on cultured chicken muscle fibers. *Proc. Natl. Acad. Sci. USA* **81,** 2265–2269.

Anthony D. T., Jacobs-Cohen R. J., Marazzi G., and Rubin L. L. (1988) A molecular defect in virally transformed muscle cells that cannot cluster acetylcholine receptors. *J. Cell Biol.* **106,** 1713–1721.

Baldwin T. J., Theriot J. A., Yoshihara C. M., and Burden S. J. (1988) Regulation of transcript encoding the 43K subsynaptic protein during development and after denervation. *Development* **104,** 557–564.

Barrantes F. J., Neugebauer D.-Ch., and Zingsheim H. P. (1980) Peptide extraction by alkaline treatment is accompanied by rearrangement of the membrane-bound acetylcholine receptor from *Torpedo marmorata*. *FEBS Lett.* **112,** 73–78.

Beam K. G., Caldwell J. H., and Campbell J. T. (1985) Na channels in skeletal muscle concentrated near the neuromuscular junction. *Nature* **313,** 588–590.

Berg D. K. and Hall Z. W. (1975) Loss of α-bungarotoxin from junctional and extrajunctional acetylcholine receptors in rat diaphragm muscle in vivo and in organ culture. *J. Physiol.* **252,** 771–789.

Becker C.-M., Hoch W., and Betz H. (1989) Sensitive immunoassay shows selective association of peripheral and integral membrane proteins of the inhibitory glycine receptor complex. *J. Neurochem.* **53,** 124–131.

Bloch R. J. (1979) Dispersal and reformation of acetylcholine receptor clusters of cultured rat myotubes treated with inhibitors of energy metabolism. *J. Cell Biol.* **82,** 626–643.

Bloch R. J. (1983) Acetylcholine receptor clustering in rat myotubes: requirement for Ca^{2+} and effects of drugs which depolymerize microtubules. *J. Neurosci.* **3,** 2670–2680.

Bloch R. J. (1986) Actin at receptor-rich domains of isolated acetylcholine receptor clusters. *J. Cell Biol.* **102,** 1447–1458.

Bloch R. J. and Froehner S. C. (1987) The relationship of the postsynaptic 43K protein to acetylcholine receptors in receptor clusters isolated from cultured rat myotubes. *J. Cell Biol.* **104,** 645–654.

Bloch R. J. and Morrow J. S. (1989) An unusual β-spectrin associated with clustered acetylcholine receptors. *J. Cell Biol.* **108,** 481–493.

Bloch R. J. and Pumplin D. W. (1988) Molecular events in synaptogenesis: nerve-muscle adhesion and postsynaptic dlfferentiation. *Am. J. Physiol.* **254,** C345–C364.

Bloch R. J., Steinbach J. H., Merlie J. P., and Heinemann S. (1986) Collagenase digestion alters the organization and turnover of junctional acetylcholine receptors. *Neurosci. Lett.* **66,** 113–119.

Boivin P. (1988) Role of the phosphorylation of red blood cell membrane proteins. *Biochem. J.* **256,** 689–695.

Bourgeois J.-P., Popot J.-L. Ryter A, and Changeux J.-P. (1978) Quantitative studies on the localization of the cholinergic receptor protein in the normal and denervated electroplaque from *Electrophorus electricus*. *J. Cell Biol.* **79,** 200–216.

Bridgman P. C., Carr C., Pedersen S. E , and Cohen J. B. (1987) Visualization of the cytoplasmic surface of *Torpedo* postsynaptic membranes by freeze-etch and immunoelectron microscopy. *J. Cell Biol.* **105,** 1829–1846.

Burden S. J. (1985) The subsynaptic 43kDa protein is concentrated at developing nerve-muscle synapses *in vitro*. *Proc. Natl. Acad. Sci. USA* **82,** 8270–8273.

Burden S. J., DePalma R. L., and Gottesman G. S. (1983) Crosslinking of proteins in acetylcholine receptor-rich membranes: association between the β-subunit and the 43 kDa subsynaptic protein. *Cell* **35,** 687–692.

Burn P. (1988) Amphitrophic proteins: A new class of membrane proteins. *Trends Biochem. Sci.* **13,** 79–83.

Burn P. and Burger M. M. (1987) The cytoskeletal protein vinculin contains transformation-sensitive, covalently bound lipid. *Science* **235**, 476–479.

