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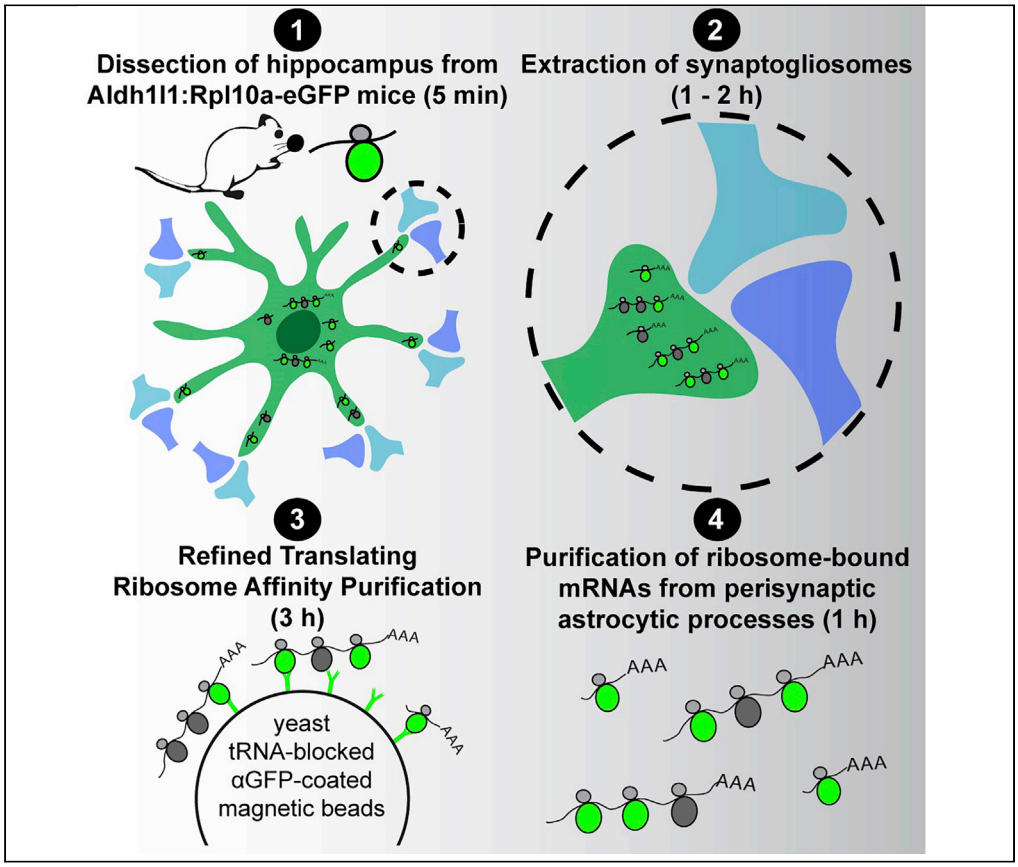
# Immunoprecipitation of Ribosome-Bound mRNAs from Astrocytic Perisynaptic Processes of the Mouse Hippocampus




Translation of distally localized mRNAs is an evolutionary mechanism occurring in polarized cells. It has been observed in astrocytes, whose processes contact blood vessels and synapses. Here, we describe a protocol for the purification of the entire pool of ribosome-bound mRNAs in perisynaptic astrocytic processes (PAPs). Our procedure combines the preparation of synaptogliosomes with a refined translating ribosome affinity purification technique. This approach can be used in any brain region to probe the physiological relevance of local translation in PAPs.

Noémie Mazaré, Marc Oudart, Giselle Cheung, Anne-Cécile Boulay, Martine Cohen-Salmon

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## HIGHLIGHTS

Astrocytes display local translation in their perisynaptic processes (PAP)

Protocol combines the preparation of synaptogliosomes with refined TRAP

Protocol allows for purification of ribosome-bound mRNAs in PAPs

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# Immunoprecipitation of Ribosome-Bound mRNAs from Astrocytic Perisynaptic Processes of the Mouse Hippocampus

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## SUMMARY

**Translation of distally localized mRNAs is an evolutionary mechanism occurring in polarized cells. It has been observed in astrocytes, whose processes contact blood vessels and synapses. Here, we describe a protocol for the purification of the entire pool of ribosome-bound mRNAs in perisynaptic astrocytic processes (PAPs). Our procedure combines the preparation of synaptogliosomes with a refined translating ribosome affinity purification technique. This approach can be used in any brain region to probe the physiological relevance of local translation in PAPs.**

**For complete details on the use and execution of this protocol, please refer to Mazaré et al. (2020).**

## BEFORE YOU BEGIN

### Preparation of the Solutions, Pre-clean, and Immunoprecipitation (IP) Tubes (Day 1)

Timing: 2–3 h

Make sure all equipment are clean and RNase-free before use: clean bench and pipettes with RNase ZAP, bake all glass materials at more than 200°C for a few hours in a dry oven.

