Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 335–337, January 1999

Commentary

The fyn art of *N*-methyl-D-aspartate receptor phosphorylation

*Carlo Sala and Morgan Sheng**

Howard Hughes Medical Institute and Department of Neurobiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Glutamate is the major excitatory neurotransmitter in the mammalian brain. Glutamatergic synaptic transmission depends on postsynaptic ion channels (ionotropic glutamate receptors) that open in response to the binding of glutamate. Of the ionotropic glutamate receptors, the *N*-methyl-D-aspartate (NMDA) class has received particular attention because it plays a role not only in synaptic transmission but also in regulating synaptic plasticity. NMDA receptors are involved in activity-dependent remodeling of synapses during brain development (1). NMDA receptors also are required for a form of activity-dependent enhancement of synaptic transmission known as long-term potentiation (LTP), which has been intensively studied as a cellular correlate of memory formation in the brain (2). The regulation of NMDA receptor activity is thus of great significance for synaptic plasticity in both the developing and mature nervous system.

Like many other neuronal ion channels, the activity of the NMDA receptor-channel is subject to modulation by tyrosine phosphorylation (3–5). In addition, NMDA receptor activation leads to tyrosine phosphorylation of other intracellular proteins (6). Thus, NMDA receptors are regulated by, as well as regulators of, protein tyrosine kinases (PTKs). What biochemical mechanisms underlie the bidirectional interaction between NMDA receptors and PTKs? The paper by Tezuka *et al.* in this issue of the *Proceedings* (7) uncovers a specific molecular link that helps to explain this relationship. They provide biochemical and genetic evidence that Fyn (a Src family PTK) directly phosphorylates the NMDA receptor. More importantly, Tezuka *et al.* show that Fyn is indirectly associated with the NMDA receptor via the binding of Fyn to the NMDA receptor anchoring protein PSD-95. These findings are the first to suggest that PSD-95 serves as a scaffold for bringing together NMDA receptors and a family of nonreceptor PTKs.

Many of the recent advances in our understanding of glutamate receptor function and regulation stemmed from the cloning of the glutamate receptor subunits. The molecular cloning of NMDA receptors led to the identification of several NMDA receptor subunits (8). The common subunit NR1 combines with different NR2 (NR2A-D) subunits to form subtypes of NMDA receptors with distinct electrophysiological and pharmacological properties. Like many other ligand-gated ion channels, phosphorylation of the NMDA receptor can modulate its channel behavior. In the case of the NMDA receptor, phosphorylation of tyrosine seems to play a regulatory role in channel gating properties (4, 5). The NR2 subunits of the NMDA receptor contain many tyrosine residues in their cytoplasmic tails, and indeed, are among the most abundant tyrosine-phosphorylated proteins at the synaptic junction (9, 10). By contrast, NR1 contains only one tyrosine residue in its cytoplasmic tail and there is no evidence of NR1 tyrosine phosphorylation *in vivo*. The following questions arise: Which PTKs phosphorylate the NMDA receptor NR2 subunits? How is this kinase-substrate specificity determined? What is the physiological significance of tyrosine phosphorylation of NR2 subunits and how is it regulated?

To date, the Src family of nonreceptor PTKs has been most closely scrutinized as potential enzymes responsible for NMDA receptor tyrosine phosphorylation. This scrutiny is partly for logistical reasons (these kinases have been intensively studied and specific molecular probes are available), but also because Src-type kinases are highly expressed in neurons, and precedents for Src phosphorylation of ion channels and neurotransmitter receptors are established (11, 12). Src family PTKs (which include Fyn, Yes, Lck, and Lyn) share a common domain structure, consisting of an N-terminal unique region with a myristoylation site, followed by an SH2 domain, an SH3 domain, and the catalytic domain at the C terminus (see Fig. 1). Within this family, Src and Fyn have been most directly implicated in tyrosine phosphorylation of NMDA receptors.

Salter and colleagues (5) showed in electrophysiological experiments in neuronal culture that activated Src increases NMDA receptor channel activity. In experiments that looked more directly at phosphorylation, Köhr and Seeburg (4) found that both Src and Fyn stimulated NMDA receptors expressed in heterologous cells (4). This phenomenon was seen with NMDA receptors containing the NR2A subunit but not the NR2B subunit, which is surprising given that both NR2A and NR2B are substrates of Fyn *in vitro* (13).

