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Morphogen Gradients: From Generation to Interpretation
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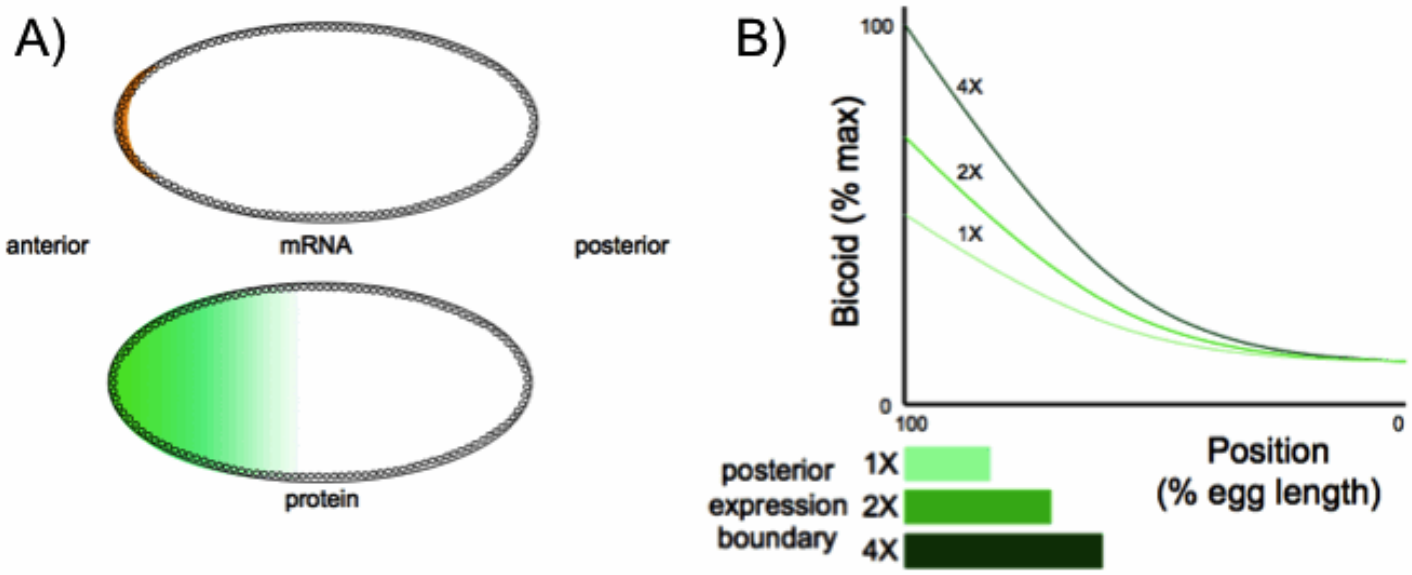

Figure S1. Bicoid morphogen.

Supplemental Figure 1 Bicoid morphogen. (A) Distribution of Bicoid mRNA (*orange*) and protein (*green*) in *Drosophila* embryos at mitotic cycle 14. Bicoid protein forms an anterior-to-posterior gradient. Nuclei indicated by circles. (B) Embryos from mothers with four copies of Bicoid (4X, twice the normal dose; *dark green*) exhibit Bicoid gradients with higher amplitudes and longer ranges than those from mothers with two copies (2X, *medium green*); embryos from mothers with only one copy of Bicoid (1X; *light green*) contain shallower gradients of lower amplitudes. The expression domain of a Bicoid reporter gene shifts depending on the number of Bicoid copies in the mother. The expression boundary shifts anteriorly in 1X embryos and posteriorly in 4X embryos. Adapted from Struhl et al. 1989.

