

The Spatial and Temporal Expression of Polymeric Immunoglobulin Receptor (pIgR) in Zebrafish Embryos.

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Introduction to pIgR

Organisms are exposed to millions of potential pathogens on a daily basis and are particularly vulnerable to infection in their mucosal surfaces, the sites within the body that are continuous with the external environment, including the respiratory tract and the gastrointestinal tract [1]. It is necessary for organisms to have a mechanism for protecting themselves from infection at these vulnerable sites. This protection mechanism is part of the mucosal immune system [1]. Antibodies in mucosal secretions play a critical role in this protection in vertebrates. The primary antibodies in the human mucosal immune system are dimeric immunoglobulin A (dIgA) and pentameric Immunoglobulin M [2]. The primary antibodies in the mucosal immune system of fish are tetrameric IgM and tetrameric IgT/IgZ [3].

Polymeric immunoglobulin receptor (pIgR) is an essential transmembrane protein that transports polymeric antibodies across epithelial cells of the mucous membranes [4].

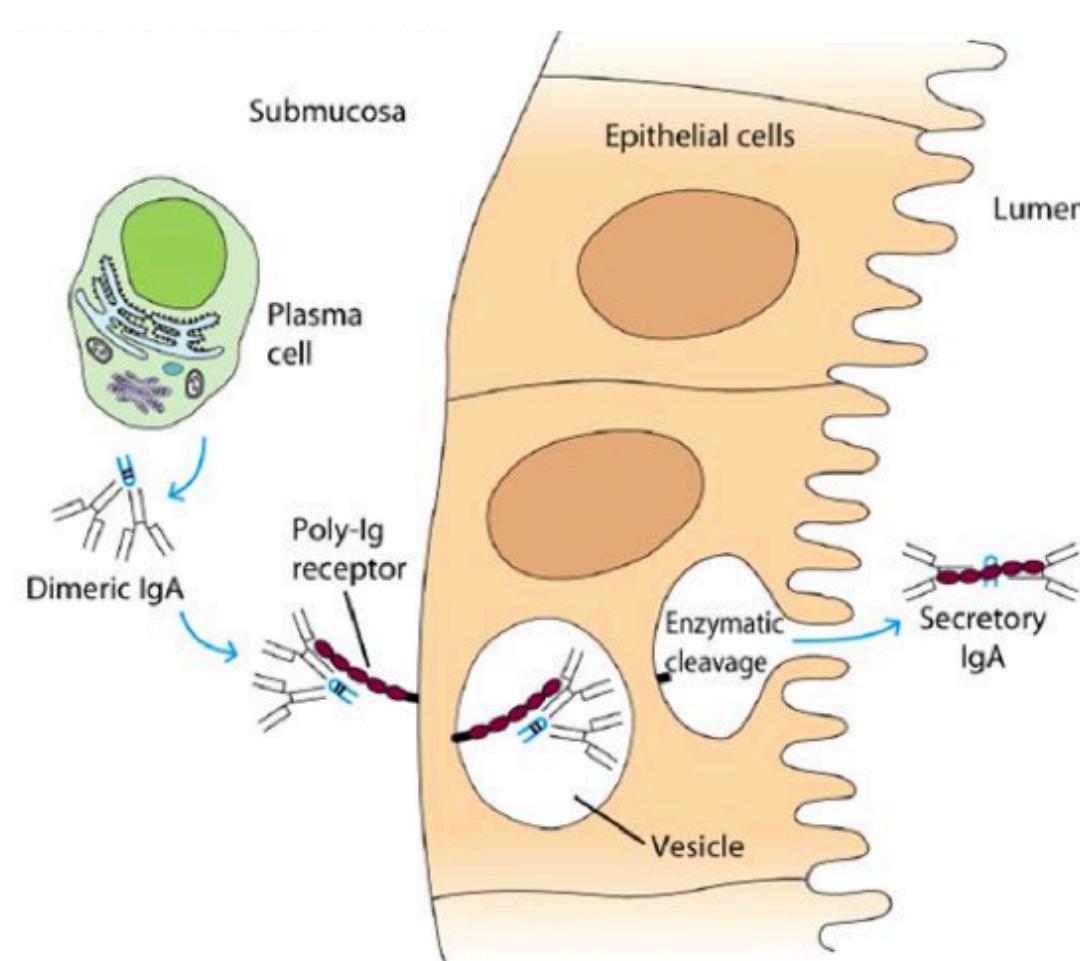


Figure 1. Formation of secretory IgA through transcytosis of dIgA across epithelial cells via pIgR.