Carr C., Fischbach G. D., and Cohen J. B. (1989) A novel M_r 87,000 protein associated with acetylcholine receptors in *Torpedo* electric organ and vertebrate skeletal muscle. *J. Cell Biol.* **109**, 1753–1764.

Carr C., McCourt D., and Cohen J. B. (1987) The 43-kilodalton protein of *Torpedo* nicotinic postsynaptic membranes: purification and determination of primary structure. *Biochemistry* **26**, 7090–7102.

Cartaud A., Courvalin J. C., Ludosky M. A. and Cartaud J. (1989) Presence of a protein immunologically related to lamin B in the postsynaptic membrane of *Torpedo marmorata* electrocyte. *J. Cell Biol.* **109**, 1745–1752.

Cartaud J., Sobel A., Rousselet A., Devaux P. F., and Changeux J.-P. (1981) Consequences of alkaline treatment for the ultrastructure of the acetylcholine-receptor-rich membranes from *Torpedo marmorata* electric organ. *J. Cell Biol.* **90**, 418–426.

Catterall W. A. (1991) Excitation-contraction coupling in vertebrate skeletal muscle: a tale of two calcium channels. *Cell* **64**, 871–874.

Chang C. C. and Huang M. C. (1975) Turnover of junctional and extrajunctional acetylcholine receptors of the rat diaphragm. *Nature* **253**, 643–644.

Changeux J.-P. (1986) Coexistence of neuronal messengers and molecular selection. *Prog. Brain Res.* **68**, 373–403.

Changeux J.-P., Babinet C., Bessereau J. L., Bessis A., Cartaud J., Daubas P., Devillers-Thiéry A., Duclert A., Hill J. A., Jasmin B., Klarsfeld A., Laufer R., Nghiêm H. O., Piette J., Roa M., and Salmon A. M. (1990) Comparmentalization of acetylcholine receptor gene expression during development of the neuromuscular junction, *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 55, pp. 381–396.

Cohen M. W. and Weldon P. R. (1980) Localization of acetylcholine receptors and synaptic ultrastructure at nerve-muscle contacts in culture: dependence on nerve type. *J. Cell Biol.* **86**, 388–401.

Daniels M P. (1990) Localization of actin, β-spectrin 43×10^3 M_r and 58×10^3 M_r proteins to receptor-enriched domains of newly formed acetycholine receptor aggregates in isolated myotube membranes. *J. Cell Sci.* **97**, 615–627.

Devreotes P. N. and Fambrough D. M. (1975) Acetylcholine receptor turnover in membranes of developing muscle fibers. *J. Cell Bol.* **65**, 335–358.

Edelstein N. G., Catterall W. A., and Moon R. T. (1988) Identification of a 33-kilodalton cytoskeletal protein with high affinity for the sodium channel. *Biochemistry* **27**, 1818–1822.

Elliott J., Blanchard S. G., Wu W., Miller J., Strader C. D., Hartig P., Moore H. P., Racs J., and Raftery M. A. (1980) Purification of *Torpedo californica* postsynaptic membranes and fractionation of their constituent proteins. *Biochem. J.* **185**, 667–677.

Fertuck H. C. and Salpeter M. M. (1976) Quantitation of junctional and extrajunctional acetylcholine receptors by electron microscope autoradiography after ^{125}I-α-bungarotoxin binding at mouse neuromuscular junctions. *J. Cell Biol.* **69**, 144–158.

Flucher B. E. and Daniels M. P. (1989) Distribution of Na^+ channels and ankyrin in neuromuscular junctions is complementary to that of acetylcholine receptors and the 43 kd protein. *Neuron* **3**, 163–175.

Frail D. E., Mudd J., Shah V., Carr C., Cohen J. B., and Merlie J. P. (1987) cDNAs for the postsynaptic 43-kDa protein of *Torpedo* electric organ encode two proteins with different carboxyl termini. *Proc. Natl. Acad. Sci. USA* **84**, 6302–6306.

Frail D. E., McLaughlin L. L., Mudd J., and Merlie J. P. (1988) Identification of the mouse muscle 43,000-Dalton acetycholine receptor-associated protein (RAPsyn) by cDNA cloning. *J. Biol. Chem.* **263**, 15,602–15,607.

Frail D. E., Musil L. S., Buonanno A., and Merlie J. P. (1989) Expression of RAPsyn (43K protein) and nicotinic acetylcholine receptor genes is not coordinately regulated in mouse muscle. *Neuron* **2**, 1077–1086.

