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PIWI Proteins-piRNA in Zebrafish Embryo Development

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Abstract: Zebrafish embryo has been a very important specimen for study of developmental biology. On the other hand the era of non-coding RNA has opened a huge arena of epigenetic studies. piRNA and PIWI proteins have attracted a lot of attention for their various regulations. Though it was previously thought that piRNA/PIWI protein are principally active in germ cell development but studies have shown these to be active even in embryonic stages influencing the crucial stages of development. This review assimilates and infers the role of piwi proteins-piRNA in zebrafish embryo development.

Keywords: Zebrafish, Embryo, Development, PIWI, piRNA, Piwil2 (zili)

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Introduction

Danio rario more frequently referred to as zebrafish has been one of the most handy model for scientists in recent time. Having a genetic similarity of about 84 per cent with human and simpler with genetic manipulation techniques, it attracts studies across a variety of fields like cancer biology, cardiovascular diseases, neurological diseases, toxicology, drug discovery and numerous others. That it can lay over 200 eggs in just 7 days that too with transparent eggs in which developmental process can be easily monitored also serves as a powerful platform for developmental biology (Khan and Alhewairini, 2019).

Similarly the neo era of non-coding RNA in

biology has given a huge attraction to the genes which do not code for protein but influence them greatly by interaction with both nucleic acids as well as proteins. Epigenetic regulation of this class of RNA is of special interest. Of the various categories of non-coding RNAs, the piwiRNAs are specialized to play vital roles in gametogenesis. Discovered in *Drosophila*, these RNA play a decisive role in transposons silencing as well as in regulation of other genes (Wu and Zamore, 2021). Piwi, abbreviated for P-element Induced WImpy testis in *Drosophila*, are proteins responsible for germ cell and stem cell differentiation. Uniquely Piwi-interacting RNA (piRNA), are 20-30 nucleotide long RNAs, associates itself with PIWI

proteins. The PIWI-piRNA complex is responsible for various DNA and histone methylation (by H3K9Me3-trimethylated lysine 9 of histone 3) along with its gene protection from transposons. This review assimilates the two important facets viz. a robust developmental model along with the influence of epigenetic modulator that controls this development. The review will limit discussion and the influence of the said non-coding RNA till the stage where the embryo is hatched as studies beyond that does not involve the most attractive feature of zebrafish embryo i.e. transparent eggs.

Zebrafish Embryo development:

To understand the actual significance of the study, it must be understood why zebrafish embryo and what are the stages of development in these organisms embryonic development? Zebrafish embryo has become a very attractive specimen in any developmental studies as its eggs are transparent and that pigmentation comes in at a later Pharyngula stage. Virtually the entire development can be studied in a stereo zoom microscope with a hatching period of only 48-72 h (Kimmel *et al.*, 1995).

Zebrafish embryo development can be broadly divided into the following stages: zygote, cleavage, blastula, gastrula, segmentation, pharyngula, and hatching periods. The stages are elaborated in Table 1. Briefly after a very short single cell stage which lasts a little over an hour, the zygote enters the cleavage stage. In this stage there is quick and multiple cell divisions and the animal pole is established. The stage ends in the 64 stage state after one and a half hour post-fertilization. The blastula phase last about 3 h and epiboly sets in with the formation of the blastoderm disc. The morphogenetic movements inaugurate the Gastrula stage which spans the next 4.45 h -5 h. This stage sees a number of changes along the germ ring around the blastoderm rim such as involution, convergence and extension related morphogenetic movements (Kimmel *et al.*, 1995). Embryonic shield, brain rudiment can also be visualized. After Gastrula the lengthy stages

appear. The first amongst these is the segmentation stage which extends though 14 h, and inaugurates the formation of various organs, the list of visualizable developments has been mentioned in Table 1. Appearance of countable V-shaped somites in the trunk region is a demarcating transformation along with the fact that heart beat measurement can be done from this stage onwards. The next 48 h is Pharyngula or the primordium ("prim") stages: Prim 5- Prim 25 with the formation of the body axis. Along with pigmentation (small dark dots appear), small flowing cells can be seen as the first signs of blood circulation. Formation and tail motility is also a feature of the stage (other details in Table 1). The larva hatches within the next 24 h (Kimmel *et al.*, 1995).

piRNA in Zebra fish embryo:

Wei *et al.* (2012), made a robust attempt to study the piRNA expressions in the four basic stages of zebrafish embryo development (256 cell stage, sphere stage, shield stage and 1dpf- day post-fertilization; see Table 1 for more details on stages). They found that these piRNAs in case of zebrafish embryos had dissimilar array of sequences with their most common size being between 26-28 nucleotide in length and were expressed widely throughout the four stages selected by the researchers. These piRNAs were different as reported with testis and ovaries as majority of them in case of the later were derived from plus stands while those detected during embryo development were from minus strands (Wei *et al.*, 2012). The expressions of the piRNAs were abundant during transition from 256 cell stages to the shield stages with the maximum being recorded in 256 cell stage and sphere stage (Wei *et al.*, 2012).