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Supplemental Figure 2 The synthesis, diffusion, and clearance (SDC) model of gradient formation. The SDC model postulates that gradient formation is the result of morphogen production from a localized source combined with diffusion and uniform linear (in this example) clearance. The gradient profile is therefore dependent on the production rate (j_0), diffusion coefficient (D), and clearance rate coefficient (k) of the morphogen. Steady-state is the time at which the concentration at every point no longer changes. In a simple 1D approximation (with an initial concentration of zero in the field, flux j_0 on the left boundary, and an embryo of length significantly larger than the decay length of the gradient), steady-state is defined by the following relationship

$$C(x) = C_0 e^{-x/\lambda}$$

where the decay length (λ) and C_0 are defined as

$$\lambda = \sqrt{\frac{D}{k}} \text{ and } C_0 = \frac{j_0}{\sqrt{Dk}}$$

(for details, see Wartlick et al. 2009). (A) Steady-state gradients with varying values of D. k and j_0 were held constant (k = 0.00025 s^{-1}, equivalent to a half-life of approximately 46 min; j_0=4 molecules s^{-1}), while D was incrementally increased by 0.1 μm^2 s^{-1}. The range of the gradient, reflected by λ, increases as D increases. λ corresponds to the point at which the dotted lines cross the x-axis. As D increases, morphogen can move farther, and the range of the gradient at steady state increases. (B) Curves from panel A normalized to C_0. λ corresponds to the point at which the dotted lines cross the x-axis. (C) Steady-state gradients with varying clearance rate coefficients (k). D and j_0 were held constant (D = 0.1 μm^2 s^{-1}; j_0=4 molecules s^{-1}), while k was incrementally increased by 0.0001 s^{-1}. The range of the gradient, reflected by λ, decreases as k increases. λ corresponds to the point at which the dotted lines cross the x-axis. As k increases, the morphogen is less stable and does not form long-range gradients. (D) Curves from panel C normalized to C_0. λ corresponds to the point at which the dotted lines cross the x-axis. Note that for GFP-Dpp, D = 0.1 μm^2 s^{-1}, k = 0.00025 s^{-1}, and j_0 = 4 molecules s^{-1} (Kicheva et al. 2007).

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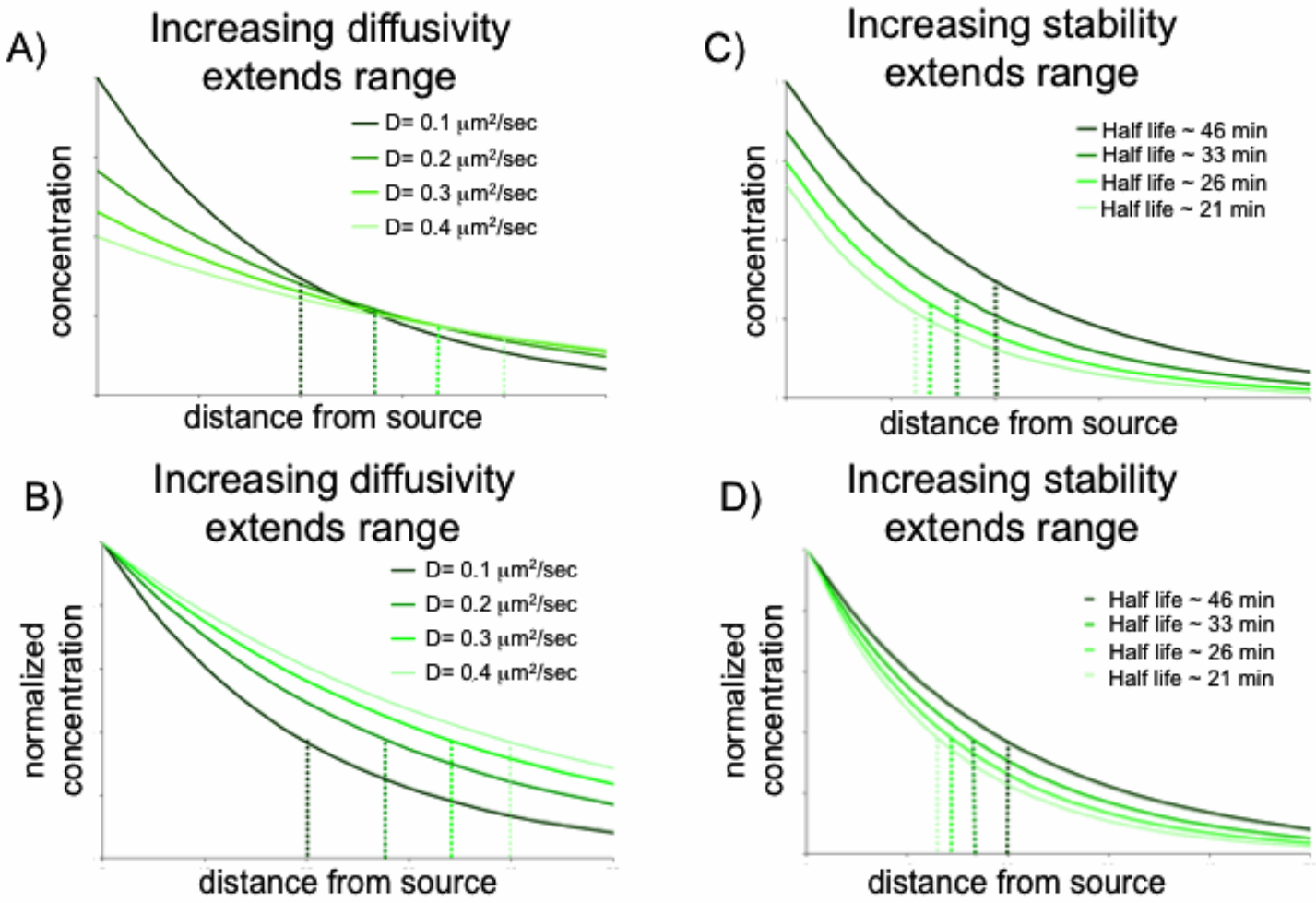

