

# Neurotransmitter Receptors

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Chemical synaptic transmission plays a fundamental role in the process of neuron-to-neuron and neuron-to-muscle communication. The type of receptors present in the plasma membrane determines in large part the nature of the response of a neuron or muscle cell to a neurotransmitter. The nature of the response can be mediated either through the direct opening of an ion channel (ionotropic receptors) or through alteration of the concentration of intracellular metabolites (metabotropic receptor) (see also Chapter 4). The response magnitude is determined by receptor number, the "state" of the receptors, and the amount of transmitter released. Finally, the response can be inhibitory or excitatory. The temporal and spatial summation of information conveyed by receptor activation determines whether the postsynaptic cell will fire an action potential or the muscle will contract. As one can see, there is remarkable flexibility and diversity in molding the response to neurotransmitter by constructing a synapse with the desired receptor types.

An ionotropic receptor is a relatively large, multisubunit complex typically composed of four or five individual proteins that combine to form an ion channel through the membrane (Fig. 10.1A). These ion channels exist in a closed state in the absence of neurotransmitter and are impermeable to ions. Neurotransmitter binding induces rapid conformational changes that open the channel, permitting ions to flow down their electrochemical gradients. Changes in membrane current resulting from ligand binding to ionotropic receptors are generally measured on a millisecond timescale. The ion flow ceases when transmitter dissociates from the receptor or when the receptor becomes desensitized, a process discussed in more detail later in this chapter.

In contrast, a GPCR is typically composed of a single polypeptide (Fig. 10.1B), although dimeric forms also exist, and exerts its effects not through the direct opening of an ion channel but rather by binding to and activating

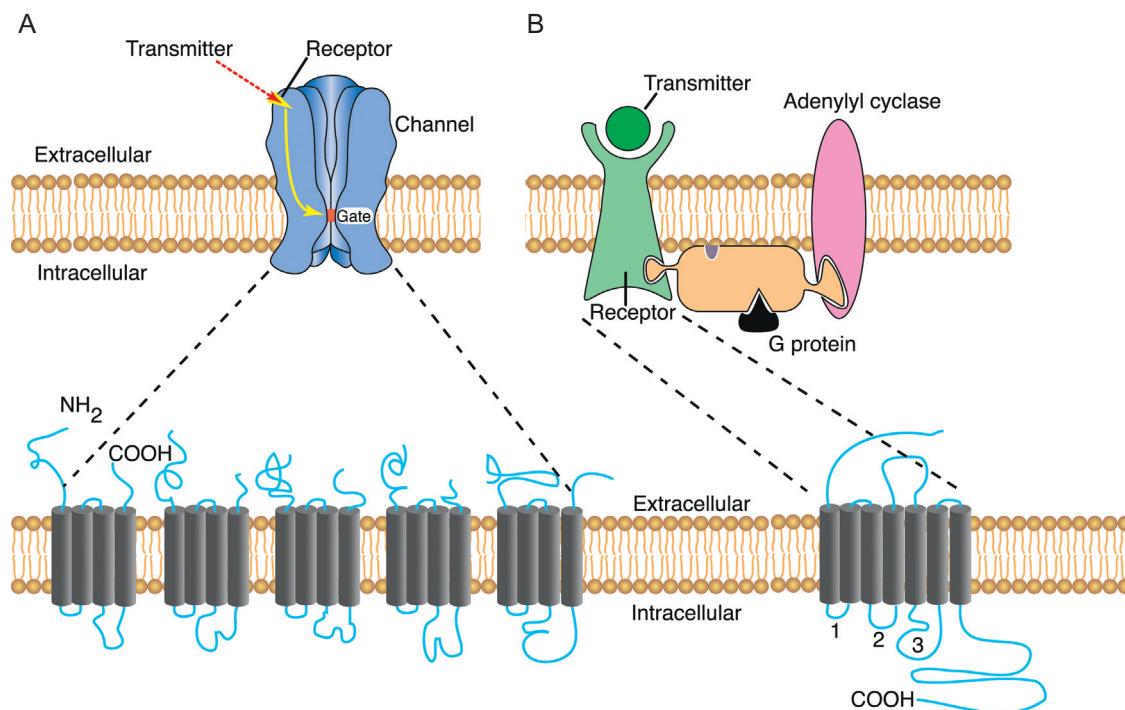
GTP-binding proteins (G proteins). Transmitters that activate GPCRs typically produce responses of slower onset and longer duration (from tenths of seconds to potentially hours) owing to the series of enzymatic steps necessary to produce a response. However, faster responses can occur through actions of activated G proteins or their associated subunits, directly on ion channels.

We consider the structure of the ionotropic receptor family first and then turn to a description of the structure of GPCRs. In each section, information is presented to establish a general structural model of each receptor type. These models are then used to guide the description of other related ionotropic receptors or GPCRs. The order in which receptor types are presented is based predominantly on structural relatedness and should not be interpreted as representing their relative importance in the function of the nervous system.

## IONOTROPIC RECEPTORS

All ionotropic receptors are membrane-bound protein complexes that form an ion-permeable pore in the membrane. By comparing the amino acid sequences of the ionotropic receptor family one can deduce that they are similar in overall structure, although two independent ancestral genes have given rise to two distinct families. One family includes the nicotinic acetylcholine receptor (nAChR), the  $\gamma$ -aminobutyric acid A (GABA<sub>A</sub>) receptor, the glycine receptor, and one subclass of serotonin receptors (Ortells and Lunt, 1995). The other family comprises the many types of ionotropic glutamate receptors (Hollmann and Heinemann, 1994).

The understanding of ionotropic receptor structure and function has expanded enormously in the past 25 years. Molecular approaches have provided elegant and extensive descriptions of gene families encoding different receptors, and systems for expressing cloned



**FIGURE 10.1** Structural comparison of ionotropic and metabotropic receptors. (A) Ionotropic receptors bind transmitter, and this binding directly translates into the opening of the ion channel through a series of conformational changes. Ionotropic receptors are composed of multiple subunits. Shown are the five subunits that together form the functional nAChR. Note that each nAChR subunit wraps back and forth through the membrane four times and that the mature receptor is composed of five subunits. (B) Metabotropic receptors bind transmitter and, through a series of conformational changes, bind to G proteins and activate them. G proteins then activate enzymes such as adenylyl cyclase to produce cAMP. Through the activation of cAMP-dependent protein kinase, ion channels become phosphorylated, which affects their gating properties. Metabotropic receptors are single subunits. They contain seven transmembrane-spanning segments, with the cytoplasmic loops formed between the segments providing the points of interaction for coupling to G proteins.

cDNAs have permitted detailed structure–function analysis of each receptor subtype. Expression of subunits independently and together has resulted in a detailed concept of the necessity and sufficiency of the multisubunit nature of the ionotropic receptor family. By employing the tools of structural biology, events associated with the opening of at least one ionotropic receptor, the nAChR, are available at nearly atomic resolution (Unwin, 1993a; Unwin, 1993b; Unwin, 1995; Unwin and Fujiyoshi, 2012). In addition, crystal structures have been obtained for glutamate (Sobolevsky et al., 2009) and P2X (Hattori and Gouaux, 2012) receptors, ushering in a new era of high-resolution structural information for describing neurotransmitter-induced opening of ion channels.

### The nAChR is a Model for the Structure of Ionotropic Receptors

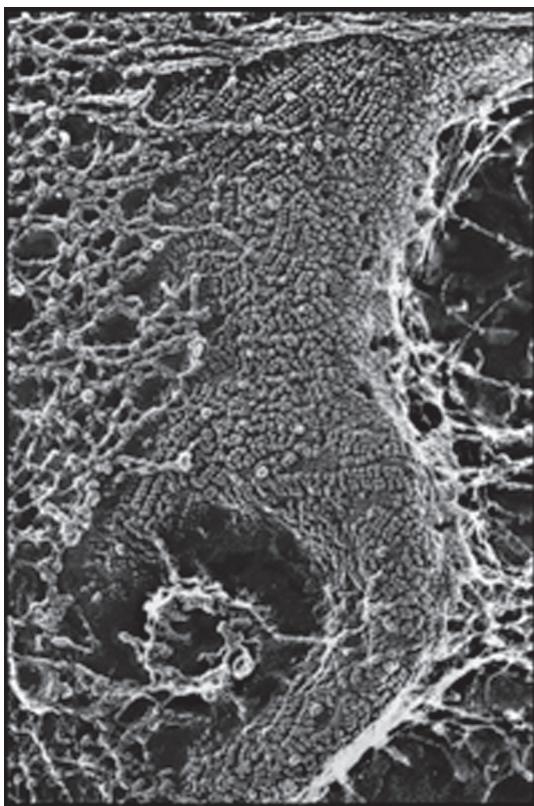
The nAChR is so named because the plant alkaloid nicotine can bind to the ACh binding site and activate the receptor. Nicotine is therefore called an *agonist* of ACh because it binds to the receptor and opens it. In contrast, *antagonists* are molecules that bind to the receptor and inhibit its function. Agonists and antagonists are

powerful tools that permit characterization of the structure and function of individual receptor subtypes.

The structure of the nAChR was the first determined, primarily because electric organs of certain species of fish, such as the *Torpedo* ray, contain nearly crystalline arrays of this molecule (Fig. 10.2). The electric organ is a specialized form of skeletal muscle that has the potential to generate large voltages (as much as 500 V in some cases) from the simultaneous opening of arrays of ion channels activated through the binding of ACh. The majority of biochemical and structural analyses of nAChRs have been done on receptors isolated from the ray electric organ. Purification of nAChRs was further aided by utilization of a toxin from snake venom called  $\alpha$ -bungarotoxin. Affinity columns constructed with  $\alpha$ -bungarotoxin bind to nAChR with high affinity and specificity, providing a means of purifying nearly homogeneous nAChRs in a single chromatographic step.

### The nAChR is a Heteromeric Protein Complex with Distinct Architecture

The structure of the nAChR is typical of ionotropic receptors. The nAChR purified as described from *Torpedo*



**FIGURE 10.2** Panoramic view of the postsynaptic membrane of an electricocyte in the *Torpedo* electric organ, revealed by "deep-etch" electron microscopy. The vaselike structure in the center of the field is the external surface of the postsynaptic membrane, which is revealed by removal of the basal lamina. Clusters and linear arrays of 8 to 9 nm protrusions can be clearly seen. These represent the AChR oligomers. To the left of the vaselike structure, a lacelike basal lamina lies above the membrane, obscuring it from view. To the right of the vaselike structure, the postsynaptic membrane has been freeze-fractured away, thus revealing an underlying meshwork of cytoplasmic filaments that supports the postsynaptic membrane and its receptors  $\times 175,000$ . Original courtesy of J. Heuser.

is composed of five subunits (see Fig. 10.1) and has a native molecular mass of approximately 290 kDa. The subunits are designated  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ , and each receptor complex contains two copies of the  $\alpha$  subunit. The subunits are homologous membrane-bound proteins that assemble in the bilayer to form a ring enclosing a central pore. Pioneering electron microscopic analyses by Nigel Unwin have provided the best image of the structural appearance of the nAChR (Fig. 10.3). The extracellular domains of the subunits together form a funnel-shaped opening that extends approximately 100 Å outward from the outer leaflet of the plasma membrane. The funnel at the outer portion of the receptor has an inside diameter of 20–25 Å (Unwin, 1993b). The funnel shape is thought to concentrate and force ions to interact with amino acids in the limited space of the pore without producing a major barrier to diffusion. This funnel narrows near the

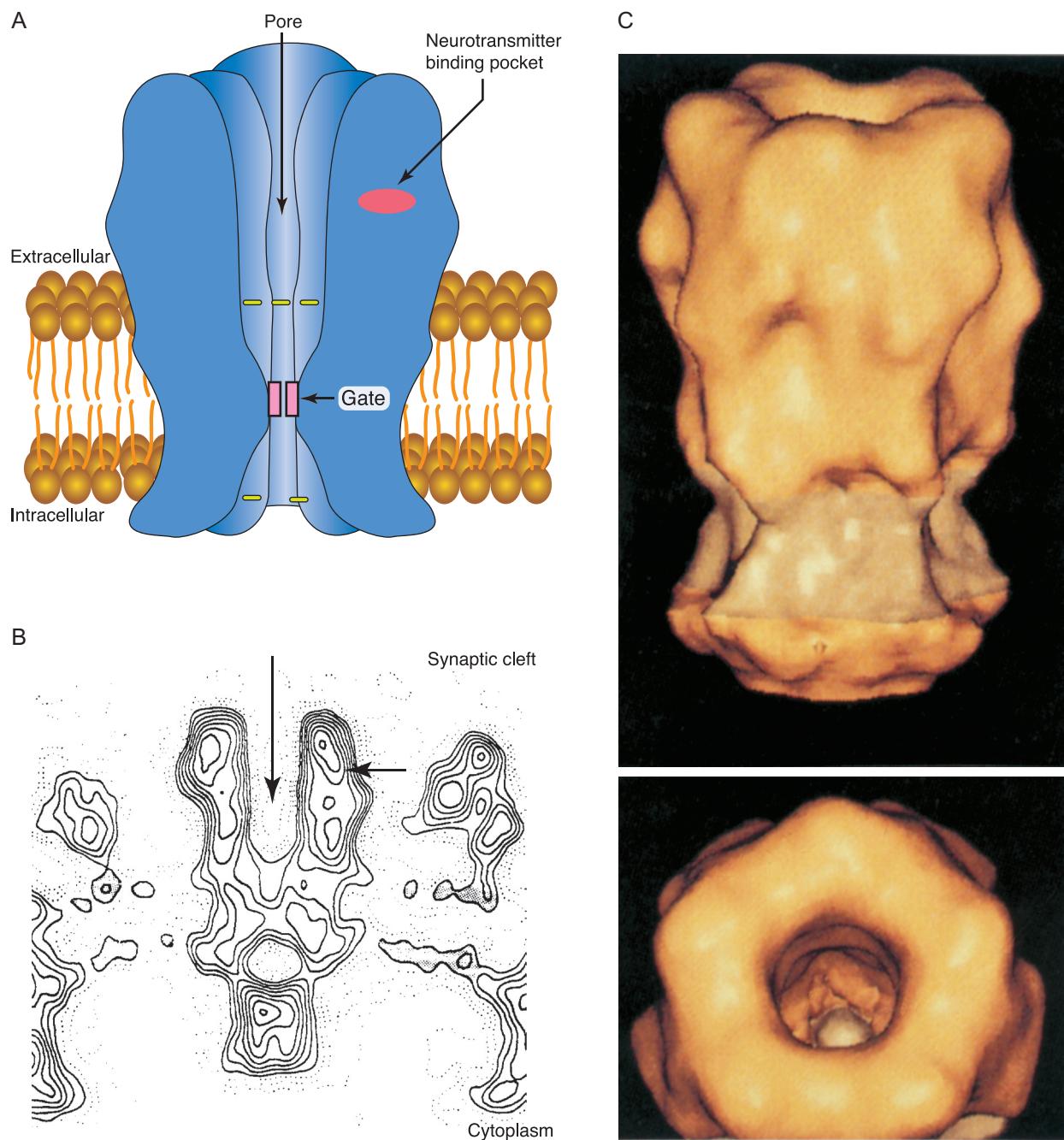
center of the lipid bilayer to form the domain of the receptor that determines the opened or closed state of the ion pore. The intracellular domain of the receptor forms short exits for ions traveling into the cell and an entrance for ions traveling out of the cell. The intracellular domain also establishes the association of the receptor with other intracellular proteins that determine the subcellular localization of the nAChR. The arrangement of the subunits in the receptor is such that the  $\beta$  subunit lies between the two  $\alpha$  subunits (Unwin, 1993b; Unwin, 1993a; Unwin, 1995; Unwin and Fujiyoshi, 2012).

### Each nAChR Subunit has Multiple Membrane-Spanning Segments

The primary amino acid sequence of each nAChR subunit was obtained by the efforts of Shosaka Numa and his colleagues (Noda et al., 1982; Noda et al., 1983). The deduced amino acid sequence from cloned mRNAs indicates that the nAChR subunits range in size from 40 to 65 kDa. A general domain structure for each subunit was derived from primary sequence data and toxin- and antibody-binding studies. Each subunit consists of four transmembrane-spanning segments referred to as TM1–TM4 (Fig. 10.4). Each transmembrane segment is composed mainly of hydrophobic amino acids that stabilize the domain within the hydrophobic environment of the lipid membrane. The four transmembrane domains are arranged in an antiparallel fashion, wrapping back and forth through the membrane. The N terminus of each subunit extends into the extracellular space, as does the loop connecting TM2 and TM3 as well as the C terminus. The amino acids linking TM1 and TM2 and those linking TM3 and TM4 form short loops that extend into the cytoplasm.

### The Structure of the Channel Pore Determines Ion Selectivity and Current Flow

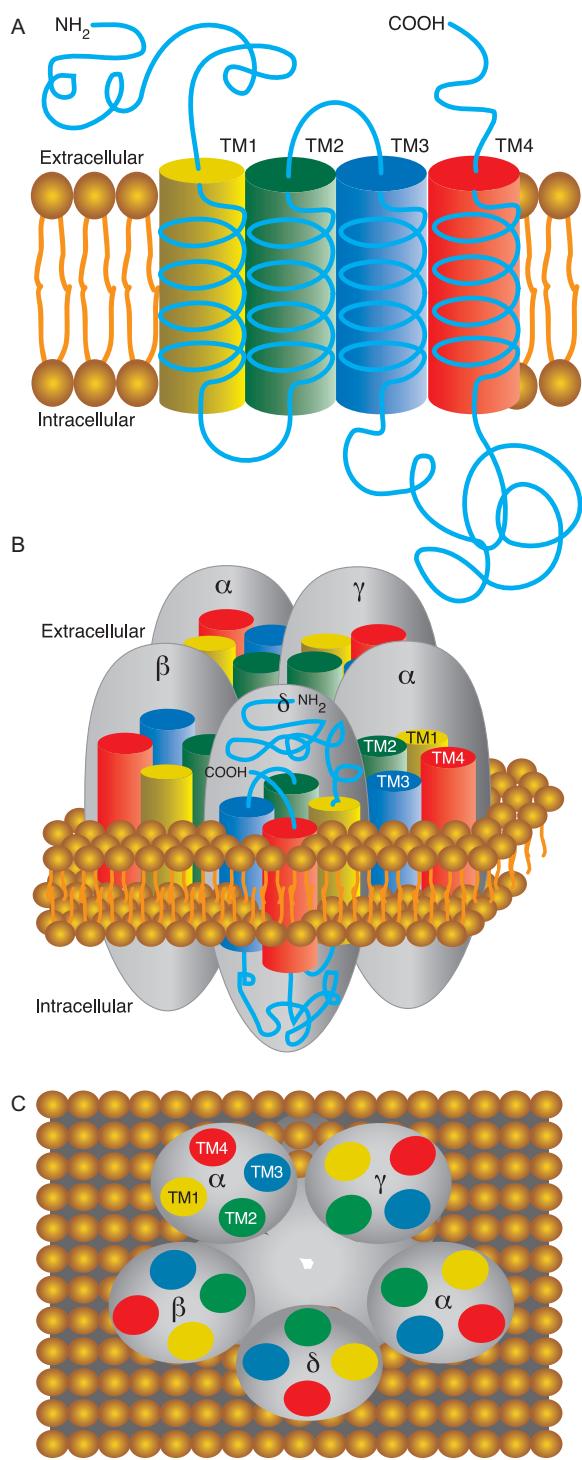
In the model shown in Fig. 10.4C, each subunit of the nAChR can be seen to contribute one cylindrical component (representing a membrane-spanning segment) that presents itself to a central cavity that forms the ion channel through the middle of the complex. The membrane-spanning segments that line the pore are the five TM2 regions, one contributed by each subunit. The amino acids that compose the TM2 segment are arranged in such a way that three rings of negatively charged amino acids are oriented toward the central pore of the channel (Fig. 10.5). These rings of negative charge appear to provide much of the selectivity filter so that only cations can pass through the central channel, whereas anions are largely excluded owing to charge repulsion (Imoto et al., 1988; Karlin,



**FIGURE 10.3** (A) Vertical section diagramming the structure of nAChR as it is believed to exist in the membrane. Note that the funnel-shaped structure narrows to a small central point referred to as the *gate*. Strategically placed rings of negatively charged amino acids on both sides of the gate form part of the selectivity filter for positively charged ions. The approximate position of the neurotransmitter binding site is shown in relation to the gate and the plasma membrane. (B) Protein density map derived from reconstructions of nAChR imaged by cryoelectron microscopy. The vertical arrow indicates the direction of ion flow from outside to inside within the funnel-shaped part of the receptor. The horizontal arrow indicates the predicted position of the neurotransmitter binding site that resides approximately 30 Å above the bilayer. The additional protein density attached to the bottom of the receptor is suggested to be a protein that anchors the nAChR to synapses. (C) Three-dimensional computer rendering of the nAChR. (Top) Side view of nAChR similar to that in (A). The darker shaded area near the bottom of the receptor delineates the approximate location where the receptor contacts the lipid bilayer. (Bottom) A view looking down into the funnel-shaped opening of the receptor. Note that the funnel narrows forming the *gate*.

1993). The nAChR is permeable to most cations, such as  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ , although monovalent cations are preferred. This mechanism for selectivity is poor in relation to the selectivity described for the family of

voltage-gated ion channels (e.g., voltage-gated  $\text{Ca}^{2+}$  channels; see Chapters 11 and 13). From analysis of the passage of various-sized cations, the dimension of the pore forming the final barrier for ion permeation was



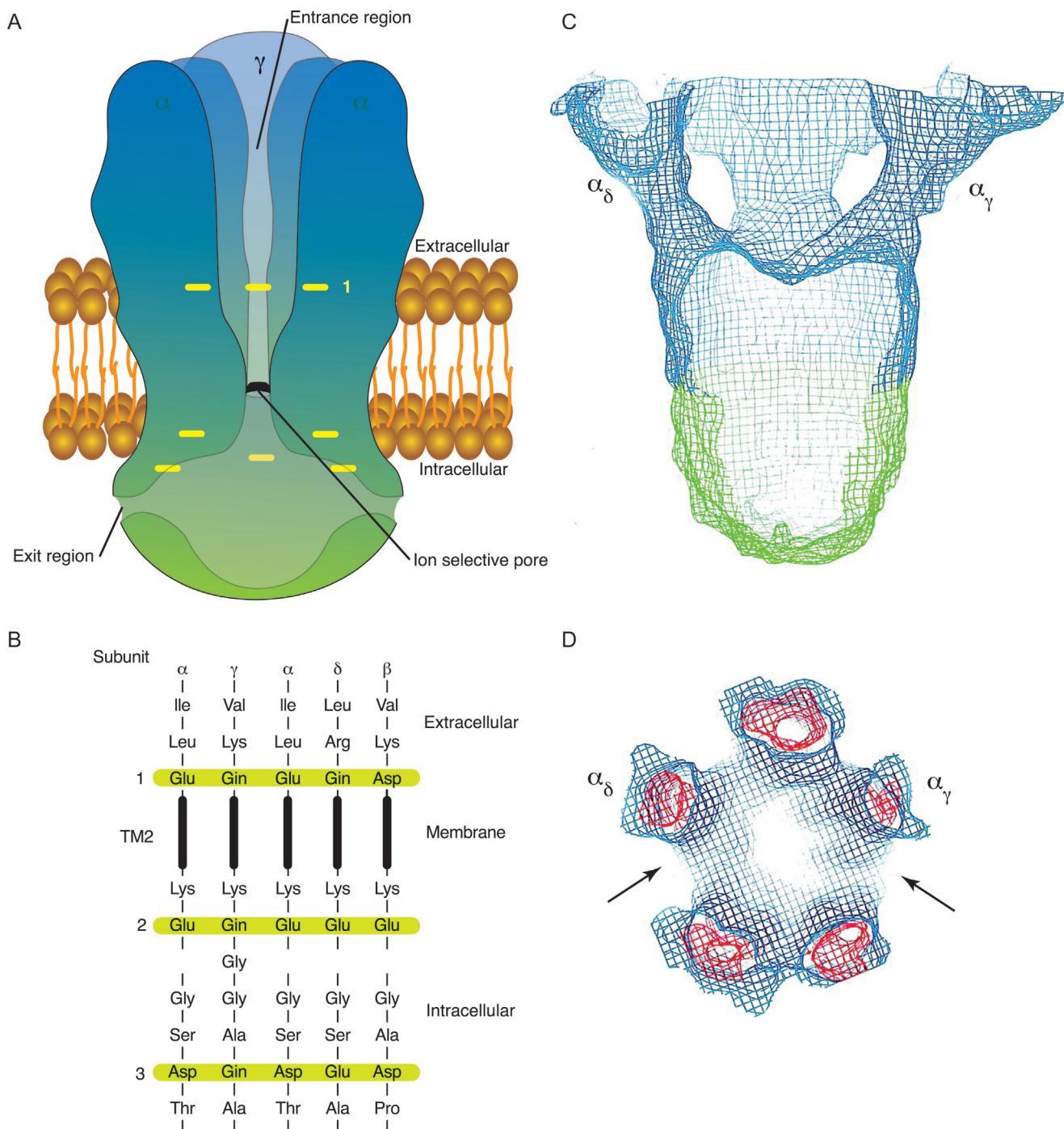
**FIGURE 10.4** (A) Diagram highlighting the orientation of the membrane-spanning segments of one subunit of nAChR. The amino and carboxy termini extend in the extracellular space. The four membrane-spanning segments are designated TM1–TM4. Each forms an  $\alpha$  helix as it traverses the membrane. (B) Side view of the five subunits in their approximate positions within the receptor complex. There are two  $\alpha$  subunits present in each nAChR. (C) Top view of all five subunits highlighting the relative positions of their membrane-spanning segments, TM1–TM4, and the position of TM2 that lines the channel pore.

estimated to be approximately 8.5 Å (Hille, 1992). This size is in excellent agreement with the measurements of 9–10 Å for the pore diameter from images of the open state of the receptor (Unwin, 1995). The restricted physical dimensions of the pore contribute greatly to the selectivity for particular ions. When the pore of the nAChR opens, positively charged ions move down their respective electrochemical gradients, resulting in an influx of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  and an efflux of  $\text{K}^+$ . A coarse filtering that also contributes to selectivity appears to be a shielding effect produced by other negatively charged amino acids surrounding the outer channel region of the receptor.

