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Title
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Permalink
https://escholarship.org/uc/item/360348ms

Journal
Science, 339(6122)

ISSN
0036-8075

Author
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Publication Date
2013-02-22

DOI
10.1126/science.1224186

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How Cells Know Where They Are

Arthur D. Lander

Development, regeneration, and even day-to-day physiology require plant and animal cells to make decisions based on their locations. The principles by which cells may do this are deceptively straightforward. But when reliability needs to be high—as often occurs during development—successful strategies tend to be anything but simple. Increasingly, the challenge facing biologists is to relate the diverse diffusible molecules, control circuits, and gene regulatory networks that help cells know where they are to the varied, sometimes stringent, constraints imposed by the need for real-world precision and accuracy.

To measure the distance from one object to another, something must traverse the intervening space. For distances of centimeters or meters, we lay down a ruler or tape measure; for objects much farther away, we might bounce off sound or radio waves and measure the return time. In the microscopic world of cells, where measurements of position typically are made over spans of a few hundred micrometers or less (Fig. 1), the things that most readily cross such distances are molecules, and the simplest way they do so is by aqueous diffusion. Unlike the propagation of light or sound waves, diffusion is not a constant-rate phenomenon—a diffusing front gets slower as it spreads—making computation of distance from arrival time tricky (although not impossible). But, given constant production at a source, diffusion can create steady-state gradients within which concentration is a proxy for distance. From this insight, it was proposed, and later demonstrated, that cells in developing animal embryos receive positional cues from diffusible molecules that indeed form stable gradients across tissues (*1*). Such molecules, dubbed morphogens, play central roles in orchestrating developmental pattern formation.

Recently, there has been debate about whether cells really receive positional information by measuring concentrations in steady-state diffusion gradients (*2*, *3*). The most serious objections have to do with reliability: In the world of cellular biochemistry, variability in synthesis and secretion, in the binding of molecules to receptors, in the activation of signaling pathways, and in gene regulation can all be quite high. Among other things, such variability can stem from the environment (e.g., unpredictable temperature or nutrition), genetics, or stochastic fluctuations in biochemical processes. Yet the positional information that cells ultimately obtain is often exceedingly reliable, particularly during development (as evidenced by the remarkably accurate symmetries and family resemblances we encounter in our own bodies). Can steady-state diffusion gradients provide that kind of reliability?

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In short, it depends. It depends on the amount and kind of variability cells face, the mechanisms by which gradients form, and how much reliability is required. Consider, for example, a sheet of cells (an epithelium) in which a diffusible morphogen is secreted at a constant rate by cells lying in a stripe (Fig. 2A). The morphogen is destroyed everywhere, through receptor-mediated uptake, at a constant proportion per time (this situation approximates what is thought to be the case in a variety of developing tissues). Eventually, a stable gradient forms in which morphogen concentration falls exponentially away from the source, the precise shape determined by the morphogen's rate of production, diffusivity, and rate of uptake and destruction. A cell's reading of morphogen concentration will then depend on its number of receptors and how much intracellular signaling occurs per occupied receptor.

Not surprisingly, if cellular location is measured from the morphogen concentration sensed by each cell, unreliability in any of these processes—morphogen production, transport, uptake, receptor synthesis, and signaling—will produce measurement errors. The type and magnitude of the error will depend on what is varying and where the cell is located. Variability that enters upstream of individual cells (e.g., in morphogen production or transport) or affects all cells equally (e.g., animal-to-animal differences or temperature change) will produce inaccuracy, that is, a shift in the locations of positional values. In contrast, cell-to-cell variability produces imprecision, that is, scatter in the

