

Perspective

Determinants Responsible for Assembly of the Nicotinic Acetylcholine Receptor

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The assembly of the four homologous, but distinct, α , β , γ , and δ subunits of the nicotinic acetylcholine receptor (nAChR) into the pentamer $\alpha_2\beta\gamma\delta$ presents a unique opportunity to delineate the individual amino acid side chains that contribute to the assembly process, and to examine the pathway responsible for subunit assembly and expression at the cell surface. It is well established that subunits assemble into the circular order of $\alpha\gamma\alpha\delta\beta$, where the γ subunit resides between the two α subunits and two binding sites are found at the $\alpha\gamma$ and $\alpha\delta$ interfaces (for detailed reviews, see Karlin and Akabas, 1995; Hucho et al., 1996). Chirality of the order of the subunits has also been proposed on the basis of cross-linking of toxins with known structures to the receptor (Machold et al., 1995; Utkin et al., 1997). The subunits are glycoproteins composed of ~ 450 – 520 amino acids that traverse the membrane four times (Karlin and Akabas, 1995); the extracellular domain is formed from the amino terminal 210 residues (Chavez and Hall, 1991; Fig. 1). Sequence elements in this domain specify both ligand recognition and the arrangement of subunits (Blount and Merlie, 1989; Sine and Claudio, 1991; Yu and Hall, 1991; Verrall and Hall, 1992; Kreienkamp et al., 1995). An extended cytoplasmic loop after the third transmembrane domain contains numerous lysine residues (Boulter et al., 1990) that may also encode signals for the stability and trafficking of the subunits.

A structural feature common to ligand-gated ion channels is the presence of multiple membrane spans extending to large extracellular and cytoplasmic domains. This separates the multisubunit ion channels from the well-studied receptors of the immune system, such as T cell-receptor subunits that traverse the membrane once. Owing to amino acid sequence similarity, hetero-oligomeric subunit composition, and the conserved positioning of disulfide loops and glycosylation sites, assembly and expression pathways should be shared by the family of ligand-gated ion channels. Therefore, the overall characteristics of assembly and expression identified for acetylcholine receptor biogenesis should be applicable to the other less well studied ligand-gated ion channels.

What is the pathway by which the subunits of the nAChR assemble in the endoplasmic reticulum (ER) and become expressed at the cell surface? Four major features in processing of the subunits appear to direct expression of the ion channel. First, subunits are inserted in the endoplasmic reticulum membrane and undergo concurrent folding transitions and other post-translational modifications throughout the assembly process. Second, unassembled subunits are susceptible to rapid degradation (Claudio et al., 1989; Blount and Merlie, 1990), and association with chaperones and assembly with neighboring subunits both enhance the stability of the emerging complex (Keller et al., 1996, 1998). Third, the subunits are ordered into the pentameric structure to compose the subunit arrangement of $\alpha\text{-}\gamma\text{-}\alpha\text{-}\beta\text{-}\delta$ (see Hucho et al., 1996); specific amino acid residues in the NH_2 -terminal domain dictate the

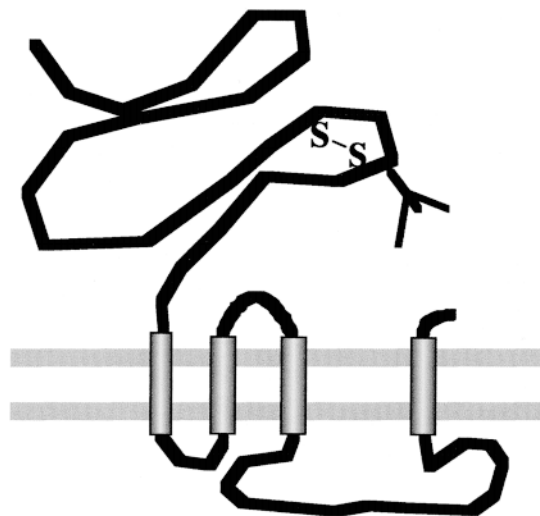


FIGURE 1. Subunit topology of the homologous acetylcholine receptor subunits. The NH_2 -terminal extracellular domain constitutes approximately half of the sequence. An extracellular disulfide loop (α Cys128-142) and glycosylation site (α Asn141) are conserved throughout the family of ligand-gated ion channels, which include the nicotinic acetylcholine, the γ -aminobutyric acid, the glycine, and the 5-hydroxytryptamine-3 receptors.

