

# EXPRESSION OF TRPV4 IN THE AMACRINE CELLS OF ZEBRAFISH RETINA DURING DEVELOPMENT

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## INTRODUCTION

Transient receptor potential (TRP) ion channels form a superfamily of non-selective cation channels that are found in almost all living organisms. TRP channels are involved in sensing mechanical/physical and chemical stimuli. Interestingly mutations in a number of different TRP channels have been linked to a variety of diseases as well as neuronal diseases and neurodegenerative disorders. TRPV4 is a Ca<sup>2+</sup> entry channel that belongs to the vanilloid sub-family, which is activated in response to different physical and chemical stimuli, and it is thought to play different roles including the regulation of body osmolarity, mechanosensation, temperature sensing, vascular regulation. Zebrafish TRPV4 is 72% identical to the human.

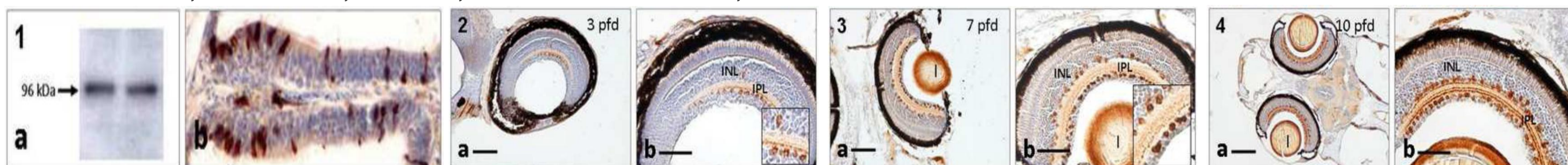
In the present study we have analyzed the expression of TRPV4 in the eye of zebrafish during development

## METHODS

Sixty zebrafish (*Danio rerio*) with ages ranging from 3 days post-fertilization (dpf) to 100 dpf (3, 7, 10, 20, 40, 100; ten animals per age group), bred under standard conditions, were obtained from CISS (Center of Experimental Ichthyopathology of Sicily). The fish were anesthetized with Tricaine methanesulfonate and sacrificed by decapitation. The heads were fixed in Bouin's fixative for 24 h, and then processed for paraffin embedding. Three freshly isolated heads from 10 dpf fish were used for Western blot.

## RESULTS

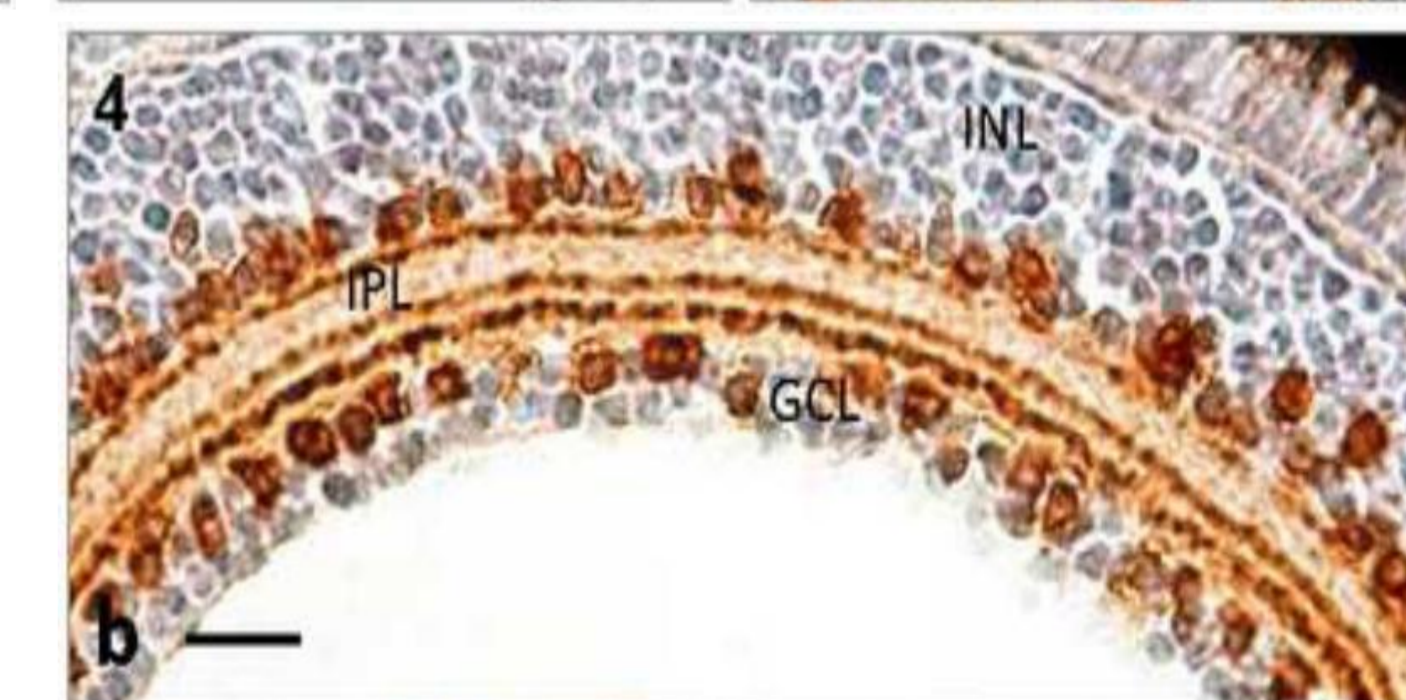
The results demonstrate expression of TRPV4 in amacrine cells in the inner and outer nuclear layers of the retina of zebrafish at all ages sampled and temporary expression in the internal plexiform layer.