1. Prepare 10% NP-40 (Igepal CA-630) by dissolving 2 mL of stock solution in 18 mL DEPC-treated $H_2O$ for a total volume of 20 mL.

   *Note:* Keep in mind that NP-40 is very viscous, which does not dilute easily into water. We thus recommend to prepare it well in advance and store it at 4°C.

2. Prepare buffer solutions (see Materials and Equipment section).

3. Prepare pre-clean and immunoprecipitation (IP) tubes (Figure 1).

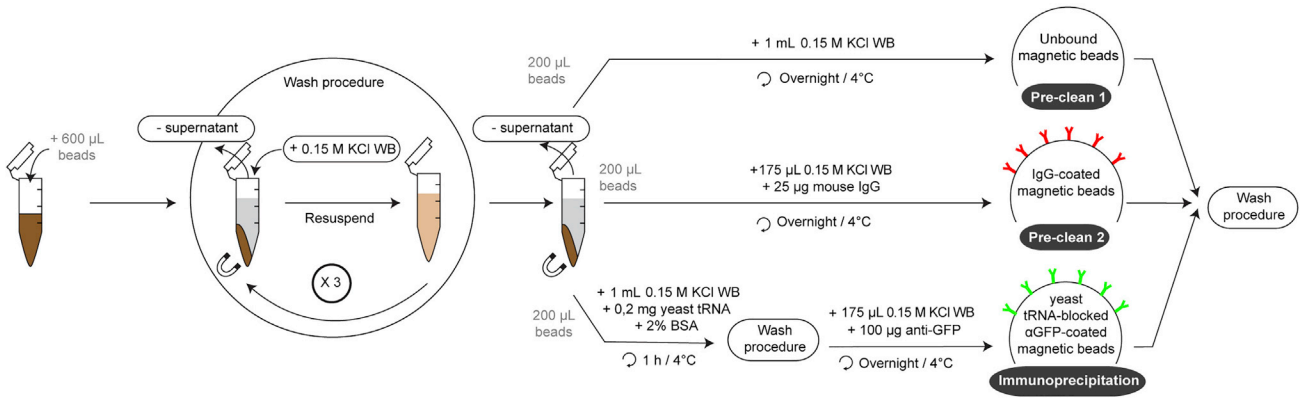


**Figure 1. Preparation of the Pre-clean and Immunoprecipitation (IP) Tubes**

*Note:* we have added two pre-cleaning steps to the initial translating ribosome affinity purification (TRAP) protocol (Heiman et al., 2014) in order to remove non-specific binding of mRNAs. Therefore, in addition to the IP tube, it is necessary to prepare a tube with no antibody (pre-clean tube 1) and a tube with non-specific mouse IgG (pre-clean tube 2). The IP tube also contains yeast tRNA and BSA in order to saturate non-specific binding sites. **We advise to prepare the tubes one day before the immunoprecipitation** and incubate with antibodies 12 h at 4°C, but this can also be performed right before the synaptogliosome extraction by incubating the tubes for 2 h at around 20°C. We recommend the use of protein G beads for the IP because mouse antibodies' binding strength to protein G is higher than protein A. Protein L could be used as an alternative to protein G but their binding efficiency was not tested for this protocol.

a. Place 600 µL Protein G beads per sample in a tube on a magnetic holder, wait 30 s for the solution to clear and remove supernatant, making sure not to disrupt the beads with the micropipette tip.
b. Add 1 mL 0.15 M KCl Wash Buffer to the tube and invert several times. If necessary, perform a short-pulse spin to remove drops from the wall and lid of the tube. Place the tube back in the magnetic holder, wait 30 s, and remove supernatant. Repeat twice more for a total of three washes.
c. Resuspend beads in 600 µL 0.15 M KCl after the last wash. Aliquot 200 µL of resuspended Protein G beads into three individual tubes.
d. Place the first tube aside (pre-clean tube 1) and keep it at 4°C. It will be used for the first pre-cleaning step.
e. Place the second tube (pre-clean tube 2) in the magnetic holder, remove the supernatant, add 25 µg mouse IgG (from the 3 mg/mL stock) to 175 µL 0.15 M KCl Wash buffer, and add this buffer to pre-clean tube 2. Resuspend the beads and **incubate 12 h at 4°C** or 2 h at around 20°C on slow tilt rotation. This will be used for the second pre-cleaning step.
f. Place the third tube (IP tube) in the magnetic holder, wait 30 s, and remove the supernatant, then proceed as follows:

