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Journal

Current Opinion in Genetics & Development, 21(6)

ISSN

0959-437X

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Publication Date

2011-12-01

DOI

10.1016/j.gde.2011.10.002

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Peer reviewed

Spatiotemporal mechanisms of morphogen gradient interpretation

Marcos Nahmad and Arthur D Lander

Few mechanistic ideas from the pre-molecular era of biology have had as enduring an impact as the morphogen concept. In the classical view, cells in developing embryos obtain positional information by measuring morphogen concentrations and comparing them with fixed concentration thresholds; as a result, graded morphogen distributions map into discrete spatial arrangements of gene expression. Recent studies on Hedgehog and other morphogens suggest that establishing patterns of gene expression may be less a function of absolute morphogen concentrations, than of the dynamics of signal transduction, gene expression, and gradient formation. The data point away from any universal model of morphogen interpretation and suggest that organisms use multiple mechanisms for reading out developmental signals in order to accomplish specific patterning goals.

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Current Opinion in Genetics & Development 2011, **21**:726–731

This review comes from a themed issue on
Genetics of system biology
Edited by Norbert Perrimon and Naama Barkai

Available online 25th October 2011

0959-437X/\$ – see front matter
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DOI [10.1016/j.gde.2011.10.002](https://doi.org/10.1016/j.gde.2011.10.002)

Introduction

A fundamental problem in developmental biology is how initially identical cells reliably achieve specific spatiotemporal patterns of differentiation. For the last half century, the most influential model has been that of the morphogen gradient. Classically, morphogens are molecules that form concentration gradients in space, and to which cells respond in distinct ways depending on the morphogen dose they encounter. In recent years, the study of morphogen gradients has been a rich topic for research, with most studies focusing either on how such gradients form, or how they are interpreted by cells (reviewed in [1–5]). Here we take up the latter question—how graded signals are translated into discrete patterns of gene expression—and review literature suggesting that morphogen gradient interpretation cannot

be entirely separated from the dynamics of signaling and morphogen gradient evolution over time.

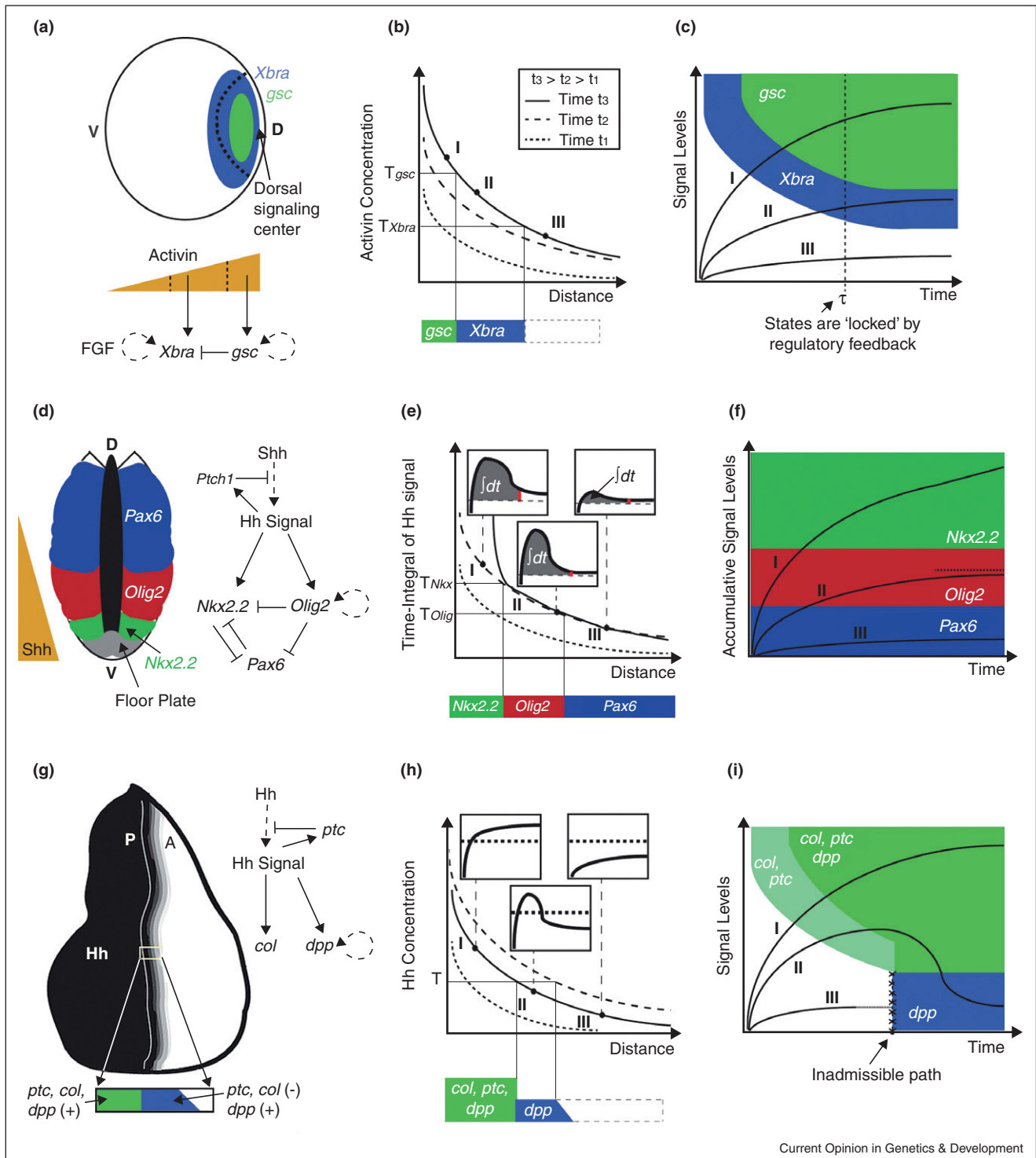
Concentration landscapes and patterns: a one-to-one relationship?