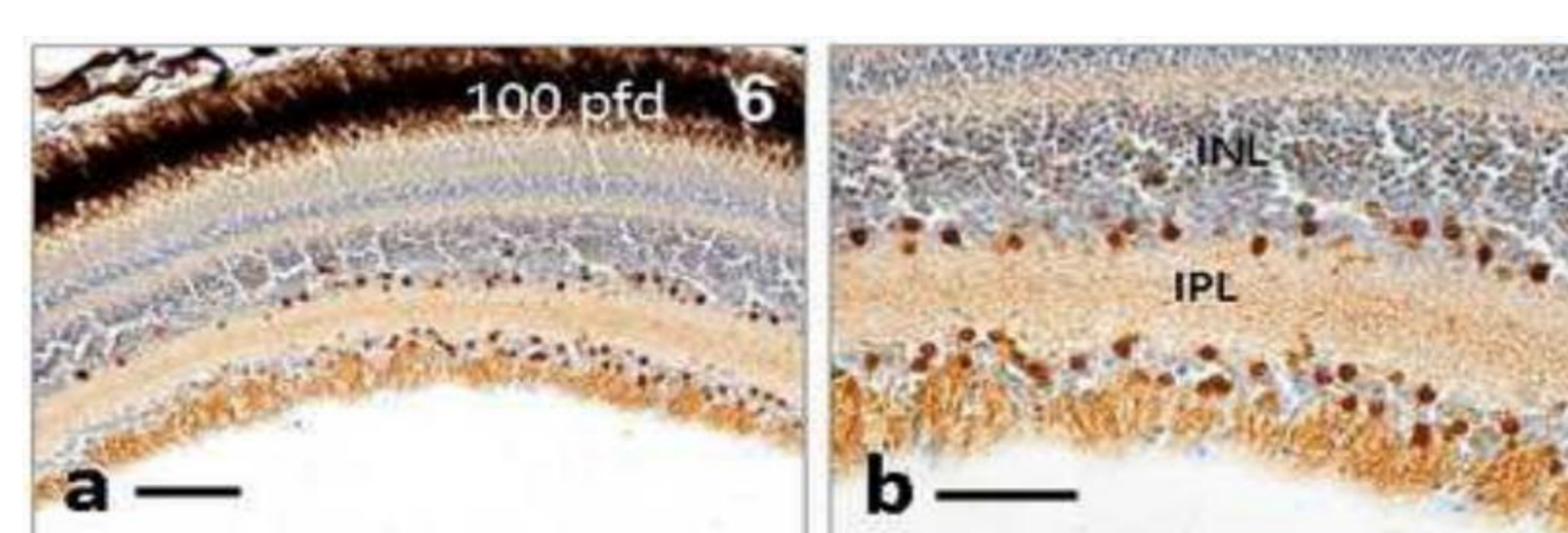
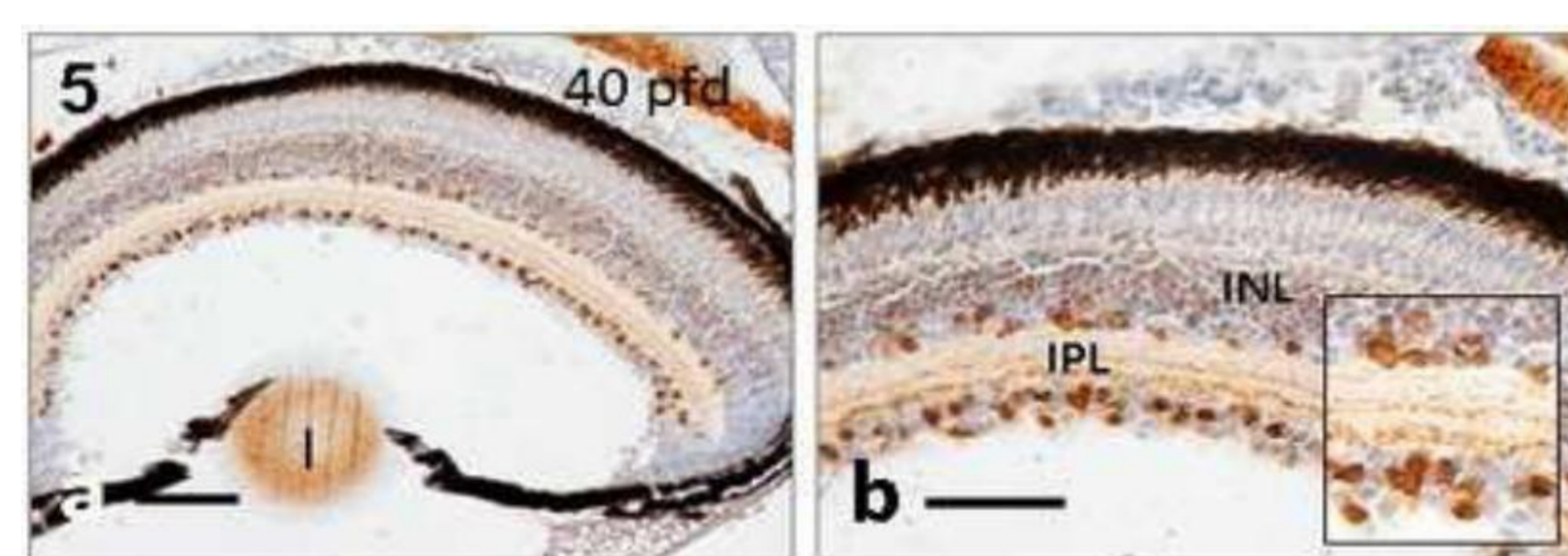
**Figure 1.-** (a) The antibody recognizes a unique protein with an estimated molecular weight of about 96 kDa, which is consistent with TRPV4. (b) Immunohistochemistry using anti-TRPV4 antibodies in the olfactory rosetta of a 40 dpf old zebrafish. Scale bar: 15  $\mu$ m.

