Published in final edited form as:
Science. 2008 April 4; 320(5872): 65–68. doi:10.1126/science.1147888.

Stochasticity and Cell Fate

Richard Losick and
Department of Molecular and Cellular Biology, The Biological Laboratories, Harvard University, 16 Divinity Ave., Cambridge, Massachusetts 02138

Claude Desplan
Center for Developmental Genetics, Department of Biology, New York University, 1009 Silver Center, 100 Washington Square East, New York NY 10003

Summary

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 as we discuss. We will also 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

Stochasticity in Bacteria and Flies

Classic 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 cells 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. The left panel shows a field of *B. subtilis* cells that contains a fusion of DNA encoding the Green Fluorescent Protein to the promoter of a gene that is under the control of the DNA-binding protein ComK, the master regulator for competence (5). Competence is a specialized state involving the expression of about 100 genes. In competence, growth ceases and the cells become capable of taking up DNA from the environment and incorporating it into the chromosome by recombination. About twenty percent of the cells are active for ComK and the rest are not. 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 entry competence is made stochastically, exit from competence and resumption of growth occurs after a relatively fixed period of time (8). Thus, competence exhibits both non-deterministic and deterministic features.

The right panel shows the retina of *Drosophila*, which has a compound eye composed of multiple unit eyes known as ommatida. In each ommatidium, a stochastic choice is made in one of the eight photoreceptor cells (called R7) to become one of two possible cell types (9). Once this choice is made, the R7 cell instructs the photoreceptors lying underneath it (called R8) to express either a blue-sensitive or a green-sensitive rhodopsin photopigment (10). Here too, the choice is made randomly: each ommatidium makes its choice independently (11).

In both, the choice is not simply the equivalent of flipping a coin. Instead, it is biased: for the bacteria, the ratio of competent to non-competent 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*) in spite of over 120 millions 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 or from cell-to-cell variations in metabolic activity, or from fluctuating levels of an external signal (13). For example, a cell might enter competence as a response to noise in the intrinsic transcription of the gene for 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 that 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) or 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 and dramatic response once a threshold has been attained. In phage lambda, this is achieved by cooperative DNA-binding interactions among repressor molecules. For 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 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 choosing cell fate stochastically 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 *E. coli* cells are found to contain a tiny subpopulation of cells that have temporarily entered non-growing or slow growing states in which they can elude the action of

antibiotics that require actively growing bacterial cells to cause killing. Thus, when a population of *E. coli* cells is treated with, for example, the antibiotic 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 non-motile cells (21). The swimming cells are active for the transcription factor σ^D, which governs motility and the production of enzymes (autolysins) that allow newly divided cells to separate from each other. Conversely, the chains of non-motile cells are inactive for σ^D. How the cells interconvert between the σ^D-ON and σ^D-OFF states is not known.

What is the biological significance 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 percent of its protein-coding sequences to olfactory receptors, representing 1,000 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 1,000 olfactory receptor genes (actually, 2,000, 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 2,000 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 and red cones in the human retina, which express the M and L opsin genes, respectively. The two M and L 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-L-M cluster is located on the X chromosome. Only one X chromosome is expressed in females due to X chromosome inactivation and 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 expressed 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.

