Kinetics of Zebrafish Dorsoventral Patterning

Ben M. Jordan[1], Patrick Müller[1]

[1]Harvard University

Abstract

The specification of cell types and morphogenesis of many biological systems are regulated by the concentrations of signalling molecules (Wolpert 1969). Many systems employ a pair of secreted short-range activators and long-range inhibitors, and these are widely used to generate complex patterns during development (Turing 1954, Mienhardt and Gierer 1979). However, the biophysical mechanisms that regulate the different ranges of activators and inhibitors have been elusive. Here, we examine the reaction kinetics and diffusivities of the Nodal/Lefty activator/inhibitor pair in sphere-stage D. rario (zebrafish) embryos to address this question. We show that the distribution profiles of fluorescent Nodal and Lefty proteins correlate with their observed activity ranges and developmental phenotypes. Using Fluorescence Recovery After Photobleaching (FRAP) microscopy assays combined with finite element method (FEM) simulations (Comsol, MATLAB, Tecplot) in realistic and embryo-specific geometries, we determine the effective diffusitivies and reaction kinetics of Nodal and Lefty. We examine several possible linear kinetics models and geometric simplifications, and find that oversimplification of the geometry can have a significant result on the resulting parameter fits, due to diffusive flux through the slice volume. Regardless of our choice of kinetics model, we find that Leftys have higher effective diffusivities than Nodals. The effective diffusitivities measured by FRAP are compared with those measured by Fluorescence Correlation Spectroscopy (FCS) by approximating the effects of cell-scale geometric and viscous tortuosities of molecules diffusing in the extracellular space. Additionally, we compare the production and degradation rates determined by FRAP with values measured using pulse-chase analysis of photoconvertible fusion proteins. Our results imply that Nodals are cleared more rapidly from the extracellular space than Leftys, and that differential diffusivity and clearance account in large part for the different activity ranges of Nodals and Leftys.

Reference

Lewis Wolpert, Pattern Formation
Alan Turing, Chemical Basis of Morphogenesis, (1954)
Gierer, A. and H. Meinhardt, A theory of biological pattern formation. Kybernetik 12, 30-39 (1972).