

A search for primitive Purkinje cells: zebrin II expression in sea lampreys (*Petromyzon marinus*)

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Abstract

Zebrin II/aldolase C is a 36 kDa polypeptide expressed by Purkinje cells in the cerebellum of elasmobranchs, teleosts, birds, and mammals, and by octavolateralis pyramidal cells in developing teleosts. To better understand the evolution of these two systems we determined if zebrin II is expressed (1) in previously described primitive Purkinje cells, and (2) in octavolateralis pyramidal cells of sea lampreys (*Petromyzon marinus*). Ammocete and adult stages were reacted with mab anti-zebrin II. In ammocetes the large pyramidal cells of the anterior octavomotor nucleus (AON) were mab anti-zebrin II immunoreactive, but immunoreactivity was not detected in the cerebellar plate. In adults there was no immunoreactivity in any portion of the brain, including the cerebellar plate and the AON. The data indicate that zebrin II immunoreactivity may prove valuable in studying the development of the octavolateralis system across vertebrates. Three explanations are proposed to account for the absence of zebrin II⁺ Purkinje cells: aldolase C is expressed in Purkinje cells but the zebrin II epitope has not yet evolved; the zebrin II epitope was present in ancestral lampreys but has since been lost; or sea lampreys do not have Purkinje cells. The evolutionary implications of these results are briefly reviewed. © 1997 Elsevier Science Ireland Ltd.

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Zebrin II is a 36 kDa polypeptide expressed by Purkinje cells in the cerebellum of elasmobranchs, teleosts, birds, and mammals, and by octavolateralis pyramidal cells in developing teleosts (Fig. 1). Cloning studies indicate that zebrin II is the respiratory isozyme aldolase C [1]. In adult lampreys, Larsell [17] described a cell type in the corpus cerebellum that he termed the 'primitive Purkinje cells of Johnson' [11]. To determine whether these putative Purkinje cells express zebrin II, sea lampreys (*Petromyzon marinus*) in both ammocete and adult stages were reacted with mab anti-zebrin II.

Systematists recognize twenty one Nearctic petromyzontiform species [5]. Jawless fishes, including lampreys, arose in the Ordovician (505–438 million years ago) and became diverse during the Devonian (408–360 million years ago). Jawless fishes gave rise to, and during the late Devonian gave way to, cartilaginous and bony fishes [3].

Adult sea lampreys and ammocetes were obtained from the National Biological Service's (currently the Biological Resources Division of the US Geological Survey's) Hammond Bay Biological Station, and from the Acme Lamprey Company (Harrison, ME, USA) and fixed by transcordial perfusion. Brains were removed, frozen sectioned, and reacted with mab anti-zebrin II according to procedures routinely conducted in our laboratories [4,8,10,14,15]. Four adults (31–37 cm total length) and four ammocetes (9–12 cm total length) were examined.

In ammocetes, large pyramidal cells in the anterior octavomotor nucleus (AON) [2,15,17,19,20,22,23,25] consistently and robustly labelled with mab anti-zebrin II (Fig. 2). Immunoreactivity was detected in the cell soma and dendrites, and was present bilaterally. Pyramidal cell zebrin II expression has been described previously in the larvae of ostariophysan teleosts [13–15]. No other cells in the brain were immunoreactive; specifically, there was no zebrin II immunoreactivity in the cerebellar plate.

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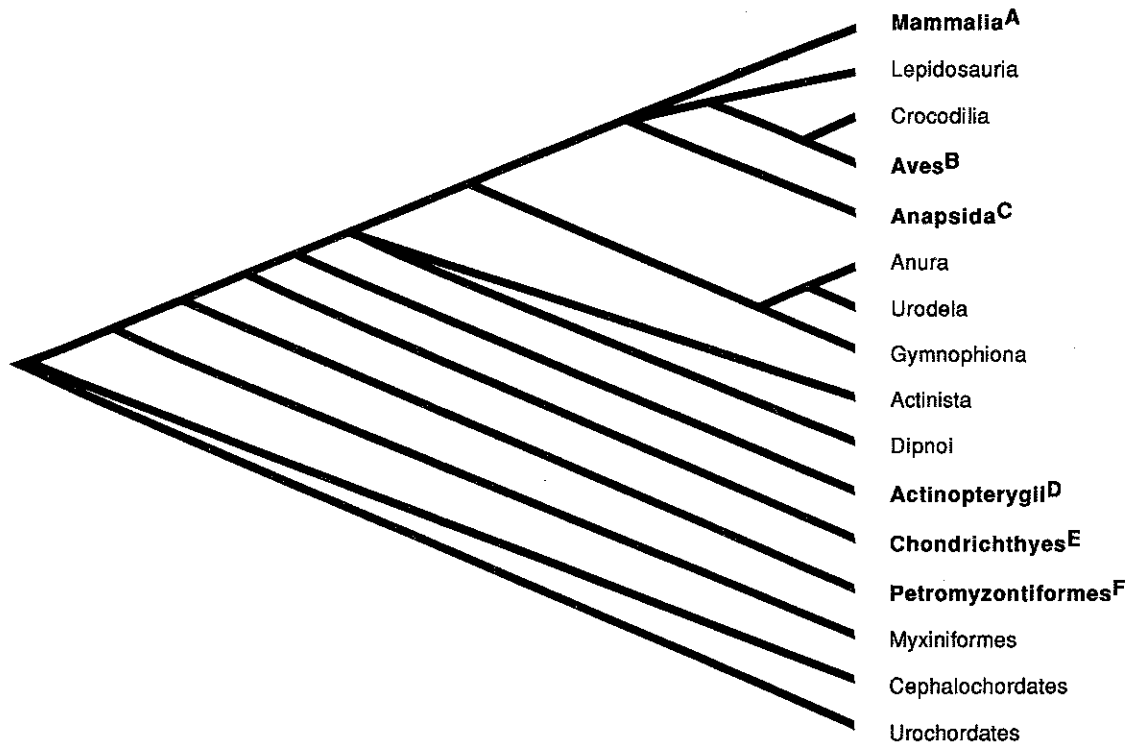


