Functional Characterization of Ifih1 and Dhx29 During Early *Xenopus laevis* Development

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Introduction

In *Xenopus laevis* oocytes an absence of transcription occurs in early development from before the resumption of meiosis in stage VI oocytes, through the maternal-zygotic transition (MZT) at almost 4000 cells, requiring new protein synthesis to be directed by stored mRNAs. Several modifications control the translation of these mRNAs including cytoplasmic polyadenylation and 2′-O methylation[1]. Mechanics of cytoplasmic polyadenylation are well established, but 2′-O methylation involvement is less clear. We are interested in the mechanism through which this modification might act in regulation of translation and are investigating candidate proteins that recognize the presence or absence of this molecular mark. The interferon induced with helicase C domain 1 (Ifih1 or Mda5) protein is known to bind RNAs based on their 2′-O methylation state[2]. The *ifih1* transcript has been identified in the stage VI oocyte, implying possible involvement in oocyte maturation, and suggesting that the Ifih1 protein could be involved in translational regulation. An alternative function may be in developmental immunology as Ifih1 is typically responsible for recognition of dsRNA and the activation of cytokines. Little is known regarding immune responses during early *Xenopus* embryogenesis, but as Ifih1 is known to target (+) ssRNA viruses, such as coronaviruses, determining the role of Ifih1 in *Xenopus laevis* development may help better understand the protein's overall function.

Observations/Results

- Ifih1 is a RIG-I-like receptor (RLR) along with Ddx58 (RIG-I) and Dhx58 (LPG2) normally acts in the immune response[3]
- *ifih1* mRNA is expressed in the oocyte and early embryo (Figures 1 and 2), but very little *ddx58* or *dhx58* mRNA
- The mRNAs for other proteins (Dhx29, Riok3 and Rnf123) known to interact with Ifih1 are expressed the oocyte and early embryo (Figures 2 and 3)

Goals

We hope to identify which of the proposed roles, translational regulation or developmental immunology, Ifih1 is fulfilling. Efforts are being made to clone the *ifih1* cDNA, which will allow rescue and overexpression of the protein in oocytes and enable us to identify potential binding partners. If Ifih1 plays the proposed role in mRNA activation and regulation, altering its expression/activity should cause observable changes in oocyte development.

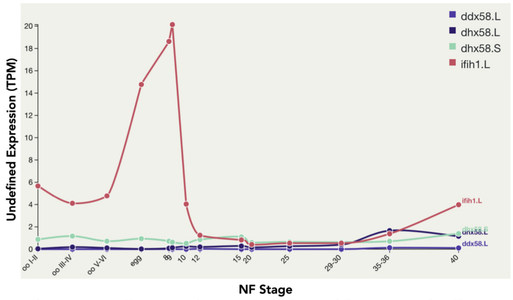

Figure 1: Expression of *ifih1*, *ddx58*, and *dhx58*. Utilizing expression data available from Xenbase[4,5], expression of *ifhi1*, *ddx58* and *dhx58* (S and L) is graphed across developmental stages showing strong expression of *ifih1* in oocytes and early embryos.

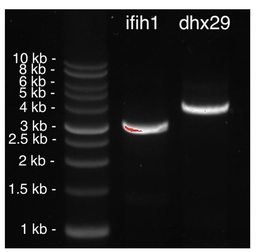

Figure 2: *ifih1* and *dhx29* RT-PCR Products. PCR amplification of the *ifih1* (3 kb) and *dhx29* (4kb) transcripts from oocyte RNA. PCR primers contain restriction sites for cloning into the pH6HNT His_6 Halo Tag vector for expression.

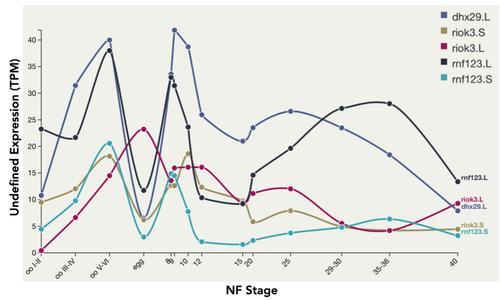

Figure 3: Expression of Ifih1 interacting factors. Utilizing expression data available from Xenbase[4,5], expression of *dhx29*, *riok3* (S and L), and *rnf123* (S and L) is graphed across developmental stages showing strong expression of these transcripts in oocytes.

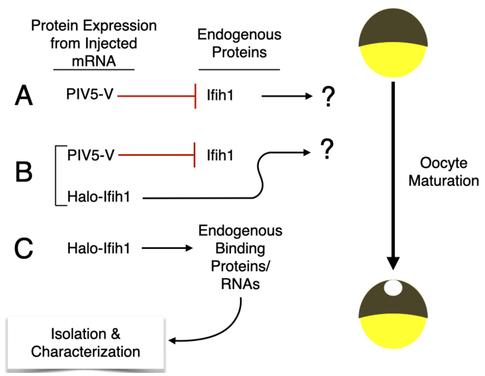

Figure 4: Experimental Approach. Characterization of Ifih1 through injection of PIV5-V (an Ifih1 inhibitor[6]) or Halo tagged *ifih1* mRNAs into oocytes.

Discussion

While research was halted due to the University shutdown, we have indication that we have cloned the *ifih1* mRNA, and have the PIV5-V ready for transcription and injection. The following considerations are guiding our experimental design:

- Ifih1 usually acts through the Mavs protein to activate interferon and cytokine transcription in response to viral infection[3]. If it is acting as an immune sentinel it will activate through a novel mechanism as there is no transcription at this stage
- The other RLR do not seem to be expressed in early development suggesting a different developmental role for this protein
- There is precedence for helicases such as Ifih1 to act as regulators of translation[7]
- Isolation of tagged Ifih1 and binding partners, (protein and mRNA) should establish the pathway that the protein is acting in during development

Literature cited

1. Kuge, H., & Richter, J. D. (1995). Cytoplasmic 3" poly(A) addition induces 5" cap ribose methylation: implications for translational control of maternal mRNA. The EMBO Journal, 14(24), 6301.
2. Züst, R., et al. (2011). Ribose 2′-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nature Immunology, 12(2), 137–143.
3. Dixit, E., and Kagan, J. C. (2013). Intracellular Pathogen Detection by RIG-I-Like Receptors. Adv Immunol. 117, 99–125
4. Xenbase (http://www.xenbase.org/, RRID:SCR_003280)
5. Session, A.M., et al. (2016). Genome evolution in the allotetraploid frog *Xenopus laevis*. Nature 538, 336-343.
6. Ramachandran, A. and Horvat, C.M. (2010). Dissociation of Paramyxovirus Interferon Evasion Activities: Universal and Virus-Specific Requirements for Conserved V Protein. J. Virol., 84 (21): 11152-11163.
7. Bourgeois, C. F., Mortreux, F., and Auboeuf, D. (2016). The multiple functions of RNA helicases as drivers and regulators of gene expression. Nat Rev Mol Cell Biol, 17, 426-438.

Acknowledgments

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Xenbase
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