Stochasticity and Cell Fate

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Fundamental to living cells is the capacity to differentiate into subtypes with specialized attributes. Understanding the way cells acquire their fates is a major challenge in developmental biology. How cells adopt a particular fate is usually thought of as being deterministic, and in the large majority of cases it is. That is, cells acquire their fate by virtue of their lineage or their proximity to an inductive signal from another cell. In some cases, however, and in organisms ranging from bacteria to humans, cells choose one or another pathway of differentiation stochastically, without apparent regard to environment or history. Stochasticity has important mechanistic requirements. We speculate on why stochasticity is advantageous—and even critical in some circumstances—to the individual, the colony, or the species.

“I, at any rate, am convinced that He does not play dice.”

Albert Einstein, 1926

Classic model systems for the study of development offer numerous examples of cellular differentiation in which cell fate is not left to chance. The generation of progeny with distinct cell fates is hard-wired into the cell cycle of Caulobacter crescentus (1). Likewise, Saccharomyces cerevisiae switches mating types (2) and Drosophila melanogaster generates neurons and glial cells by intrinsically asymmetric processes of cell division (3). Also not left to a roll of the dice is the decision to become a photoreceptor in the fly eye, which is determined by the proximity of a precursor cell to a signaling peptide (4).

In striking contrast are entry into the state of competence by Bacillus subtilis and the generation of alternative color vision photoreceptors in D. melanogaster (Fig. 1). Although these systems could not be more different, they have in common that the choice of fate is made stochastically. Figure 1A shows a field of B. subtilis cells containing DNA encoding green fluorescent protein fused to the promoter for a gene under the control of the competence regulator ComK. The cells were visualized with a red stain; the green fluorescence reveals the subpopulation of cells that are ON for ComK. The cells are 1 to 2 \( \mu m \) in length. (B) Photograph of a whole adult Drosophila retina whose R8 photoreceptors were stained with antibodies to the green-sensitive photopigment Rh6 (green) and the blue-sensitive photopigment Rh5 (blue). The horizontal distance between photoreceptors is about 10 \( \mu m \).

Each cell makes a binary choice between these two states randomly (5–7). Presumably, competence imparts a fitness advantage that outweighs the cost of producing cells that temporarily stop growing. Whereas the choice to enter competence is made stochastically, exit from competence and resumption of growth occur after a relatively fixed period of time (8). Thus, competence exhibits both nondeterministic and deterministic features.

In both examples, the choice is not simply the equivalent of flipping a coin. Instead, it is biased: For the bacteria, the ratio of competent to noncompetent cells is about 20:80, whereas for the ommatidia, the ratio of blue to green subtypes is 30:70. Interestingly, the 30:70 ratio is conserved between Drosophila and the house fly (Musca) despite more than 120 million years of evolution (12).

Noise and Bistability

Stochasticity requires both a means to generate noise and mechanisms to stabilize decisions reached in response to it. Noise can arise from multiple sources, such as variations in the activity of individual genes, cell-to-cell variations in metabolic activity, or fluctuating levels of an external signal (13). For example, a B. subtilis cell might enter competence as a response to noise in the intrinsic transcription of the gene encoding ComK (6).

Noise alone is insufficient to create binary switches between alternative cell fates. Fluctuations due to noise are generally small and transient; what is also needed are mechanisms to amplify these fluctuations and then to stabilize one choice or another. Systems of this kind are said to be bistable; that is, the system has two stable states, each of which is resistant to small perturbations and hence can persist for prolonged periods of time (14). Bistable systems often exhibit a kind of memory known as hysteresis: When a switch is thrown in one direction, it does not readily switch back when the signal is removed. Bistability ensures that once the switch is thrown, the circuit remains locked. Bistability can be achieved by positive autoregulatory loops (Fig. 2A), by double-negative loops (Fig. 2B), or by complex circuits comprising several intermediary loops (Fig. 2C) (15). A classic example is the alternative lytic and lysogenic states of the bacterial virus lambda (16). The virus is locked into lytic or lysogenic modes by mutually antagonistic repressors that inhibit each other’s synthesis. When one repressor takes over, even weakly, the system switches for long periods of time in one direction (Fig. 2D).

Bistability requires mechanisms to render the switch hypersensitive, allowing a rapid response once a threshold has been attained. In phage lambda, this is achieved by cooperative DNA-binding interactions among repressor molecules. For B. subtilis competence, production of ComK is controlled by a positive feedback loop in which ComK stimulates its own synthesis (5). Hypersensitivity is achieved by cooperative binding of ComK to its promoter. What these systems have

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in common is a hypersensitive switch that is poised on a knife edge and can flip in one direction or the other when pushed by noise.

