

## Major Vault Protein is Expressed along the Nucleus–Neurite Axis and Associates with mRNAs in Cortical Neurons

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**Major Vault Protein (MVP), the main constituent of the vault ribonucleoprotein particle, is highly conserved in eukaryotic cells and upregulated in a variety of tumors. Vaults have been speculated to function as cargo transporters in several cell lines, yet no work to date has characterized the protein in neurons. Here we first describe the cellular and subcellular expression of MVP in primate and rodent cerebral cortex, and in cortical neurons in vitro. In prefrontal, somatosensory and hippocampal cortices, MVP was predominantly expressed in pyramidal neurons. Immunogold labeled free and attached ribosomes, and structures reminiscent of vaults on the rough endoplasmic reticulum and the nuclear envelope. The nucleus was immunoreactive in association with nucleopores. Axons and particularly principal dendrites expressed MVP along individual microtubules, and in pre- and postsynaptic structures. Synapses were not labeled. Colocalization with microtubule-associated protein-2, tubulin, tau, and phalloidin was observed in neurites and growth cones in culture. Immunoprecipitation coupled with reverse transcription PCR showed that MVP associates with mRNAs that are known to be translated in response to synaptic activity. Taken together, our findings provide the first characterization of neuronal MVP along the nucleus–neurite axis and may offer new insights into its possible function(s) in the brain.**

**Keywords:** lung resistance-related protein, microtubule, mRNA transport, nuclear pore, STEP, tPA

### Introduction

Major Vault Protein (MVP) is a 100-kDa protein that complexes with small-untranslated vault RNA (vRNA), telomerase-associated protein and poly(ADP-ribose) polymerase, in what are referred to as vault particles (Kickhoefer, Siva, et al. 1999; Kickhoefer, Stephen, et al. 1999). Vaults are the largest known ribonucleoprotein particle, weighing 13 MDa and measuring approximately 65 × 40 nm, or 3 times the size of ribosomes (Kedersha and Rome 1986; Kickhoefer et al. 1993; Kickhoefer and Rome 1994). Named for their unusual morphology, vault particles have 2 identical hemispherical halves, each with a central ring enclosed by a radially symmetric 8-petaled flower (Kedersha et al. 1991; Kickhoefer et al. 1996; Mikyas et al. 2004). Ninety-six copies of MVP comprise 70–75% of the vault's mass (Kickhoefer et al. 1993). MVP is highly conserved across eukaryotic species, from slime molds to mammals, indicating that it likely plays an important role in cellular function (Kedersha et al. 1990; Herrmann et al. 1996; Kickhoefer et al. 1996; Hamill and Suprenant 1997). In particular, human and rat MVP share approximately 88% of their amino acid residues.

Studies in various cell systems have localized vaults to the cytoplasm, in association with the cytoskeleton, as well as to

the nucleus, suggesting a role in nucleocytoplasmic transport (Eichenmüller et al. 2003; Slesina et al. 2005, 2006). In addition, MVP interacts with extracellular signal-regulated kinase (ERK) following epidermal growth factor (EGF) stimulation, and may function as an ERK scaffold to modulate the activation of ERK targets (Kolli et al. 2004). MVP has also been shown to interact with the tyrosine kinase, Src, and to regulate ERK subsequent to EGF stimulation (Kim et al. 2006). In addition to its important role in cell proliferation, ERK signaling is implicated in neuronal functions, including synaptic plasticity and the consolidation of long-term memories (Crews et al. 1992; Sweatt 2004). MVP, also known as lung resistance-related protein (LRP) (Scheffer et al. 1995), is upregulated in a variety of tumors, and its expression correlates with a multidrug-resistant phenotype and poor prognosis (Mossink et al. 2003). Although the role of MVP in cancer has been extensively studied, very little is known regarding its expression and function in the brain.

We have studied MVP in normal, drug-naïve neurons to obtain insights into its cellular organization and function. Towards this aim, we combined immunogold high-resolution electron microscopy in monkey and rat cerebral cortex with immunocytochemical and molecular analyses of primary cortical cultures. We show that brain MVP is primarily cytoplasmic and associated with free and attached ribosomes, whereas a fraction of the protein is found in the nucleus. In dendrites and axons, MVP is expressed along microtubules and on primary endosomal membranes. MVP is colocalized with an array of cytoskeletal markers in neurites and growth cones of cultured neurons. Immunoprecipitation (IP) of the vault complex followed by reverse transcription–PCR (RT-PCR) shows an association with several mRNAs that are translated within dendrites, including tissue plasminogen activator (tPA) and striatal-enriched tyrosine phosphatase (STEP). Given the expression of MVP along the nucleus–neurite axis, its link to microtubules, and its association with multiple mRNAs, we suggest the possibility that MVP plays a role in the transport of mRNAs along neurites.

### Materials and Methods

#### *Antibodies and Western Blotting*

For all information regarding primary, secondary and tertiary antibodies, please refer to the Table 1. Anti-MVP was generated by Invitrogen (Carlsbad, CA) against a glutathione S-transferase (GST)-fusion protein of the human MVP C-terminus (plasmid courtesy of Dr. Anton Bennett, Yale University). Rabbits were injected with immunogen 6 times and bled 13 days after immunization. The antibody was affinity-purified by liquid-phase adsorption against the fusion protein, and recognizes a 100-kDa band in cortical homogenates. Preadsorption against the immunogen

**Table 1**

Antibodies utilized in western blot and immunocytochemistry

| Antibody     | Format   | Immunogen                     | Host   | Dilution     | Source                                   |
|--------------|--|-------------------------------|--------|--------------|--|
| Anti-ERK2    | Whole IgG, unconjugated                          | C-terminus (rat)              | Rabbit | 1:5000       | Santa Cruz Biotechnology, Santa Cruz, CA |
| Anti-FLAG    | Whole IgG, unconjugated                          | FLAG peptide                  | Mouse  | 1:1000       | Sigma-Aldrich                            |
| Anti-MAP2    | Whole IgG, unconjugated                          | Whole molecule (bovine)       | Mouse  | 1:200        | Chemicon, Temecula, CA                   |
| Anti-MVP     | Whole IgG, unconjugated                          | C-terminus aa 652-853 (human) | Rabbit | 1:500-1:1000 | Invitrogen                               |
| Anti-tau     | Whole IgG, unconjugated                          | Whole molecule (bovine)       | Mouse  | 1:500        | Cell Signaling Technology, Danvers, MA   |
| Anti-tubulin | Whole IgG, unconjugated                          | C-terminus aa 443-450 (human) | Mouse  | 1:200        | Upstate, Bilerica, MA                    |
| Anti-mouse   | Whole IgG, Alexa Fluor 488-conjugated            | Mouse Fc                      | Goat   | 1: 750       | Molecular Probes, Eugene, OR             |
| Anti-rabbit  | Whole IgG, Alexa Fluor 594-conjugated            | Rabbit Fc                     | Goat   | 1:750        | Molecular Probes                         |
| Anti-rabbit  | Whole IgG, peroxidase-conjugated                 | Rabbit Fc                     | Donkey | 1:10 000     | Amersham Biosciences, Piscataway, NJ     |
| Anti-rabbit  | F(ab) <sub>2</sub> fragment, biotinylated        | Rabbit Fc                     | Goat   | 1:800        | Jackson Immunoresearch, West Grove, PA   |
| Anti-rabbit  | Fab fragment, nanogold-conjugated                | Rabbit Fc                     | Goat   | 1:200        | Nanoprobes                               |
| Anti-biotin  | F(ab) <sub>2</sub> fragment, nanogold-conjugated | Biotin                        | Goat   | 1:500        | Nanoprobes                               |

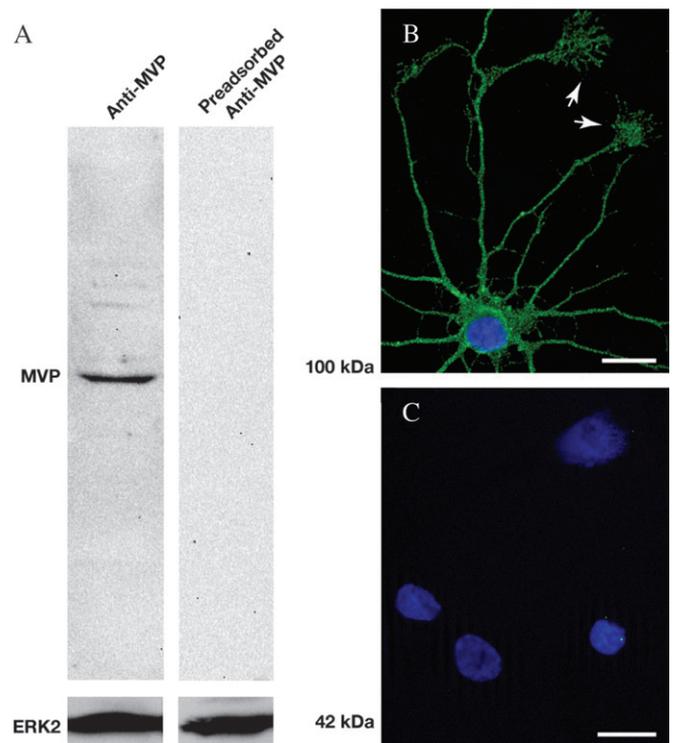
peptide (5 µg/ml) abolishes immunoreactivity (Fig. 1A). Specificity was additionally confirmed with preadsorption/omission controls in immunocytochemistry (Fig. 1B,C).

