online

18/11	15-17
25/11	9-11
25/11	15:30-17:30
27/11	16-18

In-person

2/12	9-11
03/12	11-13
04/12	11-13

Synaptic Transmission at PNS: the **Neuromuscular Junction**

1. **The Synaptogenesis**
2. The Synaptic Transmission at NMJ
3. The Safety Factor
4. Myasthenic Syndromes
5. The Tripartite Synapse

*December second: lecture on Microgravity effects at the NMJ (Alberto Griffoni)

*December 3,4: lectures given by Prof. Agenor Limon (Visiting professor from UTMB)

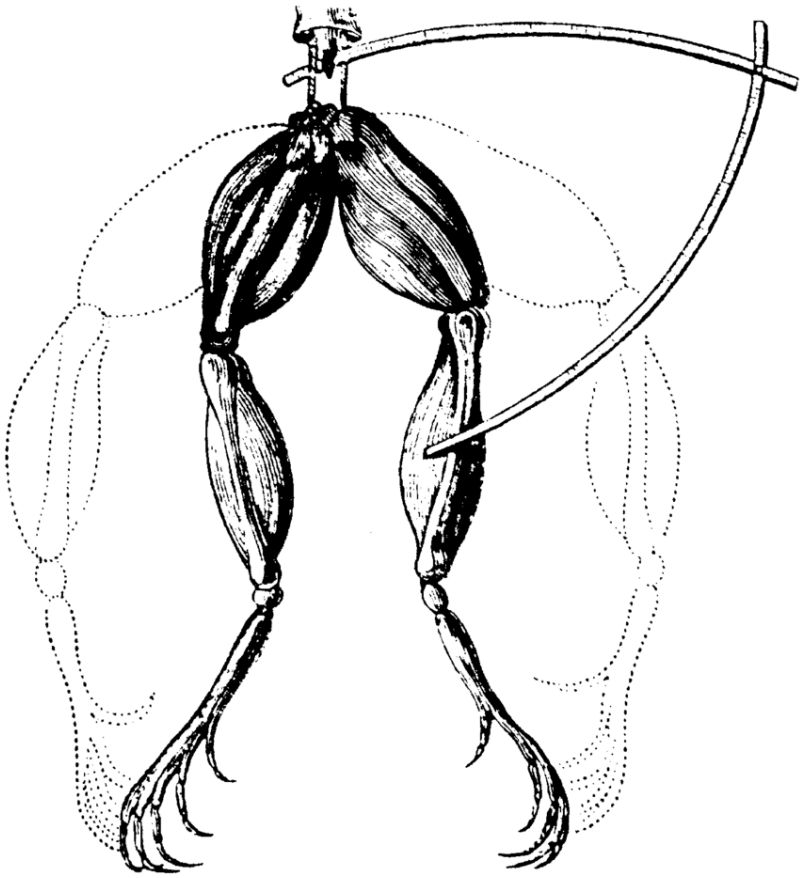
NOTE: seminar of prof Limon, December 5 (likely at 3 pm)

Mapping synaptic signatures across brain disorders: A comparative functional analysis from donor tissue to data-driven phenotypes

*Both topics will be part of the examination.

The Study of the NMJ gave:

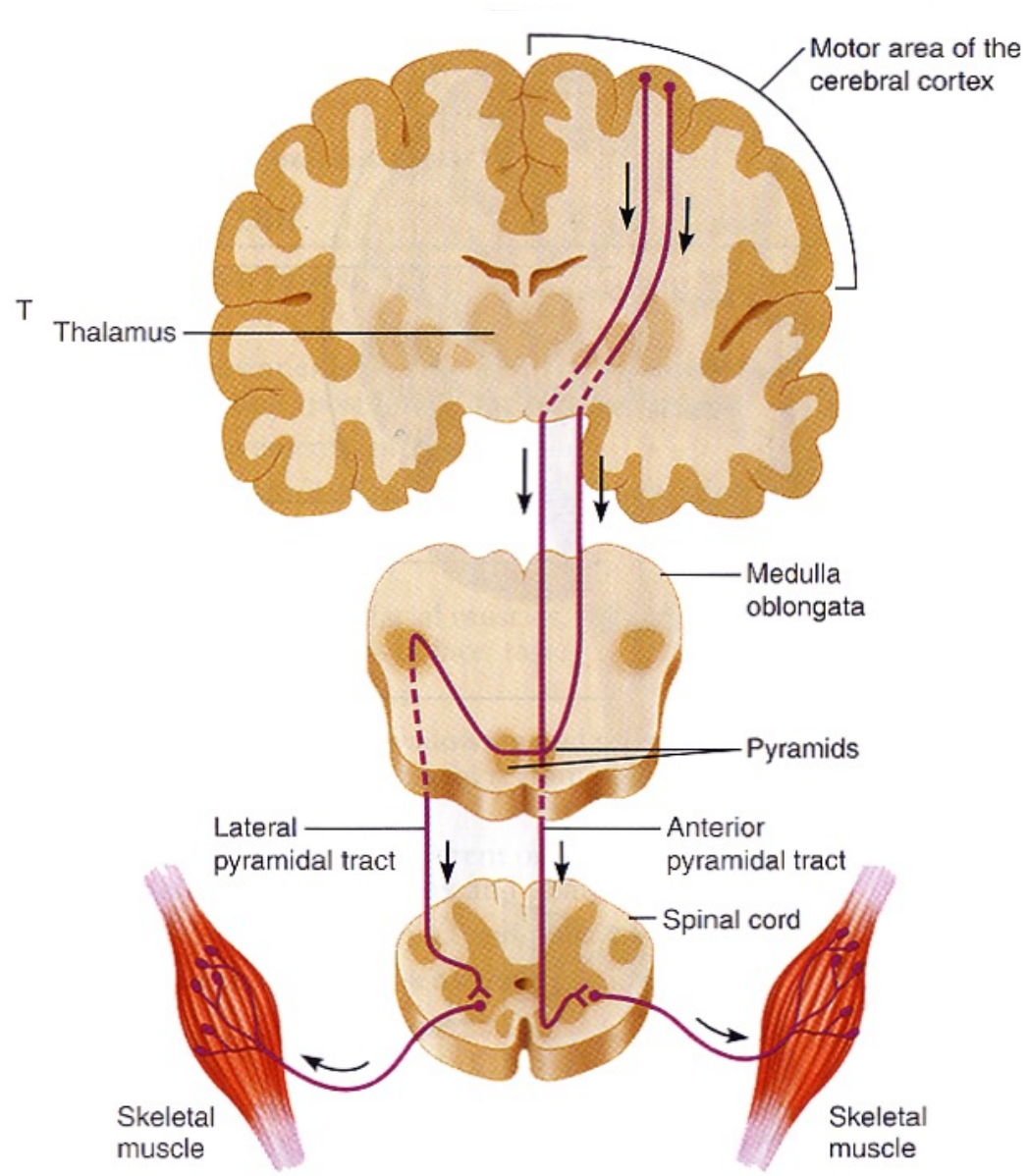
- the first concept of a membrane receptor (“receptive substance”)
- evidence for the chemical nature of synaptic transmission
- key physiological and ultrastructural evidence for the quantal mechanism of transmitter release

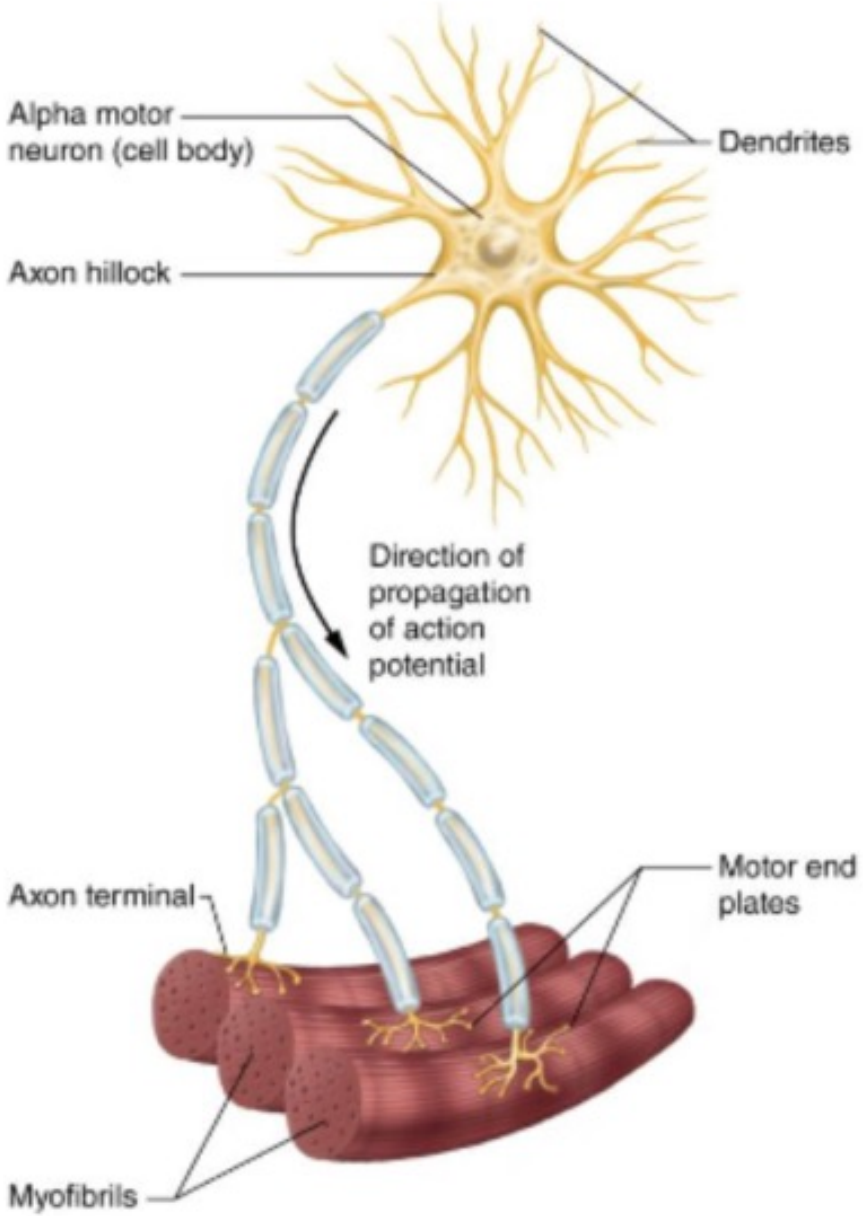


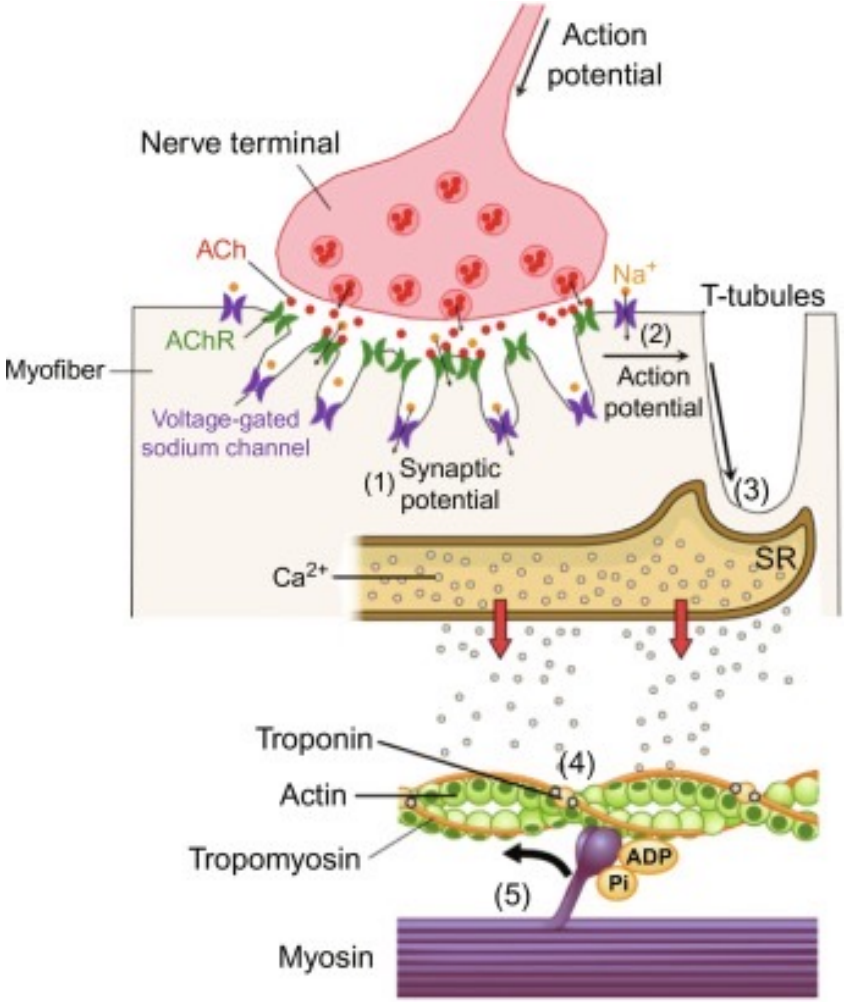
The main actors of the NMJ:

