A Stochastic Reaction–Diffusion Method for Studying the Control of Gene Expression in Eukaryotic Cells

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Motivation

- Interested in modeling biochemical networks of interacting proteins/genes within a single eukaryotic cell.
- There may not be a well–defined continuously varying concentration since the number of molecules of a given biochemical species is fundamentally integer valued.
- Hence if concentration levels are sufficiently low, system should be modelled as a discrete state process. At this level noise effects can become noticeable:
  - λ–phage
  - transcription and translation
- The well–mixed assumption is not always appropriate, spatial localization often is important, for example:
  - Egg-polarity genes in Drosophila oocyte
  - Ash1 MRNA localization in budding yeast
  - Synapse–specificity of long–term facilitation in Aplysia
  - Lipophillic hormone action through nuclear receptors

Reaction–Diffusion Master Equation

- Divide the comp. domain into a series of cells $i = 1 \ldots N$
- Assume that within each cell we can independently apply the spatially homogeneous form of stochastic chemical kinetics
- Assume that all transitions of particles between cells can be modelled as first order reactions
- Let k_{ij}^l denote the jump rate for the l'th chemical species from cell j to cell i
- Let $M_i^l(t)$ denote the integer valued random variable for the number of particles of species l in cell i, $l = 1 \ldots L$
- Let $M_i(t) = \left(M_i^1, \ldots, M_i^L\right)$ denote the chemical state vector in cell i
- Let $M(t)$ be the N by L matrix with entries $M_i^l(t)$
- Let $a_i^k(M_i(t))$ denote the probability per unit time of reaction k occurring at location i, assuming there are K total possible reactions
- Let $\nu_k = \left(\nu_k^1, \ldots, \nu_k^L\right)$ be the change in M_i due to one occurrence of reaction k
- $P(m,t) \equiv P(m) \equiv \text{Prob}\{M(t) = m | M(0) = M_0\}$

Then, the reaction–diffusion master equation is:

$$\frac{\partial P(m)}{\partial t} = \sum_{i=1}^{N}\sum_{j=1}^{N}\sum_{l=1}^{L} k_{ij}^l \left(m_j^l + 1\right) P(m + e_j^l - e_i^l) - k_{ij}^l m_i^l P(m) + \sum_{i=1}^{N}\sum_{k=1}^{K} a_i^k(m_i - \nu_k) P(m - e_i\nu_k) - a_i^k(m_i) P(m).$$

But where do the jump rates k_{ij}^l come from?

Ans: a valid master equation for the probability of a Brownian particle being in a given region gives the jump rates for the reaction–diffusion master equation.

Relation to Single Particle Brownian Motion

If there are no reactions, and only one particle, then the reaction–diffusion master equation, in terms of the probability density, p_i, to be at location x_i, reduces to

$$\frac{dp_i}{dt} = \sum_{j=1}^{N} \frac{V_j}{V_i} k_{ij}\, p_j - k_{ji}\, p_i. \qquad (1)$$

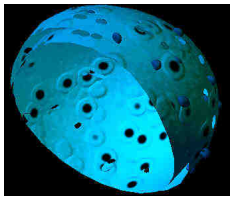
In the continuum limit, we expect this equation to become

$$\frac{\partial p(x,t)}{\partial t} = \Delta p(x,t).$$

Hence, one method to obtain the jump rates in the reaction–diffusion master equation is by creating a discretization of the Laplacian for single particle Brownian motion. Note that this discretization must have the form dictated by (1).

Nuclear Pores and Boundary Conditions

We model two boundaries for a eukaryotic cell. The exterior cell membrane is assumed to be impermeable, and so a no–flux boundary condition is used. In contrast, the nuclear membrane contains pores that allow molecules less than 9 nanometers in size to freely diffuse through. A special transport mechanism allows select molecules between 9 and 26 nanometers to pass through the pores.



