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Journal
Proceedings of the National Academy of Sciences of the United States of America, 118(28)

ISSN
0027-8424

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Publication Date
2021-07-13

DOI
10.1073/pnas.2107585118

Peer reviewed

MAGUKs are essential, but redundant, in long-term potentiation

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Contributed by Roger A. Nicoll, June 10, 2021 (sent for review April 23, 2021; reviewed by Thomas O'Dell and Eric Schnell)

This study presents evidence that the MAGUK family of synaptic scaffolding proteins plays an essential, but redundant, role in long-term potentiation (LTP). The action of PSD-95, but not that of SAP102, requires the binding to the transsynaptic adhesion protein ADAM22, which is required for nanocolumn stabilization. Based on these and previous results, we propose a two-step process in the recruitment of AMPARs during LTP. First, AMPARs, via TARPs, bind to exposed PSD-95 in the PSD. This alone is not adequate to enhance synaptic transmission. Second, the AMPAR/TARP/PSD-95 complex is stabilized in the nanocolumn by binding to ADAM22. A second, ADAM22-independent pathway is proposed for SAP102.

MAGUKs | long-term potentiation | synapse | AMPA receptors | TARPs

Discovered 50 y ago, long-term potentiation (LTP) remains the most compelling cellular model for learning and memory. It is generally agreed that NMDA receptor (NMDAR)-dependent LTP is mediated primarily by a postsynaptic modification involving the trafficking of the AMPA-type glutamate receptor (AMPAR) (1–4). During the past decade, effort has been focused on the molecular mechanisms underlying both the constitutive and activity-dependent trafficking of these receptors. A family of synaptic scaffolding proteins, referred to as membrane-associated guanylate kinases (MAGUKs) has featured prominently in these studies (5, 6). These proteins contain three PDZ domains, which are involved in protein–protein interactions. The PSD-95 family of synaptic MAGUKs include PSD-95, PSD-93, and SAP102 and are highly expressed at excitatory synapses (5, 7). In terms of AMPAR basal synaptic trafficking, all MAGUKs appear to play overlapping roles (8). Most research has focused on PSD-95. Overexpressing PSD-95 causes a roughly threefold enhancement in AMPAR excitatory postsynaptic currents (EPSCs) with no change in the NMDAR EPSC (9–13). The enhancement mimics LTP, especially with its selective effect on AMPAR EPSCs (as reviewed in ref. 3). Furthermore, PSD-95 occludes LTP, suggesting a common mechanism (9, 12). This suggests that PSD-95 is an essential step in LTP. However, LTP remains intact in cells lacking PSD-95 (14–16), raising the possibility that PSD-93 or SAP102 may play redundant roles.

PSD-95 binds to many synaptic proteins (17, 18) including transsynaptic cell-adhesion proteins (e.g., neuroligins, LRRTMs) (19–24). Of particular interest is ADAM22, a member of a large family of catalytically inactive metalloproteases (25, 26), which, via its binding to the secreted protein LGI1, governs transsynaptic nanoalignment. Deleting either ADAM22 (27) or LGI1 (28) reduces AMPAR synaptic transmission. Critical for ADAM22's function is the presence of a PDZ binding motif (PBM) at the cytoplasmic C terminus. Thus, expressing a mutated form of ADAM22, which lacks the PBM (ADAM22ΔC5) fails to rescue the defect, resulting from the deletion of ADAM22 (27). Furthermore, AMPAR responses are depressed in $ADAM22^{\Delta C5/\Delta C5}$ knockin (KI) mice (29). Previous results found that the typical enhancement in AMPAR responses seen with the overexpression of PSD-95 or the depression observed with the knockdown (KD) of PSD-95 is absent in LGI1 knockout (KO) mice (27). Interestingly, the depression observed with the KD of SAP102 remained intact (27). Complimentary results are seen with $ADAM22^{\Delta C5/\Delta C5}$ KI mice (29) where overexpression of PSD-95 failed to enhance synaptic transmission. Surprisingly, LTP was intact in these mice. What could account for the dissociation of the enhancing action of PSD-95 and LTP?