i. Add 0.2 mg yeast tRNA (0.1 mg/100 µL beads) and 2% BSA to the IP tube in 1 mL 0.15 M KCl Wash Buffer (final volume) and **incubate 1 h at 4°C** on slow tilt rotation.
ii. After 1 h, place the tube in the magnetic holder, wait 30 s for the solution to clear and remove supernatant. Add 1 mL 0.15 M KCl Wash Buffer to the tube and invert several times. If necessary, perform a short-pulse spin to remove drops from the wall and lid of the tube. Place the tube back in the magnetic holder, wait 30 s, and remove supernatant. Repeat twice more for a total of three washes.
iii. Add 50 µg mouse anti-eGFP 19C8 and 50 µg mouse anti-eGFP 19F7 in 175 µL final volume of 0.15 M KCl Wash Buffer to IP tube and **incubate 12 h at 4°C** or 2 h at around 20°C on slow tilt rotation.

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Antibodies | | |
| Mouse monoclonal anti-eGFP 19C8 and 19F7 bioreactor supernatant (50 µg each for 200 µL of Protein G beads) | Antibodies are available on request at Antibody and Bioresource Core Facility of the Sloan Kettering Institute, New York | HtzGFP-19F7 HtzGFP-19C8 |
| Chicken polyclonal anti-GFP (dilution 1:500) | Aves | Cat#1020 |
| Rabbit polyclonal anti-Homer1 (dilution 1:500) | Synaptic Systems | Cat#160003 |
| Mouse monoclonal anti-VGluT1 (dilution 1:500) | Synaptic Systems | Cat#135511 |
| Mouse IgG Isotype control | Invitrogen | 10400C |
| Critical Commercial Assays | | |
| qPCR probe: Cacna1a | Thermo Fisher | Mm00432190_m1 |
| qPCR probe: Slc12a5 | Thermo Fisher | Mm00803929_m1 |
| qPCR probe: Grin1 | Thermo Fisher | Mm00433790_m1 |
| qPCR probe: Bsn | Thermo Fisher | Mm00464452_m1 |
| qPCR probe: Slc1a2 | Thermo Fisher | Mm01275814_m1 |
| qPCR probe: RNA45S (18S) | Thermo Fisher | Mm04277571_s1 |
| Agilent RNA 6000 Pico Kit | Bioanalyser Agilent | 5067-1513 |
| RNeasy Mini kit | Qiagen | 74104 |
| Chemicals, Peptides, and Recombinant Proteins | | |
| RNase ZAP | Invitrogen | AM9782 |
| RNeasy Lysis Buffer (Buffer RLT) | QIAGEN | 79216 |
| UltraPure DEPC-treated water | Thermo Fisher | 750024 |
| Cycloheximide (CHX) | Sigma-Aldrich | C7698-5G |
| Dynabeads Protein G for immunoprecipitation | Thermo Fisher | 10004D |
| cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail | Roche | 4693159001 |
| 1,2-Diheptanoyl-sn-glycero-3-phosphocholine (07:0 PC DHPC) | Avanti | 850306P-500MG |
| tRNA from brewer's yeast | Roche | 10 109 517 001 |
| HEPES Buffer solution (1 M) | Gibco | 15630056 |
| KCl (2 M), RNase-free | Invitrogen | AM9640G |
| $MgCl_2$ (2 M) | Invitrogen | AM9530G |
| DL-Dithiothreitol (DTT) | Sigma-Aldrich | 43815-1G |
| Igepal CA-630 (NP-40) | Sigma-Aldrich | I8896-50ML |
| Bovine Serum Albumin (BSA) | Sigma-Aldrich | A4503-100G |
| Sucrose | Euromedex | 200-301 |
| Ribonuclease inhibitor recombinant 4 × 2,500 u (40 u/µL) | Euromedex | 09-0312 |
| Experimental Models: Organisms/Strains | | |
| Mouse: Tg(Aldh1l1-eGFP/Rpl10a) JD130Htz | Jackson Labs, Nathaniel Heintz Private Collection, Bar Harbor, ME | MGI:5496674 www.bactrap.org |
| Other | | |

(*Continued on next page*)

*Continued*

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Magnetic holder (Pureproteome Protein A Magnetic Bead System) | Merck Millipore | 11710353 |
| Rotoflex Tube Rotator | Argos Technologies | FV-04397-33 |
| 1 mL tissue Grinder, Dounce, with Tight Pestle | Wheaton | 357538 |
| Tissue Grinder, Potter-Elvehjem, with PTFE Pestle | DWK Life Sciences | SCERSP886000-0019 |
| ES Overhead stirrer (driver unit for the tissue grinder) | Velp Scientifica | F201A0152 |
| Microcentrifuge | Eppendorf | 5415R |
| 1.5 mL Eppendorf tubes | Eppendorf | 0030120086 |
| Nanodrop | LabTech | ND-1000 |
| Capillary electrophoresis 2100 Bioanalyzer instrument | Agilent | G2939BA |
| Bio-Rad QX200 Droplet Digital PCR System | Bio-Rad | QX200 Droplet Digital |

*Alternatives:* Any other tube rotator and magnetic tube holder will be suitable for this protocol. Any benchtop centrifuge which goes up to 20,000 × *g* can be used to perform this protocol.

