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STUDIES ON THE EXPRESSION OF OCT-4 GENE, A STEM CELL MARKER IN THE DEVELOPMENTAL STAGES OF *PTEROPHYLLUM SCALARE* (ANGEL FISH)

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Abstract: The development of mammalian embryos is controlled by regulatory genes, some of which regulate the transcription of other genes. Oct-4 is a transcription factor which is exclusively expressed in embryonic stem cells and possesses the unique property of self-renewal and ability to produce cells of all three embryonic germ layers. Oct-4 gene is a stem cell identification marker and its expression retains the pluripotency in the embryonic stem cells. The objective of this study is to assess the expression of Oct-4 gene, an ortholog of mammalian POU domain, class 5, transcription factor 3 (pou5f3) in the developmental stages of Angel fish model, *Pterophyllum scalare* by reverse- transcriptase PCR (RT-PCR). The results of the present study showed that Oct4 gene is expressed in various stages of the fertilized eggs of *P.scalare viz.,* 2,4,8 and 16–celled stages. The expression of Oct-4 was upregulated from 2-celled to 16-celled stage and down- regulated from the morula stage onwards.

Keywords: Oct4, gene expression, Angel fish, developmental stages.

Introduction

The development of mammalian embryos is controlled by regulatory genes that regulate the transcription of other genes (Scholar et al., 1990). The developmental genes include Oct4, Sox family of genes and Nanog. Oct4 belongs to the POU (Pit-Oct-Unc) transcription factor family and are exclusively expressed in embryonic stem cells. Embryonic stem (ES) cells are undifferentiated cells obtained from premature growing embryos. The distinctive feature of ES cells is the capacity for self-renewal and its ability to form all three embryonic germ layers. ES cells are used to study the growth of pluripotent cells into different cell types, and for discovering the biological functions of genes. Animal ES cells serve as a unique tool in animal genetic manipulation and generation of transgenic animals. Though putative ES cells have been reported from various animal models, only those from mice have been proved to be successful (Bejar et al., 1999). In fishes, embryonic cell cultures have been successfully

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derived from Gilt head bream (*Sparus aurata*) (Bejer et al., 1999), Zebra fish (*Danio rerio*) (Deborah, 1998) and Medaka (*Oryzias latipes*) (Hong, 1996).

There is a strong interest in developing this technology in fish (Alvarez et al, 2007). Vertebrate fishes have proved to be a reliable source for studying cellular, molecular and developmental biology as they are more closely related to humans than yeast, worms or flies. The advantages of fish models include temperature adjustable embryology, transparent accessible embryos, presence of number of molecular markers to facilitate developmental studies and short reproductive cycles (Deborah, 1998). Identification of embryonic stem cells based on expression of stem cell markers like Oct-4, which is exclusively expressed in pluripotent stem cells will help in development of cell lines of our interest (Rosner et al.1990). In the present study, expression of POU-domain containing Oct-4 gene was studied in various developmental stages of angel fish (*P. scalare*) model.

Materials and methods

Collection of Samples

Apparently healthy and sexually matured brooder set of angel fish consisting of a male and female were procured from a local fish farm in Chennai, India. The fishes were acclimatized to the lab conditions and maintained with optimum water quality conditions and *ad-libitum* feeding. Conducive conditions were provided for breeding by introducing a polished slanting slate for the deposition of eggs (Figure 1a and 1b). The fishes were observed continuously and immediately after breeding, samples of eggs were collected at various time intervals and the developmental stages were identified microscopically (Figure 2). The developmental stages and the corresponding time taken to reach various developmental stages were recorded (Table1)

Reverse Transcriptase PCR (RT-PCR)

Total RNA was extracted from the samples using a commercial kit (Biobasic, Canada) following the manufacturer's protocol. cDNA was synthesized from the total RNA (2µg) using a high capacity cDNA synthesis kit (Applied Biosystems, USA). Reverse Transcriptase PCR amplification was carried out using self-designed PCR primer set, Oct4F (Forward-5' TGCTGCAGAAGTGGGTGGAGGAAG3') and Oct4R (Reverse 5'CCGAGCTGCTGGGCGATGTG3') designed based on the sequence of Zebra fish (NC_007132). PCR amplification was carried out in a total volume of 25µl with 1X PCR master mix (Bangalore Genei, India), 40 pmoles each of forward and reverse primers and 50 ng of template DNA with an initial denaturation at 94°C for 2 min followed by 30 cycles at

94ºC for 2min; 94ºC for 45 sec; 55ºC for 45 sec; 72ºC for 30 sec and a final extension at 72ºC for 5min.