Motoneuron

Skeletal muscle fiber

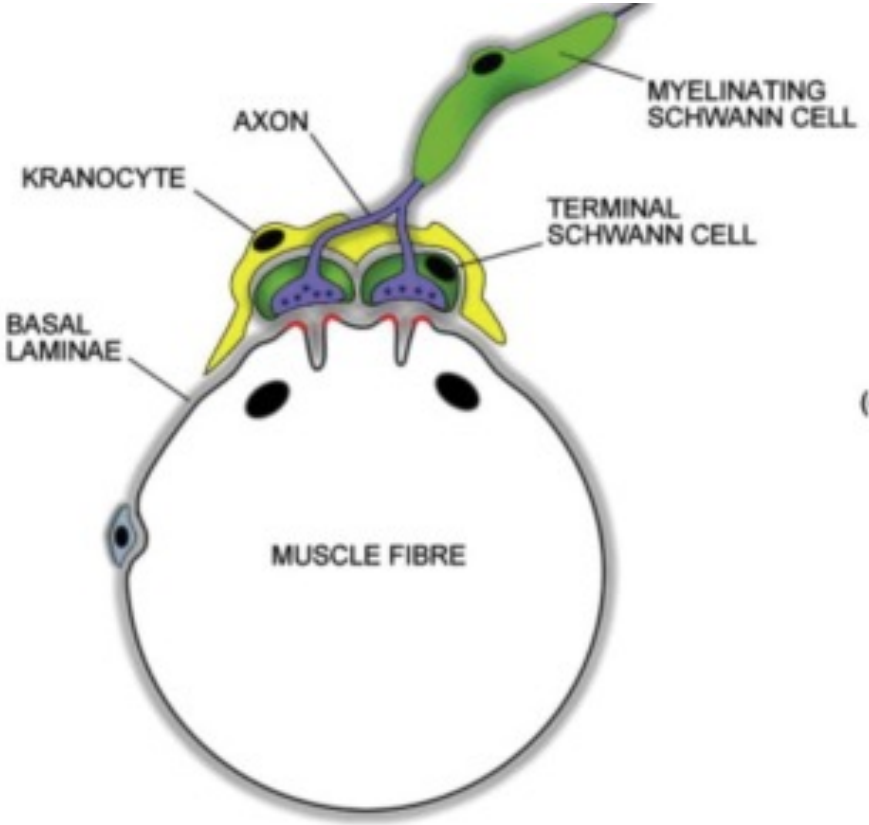


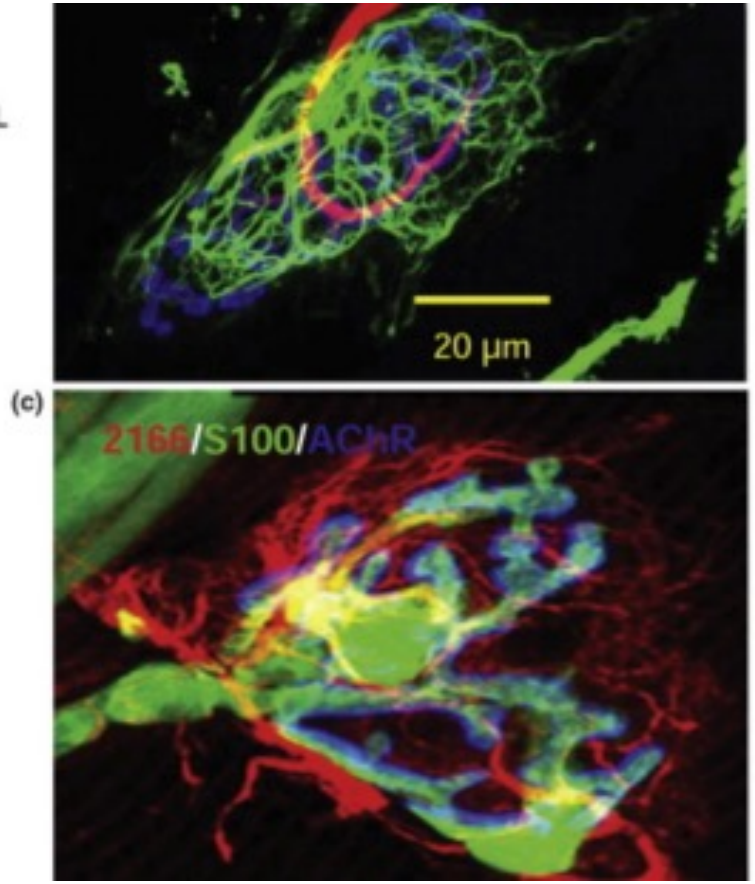

Alpha motor neuron (cell body)
Dendrites
Axon hillock
Direction of propagation of action potential
Axon terminal
Motor end plates
Myofibrils


Action potential
Nerve terminal
ACh
Na+
T-tubules
AChR
(2)
Action potential
Myofiber
Voltage-gated sodium channel
(1) Synaptic potential
(3)
SR
Ca2+
Troponin
(4)
Actin
Tropomyosin
ADP
Pi
(5)
Myosin

A modern view:

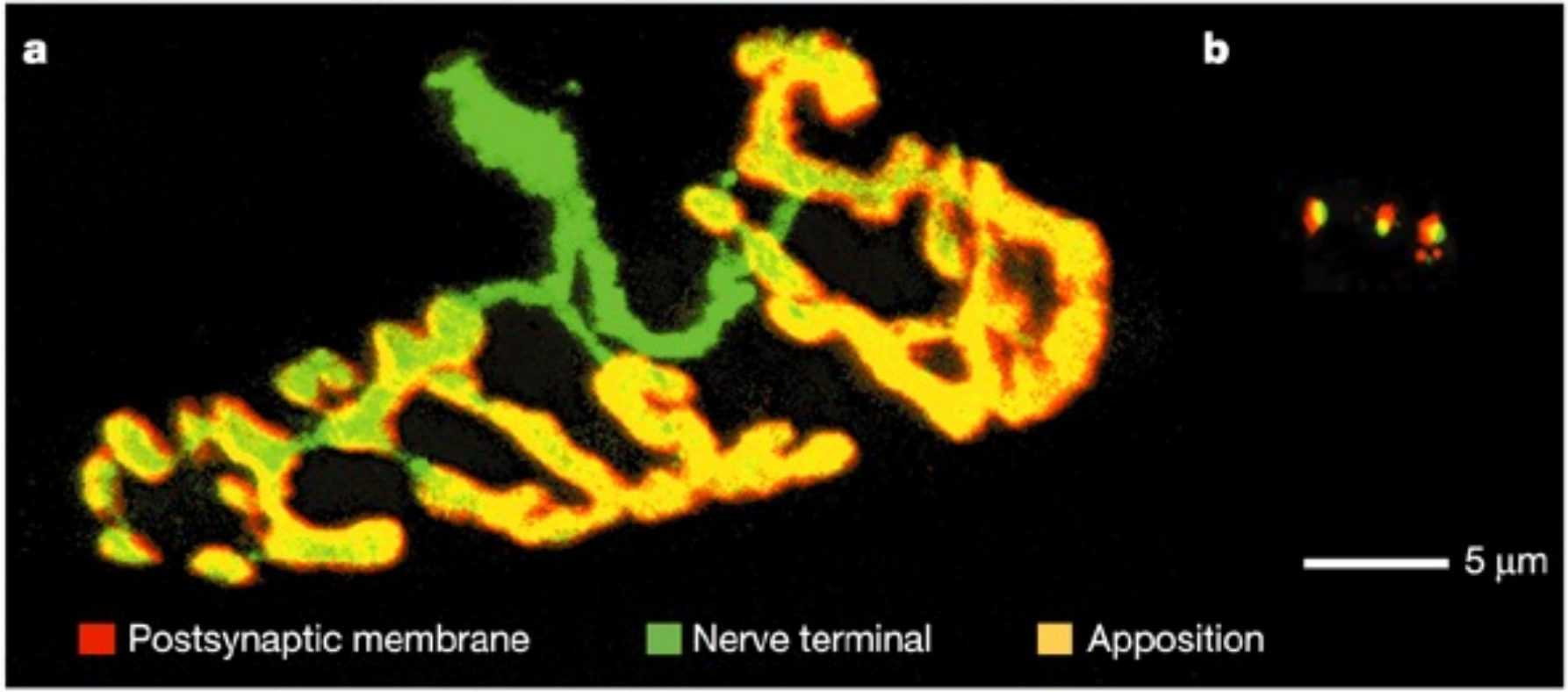
4 types of cells are present at the NMJ





RR Ribchester (2009) Current Opinion in Pharmacology, 9:297–305

Peripheral *vs* Central Synapse

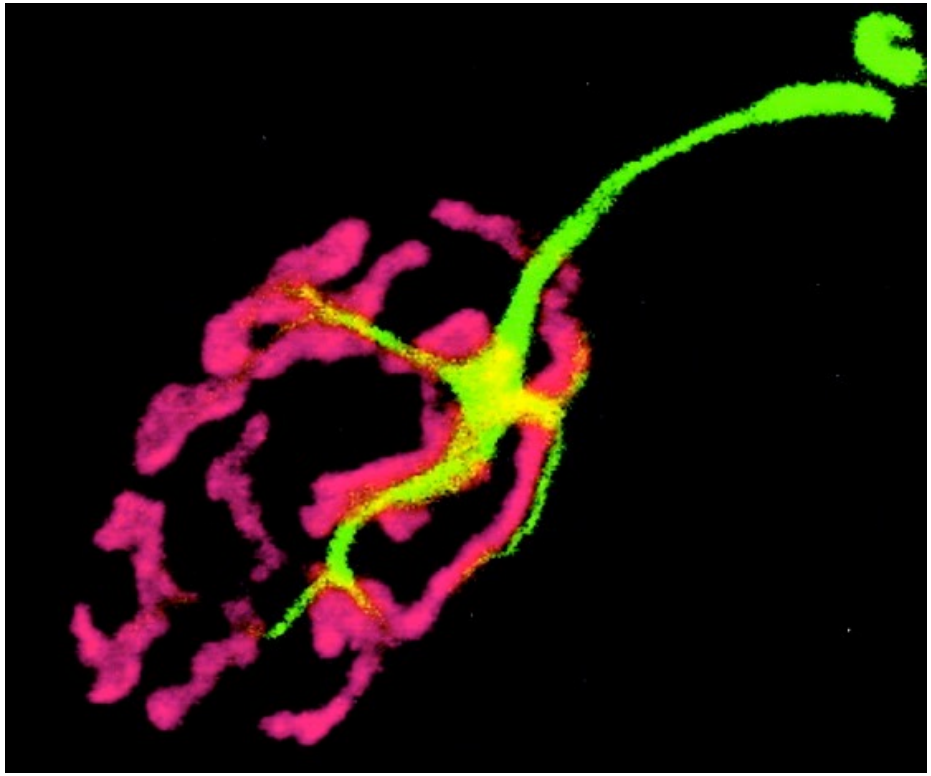


Nature Reviews | Neuroscience

1. The synaptogenesis

The aims:

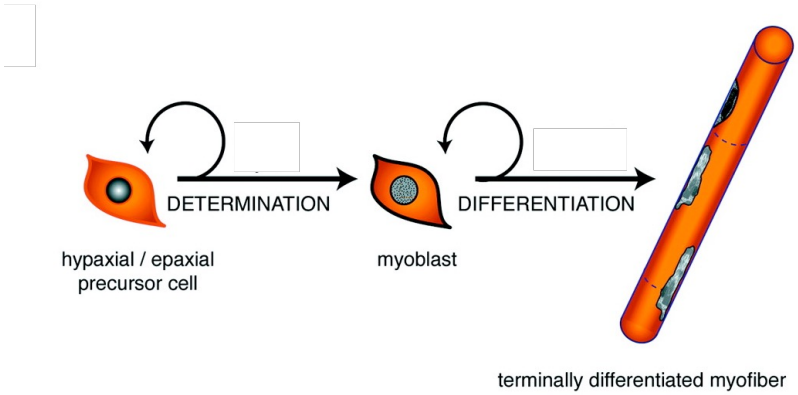
- understand the main steps of synaptogenesis
- understand the postsynaptic differentiation



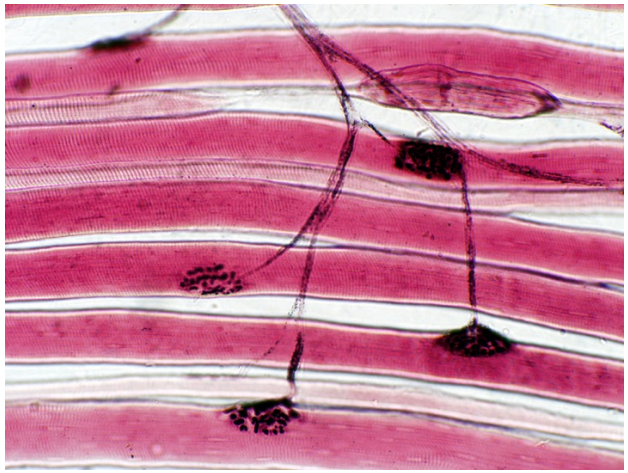
Molecules important for NMJ development and function

1) Proteins for the transmission of the nerve impulses (nAChR and VOC).
2) Proteins that organize these components into functional postsynaptic membranes (Agrin, MuSK, ErbB, Rapsin etc…).

The postsynaptic differentiation: nAChR clustarization



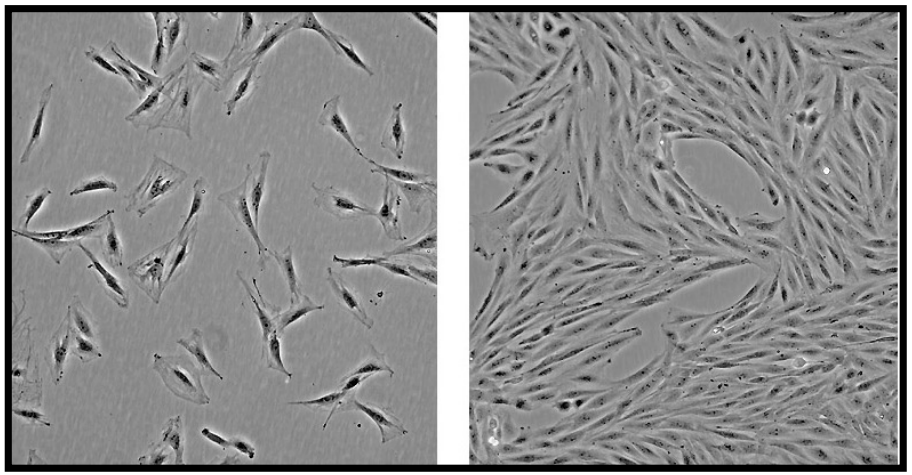
nAChR densitiy
~ 1.000/μm^2



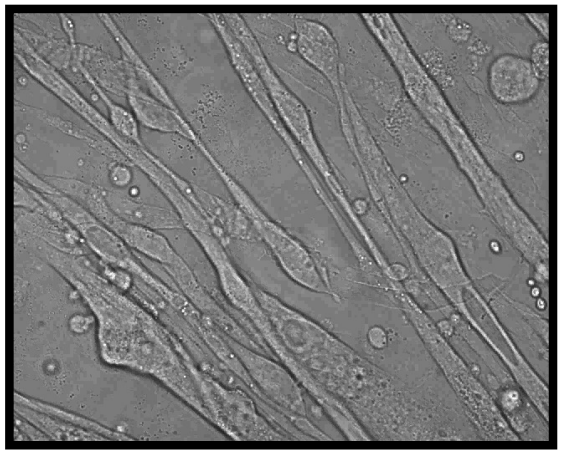
- nAChR density at the synapse ~ 10.000/μm^2
- Extrasynaptic nAChR density ~ 10/μm^2