Fig. 1. Distribution of taxa that are known to contain zebrin II antigenicity. Cladogram is based on phylogenies presented in Butler and Hodos [6]. (A) [4,8,9,16,18]. (B) [9]. (C) Lannoo, unpublished data. (D) [4,15,21]. (E) [24]. (F) [12].

In adults mab anti-zebrin II immunoreactivity was not detected in any portion of the brain, including the AON. In particular, the cerebellar plate in sea lamprey adults has been considered to be a primitive cerebellum, and large neurons in this region have been described as primitive Purkinje cells [11,17]. There was no zebrin II immunoreactivity in the cerebellar plate, a result that excludes the possibility of labelling putative Purkinje cells.

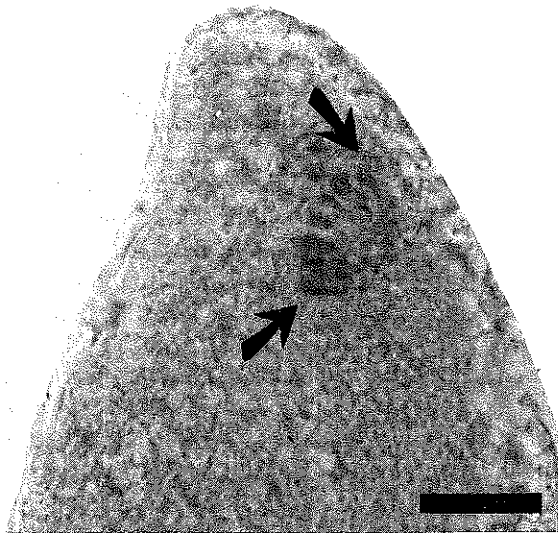


Fig. 2. Mab anti-zebrin II labelled pyramidal cells (positioned between arrows) in the anterior octavomotor nucleus of sea lamprey ammocetes. Dorsal is up, fourth ventricle is to the right. Scale bar, 50 μm.

The results here coupled with previous results [13–15] demonstrate that zebrin II is a transient marker for octavolateralis pyramidal cells in a variety of phylogenetically distinct fishes, and may therefore be a valuable marker for determining the temporal and phylogenetic aspects of octavolateralis development [14,15,19,20,23]. In particular zebrin II may assist in resolving phylogenetic relationships either across octavolateralis sensory systems [6,19,20] or within these systems [13]; similar patterns of antigen expression can reflect either common function or shared ancestry [10].

It is unlikely that zebrin II⁺ Purkinje cells existed but were unrecognized. Zebrin II Purkinje cell labelling is typically robust, and in larval fishes is stronger than pyramidal cell labelling [15]. We consider that had zebrin II⁺ Purkinje cells been present, procedures producing pyramidal cell reactivity would have labelled Purkinje cells.

Three possibilities exist to explain the lack of zebrin II expression in the cells described as primitive Purkinje cells [11,17]. The first is that Purkinje cells are present but the zebrin II epitope has not yet evolved. This lack of expression could result from the absence of aldolase C, or the expression of aldolase C lacking the zebrin II epitope.

The second possibility is that Purkinje cells are present but that zebrin II expression (either aldolase C or the zebrin II epitope) has been lost. Neonatal rodents characteristically express zebrin II in all Purkinje cells, and the development of the mature striping pattern requires the suppression of this initial expression in Purkinje cell subsets [4,8,9,15,18]. Similar transient expression has been seen in the teleost

medial valvula cerebellum [14,15]. If lamprey Purkinje cells are typical of the zebrin II⁻ class, ammocete Purkinje cells could be zebrin II⁺ during early development. Alternately, zebrin II expression may have been lost. Zebrin II immunoreactivity is absent in some derived vertebrates such as anurans (Fig. 1) and some groups of derived teleosts (Lannoo, unpublished data). The application of additional immunocytochemical markers [9] may yet demonstrate the existence of Purkinje cells if they are present but either aldolase C or the zebrin II epitope is absent.

The third possibility is that Johnson's [11,17] primitive Purkinje cells are not Purkinje cells at all. Indeed, in reviewing the known distribution of Purkinje cells one author implies that lampreys do not have Purkinje cells when she states that 'some type of Purkinje cell is present in all vertebrates above cyclostomes' [7]. If this interpretation is correct, it suggests that Purkinje cells arose during or after the late Devonian, 360 million years ago, subsequent to, and perhaps coincident with, the establishment of gnathostome (jawed) vertebrates.

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