Elucidating mechanisms underlying robustness of morphogen gradients

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Morphogen gradients play a pivotal role in most phases of developmental patterning. To ensure proper patterning, reproducible gradients are established under diverse environmental conditions and genetic backgrounds. We refer to the capacity to buffer fluctuations in gene dosage or environmental conditions as 'robustness'. By theoretical analysis of mechanisms that facilitate robustness, it is possible to unravel the machinery responsible for generating the spatial distribution of morphogens.

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Abbreviations
BMP bone morphogenetic protein
Dpp Decapentaplegic
Sog Shortened gastrulation
Tld Tolloid

Introduction

Transforming the spatial position of a naïve cell within a field into one of several distinct cell-fate decisions is the hallmark of developmental patterning. How can a cell recognize its spatial position? Clearly, communication between different cells in the field is essential. Two distinct types of communication can be envisioned. First, signaling can be restricted to short-range interactions between nearest neighbors. In principle, global tissue differentiation could still be achieved by a relay of such local signaling. Alternatively, positional information can be dictated by long-range gradients of signaling molecules [1].

A main distinction between a short- versus a long-range type of communication lies in the manner by which the signal is interpreted. In local communication, signaling induces a particular cell fate in a binary, all-or-none fashion. By contrast, in the case of long-range gradients, morphogen molecules can induce several cell fates in a concentration-dependent manner. Tissue patterning is defined by the dose-dependent response to the morphogen profile, in the sense that any shift in the morphogen distribution results in an equivalent shift in patterning, regardless of the details of the intracellular signaling cascade.

Robustness of morphogen gradients

Coding positional information strictly by quantitative morphogen levels appears to be at odds with biological reality. Although the eventual pattern is precise and reproducible, the differentiation process itself must accommodate a wide range of biological conditions. Temperature and nutrition are just two examples of widely varying environmental factors that can potentially impinge on the underlying molecular mechanisms, by altering reaction rates or protein concentrations. Moreover, genetic variability between individuals, caused by mutations and polymorphisms, may also alter gene dosage or reaction-rate constants. Developmental noise might also be generated by stochastic fluctuation in gene expression [2]. In the face of those unavoidable biological variations, the reproducibility of developmental patterning is a fascinating and unexplained outcome.

We refer to the capacity to buffer fluctuations in gene dosage or environmental conditions as 'robustness' [3] (See also discussion on robustness by Slepchenko and Terasaki in this issue.) The robustness of biological systems is emphasized by the fact that heterozygocity for most genes does not lead to an apparent phenotype. A major contemporary challenge is to elucidate the molecular paradigms that provide robustness. Relying on the enormous progress in knowledge of the molecular processes underlying development, it is now possible to characterize the robustness of specific aspects of patterning in molecular terms. First, instead of using morphological markers as the readout of the patterning processes, it is feasible to follow direct molecular markers of the patterning process itself. Such readouts include, for example, target-gene expression or the signaling response to the activation gradient. Second, the molecular components of the patterning networks and the connectivity between them are mostly known. Although the *in-vivo* parameters, including rate constants or protein concentrations, are not available, theoretical approaches can be employed to characterize the qualitative features of system dynamics on the basis of its hardware connectivity, to elucidate underlying mechanisms [4–6,7••,8••,9–11].

In this review, we describe recent progress in understanding the mechanisms used to enhance the robustness of morphogen profiles. Altering gene dosage, for example, is expected to alter patterning, but if the system is robust, the extent of this alteration will be minimized. Robustness is a relative, rather than an absolute measure and is defined with respect to a particular perturbation type. The studies described utilize a comparative approach, analyzing the relative robustness of different systems, rather than the absolute robustness of a given design. We first discuss the patterning of the dorsal region of the *Drosophila* embryo. In addition to elucidating the patterning mechanism and pinpointing the scheme used for enhancing robustness, this study exemplifies how the robustness principle can differentiate between different plausible molecular mechanisms and generate testable predictions. Subsequently, we discuss a general way for enhancing robustness to the rate of morphogen production in the canonical model of a morphogen system, where the morphogen is produced at a localized source.

