

MAP1B is a cross-linker of several proteins during axonal development.

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MAP1B, a microtubule-associated protein, is a major cytoskeletal protein during brain development. Its major function is to stabilize microtubules. It was shown that this protein is differentially phosphorylated during development and depending on its phosphorylation state it may interact with actin. With 320kDa it is also one of the largest brain MAPs, and one could speculate that the different parts of the molecule must have different functions and interact with other proteins. Here we have identified the kind of proteins that may interact with MAP1B during postnatal brain development.

We have produced recombinant MAP1B fragments and raised polyclonal antibodies against these proteins. Immunoglobulins (Ig) were purified and linked to CNBr-sepharose 4B. This anti-MAP1B-sepharose was used to precipitate MAP1B from mouse brain tissues of different postnatal ages (i.e. newborn, postnatal day 10 and adult). Analysis of co-precipitated proteins was performed by 1D and 2D gel electrophoresis. High molecular weight proteins were clearly identified in 1D gels with higher acrylamide concentration, while 2D gels were more advantageous in the identification of proteins between 100kDa to 10kDa, since various Ig proteins also located in this molecular weight range and masked other proteins (compare Figures 1 and 2).

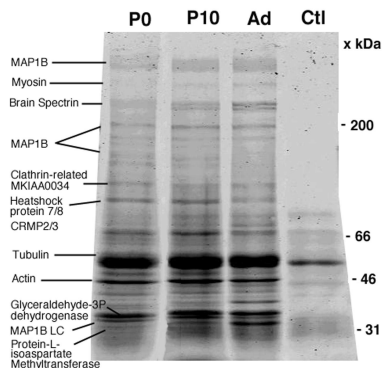
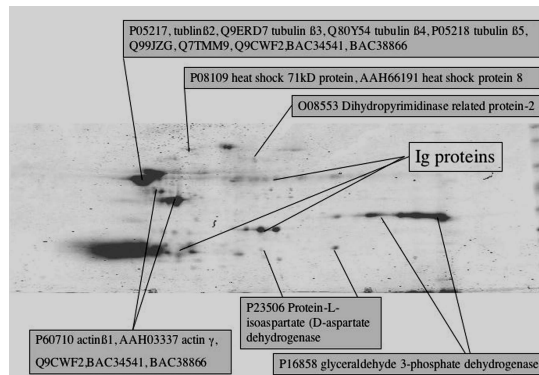


Fig. 1. Co-immunoprecipitation of MAP1B from postnatal day P0, P10 and adult (Ad) mouse brain tissues. Individual proteins were identified by MALDI-TOF. Ctl is the sample of Ig used for immunoprecipitation. This figure demonstrates differences in the association of MAP1B with other proteins during postnatal brain development.

Two to five mg anti-MAP1B immunoglobulins were bound to 1 gramme of CNBr-Sepharose 4B (according to the manufacturer's protocol). All steps were performed on ice. Eighty mg brain tissue was homogenized in two times the volume (W/V) of IP-buffer (20Mm TRIS pH 8.3, 0.8 M NaCl, 5mM MgCl₂, 1mM EGTA and protease inhibitor cocktail (Boehringer)). The same amount of IP-Buffer with 0.5% Triton X-100 was added during homogenization. The homogenate is spun for 45 min. at 100'000 x g and 4°. The supernatant was collected and incubated with 180µg anti-MAP1B Ig-sepharose for 2 hours at 4° under agitation. The samples were precipitated by centrifugation in an Eppendorf centrifuge at 1000 rpm. Pellets were washed in PBS buffer with 0.5 % Triton X-100, spun again, washed with PBS and 0.25% Triton X-100 and finally twice with 10mM Tris pH 7.5. Precipitated proteins were eluted in 30 µl electrophoresis buffer and loaded onto electrophoresis gels. Proteins were separated by 1D and 2D electrophoresis and stained by Coomassie blue. Several proteins co-precipitated with MAP1B. They were cut out of the gel and sent for MALDI-TOF identification by the local Protein Analysis Facility (PAF) in Epalinges. Not surprisingly, the full length MAP1B, MAP1B light chain and some degradation products were immunoprecipitated. It is worth to distinguish between proteins of major and minor stoichiometry in the precipitate. Major proteins were several tubulin and actin isoforms and glyceraldehydes 3-P-dehydrogenase (GPDH). A variety of proteins were of minor stoichiometry and possibly precipitated via a tubulin or actin interaction.

