Immunofluorescent biomarkers for distinguishing cell phenotypes in zebrafish somitogenesis and autonomous cellular oscillators

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Abstract

During zebrafish embryogenesis, coordinated genetic oscillations occur in a population of cells in the posterior-most tissues of the body axis, the tailbud and presomitic mesoderm (PSM), which will subdivide the embryonic body into morphological segments, called somites. It has been proved previously that single cells dispersed from tailbud will oscillate automatically. However, it remains unclear that which phenotype of the cells will present as autonomous oscillators. T-domain transcription factors Ntla and Tbx16 will both express in the period of somitogenesis but in different regions. Immunofluorescence experiments for both genes demonstrated the distribution of cells in different phenotypes in zebrafish embryo during somitogenesis. Comparison of immunofluorescence results for 5-somite stage embryos and high-somite stage embryos showed the change of PSM region. Combined with results for single-cell oscillation and statistical analysis, immunofluorescence for cell dispersals was able to tell the phenotypes of the oscillating cells.

1. Introduction

During zebrafish vertebrate embryogenesis, coordinated genetic oscillations occur in the tailbud and presomitic mesoderm (PSM) of the embryo. These oscillations generate a rhythmic spatial pattern. This "segmentation clock" is thought to subdivide the embryonic body into morphological segments, called somites[1].

It is discovered that there are many phenotypes of cell existing during somitogenesis, Progenitor cells, PSM cells and Somite cells. Making use of a transgenic zebrafish reporter line for the cyclic transcription factor *Her1*, Alex B Webb et al observed that single cells made from zebrafish tailbud were able to behave like cell-autonomous oscillators [2]. Previous work at Yang Lab showed that oscillating cells only made up a small proportion of the whole cells. To distinguish these different phenotypes, we performed immunofluorescence experiments on both embryos and cell dispersals.

Based on Webb's protocols and other former researches, two genes, T-domain transcription factors *Ntla* and *Tbx16,* were decided to be immunostained. During somitogenesis, *Ntla* expression is confined to the notochord and tailbud, while *Tbx16* is expressed in the tailbud, presomitic mesoderm and adaxial cells [3]. By immunostain these two genes, it could be able to distinguish cells in different phenotypes, which are

- PSM Cells;
- Somite;
- Progenitor Cells.

2. Materials and Methods

Ntla antibodies: anti-Ntla antibody produced in rabbit (Sigma-Aldrich), Goat Anti-Rabbit IgG H&L (Alexa Fluor® 405) preadsorbed (Abcam) ;

Tbx16 antibodies: anti-Tbx16 Mouse IgG2a (Zebrafish International Resource Center /ZIRC), Zenon™ Alexa Fluor™ 594 Mouse IgG2a Labeling Kit (ThermoFisher).

Methods: Zebrafish embryos and cell dispersals prepared from tailbud tissues are conducted through such procedures:

1. Fixation-2 hours for embryos, 20 minutes for cell dispersals;
2. Blocking-1 hour for embryos, 10 minutes for cell dispersals;
3. Primary antibody staining-2 hours for embryos, 1 hour for cell dispersals;
4. Secondary antibody staining-2 hours for embryos, 1 hour for cell dispersals;

Acknowledgements

The presenter is supported by the Pilot Scheme of Talent Training in Basic Sciences (Boling Class of Physics, Nankai University), Ministry of Education.
This work was supported by and finished at Yang Lab from the Department of Biophysics, University of Michigan.

3. Results and Discussion

Immunofluorescence Experiment Results for Tbx16

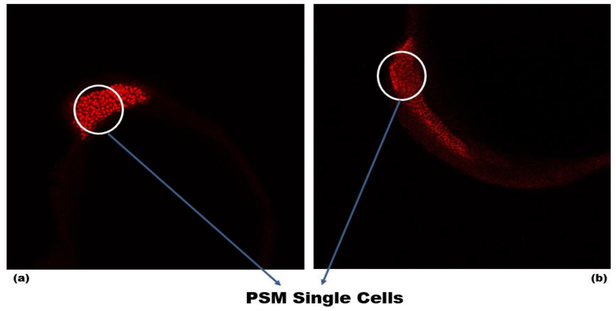


Figure 1. Tbx16 Immunofluorescence Imaging-lateral view

At First, we only performed *Tbx16* one-color immunofluorescence to test the markers.

The Figure 1 clearly shows that the Tbx16 biomarker had successfully marked some of the cells, and the single cells were clearly showed in the same figure. However, it is still hard to distinguish if these cells are PSM cells. As both of the images are token in lateral view, it was unable to see the notochord. So, the solution was to use the confocal microscope to scan the embryos in different height and take some z-stack images.

Besides, it was also a good idea to use agarose to fix the orientation of the embryos to make them to dorsal view. It should be easy to observe the notochord in dorsal view if the immunostaining was successful.

We performed z-stack imaging using confocal microscopy after fixing the embryos to dorsal view by hydrogel. Bright field in Figure 2(a) clearly showed that the orientation was dorsal view. And Figure 2(b) showed that there was a region with no fluorescent signal existed between two regions with fluorescent signal, which means that both the notochord and PSM region were clearly observed. These experiments told me that the marker and immunofluorescence labeling for PSM cells are quite successful.

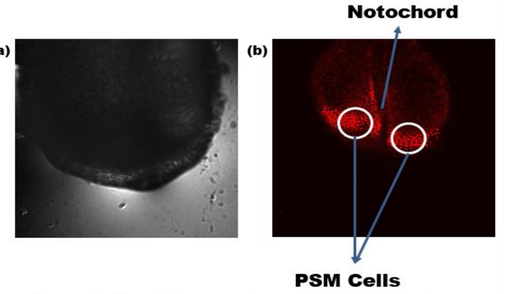
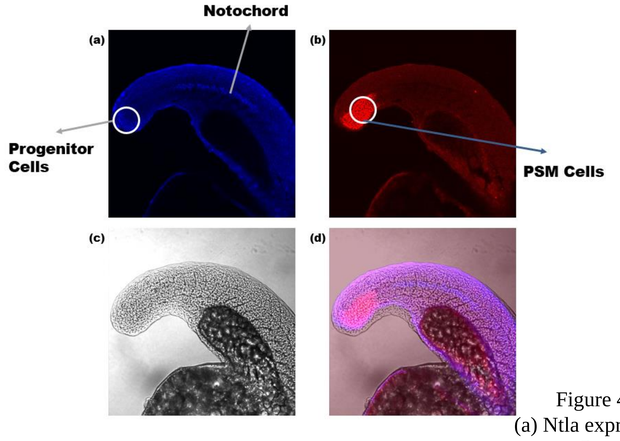


Figure 2. Tbx16 Immunofluorescence-dorsal view.
(a) bright field, (b) fluorescent signal

Immunofluorescence Experiment Results for Two-color Staining



We conducted two-color immunofluorescence labeling on some embryos that had already been in later period of somitogenesis to see if there would be any difference. The embryos chosen were in 22-somite stage.

Figure 4 shows the results for two color staining. In figure 4(a), the notochord can be clearly observed, which means the Ntla immunofluorescence labeling was successful. Meanwhile, figure 4(b) shows that PSM cells only took up a little proportion of the tailbud in later-period-somitogenesis embryos. This result somehow verified our consideration that the phenotypes of the cells may change during zebrafish somitogenesis.

Figure 4. Two-color staining for 22-somite embryo.
(a) Ntla expression, (b) Tbx16 expression, (c) bright field, (d) fluorescent signal overlapped with bright field

References

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