Froehner S. C. (1984) Peripheral proteins of postsynaptic membranes from *Torpedo* electric organ identified with monoclonal antibodies. *J. Cell Biol.* **99**, 88–96.

Froehner S. C. (1986) The role of the postsynaptic cytoskeleton in AChR organization. *Trends Neurosci.* **9**, 37–41.

Froehner S. C. (1989) Expression of RNA transcripts for the postsynaptic 43 kDa protein in innervated and denervated rat skeletal muscle. *FEBS Lett.* **249**, 229–233.

Froehner S. C. (1991) The submembrane machinery for nicotinic acetylcholine receptor clustering. *J. Cell Biol.* **114**, 1–7.

Froehner S. C., Gulbrandsen V., Hyman C., Jeng A. Y., Neubig R. R., and Cohen J. B. (1981) Immunofluorescence localization at the mammalian neuromuscular junction of the M_r 43,000 protein of

Torpedo postsynaptic membranes. *Proc. Natl. Acad. Sci. USA* **78,** 5230–5234.

Froehner S. C., Murmame A. A., Tobler M., Peng H. B., and Sealock R. A. (1987) A postsynaptic M_r 58,000 (58K) protein concentrated at acetylcholine receptor-rich sites in *Torpedo* electroplaques and skeletal muscle. *J. Cell Biol.* **104,** 1633-1646.

Froehner S. C., Luetje C. W., Scotland P. B., and Patrick J. (1990) The postsynaptic 43K protein clusters muscle nicotinic acetylcholine receptors in *Xenopus* oocytes. *Neuron* **5,** 403–410.

Fumagalli G., Balbi S., Cangiano A., and Lømo T. (1990) Regulation of turnover and number of acetylcholine receptors at neuromuscular junctions. *Neuron* **4,** 563–569.

Gibrat J.-F., Garnier J., and Robson B. (1987) Further development of protein secondary structure prediction using information theory: new parameters and consideration of residue pairs. *J. Mol. Biol.* **198,** 425–443.

Goldman D., Boulter J., Heinemann S., and Patrick J. (1985) Muscle denervation increases the level of two mRNAs coding for the acetylcholine receptor α-subunit. *J. Neurosci.* **5,** 2553–2558.

Gordon A. S., Davis C. G., and Diamond I. (1977) Phosphorylation of membrane proteins at a cholinergic synapse. *Proc. Natl. Acad. Sci. USA* **74,** 263–267.

Gordon A. S., Davis C. G., Milfay D., Kaur J., and Diamond I. (1980) Membrane-bound protein kinase activity in acetylcholine receptor-enriched membranes. *Biochim. Biophys. Acta* 600, 421–431.

Haimovich B., Schotland D. L., Fieles D. L., and Barchi R. W. (1987) Localization of sodium channel subtypes in adult rat skeletal muscle using channel-specific monoclonal antibodies. *J. Neurosci.* **7,** 2957–2966.

Hamilton S. L., McLaughlin M., and Karlin A. (1979) Formation of disulfide-linked oligomers to acetylcholine receptor in membrane from *Torpedo* electric tissue. *Biochemistry* **18,** 155–163.

Hartman D. S., Millar N. S., and Claudio T. (1991) Extracellular synaptic factors induce clustering of acetylcholine receptors stably expressed in fibroblasts. *J. Cell Biol.* **115,** 165–177.

Hill J. A., Nghiêm H.-O., and Changeux J.-P. (1991) Serine-specific phosphorylation of nicotinic receptor-associated 43K protein. *Biochemistry* 30, 5579–5585.

Hökfelt T., Holets V. R., Staines W., Meister B. Melander T., Schalling M., Schultzberg M., Freedman J., Bjorklund H., Olson L., Lindk B., Elfvin L. G., Lundberg J., Lindgren J. A., Samuelsson B., Terenius L., Post C., Everitt B., and Goldstein M. (1986) Coexistence of neuronal messengers: An overview. *Prog. Brain Res.* **68,** 33–70.

Huganir R. L. and Greengard P. (1990) Regulation of neurotransmitter receptor desensitization by protein phosphorylation. *Neuron* **5,** 555–567.