Fig. 2 Co-immunoprecipitation of MAP1B from adult mouse brain tissue. Individual proteins were separated by 2D electrophoresis and stained with Coomassie blue. The Ig proteins were also present in a control gel. The additional proteins, which co-precipitated with MAP1B were cut, digested and analyzed by mass spectrometry.



A literature research confirms that most of these proteins have a link to the cytoskeleton, development or regeneration, and our approach seems a valuable

tool to identify the interactome of MAP1B. Of particular interest are: **Tubulins**, where MAP1B stimulates microtubule assembly during development [1]. **Actin** is binding to MAP1B, depending on the state of phosphorylation of MAP1B [2]. **Myosin** is a motor protein, with an ATP binding site and essential for actin motility. **Spectrins (or fodrin)** form a membrane skeleton and are actin-binding proteins [3](Riederer et al. 1986b). **MKIAA0034** is a clathrin-related protein, MAP1B may be involved in endosome turnover or transport and synaptic vesicle recycling. **Heatshock proteins** HS7C, HSPA8, are found with clathrin and actin in slow component b fraction of axonal transport - relation to hypothyroidism. **Dihydropyrimidinase related protein-2**, DPY2, ULIP2, CRMP2 (collapsin response mediator protein 2), is involved in development and axonal guidance, regeneration and also thought to induce axon elongation by promoting microtubule assembly [4]. However, it seems to immunoprecipitate indirectly with MAP1B. **Protein-L-isoaspartate methyltransferase**, PIMT, is involved in repair of damaged tubulin in epileptic hippocampus and the β -tubulin is the major substrate [5]. **GPDH** is essential in the second phase of glycolysis, the cytoplasmic homotetramer is essential in the redox system with NAD^+ , and transports in axons together with actin [6](Yuan et al., 1999). The presence of most proteins in the precipitates was confirmed by Western blots and specific antibodies. *In conclusion*, many proteins seem linked to MAP1B. A developmental study on changes in 2D gels will be very interesting. MAP1B may act as a linker protein between microtubules and microfilaments, and may be involved in the set-up of the cytoskeleton in the growth cone, in synapses or near the cortical plasma membrane. This work was supported by the FNRS grant 31-067201.01.

References

- [1] Riederer, B, Cohen R, Matus A. MAP5: A novel brain microtubule-associated protein under strong developmental regulation. *J. Neurocytol*; 1986 15; 763-775.
- [2] Pedrotti B, Islam K. Dephosphorylated but not phosphorylated microtubule-associated protein MAP1B binds to microfilaments. *FEBS Lett* 1996 ; 388: 131-133.
- [3] Riederer BM, Zagon IS, Goodman SR. Brain spectrin(240/235) and brain spectrin(240/235E): Two distinct spectrin subtypes with different location within mammalian neural cells. *J. Cell Biol* 1986; 102: 2088-2097.
- [4] Fukata Y, Itoh T, Kimura T, et al. CRMP-2 binds tubulin heterodimers to promote microtubule assembly: a possible mechanism for CRMP-2-mediated axonal growth. *Nat. Cell Biol* 2002 ; 4: 583-591.
- [5] Lanthier J, Bouthilier A, Lapointe M, et al. Down-regulation of protein L-isoaspartyl methyltransferase in human epileptic hippocampus contributes to generation of damaged tubulin. *J Neurochem* 2002 ; 83: 581-591.
- [6] Yuan A, Mills RG, Bamberg JR., Bray JJ. Cotransport of glyceraldehydes-3-phosphate dehydrogenase and actin in axons of chicken motoneurons. *Cell Mol Neurobiol* 1999 ; 19: 733-744.