Here we show an essential role for MAGUKs in both basal synaptic transmission and in LTP. Any one of the MAGUKs can substitute for each other. However, coexpression of the MAGUKs suggests differences in their action. The synaptic enhancement seen with the coexpression of PSD-95 and PSD-93 is no greater than the enhancement observed when singly expressed. In contrast the enhancement seen with the coexpression of PSD-95 and SAP102 is additive. In addition, when MAGUK binding to ADAM22 is eliminated in $ADAM22^{\Delta C5/\Delta C5}$ KI mice, PSD-95 is no longer functional, but the action of SAP102 remains intact. Finally, KD of SAP102 in $ADAM22^{\Delta C5/\Delta C5}$ KI mice abolishes LTP. Based on these results we propose a model in which the AMPAR/TARP/PSD-95 complex binds to ADAM22, a transsynaptic adhesion protein essential for nanocolumn stability, which tethers AMPAR receptors in the nanocolumn. An additional pathway involving SAP102 would hold the AMPAR/TARP/SAP102 complex in the nanocolumn by an ADAM22-independent mechanism.

Results

To evaluate the role of MAGUKs in LTP we employed miRNA KD of the three prominent synaptic MAGUKs (PSD-95, PSD-93, and SAP102) (8). Postnatal day 0 (P0) pups were injected intracerebrally

Significance

It is now generally accepted that memories are stored, at least in part, by long-term potentiation (LTP), in which brief activation of excitatory synapses persistently enhances synaptic transmission. LTP involves the recruitment of AMPARs to the synapse, but the mechanism remains debated. This study shows that the MAGUK family of scaffolding proteins plays an essential, but redundant, role in LTP. MAGUK binding to the transsynaptic adhesion protein ADAM22 is proposed to localize AMPARs immediately across from the vesicle release site. Such a mechanism ensures reliable AMPAR activation following glutamate release.

Author contributions: X.C. and R.A.N. designed research; X.C., Y.F., and M.F. performed experiments and analyzed the data; and X.C. and R.A.N. wrote the paper.

Reviewers: T.O., University of California Los Angeles; E.S., Oregon Health and Science University.

The authors declare no competing interest.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2107585118/-/DCSupplemental.

Published July 9, 2021.

with lentiviral miRNA constructs. At P18 to P28 acute hippocampal slices were prepared and dual whole-cell recordings were made from a transfected cell and a neighboring control cell (Fig. 1*A*). In the first series of experiments, we KDed all three MAGUKs. This reduced basal AMPAR and NMDAR responses by roughly 50% (*SI Appendix*, Fig. S1*A*). This reduction is less than that previously reported and is due to the different methods of transfection (8). As discussed in Levy et al. (8), biolistic transfection is more effective than lentivirus transfection. LTP was absent in this triple KD experiment, indicating a critical role of MAGUKs in LTP (Fig. 1*B*). A potential confounding factor in studying the role of MAGUKs in LTP is that KD of MAGUKs reduces the size of NMDAR EPSCs (8). In this experiment the NMDAR response was reduced by about 50% (*SI Appendix*, Fig. S1*A*). Thus, a trivial explanation for a lack of LTP is the decreased NMDAR currents. We, therefore, carried out control experiments to determine the sensitivity of LTP to changes in the NMDAR EPSC with our induction protocol. The maximal reduction of the NMDAR EPSCs with our present MAGUK KD experiments was ~50%. We therefore found a concentration of the NMDAR antagonist D-2-amino-5-phosphonopentanoic acid (D-APV) that caused a similar reduction. As seen in *SI Appendix*, Fig. S2*A*, robust LTP was observed in the presence of 3 μM APV, which caused ~60% reduction (*SI Appendix*, Fig. S2*B*), whereas 100 μM essentially blocked LTP and the NMDAR EPSCs. This finding indicates that with our induction protocol, the activation of NMDARs is supersaturating.

To address the role of single MAGUKs in LTP we performed double KD experiments leaving one MAGUK intact. With the KD of PSD-93 and SAP102, LTP was normal, suggesting that PSD-95 on its own can support LTP (Fig. 1*C*). In these experiments basal AMPAR responses were reduced ~50% with no change in the NMDAR response (*SI Appendix*, Fig. S1*B*). LTP was also normal with the KD of PSD-95 and PSD-93, suggesting that SAP102 on its own supports LTP (Fig. 1*D*). In these experiments the AMPAR and NMDAR basal responses were reduced ~50% (*SI Appendix*, Fig. S1*C*). Together these finding imply that MAGUKs play an essential, but redundant, role in LTP.