Previously, involvement of Src and Fyn in NMDA receptor phosphorylation was inferred from experiments done *in vitro*, in heterologous cells, or in neurons treated with pharmacological agents. Tezuka *et al.* (7) now provide important *in vivo* evidence for a role of Fyn in NMDA receptor phosphorylation. In Fyn knockout mice, the tyrosine phosphorylation content of NR2A and NR2B was reduced but not abolished, suggesting that Fyn is an important, though not exclusive, kinase that acts on these substrates. It is unknown what happens to tyrosine phosphorylation of NMDA receptors in Src or Yes mice mutants. The relative contributions of Fyn, Src, and other PTKs to NMDA receptor phosphorylation *in vivo* remains to be resolved.

If NR2 subunits are physiological targets for Src/Fyn tyrosine kinases, what mechanism specifies this enzyme-substrate relationship? An emerging principle in signal transduction is that functionally interacting signaling proteins are physically associated with each other, often by being bound to a common anchoring or scaffold protein (14). Modulatory enzymes and effector molecules often are intimately associated with ion channels and receptors. In keeping with this theme, Src family PTKs can be coimmunoprecipitated with NMDA receptors from neurons (5, 7), though the mechanism underlying this association has been unclear. Tezuka *et al.* (7) now offer a novel explanation: namely, Fyn is complexed with NMDA receptors by binding to PSD-95.

PSD-95 is a PDZ domain-containing protein that binds to the conserved C-terminal sequence (−ESDV) found on the

The companion to this Commentary begins on page 435.

*To whom reprint requests should be addressed at: Massachusetts General Hospital, Howard Hughes Medical Institute/Wellman 423, 50 Blossom Street, Boston, MA 02114. e-mail: sheng@helix.mgh.harvard.edu.

PNAS is available online at www.pnas.org.

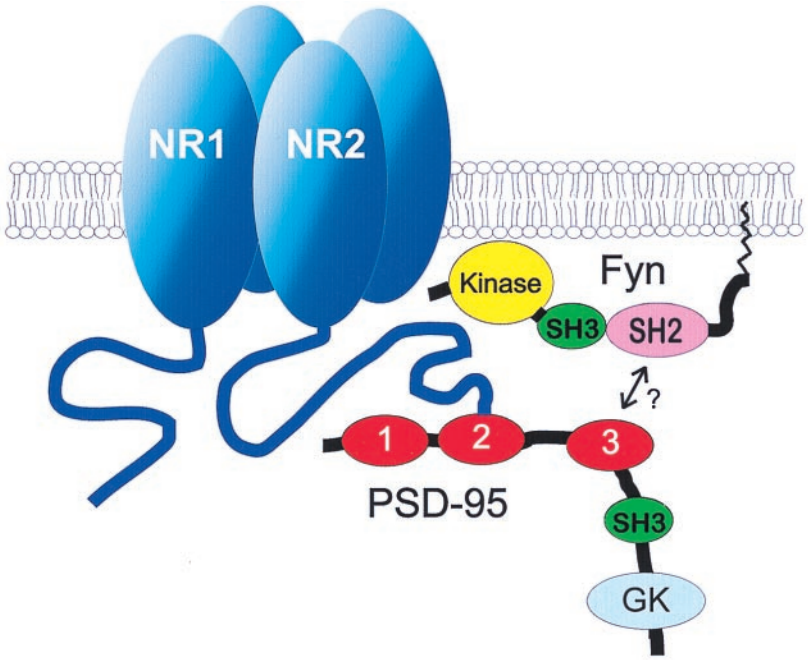

FIG. 1. A model for Fyn association with the NMDA receptor/PSD-95 complex. The NMDA receptor is represented as a tetramer of NR1 and NR2 subunits. NR2 subunit C-terminal tails interact with PDZ2 of PSD-95. Fyn binds to PDZ3 of PSD-95 possibly via its SH2 domain. Fyn also is associated with the membrane via its myristoylated N terminus. N-terminal palmitoylation of PSD-95 is not shown. PDZ domains of PSD-95 are shown numbered and in red.

