ESTABLISHMENT OF GERMLINE-SPECIFIC FLUORESCENT FRESHWATER ANGELFISH FOR TRACKING AND NUMBERS OF PRIMORDIAL GERM CELLS INVOLVED IN SEX DEVELOPMENT

Hong-Yi Gong[1,2*], Wai-Kwan Chu[1], Shih-Chin Huang[1], Ching-Fong Chang[1,2]

[1] Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan. [*] hygong@mail.ntou.edu.tw

[2] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan.

Introduction

The first cell fate determination in fish happens in the segregation of presumptive primordial germ cells (PGCs) and somatic cells in early embryogenesis. The PGCs are first located in a different position, whereas in the development of embryos, the primordial germ cells will migrate and localize to the genital ridge. The formation of primordial germ cells in fish had been proved as a preformation mechanism, however, the migration pathways and localization position of the genital ridge are not conserved between species. From the previous studies, it has been shown the 3' untranslated region (UTR) of several maternal factors of germplasm, such as *ddx4* , *nanos3* and *piwi1* are critical for mRNA stabilization. Synthesized mRNA of fluorescent protein fusion with the 3'UTR of maternal germline gene and microinjected into fish embryos are able to label and visualize the PGCs in fish. In this study, we employed a *Tol2*-mediated transgenesis system to label the PGCs of freshwater angelfish (*Pterophyllum scalare*) to reveal the migration pathway. Due to the lack of information and references of angelfish from public databases on the genome, we selected to construct the expression vector by references to the genome of Nile tilapia to amplify the tilapia *ddx4* 4.5 kb promoter to express CFP cDNA linked with a tilapia *nanos3* 1 kb 3'-UTR, to analyze the ability to track the migration behavior of PGCs in freshwater angelfish. These findings contribute valuable insights into the dynamics of PGC migration and regulation in freshwater angelfish as a cichlid model.

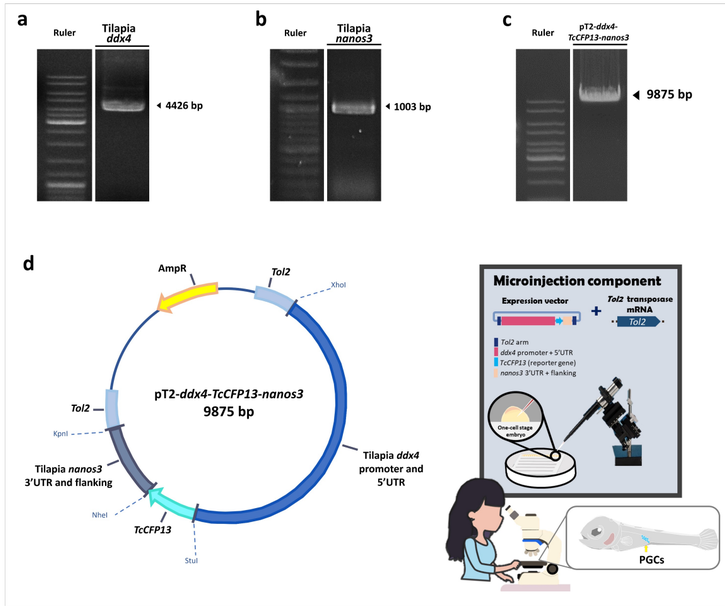


FIGURE 1. Establishment of PGCs-specific transgenic labeling system.
(a) Gel electrophoresis analysis of a 4.5 kb fragment amplified from the genome of Nile tilapia, including the promoter and 5'UTR region of the ***ddx4/vasa*** gene (b) a 1 kb fragment amplified from 3' region of **nanos3** gene in Nile tilapia (c) The confirmation of constructed expression vector **pT2-*ddx4-TcCFP13-nanos3***. (d) The schematic illustration of the transgene expression vector and the application processes of fish line establishment.