Results and discussion

RT-PCR amplification of Oct-4 gene resulted in the expected product of 193bp (Figure 3). PCR amplification of the cDNA from the samples using Oct4 primer showed that the expression of Oct-4 gene was upregulated in 2, 4 and 8 celled stages and reduced thereafter. No detectable Oct-4 expression was observed in the developing embryo. In embryos, the presence of regulatory genes like Oct-4, control the development and transcription of other genes**.** These regulators play a major role during stem cell differentiation. Evidences indicate that Oct-4 is almost exclusively expressed in ES cells (Scholer et al., 1990). Wang et al. (2011) have recorded up- regulation of Oct-4 gene in zebra fish undifferentiated embryonic stem cells and down regulation upon differentiation *in vitro*.

Experiments on mice indicated that Oct-4 is highly expressed in 1 to 2- celled stages and hence is an important factor for gene regulation in early embryos (Palmieri et al., 1994). Oct-4 expression studies (Okamoto et al., 1990) in early mouse embryo showed its presence in the nucleus of all cell stages through the morula stage. Following implantation, Oct-4 expression was limited to the primitive ectodermal cells. Ashok et al. (2009) in mouse embryos showed that Oct-4 was expressed in 8 to 16 celled morula stage. The presence of Oct-4 protein in ES cells first suggested an association with the early stages of mouse embryogenesis.

Oct-4 is also expressed in human ES cells at various developmental stages which include germ cells, whole embryos and embryonic cells. Studies indicate that Oct-4 expression increased by around thirty-fold in the ICM of human blastocysts when compared with trophoectoderm cells, hence serving as a possible tool to create and sustain human ES cells (Palmieri et al., 1994).

The results of the present study provide information on the expression of Oct-4, a stem cell marker in the embryonic stages of angel fish (*Pterophyllum scalare*). A significant number of evolutionarily conserved target genes of pou5 f1 in fish model (Zebra fish) are also involved in stem cell circuit in mammalian ES cell culture (Daria, 2011). Hence, the data from the experiments on fish models have potential to be used for ES cell technology, gene manipulation experiments and transgenic animal generation. Similar to zebra fish, angel fish model offer many advantages like easy maintenance and breeding, larger, visible eggs and easy observation of the developmental stages of eggs due to transparency. The results of this

study show that *P.scalare* angel fish would also be a suitable model for the study of pluripotency *in vivo*.

Conclusions

This study thus, indicates that Oct-4, a stem cell identification marker is expressed in various embryonic developmental stages of *Pterophyllum scalare.* It contributes to stem-ness and its potential to be used for ES cell technology for gene manipulation experiments and transgenic animal generation. Angelfish is an important ornamental fish and the export of such fishes increases the economy of country. Studies on oct-4 expression in tropical fish is limited to zebrafish. As angelfish model also shows the suitability can be taken up as a model for future studies by researchers for ES cell technologies.

List of figures

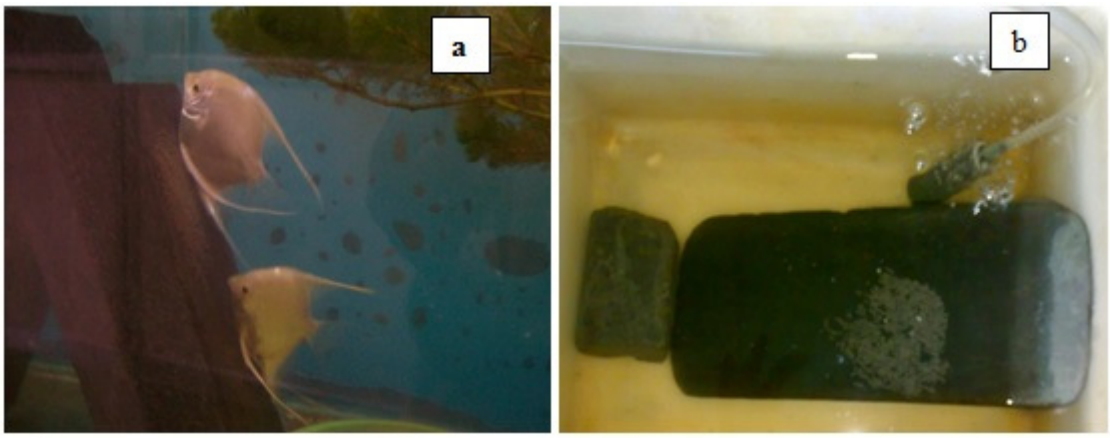

Figure 1: (a) Breeding of Angel fish (b) Polished slanting slate for the deposition of eggs.

