## 

**Figure S1. Mean and Variance Knockdown Distributions with Multiplicative Noise, Related to Figure 2.** **(A)-(H)** Histograms depicting the distribution due to multiplicative noise. The models were solved using the Euler-Maruyama method on the timespan of , and re-solved with a 90% knockdown to the associated parameter. The value was calculated from these two series, and this was repeated 100,000 times. More details outlining the experiments are found in the Transparent Methods. **(A)-(D)** 's calculated for with the respective models. **(E)-(H)** 's calculated for with the respective models.

## 

## Figure S2: Location-Independent Boundary Sharpening Experimental Diagram, Related to Figure 3. A diagram of the numerical scheme for the histogram experiments for spatial location versus threshold sharpness. The SPDE model is solved and the 50% concentration point at each location along the x-axis is found. Then the mean and the variance of these x locations are saved. This process is then repeated with reduced binding protein and the resulting mean and variance values are compared with the previous to get a value for .

## 

## Figure S3: Boundary Sharpening Disruption Extended Figures, Related to Figure 4. (A) Diagram of the extended retinoic acid (RA) model with downstream Hox-Krox signaling. The model starts with diffusive entering the cell to become and binding to to become , which then binds to produce . This induces Cyp which deactivates (and thus degrades) the intracellular . Additionally, acts as a signal to the downstream and transcription factors, which are mutually antagonistic. (B) Mean shift of the RAR signal due to Cyp knockdown. For the wildtype and Cyp setups in the extended RA model with Hox-Krox, the mean of the gradient was calculated between each of the 10 runs at the ending timepoint. (C) Sharpness Index. The y-axis corresponds to the sharpness index defined in (Zhang et al., 2012). The x-axis shows time in terms of hours postfertilization (hpf) in zebrafish. Each condition was repeated 10 times and the results were averaged.

## 

## Figure S4: Characterization of Successful Sharpening via Additional Measures, Related to Figure 5A. Binding protein production taken as 5, 15, …, 105 and Hox-Krox regulatory noise was taken as 0.1, 0.125, …, 0.3 and each pairing in the grid was solved once. The effective RA noise was calculated according to the measure from the Transparent Methods section. In (A) a successful sharpening event was characterized by having the maximum displacement between Hox and Krox dominated cells of less than 3 cell diameters. In (B) a successful sharpening event was characterized by having the mean displacement between Hox and Krox dominated cells as less than half of a cell diameter.

## 

## Figure S5: Characterization of applied to FLIM Data, Related to Figure 5B. (A) Scatter plot of the data points from (Sosnik et al., 2016). (B) Pairwise Differences. The percent change in mean and variance was calculated pairwise between each pair of higher and lower data points. A scatter plot of the results is shown. (C) Estimated CDF. Depiction of the commutative probability distribution for according to the parameter search scheme from the parameter search scheme on RMF. The simulations that produced these distributions are discussed in the Transparent Methods. Shown are the kernel density estimates from the values from the stochastic simulations. The different colored lines show the distributions for different noise types. The red line depicts experimental CDF for computed using the pairwise data points.

|  |  |
| --- | --- |
| Parameter | Value |
| , , | 0.03 |
|  | .017 |
|  | 10000 |
|  | 1 |
|  | 0.1 |
|  | 100 |
|  | 3.0 |
|  | 0.0013 |
|  | 0.0001 |
|  | 0.0001 |
|  | 0.85 |
|  | 0.85 |
|  | 0.01 |
|  | 0.1 |
|  | 1 |
|  | 1 |
|  | 0.02 |
|  | 7.5 |
|  | 3.0 |
|  | 0.4 |
|  | 4.0 |
|  | 0.4 |
|  | 0.2 |
|  | 250.46 |

**Table S1: Disruption of Downstream Boundary Sharpening Parameters, Related to Figure 4.** Parameters correspond to RMFS with Hox-Krox interactions.

## Transparent Methods

**Data and Software Availability**

|  |  |  |
| --- | --- | --- |
| Software and Algorithms | Source | Identifier |
| MATLAB 2015b | The MathWorks Inc. 2015 | https://www.mathworks.com/products/matlab.html |
| Simulations made in MATLAB | This Paper  Github: ChrisRackauckas/MINC | https://github.com/ChrisRackauckas/MINC |
| Julia | Github: Julialang/julia | https://julialang.org/ |
| DifferentialEquations.jl | Github: JuliaDiffEq/DifferentialEquations.jl | https://github.com/JuliaDiffEq/DifferentialEquations.jl |
| Simulations made in Julia with DifferentialEquations.jl | This Paper  Github: ChrisRackauckas/MINC | https://github.com/ChrisRackauckas/MINC |
| Plots.jl | Github: JuliaPlots/Plots.jl | https://github.com/JuliaPlots/Plots.jl |

**Method Details**

**Steady State Analysis**

For the SODE , we calculated the mean of using a linearization of the drift term () and solving for the unique positive steady state. To calculate the variance, we used the linearization of the Fluctuation-Dissipation Theorem where for the Jacobian of the drift at the steady-state , we have that

where is the covariance matrix at the steady state , and thus its diagonal value in column gives the variance of the th component when near the steady state. These computations were performed using Mathematica.

**Monotonicity of Variance**

Take the variance equation

Notice that

From Mathematica we see that if and only if . Therefore is monotonic in . To see that it increases, we used the Mathematica Solve function to attempt to find values for which the derivative was negative. Mathematica could find no parameter regime where this was the case. As verification, we used the Mathematica Solve function to find the values for which the derivative was positive. The function returned no constraints, indicating that this always holds.