Jasmin B. J., Cartaud A., Ludosky M. A., Changeux, J.-P., and Cartaud J. (1990) Asymmetric distribution of dystrophin in developing and adult *Torpedo marmorata* electocyte: evidence for its association with the acetylcholine receptor-rich membrane. *Proc. Natl. Acad. Sci. USA* **87,** 3938–3941.

Kellie S. and Wigglesworth N. M. (1987) The cytoskeletal protein vinculin is acylated by myristic acid. *FEBS Lett.* **213,** 428–432.

Khurana T. S., Hoffman E. P., and Kunkel L. M. (1990) Identification of a chromosome 6-encoded dystrophin-related protein. *J. Biol Chem.* **265,** 16,717–16,720.

Klarsfeld A. and Changeux J.-P. (1985) Activity regulates the level of aceylcholine receptor alpha-subunit mRNA in cultured chick myotubes. *Proc. Natl. Acad. Sci USA* **82,** 4558–4562

Klymkowsky M. W., Heuser J. E., and Stroud R. M. (1980) Protease effects on the structure of acetylcholine receptor membranes from *Torpedo californica*. *J. Cell Biol.* **85,** 823–838.

Ko P., Anderson M. J., and Cohen M. W. (1977) Denervated skeletal muscle fibers develop patches of high acetylcholine receptor density. *Science* **196,** 540–542.

Kordeli E., Cartaud J., Nghiêm H.-O., Pradel L. A., Dubreuil C., Paulin D., and Changeux J.-P. (1986) Evidence for a polarity in the distribution of proteins from the cytoskeleton in *Torpedo marmorata* electrocytes. *J. Cell Biol.* **102,** 748–761.

Kordeli E., Cartaud J., Nghiêm H.-O., Devillers-Thiéry A., and Changeux J.-P. (1989) Asynchronous assembly of the acetylcholine receptor and of the 43-kDa v_1 protein in the postsynaptic membrane of developing *Torpedo marmorata* elecrocyte. *J. Cell Biol.* **108,** 127-139

Kouzarides T. and Ziff E. (1988) Leucine zippers of *fos, jun,* and GCN4 dictate dimerization specificity and thereby control DNA binding. *Nature* **336,** 646–651.

Krikorian J. G. and Daniels M. P. (1989) Reorganization and stabilization of acetylcholine receptor aggregates on rat myotubes. *Develop. Biol.* **131,** 524–538.

Kuromi H. and Kidokoro Y. (1984) Nerve disperses preexisting acetylcholine receptor clusters prior to induction of receptor accumulation in Xenopus muscle cultures. *Dev. Biol.* **103,** 53–61.

Lacerda A. E, Kim H. S., Ruth P. Perez-Reyes E. Flockerzi V., Hofmann F., Birnbaumer L., and Brown A. M. (1991) Normalization of current kinetics by interaction between α_1 and β subunits of the skeletal muscle dihydropyridine-sensitive Ca^{2+} channel. *Nature* **352,** 527–530.

Landschulz W. H., Johnson P. F., and McKnight S. L. (1988) The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins *Science* **240,** 1759–1764.

Landschulz W. H., Johnson P. F., and McKnight S. L. (1989) The DNA binding domain of the rat liver nuclear protein C/EBP is bipartite. *Science* **243,** 1681–1688.

LaRochelle W. J. and Froehner S. C. (1986) Determination of the tissue distributions and relative concentrations of the postsynaptic 43 kDa protein and the acetylcholine receptor in *Torpedo. J. Biol. Cem.* **261,** 5270–5274.

LaRochelle W. J. and Froehner S. C. (1987) Comparison of the postsynaptic 43kDa protein from muscle cells that differ in acetylcholine receptor clustering activity. *J. Biol. Chem.* **262,** 8190–8195.

LaRochelle W. J., Ralston E., Forsayeth J. R., Froehner S. C., and Hall Z. W. (1989) Clusters of 43-kDa protein are absent from genetic variants of C2 muscle cells wth reduced acetylcholine receptor expression. *Dev. Biol.* **132,** 130–138

LaRochelle W. J., Witzemann V., Fiedler W., and Froehner S. C. (1990) Developmental expression of the 43K and 58K postsynaptic membrane proteins and nicotinic acetylcholine recepors in *Torpedo* electrocytes. *J. Neurosci.* **10,** 3460–3467.

Laufer R. and Changeux J.-P. (1989) Activity-dependent regulation of gene expression in muscle and neuronal cells. *Molec. Neurobiol.* **3,** 1–53.