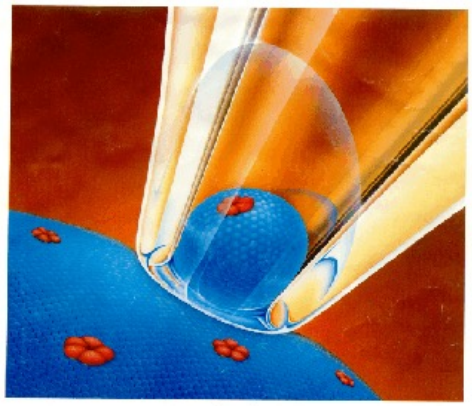
The *in vitro* Myogenesis

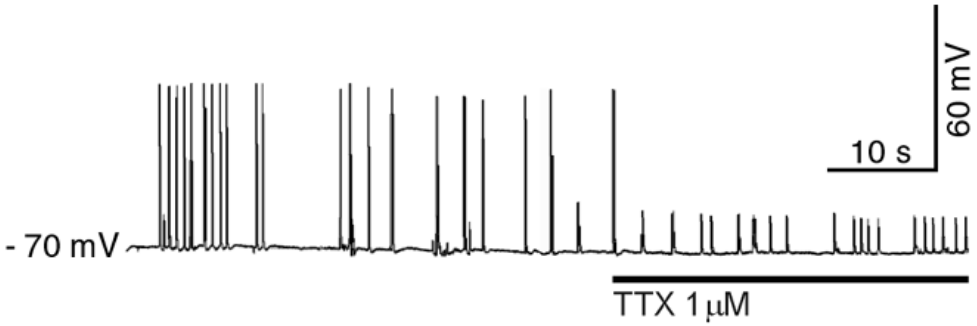
Myoblasts *in vitro*



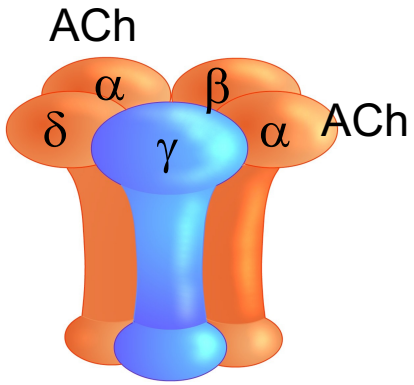
Myotubes *in vitro*



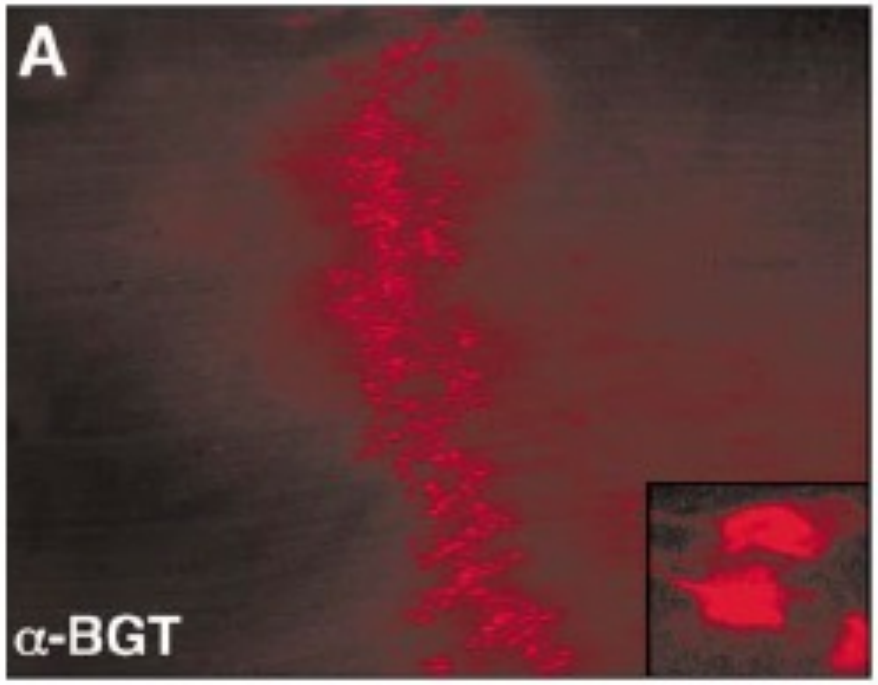




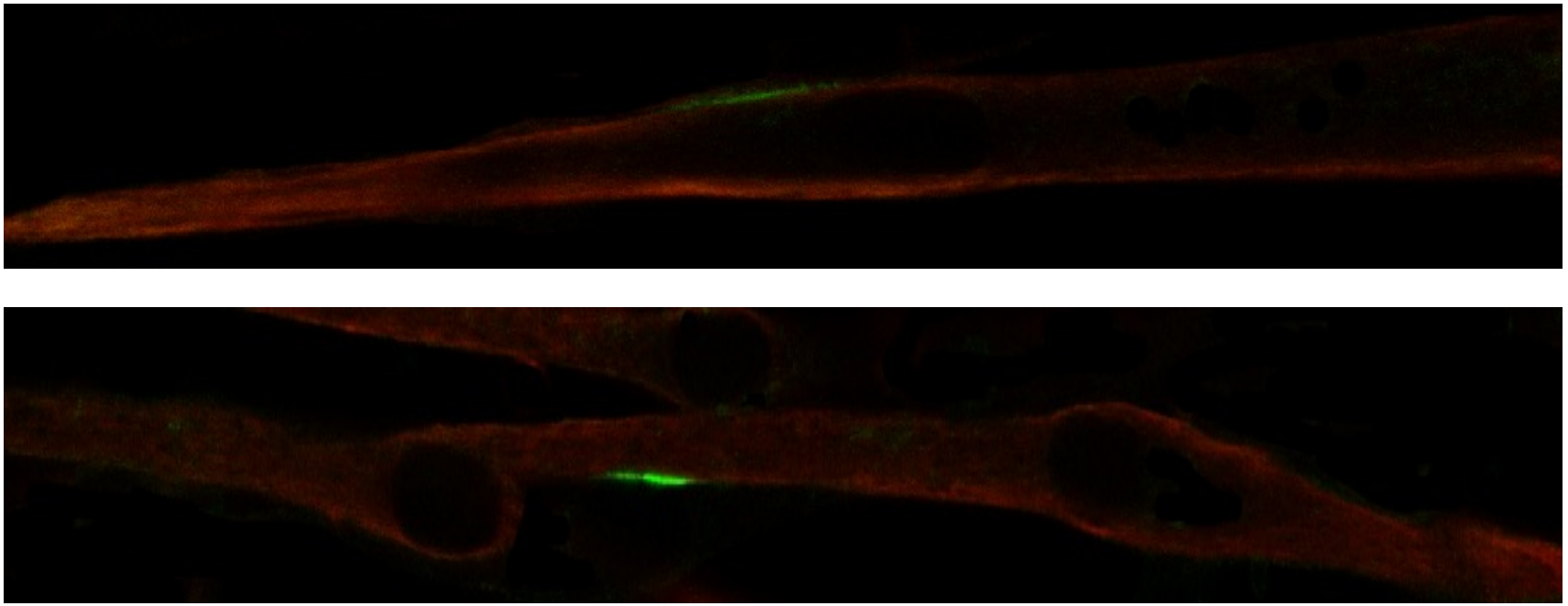
Expression and distribution of nAChRs before the arrival of the nerve



Embryonic or foetal isoform of the nAChRs



The **“Hot Spot”**
pre-patterned cluster of nAChRs



Note that:

1. Synapse are formed anywhere on the surface of the embryonic muscle; during the *in vitro* innervation, a lateral migration of nAChRs along the path of nerve-muscle contact is observed.
2. Regenerating nerves grow back to pre-existing site of synaptic differentiation.

What are the roles of the nerve terminal and
of the muscle fiber during synaptogenesis?

Main steps of the synaptogenesis:

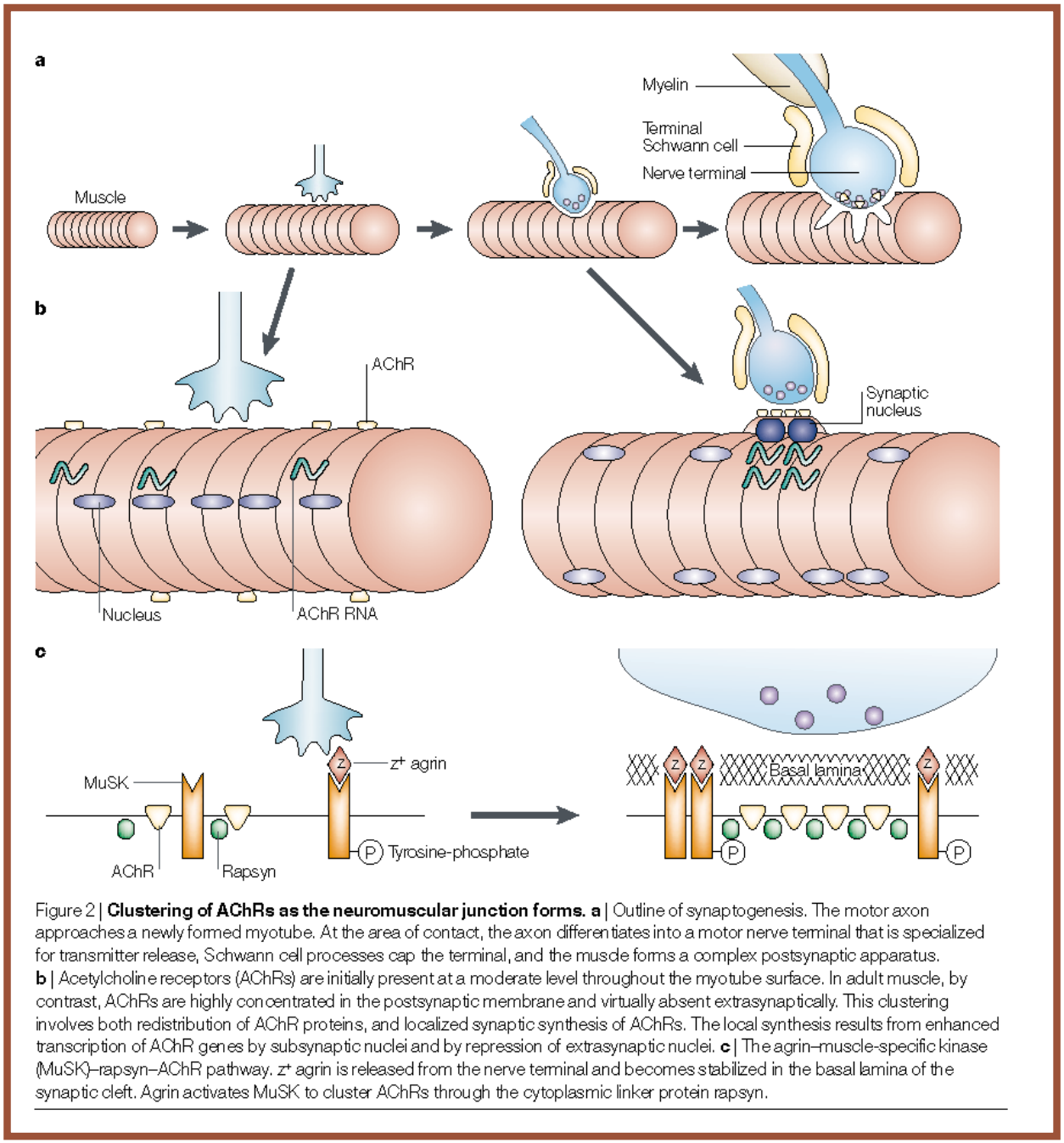


Figure 2 | **Clustering of AChRs as the neuromuscular junction forms. a** | Outline of synaptogenesis. The motor axon approaches a newly formed myotube. At the area of contact, the axon differentiates into a motor nerve terminal that is specialized for transmitter release, Schwann cell processes cap the terminal, and the muscle forms a complex postsynaptic apparatus. **b** | Acetylcholine receptors (AChRs) are initially present at a moderate level throughout the myotube surface. In adult muscle, by contrast, AChRs are highly concentrated in the postsynaptic membrane and virtually absent extrasynaptically. This clustering involves both redistribution of AChR proteins, and localized synaptic synthesis of AChRs. The local synthesis results from enhanced transcription of AChR genes by subsynaptic nuclei and by repression of extrasynaptic nuclei. **c** | The agrin–muscle-specific kinase (MuSK)–rapsyn–AChR pathway. z^+ agrin is released from the nerve terminal and becomes stabilized in the basal lamina of the synaptic cleft. Agrin activates MuSK to cluster AChRs through the cytoplasmic linker protein rapsyn.

Three different mechanisms contribute to the nAChR redistribution

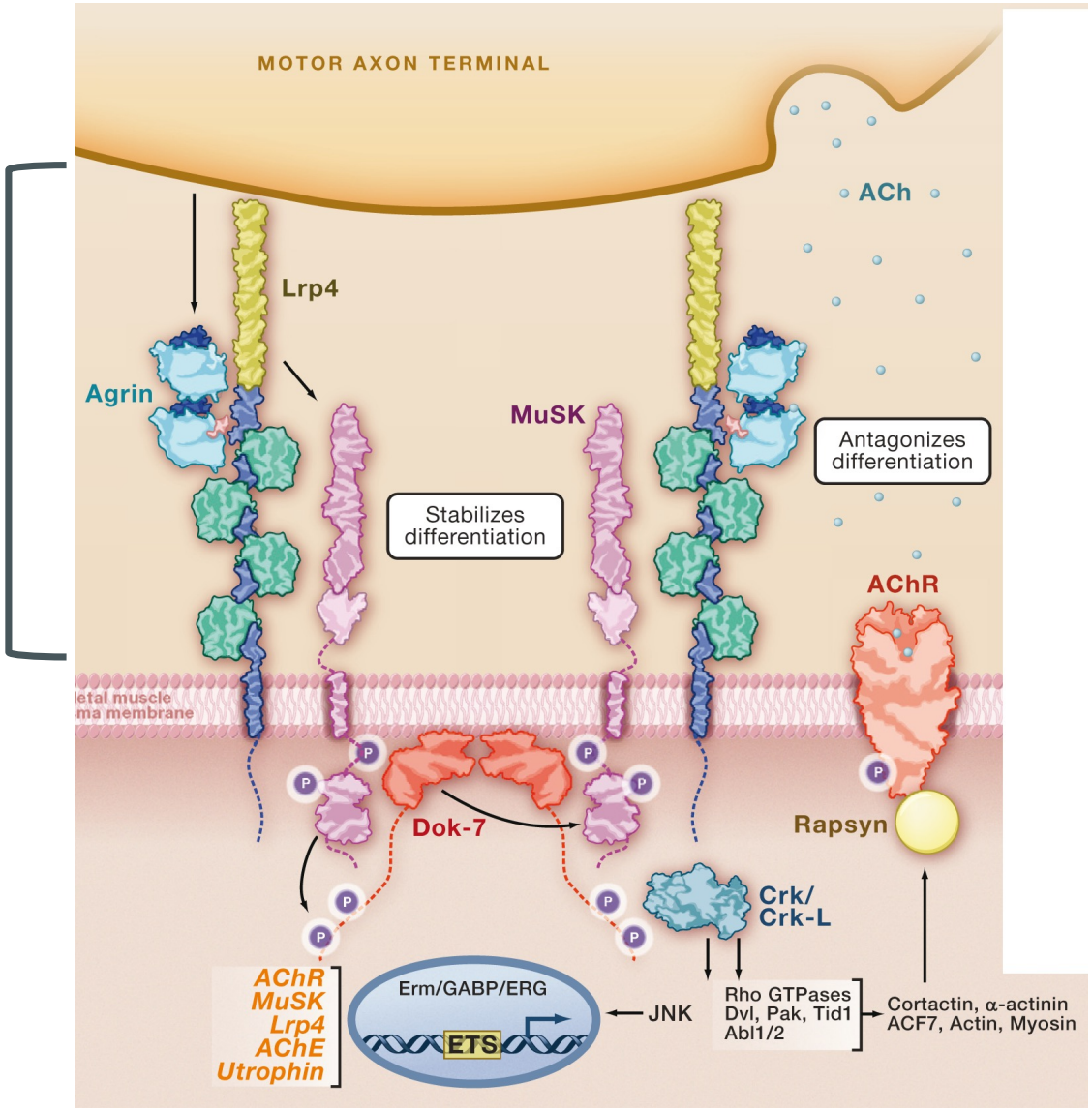
1. Clustering of diffusely distributed nAChRs in the postsynaptic membrane.
2. Transcriptional activation of nAChR subunit genes in subsynaptic nuclei.
3. Transcriptional repression of nAChR subunit genes in non synaptic nuclei.

