Commentary

Sticking together

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The receptors are tightly associated with a cytoplasmic meshwork of proteins comprising regulatory enzymes that may modify the receptor itself to alter its kinetics or location, or transmit biochemical signals deeper into the cytoplasm (6–9). The N-methyl-D-aspartate (NMDA) subtype of the glutamate receptors is an extreme example of this situation. It is equipped with unusually long carboxy-terminal “tails” that extend into the cytoplasm and associate with a variety of scaffold and signaling molecules (10).

Several recent papers, including two in PNAS, by Kneussel et al. (11) and by Chen et al. (12), have begun to clarify the mechanism of clustering of the inhibitory receptors for glycine and GABA, and the nature of the matrix of intracellular proteins associated with these receptors. A possible glycine receptor clustering protein of ~93 kDa, termed gephyrin (from the Greek word for bridge), originally was identified based on its copurification with the glycine receptor (13). Recent genetic experiments show that gephyrin is required for synaptic clustering of both glycine and GABAA receptors (8, 14). These experiments are puzzling, however, because gephyrin itself shows no significant affinity for GABAA receptors in vitro (8). A partial solution to the puzzle was offered in a set of experiments demonstrating that a small protein of ~14 kDa, termed GABARAP (GABA A receptor-associated protein) can bind to a subunit of the GABAA receptor (15) and to gephyrin (11), perhaps forming the missing physical link between the two (Fig. 1). GABARAP has an N-terminal tubulin-binding domain and is closely related to the previously described “late-acting intra-Golgi transport factor,” termed p16 (11). These findings suggest the hypothesis that GABARAP may link gephyrin and the GABAA receptor together as they are carried along microtubules and targeted to the proper membrane site (16). The propensity of gephyrin to form multimeric assemblies in vitro may be important for later stabilization of receptor complexes at the postsynaptic site.

Kneussel et al. (11) have found a fly in the hypothetical ointment, however. In cortical neurons in culture, where GABARAP previously had been shown to colocalize with GABAA receptors (15), they found that GABARAP is primarily in intracellular vesicles and does not colocalize with gephyrin. Furthermore, in the retina, GABAA receptors and gephyrin are tightly colocalized, but GABARAP shows no significant colocalization with either of them. Thus, in neurons GABARAP is not usually clustered at inhibitory synaptic sites; thus, its interaction with the GABAA receptor and gephyrin may be important for cellular functions other than receptor an-

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choring. For example, GABARAP may be involved in intraneuronal receptor sorting and targeting that precede and/or initiate receptor clustering at the synapse (16).

In light of these findings, it is curious that Chen et al. (12) find that GABARAP is targeted to the plasma membrane when expressed in heterologous cells, specifically quail fibroblast (QF6) cells in culture. Furthermore, they show that GABARAP increases the fraction of clustered GABAA receptors at the plasma membrane and colocalizes with the receptor, when they are expressed together in the same cells. Kneussel et al. (11) found that GABARAP and gephyrin become colocalized in clusters at the plasma membrane when expressed heterologously in PC12 cells. The apparent contradiction between the behavior of GABARAP in neurons and that in QF6 cells and PC12 cells illustrates the danger of extrapolating results in neurons and that in QF6 cells and PC12 cells. The apparent contradiction between the behavior of GABARAP in neurons and that in QF6 cells and PC12 cells illustrates the danger of extrapolating too literally to neurons from results in heterologous cells overexpressing a foreign protein.

Taken together, the data suggest that GABARAP is likely important for early sorting and targeting that precede and/or initiate receptor clustering at the synapse. The synaptic localization compared with diffuse GABAA receptors at the plasma membrane when expressed heterologously in PC12 cells. The apparent contradiction between the behavior of GABARAP in neurons and that in QF6 cells and PC12 cells illustrates the danger of extrapolating too literally to neurons from results in heterologous cells overexpressing a foreign protein.

Even more intriguing, however, is the finding by Chen et al. (12) that GABARAP increases the fraction of clustered GABAA receptors at the plasma membrane and colocalizes with the receptor in synaptic clusters; but, its mode of association with the GABAA receptor remains unclear.

Even more intriguing, however, is the finding by Chen et al. (12) in QF6 cells that clustered GABAA receptors have a significantly reduced affinity for GABA (from an EC50 of ~6 μM to ~20 μM) and altered kinetics of inactivation and desensitization compared with diffuse GABAA receptors. If the clustering of the receptors in heterologous cells is similar to that at synapses, the data imply that physical clustering and accompanying association with the cytoskeleton can dramatically modulate kinetic properties of receptors. Earlier work had indicated that the kinetic behavior of synaptic GABAA receptors differs from that of nonsynaptic receptors (17). However, these data were interpreted to suggest that certain combinations of GABAA receptor subunits are selectively concentrated at synapses. Indeed, this might well be the case. GABAA receptors are heteropentameric and are composed of various combinations of six α subunits, four β, four γ, one δ, and two ρ subunits (2). Most contain at least two copies of α and β subunits and at least one γ subunit. Hence, the number of distinct GABAA receptors is very large. Each combination can have slightly different kinetic properties. It is plausible that certain subunit combinations with characteristic kinetics might be more readily localized at synapses. The data of Chen et al. suggest the additional possibility that the physical clustering of receptors can itself alter receptor properties significantly. The change in affinity for GABA is in a range that could have functional consequences. A lower affinity and faster dissociation rate might permit faster channel closing at the synapse after release of one vesicle's worth of GABA.

The brain continues to show us that its range and subtlety of function are based on highly tunable properties of each processing unit. The next decade will be exciting indeed as we begin to learn how these processing units (synapses) are tuned during development, and moment by moment in adults, so that they work together to produce appropriate behavioral output.