# $Ca^{2+}$ dynamics in zebrafish hyper-dorsalization of congenital anomaly

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**Running head:** $Ca^{2+}$ dynamics in abnormal morphogenesis

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## Abstract

Intracellular calcium ion ($Ca^{2+}$) signaling is deeply involved in development, as demonstrated by the use of various $Ca^{2+}$ sensors. $Ca^{2+}$ patterns in wildtype and mutant zebrafish have been reported to date, however, not many zebrafish embryos with congenital anomaly have been studied yet. In the present study, we monitored serial $Ca^{2+}$ levels of zebrafish embryos which showed inborn hyper-dorsalization using $Ca^{2+}$ sensor, yellow cameleon, YC2.12. Our results showed clearly elevated $Ca^{2+}$ levels in the presumptive dorsal part during gastrula period and in the dorsal region during segmentation period. The results suggested that monitoring $Ca^{2+}$ levels may help to anticipate the region which influences a fate determination prior to morphogenesis.


## Introduction

Intracellular calcium ions ($Ca^{2+}$) is very important in morphogenesis (Niki et al., 1996; Berridge, Lipp & Bootman, 2000; Slusarski & Pelegri, 2007). To date, consecutive $Ca^{2+}$ dynamics of zebrafish normal development was reported with fluorescent yellow cameleon YC2.12 (Tsuruwaka et al., 2007 & 2016). We also reported that morphological changes brought about dramatic transition in $Ca^{2+}$ signaling (Tsuruwaka, Konishi & Shimada, 2015). However, $Ca^{2+}$ levels in the abnormal development have been unclear. Congenital abnormality occurs at a certain rate in developmental zebrafish; for example, hyper-dorsalization induced embryos were born at a rate of 0.0017% in our laboratory. Hyper-dorsalization embryos are caused by mutations such as *smad1*, *smad5*, *kheper*, *tkk* etc (Dick, Meier & Hammerschmidt, 1999; Muraoka et al., 2000; Nojima et al., 2010). The purpose of the present study was to analyze serial $Ca^{2+}$ patterns for morphogenesis in hyper-dorsalization using cameleon.



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## Materials and Methods

### Zebrafish and $Ca^{2+}$ imaging

Experiments were conducted as previously described (Tsuruwaka et al., 2007; Tsuruwaka, Konishi & Shimada, 2015). Briefly, 3 nL of synthetic YC 2.12 mRNA (0.5 ng/mL) was injected into blastodiscs of each single-cell embryo. After YC2.12 had conformed to be distributed ubiquitously in the whole embryo, FRET analyses were performed as followed. Fluorescence images were obtained using a Zeiss Axiovert 200 microscope equipped with a combination of two filters, i.e., CFP-CFP, YFP-YFP, and CFP-YFP filters (Carl Zeiss, Oberkochen, Germany). Amplification and numerical aperture of the objective lens were 5× and 0.16, respectively. An AxioCam MRc5 camera (Carl Zeiss) was used to photograph the images, and the image analysis was performed using Axiovert FRET version 4.4 software (Carl Zeiss). Fluorescence was quantified following the manufacturer's instructions. The experiments were performed for total 30 times. No approval was required to conduct studies on fish according to the Ministry of Education, Culture, Sports, Science and Technology, Notice No. 71 (in effect since June 1, 2006).

## Results and Discussion

### $Ca^{2+}$ dynamics during zebrafish morphogenesis

Hyper-dorsalized embryos displayed development of only dorsal-posterior side and defective head formation, which had induced by abnormal function of Wnt pathway in zebrafish and Xenopus (Nojima et al., 2004). Hyper-dorsalization induced embryos were born at a rate of 0.0017% in our laboratory (**Fig. 1**). $Ca^{2+}$ patterns showed dynamic changes during morphogenesis, especially dorsal formation (**Fig. 2**). $Ca^{2+}$ signaling is essential in the



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determination of dorsal axis (Schneider et al., 2008). To support, at 50%-epiboly stage, an elevated $Ca^{2+}$ level was observed in the presumptive dorsal region (**Fig. 2**). Since the altered $Ca^{2+}$ level was observed at early developmental stages prior to morphogenesis, monitoring of $Ca^{2+}$ dynamics may assist to predict the region which influences a fate determination.

## Conclusions

$Ca^{2+}$ patterns in the hyper-dorsalized embryo showed dynamic changes, especially the dorsal side in gastrula and segmentation periods.

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## Competing Interests

The authors declare there are no competing interests.



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## Author Contributions

• Yusuke Tsuruwaka conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures, reviewed drafts of the paper.

• Eriko Shimada performed the experiments, analyzed the data, wrote the paper, reviewed drafts of the paper.

## Animal Ethics

No approval was required to conduct studies on fish according to the Ministry of Education, Culture, Sports, Science and Technology, Notice No. 71 (in effect since June 1, 2006).