Among modern biologists, the most widely accepted notion of what a morphogen is comes from experimental studies, such as those of Stumpf [6] and Lawrence [7] in insects, as interpreted and popularized in the context of Wolpert's positional information theory [8]. Molecular identification of morphogens came much later, beginning with the maternal transcription factor Bicoid (Bcd), which acts as a cytoplasmic morphogen within the syncytial *Drosophila* embryo [9–11], and later the secreted TGF- β family member Decapentaplegic (Dpp), which patterns the *Drosophila* larval wing imaginal disc [12–14].

The subsequent molecular identification of additional families of signaling molecules—including other TGF- β s, FGFs, EGF, Hedgehogs, Wnts, and retinoids—as morphogens has depended upon experimental demonstrations that such molecules do act at a distance from their site of production and elicit distinct cellular responses in a concentration-dependent manner. The fact that cellular responses in a tissue correlate with morphogen concentration does not, however, imply that each cell simply 'reads' the concentration it experiences, nor does it tell us how different concentrations are distinguished. Indeed, given the shallowness of many morphogen gradients, it has been suggested that, using only the cell-autonomous readout of a static gradient, it might be difficult to create the reliable, sharply demarcated domains of gene expression that are commonly observed during patterning [4].

So far, the most compelling support for the view that cells simply read morphogen concentrations comes from studies of activin, a member of the TGF- β family that patterns the dorsal mesoderm in the *Xenopus* embryo (reviewed in [15]; Figure 1a). Activin controls the differential expression of the genes *gooseoid* (*gsc*) and *Xenopus brachyury* (*Xbra*) in a dose-dependent manner [16–19] (Figure 1a), with cells responding autonomously as a function of absolute receptor occupancy (about 100 activin-bound receptors are sufficient to activate *Xbra*, while approximately 300 are required for *gsc*; [20]), relatively independent of signal duration (Figure 1b). *In vivo*, the maintenance and sharpening of gene expression boundaries seems to be resolved downstream of activin signaling, at the level of target gene-interactions—for

Figure 1



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Models of morphogen gradient interpretation. (a–c), Mesoderm specification in *Xenopus* depends on activin concentrations. Activin is secreted from the dorsal signaling center, and forms a dorsal (D) to ventral (V) gradient that activates *Xbra* and *gsc* at successively increasing concentration thresholds ($T_{gsc} > T_{Xbra}$; (a)). In (b), (as well as in (e), and (h)), three gradients at successive time points are shown indicating the dynamics displayed by the gradients (legend only displayed in (b), but also applies to (e) and (h)). In the case of activin, genes are activated sequentially; cells exposed to high activin concentrations express first *Xbra* and then *gsc* (trajectory I in (c)). Then, *Xbra* is turned off by *Gsc* (a), resulting in mutually exclusive domains. Once cells have acquired a stable pattern (e.g. after time τ in the trajectories in (c)), they no longer require activin signaling and are no longer under the control of the morphogen thresholds (dotted line in (c)). Cell states are now irreversible and maintained by positive feedback loops downstream of