Figure S2. The synthesis, diffusion and clearance (SDC) model of gradient formation.

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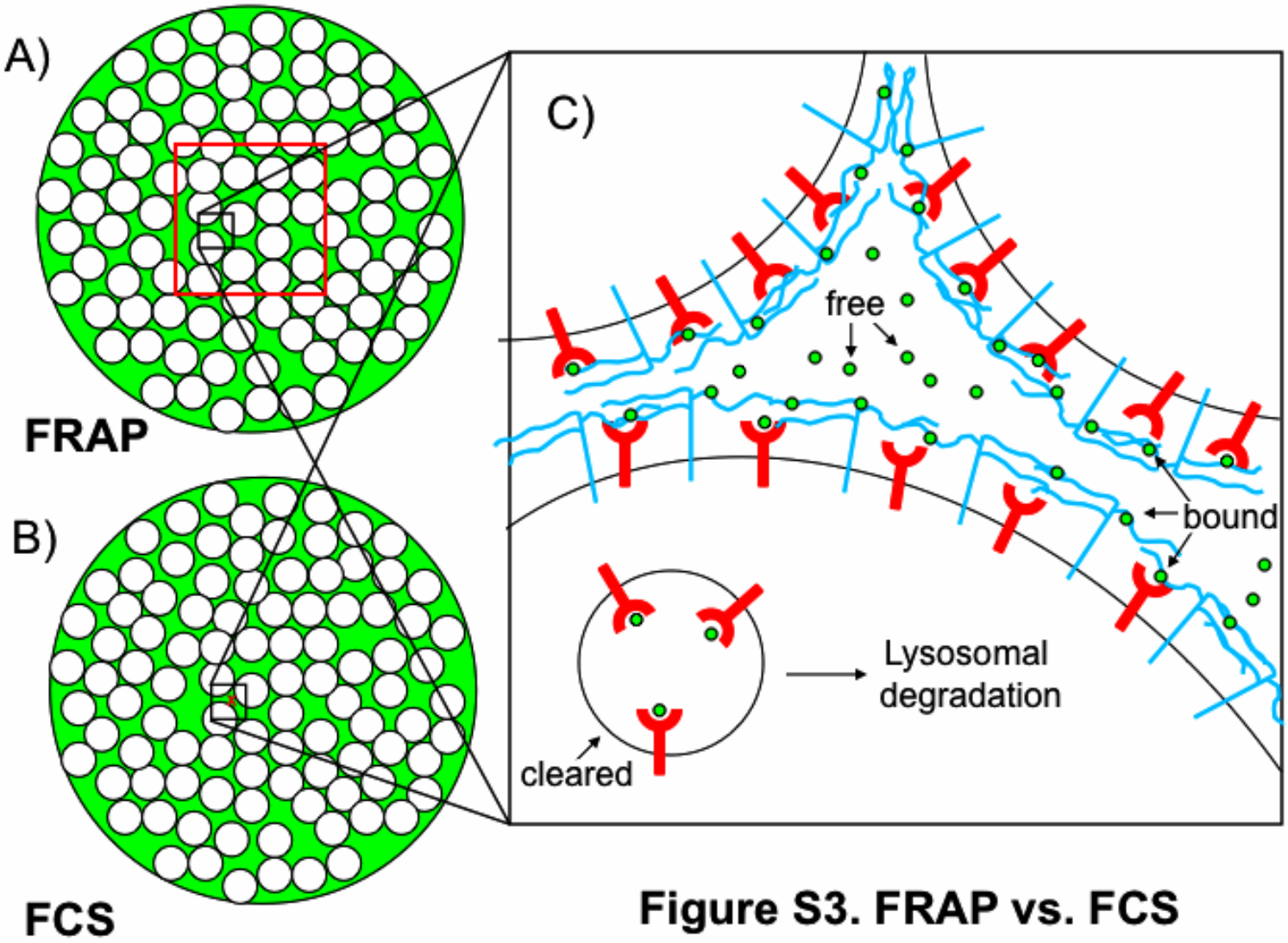

