Relationship between epithelial organization and morphogen interpretation

Review Article

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Publication date:
2022-08

Permanent link:
https://doi.org/10.3929/ethz-b-000550937

Originally published in:
Current Opinion in Genetics & Development 75, https://doi.org/10.1016/j.gde.2022.101916

Available online at www.sciencedirect.com

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Current Opinion in Genetics & Development

Relationship between epithelial organization and morphogen interpretation

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Despite molecular noise and genetic differences between individuals, developmental outcomes are remarkably constant. Decades of research has focused on the underlying mechanisms that ensure this precision and robustness. Recent quantifications of chemical gradients and epithelial cell shapes provide novel insights into the basis of precise development. In this review, we argue that these two aspects may be linked in epithelial morphogenesis.

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Current Opinion in Genetics & Development 2022, **75**:101916

This review comes from a themed issue on **Developmental mechanisms, patterning and evolution**

Edited by **Magali Suzanne** and **Miguel Torres Snchez**

https://doi.org/10.1016/j.gde.2022.101916

Introduction

Epithelia are among the first tissue types to emerge during morphogenesis. A hallmark of epithelia is apical–basal polarity (Figure 1). Beneath the apical surface, tight junctions create a watertight seal, and control the paracellular passage of ions and solutes between epithelial cells, while preventing the mixing of apical and basal–lateral membranes. The adhesion belt and further adhesive junctions along the lateral side stabilize the cell–cell contacts. On the basal-most side, epithelia bind tightly to the basal lamina. The first epithelial structure, the blastula, is a hollow sphere made of a single epithelial cell layer. At later stages, multilayered epithelia emerge. As development progresses, the simple epithelial sheets, tubes, and vesicles grow and deform into the complex shapes characteristic of organs and adult tissues. These morphogenetic changes are guided by a wide range of mechanisms that use chemical signals, mechanical constraints, and fluid-flow-induced shear stress. As animals develop from a single cell, cells must take on the correct fate at the right position and time to build a functional organism. Developmental outcomes are remarkably constant, despite environmental, inter-individual, and evolutionary changes that alter reaction time and patterning length scales [1–3], a phenomenon coined as canalization. How such developmental precision and robustness is achieved is still largely unknown. While mechanical contributions have recently received greater attention, precision of morphogenesis has, so far, mainly been studied for gradient-based patterning.

Morphogen gradient precision

The measured morphogen gradients can be approximated well either by an exponential function

$$C(x) = C_0 e^{-x/\lambda} \quad (1)$$

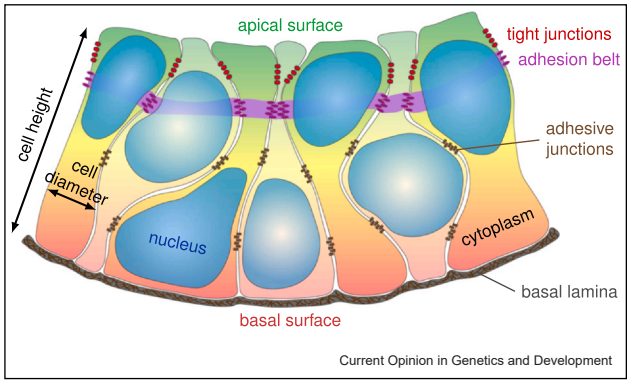
with an amplitude C_0 at the morphogen source at $x = 0$ and a decay length λ (Figure 2a), or by a power law

$$C(x) = A(x + x_0)^{-m}, \quad (2)$$

where A, m, x_0 are positive constants [4–9]. These gradient profiles emerge independent of whether morphogen transport happens via diffusion or cytonemes [10]. Diffusion-based gradients have been argued to be more precise for large gradient length, and vice versa [11]. Power-law gradients arise from ligand-enhanced degradation and are less sensitive to a variable source [12], but the shallower gradient profile far away from the source limits their usefulness [13]. It has been argued that the best cost-precision trade-off can be achieved when gradients are read out at about 2λ from the source [14], but patterning distances are much larger in the neural tube (NT) [7], and SHH-dependent responses are observed even in the very dorsal NT, which is more than 10λ away from the SHH source [15].

According to the French flag model, morphogen gradients define different tissue domains via concentration thresholds (Figure 2a) [16,17], although intracellular regulatory networks can result in more complex dependencies [18,19]. In case of a threshold-based readout, cells exposed to morphogen concentrations above the threshold take on a different fate from cells exposed to lower concentrations. Measurements in several developmental systems reveal the direct readouts of the

Figure 1



Cell polarity in a pseudostratified epithelial layer.

morphogen gradients to be smooth [7,20,17,21]. Sharp transitions require ultrasensitive readout mechanisms, which may include features such as cooperativity, zero-order ultrasensitivity, or hysteresis, among others. Bistable networks have been explored to explain sharp boundaries in development, and to engineer gradient-based patterning in synthetic biology approaches [21–26]. Also with sharp readouts, noise can still result in a transition zone with mixed cell fates (Figure 2b), resulting in misaligned boundaries in morphogenetic fields of single embryos. As the morphogen concentration declines with distance from the source, noise-driven transition zones have been suggested to widen [13]. Cell alignment along sharp boundaries can be achieved via polarized contractility, adhesion-based cell sorting, and cell competition [27–32].