However, coexpression experiments in which we tested for the interaction between MAGUKs raise the possibility that they may use distinct pathways. Expression of PSD-95 and PSD-93 together produced an approximately threefold enhancement (Fig. 2 *B* and *D*), indistinguishable from the enhancement produced by either one alone (Fig. 2*D*). In contrast, while expression of SAP102 on its own produced an approximately threefold enhancement (Fig. 2 *A* and *D*) as does PSD-95 (9, 10, 30) (Fig. 2*D*), when expressed together, the enhancement was sixfold (Fig. 2 *C* and *D*). In these experiments the NMDAR EPSCs were modestly enhanced both with SAP102 expression alone (*SI Appendix*, Fig. S3 *A* and *D*) and coexpression of SAP102 and PSD-95 (*SI Appendix*, Fig. S3 *B* and *D*). There was no change in the NMDAR EPSC with the coexpression of PSD-95 and PSD-93 (*SI Appendix*, Fig. S3 *C* and *D*). The additive effects seen with the AMPAR EPSC when PSD-95 and SAP102 are coexpressed, but not with the coexpression of PSD-95 and PSD-93, suggest PSD-95/93 share the same pathway, while SAP102 exerts its effects through a distinct pathway.

The possibility that PSD-95/93 and SAP102 act via different pathways might help explain the surprising dissociation of the action of PSD-95 and LTP in the $ADAM22^{\Delta C5/\Delta C5}$ KI mice (29). In these mice the expression of PSD-95 has no effect, but LTP remains intact. These findings challenge the essential role of PSD-95 in LTP. Might SAP102 substitute for PSD-95 in LTP? To test this possibility, we expressed SAP102 in $ADAM22^{\Delta C5/\Delta C5}$ KI mice. Indeed, the enhancing action of SAP102 in wild-type (WT) mice

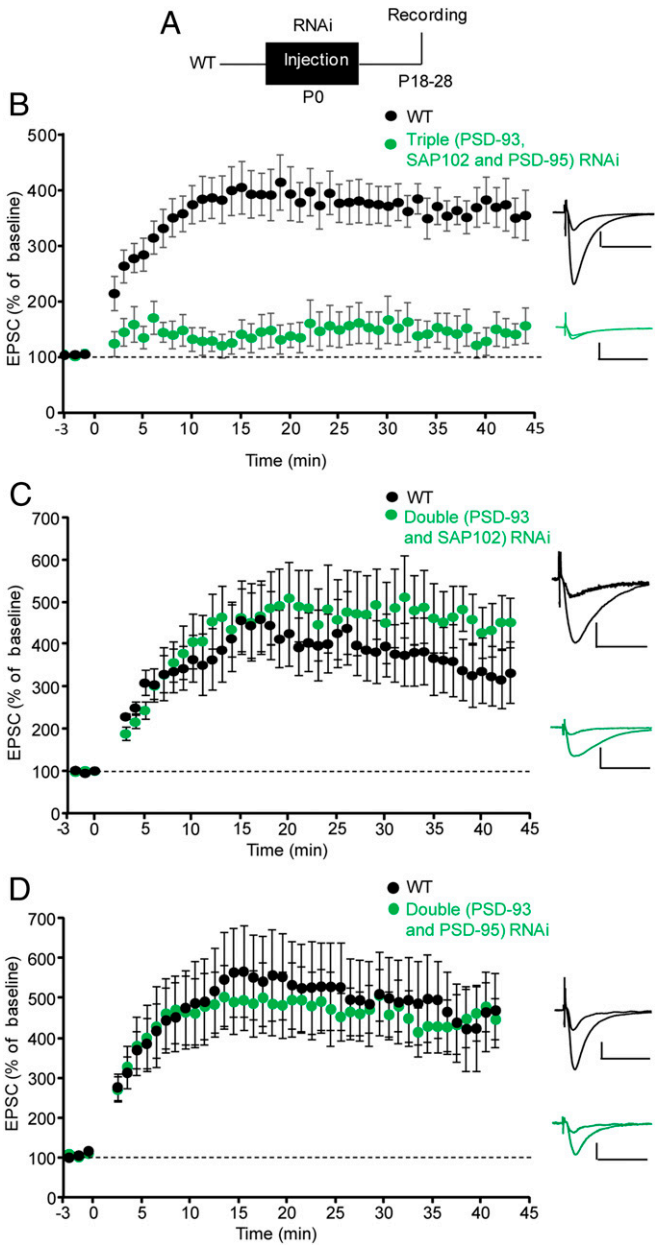

Fig. 1. MAGUKs play essential, but redundant, roles in LTP. (*A*) Schematic illustrating the timeline of the lentiviral injections and recordings. (*B*) Knockdown of all three PSD MAGUKs impairs LTP in area CA1 hippocampus ($n = 9$ control cells, 24 transfected cells, nine simultaneous recordings included). (*C*) Knockdown of PSD-93 and SAP102 does not alter LTP in the CA1 hippocampus cells ($n = 9$ control cells, 9 transfected cells. There are seven simultaneous recordings included). (*D*) Knockdown of PSD-93 and PSD-95 does not affect LTP ($n = 9$ control cells, 10 transfected cells, seven simultaneous recordings included). For all LTP graphs, control cells are shown as black-filled circles ± SEM and transfected cells are shown as green circles ± SEM. Traces show representative currents from control (in black) and transfected cells (in green) before and after LTP induction. (Scale bar: 50 pA/20 ms.)