How does the motoneuron organize the postsynaptic membrane?

Agrin-Lrp4-MuSk signaling pathway:
synaptic organizer or stabilizing agent?

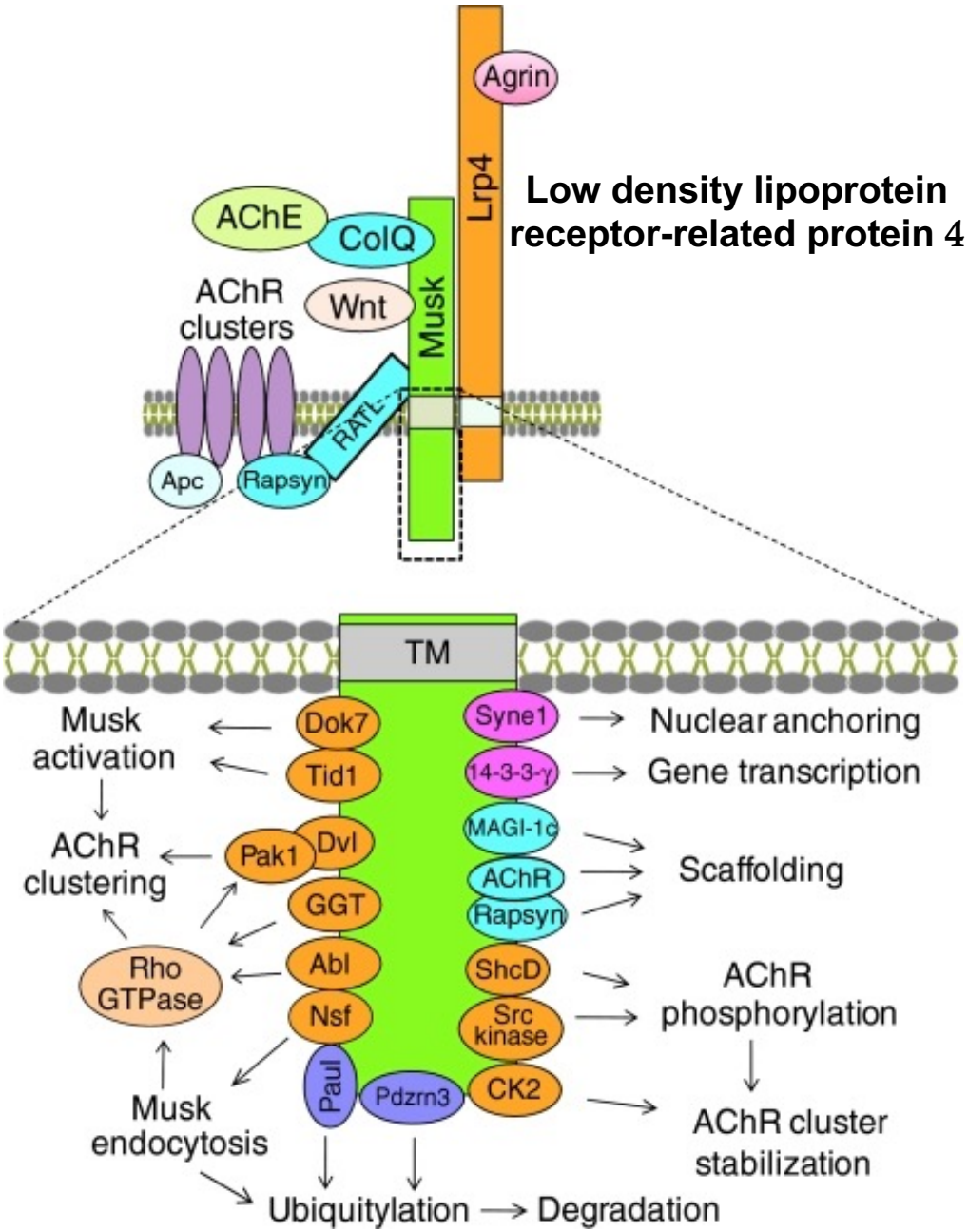
- Agrin is an heparan sulfate proteoglycan synthesized by motor neurons and it stably associates with basal lamina of the synaptic cleft.
- Agrin induces aggregation of nAChRs on cultured myotubes.

Highly specialized
basal lamina



The nAChR during formation of the NMJ

- Clustering
- Anchoring
- Stabilization



Musk appears to be a master organizer of postsynaptic development at the NMJ. Mice with mutations in the gene that encodes Musk have deficiencies in forming primitive AChR clusters or prepatterned muscle fibers, and they do not form nerve-induced AChR clusters or NMJs. Evidence suggests that Musk does not only act as a receptor and tyrosine kinase for agrin, which initiates pathways leading to postsynaptic differentiation (see Fig. 3). By interacting with additional proteins, of which a growing number is being identified, Musk might also serve as a scaffold organizer that is crucial for compartmentalized signaling. Based on their function, Musk-interacting proteins can be classified into four groups (see figure). The first group (orange) is necessary for Musk activity or downstream signaling. The second group (purple) controls agrin/Musk signaling. The function of the proteins in these two groups is discussed in Fig. 3 and its related text. The third group (blue) consists of scaffold proteins, including rapsyn (Antolik et al., 2006; Apel et al., 1997), ColQ [a protein for acetylcholinesterase (AChE) enrichment in the synaptic cleft (Cartaud et al., 2004)], the MAGUK protein MAGI-1c (Strochlic et al., 2001) and AChR (Fuhrer et al., 1997). The fourth group (pink) includes proteins that might regulate gene expression, including 14-3-3γ, a protein thought to regulate synaptic gene expression at the NMJ (Strochlic et al., 2004), and synaptic nuclear envelope 1 (Syne1), a nuclear envelope protein enriched in synaptic nuclei (Apel et al., 2000). This interaction was thought to help anchor synaptic nuclei in the synaptic region of NMJs, but although muscle nuclei in both synaptic and non-synaptic regions are disorganized in Syne1-null mutant mice, their NMJs are apparently normal (Zhang X. et al., 2007). These results indicate that the proper position of synaptic nuclei might not be as crucial as previously thought. It is worth pointing out that, unless otherwise discussed, the suggested functions of many of the Musk-interacting proteins have not been tested in vivo.

Wu et al (2010) Development. 137:1017-33

Agrin

secreted by neurons, muscles and Schwann cells

Z^+ : secreted by neurons clusters nAChRs *in vitro* 1000 times more than:

A^- :secreted by muscle and Z^- secreted by Schwann cells.

Agrin-Lrp4-MuSK signaling pathway*

→ clusterization, anchoring and and stabilization of the nAChRs

*important pharmacological target for neuromuscular disease as well aging

Rapsyn
is (one of) the MuSK effector

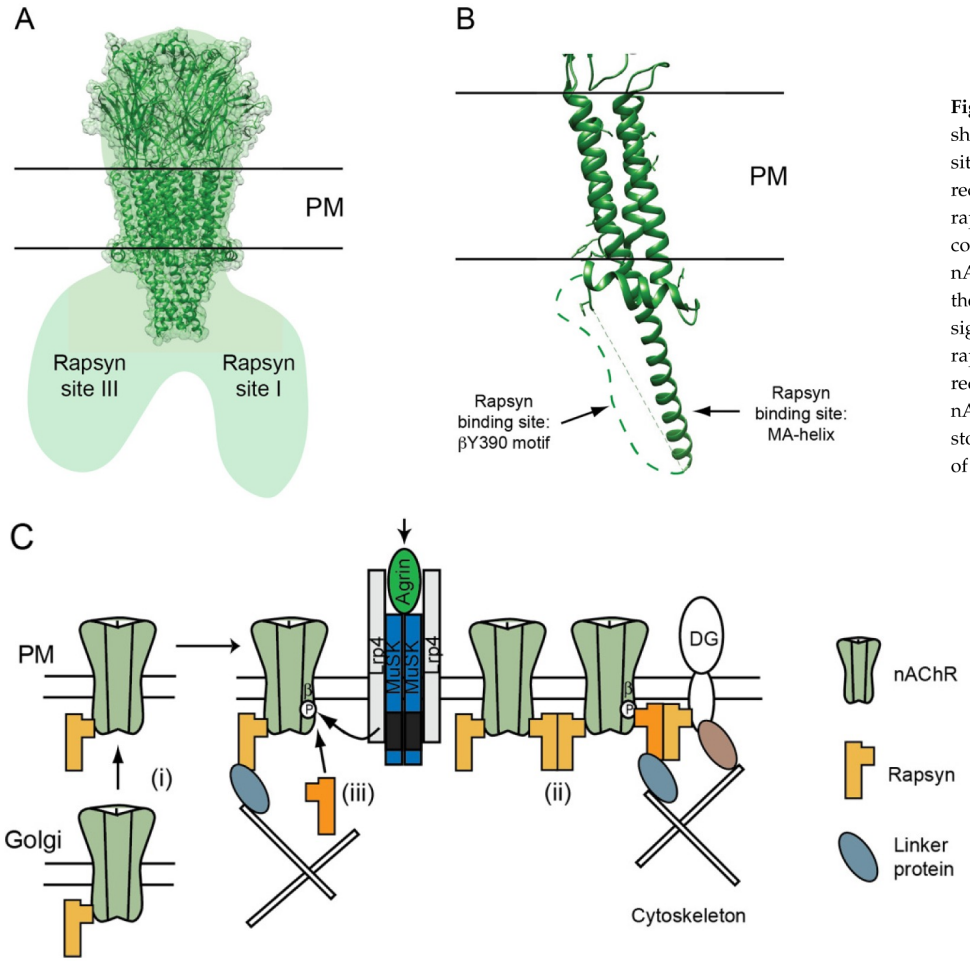
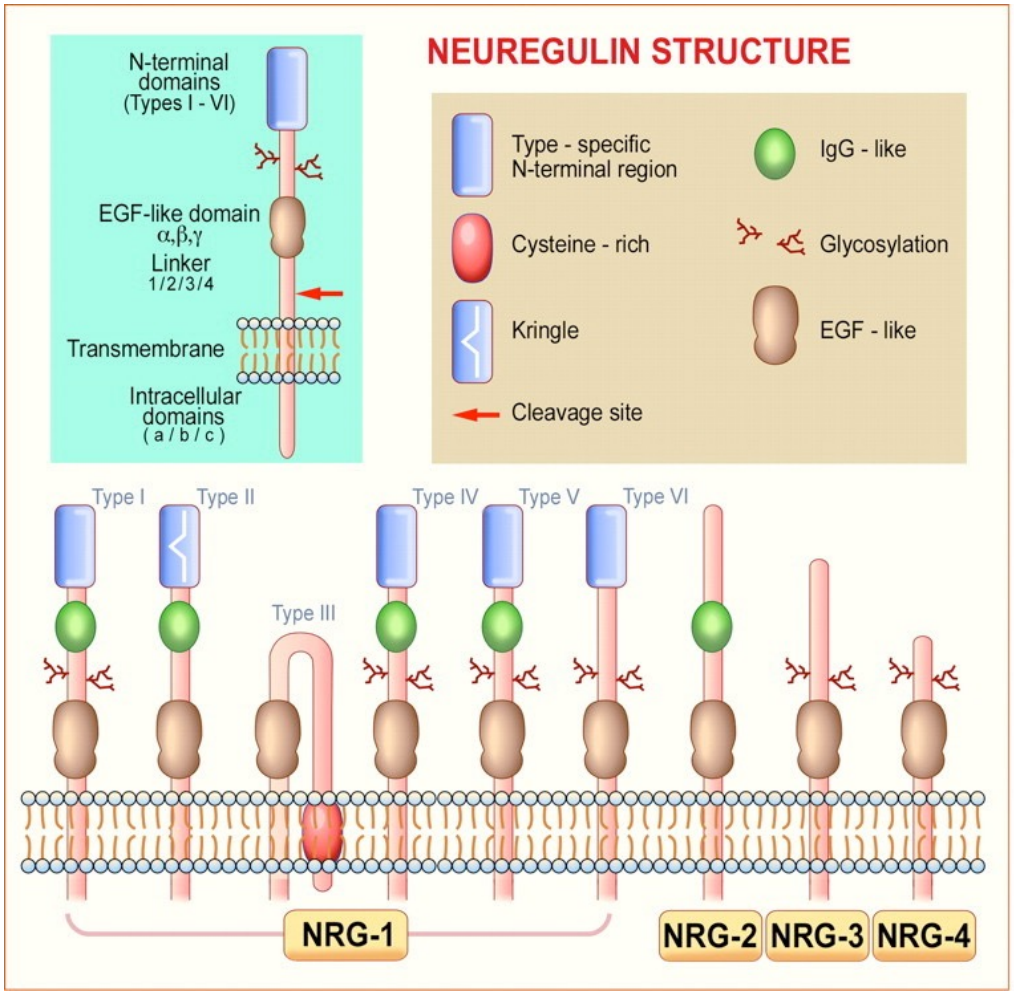
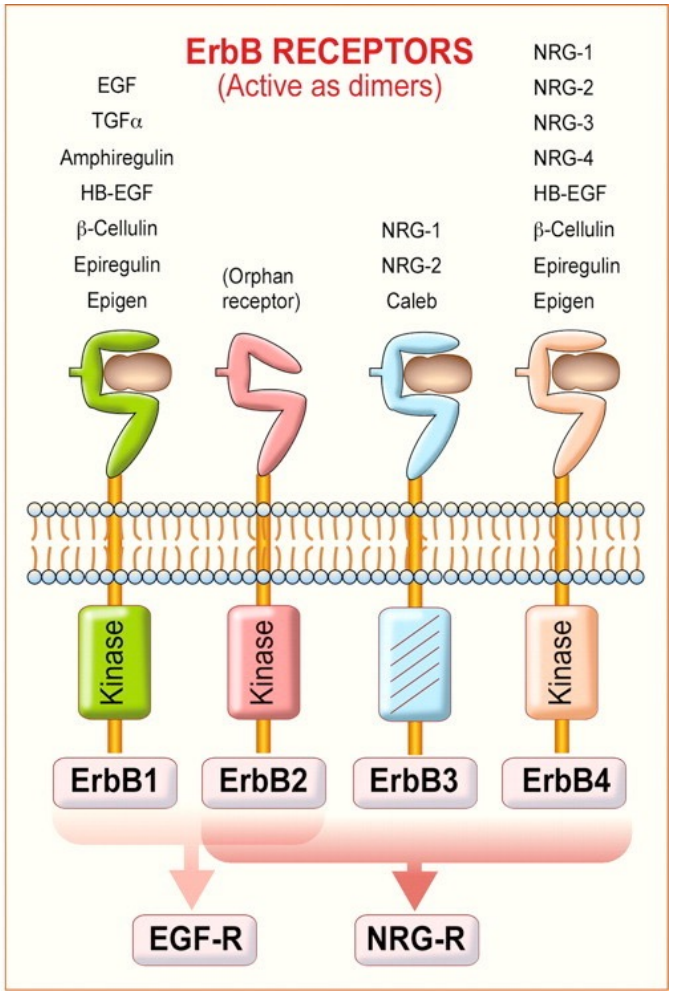