**Cell-Autonomous Choices**

Why is stochastic choice of cell fate advantageous? We address this question first in the case of stochastic choices that are made cell-autonomously. Perhaps the most attractive explanation comes from studies of stochastic switches in bacteria. Bacteria respond to adverse environmental conditions by inducing the expression of adaptive genes. Stochasticity allows bacteria to deploy specialized cells in anticipation of possible adverse changes in the environment. A striking example is the persister state, which is observed in many bacteria (17, 18). Populations of *Escherichia coli* cells are found to contain a tiny subpopulation of cells that have temporarily entered nongrowing or slow-growing states in which they can elude the action of antibiotics that can only kill actively growing bacterial cells. Thus, when a population of *E. coli* cells is treated with (for example) ampicillin, the persister cells survive by virtue of their quiescence. Cells that exit the persister state after the antibiotic treatment has ended resume growth. An appealing interpretation of this phenomenon is that *E. coli* is hedging its bets against the future possibility of encountering antibiotics. If it waited to respond until after the antibiotic was present, it would be too late to adapt and the entire population would die. Indeed, modeling shows that stochastic switching can be favored over mechanisms based on sensing when the environment changes infrequently (19, 20). The mechanism that causes cells to enter the persister state stochastically involves an imbalance between a toxin and its antitoxin encoded by a two-gene module. Normally, the antitoxin is in excess and neutralizes the toxin. However, when the toxin is in excess, cell growth is arrested but the cells are not killed. Rather, they are in stasis.

Another example of apparent bet-hedging is swimming and chaining in *B. subtilis*. Bacterial cells in exponential-phase growth are a mixture of unicellular, motile cells and long chains of nonmotile cells (21). The swimming cells are active for the transcription factor σ^P, which governs motility and the production of enzymes (autolysins) that allow newly divided cells to separate from each other. Conversely, the chains of nonmotile cells are inactive for σ^P. How the cells interconvert between the σ^ON and σ^OFF states is not known.

What is the biological importance of the alternative swimming and chaining states? An appealing possibility is that the swimmers are nomadic cells in search of new food sources, whereas the chains are sessile cells that exploit the current niche. Thus, *B. subtilis* would appear to hedge its bets against the likelihood that its current food source will be exhausted while at the same time taking full advantage of existing food.

When it comes to cell fate in metazoans, interpretations other than bet-hedging must be invoked to explain stochastic choices because all cells depend on one another. Consider the case of olfactory receptors in mammals (22). As for most sensory systems, only one type of olfactory receptor protein is produced in any given olfactory receptor neuron so as to avoid the sensory confusion that would occur if the same cell expressed more than one receptor gene. As the genome of the mouse devotes 4% of its protein-coding sequences to olfactory receptors, representing 1000 genes, the task of achieving this sensory exclusion is formidable. To meet the challenge, each neuron chooses to express one olfactory receptor gene in a stochastic manner and prevents expression of all other olfactory receptor genes in that cell (22). Thus, only one of the 1000 olfactory receptor genes (actually 2000, each gene being represented by two alleles) is randomly activated in any one cell (Fig. 3A). Here, the explanation for using stochasticity is economy: A regulatory circuit designed to choose among 2000 genes in a directed manner would need to be extraordinarily complex.

The olfactory receptor decision is made in a cell autonomous manner (22), but its mechanism remains poorly understood. A similar stochastic choice exists in the distribution of green (M) and red (L) cones in the human retina, which express the genes encoding M and L opsins, respectively. These two genes are located near each other (23). A unique locus control region (LCR) located upstream of both genes is required for their expression, but it can only activate one gene at a time (24). When the LCR connects to the L gene, the connection is stabilized and the cell becomes an L cone for the life of the cell: The M gene cannot be expressed. If the LCR associates by chance with the M gene, the M gene is expressed and the L gene is off (Fig. 3B). Given the diploid nature of mammalian cells, how does the cone cell ensure that only one gene (M or L) is expressed? The answer is that the LCR-M cluster is located on the X chromosome. Only one X chromosome is expressed in females because of X chromosome inactivation; males, of course, have only one X chromosome. Interestingly, the system has a built-in way to control the proportion of M/L cones: The gene closest to the LCR has more chances to be chosen by the LCR.

A parallel can be made between the human and *Drosophila* color vision systems. R7 color photoreceptor cells exist in alternative states that either express rh3 or rh4, which encode rhodopsin molecules that are sensitive to different hues of UV light. The rh3 and rh4 genes are not clustered on the chromosome near a common LCR. Rather, the basis for stochasticity is attributed to the expression of a transcription factor called Spineless (9). Somehow, the regulatory protein is only present in a subset of R7 cells and directs these cells to express rh4 rather than rh3. Just how Spineless becomes ex-
pressed exclusively in a subset of R7 cells is not understood.

