Corrections

NEUROSCIENCE. For the article “Unique domain anchoring of Src to synaptic NMDA receptors via the mitochondrial protein NADH dehydrogenase subunit 2,” by Jeffrey R. Gingrich, Kenneth A. Pelkey, Sami R. Fam, Yueqiao Huang, Ronald S. Petralia, Robert J. Wenthold, and Michael W. Salter, which appeared in issue 16, April 20, 2004, of Proc. Natl. Acad. Sci. USA (101, 6237–6242; first published April 6, 2004; 10.1073/pnas.0401413101), the authors would like to note the following: “The antibody that we claimed to recognize the mitochondrial protein ND4, a control in our study, actually recognizes the mitochondrial protein NdufA9. Like ND4, NdufA9 protein has a molecular weight of 39 kDa and is a subunit of NADH dehydrogenase (complex I). But unlike ND4, NdufA9 is encoded in the nucleus. Because NdufA9 is a subunit of complex I, as is ND4, NdufA9 is an appropriate control for our study. Thus, the conclusions of our article remain unchanged. Each occurrence of ND4 should be replaced by NdufA9, in the text as well as in labels of Fig. 2 A and B.” The corrected figure and legend appear below. In addition, the authors note that on page 6239, the sixth sentence of the second full paragraph, left column, “In contrast to ND2, neither the oxidoreductase protein ND4, another mitochondrial encoded component of complex I (25–27), nor Cyto1, an inner mitochondrial membrane protein component of complex IV (30), was detectable in the PSD fraction,” should read: “In contrast to ND2, neither the oxidoreductase protein NdufA9, another component of complex I (25–27), nor Cyto1, an inner mitochondrial membrane protein component of complex IV (30), was detectable in the PSD fraction.” These errors do not affect the conclusions of the article.

![Image of corrected figure and legend](https://www.pnas.org/cgi/doi/10.1073/pnas.0603447103)
EVOLUTION. For the article “Evolution of human–chimpanzee differences in malaria susceptibility: Relationship to human genetic loss of N-glycolyneuraminic acid,” by Maria J. Martin, Julian C. Rayner, Pascal Gagneux, John W. Barnwell, and Ajit Varki, which appeared in issue 36, September 6, 2005, of Proc. Natl. Acad. Sci. USA (102, 12819–12824; first published August 26, 2005; 10.1073/pnas.0503819102), the authors note that the Fig. 2 legend contained an error in the scale bar value. “(A) Representative photos from 50 microscope fields are shown. (Scale bar, 1 μm.)” should have read “(A) Representative photos from 50 microscope fields are shown. (Scale bar, 100 μm.)” Fig. 2 and its corrected legend appear below. This error does not affect the conclusions of the article.

CELL BIOLOGY. For the article “Nitrogen monoxide (NO)-mediated iron release from cells is linked to NO-induced glutathione efflux via multidrug resistance-associated protein 1,” by Ralph N. Watts, Clare Hawkins, Prem Ponka, and Des R. Richardson, which appeared in issue 20, May 16, 2006, of Proc. Natl. Acad. Sci. USA (103, 7670–7675; first published May 5, 2006; 10.1073/pnas.0602515103), the authors note that the e-mail address for Des R. Richardson should have appeared as d.richardson@med.usyd.edu.au. In addition, the institution for Ralph N. Watts and Des R. Richardson appeared incorrectly, due to a printer’s error. The online version has been corrected. The corrected affiliation line appears below.

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Fig. 2. EBA-175-RlI from Plasmodium species preferentially bind to their natural host erythrocytes. Binding assays (as in Fig. 1) were performed by using human and chimpanzee erythrocytes (RBCs) on COS cells transfected with EBA-175 or EBA-140 RlIs from P. falciparum and P. reichenowi. (A) Representative photos from 50 microscope fields are shown. (Scale bar, 100 μm.) (B) Mean erythrocyte numbers in 15 microscope fields, selected randomly. Sham, sham-transfected cells; Pfa, P. falciparum homolog; Pre, P. reichenowi homolog. Data are mean ± SE.