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were sacrificed according to the Yale University Institutional Animal Care and Use Committee (IACUC) guidelines. The brains were removed, and homogenized in TEVP (10 mM Tris, 5 mM NaF, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM EDTA [ethylenediaminetetraacetic acid], 1 mM ethylene glycol-bis[beta-aminoethyl ether] [EGTA]) with 320 mM sucrose and a protease inhibitor cocktail (Roche Diagnostics, Manheim, Germany). The sample was centrifuged at 800 g for 10 min at 4 °C yielding a pellet fraction (P1) and a supernatant fraction (S1); S1 was used for western blotting. Proteins (50 µg) were separated on 10% SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred onto nitrocellulose membrane (Millipore, Billerica, MA). We used antibody to ERK2 as loading control (Fig. 1A). Membranes were blocked with 5% nonfat dry milk, and probed with anti-MVP or anti-ERK2 overnight at 4 °C. Blots were next incubated with peroxidase-conjugated secondary antibody, and processed with chemiluminescence (Pierce Biotechnology, Rockford, IL). Protein bands were detected using a Chemi-HR16 gel imaging system (Syngene, Frederick, MD).

### MVP Immunoprecipitation

To obtain synaptic and microsomal organelle fractions, rat brains were processed as described with slight modifications (Dunah and Standaert 2001). Briefly, the S1 supernatant (see previous section) was recentrifuged at 9200 g for 15 min at 4 °C to yield the crude synaptosomal fraction (P2) and another supernatant fraction (S2). P2 underwent hypo-osmotic lysis for 30 min on ice by resuspending it in TEVP with 36 mM sucrose and protease inhibitors (Roche). The P2 lysate was then centrifuged at 25 000 g for 20 min at 4 °C to isolate the pelleted synaptic membrane fraction (LP1). S2 was spun at 100 000 g for 2 h to pellet an intracellular microsomal organelle fraction (P3). Each final pellet was gently washed in TEVP to avoid possible contamination between fractions. LP1 and P3 pellets were resuspended in TEVP, sonicated, and frozen at -20 °C until protein concentration determinations.

For MVP IP from the LP1 and P3 pelleted fractions, we prepared anti-IgG-coated protein-G sepharose beads as follows: 7.2 × 10<sup>12</sup> beads (GE Healthcare, Piscataway, NJ) were washed with phosphate-buffered saline (PBS) plus RNase inhibitor (Roche), blocked with 10% bovine serum albumin (BSA) and 10% tRNA in 2× IP buffer (100 mM N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 350 mM NaCl, 40 mM EDTA, 4 mM EGTA, 100 mM NaF, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 1% NP-40, 1% Triton X-100, 1 mM dithiothreitol) for 1 h, and incubated in anti-IgG for 1 h at 4 °C. Equal volumes of 2× IP buffer were appropriately added to LP1 and P3 fractions (final concentration 1× IP buffer), and these lysates were subsequently incubated for 1 h at 4 °C with anti-MVP or rabbit IgG-alone as a control. The lysates were then combined with anti-IgG-coated beads for an additional hour. Beads were extensively washed in 2× IP buffer before a final wash in 1× IP buffer and then resuspended in 2× Laemmli buffer (Bio-Rad, Hercules, CA) containing excess β-mercaptoethanol, for western blotting, or QIAzol Lysis Reagent, for RNA purification and RT-PCR (see below).



**Figure 1.** Characterization of the MVP antibody. (A) SDS-PAGE blot of total homogenate from cerebral cortex was probed with anti-MVP (lane 1). The antibody recognizes a 100-kDa band corresponding to the molecular mass of MVP, which is not present when anti-MVP was preadsorbed against the immunogen (lane 2). Both lanes were reprobed for ERK2 as a loading control. (B) MVP immunofluorescence throughout the soma and processes, including in growth cones (arrows). Labeling is eliminated when the culture is probed with antigen-adsorbed anti-MVP (C), or with secondary antibody alone (data not shown). Nuclei are stained blue with DAPI. Scale bars: 10 µm.

### RNA Purification and RT-PCR

RNA was isolated through guanidine-thiocyanate phenol chloroform extraction, and purified using RNeasy Lipid Tissue kit (Qiagen, Valencia, CA). cDNAs were synthesized from immunoprecipitated MVP or control IgG via random hexamer and oligo-dT primers, and subjected to PCR amplification using gene specific primers for tPA, STEP, histone H1, vRNA, and Alien RNA. As a negative control, RNase I (Roche) was added to extracted RNA samples after purification (see Fig. 10B). The primers were: tPA, 5' TCAGATGAGATGACAGG-GAAATGCC (forward) and 5' ATCATACAGTTCTCCAGCC (reverse); histone H1, 5' GGTGGCTTTCAAGAAGACCAA (forward) and 5' TGAGGCTGTTGCTGCTCCTT (reverse); STEP, 5' GGGCTCAAAT-GTCTCCTT (forward) and 5' TTCTCGTTCATCTCCTCGA (reverse);

vRNA, 5' AGCTCAGCGGTACTTC (forward), and 5' GCCCGCGGGTCTCGAAC (reverse). Each PCR reaction used HotStart Taq DNA polymerase (Qiagen), and consisted of 35 cycles of 30-s denaturation (95 °C), 1-min annealing (55 °C), and 30-s extension (72 °C). Alien primers were obtained from Stratagene (La Jolla, CA), and PCR reactions for Alien products consisted of 35 cycles of 30-s denaturation (95 °C), 1-min annealing (57 °C), and 30-s extension (72 °C).

#### **Alien and Micrococcal Nuclease Treatments**

Prior to MVP IP, Alien RNA ( $10^5$  copies) was added to P3 lysates and incubated at 4 °C for 15 min. In certain experiments (see Fig. 10D), 500 units of micrococcal nuclease (S7; Roche) were added to P3 lysates before MVP IP in the presence of 10 mM  $\text{CaCl}_2$ . Micrococcal nuclease (MNase)-treated lysates were gently mixed at room temperature for 20 min. Equal volumes of 2x IP buffer were subsequently added for a final concentration of 20 mM EDTA and 2 mM EGTA to quench the calcium-dependent MNase activity. MVP IPs were then performed as described above. In experiments where extracted RNA was treated with MNase (see Fig. 10D), 15 units of MNase were added in the presence of 5 mM  $\text{CaCl}_2$  for 20 min. RT-PCR was performed as described above. MNase is a nonspecific nuclease that predominantly cleaves single-stranded nucleic acids, but also has activity against double-stranded DNA. MNase was used instead of RNase I in these experiments because MNase activity can be regulated by the presence of calcium.

#### **Transfection of HEK Cells**

HEK 293T cells were seeded at a density of 100 000/cm<sup>2</sup> in 35-mm dishes and transfected with 1 µg pcDNA 3.1-MVP-FLAG (MVP-FLAG) plasmid (Dr Anton Bennett, Yale University) using Lipofectamine 2000 (Invitrogen). After 12 h, cells were lysed, clarified by centrifugation, and the supernatant (100 µg) was immunoprecipitated with 2 µg of anti-FLAG M2 using protein-G sepharose beads. Next, the beads were boiled in sample buffer, separated on SDS-PAGE, and processed by western blotting using MVP antibody.

#### **Immunofluorescence in Corticostriatal Cultures**

Rat embryos (E18-E19) were removed from euthanized pregnant dams via Caesarean section. The corticostriatal brain region was dissected and placed into ice-cold Hank's balanced salt solution (124 mM NaCl, 3 mM KCl, 10 mM HEPES). Tissue was trypsinized with 0.025% trypsin-EDTA at 37 °C, triturated, and resuspended in neurobasal medium with 5% fetal bovine serum (FBS) and B-27 supplement. Cells were plated on poly-D-lysine-coated 18 mm German glass coverslips at a density of 10 000/cm<sup>2</sup>, and incubated at 37 °C with 5%  $\text{CO}_2$ . After 24 h, the medium was replaced with FBS-free neurobasal medium.

Ten to 15-day-old neurons were fixed in 4% paraformaldehyde in 100 mM PBS, permeabilized with 0.2% Triton X-100, and blocked for 1 h with 10% normal goat serum (NGS) and 1% IgG-free BSA (Vector Laboratories, Burlingame, CA). Cultures were incubated overnight at 4 °C in anti-MVP, or anti-MVP and a second primary antibody against microtubule-associated protein 2 (MAP2), tubulin or tau, followed by 3 h in species-specific antibodies conjugated to Alexa Fluor 488 and/or Alexa Fluor 594 (see Table 1). Alternatively, cultures were labeled with anti-MVP, followed by 20 min in 1:100 fluorescein isothiocyanate-conjugated phalloidin (Sigma-Aldrich, St Louis, MO). Cells were mounted on slides with Vectashield hardmount (Vector) containing 4',6-diamidino-2-phenylindole (DAPI) to mark cell nuclei, and photographed under a Zeiss epifluorescent microscope equipped with ApoTome (Applied Scientific Instruments, Eugene, OR). Digital images were adjusted for brightness and contrast using Photoshop 9.0 image-editing software (Adobe Systems, San Jose, CA). Phalloidin images were pseudo-colored to keep the coloring of MVP consistent throughout.