Nuclear membrane reconstruction showing nuclear pore locations

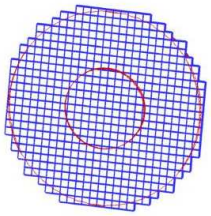
Motion through the pores is modelled as either a fixed permeability for crossing the nuclear membrane, or by a no–flux condition for membrane impermeable molecules. Jump rates for the reaction–diffusion master equation are then determined from a discretization of

$$\begin{aligned} \frac{\partial p}{\partial t} &= D\Delta p, && \text{in nucleus/cytoplasm,} \\ \frac{\partial p}{\partial \eta} &= 0, && \text{at the cell membrane,} \\ -D\frac{\partial p}{\partial \eta} &= -\rho\,[p]_n, && \text{at the nuclear membrane} \end{aligned} \qquad (2)$$

This equation describes the Brownian motion of a single particle that may pass through the nuclear membrane.

Calculating the Numerical Jump Rates

Diffusive and trans–nuclear membrane jump rates are calculated from the discretization weights of a Cartesian grid embedded boundary discretization of (2).



Cross section of spherical cell and nuclear membranes embedded in Cartesian grid.

Denote by $\alpha \in \{\text{nuc, cyt, ext}\}$ the domain of a given spatial location. The diffusive jump rate from the domain α component of cell j to the domain α component of cell i is given by our discretization to be

$$k_{ij}^{\alpha} = \frac{DA_{ij}^{\alpha}}{hV_j^{\alpha}}.$$

For cells uncut by a boundary, this reduces to

$$k_{ij}^{\alpha} = \frac{D}{h^2}.$$

The jump rate across the nuclear membrane in cell i, from domain α' to domain α is

$$k_i^{\alpha\alpha'} = \frac{A_i^B \rho}{V_i^{\alpha'}}.$$

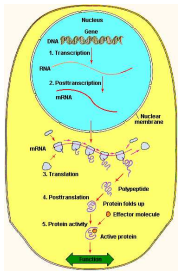
Consequences of Discretization

- method is conservative
- detailed balance is preserved

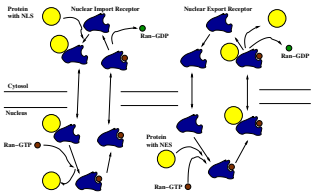
Overall Simulation Method

1. Initialization:
   (a) Given the membrane locations, calculate the area of all pieces of Cartesian cell faces intersected by a membrane, and the volume of all pieces of Cartesian cells split by a membrane.
   (b) Calculate the jump rates for all species, within all Cartesian cells containing some part of the cytoplasm or nucleus.
   (c) For each piece of a Cartesian cell, calculate the rates of all chemical reactions that can occur there. For reactions with volume dependent rates, use the volume of the piece of the Cartesian cell within which the reaction is occurring to change the rate constants to units of reciprocal time.
2. Time Evolution:
   (a) Simulate individual realizations of the stochastic process described by the reaction–diffusion master equation using the Gillespie Method. Diffusive and transmembrane solute motion are represented as first order reactions, using the jump rates calculated in step (1b). Within each component of a cell, the rates from step (1c) are used to simulate chemical reactions.
3. Output:
   (a) To estimate moments or distributions, use statistics from many simulations.

Transcription, Translation, Transport Model



Steps in eukaryotic gene expression



RanGTP import/export cycle

Transcription Model

- We follow one gene and its products
- Gene is assumed to be localized to center of nucleus
- Not an accurate eukaryotic transcriptional model, but reasonable first approximation for prokaryotic transcription

$$\begin{aligned} DNA + RNAP &\rightarrow DNA^0 && \text{at gene location} \\ DNA^{l-1} + n_i &\rightarrow DNA^l, l = 1, \ldots, M-1 && \text{at gene location} \\ DNA^{M-1} + n_M &\rightarrow mRNA_i + RNAP + DNA && \text{at gene location} \end{aligned}$$