assembly order and the insertion of subunits into the circular arrangement (Gu et al., 1991; Kreienkamp et al., 1995). Fourth, unassembled subunits and assembled intermediates of the pentameric receptor are retained in the endoplasmic reticulum; assembly of the pentamer into its circular arrangement is a requirement for export of the subunits to the Golgi, and then to the cell surface (Gu et al., 1991).

Amino Acids at the Interfaces between Subunits Direct the Assembly Pathway

As part of a larger investigation into the amino acid determinants governing ligand specificity and structure, we have employed subunit transfection into null cells not expressing the receptor to examine subunit assembly and formation of the ligand recognition sites. This approach was initially employed by Blount and Merlie (1989) to examine the basis of assembly and the non-equivalence of the two binding sites (Sine and Taylor, 1981) on the mouse receptor. Subsequent studies by Yu and Hall (1991), Verrall and Hall (1992), and Gu et al. (1991) have shown that the extracellular domain of the subunits controls the specificity of subunit oligomerization. More recent studies by Wang et al. (1996a,b) have shown that the first transmembrane domain is required to orient the subunits to assemble. These groups were able to identify specific partnering of the subunits to form oligomers and deduce a likely sequence for the assembly of the transfected subunits.

Previous studies have demonstrated that each of the homologous subunits is encoded by a separate gene (see Changeux, 1991). A signal peptide of 20 amino acids at the NH₂ terminus directs insertion into the endoplasmic reticulum (ER) such that all subunits are initially embedded and localized to the ER membrane (Anderson and Blobel, 1981). The newly synthesized subunits appear to undergo a folding transition before assembly, which appears to involve disulfide bond formation (Gelman and Prives, 1996; Fu and Sine, 1996; Green and Wanamaker, 1997). Folding before assembly may be required to expose the appropriate amino acids for subunit contact, as suggested by the extended 2-h lag period required to detect the assembled pentamer of subunits (Merlie and Lindstrom, 1983). It can be assumed that high affinity interactions between appropriate subunit interfaces occur when subunits contact, forming the assembled intermediates in biogenesis. The subunit intermediates continue to fold and other subunits are added to the newly exposed faces of the emerging oligomer. Eventually, all subunits are inserted and assembled into the enclosed pentameric arrangement. Chaperone proteins may assist in the assembly process of the receptor by stabilizing the intermediates and/or promoting folding and assembly.

Amino acid residues positioned at homologous sites in the subunits appear to direct the partnering during assembly (Kreienkamp et al., 1995; Sugiyama et al., 1996). When cells are transfected with cDNA encoding only α and γ subunits, monomers, $\alpha\gamma$ dimers, and $\alpha\gamma\alpha\gamma$ tetramers are observed, as identified by density gradient sedimentation (Blount et al., 1990; Kreienkamp et al., 1995; see Fig. 2). The tetramer $\alpha\gamma\alpha\gamma$ is not a component of the mature receptor and is an outcome of transfection with only α and γ subunits in the absence of δ or β subunits. The δ subunit does not assemble between the two γ subunits and there is a low propensity to form the unique $\delta\alpha$ interface at the nonligand binding face of the two subunits. Thus, only dimers, but not tetramers, will form upon cotransfection of α and δ . By expressing chimeras of γ or δ subunits, along with α , a region in γ responsible for tetramer formation was identified. From site-specific mutagenesis, two lysines at positions 145 and 150, unique to δ and present as neutral residues in γ and ϵ , preclude δ from associating with the non-ligand-binding faces of the α subunit. Modification of residue 152 in the α subunit, a region homologous to 143–153 in γ influences the assembly of α with δ and α with γ subunits to form dimers that associate at the ligand-binding interface (Sugiyama et al., 1996). These findings suggest that homologous residues are positioned in the same coordinate space in each subunit and are likely to be in similar contact positions in the assembly process. Upon transfections of individual subunits, well resolved peaks in the density gradients enable one to identify the individual species from expression of respective cDNAs (Kreienkamp et al., 1995; Sugiyama et al., 1996).