In addition to its function, certain domains within pIgR's structure have been conserved throughout evolutionary history.

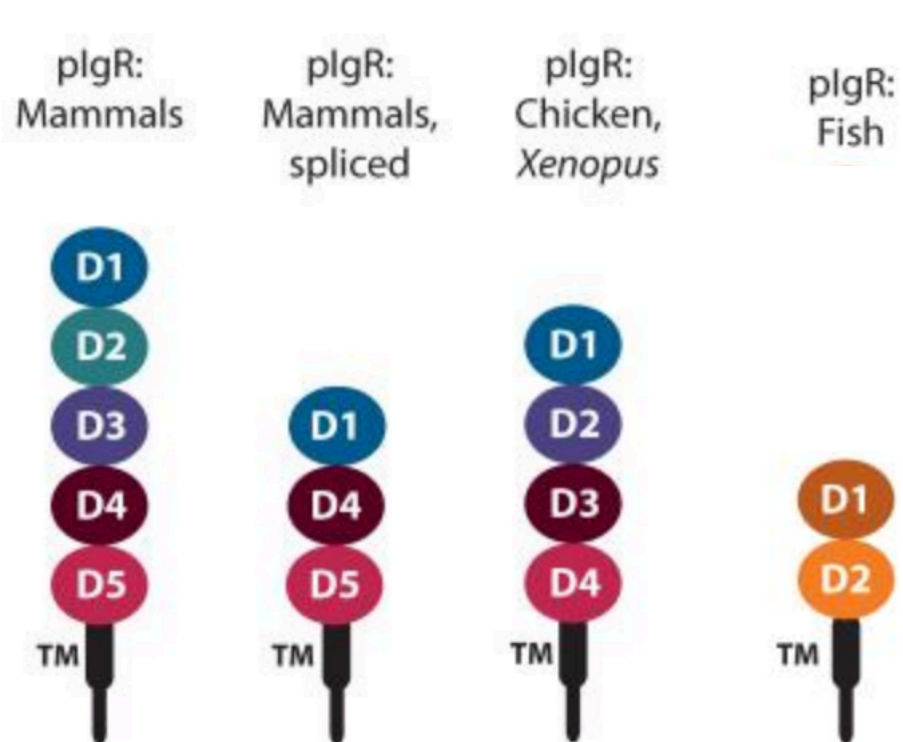


Figure 2. Structures of pIgR in various species [5].

Our research question is: what are the spatial and temporal expression patterns of pIgR in zebrafish embryos? We expect pIgR expression to begin at approximately 4 days post-fertilization (dpf). We expect the majority of pIgR expression to be concentrated in the mucosal tissues (i.e. the gills and the gastrointestinal tract).

Introduction to Zebrafish (*Danio rerio*)

The zebrafish is an important model organism for vertebrate biology because:

- Zebrafish have an **extremely large clutch size**, providing a large number of organisms for use in research [6].
- Zebrafish eggs are **externally fertilized**, allowing for the date of fertilization to be controlled [6].
- Zebrafish embryos are **virtually transparent**, allowing for their development to be precisely visualized [7].
- Zebrafish **develop rapidly**, as all of their organ systems are complete after 3 dpf.