Figure S3. FRAP vs. FCS

Supplemental Figure 3 FRAP vs. FCS. Diagrams of tissues in which cells (circles) are surrounded by morphogen (*green*). (A) In most FRAP experiments (e.g., Kicheva et al. 2007) used to measure morphogen diffusivity, large areas spanning multiple cells within a tissue are photobleached (bleached region indicated by red square). (B) In recent FCS experiments (Yu et al. 2009) used to measure morphogen diffusivity, measurements were made in small, extracellular volumes (indicated by red “X”). (C) Magnified view of the regions indicated in A and B. Morphogen (*green*) is present in multiple environments: extracellular space (free); bound to HSPGs (*blue*) or receptors (*red*) on cell surfaces (bound); or in endosomes within cells (cleared), awaiting lysosomal degradation.

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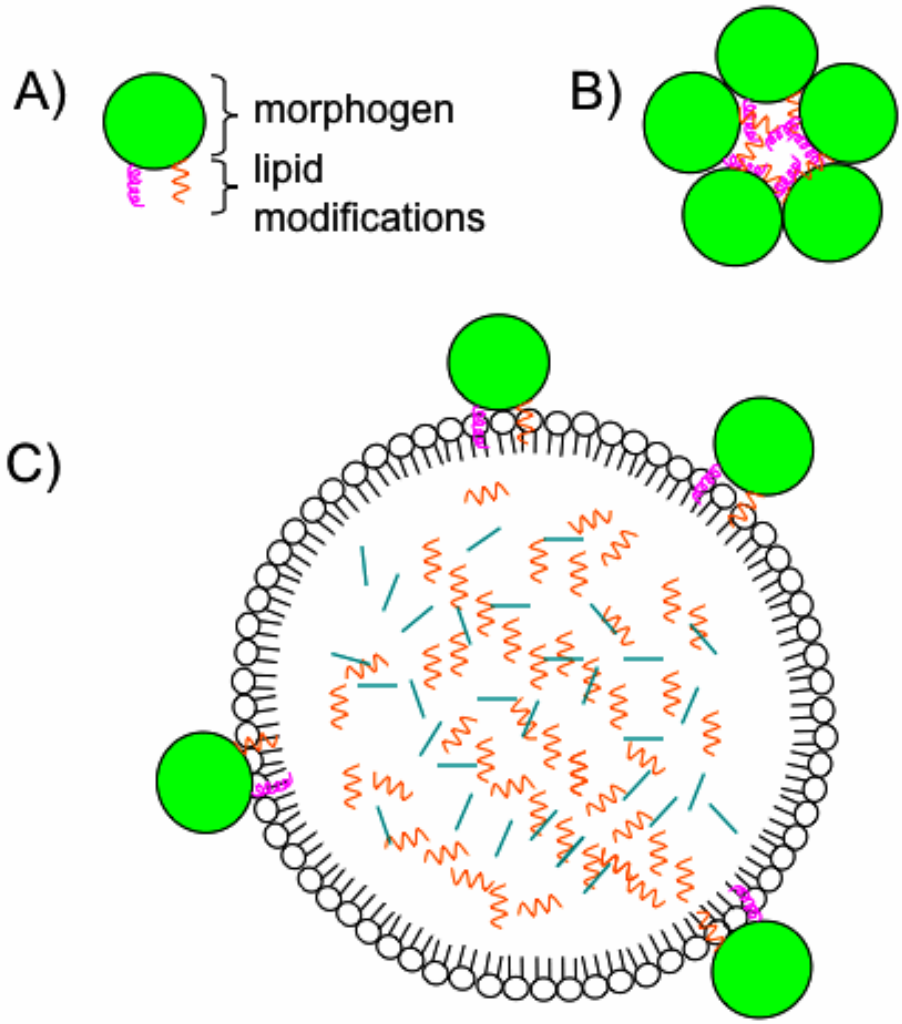