cytoplasmic tails of NMDA receptor NR2 subunits (15, 16). This interaction may be important for the clustering of NMDA receptors in the postsynaptic membrane (reviewed in refs. 17–19). NR2 proteins bind specifically to the first two PDZ domains (PDZ1 and PDZ2) of PSD-95, but not to PDZ3 (see Fig. 1). A variety of cytoskeletal linker proteins and cytoplasmic signaling proteins (including neuronal NO synthase and the ras GTPase activating protein SynGAP) also bind to PSD-95 via its PDZ and guanylate kinase domains (20–24). In this way, the multidomain PSD-95 molecule connects NMDA receptors to a variety of intracellular signaling proteins and anchors the whole complex to the postsynaptic density (reviewed in ref. 25). In a major advance, Tezuka *et al.* (7) report that Fyn forms a complex with PSD-95 when coexpressed in heterologous cells. Interestingly, Fyn association with PSD-95 depends on a region of PSD-95 that includes PDZ3; thus Fyn and NMDA receptors bind to different domains of PSD-95. Consistent with this model, PSD-95 can support a ternary complex containing NR2A and Fyn. Most importantly, PSD-95 stimulates the tyrosine phosphorylation of NR2A by Fyn, presumably by bringing kinase and substrate in close proximity.

Tezuka *et al.* (7) also performed deletion analysis of Fyn in heterologous cells to determine how it binds to PSD-95. The SH2 domain of Fyn appears to mediate association with PSD-95, but rather atypically for SH2 domains, this interaction does not depend on tyrosine phosphorylation of PSD-95. As mentioned above, Fyn appears to bind to the third PDZ domain of PSD-95. PDZ domains typically bind to C-terminal peptides or other PDZ domains (20, 26, 27). Thus the reported mode of Fyn-PSD-95 association is a curious one indeed, involving an unprecedented SH2-PDZ interaction that is also independent of tyrosine phosphorylation. In view of the unusual nature of this protein–protein interaction, more work needs to be done with purified recombinant proteins to confirm that the Fyn-PSD-95 interaction is truly direct. The apparent SH2-PDZ3 binding mode needs to be tested in greater depth. The lack of such details not withstanding, Tezuka *et al.* (7) provide the first report of a PTK binding to the PSD-95 scaffold. This finding is an important new addition to the NMDA receptor-associated PSD-95-based complex, which already contains proteins involved in NO synthesis, ras signaling, tumor suppression, and cytoskeletal attachment.

The interaction uncovered by Tezuka *et al.* (7) brings Fyn (and probably other members of the Src family) into the thick of the action at the postsynaptic membrane. Their biochemical results support earlier genetic and pharmacological evidence suggesting an important role for nonreceptor PTKs in the regulation of synaptic transmission and plasticity. NMDA receptors are stimulated by tyrosine phosphorylation and by activated Src (4, 5). LTP is associated with Src activation (28) and increased tyrosine phosphorylation of NR2B (29, 30), and is inhibited by tyrosine kinase inhibitors (31). Recent observations suggest that the potentiation of NMDA receptor activity by Src family kinases actually may mediate the induction of LTP (ref. 28, reviewed in ref. 32). Which member(s) of the Src family are of major importance in postsynaptic function? This question is a difficult one to approach except by genetics, though there are caveats in the interpretation of complex mouse knockout phenotypes. The current picture is that mice deficient for Fyn show an impairment of LTP and spatial memory (33), lending further support to Fyn's importance in synaptic function. On the other hand, Src and Yes knockout mice show no detectable difference in LTP compared with wild type (33). Nevertheless, probably multiple tyrosine kinases are involved in NMDA receptor modulation and synaptic function, as evidenced by the residual NR2A phosphorylation in Fyn-deficient mice, and by the coimmunoprecipitation of Fyn, Src, Yes, and Lyn with NMDA receptors from brain extracts (7).

The work of Tezuka *et al.* (7) raises interesting questions. How does the binding to PSD-95 affect the kinase activity of Fyn? Binding of PSD-95 to the SH2 domain might be predicted to "open up" the structure of Fyn, resulting in activation of the kinase (34, 35). What regulates the activity of Src family PTKs at postsynaptic sites? Could NMDA receptor activation itself stimulate the associated PTKs? Do other members of the Src family exploit similar binding mechanisms to associate with the

PSD-95 complex? Src family PTKs and PSD-95 undergo N-terminal myristoylation and palmitoylation, respectively (36). Are these fatty acid modifications important for the association of kinase and PSD-95 at the postsynaptic membrane? Pursuing these questions will provide further insights into the molecular and functional organization of postsynaptic signaling complexes. They also should uncover new clues about the physiological significance of tyrosine phosphorylation of NMDA receptors, the function of which remains elusive.

