Stochastic factors in higher-order biocomplexity
(or, how noise can be good)

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Introduction


Stochastic models approximate random behavior:
1) uncertainty about initial conditions.
2) sensitivity to initial conditions.
3) incomplete descriptions (makes behavior unpredictable).
4) fundamental descriptions (probabilistic description of aggregate behavior).

Why use these models?
* in a perfect world, iid deterministic variables. Stochastic process: collection of imprecisely-measurable variables with noisy inputs.

* forecast (predictive) and structural (processes that produce phenomena) models. Good for understanding spontaneous events (e.g. mutation).

* more versatile than deterministic models (model variables with chaotic behavior).
Noise is there, get used to it

ODE plus white noise: macroscopic approach to approximating synaptic conductance (Monte Carlo discrete Markov Chain approach – MCMC - also used).

* both approaches emphasize role of stochasticity in a process (e.g. neuronal firing).


* equation describing voltage-gated ion channels. Original equation is deterministic (model of Cerebellar granule cell function).

\[
\frac{dX(t)}{dt} = (\alpha_x(V_m) - (1-X(t)) - \beta_x(V_m) X(t)) dt + \sigma dW
\]


\[
\frac{dX(t)}{dt} = (\alpha_x(V_m) - (1-X(t)) - \beta_x(V_m) X(t)) + \sigma \xi(t)
\]

Brownian noise term

variance

White noise term
Noise is there, is it useful?

Noise (characterized as a component of variance) shapes dynamical system output.

Example #1: transcriptional noise (based on empirical observations):

Single gene measurements, does not account for additive, multiplicative effects on cells, populations, and tissues.

Example #2: Stochastic resonance. Random signal $r$ can augment stationary signal $x$.

Random signal (stochastic) + sine wave (deterministic) = semi-periodic output.

* useful for understanding phenomena on the edge of chaos.

* noise might facilitate organized spontaneity, or otherwise drive system away from equilibrium.

Noise 1) relative deviation: $\gamma = \delta / \bar{x}$ (see Nat. Rev. Genetics, 6, 451, 2005).

Noise 2) noise strength: $\phi = \delta^2 / \bar{x}$ (see Nat. Rev. Genetics, 6, 451, 2005).
Noise can be a good thing!


Noise as input to a neuron:

* higher η, φ values as input leads to variability in firing rate output (right).

* in turn leads to modulation of rate code, which characterizes information content in nervous system (left).

Cell is modeled as a discrete unit (black box?) with electrical potential as phenotype

* molecular determinants of action potential gating not taken into account.
Story of Cellular Reprogramming

What is cellular reprogramming?
The creation of iPS (or induced pluripotent stem) cells from differentiated cells (fibroblasts, neurons).

Pluripotent cells: capable of differentiating into any cell type.

* four transcription factors (Oct4, Sox2, c-Myc, and Nanog) can be used to “reprogram” differentiated cells to pluripotent (stem-cell like).

* Not stem cells per se, must acquire stem cell characteristics (days to weeks).

* cells can be used as delivery system for gene therapy, or as way to repair damaged tissue (integrate into cell population).

Shinya Yamanaka: discovered four factor trigger using a “high-throughput” genetic screen.
Examples of iPS cells in culture

Infected fibroblasts (not pluripotent)
iPS colony (single)
iPS colony (multiplexed)
piPS colony w/surrounding differentiation
Crescent-shaped proto-colony
Story of cellular reprogramming (con’t)

Hypothesis: Cellular reprogramming can be driven by noise.
* noise in the form of transcriptional variance and other stochastic processes can trigger, drive reprogramming process in vitro.

* presence of Oct4, Sox2, and NANOG suppress differentiation genes and activate stem cell genes (modules).

Example: MacArthur et.al, PLoS One, 3(8), e3086.

Computational approaches to gene expression include adding noise (stochastic element) to model.

* non-specific noise in expression of four factors, other genes can trigger reprogramming.