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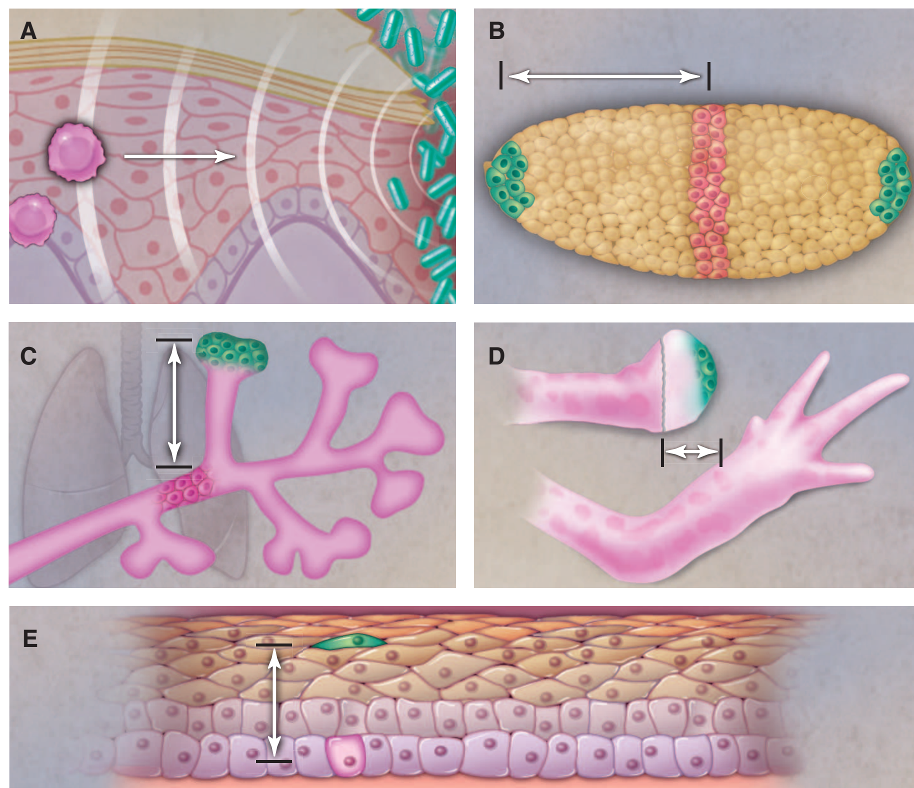

Fig. 1. A gallery of positional tasks. A leukocyte (**A**) may need to know in which direction to head to find the site of an infection but not the absolute distance to it. A cell in an early embryo (**B**) may need to know absolute location with respect to one or the other end of the embryo so that it differentiates into a spatially appropriate cell type, whereas a cell in a tissue undergoing branching morphogenesis (**C**) may need to know only the rough location with respect to the nearest branch point or vessel. In regenerating tissues (**D**), cells need to know their position with respect to a site of injury or amputation, whereas in tissues or organs with laminar structures (**E**) cells may need only know whether they are in the appropriate layer.

positional measurements that cells at equivalent locations make. Inaccuracy is often best quantified by a sensitivity coefficient, a unitless number that captures the fold change in the location of a positional value per any given fold change in some upstream process. Imprecision is most readily quantified as a transition width, the distance away from any position *x* that one must move before no more than some maximum proportion of cells (e.g., 15%) conclude that they are on the wrong side of *x* from where they actually are (*4–6*).

Sensitivity coefficients for perturbations of morphogen and receptor production and estimated transition widths associated with stochastic fluctuations in receptor occupancy are plotted in Fig. 2B for morphogen gradients such as the one in Fig. 2A by using assumptions about receptor abundance estimated from the *Drosophila* wing disc (*6*). Sensitivity to morphogen production declines with distance from the morphogen source, whereas sensitivity to receptor production falls to zero and then rises to an asymptotic value. The transition width caused by fluctuation in receptor occupancy ("binding noise") rises with distance from the morphogen source, because lower receptor occupancy produces larger stochastic fluctuations (*6*). A similar rise occurs for fluctuations driven by other sources of cell-to-cell variability (*5*).

In essence, there is some effect of every form of variability at virtually every location, but which errors matter in practice depend on where positional measurements need to be made, how much error can be tolerated, and how much different inputs actually vary. Only in a few cases are hard data on in vivo variability available [e.g., (*5*, *7*, *8*)], but the fact that mutants that are heterozygous null for genes encoding morphogens, receptors, or components of signaling pathways (i.e., putative 50% reductions in input) usually display developmental patterns that are shifted only slightly (e.g., <20%) suggests that sensitivity coefficients for developmental morphogen gradients are often <0.26 (ln1.2/ln2) (*6*). Similarly, from looking at the phenotypic effects of mutations that lead to broadening of the widths of gene expression stripes established by morphogen gradients [e.g., (*9*)], we can estimate bounds on tolerable transitions widths (Fig. 2B). Such limits reveal a surprising fact: In simple, steady-state morphogen diffusion gradients, there is no location at which cells simultaneously achieve substantial robustness to variation in morphogen production, variation in receptor production, and cell-to-cell variability in receptor occupancy.