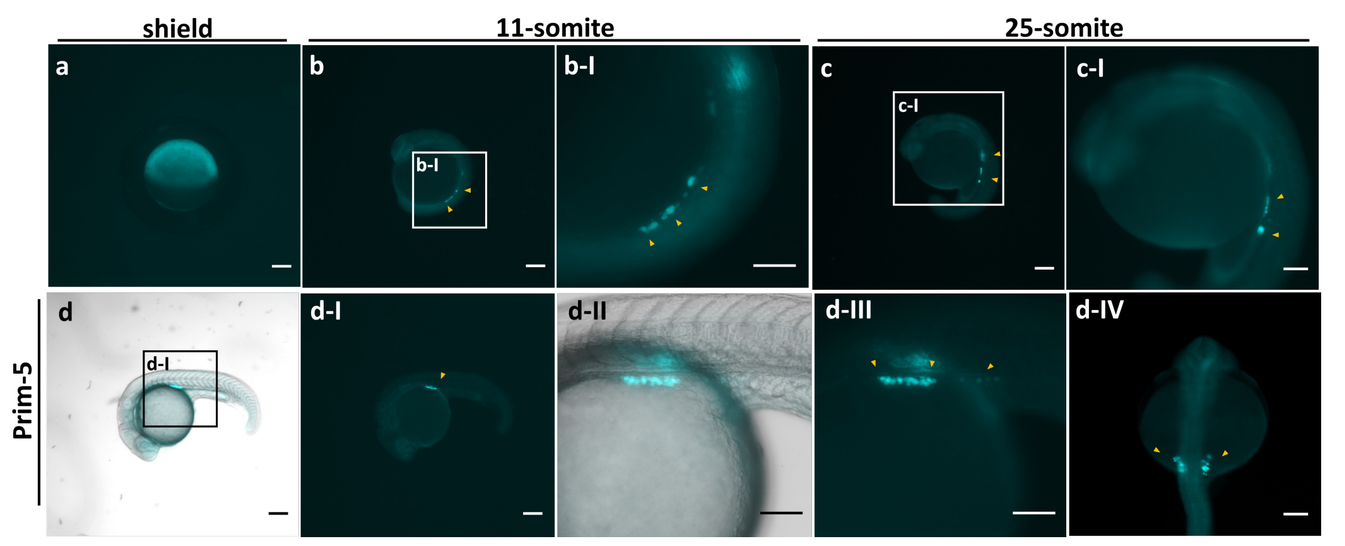


FIGURE 2. Micrograph visualizing the PGCs migration in Tg(*ddx4:TcCFP13-nanos3*) transgenic zebrafish. Arrows in yellow indicate the cyan fluorescent signal positive cells. Bar: 200µm (d), 100µm (a, b, c).

