THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Supplementary Material

corresponding to:

Pax7 identifies neural crest, chromatophore lineages and pigment stem cells during zebrafish development

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Full Text for this paper is available at: https://doi.org/10.1387/ijdb.062217al

PODCAST TEXT

John Trinkaus wrote in his classic book "Cells into Organs: The Forces that Shape the Embryo" that: "During vertebrate morphogenesis, the most famous, the most studied, and the most impressive invasive movement of migratory cells in cell streams is that of the neural crest".

The neural crest is a population of embryonic multipotent stem cells that migrate throughout the embryo to give rise to a wide variety of cell types. They form neurons and glia of the peripheral nervous system, mesenchyme, smooth muscle, adrenal medulla cells, pigment cells of the skin, and bone, cartilage and connective tissue of the head. Because of its multi-differentiation potential, there is a great diversity of disorders including albinism, melanoma, neuroblastoma, Waardenburg syndrome, and the Hirschsprung disease.

The Paired box (Pax) gene family encodes a group of transcription factors with essential roles in development, stemness and oncogenesis. Pax3 and Pax7 are members of one of the four Pax subfamilies.The zebrafish (Danio rerio) have become one of the favourite models in development, disease, biotechnology and stem cell biology. Zebrafish are prolific, and their embryos are transparent and develop rapidly.

Although many Danio rerio mutations cause neural crest phenotypes, very little is known about the expression of Pax3 and Pax7 genes in zebrafish neural crest. In a seminal paper by Seo and collaborators, at the University of Bergen (Norway), zebrafish Pax3 and Pax7 genes were analyzed. Since no expression of Pax7 was detected in neural crest cells throughout embryogenesis, they suggested the need for further study to reveal neural crest expression at the protein and RNA level.

In the present study, we report that cranial and trunk neural crest express the paired box protein Pax7, thus revealing a novel neural crest marker in zebrafish. Pax7 recognizes both premigratory and migratory neural crest cells.

With development, Pax7 expression occurrs in migrating trunk neural crest cells, which were located both on the medial and lateral migration pathways. Because the neural crest frequently appears as streams of adjacent cells, nuclear expression of the transcription factor Pax7 provides a reliable method for neural crest identification, analysis and counting.

During development, amniotes are either protected by shells (reptiles and birds) or by developing inside of the female (mammals). On the other hand, anamniotes, such as amphibians and fish lack this type of protection and their embryos develop under the risks of the external environment. To increase their chance for survival, anamniotes utilize the Rohon-Beard system for early sensory function.

We compared the immunostaining patterns of the Rohon-Beard sensory neuron marker, HNK-1 and that of Pax7. Our observations confirm the sequential steps of Rohon-Beard neurogenesis and neurocristogenesis in the zebrafish (for more details, see papers by Robert Cornell and Judith Eisen).

Animal coloration patterns are essential for survival and evolution. Mammals and birds have only one class of chromatophore: the melanocyte. The zebrafish Danio rerio, on the other hand, has three types of chromatophores: black melanophores, yellow xanthophores, and iridescent iridophores.

In our report, we present evidence that Pax7 is a common transcription factor of the zebrafish cromathophore lineages and that its temporal control is related to the timing of differentiation of the three pigment cell precursors. Therefore, these findings are consistent with the tight association between downregulation of Pax genes and differentiation.

In the melanophore lineage, Pax7 downregulation takes place after the beginning of melanin deposition. Hence, there is a notable difference between Pax7 regulation and deposition of melanin pigment in vertebrate pigmentation models.

These findings in zebrafish Pax7 open up new avenues to understand how different vertebrate pigmentation models orchestrate Pax3/Pax7 and other factors in controlling melanin formation at the proper time and place.

In zebrafish, several studies have demonstrated that metamorphic pigment cells differentiate de novo from latent stem cells. The lack of molecular markers for zebrafish pigment stem cells have been claimed. During larva to adult transition, we show that pigment stem cells recapitulate the expression of Pax7. Recent studies showed that Pax7 is a reliable skeletal muscle stem (satellite) cell marker across vertebrates. Therefore, we evidence that the evolutionary conserved stem cell transcription factor Pax7 is also expressed in zebrafish pigment stem cells.

Taken together, this study provides the basis for future analysis of Pax7 gene function in zebrafish development and the genetic networks of 'stemness'.