was still present in the $ADAM22^{\Delta C5/\Delta C5}$ KI mice (see Fig. 3 *A* and *B*). We also compared the effect of SAP102 and PSD-95 KD in $ADAM22^{\Delta C5/\Delta C5}$ KI mice. While KD of PSD-95 had no effect (Fig. 3 *D* and *E*), KD of SAP102 strongly depressed AMPAR responses (Fig. 3 *C* and *E*). Since the action of SAP102 remains intact in $ADAM22^{\Delta C5/\Delta C5}$ KI mice, we next examined the possibility that LTP observed in $ADAM22^{\Delta C5/\Delta C5}$ KI mice might depend on

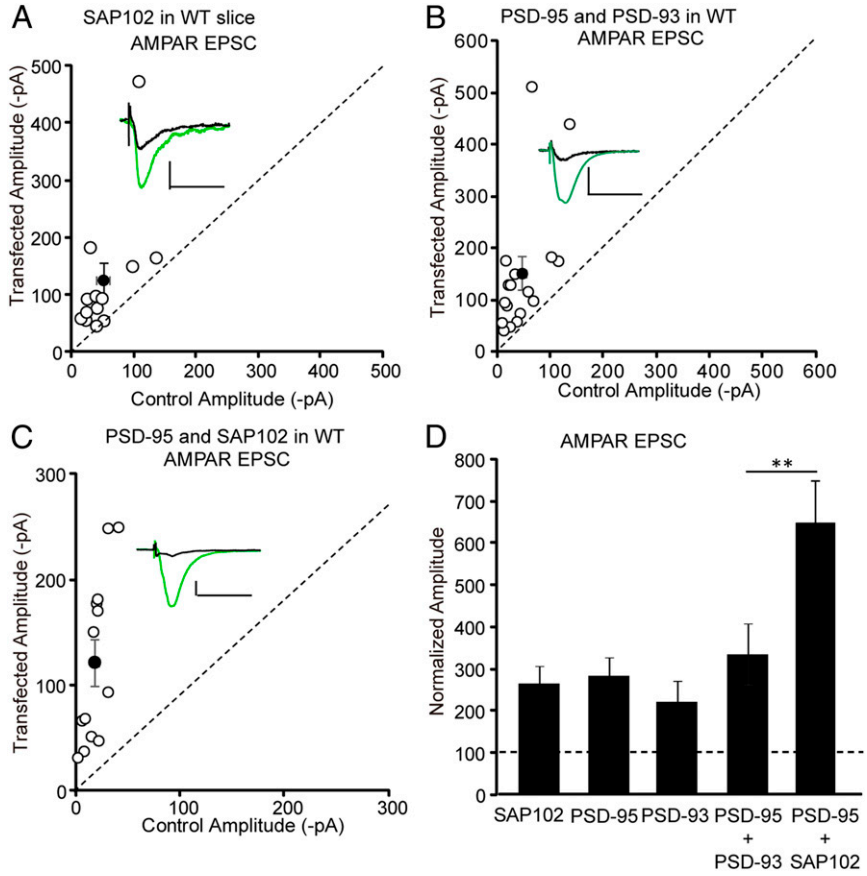

Fig. 2. Coexpression of PSD-95 and SAP102 shows an additive enhancement in basal transmission, while coexpression of PSD-95 and PSD-93 does not. (*A*) Overexpression of SAP102 causes a threefold enhancement in AMPAR EPSCs ($P < 0.0005$, $n = 13$). (*B*) Coexpression of PSD-95 and PSD-93 also causes a threefold enhancement in AMPAR EPSCs ($P < 0.0005$, $n = 17$). (*C*) Coexpression of PSD-95 and SAP102 causes roughly a sixfold enhancement in AMPAR EPSCs ($P < 0.0005$, $n = 14$). For all scatterplots, open circles represent individual paired recordings, while filled circles represent means ± SEM. Traces show representative currents for each condition, with WT in black and transfected cells in green. (Scale bar: 50 pA/20 ms.) (*D*) Bar graph showing the effect of overexpression of SAP102, PSD-95, PSD-93, PSD-95 and PSD-93, or PSD-95 and SAP102 in AMPAR EPSCs as percent of control (means ± SEM). Only PSD-95 and SAP102 coexpression shows an additive enhancement (**$P < 0.005$). The data for PSD-95 is reproduced from ref. 29 for comparison and the data for PSD-93 is reproduced from ref. 30 for comparison.