Lazarides E. and Woods C. (1989) Biogenesis of the red blood cell membrane skeleton and the control of erythroid morphogenesis. *Annu. Rev. Cell Biol.* **5,** 427–452.

Lidov H. G. W., Byers T. J., Watkins S. C., and Kunkel L. M. (1990) Localization of dystrophin to postsynaptic regions of central nervous system cortical neurons. *Nature* **348,** 725–727.

Lo M. M. S., Garland P. B., Lamprecht J., and Barnard E. (1980) Rotational mobility of the membrane-bound acetylcholine receptor of *Torpedo* electric organ measured by phosphorescence depolarization. *FEBS Lett.* **111,** 407–412.

Love D. R., Hill D. F., Dickson G., Spurr N. K., Byth B. C., Marsden R. F., Walsh F. S., Edwards Y. H., and Davies K. E. (1989) An autosomal transcript in skeletal muscle with homology to dystrophin. *Nature* **339,** 55–58.

McCormack K., Campanelli J. T., Ramaswami M., Mathew M. K., Tanouye M. A., Iversen L. E., and Rudy B. (1989) Leucine-zipper motif update. *Nature* **340,** 103.

McKnight S. L. (1991) Molecular zippers in gene regulation. *Sci. Am.* **264(4),** 54-64.

Merlie J. P., Isenberg I. E., Russell S. D., and Sanes J. R. (1984) Denervation supersensitivity in skeletal muscle: Analysis with a cloned cDNA probe. *J. Cell Biol.* **99,** 332–335.

Miles K. and Huganir R. L. (1988) Regulation of nicotinic acetylcholine receptors by protein phosphorylation. *Molec. Neurobiol.* **2,** 91-124.

Miles K., Greengard P., and Huganir R. L. (1989) Calcitonin gene-related peptide regulates phosphorylation of the nicotinic acetylcholine receptor in rat myotube. *Neuron* **2,** 1517-1524.

Mishina M., Kurosaki, Tobimatsu T., Morimoto Y., Noda M., Yamamoto T., Terao M., Lindstrom J., Takahashi T., Kuno M., and Numa S. (1984) Expression of functional acetylcholine receptor from cloned cDNAs. *Nature* **307,** 604–608.

Mitra A. A. K., McCarthy M. P., and Stroud R. M. (1989) Three-dimensional structure of the nicotinic acetylcholine receptor and location of the major associated 43-kD cytoskeletal protein, determined at 22 Å by low dose electron microscopy and X-ray diffraction to 12. 5 Å. *J. Cell Biol.* **109,** 755–774.

Moody-Corbett F. and Cohen M. W. (1982) Influence of nerve on the formation and survival of acetylcholine receptor and cholinesterase patches on embryonic *Xenopus* muscle cells in culture. *J. Neurosci.* **2,** 633–646.

Musil L. S., Carr C., Cohen J. B., and Merlie J. P. (1988) Acetylcholine receptor-associated 43K protein contains covalently bound myristate. *J. Cell Biol.* **107,** 1113–1121.

Musil L. S., Frail D. E., and Merlie J. P. (1989) The mammalian 43kD acetylcholine receptor-associated protein (RAPsyn) is expressed in some nonmuscle cells. *J. Cell Biol.* **108,** 1833–1840.

Neubig R. R., Krodel E. K., Boyd N. D., and Cohen J. B. (1979) Acetylcholine and local anesthetic binding to *Torpedo* nicotinic postsynaptic membranes after

removal of nonreceptor peptides *Proc. Natl. Acad. Sci. USA* **76,** 690–694.

Nghiêm H.-O., Cartaud J., Dubreuil C., Kordeli C., Buttin G., and Changeux J.-P. (1983) Production and characterization of a monclonal antibody directed against the 43,000 dalton v_1 polypeptide from *Torpedo marmorata* electric organ. *Proc. Natl. Acad. Sci. USA* **80,** 6403–6407.

Nghiêm H.-O., Hill J., and Changeux J.-P. (1991) Developmental changes in the subcellular distribution of the 43K (v_1) polypeptides in *Torpedo marmorata* electrocyte: support for a role in acetylcholine receptor stabilization. *Development* **113,** 1059–1067.