Robustness can distinguish between molecular mechanisms

The need to maintain robustness largely limits the possible design of patterning networks. An approach for distinguishing between different patterning mechanisms on the basis of their compatibility with the robustness principle was employed recently in the study of the BMP-mediated patterning of the dorsal ectoderm of the *Drosophila* embryo [8••]. The dorsal region of the *Drosophila* embryo is patterned by a network of extracellular proteins, which establishes a gradient of BMP activation [12,13]. Although the components of this network have been characterized in detail, how their function is integrated to generate a robust gradient remained unclear. Moreover, in this system the morphogens are uniformly expressed in a broad range, but the final activation pattern is graded and restricted. Any model proposed should explain this feature as well.

To understand the patterning mechanism, a general rigorous model that is based on the available molecular knowledge was formulated. This theoretical model, composed of a set of reaction–diffusion equations, was kept as general as possible to allow for a broad spectrum of possible interactions. An extensive computational screen was then applied to identify the specific networks — with a particular realization of parameters, namely rate constants and gene dosages — that produce patterning and are robust. As expected, the results showed that although most networks can indeed produce proper patterning, only a small fraction (<0.3% for the robustness threshold used) are actually robust to twofold changes in gene dosage.

The subset of robust networks displayed several unique properties. First, in all these networks the steady-state profile of the morphogen (Screw or Dpp) followed a power-law with n=2 as the exponent. By contrast, in the large majority of networks not preserving robustness, the ligand was at a first approximation uniformly distributed and the activation profile was exponential. Those results indicate the uniqueness of the robust solutions.

Insight into the machinery generating robustness was provided by examining the molecular features of the robust solutions. It was found that for all the robust cases, the ligand was diffusible only upon binding to the inhibitor protein (Sog). In addition, in all the robust cases, free Sog was not cleaved by the protease Tolloid (Tld). Rather, the binding of Sog to the ligand significantly increased its cleavage by Tld. A reduced model that incorporates those two results was solved analytically to elucidate the underlying patterning mechanism. Indeed, it was found that the transport of BMP into the dorsal midline by Sog is the key event in this patterning process, in accordance with one of the previously proposed mechanisms [14–16]. The robustness mechanism relies on the ability to store an excess of signaling ligand molecules in a restricted spatial domain where Sog is largely absent.

This analysis thus identified two principal molecular features that are essential for robust network design: first, free Sog is not cleaved efficiently, an assumption that is supported by the *in-vitro* finding that Sog cleavage by Tld requires the binding of Sog to the ligand [17,18]. Second, the diffusion of free ligand is restricted. This last property was the main prediction of the theoretical studies. Importantly, in follow-up experiments, it was demonstrated that, indeed, Dpp is widely diffusible in the presence of Sog, but remains tightly localized in its absence, thus validating a central prediction of the theoretical study [8••].

Interplay between robustness and long-range morphogen signaling

While the above mechanism of patterning by BMPs is conserved in evolution [15], the strategy of generating morphogen gradient by shuttling a uniformly-expressed ligand to the midline is an exception. More typically, the morphogen is secreted by specialized cells located at the center of the field. A gradient of morphogen concentration is then established through diffusion, transport and degradation of the morphogen within the field. In the absence of feedback, if a morphogen is degraded at a fixed rate, the morphogen concentration will decay exponentially with the distance from the source.

A recent study of robustness in this more general system revealed that conditions that favor long-range signaling limit the capacity to buffer fluctuations in morphogen-production rate [7••]. Using both numerical simulations and theoretical analysis, it was found that an exponential

profile cannot, at the same time, buffer fluctuations in morphogen production rate and define long-range gradients. The former requires a rapid decline of morphogen levels close to the source whereas the latter relies upon a gradual decay in morphogen levels. In the absence of feedback, exponential profiles decay at the same rate throughout the field. Rapid decay close to the source also implies rapid decay everywhere, limiting the signaling range. This interplay severely limits the applicability of exponential profiles for coding biologically relevant morphogen gradients.

How can a morphogen compensate for variability in levels at its source? A numerical screen was initiated to search for morphogen networks that buffer fluctuations in morphogen production rate, yet maintaining the capacity for long-range signaling. A search in a wide-range of parameter space revealed several network designs that break the interplay between long-range signaling and robustness. Importantly, the crucial aspect was an effective feedback regulation of morphogen degradation rate, leading, in regions of high morphogen signaling, to an enhanced degradation.