**Mathematical Models and Steady-State Results**

**General Master Equation (SM)**

The SM model can be written in the general master equation framework as:

where with being the number of RA particles and being the number of RA-RAR particles, and being the step operators (, implying is the annihilation of RA) for RA and RA-RAR respectively. Following (Wang et al., 2008; Toral & Colet, 2014), we write the general master equation in the form:

where is a reaction rate for the operation of losing RA and RA-RAR, and calculate the identities via algebraic manipulation:

(For example: multiply by , then re-define to be shifted by and simplify. The others follow from similar manipulations). This gives the system of ODEs:

Setting the derivatives to zero, we receive the steady-state values (calculations in the Mathematica notebooks):

We note that the derivatives of the variance and covariance equations by are non-zero for all positive parameter values. Thus both equations are increasing functions of .

**Simple Model with Feedback (SMF)**

where Importantly we note that and are independent of and .

**Intermediate Model (IM)**

,

The variance equations are too large to fit in normal text and are thus contained in the respective Mathematica notebooks. Notice that when or , the respective terms cancel out of and

**Intermediate Model (IM) with More Signaling Steps and a Separate Pool for Cyp Degradation**

,

,

The mean and variance equations are too large to fit in normal text and are thus contained in the respective Mathematica notebooks. Notice that when or , the respective terms cancel out of and .

**Retinoic Acid Model (RM)**

The variance equations are too large to fit in normal text and are thus contained in the respective Mathematica notebooks. Notice and are independent of . By substitution we have that

**Retinoic Acid Model with Binding Protein Feedback (RMF)**

The equations are the same as RM except for:

The steady-state analysis results are too large to fit in normal text and are thus contained in the Mathematica notebooks.

**Spatial Retinoic Acid Model**

The equations are the same as RM with BP feedback except

where where is the Heaviside step function denoting is the edge of production,

and

where each is an uncorrelated Gaussian white noise. The spatial domain was a two-dimensional box with the x-domain [-100,400] and the y-domain [0,50] with units of . The problem was discretized to ODEs via the method of lines with a second-order discretization of the Laplacian and . For all sections, we fixed . The boundary was reflective on all ends except the right boundary, which was leaky with parameter 0.002.

When the Hox-Krox interactions are included, those portions of the system are defined by:

**Numerical Parameter Search in Knockdown Experiments**

The scheme is:

1. For every parameter , take uniformly, and let .
2. Solve the model for 200 seconds with the initial condition at the steady-state value. Calculate the mean and variance.
3. Knock down the associated parameter by 90% and redo step 2.
4. Calculate the value .

The values are given in Table 1. 100,000 simulations were run per model. The simulation was solved using the Euler-Maruyama method with a and the mean/variance was calculated. For models without the explicit binding protein, the parameter γ was the one affected. For models with the explicit binding protein, the production parameter was the one affected. The simulation was solved using the same solver settings and the same Brownian path (the same Brownian path was used to simulate the embryo with the same conditions but with a different epigenetic makeup). Cyp was knocked down (from the parameter set that did not include the BP knockdown) by a 90% decrease to α. The same solver settings and the same Brownian path were used and the mean/variance was calculated. Using the mean/variance calculations for these three runs, a ζ was calculated for the BP-knockdown and a ζ was calculated for the Cyp-knockdown. Note that the percentages were calculated relative to the larger quantity, e.g.

to ensure a value between 0 and 1.

**Numerical Parameter Search in Spatial Knockdown Experiments**

100 simulations were run, with random parameter sets chosen by taking uniformly from [-2,2] and letting. was chosen so that the parameter range covers all of the most likely parameters, but slightly biased in order to decrease the amount of time to steady state to make the problem computationally feasible (boosting degradation, and increasing production so as to keep the total concentrations at reasonable levels). The simulations were accelerated using NVIDIA GTX 970 and GTX 980Ti GPUs via MATLAB's CUDA interface. The base values are given in Table 1.

The simulation was first solved to steady-state without noise at the highest possible , and then the model was solved using a more stable variant of a second order Runge-Kutta method via a method of lines discretization with for 100 seconds, roughly matching the experimental setup of Sosnik et al. 2016. We note that the results are robust to the choice of final time point being an order magnitude less or greater, indicating convergence of the stochastic model to a quasi-steady distribution. The model was first solved using and then with a 90% knockdown of . At the end of each run, the 60% threshold from the non-knockdown control was used to set the boundary location. For each y, the lowest x above the threshold was chosen as the boundary location. The mean and the variance of these x values was used as the boundary mean and variance. This scheme is diagrammed in Figure S2.

**Spatial Boundary Sharpening Experiments**

The boundary sharpening experiments were solved using a method of lines approach. The steady-state gradient was first established by turning off the noise and solving the discretized PDE using an adaptive second order Rosenbrock method from DifferentialEquations.jl. Then the SPDE was solved for 500 seconds using the adaptive SRIW1 method from (Rackauckas and Nie 2017). After that, the Hox-Krox interactions were initiated, starting with a random steady state where Krox was zero and Hox started with each point in space having where is a uniform random number. This was solved to steady state using the Tsit5 algorithm from DifferentialEquations.jl and then solved with noise for 10,000 seconds using the adaptive SRIW1 method.

For the boundary sharpening experiments, parameters were chosen to conform to regimes specified in previous models. The parameters were chosen as detailed in Table S1. From the results, the effective RA noise was calculated as the variance of RA at which was the Hox-Krox boundary in the absence of noise.

**Determination From Data**

To determine from the data of Sosnik et al., 2016, the relative concentration values for free intracellular RA had to be converted to a sensible absolute concentration value in some arbitrary units by determining a 0. This background was discarded by subtracting out the mean relative abundance of the control experiment, which was .3132. The 0-adjusted values are given in in the accompanying MATLAB script. Since the embryos have no preferred pairing, a separate was estimated from each pairwise interaction between knockdowns.