Requirements for efficient cell-type proportioning: regulatory timescales, stochasticity and lateral inhibition

B Pfeuty and K Kaneko

1 Université de Lille, CNRS, Laboratoire de Physique des Lasers, Atomes, et Molécules, F-59000, Lille, France
2 Department of Pure and Applied Sciences, University of Tokyo, Tokyo 153-8902, Japan
E-mail: benjamin.pfeuty@univ-lille1.fr

Keywords: bistability, differentiation, feedback, development, canalization, epigenetic

Abstract
The proper functioning of multicellular organisms requires the robust establishment of precise proportions between distinct cell types. This developmental differentiation process typically involves intracellular regulatory and stochastic mechanisms to generate cell-fate diversity as well as intercellular signaling mechanisms to coordinate cell-fate decisions at tissue level. We thus surmise that key insights about the developmental regulation of cell-type proportion can be captured by the modeling study of clustering dynamics in population of inhibitory-coupled noisy bistable systems. This general class of dynamical system is shown to exhibit a very stable two-cluster state, but also metastability, collective oscillations or noise-induced state hopping, which can prevent from timely and reliably reaching a robust and well-proportioned clustered state. To circumvent these obstacles or to avoid fine-tuning, we highlight a general strategy based on dual-time positive feedback loops, such as mediated through transcriptional versus epigenetic mechanisms, which improves proportion regulation by coordinating early and flexible lineage priming with late and firm commitment. This result sheds new light on the respective and cooperative roles of multiple regulatory feedback, stochasticity and lateral inhibition in developmental dynamics.

Introduction
The development of multicellular organisms relies on sophisticated collective behaviors of interacting cells, such as aggregation [1], segmentation [2] or cell-type diversification [3]. One intriguing collective phenomenon has been termed canalization [4] and refers to the developmental ability of a multicellular organism to produce the same end-result, such as the proportion between distinct cell types, regardless of variability of its environment or genotype. Cell-type proportions are primarily the result of a sequential differentiation process wherein multipotent cells select between two distinct fate-restricted cell subtypes. These binary cell-fate decisions are regulated by the interplay between intracellular regulatory mechanisms required to generate diverse cell-type attractors from a single precursor cell-type and intercellular signaling mechanisms required to coordinate fate decisions in population of cells (table 1). On the one hand, differentiation regulatory networks typically involve positive feedback loops such as self-activation, mutual activation or inhibition, or more elaborate motifs [5], which may also operate on different timescales [6]. On the other hand, cell-fate choice is influenced by intercellular coupling via juxtacrine or paracrine signaling, notably through a lateral inhibition mechanism which prevents coupled cells from differentiating into the same type [7].

The two systems where regulation of cell-type proportions have received the most attention are the multicellular development of the social amoeba Dictyostelium and early lineage specification during mammalian embryogenesis. Vegetative Dictyostelium cells exposed to starving conditions aggregate to form slugs and, eventually, fruiting bodies, which contain spores and stalk cells with a relatively precise ratio of about 4:1 [8, 9]. During this process, starving cells start to differentiate into a heterogeneous population of prespore or prestalk cells where prespore cells elicit Dif1 signals that promote other cells to acquire the stalk fate [10, 11]. The spore and stalk phenotypes are

© 2016 IOP Publishing Ltd
Table 1. Examples of developmental fate decisions based on intracellular positive feedback loops and intercellular inhibitory signaling. $p/j$: paracrine/juxtacrine signaling.

| Cell lineages (organism)          | Positive feedback                        | Signaling                          | References |
|----------------------------------|------------------------------------------|------------------------------------|------------|
| Spore/Stalk (Dictyostelium)       | Sdfl $\rightarrow$ DkhA                  | Dif$^\beta$                         | [10, 13]   |
| Tricho-/Atrichio-blasto (Arabidopsis) | Wer $\rightarrow$ GB3                    | CPC$^\alpha$                        | [19, 20]   |
| Anchor/Ventral uterine (C. Elegans) | Lin12 $\rightarrow$ Lin12               | Lin12-Lag2$^\alpha$                 | [21]       |
| Neuro-/epidermo-blasto (Drosophilia) | AS-C$^\alpha$-E(Spl)-C                 | Delta-Notch$^\beta$                 | [22]       |
| Motile/Primary cilia (Vertebrate) | Foxj1 $\rightarrow$ Rfx1                | Jagged-Notch$^\alpha$                | [23]       |
| Hypo-/Epi-blasto (Mammal)        | Gata6 $\rightarrow$ Nanog               | Fg2-Fgfr$^\beta$                      | [15]       |
| Neuron/Neural stem (Mammal)      | Ngn1 $\rightarrow$ Cyclins              | Delta-Notch$^\alpha$                 | [24]       |

later stabilized at the mound and fruiting body stages through positive feedback generated by intracellular or autocrine signaling mechanisms [12, 13]. Quite similar developmental features are observed in early mammalian embryos where the small pool of cells (16-32 cell stage) of the inner cell mass is equally segregated in two populations of primitive endoderm (Gata6-positive) and epiblast (Nanog-positive) lineage cells [14]. Cells are first biased to a specific lineage in a reversible manner as Nanog-positive cells contribute to increase extracellular Fgf4 levels that influence Fgf4-bound cells by preventing accumulation of Nanog and promoting accumulation of Gata6 [15]. Cell fate is later stabilized in an irreversible manner [16] through the activation of stabilizing intracellular feedback mechanisms [15, 17] and cell motility and sorting processes [18].