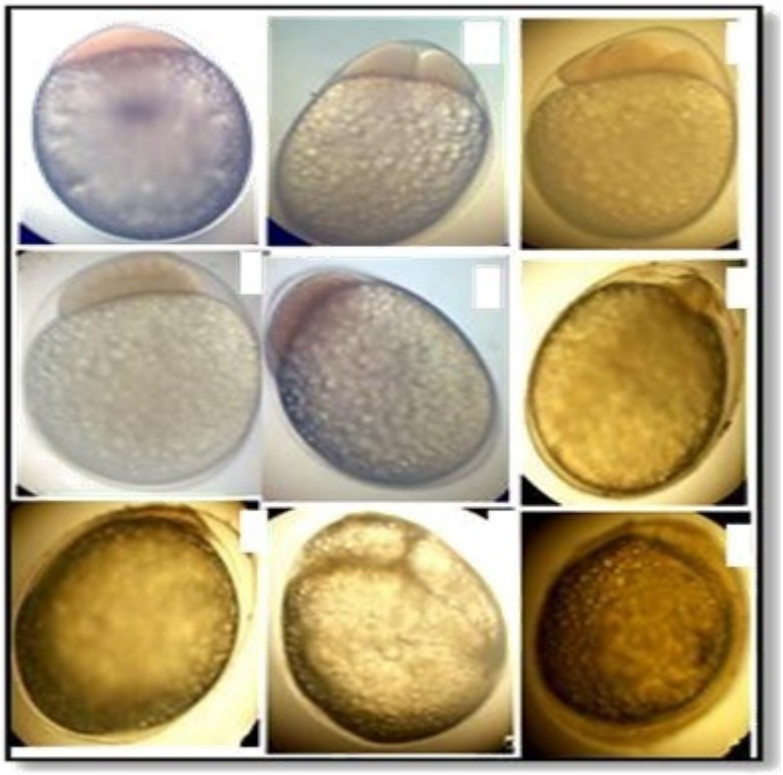

Figure 2: Microphotograph of the various developmental stages of Angel fish (10 X magnification); (1,2 3- 2,4 and 8 celled stages; 4,5 and 6- 16, 32 and multicellular stages; 7, 8 and 9- Embryonic developmental stages

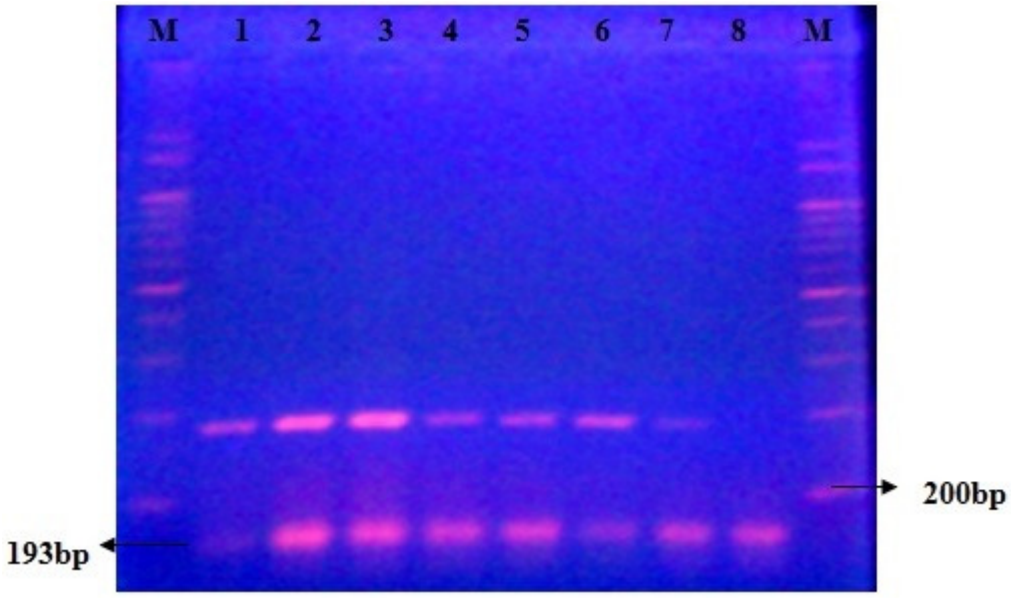

Figure 3: Expression of oct-4 gene in the developmental stages of angel fish
Lanes: M - 100bps marker. **Lane 1**:2 celled; **Lane 2**:4 celled; **Lane 3**:8 celled; **Lane 4**:16 celled; **Lane 5- 32 celled; Lane 6: Morula 7**: Gastrula **Lane 8**: developing embryo

Table 1. Developmental stages of *Pterophyllum scalarae.*

S.no.	Developmental stage	Time after spawning H mins
1	2-celled	01 45
2	4-celled	02 30
3	8-celled	03 15
4	16-celled	04 30
5	32 celled	06 45
6	Morula	27
7	Gastrula	30
8	Developing embryo	60 h

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