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Unique domain anchoring of Src to synaptic NMDA receptors via the mitochondrial protein NADH dehydrogenase subunit 2

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Src is the prototypic protein tyrosine kinase and is critical for controlling diverse cellular functions. Regions in Src define structural and functional domains conserved in many cell signaling proteins. Src also contains a region of low sequence conservation termed the unique domain, the function of which has until now remained enigmatic. Here, we show that the unique domain of Src is a protein–protein interaction region and we identify NADH dehydrogenase subunit 2 (ND2) as a Src unique domain-interacting protein. ND2 is a subunit of complex I in mitochondria, but we find that ND2 interacts with Src outside this organelle at excitatory synapses in the brain. ND2 acts as an adapter protein anchoring Src to the N-methyl-D-aspartate (NMDA) receptor complex, and is crucial for Src regulation of synaptic NMDA receptor activity. By showing an extramitochondrial action for a protein encoded in the mitochondrial genome, we identify a previously unsuspected mechanism by which mitochondria regulate cellular function, suggesting a new paradigm that may be of general relevance for control of Src signaling.

The nonreceptor protein tyrosine kinase Src is a ubiquitous enzyme with key roles in diverse developmental, physiological and pathological processes (1, 2). Domains identified in Src, the Src homology 3 (SH3) domain, the SH2 domain, and the SH1, or catalytic, domain, are signature regions that define highly conserved protein modules found in a wide variety of signaling proteins (3). In addition to the highly conserved regions, Src also contains a region of low sequence conservation and unknown function termed the unique domain.

The CNS is a major site of expression of Src, and numerous functions are ascribed to Src in the developing and adult CNS. A growing body of evidence indicates that in the CNS a key function of Src is to regulate glutamatergic neurotransmission and synaptic plasticity (4). At glutamatergic synapses, Src modulates the activity of a number of receptors and synaptic plasticity (4). A growing body of evidence indicates that in the CNS a key function of Src is to regulate glutamatergic neurotransmission and synaptic plasticity (4). At glutamatergic synapses, Src modulates the activity of a number of receptors and synaptic plasticity (4). Thus, in the present study, we set out to search for proteins that may interact with the Src unique domain and may thereby mediate the interaction between Src and NMDARs.

Materials and Methods

Detailed methods are found in ref. 22 and Supporting Text, which is published as supporting information on the PNAS web site.

Yeast Two-Hybrid Screen. cDNAs encoding amino acids 4–82 (the Src unique domain) and amino acids 4–150 (the Src unique and SH3 domains) of murine n-Src were ligated into pEG202 (23) to create two expression vectors encoding in frame LexA fusions containing the Src unique domain. To create the selection strains for screening, each bait plasmid was individually transformed into the yeast strain EGY48. The selection strains were transformed with a representative activation-tagged cDNA prey fusion library constructed by using ~1-kb EcoRI fragmented poly(A)(+) RNA from human fetal brain. Yeast transformed with the prey library (~1.1 × 10⁶ clones) were screened by double selection on 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) Leu medium. Prey cDNAs encoding proteins that interacted with the bait were isolated and sequenced.

Src, Fyn, and NADH Dehydrogenase Subunit 2 (ND2) Recombinant Proteins. The cDNAs encoding the SH3 and SH2 domains of mouse n-Src and Fyn were PCR subcloned, ligated in frame into pGEX4T-1 (Amersham Pharmacia). These plasmids, as well as plasmids encoding the unique domains of Src and Fyn in pGEX2T'6, were transformed into BL21 bacteria, and GST fusion proteins were purified by glutathione affinity chromatography. To create the ND2.1, ND2.2, and ND2.3 GST fusion proteins, cDNAs encoding amino acids 239–321 (ND2.1–GST), amino acids 189–238 (ND2.2–GST), and amino acids 1–188 (ND2.3–GST) of human ND2 were PCR subcloned and ligated into pGEX4T-1. Using PCR-based single-nucleotide mutagenesis, all cDNAs encoding ND2 fusion proteins were corrected for premature termination. Prey cDNAs encoding ND2 fusion proteins were corrected for premature translation termination and protein truncation.