What Price Reliability?

Might cells improve robustness by altering the mechanisms by which gradients form or are sensed? Evidence suggests that they do. For example, some cells increase morphogen degradation in response to morphogen signaling (so-called self-enhanced degradation), which decreases sensitivity to variation in morphogen production (*10*). But this benefit comes at the expense of making gradients shallower, the result of which is greater imprecision for any given amount of cell-to-cell variability (*6*). Of course, precision can be improved by operating in regimes of higher total morphogen (increased receptor occupancy translates into reduced stochastic fluctuations in binding), but this quickly leads to receptor saturation near the morphogen source, which greatly increases sensitivity to variation in morphogen production rate (*6*). Sensitivity to morphogen production rate can be lowered by measuring morphogen concentrations before the gradient reaches a steady state (*2*), but this carries the price of making cells highly dependent on the precise time at which they make their measurement (i.e., they gain a more accurate ruler at the expense of needing an accurate clock).

These scenarios illustrate a basic engineering principle: Strategies that improve performance in one arena typically degrade it in another. Often such trade-offs arise out of a linkage between the number of degrees of freedom available for combating unreliability and the number of sources of unreliability. For example, assume that we could insert into the morphogen gradient in Fig. 2 an additional component, a "nonreceptor" molecule that binds, internalizes, and degrades the morphogen without participating in signaling [such a role has been suggested for cell-surface proteoglycans (*11*, *12*)]. Now the curve of sensitivity of positional measurement to receptor production can easily be shifted so that it overlaps with the curve of sensitivity to morphogen production (i.e., the system can now be simultaneously robust, at many locations, to variation in both morphogen and receptor production). Unfortunately, addition of a new component adds a new potential source of unreliability—variability in the production of the nonreceptor. Not surprisingly, just those conditions that shift the receptor-sensitivity curve to a more desirable location correspond to conditions that make nonreceptor sensitivity high everywhere. In other words, whenever we add something new, we risk trading sensitivity to what was there for sensitivity to what we added.