Systems Biology of Stemness

There are many different types of stem cells with widely different characteristics.

**Box 4 | A stochastic multi-stable switch**

Consider the simple motif in which two transcription factors activate their own expression and mutually repress each other's expression (see the figure, part a). This type of feedback naturally gives rise to multi-stability and provides the cell with the ability to make all-or-none fate decisions in response to external cues. The following stochastic differential equations describe the expression levels of two transcription factors ($x_1$ and $x_2$) that are interacting in this way:

$$dx_1 = \frac{x_1^3}{K_1 + x_1^3 + x_2^3} - b_1x_1 + \sigma_1 dW$$

$$dx_2 = \frac{k_2x_2^3}{K_2 + k_2x_2^3 + x_1^3} - b_2x_2 + \sigma_2 dW$$

In these equations $k_1$, $k_2$, and $K_1$ are the (normalized) rate constants at which transcription factors bind to promoters; $K_2$ and $K_2$ are (normalized) dissociation rate constants; $b_1$ and $b_2$ are (normalized) decay rate constants; $\sigma_1$ and $\sigma_2$ are constants determining the amplitude of noise in the system; and $W$ denotes a Weiner process (Brownian motion). In this simple illustrative case we have assumed that each transcription factor binds cooperatively to its own promoter and to that of the other transcription factor as a homodimer (which is why $x$ is raised to the power of two). In the absence of molecular noise ($\sigma_1, \sigma_2 = 0$) this model has many coexisting steady state attractors (for appropriate parameter regimes). In the presence of molecular noise ($\sigma_1, \sigma_2 > 0$), individual cells do not settle at a single attractor but instead stochastically switch between distinct states at a rate that depends on the amplitude of molecular noise. However, over time the joint probability density $p(x_1, x_2)$ (that is, the probability of finding a cell with expression levels of $(x_1, x_2)$) settles to a stationary state, and a robust distribution of cell types is achieved. The figure (part b) shows the stationary probability distribution for a representative simulation of this system: red hot spots indicate preferred genetic configurations at which cells will accumulate, and blue indicates low probability configurations.

**Stemness = what do the diversity of stem cells types have in common?**

* pluripotency, gene regulation profiles.

* multi-stability (ability to change state in response to environmental, viral cues).

**Switch that governs this transformation may be stochastic:**

* Two factors activate their own expression, mutually repress each other (all-or-nothing response).

* Weiner process (additive) = stochasticity. At $\sigma = 0$, switch between fate at rate $r$. 

$$\sigma = 0$$
Systems Biology of Stemness (con’t)

Stemness is maintained by a network centered on NANOG, which is controlled by the four factors:

Left: protein-protein interaction network of genes upregulated when cell is in a “stem” state (based on ChIP experiments).

Right: genes on A side of figure downregulated, genes on B side upregulated when middle box (3 of 4 factors + NANOG) circuit is activated.

Figure 1. Stem cell regulatory networks. a) Schematic showing high-confidence protein–protein interactions between NANOG and NANOG-associated proteins, as derived from HIPPIPS 17, 137, 138, 139. b) Schematic showing the stem cell transcriptional regulatory circuit. This network was reconstructed from the data presented in various recent high-throughput chromatin immunoprecipitation (ChIP) experiments, as indicated in the A side of figure. Both networks are rich in regulatory loops (see also box 2, suggesting a complex system with the ability to exhibit a wide range of context-dependent dynamic behaviours. Factors present in both the NANOG interactome and the core transcriptional network are shown in red. Note that there is great overlap between these two networks (with shared factors being the most central elements of both networks), suggesting that the core transcription factors regulate each other’s expression in a coordinated, combinatorial manner, involving both protein-protein and protein-DNA interactions. See Integrated Stem Cell Molecular Interactions database for an interactive version of this network.