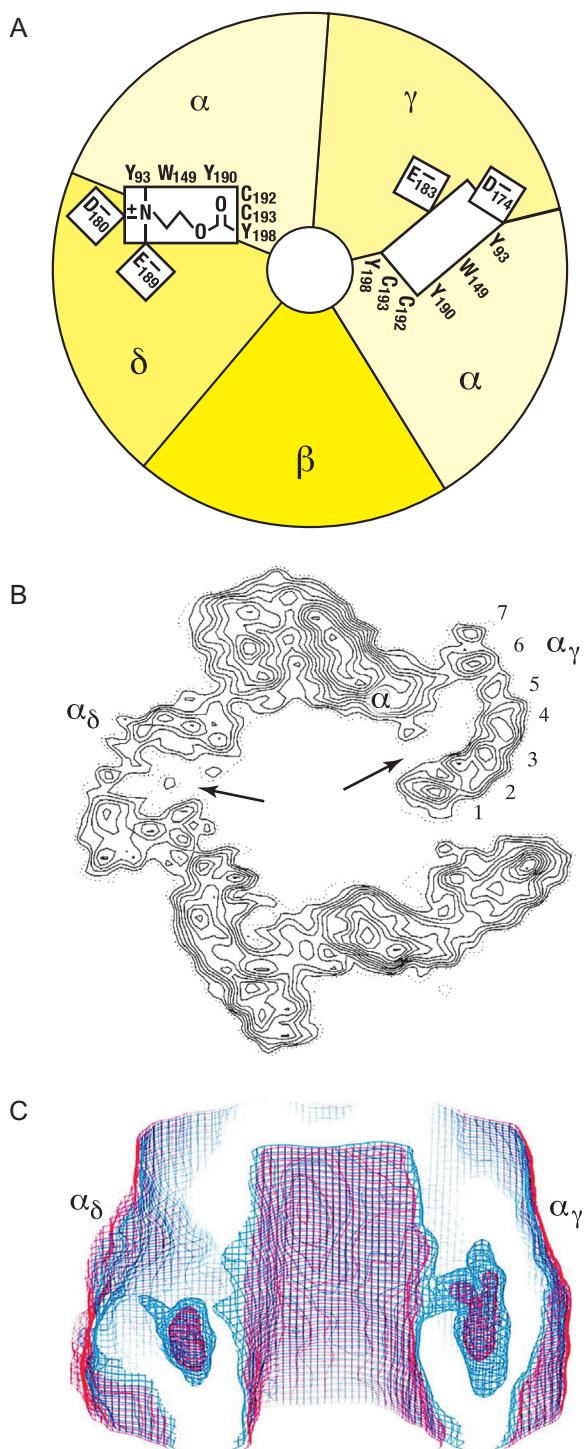
Ions do not directly enter or exit through the central pore of the cytoplasmic side of the nAChR. Two narrow openings are present on the lateral aspects of the cytoplasmic portion of the receptor through which ions must exit or gain access to the central pore (Figs. 10.5A, C, and D) (Miyazawa et al., 1999).  $\alpha$ -Helical rods extending down from each subunit form an inverted pentagonal cone to produce these openings. Although too large (8 × 15 Å) to be a significant barrier to ion flow, these lateral pores can serve as an additional filtering step for the selectivity of certain ions.

### There are Two Binding Sites for ACh on the nAChR

Each nAChR complex has two ACh binding sites that reside in the extracellular domain and lay approximately 30 Å from the outer leaflet of the membrane (see Fig. 10.3). The ACh binding site is formed for the most part by six amino acids in the  $\alpha$  subunits; however, amino acids in both the  $\gamma$  and the  $\delta$  subunits also contribute to binding (Karlin, 1993) (Fig. 10.6A). Mutations introduced at these critical amino acids in the  $\alpha$  subunit significantly attenuate ligand binding. The two binding sites are not equivalent because of the receptor's asymmetry due to the different neighboring subunits (either  $\gamma$  or  $\delta$ ) adjacent to the two  $\alpha$  subunits. Significant cooperativity also exists within the receptor molecule, and so binding of the first molecule of ACh enhances binding of the second (Changeux et al., 1984). For as yet not well understood reasons, ACh molecules must enter the pore and traverse small channels to gain access to their binding sites (Miyazawa et al., 1999) (Figs. 10.6B and 10.6C). It is speculated that similar attractive forces that bring positively charged ions into the pore also attract the positively charged ACh molecules favoring entrance into the channels that lead to the ACh binding sites. Two adjacent Cys residues (Cys-192 and Cys-193) in each  $\alpha$  subunit form a disulfide bond that also appears to contribute to the stability of the ACh binding pocket.



**FIGURE 10.5** (A) Vertical section highlighting the relative positions of the three rings of negatively charged amino acids that help form the cation selectivity of nAChR. The regions where ions exit or enter from the intracellular side of the receptor are disposed laterally at the base of the receptor. (B) Amino acid sequence of each of the TM2 membrane-spanning segments of the five nAChR subunits. Numbers 1–3 correspond to the positions of the amino acids taking part in the formation for the three rings of negatively charged amino acids that determine the cation selectivity of the pore. Aspartate (Asp) and glutamate (Glu) are negatively charged amino acids. (C) Wireframe portrayal of the protein density distribution of the intracellular portion of nAChR. The front portion of the receptor was cut away to reveal the inverted cone-shaped cavity of the intracellular domain. The green wireframe represents protein density contributed by the anchoring protein rapsyn. (D) Wireframe portrayal of the protein density distribution of the intracellular domain of nAChR looking downward from within the receptor. The arrows indicate the major gaps in the lateral walls of the receptor where ions enter and exit.



**FIGURE 10.6** (A) Diagram of the relative positions of amino acids that form the Ach binding site in nAChR. The view is from above the receptor looking down into the pore. Each subunit is represented by a wedge. At the left, Ach is shown bound to its site at the interface between the α and δ subunits. The length of the binding site is shown slightly contracted relative to the site (between the α and γ subunits) without bound Ach. Critical amino acids for transmitter binding are indicated. Residues shown in boxes are amino acids predicted to make contact with the positively charged part of the Ach molecule. Note that many of the residues important for Ach binding are contributed

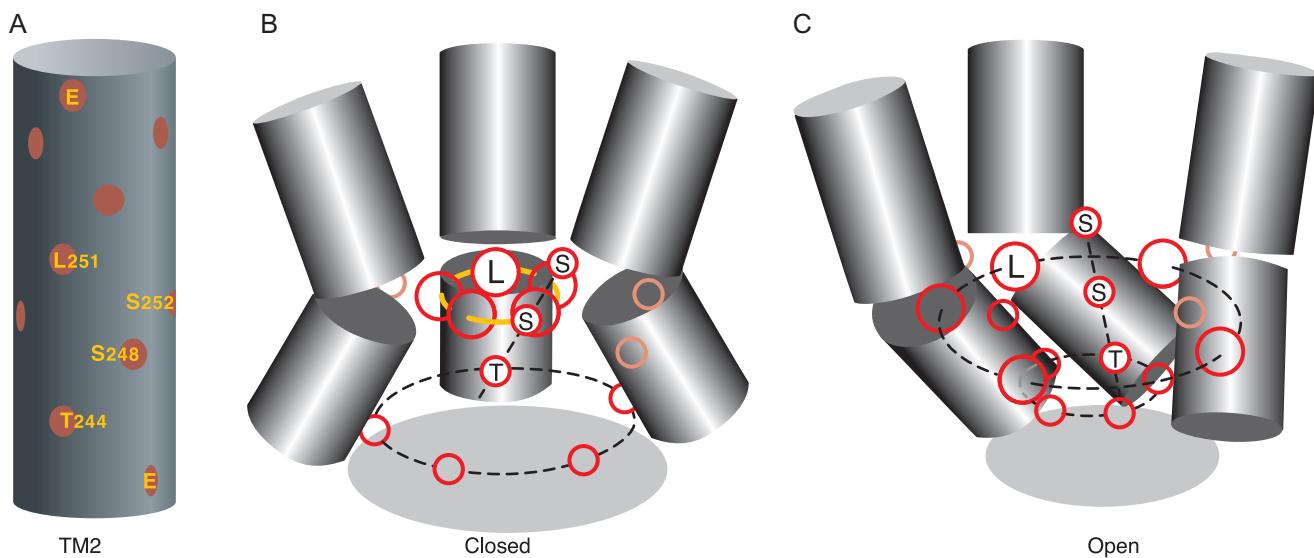
(Fig. 10.6A). These Cys residues are highly conserved in most ionotropic receptors and form an essential bond for stabilizing high-affinity neurotransmitter binding. α-bungarotoxin binds to the α subunit in close proximity to the two adjacent Cys residues (Karlin, 1993).

### Opening of the nAChR Occurs through Concerted Conformational Changes Induced by ACh Binding

When the nAChR binds two molecules of ACh, the channel opens almost instantaneously (time constants for opening are approximately 20 µs (Colquhoun and Sakmann, 1985; Colquhoun and Ogden, 1988), thus permitting the passage of ions. A model developed from electron micrographic reconstructions of the nicotine-bound form of the nAChR indicates that the closed-to-open transition is associated with a subtle rotation of the TM2 segments (Unwin, 1995; Unwin and Fujiyoshi, 2012) (Fig. 10.7). The TM2 segments are helical and exhibit a mild kink in their structure that forces a Leu residue from each segment into a tight ring that effectively blocks the flow of ions through the central pore of the receptor. When the TM2 segments rotate because of ACh binding, the kinks also rotate, relaxing the constriction formed by the Leu ring, and ions can then permeate the pore. The rotation also orients a series of Ser and Thr residues (amino acids with a polar character) into the central area of the pore, which facilitates the permeation of water-solvated cations.

The main features of the nAChR transition from a closed to open state are summarized in Figure 10.8. ACh gains access to its binding sites by entering the central pore of the receptor where it then enters small channels that provide access to the binding sites. Once both binding sites are occupied, the receptor rapidly opens to permit ion flow. It is the twisting of the TM2 segments induced by ACh binding that gates open the ion pore. In addition to negatively charged rings spaced within the central channel, charge screening also occurs in the lateral openings of the cytoplasmic domain of the receptor (Fig. 10.8). The ion selectivity of the nAChR for positively charged ions ( $\text{Na}^+$  and  $\text{K}^+$  mainly) comes from the charge screening at these different levels and the physical constriction of the gate of the pore.

by the α subunit. Cysteine (Cys) residues at positions 192 and 193 form a disulfide bond essential for stabilizing the Ach binding pocket. (B) A top-down view of the protein density map of nAChR sliced through the area where the Ach-binding areas reside. Note that Ach molecules must gain access to their binding sites through channels whose openings are on the inside of the funnel-shaped portion of the receptor. (C) Lateral view of a wireframe portrayal of nAChR at the level of the Ach binding sites. A portion of the receptor was cut away to highlight the fact that the cavities where Ach bind must be accessed from the pore of the receptor through short channels.



**FIGURE 10.7** (A) Relative positions of amino acids in the TM2 segment of one of the nAChR  $\alpha$  subunits modeled as an  $\alpha$  helix. The glutamate residues (E) that form parts of the negatively charged rings for ion selectivity are shown at the top and bottom of the helix. (B) Arrangement of three of the TM2 segments of nAChR modeled with the receptor in the closed (Ach-free) configuration. In the closed configuration, leucine (L) residues form a right ring in the center of the pore that blocks ion permeation. (C) Arrangement of the three TM2 segments after Ach binds to the receptor. In the open configuration, the construction formed by the ring of leucine (L) residues opens as the helices twist about their axes. Note that the polar serine (S) and threonine (T) residues align when Ach binds, which apparently help the water-solvated ions travel through the pore. Adapted with permission from [Unwin \(1995\)](#) (Macmillan Publishers Ltd.).

### The Muscle Form of the nAChR is Very Similar to the nAChR from *Torpedo*

The nAChRs at the neuromuscular junction are a concentrated collection of homogeneous receptors having a structure similar to that of the nAChR from the *Torpedo* electric organ. This similarity is not surprising, because the electric organ is a specialized form of muscle tissue. The adult form of the muscle receptor has the pentameric structure  $\alpha_2\beta\gamma\delta$ . An embryonic form of the receptor has an analogous structure, except that the  $\gamma$  subunit is replaced by a unique  $\epsilon$  subunit. The embryonic and adult subunits of both mouse and bovine muscle receptors have been cloned and expressed in heterologous systems, such as the *Xenopus laevis* oocyte (Box 10.1) (Mishina et al., 1984), and the receptors differ in both channel kinetics and channel conductance. These differences in channel properties appear to be necessary for the proper function of the nAChRs as they undergo the transition from developing to mature neuromuscular junction synapses.

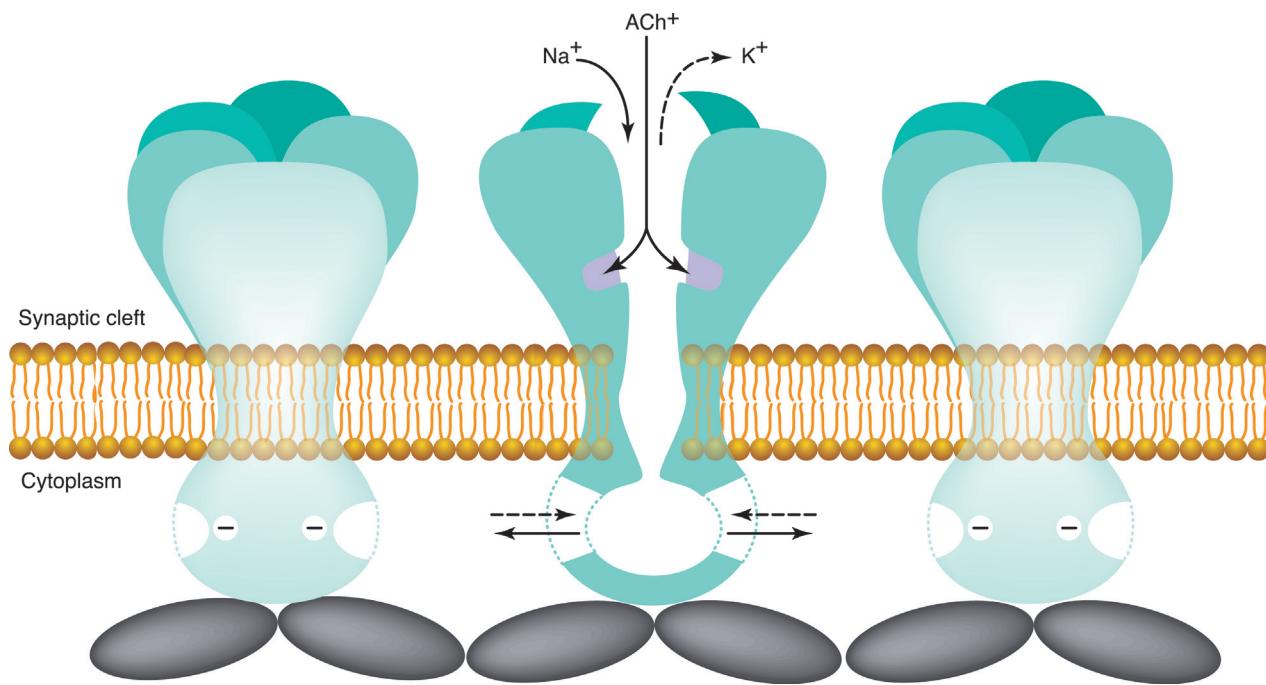
### The nAChR is Synthesized as a Membrane-bound Protein and has Well-Ordered Assembly

The pathway of the nAChR assembly in muscle is a tightly regulated process. For example, the five subunits of the nAChR have the potential to randomly assemble into 208 different combinations. Nevertheless, in vertebrate muscle, only one of these configurations ( $\alpha_2\beta\gamma\delta$ ) is

typically found in mature tissue, indicating a very high degree of coordinated assembly and little structural variability (Paulson et al., 1991; Green and Claudio, 1993). The well-ordered assembly of specific intermediates is essential for this coordinated process, and the intermediates formed appear to start with a dimer between  $\alpha$  and either  $\epsilon$  ( $\gamma$  in mature muscle) or  $\delta$ . The heterodimers then bind to  $\beta$  and to each other to form the final receptor (Gu et al., 1991). An alternative pathway in which  $\alpha$ ,  $\beta$ , and  $\gamma$  first form a trimer has also been proposed (Green and Claudio, 1993). All this assembly takes place within the endoplasmic reticulum. During intracellular maturation, each subunit is glycosylated, and, if glycosylation is inhibited, the production of mature nAChRs decreases. Two highly conserved disulfide bonds in the N-terminal extracellular domain are essential for efficient assembly of the mature receptor. The first is between two adjacent Cys residues (Cys-192 and Cys-193) and, as noted, resides very close to the ACh binding site on the receptor (see Fig. 10.6). The second disulfide bond is between two Cys residues 15 amino acids apart, forming a loop in the extracellular domain.

### nAChRs are Concentrated and Anchored in the Postsynaptic Membrane

The nAChRs are highly concentrated at the neuromuscular junction, which ensures rapid and reliable communication between the presynaptic motor neuron



**FIGURE 10.8** Summary figure highlighting the structural features of nAChR. The ACh-free form of the receptor remains closed to ion flow. ACh gains access to its binding site by entering the outer portion of the central pore of nAChR, which produces a relaxation of the central pore and an expansion of the holes in the lateral walls of the intracellular portion of the receptor. The protein rapsyn, represented by the gray ovals, anchors nAChR to synapses by interacting with the intracellular domain.

and postsynaptic muscle cell to induce contraction. The clustering of nAChRs begins during development and is mediated in part by the release of agrin from the motor neuron that binds to a tyrosine kinase in the postsynaptic membrane called *MuSK* (Sanes, 1997; Willmann and Fuhrer, 2002). MuSK activation recruits members of the Src family of kinases that phosphorylates the nAChR leading to increased interactions with a multitude of postsynaptic signaling complexes ultimately leading to the recruitment of rapsyn. Rapsyn is a multidomain protein that binds directly to cytoplasmic tails of the nAChR subunits along with other proteins, including itself. Rapsyn thus forms the critical scaffolding function that clusters nAChRs at the neuromuscular junction. Rapsyn binds tightly to the nAChR and can be identified in reconstructed images of the receptor (Figs. 10.3C and 10.8). In addition to the nAChR, rapsyn also binds to other important muscle proteins including MuSK and the dystrophin–utrophin glycoprotein complex (dystrophin is the molecule that when mutated leads to certain types of muscular dystrophy (Sanes, 1997)). As the junctions mature, a complex web of protein–protein interactions occurs, including association with the underlying actin cytoskeleton (Fig. 10.9). Activity of the nAChR itself is also critical for its stabilization at the neuromuscular junction. The lifetime of the nAChR decreases from 14 d to less than a day following block of the receptor with  $\alpha$ -bungarotoxin. In total, a

complex interplay of signals coming from the presynaptic motor neuron is integrated by the postsynaptic receptors to concentrate and stabilize nAChRs at the neuromuscular junction.

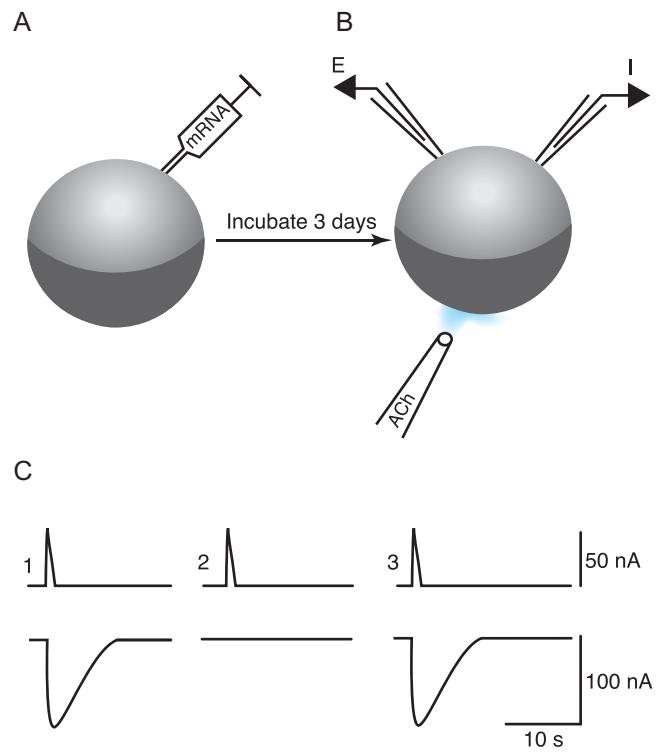
### Phosphorylation is a Common Posttranslational Modification of Receptors

Many ionotropic receptors, such as the nAChR, are phosphorylated, although the functional significance of the phosphorylation is not always evident. The nAChR is phosphorylated by at least three protein kinases: cAMP-dependent protein kinase (PKA) phosphorylates the  $\gamma$  and  $\delta$  subunits;  $\text{Ca}^{2+}$ -phospholipid-dependent protein kinase (PKC) phosphorylates the  $\delta$  subunit; and an unidentified tyrosine kinase that phosphorylates the  $\beta$ ,  $\gamma$ , and  $\delta$  subunits (Huganir and Greengard, 1990). The phosphorylation sites are all found in the intracellular loop between the TM3 and TM4 membrane-spanning segments. Phosphorylation by these three protein kinases appears to increase the rapid phase of desensitization of the receptor. Desensitization of receptors is a common observation, and this process limits the amount of ion flux through a receptor by producing transitions into a closed state (one that does not permit ion flow) in the continued presence of neurotransmitter. For the nAChR, the rate of desensitization has a time constant of

## BOX 10.1

## THE XENOPUS OOCYTE

The *Xenopus* oocyte has been used extensively to study the properties of cDNAs encoding receptor subunits and their mutated forms. In addition, the oocyte has been used to study how combinations of different subunits interact to produce receptors with different properties. The large size and efficient translational machinery of *Xenopus* oocytes make them ideal for electrophysiological analyses of cDNAs encoding prospective receptors and channels. For example, mRNAs produced by *in vitro* transcription of cDNAs encoding each of the individual nAChR subunits were introduced into oocytes by micro-injection (A). Several days later, the oocytes were voltage-clamped to study the properties of the expressed channels (B). When ACh was applied through a separate pipet, a significant inward current was detected in the oocyte (C, panel 1). The response was specifically blocked by addition of an antagonist, tubocurarine (C, panel 2), and the block was reversed by a 15-min wash (C, panel 3). Details of this study indicate that all four of the nAChR subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) were required for ACh to produce an electrophysiological response (Mishina et al., 1984). More recently, the patch-clamp technique has also been applied to oocyte expression of receptors to analyze the behavior of single channels.



approximately 50–100 ms. This rate appears to be too slow to have much significance in shaping the synaptic response at the neuromuscular junction, where the response typically lasts from 5 to 10 ms. This slow desensitization is not true of the brain forms of the nAChR and is discussed further in a later section of this chapter.