Zebrafish piwi proteins piwil1 (ziwi) and piwil2 (zili):

In Zebrafish piwil1 (ziwi) and piwil2 (zili) are the two Piwi protein homologs (Houwing *et al.*, 2007; Houwing *et al.*, 2008) and were first considered to be expressed in germ cells only. According to

Table 1: Various developmental stages of Zebrafish Embryo

Stage	Approximate duration	Major Events
Zygote 1-2 cell stage	0 – 75 mins	The fertilized eggs undergo a single zygotic cell cycle. Formation of animal pole and blastodisc by cytoplasm flow.
Cleavage 4-64 cell stage	75 mins – 2h 15 mins	Rapid cell divisions through resulting in 64 cells in the animal pole arranged along the curved blastodisc.
Blastula 128 -2000 cell stage Upto 30% epiboly	2h 15 mins - 5h 15 mins	Cell cycles are less rapid than previous phase with asynchronous ones in the middle phase of blastula. Epiboly begins along with yolk cell bulging at animal pole. Blastoderm appears as inverted cup with even thickness. 256 cell stage is in the beginning and sphere stage towards the end.
Gastrula 50% epiboly to 100% epiboly	5h 15 mins – 10 h	Involution, convergence and extension related morphogenetic movements begin appearance of germ ring around the blastoderm rim. Body axis develops. Embryonic shield, brain rudiment can be visualised.
Segmentation 1 somite to 26 somite	10h – 24 h	As the name symbolizes this phases exhibits visual recordable formation of various organs. Development of V-shaped somites in the trunk region along with pharyngeal arch (primordial) , nuromeres, optic vesicle, lens and well extended tail can be visualized. Heart beat can also be seen and recorded from this stage onwards.
Pharyngula primordium (“prim”) stages :Prim 5 – Prim 25	24h-48 h	This is the Phylotypic stage of zebrafish. The body axis for the first time separates the yolk outline and straightens. Pigmentation increases and circulation can be visible under microscopic magnification. Tail formation and its motility can be seen towards the end of this phase. Further development as mandibular and hyoid arches; foregut development; olfactory cilia formation; thickened otic vesicle walls can be visualized.
Hatching Period/ Larva	48h-72 h	In this stage there is accelerated morphogenesis of all primary organs and systems. Cartilage formation in head and pectoral fins take place with the inflation of the swim bladder. Mouths are wide open and eyes remain protruded and big. Hatching occurs. Larvae seek food and are active in avoidance.

Houwing *et al.* (2007, 2008) ziwi major role lies in germ cell maintenance while zili is essential for meiosis and germ cell differentiation (Tan *et al.*, 2002; Houwing *et al.*, 2007, 2008). Credit goes to

Tan *et al.* (2002) for identification and localization of (ziwi). piRNAs are mainly expressed in testes and ovaries of zebrafish, however, it has been reported that zebrafish without PIWI protein

expressions as in *ziwi*-mutant zebra fish are associated with apoptosis of adult germ cells thus are basically sterile (Houwing *et al.*, 2008).

Piwil2 influence on Fibroblast growth factor:

Fibroblast growth factor more commonly referred by its acronym Fgf, is signaling moiety that controls multiple phenotypic traits. Present in almost all forms of animal life, they are known to influence various cellular processes like proliferation, differentiation, homeostasis and migration (Cao *et al.*, 2004).

Zebrafish acerebellar mutants carry a mutation in the *fgf8a* locus and reduction of somatic mesoderm (Reifers *et al.*, 1998). Brachyury is a down stream transcription factor of Fgf and has its zebrafish homolog as loss-of-function mutation of *ntl* (no tail gene) and is also a Fgf mediator of mesoderm formation. (Halpern *et al.*, 1993).

Zhao *et al.* (2010) using *piwil2* knockdown model found that fibroblast growth factor 8a (*fgf8a*) is positively correlated with *piwil 2*, in fact introducing *piwil2* mRNA promoted *fgf8a* expression. Thus, *piwil2* knockdown model exhibited inhibition of dorlasized phenotype resulting in the oval shape towards the end of epiboly. Moreover, as Fgf regulates *ntl*, knockdown of *piwil2* inhibited *ntl*. It has been further proved that though *Piwil2* positively regulates *ntl* during the bud stage, it negatively regulates the same factor during Pharyngula stage.

