have also been used as reworkable underfills (*7*). Another approach is to use blowing agents that decompose at high temperature, giving off a large amount of gas. By blending the blowing agents into epoxy resins, underfills can be made reworkable (*8*).

Another drawback of conventional underfill is the long and tedious dispensing and curing process. A wafer level underfill material is in development to address this problem (*9*). Instead of dispensing the conventional underfill into the gap between the chip and the substrate after solder reflow, wafer level underfill can be applied directly onto the wafer. The wafer is then diced into individual chips for further assembly onto the substrate; final curing of underfill and solder joint connection occur simultaneously during solder reflow.

An approach that does not use underfill has also been pursued to enhance the reliability of flip chips on organic substrates. A polymer stress buffer layer is applied and flexible metal leads are built from the metal terminal on the chip (*10*). At the end of the leads, solder bumps are formed for flip chip interconnection. Through the deformation of the low-modulus buffer layer and the flexible leads, the thermal stress on the solder joints is released and the fatigue life of the solder joints is improved. The stress buffer layer, flexible leads, and solder bumps can all be fabricated on the wafer level. Because no underfill is used, the chip can be reworked easily.

Currently, only about 1% of all IC chips are assembled with flip chip technology, but given the rapid advances in microelectronics and electronic packaging, the application of flip chip technology is expected to increase dramatically in the near future. For small chips with low I/O counts, flip chips with stress buffer layers will play a major role, but for large chips with high I/O counts such as microprocessors, the stress buffer layer cannot ensure sufficient reliability. Wafer level reworkable underfill may enable low cost, high reliability flip chip assembly for these applications.


**References**

1. R. R. Tummala, E. J. Rymaszewski, A. G. Klopfenstein, *Microelectronics Packaging Handbook* (Chapman & Hall, New York, 1997).
2. C. P. Wong, Ed., *Polymers for Electronic and Photonic Applications* (Academic Press, San Diego, CA, 1993).
3. L. F. Miller, *IBM J. Res. Dev.* **13**, 239 (1969).
4. T. Inoue *et al.*, in *Proceedings of 41st Electronic Components and Technology Conference* (IEEE, Piscataway, NJ, 1991), pp. 704–709.
5. D. Suryanarayana *et al.*, in *Proceedings of 40th Electronic Components and Technology Conference* (IEEE, Piscataway, NJ, 1990), pp. 338–343.
6. S. L. Buchwalter, L. L. Kosbar, *J. Polym. Sci. Part A Polym. Chem.* **34**, 249 (1996).
7. S. Yang *et al.*, *Chem. Mater.* **10**, 1475 (1998).
8. L. Wang, C. P. Wong, *IEEE Trans. Adv. Packag.* **22** (no. 1), 46 (1999).
9. Q. Tong *et al.*, in *Proceedings of 50th Electronic Components and Technology Conference* (IEEE, Piscataway, NJ, 2000), pp. 101–106.
10. P. Garrou, *IEEE Trans. Adv. Packag.* **23**, 202 (2000).


PERSPECTIVES: NEUROBIOLOGY

# A *Stargazer* Foretells the Way to the Synapse

**Terunaga Nakagawa and Morgan Sheng**

The chemical messenger glutamate moves from cell to cell, enabling excitatory neurons in the brain to communicate with each other. This neurotransmitter is released into the synapse by the presynaptic neuron, travels across the synapse, and then binds to glutamate receptors on the surface of the postsynaptic cell (see the figure). One class of glutamate receptor, the AMPA receptor, moves rapidly into and out of the postsynaptic membrane. The number of AMPA receptors in the postsynaptic membrane controls the strength of excitatory transmission between neurons and perhaps also the storage of memories in the brain. Consequently, there is much interest in elucidating how AMPA receptors make their way from their site of synthesis in the neuronal cytoplasm to distant postsynaptic membranes located at the end of neuronal processes called dendrites. Our current knowledge of this process is rudimentary, based largely on identification of the proteins that interact with the cytoplasmic carboxyl-terminal tails of AMPA receptor subunits (GluR) in the postsynaptic membrane. Chen *et al.* (*1*) report in yesterday's *Nature* that a transmembrane protein called stargazin (which is defective in the *stargazer* mutant mouse) is critical for bringing AMPA receptors to the cell surface and for targeting them specifically to postsynaptic sites. Their work reveals the unexpected involvement of stargazin in AMPA receptor trafficking, distinguishes two steps in the synaptic targeting of AMPA receptors, and suggests intriguing connections between AMPA receptors and calcium channels.