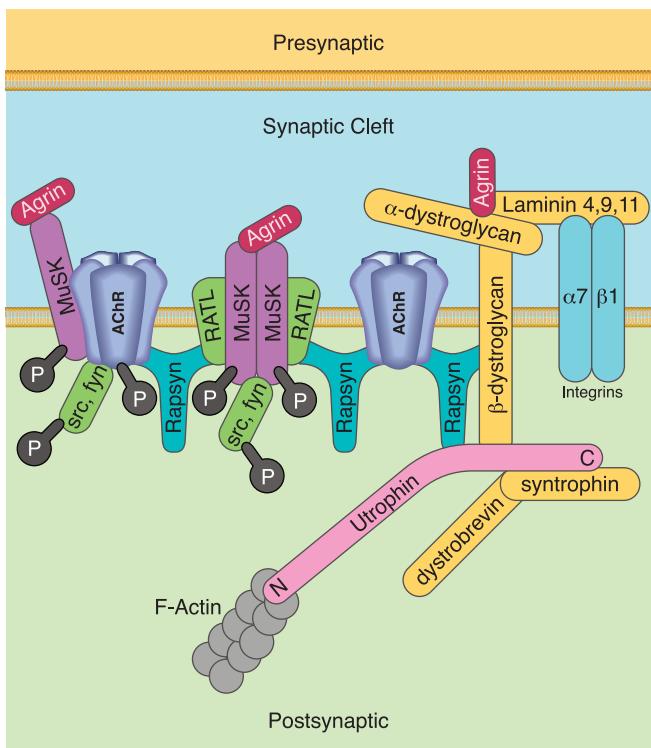
### The Structures of Other Ionotropic Receptors are Variations of the nAChR Structure

On the basis of similarity of structure, clear evolutionary relationships exist for the family of ionotropic receptors. Figure 10.10 shows an evolutionary tree for the family of ionotropic receptors with nAChR at the top. The 5-hydroxytryptamine (5-HT<sub>3</sub>, serotonin) receptor is closely related to the nAChR and then a major subdivision separates the cation permeable receptors from those permeable to anions (e.g., Cl<sup>-</sup>). The latter group includes the GABA<sub>A</sub> and glycine receptors, and the former includes the nAChR and 5-HT<sub>3</sub> receptors. More distantly related are the family of glutamate receptors that appear to have evolved from a separate

ancestral gene. Overall, one can begin to appreciate that structural similarities at the amino acid sequence level can predict a degree of functional similarity. Each of these receptor types is described in the next section.

### Neuronal nAChRs Contain Two Types of Subunits

Structurally, neuronal nAChRs are similar to, yet distinct from, the *Torpedo* isoform of the receptor (Fig. 10.10 and Fig. 10.11). For example, the neuronal nAChR appears to have only two types of subunits,  $\alpha$  and  $\beta$ , that combine to produce the functional receptor, and the majority of these receptors do not bind to  $\alpha$ -bungarotoxin. At least nine different  $\beta$  subtypes ( $\alpha 1$  being the muscle  $\alpha$  subunit) have been identified, and some are species specific ( $\alpha 8$  is found only in chicken, and  $\alpha 9$  is found only in rat). Four different  $\beta$  subtypes ( $\beta 1$  being the muscle  $\beta$  subunit) have been identified. The neuronal  $\beta$  subunits are not closely related to the muscle  $\beta 1$  subunit and are sometimes referred to simply as non- $\alpha$  subunits. One structural feature that distinguishes neuronal  $\alpha$  subunits



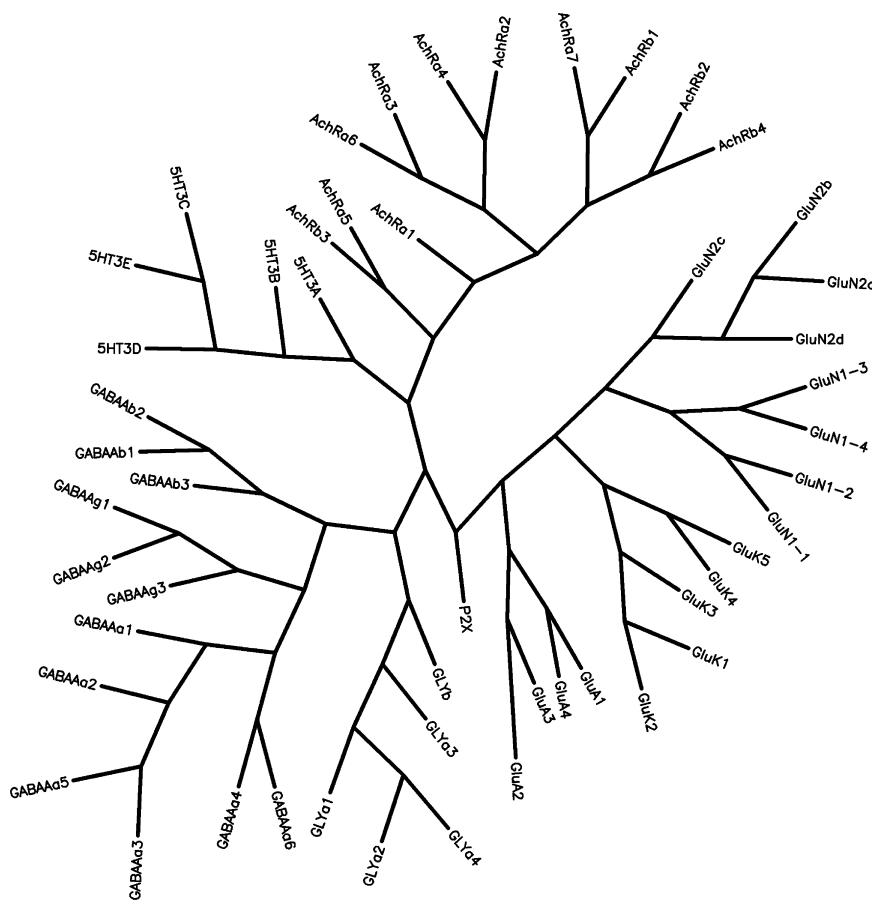
**FIGURE 10.9** Diagram of nAChR clustering at the neuromuscular junction. Rapsyn is a major anchoring protein at the neuromuscular junction that binds to itself and to the nAChR that concentrates and stabilizes nAChRs. The development and stabilization of the neuromuscular junction is mediated by a number of signaling cascades, only a few of which are shown. For example, agrin released from the presynaptic motor neuron binds to a number of proteins associated with the postsynaptic membrane including the tyrosine kinase MuSK (muscle-specific kinase). MuSK activation by agrin recruits and activates the soluble tyrosine kinases Src and Fyn, which further modify a number of proteins. RATL (rapsyn-associated linker protein) is a membrane-bound protein that binds to both MuSK and to rapsyn to anchor MuSK at the neuromuscular junction. Agrin also interacts with the dystroglycans that make up the dystrophin complex important for the maintenance of the neuromuscular junction. Rapsyn also binds to the utrophin complex that anchors the overlying protein complex to the actin cytoskeleton. Adapted from Willmann and Fuhrer (2002).

from  $\beta$  subunits is the presence of particular Cys residues in the extracellular domain. Two of these Cys residues are adjacent to one another in the  $\alpha$  subunit and form a disulfide bond. The  $\beta$  subunits do not have these adjacent Cys residues. Because these Cys residues are critical for ACh binding, the  $\alpha$  subunits of neuronal AChRs, like muscle  $\alpha$  subunits, contain the main contact points for ACh binding (Fig. 10.11). All the  $\alpha$  and  $\beta$  genes encode proteins with four transmembrane-spanning segments (TM1–TM4). Although the physical structure of the neuronal AChR has not been well characterized, it appears that each functional receptor is a pentameric assembly.

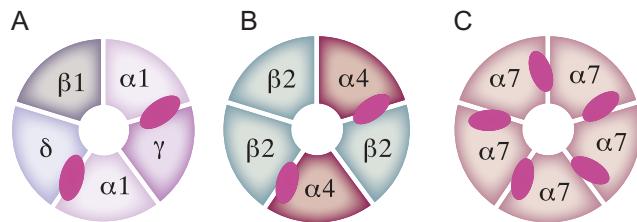
## Structural Diversity of Neuronal nAChRs Produces Channels with Unique Properties

Neuronal nAChRs have diverse functions and are the receptors responsible for the psychophysical effects of nicotine. One major function of nAChRs in the brain is to modulate excitatory synaptic transmission through a presynaptic action (McGehee et al., 1995). The diversity in function can be related to the heterogeneous structure contributed by the thousands of possible combinations between the different  $\alpha$  and  $\beta$  subunits. Control mechanisms for receptor assembly in neurons are not as stringent as those of nAChR in *Torpedo* and muscle. Functional neuronal nAChRs can be assembled from a single subunit (as in  $\alpha 7$ ,  $\alpha 8$ , and  $\alpha 9$ ), and a single type of  $\alpha$  subunit can be assembled with multiple types of  $\beta$  subunits (e.g.,  $\alpha 3$  with  $\beta 2$  or  $\beta 4$  or both) and vice versa (Fig. 10.11). These additional possibilities produce a staggering array of potential receptor molecules, each with distinct properties including differences in single-channel kinetics and rates of desensitization. This type of diversity is not unique to neuronal nAChRs. For most receptor classes studied in detail, diversity is the rule and not the exception. Unique subunit composition may also play roles in targeting the receptors to different intracellular locations.

Neuronal nAChRs exhibit a range of single-channel conductances between 5 and 50 pS, depending on the tissue analyzed or the specific subunits expressed. Most, but not all, are blocked by neuronal bungarotoxin, a snake venom distinct from  $\alpha$ -bungarotoxin. All the neuronal nAChRs are cation-permeable channels that, in addition to permitting the influx of  $\text{Na}^+$  and the efflux of  $\text{K}^+$ , permit an influx of  $\text{Ca}^{2+}$ . This  $\text{Ca}^{2+}$  permeability is greater than that for muscle nAChR (Vernino et al., 1992) and is variable among the different neuronal receptor subtypes. Indeed, some receptors have very high  $\text{Ca}^{2+}$ – $\text{Na}^+$  permeability ratios; for example,  $\alpha 7$  nAChRs exhibit a  $\text{Ca}^{2+}$ – $\text{Na}^+$  permeability ratio of nearly 20 (Seguela et al., 1993). The  $\text{Ca}^{2+}$  permeability of the  $\alpha 7$  nAChR can be eliminated by the mutation of a single amino acid residue in the second transmembrane domain (Glu-237 for Ala) without significantly affecting other aspects of the receptor (Bertrand et al., 1993). This key Glu residue must lie within the pore of the receptor and presumably enhances the passage of  $\text{Ca}^{2+}$  ions through an interaction with its negatively charged side chain. Activation of  $\alpha 7$  receptors through the binding of ACh could therefore produce a significant increase in the level of intracellular  $\text{Ca}^{2+}$  without the opening of voltage-gated  $\text{Ca}^{2+}$  channels. Subunits  $\alpha 7$ ,  $\alpha 8$ , and  $\alpha 9$  are also the  $\alpha$ -bungarotoxin-binding subtypes of neuronal nAChRs. Other neuronal isoforms exhibit  $\text{Ca}^{2+}$ – $\text{Na}^+$  permeability ratios of about 1.0–1.5.



**FIGURE 10.10** Evolutionary relationships of the ionotropic receptor family. The tree was constructed by aligning the protein sequences from each receptor family with the ClustalW program. Based on the alignment, the phylogenetic relationship was inferred with the maximum parsimony method and the tree was constructed using the Phylogeny Inference Package v3.6 (distributed by J. Felsenstein, Department of Genome Sciences, University of Washington, Seattle, WA). Dr. Yin Liu (Department of Neurobiology and Anatomy, University of Texas Health Science Center-Houston, Houston, TX) kindly provided the phylogenetic tree and figure.



**FIGURE 10.11** Diagrams of top-down views of AChR from muscle (A), one of the neuronal AChRs composed of  $\alpha_2$  and  $\beta_4$  subunits (B), and the homomeric form of neuronal AChR produced by assembly of  $\alpha_7$  subunits (C). Purple ovals represent the ACh binding sites on each receptor complex. The ACh receptors from brain are diverse in both structure and properties due to the variety of different receptor complexes produced from subunit mixing.

### Neuronal nAChRs Desensitize Rapidly

For the nAChR from muscle, desensitization is minor and probably not physiologically significant in determining the shape of the synaptic response at the neuromuscular junction. However, for some neuronal nAChRs, desensitization plays a major role in determining the effects of the actions of ACh. Receptors composed of  $\alpha_7$ ,  $\alpha_8$ , and certain  $\alpha-\beta$  combinations exhibit desensitization time constants between 100 and 500 ms, whereas others

exhibit desensitization constants between 2 and 20 s. Given the diverse functions of neuronal nAChRs, the variable rates of desensitization likely play important roles whereby this inherent property of the receptor shapes the physiological response generated from binding ACh.

### One Serotonin Receptor Subtype, 5-HT<sub>3</sub>, is Ionotropic and is a Close Relative of the nAChR

Serotonin (5-hydroxytryptamine, 5-HT) is historically thought of as a transmitter that binds to and activates GPCRs (described in more detail later). The 5-HT<sub>3</sub> subclass is an exception forming an ionotropic receptor activated by binding serotonin that is evolutionarily related to the nAChR (Fig. 10.10). The 5-HT<sub>3</sub> receptor is permeable to Na<sup>+</sup> and K<sup>+</sup> ions and is similar in many ways to the nAChR in that both desensitize rapidly and are blocked by tubocurarine. From expression studies of the cloned cDNA (Maricq et al., 1991), it appears that the 5-HT<sub>3</sub> receptor is a homomeric complex composed of five copies of the same subunit. The deduced amino acid sequence of the cDNA indicates that the protein is 487 amino acids long (56 kDa) and has a structure most

analogous to the  $\alpha 7$  subtype of neuronal nAChRs, which also forms a homo-oligomeric receptor.

The 5-HT<sub>3</sub> receptor is mostly impermeable to divalent cations. For example, Ca<sup>2+</sup> is largely excluded from permeation and, in fact, effectively blocks current flow through the pore, even though the predicted pore size of the channel (7.6 Å) is approximately the same as that for the nAChR (8.4 Å). Apparently, other physical or electrochemical barriers limit the capacity of divalent ions to permeate the 5-HT<sub>3</sub> pore. Dose-response studies indicate that at least two ligand binding sites must be occupied for the channel to open; however, the binding of agonist and/or opening of the channel appears to be approximately 10 times slower than for most other ligand-gated ion channels. The functional significance or physical explanation of this slow opening is not known. The native 5-HT<sub>3</sub> receptor also exhibits desensitization (time constant 1–5 s), although the rate varies widely, depending on the methodology used for analysis and the source of receptor. Interestingly, this desensitization can be significantly slowed or enhanced by single amino acid substitutions at a Leu residue in the TM2 transmembrane-spanning segment of the subunit (Yakel et al., 1993).

The 5-HT<sub>3</sub> receptors are sparsely distributed on primary sensory nerve endings in the periphery and widely distributed at low concentrations in the mammalian CNS. The 5-HT<sub>3</sub> receptor is clinically significant because antagonists of 5-HT<sub>3</sub> receptors have important applications as antiemetics, anxiolytics, and antipsychotics.

### GABA<sub>A</sub> Receptors are Related in Structure to the nAChR but Exhibit an Inhibitory Function

Synaptic inhibition in the mammalian brain is mediated principally by GABA receptors. The most widespread ionotropic receptor activated by GABA is designated GABA<sub>A</sub>. The subunits composing the GABA<sub>A</sub> receptor have sequence homology with the nAChR subunit family, and the two families have presumably diverged from a common ancestral gene. In fact, the general structures of the two receptors appear to be quite similar. The GABA<sub>A</sub> receptor is composed of multiple subunits, forming a heteropentameric complex of approximately 275 kDa. Five different types of subunits are associated with GABA<sub>A</sub> receptors and are designated  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , and  $\epsilon$ . An additional subunit,  $\rho$ , is found predominantly in the retina, whereas the other subunits are widely distributed in the brain. Each subunit group also has different subtypes; for example, six different  $\alpha$ , four  $\beta$ , four  $\gamma$ , and two other  $\rho$  subunits have been identified. The predicted amino acid sequences indicate that each of these subunits has a molecular mass ranging between 48 and 64 kDa. Like

neuronal nAChR, these subunits mix in a heterogeneous fashion to produce a wide array of GABA<sub>A</sub> receptors with different pharmacological and electrophysiological properties. The predominant GABA<sub>A</sub> receptor in brain and spinal cord is  $\alpha 1$ ,  $\beta 2$ , and  $\gamma 2$ , with a likely stoichiometry of two  $\alpha 1$ s, two  $\beta 2$ s, and one  $\gamma 2$ . Expression of subunit cDNAs in oocytes indicates that the  $\alpha$  subunit is essential for producing a functional channel. The  $\alpha$  subunit also appears to contain the high-affinity binding site for GABA (Sieghart, 1992).

The ion channel associated with the GABA<sub>A</sub> receptor is selective for anions (in particular, Cl<sup>-</sup>), and the selectivity is provided by strategically placed positively charged amino acids near the ends of the ion channel (Barnard et al., 1988). When GABA binds to and activates this receptor, Cl<sup>-</sup> flows into the cell, producing a hyperpolarization by moving the membrane potential away from the threshold for firing an action potential. The neuronal GABA<sub>A</sub> receptor exhibits multiple conductance levels, with the predominant conductance being 27–30 pS. Measurements and modeling of single-channel kinetics suggest that two sequential binding sites exist for anions within the pore (Bormann, 1988).

### The GABA<sub>A</sub> Receptor Binds Several Compounds That Affect its Properties

The GABA<sub>A</sub> receptor is an allosteric protein, its properties being modulated by the binding of a number of compounds. Two well-studied examples are barbiturates and benzodiazepines, both of which bind to the GABA<sub>A</sub> receptor and potentiate GABA binding. The net result is that in the presence of barbiturates or benzodiazepines or both, the same concentration of GABA will increase inhibition. Benzodiazepine binding is conferred on the receptor by the  $\gamma$  subunit (Pritchett et al., 1989), but the presence of the  $\alpha$  and  $\beta$  subunits is necessary for the qualitative and quantitative aspects of benzodiazepine binding. The benzodiazepine binding site appears to lie along the interface between the  $\alpha$  and  $\gamma$  subunits, and only certain subtypes are sensitive to benzodiazepines. Benzodiazepine binding to GABA<sub>A</sub> receptors requires  $\alpha 1$ ,  $\alpha 2$ , or  $\alpha 5$  and  $\gamma 2$  or  $\gamma 3$ ; other subunit combinations are insensitive to benzodiazepines (Rudolph et al., 1999).

Picrotoxin, a potent convulsant compound, appears to bind within the channel pore of the GABA<sub>A</sub> receptor and prevent ion flow (Sieghart, 1992). Single-channel experiments indicate that picrotoxin either slowly blocks an open channel or prevents the GABA receptor from undergoing a transition into a long-duration open state. Apparently, barbiturates produce similar changes in channel properties, but they potentiate rather than inhibit GABA<sub>A</sub> receptor function. Bicuculline, another potent convulsant, appears to inhibit GABA<sub>A</sub> receptor

channel activity by decreasing the binding of GABA to the receptor. Steroid metabolites of progesterone, corticosterone, and testosterone also appear to have potentiating effects on GABA currents that are similar in many ways to the action of barbiturates; however, the binding sites for these steroids and the barbiturates are distinct. Finally, penicillin directly inhibits GABA receptor function, apparently by binding within the pore and thus being designated an open channel blocker. The physiological effects of compounds such as picrotoxin, bicuculline, and penicillin are striking. Each of these compounds at a sufficiently high concentration can produce widespread and sustained seizure activity. Conversely, many, but not all, of the sedative properties associated with barbiturates and benzodiazepines can be attributed to their ability to augment inhibition in the brain by enhancing GABA's inhibitory potency.

Interestingly,  $\rho$ -subunit-containing GABA receptors, found in abundance in the retina, are pharmacologically unique. They are resistant to bicuculline's inhibitory action, although they remain sensitive to blockage by picrotoxin. In addition, these retinal receptors are not sensitive to modulation by barbiturates or benzodiazepines. Thus,  $\rho$  subunit-containing receptors are distinct from GABA<sub>A</sub> receptors and similar to receptors earlier designated GABA<sub>C</sub> (Bormann and Feigenspan, 1995).

### Glycine Receptors are Closely Related to GABA<sub>A</sub> Receptors

Glycine receptors are the major inhibitory receptors in the spinal cord (Betz, 1991), although they also are present within the brain, particularly in the brain stem, and provide inhibitory functions similar to the GABA<sub>A</sub> receptor. Glycine and GABA<sub>A</sub> receptors both open ion channels selectively permeable to the anion Cl<sup>-</sup> and are evolutionarily related (see Fig. 10.10). The structure of the glycine receptor is indicative of this similarity in properties. The native complex is approximately 250 kDa and is composed of two main subunits,  $\alpha$  (48 kDa) and  $\beta$  (58 kDa). The receptor appears to be pentameric, most likely composed of three  $\alpha$  and two  $\beta$  subunits. Apparently, three molecules of glycine must bind to the receptor to open it to ion flow (Young and Snyder, 1974), suggesting that the  $\alpha$  subunit contains the glycine binding site. The glycine receptor has an open-channel conductance of approximately 35–50 pS, similar to that of the GABA<sub>A</sub> receptor. A potent antagonist of the glycine receptor is the compound strychnine.

Four distinct  $\alpha$  subunits and one  $\beta$  subunit of the glycine receptor have been cloned. Each exhibits the typical predicted four transmembrane segments, and they are approximately 50% identical with one another at the amino acid level. Expression of a single  $\alpha$  subunit in

oocytes is sufficient to produce functional glycine receptors, indicating that the  $\alpha$  subunit is the pore-forming unit of the native receptor. The  $\beta$  subunits play exclusively modulatory roles, affecting, for example, sensitivity to the inhibitory actions of picrotoxin.  $\beta$  subunits are widespread in the brain, and their distribution does not specifically colocalize with glycine receptor  $\alpha$ -subunit mRNA. The  $\beta$  subunits may serve other functions independent of their association with glycine receptor.

### Clustering of GABA<sub>A</sub> and Glycine Receptors

During maturation of inhibitory synapses, gephyrin clusters beneath the postsynaptic membrane and recruits and localizes GABA<sub>A</sub> or glycine receptors. Gephyrin appears to serve an analogous function for the stabilization of inhibitory receptors that rapsyn plays for nAChRs. Gephyrin is a multidomain protein that interacts with the cytoplasmic domains of the GABA<sub>A</sub> or glycine receptor subunits (Moss and Smart, 2001, Saiyed et al., 2007). Like rapsyn, gephyrin has the capacity to interact with itself and other proteins in addition to the receptors and these interactions serve to restrict the lateral mobility of GABA<sub>A</sub> and glycine receptors in the plasma membrane. Ultimately these interactions promote the formation of postsynaptic inhibitory specializations.

### Certain Purinergic Receptors are Also Ionotropic

Purinergic chemical transmission is distributed throughout the body, and the receptor subtypes and myriad effects are considered in greater detail in the later section on GPCRs. Purinergic receptors bind to ATP (or other nucleotide analogs) or its breakdown product adenosine. ATP is released from certain synaptic terminals in a quantal manner and often packaged within synaptic vesicles containing another neurotransmitter, the best described being acetylcholine and the catecholamines.