1. Constantine-Paton, M., Cline, H. T. & Debski, E. (1990) *Annu. Rev. Neurosci.* **13,** 129–154.
2. Bliss, T. V. P. & Collingridge, G. L. (1993) *Nature (London)* **361,** 31–39.
3. Wang, Y. T. & Salter, M. W. (1994) *Nature (London)* **369,** 233–235.
4. Köhr, G. & Seeburg, P. H. (1996) *J. Physiol.* **492,** 445–452.
5. Yu, X., Askalan, R., Keil, G. & Salter, M. (1997) *Science* **275,** 674–678.
6. Bading, H. & Greenberg, M. E. (1991) *Science* **253,** 912–914.
7. Tezuka, T., Umemori, H., Akiyama, T., Nakanishi, S. & Yamamoto, T. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 435–440.
8. Hollmann, M. & Heinemann, S. (1994) *Annu. Rev. Neurosci.* **17,** 31–108.
9. Moon, I. S., Apperson, M. L. & Kennedy, M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3954–3958.
10. Lau, L. F. & Huganir, R. L. (1995) *J. Biol. Chem.* **270,** 20036–20041.
11. Moss, S., Gorrie, G., Amato, A. & Smart, T. (1995) *Nature (London)* **377,** 344–348.
12. Holmes, T. C., Fadool, D. A., Ren, R. & Levitan, I. B. (1996) *Science* **274,** 2089–2091.
13. Suzuki, T. & Okamura-Noji, K. (1995) *Biochem. Biophys. Res. Commun.* **216,** 582–588.
14. Pawson, T. & Scott, J. D. (1997) *Science* **278,** 2075–2080.
15. Kornau, H.-C., Schenker, L. T., Kennedy, M. B. & Seeburg, P. H. (1995) *Science* **269,** 1737–1740.
16. Niethammer, M., Kim, E. & Sheng, M. (1996) *J. Neurosci.* **16,** 2157–2163.
17. Sheng, M. (1996) *Neuron* **17,** 575–578.
18. Ziff, E. B. (1997) *Neuron* **19,** 1163–1174.
19. Kornau, H., Seeburg, P. & Kennedy, M. (1997) *Curr. Opin. Neurobiol.* **7,** 368–373.
20. Brenman, J. E., Chao, D. S., Gee, S. H., McGee, A. W., Craven, S. E., Santillano, D. R., Wu, Z., Huang, F., Xia, H., Peters, M. F., *et al*. (1996) *Cell* **84,** 757–767.
21. Kim, E., Naisbitt, S., Hsueh, Y.-P., Rao, A., Rothschild, A., Craig, A. M. & Sheng, M. (1997) *J. Cell Biol.* **136,** 669–678.
22. Kim, J. H., Liao, D., Lau, L. F. & Huganir, R. L. (1998) *Neuron* **20,** 683–691.
23. Chen, H. J., Rojas-Soto, M., Oguni, A. & Kennedy, M. B. (1998) *Neuron* **20,** 895–904.
24. Niethammer, M., Valtschanoff, J. G., Kapoor, T. M., Allison, D. W., Weinberg, R. J., Craig, A. M. & Sheng, M. (1998) *Neuron* **20,** 693–707.
25. Craven, S. & Bredt, D. (1998) *Cell* **93,** 495–498.
26. Doyle, D. A., Lee, A., Lewis, J., Kim, E., Sheng, M. & MacKinnon, R. (1996) *Cell* **85,** 1067–1076.
27. Songyang, Z., Fanning, A. S., Fu, C., Xu, J., Marfatia, S. M., Chishti, A. H., Crompton, A., Chan, A. C., Anderson, J. M. & Cantley, L. C. (1997) *Science* **275,** 73–77.
28. Lu, Y. M., Roder, J. C., Davidow, J. & Salter, M. W. (1998) *Science* **279,** 1363–1367.
29. Rosenblum, K., Dudai, Y. & Richter-Levin, G. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 10457–10460.
30. Rostas, J., Brent, V., Voss, K., Errington, M., Bliss, T. & Gurd, J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 10452–10456.
31. O'Dell, T., Kandel, E. & Grant, S. (1991) *Nature (London)* **353,** 558–560.
32. Salter, M. (1998) *Biochem. Pharmacol.* **56,** 789–798.
33. Grant, S., O'Dell, T., Karl, K., Stein, P., Soriano, P. & Kandel, E. (1992) *Science* **258,** 1903–1910.
34. Brown, M. T. & Cooper, J. A. (1996) *Biochem. Biophys. Acta* **1287,** 121–149.
35. Xu, W., Harrison, S. & Eck, M. J. (1997) *Nature (London)* **385,** 595–602.
36. Topinka, J. R. & Bredt, D. S. (1998) *Neuron* **20,** 125–134.