example, Gsc-mediated repression of *Xbra* [21], and a positive feedback loop between *Xbra* and FGF signaling that amplifies expression of *Xbra* in its own domain [22,23] (Figure 1a). Such network interactions are sufficient to ‘lock’ the regulatory state of cells, so that fates can be maintained when signaling levels drop below required thresholds, or after morphogen exposure is lost [19,24] (Figure 1c).

Is activin’s function as a simple dose-dependent inducer of genes typical of all morphogens? Or is positional information sometimes encoded in other ways? It is inevitable that morphogen gradients change over time, initiating, spreading, and eventually being shut down. Moreover, cells that respond to morphogens often divide, move and differentiate, so that their relationships even with static morphogen gradients would be expected to change over time. Finally, the signals generated within cells by morphogens may themselves be dynamic, due, for example, to feedback or feed-forward effects. If cells normally experience morphogen gradients in such a spatiotemporally varying a manner, the question arises as to whether the information encoded in such dynamics is used in any meaningful way, and if so, to what end? Below we discuss some insights into these questions that have come out of the study of several morphogen systems, most notably the patterning of the vertebrate neural tube and the *Drosophila* wing disc by morphogens of the Hedgehog family.

Signal integration: the case for duration-encoding

Even cells that encounter similar morphogen levels at the time we observe them may have had very different histories of morphogen exposure. One measure of that history—the duration of exposure—can potentially be recorded by cells in the form of a signaling intermediate or gene product whose level accumulates during the period of morphogen exposure. That level, reflecting the time-integral of morphogen signaling, would normally be sensitive to both the amount and duration of morphogen

exposure, but given equal levels of morphogen signaling, would directly reflect the duration.

There are several patterning systems in which it has been proposed that positional information is encoded in the duration of morphogen signaling. These include the patterning of rhombomeres in the zebrafish hindbrain by retinoic acid [25,26]; the specification of digits in the vertebrate limb in response to Sonic Hedgehog (Shh) signaling [27]; the specification of olfactory and lens placodal cells in the chick embryo in response to BMPs [28]; and the dorsoventral patterning of the chick neural tube by Shh [29].

The last of these examples is so far the best understood mechanistically, thanks to several recent studies [29,30,31*,32,33*]. In the developing vertebrate spinal cord, Shh is produced at the ventral midline of the neural tube and forms a ventral-to-dorsal concentration gradient responsible for assigning positional identities to neural progenitor cells (Figure 1d). Although levels of Shh correlate with the establishment of neural progenitor domains, experimental evidence indicates that duration of exposure to Shh plays a crucial role in establishing cell fates. For example, exposure to moderate Shh levels for about 6 h causes cells to express the motor neuron marker, *olig2*, but longer exposures eventually lead them to upregulate the V3 interneuron marker, *Nkx2.2*, which represses the *olig2* fate in the ventral-most region of the neural tube, where Shh levels are highest [29] (Figure 1d). The gene regulatory mechanisms underlying this behavior have been partly worked out: Cells receiving and accumulating Shh signaling can immediately activate *Olig2* expression, but *Nkx2.2* activation cannot proceed because it is repressed by *Pax6*; once *Olig2* accumulates sufficiently it represses *Pax6* and *Nkx2.2* expression proceeds. Thus, signal accumulation is recorded by a feed-forward gene-regulatory loop (Figure 1d). Consistent with this model, patterns of *Olig2* and *Nkx2.2* are normally established sequentially, and on the time scale over which *Pax6* becomes restricted to dorsal regions (Figure 1e,f; [31*]).