Piwil2 influence on TGF beta and BMP signaling:

Though it was earlier thought that *Piwil 2* (*Zili*), in case of zebrafish is expressed in primordial germ cells (PCD), a group lead by Yongxin Ma proved that *zili* functions in early embryonic stages (Sun *et al.*, 2010). Ma group extensively studied the expression of *zili* in embryological stages and has reported that *zili* is expressed as early as in a single cell stage and in all cells during blastula stage. During gastrulation, *zili* was found to be concentrated at the axis. During segmentation phase *zili* was found to gain strong expressions in

brachials and pharyngeal arches, however, after prim 5 stage of Pharyngula the expression of *zili* decreases only to reappear at PGCs post 3 day fertilization i.e. in larval stages.

Mechanistic study by Ma group revealed that *zili* inhibited the signaling of multifunctional cytokine namely Transforming growth factor beta (TGF- β). TGF- β is a pivotal cytokine in various developmental processes spanning through early germ cell patterning in pregastrula stage of an embryo to tissue healing/regeneration and homeostasis in adult forms. TGF β binds to its receptor TGFR1 or TGFR2 on the cell surface and has its downward signaling moities in the form of Smad(s). Receptor activated Smads are known as R-Smads which upon phosphorylation form complexes with Smad4 and are transported to the nucleus. The Smad complex associates with various transcriptional factors and regulate transcription. It was found that *zili* (*Piwil 2*) through protein interaction with Smad 4 averted association of Smad2/3/4 and Smad1/5/9/4 complexes. Furthermore, researchers had ectopically expressed *zilli* and found under such over-expression caused eyes fusion and suppression of mesodermal gene expression. Such result suggests *zilli* inhibits Nodal signaling and mesoderm formation.

Sun *et al.* (2010) in their study had further traced the logistics of how *zili* inhibited the phosphorylated R-Smad complex formation. In this quest they reported that *zili* primarily inhibits the transcriptional effects of the ligands of Nodal and BMP (Bone morphogenetic protein) namely *sqt*, *cyc*, and *bmp2b*. The abrogation of such signaling lead to decrease in expression of sonic hedgehog (*shh*), goosecoid (*gsc*) and *vent*. Finally *zilli* competitively binds with Smad 4 repressing the other Smads forming complexes with Smad 4. They also found that *zili*/Smad 4 complex is ultimately trans located to the nucleus.

Taking cue from these results especially the fact BMP signaling is being affected as BMP signaling is crucial for dorsal-ventral (DV)

Fig. 1: PIWI interacting RNA activity classically involves both the protein as well as the RNA component that is PIWI proteins i.e. piwil2 (zili) as well as piRNAs. A schematic representation of the occurrence of the two components has been made to try to understand the stage wise role Piwi-piRNA possible activity.

patterning in vertebrate embryos, Li *et al.* (2011) provided further information. Dorso-ventral formation (back to belly) is an important developmental process in a developing embryo and is prominent from early gastrula stages. Briefly the mesoderm differentiation takes place from dorsal to ventral into prechordal plate and notochord then to somites, followed by kidney lateral plate mesoderm and finally the ventral blood islands (De Robertis and Kuroda, 2004).

The genes involved are mainly Bmp2b, vox vent for ventral formation while ntl, snail and shh influence the dorsal formation (Marlow 2010; Li *et al.*, 2011). Li *et al.* (2011) reported that zili knock down models experiences decrease in the dorsal gene regulators namely ntl, snail and shh, while the ventral regulators, tbx6, vox, and vent, are increased during gastrulation. Supporting this notion was the fact that when zili was overexpressed the reverse effects were observed. Probing into the moieties in the Bmp pathway the researchers reported increase of expression of bmp2b and bmp4 on one hand while decreasig Bmp antagonists chordin (chd), noggin (nog1), and follistatin (fst), on the other.

Furthermore, exploring genetic analysis and interaction Li *et al.* (2011) showed that zili knockdown induced Bmp signaling through bmp2b-mediated Bmp pathway while its over-expression achieved the opposite. Taking into considerations of the findings of Sun *et al.* (2010) and Li *et al.* (2011) it can be said that zili definitely affects the DV patterning in the early stages of embryo development in zebrafish.