In both developmental systems, cell-type diversification and proportioning relies on the interplay between intracellular regulatory mechanisms and intercellular inhibitory signals, and tends to occur as a two-step differentiation process. In contrast, positional information or cell division are not critical factors in regulating these developmental differentiation processes, though it can be the case for other developmental lineage decisions [25–26]. An important issue is then to identify which regulatory features are minimally required for efficient proportioning process [27–30]. To address this issue, we derive a model consisting of a population of inhibitory-coupled, noisy and bistable cells, which recapitulates the main intracellular and intercellular properties described above. The rigorous and exhaustive analysis of this class of model allows to identify the major obstacles to efficient cell-type proportioning depending on the strengths of noise, intracellular feedback and intercellular coupling. We further identify a universal strategy based on dual-time regulatory feedback loops to circumvent these obstacles. Finally, we discuss how this strategy is implemented in biological systems, notably through the dichotomy between transcriptional and epigenetic regulation, and how other mechanisms may further improve the cell-type proportioning process or coordinate it with growth or patterning processes.

**Methods**

**Effective one-dimensional model for binary cell-fate decision**

Bistable behaviors occurring in a wide range of physical systems are often studied as effective one-dimensional models. Although protein networks are generally characterized by a sophisticated signaling and regulatory architecture, the mechanism underlying cellular bistability typically relies on a core positive feedback loop, such as mutual inhibition or mutual activation between two proteins. The dynamics of circuits featured with simple positive feedback architecture can potentially be reduced to a one-dimensional model by using timescale separation arguments. Adiabatic elimination of fast modes is straightforward when a fast variable can be explicitly identified (e.g., mRNA levels), but can also be done by appropriate changes of variables. This is, for instance, the case for the toggle-switch circuit where two protein species of concentration $p_A$ and $p_B$ are activated by some signal $sA_B$, interacts through mutual inhibition of strength $\mu$, and are eventually subject to self-activation of strength $\alpha$ (figure 1(A)):

$$
\frac{dp_A}{dt} = \frac{s_A + \alpha p_A^2}{1 + \alpha} - (1 + \mu p_B^2)p_A
$$

$$
\frac{dp_B}{dt} = \frac{s_B + \alpha p_B^2}{1 + \alpha} - (1 + \mu p_A^2)p_B
$$

This dynamical system, often used as a generic model for binary differentiation decisions [31, 32], shows a transition from monostability to bistability as $s_{A,B}$ increases. In the fully symmetric case $s_A = s_B$, the symmetric state $p_A = p_B$ is destabilized through a pitchfork bifurcation (supercritical or subcritical depending on $\alpha$ and $\mu$) giving rise to two coexisting stable states $A$ ($p_A > p_B$) and $B$ ($p_B > p_A$). The coexistence of two stable fixed points separated by a saddle point $p_B$ defines a 1D invariant manifold denoted $M$ that corresponds to the unstable manifold of the saddle point and extends to the stable manifold tangent to one eigenvector of the stable fixed points (blue line of figure 1(B)). In contrast, the stable manifold of the saddle point (red line of figure 1(B)) divides the phase plane into two basins of attraction. For large enough $\mu$, trajectories from any initial condition quickly approach close to $M$ and, then,
slowly evolve along $\mathcal{M}$ toward $\mathcal{A}$ or $\mathcal{B}$. Timescale separation between fast and slow dynamics arises from the existence of a fast eigenvalue $\lambda_f$ and a slow eigenvalue $\lambda_s (|\lambda_f| \ll |\lambda_s|)$ in the eigenspectrum of the linearized system along $\mathcal{M}$ (top panel of figure 1(C)). The slower dynamics restricted to $\mathcal{M}$ can be captured by a scalar field $f(x)$ where $x \approx p_B - p_s$, which is colinear to the slow eigenvector tangent to $\mathcal{M}(p_B)$ in the symmetric case. The reduced scalar field $f(x)$ on $\mathcal{M}$ can be numerically computed, but also approximated by a third-order polynomial by applying center manifold reduction in the neighborhood of the saddle $p_B$ (bottom panel of figure 1(C)). Indeed, by using appropriate linear coordinate transformations $\tilde{z} = T^{-1}(\tilde{p} - \tilde{p}_B)$, the vector field given by equations (1) and rewritten as $\tilde{p} = A(\tilde{p} - \tilde{p}_B) + \tilde{N}(\tilde{p} - \tilde{p}_B)$ becomes:

$$\frac{d\tilde{z}}{dt} = f(\tilde{z}) + \tilde{N}(\tilde{z})$$

where $T$ is the Jordan transformation matrix of $A$, $J = \text{diag}(\lambda_f, \lambda_s)$ and $\tilde{N}(\tilde{z}) = T^{-1}N(T\tilde{z})$. Representing $\mathcal{M}$ by the mapping $z_f = h(z)$ allows to rewrite equation (2) as $\dot{z}_f = \lambda_s z_f + \tilde{N}(z_f, h(z_f))$ and truncation of the Taylor expansion up to third-order term lead to the normal form equation:

$$\frac{dz_f}{dt} = \lambda_0 + \lambda_1 z_f - \lambda_2 z_f^2 + \lambda_3 z_f^3$$