Abbreviations: SH3, Src homology 3; NMDA, N-methyl-D-aspartate; NADH, NADH dehydrogenase; LTP, long-term potentiation; ND2, NADH dehydrogenase subunit 2; PSD, postsynaptic density; HA-HPR, streptavidin–horseradish peroxidase; AMPA, α-amino-3-hydroxy-5-methylisoxazolepropionic acid; Cyto1, cytochrome c oxidase subunit 1; CAP, chloramphenicol; neFPCs, miniature excitatory postsynaptic currents.

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The plasmids were transformed into bacteria, and GST fusion proteins were purified as above.

**Cellular Fractionation.** Postsynaptic density (PSD) proteins (24) were prepared from rat brain as described (22). Cellular fractionation of rat brain tissue into nuclear, light mitochondrial, microsomal, and cytosolic fractions was performed by differential centrifugation of tissue homogenate in 0.25 M sucrose/10 mM Hepes-NaOH/1 mM EDTA, pH 7.4, with 2 μg each of aprotinin, pepstatin A, and leupeptin (Sigma) at 4°C. All pellets from individual fractions were resuspended in RIPA buffer (50 mM Tris, pH 7.6/150 mM NaCl/1 mM EDTA/1% Nonidet P-40/2.5 mg/ml NaDOC/1 mM NaVO₄/1 mM PMSF/2 μg/ml each of protease inhibitors). The light mitochondrial fraction was used in subsequent experiments. For immunoblots 50 μg of total protein was loaded per lane, resolved by SDS-PAGE, transferred to nitrocellulose membranes, and probed with anti-ND2, anti-Cytol (cytochrome c oxidase subunit 1), and anti-ND4 (mouse monoclonals, Molecular Probes), anti-PSD95 (mouse monoclonal clone 7E3-1B8, Oncogene Research Products, Cambridge, MA), anti-NR1 (mouse monoclonal clone 54.1, Pharmingen), anti-Src, or anti-synaptophysin (mouse monoclonal, Sigma).

**Dot Blotting.** Src40–58 and scrambled Src40–58 peptides were biotinylated by incubating with Sulfo-NHS-Biotin (Pierce) for 30 min at room temperature. The biotinylation reaction was then quenched by the addition of TrisHCl (pH 8.0) to a final concentration of 20 mM. Purified recombinant fusion proteins (∼20 μg each) were dotted onto nitrocellulose and dried overnight. Membranes were blocked with 5% BSA in PBS (pH 7.5) for 1 h, after which biotinylated peptides (20 μg/ml) diluted 1:1,000 in fresh 5% BSA in PBS were added. The membranes were incubated with the peptides for 1 h, washed, and probed with streptavidin–horseradish peroxidase conjugate (SA-HRP). Bound probe was then detected on film by using an enhanced chemiluminescence kit.

**Cultured Hippocampal Neurons.** Fetal hippocampal neurons were prepared, cultured, and used for electrophysiological recordings 12–17 days after plating.

**Results**

**ND2 Is a Src Unique Domain-Binding Protein.** To search for proteins that interact with the Src unique domain, we did a yeast two-hybrid screen using bait constructs containing the Src unique domain. In two independent screens, we isolated cDNA fragments encoding overlapping regions within ND2 (Fig. 1 L1), a 347-aa protein that is a subunit of the inner mitochondrial membrane enzyme NADH dehydrogenase (complex I). ND2 is one of a group of seven oxidoreductase proteins that are encoded in the mitochondrial genome and which assemble with 35 nuclear encoded subunits to form complex I. ND2 on its own lacks enzymatic activity (25–27).