Figure S4. Morphogen vehicles.

Supplemental Figure 4 Morphogen vehicles. (A) Many morphogens (*green*) are lipid-modified (*orange and pink*). (B) Morphogens may cluster. (C) Some morphogens may be transported in lipoprotein particles, membranous vehicles composed of a phospholipid monolayer surrounding esterified cholesterols (*orange*) and triglycerides (*blue*), complexed with a protein scaffold (not shown). The concentration of morphogen molecules per lipoprotein particle is unknown. Adapted from Eaton (2008).

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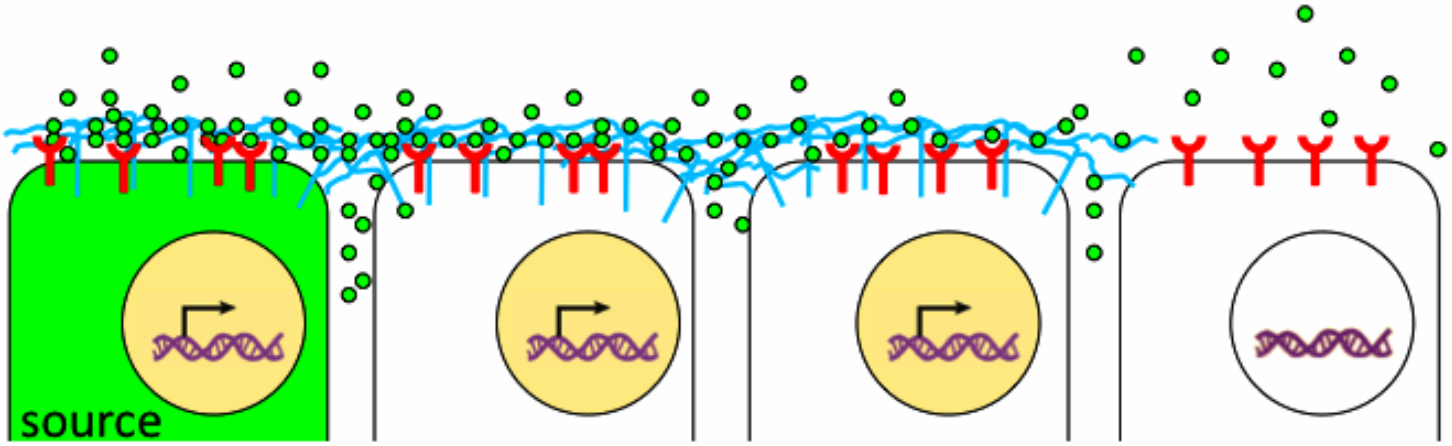

Figure S5. HSPGs concentrate morphogen at cell surfaces.