For $\alpha = 0, \lambda_0 = 0$ and $\lambda_3 = \rho_0^2 \mu^2(2\lambda_f - \lambda_s) - \mu/2$ is obtained by solving the invariance equation $z_f = h'(z_f)z_f$. Applying the invariant manifold reduction approach in the non-symmetric case ($s_B = s_0$) introduces a constant symmetry-breaking term $\lambda_0 = \lambda_1(p_{A,0} - p_{B,0}) - \lambda_2(p_{A,0} - p_{B,0})^3$ in equation (3) that favors the stability of $\mathcal{A}$ at the expense of that of $\mathcal{B}$. The scaling transformation $x = z_f/\lambda_0^{3/2}$ and the introduction of a Gaussian white noise term finally leads to the reduced one-dimensional system:

$$\dot{x} = s + \rho x - x^3 + \sqrt{2D} \zeta(t)$$

where $s$ is a symmetry-breaking parameter, $\rho$ the strength of intracellular positive feedback and $D$ the variance of the noise.

**Effective population model of inhibitory-coupled bistable cells**

A simple population model can be built by considering $N$ cells $i$ that are described by the 1D intracellular dynamics of equation (4) and are coupled each other through intercellular signaling. A common coupling term used for this general class of globally-coupled bistable systems is $\pm \gamma (x_i - x_j)$ which mediates activatory/tractive (for $-$) or inhibitory/repressive (for $+$) coupling [33, 34]. Furthermore, interaction delays can be incorporated as an explicit delay [34] or mediated by an intermediate mean field signal variable. From a biological viewpoint, although an explicit delay is often used for models of Delta-Notch signaling [35], a diffusive signal is assumed to be more relevant to describe paracrine signaling mediated by diffusible factors (Dif1, Fgf, CPC) or juxtacrine signaling in the presence of mixing processes due to cell movement [36]. Accordingly, we consider the following population model:
with \( f(x) = s + \rho x - x^3 \). The aforementioned interaction \( \gamma(x_i - m) \) (with \( \gamma > 0 \)) contains two terms: the first term is absorbed in the positive feedback parameter \( \rho \) and, thus, contributes to bistability [37] while \(-\gamma m\) mediates a global negative feedback of timescale \( \tau_m \) related to the time-consuming processes of synthesis, degradation, regulation or diffusion of the signaling components. For the following, it is convenient to define the parameterized potential, 
\[
U(x, m) = \int_{\mathbb{R}} [f(x) - \gamma m] \, dx
\]
which exhibits two wells \( x_j(m) \) (\( j = A \) or \( B \)) and a saddle \( x_S(m) \) for \( \gamma m \in [\xi, \bar{\xi}] \) with basins \( \Omega_j \), curvatures \( U''(A, B; \gamma)(m) \) and barrier heights \( \Delta_{A,B}(m) \). The Kramers escape rate from the well \( j \) = \( A \) or \( B \) is given by:
\[
r_j(m) = \frac{\sqrt{\left| U''(m) \right| U''(\gamma)(m)}}{2\pi} \exp \left( -\frac{\Delta_{j}(m)}{D} \right).
\]

### Results

#### Steady-state proportion between two cell-type clusters

To investigate the dynamic and steady-state properties of the cell population model (equations (5)), it is convenient to consider the continuum limit \( N \rightarrow +\infty \) for which the model can be reformulated in terms of probability distribution function \( P(x, t) \):

\[
\frac{\partial P}{\partial t} = \frac{\partial}{\partial x} \left( \frac{d}{dx} U(x, m) \right) P + D \frac{\partial^2}{\partial x^2} P
\]
\[
\tau_m \frac{dm}{dt} = \int_{\mathbb{R}} x \, P(x, t) \, dx - m
\]