Other studies with subunit chimeras between γ and ϵ subunits and with mutations in the β subunit have identified other regions responsible for assembly (Yu and Hall, 1994; Kreienkamp et al., 1995). Identification of residues involved in the assembly of subunits and binding of selective ligands, coupled with labeling by site-directed chemical and antigenic labels, has led to the refinement of a homology model of the structure of the extracellular domain of the receptor (Tsigelny et al., 1997).

By identifying which combinations of subunits form stable complexes in cells transfected to express oligomeric assemblies of the receptor subunits, Kreienkamp et al. (1995) and others (Blount and Merlie, 1989; Blount et al., 1990; Saedi et al., 1991; Gu et al., 1991) have proposed an assembly pathway illustrated in Fig. 2. Specific contacts between two subunits tethered to the ER membrane enable the subunits to form $\alpha\delta$ and $\alpha\gamma$ dimers, with $\alpha\delta$ being the more stable dimer (Sugiyama et al., 1996). The $\alpha\gamma$ dimer in turn associates with the β subunit and the $\alpha\delta$ dimer to form the ion channel pentamer. From this scheme, it is apparent

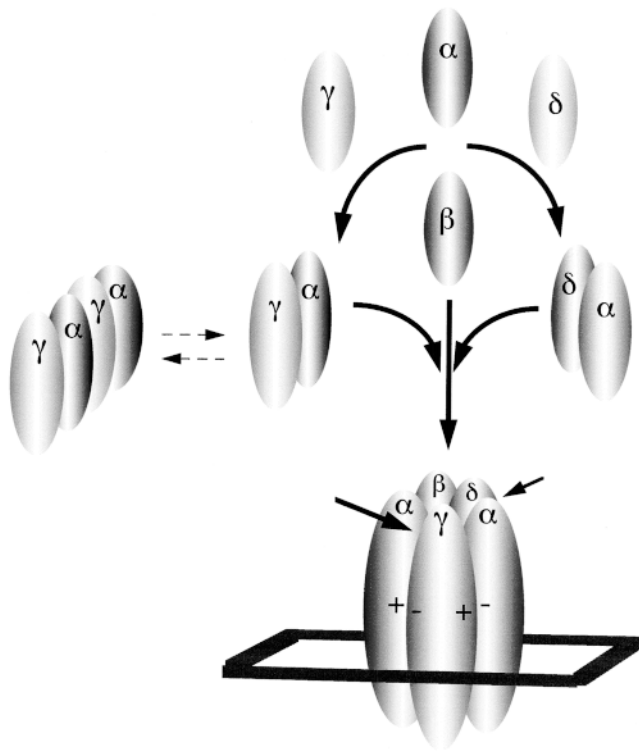


FIGURE 2. Proposed assembly pathway for nAChR, as deduced by identification of subunit intermediates after transfection. Arrows in the assembled pentamer point to the ligand recognition sites; (+) and (-) designate the counterclockwise and clockwise faces, respectively. The $\alpha\gamma\alpha$ tetramers only are detected in the absence of β and δ . The epsilon subunit is expressed in the innervated receptor in adult muscle and takes the place of the γ subunit in this scheme. The assembling subunits are associated with the ER membrane, transferred to the Golgi as a pentamer, and then to the cell surface (shown as the rectangle).

why a sequence in γ or ϵ that allows $\alpha\gamma\alpha$ or $\alpha\epsilon\alpha$ tetramer formation from dimers would be at a homologous position to residues in the α subunit that influences $\alpha\delta$ or $\alpha\gamma$ dimer formation.