Supplemental Figure 5 Heparan sulfate proteoglycans (HSPGs) concentrate morphogen at cell surfaces. HSPGs (*blue*) concentrate morphogen near the cell surface, promoting productive receptor (*red*)–morphogen (*green*) interactions that lead to transcriptional responses (*yellow*). When a cell (*far right*) is unable to produce HSPGs, morphogen can no longer be tethered to the cell surface, and the probability that diffusing morphogen molecules will contact receptors is lower for this cell than for the others. Adapted from Belenkaya et al. (2004).

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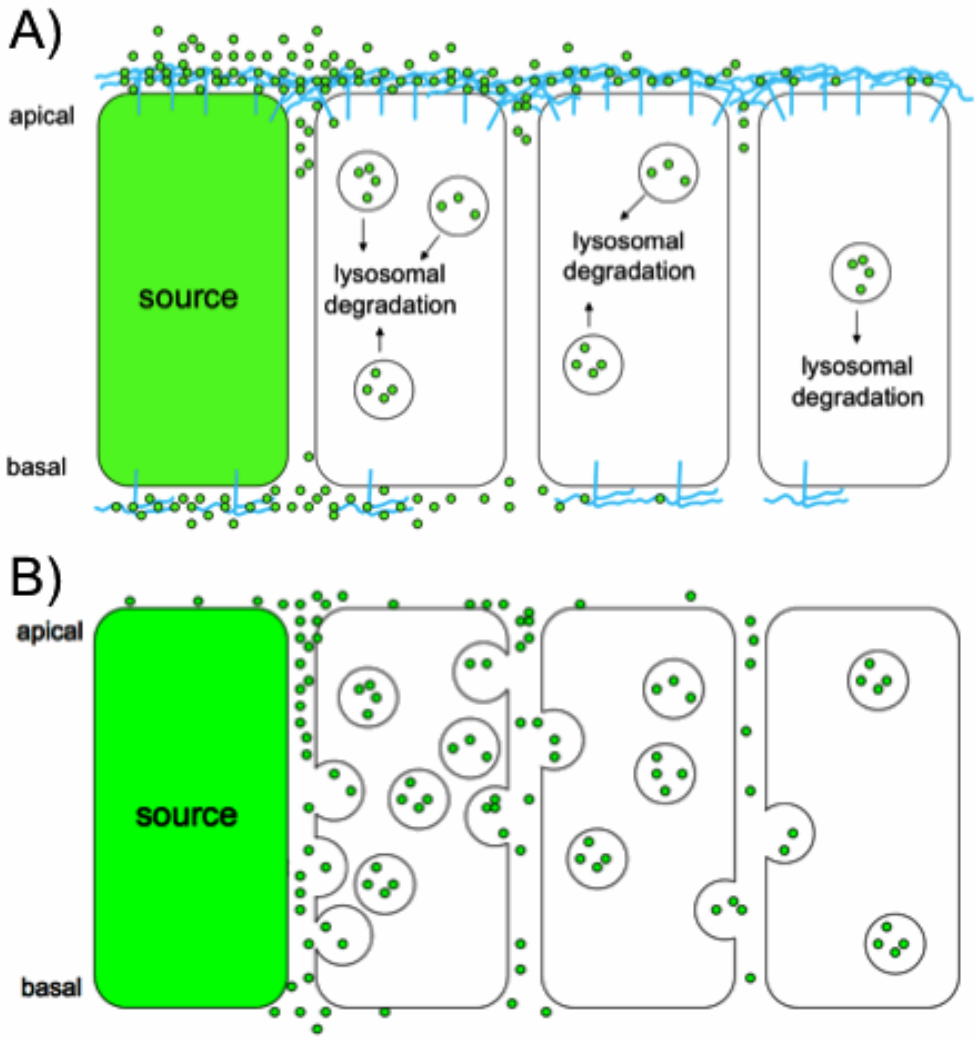

Figure S6. Morphogen routes.