What is the meaning of stochasticity in the choice of photoreceptor cells in the eye of the fly or of a human? Because the retina in these two very different eyes is composed of many photoreceptors of different types, stochasticity is a simple mechanism to distribute two kinds of photoreceptors (in a particular ratio) across a large field and to avoid repetitive patterns that might limit the ability of the eye to perceive corresponding patterns in the visual field.

**Nonautonomous Choices**

In the preceding examples, a cell decides its fate stochastically in a manner that is independent of other cells. In some cases, the choice the cell makes influences the fate of nearby cells. Nonetheless, the original cell fate decision is made independently of its neighbors. Not all stochastic decisions are cell-autonomous; sometimes the decision is the result of back-and-forth interactions between two (or more) cells. In animals, the simplest system of cell nonautonomous decision-making is the choice between the anchor cell (AC) and the ventral uterine (VU) cell fates in the nematode *Caenorhabditis elegans* (25). Two neighboring precursor cells of the gonad can choose either fate. The two cells are the products of two parallel lineages that arose from a common ancestor several divisions earlier. However, small differences in the cell cycle of cells in these lineages lead one or the other of the two precursors to be born first. The first-born cell is biased to become the VU cell, but it does not make this decision alone. Rather, the decision-making process involves inhibitory lateral interactions between the two cells via the LIN-12 signaling pathway (known as the Notch pathway in flies and vertebrates).

LIN-12 is a receptor. Its ligand LAG-2 stimulates the activity of the LIN-12 pathway, resulting in the production of additional LIN-12 receptors. This causes the cell to become hypersensitive to the ligand. Meanwhile, high levels of LIN-12 activity decrease the production of the ligand (Fig. 4A). Therefore, a cell that is activated for LIN-12 has diminished capacity to stimulate its neighbor (25). As with the paradigm of bistable processes that are noise-driven, stochasticity in birth order (developmental noise) tips the switch in one direction or the other. This bias is then amplified and locked in by lateral interactions between the two cells. The first-born cell exhibits somewhat higher LIN-12 activity than its neighbor and hence has diminished levels of the LAG-2 ligand. LAG-2 signaling from the second-born neighbor results in yet higher levels of LIN-12 and yet lower levels of ligand in the first cell (25). This sets up a self-reinforcing cycle of lateral inhibition in which the first-born cell achieves higher and higher levels of LIN-12 and the second-born cell, not receiving any stimulation from its neighbor, has lower and lower LIN-12 activity. High LIN-12 activity leads to the VU fate and low activity to the AC fate.

Lateral inhibition is also the basis for nonautonomous cell fate determination in the epidermis of *Drosophila*. One cell in a proneural cluster of equivalent cells becomes a neuroblast, and it must do so to the exclusion of all the other cells in the cluster, which become epidermal cells (3, 26). Flies use the same system as worms to achieve this (Fig. 4A). Notch is the LIN-12 equivalent in flies and its ligand is called Delta, the equivalent of worm LAG-2. The neuroblast fate arises stochastically by transcription noise leading to a very small increase in the capacity of one cell in the cluster to produce more Delta and hence stimulate the Notch pathway a little more in all of its neighbors. This signaling stimulates Notch production in the neighbors, increasing their sensitivity to Delta and, as in the AC/VU example, setting up a self-reinforcing cycle (Fig. 4A).

Meanwhile, the cell that, as a result of noise, exhibited an elevated capacity to signal attains a state of low Notch activity and hence becomes a neuroblast. Each cell in the cluster is competent to become a neuroblast, because killing the neuroblast—and thereby relieving lateral inhibitory signaling—allows another random cell to start the bistable loop again and to adopt the neuroblast fate (3, 26).

An equivalent example of cell-nonautonomous decision making is not known in bacteria. But the phenomenon of "cannibalism" combines stochastic decision-making with reciprocal intercellular interactions (27). When grown under conditions of nutrient limitation, *B. subtilis* enters an elaborate developmental process that culminates in the formation of a dormant spore. Entry into sporulation is governed by the regulatory protein Spo0A, whose activation is governed by a bistable switch (28). Thus, only some cells in the population (about half) are ON for Spo0A and the others are OFF. The Spo0A-ON cells produce toxins that kill the Spo0A-OFF siblings. The dying siblings, in turn, release nutrients that limit further Spo0A activation in the Spo0A-ON cells, thereby arresting sporulation or even reversing it. This phenomenon can also be interpreted as hedging: Uncertain as to whether they are experiencing a temporary shortage of nutrients or the onset of a prolonged famine, the bacteria stall for as long as possible before committing to spore formation, even at the expense of fratricide. In the Notch signaling systems, intercellular interactions reinforce alternative cell fate decisions. By contrast, in the cannibalistic bacterial system, the reverse is true, as the remaining cells are delayed in committing to the spore fate.