## MATERIALS AND EQUIPMENT

Buffers should be prepared right before experiments on day 1 and stored at 4°C during the experiment time.

### 0.15 M KCl Wash Buffer

| Reagent | Final Concentration | Amount |
|---|---|---|
| HEPES Buffer Solution (1 M, pH 7.2–7.5) | 10 mM | 250 µL |
| $MgCl_2$ (1 M) | 5 mM | 125 µL |
| KCl (2 M) | 150 mM | 1,875 µL |
| NP-40 (10%) | 1% | 2.5 mL |
| DEPC-treated water | n/a | 20.25 mL |
| **Total** | **n/a** | **25 mL** |

### Synaptogliosome Buffer (SB)

| Reagent | Final Concentration | Amount |
|---|---|---|
| Sucrose | 0.32 M | 1.1 g |
| HEPES Buffer Solution (1 M, pH 7.2–7.5) | 10 mM | 100 µL |
| DEPC-treated water | n/a | 9.9 mL |
| **Total** | **n/a** | **10 mL** |

### TRAP Homogenization Buffer

| Reagent | Final Concentration | Amount |
|---|---|---|
| HEPES Buffer Solution (1 M, pH 7.2–7.5) | 10 mM | 100 µL |
| $MgCl_2$ (1 M) | 5 mM | 50 µL |
| KCl (2 M) | 150 mM | 750 µL |
| DEPC-treated water | n/a | 9.1 mL |
| **Total** | **n/a** | **10 mL** |

**0.35 M KCl Wash Buffer**

| Reagent | Final Concentration | Amount |
|---|---|---|
| HEPES Buffer Solution (1 M, pH 7.2–7.5) | 10 mM | 200 µL |
| $MgCl_2$ (1 M) | 5 mM | 100 µL |
| KCl (2 M) | 350 mM | 3.5 mL |
| NP-40 (10%) | 1% | 2 mL |
| DEPC-treated water | n/a | 14.2 mL |
| **Total** | **n/a** | **20 mL** |

## STEP-BY-STEP METHOD DETAILS

### Synaptogliosome Extraction (Day 2)

Timing: 1–2 h

This step aims at purifying synaptogliosomes (Figure 2A). Synaptogliosomes are composed of pre- and postsynaptic compartments along with the PAP, which surrounds the synapse. This protocol enables the conservation of these compartments' cellular contents, including mRNAs and ribosomes. The following protocol has been developed on **hippocampal tissue** from **adult (P60 and older) mice**. Performing this protocol on younger brain tissue and on different brain regions might require adjustments in homogenization volume and sucrose concentrations.

*Note:* All steps are performed on ice, in RNase-free conditions.

1. Prepare CHX at stock concentration 100 mg/mL in MetOH; Prepare DTT at stock concentration 100 mM in DEPC-treated water.
2. Set centrifuge to 4°C.

*Note:* DTT stock solution can be prepared beforehand and stored as aliquots at −20°C for several months. Once defrosted, aliquots cannot be re-used. CHX should be prepared right before use.

3. Add the following to 10 mL synaptogliosome buffer (SB) right before use:
   a. 1 tablet of cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail
   b. 10 µL CHX (Final: 100 µg/mL)
   c. 5 µL 1 M DTT (Final: 0.5 mM)
   d. 10 µL Ribonuclease inhibitor (Final: 1 µL/mL)
4. Add 800 µL SB in a 1 mL tissue Grinder kept on ice.
5. Extract mouse brain and dissect hippocampus. Place one hippocampus directly in the tissue grinder, place the other one in a small cell culture dish filled with SB.
6. Homogenize one hippocampus by applying 20–30 strokes with a tight glass pestle.

⚠ CRITICAL: Make sure the pestle does not rise above solution to avoid forming air bubbles.

7. Transfer the homogenate to 1.5 mL tubes and keep on ice.
8. Repeat step 5 with the second hippocampus.
9. Spin the two tubes at 900 × *g* for 15 min at 4°C in microcentrifuge.
10. Collect supernatant (S1) fractions and transfer to new tubes. Discard pellet (P1).
11. Spin S1 at 16,000 × *g* for 15 min at 4°C.
12. Discard supernatants and resuspend pellets (P2) with 600 µL synaptogliosomes buffer (SB).
13. Spin again at 16,000 × *g* for 15 min at 4°C.

