

available at www.sciencedirect.comwww.elsevier.com/locate/brainres**BRAIN
RESEARCH****Research Report****Early born lineage of retinal neurons express class III β -tubulin isotype****Rajesh K. Sharma*, Peter A. Netland**

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ABSTRACT

Aim: Class III β -tubulin, a constituent of neuronal microtubules, has been frequently used as a marker for the neuronal lineage in developmental biology. In retina, it is often used as a marker for ganglion cells. We investigated the developmental expression of this protein in retina and identified the cell types expressing it to gain a better understanding of whether preferred expression of this isotype in certain retinal neurons plays a cell specific role, or whether it is only a part of an intrinsic developmental program. **Methods:** Immunohistochemistry was done using an antibody against class III β -tubulin and other retinal cell specific markers in adult retinæ of mice. Rabbit and human retinæ were used to investigate if there are any species-specific differences. **Results:** Class III β -tubulin was found in ganglion cells, certain amacrine cells, some horizontal cell processes and cone photoreceptors. Class III β -tubulin was already expressed in the earliest developmental stage studied (Embryonic day 14) in developing nerve fiber layer but became distinct at the day of birth when immunoreactive cells were located in the ganglion cell layer (ganglion and displaced amacrine cells), proximal parts of neuroblastic/inner nuclear layer (amacrine cells) and distal part of neuroblastic/outer nuclear layer (photoreceptors). In one animal, class III β -tubulin containing bodies were found in the retinal pigment epithelium cells. **Conclusions:** Class III β -tubulin is not solely expressed by ganglion cells and, therefore, cannot be used as an exclusive marker for these cells. Results show that the expression of class III β -tubulin was not related to cell morphology or cell function, but rather to the cell lineage (early born retinal neurons) suggesting that the expression of class III β -tubulin in certain cell types may be due to the cell specific developmental program.

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1. Introduction

Eukaryotic cytoskeleton is made up of three distinct components: microtubules, intermediate filaments and microfilaments. Microtubules are vital for cellular functions. They form tracks for trafficking membrane-bound organelles and are essential for the neurite growth during development and regeneration. Although the neuronal and non-neuronal micro-

tubules are composed of the same basic constituents, neurons contain different isotypes of tubulins, post-translational modifications and microtubule-associated proteins (Heidemann, 1996). Multiple genes exist for both α - and β -tubulin. Most α - and β -tubulin isotypes are universally expressed; however, some are preferentially expressed in certain tissue types. Most notably, class III and IVa β -tubulins are neuron-specific. The reason for preferred expression of various isotypes is not clear.

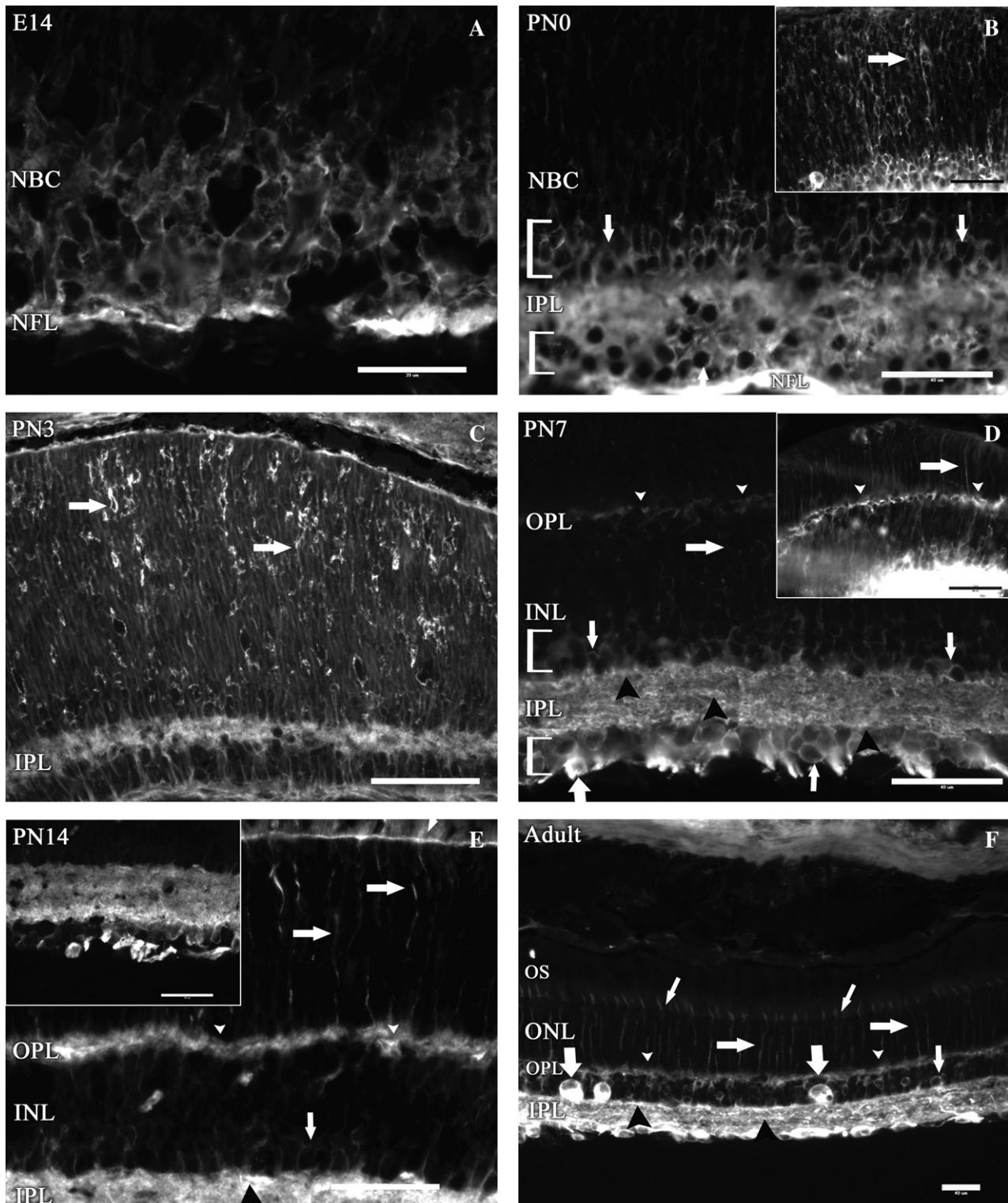
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The class III β -tubulin isotype is one of the six tubulin isotypes expressed in mammalian tissue (Sullivan and Cleveland, 1986). Its expression is associated with early neural differentiation (Easter et al., 1993; Lee et al., 1990; Moody et al., 1989). Retinal ganglion cells, that are involved in glaucoma, have been identified by class III β -tubulin in vitro (Fournier and McKerracher, 1997; Hu et al., 2006). Whether

this isotype is also expressed by other retinal neurons is not well studied.

In this study, we have investigated the developmental expression of class III β -tubulin in mouse retina and compared its expression in mice, rabbit, and human retinæ. Our results provide a clue as to whether cell-specific expression of class III β -tubulin is because of its suitability with neuronal



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