Non-autonomous 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 other nearby cells. Nonetheless, the original cell fate decision is made independently of its neighbors. But 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 non-autonomous decision making is the choice between the Anchor Cell (AC) and the Ventral Uterine Cell (VU) fates in the nematode *C. 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 receptor. 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 actions between the two cells. The first born 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 non-autonomous cell fate determination in the epidermis of *Drosophila*. One cell in a pro-neural 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, due to noise, exhibited an elevated capacity to signal attains a state of low Notch activity and hence becomes a neuroblast. All cells in the cluster are competent to become a neuroblast since 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 non-autonomous 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 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 bet 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 reinforced alternative cell fate decisions. In 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 in 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, two photoreceptors named 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 superimposed on circuitry that in other contexts (*e.g.* the choice between VU and AC fates in worms and neuroblast commitment in flies) is noise-driven, are gradients of signaling proteins (*e.g.* Wnt) that drive the decision to the R4 fate (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) 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 micro RNA (miR-273) inhibits translation of the mRNA for a transcription factor (DIE-1), which, itself turns on the synthesis of another micro RNA (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 lytic and lysogenic states of phage lambda (Fig. 2D). Thus, when COG-1 is ON, the synthesis of miR-273 blocks the production of the transcription factor (DIE-1) for the opposite cell fate (Fig. 2C) (just as one lambda repressor blocks the synthesis of the other repressor). Conversely, when DIE-1 is ON, it determines the right-hand fate and induces the synthesis of *lsy-6* that prevents the accumulation of the transcription factor COG-1. In contrast to the stochasticity that drives the phage lambda double-negative loop, the choice between the left-hand (ASE-L) and right-hand (ASE-R) fates is instructed by the lineage of the two neurons; ASE-R is always on the right and ASE-L always on the left (15)!

Why then have 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 directional asymmetry, 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.

Acknowledgements

We thank J. Hahn and D. Dubnau for the image of Fig. 1A, J. Blau, D. Dubnau, M. Elowitz, O. Hobert, R. Johnston, E. Kussell, E. O’Shea, A. Schier, L. Shapiro, G. Suel, A. Tomlinson and G. Yuan for comments on the manuscript. This work was supported by NIH grant GM18568 to RL and EY13010 to CD.

References

1. Holtzendorff J, et al. Science May 14;2004 304:983. [PubMed: 15087506]
2. Rine J, Jensen R, Hagen D, Blair L, Herskowitz I. Cold Spring Harb Symp Quant Biol 1981;45 Pt 2:951. [PubMed: 6266770]
3. Campos-Ortega JA. Curr Biol 1997;7R726
4. Freeman M. Cell 1996;87651
5. Maamar H, Dubnau D. Mol Microbiol May;2005 56615
6. Maamar H, Raj A, Dubnau D. Science Jul 27;2007 317526
7. Suel GM, Garcia-Ojalvo J, Liberman LM, Elowitz MB. Nature Mar 23;2006 440545
8. Suel GM, Kulkarni RP, Dworkin J, Garcia-Ojalvo J, Elowitz MB. Science Mar 23;2007 3151716
9. Wernet MF, et al. Nature Mar 9;2006 440174
10. Mikeladze-Dvali T, et al. Cell Sep 9;2005 122775
11. Bell ML, Earl JB, Britt SG. J Comp Neurol May 1;2007 50275
12. Franceschini N, Kirschfeld K, Minke B. Science 1981;2131264
13. Raser JM, O’Shea EK. Science Sep 23;2005 3092010
14. Ferrell JE Jr. Curr Opin Cell Biol Apr;2002 14140

15. Johnston RJ Jr, Chang S, Etchberger JF, Ortiz CO, Hobert O. Proc Natl Acad Sci U S A Aug 30;2005 10212449
16. Ptashne M. Curr Biol Apr 3;2007 17R233
17. Lewis K. Nat Rev Microbiol Jan;2007 548
18. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. Science Sep 10;2004 3051622
19. Thattai M, van Oudenaarden A. Genetics May;2004 167523
20. Kussell E, Leibler S. Science Sep 23;2005 3092075
21. Kearns DB, Losick R. Genes Dev Dec 15;2005 193083
22. Mombaerts P. Curr Opin Neurobiol Feb;2004 1431
23. Nathans J. Neuron Oct;1999 24299
24. Smallwood PM, Wang Y, Nathans J. Proc Natl Acad Sci U S A Jan 22;2002 991008
25. Greenwald I. Genes Dev Jun 15;1998 121751
26. Doe CQ, Skeath JB. Curr Opin Neurobiol Feb;1996 618
27. Gonzalez-Pastor JE, Hobbs EC, Losick R. Science Jul 25;2003 301510
28. Veening JW, Hamoen LW, Kuipers OP. Mol Microbiol Jun;2005 561481
29. Wehrli M, Tomlinson A. Development 1995;1212451
30. Yang CH, Axelrod JD, Simon MA. Cell Mar 8;2002 108675
31. Palmer AR. Proc Natl Acad Sci U S A Dec 10;1996 9314279

