Review

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How to build a robust intracellular concentration gradient

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Concentration gradients of morphogens are critical regulators of patterning in developmental biology. Increasingly, intracellular concentration gradients have also been found to orchestrate spatial organization, but inside single cells, where they regulate processes such as cell division, polarity and mitotic spindle dynamics. Here, we discuss recent progress in understanding how such intracellular gradients can be built robustly. We focus particularly on the Pom1p gradient in fission yeast, elucidating how various buffering mechanisms operate to ensure precise gradient formation. In this case, a systems-level understanding of the entire mechanism of precise gradient construction is now within reach, with important implications for gradients in both intracellular and developmental contexts.

Gradients on multiple scales

The concept of a concentration gradient has been pivotal in explaining how development in biology is regulated spatially [1–6]. Typically, a spatially varying concentration of a morphogen protein drives spatially differentiated gene expression through a concentration thresholding mechanism, whereby morphogen concentrations above a particular threshold can, for example, activate the expression of a specific gene. In this manner, a continuously varying concentration in space can be converted into a discrete pattern of gene expression territories.

Classical morphogens often act over relatively long distances; the intensively studied Bicoid morphogen, important for anterior cell fate specification in the early *Drosophila* embryo, extends for hundreds of microns away from its anterior source [7]. At slightly shorter length scales, the morphogen Decapentaplegic (Dpp) extends tens of microns away from the anterior–posterior compartment border in the center of the *Drosophila* wing imaginal disc [8,9]. In both cases, the gradients span many cells (or nuclei for Bicoid in the syncytial blastoderm), generating long-range patterning potential. The mechanism of formation of these gradients is thought to involve localized protein production, followed by effective diffusion away from the source, and then eventual degradation (Box 1). The Bicoid system largely supports this mechanism; although an underlying spatial *bicoid* mRNA gradient contributes to the Bicoid protein gradient, protein movement is also required [10,11]. For Dpp, although the system is governed by effective diffusive transport on longer length scales, the mechanism of morphogen movement on shorter scales is still controversial [12].

Over the past 10 years or so, studies have shown that concentration gradients are not the exclusive preserve of developmental biology, but can also have a crucial role in spatial organization inside single cells [13–17]. Brown and Kholodenko showed theoretically that concentration gradients could exist inside an individual cell with biophysically relevant parameters [13]. Hence, an individual cell was not so small that homogeneous concentrations were inevitable (Box 1). Since then, a host of different gradients have emerged, ranging from cell division regulators [18,19] and cell fate determinants [20,21] to mitotic spindle organizers [22–24]. Even some of the smallest bacterial cells contain gradients with spatial extents of only around a micron [25,26]. An important difference exists between intracellular gradients and their developmental cousins, however: the role of degradation. In developmental systems, the morphogen protein is typically degraded [27,28] and this, together with diffusion, is believed to be responsible for the decrease in morphogen concentration as a function of distance away from the source. In intracellular systems, the lifetimes required for this mechanism to generate a meaningful gradient are too short to be realistic (Box 1). Instead, it is typically a modification of the protein (e.g. its phosphorylation status) that is modulated as part of a gradient. In this manner, a typical protein with a lifetime of hours or more can potentially participate in (phospho-)gradient formation many times before being degraded (Box 1).

Making development precise

The study of developmental morphogen gradients has recently been reinvigorated by a novel focus on noise and precision [15,29–32]. All biological systems inevitably contain sources of noise, which can potentially corrupt the ability of the system to generate reliable outcomes. Noise can broadly be separated into two classes: extrinsic and intrinsic. Here, extrinsic noise refers to fluctuations from one gradient to another in two different cells or embryos (Figure 1a). This might arise, for example, from different amounts of mRNA laid down from one embryo to another, leading to differing gradient profiles. Intrinsic noise refers to fluctuations inherent even within a single copy of the gradient (Figure 1b). In the latter case, noise arises from the inevitable biochemical fluctuations inherent in processes such as diffusion that are needed to make a gradient (Box 2) and which will be particularly prevalent at low molecular copy numbers. Fluctuations, both extrinsic and

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Box 1. Making a simple concentration gradient

Morphogen gradients in a developmental biology context often rely on local protein production, followed by diffusion and eventual degradation. The symmetry breaking necessary for gradient formation therefore relies on a localized source, which can be provided by previous localization of morphogen mRNA. Provided each morphogen protein is degraded independently at a constant rate, a simple mathematical analysis reveals a concentration profile that decays exponentially with distance from the (planar) source. The decay length of the gradient (the distance over which it decays to 1/e of its highest value) has also been measured in several cases (and is approximately 100 μm for Bicoid and 20 μm for Dpp). Modulating the decay process can produce qualitatively different gradient shapes; for example, if a dimerization reaction is required for decay (an example of self-enhanced degradation), a power law decay results at large distances. In this case, at large distances from a planar source, the concentration decays with distance as 1/(distance)$^{\alpha}$, where α is equal to two for the dimerization reaction.