(Figure 1 Legend Continued) activin signaling ((a); dotted lines in the regulatory networks denote that the interaction may be not direct). (d–g), In the developing spinal cord, Shh is secreted in the floor plate in the ventral-most cells of the neural tube and establishes the expression of *Nkx2.2*, *Olig2*, and *Pax6*, among other neural markers, in specific domains of the neural tube (d). Not shown in (d–f), for the sake of simplicity, is that low levels of *Pax6* persist in *Olig2*-expressing cells. As in the case of activin, the neural progenitor markers *Olig2* and *Nkx2.2* are also established sequentially (trajectory I in (f)), but in this case, the order of activation depends on the integration of Hh signaling over time (e). This is accomplished by a feed-forward loop in which activation of *Nkx2.2* requires both sustained Shh exposure and *Olig2*-mediated repression of the *Pax6* inhibitor (d). In (e), the time-integral (area under signal vs. time curves; see insets) is higher closer to the Shh source, because ‘desensitization’ of cells is slower when exposed to higher Shh concentrations. In this model, cell fates are determined by thresholds in accumulated signal levels (f). In (f), it is assumed that the ‘time-integral’ of the signal is bounded, at least in cells marked by *Olig2* (dotted line in (f)), although it is unclear how this is accomplished. (g–i) In the *Drosophila* wing disc, Hh is expressed in the posterior (P) compartment and establishes a short-range gradient into the anterior (A) compartment (g). *col* (and *ptc*) and *dpp* become expressed in different domains as a consequence of a refinement in the Hh gradient (h). This ‘shift’ results from increased Hh sequestration and destruction by *Ptc* as *ptc* becomes upregulated by Hh signaling (g). Thus, unlike cells exposed to sustained signaling (I), or not receiving Hh at all (III), some cells in the middle of the gradient (II) receive Hh signaling only transiently (insets in (h)). This model of signal interpretation (represented by the state-space in (i)) only requires a single switching threshold, T. Unlike in the previous examples (panels (c) and (f)), *col/ptc* are turned on before *dpp* (i), despite the fact that cells are exposed to increasing concentrations. In (i), the white and blue domains are not connected (i.e. trajectory I is assumed to reach the white-blue boundary in infinite time).

Interestingly, if one examines a direct readout of Shh signaling (a reporter for GLI transcription factor activity) within moderately short times after exposure of cultured neural plate explants, rather small differences are observed between the effects of significantly different doses of the morphogen. Only later do large differences in signaling emerge, owing to temporal adaptation [29]. This adaptation reflects two peculiarities of Hh signaling: The first is that the normal function of the Hh receptor Patched (Ptc; Ptc1 in vertebrates) is to maintain signaling in an inactive (OFF) state, while binding of Hh to Ptc relieves this repression and activates signaling (in effect, signaling is a function of the total number of *unoccupied* receptors). The second feature is that, in all systems examined so far, *ptc* is always a transcriptional target of Hh signaling, being strongly upregulated by members of the Hh family in both *Drosophila* and vertebrates (Figure 1d,g). Together these phenomena help explain the slow desensitization of Shh-treated cells in the neural tube [29].

One potential disadvantage of encoding positional information in the time-integral of a morphogen signal is that the integral of a sustained signal grows continuously over time, making the positional value that a cell reads potentially very sensitive to the exact time it chooses to read it (Figure 1e). For duration-encoded signals to provide robust patterning information, either morphogen signaling needs to be turned off, cells need to move away from the morphogen, or cell-intrinsic mechanisms need to 'lock in' cell fates despite the presence of a continuously changing morphogen signal (Figure 1f). One advantage offered by temporal adaptation of morphogen signals is that, by slowing down the rate of signal integration, patterning can be made less sensitive to the timing of these events.

The importance of morphogen gradient dynamics

A general consequence of temporal adaptation is that it creates a dynamic output even in response to a constant input. Because adaptation to Shh in the neural tube appears to vary inversely with Shh concentration [29]—a curious finding, which may relate to indirect effects of Shh target genes on Shh sensitivity [33*]—sustained high-Shh exposure results in much longer-duration signaling than sustained low-Shh exposure [29,31*]. Thus, cells in the neural tube might usefully employ duration-encoding as a way to translate small changes in static Shh levels into large changes in signaling.

However, there are good reasons to suspect that Shh levels are not static. Because Ptc appears to be the primary receptor for Hh clearance, in both vertebrates and invertebrates, the very same transcriptional feedback that causes cell-autonomous adaptation of Hh signals should also be expected to cause changes to the shapes of Hh gradients themselves [34]. Although such gradient

dynamics have been modeled in the vertebrate neural tube [35], they have been most extensively studied, and subjected to experimental verification, in the *Drosophila* wing disc [34,36*].