Two subtypes of ATP-binding purinergic receptors (P2x and P2z) were discovered to be ionotropic receptors and, interestingly from an evolutionary perspective, they lie between the anion permeable receptors (GABA<sub>A</sub> and glycine) and the glutamate receptors (Fig. 10.10). P2x receptors appear to mediate a fast depolarizing response in neurons and muscle cells to ATP by the direct opening of a nonselective cation channel. cDNAs encoding the P2x receptor indicate that its structure comprises only two transmembrane domains, with some homology in its pore-forming region with K<sup>+</sup> channels (Brake et al., 1994; Valera et al., 1994). The crystal structure of the P2x receptor reveals that it has a

unique architecture (Hattori and Gouaux, 2012). Each subunit contributes two TM domains and three subunits congregate together to form the native receptor. ATP binding causes the expansion of the pore region in an iris-like manner by twisting of the TM helices to allow permeation of cations (Hattori and Gouaux, 2012). The P2z receptor also is a ligand-gated channel that permits permeation of either anions or cations and even molecules as large as 900 Da.

### Glutamate Receptors are Derived from a Different Ancestral Gene and are Structurally Distinct from Other Ionotropic Receptors

Glutamate receptors are widespread in the nervous system, where they are responsible for mediating the vast majority of excitatory synaptic transmission in the brain and spinal cord. Early studies suggested that the glutamate receptor family was composed of several distinct subtypes. In the 1970s, Jeffrey Watkins and his colleagues significantly advanced this field by developing agonists that could pharmacologically distinguish between different glutamate receptor subtypes. Four of these agonists—*N*-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA), kainate, and quisqualate—are distinct in the type of receptors to which they bind and have been used extensively to characterize the glutamate receptor family (Hollmann and Heinemann, 1994; Watkins et al., 1990). A convenient distinction for describing the ionotropic glutamate receptors has been to classify them as either NMDA or non-NMDA subtypes, depending on whether they bind the agonist NMDA. Non-NMDA receptors also bind the agonist kainate or AMPA. Both NMDA and non-NMDA receptors are ionotropic. Quisqualate is unique within this group in having the capacity to activate both ionotropic and GPCR glutamate receptor subtypes (Hollmann and Heinemann, 1994). The nomenclature describing members of the glutamate receptor family has undergone transitions over the years but appears to have converged; receptors binding AMPA are termed GluA1-GluA4, those binding kainate are GluK1-GluK5, those binding NMDA are GluN1, GluN2A-GluN2D, and GluN3A-GluN3B and those that activate G-proteins are designated mGluR1-mGluR8 (Traynelis et al., 2010). The evolutionary relationships of the ionotropic glutamate receptors with each other and with other members of the ionotropic receptor family are shown in Figure 10.10.

### Non-NMDA Receptors are a Diverse Family

In 1989, Stephen Heinemann and his colleagues reported the isolation of a cDNA that produced a

functional glutamate-activated channel when expressed in *Xenopus* oocytes (Hollmann et al., 1989). The initial glutamate receptor was termed GluR-K1, and the cDNA encoded a protein with an estimated molecular mass of 99.8 kDa. Not long after this original report, Heinemann's group (Boulter et al., 1990), Peter Seuberg's group (Keinanen et al., 1990), and Richard Axel's group (Nakanishi et al., 1990) independently reported the isolation of families of glutamate receptor subunits, termed GluR<sub>1</sub>-GluR<sub>4</sub> by Heinemann's group and GluRA–GluRD by Seuberg's group. Each GluR subunit consists of approximately 900 amino acids and has four predicted membrane-spanning segments (TM1–TM4). However, there is an important distinction in the TM2 domain making the GluRs distinct from the nAChR family. Native GluRs are tetrameric with an approximate molecular mass of 600 kDa (Blackstone et al., 1992; Wenthold et al., 1992) making them almost twice the size of nAChR, mostly because of the large extracellular domain where glutamate binds.

### Unique Properties of Non-NMDA Receptors are Determined by Assembly of Different Subunits

When the cDNAs encoding these receptors were expressed in either oocytes or HeK-293 cells, application of the non-NMDA receptor agonist AMPA produced substantial inward currents. In these same experiments, the agonist kainate was demonstrated to produce larger currents, mainly because of rapid and significant desensitization of the receptor when AMPA was used as the agonist. A striking observation was made from these expression studies. When the GluA2 subunit alone was expressed in the oocytes, little current was obtained when the preparation was exposed to agonist, unlike the large currents found when either GluA1 or GluA3 was expressed (Boulter et al., 1990; Nakanishi et al., 1990; Verdoorn et al., 1991). GluA2 subunits by themselves appear to form poorly conducting receptors. However, when GluA2 is expressed with either GluA1 or GluA3, the behavior of the heteromeric receptor is distinctly different. Current–voltage relationships (i.e., *I/V* plots) indicate that when GluA1 and GluA3 are expressed alone or together, they produce channels with strong inward rectification. Coexpression of GluA2 with either GluA1 or GluA3 produces channels with little rectification and near-linear *I/V* plots. Further analyses (Hollmann and Heinemann, 1994) indicated that GluA1 and GluA3, either independently or when coexpressed, exhibited channels permeable to Ca<sup>2+</sup>. In contrast, any combination of receptor that included the GluA2 subunit produced channels impermeable to Ca<sup>2+</sup>. Clearly,

the properties of glutamate receptors can be quite different and initiate unique intracellular responses, depending on the subunit composition expressed in a particular neuron. The replacement of a single amino acid (Arg for Gln) in the second transmembrane region of the GluA2 subunit (see Fig. 10.13B for identification of this amino acid) was shown to switch its behavior from a non- $\text{Ca}^{2+}$ -permeable to a  $\text{Ca}^{2+}$ -permeable channel (Hume et al., 1991; Burnashev et al., 1992). Apparently, an Arg at this position blocks  $\text{Ca}^{2+}$  from traversing the pore formed in the center of the GluR channel.

### Functional Diversity in GluRs is Produced by mRNA Splicing and RNA Editing

Analysis of the mRNAs encoding GluR subunits indicated that each could be expressed in one of two splice variants, termed *flip* and *flop* (Sommer et al., 1990). These flip and flop modules are small (38 amino acid) segments just preceding the TM4 transmembrane domain in all four GluR subunits. The receptor channel expressed from these splice variants has distinct properties, depending on which of the two modules is present. The flop-containing receptors exhibit significantly greater magnitudes of desensitization during glutamate application. Therefore, GluRs with flop modules express smaller steady-state currents than GluRs with flip modules. Both flip- and flop-containing GluRs are widely expressed in the brain with a few exceptions. One unique cell type appears to be pyramidal CA3 cells in the rat hippocampus, where the GluRs are deficient in flop modules. In neighboring CA1 pyramidal cells and dentate granule cells, flop-containing GluRs appear to dominate. The significance of these splice variations for information processing in the brain is not known, but the physiological prediction would be that CA3 neurons exhibit larger steady-state glutamate-activated currents owing to decreased desensitization from the absence of flop modules.

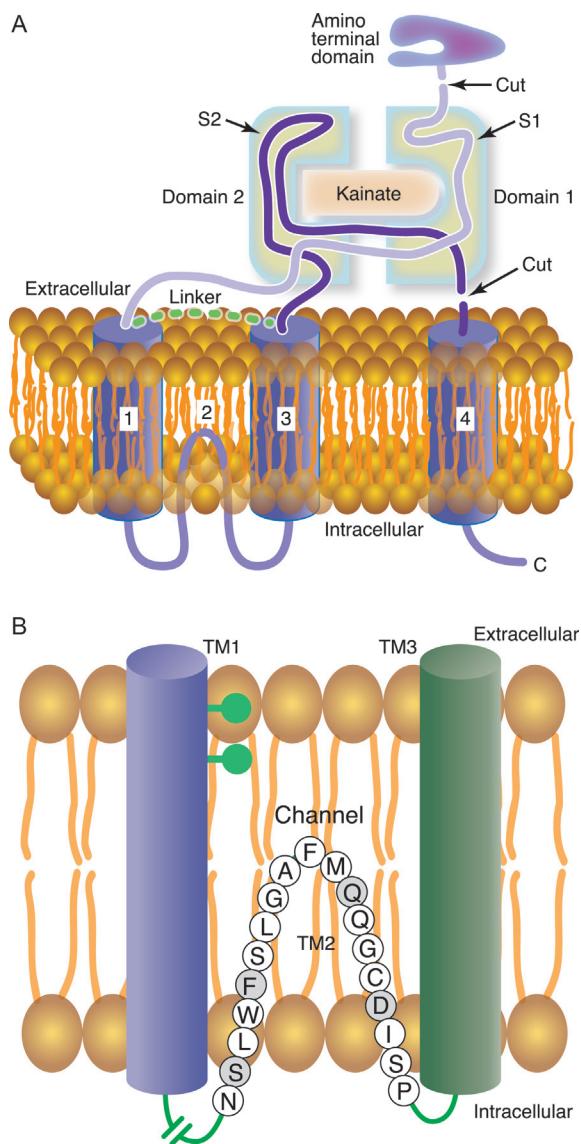
Typically, there is fidelity in the process of transcribing DNA into mRNA and then into protein so that the nucleotides present in the DNA are accurate predictors of the amino acid sequence of the protein. However, Peter Seuberg and his colleagues discovered a novel mechanism in the neuronal nucleus that edits mRNAs posttranscriptionally, and at least three of the four GluR subunits are subjected to this editing mechanism (Sommer et al., 1991). In fact, one of the sites edited is the critical Arg residue regulating  $\text{Ca}^{2+}$  permeability in the GluA2 subunit. At another edited site, Gly replaces Arg-764 in the GluA2 subunit, and this editing also takes place in GluA3 and GluA4. The Arg-to-Gly conversion at amino acid 764 produces

receptors that exhibit significantly faster rates of recovery from the desensitized state (Lomeli et al., 1994). The extent to which other receptors or other protein molecules undergo this form of RNA editing is an area rich for investigation. At a minimum, this editing mechanism produces dramatic differences in the function of GluRs.

### Glutamate Receptors Do Not Conform to the Typical Four Transmembrane-Spanning Segment Structures Described for the nAChR

The field of glutamate receptor structure/function is advancing at a rapid pace fostered by the crystallization of the GluA2 receptor (Sobolevsky et al., 2009) and report of the 3D structure of the native GluRs determined by electron microscopy reconstructions (Nakagawa et al., 2005). These studies reveal the overall architecture of the GluR complex and provide some global insights into how glutamate binding leads to channel opening. These models can be additionally refined by the significant amount of work analyzing crystal structures of the isolated GluR ligand-binding domain with and without the presence of bound agonists and antagonists (see Traynelis et al., 2010 and Kumar and Mayer, 2013 for review).

The GluR has a large extracellular domain composed of the amino-terminal domain (ATD) important for receptor assembly and trafficking and the ligand-binding domain (LBD) that serves as the binding site for glutamate (Fig. 10.12A). These domains form a structurally unique dimer of dimer arrangement (Fig. 10.13) in the extracellular domain that transitions into a four-fold symmetry of the pore forming region of the TM domains. Thus, GluRs are tetramers. Unlike the TM segments of the nAChR that each completely traverses the membrane, the TM2 segment of GluRs forms a re-entrant loop within the membrane (Fig. 10.12A and 10.12B) and enters back into the cytoplasm, similar in some ways to the pore-forming domain (P segment) of voltage-activated  $\text{K}^+$  channels. An enlargement of this TM2 segment (Fig. 10.6B) highlights the amino acids conserved in the GluRs and further shows the positions of the critical Gln residue responsible for  $\text{Ca}^{2+}$  permeability of the receptor. Like many ionotropic receptors, the GluR family also undergoes transitions into a closed (desensitized) state in the continued presences of glutamate (Fig. 10.13). As discussed above, desensitization is regulated by the specific subunits incorporated into the receptor and plays an important role in shaping the electrophysiological response to glutamate release at synapses.



**FIGURE 10.12** (A) Model of one of the subunits of the ionotropic glutamate receptor. The ionotropic glutamate receptors have four membrane-associated segments; however, unlike nAChR, only three of them completely traverse the lipid bilayer. TM2 forms a loop and re-enters into the cytoplasm. Thus, the large N-terminal region extends into the extracellular space, while the C terminus extends into the cytoplasm. Two domains in the extracellular segments associate with each other to form the binding site for transmitter, in this example kainate, a naturally occurring agonist of glutamate. (B) Enlarged area of the predicted structure and amino acid sequence of the TM2 region of the glutamate receptor, GluR<sub>3</sub>. TM1 and TM3 are drawn as cylinders in the membrane flanking TM2. The residue that determines the Ca<sup>2+</sup> permeability of the non-NMDA receptor is the glutamine residue (Q) highlighted in gray. In NMDA receptors, an asparagine residue at this same position is the proposed site of interaction with Mg<sup>2+</sup> ions that produce the voltage-dependent channel block. The serine (S) and phenylalanine (F) also shaded in gray are highly conserved in the non-NMDA receptor family. The aspartate (D) residue is also conserved and thought to form part of the internal cation binding site. The break in the loop between TM1 and TM2 indicates a domain that varies in length among ionotropic glutamate receptors. Adapted from Wo and Oswald (1995).

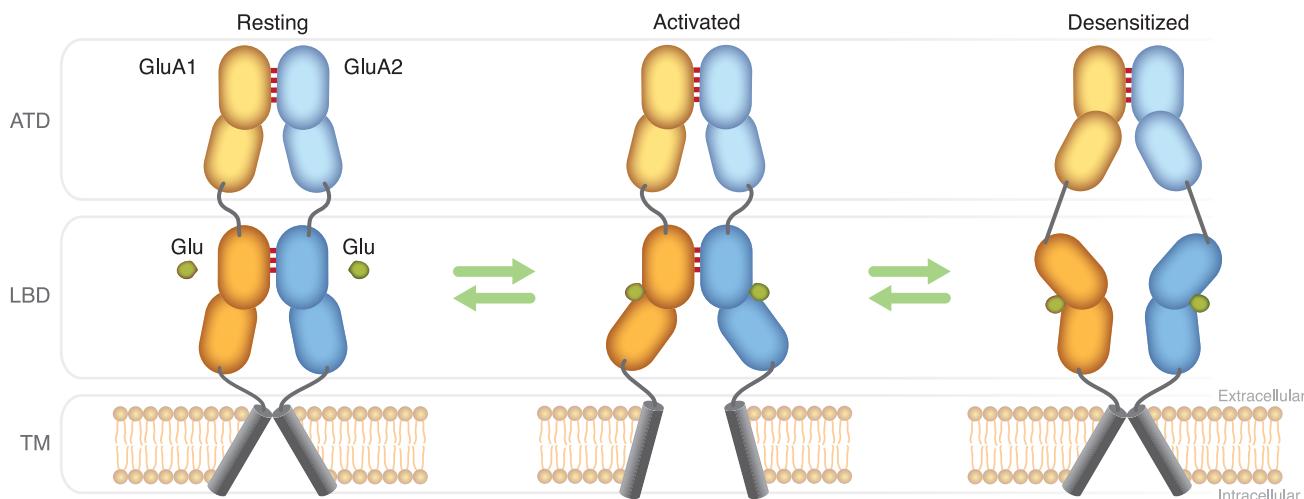
### Other Non-NMDA GluRs have Poorly Characterized Functions

GluK1-GluK5 subunits form a second non-NMDA receptor subfamily (Fig. 10.10) that is activated by the agonist kainate but whose contribution to producing functionally distinct receptors is less well-understood (Traynelis et al., 2010). Their overall structure is similar to that of GluA1–4, and they exhibit about 40% sequence homology; however, their agonist-binding profile and their electrophysiological properties are distinct. GluK1 and GluK2 can form either homo- or heterotetramers; however, GluK4 and GluK5 require coexpression with one of the GluK1-GluK3 family members. They are generally expressed at lower levels in the brain than the GluA1–4 subtypes (Hollmann and Heinemann, 1994). GluK4 is expressed at significant concentrations in only two cell types, hippocampal CA3 and dentate granule cells. GluK5 exhibits distinct properties when combined with other GluR subunits. For example, coexpression of GluK2 and GluK5 produces functional receptors that respond to AMPA, although neither subunit itself responds to this agonist.

Another GluR class includes GluD1 and GluD2 receptors that can form homotetrameric receptors, but do not seem to form heteromers with other GluRs. They are also resistant to activation by all known agonists capable of activating other members of the GluR family (Traynelis et al., 2010). Their physiological role remains poorly characterized.

### The NMDA Receptors are a Family of Ligand-Gated Ion Channels that are Also Voltage Dependent

NMDA receptors appear to be at least partly responsible for aspects of development, learning, and memory and neuronal damage due to brain injury. The particular significance of this receptor to neuronal function comes from two of its unique properties. First, the receptor exhibits associativity, defined in this case as the need for the receptor to have glutamate bound and the membrane be depolarized for the pore to open to let ions flow. This behavior is due to a Mg<sup>2+</sup>-dependent block of the receptor at normal membrane resting potentials (Ascher and Nowak, 1988, Mayer and Westbrook, 1987) and gives rise to the dramatic voltage dependence of the channel. Second, the receptor permits a significant influx of Ca<sup>2+</sup>, and increases in intracellular Ca<sup>2+</sup> activate a variety of processes that alter the properties of the neuron. Excess Ca<sup>2+</sup> is also toxic to neurons, and the hyperactivation of NMDA receptors is thought to contribute to a variety of neurodegenerative disorders.



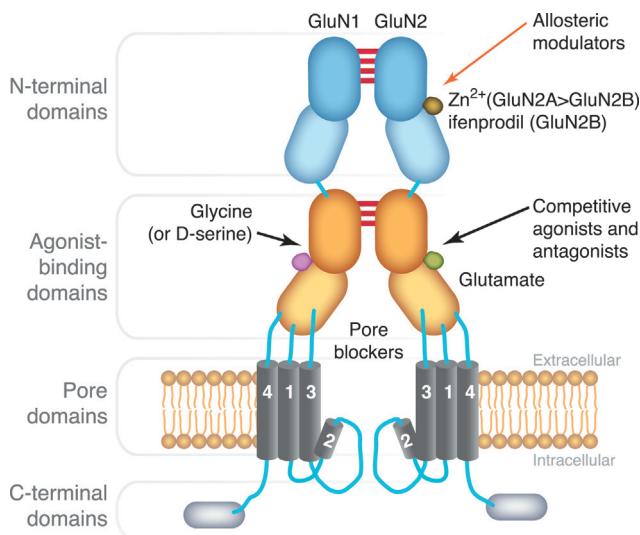
**FIGURE 10.13** Diagram of the ionotropic glutamate receptor highlighting the amino-terminal domain (ATD), the ligand binding domain (LBD) and the transmembrane domains (TM). Only two of the four subunits that compose each receptor are shown for simplicity. Note, as highlighted in Fig. 10.12, that the second of four transmembrane domains (not detailed here) in each subunit forms a reentrant loop. When glutamate binds to the LBDs, a conformational change ensues leading to the opening of the pore that lies within the TM domains (activated). In the continued presence of glutamate, the receptor can undergo additional conformational changes that break the dimer interface, closing the ion pore (desensitized). Adapted from *Kumar and Mayer* (2013).

Many pharmacological compounds produce their effects through interactions with the NMDA receptor. For example, certain hallucinogenic compounds, such as phencyclidine (PCP) and dizocilpine (MK-801), are effective blockers of the ion channel integral to the NMDA receptor (Pore blockers; Fig. 10.14). These potent antagonists require the receptor channel to be open to gain access to their binding sites and are therefore referred to as open-channel blockers. They also become trapped when the channel closes and are therefore difficult to wash out of the NMDA receptor's channel. Competitive antagonists for the glutamate-binding site (Fig. 10.14) also have been developed, and some of the best-known are AP-5 and AP-7. These and other antagonists specific for the glutamate binding site also produce hallucinogenic effects in both animal models and humans. NMDA remains a specific agonist for this receptor; however, it is about one order of magnitude less potent than L-glutamate for receptor activation. L-glutamate is the predominant neurotransmitter that activates the NMDA receptor; however, L-aspartate can also activate the receptor, as can an endogenous dipeptide in the brain, N-acetylaspartylglutamate (Hollmann and Heinemann, 1994). Opening of the NMDA receptor also requires that a site on the GluN1 subunit be bound with glycine (Fig. 10.14). The level of glycine found in the extracellular space normally is sufficient to occupy this site.

### NMDA Receptor Subunits Show Similarity to Non-NMDA Receptor Subunits

The primary structure of the NMDA receptor was revealed in 1990 when Nakanishi and his colleagues isolated the first cDNA encoding a subunit of the NMDA receptor (Moriyoshi et al., 1991). The first cloned subunit was aptly named NMDAR<sub>1</sub> (now termed GluN1), and the deduced amino acid sequence indicated a protein of approximately 97 kDa, similar to other members of the GluR family. Four potential transmembrane domains were identified, and the current assumption is that four individual subunits compose the macromolecular NMDA receptor complex. However, recall that the transmembrane organization of GluR subunits indicates that TM2 does not fully traverse the membrane and instead forms a re-entrant loop. NMDA receptor subunits also conform to this model (Fig. 10.14). The TM2 segment of each subunit clearly lines the pore of the NMDA receptor, as does the TM2 segment of the GluR subunits. In fact, a single Asn residue, analogous to that in the GluA2 subunit, regulates the Ca<sup>2+</sup> permeability of the NMDA receptor (Burnashev et al., 1992, Mori et al., 1992). Mutation of this Asn residue markedly reduces Ca<sup>2+</sup> permeability.

Three of the best-characterized facets of the NMDA receptor were found when the GluN1 subunit was initially expressed by itself in oocytes, although currents



**FIGURE 10.14** Diagram of an NMDA receptor highlighting binding sites for numerous agonists, antagonists, and other regulatory molecules. The NMDA is a tetramer and two of the subunits (GluN1 and GluN2) of the complex are shown for simplicity. The N-terminal domains are the portions of the receptor extending furthest away from the membrane and include binding sites for a number of allosteric modulators. The agonist binding domains contain the glutamate and glycine binding sites; GluN1 and or GluN3 (not shown) subunits provide the glycine-binding site while the GluN2 subunit provides the glutamate-binding sites. The four transmembrane segments compose the pore domains and note that transmembrane segment 2 forms a reentrant loop typical of the glutamate receptor family. Molecules like Mg<sup>2+</sup>, and other pore blocking antagonists, bind to the pore to prevent ion flow. The location of these sites is a crude approximation for the purpose of discussion. Adapted from Paoletti and Neyton (2007).

were relatively small. These characteristics are (1) a Mg<sup>2+</sup>-dependent voltage-sensitive ion channel block, (2) a glycine requirement for effective channel opening, and (3) Ca<sup>2+</sup> permeability (Moriyoshi et al., 1991). As described later, other NMDAR subunits contribute to assembly of the receptors thought to exist in the nervous system. However, these initial expression studies were complicated by the apparent endogenous expression of other GluN-like subunits. No glutamate-stimulated current is found when GluN1 is expressed in mammalian cells devoid of other GluN subunits and the NMDAR is now recognized to exist in the nervous system as obligate heteromers of GluN1 mixed with other subunits (Traynelis et al., 2010).

Several other members of the NMDA receptor family have been cloned (GluN2A–2D and GluN3A and GluN3B), and their deduced primary structures are highly related to each other and to other GluRs (see Fig. 10.10). These GluN receptor subunits do not form channels when expressed singly or in combination unless they are co-expressed with GluN1 (Kutsuwada

et al., 1992; Meguro et al., 1992; Monyer et al., 1992). Apparently, GluN1 serves the essential role for the formation of a functional pore to permit the flow of ions. The native NMDA receptor is thought to be composed of two GluN1 subunits and either two GluN2 subunits or a combination of GluN2 and GluN3 subunits. The GluN1 or GluN3 subunits provide the glycine-binding site for the receptor, while the GluN2 subunits contain the glutamate binding sites (Fig. 10.14) (Traynelis et al., 2010). The C-terminal domains of GluN2A-GluN2D are quite large relative to the GluN1 C-terminus and appear to play roles in altering channel properties and in determining the subcellular localization of the receptors. All the NMDAR subunits have an Asn residue at the critical point in the TM2 domain essential for producing a receptor with Ca<sup>2+</sup> permeability. This Asn residue also appears to form at least part of the binding site for Mg<sup>2+</sup>, indicating that the sites for Mg<sup>2+</sup> binding and Ca<sup>2+</sup> permeation overlap (Burnashev et al., 1992; Mori et al., 1992).

