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# In Search of Turing In Vivo: Understanding Nodal and Lefty Behavior

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The Turing reaction-diffusion model proposes that short-range activators and long-range inhibitors can generate complex patterns. In a *Science* study, Müller et al. (2012) assess behavioral determinants of Nodal and Lefty, TGFβ-related molecules that constitute an activator/inhibitor system, and provide evidence that the factors indeed form a reaction-diffusion system.

Who could have predicted that a mathematical model created 60 years ago to provide a mechanistic basis for explaining how autonomous spatial patterns form would have such large impacts on developmental biology decades later? The reaction-diffusion model, developed by Alan Turing (Turing, 1952) and later refined by Hans Meinhardt (Meihardt, 1982), proposes that two diffusing and interacting molecules, one acting as an activator while the other acts as its inhibitor, can create a network based on short-range activation and long-range inhibition to generate complex spatial patterns (Kondo and Miura, 2010). To create this network, the two molecules must fulfill two requirements. First, the activator must stimulate the production of itself and of the inhibitor. Second, the inhibitor must diffuse faster than the activator. The reaction-diffusion model remained little more than theoretical musings for a long time, but the discovery of its relevance to the determination of skin pigmentation patterning in fish (Kondo and Asai, 1995) brought renewed interest from developmental biologists.

Of the known molecules key to developmental regulation, one of the best candidates proposed to form an activator-inhibitor pair conforming to the reaction-diffusion model are two TGFβ-related factors, Nodal and Lefty. Indeed, the Nodal/Lefty system exhibits short-range amplification and long-range inhibition behavior, consistent with that predicted by the reaction-diffusion model. This behavior of the system has been suggested to play an essential role in left-right symmetry breaking during mouse embryogenesis by converting an initial small difference between the left and right sides into a robust asymmetry (Nakamura et al., 2006). In a recent study published in *Science*, Müller et al. (2012) directly examine the distribution and behavior of Nodal and Lefty molecules during zebrafish embryogenesis to directly test whether they form a true reaction-diffusion system and to assess the overall relevance of the reaction-diffusion model to the regulation of this biological process.

Nodal and Lefty, both secreted factors, control key events during embryonic patterning, such as left-right asymmetry, formation and patterning of the mesoderm (mesoendoderm), and establishment of anterior-posterior polarity (Shen, 2007). Nodal is a typical TGFβ-related factor that can transduce signals through its receptors and intracellular effectors, whereas Lefty is an atypical TGFβ-related factor that is twice as large and inhibits Nodal signaling through multiple ways such as competitive binding to Nodal receptor. In addition, the expression of both genes can be regulated by Nodal itself. Thus, *Nodal* and *Lefty* genes have similar Nodal-responsive (FoxH1-dependent) enhancers in the intron and 5′-upstream region, respectively, that mediate positive and negative feedback regulation. Although Nodal and Lefty fulfill the first requirement of the reaction-diffusion model—that the activator stimulates the production of itself and of the inhibitor—it was unclear whether they satisfy the second requirement regarding their relative diffusion rates. Previous work has provided indirect evidence suggesting that Lefty, the inhibitor, travels faster than Nodal, the activator (Sakuma et al., 2002). However, the exact behavior of secreted Nodal and Lefty proteins and their regulation remained unknown.

Differential diffusion rates between the two components of a reaction-diffusion system can be achieved by at least two mechanisms: the inhibitor may undergo slower clearance (slower degradation) than the activator, or the inhibitor molecule may move faster than the activator. To assess what parameters determine the behaviors of Nodal and Lefty proteins in vivo, Müller et al. (2012) directly examined the distribution, clearance (half-life), and diffusivity of Nodal and Lefty proteins in the zebrafish embryo. To determine distribution, Nodal-GFP or Lefty-GFP fusion protein was expressed in a local region of the embryo, and the level of GFP fluorescence was determined at various distances from the source of expression. The authors found that Lefty proteins exhibited a distribution over a longer distance than Nodal. To examine extracellular clearance of Nodal and Lefty proteins, the authors developed a carefully designed assay based on pulse labeling enabled by Nodal and Lefty proteins fused with a green-to-red photoconvertible protein Dendra2. The half-life of a protein can then be estimated by monitoring the level of red signal after photoactivation. Müller et al. (2012) observed that Lefty proteins were slightly more stable than Nodal proteins, but the difference in the half-lives alone was too small to explain the difference in protein distribution. Finally, they measured the diffusion coefficients of Nodal and Lefty proteins by fluorescence recovery after photobleaching. Lefty proteins were found to diffuse much faster than Nodals.