In both systems, modeling led to the hypothesis that Hh gradients should undergo biphasic dynamics, first spreading out broadly (when Ptc levels are relatively low), and then retracting back toward the morphogen source (as Ptc is upregulated in response to signaling), ultimately producing a much shorter gradient with a much steeper slope. Experimental evidence in the wing disc supports the view that this refinement, or 'spatial overshoot', indeed takes place [36*]. As a result of this overshoot, there is a domain of cells that is only transiently exposed to Hh signaling, sandwiched between cells that receive the signal continuously, and cells that never receive the signal [36*] (Figure 1h). In order for such a situation to produce nested patterns of gene expression, it was proposed, and experimentally verified, that some Hh targets, such as *dpp*, can be maintained by the memory of earlier Hh signaling (i.e. wing disc cells integrate and retain an earlier signal), while others (such as *collier (col)* and *ptc* itself) simply read out the current level of Hh signaling [36*] (Figure 1i). As in the chick neural tube, the end result is that cell fates that require continuous morphogen exposure lie closest to the morphogen source, and those that do not, lie further away. A major difference, however, is that—because of the remodeling of the morphogen gradient over time—cells nearest the Hh source in the wing disc display fates associated with responding to the current (not time-integrated) morphogen signal, whereas in the neural tube the cells closest to the Shh source display fates associated with long-time integration of the signal (Figure 1f,i).

A second difference between the wing disc and neural tube models is that, in the wing disc, because of the gradient dynamics, there is no need for cells to encode any information about Hh concentration other than whether it is 'high' or 'low', that is, only a single, binary signaling threshold is required. By contrast, the temporal adaptation model for the neural tube depends upon different signaling strengths producing different durations of signaling.

Looking ahead: anticipating a greater role for dynamics in patterning

The ability of the wing disc Hh gradient to specify multiple cell fates at distinct locations despite an absence of multiple response thresholds illustrates a general point: positional information can be encoded by morphogen gradients in multiple ways. Not only is there a reason to believe, as discussed above, that the time integrals of morphogen signals play a crucial role in patterning, there is also reason to believe that cells can measure the time derivatives of morphogen signals.

For example, it has been proposed that the relative rate of rise of Dpp concentration, rather than Dpp concentration itself, drives cell growth in the *Drosophila* wing disc [37]. In addition, recent studies indicate that signaling dynamics downstream of both Wnts and EGF (both serve as morphogens) are temporally adaptive [38,39]. Within the context of a dynamically changing morphogen gradient, such adaptivity implies that cells are intrinsically capable of reading out the relative time derivative of the gradient. For Wnts and EGFs, the mechanistic basis for such behavior is thought to be an adaptive circuit known as the incoherent feed-forward loop [40], in which a stimulus first activates and then, through a parallel pathway, represses a response. In general, any adaptive circuit has the potential to make responses sensitive to the time-derivatives of stimuli. For example, in the *Drosophila* wing disc Hh gradient (Figure 1g), in which the dynamics of gradient expansion and contraction are driven by an adaptive circuit (mediated by Hh-dependent *ptc* upregulation), the spatial extent of *dpp* expression will be a function of the rate at which Hh spreads relative to the rate at which *ptc* is upregulated.

Just as we are increasingly learning that cells can respond to the time-derivatives and time-integrals of morphogen signaling, evidence is also emerging that they may measure space-derivatives and space-integrals as well. For example, in the Dpp gradient of the *Drosophila* wing disc, there is good evidence that some cellular responses are driven by measurements that cells make of the difference between their own Dpp signaling and that of their neighbors, effectively a readout of spatial slope [41,42]. A measurement of the spatial integral of a morphogen gradient can be obtained whenever a morphogen induces (or represses) expression of something that diffuses rapidly throughout a morphogen field, such that its level reflects the total (integrated) amount of morphogen. This sort of mechanism has recently been suggested to underlie the scaling of morphogen gradients with tissue size, in cases in which the rapidly diffusing molecule feeds back upon the length scale of the morphogen gradient itself [43,44].

In coming years, it seems likely that many additional examples will emerge of dynamic morphogen gradients, in which cells measure and respond not just to morphogen levels, but to temporal and spatial derivatives and integrals. The challenge for the future will be not only to identify the mechanisms underlying such processes, but to understand how they may contribute to making patterning more precise, robust, or flexible.

Acknowledgements

The authors acknowledge support from NIH grants GM067247 and GM076516. MN also acknowledges postdoctoral support from CONACyT (Mexico).

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