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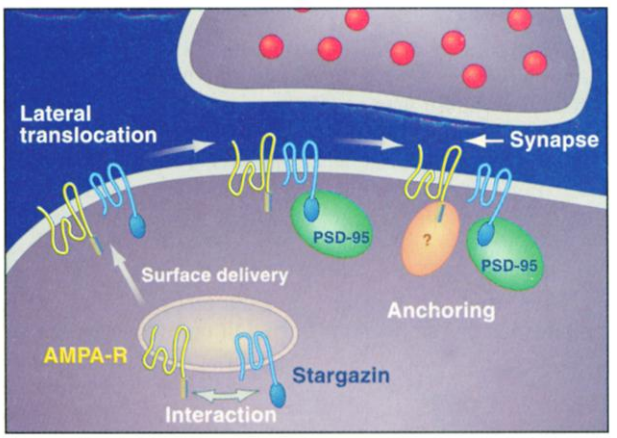
The *stargazer* mutant mouse exhibits unusual head-tossing movements, an ataxic gait, and epileptic seizures. The epileptic phenotype has been attributed to hyperexcitability in cortical networks (*2*), and the ataxia to aberrant development of cerebellar granule neurons (*3*). The *stargazer* mutation disrupts the 38-kD stargazin protein, which has four predicted transmembrane domains and is homologous to the γ subunit of muscle voltage-gated calcium channels (*4*). In addition to its structural similarity to muscle calcium channels, stargazin alters the activity of a neuronal voltage-dependent calcium channel in vitro. Thus, stargazin may be the γ2 subunit of neuronal calcium channels, equivalent to the γ1 subunit of muscle calcium channels (*4*). The main cerebellar defect in *stargazer* mice is found in granule cells of the cerebellum, which display retarded differentiation (*3*) and almost complete loss of AMPA receptor synaptic responses (*1*, *5*), despite normal expression of AMPA receptor messenger RNA and protein. Yet, calcium channel activity seems relatively unaffected in these cells (*1*). In their new work, Chen *et al.* provide the connection between stargazin, AMPA receptor trafficking, and defective synaptic transmission in *stargazer* cerebellar granule cells (*1*).

Like other transmembrane receptors, AMPA receptors are presumably synthesized in the endoplasmic reticulum, processed in the Golgi apparatus, and transported to the cell surface in membrane vesicles (see the figure). In neurons, the problem is more complicated because AMPA receptors are transported to dendrites (rather than to axons) and ultimately become concentrated in a small patch of postsynaptic membrane (rather than being diffusely sprinkled across the surface of dendrites). How AMPA receptors make their way to the synapse is a key question. So far, we know only that the carboxyl-terminal tails of AMPA receptor subunits—which bind to proteins containing specialized protein interaction domains called PDZ domains—appear to be important for the localization or stabilization of AMPA receptors in the postsynaptic membrane (*6*–*9*).

With everyone investigating the interaction between AMPA receptor subunits and cytoplasmic proteins, no one expected that a transmembrane protein would be involved in AMPA receptor trafficking. But Chen *et al.* now report that cell surface expression and synaptic clustering of AMPA receptors is abolished in the cerebellar granule cells of *stargazer* mice, and that this mutant phenotype can be rescued by transfecting wild-type stargazin into these cells in vitro (*1*). Deleting the cytoplasmic carboxyl terminus of stargazin

(which binds to the PDZ domains of PSD-95, a postsynaptic scaffold protein) still allowed rescue of the surface expression, but not the synaptic localization, of AMPA receptors.

The dissociation of synaptic and nonsynaptic delivery of AMPA receptors is an important conclusion of the Chen *et al.* work. It suggests a two-step model in which stargazin first conveys AMPA receptors to the neuronal surface and then sweeps them laterally into postsynaptic sites, the second step requiring an interaction of the carboxyl-terminus of stargazin with PSD-95 (or a similar synaptic anchoring protein). However, it is also possible that stargazin delivers AMPA receptors directly into the synapse, and that they disperse extrasynaptically if stargazin is unable to dock with PSD-95. Either way, stargazin presumably must bind to AMPA receptors to perform its task. Indeed, stargazin can interact with AMPA receptors when it is coexpressed in non-neuronal cultured cells (*1*). But, Chen *et al.* were unable to demonstrate a direct association in vivo, perhaps because the interaction between stargazin and AMPA receptors is weak or transient.