When normalized by the clearance rate, Lefty proteins travel about 14 times faster than Nodals. This difference is large enough compared with the theoretically sufficient difference between inhibitor and activator diffusion rates postulated by previous models (Kondo and Asai, 1995; Nakamura et al., 2006) to suggest that differential diffusivity is the major determinant of differences between Nodal and Lefty behaviors.

These observations that support the conclusion that differential diffusion rates are a key parameter in determining Nodal/Lefty in vivo distribution and thus contribute to their roles in short-range activation and long-range inhibition also nicely explain previous observations made in embryos. When left-right asymmetry is established in the lateral plate mesoderm (LPM) (Figure 1) (Nakamura et al., 2006), for example, an initially low level of *Nodal* expression in the left LPM will further activate its own expression and induce *Lefty2* expression within the region. It will also travel to the midline, where it will induce expression of *Lefty1*. A faster diffusion rate for Lefty2 and Lefty1 proteins will allow them to "travel ahead" of Nodal beyond the left LPM and the midline to mediate inhibition of *Nodal* expression in the right LPM, thereby promoting asymmetry in the tissue. Ultimately, Lefty will inhibit Nodal expression at the left LPM, the midline, and the right LPM, thus ensuring that *Nodal* and *Lefty* expression will quickly disappear from the LPM and midline after a certain period (~5–6 hr in the mouse embryo).

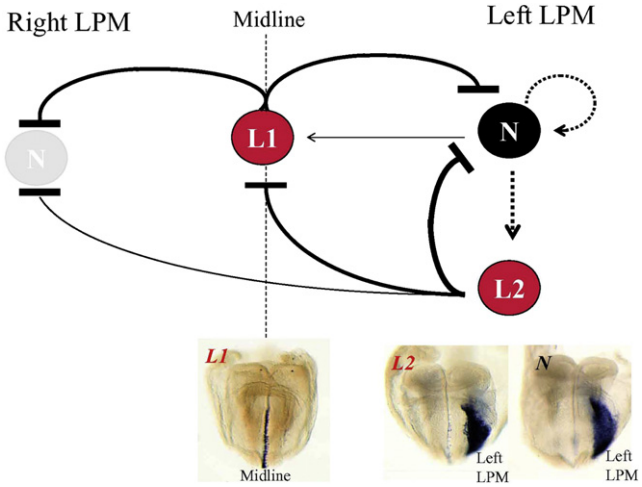


**Figure 1. L-R Asymmetric Expression of *Nodal* and *Lefty***
Short-range activation and long-range inhibition by the activator Nodal (N) and the inhibitors Lefty1 (L1) and Lefty2 (L2) establishes left-right asymmetry in the mouse embryo. Arrows with the dotted line indicate gene activation, whereas solid lines denote diffusion of a protein. Typical expression patterns of *Nodal*, *Lefty1*, and *Lefty2*, as revealed by in situ hybridization, are shown below the genetic diagram. Although *Nodal* and *Lefty* expression are very dynamic, these images represent the stage at which each gene is expressed at the maximum level.

It remains an open question why Lefty proteins diffuse faster than Nodal. In general, the diffusion coefficient of a molecule depends on its own properties and environment (for example, solvent and temperature). Similarly, the diffusivities of Nodal and Lefty proteins are determined by their biophysical property, as well as by environmental factors (such as interactions with other molecules). For instance, Nodal can travel over a long range (from the node to the lateral plate mesoderm on the left side in the mouse embryo) in an extracellular matrix (ECM)-dependent manner (Oki et al., 2007). Rapid and differential transport of Nodal and Lefty proteins in the *Xenopus* embryo also depends on the ECM (Marjoram and Wright, 2011), suggesting that diffusivities of Nodal and Lefty may change, depending on the environment. In this regard, it would be ideal (although technically challenging) to examine the behaviors of a protein in a physiological region (instead of an ectopically expressed region). Nonetheless, the work of Müller and colleagues (2012) provides very strong evidence that the Nodal and Lefty activator-inhibitor pair is an in vivo example of the relevance of a simple reaction-diffusion model to developmental pattern formation. It is possible that there are many other pairs of signaling molecules that act in a short-range amplification/long-range inhibition manner; Nodal and Lefty may be just the tip of the iceberg.

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