mRNA Export Model

- $mRNA$ freely diffuses within nucleus, only exits through $RanGTP$ export system
- Non–standard pathway for $mRNA$ export, but is used, ex:
  - incompletely spliced HIV structural protein mRNAs
- Here NR is nuclear receptor, Rt is $RanGTP$, and Rb is $RanBP1$
- $mRNA$–NR–Rt is the only membrane permeable state
- Transport proteins modelled as fixed background concentration

$$\begin{aligned} mRNA_i + NR\text{–}Rt_i &\rightarrow mRNA\text{–}NR\text{–}Rt_i && \text{within nucleus} \\ mRNA\text{–}NR\text{–}Rt_i + Rb_i &\rightleftarrows mRNA\text{–}NR\text{–}Rt\text{–}Rb_i && \text{within cytoplasm} \\ mRNA\text{–}NR\text{–}Rt\text{–}Rb_i &\rightarrow mRNA_i + NR_i + RanGDP_i && \text{within cytoplasm} \end{aligned}$$

Translation and mRNA Decay Model

- Within the cytoplasm mRNA can be degraded, translated, and freely diffuse
- Assume fixed background concentration of ribosomes, uniformly distributed throughout cytoplasm
- P is the protein product

$$\begin{aligned} mRNA_i + Ribosome_i &\rightarrow mRNA_i^0 && \text{within cytoplasm} \\ mRNA_i^{l-1} + a_i &\rightarrow mRNA_i^l, l = 1, \ldots, N-1 && \text{within cytoplasm} \\ mRNA_i^{N-1} + a_N &\rightarrow P_i + mRNA_i && \text{within cytoplasm} \\ mRNA_i &\rightarrow \varnothing && \text{within cytoplasm} \end{aligned}$$

Nuclear Import Model

- Protein can freely diffuse within cytoplasm and nucleus, but must be actively transported to cross nuclear membrane
- Protein can be degraded anywhere within cell
- Only P–NR may pass through the nuclear membrane

$$\begin{aligned} P_i &\rightarrow \varnothing && \text{everywhere} \\ P_i + NR_i &\rightarrow P\text{–}NR_i && \text{within cytoplasm} \\ P\text{–}NR_i + Rt_i &\rightarrow P_i + NR\text{–}Rt_i && \text{within nucleus} \end{aligned}$$

Gene Regulation Model

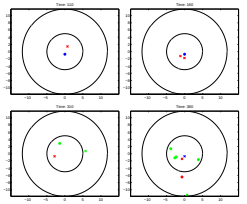
- Protein product of gene can feedback and inhibit transcription

$DNA + P_i \rightleftarrows DNA_{\text{rep}}$ at gene regulatory site

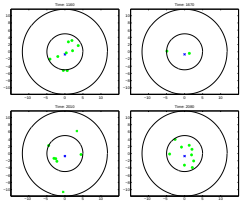
2D Model Results

The nuclear and cell membranes are approximated as concentric circles.

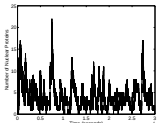
In the following figures, a blue star denotes the unbound DNA, and a blue "x" that the DNA is repressed. During transcriptional states the DNA is not displayed. Red stars denote mRNA, and red "x's" mRNA bound to nuclear receptor and RanGTP. mRNAs coupled to nuclear receptors, RanGTP, and RanBP1 are not present in the images shown. During translation mRNAs are not displayed. Green stars denote proteins, and a green "x" represents protein bound to nuclear receptor. Time is in seconds.



Evolution of one realization of the model over several minutes showing accumulation of nuclear protein.



Evolution of one realization of the model over a half hour showing oscillation in nuclear protein level.



Total number of nuclear proteins in one realization over 30,000 seconds.

References

[1] S. A. Isaacson and C. S. Peskin (2004). *Incorporating diffusion in complex geometries into stochastic chemical kinetics simulations*, Siam J. Sci. Comp., submitted.

Movies at http://www.math.nyu.edu/~isaacsas