Theoretical analysis was used to better characterize the interplay between robustness and long-range signaling, and to elucidate how feedback regulation of morphogen degradation rate assists in satisfying these two opposing requirements. It was found that the capacity to buffer fluctuations in morphogen synthesis rate is controlled at the source vicinity. Specifically, this capacity is determined by the rate of morphogen degradation at the source, but is completely independent of its rate of decay across the field. By contrast, the signaling range is determined by the average decay across the field. Feedback mechanisms ensure rapid morphogen decay close to its source, but maintain a moderate decay elsewhere.

Importantly, for such a mechanism to work, the enhancement of morphogen degradation close to its source has to be obtained through activity-dependent feedback rather than by some fixed, pre-set position-dependent control. In the absence of activity-dependent feedback mechanisms, a change in morphogen production rate will cause a proportional change in the morphogen level at each spatial position. Thus, position-dependent degradation rate cannot be used to enhance buffering capacity.

Such self-enhanced morphogen degradation may indeed be a prominent mechanism employed by several morphogen systems. For example, ligand is often degraded through receptor-mediated endocytosis. Feedback regulation of receptor expression by morphogen signaling can thus be used to control morphogen degradation. Hedgehog, for example, is a prominent morphogen that functions in numerous developmental contexts [19]. In all contexts examined, the expression of its receptor Patched is strongly induced by Hedgehog signaling [20,21]. As Hedgehog is degraded primarily through endocytosis, such feedback indeed results in enhanced ligand degradation in regions of prominent signaling, in accordance with the model.

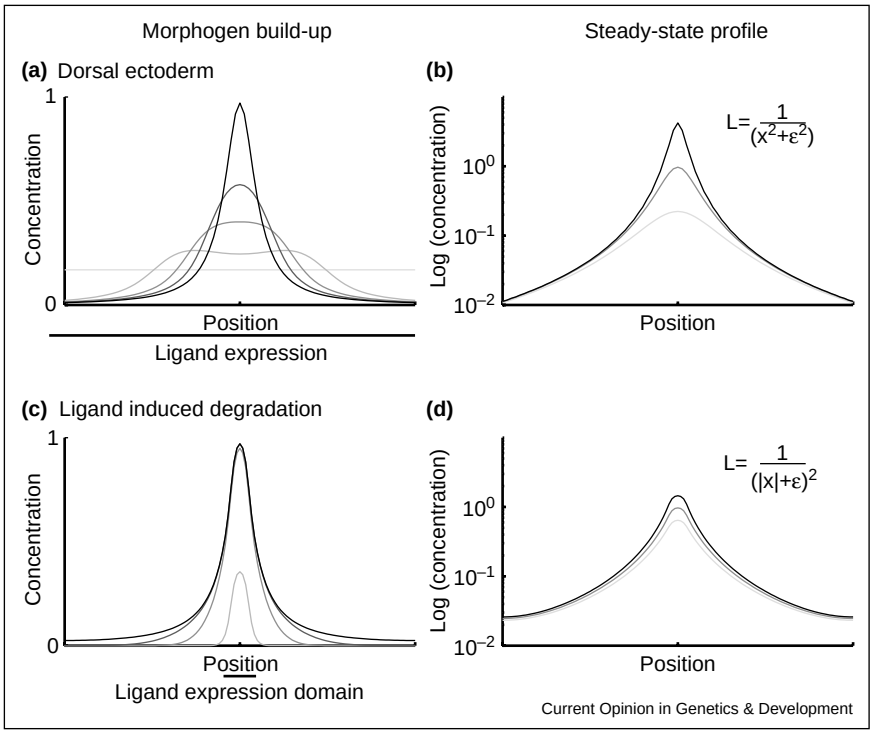
Increasing ligand degradation through elevation of receptor expression is just one example of how degradation can be regulated. Other possibilities could employ induction of protease(s) degrading the ligand or through induction of other components impinging on the extracellular matrix. Irrespective of the actual mechanism used, the outcome is the same: feedback enhancement of morphogen degradation in regions of high morphogen activity can significantly reduce the sensitivity to the rate of morphogen production.

The work described here analyzed robustness at the level of generating the morphogen profiles. It is interesting to note that two very different molecular mechanisms resulted in the same solution to the distribution of the morphogen (Figure 1). In the case of patterning the dorsal ectoderm, a uniformly expressed ligand was shuttled to produce a graded distribution decaying faster at the midline than at the more distant regions. In the case of a generic morphogen gradient, the ligand is produced by a restricted group of cells, and self-enhanced ligand degradation leads to a distribution profile that follows the same rules. In both cases, the resulting morphogen profile is the most resistant pattern to fluctuations.