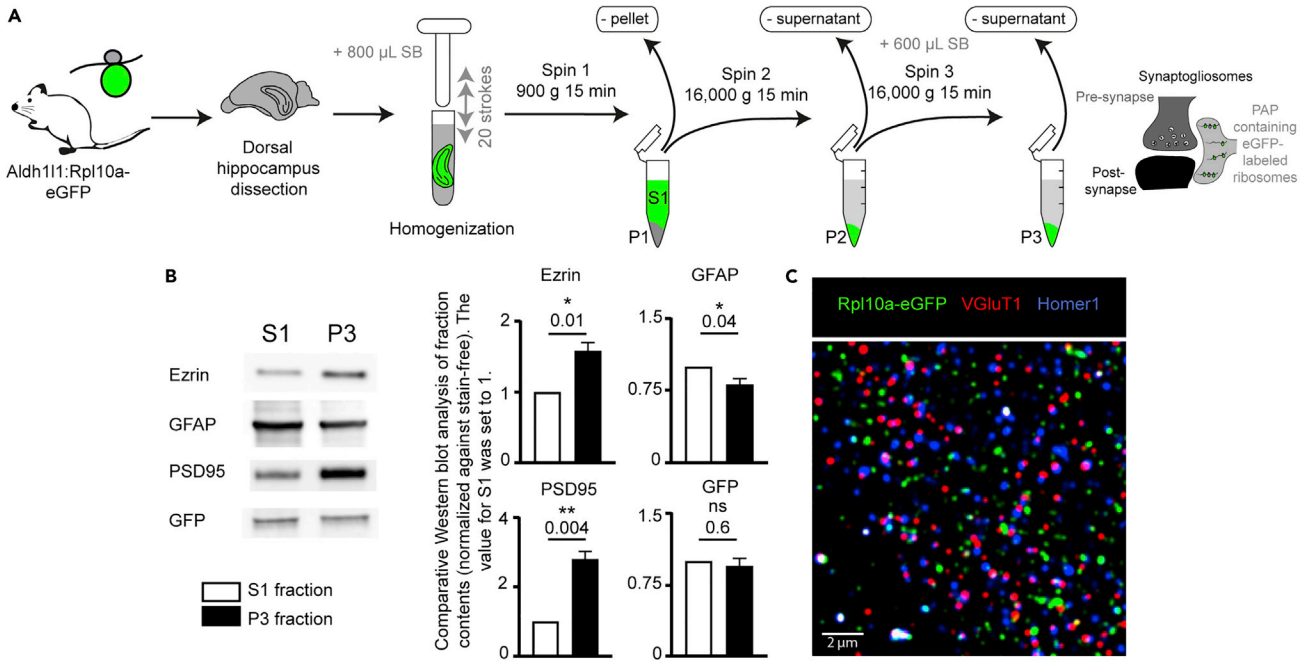


**Figure 2. Synaptogliosomes Preparation**

(A) Flowchart of the synaptogliosome preparations from the hippocampus of Aldh1l1:L10a-eGFP transgenic mice.

(B) Western blot comparison of S1 and P3 fractions. *p = 0.05, **p = 0.01, and ns (not significant) in a two-tailed paired t test. The data are presented as mean ± SEM (n = 4). From Mazaré et al. (2020).

(C) A confocal immunofluorescence microscopy image of synaptogliosomes from an Aldh1l1:L10a-eGFP mouse. The ribosomes in PAPs were immunolabelled with eGFP (green). Pre- and postsynaptic areas were immunolabelled for VGluT1 (red) and Homer1 (blue), respectively. Scale bar: 2 µm. From Mazaré et al. (2020).

14. Meanwhile, add Ribonuclease inhibitor (1 µL/mL final), CHX (100 µg/mL final) and DTT (0.5 mM final) to TRAP homogenization buffer.
15. At the end of the centrifugation, discard supernatants. The pellets (P3) contain the synaptogliosomes fraction.
16. Resuspend the first pellet in 500 µL of TRAP homogenization buffer and take this homogenate to resuspend the second pellet. The preparations from both hippocampi are now pooled (500 µL total volume).

*Note:* It is necessary to test the quality of the synaptosome. It can be done by Western blot (Figure 2B). Compared with the first supernatant obtained from hippocampal homogenate (S1), the synaptogliosome fraction (P3) should contain a lower level of GFAP and higher levels of the postsynaptic protein PSD95 and the cytosolic PAP protein Ezrin (Derouiche and Frotscher, 2001; Lavialle et al., 2011). Rpl10a-eGFP should also be detected in this fraction, indicating that the P3 fraction comprises astrocytic ribosomes (Figure 2B). The presence of astrocyte ribosomes and synapses in the P3 fraction can also be visualized via the immunofluorescent detection of eGFP, VGluT1 (pre-synapse) and Homer1 (post-synapse) (Figure 2C). Histone 3, a nuclear marker, is present in the total tissue fraction but should be absent from both S1 and P3 fractions (not shown).