The stationary solutions of equations (8) satisfy the self-consistent equation [33]:
\[
m = \int_{\mathbb{R}} x \, P(x, m) \, dx \equiv q(m)
\]
where \( P(x, m) = Z(m)^{-1} \exp(-U(x, m)/D) \) (\( Z \) is the normalization prefactor). For \( \gamma > 0 \), equation (9) has a unique stationary solution \( m \) that is associated with a bimodal steady-state distribution \( P(x) = P(x, m) \) for \( \rho \) large enough and \( s \) small enough. Several other steady-state quantities can be defined the same way; potential \( U(x) \), fixed points \( x_{\gamma} \), attraction basins \( \Omega_j \), Kramers escape rates \( \tilde{r}_j \), cluster sizes \( \bar{P}_j = \int_{\Omega_j} P(x) \, dx \) and cluster proportion
\[
\bar{\tilde{P}}_A - \tilde{P}_B.
\]
This two-cluster steady state \( m \) is stable for any \( \gamma, D \) and \( \tau_m \) values, which can be demonstrated in some limits by computing the lowest stability exponent. In the limit \( \tau_m \rightarrow +\infty \), the probability distribution follows adiabatically the slow population dynamics and the stability of the two-cluster steady state is determined by the eigenvalue \( q'(m) - 1 \) (equation (9)) that is always negative for inhibitory coupling as \( q'(\bar{m}) < 0 \) for \( \gamma > 0 \). For noise small enough so that the escape time from one well is large compared with the intrawell relaxation time, the slow time evolution of the mean field \( m \) and proportion \( R = \bar{P}_A - \bar{P}_B \) depends on Kramers escape rate \( \tau_j(m) \) (equation (7)) and well position \( x_j(m) \) as,
\[
\tau_m \frac{dm}{dt} = r_A(m) - r_B(m) + \bar{r}_A(m) + \bar{r}_B(m)
\]
\[
\tau_m \frac{dR}{dt} = (x_A(m) + R) + x_B(m)(1 - R))/2 - m
\]

The steady state satisfies \( \bar{R} = \frac{2m - \bar{q}_A - \bar{q}_B}{\bar{q}_A - \bar{q}_B} = g(\bar{m}) \) and the eigenvalues of the linearized system around \( \bar{m}, \bar{R} \) are found to be always negative for inhibitory coupling as \( \bar{r}_A(m) > 0 \) and \( \bar{r}_B(m) < 0 \) for \( \gamma > 0 \).

The cell-type proportioning process requires that a broad range of initial conditions converges to the two-cluster steady state, but also rapidly enough with respect to the developmental time \( \tau \) (\( \gg \{1, \tau_m\} \)) and stable enough with respect to noise-induced interwell hopping. Although we have shown that a population of inhibitory-coupled noisy bistable cells exhibits an always stable two-cluster steady state, we further reveal the existence of three distinct regimes that may obstruct efficient proportioning (figure 2); (i) frozen metastability that leads to critically slow relaxation dynamics and compromises precise proportions in finite time; (ii) steady-state hopping that hinders robust cell-type acquisition against noise; (iii) collective oscillations that compromise stable proportions over time. These regimes occur for specific ranges of parameters (figures 2(A)-(C)) and are illustrated in the extreme case of an initial condition where all cells start in a state strongly shifted to high x levels and biased toward A fate (figures 2(D)-(G)).

### Trade-off between precise proportion and robust commitment

For \( D \) low enough and \( \tau_m \) not too large, the relaxation rate toward a steady state \( \{m, \bar{R}\} \) is governed at long time by the slowest relaxation modes determined by the Kramers transition rates. At this slow timescale assumed to be larger than \( \tau_m \) \( m \) adiabatically follows the change of proportion
\[
R(t) = \int_{\Omega_A} P(x, t) \, dx - \int_{\Omega_B} P(x, t) \, dx
\]
which is driven by the Kramers transition rates according to equation (10) with \( m = g^{-1}(R) \). The rate of the monotonous perturbation decay \( \delta R(t) = |R(t) - \bar{R}| \) is mostly proportional to the largest Kramers escape rate that typically decreases by several orders of magnitude as \( \delta R \) diminishes. As a result, when the system has reached a critical distance \( \delta R \) from the steady state \( \bar{R} \), the relaxation can become critically slow (frozen) as individual cells are trapped in a...
metastable state for a very long time ($\gg \tau$). This frozen relaxation is prone to establish a spurious and biologically irrelevant proportion $R(\tau)$ that can be very different from the biologically relevant steady-state proportion $R$ and thus leads to a proportion error $\delta R(\tau)$. Given that the mapping $\mathcal{H}(\delta R(t_0)) = \delta R(t_0 + \tau)$ has a decreasing derivative function indicating a subexponential decay (constant derivative indicates an exponential decay), one can define a maximum proportion error or bias $\mathcal{H}(\delta R_t)$ below which frozen relaxation at population level and metastability at individual level co-occur:

$$\mathcal{H}(\delta R_t, \tilde{\rho}) = \delta R_t(1 - \epsilon)$$

where $\epsilon \ll 1$ and $\tilde{\rho} = [D, \gamma, s, \rho]$. For $s = 0$, this maximal proportion error associated with metastability can be different for positive and negative perturbations and needs to be normalized as $\delta R_t/(1 + |\tilde{\rho}|)$. If $\mathcal{H}(\delta R_t(m), \tilde{\rho}) = (1 - \epsilon)$ is always negative or always positive for $m \in [n/\gamma, n_2/\gamma]$, then $\delta R_t(\tilde{\rho}) = 0$ or $\delta R_t(\tilde{\rho}) = 1$, respectively. Expectedly, $\delta R_t$ is the highest for $D$ and $\gamma$ small and $s$ large (figures 2A, B, D). Increasing $\gamma$ diminishes the range of proportion biases and errors $\delta R_t$, by narrowing the range of $m$ values associated with bistability ($m \in [n/\gamma, n_2/\gamma]$). Higher noise (or lower barrier height) also reduces $\delta R_t$ by increasing the perturbation decay rate as $\tau \propto \exp(-\Delta/D)$.