#### **Neuronal Transfection and Immunofluorescence**

Cortical neuronal cultures were prepared from rat embryos as detailed above. Cultures were seeded at a density of 20 000 cells/cm<sup>2</sup>, and maintained in neurobasal medium with B27 supplement. After 10-12

days, they were transfected with 2 µg MVP-FLAG plasmid using Lipofectamine 2000, and incubated for 6 h at 37 °C in 5%  $\text{CO}_2$ . The transfection medium was changed and incubation was continued in fresh neurobasal medium for another 12 h. Cells were then double-labeled with anti-MVP and anti-FLAG M2, and visualized as described above.

#### **Light and Electron Microscopic Localization of MVP in Cerebral Cortex**

Three adult rhesus macaques and 6 adult Sprague-Dawley rats were used for the studies in cerebral cortex. All procedures were in accordance with the Yale IACUC. Primates were sacrificed as described in Paspalas and Goldman-Rakic (2004), with transcardial perfusion of 4% paraformaldehyde/0.05-0.1% glutaraldehyde in 100 mM PB. Brains were sectioned coronally at 60 µm, cryoprotected and stored at -80 °C. Rat brains were obtained identically but used immediately after perfusion. Sections of the monkey dorsolateral prefrontal cortex (PFC; Walker's area 46) as well as sections of the rat somatosensory cortex (S1) and the underlying hippocampus (area CA1) were processed for MVP-immunocytochemistry. To facilitate penetration of immunoreagents, all sections went through 3 freeze-thaw cycles in liquid nitrogen. Nonspecific reactivity was suppressed with a protein blocker as described above.

For light microscopy, sections were incubated for 36 h in anti-MVP in 50 mM Tris-buffered saline (TBS), plus 0.05% Triton X-100, 2% NGS, and 1% BSA, and for another 3 h in biotinylated F(ab')<sub>2</sub>. To probe the biotinylated fragments, we used avidin-biotin-peroxidase complexes (ABC Elite, Vector) for 1 h or, alternatively, anti-biotin F(ab')<sub>2</sub> conjugated to 1.4 nm gold cluster (nanogold) for 2 h. MVP was visualized with diaminobenzidine as a chromogen or with silver enhancement of nanogold after glutaraldehyde fixation (HQ Silver, Nanoprobes, Yaphank, NY; see Paspalas and Goldman-Rakic 2004, 2005).

For electron microscopy, sections were placed in anti-MVP for 48 h, followed by biotinylated F(ab')<sub>2</sub>, both diluted in TBS with 2% NGS, 0.1% acetylated BSA-C (Aurion, Wageningen, The Netherlands), and 0.01% Tween 20. Next we applied nanogold-anti-biotin F(ab')<sub>2</sub>, and used silver autometallography to enhance the nanogold (Paspalas and Goldman-Rakic 2005). This procedure produces multiparticle aggregates (see also below). An alternative approach was to directly complex MVP antibody with nanogold-Fab', followed by silver enhancement. Having a stoichiometry close to 1 (i.e., one particle per IgG fragment), the 2-layer procedure is expected to yield single immunoparticles (compare Fig. 7D-G).

A third procedure, modified from Paspalas and Goldman-Rakic (2004), was used to control for specificity. Anti-MVP was complexed with nanogold-Fab' but the signal was enhanced with gold (Gold-Enhance; Nanoprobes). Nanogold-Fab' was once again introduced and postfixed, and a second gold autometallographic reaction was used to enhance the newly introduced nanogold as well as the already present immunoparticles of the first series. Sequential enhancement of nanogold yields 2 nonoverlapping particle size-groups (e.g., Fig. 9; for technical account, see Paspalas and Goldman-Rakic 2004).

Omission of the bridging biotinylated antibodies abolished immunoreactivity. Similarly, peroxidase labeling was eliminated when blocking the biotinylated probes with avidin/biotin (Vector). To control for self-nucleation of the metallographic developer, nanogold was omitted, whereas the sections were routinely processed for silver or gold autometallography for 15 min at room temperature. In labeling MVP, we never exceeded 12 min at 4 °C. For primary antibody characterization, please see "Antibodies and Western Blotting."

Sections were processed for electron microscopy as previously described (Paspalas and Goldman-Rakic 2004, 2005). Layers II-IV and V-VI of PFC, and layers II-VI of S1 as well as all strata of CA1 were sampled for re-sectioning and analysis under a JEM1010 (Jeol, Tokyo, Japan) transmission electron microscope at 80-100 kV. MVP structures were digitally captured at 45 000-350 000× magnification (Gatan, Pleasanton, CA), and individual panels were adjusted for brightness and contrast. For profile identification, we adopted the criteria summarized in Peters et al. (1991).

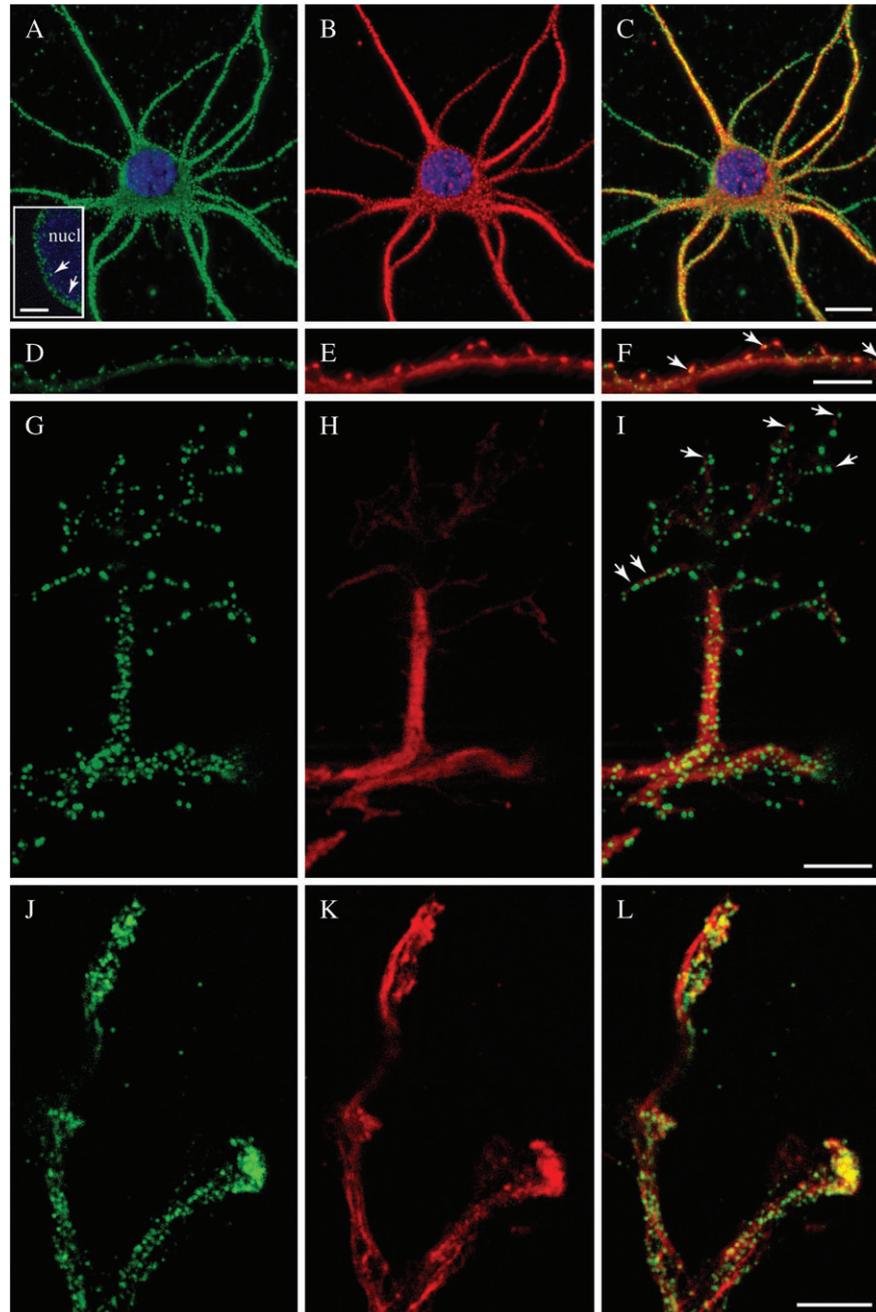
## Results

### *MVP in Primary Neuronal Culture*

#### *MVP Localizes to the Cytoskeleton and the Nucleus in Developing Neurons*

To better appreciate the expression pattern of MVP *in vitro*, we utilized an array of cytoskeletal specific markers. Dual immunofluorescence for MVP and the dendritic microtubule marker MAP2 revealed extensive colocalization in somata and

presumed dendritic processes (Fig. 2A-C). MVP puncta were numerous in the cytoplasm, and localized in small numbers within the nucleoplasm around the edge of the nucleus. To exclude image merging with the overlying or underlying labeled perikaryon, 0.25- $\mu$ m z-stack images were taken through the nucleus (Fig. 2A, inset). Control experiments with z-stack imaging did not yield nuclear labeling for the purely cytoplasmic proteins tau and tubulin (data not shown), which indicates that the fluorophores did not cross-react with the nucleoplasm. MVP was also expressed in fine varicose neurites,