Deviations in the readout position, x_θ, between embryos, i, are referred to as positional error (Figure 2c)

$$\sigma_x = \mathrm{SD}\{x_{\theta,i}\}. \quad (3)$$

The positional error has been reported to be smaller for the readout than for the gradient [4,33,34,7], resulting in a quest for precision-enhancing mechanisms. Spatial and temporal averaging have been proposed to enhance precision in the *Drosophila* blastoderm syncytium [4], and the downstream-gap gene network has been suggested to act as an optimal decoder of upstream positional information by integrating maternal inputs across the embryo [35,36]. Similarly, optimal decoding of the opposing SHH and BMP gradients has been proposed to explain the high precision of the progenitor-domain boundaries in the center of the mouse NT [7]. However, the gradient variability had been overestimated, and single gradients would be precise enough to pattern the center [37].

Recent work highlights the importance of dynamics for patterning precision in the *Drosophila* blastoderm. Precise patterning can be achieved faster when cellular decision times vary, depending on the statistical realization of the noisy signal, as formulated in Wald's sequential probability-ratio test [38], and the transient dynamics that emerge from the complex regulatory interactions in the gap gene network play an important role in patterning precision and canalization [19].

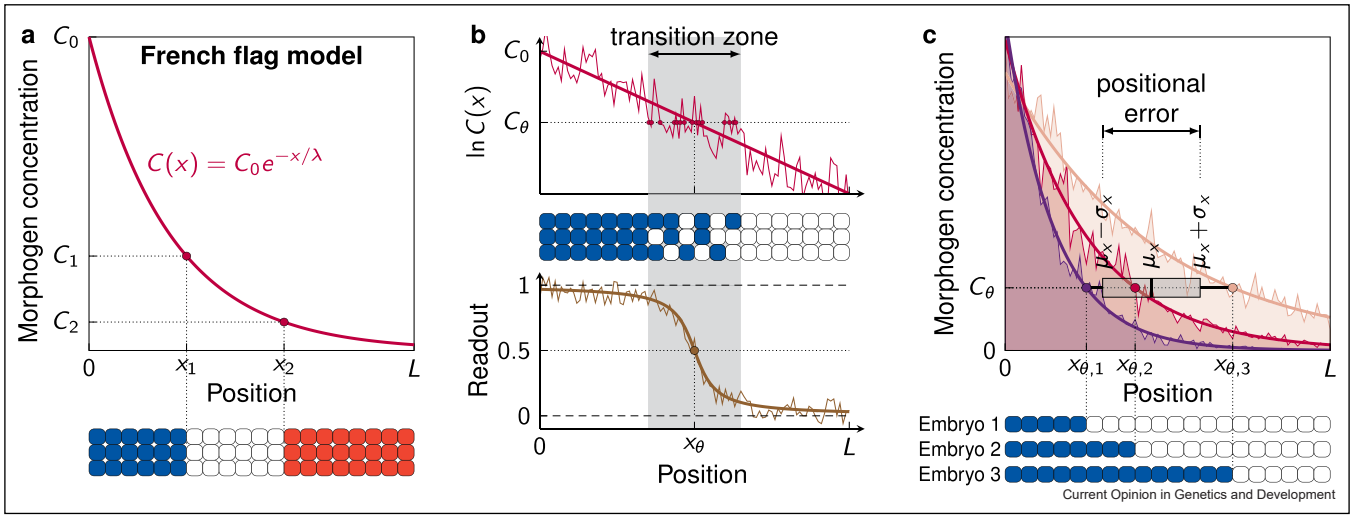
As concentration gradients spanning orders of magnitude are more difficult to image in their entirety than their sigmoidal readouts (Figure 2b), the possibility remains that the reported higher gradient variability reflects technical errors. For the reported molecular noise levels in morphogen production, decay, and transport, cell-based simulations predict a gradient variability that is consistent with the observed precision of readouts, even at very large distances from the source [37,39]. Morphogen gradients may thus be more precise than thought and provide sufficient positional information on their own.