An alternative pathway was proposed by Green and colleagues using stably incorporated *Torpedo* receptor subunits in mammalian cells to study the assembly steps (Green and Claudio, 1993; Green and Wanamaker, 1997, 1998). By following subunit incorporation with pulse labeling and coimmunoprecipitation, they propose a more complex assembly scheme. In this pathway, the first recognized intermediate in the assembly process is a rapidly forming $\alpha\beta\gamma$ trimer (Green and Claudio, 1993). Subsequently, a δ subunit and then an additional α subunit are added to the complex (Green and Claudio, 1993). The model proposed by Green is based primarily on the identification of subunit combinations in cells grown at 20°C to slow rates of the assembly process, although a similar assembly pathway for mouse $\alpha\beta\delta\epsilon$ subunits expressed at 37°C has been described (Green and Claudio, 1993). In addition to the

order of subunit assembly, another major distinction between the two schemes is that the δ subunit and the second α subunit insert between subunits into the emerging receptor complex (Green and Wanamaker, 1998). In contrast, in Fig. 2 it is assumed that subunits are added to exposed interfaces, and that the ion channel encloses as the last subunit joins the complex.

Why so different assembly pathways? First, dissimilar assembly schemes were deduced by transfecting and expressing various combinations of receptor subunits in separate batches of cells grown at 37°C and identifying the stable assembled intermediates to reconstruct steps in the assembly process, in comparison with expressing all subunits simultaneously at 20°C and following subunit incorporation with metabolic labeling and immunoprecipitation. Kinetically rapidly forming intermediates, such as the α - δ and α - γ dimers, may be undetectable by this method. Differences in temperatures employed to grow cells assembling the receptor and conditions for receptor solubilization may further contribute to discrepancies in detecting subunit intermediates such as the $\alpha\beta\delta\gamma$ tetramer. Second, the assembly pathway for *Torpedo* $\alpha\beta\delta\gamma$ (and mouse $\alpha\beta\delta\epsilon$) sequences employed by Green and Claudio (1993) and Green and Wanamaker (1996) may show some different assembly characteristics than the combination of mouse $\alpha\beta\delta\gamma$ sequences employed in our studies, because the amino acid sequences of the subunits appear to govern the order of assembly (Kreienkamp et al., 1995).

Subunit Stability, Processing and Degradation

Nascent subunit peptides residing in the ER are subject to posttranslational modifications, folding, assembly, and degradation. Although unassembled α subunits are rapidly degraded (Blount and Merlie, 1990; Claudio et al., 1989), our recent studies suggest that association with the chaperone protein, calnexin, substantially reduces the degradation (Keller et al., 1996, 1998). Our studies also reveal that the degradative route for unassembled subunits dissociated from calnexin is the ubiquitin-proteasome pathway (Keller et al., 1998).

Calnexin, as a transmembrane spanning protein, has the capacity to protect the threaded receptor subunits

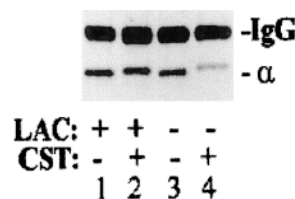


FIGURE 3. The proteasome-specific inhibitor lactacystin (LAC) inhibits degradation of the receptor α subunit expressed in the presence of castanospermine (CST). The mobility of α subunits expressed in CST is decreased due to the conjugation of the larger untrimmed oligosaccharide. These data reveal that altering oligosaccharide processing increases degradation of the receptor α subunit by the proteasome.