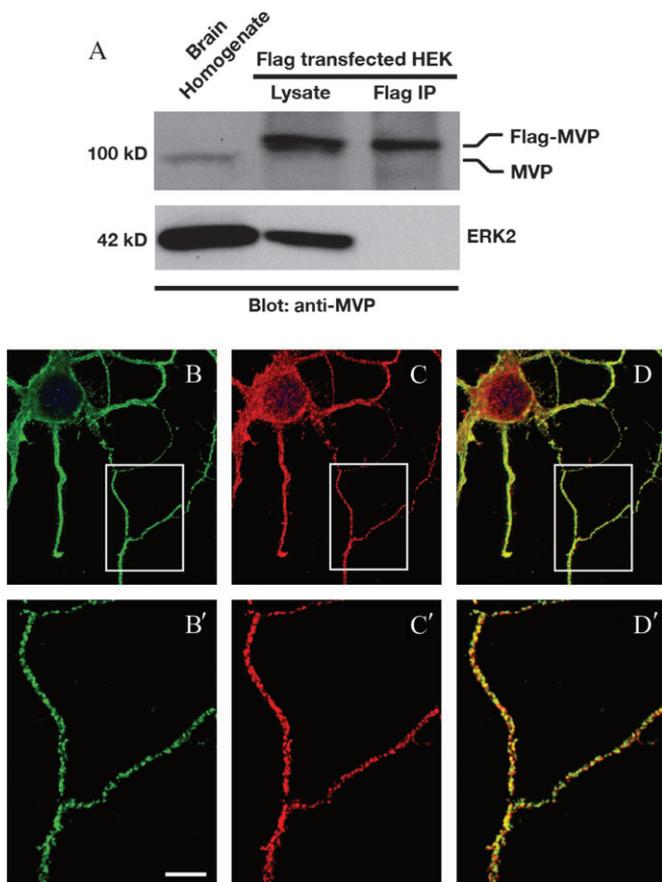


**Figure 2.** Cellular expression of MVP (green) in rat corticostriatal cultures. (A-C) MVP colocalization with MAP2 (red) in immature dendrites. z-stack imaging reveals a fraction of MVP (arrows in inset) in the peripheral nucleoplasm (nucl). (D-F) MVP is colocalized (arrows in F) with the axonal marker tau (red) in fine varicose neurites, presumably axons; in developing neurons, tau may also be expressed by immature dendrites. G through I depict an elaborate growth cone, visualized with tubulin (red). MVP puncta are present in the neuritic stem, and penetrate the filopodia of the growth cone (arrows in I). (J-L) To confirm localization of MVP in growth cones, we performed double labeling with phalloidin, a toxin that binds F-actin (red). Scale bars: (A-C) 4  $\mu$ m; (A) inset, 1  $\mu$ m; (D-I) 2  $\mu$ m; (J-L) 1  $\mu$ m.

involving both the varicosities and intervaricose segments. Figure 2D–F shows such MVP processes spiraling around a thicker stem, likely an immature dendrite, and coexpressing the axonal microtubule protein tau. In neuritic tips, MVP antibodies labeled growth cones, identified by colocalization with tubulin (Fig. 2G–I) and the actin-specific marker, phalloidin (Fig. 2J–L). MVP puncta filled the neuritic stem and extended well into the filopodia of the growth cones.

#### Exogenous MVP Localizes with Endogenous MVP

We further established the localization pattern of MVP by transfecting a FLAG-tagged MVP construct, and comparing its labeling with that of endogenous MVP. First we compared the mobility of FLAG-MVP expressed in HEK cells against endogenous MVP from cerebral cortex. Total cell lysate as well as FLAG IP samples from transfected HEK cells were immunoblotted with anti-MVP (Fig. 3A). Note the expected slight increase in mobility due to the 11 amino acid FLAG sequence. We next examined whether endogenous MVP is colocalized



**Figure 3.** Comparison of FLAG-tagged exogenous MVP to endogenous MVP. (A) Total homogenate from cerebral cortex was compared with total lysate from FLAG-MVP-transfected HEK 293T cells as well as with an anti-FLAG IP from transfected cells. Note that FLAG-MVP is expressed in the transfected cells, and the FLAG-immunoprecipitated sample is recognized by anti-MVP. The exogenous MVP band runs higher as compared with endogenous MVP due to the 11 amino acid FLAG tag. ERK2 was used to determine protein loading. (B–D) Cortical cultures were transfected with FLAG-MVP, and probed with anti-MVP (green) and anti-FLAG (red). (B'–D') The selected neurite is magnified to show extensive colocalization of transfected FLAG-MVP with endogenous MVP. Immunoreactivity to FLAG epitope was not detected in non-transfected cultures or with secondary antibody alone (data not shown). Scale bars: (B') 2  $\mu$ m, applies to (C') and (D').

with FLAG-MVP after transfection of cortical cultures. FLAG-MVP exhibited a similar cellular distribution and punctate appearance as endogenous MVP (Fig. 3B–D). In the enlarged images of the neurite shown in Figure 3B'–D', very few red puncta were noticed in the merged panel, suggesting that the majority of transfected MVP is colocalized with endogenous MVP.

Collectively, these findings indicate that neuronal MVP is expressed in the perikaryon and in association with the cytoskeleton in neurites, whereas a fraction of the protein is localized in the nucleus. The characteristic punctate labeling for MVP reflects the fact that virtually all cellular MVP is assembled into highly stable vault particles (reviewed in Introduction). It therefore should be clarified that MVP labeling coincides with vault particle labeling, although the vault structure per se is not readily visualized in transmission immunoelectron microscopy (see next sections).

#### MVP in Brain Neurons

We investigated the expression of MVP in the cerebral cortex of monkey (PFC) and rat (S1 and area CA1). Light microscopic findings are shown in the separate columns of Figure 4. The ultrastructural data were pooled in Figures 5 through 9, as the subcellular distribution did not differ between cortical areas and species. Figure 10 summarizes the association of MVP with mRNAs in both synaptic (LP1) and intracellular microsomal organelle (P3) fractions.

#### MVP is mainly Expressed in Pyramidal Neurons

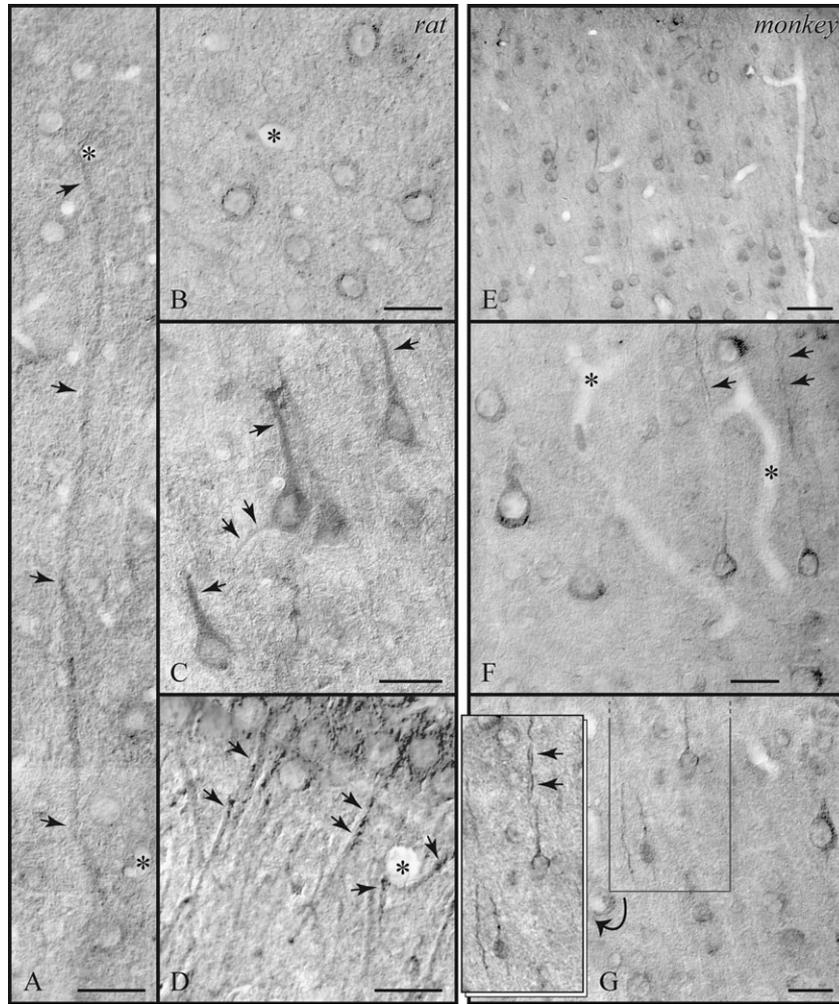
The S1 exhibited strong immunoreactivity, particularly in layers II/III and V, where MVP was associated with pyramidal perikarya and their processes. Labeled perikarya of presumably nonpyramidal neurons were also found but were clearly outnumbered by immunoreactive pyramids, and exhibited fewer faint labeled processes. Unlike pyramidal apical dendrites, which in certain cases could be traced across layers (Fig. 4A), basal dendrites were typically labeled close to the soma (Fig. 4B,C). Neurons detected with immunoperoxidase acquired a homogenous appearance, including faint nuclear labeling, whereas immunogold yielded a sharper image (compare Fig. 4B to C), where a narrow perinuclear rim and the processes as well as the neuropil contained punctate or thread-like reactive structures.