Although noise-induced interwell hopping is beneficial to avoid frozen metastability and frozen relaxation that prevents from reaching the desired proportion state, it is biologically unlikely when equilibrium is reached as stochastic transdifferentiation is not observed and harmful in fully developed organisms. We thus define a biologically irrelevant hopping regime (figures 2A, B) for which non-negligible noise-induced interwell hopping occur during time interval $\tau$ (figure 2E), which occurs for a noise larger than $D_{HI}$ given by

$$(R + 1)D_A(D_{HI}) \tau = 1.$$  

It is important to note that the hopping regime necessarily coincides with an absence of metastability $\delta R_t = 0$. Therefore, a noise-dependent trade-off exists between frozen metastability and steady-state hopping, such that efficient proportioning illustrated in figure 2G requires a narrow and specific range of noise level (figure 2A) relative to the positive feedback strength (figure 2B).
Oscillations driven by lateral inhibitory coupling

Although increasing the strength of inhibitory coupling reduces the range $\delta R_c$ of spurious and frozen proportion pattern, it can also give rise to collective oscillations in the presence of large enough coupling delays. Some hints regarding the stability of these collective oscillations can be gained by investigating the model without noise ($D = 0$) and with identical elements ($x_i \rightarrow x$) without noise ($D = 0$):

$$\frac{dx}{dt} = s - \gamma m + \rho x - x^3$$
$$\tau_m \frac{dm}{dt} = x - m \tag{13}$$

This class of two-dimensional dynamical system, which has been studied in details in [38], clearly shows a negative feedback loop where $x$ activates $m$ that inhibits $x$ with a strength $\gamma$. The nullcline $\tilde{x}(m)$ obtained by solving $f(x) - \gamma m = 0$ has a Z shape for $m \in [n/\gamma, r_2/\gamma]$, such that stable hysteretic oscillations occur for large enough coupling strength and delay for which all the fixed points of the deterministic system are unstable. For $s = 0$, there is a single fixed point ($x = 0$ and $m = 0$) for $\gamma > \rho$, and such fixed point is unstable for $\tau_m > 1/\rho$. Stable oscillations occur in the presence of a single unstable fixed point when inhibitory coupling is strong enough ($\gamma > \rho$) and slow enough ($\tau_m > 1/\rho$), but also in the presence of three unstable fixed points for $\gamma < \rho$. In the presence of moderate levels of noise, numerical simulations show the existence of a stable one-cluster oscillatory state that coexists with the two-cluster steady state and is destabilized toward this state above a critical noise level (figure 2(A),(F)). Note that such oscillatory behavior quite differs from the case of explicit delays for which the two-cluster steady state can be destabilized toward multiple oscillatory states [34].

A dual-time positive feedback improves proportion regulation

Population of inhibitory-coupled bistable cells exhibits a stable two-cluster state that nevertheless tends to be either weakly stable at single-cell level with respect to noise-induced hopping or weakly attracting at the population level as slow relaxation or oscillations are prone to occur. To resolve this antagonism, we propose a solution based on the existence of multiple regulatory timescales, which allows to control separately the relaxation and the steady-state properties. Indeed cellular differentiation relies not only on transcriptional positive feedback loops, but also on slower positive feedback loops for instance mediated by epigenetic mechanisms that stabilize gene expression pattern [39–41]. To describe such slower reinforcement mechanism, the model of the intracellular dynamics described by $x$ in equation (5) is supplemented with a slow variable $y_i$ as follows:

$$\frac{dx_i}{dt} = f(x_i) + \frac{\beta}{\tau_y} y_i - \gamma m + \sqrt{2D_x} \zeta_{c,i}(t)$$
$$\tau_y \frac{dy_i}{dt} = \sqrt{\beta \tau_y} x_i - y_i + \sqrt{2D_y \tau_y} \zeta_{r,i}(t) \tag{14}$$