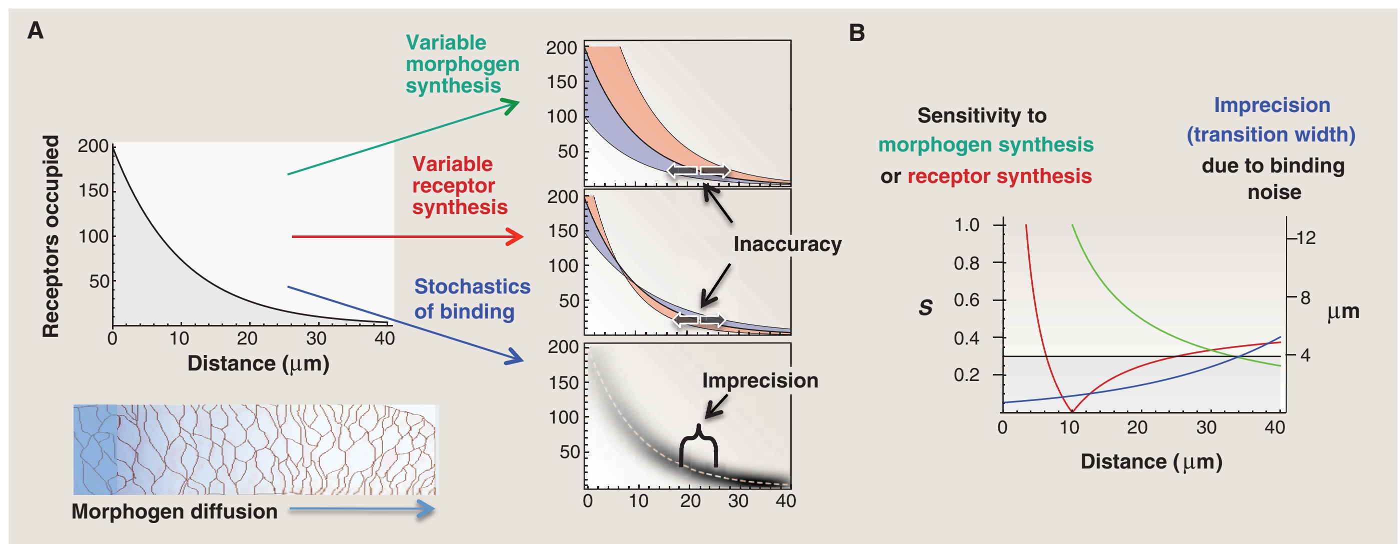

Fig. 2. Effect of input variability on the reliability of diffusion gradients. **(A)** Diffusion of molecules through intracellular spaces, when coupled to receptor-mediated uptake, produces steady-state gradients from which cells can ascertain their positions. But variability in processes that contribute to gradient formation or interpretation will necessarily lead cells to make mistakes. Their errors may be classified as either inaccuracy, whereby the average cell at a given location obtains an incorrect positional value, or imprecision, whereby there is cell-to-cell variability in the positional information obtained by cells at equivalent positions (the latter effectively converts the gradient of positional information into a probability cloud, rather than a sharp curve). **(B)** The most important potential sources of unreliability are different at different locations along a gradient [values shown are based on the gradient in (A)].

The same problem surfaces when we examine the suggestion that morphogen gradients improve accuracy by abandoning simple morphogen diffusion in favor of active transport (*13*). Sensitivity to variation in morphogen production can certainly be reduced but only at the expense of creating new sensitivity to variation in the level of whatever carrier is responsible for mediating the active transport (e.g., receptors). In fact, observations suggesting that active transport plays a role in morphogen-gradient formation in animal tissues (*14–16*) have increasingly been challenged (*17–20*). The situation is clearly different in plants, which lack the contiguous intercellular spaces of animal tissues, so that long-range transport of water-soluble molecules typically necessitates passage through cells, enabled by plasma membrane carriers and active transporters (*21*, *22*).

Of course, relying on diffusion for transport does make morphogen gradients highly sensitive to variations in diffusivity, but the beauty of diffusion is its reliability. Diffusion coefficients are only weakly sensitive to temperature and are independent of the metabolic state of the cell. Effective diffusivity is surprisingly insensitive to randomly placed obstacles, even when densely packed (*23*), and transient binding or trapping by "sticky" molecules in the environment only affects the rate at which morphogen gradients form, not their steady-state shape (*2*, *18*, *19*).

This discussion highlights an important point: The goal of good design is not to make sensitivity go away—because of performance trade-offs every system will always be sensitive to something—but rather to shift it onto the things that are reliable and away from those that are not. Of course what is reliable may be idiosyncratic. For example, tissues with small cells that have few receptors will experience larger stochastic fluctuations in receptor occupancy than tissues with larger cells and many receptors, placing a greater premium on insensitivity to receptor density in one case versus the other. Likewise, embryos that develop in eggs laid on land should place a greater premium on resistance to temperature fluctuation than mammalian embryos or embryos that develop in marine environments.

An interesting example is provided by the retinoic acid (RA) gradient that patterns the vertebrate hindbrain. Because RA is a small molecule derived in a few enzymatic steps from dietary vitamin A, we expect its abundance to vary greatly from embryo to embryo, much more than is the case for morphogens encoded by the genome. It should thus come as no surprise that numerous control mechanisms that reduce or counteract such variability—including feedback regulation of RA biosynthesis, degradation, and delivery to intracellular receptors—are observed in the hindbrain (*24*). Consistent with the presence of these (and other) control mechanisms, large experimentally induced fluctuations (>10-fold) in total RA levels have surprisingly little effect on the positional information that hindbrain cells obtain from RA (*25*). From the standpoint of the organism, a reduced sensitivity to variation in abundance of RA undoubtedly increases sensitivity to amounts of other components [e.g., cellular RA-binding proteins and cytochrome P450 and RA receptors (*26*, *27*)]. This is an acceptable trade, because amounts of those other components are likely to be more reliable.