**Figure 2.-** Scattered TRPV4 positive somas were found aligned in the INL and a few cells in the ONL expressed TRPV4

**Figure 3.-** The expression of TRPV4 increased in both the INL and ONL, but more cells expressed TRPV4 in the INL than in the ONL. TRPV4-positive cells were distributed forming monolayers.



**Figure 4.-** The expression of TRPV4 in the retina peaks at 30 dpf, when the number of cells that express TRPV4 was similar in the INL and ONL. TRPV4 was found to be expressed in 3 strata of the IPL: 2 in the inner ON sublayer and 1 in the OFF sublayer



**Figure 5 and 6.-** The number of TRPV4-positive amacrine cells remained constant compared with 20 dpf-old animals, however, the TRPV4-positive cells were distributed irregularly rather than forming a monolayer in the INL and ONL. **40 dpf:** We also observed a progressive decrease in the levels of expression of TRPV4 in the strata of the IPL. **100 dpf:** TRPV4 was detected at low levels in the two strata of the ON sublayer but not in the OFF sublayer. TRPV4 was completely absent from the IPL in 100 dpf zebrafish.

## CONCLUSIONS

In these studies we demonstrated for the first time expression of TRPV4 in amacrine cells in the retina of zebrafish at different stages of development. We chose to perform these studies in zebrafish because it is the current model being used to study human congenital ocular and visual disorders. It is possible to speculate that TRPV4 may regulate the activity of amacrine cells through modulation of calcium influx in response to external stimuli

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