on the cytoplasmic, transmembrane, and luminal domains. It displays features of a lectin because it recognizes an oligosaccharide structure of one terminal glucose linked to mannose residues in the chain, an early structural intermediate in the processing pathway of nascent N-linked oligosaccharides (Zapun et al., 1997). The alkaloid castanospermine inhibits the processing enzymes that trim the nascent oligosaccharide into this structure primarily recognized by calnexin (Helenius et al., 1997; Trombetta and Helenius, 1998). Experiments by Chang et al. (1997) and Keller et al. (1998) have demonstrated that treatment with castanospermine increases the degradation of the receptor α subunit (see Fig. 3, compare lanes 3 and 4), implying that calnexin enhances stability of the associated subunit. The chaperones, ERp57 and calreticulin, which may be cryptically associated with the receptor subunit (Keller et al., 1998), may also contribute to the stabilization. In agreement with these observations with castanospermine treatment, earlier studies altering oligosaccharide expression and processing have also revealed decreased stability of the receptor α subunit (Smith et al., 1986; Blount and Merlie, 1990). Degradation caused by castanospermine treatment can be inhibited with the proteasome inhibitor lactacystin (Fig. 3), suggesting that subunits with weak calnexin association are targeted to proteasomal hydrolysis.

By stabilizing the α subunit and thereby reducing dislocation into the ubiquitin-proteasome pathway, cal-

nexin facilitates the incorporation of α subunit into the oligomeric receptor. In contrast to the isolated subunit, degradation of assembled $\alpha\delta$ subunits is not substantially altered when expressed in the presence of castanospermine, suggesting that, similar to the association with calnexin, assembly of the subunits themselves also promotes their stabilization. As subunits assemble, the neighboring subunit assumes the role of a chaperone stabilizing intermediates in the formation of the assembling receptor.

Fig. 4 summarizes our current view of the processes involving calnexin association, ubiquitination, and subunit assembly in the control of receptor synthesis. Calnexin (Fig. 4, CN) is attached to monomeric subunits primarily at the terminal glucose (G) residue in the oligosaccharide. Nevertheless, as a transmembrane protein, it might also protect the receptor subunits at the cytoplasmic, transmembrane, or extracellular surfaces. Exposed lysine residues (Fig. 4, K) are recognized by the ubiquitin conjugation machinery, which enables attachment of polyubiquitin chains (UUU) to these sites. Owing in part to subunit association with calnexin, which contains an ER retention sequence (Rajagopalan and Brenner, 1994), and the tendency for dislocation of polyubiquitin-tagged glycoproteins to the cytoplasm (Kopito, 1997; Suzuki et al., 1998), unassembled subunits are not exported to the Golgi. Instead, detachment of calnexin further targets the unassembled subunit to proteasomal degradation. The assembly of re-

