PHOSPHORYLATED MICROTUBULE ASSOCIATED PROTEIN (MAP1B-PHOS) IN THE RETINA DURING REGENERATION OF THE FISH OPTIC NERVE

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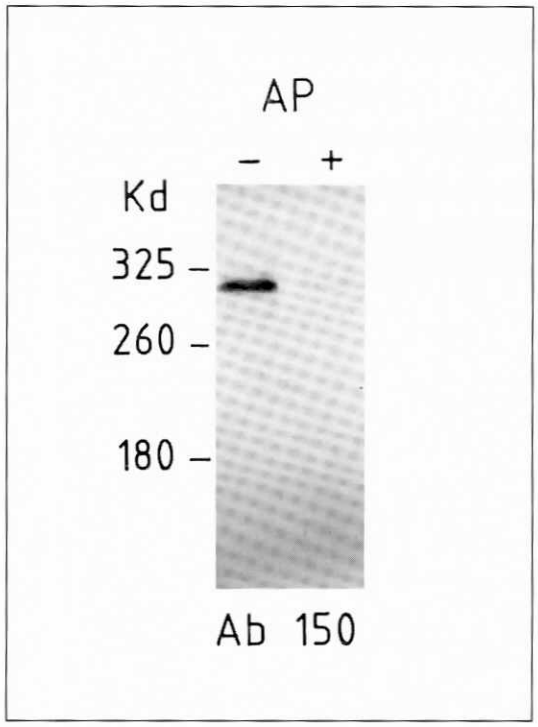
The fish visual system retains a considerable level of plasticity. Fish optic axons can regenerate after optic nerve damage, re-establishing their appropiate pattern of synaptic connections with the neurons of the optic tectum (Sperry, 1948) heading to the recovery of visual function. The characteristics of the regenerating fish retina ganglion cells have been extensively studied and one of the relevant changes produced in the regenerating cells is the rapid increase in the supply of virtually all the major clases of axonally transported proteins, including cytoskeletal elements. On the other hand, microtubule associated proteins (MAPs) are the principal components which confer stability to microtubule (Matus, 1988). It is likely that MAPs play an important role in the regulation and organization of the neural cytoskeleton during neurite outgrowth, plasticity and regeneration. The MAPs are a heterogeneus group of proteins involved in microtubule assembly regulation , cytoskeletal organization and organelle transport. MAP1B is one of the MAPs that has been characterised in its expression during brain development in mamals (Rieder and Matus, 1985) and regeneration (Fawcett et al., 1994), indicating that the levels of this protein are relatively high early in development and decrease in the adult. There is also evidence that MAP1B can be modified by two modes of phosphorylation, mode 1 (catalyzed by a proline dependent protein kinase) and mode 2 (catalyzed by casein kinase 2) (Ulloa et al., 1993).



Since the retina of the teleost grows continually throughout life and the optic nerve can regenerate after lesion, we have used the tench (Tinca tinca, L), a teleost fish as an animal model to study the expression of MAP1B-Phos during growth and regeneration of retina ganglion axons. MAP1B (mode1) specifically recognized by the monoclonal antibody 150 (mAB 150) used in the present study was located in the distal growing segment of developing axons.

Thus, we have performed Western blot and immuno-histochemistry analysis using mAB 150 to evaluate the expression of MAP1B-Phos in the retina of fish at different survival times after optic nerve crush. The thick axons of the horizontal cells H1 and of a few ganglion cells and their axons showed immunoreactivity to MAP1B-Phos antibody in control animals. Immunoreactivity was increased after the crush, mainly in the retina ganglion cell bodies where there was a clear increaseinf immunoreactivity. However, the pattern of immunoreactivity within the horizontal cells does not change after different periods of time post-lesion.

In a previous report (Vecino et al., 1996) we have shown that within the control optic nerves, only the developing growing axons were immunoreactive. After optic nerve crush, all axons regenerate and all are immunoreactive to mAb 150. Two hundred days postcrush, the optic nerve recovered its nomal organization except that, most of the axons were still labelled. Within the control optic tectum very few immunoreactive terminals were seen, but from 60 to 90 days after lesion, there was an increase in the number of labelled terminals found in the contralateral stratum opticum. These data show that MAP1B-Phos is present in the growing and regenerating axons of the fish retinal ganglion cells, suggesting a role for this protein in axon outgrouth in the central nervous system.