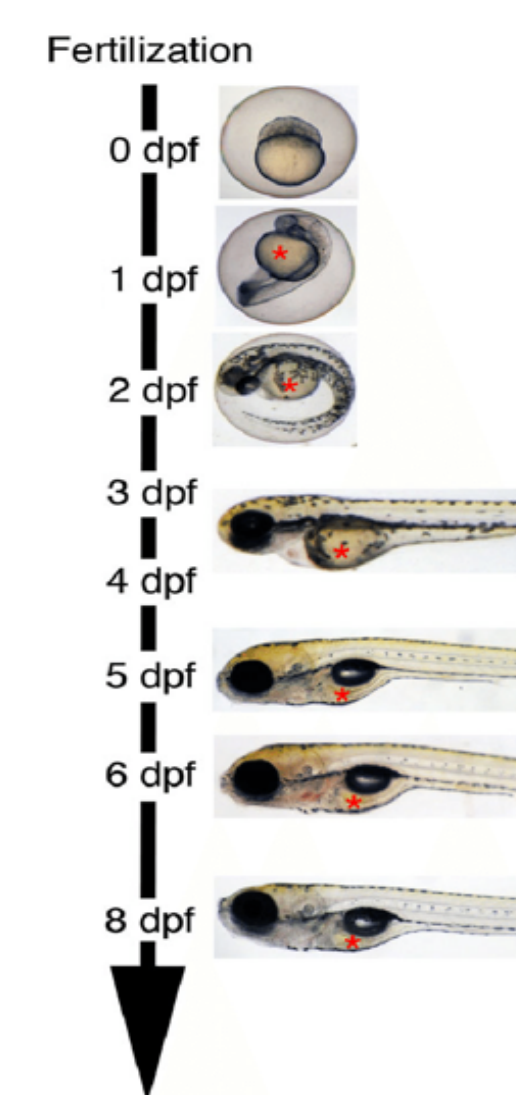


Figure 3. The typical development timeline of zebrafish embryos from zero to eight dpf [8] Red asterisk indicates the yolk sac.

Materials and Methods

Fish Husbandry. Zebrafish embryos were obtained from Dr. Kana Lewis at the UCSD Department of Biology at 0 dpf. Embryos were raised at 28°C. A subset of the embryos were sacrificed each day and stored in RNAlater (for use in RT-PCR) or fixed in paraformaldehyde (for use in *in-situ* hybridizations).

RT-PCR. RNA was isolated from zebrafish embryos using the Qiagen RNeasy mini-kit and converted into cDNA via a reaction catalyzed by SuperScript™ II Reverse Transcriptase. The cDNA was amplified via polymerase chain reaction (PCR). Specificity for target genes was accomplished by the inclusion of specific primers for EF1 α , Rag1, and pIgR. PCR products were visualized via gel electrophoresis.

***In-situ* hybridizations.** Embryos were rehydrated and treated with Proteinase K to make them permeable to the digoxigenin (DIG)-labeled RNA probe. Embryos were incubated with the probe overnight. High stringency washes were performed to remove any unbound probe. The embryos were incubated with antibodies specific for DIG and, subsequently, NBT/BCIP substrate, which reacted with the enzyme on the anti-DIG antibodies. Color development (representing gene expression) was assessed.

Results

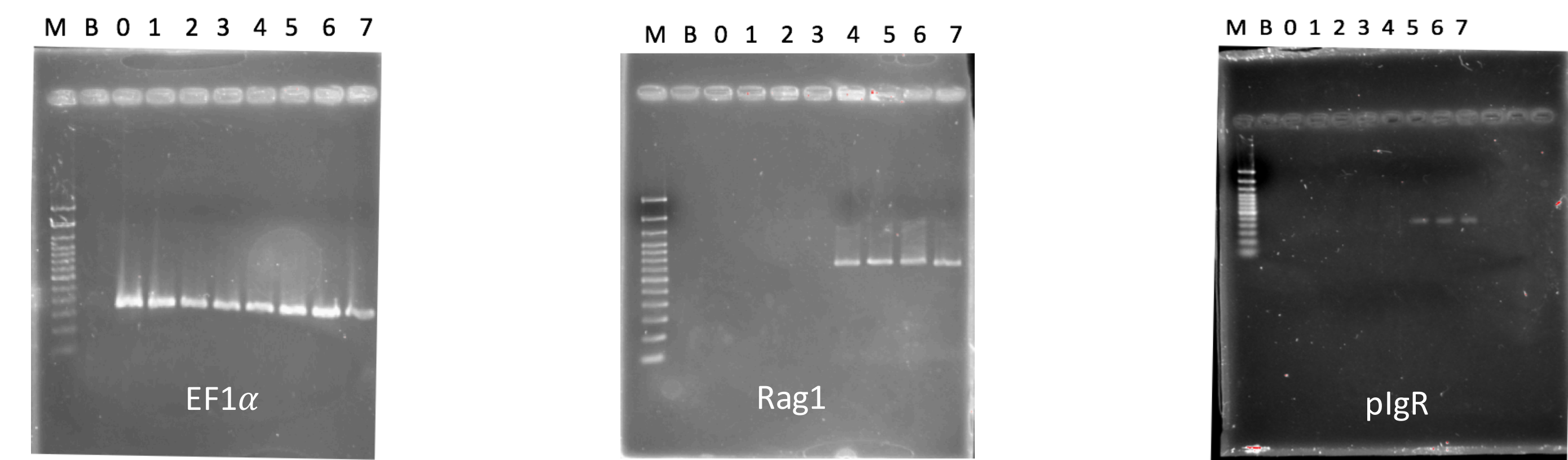


Figure 4. 1.5% agarose gels of RT-PCR reactions from zebrafish embryos days 0-7 dpf. Gels were stained with ethidium bromide and visualized under UV light.