The distribution of GluN2 subunits generally is more restricted than the homogeneous distribution of GluN1, with the exception of GluN2A, which is expressed throughout the nervous system. GluN2C is restricted mostly to cerebellar granule cells, whereas 2B and 2D exhibit broader distributions. As noted, the large size of the C terminus of the GluN2 subunit suggests a potential role in association with other proteins, possibly to target or restrict specific NMDA receptor types to areas of the neuron (see the following). Mechanisms related to receptor targeting now are becoming better understood and will play major roles in determining the efficacy of synaptic transmission (Ehlers et al., 1995; Kornau et al., 1995).

### Additional NMDA Receptor Diversity Occurs Through RNA Splicing

At least eight splice variants have now been identified for the GluN1 subunit and these variants produce differences, ranging from subtle to significant, in the properties of the expressed receptor (Hollmann and Heinemann, 1994; Traynelis et al.; 2010, Kumar and Mayer, 2013). For example, GluN receptors lacking a particular N-terminal insert, owing to alternative splicing, exhibit enhanced blockade by protons and exhibit responses that are potentiated by Zn<sup>2+</sup> in micromolar concentrations. Zn<sup>2+</sup> has classically been described as an allosteric regulator of the NMDA receptor that can significantly block its activation (Fig. 10.14). Clearly, the particular splice variant incorporated into the receptor complex affects the types of physiological

response generated. Spermine, a polyamine found in neurons and in the extracellular space, also slightly increases the amplitude of NMDA responses, and this modulatory effect also appears to be associated with a particular splice variant.

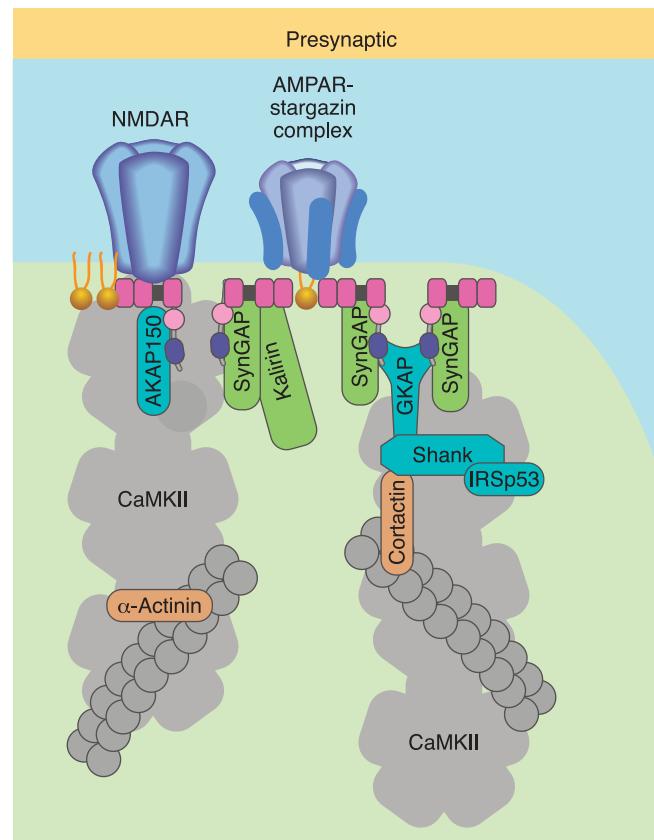
## NMDA Receptors Exhibit Complex Channel Properties

Based on the characteristics described above, one can predict that the biophysical properties of the NMDA receptor would be complex and this is the case (Mayer and Westbrook, 1987; Ascher and Nowak, 1988). The single-channel conductance has a main level of 50 pS; however, subconductances are evident, and different subunit combinations produce channels with distinct single-channel properties. A binding site for the  $\text{Ca}^{2+}$ -binding protein calmodulin has also been identified on the GluN1 subunit (Ehlers et al., 1996). Binding of  $\text{Ca}^{2+}$ -calmodulin to NMDA receptors produces a four-fold decrease in open-channel probability.  $\text{Ca}^{2+}$  influx through the NMDA receptor could induce calmodulin binding and lead to an immediate short-term feedback inhibition, decreasing ion flow through the receptor.

## Glutamate Receptors Cluster at Synapses

Glutamate receptors are concentrated at excitatory synapses through interactions with underlying scaffolding molecules (Fig. 10.15). These scaffolding molecules and a host of other structural and signaling proteins form an electron-dense structure at excitatory synapses called the *postsynaptic density* (PSD; see also Chapter 2). One of the best known of these scaffolds is PSD-95, a multidomain protein enriched at excitatory synapses that helps organize receptors and signaling molecules. Through one of its multiple PDZ domains, PSD-95 binds directly to the cytoplasmic domain of GluN2 subunits and anchors them tightly to the PSD (Sheng and Hoogenraad, 2007). In fact, biochemically isolated PSDs stripped of the overlying membrane retain GluNs, indicating the interaction is mediated by tight protein-protein binding. PSD-95 and GluNs are considered to be "core" PSD components, as their concentration is relatively consistent between PSDs (Swulius et al., 2010). A number of other PSD-enriched proteins have been identified to bind to the C-terminal domain of GluN receptor subunits, including the  $\text{Ca}^{2+}$ -calmodulin-activated protein kinase-CaMKII (Fig. 10.15).

GluA receptors do not interact directly with PSD-95, and their relative concentration in the PSD is dynamic. The up-and-down regulation of GluAs number in the postsynaptic membrane alters the efficiency of synaptic transmission and provides a mechanism for the



**FIGURE 10.15** Diagram of glutamate receptor clustering at an excitatory synapse. The NMDA receptor interacts directly with PSD-95 through binding to one of PSD-95's three PDZ domains (the PDZ domains of PSD-95 are shown as pink squares). The AMPAR is associated with a protein called *stargazin* and stargazin interacts with one of the PDZ domains of PSD-95. Only a few of the many other signaling and scaffolding proteins at excitatory synapses are shown. AKAP150 is an A-kinase-anchoring protein of 150 kDa that binds to protein kinase A and other proteins; SynGAP is an abundant synaptic-associated Ras GTP-ase-activating protein that interacts with PSD-95; GKAP is a guanylate kinase-associated protein that interacts with PSD-95; and CaMKII is an abundant  $\text{Ca}^{2+}$ -calmodulin-activated protein kinase that interacts directly with the NMDAR. CaMKII also interacts with itself and with  $\alpha$ -actinin, which is an actin-binding protein. This web of protein–protein interactions forms the electron-dense structures called the *postsynaptic densities* visible in electron micrographs of excitatory synapses. Adapted from Sheng and Hoogenraad (2007).

expression of plasticity at excitatory synapses. As such, identifying factors that influence the trafficking of GluA receptors is an intensely studied area. One such factor is the protein stargazin that was discovered as a structural component of isolated GluA receptors (Nicoll et al., 2006; Ziff, 2007). Stargazin is one member of a family of proteins called TARP<sub>s</sub>, for transmembrane AMPA receptor regulatory proteins, that are important for the maturation and targeting of GluA receptors to excitatory synapses (Fig. 10.15). TARP<sub>s</sub> appear to bind stoichiometrically to GluA receptors and can be identified

as additional protein density in electron microscopic reconstructions of isolated GluA receptors (Nakagawa et al., 2005). This situation is analogous to that described for rapsyn binding to the nAChR. However, TARPs are themselves membrane-spanning proteins, having four predicted transmembrane domains. TARPs also have a domain that interacts with PSD-95 (or other PDZ-containing proteins), and it is this interaction that leads to the association of GluA receptors with synaptic specializations. TARPs also affect the maturation and channel properties of GluA receptors in addition to their role in synaptic targeting (Nicoll et al., 2006). Interestingly, TARP binding decreases when glutamate binds to the receptor, suggesting interplay exists between receptor use and TARP-mediated localization. There are other well-documented proteins that interact with the C-terminal domain of GluA receptor subunits, some in an isoform-specific manner. This list includes GRIP/ABP, PICK-1, NSF, and SAP-97, and these proteins have been implicated in the maturation and trafficking of GluA receptors. It is also well documented that specific residues in the C-terminal domain of GluA receptors are phosphorylated, and phosphorylation regulates channel properties and surface expression (Traynelis et al., 2010). These posttranslational modifications and interactions with other proteins must work in concert for the proper localization, stability, and function of GluA receptors at synapses.

## Summary

A general model for ionotropic receptors has emerged mainly from analyses of nAChR. Ionotropic receptors are large membrane-bound complexes generally composed of five subunits. The subunits each have four transmembrane domains, and the amino acids in the transmembrane segment TM2 form the lining of the pore. Transmitter binding induces rapid conformational changes that are translated into an increase in the diameter of the pore, permitting ion influx. Cation or anion selectivity is obtained through the coordination of specific negatively or positively charged amino acids at strategic locations in the receptor pore. How well the details of structural information obtained for nAChR will generalize to other ionotropic receptors awaits structural analyses of these other members. However, it is already clear that glutamate receptors are structurally distinct from the nAChR family of receptors and a major advancement in defining these differences came from the successful crystallization of the GluR2 receptor. This and previous results showed that the TM2 domain of glutamate receptors forms a hairpin instead of traversing the membrane completely, causing the remainder of the receptor to adopt a

different subunit topology than that described for the nAChR family. Glutamate receptors are also composed of four, not five, subunits. These differences are perhaps not surprising given that the nAChR family and the glutamate receptor family appear to have arisen from two different ancestral genes.

## G-PROTEIN-COUPLED RECEPTORS

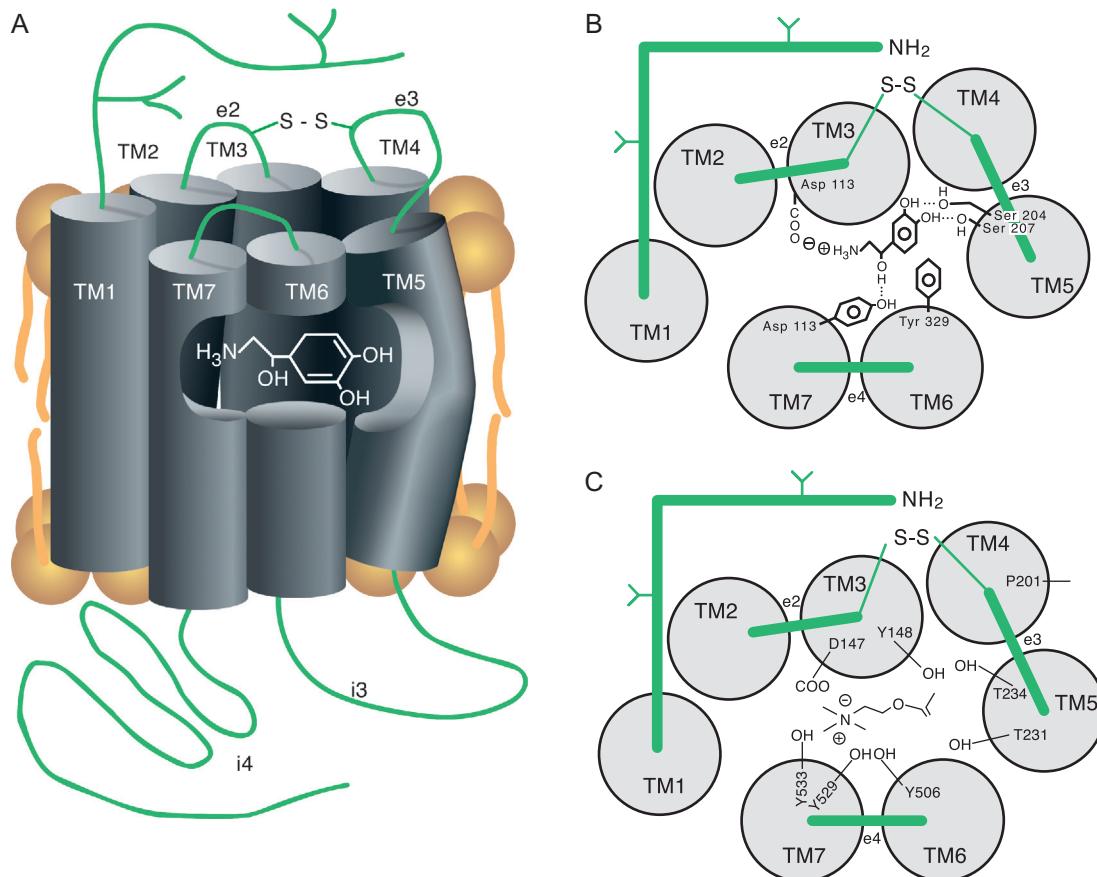
The number of members in the G-protein coupled receptor family is enormous, with over 800 identified from sequencing of the human genome. Historically, the term *metabotropic* was used to describe the fact that intracellular metabolites are produced when these receptors bind ligand. However, there are now clearly documented cases where the activation of “metabotropic” receptors does not produce alterations in metabolites but instead produces effects by interacting with G-proteins that directly alter the behavior of ion channels. Thus, these receptors are now referred to as G-protein-coupled receptors (GPCRs). Currently, the GPCR family can be divided into three subfamilies on the basis of their structures: (1) the rhodopsin–adrenergic receptor subfamily, (2) the secretin–vasoactive intestinal peptide receptor subfamily, and (3) the metabotropic glutamate receptor subfamily (Strader et al., 1995).

When a GPCR is activated it couples to a G protein, initiating the exchange of GDP for GTP, activating the G protein (Fig 10.1B). Activated G proteins then couple to many downstream effectors, and most alter the activity of other intracellular enzymes or ion channels. Many of the G-protein target enzymes produce diffusible second messengers that stimulate further downstream biochemical processes, including the activation of protein kinases (see Chapter 4). Time is required for each of these coupling events, and the effects of GPCR activation are typically slower in onset than those observed following activation of ionotropic receptors. Because there is a lifetime associated with each intermediate, the effects produced by activation of GPCRs are also typically longer in duration than those produced by activation of ionotropic receptors. Most small neurotransmitters, such as ACh, glutamate, serotonin, and GABA, can bind to and activate both ionotropic receptors and GPCRs. Thus, each of these transmitters can induce both fast responses (milliseconds), such as typical excitatory or inhibitory postsynaptic potentials, and slow-onset and longer-duration responses (from tenths of seconds to, potentially, hours). Other transmitters, like neuropeptides, produce their effects largely by binding only to GPCRs. These effects across multiple time domains provide the nervous system with a rich source for temporal information processing that is subject to constant modification.

## GPCR Structure Conforms to a General Model

A GPCR consists of a single polypeptide with a generally conserved structure. The receptor contains seven membrane-spanning helical segments that wrap back and forth through the membrane (Fig. 10.16). G-protein-coupled receptors are homologous to rhodopsin from both mammalian and bacterial sources, and detailed structural information on rhodopsin has been used to provide a framework for developing a general model for GPCR structures (Henderson et al., 1990; Palczewski et al., 2000). However, there are now crystal structures available for the human adenosine (A<sub>2a</sub>) receptor, the human D<sub>3</sub> receptor, the human  $\beta 2$  adrenoreceptor and the avian  $\beta 1$  adrenoreceptor that provide important comparative information to describe the unique and conserved features of GPCR structure.

The most conserved feature of GPCRs is the seven membrane-spanning segments; however, other generalities can be made about their structure. The N terminus of the receptor extends into the extracellular space, whereas the C terminus resides within the cytoplasm (Fig. 10.16). Each of the seven transmembrane (TM) domains between the N and C termini consists of approximately 24 mostly hydrophobic amino acids. These seven TM domains associate together to form an oblong ring within the plasma membrane (Fig. 10.16B). Between each transmembrane domain is a loop of amino acids of various sizes. The loops connecting TM1 and TM2, TM3 and TM4, and TM5 and TM6 are intracellular and labeled i<sub>1</sub>, i<sub>2</sub>, and i<sub>3</sub>, respectively, whereas those between TM2 and TM3, TM4 and TM5, and TM6 and TM7 are extracellular and labeled e<sub>1</sub>, e<sub>2</sub>, and e<sub>3</sub>, respectively (see Fig. 10.16A).



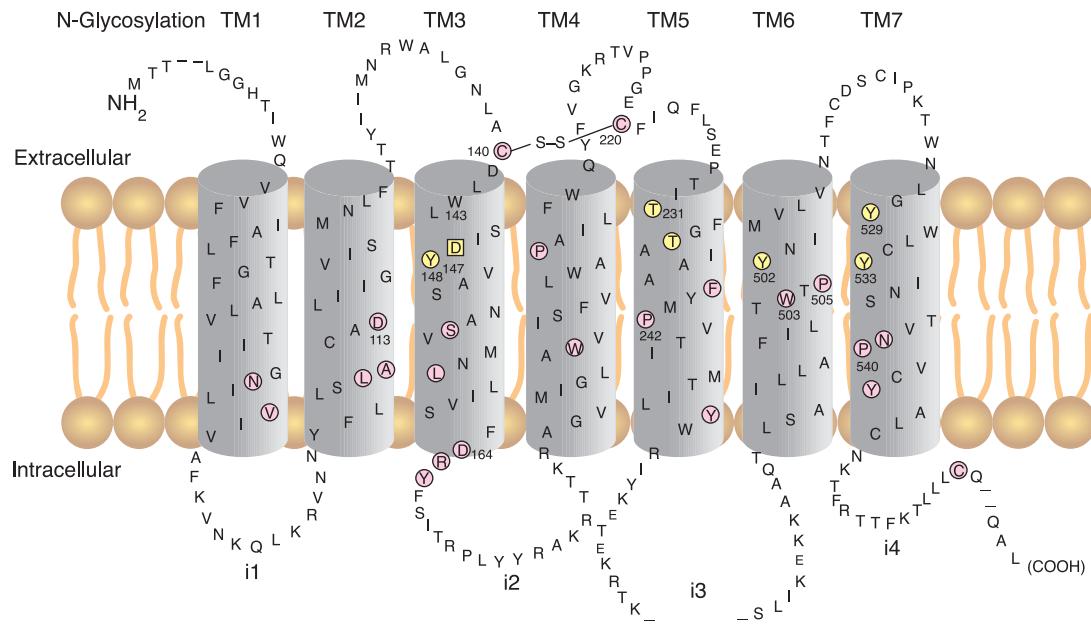
**FIGURE 10.16** (A) Diagram showing the approximate position of the catecholamine binding site in  $\beta$ AR. The transmitter binding site is formed by amino acids whose side chains extend into the center of the ring produced by the seven transmembrane domains (TM1–TM7). Note that the binding site exists at a position that places it within the plane of the lipid bilayer. (B) A view looking down on a model of  $\beta$ AR identifying residues important for ligand binding. The seven transmembrane domains are represented as gray circles labeled TM1 through TM7. Amino acids composing the extracellular domains are represented as green bars labeled e<sub>1</sub> through e<sub>4</sub>. The disulfide bond ( $-S-S-$ ) that links e<sub>2</sub> to e<sub>3</sub> also is shown. Each of the specific residues indicated makes stabilizing contact with the transmitter. (C) A view looking down on a model of mAChR identifying residues important for ligand binding. Stabilizing contacts, mainly through hydroxyl groups ( $-OH$ ), are made with the transmitter on four of the seven transmembrane domains. The chemical nature of the transmitter (i.e., epinephrine versus ACh) determines the type of amino acids necessary to produce stable interactions in the receptor binding site (compare B and C). Adapted from Strosberg (1990).

## The Neurotransmitter Binding Site is Buried in the Core of the Receptor

The neurotransmitter binding site for many GPCRs (excluding the metabotropic glutamate, GABA<sub>B</sub>, and neuropeptide receptors) resides within a pocket formed in the center of the seven membrane-spanning segments (Fig. 10.16A). In the  $\beta$ AR, this pocket resides  $\sim 11 \text{ \AA}$  into the hydrophobic core of the receptor, placing the ligand binding site within the plasma membrane lipid bilayer (Kobilka, 1992; Mizobe et al., 1996; Rosenbaum et al., 2009). Strategically positioned charged and polar residues in the membrane-spanning segments point inward into a central pocket that forms the binding site for the ligand. For example, Asn residues in the second and third segments, two Ser residues in the fifth segment, and a Phe residue in the sixth segment provide major contact points in the  $\beta$ AR binding site for the transmitter (Kobilka, 1992) (Fig. 10.16B). Replacing the Asp in TM3 with a Glu reduced transmitter binding by more than 100-fold, and replacement with a less conserved amino acid, such as Ser, reduces binding by more than 10,000-fold. Two Ser residues in TM5 are also essential for efficient transmitter binding and receptor activation, as are an Asp residue in TM2 and a Phe residue in TM6. In total, the two Asp, two Ser, and one Phe residues are highly conserved in all receptors that bind catecholamines. Variations in the

amino acids at these five positions appear to provide the specificity between binding of different transmitters to the individual GPCRs.

The neurotransmitter binding site of the mAChRs, like that of  $\beta$ 2AR, has been investigated in great detail (Fig. 10.16C). The Asp residue in TM3 is also critical for ACh binding to the mAChRs. Mutagenesis studies indicate important roles for Tyr and Thr residues in TM3, TM5, TM6, and TM7 in contributing to the ligand-binding site for ACh. Interestingly, many of these mutations do not affect antagonist binding, indicating that distinct sets of amino acids participate in binding agonists and antagonists. When the TM domains are examined from a side view (Fig. 10.17), all the key amino acids implicated in agonist binding lie at about the same level within the core of the receptor structure, buried approximately 10–15  $\text{ \AA}$  from the surface of the plasma membrane (yellow boxed amino acids). An additional amino acid identified as indirectly influencing agonist binding of the mAChR is a Pro residue in TM4 (P201 in Fig. 10.16B). This residue is also highly conserved among the GPCRs, and structural predictions suggest that it affects ligand binding not by interacting with agonist directly but by stabilizing a conformation essential for high-affinity binding. Structural predictions also place this Pro residue in the same plane as the Asp, Tyr, and Thr residues that form the ligand-binding site of the mAChR (Fig. 10.17).



**FIGURE 10.17** Amino acid sequence and predicted domain topology of the M3 isoform of mAChR. The transmembrane domains are TM1–TM7. The NH<sub>2</sub> terminus of the protein is at the left and extends into the extracellular space. The COOH terminus is intracellular and is at the right; i1 to i4 are the four intracellular domains. The conserved disulfide bond ( $-S-S-$ ) connects extracellular loop 2 to loop 3. The dashes in the amino acid sequence represent inserts of various lengths that are not shown. Conserved amino acids for all members of the G-protein-coupled receptor family of receptors are marked in purple. The amino acids taking part in ACh binding to the receptor are highlighted in yellow. Note that all amino acids associated with ligand binding lie in approximately the same horizontal plane across the receptor. Adapted from Wess (1993).

## Transmitter Binding Causes a Conformational Change in the Receptor and Activation of G Proteins

Proposed models for GPCR activation assume that the receptor can spontaneously isomerize between the inactive and active states (Premont et al., 1995; Perez et al., 1996). Only the active state interacts with G proteins in a productive fashion. This isomerization is analogous to the spontaneous isomerization proposed for ion channels as they oscillate between open and closed states. At equilibrium, in the absence of agonist, the inactive state of GPCRs is favored, and little G-protein activation occurs. Agonist binding stabilizes the active conformation and shifts the equilibrium toward the active form, and G-protein activation ensues. Conversely, receptor antagonists block G-protein activation through two proposed mechanisms: (1) negative antagonism in which antagonists bind to the inactive state of the receptor, thus favoring an equilibrium with the inactive form; and (2) neutral antagonism in which antagonists bind to both the active and inactive forms, thus stabilizing both and preventing a complete transition into the active form. This kinetic model indicates that agonist binding is not necessary for the receptor to undergo a transition into the active state; instead, it stabilizes the activated state of the receptor. Observations of both spontaneously arising and engineered mutants of  $\beta$ ARs and  $\alpha$ ARs support this proposed model. Specific amino acid replacements produce receptors that exhibit constitutive activity in the absence of agonists (Premont et al., 1995; Perez et al., 1996). The amino acid changes stabilize the active conformation of the molecule in a state more similar to the agonist-bound form, leading to productive interactions with G proteins in the agonist-free state. Stabilization of intermediate states by pharmacological agents can explain how certain drugs produce full agonist, partial agonist, neutral antagonist or inverse agonist behavior (Rosenbaum et al., 2009).