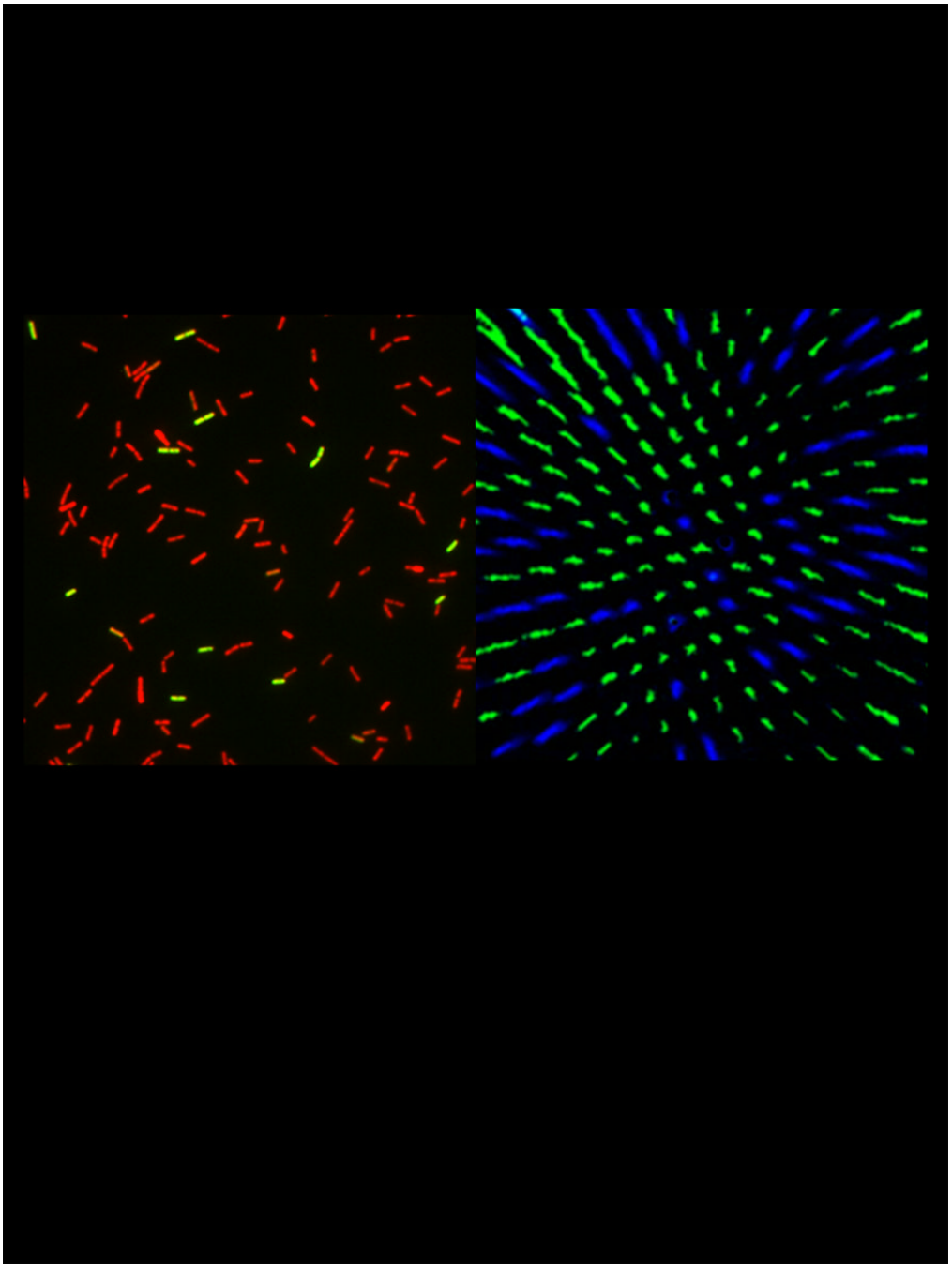

Figure 1. Stochastic distribution of cell fates in bacteria and in insect photoreceptors

Left panel: Fluorescence micrograph showing cells of *B. subtilis* containing a fusion of the coding sequence for GFP 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.