In hippocampal CA1, the neurons of stratum pyramidale and their dendrites within stratum radiatum were strongly immunoreactive. Again, immunogold revealed the punctate distribution of MVP in a perinuclear rim, and along the principal dendrites (Fig. 4D). Strata oriens and lacunosum moleculare (not shown) were diffusely labeled.

All layers of the PFC were MVP immunoreactive, with the highest expression in the pyramids of layers III and V (Fig. 4E). The latter exhibited puncta marking the perinuclear rim and distinctly labeling apical dendrites as found in S1. Note the thread-like reactive structures in the neuropil, consistent with labeling of individual stems of dendrites running towards the pia (Fig. 4F,G).

#### Somatic MVP is Expressed in the Cytoplasm and the Nucleoplasm

In perikarya, MVP was predominantly associated with the rough endoplasmic reticulum and free ribosomes, including polysomal clusters (Fig. 5). Top insets in Figure 5A capture



**Figure 4.** Cellular expression of MVP in rat (A–D) and monkey (E–G) cerebral cortex; immunogold labeling, except for A and C (immunoperoxidase). (A) Illustrates a layer V pyramid in S1 with an apical dendrite (arrows) spanning layer IV. In (B) and (C), immunoreactivity reveals pyramidal perikarya in layer III; arrows in (C) point to apical and basal dendrites. (D) In hippocampus proper (CA1), MVP is strongly expressed in the perikarya of stratum pyramidale and the stratum radiatum dendrites (arrows). A similar pattern is found in PFC, with labeling of pyramidal perikarya and apical dendrites (E). Note in (F) and (G), inset, the thread-like labeling of dendrites (arrows), corresponding to microtubule immunoreactivity (see Fig. 8). Asterisks mark vascular profiles. Scale bars: 10  $\mu\text{m}$ , except for E, 30  $\mu\text{m}$ .

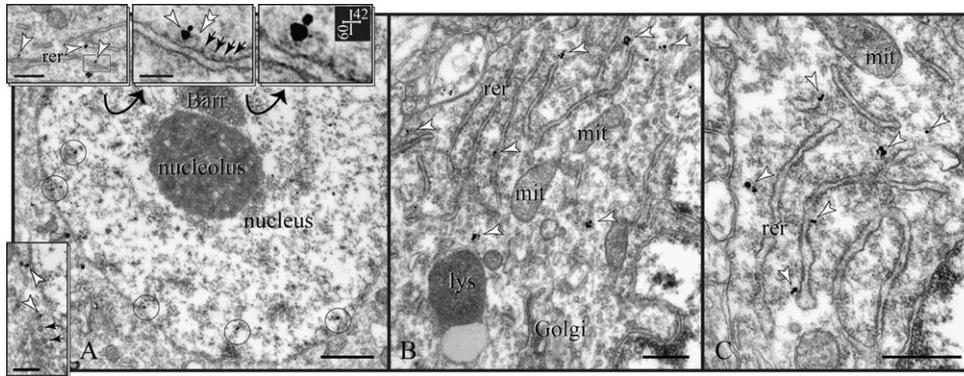
a rare immunoreactive structure on a reticular cistern that corresponds to the form and size of a vault or 3 times the size of the attached ribosomes. Localization on the plasmalemma (see Fig. 5B,C) was almost never detected, and likewise the Golgi, mitochondria and lysosomes, including multivesicular bodies, were not labeled. The nucleoplasm, which in Figure 5A occupies most of the field, was immunoreactive towards its outer rim. Nevertheless, nuclear labeling was a fraction of the total gold particles that labeled the soma.

To determine the contribution of nuclear immunoreactivity to the soma immunoreactivity, we examined 60 MVP-labeled pyramidal somata in PFC of 2 brains (30 somata/brain; 6 blocks/brain), and counted gold particles in the cytoplasm vs. the nucleoplasm. Care was taken to sample neurons sectioned through the center of the nucleus. The majority of immunoparticles (899 of 1071 particles;  $83.9 \pm 6.95\%$  SD) labeled the cytoplasm. If we extend the analysis to include the most proximal portions of tapering primary dendrites (diameter  $\geq 3 \mu\text{m}$ ), the nuclear contribution to the perisomatic region immunoreactivity diminishes (fewer than 2% of all gold label appears in the outer rim of the nucleus).

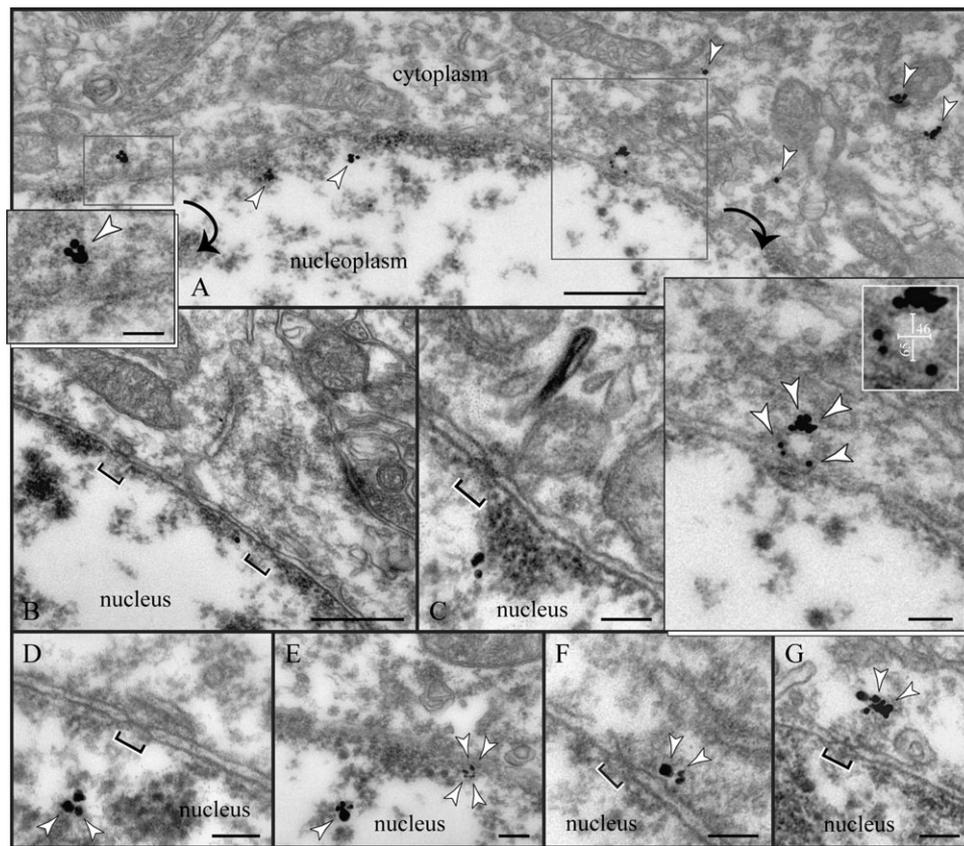
#### *MVP Associates with the Nuclear Envelope*

In the outer nuclear rim, label was invariably associated with masses of heterochromatin. One could argue that MVP was absorbed on the surface of heterochromatin prior to fixation, and that its nuclear localization is an antigen migration artifact. Nevertheless, the nucleolar complex was immunonegative, as was the heterochromatin towards the center of the nucleus (Fig. 5A). Note also that nuclear MVP was detected in cultured neurons with a very different fixation protocol (Fig. 2A). More importantly, the cytoplasmic and the nucleoplasmic face of the nuclear envelope exhibited immunoreactivity in association to nucleopores.