## The Third Intracellular Loop Forms a Major Determinant for G-Protein Coupling

Extensive studies using site-directed mutagenesis and the production of chimeric molecules have revealed the domains and amino acids essential for G-protein coupling to GPCRs. Receptor domains within the second (i2) and third (i3) intracellular loops (Fig. 10.17) appear largely responsible for determining the specificity and efficiency of coupling for adrenergic and muscarinic cholinergic receptors and are the likely sites for G-protein coupling of the entire GPCR family. In particular, the 12 amino acids of the N-terminal region of the third intracellular loop significantly affect the specificity of G-protein coupling. Other regions in the C terminus of the third intracellular loop and the N-terminal region

of the C-terminal tail appear to be more important for determining the efficiency of G-protein coupling than for determining its specificity (Kobilka, 1992). The third intracellular loop varies enormously in size among the different G-protein-coupled receptors, ranging from 29 amino acids in the substance P (a neuropeptide) receptor to 242 amino acids in mAChR (Strader et al., 1994). The intracellular loop connecting TM5 and TM6 is the main point of receptor coupling to G proteins, and ligand binding to amino acids in TM5 and TM6 may be responsible for triggering G protein–receptor interaction by transmitting a conformational change to the third intracellular loop (i3).

## Specific Amino Acids are Involved in Transducing Transmitter Binding into G-Protein Coupling

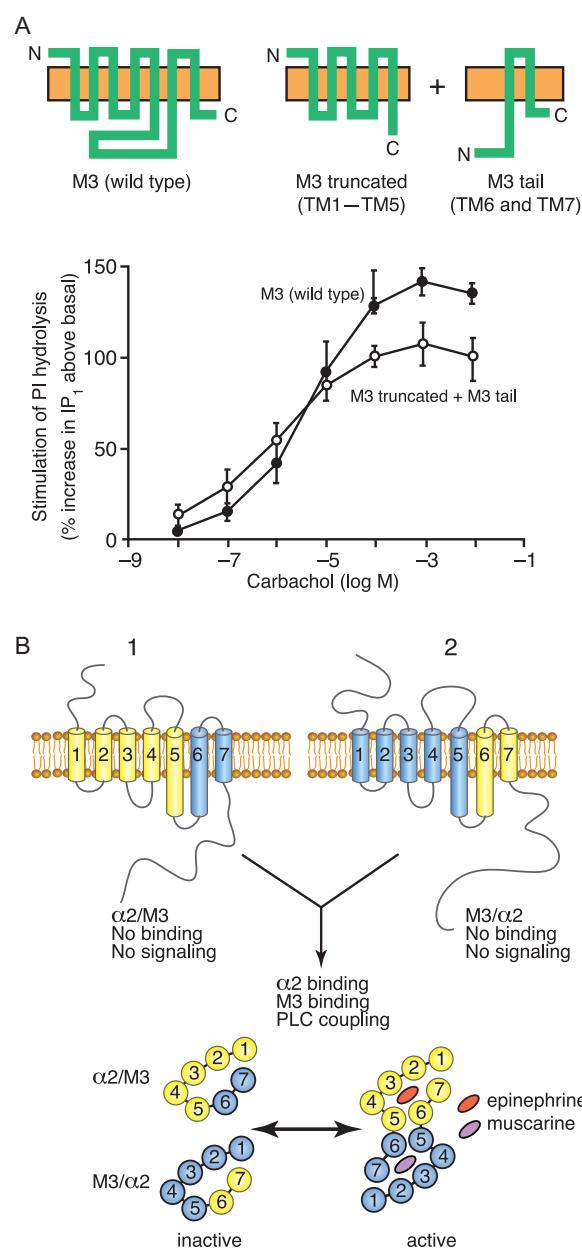
Residues associated with transmitting the conformational change induced by ligand binding to the activation of G proteins have been investigated with the use of mAChRs. These studies revealed that an Asp residue in TM2 (D113 in Fig. 10.17) is important for receptor activation of G proteins, and altering the Asp by site-directed mutagenesis has a major negative effect on G protein–receptor activation (Fraser et al., 1988; Fraser et al., 1989). A Thr residue in TM5 (T231 in Fig. 10.17) and a Tyr residue in TM6 (Y502 in Fig. 10.17) also are essential. Because these residues are connected by i3, they are assumed to play fundamental roles in transmitting the conformational change induced by ligand binding to the area of i3 essential for G-protein coupling and activation. When mutated, a Pro residue on TM7 (P540 in Fig. 10.17; part of a conserved -Asn-Pro-X-X-Tyr- motif) produces a major impairment in the ability of the TM3 segment to induce G-protein coupling. The specifics of these earlier mutagenesis studies have largely been confirmed by comparison of different crystallized forms of the GPCRs and the term “rotamer toggle switch” is used to capture the conformational transition from the inactive to active conformations. There is also a highly conserved sequence -Asp-Glu-Arg-Tyr- in TM3 and a Glu residue in TM6 that forms a series of interactions termed the “ionic lock” that help stabilize the inactive conformation (Rosenbaum et al., 2009). Disruption of this “lock” is thought to be an important step in inducing the active conformation capable of productive G-protein interactions.

As mentioned earlier, GPCRs are most often single polypeptides; however, they are clearly separable into distinct functional domains. For example, the  $\beta$ 2AR can be physically split, with the use of molecular techniques, into two fragments, one fragment containing TM1–TM5 and the other containing TM6 and TM7. In isolation,

neither of these fragments can produce a functional receptor; however, when coexpressed in the same cell, functional  $\beta$ 2ARs that can bind ligand and activate G proteins are produced (Fig. 10.18). This remarkable experiment indicates that physical contiguity in the primary sequence is not essential for producing functional  $\beta$ 2ARs, but it does emphasize the contribution of domains in the separate fragments (TM1–TM5 and TM6, TM7) to both ligand binding and G-protein coupling. Like the  $\beta$ 2AR, the m2 and m3 members of the mAChR family can form functional receptors even if split into two separate domains. A fragment containing the first five TM domains, when expressed with a fragment containing TM6 and TM7, forms a functional receptor (Strosberg, 1990) (Fig. 10.18).

### GPCRs also Exist as Homo- or Hetero-Oligomers

The observation that GPCRs can be physically split through genetic engineering and when recombined produce functional channels provided the first hint that full-length GPCRs might also oligomerize with each other into functional molecules. A test of such a hypothesis was accomplished by making chimeric receptors composed of TM domains 1–5 of  $\alpha$ 2-AR and TM domains 6 and 7 of the m3 muscarinic receptors and vice versa (Fig. 10.18B) (Maggio et al., 1993). When either of these chimeric molecules was expressed in isolation, neither formed a functional receptor. However, when coexpressed, receptors were formed that bind both muscarinic and adrenergic ligands, and ligand binding led to functional activation of downstream effectors. Through domain swapping the ligand binding sites for both receptor ligands were reconstituted by oligomerization of the two chimeric receptors into one bifunctional chimeric dimer (Fig. 10.18B). Oligomerization of GPCRs is also supported by crosslinking and immunoprecipitation experiments and with experiments examining the direct biophysical association of the receptors in living cells (Maggio et al., 1993; Lee et al., 2000; Overton and Blumer, 2000; Salahpour et al., 2000). The oligomerization of GPCRs is adding a new layer of complexity and diversity to the study of these receptors and the functional impacts of GPCR oligomerization are just beginning to be appreciated. Important functional consequences could relate to alterations in (1) ligand binding, (2) efficiency and specificity of coupling to downstream effectors, (3) subcellular localization, and (4) receptor desensitization. The evolving and apparently widespread nature of direct receptor–receptor interactions leads one to believe that our current understanding of neurotransmitter receptors and their biological impact will be undergoing continual modifications for many years to come.



**FIGURE 10.18** (A) mAChR can be split into two physically separated domains that, when added back together, retain the ability to bind transmitter and activate G proteins. (Upper left) Model of full-length mAChR; (upper right) two engineered pieces of the receptor. The graph indicates that, when coexpressed in the same cells, the two fragments can produce a functional mAChR that responds to the agonist carbachol producing activation of G protein and subsequent activation of an enzyme that hydrolyzes phosphatidylinositol (PI). Adapted from Wess (1993). (B) Some GPCRs can function as dimers. In this example, chimeric receptors were produced between  $\alpha$ 2AR ( $\alpha$ 2) and mAChR(M3) by swapping certain TM domains through genetic engineering. When  $\alpha$ 2/M3 or M3/ $\alpha$ 2 are expressed separately, they are not active. However, if both chimeric molecules are expressed in the same cells, they form receptors that can be activated by either epinephrine or muscarine. The bottom panel shows a top-down view of how this domain swapping might occur when two molecules dimerize to produce receptors that can respond to both transmitters.

## G-Protein Coupling Increases the Affinity of the Receptor for Neurotransmitter

The affinity of GPCR for neurotransmitter increases when the receptor is coupled to the G protein. This positive feedback effectively increases the lifetime of the neurotransmitter-bound form of the receptor by decreasing its dissociation rate. An excellent demonstration of this effect comes from studies using engineered  $\beta$ ARs that are constitutively active in their ability to couple to G proteins. These mutant receptors show significantly increased affinity for neurotransmitter (Perez et al., 1996). When G protein dissociates, the agonist-binding affinity of the receptor returns to its original state. Changes induced by ligand binding apparently stabilize the receptor in a conformation with both higher affinity for ligand and higher affinity for coupling to G proteins.

### Specificity and Potency of G-Protein Activation are Determined by Several Factors

GPCRs associate with G proteins to transduce ligand binding into intracellular effects. This coupling step can lead to diverse responses, depending on the type of G protein and the type of effector enzyme present. In addition, ligand binding to a single subtype of GPCR can activate multiple G-protein-coupled pathways. Activated  $\alpha$ 2ARs have been shown to couple to as many as four different G proteins in the same cell (Strader et al., 1994). Some of the specificity for G-protein activation can be determined by the specific conformations assumed by the receptor, and a single receptor can assume multiple conformations. For example,  $\alpha$ 2ARs can isomerize into at least two states. One state interacts with a G protein that couples to phospholipase C, and a second state interacts with G proteins that couple to both phospholipase C and phospholipase A2 (Perez et al., 1996). Thus, a single GPCR can produce a diversity of responses, making it difficult to assign specific biological effects to individual receptor subtypes in all settings.

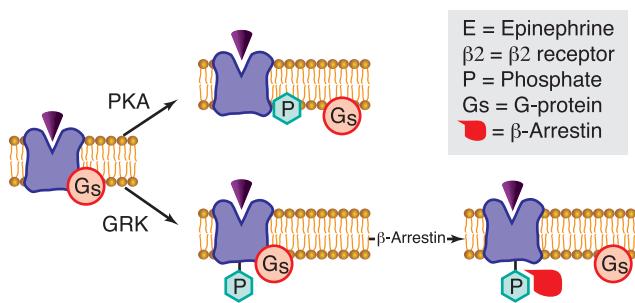
Activated GPCRs are free to couple to many G-protein molecules, permitting a significant amplification of the initial transmitter-binding event (Cassel and Selinger, 1977). This catalytic mechanism is referred to as collision coupling (Tolkovsky et al., 1982), whereby a transient association between the activated receptor and the G protein is sufficient to produce the exchange of GDP for GTP, activating the G protein. Because enzymes such as adenylyl cyclase appear to be tightly coupled to the G protein, the rate-limiting step in the production of cAMP is the number of productive collisions between the receptor and the G protein. A constant GTPase activity hydrolyzes GTP, bringing the

G protein and therefore the adenylyl cyclase back to the basal state. Transmitter concentration clearly plays a role in the number of activated receptors present at any given time, and GPCRs exhibit saturable dose-response curves. This apparent maximal rate is achieved when all of the G protein–cyclase complexes have become activated (more accurately, when the rate of formation is maximal with respect to the rate of GTP hydrolysis). A less intuitive consequence from these models is that receptor number can significantly affect the concentration of transmitter that produces a half-maximal response of cAMP accumulation. The larger the receptor numbers the greater the probability that a productive collision will occur between an agonist-bound receptor and the G protein. Experimental evidence for this prediction was obtained for  $\beta$ AR expressed at various levels in eukaryotic cells. Increasing concentrations of  $\beta$ AR produced a decrease in the concentration of neurotransmitter required to produce half-maximal production of cAMP. Apparently, the cell can adjust the magnitude of its response by adjusting the number of receptors available for transmitter interaction. In addition, the important process of receptor desensitization can also regulate the number of receptors capable of productive G-protein interactions.

### Receptor Desensitization is a Built-In Mechanism for Decreasing the Cellular Response to Transmitter

Desensitization is a very important process whereby cells can decrease their sensitivity to a particular neurotransmitter to prevent saturation of the system. Desensitization involves a complex series of events (Kobilka, 1992; Clark et al., 1999; Rosenbaum et al., 2009). For GPCRs, functional desensitization is defined as an increase in the concentration of neurotransmitter required to produce half-maximal stimulation of, for example, adenylyl cyclase. In practical terms, desensitization of receptors produces less response for a constant amount of transmitter.

There are two known mechanisms for desensitization (see Figure 10.19). One mechanism is a decrease in response brought about by the covalent modifications produced by receptor phosphorylation and is quite rapid (seconds to minutes). The other mechanism is the physical removal of receptors from the plasma membrane through a mechanism of receptor-mediated endocytosis and tends to require greater periods (minutes to hours). The latter process can be either reversible (sequestration) or irreversible (downregulation), when the receptors are removed from the cell through degradation (Figure 10.19).



**FIGURE 10.19** Different modes of desensitization of GPCRs. This diagram indicates that the epinephrine (E)-bound form of  $\beta_2$ AR normally couples to the G protein  $G_s$ . PKA can phosphorylate the receptor, leading to an inhibition of binding to  $G_s$ . G-protein receptor kinase (GRK) also can phosphorylate the receptor; however, this phosphorylation does not directly interfere with binding to  $G_s$ . GRK phosphorylation is needed for the binding of another protein,  $\beta$ -arrestin, which, by its association with the receptor, prevents  $G_s$  from binding. Adapted from Kobilka (1992).

### The Rapid Phase of GPCR Desensitization is Mediated by Receptor Phosphorylation

Desensitization of  $\beta$ ARs appears to involve at least three protein kinases: PKA, PKC, and/or a G-protein receptor kinase (GRK). Phosphorylation of ARs by PKA does not require that agonist be bound to the receptor and appears to be a general mechanism by which the cell can reduce the effectiveness of all receptors, independent of whether they are in the neurotransmitter-bound or unbound state (Fig. 10.19). This process is also referred to as heterologous desensitization because the receptor does not require bound neurotransmitter (for simplicity PKA is shown phosphorylating only the agonist-bound form of the receptor in Fig. 10.19). PKA and PKC phosphorylate sites on the third intracellular loop and possibly the C-terminal cytoplasmic domain. Phosphorylation of these sites functionally interferes with the receptor's ability to couple to G proteins, thus producing the desensitization (Fig. 10.19). Whether the same sites on  $\beta$ AR are phosphorylated by both PKA and PKC is controversial. Some researchers conclude that the effects of phosphorylation by either kinase on decreasing coupling of the receptor to G proteins are similar (suggesting that the sites phosphorylated are similar) (Huganir and Greengard, 1990). Others find that the effects are additive (Yuan et al., 1994). Although the details of the role played by each of these kinases are ambiguous, phosphorylation by either enzyme desensitizes the receptor.

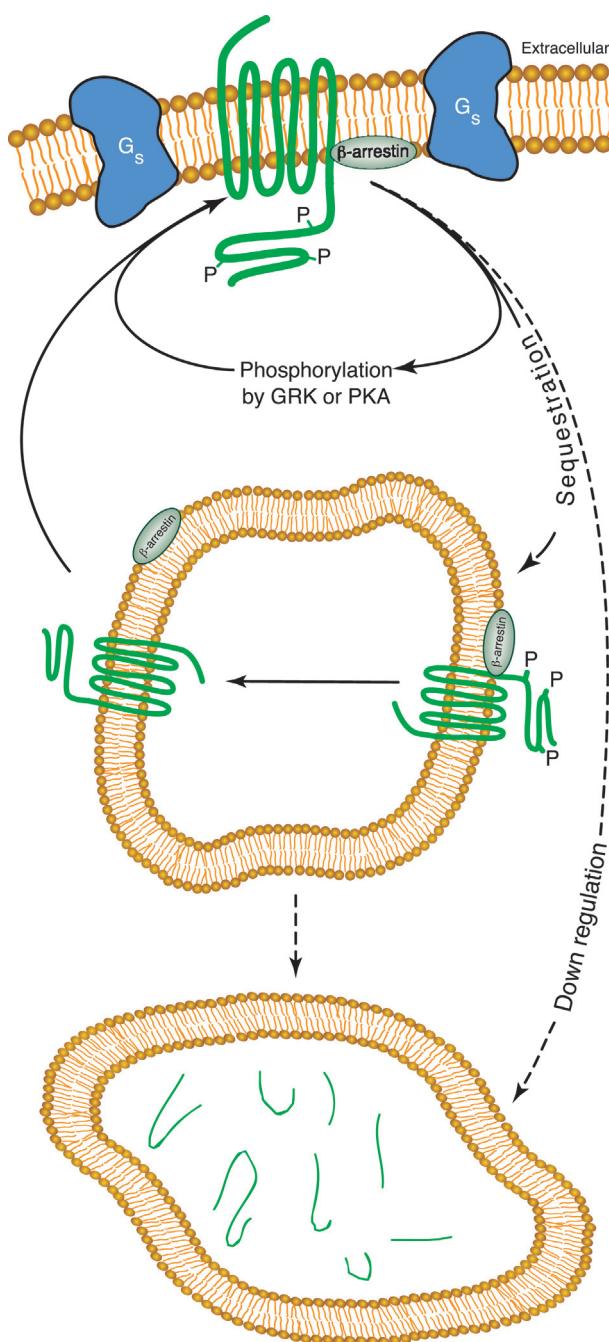
GRKs can also phosphorylate GPCRs and lead to receptor desensitization (Inglese et al., 1993; Sterne-Marr and Benovic, 1995). Six members of the GRK family of kinases have been identified: rhodopsin kinase (GRK1),  $\beta$ ARK (GRK2), and GRK3 through GRK6 (Premont et al., 1995; Sterne-Marr and Benovic, 1995; Huang and

Tesmer, 2011). GRK2 is a Ser- and Thr-specific protein kinase initially identified by its capacity to phosphorylate the  $\beta$ AR. GRK2 phosphorylates only the neurotransmitter-bound form of the receptor, usually when neurotransmitter concentrations reach the micromolar level, as typically found in the synaptic cleft. This process is referred to as homologous desensitization because the regulation is specific for those receptor molecules that are in the transmitter-bound state. Phosphorylation of  $\beta$ AR by GRK2 does not interfere with coupling to G proteins. Instead, an additional protein, arrestin, binds the GRK2-phosphorylated form of the receptor, thus blocking receptor–G-protein coupling (Fig. 10.19). This process is analogous to the desensitization of the light-sensitive receptor molecule rhodopsin produced by GRK1 phosphorylation and the binding of arrestin. The phosphorylation sites on  $\beta$ AR for GRK2 reside on the C-terminal cytoplasmic domain and are distinct from those phosphorylated by PKA.

The cycle of homologous desensitization starts with the activation of a GPCR, which induces activation of G proteins and dissociation of the  $\beta\gamma$  subunit complex from  $\alpha$  subunits. At least one role for the  $\beta\gamma$  complex appears to be to bind to GRKs, which leads to their recruitment to the membrane in the area of the locally activated G-protein–receptor complex. The recruited GRK is then activated, leading to phosphorylation of the agonist-bound receptor and subsequent binding of arrestin. Arrestin binds to the same domains on the receptor necessary for coupling to G proteins, thus terminating the actions of the activated receptor (Fig. 10.20). The ensuing process of sequestration follows GPCR phosphorylation and arrestin binding.

### Desensitization can also be Produced by Loss of Receptors from the Cell Surface

Desensitization of GPCRs is also produced by removal of the receptor from the cell surface. This process can be either reversible (sequestration or internalization) or irreversible (downregulation). Sequestration is the term used to describe the rapid (within minutes) but reversible endocytosis of receptors from the cell surface after neurotransmitter application (Fig. 10.20). Neither G-protein coupling nor receptor phosphorylation appears to be absolutely essential for this process, but phosphorylation by GRKs clearly enhances the rate of sequestration (Ferguson et al., 1995). The binding of arrestins to the phosphorylated receptor also enhances sequestration (Ferguson et al., 1996). Thus, arrestin binding appears to promote not only rapid desensitization by disrupting the receptor–G-protein interaction but also receptor sequestration. Because the receptor can be functionally uncoupled from the G protein



**FIGURE 10.20** Additional intracellular pathways associated with desensitization of GPCRs. GPCRs are phosphorylated (noted with P) on their intracellular domains by PKA, GRK, and other protein kinases. The phosphorylated form of the receptor can be removed from the cell surface by a process called *sequestration* with the help of the adapter protein  $\beta$ -arrestin; thus, fewer binding sites remain on the cell surface for transmitter interactions. In intracellular compartments, the receptor can be dephosphorylated and returned to the plasma membrane in its basal state. Alternatively, the phosphorylated receptors can be degraded (downregulated) by targeting to a lysosomal organelle. Degradation requires replenishment of the receptor pool through new protein synthesis. Adapted from Kobilka (1992).

through the rapid phosphorylation-dependent phase of desensitization, the physiological role(s) of sequestration remains an open issue, although decreasing the number of receptor molecules on the cell surface would contribute to the overall process of desensitization to neurotransmitter. Receptor cycling through intracellular organelles is a trafficking mechanism that leads to an enhanced rate of dephosphorylation of the phosphorylated receptor, returning it to the cell surface in its basal state (Fig. 10.20) (Barak et al., 1994).

Downregulation occurs more slowly than sequestration and is irreversible (Fig. 10.20). The early phase (within 4 h) may involve both PKA-dependent and PKA-independent processes (Bouvier et al., 1989; Proll et al., 1992). This early phase of downregulation is apparently due to receptor degradation after endocytic removal from the plasma membrane. The later phases (>14 h) of downregulation appear to be further mediated by a reduction in receptor biosynthesis through a decrease in the stability of the receptor mRNA (Bouvier et al., 1989) and a decreased transcription rate.

### Other Posttranslational Modifications are Required for Efficient GPCR Function

Like many proteins expressed on the cell surface, GPCRs are glycosylated, and the N-terminal extracellular domain is the site of carbohydrate attachment (Fig. 10.16). Glycosylation does not appear to be essential to the production of a functional ligand-binding pocket (Strader et al., 1994), although prevention of glycosylation decreases membrane insertion and alters intracellular trafficking of the  $\beta 2$ AR.

Another important structural feature of most GPCRs is the disulfide bond formed between two Cys residues present on the extracellular loops (e2 and e3; Figs. 10.16 and 10.17). This disulfide bond stabilizes a restricted conformation of the mature receptor by covalently linking the two extracellular domains, and this conformation favors ligand binding. Disruption of this disulfide bond significantly decreases neurotransmitter binding (Kobilka, 1992).

A third Cys residue, in the C-terminal domain of GPCRs (Fig. 10.17, pink-circled C in i4), can serve as a point for covalent attachment of a fatty acid (often palmitate). Fatty acid attachment stabilizes an interaction between the C-terminal domain of a GPCR and the membrane (Casey, 1995). The full consequences of this posttranslational modification are not understood, because replacing the normally palmitoylated Cys with an amino acid that cannot be acylated appears to have little effect on receptor binding; however, G-protein coupling may not be as efficient (O'Dowd et al., 1989).

## GPCRs Can Physically Associate with Ionotropic Receptors

There is now good evidence that GPCRs and ionotropic receptors can interact directly with each other (Liu et al., 2000). GABA<sub>A</sub> receptors (ionotropic) were shown to couple to dopamine (D<sub>5</sub>) receptors through the second intracellular loop of the  $\gamma$  subunit of the GABA<sub>A</sub> receptor and the C-terminal domain of the D<sub>5</sub>, but not the D<sub>1</sub>, receptor. Dopamine binding to D<sub>5</sub> receptors produced downregulation of GABA<sub>A</sub> currents, and pharmacologically blocking the GABA<sub>A</sub> receptor produced decreases in cAMP production when cells were stimulated with dopamine. It further appeared that ligand binding to both receptors was necessary for their stable interaction. Whether this form of receptor regulation is unique to this pair of partners or is a widespread phenomenon remains an open question requiring further investigation.