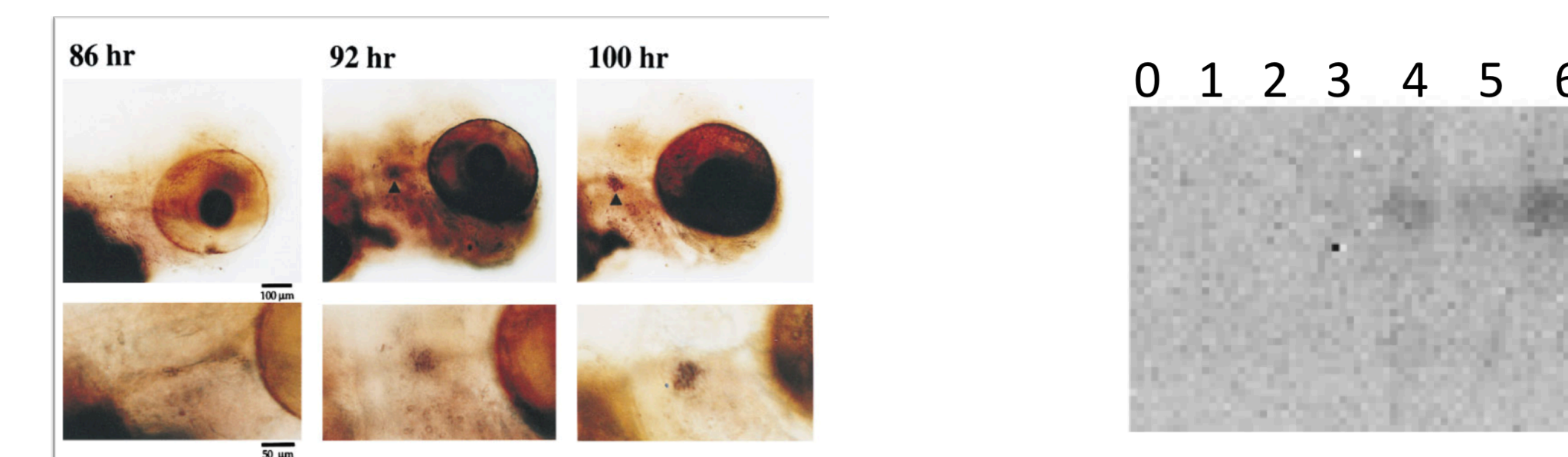


Figure 5. Rag1 expression was detected by Willett et al. at ~4 dpf by *in-situ* hybridization (left) and northern blotting (right) [9,10]. We are attempting to reproduce these *in-situ* hybridization results for use as a positive control in our own study.

Conclusions and Future Directions

We detected pIgR expression beginning at 5 dpf. Rag1 expression was observed beginning at 4 dpf, consistent with other reports in the literature [9,10]. Rag1 (recombination activating gene 1) generates antibodies with a broad range of antigen affinities and is therefore an essential component of the adaptive immune system. EF1 α was expressed at a high and constant level throughout the first seven days post-fertilization, consistent with previous studies [11]. EF1 α is a housekeeping gene necessary for protein synthesis and serves as our positive control to ensure the success of RT-PCR.

We now know that pIgR is expressed beginning at 5 dpf. This is the stage in development at which zebrafish embryos begin to be externally fed, as they are no longer able to rely on their yolk sac for nutrition (Figure 2). In order to account for this feeding, the digestive tract is expected to be sufficiently developed. As pIgR is generally expressed in mucosal surfaces (i.e. the digestive tract), it would make sense that its expression begins in conjunction with the physiological development of these mucosal surfaces. However, to determine the tissues in which pIgR is expressed, we need to use *in-situ* hybridizations.

We are currently establishing appropriate Proteinase K and stringency conditions. The spatial expression pattern of Rag1 will be utilized as a positive control in our *in-situ* hybridizations, as Willett et al. demonstrated that Rag1 is expressed in the thymus of zebrafish embryos, which is located near the gill region and develops as bilateral symmetric lobes (Figure 5) [9]. Replicating the demonstrated expression pattern of Rag1 would ensure the success of our *in-situ* hybridizations.

Acknowledgments

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