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**Figure 1**

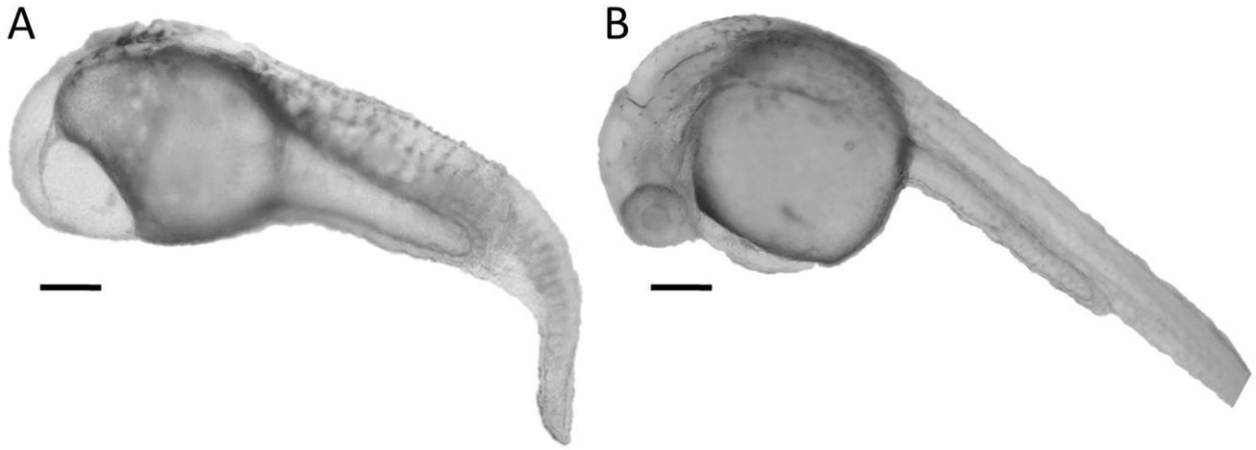


**Fig. 1. Phenotype of hyper-dorsalization in zebrafish embryo at prim-10 stage.** Lateral view with anterior to the left. Abnormal anterior formation was shown in (A) hyper-dorsalized embryo. (B) wildtype; Scale bars, 200 µm.



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**Figure 2**

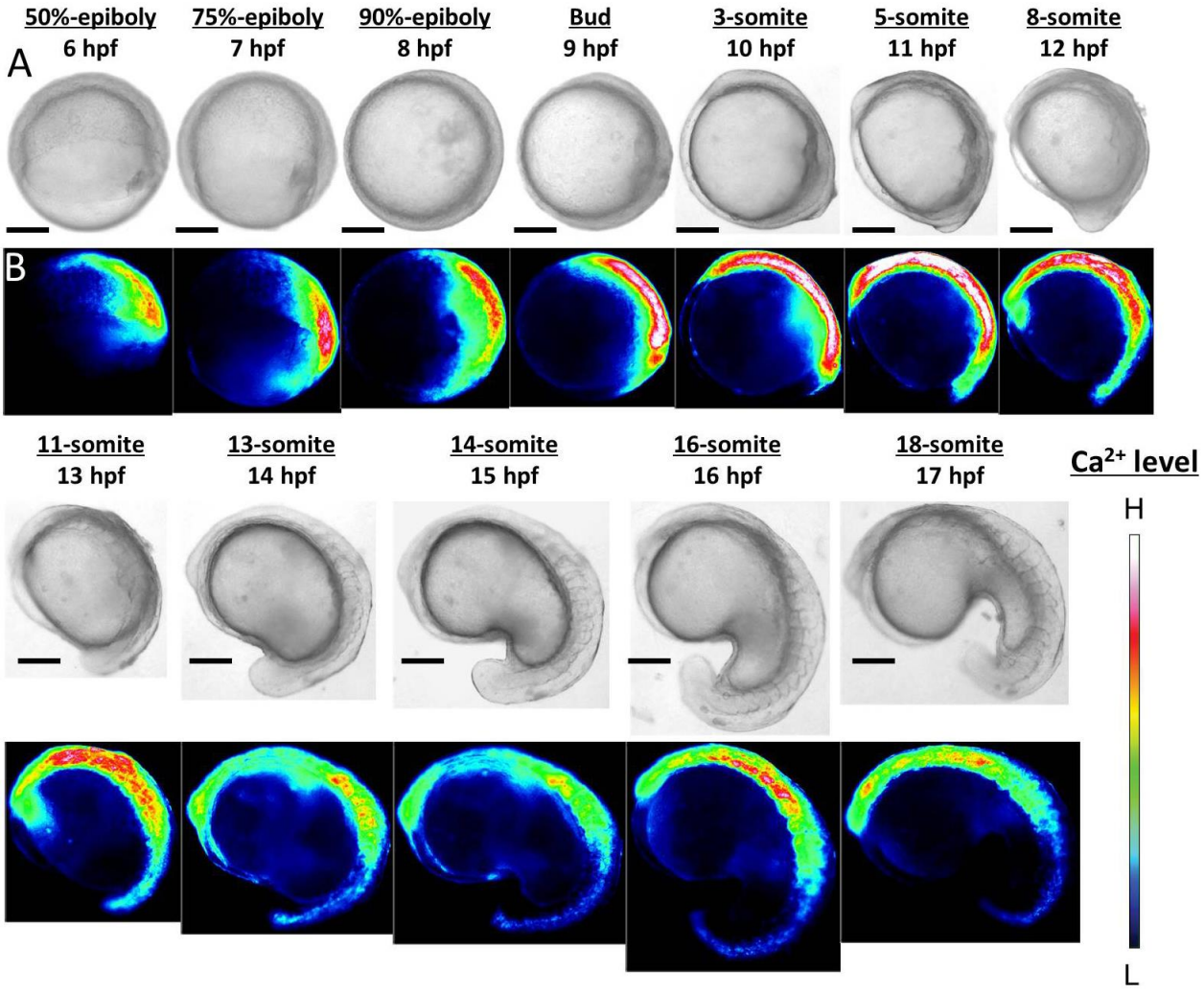


**Fig. 2. $Ca^{2+}$ dynamics of the hyper-dorsalized embryos in the gastrula and segmentation periods.** (A) bright field image; (B) color-coded image; scale bar, 200 μm. The color-coded image shows $Ca^{2+}$ levels as white (high $Ca^{2+}$) and blue (low $Ca^{2+}$).



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