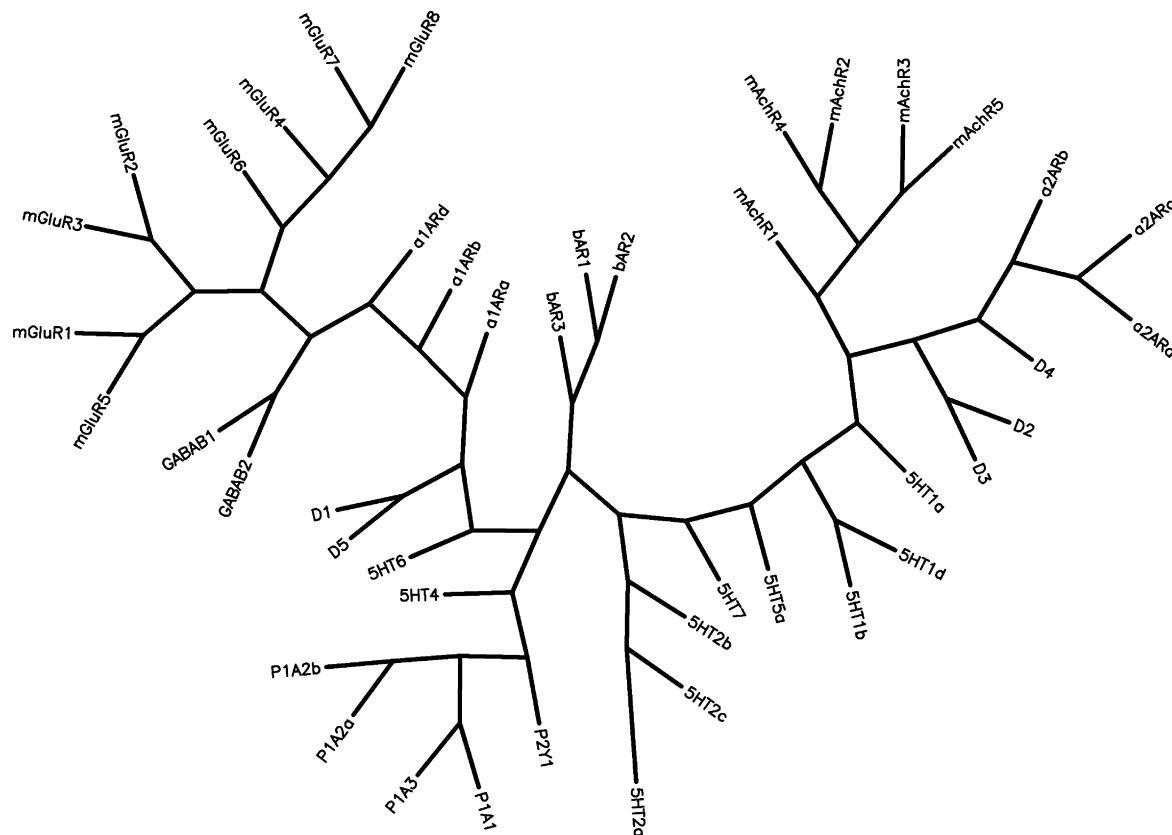
## GPCRs all Exhibit Similar Structures

The family of GPCRs exhibits structural similarities that permit the construction of "trees" describing the

degree to which they are evolutionarily related (Fig. 10.21). Some remarkable relations become evident in such an analysis. For example, the D<sub>1</sub> and D<sub>5</sub> subtypes of dopamine receptors are more closely related to  $\alpha 1\text{AR}$  than to the D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> dopamine receptors. Similarly, the D<sub>1</sub> and D<sub>5</sub> receptors are more closely related to the  $\alpha 2\text{ARs}$  than the  $\alpha 1$  and  $\alpha 2\text{ARs}$  are related to each other. The similarities and differences among GPCR families are highlighted in the remainder of this chapter.

### Muscarinic ACh Receptors

Muscarine is a naturally occurring plant alkaloid that binds to and activates muscarinic subtypes of AChRs. mAChRs play a dominant role in mediating the actions of ACh in the brain, indirectly producing both excitation and inhibition through binding to a family of unique receptor subtypes. mAChRs are found both presynaptically and postsynaptically, and ultimately, their main neuronal effects appear to be mediated through alterations in the properties of ion channels. Presynaptic mAChRs take part in important feedback loops that regulate neurotransmitter release.



**FIGURE 10.21** Evolutionary relationship of the GPCR family. The tree was constructed by aligning the protein sequences from each receptor family with the ClustalW program and the phylogenetic relationship was inferred with the maximum parsimony method as described in the legend for Fig. 10.10. Dr. Yin Liu (Department of Neurobiology and Anatomy, University of Texas Health Science Center-Houston, Houston, TX) kindly provided the phylogenetic tree and figure.

ACh released from the presynaptic terminal can bind to mAChRs on the same nerve ending, thus activating enzymatic processes that modulate subsequent neurotransmitter release. This modulation is typically an inhibition; however, activation of the m5 AChR produces an enhancement in subsequent release. These autoreceptors are an important regulatory mechanism for short-term (milliseconds to seconds) modulation of neurotransmitter release.

The family of mAChRs now includes five members (m1–m5; right side of Fig. 10.21), ranging from 55 to 70 kDa, and each of the five subtypes exhibits the typical architecture of seven TM domains. Much of the diversity in this family of receptors resides in the third intracellular loop (i3) responsible for the specificity of coupling to G proteins. The m1, m3, and m5 mAChRs couple predominantly to G proteins that activate the enzyme phospholipase C. The m2 and m4 receptors couple to G proteins that inhibit adenylyl cyclase, as well as to G proteins that directly regulate K<sup>+</sup> and Ca<sup>2+</sup> channels. As is the case for other GPCRs, the domain near the N terminus of the third intracellular loop is important for the specificity of G-protein coupling. This domain is conserved in m1, m3, and m5 AChRs but is unique in m2 and m4. Several other important residues also have been identified for G-protein coupling. A particular Asp residue near the N terminus of the second intracellular loop (i2) is important for G-protein coupling, as are residues residing in the C-terminal region of the i3 loop.

The major mAChRs found in the brain are m1, m3, and m4, and each is diffusely distributed. The m2 subtype is the heart isoform and is not highly expressed in other organs. The genes for m4 and m5 lack introns, whereas those encoding m1, m2, and m3 contain introns, although little is known concerning alternatively spliced products of these receptors. Atropine is the most widely used antagonist for mAChR and binds to most subtypes, as does N-methylscopolamine. The antagonist pirenzipine appears to be relatively specific for the m1 mAChR, and other antagonists such as AF-DX116 and hexahydrosiladifenidol appear to be more selective for the m2 and m3 subtypes.

### Adrenergic Receptors

The catecholamines epinephrine (adrenaline) and norepinephrine (noradrenaline) produce their effects by binding to and activating adrenergic receptors. Interestingly, epinephrine and norepinephrine can both bind to the same adrenergic receptor. Adrenergic receptors are currently separated into three families,  $\alpha_1$ ,  $\alpha_2$ , and  $\beta$ . The  $\alpha_1$  and  $\alpha_2$  families are further subdivided into three subclasses each. Similarly, the  $\beta$  family also contains three subclasses ( $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ ; see Fig. 10.21). The main adrenergic receptors in the brain are the  $\alpha_2$

and  $\beta_1$  subtypes. The  $\alpha_2$ ARs have diverse roles, but the function that is best characterized (in both central and peripheral nervous tissue) is their role as autoreceptors (i.e., presynaptic receptors that bind transmitter and alter the release apparatus so that subsequent release is modulated, usually, in an inhibitory fashion). Different adrenergic receptor subtypes bind to G proteins that can alter the activity of phospholipase C, Ca<sup>2+</sup> channels, and, probably best studied, adenylyl cyclase. For example, activation of  $\alpha_2$ ARs produces inhibition of adenylyl cyclase, whereas all  $\beta$ ARs activate the cyclase.

Only a few agonists or antagonists cleanly distinguish the adrenergic receptor subtypes. One of them, isoproterenol, is an agonist that appears to be highly specific for  $\beta$ ARs. Propranolol is the best-known antagonist for  $\beta$  receptors, and phentolamine is a good antagonist for  $\alpha$  receptors but weakly binds at  $\beta$  receptors. The genomic organization of the different AR subtypes is unusual. Like many GPCRs,  $\beta_1$ ARs and  $\beta_2$ ARs are encoded by genes lacking introns.  $\beta_3$ ARs, which apparently have a role in lipolysis and are poorly characterized, are encoded by an intron-containing gene, as are  $\alpha$ ARs, providing an opportunity for alternative splicing as a means of introducing functional heterogeneity into the receptor.

### Dopamine Receptors

Some 80% of the dopamine in the brain is localized to the corpus striatum, which receives major input from the substantia nigra and takes part in coordinating motor movements. Dopamine is also found diffusely throughout the cortex, where its specific functions are many-fold. For example, many neuroleptic drugs appear to exert their effects by blocking dopamine binding, and imbalances in the dopaminergic system have long been associated with neuropsychiatric disorders.

Dopamine receptors are found both pre- and postsynaptically, and their structure is homologous to that of the receptors for other catecholamines (Bunzow et al., 1988; Civelli et al., 1993). Five subtypes of dopamine receptors can be grouped into two main classes, D<sub>1</sub>-like and D<sub>2</sub>-like receptors. D<sub>1</sub>-like receptors include D<sub>1</sub> and D<sub>5</sub>, and D<sub>2</sub>-like receptors include D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> (see Fig. 10.21). The main distinction between these two classes is that D<sub>1</sub>-like receptors activate adenylyl cyclase through interactions with G<sub>s</sub>, whereas D<sub>2</sub>-like receptors inhibit adenylyl cyclase and other effector molecules by interacting with G<sub>i</sub>/G<sub>o</sub>. D<sub>1</sub>-like receptors are also slightly larger in molecular mass than D<sub>2</sub>-like receptors. An additional point of interest, as noted previously, is that D<sub>5</sub> receptors selectively bind to GABA<sub>A</sub> receptors, impacting their function and vice versa (Liu et al., 2000). The deduced amino acid sequence for the entire family ranges from 387 amino acids (D<sub>4</sub>) to 477 amino acids (D<sub>5</sub>). The main structural differences between

D<sub>1</sub>-like and D<sub>2</sub>-like receptors are that the intracellular loop between the sixth and seventh TM segments is larger in the D<sub>2</sub>-like receptors, and the D<sub>2</sub>-like receptors have smaller C-terminal intracellular segments. Two isoforms of the D<sub>2</sub> receptor have been isolated and are called D<sub>2</sub> long and D<sub>2</sub> short; alternative splicing generates these isoforms. D<sub>2</sub> long contains a 29-amino-acid insert in the large intracellular loop between the fifth and sixth membrane-spanning segments. Functional or anatomical differences have not yet been fully resolved for the short and long forms of D<sub>2</sub>.

D<sub>1</sub>-like receptors, like βARs, are transcribed from intronless genes (Dohlman et al., 1987). Conversely, all D<sub>2</sub>-like receptors contain introns, thus providing for possibilities of alternatively spliced products. Posttranslational modifications include glycosylation at one or more sites, disulfide bonding of the two Cys residues in e2 and e3, and acylation of the Cys residue in the C-terminal tail (analogous to the β2AR). The dopamine binding site includes two Ser residues in TM5 and an Asp residue in TM3, analogous to βAR. The recent crystallization of the D3 receptor (Chien et al., 2010) supports the importance of these amino acid interactions with ligand, confirms the similarity to the β2AR, and provides the potential for future subtype specific drug design.

Because of dopamine's presumed role in neuropsychiatric disorders, enormous effort has been focused on developing pharmacological tools for manipulating this system. Dopamine receptors bind amphetamines, bromocriptine, lisuride, clozapine, melperone, fluperlapine, and haloperidol. Because these drugs do not show great specificity for receptor subtypes, their usefulness for dissecting effects specifically related to binding to one or another dopamine receptor subtype is limited. However, their role in the treatment of human neuropsychiatric disorders is enormous.

### Purinergic Receptors

Purinergic receptors bind to ATP (or other nucleotide analogs) or its breakdown product adenosine. Although ATP is a common constituent found within synaptic vesicles, adenosine is not and is therefore not considered a "classic" neurotransmitter. However, the multitude of receptors that bind and are activated by adenosine indicates that this molecule has important modulatory effects on the nervous system as well as in the periphery. Situations of high metabolic activity that consume ATP and situations of insufficient ATP-regenerating capacity can lead to the accumulation of adenosine. Because adenosine is permeable to membranes and can diffuse into and out of cells, a feedback loop is established in which adenosine can serve as a local diffusible signal that communicates the metabolic status of the neuron to surrounding cells, and vice

versa (Junger, 2011). In addition, all mammalian cells express extracellular ectonucleotidases that rapidly metabolize nucleotides into adenosine, providing an additional extracellular source of transmitter for the activation of adenosine receptors.

The original nomenclature describing purinergic receptors defined adenosine as binding to P1 receptors and ATP as binding to P2 receptors. Families of both P1 and P2 receptors have since been described, and adenosine receptors are now identified as A-type purinergic receptors, consisting of P1A1, P1A2a, P1A2b, and P1A3. ATP receptors are designated as P type and consist of P2x and P2Y subtypes, the latter is a GPCR (Junger, 2011). Recall that P2x subtypes are ionotropic receptors (Fig. 10.10).

A-type receptors (P1A) exhibit the classic arrangement of seven transmembrane-spanning segments but are typically smaller than most GPCRs, ranging in size between 35 and 46 kDa. The ligand binding site of A-type receptors is unique in that the ligand, adenosine, has no inherent charged moieties at physiological pH. A-type receptors appear to use His residues as their points of contact with adenosine, and, in particular, a His residue in TM7 is essential, because its mutation eliminates agonist binding. Other His residues in TM6 and TM7 are conserved in all A-type receptors and may serve as other points of contact with agonists. Work with chimeric A-type receptors has further substantiated the importance of residues in TM5, TM6, and TM7 for ligand binding. A<sub>1</sub> receptors are highly expressed in the brain, and their activation downregulates adenylyl cyclase and increases phospholipase C activity. A<sub>2a</sub> and A<sub>2b</sub> receptors are not as highly expressed in nervous tissue and are associated with the stimulation of adenylyl cyclase and phospholipase C, respectively. The A<sub>3</sub> subtype exhibits a unique pharmacological profile in that binding of xanthine derivatives, which block adenosine's action competitively, is absent. Very low levels of the A<sub>3</sub> receptor are found in brain and peripheral nervous tissue. The A<sub>3</sub> receptor appears to be coupled to the activation of phospholipase C.

The human A<sub>1</sub> receptor has a unique mode of receptor expression (Olah and Stiles, 1995). Introns in the 5' untranslated sequence of the mRNA, spliced in a tissue-specific manner, are capable of affecting the translational efficiency of the mRNA. Two extra start codons upstream from the start codon that initiates translation of the A<sub>1</sub> receptor exert a negative effect on translation. Mutating these two extra start codons can relieve the translational repression. This process is an effective way of controlling the level of receptor expression and may serve as a more general model for translational regulation for other mRNAs.

The P-type receptors, P2Y1-P2Y7, are also typical GPCRs, mostly localized to the periphery. However,

direct effects of ATP have been detected in neurons, and often the response is biphasic; an early excitatory effect followed, with its breakdown to adenosine, by a secondary inhibitory effect. Interestingly, P-type receptors exhibit a higher degree of homology to peptide-binding receptors than they do to the A-type purinergic receptors. As in A-type receptors, P-type receptors have a His residue in the third transmembrane domain and presumably bind to nucleotides using a similar constellation of amino acids as other members of the class A GPCRs.

### **Serotonin Receptors**

Serotonin-containing cell bodies are found in the raphe nucleus in the brain stem and in nerve endings distributed diffusely throughout the brain (Julius, 1991). Serotonin has been implicated in sleep, modulation of circadian rhythms, eating, and arousal. Serotonin also has hormone-like effects when released in the bloodstream, regulating smooth muscle contraction and affecting the platelet-aggregating and immune systems.

Serotonin receptors are classified into seven subtypes: 5-HT<sub>1</sub> to 5-HT<sub>7</sub>, with a further subdivision within subtypes (Fig. 10.21). Evolutionarily, 5-HT<sub>4</sub> and 5-HT<sub>6</sub> are more closely related to each other than the other 5-HT GPCRs (Fig. 10.21). Recall that the 5-HT<sub>3</sub> receptor is ionotropic (Fig. 10.10). The other 5-HT receptors exhibit the typical seven transmembrane-spanning segments, and all couple to G proteins to exert their effects. For example, 5-HT<sub>1a</sub>, 1b, 1d, and 5-HT<sub>4</sub>, 5, 6 and 7 either activate or inhibit adenylyl cyclase. 5-HT<sub>1c</sub> and 5-HT<sub>2</sub> receptors preferentially stimulate activation of phospholipase C to produce increased intracellular levels of diacylglycerol and inositol 1,4,5-trisphosphate.

Serotonin receptors can also be grossly distributed into two groups on the basis of their gene structures. Both 5-HT<sub>1c</sub> and 5-HT<sub>2</sub> are derived from genes that contain multiple introns. In contrast, similar to the βAR family, 5-HT<sub>1</sub> is coded by a gene lacking introns. Interestingly, 5-HT<sub>1a</sub> is more closely related ancestrally to the βAR family than it is to other members of the serotonin receptor family and was originally isolated by using the cDNA for the β2AR as a molecular probe (Kobilka et al., 1987). This observation helps explain some pharmacological data suggesting that both 5-HT<sub>1a</sub> and 5-HT<sub>1b</sub> can bind certain adrenergic antagonists.

### **Glutamate GPCRs**

The GPCRs that bind glutamate (metabotropic glutamate receptors, or mGluRs) are similar in general structure in having seven transmembrane-spanning segments to other GPCRs; however, they are divergent enough to have originated from a separate evolutionary-derived receptor family (Hollmann and Heinemann, 1994; Nakanishi, 1994; Niswender and Conn, 2010). In fact,

sequence homology between the mGluR family and the other GPCRs is minimal except for the GABA<sub>B</sub> receptor (Fig. 10.21) and these two receptor families along with Ca<sup>2+</sup>-sensing and taste receptors are now grouped together as class C GPCRs. The mGluR family is heterogeneous in size, ranging from 854 to 1179 amino acids. Both the N-terminal and C-terminal domains are unusually large for G-protein-coupled receptors. One great difference in the structures of mGluRs is that the binding site for glutamate resides in the large N-terminal extracellular domain (Armstrong and Gouaux, 2000; O'Hara et al., 1993). Several crystal structures of this domain in different states have been obtained. The binding site is called the Venus Fly trap Domain (VFD) to anecdotally capture the structural transition of the open to closed nature of the domain when bound to glutamate. Recall that in Class A GPCRs, the ligand-binding pocket sits within the TM domains of the receptor. In addition, mGluRs exist as functional dimers in the membrane in contrast to the single-subunit forms of most other GPCRs (Kunishima et al., 2000; Niswender and Conn, 2010). Because of this dimer structure, the mGluRs can exist in states with either one or two molecules of glutamate bound. Maximal activity requires that two glutamate molecules are bound, but there remains some controversy about whether a single molecule of glutamate can activate the receptor. The second intracellular loop (i2), as opposed to the third intracellular loop in class A GPCRs, along with the large C-terminal domain are thought to be the major determinants responsible for G-protein coupling. The C-terminal domain also serves as the site for interactions with other intracellular scaffolding and regulatory molecules. These significant structural distinctions support the idea that mGluRs evolved separately from other GPCRs.

Currently, eight different mGluRs can be subdivided into three groups on the basis of sequence homologies and their capacity to couple to specific enzyme systems. Group I includes both mGluR1 and mGluR5 and these receptors are found predominantly postsynaptic and activate G-proteins that couple to phospholipase C or adenylyl cyclase (Aramori and Nakanishi, 1992). Group II includes mGluR2 and mGluR3 that are found pre- and postsynaptic, inhibit adenylyl cyclase, inhibit Ca<sup>2+</sup>-channels and activate K<sup>+</sup>-channels. Group III includes mGluR4-mGluR8 and produce functional effects similar to Group II mGluRs. mGluR6 is unique and is found only in the retina where it stimulates the enzyme cGMP phosphodiesterase.

Group I mGluRs are selectively activated by the agonist (S)-3,5-dihydroxyphenylglycine [(S)-3,5-DHPG]. Group II mGluRs are selectively activated by (2S,2'R,3'R)-2-(2',3')-dicarboxycyclopropyl)glycine DCG-IV and (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (2R,4R-APDC) and group II mGluRs are selectively

activated by L-(+)-2-amino-4-phosphonobutyric acid (L-AP4). Numerous other pharmacological compounds have also been developed that can selectively activate members within some of these mGluR classes.

The mGluRs are widespread in the nervous system and are found both pre- and postsynaptically. Presynaptically, they serve as autoreceptors and appear to participate in the inhibition of neurotransmitter release. Their postsynaptic roles appear to be quite varied and depend on the specific G protein to which they are coupled. mGluR<sub>1</sub> activation has been implicated in long-term synaptic plasticity at many sites in the brain, including long-term potentiation in the hippocampus and long-term depression in the cerebellum (see Chapter 18).

### GABA<sub>B</sub> Receptors

GABA<sub>B</sub> receptors are found throughout the nervous system, where they are sometimes colocalized with ionotropic GABA<sub>A</sub> receptors. GABA<sub>B</sub> receptors are present both pre- and postsynaptically. Presynaptically, they appear to mediate inhibition of neurotransmitter release through an autoreceptor-like mechanism by activating K<sup>+</sup> conductances and diminishing Ca<sup>2+</sup> conductances. In addition, GABA<sub>B</sub> receptors may affect K<sup>+</sup> channels through a direct physical coupling to the K<sup>+</sup> channel, not mediated through a G-protein intermediate. Postsynaptically, GABA<sub>B</sub> receptor activation produces a characteristic slow hyperpolarization (termed the slow inhibitory postsynaptic potential) through the activation of a K<sup>+</sup> conductance. This effect appears to be through a pertussis-toxin-sensitive G protein that inhibits adenylyl cyclase.

The cloning of the GABA<sub>B</sub> receptor (GABA<sub>B</sub>R<sub>1</sub>) revealed that it has high sequence homology to the family of glutamate GPCRs but shows little similarity to other G-protein-coupled receptors. The large N-terminal extracellular domain of the GABA<sub>B</sub> receptor is the presumed site of GABA binding. With the exception of this large extracellular domain, the GABA<sub>B</sub> receptor structure is typical of the GPCR family, exhibiting seven TM domains. The initial cloning of the GABA<sub>B</sub> receptor was made possible by the development of a high-affinity, high-specificity antagonist termed CGP64213. This antagonist is several orders of magnitude more potent at inhibiting GABA<sub>B</sub> receptor function than the more widely known antagonist baclofen. Baclofen, an analog of saclofen, remains the best agonist for activating GABA<sub>B</sub> receptors.

Functional GABA<sub>B</sub> receptors exist as obligate dimers in the membrane (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998). Expression of the cloned GABA<sub>B</sub>R<sub>1</sub> isoform does not produce significant functional receptors. However, when coexpressed with the GABA<sub>B</sub>R<sub>2</sub> subunit, receptors that are indistinguishable

functionally and pharmacologically from those in brain were produced. GABA binds to the GABA<sub>B1</sub> subunit to activate the receptor and the GABA<sub>B2</sub> subunit is responsible for coupling to G-proteins (Kniazeff et al., 2011). As noted earlier in this chapter (Fig. 10.18B), GPCRs can interact with themselves and other receptors. It is good to keep in mind that these types of direct receptor interactions may be more widespread than currently appreciated.

### Peptide Receptors

Neuropeptide receptors are an immense family. Because of their diversity, they cannot be covered in detail in this chapter. Despite their diversity, none of the receptors that bind peptides appear to be coupled directly to the opening of ion channels. Neuropeptide receptors exert their effects either through the typical pathway of activation of G proteins or through a pathway related to activation of an intrinsic tyrosine kinase activity associated with the receptor.