## Translating Ribosome Affinity Purification (TRAP)

Timing: 3 h

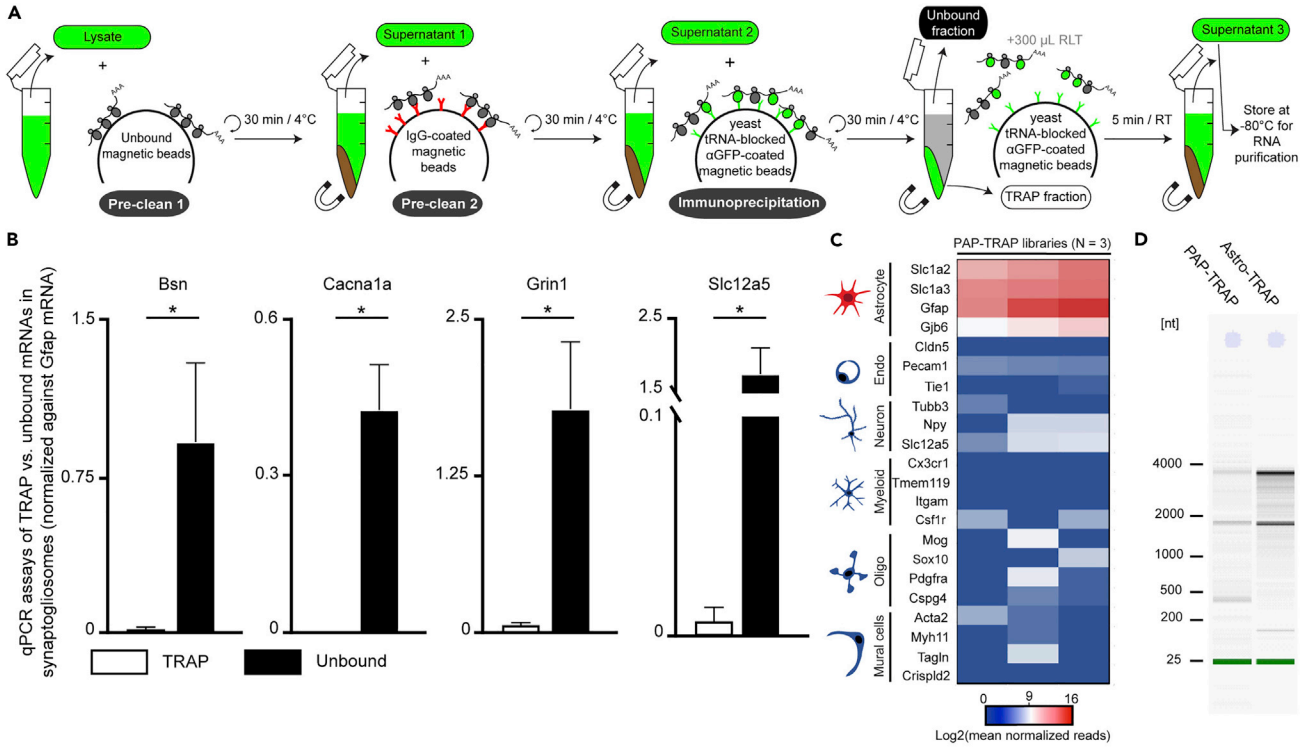


**Figure 3. Optimized Translating Ribosome Affinity Purification (TRAP)**

(A) Flowchart for TRAP of ribosome-bound mRNAs extracted from Aldh1L1:L10a-eGFP transgenic mice.

(B) qPCR comparison of a set of synapse-enriched mRNAs between the Unbound versus TRAP fraction of hippocampal synaptogliosomes. *p = 0.05 in a one-tailed Mann-Whitney test. The data are presented as mean ± SEM (n = 3). From Mazaré et al. (2020).

(C) Purity heat map of RNA-seq data for TRAP on synaptogliosomes for a selection of mRNAs specific for each type of brain cell. The centered value represents mRNAs with log2 (500 normalized reads), which corresponds to our threshold for the presence of an mRNA. Each column represents an independent cDNA library (n = 3 libraries). From Mazaré et al. (2020),

(D) Capillary electrophoresis of RNAs extracted from synaptogliosomes from dorsal hippocampi (PAP-TRAP). As a comparison, capillary electrophoresis of RNAs extracted from whole dorsal hippocampi (Astro-TRAP) is also shown.

This step consists in extracting astroglial ribosome-bound mRNAs from the previously isolated synaptogliosomes from Tg(Aldh1l1-eGFP/Rpl10a) JD130Htz mice (Figure 3A).