Because yeast two-hybrid screens may reveal false positive protein–protein interactions, we investigated whether the interaction between Src and ND2 was observed with an independent methodological approach. We tested for direct binding *in vitro* between ND2 and Src by using recombinant proteins. We made a series of GST fusion proteins comprised of portions of ND2 that spanned the overlapping region found with the yeast two-hybrid screen (Fig. 1 A). Importantly, cDNAs encoding ND2 fusion proteins were corrected for differences between mitochondrial and nuclear codons so that the sequence of the ND2 portion of the fusion proteins was that which would be produced by mitochondrial translation. We tested each of these proteins individually for interaction with the Src unique domain. We found that a GST fusion protein containing amino acids 239–321 of ND2 (ND2.1–GST) bound to the unique domain of Src (Fig. 1 B). In contrast, fusion proteins containing amino acids 189–238 (ND2.2–GST) or 1–188 (ND2.3–GST) of ND2 did not bind to the Src unique domain. These results, together with those from the yeast two-hybrid screen, show that ND2 is a Src unique domain-binding protein. Our results indicate further that the Src-binding portion of ND2 is contained within the region of amino acids 239–321. This region of ND2 has low sequence conservation in the unique domain, among mitochondrially encoded oxidoreductase proteins and is outside the so-called “oxidoreductase domain,” a signature region present in all mitochondrially encoded subunits of NADH dehydrogenase (25–27) and some antiporters (28).

We wondered whether binding of ND2 might generalize to other domains of Src or to other Src family tyrosine kinases. However, we found that ND2.1–GST did not bind to either of the prototypic protein–protein interaction domains of Src, the SH2 or SH3 domains (Fig. 1 C). To examine the potential interaction of ND2 with other kinases of the Src family, we tested recombinant domains of Fyn. Although Fyn is closely related to Src, it has low sequence conservation in the unique domain (1–3). We found that ND2.1–GST did interact *in vitro* with the Fyn unique domain; nor did it bind to the SH2 or SH3 domains of

![Image](https://www.pnas.org/cgi/doi/10.1073/pnas.041413101)
To investigate the possibility that Src and ND2 may interact in vivo, we immunoprecipitated brain lysates with antibodies directed against ND2 (anti-ND2) or against Src (anti-Src). We found that immunoprecipitating Src led to coimmunoprecipitation of ND2 and, conversely, immunoprecipitating with anti-ND2 resulted in coimmunoprecipitation of Src (Fig. 1D). In contrast, anti-ND2 did not coimmunoprecipitate Fyn and neither ND2 nor Src was immunoprecipitated with nonspecific IgG. As an independent immunoprecipitation control, we found that ND2 was coimmunoprecipitated by anti-Src from Src−/− fibroblasts but not from Src+/+ fibroblasts (Fig. 1E). Thus, in addition to finding the ND2-Src unique domain interaction in the yeast two-hybrid screens and in vitro binding assays, we found that ND2 and Src coimmunoprecipitated with each other, leading us to conclude that ND2 is a Src unique domain binding protein that may interact with Src in vivo.