Box 1 | The core embryonic stem cell transcriptional circuit

Systems Biology of Stemness (con’t)

Fitness or energy landscape used to characterized transitional and end states during reprogramming.

* left: navigation along minima during process (characterize molecular changes).

* right: stable states = minima and maxima. Characterize piPS cells in relation to differentiated and stem-like cell type.

Figure 2 | Cellular reprogramming as navigation through a complex attractor landscape. In a complex cellular attractor landscape there might be many coexisting stationary attractors (here represented as local minima), each of which might be associated with a unique molecular signature. In this view, cellular reprogramming corresponds to guiding the cell through the landscape from one local minimum to another (shown by the dotted arrows). As there might be many distinct paths between minima (both direct and through intermediary minima), reprogramming from one cell type to another might be achieved through numerous different routes. MacArthur et al. Nature Reviews Molecular Cell Biology, 10, 673 (2009).
Competing models for reprogramming (stochastic vs. deterministic):

1) stochastic: transformation occurs according to a **variable** latency.
   * time from trigger to transformation is variable.

2) deterministic: transformation occurs according to a **uniform** latency.
   * time from trigger to transformation is uniform.

Elite models argue that only a subset (1/n) of cells will reprogram (innate ability).
   * elite model is independent of stochasticity vs. uniformity.

Three components to the stochastic model of reprogramming:

1) basic assumption: given N cells, one-step reprogramming process occurs with constant cell-intrinsic rate $k$.

\[
\begin{align*}
N(t) & \xrightarrow{k} \text{iPS cell} \\
B & \rightarrow k
\end{align*}
\]

2) latency: interval $t_p$ defined as time between $t_n$ (when first cell in population N is reprogrammed) and $t_{n+1}$ when daughter cells grow to reach detection threshold. From “first settler” to viable colony.

3) scaling: at any $t_n$, population of cells in a well, $N(t)$, scales at rate which first reprogramming event takes place. Cumulative PDF:

\[
P(t + t_p) \approx 1 - e^{-kt} \text{ where } t = \int_0^t N(t')dt' \\
\approx 1 - e^{-kN_{\text{eff}}(t-t_0)} \text{ for } t > t_0
\]
Population-averaged doubling times ($t_d$) derived from overexpression lines on Doxycycline. Rescaling time by $t_d = \text{number of cell divisions that occur during latency period (each cell division – opportunity to transform)}$. 

Figure 3 | Cell-division-rate-dependent and -independent acceleration of reprogramming. a, Average induction levels for transgenes in different NGFP1 cell populations. $n$ indicates number of populations sampled per group, presented as mean ± s.d. b, Growth curves for cells on doxycycline. Exponential growth (dashed line) described the data well ($R^2 = 0.97–1.0$), and the population-averaged doubling times ($t_d$) were calculated from these fits (Supplementary Fig. 9). c, As in Fig. 2b, latencies for reprogramming various clonal B-cell-derived populations. NGFP1-p53$^{kd}$, NGFP1-p21$^{kd}$ and NGFP1-Lin28$^{OE}$ wells were statistically distinct from the NGFP1 and NGFP1-control hairpin wells ($P < 0.0001$, log-rank test for dissimilarity). 

d, Rescaling time by $t_d$ provides an estimate for the number of cell divisions occurring during latency. $C_{d}$, population-averaged number of cell divisions on doxycycline before Nanog–GFP detection. No statistical difference between groups was observed after rescaling time by $t_d$ ($P > 0.1$). e, f, As in c, d, but for NGFP1-Nanog$^{OE}$ wells. $n$ indicates number of populations monitored.
Scaling separates out effects of intrinsic ability to reprogram and contributions of population size.

* rate of cell division $\sim$ population size $\sim$ reprogramming ability.
Example of scaling (more instances of transformation with more cells):

\[ N = 10^3 \]  

time to reach > 90% reprogrammed cells in well longer.

\[ N = 10^6 \]  

time to reach > 90% reprogrammed cells in well shorter.
Regulatory interactions among genes in core pluripotency module:

* Oct4-Sox2 and Oct4-Sox2-NANOG repress both Sox9 and PPAR-γ (seen in neural and bone cells, respectively).