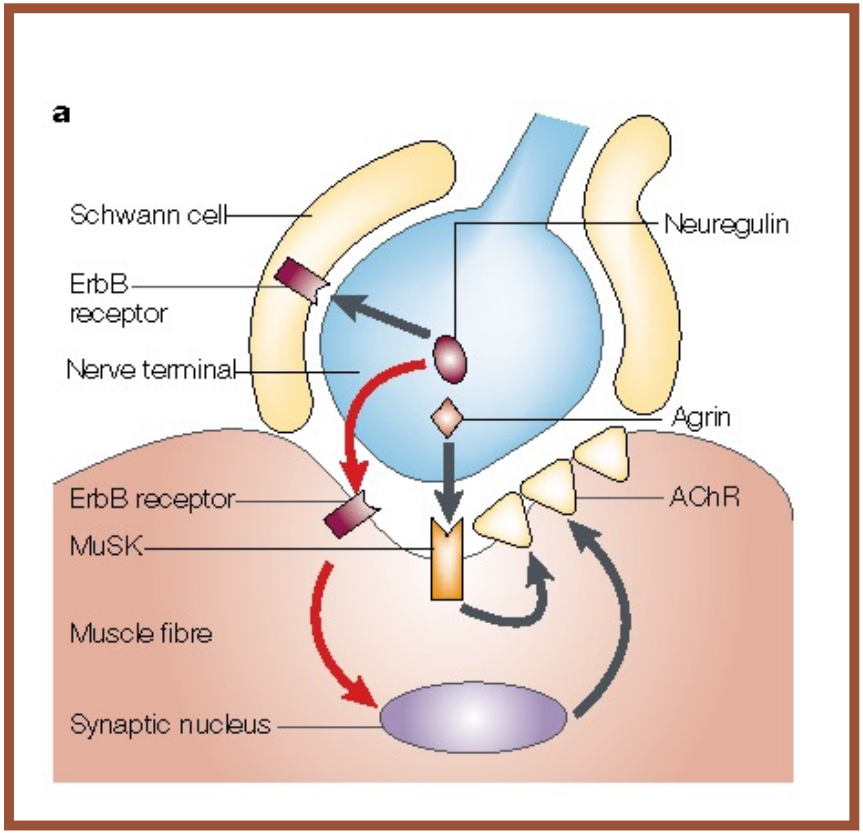
Figure 2. nAChR–rapsyn binding sites. (**A**) Structural studies on Torpedo synaptic membranes showed that each nAChR is associated with 1-3 rapsyn molecules, which bind at two homologous sites on adjacent subunits (site I and II), and a non-homologous site on the opposing face of the receptor (site III) [80]. (**B**) Complementary studies using subunit loop chimeric proteins identified rapsyn binding sites on the MA-helices and a phosphorylation-dependent binding site on the conserved βY390 motif. (**C**) These findings suggest a revised model where rapsyn localizes the nAChR in the postsynaptic membrane via multiple, regulated interactions. Rapsyn associates with the nAChR in the Golgi apparatus (i) and they exist as pre-formed complexes on the PM. Agrin-MuSK signaling induces their co-clustering via rapsyn dimerization (ii) and by binding of an additional rapsyn molecule to the phosphorylated βY390 motif (iii). Recruitment of additional rapsyn may require a chaperone protein (not shown). These two mechanisms create rapsyn bridges between nAChRs and also anchor receptors to transmembrane and cytoskeletal scaffolding proteins. Thus, the stoichiometry of rapsyn/nAChR complexes is an important determinant of the density and stability of nAChRs in the postsynaptic membrane.

Ferns M (2021). Molecules 26:3065

Neuregulin

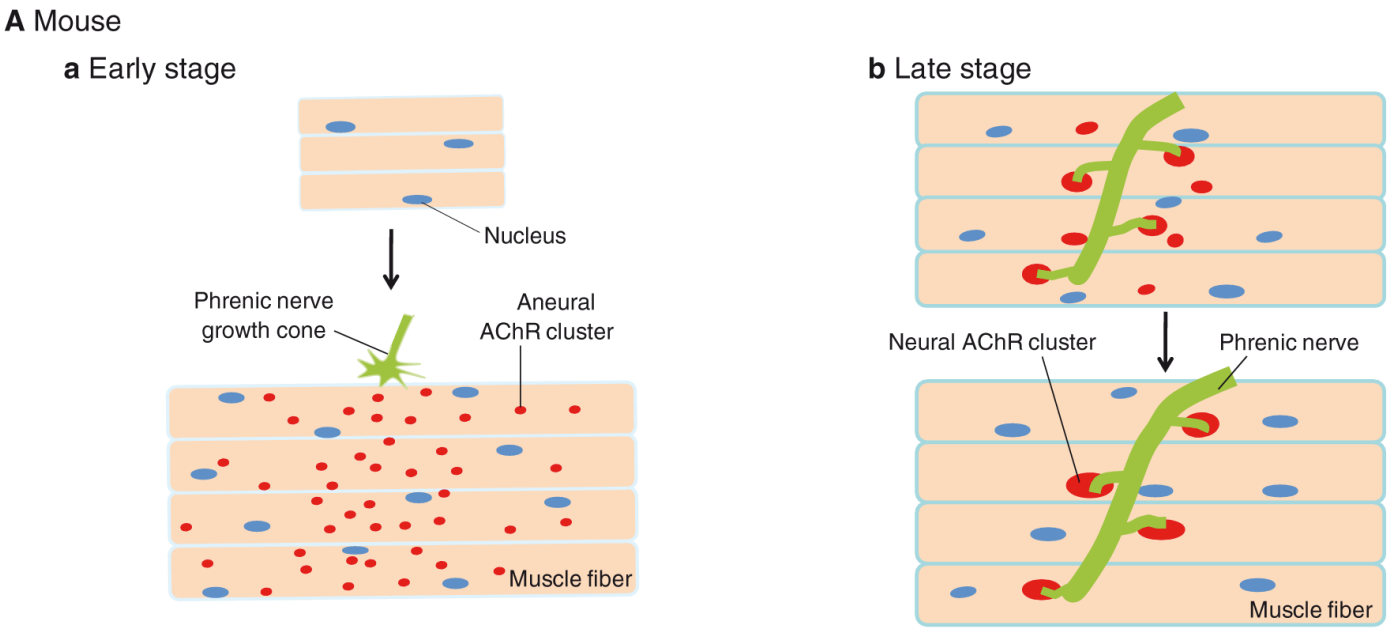

NEUREGULIN STRUCTURE
N-terminal domains (Types I - VI)
EGF-like domain α,β,γ
Linker 1/2/3/4
Transmembrane
Intracellular domains (a / b / c)
Type - specific N-terminal region
Cysteine - rich
Kringle
Cleavage site
IgG - like
Glycosylation
EGF - like
Type I
Type II
Type III
Type IV
Type V
Type VI
NRG-1
NRG-2
NRG-3
NRG-4


ErbB RECEPTORS
(Active as dimers)
EGF
TGFα
Amphiregulin
HB-EGF
β-Cellulin
Epiregulin
Epigen
(Orphan receptor)
NRG-1
NRG-2
Caleb
NRG-1
NRG-2
NRG-3
NRG-4
HB-EGF
β-Cellulin
Epiregulin
Epigen
Kinase
Kinase
Kinase
ErbB1
ErbB2
ErbB3
ErbB4
EGF-R
NRG-R

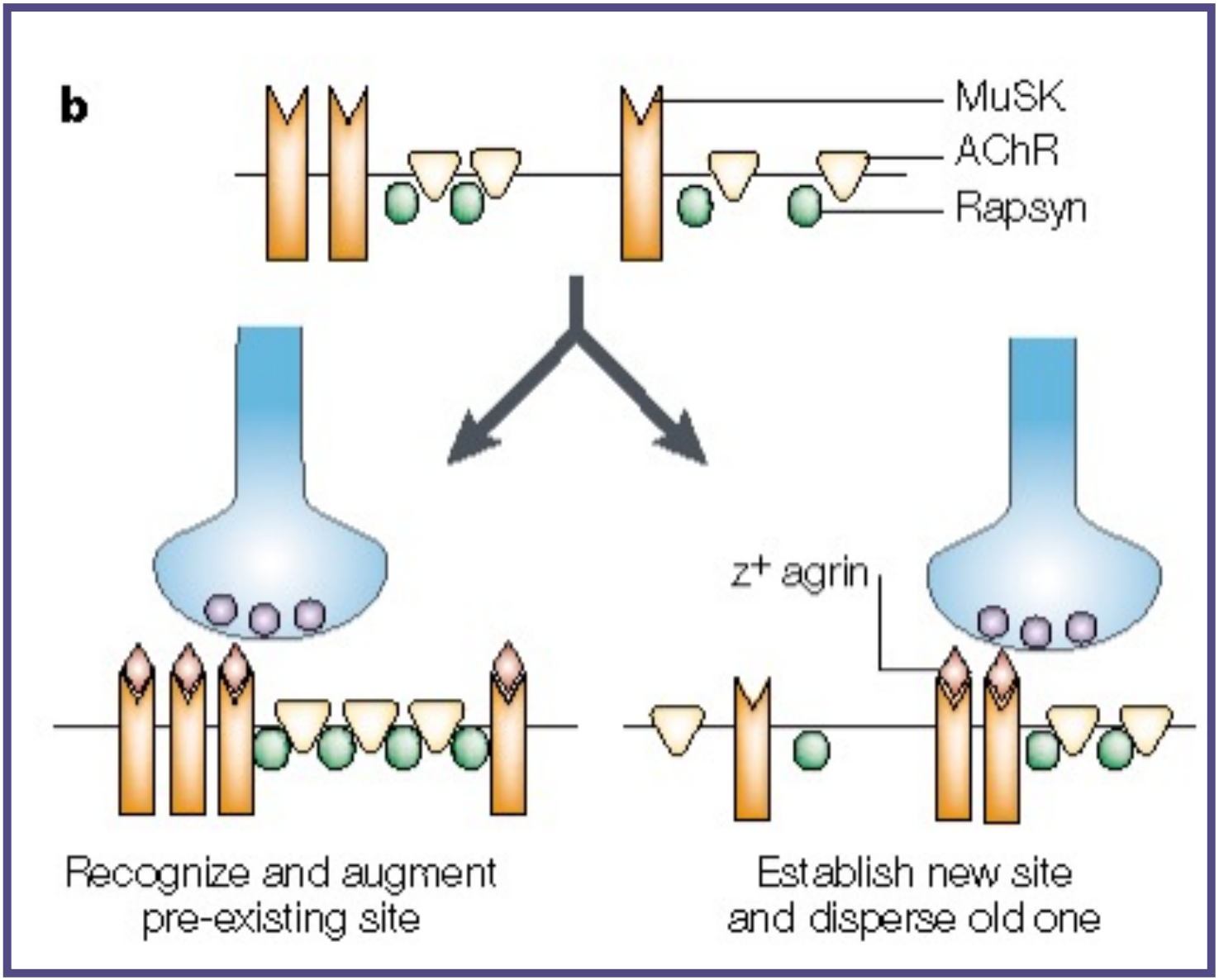


Neuregulin1 is a growth factor that acts in parallel with agrin (the secreted isoform is named ARIA: AChR-inducing activity). It binds ErbB receptors (tyrosine kinases) expressed by postsynaptic muscle membrane and Schwann cells.

Neuromuscular synapse formation in vertebrates



(A) In mice, muscle fibers of the diaphragm form primitive, aneural AChR clusters prior to the arrival of phrenic nerve terminals. The clusters are distributed in a broad, poorly defined region in the middle of muscle fibers, a phenomenon called prepatterning (a). Innervation leads to the appearance of large AChR clusters in the synaptic region and to the disappearance of primitive clusters in non-synaptic areas (b).



“Myocentric model”

“Neurocentric model”

Postsynaptic differentiation without nerve

1. nAChR aggregates are present in aneural muscle at the central endplate band.
2. Postsynaptic site are transiently present in agrin mutant embryos.
3. Nuclei associated with nAChR clusters are becoming transcriptionally specialize in aneural muscles.
4. Neuregulin mutants showed mild postsynaptic defect.