Supplemental Figure 6 Morphogen routes. Illustration of potential morphogen routes in the *Drosophila* wing disc epithelium. Such routes also may be applicable to other tissues. (A) Extracellular morphogen movement. Morphogen (*green*) moves through the extracellular matrix [depicted as heparan sulfate proteoglycans (HSPGs), *blue*] and/or through areas outside of the extracellular matrix. As morphogen moves through a target tissue, it is internalized and trafficked to the lysosome pathway for degradation. For Hh, distinct apical and basolateral gradients exist. (B) Intracellular morphogen movement. Morphogen moves through tissues by transcytosis, in which cells take up and release morphogen. Some morphogen is still targeted to the lysosome pathway and degraded (not shown).

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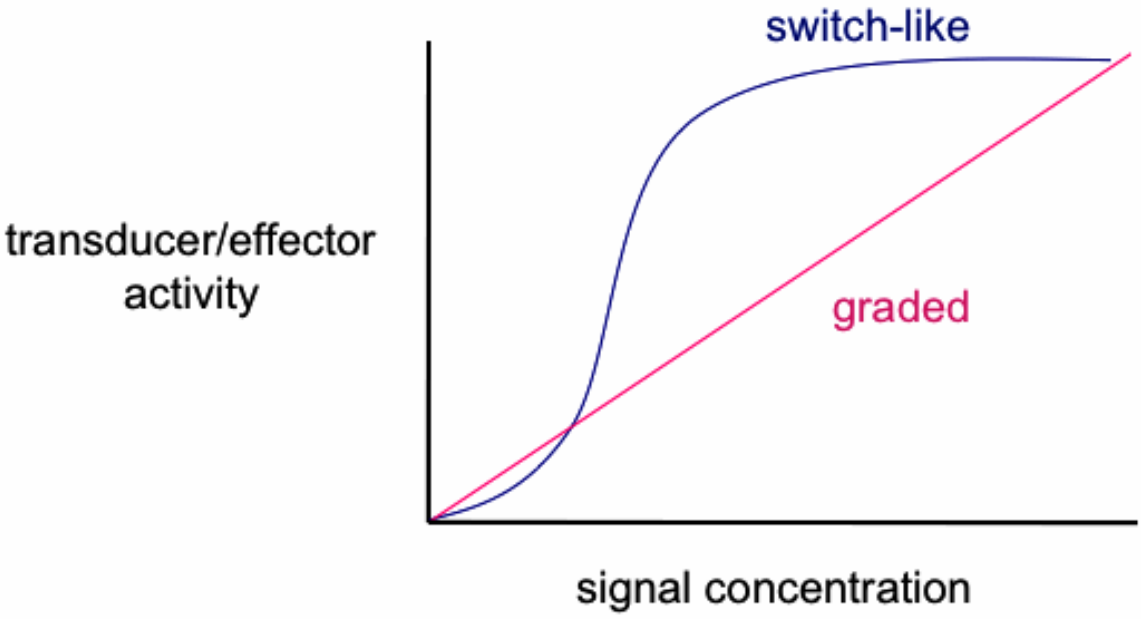

Figure S7. Graded vs. switch-like signal transduction

Supplemental Figure 7 Graded versus switch-like signal transduction. In a system that responds to a signal with switch-like activity (*blue*), increasing signal concentration causes an all-or-nothing response (i.e., transducers or effectors are either completely on or completely off). In contrast, the activity of transducers/effectors in systems that respond in a graded manner (*pink*) increases linearly as the signaling concentration increases.