the presence of SAP102. In agreement with previous results (31), KD of SAP102 with P0 injection of SAP102 RNAi (Fig. 4*A*) had no effect on LTP in WT mice (Fig. 4*B*). However, in *ADAM22*$^{\Delta C5/\Delta C5}$ mice, knockdown of SAP102 abolished LTP. (Fig. 4*C*). These findings further strengthen the model that MAGUKs signal through two parallel pathways: an ADAM22-dependent pathway (PSD-95/93) and an independent pathway (SAP102).

Discussion

The linkage of MAGUKs to LTP has a long history. The enhancement of synaptic transmission observed with the overexpression of PSD-95 faithfully mimics LTP (9–13). Furthermore, this enhancement occludes with LTP (9, 12), strongly implying that they share a common mechanism. However, LTP remains intact in cells lacking PSD-95 (14–16) or SAP102 (31). These negative results strongly challenge the model that MAGUKs are an essential step in LTP. This conundrum forms the basis for the current study.

We first addressed the possibility that the normal LTP observed in the absence of PSD-95 or SAP102 might be due to redundancy. Indeed, deleting all three of the MAGUKs (PSD-95, PSD-93, and SAP102) eliminated LTP, establishing an essential role for MAGUKs in LTP. However, PSD-95 on its own and SAP102 on its own can support LTP. Thus, for LTP the MAGUKs can substitute for one another. In previous experiments we found that, while SAP102 plays a major role in AMPAR trafficking in neonates (13), when it is expressed at high levels, its levels decline during maturation and PSD-95 largely replaces the role of SAP102 (13). However, when PSD-95 is deleted SAP102 is upregulated and compensates for the loss of PSD-95 (30). Thus, we propose that in WT conditions PSD-95 is the primary MAGUK.

However, when we overexpress SAP102 it can add to the enhancement observed with PSD-95. Specifically, when PSD-95 and PSD-93 are coexpressed the net enhancement (approximately threefold) is no greater than when they are expressed individually. This could have a number of explanations, such as saturating a common target. However, by contrast when SAP102 is coexpressed with PSD-95 the responses were additive. These findings raise the possibility that PSD-95 and SAP102 act through parallel pathways, although under WT conditions, PSD-95 is the primary player.

With the findings that MAGUKs are essential, but redundant, for LTP and that they may use parallel signaling pathways, we next turned to the seemingly incompatible finding that in the *ADAM22*$^{\Delta C5/\Delta C5}$ KI mice, LTP is normal, but the action of PSD-95 is absent. Might SAP102 fulfill the role of PSD-95 in these mice? Indeed, the enhancing action of SAP102 remained intact in *ADAM22*$^{\Delta C5/\Delta C5}$ KI