where $\tau_y$ and $\beta > 0$ are respectively the timescale and the strength of the so-called epigenetic feedback. The choice of linear coupling terms between variables $x$ and $y$ and the further assumption that $D_x = D_y = D$ are not critical, but very convenient as a two-dimensional potential $U(x, y, m) = U(x, m) + \sqrt{\beta \gamma} x y - \frac{1}{2} \tau_y y^2$ can be defined and stationary solutions $\bar{P}(x, y)$ and $\bar{m}$ can be obtained by replacing $U(x, m)$ with $U(x, y, m)$ in equation (8). Using a timescale separation argument, an epigenetic feedback that is slow enough ($1 \ll \tau_y < \tau$) and inactivated at early time ($y(0) \sim 0$) allows to reduce the occurrence of frozen metastability, steady-state hopping and collective oscillations (figure 3). On the one hand, the frozen metastability regime is mostly determined at early time by transition rates associated with the fast system and potential $U(x, 0, m)$ whose saddle barrier can be lowered by decreasing $\rho$. On the other hand, the hopping regime is determined by the steady-state potential $\bar{U}(x, y)$ whose saddle barrier can be heightened by increasing $\beta$. Furthermore, oscillations are precluded in the fast system due to the low ratio $\Delta(\rho)/D$ (for $\rho$ small) as well as in the slow system if one assumes a slower timescale for the epigenetic positive feedback than for the global negative feedback (for $\tau_y > \tau_m$). As a result, dual fast-slow positive feedback can significantly extend the domain of efficient proportioning by controlling separately and, thus, reducing simultaneously the respective domains of frozen metastability, hopping and oscillations (figures 3(A),(E)).

The efficiency of this proportioning mechanism therefore relies on the existence of two relaxation phases well-separated in time (figures 3(B),(C)). In a short timescale, fast intracellular feedback, noise level and intercellular coupling strength contribute to a full relaxation of the fast modes toward the steady state associated with low $y$ values and low barrier height, while further activation of the slow epigenetic feedback stabilizes both cell fates and cluster size proportions. Although introducing a slow feedback is critical for stabilizing cell-fate decisions, keeping a fast positive feedback mechanism is still required for rapid symmetry-breaking and cell-fate diversification, otherwise the cell-type proportioning would occur at the timescale of the slow positive feedback. This biphasic relaxation mechanism can operate to set any final proportion $R(\tau)$ value between 0 (equal proportion) and 1 (all-or-none proportion) by tuning the symmetry-breaking parameter $s$ (figure 3(D)). A minor caveat is that $R$ depends on $D$ (in proportion to $s$) while an initial epigenetic bias may lead to significant proportion errors associated with high $\delta R(\tau)$ values.
Overall, this proportioning mechanism requires a specific hierarchy of the system timescales where $1/\tau_0^{(x,y)} > \tau \sim \tau_0 > 1/\tau_0^{(x,0)} > \tau_x \sim \tau_m$, which is consistent with the typical biological time scales ranging from less than an hour for intercellular signaling [42], hours for protein expression changes through synthesis and degradation processes, few hours to days for epigenetic regulatory events [40, 43] and days for the developmental time scale.

**Discussion**

Using a generic modeling framework, we have identified a minimal set of mechanisms required to perform
an efficient proportion control of distinct differentiated cell types emerging during multicellular development. In a population of interacting bistable cells, noise and inhibitory coupling need to be finely tuned to reach a collective state characterized by a precise proportion between robust cell types. Fine-tuning is due to the antagonistic requirements between the respective tasks of diversification, proportioning and stabilization of cell-state attractors. Stochasticity is important to create small differences between identical cells [44, 45] and to escape from metastable cell states, but is detrimental for robust cell-type specification. For its part, lateral inhibition contributes to amplify small cellular differences [37, 44] and is critical for rapid relaxation within a narrow proportion range, whereas it promotes oscillatory behaviors or becomes counterproductive after cell sorting occurs. A solution to these antagonisms consists in the existence of dual positive feedback loops operating at distinct timescales, which coordinate to orchestrate in time the emergence of diverse cell types, the precise regulation of their proportion and their robust and quasi irreversible fate commitment.

The importance of a multiphasic differentiation process is supported by the common observation of a lineage priming phase before the irreversible lineage-restricted fate commitment. This developmental stage is typically characterized by a dynamic expression of differentiation factors during which pre-commitment decisions are reversible, such as in Amoeba [46, 47] or mammal embryos [48, 49]. In this process, the cooperation between transcriptional noise and feedback is critical to provide flexible fate switching abilities and quickly reach steady-state proportions. The further transition from reversible lineage priming to irreversible fate determination happens to involve the activation of a delayed positive feedback mechanism, which is most likely mediated by epigenetic regulatory processes [50], but also by the activation of autocrine signaling pathways [13] or by the spatial segregation of cell subpopulations [18]. Importantly, our model suggests the possibility to autonomously schedule this transition between reversible and irreversible commitment, as late epigenetic activation occurs when single-cell dynamics have been stabilized to some extent, thereby reflecting that some steady-state proportion has been reached. Although this biphasic differentiation dynamics can establish precise, symmetric or asymmetric proportions for a wide range of initial conditions and noise levels, it remains sensitive to two classes of perturbations. On the one hand, parametric perturbations of signaling or intracellular dynamics (parameter s in the model studied here) can affect the proportions, as it has been shown when manipulating extracellular levels of Dif1 in Dictostelium [51] or of Fgf4 in mammalian embryos [52, 53]. On the other hand, transient perturbations of proportion after cell removal or death during the late stabilization phase can lead to biased proportions, which accounts for the error tolerance zone observed in Dictostelium [54] or for the use of a pool of undifferentiated stem cells to restore the proportions in plants or animals [55]. Last, the existence of a multiphasic differentiation dynamics that sets the arrow of the developmental time is consistent with the notion that the reprogramming efficiency of somatic cells to induced pluripotent stem cells requires a well-scheduled addition of selected factors [56] that operate at both epigenetic and genetic levels [41, 57].