In an intracellular context, the question arises of whether a cell is large enough to support an intracellular gradient, or whether internal concentrations are necessarily homogeneous. A molecule with a diffusion constant D and a lifetime τ, will typically move a distance on the order of the decay length $d \propto \sqrt{(D\tau)}$ before being degraded. For a diffusion constant on the order of $D \sim 1\ \mu m^2 s^{-1}$, and a protein lifetime of hours, the characteristic decay length d will be far larger than typical cellular dimensions. However, the distinguishing feature of a gradient protein could be a modification of the protein; for example, by phosophorylation. In that case, the effective lifetime of the protein with the appropriate modification could be far shorter. For example, the protein could be dephosphorylated at the same time as being localized at a specific site within the cell (as is the case for Pom1p in fission yeast). The protein could then diffuse away while being continuously subject to attempted phosphorylation. If the latter process occurred on a timescale of approximately 1 s, with $D \sim 1\ \mu m^2 s^{-1}$ a gradient with a typical d of the order of 1 μm would emerge, which is significantly smaller than typical eukaryotic cell sizes and comparable with the size of bacteria. Hence, intracellular gradients are not only possible, but can be expected to be fairly ubiquitous. Once phosphorylated, the gradient protein could then be recycled for reuse in the gradient, a much more energy-efficient mechanism for gradient maintenance than rapid degradation combined with protein re-synthesis.

intrinsic, will degrade the precision of the positional information provided by a gradient (Figure 1).

Various mechanisms are available to potentially buffer these sources of noise, thereby increasing the precision of the resulting positional information. Time-averaging will clearly reduce positional error introduced by intrinsic fluctuations [15,31] (Box 2), as can spatially averaging the gradient read-out provided by neighboring cells or nuclei in a developmental biology context [31,33]. Time-averaging is performed by a downstream signal-processing network, with time scales determined by the transcript and/or protein lifetimes of the target gene (e.g. for Bicoid, this would be the lifetimes of the products of its target gene *hunchback*). Extrinsic noise can be reduced by changing the degradation process involved in the removal of the morphogen protein, thereby altering the gradient shape (see [34] and [29] for alternative methods). For example, it was demonstrated that morphogens with self-enhanced degradation exhibit, at large distances, profiles that decay as power laws (Box 1) [32]. Theoretical analysis has shown that such profiles can potentially better buffer extrinsic noise in morphogen production rates compared with profiles with standard (i.e. linear) morphogen degradation, which generates exponentially decaying profiles. More generally, if both extrinsic fluctuations in morphogen production rates and intrinsic fluctuations need to be buffered, the gradient shape that maximizes precision depends on which source of noise is most important [35]. Power law profiles are potentially best for systems dominated by extrinsic noise in morphogen production (as expected from [32]), straight line profiles for intrinsic noise and exponential profiles for systems where both types of noise are important [35,36].

So far, this type of analysis has been mostly applied to developmental morphogen systems. Here, we review how similar ideas concerning robustness and precision are

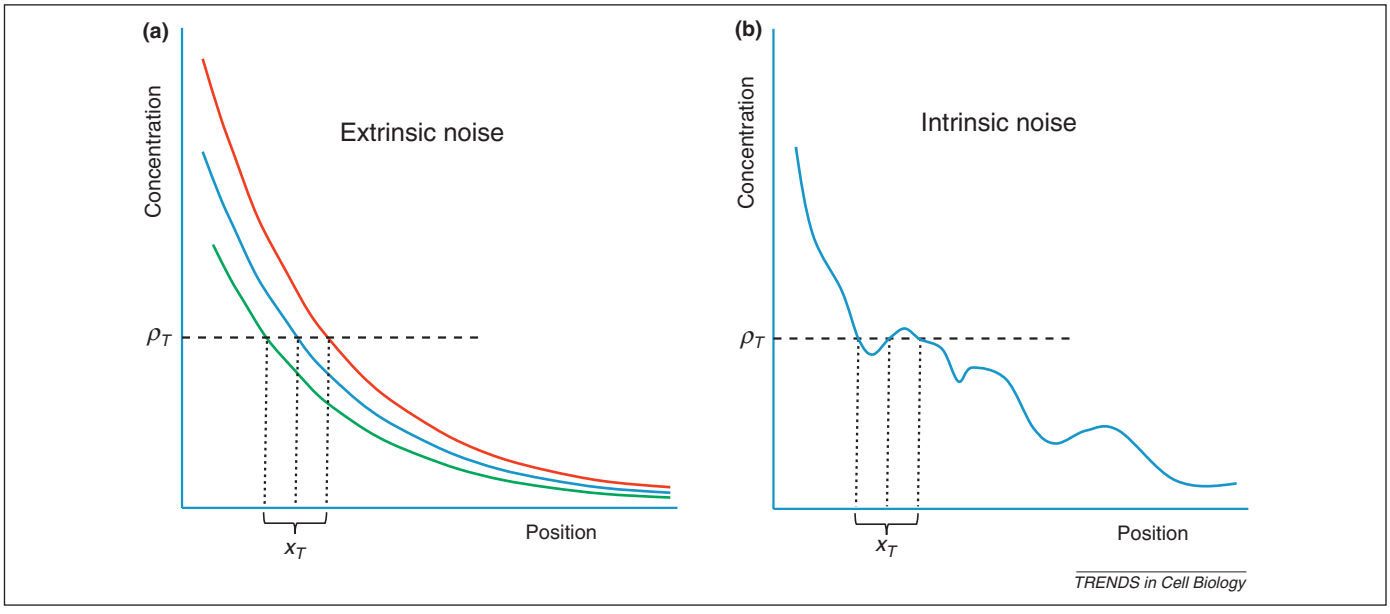

Figure 1. Extrinsic and intrinsic noise both affect the precision of the positional information provided by concentration gradients. (**a**) Extrinsic noise in the morphogen production rate leads to a varying profile from one gradient to another. Variation in the position at which the gradient concentration drops through a critical level (ρ_T) leads to imprecision in the specification of position x_T. (**b**) Similarly, intrinsic noise within a single gradient also leads to imprecise positional information.