Conclusion

In light of the assimilated information it can be equivocally stated that PIWI RNA is not restricted to the development of testis and neither ovaries nor its activity confined in germ cells. That Piwi RNA is active and responsible in control and development of zebrafish embryo is beyond doubt. However, it must be kept in mind that most of the work that has been done are either on piRNAs or PIWI proteins in zebrafish embryo development. However, a classical PIWI interacting RNA activity should involve both the protein as well as the RNA component that is PIWI proteins i.e. piwil1 (ziwi) and piwil2 (zili) as well as piRNAs. Since no work in this light has been produced as yet, a schematic representation of the occurrence of the two

components has been made to try to understand the stage wise role Piwi-piRNA possible activity (Fig.1). As per available data it seems most probable that such interactive activity may be initiated in low extent in the early Blastula phase (256 cell stage) with highest probability of activity in the Pharyngula stages with vacant phases in segmentation and beyond Pharyngula till primordial germs cells come in activity with the piwil1 (ziwi) proteins. It is interesting but more work in the related field needs to be done for a more concrete evidence based picture.

References

- Cao Y, Zhao J, Sun Z, Zhao Z, Postlethwait J and Meng A. (2004) fgf17b, a novel member of Fgf family, helps patterning zebrafish embryos. *Dev Biol.* 271: 130-143.
- De Robertis EM and Kuroda H. (2004) Dorsal-ventral patterning and neural induction in *Xenopus* embryos. *Ann Rev Cell Develop Biol.* 20: 285-308.
- Farmanur RK and Saleh SA. (2019) Zebrafish (*Danio rerio*) as a Model Organism. In: *Current Trends in Cancer Management*, (eds.) Streba L., Ionut Gheonea D. and Schenker M., <https://www.intechopen.com/chapters/64178>.
- Halpern ME, Ho RK, Walker C and Kimmel CB. (1993) Induction of muscle pioneers and floor plate is distinguished by the zebrafish no tail mutation. *Cell* 75: 99-111.
- Houwing S, Berezikov E and Ketting RF. (2008) Zili is required for germ cell differentiation and meiosis in zebrafish. *EMBO J.* 27(20): 2702-2711.
- Houwing S, Kamminga LM, Berezikov E, Cronembold D, Girard A, van den Elst H, Filippov DV, Blaser H, Raz E, Moens CB, Plasterk RH, Hannon G J, Draper BW and Ketting RF. (2007) A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. *Cell* 129(1): 69-82.
- Kimmel CB, Ballard WW, Kimmel SR, Ullmann B and Schilling TF (1995) Stages of embryonic development of the zebrafish. *Dev Dyn.* 203(3): 253-310.
- Li D, Sun H, Deng W, Tao D, Liu Y and Ma Y. (2011) Zili antagonizes Bmp signaling to regulate dorsal-ventral patterning during zebrafish early embryogenesis. *Zool Sci.* 28(6): 397-402.
- Marlow FL. (2010) *Maternal Control of Development in Vertebrates: My Mother Made Me Do It!* San Rafael (CA): Morgan & Claypool Life Sciences; 2010. <https://www.ncbi.nlm.nih.gov/books/NBK53192/>
- Reifers F, Bohli H, Walsh EC, Crossley PH, Stainier DY and Brand M. (1998) Fgf8 is mutated in zebrafish acerebellar (ace) mutants and is required for maintenance of midbrain-hindbrain boundary development and somitogenesis. *Development* 125: 2381-2395.
- Sun H, Li D, Chen S, Liu Y, Liao X, Deng W, Li N, Zeng M, Tao D and Ma Y. (2010) Zili inhibits transforming growth factor-beta signaling by interacting with Smad4. *J Biol Chem.* 285(6): 4243-4250.
- Tan CH, Lee TC, Weeraratne SD, Korzh V, Lim TM and Gong Z. (2002) Ziwi, the zebrafish homologue of the *Drosophila* piwi: co-localization with vasa at the embryonic genital ridge and gonad-specific expression in the adults. *Mechanisms Develop.* 119 (Suppl 1): S221-S224.
- Wei C, Salichos L, Wittgrove CM, Rokas A and Patton JG. (2012) Transcriptome-wide analysis of small RNA expression in early zebrafish development. *RNA* 18(5): 915-929.
- Wu PH and Zamore PD. (2021) Defining the functions of PIWI-interacting RNAs. *Nat Rev Mol Cell Biol.* 22: 239-240.
- Zhao J, Sun H, Deng W, Li D, Liu Y, Lu Y, Liu Y, Tao D, Zhang S and Ma Y. (2010) Piwi-like 2 mediates fibroblast growth factor signaling during gastrulation of zebrafish embryo. *Tohoku J Exper Med.* 222(1): 63-68.