The peptide-binding domain of neuropeptide receptors includes residues in both the large N-terminal extracellular domain and the transmembrane domain (Strader et al., 1995). These additional stabilizing contacts presumably provide the receptors with their remarkably high affinity for neuropeptides (in the nanomolar concentration range). For example, residues in the first and second extracellular domains, as well as those in at least four of the TM domains of the NK1 neurokinin receptor, interact with substance P to form stabilizing contacts. Many small-molecule antagonists are known to inhibit activation of the NK1 neurokinin receptor, and these antagonists bind to some, but not all, of the same amino acids in the TM segments as does substance P. The possible mechanisms for inhibition of the peptide receptors range from complete structural overlap between agonist and antagonist binding to complete allosteric exclusion (Strader et al., 1995). Knowledge of the activated structure of the neuropeptide receptors provides remarkable opportunities for future drug design.

### Summary

GPCRs are single polypeptides composed of seven transmembrane-spanning segments. In general, the binding site for neurotransmitter is located within the core of the circular structure formed by these segments. Transmitter binding produces conformational changes in the receptor that expose parts of the i3 region, among others, for binding to G proteins. G-protein binding increases the affinity of the receptor for transmitter. Desensitization is common among GPCRs and leads to decreased response of the receptor

to neurotransmitter by several distinct mechanisms. The mGluRs and GABA<sub>B</sub> receptors are structurally distinct from other GPCRs; they both have large N-terminal extracellular domains that form the binding site for neurotransmitter and their functional forms are dimeric. Otherwise, the basic structure of mGluRs and GABA<sub>B</sub> receptors appears to be similar to that of the rest of the GPCR family.

## References

- Aramori, I., Nakanishi, S., 1992. Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. *Neuron*. 8, 757–765.
- Armstrong, N., Gouaux, E., 2000. Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. *Neuron*. 28, 165–181.
- Ascher, P., Nowak, L., 1988. The role of divalent cations in the N-methyl-D-aspartate responses of mouse central neurones in culture. *J. Physiol.* 399, 247–266.
- Barak, L.S., Tiberi, M., Freedman, N.J., Kwatra, M.M., Lefkowitz, R.J., Caron, M.G., 1994. A highly conserved tyrosine residue in G protein-coupled receptors is required for agonist-mediated beta 2-adrenergic receptor sequestration. *J. Biol. Chem.* 269, 2790–2795.
- Barnard, E.A., Darlison, M.G., Fujita, N., Glencorse, T.A., Levitan, E.S., Reale, V., et al., 1988. Molecular biology of the GABA<sub>A</sub> receptor. *Adv. Exp. Med. Biol.* 236, 31–45.
- Bertrand, D., Galzi, J.L., Devillers-Thiery, A., Bertrand, S., Changeux, J.P., 1993. Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal alpha 7 nicotinic receptor. *Proc. Natl. Acad. Sci. U.S.A.* 90, 6971–6975.
- Betz, H., 1991. Glycine receptors: heterogeneous and widespread in the mammalian brain. *Trends Neurosci.* 14, 458–461.
- Blackstone, C.D., Moss, S.J., Martin, L.J., Levey, A.I., Price, D.L., Huganir, R.L., 1992. Biochemical characterization and localization of a non-N-methyl-D-aspartate glutamate receptor in rat brain. *J. Neurochem.* 58, 1118–1126.
- Bormann, J., 1988. Electrophysiology of GABA<sub>A</sub> and GABA<sub>B</sub> receptor subtypes. *Trends Neurosci.* 11, 112–116.
- Bormann, J., Feigenspan, A., 1995. GABAC receptors. *Trends Neurosci.* 18, 515–519.
- Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C., et al., 1990. Molecular cloning and functional expression of glutamate receptor subunit genes. *Science*. 249, 1033–1037.
- Bouvier, M., Collins, S., O'Dowd, B.F., Campbell, P.T., De Blasi, A., Kobilka, B.K., et al., 1989. Two distinct pathways for cAMP-mediated down-regulation of the beta 2-adrenergic receptor. Phosphorylation of the receptor and regulation of its mRNA level. *J. Biol. Chem.* 264, 16786–16792.
- Brake, A.J., Wagenbach, M.J., Julius, D., 1994. New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature*. 371, 519–523.
- Bunzow, J.R., Van Tol, H.H., Grandy, D.K., Albert, P., Salon, J., Christie, M., et al., 1988. Cloning and expression of a rat D2 dopamine receptor cDNA. *Nature*. 336, 783–787.
- Burnashev, N., Schoepfer, R., Monyer, H., Ruppersberg, J.P., Gunther, W., Seeburg, P.H., et al., 1992. Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. *Science*. 257, 1415–1419.
- Casey, P.J., 1995. Protein lipidation in cell signaling. *Science*. 268, 221–225.
- Cassel, D., Selinger, Z., 1977. Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolysis at the regulatory site. *Proc. Natl. Acad. Sci. U.S.A.* 74, 3307–3311.
- Changeux, J.P., Devillers-Thiery, A., Chemouilli, P., 1984. Acetylcholine receptor: an allosteric protein. *Science*. 225, 1335–1345.
- Chien, E.Y., Liu, W., Zhao, Q., Katritch, V., Han, G.W., Hanson, M.A., et al., 2010. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science*. 330, 1091–1095.
- Civelli, O., Bunzow, J.R., Grandy, D.K., 1993. Molecular diversity of the dopamine receptors. *Annu. Rev. Pharmacol. Toxicol.* 33, 281–307.
- Clark, R.B., Knoll, B.J., Barber, R., 1999. Partial agonists and G protein-coupled receptor desensitization. *Trends Pharmacol. Sci.* 20, 279–286.
- Colquhoun, D., Ogden, D.C., 1988. Activation of ion channels in the frog end-plate by high concentrations of acetylcholine. *J. Physiol.* 395, 131–159.
- Colquhoun, D., Sakmann, B., 1985. Fast events in single-channel currents activated by acetylcholine and its analogues at the frog muscle end-plate. *J. Physiol.* 369, 501–557.
- Dohlman, H.G., Caron, M.G., Lefkowitz, R.J., 1987. A family of receptors coupled to guanine nucleotide regulatory proteins. *Biochemistry*. 26, 2657–2664.
- Ehlers, M.D., Tingley, W.G., Huganir, R.L., 1995. Regulated subcellular distribution of the NR1 subunit of the NMDA receptor. *Science*. 269, 1734–1737.
- Ehlers, M.D., Zhang, S., Bernhardt, J.P., Huganir, R.L., 1996. Inactivation of NMDA receptors by direct interaction of calmodulin with the NR1 subunit. *Cell*. 84, 745–755.
- Ferguson, S.S., Menard, L., Barak, L.S., Koch, W.J., Colapietro, A.M., Caron, M.G., 1995. Role of phosphorylation in agonist-promoted beta 2-adrenergic receptor sequestration. Rescue of a sequestration-defective mutant receptor by beta ARK1. *J. Biol. Chem.* 270, 24782–24789.
- Ferguson, S.S., Downey 3rd, W.E., Colapietro, A.M., Barak, L.S., Menard, L., Caron, M.G., 1996. Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. *Science*. 271, 363–366.
- Fraser, C.M., Chung, F.Z., Wang, C.D., Venter, J.C., 1988. Site-directed mutagenesis of human beta-adrenergic receptors: substitution of aspartic acid-130 by asparagine produces a receptor with high-affinity agonist binding that is uncoupled from adenylate cyclase. *Proc. Natl. Acad. Sci. U.S.A.* 85, 5478–5482.
- Fraser, C.M., Wang, C.D., Robinson, D.A., Gocayne, J.D., Venter, J.C., 1989. Site-directed mutagenesis of m1 muscarinic acetylcholine receptors: conserved aspartic acids play important roles in receptor function. *Mol. Pharmacol.* 36, 840–847.
- Green, W.N., Claudio, T., 1993. Acetylcholine receptor assembly: subunit folding and oligomerization occur sequentially. *Cell*. 74, 57–69.
- Gu, Y., Forsayeth, J.R., Verrall, S., Yu, X.M., Hall, Z.W., 1991. Assembly of the mammalian muscle acetylcholine receptor in transfected COS cells. *J. Cell Biol.* 114, 799–807.
- Hattori, M., Gouaux, E., 2012. Molecular mechanism of ATP binding and ion channel activation in P2X receptors. *Nature*. 485, 207–212.
- Henderson, R., Baldwin, J.M., Ceska, T.A., Zemlin, F., Beckmann, E., Downing, K.H., 1990. Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. *J. Mol. Biol.* 213, 899–929.
- Hille, B., 1992. Ionic Channels of Excitable Membranes. Sinauer, Sunderland, MA.
- Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. *Annu. Rev. Neurosci.* 17, 31–108.

- Hollmann, M., O'Shea-Greenfield, A., Rogers, S.W., Heinemann, S., 1989. Cloning by functional expression of a member of the glutamate receptor family. *Nature*. 342, 643–648.
- Huang, C.C., Tesmer, J.J., 2011. Recognition in the face of diversity: interactions of heterotrimeric G proteins and G protein-coupled receptor (GPCR) kinases with activated GPCRs. *J. Biol. Chem.* 286, 7715–7721.
- Huganir, R.L., Greengard, P., 1990. Regulation of neurotransmitter receptor desensitization by protein phosphorylation. *Neuron*. 5, 555–567.
- Hume, R.I., Dingledine, R., Heinemann, S.F., 1991. Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science*. 253, 1028–1031.
- Imoto, K., Busch, C., Sakmann, B., Mishina, M., Konno, T., Nakai, J., et al., 1988. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature*. 335, 645–648.
- Inglese, J., Freedman, N.J., Koch, W.J., Lefkowitz, R.J., 1993. Structure and mechanism of the G protein-coupled receptor kinases. *J. Biol. Chem.* 268, 23735–23738.
- Jones, K.A., Borowsky, B., Tamm, J.A., Craig, D.A., Durkin, M.M., Dai, M., et al., 1998. GABA<sub>B</sub> receptors function as a heteromeric assembly of the subunits GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2. *Nature*. 396, 674–679.
- Julius, D., 1991. Molecular biology of serotonin receptors. *Annu. Rev. Neurosci.* 14, 335–360.
- Junger, W.G., 2011. Immune cell regulation by autocrine purinergic signalling. *Nat. Rev. Immunol.* 11, 201–212.
- Karlin, A., 1993. Structure of nicotinic acetylcholine receptors. *Curr. Opin. Neurobiol.* 3, 299–309.
- Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., et al., 1998. GABA<sub>B</sub>-receptor subtypes assemble into functional heteromeric complexes. *Nature*. 396, 683–687.
- Keinanen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T.A., et al., 1990. A family of AMPA-selective glutamate receptors. *Science*. 249, 556–560.
- Kniazeff, J., Prezeau, L., Rondard, P., Pin, J.P., Goudet, C., 2011. Dimers and beyond: the functional puzzles of class C GPCRs. *Pharmacol. Ther.* 130, 9–25.
- Kobilka, B., 1992. Adrenergic receptors as models for G protein-coupled receptors. *Annu. Rev. Neurosci.* 15, 87–114.
- Kobilka, B.K., Frielle, T., Collins, S., Yang-Feng, T., Kobilka, T.S., Francke, U., et al., 1987. An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. *Nature*. 329, 75–79.
- Kornau, H.C., Schenker, L.T., Kennedy, M.B., Seeburg, P.H., 1995. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science*. 269, 1737–1740.
- Kumar, J., Mayer, M.L., 2013. Functional insights from glutamate receptor ion channel structures. *Annu. Rev. Physiol.* 75, 313–337.
- Kunishima, N., Shimada, Y., Tsuji, Y., Sato, T., Yamamoto, M., Kumakura, T., et al., 2000. Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature*. 407, 971–977.
- Kutsuwada, T., Kashiwabuchi, N., Mori, H., Sakimura, K., Kushiya, E., Araki, K., et al., 1992. Molecular diversity of the NMDA receptor channel. *Nature*. 358, 36–41.
- Lee, S.P., O'Dowd, B.F., Ng, G.Y., Varghese, G., Akil, H., Mansour, A., et al., 2000. Inhibition of cell surface expression by mutant receptors demonstrates that D2 dopamine receptors exist as oligomers in the cell. *Mol. Pharmacol.* 58, 120–128.
- Liu, F., Wan, Q., Pristupa, Z.B., Yu, X.M., Wang, Y.T., Niznik, H.B., 2000. Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. *Nature*. 403, 274–280.
- Lomeli, H., Mosbacher, J., Melcher, T., Hoger, T., Geiger, J.R., Kuner, T., et al., 1994. Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. *Science*. 266, 1709–1713.
- Maggio, R., Vogel, Z., Wess, J., 1993. Coexpression studies with mutant muscarinic/adrenergic receptors provide evidence for intermolecular "cross-talk" between G-protein-linked receptors. *Proc. Natl. Acad. Sci. U.S.A.* 90, 3103–3107.
- Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M., Julius, D., 1991. Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. *Science*. 254, 432–437.
- Mayer, M.L., Westbrook, G.L., 1987. Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. *J. Physiol.* 394, 501–527.
- McGehee, D.S., Heath, M.J., Gelber, S., Devay, P., Role, L.W., 1995. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science*. 269, 1692–1696.
- Meguro, H., Mori, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., et al., 1992. Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature*. 357, 70–74.
- Mishina, M., Kurosaki, T., Tobimatsu, T., Morimoto, Y., Noda, M., Yamamoto, T., et al., 1984. Expression of functional acetylcholine receptor from cloned cDNAs. *Nature*. 307, 604–608.
- Miyazawa, A., Fujiyoshi, Y., Stowell, M., Unwin, N., 1999. Nicotinic acetylcholine receptor at 4.6 Å resolution: transverse tunnels in the channel wall. *J. Mol. Biol.* 288, 765–786.
- Mizobe, T., Maze, M., Lam, V., Suryanarayana, S., Kobilka, B.K., 1996. Arrangement of transmembrane domains in adrenergic receptors. Similarity to bacteriorhodopsin. *J. Biol. Chem.* 271, 2387–2389.
- Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., et al., 1992. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science*. 256, 1217–1221.
- Mori, H., Masaki, H., Yamakura, T., Mishina, M., 1992. Identification by mutagenesis of a Mg<sup>2+</sup>-block site of the NMDA receptor channel. *Nature*. 358, 673–675.
- Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N., Nakanishi, S., 1991. Molecular cloning and characterization of the rat NMDA receptor. *Nature*. 354, 31–37.
- Moss, S.J., Smart, T.G., 2001. Constructing inhibitory synapses. *Nat. Rev. Neurosci.* 2, 240–250.
- Nakagawa, T., Cheng, Y., Ramm, E., Sheng, M., Walz, T., 2005. Structure and different conformational states of native AMPA receptor complexes. *Nature*. 433, 545–549.
- Nakanishi, N., Shneider, N.A., Axel, R., 1990. A family of glutamate receptor genes: evidence for the formation of heteromultimeric receptors with distinct channel properties. *Neuron*. 5, 569–581.
- Nakanishi, S., 1994. Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. *Neuron*. 13, 1031–1037.
- Nicoll, R.A., Tomita, S., Bredt, D.S., 2006. Auxiliary subunits assist AMPA-type glutamate receptors. *Science*. 311, 1253–1256.
- Niswender, C.M., Conn, P.J., 2010. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* 50, 295–322.
- Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Furutani, Y., Hirose, T., et al., 1982. Primary structure of alpha-subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequence. *Nature*. 299, 793–797.
- Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikuyama, S., Furutani, Y., et al., 1983. Structural homology of *Torpedo californica* acetylcholine receptor subunits. *Nature*. 302, 528–532.
- O'Dowd, B.F., Hnatowich, M., Caron, M.G., Lefkowitz, R.J., Bouvier, M., 1989. Palmitoylation of the human beta 2-adrenergic receptor. Mutation of Cys341 in the carboxyl tail leads to an uncoupled nonpalmitoylated form of the receptor. *J. Biol. Chem.* 264, 7564–7569.

- O'Hara, P.J., Sheppard, P.O., Thogersen, H., Venezia, D., Haldeman, B.A., McGrane, V., et al., 1993. The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron*. 11, 41–52.
- Olah, M.E., Stiles, G.L., 1995. Adenosine receptor subtypes: characterization and therapeutic regulation. *Annu. Rev. Pharmacol. Toxicol.* 35, 581–606.
- Ortells, M.O., Lunt, G.G., 1995. Evolutionary history of the ligand-gated ion-channel superfamily of receptors. *Trends Neurosci.* 18, 121–127.
- Overton, M.C., Blumer, K.J., 2000. G-protein-coupled receptors function as oligomers in vivo. *Curr. Biol.* 10, 341–344.
- Palczewski, K., Kumashiro, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., et al., 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science*. 289, 739–745.
- Paoletti, P., Neyton, J., 2007. NMDA receptor subunits: function and pharmacology. *Curr. Opin. Pharmacol.* 7, 39–47.
- Paulson, H.L., Ross, A.F., Green, W.N., Claudio, T., 1991. Analysis of early events in acetylcholine receptor assembly. *J. Cell Biol.* 113, 1371–1384.
- Perez, D.M., Hwa, J., Gaivin, R., Mathur, M., Brown, F., Graham, R.M., 1996. Constitutive activation of a single effector pathway: evidence for multiple activation states of a G protein-coupled receptor. *Mol. Pharmacol.* 49, 112–122.
- Premont, R.T., Inglese, J., Lefkowitz, R.J., 1995. Protein kinases that phosphorylate activated G protein-coupled receptors. *FASEB J.* 9, 175–182.
- Pritchett, D.B., Sontheimer, H., Shivers, B.D., Ymer, S., Kettenmann, H., Schofield, P.R., et al., 1989. Importance of a novel GABA<sub>A</sub> receptor subunit for benzodiazepine pharmacology. *Nature*. 338, 582–585.
- Proll, M.A., Clark, R.B., Goka, T.J., Barber, R., Butcher, R.W., 1992. Beta-adrenergic receptor levels and function after growth of S49 lymphoma cells in low concentrations of epinephrine. *Mol. Pharmacol.* 42, 116–122.
- Rosenbaum, D.M., Rasmussen, S.G., Kobilka, B.K., 2009. The structure and function of G-protein-coupled receptors. *Nature*. 459, 356–363.
- Rudolph, U., Crestani, F., Benke, D., Brunig, I., Benson, J.A., Fritschy, J.M., et al., 1999. Benzodiazepine actions mediated by specific gamma-aminobutyric acid A receptor subtypes. *Nature*. 401, 796–800.
- Saiyed, T., Paarmann, I., Schmitt, B., Haeger, S., Sola, M., Schmalzing, G., et al., 2007. Molecular basis of gephyrin clustering at inhibitory synapses: role of G- and E-domain interactions. *J. Biol. Chem.* 282, 5625–5632.
- Salahpour, A., Angers, S., Bouvier, M., 2000. Functional significance of oligomerization of G-protein-coupled receptors. *Trends Endocrinol. Metab.* 11, 163–168.
- Sanes, J.R., 1997. Genetic analysis of postsynaptic differentiation at the vertebrate neuromuscular junction. *Curr. Opin. Neurobiol.* 7, 93–100.
- Seguela, P., Wadiche, J., Dineley-Miller, K., Dani, J.A., Patrick, J.W., 1993. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. *J. Neurosci.* 13, 596–604.
- Sheng, M., Hoogenraad, C.C., 2007. The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annu. Rev. Biochem.* 76, 823–847.
- Sieghart, W., 1992. GABA<sub>A</sub> receptors: ligand-gated Cl<sup>-</sup> ion channels modulated by multiple drug-binding sites. *Trends Pharmacol. Sci.* 13, 446–450.
- Sobolevsky, A.I., Rosconi, M.P., Gouaux, E., 2009. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature*. 462, 745–756.
- Sommer, B., Keinanen, K., Verdoorn, T.A., Wisden, W., Burnashev, N., Herb, A., et al., 1990. Flip and flop: a cell-specific functional switch in glutamate-operated channels of the CNS. *Science*. 249, 1580–1585.
- Sommer, B., Kohler, M., Sprengel, R., Seuberg, P.H., 1991. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell*. 67, 11–19.
- Sterne-Marr, R., Benovic, J.L., 1995. Regulation of G protein-coupled receptors by receptor kinases and arrestins. *Vitam. Horm.* 51, 193–234.
- Strader, C.D., Fong, T.M., Tota, M.R., Underwood, D., Dixon, R.A., 1994. Structure and function of G protein-coupled receptors. *Annu. Rev. Biochem.* 63, 101–132.
- Strader, C.D., Fong, T.M., Graziano, M.P., Tota, M.R., 1995. The family of G-protein-coupled receptors. *FASEB J.* 9, 745–754.
- Strosberg, A.D., 1990. Biotechnology of beta-adrenergic receptors. *Mol. Neurobiol.* 4, 211–250.
- Swulius, M.T., Kubota, Y., Forest, A., Waxham, M.N., 2010. Structure and composition of the postsynaptic density during development. *J. Comp. Neurol.* 518, 4243–4260.
- Tolkovsky, A.M., Braun, S., Levitzki, A., 1982. Kinetics of interaction between beta-receptors, GTP protein, and the catalytic unit of turkey erythrocyte adenylate cyclase. *Proc. Natl. Acad. Sci. U.S.A.* 79, 213–217.
- Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., et al., 2010. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.* 62, 405–496.
- Unwin, N., 1993a. Neurotransmitter action: opening of ligand-gated ion channels. *Cell*. 72 (Suppl), 31–41.
- Unwin, N., 1993b. Nicotinic acetylcholine receptor at 9 Å resolution. *J. Mol. Biol.* 229, 1101–1124.
- Unwin, N., 1995. Acetylcholine receptor channel imaged in the open state. *Nature*. 373, 37–43.
- Unwin, N., Fujiyoshi, Y., 2012. Gating movement of acetylcholine receptor caught by plunge-freezing. *J. Mol. Biol.* 422, 617–634.
- Valera, S., Hussy, N., Evans, R.J., Adamo, N., North, R.A., Surprenant, A., et al., 1994. A new class of ligand-gated ion channel defined by P2x receptor for extracellular ATP. *Nature*. 371, 516–519.
- Verdoorn, T.A., Burnashev, N., Monyer, H., Seuberg, P.H., Sakmann, B., 1991. Structural determinants of ion flow through recombinant glutamate receptor channels. *Science*. 252, 1715–1718.
- Vernino, S., Amador, M., Luetje, C.W., Patrick, J., Dani, J.A., 1992. Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. *Neuron*. 8, 127–134.
- Watkins, J.C., Krosgaard-Larsen, P., Honore, T., 1990. Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharmacol. Sci.* 11, 25–33.
- Wentholt, R.J., Yokotani, N., Doi, K., Wada, K., 1992. Immunochemical characterization of the non-NMDA glutamate receptor using subunit-specific antibodies. Evidence for a heterooligomeric structure in rat brain. *J. Biol. Chem.* 267, 501–507.
- Wess, J., 1993. Molecular basis of muscarinic acetylcholine receptor function. *Trends Pharmacol. Sci.* 14, 308–313.
- White, J.H., Wise, A., Main, M.J., Green, A., Fraser, N.J., Disney, G.H., et al., 1998. Heterodimerization is required for the formation of a functional GABA<sub>B</sub> receptor. *Nature*. 396, 679–682.
- Willmann, R., Fuhrer, C., 2002. Neuromuscular synaptogenesis: clustering of acetylcholine receptors revisited. *Cell Mol. Life. Sci.* 59, 1296–1316.
- Wo, Z.G., Oswald, R.E., 1995. Unraveling the modular design of glutamate-gated ion channels. *Trends Neurosci.* 18, 161–168.
- Yakel, J.L., Lagrutta, A., Adelman, J.P., North, R.A., 1993. Single amino acid substitution affects desensitization of the 5-hydroxytryptamine

- type 3 receptor expressed in *Xenopus* oocytes. Proc. Natl. Acad. Sci. U.S.A. 90, 5030–5033.
- Young, A.B., Snyder, S.H., 1974. The glycine synaptic receptor: evidence that strychnine binding is associated with the ionic conductance mechanism. Proc. Natl. Acad. Sci. U.S.A. 71, 4002–4005.
- Yuan, N., Friedman, J., Whaley, B.S., Clark, R.B., 1994. cAMP-dependent protein kinase and protein kinase C consensus site mutations of the beta-adrenergic receptor. Effect on desensitization and stimulation of adenylylcyclase. J. Biol. Chem. 269, 23032–23038.
- Ziff, E.B., 2007. TARPs and the AMPA receptor trafficking paradox. Neuron. 53, 627–633.