**The benefits of stargazin.** Synaptic targeting of AMPA receptors. The transmembrane protein stargazin interacts with AMPA receptors in an intracellular compartment of the neuron and promotes their delivery to the neuronal surface. The carboxyl terminus of stargazin binds specifically to an anchor protein called PSD-95 and mediates recruitment of the stargazin–AMPA receptor complex to postsynaptic sites. Additional interactions between the carboxyl terminus of AMPA receptor subunits and other anchoring proteins stabilize AMPA receptors in the postsynaptic membrane.

Why is the AMPA receptor defect observed in cerebellar granule cells but not in other regions of the *stargazer* mouse brain? The authors propose that members of the stargazin/γ-subunit family expressed in other types of neurons compensate for the loss of stargazin in these cells. Consistent with this notion, overexpression of a stargazin protein lacking a carboxyl terminus inhibited synaptic targeting of AMPA receptors in hippocampal neurons (*1*); presumably mutant stargazin had a "dominant interfering" effect on other stargazin-related proteins in the cell. This result suggests that proteins of the stargazin family are universally important for AMPA receptor trafficking.

The stargazin study raises several questions. How does stargazin bind to the AMPA receptor? Where does this interaction take place in the neuron, and how is it regulated? Synaptic accumulation of AMPA receptors is already known to depend on interactions of the cytoplasmic tails of their GluR1 and GluR2 subunits with the PDZ domains of other proteins (*6–9*). How do the direct interactions of AMPA receptor subunits fit into the *stargazer* model? Perhaps stargazin merely ushers AMPA receptors to the synapse. Once there, AMPA receptors might be released from stargazin to bind to a different set of PDZ-domain proteins that then anchor them in the postsynaptic membrane (see the figure).

Although it appears unimportant for synaptic delivery of another class of glutamate receptor (the NMDA receptor), stargazin is unlikely to be involved solely in the trafficking of AMPA receptors. Which other membrane proteins does stargazin convey to the cell surface and to synapses? And what is the connection between stargazin and neuronal calcium channels? Identifying other components of the AMPA receptor–stargazin protein complex should help to answer these questions and should shed further light on how membrane trafficking contributes to the formation and plasticity of synapses.

**References**

1. L. Chen *et al.*, *Nature*, in press.
2. E. Di Pasquale, K. D. Keegan, J. L. Noebels, *J. Neurophysiol.* **77**, 621 (1997).
3. X. Qiao *et al.*, *J. Neurosci.* **18**, 6990 (1998).
4. V. A. Letts *et al.*, *Nature Genet.* **19**, 340 (1998).
5. K. Hashimoto *et al.*, *J. Neurosci.* **19**, 6027 (1999).
6. M. Sheng, D. Pak, *Annu. Rev. Physiol.* **62**, 755 (2000).
7. R. Malinow, Z. F. Mainen, Y. Hayashi, *Curr. Opin. Neurobiol.* **10**, 352 (2000).
8. R. H. Scannevin, R. L. Huganir, *Nature Rev. Neurosci.* **1**, 133 (2000).
9. P. Osten *et al.*, *Neuron* **27**, 313 (2000).

PERSPECTIVES: CELL CYCLE

# License Withheld—Geminin Blocks DNA Replication

**Zoi Lygerou and Paul Nurse**

For cells to survive they must receive a complete copy of their genome every time they divide. Two events enable dividing cells to achieve this goal—S phase (during which the DNA of the chromosomes is replicated) and M phase or mitosis (during which the replicated chromosomes segregate into the two newly divided cells). To ensure genomic stability, S phase is tightly regulated so that replication of the chromosomes is initiated only once in each cell cycle. A process called licensing ensures that chromatin becomes competent for a further round of DNA replication only after passage through mitosis (*1*). Building on the secure foundations provided by studies of prokaryotes and viruses, work in budding and fission yeasts and with frog egg extracts has identified and characterized many of the components that tightly regulate S-phase onset. Reports by Wohlschlegel *et al.* on page 2309 of this issue (*2*) and Tada *et al.* (*3*) in *Nature Cell Biology* now connect the activities of two of these components—the positive regulator Cdt1 and the negative regulator Geminin—and provide further insight into the licensing of DNA replication in human and frog cells.

The mechanisms leading to the initiation of DNA replication depend on the sequential association of proteins with chromatin. A collection of proteins called the origin recognition complex (ORC) (*4*), which is thought to bind to origins of replication in the chromatin, is associated with chromatin throughout the cell cycle (see the figure). This association is necessary for the binding of other replication proteins but does not appear to regulate the timing of S-phase onset. The onset of S phase appears to be controlled by six proteins that form the MCM (minichromosome maintenance) complex. As the cells exit from mitosis, the MCM interacts with chromatin and licenses the DNA for replication. Although the molecular basis

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