On the cytoplasmic aspect of the envelope, we labeled spherical structures identical to those found on reticular endomembranes (compare Fig. 6A to Fig. 5A). Their size and form is reminiscent of the vaults identified on the surface of isolated cell nuclei by Chugani et al. (1993). Note, however, that such perinuclear structures could only be discerned when multiple immunoparticles decorated their surface, as their core was not electron opaque. In favorable section planes (e.g., Fig. 6B,C), an analogous structure was found to be “plugging”



**Figure 5.** MVP expression in neuronal somata. (A–C) Immunogold (arrowheads) is associated with the rough endoplasmic reticulum (rer) and free ribosomes. The correlated insets in (A) illustrate a vacuolar structure (double arrowhead) on a *rer* cistern (arrows point to attached ribosomes). Its form, size and MVP immunoreactivity are reminiscent of a vault particle. The plasmalemma, mitochondria, lysosomes and Golgi complexes are not immunoreactive, with the exception of smooth vesicles on the *trans*-Golgi (lower inset in A; arrows point to coated vesicles). Nucleoplasmic labeling (see also Fig. 6) can be observed within circles in (A); the nucleolus and a Barr body are immunonegative. 1ys, lysosome; mit, mitochondrion. Scale bars: (A) 800 nm; (A) left insets, (B, C) 400 nm; (A) middle inset, 100 nm.



**Figure 6.** MVP association with the nuclear envelope. (A) Two vesicular structures (enlarged in insets) onto the cytoplasmic aspect of the envelope display MVP immunoreactivity (arrowheads); a nucleopore cannot be discerned at this plane. The labeled structure in right inset is identified as a vault particle based on descriptions in other cell systems (see Discussion). (B and C) Illustrate similar spherical structures “plugging” nucleopores. However, they are not labeled and may correspond to fibrils attached to the cytoplasmic ring of the pore. (D–G) Depict MVP-particles (arrowheads) next to the nuclear envelope and in association with nucleopores (brackets) on either side. Scale bars: (A), (B), 400 nm; (A) insets, (C–G) 100 nm.

nucleopores, an image that likely corresponds to the outer ring fibrils of the pore complex. These “plugs” were not immunoreactive, and therefore it is not known whether they may represent vaults that have escaped detection against MVP (see Discussion). More typically, labeling was found in the vicinity of

nucleopores on either side of the envelope, or was embedded in the envelope in oblique section planes (Fig. 6D–G).

To test if MVP is indeed enriched near nucleopores, we counted single immunoparticles (produced with the two-layer procedure) within an arbitrary 200-nm-wide rim adjacent to

the nuclear envelope on either side. Particles were categorized as pore-associated ( $\leq 200$  nm from the center of a pore) and as pore-nonassociated ( $>200$  nm) in 50 pyramids of PFC (25 neurons/brain, 5 blocks/brain). Almost 27% (44 of 164) of immunoparticles within the cytoplasmic rim were pore-associated. In the nuclear rim, pore-associated particles made up 12% (28 of 230). The relative enrichment of MVP immunoreactivity near pores on the cytoplasmic side of the envelope likely reflects a strong predilection of MVP for this side of nuclear pores, in spite of the much higher particle density in the nucleoplasmic rim.

#### *MVP is Predominantly Expressed in Dendrites*

Out of 2250 labeled cellular profiles pooled from all layers of PFC (2 brains, 8 blocks/brain), 1302 (58%) were dendritic profiles, including large stems, fine branches and spines. Figure 7 shows examples of MVP-labeled dendrites in PFC (A–C) and S1 (D, E). Similar to the soma, immunoparticles in larger dendrites were rarely localized on plasma membranes. Rather, they were found intracellularly in association to microtubules and vesicular endomembranes of the endosomal line. An immunoreactive endosome-clathrin vesicle fusion can be seen in Figure 7B (for a depiction of the endocytotic profiles, see Paspalas et al. 2006). Of course the possibility of MVP also being localized on the smooth endoplasmic reticulum (ser) cannot be excluded by using ultrastructural criteria alone and without employing organelle-specific markers. Nevertheless, ser elements in the soma as well as spine apparatus were not immunoreactive, whereas MVP was previously reported to participate in endosomal sequestration (Herlevsen et al. 2007).

In smaller branches and in spines, label could be found both intracellularly and next to the plasmalemma. Those seemingly

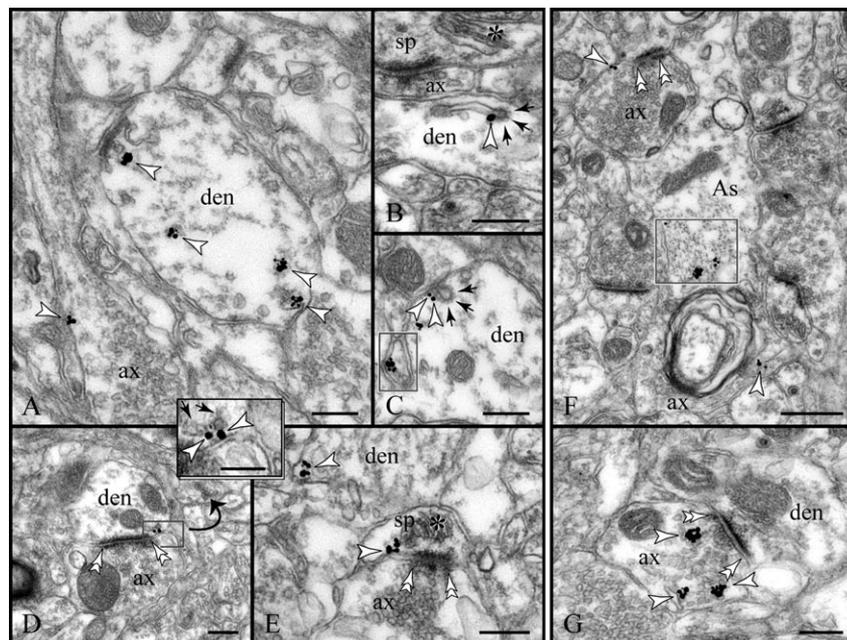
membrane-associated immunoparticles were often still attached to minute intracellular profiles representing cross-sectioned microtubules or endoplasmic vesicles (Fig. 7D). Note that in postsynaptic dendrites and spines, MVP was never localized within synaptic specializations (Fig. 7D,E).

MVP expression in axons resembled the labeling pattern in dendrites but axons accounted for 21% of MVP profiles in PFC neuropil (376 out of 2250 profiles). However, prevalence in axons is likely underestimated, as many of the nonidentified profiles (8%; 183 of 2250) were most probably nonmyelinated intervaricose segments of axons (e.g., Fig. 7C, frame). In varicosities, the immunoparticles intermingled with the synaptic vesicles and rarely appeared next to the axolemma (Fig. 7E,G). Similar to postsynaptic dendrites, MVP in pre-synaptic axons was not associated with synaptic specializations (Fig. 7G).

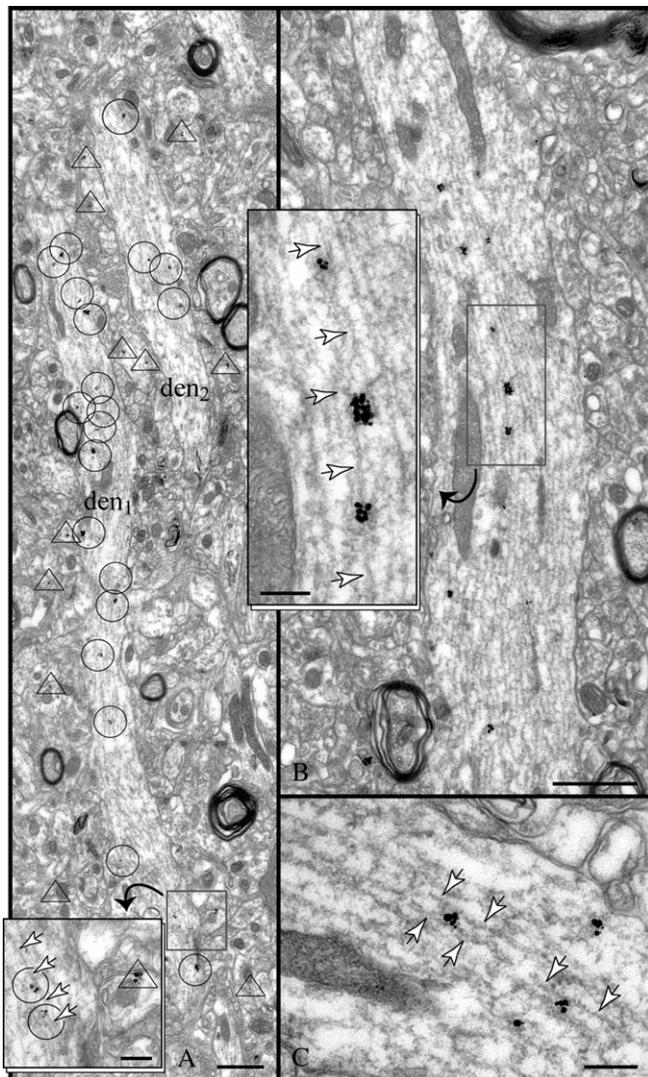
We detected low levels of MVP immunoreactivity in astrocytic profiles, consistent with the fact that cortical astrocytes were not visualized with light microscopy but may partially account for the punctate labeling in the neuropil. Particles labeled major astrocytic processes (Fig. 7F), including low expression in perivascular glia. Their parent perikarya displayed sparse reactivity in various endomembranes (data not shown).

