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Supplemental Material

Co-Localization of Reelin and Proteolytic AβPP Fragments in Hippocampal Plaques in Aged Wild-Type Mice

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SUPPLEMENTARY METHODS AND MATERIALS

Cell culture, transfection, and Western blotting

Human embryonic kidney cells (HEK293) were cultured in Dulbecco's modified eagle medium (DMEM, Invitrogen Inc.) supplemented with 10% fetal calf serum (FCS, Invitrogen Inc, Carlsbad, CA, USA) at 37°C, 5% CO₂, 95% humidity on plastic culture dishes (Falcon, Switzerland) coated with polyornithine (10 μ g/ml, Sigma-Aldrich, Buchs, Switzerland) and laminin (5 μ g/ml, Invitrogen Inc.). Transfection of 1 μ g of human A β PP-Citrine [1] plasmid DNA was performed using Polyethyleneimine Reagent (PEI, Polysciences Inc., Warrington, PA, USA) according to the manufacturer's protocol. After 48 h, whole cell extracts were isolated in 120 mM NaCl, 50 mM Tris (pH = 8.0) lysis buffer containing 0.5% (v/v) Nonidet P-40 without protease or phosphatase inhibitors. Dissected hippocampi from 15 month-old wild-type mice (n =3) were lysed in same buffer including 2 mM EDTA, pH = 8.0, 0.5% Deoxycholate, 0.1% SDS, 0.5 mM

DTT (RIPA) with and without protease/phosphatase inhibitors. For both procedures, cell debris was removed by centrifugation for 15 min at 14,000 rpm and protein concentration determined using Bradford protein assay. Cell or brain extracts were incubated in pepsin (0.15 mg/ml in 0.2 N HCl) or vehicle for 0, 10 min, 30 min, or 1 h at 37°C and the reaction stopped with 2xSDS PAGE sample buffer. Additional controls included the incubation of the samples at 37°C for 1 h in PBS buffer. Extracts were separated on 10-20% Novex NuPAGE Tris-Tricine gels, blotted on Protran BA 79 membranes (0.1 µm; Schleicher & Schuell, Dassel, Germany), blocked in TBS containing 5% western blocking reagent (Roche Diagnostics GmbH, Mannheim, Germany) for 1 h at RT, incubated with primary antibodies (mouse anti-A β 6E10 and rabbit anti-C-A β PP; 1:500 each, mouse anti-N-A β PP and rabbit A $\beta_{1-40/42}$; 1:1000 each), and visualized by the enhanced chemiluminescence reaction (Amersham Biosciences).

Reference

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Supplementary Figure 1. Pepsin but not the acidic environment is responsible for the antigen retrieval effect. A-C) Double-immunofluorescence of perfusion-fixed brain sections of 15 month-old wild-type mouse tissue using anti-Reelin (green, A', B', C') and anti-A β (red, A", B", C") antibodies processed without pepsin (A), with 0.2 N HCl for 60 min at 37°C (B) and with pepsin for 60 min at 37°C (C). Note, that the treatment with 0.2 N HCl alone had no influence on the intensity and detectability of A β - and Reelin-immunoreactivity. D-E) Biochemical analysis of the proteolytic processing of murine and human A β PP following pepsin treatment. D) Western blot using rabbit anti-A $\beta_{40/42}$ antibody on hippocampal brain lysates and obtained from 15 month-old wild-type mice (left, n = 3) and HEK cell extracts transfected with human wild-type A β PP (right). Samples were incubated with 0.15 mg/ml pepsin in 0.2 N HCl at 37°C for 0, 10, 30, or 60 min or vehicle alone. Anti-C-A β PP antibodies gave a similar picture (data not shown). E) Western blot using mouse anti-A β_{1-17} (6E10) antibody on HEK cell extracts transfected with rodent A β PP and its proteolytic fragments as shown on the right using hippocampal brain lysates (HIP). No signal was obtained using the N-A β PP antibody following pepsin incubation, demonstrating the proteolytic digestion starting from the N-terminus of A β PP (data not shown). No difference in A β PP processing was observed following incubation of the cell extracts in PBS at 37°C for 60 min in the absence of pepsin and HCl (right lane).