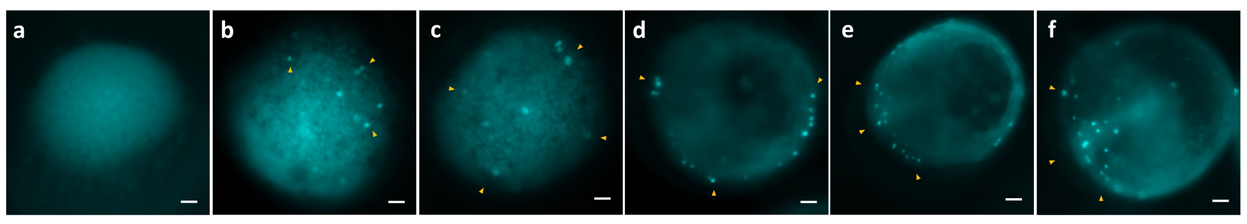
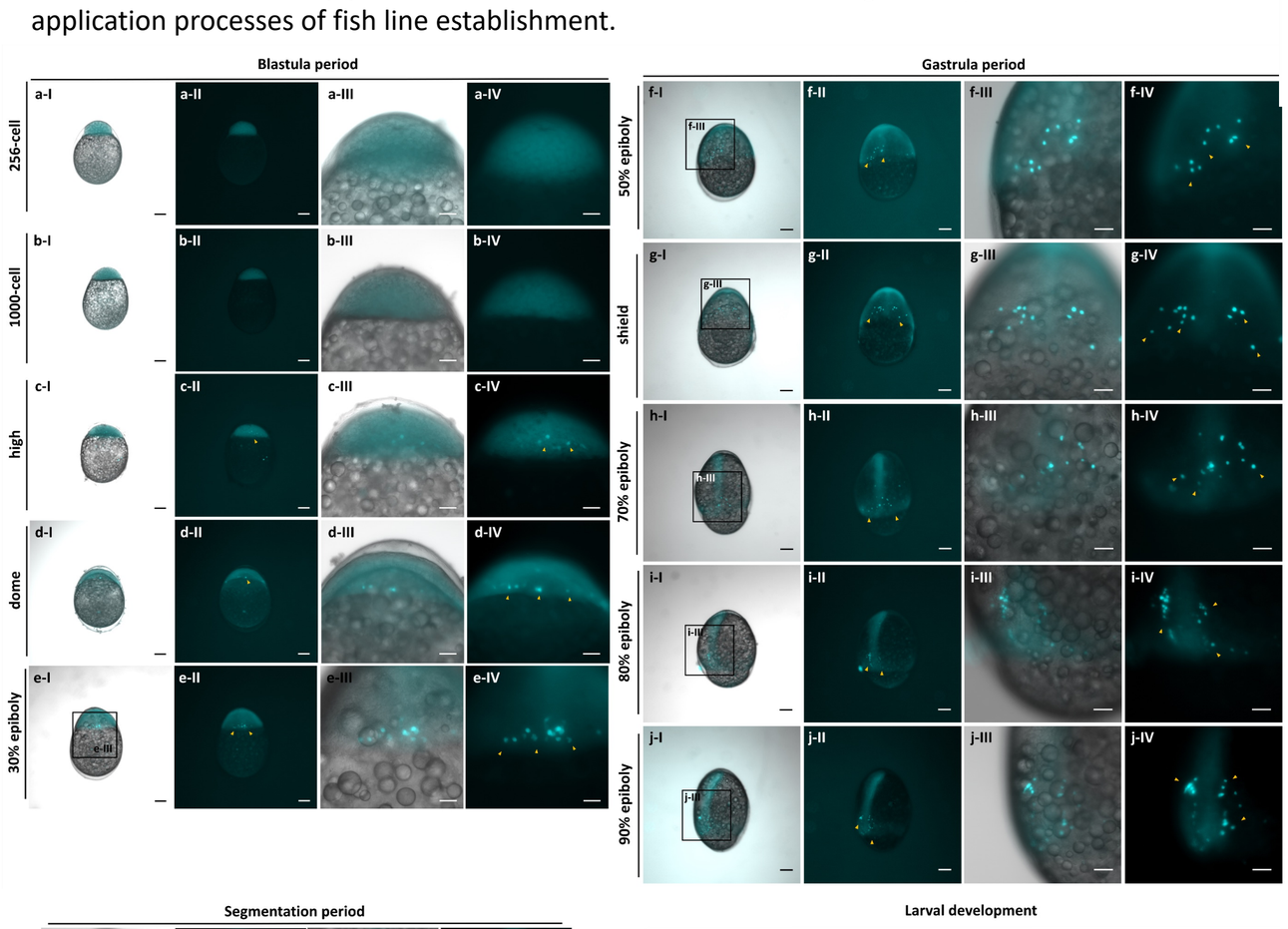


FIGURE 3. Micrograph visualizing the PGCs migration in animal pole view during early embryonic development periods in transgenic freshwater angelfish Tg(*ddx4:TcCFP13-nanos3*). The cyan fluorescent signal present in specific cells were firstly observed from 1000-cell stage of embryonic development in freshwater angelfish. Arrows in yellow indicate the PGCs in freshwater angelfish. (a) 512-cell stage, (b) 1000-cell stage, (c) high stage, (d) dome stage, (e) 50% epiboly, (f) germ ring stage. Bar: 100µm