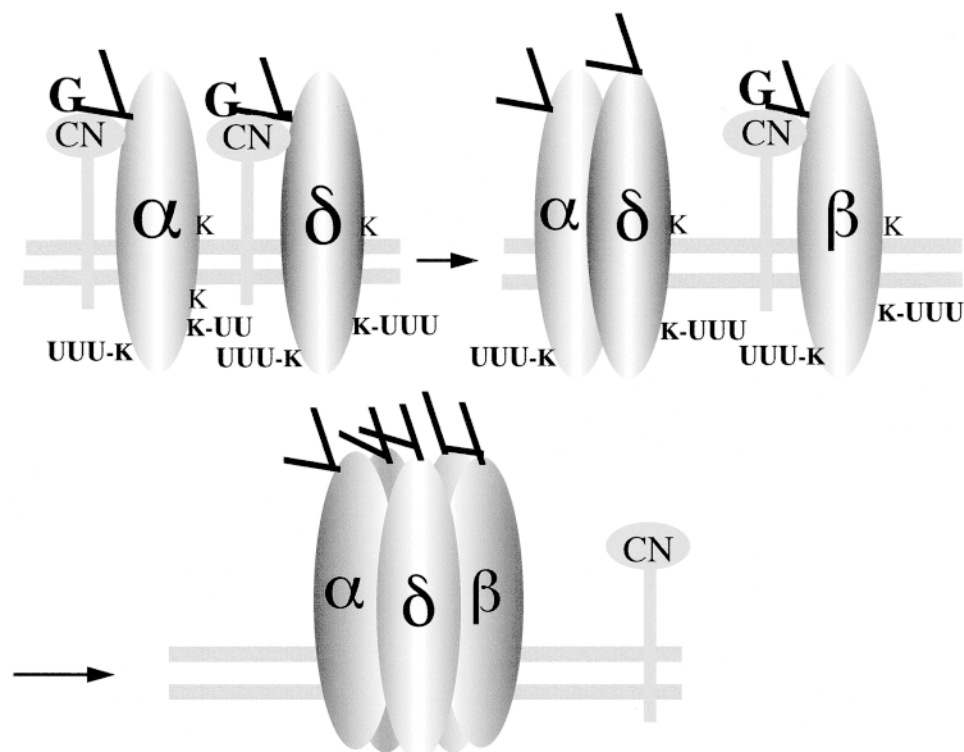


FIGURE 4. Assembly of the receptor subunits into a pentamer (see text for details).

ceptor subunits may cover lysine residues at the interfaces between the subunits, which should occlude the ubiquitin conjugation machinery. As subunits assemble, ubiquitin tagging should be reduced and the nascent assembled receptor subunits should become more stable.

Trafficking of the Subunits from the ER to the Golgi and Cell Surface

A question that emerges from these studies is: what regulates sequestering of unassembled subunits and eventual export of the assembled complex to the Golgi and cell surface? Ubiquitination and calnexin could also have roles in the trafficking of unassembled subunits to the Golgi, due to their inhibitory influence on subunit transport into the secretory pathway. When subunits assemble, calnexin dissociates (Keller et al., 1996), and the tendency of unchaperoned subunits to dislocate into the degradative pathway should be reduced. Full assembly and export of the subunits into the secretory pathway leading to the Golgi should then be favored.

In summary, by transfecting cells with subunit combinations that are components of the receptor, we and others have identified amino acid residues responsible for subunit assembly and cellular factors that assist in the assembly and expression mechanisms. Additionally, by expressing these subunits in mammalian cells under normal physiological conditions, our findings should be representative of nAChR biosynthesis in vivo. The transient transfection system, where mutations affecting receptor subunit associations can be systematically studied, should continue to provide a valuable approach to understanding subunit assembly. Moreover, studies examining the influence of defined cellular proteins such as calnexin on the assembly and expression of the receptor should likewise yield important new findings on receptor synthesis.

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REFERENCES

Anderson, D.J., and G. Blobel. 1981. In vitro synthesis, glycosylation and membrane insertion of the four subunits of *Torpedo* acetylcholine receptor. *Proc. Natl. Acad. Sci. USA.* 78:5598–5602.

Blount, P., M.M. Smith, and J.P. Merlie. 1990. Assembly intermediates of the mouse muscle nicotinic acetylcholine receptor in stably transfected fibroblasts. *J. Cell Biol.* 111:2601–2611.

Blount, P., and J.P. Merlie. 1989. Molecular basis of the two non-equivalent ligand binding sites of the muscle nicotinic acetylcholine receptor. *Neuron.* 3:349–457.

Blount, P., and J.P. Merlie. 1990. Mutational analysis of muscle nicotinic acetylcholine receptor subunit assembly. *J. Cell Biol.* 111:2612–2622.

Boulter, J., A. O'Shea Greenfield, R. Duvoisin, J. Connolly, E. Wada, A. Jensen, P. Gardner, M. Ballivet, E. Deneris, D. McKinnon, et al. 1990. $\alpha 3$, $\alpha 5$, and $\beta 4$: three members of the rat neu-

ronal nicotinic acetylcholine receptor-related gene family form a gene cluster. *J. Biol. Chem.* 265:4472–4482.