Other sophisticated regulatory processes influence cell-fate decisions and, indirectly, the proportioning process. Notably, stem or progenitor cells are subjected to a highly dynamic control of differentiation factors, through ultradian oscillations [58], cell-cycle progression [24, 59], or asymmetric division [60]. All these processes are prone to contribute to dynamic cellular heterogeneity in term of cell-fate propensity, which further promotes both rapid and divergent fate decisions and, thus, minimizes the risk of too slow and failed differentiation without requiring other sources of stochasticity [24, 61]. Although the intracellular mechanisms that switch complex oscillatory dynamics toward diverse steady states can be very complicated, low-dimensional dynamical models can nevertheless be used to address these issues [62, 63].

The developmental regulation of cell-type proportions also uses mechanisms involved, in the first place, in tissue growth and patterning. The fact that fate-restricted progenitor cells keep the ability to proliferate suggests that proportioning can also be controlled through the relative proliferation rates rather than interconversion rates between distinct types of progenitors [26]. In fact, spatial regulation of cell-type specification adds a whole dimension to the issue of proportioning. Spatial arrangement of cell types into domains often occurs after the proportioning process through cell motility and sorting process [18, 64], but it may also take place simultaneously through a spatial control of fate decision depending on positional informations relative to compartment’s boundaries and signaling centers [25, 65]. Yet, the presence of morphogen gradient does not preclude the need for lateral signaling to regulate domain size and sharpen domain boundary against various sources of noises [66].

**Conclusion**

This study highlights how the developmental establishment of diverse cell types requires a well-orchestrated interplay between intracellular, intercellular and stochastic mechanisms. The challenge lies in reconciling the competing demands of flexible decisions during early cell-type diversification and proportioning and of robust lineage-restricted fate commitment for specialization purposes. The existence of multiple regulatory timescales appear critical to dissociate the positive feedback mechanisms that cooperate with noise and lateral
inhibition to promote cellular heterogeneity and tune proportions, to those required to lock fate decisions regardless intercellular signals and noise levels.

Acknowledgments

We thank Nen Saito for stimulating discussions. BP acknowledges support of CANON foundation in Europe fellowship. KK acknowledges support of MEXT Japan.

