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Developmental Biology: Morphogen in a Dish

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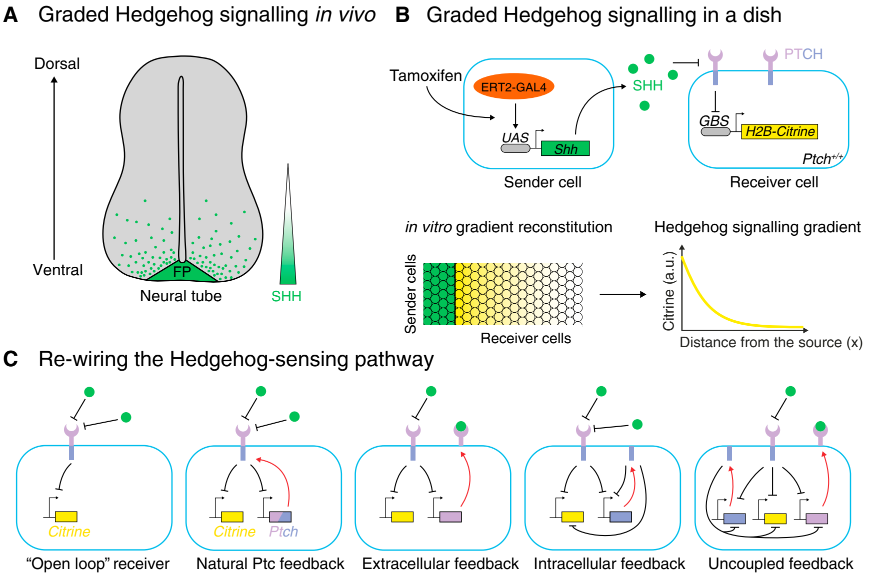
Reconstitution of a Hedgehog morphogen gradient *in vitro* and *in silico* reveals the architectural features of the signal transduction pathway that ensure rapid formation of a robust signalling gradient.

Morphogens are signalling molecules that are typically produced by a localized group of cells to form concentration gradients in surrounding tissues. Morphogen gradients are transduced into signalling gradients, leading to activation of target genes in a concentration-dependent manner and the subsequent orderly establishment of cell fates that characterise biological tissues. The idea that morphogens could organise patterns, which was initially predicted by classical embryologists [1,2], gained ground with the identification of actual morphogens through molecular genetics [3,4]. The elegance of this concept has ensured its enduring quality in developmental biology. However, behind its simplicity lie several questions. Lewis Wolpert, who famously coined the term 'positional information' [5], later said that "diffusible gradients are too messy" [6]. Whether this statement is accurate or not, it highlights the need to understand how morphogen gradients, which must be sensitive to the vagaries of intrinsic and environmental conditions, are reliably interpreted. To understand how morphogen gradients are formed and interpreted, quantitative approaches, combined with perturbation analysis, are needed [7]. The availability of reporter genes and tagged components of signal transduction, along with progress in imaging technology and tissue culture, has made the study of morphogens ever more rigorous. However, the complex structure of native tissues and the confounding effects of additional cross-regulating pathways *in vivo* make identifying the features that enable robust interpretation of morphogen gradients a significant challenge. To overcome these difficulties, developmental biologists are beginning to develop *in vitro* culture methods [8] that preserve the pathway under investigation while simplifying the system and improving accessibility for high-quality live imaging. Although the physiological significance of such systems needs to be validated *post-hoc*, they offer unparalleled opportunities for experimental control of key parameters, quantitative measurements, and realistic mathematical modelling. With this experimental philosophy in mind, Li and colleagues [9] in a recent study reconstituted a morphogen gradient in a lawn of cultured cells and investigated how the architecture of signal transduction impacts on the signalling gradient.

Li and colleagues [9] engineered cultured cells to express *Sonic Hedgehog* (*Shh*), which was identified as an extracellular morphogen in 1994 [10], and placed these so-called sender cells in a field of receiving cells (Figure 1). They were not able to directly visualise the SHH



Current Biology

Figure 1. Reconstitution of a HH signalling gradient and rewired variants of signal transduction.

(A) In the vertebrate neural tube, SHH is secreted from the floor plate (FP) to specify neuronal identities in a concentration-dependent manner. (B) Reconstitution of a SHH gradient in cultured cells. Sender cells produce SHH in a tamoxifen-dependent manner. Graded activation of SHH signalling is detected in receiver cells with a Histone-Citrine reporter controlled by 8 GLI binding sites (GBS). (C) Re-wired variants of the SHH-sensing pathway. PTCH normally mediates negative feedback through intracellular signal inhibition (I) and extracellular ligand sequestration (E). The contribution of these mechanisms and the importance of their coupling for gradient robustness can be tested with the re-wired variants *in silico* and *in vitro*.

protein but inferred that it formed a gradient from the graded pattern of signalling activity. Therefore, a signalling gradient can be reconstituted in this simplified system.