Relationship between cellular organization and patterning precision

Large cell diameters in the patterning direction increase gradient variability via their impact on morphogen production, removal, and transport, while spatial averaging over the cell surface or via cilia- or cytoneme-based sensing has only a small impact in the cell-based simulations (Figure 3a). Here, it is only important to limit the cell diameter in patterning direction, but not necessarily in the orthogonal directions. Interestingly, in the developing lung, where no gradient-based patterning has been described, the apical surfaces are elongated in the direction of preferential outgrowth [41], while in the NT, where gradients pattern the tissue along the axis of preferential outgrowth, this is not the case [42,43]. Many ligands are sensed also on the basal–lateral side [44,45], but the same principles that apply apically also apply basal-laterally: also in this case, smaller cell diameters in the patterning direction will result in less variability. As morphogens must diffuse through the intercellular space to reach the lateral sides, the effective diffusion path is, however, longer than the beeline [46]. This phenomenon is commonly referred to as tortuosity, and alters the effective macroscopic morphogen diffusivity.

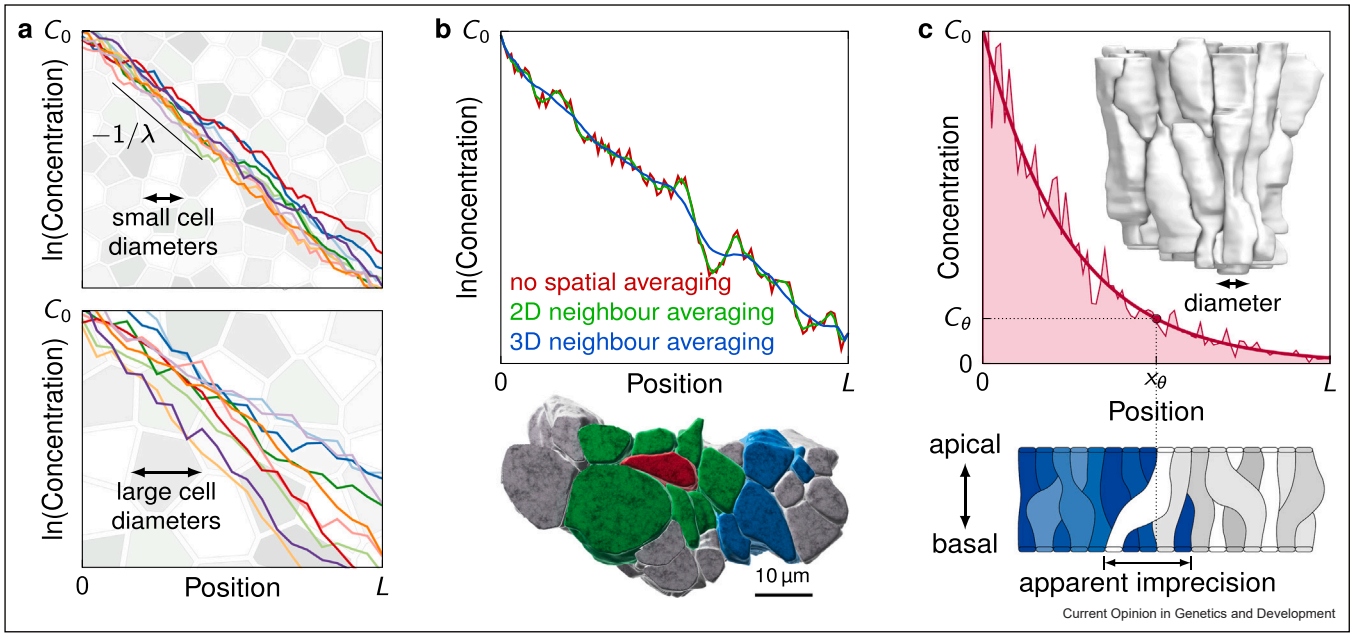
The apical surfaces are small in all epithelia that are known to employ gradient-based patterning and that have been quantified [39]. A small apical surface is not necessarily a reflection of small overall cell size, as measured by its volume. For one, tissue curvature can result in smaller apical surfaces (Figure 1a). In the NT, the SHH-sensing cilium is indeed located on the inner, apical surface [47], while in the flat *Drosophila* wing and eye discs, cells sense Hh along the entire apical–basal axis [45]. Finally, several morphogens, including SHH