Summarizing:

- early aspects of synaptogenesis are nerve activity-independent
- the synaptic maintenance, stabilization and maturation are nerve activity-dependent

Maturation of the NMJ (after birth)

There are 5 sets of alterations involving AChRs:

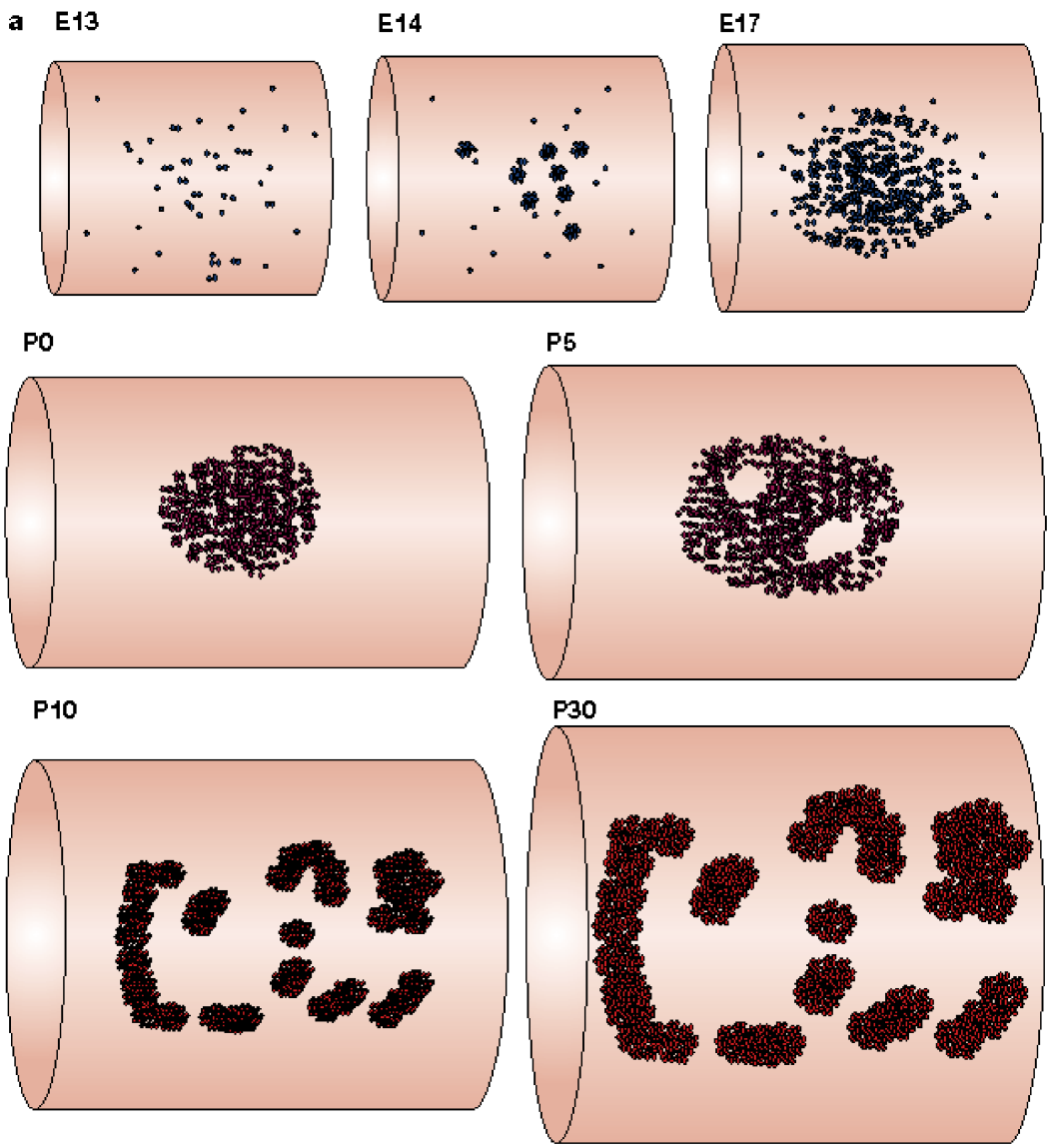
1. Shape: from oval plaque to a pretzel-like set of branches

2. Topography: from flat to invaginated surface

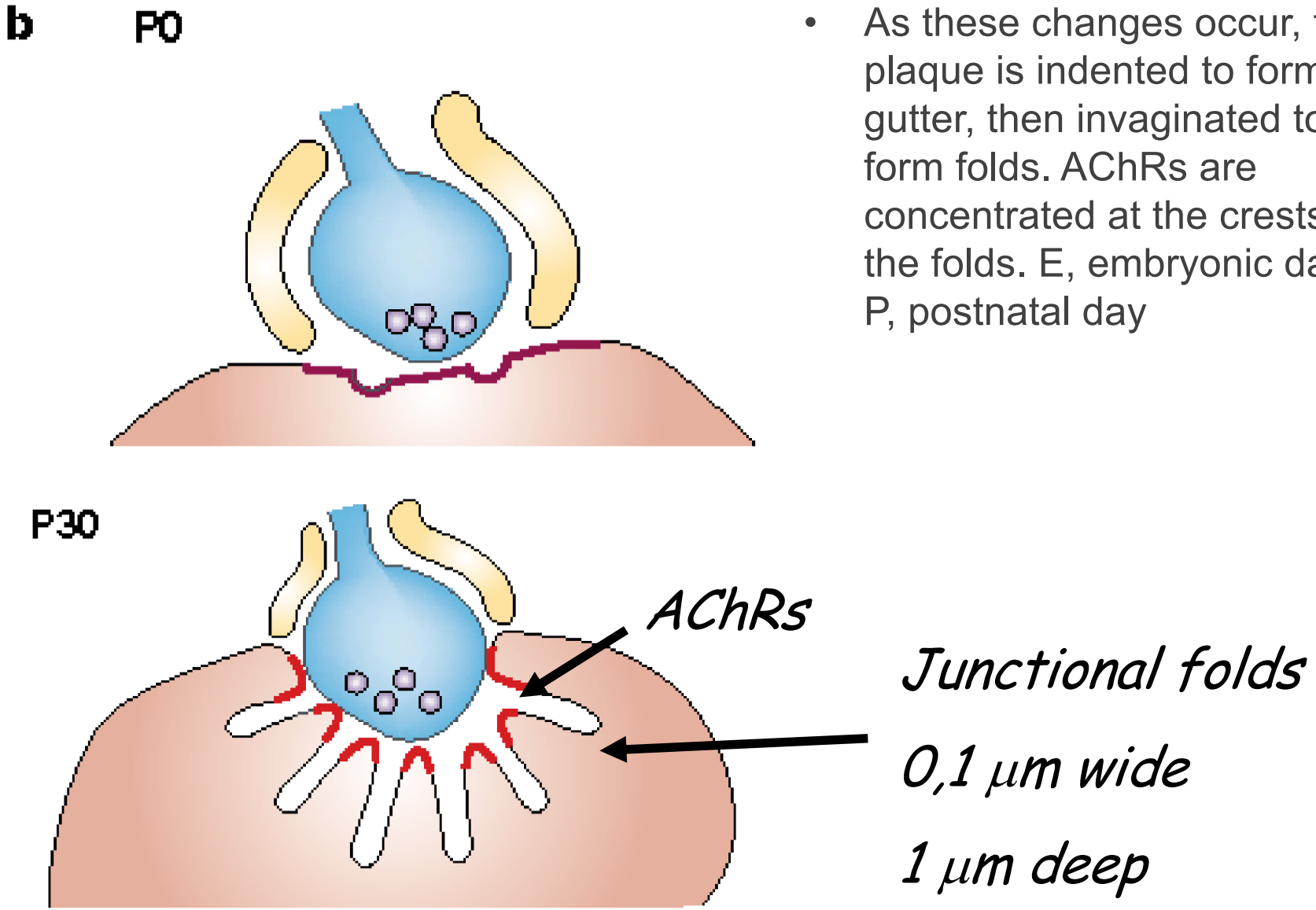
3. The extracellular matrix and cytoskeletal constituents: the composition of basal lamina cytoskeletal apparatus changes quantitatively and qualitatively

4. Channel function: a shift in nAChR subunit composition leads to a change in its Ca^{2+} permeability

5. Ion and ligand-gated channels segregate into discrete alternating domains



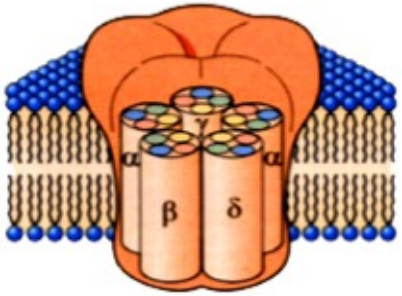
Maturation of the postsynaptic apparatus. a | Acetylcholine receptor (AChR) microclusters coalesce to form a loose aggregate. Late in embryogenesis, the aggregate consolidates to form a plaque: its borders sharpen, its length decreases, and AChR density increases. Postnatally, the plaque becomes perforated to eventually form a pretzel-like array of branches. The branches then expand in an intercalary fashion as the muscle grows. Change in AChR colour denotes the switch from γ- to ε-containing AChRs.

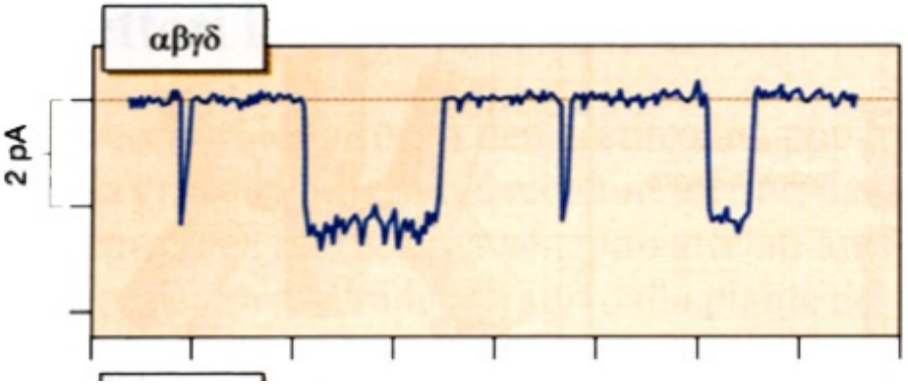


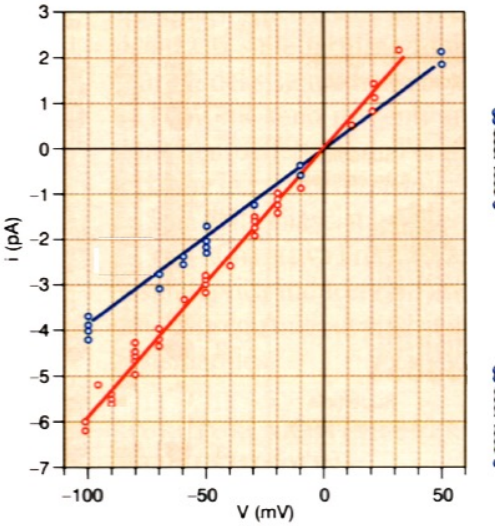
- As these changes occur, the plaque is indented to form a gutter, then invaginated to form folds. AChRs are concentrated at the crests of the folds. E, embryonic day; P, postnatal day

nAChRs grow up

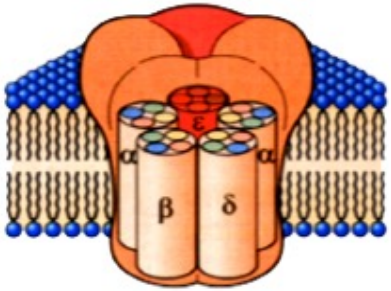
Fetal/Embryonic isoform: γ sunbunit

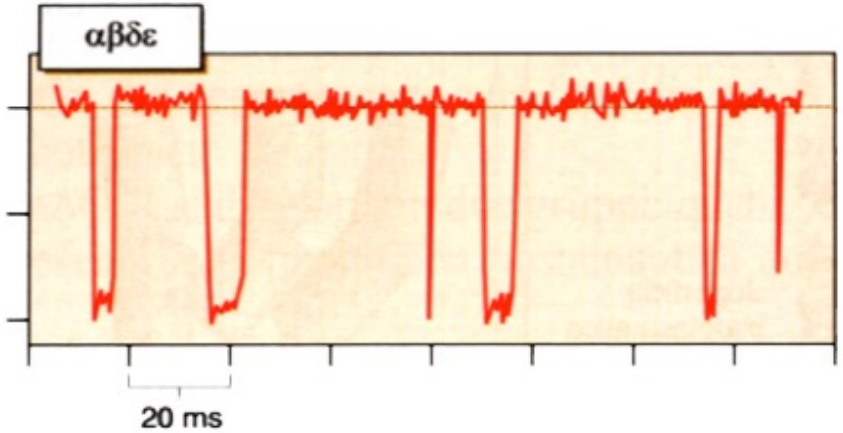






Adult isoform: ε subunit





The γ -subunit is required for the proper maturation of the neuromuscular synapse; in the adult γ -containing nAChRs are found after denervation and in some congenital myasthenic syndromes and myogenic disorders.

The embryonic receptor has higher agonist affinity, smaller single-channel conductance, longer open-channel lifetime, lower Ca^{2+} permeability and lower probability of opening constitutively than the adult ε –containing nAChR.