Nitkin R. M., Smith M. A., Magill C., Fallon J. R., Yao Y. -M. M., Wallace B. G., and McMahan U. J. (1987) Identification of agrin, a synaptic organizing protein from *Torpedo* electric organ. *J. Cell Biol.* **105,** 2471–2478.

Olek A. J., Krikorian J. G., and Daniels M. P. (1986) Early stages in the formation and stabilization of acetylcholine receptor aggregates on cultured myotubes: sensitivity to temperature and azide. *Dev. Biol.* **117,** 24–34.

Olek A. J., Pudimat P. A., and Daniels M. P. (1983) Direct observation of the rapid aggregation of acetylcholine receptors on identified cultured myotubes after exposure to embryonic brain extract. *Cell* **34,** 255–264.

Orida N. and Poo M.-M. (1978) Electrophoretic movement and localization of acetylcholine receptors in the embryonic muscle cell membrane. *Nature* **275,** 31–35.

Peng H. B., Cheng P.-C., and Luther P. W. (1981) Formation of ACh receptor clusters induced by positively charged latex beads. *Nature* **292,** 831–834.

Peng H. B. and Cheng P.-C. (1982) Formation of postsynaptic specializations induced by latex beads in cultured muscle cells. *J. Neurosci.* **2,** 1760–1774.

Peng H. B. and Froehner S. C. (1985) Association of the postsynaptic 43K protein with newly formed acetylcholine receptor clusters in cultured muscle cells. *J. Cell Biol.* **100,** 1698–1705.

Peng H. B., Lauren L. P., and Chen Q. (1991) Induction of synaptic development in cultured muscle cells by basic fibroblast growth factor. *Neuron* **6,** 237–246.

Peng H. B. and Poo M.-M. (1986) Formation and dispersal of acetylcholine receptor clusters in muscle cells. *Trends Neurosci.* **9,** 125–129

Phillips W. D., Kopta C., Blount P., Gardner P. D., Steinbach J. H., and Merlie J. P. (1991) ACh receptor-rich membrane domains organized in fibroblasts by recombinant 43-kilodalton protein. *Science* **251,** 568-570.

Porter S. and Froehner S. C. (1985) Interaction of the 43K protein wth components of *Torpedo* post-synaptic membranes. *Biochemistry* **24,** 425-432.

Prives J., Fulton A. B., Penman S., Daniels M. P., and Christian C. N. (1982) Interaction of the cytoskeletal framework with acetylcholine receptors on the surface of embryonic muscle cells in culture. *J. Cell Biol.* **92,** 231–236.

Pumplin D. W. and Bloch R. J. (1987) Disruption and reformation of the acetylcholine receptor clusters of cultured rat myotubes occur in two distinct stages. *J. Cell Biol.* **104,** 97–108.

Rasmussen R., Benvegnu D., O'Shea E. K., Kim P. S., and Alber T. (1991) X-ray scattering indicates that the leucine zipper is a coiled coil. *Proc. Natl. Acad. Sci. USA* **88,** 561–564.

Rich M. M. and Lichtman J. W. (1989) *In vivo* visualization of pre- and postsynaptic changes during synapse elimination in reinnervated mouse muscle. *J. Neurosci.* **9,** 1781–1805.

Robitaille R., Adler E. M., and Charlton M. P. (1990) Strategic location of calcium channels at transmitter release sites of frog neuromuscular synapses. *Neuron* **5,** 773–779.

Ross A., Rapuano M., and Prives J. (1988) Induction of phosphorylation and cell surface redistribution of acetylcholine receptors by phorbol ester and carbamylcholine in cultured chick muscle cells. *J. Cell Biol.* **107,** 1139–1145.

Rotzler S. and Brenner H. R. (1990) Metabolic stabilization of acetylcholine receptors in vertebrate neuromuscular junction by muscle activity. *J. Cell Biol.* **111,** 655-661.

Rotzler S., Schramek H., and Brenner H. R. (1991) Metabolic stabilization of endplate acetylcholine receptors regulated by Ca^{2+} influx associated with muscle activity. *Nature* **349,** 337–339.

Rousselet A., Cartaud J., Devaux P. F., and Changeux J.-P. (1982) The rotational diffusion of the acetylcholine receptor in *Torpedo marmorata* membrane fragments studied with a spin-labelled alpha-toxin: importance of the 43,000 protein(s). *EMBO J.* **1,** 439–445.