Right panel: Photograph showing a whole adult *Drosophila* retina whose R8 photoreceptors were stained with antibodies against the green-sensitive photopigment Rh6 (green) and the blue-sensitive photopigment Rh5 (blue).

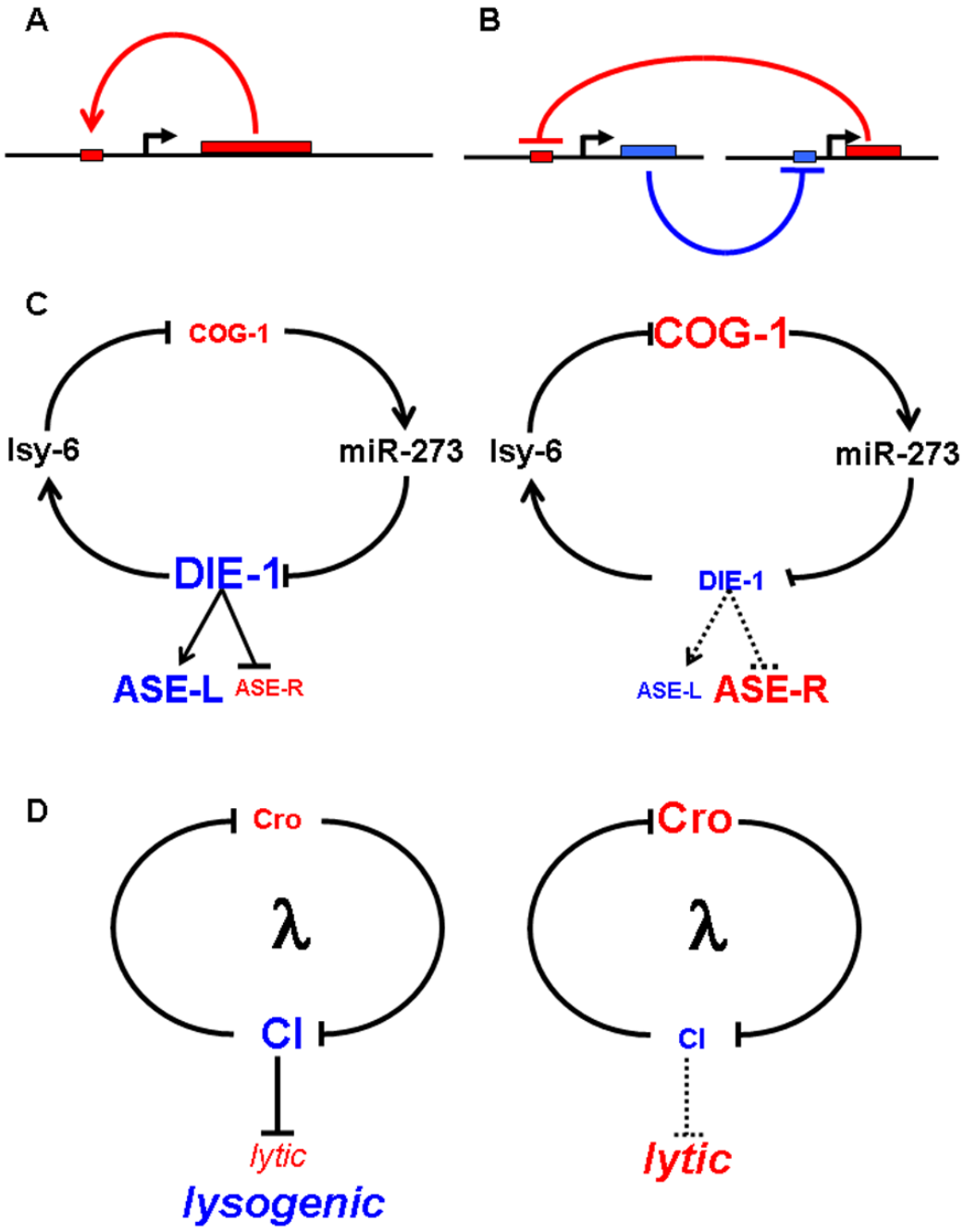

Figure 2. Regulatory circuits exhibiting bistability

Panels ***A*** *and* ***B*** illustrate two kinds of regulatory circuits that can exhibit bistability. Shown in A is a positive feedback loop in which an activator (as in the example of the activator of competence ComK) stimulates the transcription of its own gene. Hypersensitivity is achieved by cooperativity among activator molecules in binding to the promoter region for the gene (not illustrated). Shown in B is a double-negative regulatory circuit in which two repressors (as in the example of the phage lambda CI and Cro repressors) antagonize the transcription of each other's gene. Hypersensitivity is achieved by cooperativity among repressor molecules in binding to operator sites in DNA.

Panel ***C*** illustrates an example of a double-negative regulatory circuit that governs the alternative neuronal ASE-L and ASE-R fate in *C. elegans.* In this case, the two transcriptional regulators (COG-1 and DIE-1) antagonize each other's synthesis indirectly through the action of the micro RNAs lsy-6 and miR-273, which block the translation of the mRNAs for COG-1 and DIE-1, respectively. Neurons have the ASE-L fate when DIE-1 levels are high and COG-1 levels are low (left-hand cartoon) and the ASE-R fate when the opposite is the case (right-hand cartoon).

Panel ***D*** illustrates the case of the classic example of the double-negative circuit (see panel B above) governing the alternative lytic and lysogenic states of phage lambda. When the lambda repressor CI is at high levels it represses the gene for the Cro repressor and genes involved in lytic growth (left-hand cartoon). Hence the phage is held in the dormant, lysogenic state. Conversely, when Cro is at high levels it represses the gene for CI under which condition genes involved in lytic growth are freely expressed (right-hand cartoon).