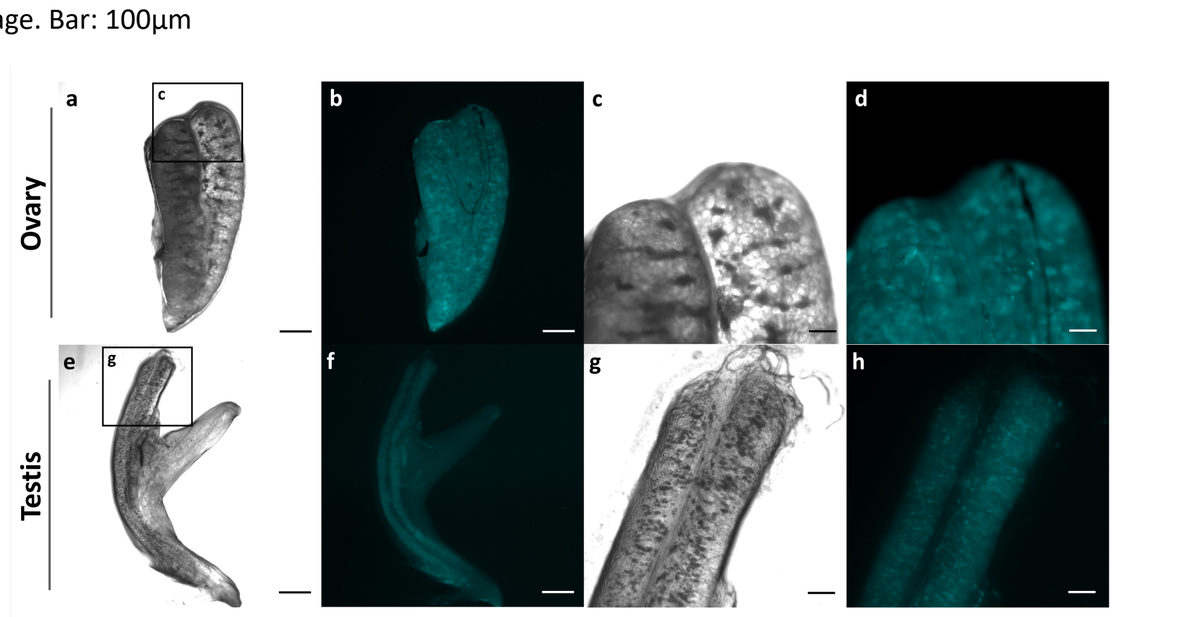


FIGURE 5. Micrograph visualizing the differentiated gonads of juvenile transgenic freshwater angelfish Tg(*ddx4:TcCFP13-nanos3*). Cells-like expression pattern indicated the germ cells were labeled. Bar: 1000µm (a, b, e, f), 200µm (c, d, g, h).