Saitoh T. and Changeux J.-P. (1980) Phosphorylation *in vitro* of membrane fragments from *Torpedo marmorata* electric organ: effect on membrane

solubilization by detergents. *Eur. J. Biochem.* **105,** 51–62.

Saitoh T., Wennogle L. P., and Changeux J.-P. (1979) Factors regulating the susceptiblity of the acetylcholine receptor protein to heat inactivation. *FEBS Lett.* **18,** 489–494.

Schmitt B., Knaus P., Becker C.-M., and Betz H. (1987) The M_r 93,000 polypeptide of the postsynaptic glycine receptor complex is a peripheral membrane protein. *Biochemstry* **26,** 805–811.

Schuetze S. M. and Role L. W. (1987) Developmental regulation of nicotinic acetylcholine receptors. *Annu. Rev. Neuroci.* **10,** 403–457.

Sealock R., Wray B. E, and Froehner S. C. (1984) Ultrastructural localization of the M_r 43,000 protein and the acetylcholine receptor in *Torpedo* postsynaptic membranes using monoclonal antibodies. *J. Cell Biol.* **98,** 2239–2244.

Seitanidou T., Triller A., and Korn H. (1988) Distribution of glycine receptors on the membrane of a central neuron: an immunoelectron microscopy study. *J. Neurosci.* **8(11),** 4319–4333.

Shyng S.-L., Xu R., and Salpeter M. M. (1991) Cyclic AMP stablizes the degradation of original junctional acetylcholine receptors in denervated muscle. *Neuron* **6,** 469–475.

Sobel A., Weber M., and Changeux J.-P. (1977) Large scale purificaion of the acetylcholine receptor protein in its membrane-bound and detergent-extracted forms from *Torpedo marmorata* electric organ. *Eur. J. Biochem.* **80,** 215–224.

Srinivasan Y., Elmer L., Davis J., Bennett V., and Angelides K. (1988) Ankyrin and spectrin associate with voltage-dependent sodium channels in brain. *Nature* **333,** 177–180.

St. John P. A., Froehner S. C., Goodenough D. A., and Cohen J. B. (1982) Nicotinic postsynaptic membranes from *Torpedo*: sidedness, permeability to macro-molecules, and topography of major polypeptides. *J. Cell Biol.* **92,** 333–342.

Staufenbiel M. and Lazarides E. (1986) Ankyrin is fatty acid acylated in erythrocytes. *Proc. Natl. Acad. Sci. USA* **83,** 318–322.

Steinbach J. H. (1981) Developmental changes in acetylcholine receptor aggregates at rat skeletal neuromuscular junctions. *Dev. Biol.* **84,** 267–276.

Toyoshima C. and Unwin N. (1988) Ion channel of acetylcholine receptor reconstructed from images of postsynaptic membranes. *Nature* **336,** 247–250.

Triller A., Cluzeaud F., Pfeiffer F., Betz H., and Korn H. (1985) Distribution of glycine receptors at central synapses: an immunoelectron microscopy study. *J. Cell Biol.* **101,** 683–688.

Triller A., Seitanidou T., Franksson O., and Korn H. (1990) Size and shape of glycine receptor clusters in a central neuron exhibit a somato-dendritic gradient. *New Biologist* **2(7),** 637–641.

Tsui H.-C. T., Cohen J. B., and Fischbach G. D. (1990) Variation in the ratio of acetylcholine receptors and the M_r 43,000 receptor-associated protein in embryonic chick myotubes and myoblasts. *Dev. Biol.* **140,** 437–446.

Walker J. H., Boustead C. M., and Witzemann V. (1984) The 43K protein, v_1, associated with acetylcholine receptor containing membrane fragments is an actin-binding protein. *EMBO J.* **3,** 2287–2290.

Wallace B. G., Qu Z., and Huganir R. L. (1991) Agrin induces phosphorylation of the nicotinic acetylcholine receptor. *Neuron* **6,** 869–878.

Wennogle L. P. and Changeux J.-P. (1980) Transmembrane orientation of proteins present in acetylcholine receptor-rich membranes from *Torpedo marmorata* studied by selective proteolysis. *Eur. J. Biochem.* **106,** 381–393.

Witzemann V., Richardson G., and Boustead C. (1983) Characterization and distribution of acetylcholine receptors and acetylcholinesterase during electric organ development in *Torpedo marmorata*. *Neuroscience* **8,** 333–349.