Figure1. Characterization of mAb 150 which recognizes a phosphorylated epitope present in a tench brain microtubule-asociated protein. MAP extracts devoid of endogenous protein kinases obtained from adult tench brain was either untreated (-AP) or treated (+AP) with alkaline phosphatase.The electrophoretical mobility is indicated by markers . The antibody recognizes a specific phosphorylated epitope in denatured gel.

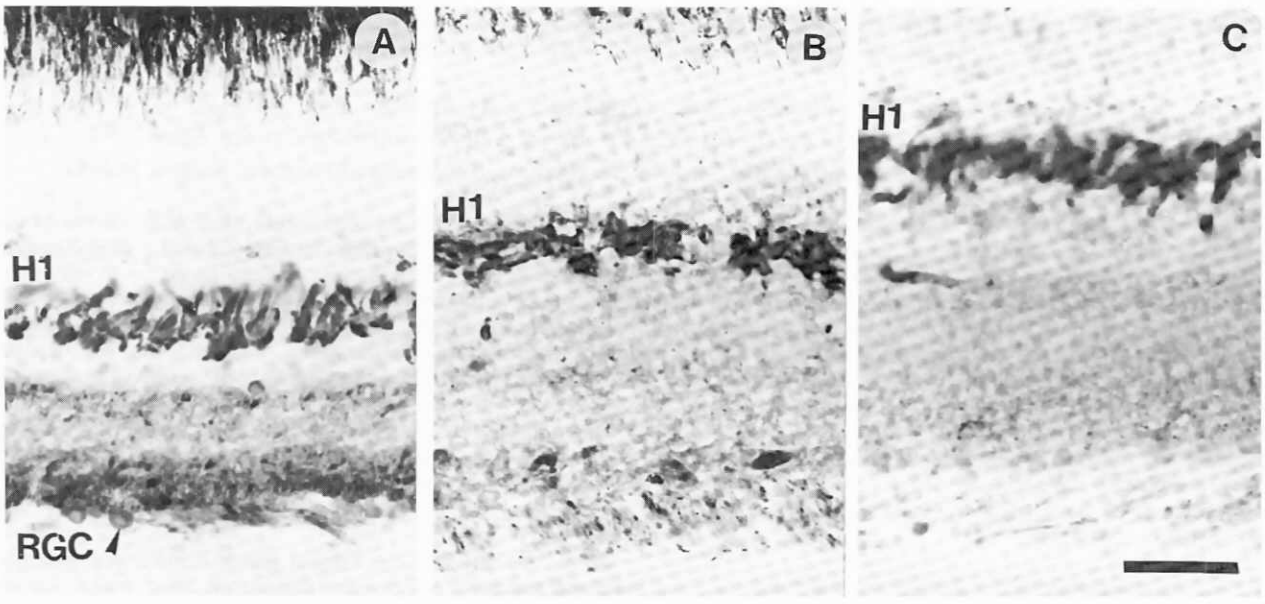


Figure 2.A. MAP1B-Phos in peripheral control retina . B. 14 days after crush central retina. and C. 120 days after crush tench retina. Note the strong immunolabelling in the horizontal cell axons (arrows) the labelling in the ganglion cell layer (GCL). Scale Bar 50 μm.

REFERENCES

Fawcett et al., (1994) Regenerating sciatic nerve axons contain the adult rather than the embryonic pattern of microtubule associated proteins. Neurosci., 5:1302-1311.
Matus, A. (1988) Microtubule associated proteins. Ann. Rev. Neurosci., 11: 29-44.
Rieder, B., and Matus, A. (1985) Differential expression of distinct microtubule-associated proteiens during brain development .Proc. Natl. Acad. Sci. USA. 82:6006-6009.
Sperry, R. W. (1948) Patterning of central synapses in regeneration of the optic nerve in teleosts. Physiol. Zool., 21: 351-361.
Ulloa et al., (1993) Heterogeneity in the phosphorylation of microtubule-associated protein MAP1B during rat brain development. J. Neurochem. 61:961-972.
Vecino et al., (1996) Analysis of fish optic nerve regeneration using MAP1B-Phosphorylated as a marker. Eur. Jour. Neurosci. (submitted)