17. Get the pre-clean 1, pre-clean 2, and IP tubes.
18. Place the tubes in the magnetic holder, wait 30 s, and remove supernatants. Add 1 mL 0.15 M KCl Wash Buffer to each tube and invert several times. If necessary, perform a short-pulse spin to remove drops from the wall and lid of the tube. Place the tubes back in the magnetic holder, wait 30 s, and remove supernatants. Repeat twice more for a total of three washes for each tube. Store the tubes on ice in 0.15 M KCl Wash Buffer.

⚠ CRITICAL: Make sure you leave at least 200 µL 0.15 M KCl Wash Buffer on tubes to avoid the beads from drying.

19. Add Ribonuclease inhibitor (1 µL/mL final), CHX (100 µg/mL final) and DTT (0.5 mM final) to the remaining 0.15 M KCl Wash Buffer and 0.35 M KCl Wash Buffer.
20. Transfer the homogenate to the 1 mL tissue grinder Potter-Elvehjem kept on ice.
21. Connect the pestle to the ES overhead stirrer, preferably installed in a cold room, and insert it into glass tube. Gently rise the speed to 900 rpm and perform 20 strokes. Transfer to a 1.5 mL tube on ice.

⚠ CRITICAL: Make sure the pestle does not rise above solution to avoid forming air bubbles.

⚠ CRITICAL: Ensure that the homogenate stays at 4°C during the whole homogenization procedure.

22. Spin at 2,000 × *g* for 10 min at 4°C.
23. Transfer the supernatant to a new tube and add NP-40 to 1% final concentration and DHPC to 30 mM final concentration.
24. Mix by inversion and incubate for 5 min on ice.
25. Spin at 20,000 × *g* for 15 min at 4°C.
26. Place pre-clean 1 tube on the magnetic holder, wait 30 s for the solution to clear and discard extra 0.15 M KCl Wash buffer.
27. Transfer the supernatant to the pre-clean tube 1 and incubate for 30 min at 4°C on slow tilt rotation using the Rotoflex Tube Rotator.
28. Place the pre-clean tube 2 on the magnetic holder, wait 30 s for the solution to clear and discard extra 0.15 M KCl Wash buffer.
29. Place the pre-clean tube 1 on the magnetic holder, wait 30 s for the solution to clear and transfer the supernatant from the pre-clean tube 1 to the pre-clean tube 2. Incubate for 30 min at 4°C on slow tilt rotation using the Rotoflex Tube Rotator.
30. Place the IP tube on the magnetic holder, wait 30 s for the solution to clear and discard extra 0.15 M KCl Wash buffer.
31. Place pre-clean 2 tube on the magnetic holder, wait 30 s for the solution to clear and transfer the supernatant to the IP tube. Add 20 μL of 300 mM DHPC, resuspend and incubate for 30 min at 4°C on slow tilt rotation using the Rotoflex Tube Rotator.
32. Collect IP beads with magnetic holder, wait 30 s until the solution clears, then place the supernatant, which contains the unbound fraction, in a new tube.
33. Store the unbound fraction at −80°C.

*Note:* Astroglial ribosome-bound mRNAs are now bound to the anti-GFP-coated magnetic beads.

*Note:* After immunoprecipitation, the 0.35 M KCl Wash Buffer is used for a more stringent wash.

34. Add 1 mL 0.35 M KCl Wash Buffer to the IP beads and invert several times. If necessary, perform a short-pulse spin to remove drops from the wall and lid of the tube. Place the IP tube back in the magnetic holder, wait 30 s, and remove supernatants. Repeat twice more for a total of three washes.
35. Place the IP tube back in the magnetic holder, wait for the solution to clear and discard extra 0.35 M KCl Wash buffer, making sure all liquid is removed.
36. Add 300 μL RLT (RNeasy lysis buffer), remove the magnetic holder, resuspend beads and incubate for 5 min at around 20°C.

*Note:* The ribosome-bound mRNAs are now detached from the beads and free in the supernatant.

37. Collect beads with the magnetic holder, wait until the solution clears and transfer the supernatant to a new 1.5 mL tube.

⚠ CRITICAL: Make sure no beads are transferred with the RLT supernatant.

**Pause point:** Ribosome-bound mRNAs can be stored at −80°C in RLT for several months as well as the unbound fraction.

38. Proceed to mRNA extraction following the RNAeasy Mini kit standard protocol (https://www.qiagen.com). Elute RNA in 30 µL of RNase-free water.

*Note:* Yeast tRNAs are about 70 bp length and are therefore lost during the RNA purification step. It is possible to test the purity of the ribosome-bound PAP mRNA extraction with regards to neuronal transcripts by qPCR, comparing in the unbound versus TRAP fraction the level of mRNAs such as Grin1, Slc12a5, Cacna1a, or Bsn, which have previously been detected in the synapses or in the neuropil of the hippocampus (Cajigas et al., 2012; Hafner et al., 2019) (Figure 3B). The level of mRNAs from other neural cell types, including oligodendrocytes, vascular cells (endothelial cells, and mural cells (pericytes and vascular smooth muscle cells)) and microglia, were also verified by RNA-Seq and found to be below the threshold of detection (Figure 3C).