**ND2 Is Present in PSDs in Brain.** In the CNS a prominent subcellular location for Src is in the PSD (5), a subsynaptic specialization at glutamatergic synapses comprised of α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA-) and NMDA-type glutamate receptors together with scaffolding, signaling, and regulatory proteins (29). Because Src regulates subsynaptic NMDARs (5), we predicted that if ND2 is the protein mediating the interaction between NMDARs and the Src unique domain, then ND2 would be present in the PSD. Therefore, we prepared PSD proteins from rat brain homogenate by sequential fractionation and determined whether ND2 was present in this fraction. Characteristic of a bona fide PSD fraction, this fraction contained postsynaptic proteins including PSD-95 and NMDAR subunit proteins, but lacked the presynaptic protein synaptophysin (Fig. 2A). We found that ND2 was present in the PSD fraction, and we estimated the amount of ND2 in this fraction was ~15% of that in total brain homogenate. In contrast to ND2, neither the oxidoreductase protein ND4, another mitochondrial encoded component of complex I (25–27), nor Cyto1, an inner mitochondrial membrane protein component of complex IV (30), was detectable in the PSD fraction. Conversely, Cyto1 and ND4, as well as ND2, were readily detected in proteins from brain mitochondria (Fig. 2B). It is possible that the protein detected in the PSD fraction was not ND2, but a protein of the same molecular size that was spuriously recognized by anti-ND2. However, incubating anti-ND2 with the antigen used to derive the antibody prevented the immunoblot signal (Fig. 2C). Moreover, a second antibody directed toward a distinct epitope in a region of ND2 remote from that of the anti-ND2 epitope also detected ND2 in both PSD and mitochondrial preparations (Fig. 2D). Thus, ND2 was found in the PSD fraction by two separate antibodies, and this was not caused by a general contamination with mitochondrial proteins because neither Cyto1 nor ND4 were detected in the PSD.

We also tested for the presence of ND2 in PSDs by postembedding immunogold electron microscopy in the CA1 stratum radiatum of rat hippocampus (31, 32). In this approach, the tissue is fixed immediately after death to preserve protein localization before sectioning. We found ND2 labeling, as visualized by secondary antibody conjugated to 10-nm gold particles, in the PSD and the postsynaptic membrane in dendritic spines of CA1 neurons (Fig. 2E), as well as over mitochondria (see Fig. 6, which is published as supporting information on the PNAS web site). ND2 labeling was enriched in the postsynaptic membrane ~30-fold as compared with the plasma membrane in the remainder of the dendritic spine (0.37 particles per PSD/section versus 0.012, P < 0.005) and there was no obvious accumulation of ND2 labeling along the plasma membrane of the dendritic shaft.

Because mitochondria are excluded from dendritic spines (33), the ND2 labeling observed in the PSD and postsynaptic membrane was not caused by mitochondrial labeling. Thus, these results show that ND2 is present in the biochemically defined PSD protein fraction and is localized at PSDs in CA1 neurons.

**ND2 Interacts with Src at the NMDA Receptor Complex in PSDs.** Because our results show that ND2 is present in PSDs from brain, we examined whether ND2 interacts with Src in PSDs. We found that immunoprecipitating ND2 from the PSD fraction led to coimmunoprecipitation of Src and vice versa (Fig. 3A), showing that ND2 and Src interact postsynaptically at glutamatergic synapses. Moreover, Src was pulled from the PSD fraction by the fusion protein ND2.1-GST, but not by either ND2.2– or ND2.3–GST (Fig. 3B). Thus, as we found with Src–ND2 binding in vitro, these results indicate that amino acids 239–321 of ND2 are both necessary and sufficient for ND2 to interact with Src in the PSD.

The hypothesis that ND2 is the protein mediating the interaction between Src and NMDARs requires that, in addition to being present in the PSD and interacting there with Src, ND2 is
part of the NMDAR protein complex. To determine whether ND2 is a component of the NMDAR complex, we used an antibody directed against the core NMDAR subunit NR1 (8) to immunoprecipitate NMDAR complexes from the PSD fraction and probed the coimmunoprecipitates with anti-ND2. We found that ND2 coimmunoprecipitated and, conversely, immunoprecipitating with anti-ND2 led to coimmunoprecipitation of NR1 (Fig. 4A). Neither ND2 nor NR1 was immunoprecipitated by nonspecific IgG, and ND2 did not coimmunoprecipitate with the potassium channel Kv3.1 (Fig. 4B), a negative control for nonspecific immunoprecipitation of postsynaptic proteins, therefore we concluded that ND2 is an NMDAR complex protein. Importantly, neither ND4 nor Cytol was detected in coimmunoprecipitates of NR1 (not shown) indicating that mitochondrial proteins in general are not part of the NMDAR complex. Moreover, ND2 did not coimmunoprecipitate with GluR2, γ-aminobutyric acid type A receptor α (GABAARα), or GABAARβ2/3 (Fig. 4B) showing that ND2 is not a detectable component of AMPA receptor or GABA receptor complexes. Thus, although ND2 is a part of NMDAR complexes, it is not generally a component of neurotransmitter receptor complexes.