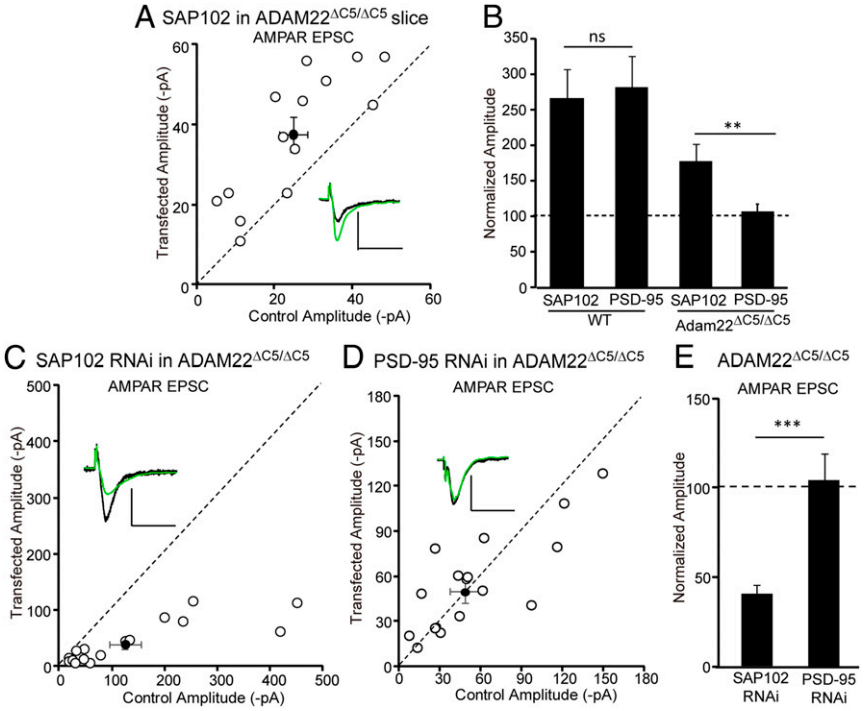

Fig. 3. Expression of SAP102, but not PSD-95, in *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice enhances basal transmission. (*A*) Overexpression of SAP102 in *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice, significantly increases AMPAR EPSCs ($P < 0.005$, $n = 14$). For all scatterplots, open circles represent individual paired recordings, while filled circles represent means ± SEM. Traces show representative currents for each condition, with WT in black and transfected cells in green. (Scale bar: 50 pA/20 ms.) (*B*) Bar graph showing SAP102 still enhances AMPAR EPSCs in *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice, while PSD-95 overexpression has no effect (**$P < 0.005$). PSD-95 data in WT and *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice are reproduced from ref. 29 for comparison and WT SAP102 data are reproduced from Fig. 2 for comparison. ns, no significant difference. (*C*) Knockdown of SAP102 in *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice, reduces AMPAR EPCSs by 50% ($P < 0.0005$, $n = 16$). Scatterplots and sample traces are analogous to those in *A*. (*D*) Knockdown of PSD-95 in *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice has no effect on AMPAR EPSCs. Scatterplots and sample traces are analogous to those in *A*. (*E*) Bar graph showing knockdown of SAP102 in *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice decreases AMPAR EPSCs, while knockdown of PSD-95 does not (***$P < 0.0005$).

mice, and depleting SAP102 in *ADAM22*$^{\Delta C5/\Delta C5}$ KI mice eliminated LTP.

What might explain the finding that the action of PSD-95 requires ADAM22, but the action of SAP102 is independent? One possibility is that PSD-95 binds to ADAM22, but SAP102 cannot. The finding that immunoprecipitations and Western blotting abundantly pull down PSD-95 and PSD-93, but not SAP102 (25, 28) (*SI Appendix*, Fig. S4 *A* and *B*), is consistent with this possibility. Low expression level of SAP102 cannot explain this difference since its level of expression is similar to that of PSD-93 (*SI Appendix*, Fig. S4*C*). However, full length ADAM22 and full-length PSD-95, PSD93, or SAP102 all interacted similarly when expressed in HEK293T cells (*SI Appendix*, Fig. S4*D*). This suggests that MAGUKs are physically segregated in the PSD. Superresolution microscopy and immunogold electron microscopy (EM) labeling studies suggest that this is the case (32). The nature of the ADAM22-independent pathway is unclear. However, it has been proposed that, unlike PSD-95, SAP102 regulation of synaptic transmission requires the AMPAR auxiliary subunit cornichon-2 (CNIH-2) (33). The mechanism by which CNIH-2 mediates its effects is unclear, because CNIH-2 lacks a PBM.

It is important to note that other transsynaptic adhesion proteins, i.e., neuroligin-1 (34–36) and LRRTMs (37, 38), are required for LTP. However, their roles appeared to be distinct from that of ADAM22, which depends on its cytoplasmic PBM. Surprisingly, despite the fact that both neuroligin-1 and LRRTMs contain C-terminal PBMs, deleting the PBM of neuroligin-1 (34, 35) and that of LRRTMs (37) does not affect LTP. Rather, mutations in the extracellular domain that prevent binding to presynaptic neurexins disrupt LTP. We propose that all three transsynaptic adhesion proteins play a similar role in maintaining the nanocolumn that is required for LTP expression. Deleting ADAM22, LGI1, or just the PBM of ADAM22 disrupts the nanocolumn (27, 29). Overexpression of PSD-95 in these conditions still accumulates in the PSD, but has no effect on synaptic transmission. This finding is remarkably similar to those of Sinnen et al. (39), who found that, while optically induced association of AMPARs with PSD-95 increased the number of AMPARs in the PSD, it did not enhance synaptic transmission, supporting a sub-PSD nanodomain arrangement.