## EXPECTED OUTCOMES

RNA yield from TRAP performed on synaptogliosomes from two mouse hippocampi is compatible with RNA-seq and qPCR techniques (Here, we used the droplet digital PCR (ddPCR) technology, for conditions regarding this qPCR technique, visit the Bio-Rad website, https://www.bio-rad.com/fr). Expected Nanodrop RNA concentration for unbound samples range from 10 to 25 ng/µL for synaptogliosomes from two hippocampi. TRAP samples present RNA concentrations around 2 ng/µL. Ribosomal RNAs bands at 2 kb (RNA 18S) and 4 kb (RNA 28S) should be visible by capillary electrophoresis (Figure 3D).

See Figures 2B and 2C for expected outcomes on synaptogliosome protein content (Figure 2B) and immunostaining (Figure 2C). Figure 2B shows that compared with the first supernatant obtained from hippocampal homogenate (S1), the synaptogliosome fraction (P3) contains low levels of GFAP, high levels of the postsynaptic protein PSD95 and the cytosolic PAP protein Ezrin. Rpl10a-eGFP is also detected in this fraction indicating that the P3 fraction comprises ribosome-containing PAPs. Figure 2C shows the presence of astrocyte eGFP-tagged ribosomes in the P3 fraction via the immunofluorescent detection.

See Figures 3B and 3C for expected outcomes on TRAP purity. Figure 3B shows that libraries obtained by TRAP are enriched in astrocyte-specific genes and depleted in genes specific to neurons, myeloid cells, oligodendrocytes, mural cells (vascular smooth muscle cells and pericytes), and endothelial cells. Figure 3C shows that compared to the unbound fraction, all neuronal mRNAs are barely present in the PAP-TRAP fraction, confirming the absence of neuronal contamination.

## LIMITATIONS

This protocol was optimized for isolation of synaptogliosomes from two dorsal or whole hippocampi. It works in the same conditions for entire hippocampi for the extraction of polysomes from whole astrocytes. If you choose another part of the brain, the yield and purity of the synaptogliosomes extraction may change. You may need to adjust the volumes for extraction to the quantity of tissue, as well as the number of strokes for homogenization.

## TROUBLESHOOTING

### Problem 1

No RNA is detected, suggesting a degradation of mRNAs (all steps).

### Potential Solution

- Make sure you perform all experiments in clean, RNase-free conditions. Autoclave tissue grinder before use, disinfect pipettes and lab benches with RNase ZAP. Change your gloves regularly over the course of the experiment.

- Do not increase incubation times. mRNAs are very fragile, unstable structures, and the longer the experiment, the more mRNA you will lose. 30 min for each pre-clean step and immunoprecipitation step is enough to obtain a good, uncontaminated RNA content.

### Problem 2

Contamination by neuronal transcripts is observed (Preparation of the solutions, pre-clean and immunoprecipitation (IP) tubes (day 1)).

### Potential Solution

Make sure that all pre-clean steps have been properly performed. Neuronal contamination can be assessed by qPCR or RNA-seq.

### Problem 3

RNA yield below Nanodrop threshold of detection (step 38).

### Potential Solution

The RNA yield for synaptogliosomes is usually lower than for a whole tissue RNA extraction, because PAP RNAs represent a small fraction of all astroglial RNAs. Our IP conditions have been set with highly specific mouse monoclonal anti-GFP antibodies (Heiman et al., 2014), **which should not be replaced by other antibodies**. In addition, it is very important to perform all experiments with fresh CHX, which guarantees the integrity of the polysomes.

## RESOURCE AVAILABILITY

### Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Martine Cohen-Salmon, martine.cohen-salmon@college-de-france.fr.

### Materials Availability

The transgenic mouse line Tg(Aldh1l1-eGFP/Rpl10a) JD130Htz used in this study has been deposited to Jackson Labs, Heintz Private Collection, Bar Harbor, ME [MGI 5496674].

### Data and Code Availability

RNA sequencing data of our initial TRAP experiments on astrocytes and PAP of the dorsal hippocampus are recorded in the Gene Expression Omnibus (GEO) website with the record reference GSE143531.

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## AUTHOR CONTRIBUTIONS

Conceptualization, M.C.-S.; Methodology, M.C.-S., N.M., G.C., and A.-C.B.; Investigation, A.-C.B., N.M., M.O., and G.C.; Writing – Original Draft, N.M.; Funding Acquisition, M.C.-S.; Supervision, M.C.-S.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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