SHH triggers downstream signalling by binding to Patched (PTCH). This receptor is unusual in that its default role (in the absence of ligand) is to prevent downstream signalling. It does so by inhibiting another transmembrane protein, Smoothened (SMO), which would otherwise constitutively trigger signal transduction and activation of the transcriptional effector GLI. Thus, SHH activates signalling by relieving the inhibitory activity of PTCH, a mode of action that Li and colleagues [9] refer to as 'double-negative'. This is in contrast to the more conventional double-positive architecture, whereby an activated receptor activates downstream mediators. As expected, mutations in *Ptch* trigger runaway SHH signalling and are indeed frequently associated with cancer [11]. Another important feature of *Ptch* is that it is a transcriptional target of SHH signalling, providing a mechanism for negative feedback as well as for limiting ligand spread by sequestration [12].

To experimentally assess the importance of negative feedback, Li and colleagues [9] made *Ptch* expression independent of SHH signalling. This was achieved by replacing the endogenous gene in cultured cells with an inducible version, thus creating an 'open-loop' architecture. Since, in their system, expression of *Shh* was also tuneable, they were able to uncouple the expression of these two genes and generate a matrix of different expression levels for *Shh* and *Ptch*. They found that, as ligand expression increased (which they assume to correlate with surface protein levels), so did signal amplitude and gradient length scale, while the reverse was seen upon increasing receptor expression. Thus, the open-loop architecture provides poor robustness to changes in ligand and receptor levels, as expected intuitively. Remarkably however, the gradient properties were determined by the ratio between *Shh* and *Ptch* expression, a behaviour described as 'ratiometric'.

Li and colleagues [9] created a simplified mathematical toy model to analyse the cellular signalling response to a fixed extracellular gradient. They compared the double-negative and double-positive open-loop architectures, and found that ratiometric behaviour is an intrinsic feature of the double-negative regulatory logic. No such behaviour arises from the more conventional double-positive architecture, wherein downstream signalling depends on the levels of ligand-bound receptors. It is important to note that this conclusion relies on the assumption that signalling activity depends on the levels of unligated PTCH, in accordance with the so-called 'free-receptor model', which was derived from experiments in cell culture [9,13]. This may not be universally true as *in vivo* genetic data suggested the distinct 'ratio model' according to which signalling activity would be determined by the ratio between ligand-bound and free PTCH [14]. From subsequent analysis, Li and colleagues [9] then developed a more detailed model incorporating the effect of PTCH on the distribution and degradation of SHH. This model was fitted against the measured temporal response of open-loop cells to various levels of Shh, and gradient formation dynamics, and found to still exhibit ratiometric behaviour.

With their *in vitro* and *in silico* models, Li and colleagues [9] systematically investigated the benefits of feedback. They first found that feedback renders the length scale and amplitude of the signalling gradient less sensitive to changes in ligand levels, as shown previously [15]. In addition, feedback enables the signalling gradient to reach steady state faster and improves its 'useful range' by preserving a near-linear shape. Using a version of their mathematical model that incorporates the effect of signalling on the production of receptors, they were also able to explore the relative effects of PTCH's two roles — inhibition of Smoothened by the intracellular domain and ligand sequestration by the extracellular domain. This allowed them to predict that intracellular feedback provides amplitude robustness while ligand sequestration contributes to length scale robustness. To validate the first prediction, they took advantage of a previously mutated form of PTCH ($\text{PTCH}^{\Delta\text{loop}}$) which no longer binds SHH but can still perform intracellular inhibition [16]. They programmed receiver cells to express $Ptch^{\Delta loop}$ in response to signalling, thus reconstituting ‘signal inhibition-only’ feedback. As predicted by the model, these cells produced gradients whose amplitudes (but not length scales) were robust to ligand levels. In the wild type, the two functions of PTCH are coupled by being linked in a single protein. Remarkably, as the authors show *in silico*, such coupling is essential for optimal performance.

The new study by Li and colleagues [9] emphasises the importance of double-negative signal transduction in the establishment of a robust morphogen signalling gradient. Yet, there is no experimental indication that morphogen systems operating through double-positive signalling (such as BMPs) are less robust. An important task for the future will be to identify the relevant architectural features of these pathways. In the case of SHH, PTCH soaks up ligand near the source, thus reducing gradient length scale when signalling activity increases, basically buffering the gradient against changes in ligand production. In double-positive pathways, signalling activity depends on the levels of ligand-bound receptors and typically represses receptor expression. While such receptor downregulation contributes to negative feedback, it is expected to extend the gradient, i.e. making the gradient length scale overly sensitive to changes in ligand levels. Therefore, robustness of double-positive signalling gradients has to arise from additional mechanisms. This could be achieved by signalling-dependent expression of additional, non-signalling, receptors (such as other extracellular modulators) [17] or negative feedback though proteins acting further downstream in the signal transduction cascade [18].

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Decision Making: How Fruit Flies Integrate Olfactory Evidence

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New studies show that, as in mammals, perceptual decision-making behavior in fruit flies involves the integration of sensory information that accumulates over time; this involves a process of dendritic integration that depends on the transcription factor FoxP.

Is this milk still good? Sometimes the smell tells you instantly — yuk, no! — but in less obvious cases you may need a few sniffs to decide. Many animals behave like this, gathering sensory cues over time until the choice is clear. The time it takes to make an accurate decision is proportional to the quality of the sensory information; easy perceptual decisions can be made quickly, but harder problems, when the sensory information is more ambiguous, require more time [1,2]. While electrophysiological and functional imaging investigations in