References

[1] Gregor T, Fujimoto K, Masaki N and Sawai S 2010 Science 328 1023–5
[2] Sorodomi D, Jorg D J, Morelli L G, Richmond D L, Schindelin J, Julicher F and Oates A C 2014 Science 345 222–5
[3] Chang H H, Hemberg M, Barahona M, Inger D E and Huang S 2008 Nature 453 544–7
[4] Waddington C 1942 Nature 150 563–5
[5] Guantes R and Poyatos J F 2008 PLoS Comput. Biol. 4 e1000235
[6] Brandman O 2005 Science 310 496–8
[7] Greenwald L and Rubin G M 1992 Cell 68 271–81
[8] Bonner T and Slikin M 1949 Am. J. Bot. 36 727–34
[9] Sakai Y 1973 Dev. Growth Differ. 15 11–9
[10] Kay R R and Thompson C R 2001 Development 128 4959–66
[11] Williams J G 2006 Rep. 7 694–8
[12] Katoh M, Shaw C, Xu Q, Van Driessche N, Morio T, Kuwayama H, Obara S, Urushihara H, Tanaka Y and Sahuksy G 2004 Proc. Natl Acad. Sci. USA. 101 7005–10
[13] Anjard C and Loomis W F 2005 Proc. Natl Acad. Sci. USA. 102 7607–11
[14] Gardner R L and Rossant J 1979 J. Embry. Exp. Morph. 52 141–52
[15] Frankenberg S, Gerbe F, Besonnard S, Belville C, Pouchin P, Bardot O and Chazaud C 2011 Dev. Cell 21 1005–13
[16] Grabarek J, Zyzynska K, Saiz N, Piliszek A, Frankenberg S, Nichols J, Hadjantonakis A K and Plissa B 2012 Development 139 129–39
[17] Besonnard S, De Mot L, Gonze D, Barriol M, Dennis C, Goldbeter A, Dupont G and Chazaud C 2014 Development 141 3637–48
[18] Xenopoulos P, Kang M, Puliafito D, DiTalia S and Hadjantonakis A K 2015 Cell Rep. 10 1508–20
[19] Lee M C and Schiebel E 2002 Plant cell 14 611–8
[20] Schellmann S, Schmitz P, Kirik V, Wada T, Okada K, Beermann A, Thumbfrat J, Jürgens G and Hülskamp M 2002 EMBO J. 21 5136–46
[21] Wilkinson H a, Fitzgerald K and Greenwald I 1994 Cell 79 1187–98
[22] Heitzler P, Bourouis M, Ruel L, Carteret C and Simpson P 1996 Development 122 161–71
[23] Choksi S P, Lauter G, Swoboda P and Roy S 2014 Development 141 1427–41
[24] Pfeuty B 2015 Development 142 677–85
[25] Babst A and Rossant J 2008 Dev. Biol. 313 614–29
[26] Kicheva A, Bollenbach T, Ribeiro A, Valle H, Rovell-Badge R, Episkopou V and Briscoe J 2014 Science 345 1254927
[27] Kaneko K and Yomo T 1994 Physica D 75 89–102
[28] Mizuguchi T and Sano M 1995 Phys. Rev. Lett. 75 966–9
[29] Vakulenko S M, Reinitz J and Radulescu O 2009 Phys. Rev. Lett. 103 168102
[30] Nakajima A and Kaneko K 2008 J. Theor. Biol. 253 779–87
[31] Huang S, Guo Y, Enver T and May G 2007 Dev. Biol. 305 695–713
[32] Wang J, Zhang K, Xu L and Wang E 2011 Proc. Natl Acad. Sci. USA 108 8257–62
[33] Desai R and Zwanzig R 1978 J. Stat. Phys. 19 1–24
[34] Huber D and Tsirion S 2003 Phys. Rev. Lett. 91 266001
[35] Lewis J 2003 Curr. Biol. 13 1398–408
[36] Uriu K and Morelli I 2014 Biophys. J. 107 514–26
[37] Matsuda M, Koga M, Woltjen K, Nishida E and Eibiswurz SY 2015 Nat. Commun. 6 6195
[38] Boissonneaud J and De Kepper P 1980 J. Phys. Chem. 84 501–6
[39] Dodd I R, Michielssen M A, Spenner K and Thon G 2007 Cell 129 813–22
[40] Sasaki M, Kawai Y, Makishi K, Itoh K and Terada T P 2013 PLoS Comput. Biol. 9 e1003380
[41] Miyamoto T, Furusawa C and Ankeo K K 2015 PLoS Comput. Biol. 11 e1004476
[42] Herrgen L, Ares S, Morelli L G, Schröter C, Julicher F and Oates A C 2010 Curr. Biol. 20 1244–53
[43] D’Urso A and Bricker J H 2014 Trends Genet. 30 230–6
[44] Losick R and Desplan C 2008 Science 320 65–8
[45] Meyer H M and Roeder A H K 2014 Front. Plant. Sci. 5 1–4
[46] Takasaki M, Noce T and Takeuchi 1983 Proc. Natl Acad. Sci. USA 80 5340–4
[47] Chattwood A and Thompson C R L 2011 Dev. Growth Diff. 53 558–66
[48] Martinez Arias A and Brickman J M 2011 Curr. Opin. Genet. Cell Biol. 23 650–6
[49] Martinez-Arias A, Nichols J and Schroter C 2013 Development 140 3499–510
[50] Hemberger M, Dean W and Reik W 2009 Nat. Rev. Mol. Cell. Biol. 10 526–37
[51] Parkinson K, Buttery N J, Wolf J B and Thompson C R L 2011 PLoS Biol. 9 e1001039
[52] Yamana Y, Lanner F and Rossant J 2010 Development 137 713–24
[53] Krawchuk D, Homma-Yamana N, Anani S and Yamana Y 2013 Dev. Biol. 384 65–71
[54] Ráfols I, Sawada Y, Aragai A, Maeda Y and MacWilliams H K 2001 Differentiation 67 107–16
[55] Birnbaum K and Sanchez Alvarado A 2008 Cell 132 697–710
[56] Gaeta X, Xie Y and Lowry W 2013 Nat Cell. Biol. 15 725–7
[57] Ashwin S and Sasaki M 2013 Sci. Rep. 3 16746
[58] Imayoshi J, Isomura A, Harima Y, Kawaguchi K, Kori H, Miyachi H, Fujisawa T, Ishidate F and Kageyama R 2013 Science 342 1203–8
[59] Pauklin S and Vallier L 2013 Cell 155 135–47
[60] Zhong W 2008 Curr. Opin. Neurobiol. 18 472–8
[61] Furusawa C and Kaneko K 2001 J. Theor. Biol. 209 395–416
[62] Ullner E, Zaikin A, Volkov E I and Garcia-Ojalvo J 2007 Phys. Rev. Lett. 99 148103
[63] Pfeuty B and Kaneko K 2014 Phys. Rev. E 89 022707
[64] Nicol A, Kappel W, Levine H and Loomis W F 1999 J. Cell. Sci. 112 3923–4
[65] Wolpert L 1969 J. Theor. Biol. 25 1–47
[66] Xiong F et al 2013 Cell 153 550–61