Quality Through Quantity

Shifting sensitivities is not the only way to improve positional reliability; by making combinations of measurements, cells may exploit strategies such as pooled sampling and disturbance compensation. The former refers to the way scientists typically tame noisy data: We average (pool) repeated measurements. For this to be effective, successive measurements must be independent; that is, disturbances that corrupt one measurement need to be uncorrelated with those that corrupt the next.

Cells naturally pool measurements by letting intracellular signals accumulate over time (temporal integration). For example, morphogen-receptor complexes may be internalized and continue to signal within endosomes. Or the half-lives of protein products produced in response to morphogen signaling may exceed the duration of signaling complexes. In such cases, steady-state morphogen responses will reflect the average morphogen concentration over a time on the order of the half-life of signaling endosomes or downstream protein products, whichever is greater.

In this way, many short-lived disturbances are easily averaged out (for example, fluctuations in the extracellular levels of freely diffusing molecules tend to relax on a time scale of seconds). But some disturbances may have long time scales. For example, stochastic fluctuation in receptor occupancy resulting from the probabilistic nature of binding will reflect the time scales of receptor dynamics, which generally need to be long (e.g., hours) so as not to interfere with the formation of long-range diffusion gradients (*6*, *28*). Whether cells have sufficient time to average away such noise may thus depend on how quickly they need to obtain their positional information. In other words, cells face a speed-accuracy trade-off (Fig. 3).

A drawback of simple temporal integration is that a cell must commit to a particular time scale of integration (e.g., a particular half-life of the accumulating molecule). Even if disturbances happen on faster time scales, cells must wait a fixed time before determining their locations [lest their measurements be inaccurate (Fig. 3C)]. One way to get around this is to exploit noise-induced switching (*29*), a phenomenon that occurs when noisy inputs act on hysteretic switches—devices that switch from off to on at different thresholds from which they switch from on to off. Between the two thresholds, any transient fluctuation that flips the switch from off to on will tend to leave it in the on state until a large enough fluctuation in the other direction occurs to flip it back. In effect, the on state is "remembered" for a time related to the rate at which fluctuations occur. Because of this memory, hysteretic switches achieve temporal averaging on a time scale set by the noise itself (Fig. 3D).

There are other reasons why switches are a useful thing to include in the machinery with which cells read position: Diffusible carriers of positional information (morphogens) are usually smoothly graded in space, but cells often need to make binary decisions (e.g., to differentiate or not). Response circuits that are switchlike, that is, ultrasensitive, are thus essential. What is interesting is how many of the switches that operate downstream of positional cues turn out to be of the hysteretic type. Examples include gene regulatory switches driven by Bicoid signaling in the *Drosophila* embryo; sonic hedgehog, fibroblast growth factors (FGFs), and RA in the vertebrate spinal cord; and RA in the vertebrate hindbrain (*30–34*). In several of these cases, modeling strongly suggests that noise-induced switching plays a key role in producing sharp, positionally accurate gene expression borders (*30*, *34*).

Pooled sampling does not necessarily mean repeating the same measurement; it can also mean pooling different kinds of data. For example, some cells can get positional information from more than one morphogen at the same time. In the vertebrate hindbrain, the posterior-to-anterior RA gradient is supplemented by FGF and Wnt gradients in the same orientation (*35*); in the early *Drosophila* embryo, the Bicoid gradient works together with independent gradients of Caudal and maternal Hunchback (*7*); in the bone morphogenetic protein gradients that provide dorsoventral positional information to early invertebrate and vertebrate embryos, there are always multiple ligands (*36*, *37*).

Measurements can also be pooled over space instead of time; that is, neighboring cells can share information. In syncytia, such as the early *Drosophila* embryo, some spatial integration happens simply by virtue of diffusion of downstream effectors of morphogen signaling from one nucleus to another (*38*). In cellularized systems, mechanisms of spatial pooling are less well understood. One fascinating example occurs in the *Drosophila* wing imaginal disc, where the spatial extent of expression of the transcription factor *vestigial* (*vg*, which directs cells to adopt a wing fate) is determined by the Wingless (Wg) morphogen gradient. Because this gradient is quite steep, Wg concentrations are thought to be very low at the edges of the *vg* domain, exposing receptor occupancy to large, slow, stochastic fluctuations. Ordinarily, this should make the formation of a sharp, reliable gene expression border very difficult, but cells communicate with their neighbors (via the Fat signaling pathway) so that Wg is only permitted to turn on *vg* in a cell adjacent to one already expressing *vg* (an example of spatial pooling). Moreover, once they turn it on it remains on independent of Wg (i.e., the switch is hysteretic, so they accomplish temporal integration as well) (*39*). The combined effect is to

produce a wave of *vg* expression that spreads from the Wg source outward, coming to a near halt at a reliable position (Fig. 3D, curve g).