Chang, W., M.S. Gelman, and J.M. Prives. 1997. Calnexin-dependent enhancement of nicotinic acetylcholine receptor assembly and surface expression. *J. Biol. Chem.* 272:28925–28932.

Changeux, J.P. 1991. Compartmentalized transcription of acetylcholine receptor genes during motor endplate epigenesis. *New Biol.* 3:413–429.

Chavez, R., and Z.W. Hall. 1991. The transmembrane topology of the amino terminus of the α subunit of the nicotinic acetylcholine receptor. *J. Biol. Chem.* 266:15532–15538.

Claudio, T., H.L. Paulson, W.N. Green, A.F. Ross, D.S. Hartman, and D. Hayden. 1989. Fibroblasts transfected with *Torpedo* acetylcholine receptor beta-, gamma-, and delta-subunit cDNAs express functional receptors when infected with a retroviral alpha recombinant. *J. Cell Biol.* 108:2277–2290.

Fu, D.X., and S.M. Sine. 1996. Asymmetric contribution of the conserved disulfide loop to subunit oligomerization and assembly of the nicotinic acetylcholine receptor. *J. Biol. Chem.* 271:31479–31484.

Gelman, M.S., and J.M. Prives. 1996. Arrest of subunit folding and assembly of nicotinic acetylcholine receptors in cultured muscle cells by dithiothreitol. *J. Biol. Chem.* 271:10709–10714.

Green, W.N., and T. Claudio. 1993. Acetylcholine receptor assembly: subunit folding and oligomerization occur sequentially. *Cell.* 74:57–69.

Green, W.N., and C.P. Wanamaker. 1997. The role of the cystine loop in acetylcholine receptor assembly. *J. Biol. Chem.* 272:20945–20953.

Green, W.N., and C.P. Wanamaker. 1998. Formation of the nicotinic acetylcholine receptor binding sites. *J. Neurosci.* 18:5555–5564.

Gu, Y., P. Camacho, P. Gardner, and Z.W. Hall. 1991. Identification of two amino acid residues in the epsilon subunit that promote mammalian muscle acetylcholine receptor assembly in COS cells. *Neuron.* 6:879–887.

Helenius, A., E.S. Trombetta, D.N. Herbert, and J.F. Simons. 1997. Calnexin, calreticulin and the folding of glycoproteins. *Trends Cell Biol.* 7:193–200.

Hucho, F., V.I. Tsetlin, and J. Machold. 1996. The emerging three-dimensional structure of a receptor. The nicotinic acetylcholine receptor. *Eur. J. Biochem.* 239:539–557.

Karlin, A., and M. Akabas. 1995. Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. *Neuron.* 15:1231–1244.

Keller, S.H., J. Lindstrom, and P. Taylor. 1996. Involvement of the chaperone protein calnexin and the beta-subunit in the assembly and cell surface expression of the receptor. *J. Biol. Chem.* 271:22871–22877.

Keller, S.H., J. Lindstrom, and P. Taylor. 1998. Inhibition of glucose trimming with castanospermine reduces calnexin association and promotes proteasome degradation of the alpha-subunit of the nicotinic acetylcholine receptor. *J. Biol. Chem.* 273:17064–17072.

Kopito, R.R. 1997. ER quality control: the cytoplasmic connection. *Cell.* 88:427–430.

Kreienkamp, H.-J., R.K. Maeda, S. Sine, and P. Taylor. 1995. Inter-subunit contacts governing assembly of the mammalian nicotinic acetylcholine receptor. *Neuron.* 14:636–644.

Machold, J., C. Weise, Y. Utkin, V. Tsetlin, and F. Hucho. 1995. The handedness of the subunit arrangement of the nicotinic acetylcholine receptor from *Torpedo californica*. *Eur. J. Biochem.* 234:423–430.

Merlie, J.P., and J. Lindstrom. 1983. Assembly in vivo of mouse muscle acetylcholine receptor: identification of an alpha subunit species that may be an assembly intermediate. *Cell.* 34:747–757.