We propose the following model for the recruitment of AMPARs in LTP (Fig. 5), which is an extension of the PSD centric model of LTP (2, 40). It incorporates the recent proposal of nanocolumns (29, 39, 41–45) in which AMPARs are clustered opposite to release sites by transsynaptic adhesion complexes, including ADAM22/LGI1 (27, 29). We focus on PSD-95 since it is the most abundant MAGUK. AMPARs are not stand-alone receptors but are associated with a family of auxiliary proteins referred to as transmembrane AMPAR regulatory proteins (TARPs) (46–48). The C-terminal domain of TARPs contains a PBM that interacts with PDZ domains of PSD-95 (10, 49, 50). This interaction is essential for basal synaptic trafficking of AMPARs and LTP. The sequence of events for LTP is as follows: 1) NMDAR activation elevates spine Ca^{2+}, which activates CaMKII. 2) CaMKII causes a modification in the PSD such that it unmasks PSD-95, which far outnumber AMPARs. The nature of this unmasking remains to be established. 3) Diffusing AMPAR/TARPs bind to the newly exposed PSD-95 in the PSD. Consistent with this model is the enhancement observed with the overexpression of PSD-95. In this situation PSD-95 would outnumber any masking

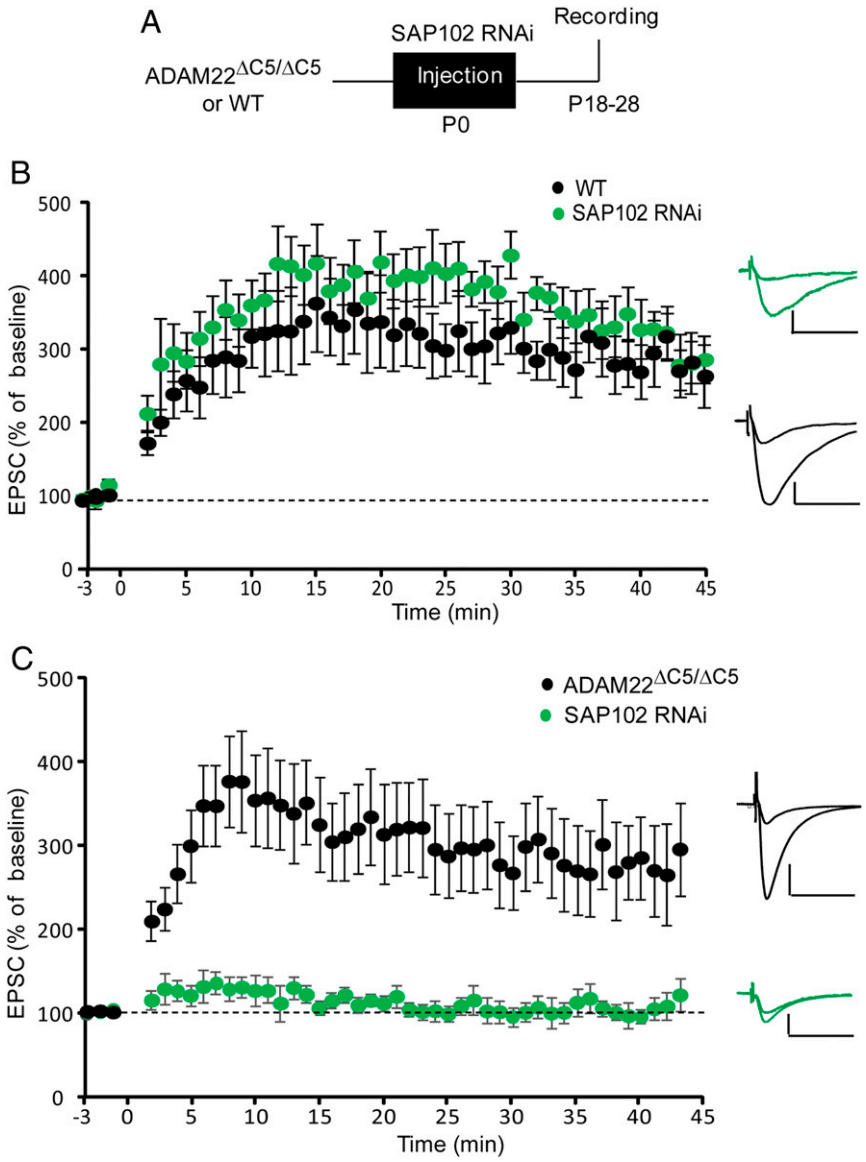

Fig. 4. SAP102 is required for LTP in *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice. (*A*) Schematic illustrating the timeline of the lentiviral injections and recordings. (*B*) Knockdown of SAP102 does not alter LTP in WT CA1 hippocampus cells ($n = 8$ control cells, 9 transfected cells, 8 simultaneous recordings included). (*C*) Knockdown of SAP102 abolishes LTP in *ADAM22*$^{\Delta C5/\Delta C5}$ knockin mice ($n = 9$ control cells, 18 transfected cells, 9 simultaneous recordings included). For all LTP graphs, control cells are shown as black-filled circles ± SEM and transfected cells are shown as green circles ± SEM. Traces show representative currents from control (in black) and transfected cells (in green) before and after LTP induction. (Scale bar: 50 pA/20 ms.)

proteins so AMPAR/TARP can bind to unmasked PSD-95. Thus, LTP and PSD-95 overexpression is assumed to accomplish the same thing—an increase in the number of unmasked PSD-95. 4) The AMPAR/TARP/PSD-95 complex then binds to ADAM22, which is essential for the stability of the nanocolumn (29), holding the AMPAR/TARP/PSD-95 complex in the nanocolumn. A secondary pathway, which normally plays a minor role, involves SAP102, which would deliver the AMPAR/TARP/SAP102 complex to the nanocolumn by an ADAM22-independent pathway. The nature of this pathway remains to be determined. It will be of interest to identify the molecular players involved in this SAP102 trafficking pathway.