Fixing Errors Before They Happen

Making multiple measurements that are independent with respect to disturbances is not always feasible. In such cases, cells may exploit a different strategy, disturbance compensation. This becomes available whenever multiple measurements are affected by a common disturbance and there is a priori knowledge of the nature of their mutual dependence. A particularly simple example is ratiometric measurement: For instance, if a morphogen's rate of production rises in a certain way with temperature, then measuring its abundance relative to that of some other molecule that rises in the same way with temperature will produce a temperature-corrected reading.

Ratiometric measurement can be implemented in a surprising number of ways. For example, cell-to-cell variation in receptor occupancy resulting from noisy receptor expression can be nullified by measuring the ratio of occupied to unoccupied receptors rather than receptor occupancy per se. Indeed, amounts of the morphogen Hedgehog appear to be measured in just this way (*40*). Similarly, when morphogens with opposing actions are produced at opposite ends of a field of cells, the net signal that cells receive can cancel out perturbations that affect both morphogens equally (*41*).

Any instance in which perturbations to a system have more than one predictable effect creates an opportunity for disturbance compensation. For example, in a morphogen gradient set up by

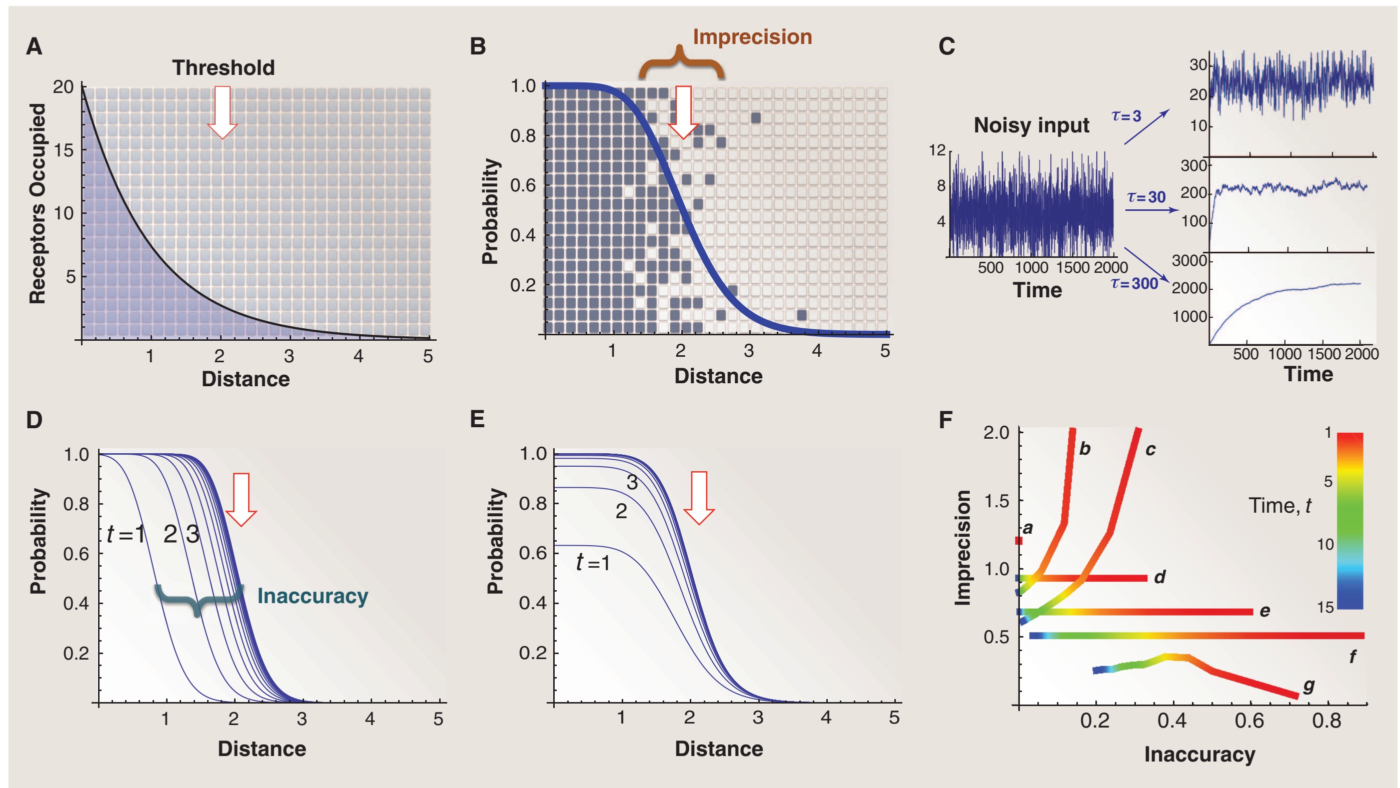

Fig. 3. The costs of pooling information. Even when average receptor occupancy accurately reflects position (**A**), any single measurement made by a cell will be corrupted by detection noise, including (but not limited to) stochastic variation in receptor occupancy. (**B**) Dark squares correspond to cells with instantaneous receptor occupancy above an arbitrary threshold (here equal to 2), and the curve depicts, given just the sampling noise in the receptor occupancy levels in (A), the probability of individual cells exceeding this threshold as a function of distance. Imprecision is the zone over which a cell's measurement has a substantial probability of being on the "wrong" side of the threshold value, given its location. (**C**) A common form of temporal integration occurs when noisy signals drive the accumulation of a downstream element (e.g., a second messenger or a transcription factor) that turns over more slowly than the noise fluctuations. The simulation illustrates what happens when a signal with random (Gaussian) noise drives accumulation of a factor with a half-life of 3, 30, or 300 times the characteristic noise time. The longer the integration, the lower the noise, but the longer it takes for the signal to approach the correct steady-state value. (**D**) Trade-offs between noise reduction and speed necessarily also apply to cells that read