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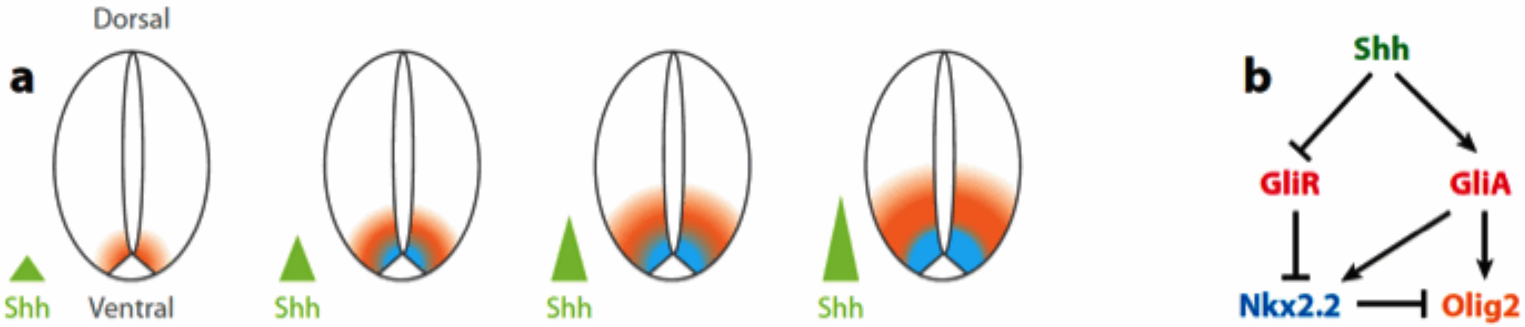

Figure S8. Spatiotemporal dynamics of gene expression in response to Shh signaling.

Supplemental Figure 8 Spatiotemporal dynamics of gene expression in response to Sonic hedgehog (Shh) signaling. (A) The ventral-to-dorsal Shh gradient in the developing neural tube expands over the course of several hours (indicated by *green triangles*). Early in the expansion (*left*), the gradient range is short, and Olig2 (*orange*) is expressed ventrally. As the gradient evolves, the Olig2 expression domain expands dorsally, and expression of Nkx2.2 (*blue*) initiates near the Shh source. Because Nkx2.2 represses Olig2 expression, as the Nkx2.2 domain expands dorsally, the ventral Olig2 domain is reduced. This incoherent feed-forward loop results in a domain of Nkx2.2 expression near the Shh source and a domain of Olig2 expression abutting the Nkx2.2 domain. (B) Gene network architecture. Shh signaling stabilizes Gli^A, the transcriptional effector of Shh signaling. Gli^R, the processed form of Gli^A, inhibits expression of Shh target genes. Nkx2.2 indirectly reduces Gli^R levels, thereby enhancing Shh signal transduction in Nkx2.2-expressing cells.

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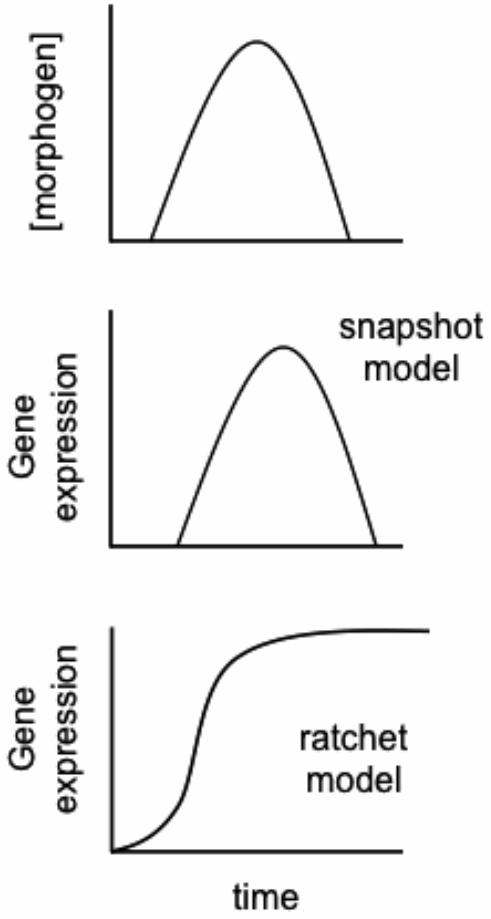

Figure S9. The ratchet and snapshot models.

Supplemental Figure 9 The ratchet and snapshot models. Cells are exposed to increasing and then decreasing levels of morphogen (*top*). Cells that respond according to the snapshot model (*middle*) constantly adjust their response and respond only to current morphogen levels. These cells do not have a memory of former morphogen levels and stop expressing target genes when morphogen is removed. Cells that respond according to the ratchet model (*bottom*) adjust their response while morphogen levels increase. These cells have a memory of the highest morphogen levels encountered and continue to express target genes after morphogen removal.