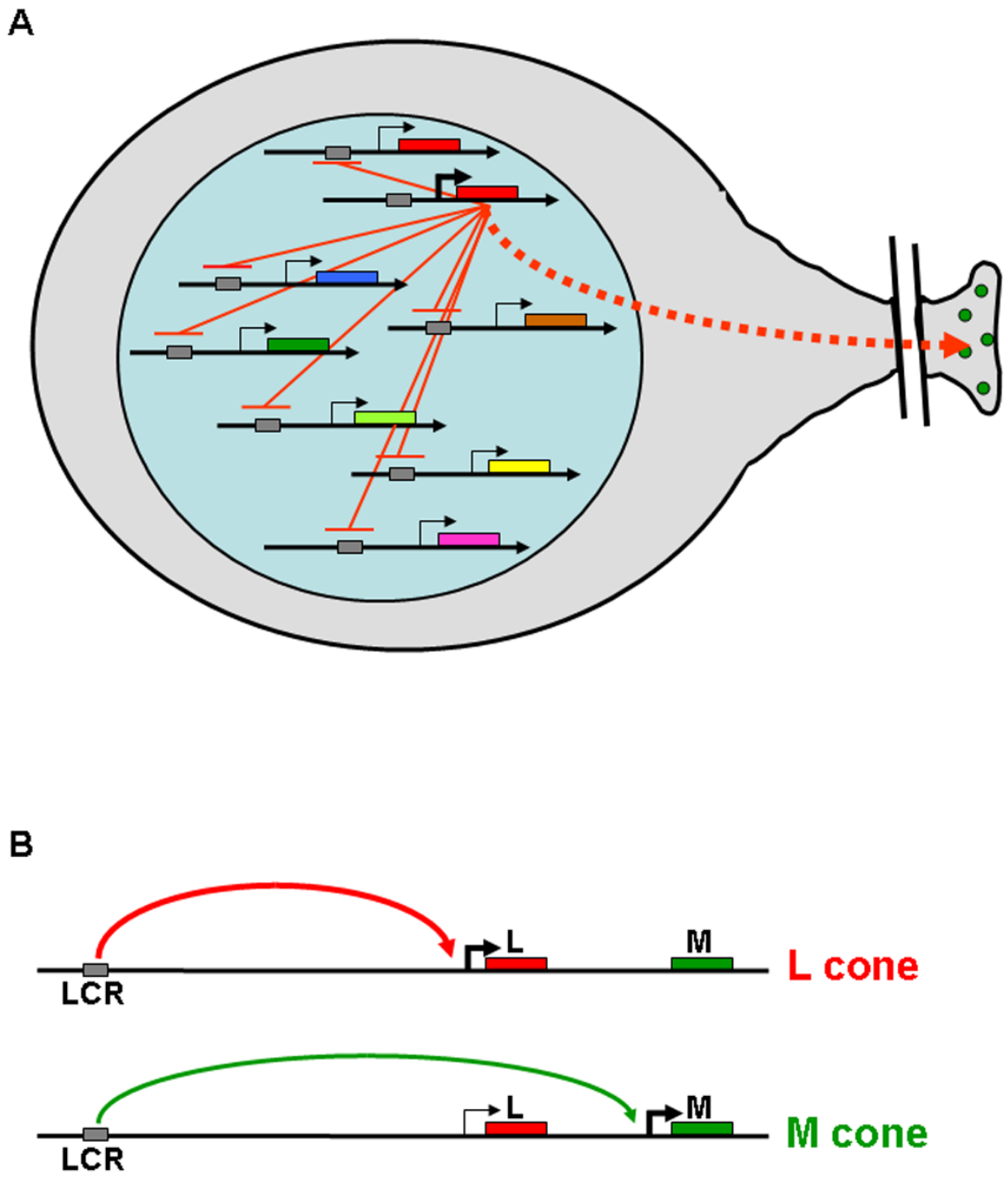

Figure 3. Cell-autonomous cell fate decisions

Panel ***A*** illustrates 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).

Panel ***B*** illustrates 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, due to X-chromosome inactivation.