morphogen concentrations. In this simulation, noisy signaling like that in (B) drives production of a molecule with a half-life twice the characteristic time t of fluctuations in receptor occupancy [axes as in (B)]. Imprecision is reduced by ~45%, but only after sufficient integration time ($t > 4$) is the threshold value of morphogen signaling crossed near its steady-state location. (**E**) Here, pooling was achieved by noise-induced switching, in which hysteretic cellular decisions drive cells back and forth across threshold values in a manner driven by the noise itself. (**F**) The trade-offs between precision, accuracy, and timing are different for different strategies. Several simulations like those in (D) and (E) are summarized by using curves (a to g) to plot the time evolution of imprecision and inaccuracy, with color used to depict time (that is, simulations start at red and end at blue; time is expressed in units of the characteristic noise time, i.e., the time it takes noise to decorrelate). Imprecision is in units of transition width (normalized to morphogen gradient length scale), and accuracy as the fractional approach of the location of median probability to that at $t = \infty$ (b to f) or $t = 100$ (g). Without any pooling (a), imprecision is high, but reads are accurate from the outset. With noise-induced switching (b and c), precision is initially very poor but eventually exceeds that in a; the detailed trajectories depend on whether the hysteretic window is large (0 to 4 in b); or more modest (1 to 3 in c). With temporal integration (d to f), precision improves steadily with longer integration times (d, e, and f depict $\tau = 1$, 2, and 4, respectively), but for dramatic improvement it can take very long to achieve accuracy. Curve g simulates the spatiotemporal strategy used by the fruit fly wing disc to measure the Wg gradient (see text); here very high precision can be achieved, with inaccuracy that declines at a rate determined by how fast information about *vg* expression is relayed from cell to cell (in the simulation used to generate the figure, a rate of spread of six cells per time unit was used).

diffusion and receptor-mediated uptake, changes in receptor production do not just alter the amount of morphogen detected by each cell (as depicted by the red curve in Fig. 2B), they also alter the slope of the gradient. If cells can directly measure that slope, its change can be used to correct measurements of position obtained from morphogen levels. In fact, cells in the *Drosophila* wing disc do measure the slope of the Decapentaplegic (Dpp) morphogen gradient, albeit indirectly, because the Fat signaling pathway responds to cell-to-cell differences in perceived morphogen (*42*). Whether this information is actually exploited to correct for disc-to-disc variations in receptor levels remains to be seen.