Figure 2



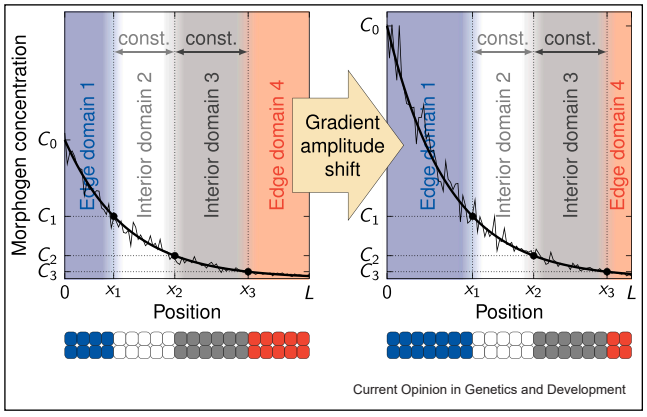
Morphogen gradient precision. **(a)** The French flag model and the French flag patterning problem. **(b)** Transition zones are not necessarily sharp. The noisy gradient reaches the readout threshold $C(x) = C_\theta$ at several positions (purple dots). The readout position x_θ (brown dot) is set as the position where the averaged morphogen concentration (pink line) equals the Hill constant of the sigmoidal readout (brown line); alternative definitions exist. **(c)** Variability in the gradients translates into different readout positions. μ_x is the mean readout position of the three gradients.

Figure 3



Relationship between cellular organization and patterning precision. **(a)** Morphogen gradients across tissues with small cell diameters are less variable than with large cell diameters. **(b)** Spatial concentration averaging over cell neighborhoods reduces gradient variability only little; long-range 3D cell contacts may increase this effect. Colors as below in the epithelium. Bottom: Nonlocal cell neighborship in the mouse lung epithelium as seen on the apical surface, reproduced with modifications from Ref. [40]. Green and blue cells are in direct contact with the red cell somewhere along the apical–basal axis, even though on the surface, only green neighbors are apparent. **(c)** The complex noncolumnar shape of cells in pseudostratified epithelia may give rise to an apparent imprecision of domain boundaries (blue/white). Inset: Epithelial cells in the monolayer epithelium of the developing mouse lung. Dataset from Ref. [40]. All noisy gradients are artificially created for illustration.

Figure 4



Precision of progenitor cell numbers. In interior patterning domains (white, gray), cell numbers (bottom) are preserved under changes in the amplitude C_0 of exponential morphogen gradients.

and ligands of the TGF-β and the WNT family, have been shown to increase the apical–basal height of epithelial cells, and thus shrink the cell diameter, via their impact on actin polymerization, myosin localization, and activity [48–50]. Morphogen signaling itself may thus result in small epithelial cell diameters — a relationship that deserves further clarification.

For narrow cells, the nucleus — albeit deformable — is wider than the average cell diameter (Figure 1), and the nuclei disperse along the apical–basal axis, a phenomenon commonly referred to as pseudostratification [40,51]. During mitosis, the nuclei must locate to the apical surface. As there is insufficient space to accommodate all nuclei apically, they move toward the basal side during the G1 phase, and back to the apical surface during the G2 phase in a process termed interkinetic nuclear migration (IKNM) [51]. The evolutionary driving force behind the emergence of pseudostratification has so far remained elusive, but may now be explicable with the importance of slim cells for high patterning precision.

Cells in pseudostratified epithelia change their neighbor relationships several times along the apical–basal axis in an effort to minimize the surface area that covers their complex cell shapes [40]. They are thus in contact with many more cells than what is apparent on the apical or basal cell surface (Figure 3b), and potentially sense ligands over a wider distance. The neighbor relationships change dynamically over time, facilitating the sorting of epithelial cells to create sharper boundaries [32,40].

Going forward, it will be important to analyze expression domain boundaries in 3D. Cells or nuclei that may appear as if they were in the 'wrong' position in 2D slices may actually be in the correct domain (Figure 3c). Given the fluidity of cell contacts, establishing sharp boundaries may not be as relevant as producing cells of a certain type in correct numbers, as determined by the size of individual domains. The size of interior domains is more precise than the position of their domain boundaries if the gradient shape remains exponential because changes in the gradient amplitude shift the position of the domain without altering its size (Figure 4) [37].

Conclusion and outlook

Patterning precision has long been analyzed with a focus on the information content of chemical gradients. It is becoming increasingly evident that tissues achieve high patterning precision not only by minimizing molecular noise in chemical reactions, but also by controlling cell and tissue geometry [2,39]. Mechanical stress patterns that depend on the cellular contractility and substrate stiffness may also contribute to tissue patterning [52]. Finally, tissue patterning and growth are intricately linked. Morphogens control not only patterning, cell differentiation, and cell shapes, but also the tissue growth rate [6]. How embryos control tissue size and how patterns scale with domain size remains a field of intense inquiry (e.g. [9,53–57]). Synthetic gradients [58,53,59] and computational frameworks that enable high-resolution 3D cell-based tissue simulations [60] are promising tools to understand how nature achieves robust and reliable patterning to an extent that the same molecular patterning mechanism can be reused in evolution, despite large changes in tissue size and developmental rate.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was funded by Swiss National Science Foundation (SNF) Sinergia Grant CRSII5 70930. We thank Kevin Yamauchi for helpful comments, and apologize to those authors whose work we could not discuss due to length and citation constraints.

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