**ND2 Acts as an Adapter Protein for Src.** Amino acids 40–58 within the Src unique domain were implicated in the binding of Src to the interacting protein in the NMDAR complex (5–7) and thus ND2 was predicted to bind to this region of Src. We tested this prediction in vitro by using a peptide with the sequence of amino acids 40–58 (Src40–58) that we found to bind directly to ND2.1–GST (Fig. 4C). In contrast, a peptide with identical amino acid composition, but a scrambled sequence (scrambled Src40–58), did not bind to ND2.1–GST. Neither Src40–58 nor scrambled Src40–58 bound to ND2.2–GST, ND2.3–GST, or GST alone (Fig. 4C). We next examined the effect of Src40–58 on the interaction between Src and ND2 and found that incubating ND2.1-GST with Src40–58 prevented pull down of the Src unique domain protein in vitro (Fig. 4D). Conversely, scrambled Src40–58 did not affect the interaction between ND2.1-GST and the Src unique domain. Incubating PSD proteins with Src40–58 prevented the coimmunoprecipitation of ND2 by anti-Src but this was unaffected by scrambled Src40–58 (Fig. 4E). Importantly, Src40–58 did not affect the immunoprecipitation of Src from PSDs. Thus, we conclude that amino acids 40–58 of Src interact with the region spanned by ND2.1, thereby mediating the binding between the Src unique domain and ND2.

Because ND2 alone is not catalytically active (25–27), we investigated its functional role in the NMDAR complex. ND2 might be a phosphorylation target for Src, but we found that ND2 immunoprecipitated from PSD protein fractions was not detectably phosphorylated on tyrosine. Moreover, inclusion of ND2.1–GST did not alter the catalytic activity of Src in vitro (not shown), consistent with binding of ND2 to the unique domain rather than to the regulatory or catalytic domains. Thus, it is unlikely that ND2 is a target of Src or a regulator of Src kinase activity.

These results led us to consider a role for ND2 in the association of Src with the NMDAR complex. Antibody directed against the core NMDAR subunit NR1 was used to immunoprecipitate NMDAR complexes from PSDs, and the coimmunoprecipitates were probed with anti-Src. We found that the coimmunoprecipitation of Src with NMDARs (Fig. 4F, Left) was suppressed by Src40–58, but not scrambled Src40–58, and by ND2.1 (Fig. 4F, Right), indicating that the association of Src with NMDAR complexes depends on the interaction with ND2. In contrast, the coimmunoprecipitation of ND2 with NMDARs was not affected by Src40–58 (Fig. 4G), implying that binding ND2 to Src is not required for ND2 to associate with NMDAR complexes. Taking these results together, we conclude that ND2 may function as an adapter protein that anchors Src in the NMDAR complex.