#### *MVP Associates with the Microtubules in Dendrites and Axons*

The enrichment of MVP in the dendritic compartment and its association with the microtubules is best perceived in longitudinal sections of dendrites (Fig. 8A). The immunoparticles adorned microtubules in large dendritic stems, such that a single or numerous particles (or particle clusters) would label



**Figure 7.** MVP expression (arrowheads) in the neuropil. (A–E) Labeling in dendrites is associated with microtubules (see also Fig. 8) and vesicular endomembranes, including profiles as the one shown in (B). This early endosome has a coated tip (arrows) resulting from fusion with a clathrin-coated vesicle; compare to (C) showing one such immunoreactive vesicle (arrows). In fine dendrites (D) and in spines (E), particles also appear on plasma membranes, often still associated to microtubules (arrows in D, inset), but not within synaptic specializations (double arrowheads). (F, G) MVP was localized on both varicose and intervaricose (see also frame in C) segments of axons. Synaptic specializations (double arrowheads) were not labeled. An astrocytic process (As) in (F) displays MVP among the gliofilaments (frame). Asterisks mark spine apparatus. ax, axon; den, dendrite; sp, spine. Scale bars: (A–E), (G), 200 nm; (D) inset, 100 nm; F, 400 nm.



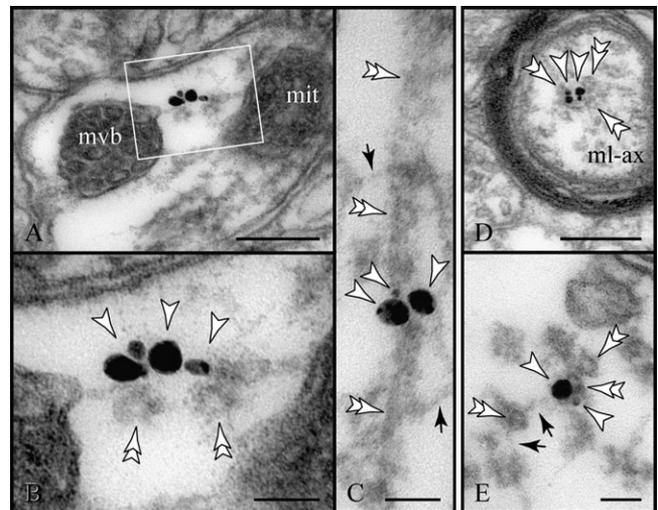
**Figure 8.** Association of MVP with dendritic microtubules (arrows). (A) Low magnification montage shows enrichment of the MVP signal in 2 longitudinally sectioned dendrites (circles in den1, den2); labeling in the remaining field is shown in triangles. The inset illustrates a detail of one labeled dendrite and adjacent neuropil. (B, C) MVP is specifically localized along individual microtubules. Note that a single microtubule could be labeled with numerous particles along several hundreds of nanometers. Scale bars: (A, B) 800 nm; insets, (C) 200 nm.

an individual microtubule along its path (Fig. 8B,C). It is most likely that immunoreactive microtubules within a dendrite are the electron microscopic equivalent of the fine filamentous labeling observed in the 60  $\mu\text{m}$ -thick sections (see Fig. 4F,G).

High-power electron microscopy (100 kV accelerating voltage) confirmed the explicit association of MVP with cross-sectioned and longitudinally sectioned microtubules in dendrites (Fig. 9A-C), and likewise in axons, where the particles tagged loose microtubule bundles in myelinated and in nonmyelinated intervaricose segments (Fig. 9D,E).

#### MVP Associates with mRNAs in Neurons

Given the specific association of MVP with the neuronal cytoskeleton (this work), and the speculative function of vaults in intracellular transport (see Suprenant 2002), we next sought to determine whether MVP might associate with mRNAs in neurons. To this end, we immunoprecipitated MVP from

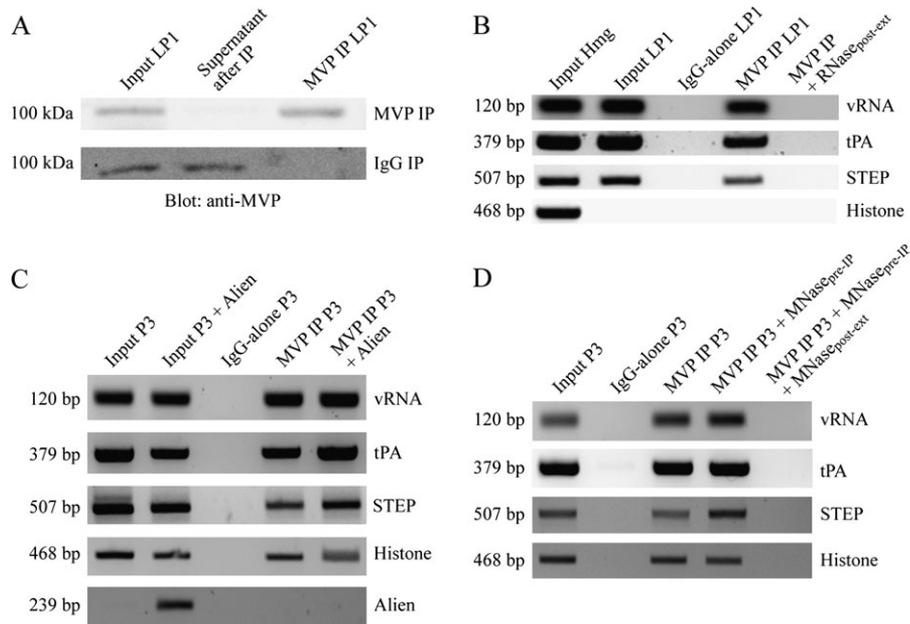


**Figure 9.** High-power micrographs capturing the direct link of MVP (arrowheads) to single microtubules (double arrowheads). Immunoparticles distinctly tag the microtubules in cross-sectioned (A, B) as well as in longitudinally sectioned (C) dendrites, and likewise label cross-sectioned microtubules in myelinated (D) and nonmyelinated preterminal segments (E) of axons. Arrows point to microfilament side arms bridging the dendritic and axonal microtubules. The immunocomplexes used here have a presumed maximum linear dimension of about 15 nm, which may translate into a distance of the gold core from the epitope. To demonstrate specificity, the micrographs illustrate material labeled with sequential enhancement of nanogold (see Methods). mit, mitochondrion; ml-ax, myelinated axon; mivb, multivesicular body. Scale bars: (A, D) 200 nm; (B, C, E) 50 nm.

synaptosomal-enriched fractions (LP1) (Fig. 10A) and then performed RT-PCR on immunoprecipitates (Fig. 10B). First, immunoblots were obtained to establish that the predicted 100-kDa MVP band was indeed detected in immunoprecipitated samples (Fig. 10A, right lane). We ran the supernatant after IP and found that little, if any, MVP remained (Fig. 10A, middle lane), indicative that the majority had been pulled down. In the IgG-alone control, MVP was not pulled down but remained in the supernatant (Fig. 10A, right and middle lanes, respectively).

We then conducted RT-PCR on immunoprecipitated MVP from LP1 fractions (Fig. 10B). First, we examined vRNA because its association with MVP via TEP1 has been previously established (Kickhoefer et al. 1993). We detected a fragment of the expected size (Fig. 10B, top row). Sequencing identified this fragment as vRNA (data not shown). We next selected probes against mRNAs, namely tPA and STEP, known to be translated after synaptic stimulation. Again, RT-PCR produced fragments of the expected sizes (Fig. 10B, middle rows). Sequencing identified the fragments as tPA and STEP (data not shown). All fragments disappeared with RNase treatment after RNA extraction (RNase<sub>post-ext</sub>) and prior to RT-PCR. The IgG-control lanes also produced no detectable RT-PCR products. Histone mRNA is not expressed in LP1 fractions (Fig. 10B, bottom row, second lane). In agreement, we found that histone was not associated with MVP in this fraction (Fig. 10B). To ensure copies of the messages were originally present in the starting material, RT-PCR for each of the 4 samples was also conducted from brain homogenates (Input Hmg) (Fig. 10B, first lane).

We found that MVP also associates with vRNA, tPA, and STEP mRNAs when immunoprecipitated from intracellular microsomal



**Figure 10.** MVP association with mRNAs. (A) Synaptosomal fractions (LP1) were immunoprecipitated with anti-MVP or anti-IgG, and probed with MVP antibody. MVP is selectively immunoprecipitated by anti-MVP but not anti-IgG. (B) RT-PCR of different mRNAs, with and without RNase treatment after RNA extraction (RNase<sub>post-ext</sub>). RT-PCR was performed from inputs of total brain homogenate, inputs of LP1 fractions, IgG-alone IP, LP1 IP, and RNase-treated LP1 IP post-RNA extraction (lanes 1 through 5). vRNA, tPA and STEP mRNAs are found to associate with the MVP IP while being absent from IgG-control IP. In addition, histone H1 mRNA was not detected by RT-PCR in LP1 fractions. (C) vRNA, tPA, and STEP mRNAs are also associated with MVP immunoprecipitated from intracellular microsomal organelle (P3) fractions. Histone mRNA is abundantly expressed in P3 fractions, and MVP associates with histone mRNA in that fraction. MVP does not associate with Alien RNA added to P3 inputs prior to IP. (D) mRNAs remain associated with MVP even when P3 inputs are treated with MNase prior to IP (MNase<sub>pre-IP</sub>). MNase treatment after RNA extraction (MNase<sub>post-ext</sub>) completely eliminated detection of RT-PCR products (last lane).

organelle (P3) fractions (Fig. 10C). Histone mRNA was associated with MVP immunoprecipitates obtained from P3 fractions, a fraction where histone mRNA is abundantly expressed (Fig. 10C). These findings suggest that MVP associates with mRNAs only in fractions where they are abundantly localized and raise the possibility that vaults containing selectively localized mRNAs are targeted to different subcellular compartments.