The structure of the nAChR in skeletal muscle

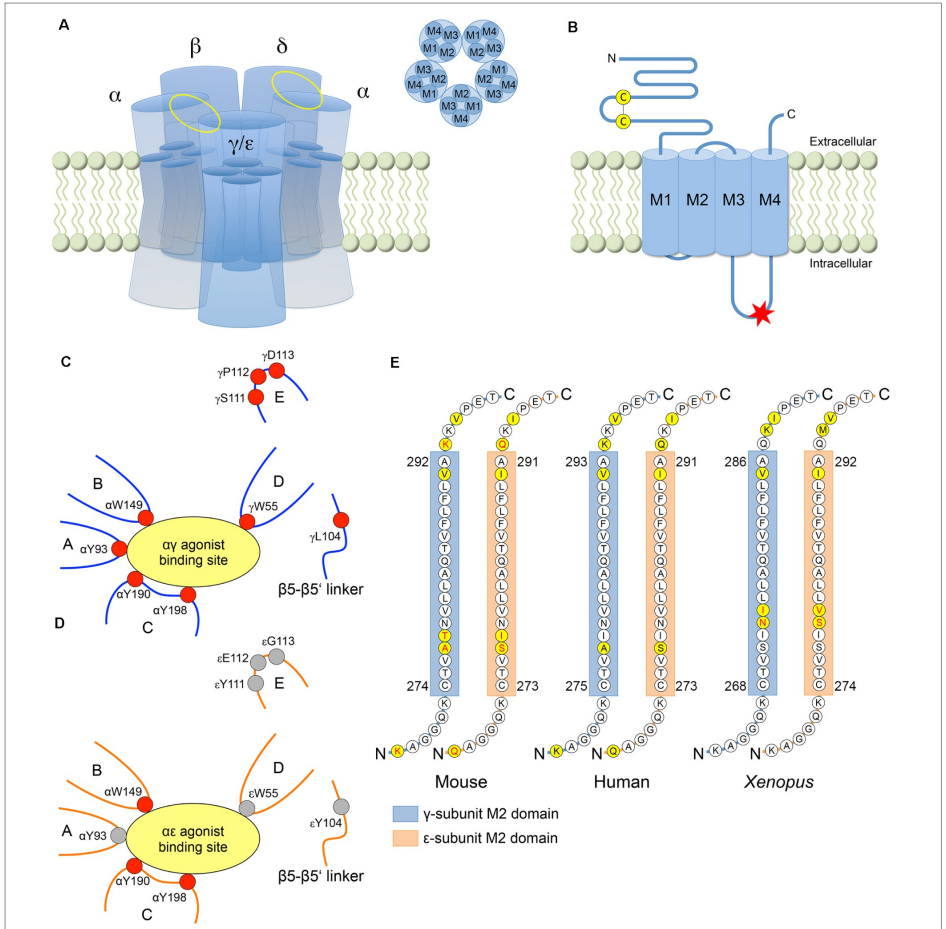
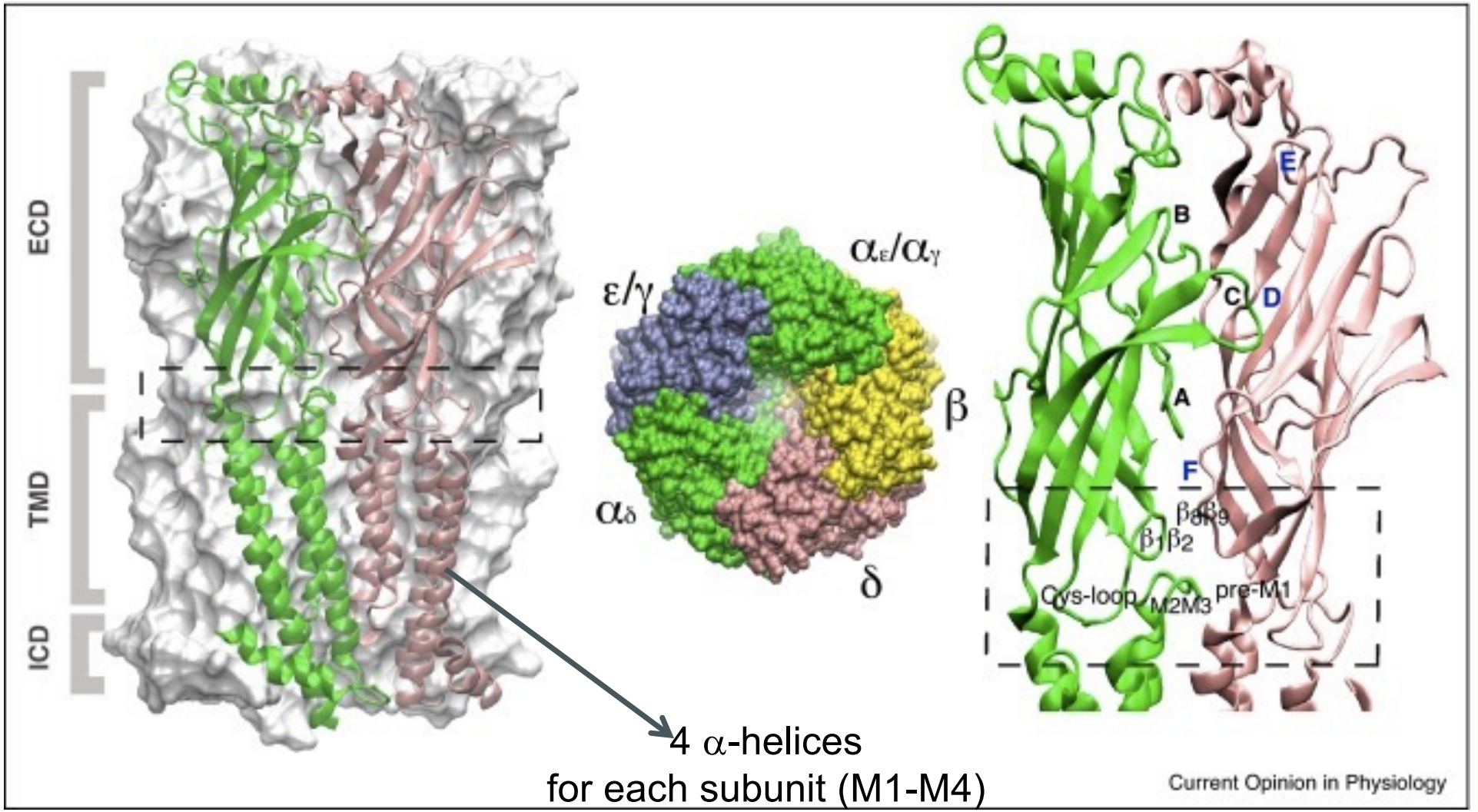


FIGURE 1 | Structural differences between the γ- and ε-subunit. **(A)** AChR consisting of five subunits with the fetal specific γ-subunit or the adult specific ε-subunit. Each subunit has four helical transmembrane domains M1-M4. The αγ/αε and αδ agonist binding sites are located between the corresponding subunits (yellow ellipses). **(B)** AChR subunit with N-terminal extracellular domain containing the Cys-loop, four transmembrane domains and a cytoplasmic loop domain between M3 and M4. In human, a consensus sequence for phosphorylation by protein kinase A (RRXSX, where X is any amino acid) is only present in the M3-M4 loop of the ε-subunit (red asterisk) without any corresponding sequence in the γ-subunit (residues 370–374 in the ε-subunit, i.e., RRASS). **(C)** At the αγ binding site, the favorable free energy resulting from the change of ACh affinities upon transformation of the AChR from the resting into the open-channel state is provided by the five aromatic core residues αY93, αW149, αY190, αY198, and γW55 from discontinuous sections of the primary sequence that rearrange close to the binding site upon agonist binding. The relative conformation of the aromatic core is stabilized by four other residues (γL104, γS111, γP112, and γD113) increasing the affinity of the αγ binding site to the agonist. **(D)** At the αε binding site, acetylcholine binding energy is provided by only three (instead of five) aromatic core residues (αW149, αY190, and αY198), resulting in a lower affinity to the agonist (with residues contributing to acetylcholine binding energy in red). **(E)** The majority of the amino acid residues of the pore forming M2 domain is highly conserved across paralogs and species. The γ- and ε-subunits differ at only three amino acid residues in mouse and *Xenopus*, and at two residues in human (yellow circles). The AChR isoform specific conductance was shown to be determined by amino acid residues within the M2 domain (in mouse and *Xenopus*) and both flanking regions (in mouse) (amino acids in red).

frontiers in Molecular Neuroscience

*Hakan Cetin[1,2], David Beeson[2], Angela Vincent[2] and Richard Webster[2]**

REVIEW
published: 08 September 2020
doi: 10.3389/fnmol.2020.581097



nAChR structure. (Left) Side view of the model nAChR, corresponding to the structure of the a4b2 nAChR (PDB code: 5KXI) [94]. Two adjacent subunits are shown in color. The three main domains, extracellular (ECD), transmembrane (TMD) and intracellular (ICD), are marked. Most of the ICD is not shown since it was removed to obtain well-diffracting crystals [99]. The square shows the location of the coupling region. (Center View of the nAChR from the top. The disposition of muscle nAChR subunits is shown. (Right) Side view of the extracellular domain and coupling region. The principal face of the binding site is formed by loops A, B and C from the a-subunit (shown in green), and the complementary face is formed by loops D, E and F of e/g or d subunits. Main loops of the coupling region include b1b2, Cys-loop (b6b7) and b8b9 loops, and terminus of b10 strand from the ECD and the pre-M1 and M2M3 linker from the TMD.

Bouzat & Mukhtasimova, 2018

Fetal *vs* Adult isoform

TABLE 1 | Functional differences between the fetal and adult AChR.

	Fetal AChR	Adult AChR	References	Species
Subunits	$\alpha_2\beta\delta\gamma$	$\alpha_2\beta\delta\varepsilon$		
Time of occurrence	Up to 2nd postnatal week	Thereafter	Sakmann and Brenner, 1978	Rat
	Up to 31st prenatal week, with low-level expression from sub- and perisynaptic nuclei thereafter in human	Thereafter	Hesselmans et al., 1993	Human
Location in adult muscle	Extrajunctional and junctional	Junctional	Berg et al., 1972	Rat
			Brenner et al., 1990	Rat
			Gu and Hall, 1988	Rat
Expression in extraocular muscles	En grappe endplates	En plaque endplates	Fraterman et al., 2006	Rat
	En grappe endplates	En plaque endplates	Missias et al., 1996	Mouse
	En grappe/en plaque endplates	En grappe/en plaque endplates	Kaminski et al., 1996	Rat
Conductance	40 pS	60 pS	Mishina et al., 1986	Calf
	53 pS	75 pS	Bouzat et al., 1994	Mouse
Open time	7.2 ms	2.3 ms	Mishina et al., 1986	Calf
	5.7 ms	1.6 ms	Kopta and Steinbach, 1994	Mouse
	7.9 ms	4.1 ms	Newland et al., 1995	Human
Ca^{2+} permeability	Lower	Higher	Villarroel and Sakmann, 1996	Rat
			Fucile et al., 2006	Human
			Ragozzino et al., 1998	Mouse
Resting affinity to ACh, k_D	0.75 nM	22.11 nM	Nayak et al., 2014)	Mouse
Resting affinity to choline k_D	1.29 μM	27.30 μM	Nayak et al., 2014	Mouse
Affinity to ^{125}I-α-bungarotoxin, k_D	0.04 nM	0.10 nM	Vincent et al., 1998	Human

frontiers in Molecular Neuroscience

*Hakan Cetin[1,2], David Beeson[2], Angela Vincent[2] and Richard Webster[2]**

REVIEW
published: 08 September 2020
doi: 10.3389/fnmol.2020.581097

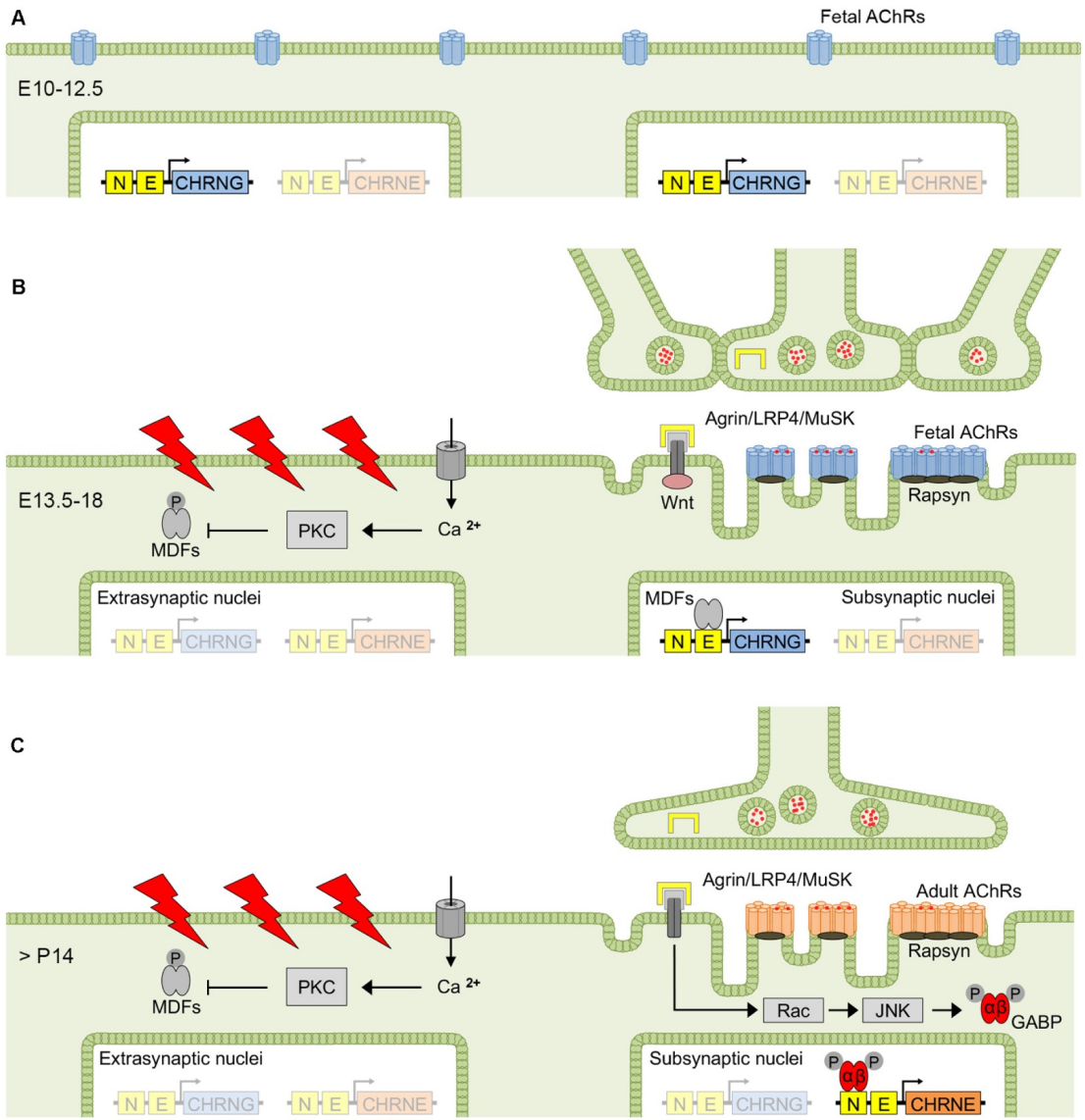


FIGURE 2 | Factors determining fetal and adult AChR expression at the NMJ. Before innervation, fetal AChRs are evenly distributed at the surface of mouse myotubes **(A)**, but aggregate to clusters at the myotube center between E13.5 and E18 (i.e., prepatterning), when myotubes start to become innervated **(B)**. The exact mechanism of prepatterning is unclear but was suggested to be nerve-independent and require MuSK, LRP4 and Wnt signaling. The first 2 weeks after birth are dominated by synapse elimination resulting in singly innervated muscle fibers, and electrical activation and nerve-released agrin determine the conversion from fetal to adult AChRs, with the latter predominantly expressed in the adult muscle **(C)**. AChR, acetylcholine receptor; GABP, GA-binding protein; JNK, c-Jun NH_2-terminal kinase; MDF, myogenic determination factor; MuSK, muscle-specific kinase; LRP4, low-density lipoprotein related protein 4; PKC, protein kinase C.

*Hakan Cetin[1,2], David Beeson[2], Angela Vincent[2] and Richard Webster[2]**

REVIEW
published: 08 September 2020
doi: 10.3389/fnmol.2020.581097

nAChRs on the move:

the tournover for adult is up to 14 days while for fetal only 1 day. The nAChR stability at the endplate depends on synaptic activity.