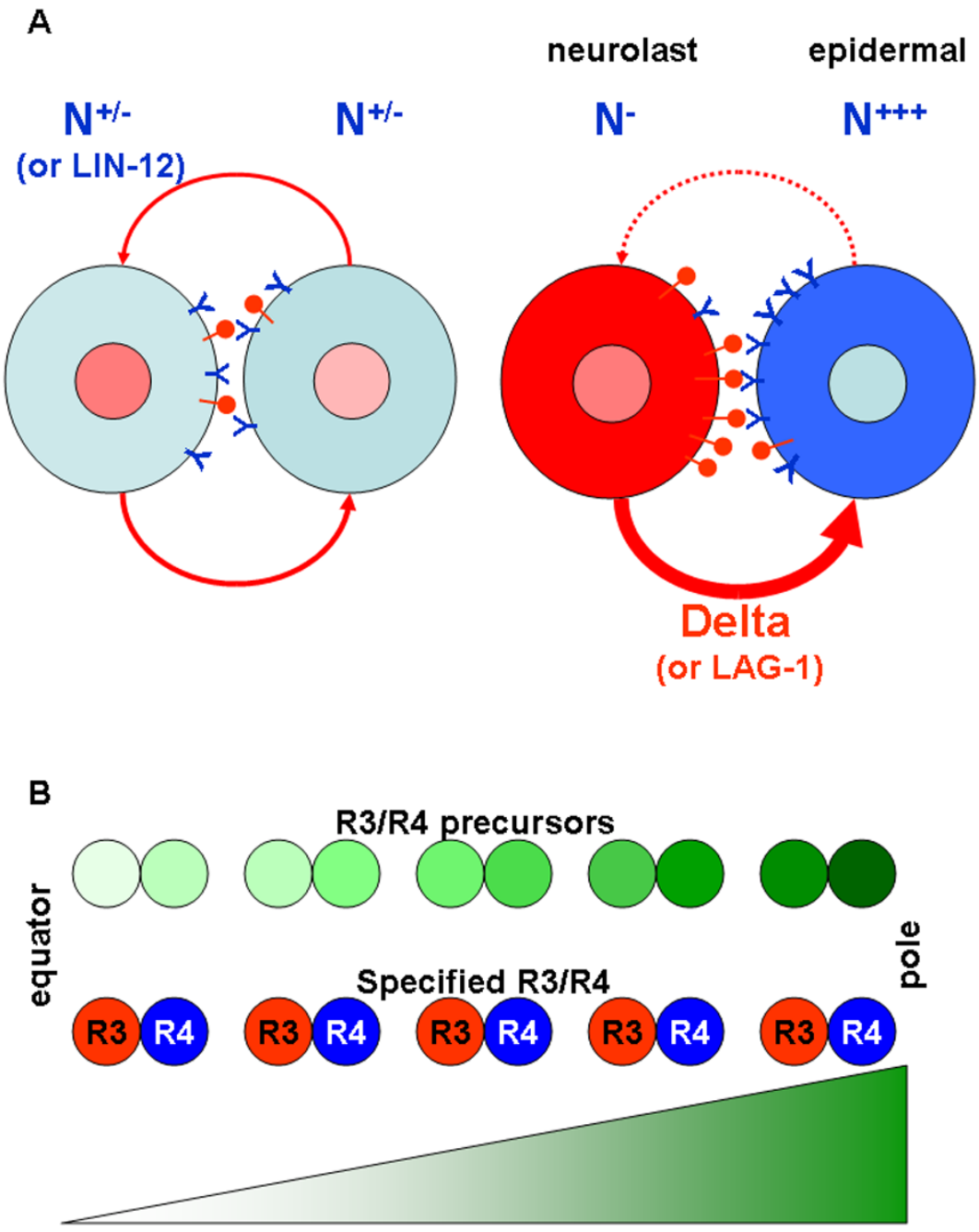

Figure 4. Cell-non-autonomous cell fate decisions

Panel ***A*** illustrates lateral inhibition by the Notch (LIN-12) regulatory system in which a stochastic decision by one cell prevents its neighbor(s) from making the same decision. Two neighboring epidermal cells of *Drosophila* start with the same potential to become neuroblasts, both initially exhibiting low Notch activity ($\mathbf{N}^{+/-}$) (left-hand cartoon). Variations in gene expression in the precursor cells leads one cell (dark pink nucleus) to increase production of the Notch ligand Delta (red lollipop) and to decrease production of the Notch receptor (blue Y) (right-hand cartoon). This asymmetry sets in motion a self-reinforcing cycle in which one cell (N^{-}) becomes less and less sensitive to the Delta ligand and more and more active in producing ligand whereas the other cell (N^{+++}) becomes more and more sensitive to ligand but

less active in producing it. The N^{-} cell becomes a neuroblast while the N^{+++} cell remains an epidermal cell.

Panel ***B*** illustrates a Notch-Delta regulatory switch that is biased in one direction by gradients of signaling molecules. Two neighboring photoreceptor cells, R3 and R4, in the fly compete as in (**A**) to acquire their cell fate. High Notch leads to the R4 cell fate while low Notch leads to the R3 fate. Pairs of R3/R4 precursors are in a gradient of a signaling molecule (*e.g.* wingless, green). In each pair, the cell positioned at the polar side receives more signal than its more equatorial neighbor, thus biasing it to becoming R4. The decision is then reinforced by lateral inhibition: all equatorial cells become R3 and all polar cells become R4.