Cells control pattern formation by changing their sensitivity to a morphogen gradient

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***Short Abstract* — How equipotent cells interpret morphogen gradients to establish precise spatial cell fate patterns is a general and unresolved question. We quantified how a LIN-3/EGF morphogen gradient induces expression of Notch ligands in Vulva Precursor Cells (VPCs) during *Caenorhabditis elegans* vulva development. We found that LIN-3 upregulates expression of the Notch ligands *lag-2* and *apx-1* with distinct temporal patterns. Combining experiments and mathematical modeling, we found that the observed Notch ligand expression dynamics was driven not by changes in LIN-3 level, but instead by increased sensitivity of each VPC to LIN-3, a mechanism that provides robustness against changes in morphogen dosage.**

***Keywords* — Pattern formation, cell fate decisions, developmental biology, robustness.**

Spatial cell fate patterns are often induced during embryonic development by spatial concentration gradients of signaling proteins called morphogens [1]. It is increasingly clear that the amplitude and spatial extent of morphogen gradients as well as the downstream signaling in the receiving cells can change substantially during induction, potentially providing robustness to biochemical noise, variations in morphogen dosage and changes in tissue size [2,3].

During *C. elegans* vulva development, a spatial LIN-3/EGF gradient excreted from the anchor cell (AC) induces different cell fates in the Vulva Precursor cells (VPCs), labeled P3.p to P8.p, in a distance-dependent manner [4]: P6.p, closest to the AC, adopts the 1° fate, whereas the more distant neighbors P5.p and P7.p adopt the 2° fate. The remaining cells assume 3° fate. Notch signaling between VPCs is required to restrict 1° fate to a single VPC. Using smFISH, a novel technique that make it possible to visualize individual mRNA molecules [5], we quantified Notch ligand expression in each VPC with single mRNA resolution during vulva induction. We found that upregulation of the Notch ligands *lag-2* and *apx-1* by LIN-3 followed a distinct temporal pattern over the course of ~10 hours: first, in the early stage of induction only *apx-1* was expressed, initially in several VPCs but then restricted exclusively to the prospective 1° cell, P6.p. Then, during the late induction stage both *lag-2* and *apx-1* expression in P6.p increased dramatically. Moreover, we found that in P6.p this temporal pattern of Notch ligand expression was highly robust to changes in LIN-3 dosage. Finally, we measured the shape of the LIN-3 gradient indirectly, by measuring Notch ligand expression in mutants where Notch signaling between VPCs was abolished. We found that we could fit our experimental observations using a simple mathematical model with three free parameters: the decay length of the LIN-3 gradient, the production rate of excreted LIN-3 and the sensitivity of the VPCs to the external LIN-3 signal. The model showed that the observed Notch ligand expression dynamics was driven not by changes in LIN-3 excretion, but instead by increased sensitivity of each VPC to external LIN-3, as we subsequently confirmed experimentally. In addition, the model indicated that the LIN-3 gradient itself narrowed significantly over the course of induction. Our results show that the induction of spatial gene expression patterns by morphogen gradients not only depends on the shape of the gradient but also on the modulation of the morphogen-induced signal in the receiving cells.

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