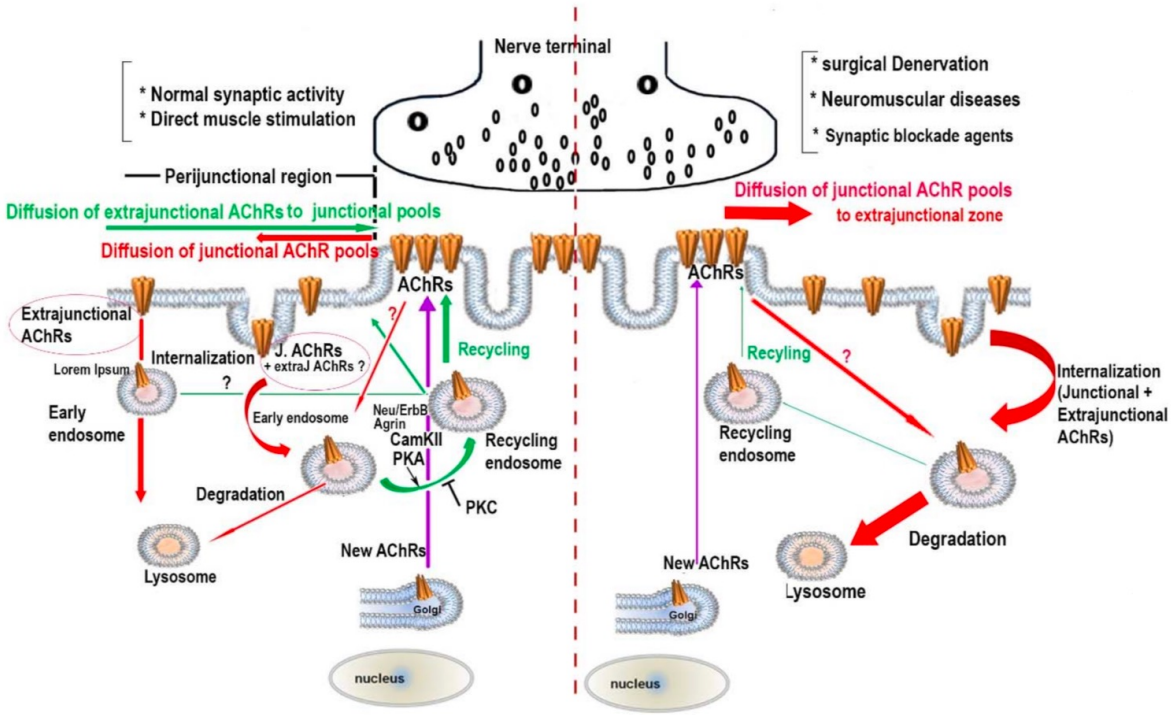


Figure 1. The metabolic stability of nAChRs at the functional and impaired peripheral cholinergic neuromuscular junction. A schematic diagram shows possible ways by which AChR pools (junctional and extrajunctional) are removed from and inserted into the neuromuscular junction under normal and pathological situations. Possible signaling molecules that mediate the recycling of AChRs are also represented.

Anatomy of the neuromuscular junction

- presynaptic nerve terminal that is capped by a terminal Schwann cell;
- synaptic basal lamina that occupies the synaptic cleft;
- the specialized postsynaptic membrane of the muscle.

Perisynaptic (or Terminal) Schwann cells

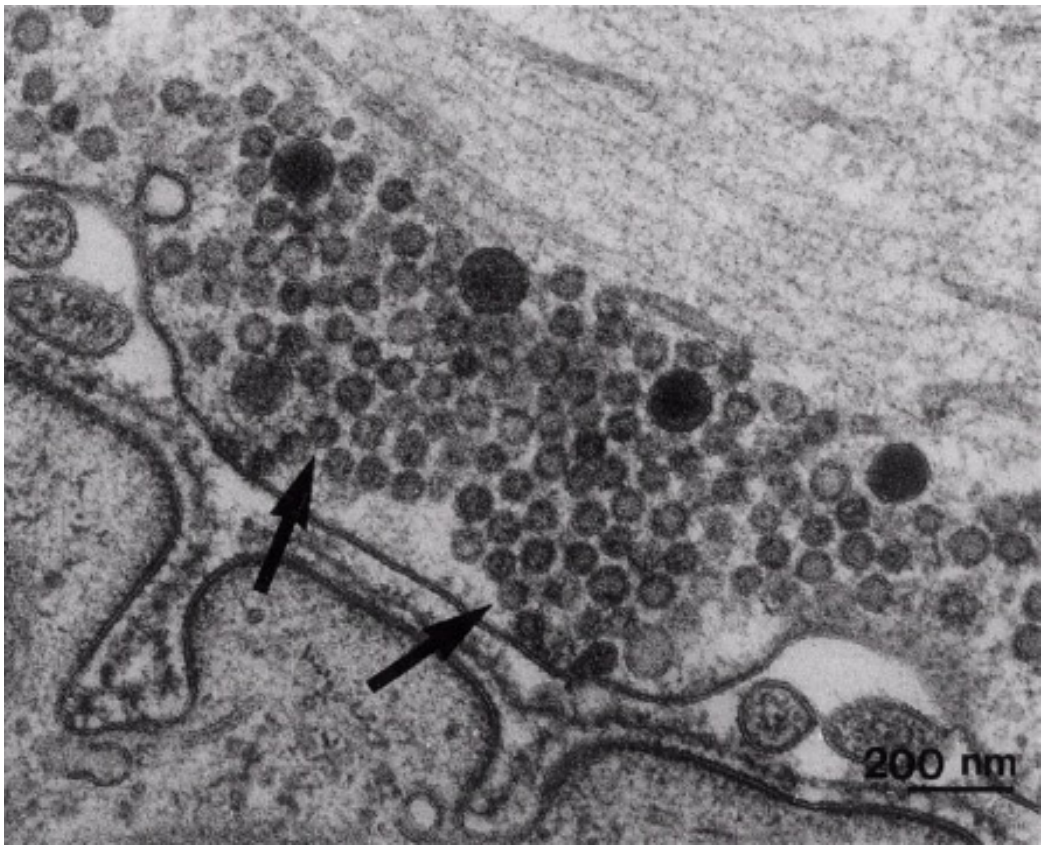
They are involved in:

- modulation of synaptic transmission
- nerve terminal growth and maintenance
- axonal sprouting
- nerve regeneration

They sense neuronal activity by a intracellular calcium increase

Presynaptic region

- Synaptic vesicles
- Active zone
- Calcium channels
  (P/Q type)



Stabilization of the post-synaptic elements

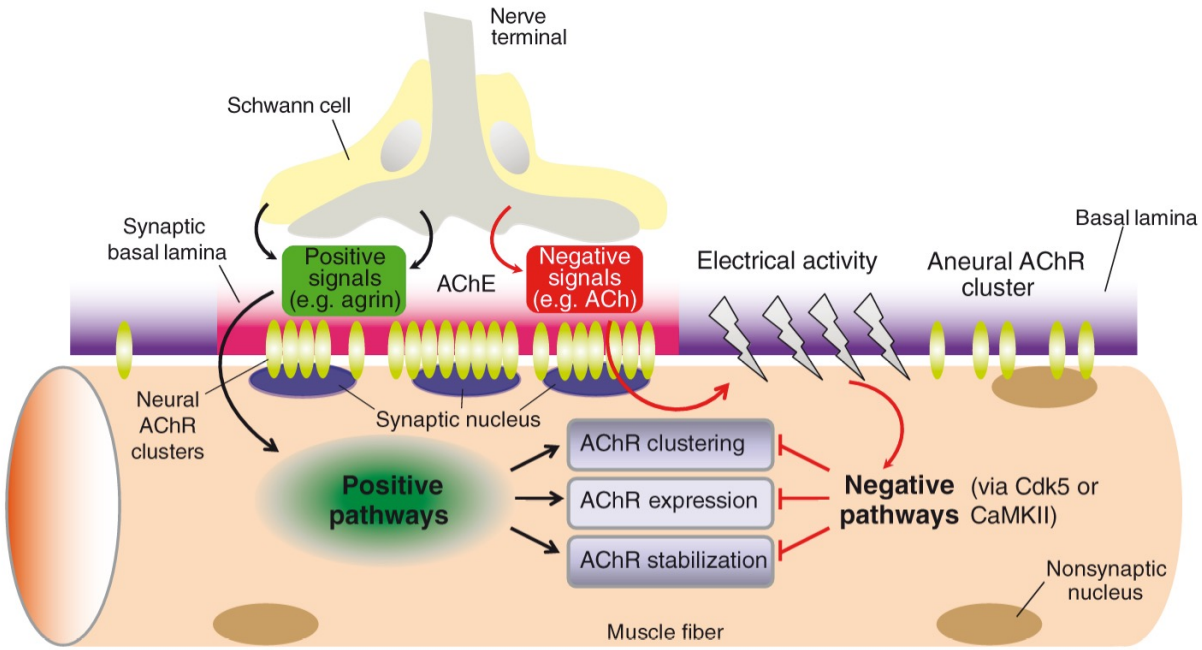


Fig. 2. Coordinated action of positive and negative signals in NMJ assembly. At least three cellular mechanisms contribute to the high density of AChRs at the NMJ. First, AChR might redistribute from primitive clusters to the synaptic area, either by lateral movement, by diffusion in the plasma membrane or by endo- and exocytosis. Second, muscle fibers are multi-nucleated cells, and only the nuclei beneath the postsynaptic membrane (synaptic nuclei) are actively transcribing the AChR subunit genes, contributing to synapse-specific AChR expression. Third, AChR turnover rate is reduced at mature NMJ or when clustered (as shown by the half-life of AChRs at the NMJ at 8-14 days compared with 17-24 hours for non-clustered or embryonic AChR). Motor nerves activate muscle fibers by releasing ACh, a negative signal, which activates AChR. Muscle activation stimulates the serine/threonine kinases cyclin-dependent kinase 5 (Cdk5) and Ca^{2+}/calmodulin-dependent kinase II (CaMKII) to inhibit AChR clustering, to suppress AChR expression and to destabilize AChR clusters in entire muscle fibers. At the same time, nerves also release positive signals, such as agrin, which counteract the effects of negative signals, resulting in a high AChR concentration at the NMJ.

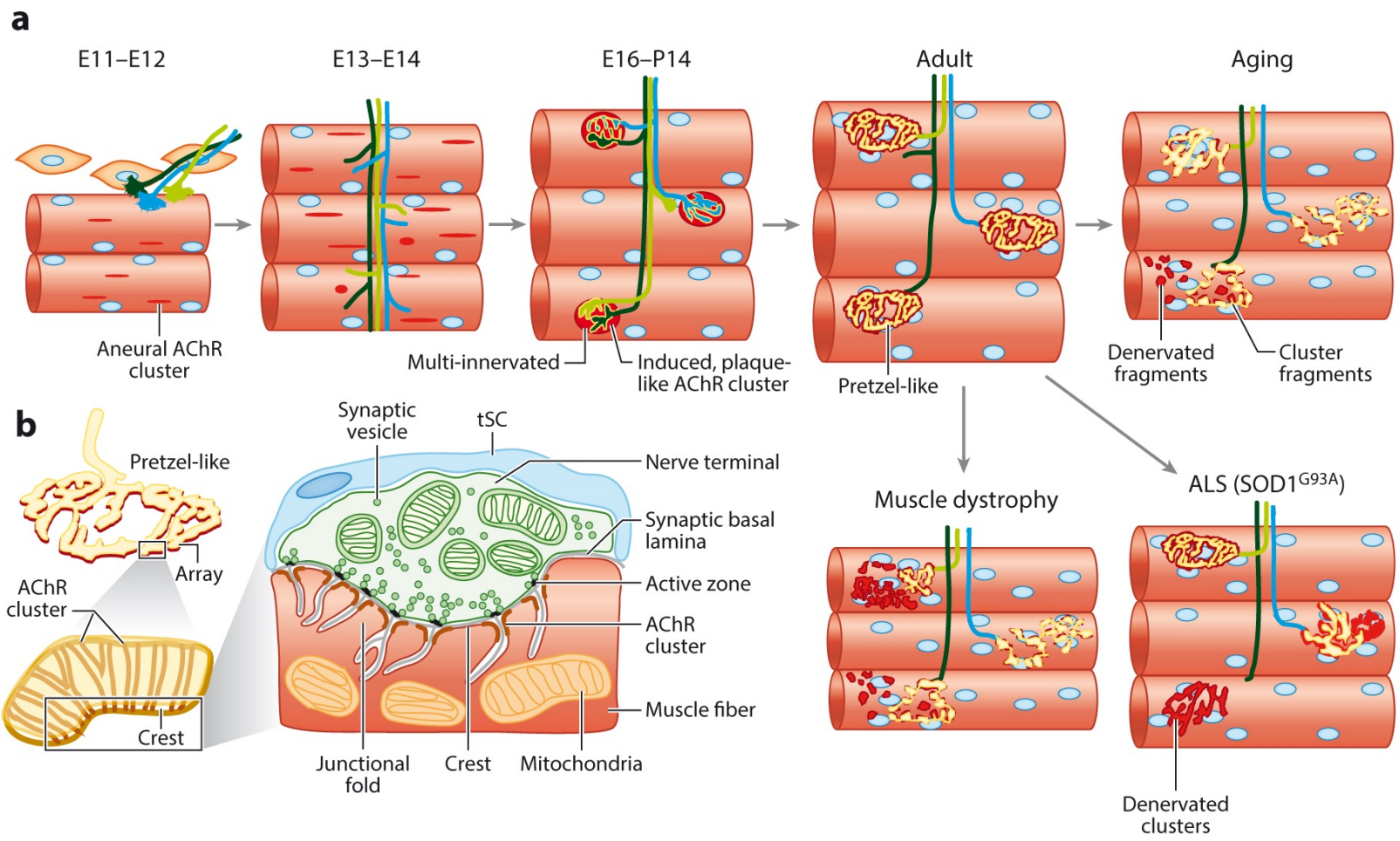


Figure 1

NMJ development in mice. (*a*) Prior to the arrival of nerve terminals, myotubes form primitive, small, thin AChR clusters that are distributed in a broad middle region (axons E11–E12; E13–E14). Nerve-induced clusters are initially oval plaques, often innervated by multiple axons (E16–P14). As NMJs mature, AChR clusters become perforated and complex, resembling pretzels with arrays or branches that are innervated by one axon per NMJ (adult). AChR clusters become fragmented and denervated in aged mice and in muscular dystrophic mice. In $SOD1^{G93A}$ mice, some AChR clusters are denervated. (*b*) NMJ structures at different magnifications. Abbreviations: AChR, acetylcholine receptor; ALS, amyotrophic lateral sclerosis; NMJ, neuromuscular junction; tSC, terminal Schwann cell.