**Loss of ND2 in Neurons Prevents the Regulation of NMDA Receptor Activity by Src.** We reasoned that if ND2 is a Src adapter protein then loss of ND2 should prevent the up-regulation of NMDAR activity by endogenous Src (5). We tested this by recording miniature excitatory postsynaptic currents (mEPSCs) from cultured hippocampal neurons (34). In these neurons, the NMDAR-mediated component of mEPSCs is increased by
activating endogenous Src via intracellular administration of a high-affinity activating phosphopeptide EPO(pY)EEIPIA (35) and is reduced by applying the Src activator and Src40–58 peptide. The criteria for identifying this protein, as inferred from previous work (4, 5), are as follows: the protein must bind directly to the Src unique domain through amino acids 40–58; this binding must be prevented by the Src40–58 peptide; the protein must be present at excitatory synapses and must be a component of the NMDAR complex; and lack of the protein must prevent the up-regulation of NMDAR activity by endogenous Src. In the present study, we provide multiple, converging lines of evidence leading to the conclusion that ND2 is the protein mediating the interaction between NMDARs and the Src unique domain.

ND2 is mitochondrially encoded and translated, yet we find it within PSDs of glutamatergic synapses in the brain. We did not detect other mitochondrial proteins (ND4 or Cyto1) in the PSD fraction, implying that this fraction is not contaminated nonspecifically by mitochondrial proteins. Furthermore, we find ND2 immunoreactivity by immunogold electron microscopy within identified PSDs in dendritic spines of CA1 neurons. In this preparation, immobilization of proteins by tissue fixation precludes the possibility that ND2 could have relocated from mitochondria to the PSD during processing. Moreover, because dendritic spines are devoid of mitochondria (33), ND2 immunoreactivity cannot be accounted for by mitochondria abutting the PSD. Taken together, these findings indicate that ND2, but not the entire complex I, is normally present within the PSD.

Discussion

The principal goal of the present study was to identify the protein mediating the interaction between NMDARs and the unique domain of Src. The criteria for identifying this protein, as inferred from previous work (4, 5), are as follows: the protein must bind directly to the Src unique domain through amino acids 40–58; this binding must be prevented by the Src40–58 peptide; the protein must be present at excitatory synapses and must be a component of the NMDAR complex; and lack of the protein must prevent the up-regulation of NMDAR activity by endogenous Src. In the present study, we provide multiple, converging lines of evidence leading to the conclusion that ND2 is the protein mediating the interaction between NMDARs and the Src unique domain.

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Many enzymes in the PSD may be involved in regulating synaptic function (39), including glycolytic enzymes capable of generating ATP (40). However, without the remaining components of complex I, it is unlikely that ND2 functions catalytically in the PSD.

Thus, in addition to its localization in mitochondria and function as a component of complex I, the present results indicate that ND2 has a second location and function outside mitochondria. Mitochondria are intimately linked to overall cellular functioning through generation of ATP by oxidative phosphorylation, are key for sequestration of intracellular calcium (41, 42), and participate in programmed cell death (43, 44). Some mitochondrial proteins are present at extramitochondrial sites (45, 46), but our evidence reveals a function for a mitochondrial protein outside this organelle; that is, ND2 acts as an adapter protein that anchors Src within the NMDAR complex, where it thereby allows Src to up-regulate NMDAR activity.

Up-regulating the activity of NMDARs is a major function of Src in neurons in the adult CNS (6, 22, 47), and this mediates the induction of long-term potentiation of excitatory synaptic transmission in CA1 hippocampal neurons (4). Our present findings imply that the ND2-Src interaction is essential for LTP induction because LTP in CA1 neurons is prevented by Src40–58 and by anti-Src1, an antibody that recognizes this amino acid sequence within the Src unique domain and prevents binding of the Src unique domain to ND2.1 in vitro (J.R.G. and M.W.S., unpublished data). LTP at Schaffer collateral-CA1 synapses is the prototypic example of NMDAR-dependent enhancement of excitatory synaptic transmission observed at numerous types of glutamatergic synapses throughout the CNS (48). In addition, Src is implicated in NMDAR-dependent seizures (49), chronic pain (50), and neurotoxicity (51). Thus, our discovery of the Src–ND2 interaction at NMDARs defines a protein–protein interaction of general relevance to regulation of neuronal function, synaptic plasticity, and pathophysiology in the CNS.

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