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#### Abstract

Histones are well-known components of the nucleosome. Among core histones H2A, H2B, H3 and H4, the H2A family exhibits the greatest diversity including the largest number of various variants such as H2A.X, H2A.Z, MacroH2A and H2A.Bbd. In general, H2A variants differ mostly in their C-terminus, regarding both length and amino acid sequences. The importance of H2A variants in nucleosome stability and chromatin structure has been well established. Although a lot of progress was made regarding antibacterial peptides derived from the N-terminus of histone H2A in the past decade, the nucleotide polymorphism of H2A and their functions of H2A variants in pathogen infection are largely unknown. In the present study, we found that piscine H2A exhibited abundant nucleotide polymorphism. 15 H2A variants were cloned from zebrafish. Although only 1 to 2 amino acids differ, the similarities among these H2A variants are 90.1–99.5%. Strikingly, nucleotide polymorphism of H2A influenced the antibacterial and antiviral activities. Thus, our results provide insights into the functional differences of H2A variants in pathogen infection.

**keywords:** Histone H2a; nucleotide polymorphism; viral infection; bacterial infection

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#### P-052.

##### The effects of IFN $\gamma$ , IL-1 $\beta$ , and TNF- $\alpha$ on IFN $\gamma$ -IFN $\gamma$ R1/R2 pathway in macrophages from rainbow trout (*Oncorhynchus mykiss*)

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#### Abstract

In vertebrates, the cytokines: Interleukin-1 $\beta$  (IL-1 $\beta$ ), Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) and Interferon- $\gamma$  (IFN $\gamma$ ) are proinflammatory cytokines crucial for the inflammatory response. The cytokine IL-1 $\beta$  is important for inducing neutrophilia and imparting a signal required for optimal T and B cell function. The cytokine TNF- $\alpha$  is key for T and macrophage cell activation and has an important role in the induction of inflammatory mediators, such as nitric oxide and prostaglandins. While the IFN $\gamma$  plays a central role controlling the host response to viral or bacterial infection, through the activation of the JAK/STAT pathway and the induction of iNOS in M1 macrophages. Studies described that IFN $\gamma$  is capable of inhibits IL-1 $\beta$  and induce TNF- $\alpha$  in murine macrophages. Also, nitric oxide regulates IL-1 $\beta$  production in murine macrophages. In rainbow trout, the existence of IFN $\gamma$  receptors (IFN $\gamma$ R1/R2) has been demonstrated, and IL-1 $\beta$ , TNF- $\alpha$ , IFN $\gamma$  shows similar functions to their vertebrate's counterparts. However, there are no studies about the combinatory effect that different cytokines have on the IFN $\gamma$  pathway and cytokine expression in rainbow trout. The aim of this work was to characterize at transcriptional level the IFN $\gamma$ -IFN $\gamma$  R1/R2 pathway, IL-1 $\beta$  and Interleukin-6 (IL-6) expression in trout macrophages. For this, a cell line of monocytes/macrophages from rainbow trout were stimulated with recombinant IFN $\gamma$ /IL-1 $\beta$  and IFN $\gamma$ /TNF- $\alpha$  during 4-6 and 12 hours. The results showed that IFN $\gamma$ R1 and TRIM8 were downregulated. While IFN $\gamma$ R2 has an oscillatory response and was not detected at 12 hours, also STAT1 was upregulated mainly at 12 hours for IFN $\gamma$ /IL-1 $\beta$  treatment. The IFN $\gamma$ /TNF $\alpha$  treatment showed an upregulation of STAT1 for all the hours, mainly at 4 hours, contrasting with the expression observed

for the IFN $\gamma$ /IL-1 $\beta$ . iNOS expression was downregulated in IFN $\gamma$ /IL-1 $\beta$  at 12 hours. The evaluation of the cytokines showed downregulation of IL-1 $\beta$  at 6-12 hours and IL-6 at 4-6-12 hours for IFN $\gamma$ /IL-1 $\beta$  experiment. However, in IFN $\gamma$ /TNF- $\alpha$  just a downregulation of IL-6 was observed. An experiment with just IL-1 $\beta$  showed that IFN $\gamma$ R1 was upregulated at 12 hours. The results obtained suggest a different mechanism of regulation for IFN $\gamma$ -IFN $\gamma$  R1/R2 pathway and a difference in cytokine regulation, mainly with IL-6, a cytokine that was upregulated in mammals when IFN $\gamma$  is used with IL-1 $\beta$ /TNF- $\alpha$ .

**keywords:** Cytokines - IFN $\gamma$  - IL-1 $\beta$  - IFN $\gamma$ -IFN $\gamma$  R1/R2 pathway - Rainbow trout

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#### P-053.

##### Rainbow trout shape-shifted red blood cells

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#### Abstract

Rainbow trout Ficoll-purified red blood cells (RBCs) cultured *in vitro* undergo morphological changes, especially when exposed to stress, and derive into a cell stage that we have coined shape-shifted RBCs (shRBCs). We have characterized these shRBCs using transmission electron microscopy (TEM) micrographs, Wright-Giemsa staining, cell markers immunostaining, and transcriptomic and proteomic evaluation. shRBCs displayed reduced density of the cytoplasm, hemoglobin loss, decondensed chromatin in the nucleus. Also, shRBCs displayed striking expression of the B lymphocyte molecular marker IgM. shRBCs were transiently observed in heat-stressed rainbow trout bloodstream for three days. Functional network analysis of combined transcriptomic and proteomic studies resulted in the identification of proteins involved in pathways related to the regulation of cell morphogenesis involved in differentiation, cellular response to stress, and immune system process. In this regard, it has long been suggested that primitive nucleated erythroid cells in the bloodstream of mammals are more similar to nucleated red cells of fish, amphibians, and birds than the red cells of fetal and adult mammals. In addition, shRBCs increased interleukin 8 (IL8), interleukin 1 $\beta$  (IL1 $\beta$ ), interferon  $\gamma$  (IFN $\gamma$ ), and natural killer enhancing factor (NKEF) protein production in response to viral hemorrhagic septicemia virus (VHSV). Also, shRBCs conditioned medium triggered cytokine signaling in trout pronephros stroma (TPS-2) cell line. In conclusion, shRBCs may represent a novel cell stage that participates in roles related to immune response mediation, homeostasis, and the differentiation and development of blood cells.

**keywords:** Rainbow trout; shape-shifted red blood cells; VHSV; transcriptome; proteome

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