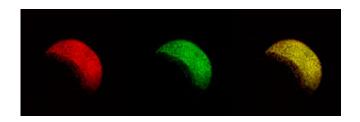


Imaging live zebrafish embryos reveals in real time how the basic body plan is laid out

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Zebrafish embryo expressing fluorescently-labeled Oct4 at gastrula stage.. Credit: M. Perez-Camps et al.

A team from A*STAR's Institute of Medical Biology and Institute of Molecular and Cell Biology in Singapore show how the gene-regulating proteins Pou5f3 and Nanog determine the organization of body structures in zebrafish embryos. Their work shows how precise the orchestration of molecular events behind normal embryonic development, and why it can easily go wrong.

Small and transparent zebrafish embryos are an increasingly popular model organism for imaging the earliest stages of animal development. The first step in laying down an animal's body plan occurs when a simple ball of embryonic cells form three distinct layers—the ectoderm, mesoderm and endoderm—in a process called gastrulation. The regulation of genes by proteins called 'transcription factors' is crucial for instructing cells to form these layers and for their subsequent differentiation into specialized cells that form the body tissues.



Using the latest imaging technologies: Fluorescence Lifetime Imaging Microscopy (FLIM) and Fluorescent Correlation Microscopy (FCS), the authors tracked the activity of fluorescently-labeled Pou5f3 during gastrulation, in living embryos. "These state-of-the-art techniques, allow us to better assess the dynamic changes that drive stem cell specifications in vivo" explained Bruno Reversade who led the study.

The team found the highest levels of DNA-bound (active) Pou5f3 in mesodermal cells where it also interacted with Nanog. The Pou5f3–Nanog complexes were restricted to a particular area of the mesoderm and removal of either Pou5f3 or Nanog disrupted the formation of distinct ectoderm, mesoderm and endoderm layers. These findings suggest that the Pou5f3–Nanog complex is required for specifying the cells that form these layers and thus, the development of tissues that will eventually form the top side and under side of the fish.

They also show that the activity of the Pou5f3–Nanog complexes is restricted by the transcription factor Sox32, which competes with Nanog for Pou5f3 binding in the endoderm.

Interestingly, results in mutant zebrafish suggest that the conserved hormone elabela controls levels of Sox32, allowing the formation of Pou5f3–Nanog complexes and the expression of genes involved in bone morphogenetic protein (BMP) signaling, which is essential for tissue specification.

Together these findings highlight a new mechanism through which Pou5f3–Nanog complexes modulate BMP activity during early development. The tight regulation of transcription factors described in this study is likely to be conserved across vertebrates.

More information: Mireia Perez-Camps et al. Quantitative imaging reveals real-time Pou5f3–Nanog complexes driving dorsoventral



mesendoderm patterning in zebrafish, *eLife* (2016). <u>DOI:</u> 10.7554/eLife.11475

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