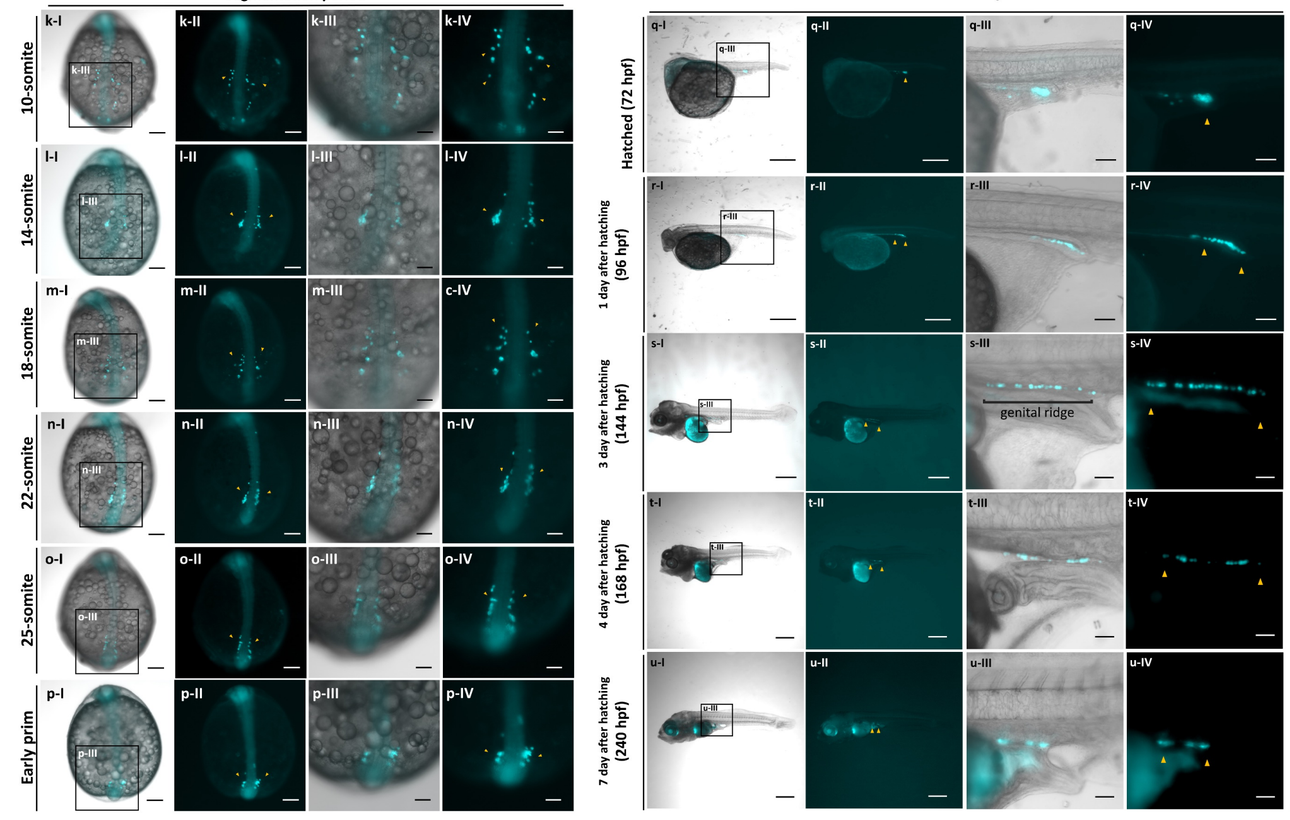


FIGURE 4. Micrograph visualizing the complete PGCs migration process in transgenic freshwater angelfish Tg(*ddx4:TcCFP13-nanos3*). The complete migration route and cell behaviors of PGCs were labeled successfully until larval stage. Arrows in yellow indicate the PGCs in freshwater angelfish. Micrograph of embryonic development period, including (a)-(e) lateral view of embryo during blastula period, (f)-(j) dorsal view of embryo during gastrula period, (k)-(p) dorsal view of embryo during segmentation period, (q)-(u) side view of embryo during larval development. Bar: 500µm (q)-(u) I-II, 200µm (a)-(p) I-II, 100µm (a)-(u) III-IV.