Error-correcting mechanisms downstream of morphgen distribution

Additional downstream mechanisms could be used to buffer fluctuations in morphogen distribution. Recently, Houchmandzadeh *et al.* reported a fascinating example of error-correction at the level of gene-expression domains, by characterizing the embryo-to-embryo variability in the spatial expression of the *bicoid* and *hunchback* genes in the early *Drosophila* embryo [22••]. *Bicoid* is a maternally encoded transcription factor whose graded distribution in the early embryo initiates the anterior–posterior polarity. *hunchback* is one of the zygotic genes whose expression is induced by Bicoid. *hunchback* transcription is induced at a sharp border in responses to the graded distribution of Bicoid. Remarkably, although the Bicoid gradient displayed a high variability between wild-type embryos, the downstream *hunchback* expression domains were markedly constant. Moreover, the *hunchback* domain was insensitive to changes in temperature and was scaled properly with embryo size. Neither property was displayed by the upstream Bicoid gradient. Those results provide an important example for the capacity to achieve buffering and size compensation at the level of expression of individual genes, in spite of fluctuations in the level of the morphogen that induces their expression.

Figure 1



Similarities and differences between two robust morphogen gradients. The two robust mechanisms described in the text rely on different sources of asymmetry and employ distinct kinetics, but culminate in similar steady-state morphogen profiles. **(a,b)** In the robust model for generating graded BMP activation in the dorsal region of the *Drosophila* embryo, the morphogen (Dpp or Screw) is expressed throughout the dorsal region and the asymmetry is provided by the expression of an inhibitor (Sog) in the flanking regions. The figure displays the dorsal domain. Sog expression abuts this region on either side. Within the robust model, the main function of Sog is to transport the morphogen into the dorsal region, thus gradually increasing morphogen accumulation in the dorsal midline. The build-up of morphogen distribution for different times (0, 1, 2, 3 in normalized units from lighter to darker shade, respectively) is shown in (a), while its steady state distribution is shown in (b). **(c,d)** Robustness to morphogen-production rate can be achieved by a mechanism that enhances morphogen degradation in regions of high morphogen activation. In this model, morphogen is secreted by a localized source, providing the initial asymmetry. In contrast to the previous model, here the morphogen profile broadens with time. The build-up of the morphogen distribution is shown in (c) for different times (10^{-3}, 10^{-2}, 0.03 and 1 in normalized units from lighter to darker shade, respectively), whereas its steady-state profile is shown in (d). In both models, the steady-state profile decays rapidly close to its peak but more gradually further away. The steady-state distributions in (b,d) are shown for different rates of morphogen production (0.5, 1 and 2 in arbitrary units from lighter to darker shade, respectively). In both cases, profiles corresponding to different production rates differ only in a region close to the source, but are superimposed in most of the field, indicating their robustness.

Although *hunchback* expression can compensate for the relatively small naturally occurring fluctuations in Bicoid levels, changes in *bicoid* gene dosage induce a measurable shift in its expression domain [23,24]. Additional error-correction mechanisms function later in development to minimize those alterations and retain a wild-type phenotype. For example, it was shown that the anterior size, which is initially too large in mutant overexpressing Bicoid, develops to a normal-sized head in the larvae, and that increased apoptosis plays a role in this compensation [25].

Robustness was also investigated in the context of short-range signaling [4–6]. Computer simulations of a model of the segment polarity network revealed that this network can maintain the proper pattern for a wide range of parameters [5]. More recent theoretical studies have revealed that the observed robustness is caused by two positive feedback loops that produce a bi-stable behavior [26•]. Indeed, bi-stability may be a general strategy for stabilizing short-range differentiation decisions.

Conclusions and outlook

Robustness to fluctuations in gene dosage is one aspect of the general capacity to sustain developmental stability despite genetic and environmental fluctuations, which has fascinated developmental biologists since the early studies of embryonic development. Over 60 years ago, CH Waddington coined the concept of 'canalization', referring to the capacity of the wild-type to overcome genetic and environmental variation [27]. In the absence of molecular data, early work characterized canalization by comparing the variability of the wild-type phenotype in genetically heterogeneous populations, to the variability

of the phenotype in either a mutant background or under extreme environmental conditions. The advance in understanding the molecular machinery underlying robustness to changes in gene dosage may open the way for elucidating the molecular machinery underlying other aspects of canalization as well.

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