* Sox9 and PPAR-γ are co-repressive of each other and Oct4, Sox2, NANOG.

Tip of the iceberg viewpoint:
* reprogramming is a critical process that is triggered by the right state of this core module.

What role does stochasticity play? Oct4/Sox2 and NANOG vs. rest of genome.
Paper #2: PLoS One (con’t)

Free (F) and bound (B) binding sites for the promoters of PG and LSMG.

* bound by individual transcription factors (P_n, L_n) or complexes (C_n).

C_1 = Oct4-Sox2 complex, C_2 = Oct4-Sox2-NANOG complex.

K, kc_1, kc_2 = equilibrium dissociation constants.

LSMGs bind co-operatively to each others promoters with binding site affinity 2.

* ensures that transcriptional FB loops are nonlinear.
LSMGs – lineage master specifying genes.
* \( L_1 \) (RUNX2), \( L_2 \) (SOX9), \( L_3 \) (PPAR-\( \gamma \)), ……\( L_n \) = lineage specifying genes.

PGs – pluripotency genes.
* \( P_1 \) (Oct4), \( P_2 \) (Sox2), \( P_3 \) (NANOG), ……\( P_n \) = pluripotency genes.

Equilibrium expression level = \((L_1+L_2+L_3) + (P_1+P_2+P_3)\).

Stemness: all gene types expressed at very low level (Frame A).

Increase in expression level related to amplitude of noise in gene expression (amplitude = trigger for expression).
Noise in the expression of genes with specific function is what is crucial to triggering reprogramming:

Noise on PGs, Oct4, or Sox2 alone = reprogramming. Noise on LSMGs alone = none.

Less amplitude in noise function required in Frame A for reprogramming (more efficient).
External stimuli (applied to pluripotent cells to produce differentiated cells):

* BMP4 (growth factor)
* RA (retinoic acid)

As concentration increases:

* equilibrium gene expression level is shown to drop exponentially in pluripotent cells (leads to instability).

* terminally-differentiated cells remain stable, and equilibrium expression level increases linearly.

* bi- and tri-potent cells also unstable at high levels of external stimulus.
Evolutionary Capacitance


Activity: “buffers” genotypic variation (multiple genotypes = single phenotype).

Inactivity: triggered by environment stress (overwhelms function, results in diverse phenotypic effects). Multiple pleiotropic effects.

*Drosophila* (fruit fly) larvae example:

Buffering in context:
Left: larvae exposed to mild heat shock, then to severe heat shock (expression).

Right: larvae exposed to severe heat shock only (no expression).

Inducible tolerance ~ expression of proteins in $Hsp$ family (protective function).
Evolutionary Capacitance (con’t)

**One take-home message:**
Accumulation of variation (random mutation, etc.) during phenotypic buffering ~ Loss-of-function mutants with higher fitness.

**At left:**
Power-law distribution of distance (from buffered phenotypes), very few viable individuals at large phenotypic distance (see ‘lethal’ category).

**Theoretically:**
Evolutionary capacitance can be described as a population-level phenomenon using principle of facilitated variation.
Facilitated variation (FV) can be described mathematically as

\[ FV = \left( \frac{M_n}{M_l} \right) \times \left( \frac{D_n}{D_l} \right) \]

* \( M_n \) are the number of non-lethal mutants.
* \( M_l \) is the number of lethal mutants.
* \( D_n \) is the phenotypic distance of non-lethal mutants from the wildtype.
* \( D_l \) is the phenotypic distance of lethal mutants from the wildtype.

As variation is facilitated (noise?), both \( M_n \) and \( D_n \) become large.

* non-wildtype variants become more abundant and more dissimilar as FV is maximized.