To determine if MVP immunoprecipitates associate non-specifically with RNAs, an Alien RNA was added to P3 inputs prior to IP (Fig. 10C, last row). We found that Alien RNA does not associate with MVP immunoprecipitates, suggesting that MVP does not bind nonspecifically to RNAs. Moreover, association of vRNA, tPA, STEP, and histone mRNAs with MVP immunoprecipitates was preserved when P3 inputs were treated with micrococcal nuclease (MNase) prior to IP (MNase<sub>pre-IP</sub>) (Fig. 10D, fourth lane). Confirmation of effective MNase activity was obtained by showing that MNase treatment of extracted RNA (MNase<sub>post-ext</sub>) completely eliminated the PCR products (Fig. 10D, last lane). These findings suggest that mRNAs are protected from MNase while associated inside the vault structure, but are degraded by MNase once they are extracted.

## Discussion

MVP has been extensively studied in various cell expression systems because of its possible contribution to multidrug resistance in cancer (Scheffer et al. 2000; Mossink et al. 2003). Brain studies have mainly been focused on epilepsy and malignancies (Aronica et al. 2003, 2004; Sisodiya et al. 2003; van Vliet et al. 2004), and one study introduced MVP as

a marker for developing microglia (Chugani et al. 1991). Our results demonstrate that MVP is abundantly expressed in primate and rodent cerebrocortical neurons, and in cultured corticostriatal neurons, with remarkably similar expression patterns. We report that neuronal MVP is localized along the nucleus–neurite axis “targeting” the microtubules, and intracellular organelles, including free and attached ribosomes. Moreover, we demonstrate that neuronal MVP is associated with several mRNAs that are translated in response to synaptic activity. Given that vaults have been previously postulated as transport-associated modules (see Suprenant 2002), the present findings suggest a possible role for MVP in mRNA transport in neurons.

Our data show that MVP is highly expressed in developing neurons. The protein is distributed in the soma, including the nucleus, and the processes up to the level of filopodia at the tip of neurites. This is consistent with previous studies reporting MVP in axons and nerve terminals of electric eels (Herrmann et al. 1996), and in the neurite tips of PC12 cells (Slesina et al. 2006). Thus it appears that expression along cell processes is a conserved feature of MVP in various neuronal types and across species. In immature dendrites and axons, MVP is localized to the cytoskeleton, suggesting that it may be involved in active cytoplasmic transport in developing neurons. In fact, MVP has been reported to undergo fast axonal transport in electric rays (Herrmann et al. 1998; Li et al. 1999). Furthermore, MVP transport is microtubule-dependent in non-neuronal cells, with the cap of the vault attaching to the microtubule itself (Eichenmüller et al. 2003; van Zon et al. 2006).

Our ultrastructural studies in primate and rodent cerebral cortex reveal an association of MVP with microtubules in

axons, and particularly in dendrites where several immunoparticles were often seen labeling a single microtubule or microtubule bundle in a 50-nm-thick section of a stem. These results point to a “targeted” association with individual microtubules rather than a general, nonselective preference of MVP for the cytoskeleton. In the perikaryon, MVP is localized on free and attached ribosomes, and in vault-like structures on the rough endoplasmic reticulum. The association of MVP with ribosomes and the endoplasmic reticulum is the aim of future work, because it remains unknown whether the presence of MVP in the translation machinery represents newly synthesized protein destined for assembly into vaults at a later stage. Another hypothesis we favor (see below) is that MVP shuttles mRNA cargo to sites of translation.

Similarly, a fraction of MVP is found in the nucleus. Nuclear MVP has previously been documented in sea urchin cells (Hamill and Suprenant 1997), and in human U373 cells (Slesina et al. 2005) where it reportedly forms 5% of the total MVP (2% of the perisomatic MVP in this work), suggesting that the protein may shuttle between the cytoplasm and the nucleoplasm. It is worth noting that Slesina et al. (2005) employed cryo-immunoelectron microscopy that is devoid of antigen migration artifacts (see our comments in Results). Another important finding from our work is that MVP-immunoreactive vault-like particles are attached to the cytoplasmic aspect of the nuclear envelope. Early work has elegantly localized vaults on isolated nuclei of liver cells (Chugani et al. 1993), and those structures are of the exact same size and form as those we have identified in brain neurons (Fig. 6A). We did not find evidence that MVP-reactive vaults coincide with the central plug/transporter of the nuclear pore (see discussion in Chugani et al. 1993), as nucleopores were not labeled in our material (Fig. 6B). Rather, immunoparticles were clustered near the pores as commonly found with cryoelectron microscopy (Slesina et al. 2005). One possibility is that MVP present in nuclear pore complexes retains a structure that is not accessible by antibodies in pre-embedding immunocytochemistry of brain tissue. In support of this hypothesis, Chugani et al. (1993) also failed to detect immunoreactive vaults in the nuclear pore complex *in situ*, yet they report that one third of vaults on the nuclear envelope correspond to pore complexes in isolated fixed nuclei. It remains incompletely understood whether vaults constitute an integral component of the nuclear pore complex in neurons or if they are only intermittently associated when crossing nucleopores (see Slesina et al. 2005) to meet physiological demands. One example of intermittent association is that vaults associate with the estrogen receptor in the nucleus of MCF-7 breast cancer cells, and this association increases in response to estradiol stimulation (Abbondanza et al. 1998).

We also report that MVP associates with STEP and tPA mRNAs. These are messages that are translated in synaptosomal fractions after synaptic stimulation. STEP is a brain-specific tyrosine phosphatase implicated in the regulation of synaptic plasticity (Paul et al. 2003, 2007; Snyder et al. 2005). STEP is translated in corticostriatal slices and cell cultures after  $\beta$ -adrenergic receptor stimulation (Hu et al. 2007) as well as in the lateral amygdala within minutes after fear conditioning training (Paul et al. 2007). tPA has also been implicated in the regulation of synaptic plasticity, as its inhibition impairs both late-phase LTP and long-term memory consolidation (Seeds et al. 2003; Pang and Lu 2004). tPA mRNA binds to cytoplasmic

polyadenylation element binding protein-1, an interaction that blocks the translation of tPA. Moreover, tPA is rapidly translated after glutamate stimulation (Shin et al. 2004).

An important consideration of this study was to establish that mRNAs specifically associate to MVP in our preparation. One way we addressed this issue was by the addition of Alien RNA to input material prior to the IP and RT-PCR. Alien RNA did not associate with MVP or IgG in these experiments. We also added MNase prior to MVP IP. We reasoned that the vault structure might protect its “mRNA cargo” from MNase treatment and that any nonspecific mRNAs associated with the external side of the closed vault particle would be digested. We found that MVP-associated mRNAs were protected from MNase treatment and could be extracted after inactivation of the MNase. Taken together, these results strongly suggest that the STEP and tPA mRNAs are selectively associated within the MVP complex. Further experiments are now underway to determine whether additional mRNAs also associate with MVP and whether MVP associates with mRNAs in other tissues.

Another noteworthy finding from this work is that histone mRNA associates with MVP in microsomal organelle fractions but not in synaptosomal fractions. This raises the intriguing possibility that MVP is itself targeted to specific subcellular compartments, and this targeting likely depends on the mRNA cargo that it carries. Future work is required to more rigorously address this question.

In line with the postulated function of vaults as nucleocytoplasmic shuttles (Suprenant 2002), we propose that MVP could similarly be involved in transport mechanisms in developing and adult neurons. The question naturally arises as to what the vault’s cargo might be. We have presented evidence that MVP associates with mRNAs that are translated at dendrites. Although we have not directly demonstrated that neuronal MVP is involved in mRNA transport to sites of local synthesis, our data point to this hypothesis and set the ground for future work on vault biology.

## Funding

National Association of Research on Schizophrenia and Depression (NARSAD); and by the National Institutes of Health grants (MH01527 and MH52711) to P.J.L.; Brown-Coxe Fellowship supported D.V.V.; and NIMH (5T32MH18258) Training Program in Childhood Neuropsychiatric Disorders supported S.M.G.-G.

## Notes

We are grateful to Dr Anton Bennett for donating the FLAG-MVP construct and the GST-fusion protein. We are appreciative of Dr Sandra Wollin for technical advice on the micrococcal nuclease experiments. We also thank Dr Janice Naegele for comments on the manuscript.

*Conflict of Interest:* None declared.

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