- Rajagopalan, S., and M.B. Brenner. 1994. Retention of unassembled components of integral membrane proteins by calnexin. *Science*. 263:387–390.
- Saedi, M., W.G. Conroy, and J. Lindstrom. 1991. Assembly of *Torpedo* acetylcholine receptor in *Xenopus* oocytes. *J. Cell Biol.* 112:1007–1015.
- Sine, S.M., and T. Claudio. 1991. Gamma- and delta-subunits regulate the affinity and the cooperativity of ligand binding to the acetylcholine receptor. *J. Biol. Chem.* 266:19369–19377.
- Sine, S.M., and P. Taylor. 1981. Relationship between reversible antagonist occupancy and the functional capacity of the acetylcholine receptor. *J. Biol. Chem.* 256:6692–6699.
- Smith, M.M., S. Schlesinger, J. Lindstrom, and J.P. Merlie. 1986. The effects of inhibiting oligosaccharide trimming by 1-deoxynojirimycin on the nicotinic acetylcholine receptor. *J. Biol. Chem.* 261:14825–14832.
- Sugiyama, N., A.E. Boyd, and P. Taylor. 1996. Anionic residue in the alpha-subunit of the nicotinic acetylcholine receptor contributing to subunit assembly and ligand binding. *J. Biol. Chem.* 271:26575–26581.
- Suzuki, T., Q. Yan, and W.J. Lennarz. 1998. Complex two-way traffic of molecules across the membrane of the endoplasmic reticulum. *J. Biol. Chem.* 273:10083–10086.
- Trombetta, E.S., and A. Helenius. 1998. Lectins as chaperones in glycoprotein folding. *Curr. Opin. Struct. Biol.* 8:587–592.
- Tsigelny, I., N. Sugiyama, S.M. Sine, and P. Taylor. 1997. A model of the nicotinic receptor extracellular domain based on sequence identity and residue location. *Biophys. J.* 73:52–66.
- Wang, Z.Z., S.F. Hardy, and Z.W. Hall. 1996a. Assembly of the nicotinic acetylcholine receptor. The first transmembrane domains of truncated alpha and delta subunits are required for heterodimer formation in vivo. *J. Biol. Chem.* 271:27575–27584.
- Wang, Z.Z., S.F. Hardy, and Z.W. Hall. 1996b. Membrane tethering enables an extracellular domain of the acetylcholine receptor alpha-subunit to form a heterodimeric ligand-binding site. *J. Cell Biol.* 135:809–817.
- Utkin, Y.N., A.V. Krivoshein, V.L. Davydov, I.E. Kasheverov, P. Franke, I.V. Maslennikov, A.S. Arseniev, F. Hucho, and V.I. Tselintin. 1997. Labeling of *Torpedo californica* nicotinic acetylcholine receptor subunits by cobratoxin derivatives with photoactivatable groups of different chemical nature at Lys23. *Eur. J. Biochem.* 253:229–235.
- Verrall, S., and Z.W. Hall. 1992. The N-terminal domains of acetylcholine receptor subunits contain recognition signals for the initial steps of receptor assembly. *Cell*. 68:23–31.
- Yu, X.M., and Z.W. Hall. 1991. Extracellular domains mediating epsilon subunit interactions of muscle acetylcholine receptor. *Nature*. 352:64–67.
- Yu, X.M., and Z.W. Hall. 1994. Amino- and carboxyl-terminal domains specify the identity of the delta subunit in assembly of the mouse muscle nicotinic acetylcholine receptor. *Mol. Pharmacol.* 46:964–969.
- Zapun, A., S.M. Petrescu, P.M. Rudd, R.A. Dwek, D.Y. Thomas, and J.J. Bergeron. 1997. Conformation-independent binding of monoglycosylated ribonuclease B to calnexin. *Cell*. 88:29–38.