Even the noise in a signal can drive disturbance compensation, provided there is a known correlation between noise strength and signal strength (generally true for sampling noise, as in the biochemical fluctuations in ligand-receptor interactions). That is because hysteretic switches not only time-integrate in response to noise, they tend to shift their input-output relationships as a function of the noise strength (*29*). Indeed, some of the improvement in precision provided by noise-induced switching (Fig. 3D, curves b and c) reflects this effect.

Measurement Through Self-Organization

In the earlier discussion of how *vg* expression is controlled in the *Drosophila* wing disc, the morphogen Wg was presented as the source of positional information, with cell-cell interactions serving to help cells read Wg more reliably. We could, however, flip this depiction on its head and argue that the cell-cell interactions themselves carry the positional information. That is because the arrival of a spreading wave of cell-to-cell signaling [a "juxtacrine relay" (*43*)] can itself be used to measure distances in much the same way that the time of arrival of thunder allows us to judge distance from a lightening strike. From this viewpoint, we might see the Wg gradient as merely biasing the rate at which the wave travels so as to improve the accuracy with which position is determined from it.

There are, in fact, many ways in which short-range cell-cell interactions can make long-range things happen reliably. Systems that do this are called spatially self-organizing, because spatial order emerges directly from collective, or pooled, interactions. Both the Fat and the Notch pathways have the potential to drive self-organization based on interactions between cells and their immediate neighbors (*22*, *42*, *44*). Self-organization over longer ranges can be achieved through the local production of secondary morphogens and inhibitors that may interfere to create spontaneous patterns (e.g., Turing patterns) with characteristic length scales (*45*). When allowances are made for cell rearrangement, even differences in cell-cell adhesion will drive spontaneous, large-scale spatial organization (*46*).

Of course self-organization cannot tell cells where they are relative to a fixed reference point unless that location is somehow linked to the self-organization process. Such coupling can come from boundary conditions [as occurs when bacterial cells use self-organization to locate their own midpoints (*47*)], but the control of *vg* expression in the wing disc tells us that in tissues it can also come directly from a long-range morphogen gradient. These observations suggest that we risk being narrow-minded when we think of cells as first measuring their positions and then acting in a position-specific way (e.g., organizing into patterns). We may find that, more often than not, the spatial control of morphogenesis is inextricable from the process of morphogenesis itself.

On the Horizon

In biology, simple questions rarely have simple answers, and "how do cells know where they are?" is no exception. I have focused here on the problem that, despite the existence of straightforward ways for cells to measure position, making measurements sufficiently accurate and precise is inherently challenging. Although recent years have seen considerable progress in identifying mechanisms for encoding and detecting positional information, as well as for achieving reliability, there is much we still do not know. For example:

1) How many of the "rulers" that cells use have we found? Are they mainly diffusing molecules, or are migrating cells, mechanical signals, or even electrical fields just as important?

2) How collaborative are most cases of positional sensing? As we have seen, cells gain reliability by pooling information with neighbors, or even participating in large-scale, spatial self-organization. How often do they do so, and in what contexts?

3) What are the primary sources of unreliability that constrain how cells in different contexts measure position? To answer this question requires that we not only quantify natural variability but also learn about the environments in which organisms evolved.

4) To what extent can known sources of uncertainty and variability provide a satisfactory (constraining) explanation for position-sensing mechanisms we observe? In particular, how many of the multiple morphogens, feedback loops, and complex gene regulatory circuits that we find in real systems can we explain by their positive influence on reliability?

Tackling these questions may force us to explore new research directions, but if we wish to truly understand how cells read position, there is really no better position to be in.

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Acknowledgments: Supported by NIH grants P50GM076516 and R01GM067247.

Supplementary Materials

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Materials and Methods
References

10.1126/science.1224186