**Bistable-Like Switches That Are Hard-Wired by Upstream Events**

Not all switches that exist in alternative stable states are driven by noise. Hypersensitive switches that include loops can also be used to lock a cell into one or another fate, but the decision is not left to chance. This is often the case when the deterministic signal is very weak and needs to be reinforced. For instance, in the fly eye, the photoreceptors R3 and R4 are derived from seemingly identical cells. Once again, competition for Notch activation leads to a critical distinction between the R3 or R4 fates, and this distinction is crucial to promote the correct orientation of the ommatidium (29). However, in each of the 800 ommatidia, it is always the cell closer to the equator that becomes R3, the polar one becoming R4 (Fig. 4B). This is because gradients of signaling proteins (e.g., Wnt) that drive the decision to the R4 fate are superimposed on circuitry that, in

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**Fig. 3.** Cell-autonomous cell fate decisions. (A) Cell-autonomous stochasticity in a mouse olfactory neuron. The neuron expresses one olfactory receptor gene (red) to the exclusion of all others (blue, brown, dark or light green, yellow, or pink), including the other allele of the "red" gene. The olfactory neuron somehow instructs its target neuron in the olfactory bulb of its choice (dashed arrow). (B) Cell-autonomous stochasticity in an Old World primate color vision cone photoreceptor. The choice of a cone photoreceptor to become M (green-sensitive) or L (red-sensitive) depends on the ability of a single locus control region (LCR) located upstream of the L and M genes to contact one of the two genes. If the LCR contacts the M gene, the cone becomes an M cone, and similarly for the L gene. This ensures that only one gene is expressed in each cone. As the LCR-M-L cluster is located on the X chromosome, only one copy is present in males and only one is active in females because of X-chromosome inactivation.
other contexts (e.g., the choice between VU and AC fates in worms; neuroblast commitment in flies), is noise-driven (29, 30).

The Wnt protein is at its highest concentration at the north and south poles and at its lowest at the equator. Interestingly, it is not the absolute value of Wnt that matters. Rather, it is the relative difference in the level of signaling perceived, directly or indirectly, between the precursors of R3 and R4 that determines the outcome (29). Thus, for each ommatidium, the precursor cell closest to the pole (where Wnt levels are higher) becomes R4, and the one closest to the equator (where Wnt is relatively lower) becomes R3 (Fig. 4B).

Another example of a bistable-like switch in which the outcome is hard-wired is the establishment of left-right asymmetry between the two neurons (ASE) that sense either Na+ or Cl− in C. elegans (15). The switch consists of a complex regulatory loop in which a microRNA (miR-273) inhibits translation of the mRNA for a transcription factor (DIE-1), which itself turns on the synthesis of another microRNA (lsy-6) (Fig. 2C). Closing the loop, lsy-6 blocks the synthesis of the transcription factor (COG-1) that is responsible for directing miR-273 synthesis. The left and right fates of ASE are specified by DIE-1 and COG-1, respectively (Fig. 2C). The ASE switch has the same logic as the double-negative loop that governs the alternative lambda double-negative loop, the choice between the two neurons; ASE-R is always on the right and ASE-L always on the left (15).

Why, then, has a system that resembles a bistable switch? Perhaps the ASE system derives from an ancestral worm that made the choice between the right- and left-hand fates stochastically.

If so, only half of the ancestral animals would have had both ASE-R and ASE-L. If having a given neuron on the left, or on the right, proved advantageous, the system might have evolved through “genetic assimilation” into directionality, in which it is always the same cell type that is on the right, and the other on the left (31). Even though upstream signals dictate the outcome in the contemporary nematode, the circuitry of what once was a noise-driven switch might have been maintained in evolution as a way to lock in the decision robustly.

Conclusions
Most organisms exhibit characteristics that are reproducibly inherited from generation to generation, which strongly implies that development is hard-wired. However, certain developmental decisions are left to chance, sometimes out of necessity (when the choices are too many to be tightly controlled), or sometimes when it benefits the community to hedge its bets. In yet other cases, particular developmental outcomes are imposed on systems that are otherwise intrinsically stochastic. Nature knows how to make deterministic decisions, but, in contrast to Einstein’s view of the universe, she also knows how to leave certain decisions to a roll of the dice when it is to her advantage.

References and Notes
1. J. Holtzendorf et al., Science 304, 983 (2004); published online 15 April 2004 (10.1126/science.1095191).
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