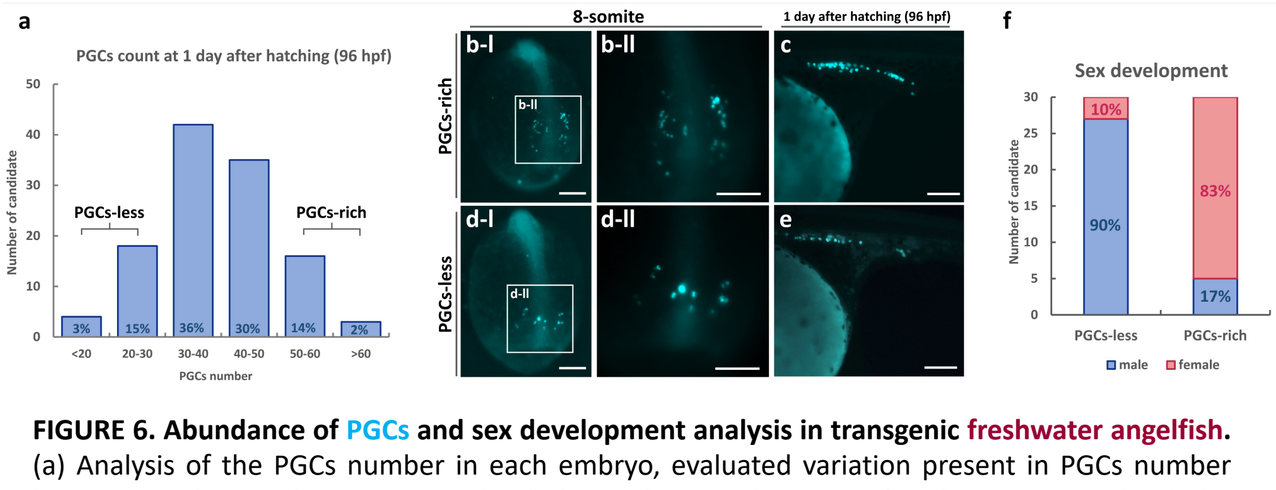
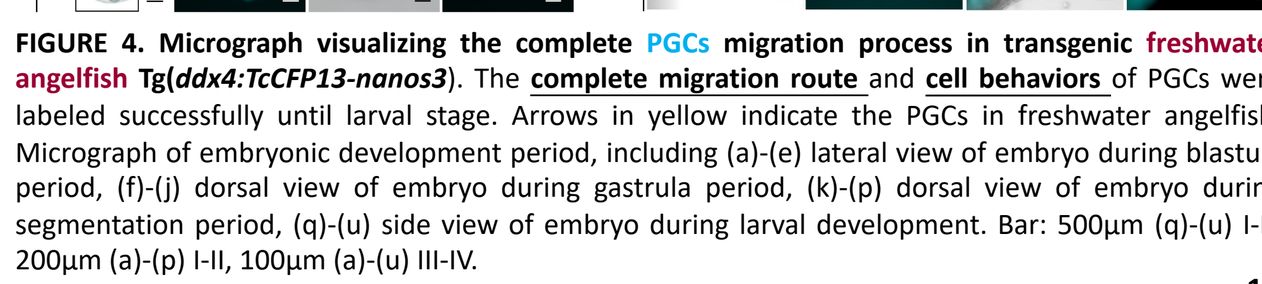


FIGURE 6. Abundance of PGCs and sex development analysis in transgenic freshwater angelfish. (a) Analysis of the PGCs number in each embryo, evaluated variation present in PGCs number between embryos. (b-c) Micrograph of the embryo with PGCs-rich. (d-e) Micrograph of the embryo with PGCs-less. (f) Sex ratio analysis was proceeded in the mature stage of the individuals in both groups. Bar: 200µm (b, c, d, e).



Conclusion

We successfully established a germline-specific transgenic line to visualize the *in vivo* migration of PGCs in freshwater angelfish. The abundance of PGCs was found to be highly correlated with the sex development in freshwater angelfish. Our investigation also revealed multistage migration activities that exhibited variations compared to the model species, zebrafish. Here are key findings:

1. **Extended migration period:**
   - In zebrafish, PGCs complete their localization into the genital ridge within 24 hpf during embryogenesis. In contrast, PGCs in freshwater angelfish continue their migration and achieve localization within 168 hpf (4 dpf).
2. **Conserved migration characteristics:**
   - Early migration of PGCs in both freshwater angelfish and zebrafish demonstrates conserved cell migration characteristics. Rotation movements along the blastodisc margin and migration towards the dorsal side are highly similar.
3. **Unique migration route and cell migration characteristic:**
   - Freshwater angelfish PGCs exhibit a traverse movement, passing through the embryo with the middle of the dorsal and tail bud direction between two sides of the dorsal. This is a unique characteristic not observed in zebrafish. Significant differences in the principles of cell cluster movement were defined between the high stage to 12-somite and 16-somite to complete localize stages. A disciplined migration characteristic is observed in the later period of PGCs migration in freshwater angelfish.
4. **Complex regulation mechanisms:**
   - We hypothesize that PGCs in freshwater angelfish are governed by complex regulation mechanisms. This is evidenced by their continuous migration towards the genital ridge during organogenesis, including intestinal system development.
5. **Opportunities:**
   - The longer period of PGCs migration in freshwater angelfish provides researchers with more opportunities to study and conduct reverse genetics approach on PGC biology and application to infertility control of transgenic ornamental fish and edible fish.