Experimental Procedures

Experimental Constructs. The MAGUK miRNA construct targeting PSD-93, PSD-95, and SAP102 was made using sequences that have been previously characterized (8, 13). See *SI Appendix, Supplemental Experimental Procedures* for details.

Lentiviral Production and P0 Injection. Lentiviral particles for the viral expression of MAGUKs RNAi were produced in HEK293T cells and injected bilaterally into the CA1 region of the hippocampus of P0 mice pups. See *SI Appendix, Supplemental Experimental Procedures* for details.

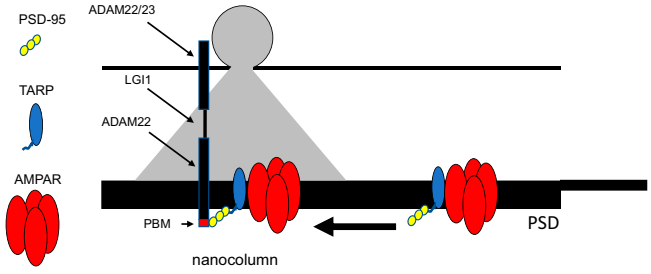

Fig. 5. Summary diagram. We propose a two-step model in AMPAR trafficking. AMPARs are first captured at the PSD by PSD-95. They then bind to ADAM22, which stabilizes them in the nanocolumn. See *Discussion* for more detail.

Hippocampal Slice Preparation. Acute slices were prepared from P18 to P28 mice after P0 injection. Hippocampal organotypic slice cultures were prepared from 6- to 8-d-old mice and transfected using biolistics (51). Detailed procedures can be found in *SI Appendix, Supplemental Information*.

Electrophysiological Recording. Synaptic currents were elicited by stimulation of the Schaffer collaterals when recording from CA1 cells. AMPAR- and NMDAR-mediated responses were collected in the presence of 100 μM picrotoxin to block inhibition. LTP was induced via a pairing protocol of 2-Hz stimulation for 90 s at a holding potential of 0 mV, after recording a 3- to 5-min baseline, but not more than 6 min after breaking into the cell. All LTP experiments were carried out in acute slices. Simultaneous dual whole-cell recordings were made in a transfected CA1 pyramidal cell and a neighboring wild-type cell. In some cases, one of the paired cells was lost during the experiment, in which case the recordings were considered until that point. In cases where one cell was lost, the remaining cell was considered for the averages.

Data Availability. All study data are included in the article and/or supporting information.

ACKNOWLEDGMENTS. We thank the R.A.N. laboratory for comments on the manuscript and Eric Dang for technical assistance. This work was supported by grants from the Ministry of Education Culture, Sports, Science, and Technology Grants 19H03331 and 19K22439 (to Y.F.), and Grants 19H04974, 19K22548, 20H04915, and 20H00459 (to M.F.); the Daiko Foundation (Y.F.); NIH Grant R01MH117139 (to R.A.N.); and the Hori Sciences and Arts Foundation (M.F.).

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