Modeling of mRNA Localization in Xenopus egg cells

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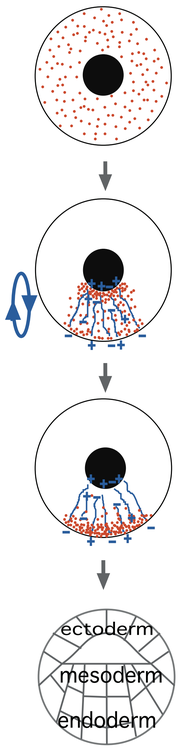
mRNA Localization



mRNA (messenger RNA, red dots) are collections of RNA particles. Maternal mRNA (from mother to egg cell) influences gene expression in the growing organism. At the beginning of egg formation, mRNA is distributed throughout the cell cytoplasm.

Molecular motor proteins then move mRNA from the nucleus (black circle) along tubular polymers called microtubules (MTs, in blue). Kinesin moves the RNA to the + ends of MTs, and dynein to the − ends.
→ Bidirectional transport may play a role.

After more than 24h, the mRNA becomes fully localized at the bottom of the cell.
→ Anchoring mechanism may play a role.
Failure to fully localize mRNA leads to compromised development of the cell.
→ Width of accumulation at the bottom may determine success of development.

- mRNA localization is **essential** in forming a top - bottom axis in the oocyte.
- This **axis polarity** ensures that layers in the early formation of the embryo are properly specified.

Experiments and Key parameters

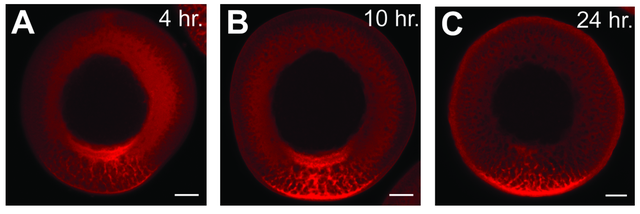


Figure: Localization of mRNA 4, 10 and 24 hours after injection and culture in an egg cell [2].

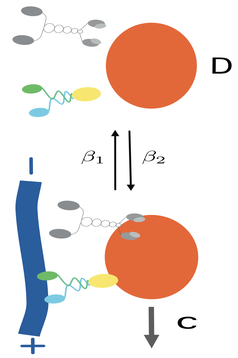
Key parameters

- Free mRNA particles diffuse in the cytoplasm with **diffusion coefficient** $\boldsymbol{D}$.
- Motor-mRNA complexes move along the MTs with **speeds** $\boldsymbol{c_{+/-}}$(down/up).
- Unbinding $\boldsymbol{\beta_1}$ and binding $\boldsymbol{\beta_2}$ **rates** from/to MTs are not well known but provide the connection between moving and diffusing or paused particles.
- mRNA at the bottom remains **anchored** to the cortical skeleton.

Data from **FRAP** (fluorescence recovery after photobleaching) experiments captures the above processes.

Discrete Model

- We set up a one-dimensional discrete model of the key processes for the vertical direction of transport from the nucleus to the cell bottom.
- Red particles are mRNA complexes to which two types of motors can bind.
- We assume motor-mRNA particles that are not bound to MTs diffuse.
- Motor-mRNA particles bound to MTs are assumed to have net directionality toward the cell bottom.



Model Results

- mRNA particles start in the top 5% of a vertical grid (top/nucleus → bottom).
- We assume mRNA particles are initially in equilibrium proportions of the 2 states.
- Particles spend time drawn from $Exp(\beta_2)$ for diffusion and $Exp(\beta_1)$ for convection.
- The particles switch state at the end of each exponentially-distributed assigned time.

Fraction of mRNA

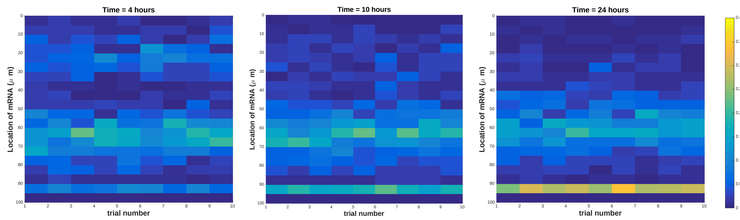
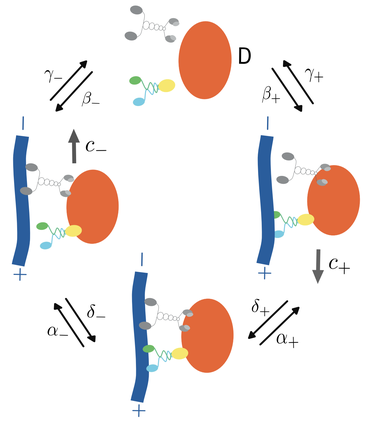


Figure: Predicted Localization after 4, 10 and 24 hours. Different trials (1-10) are plotted versus vertical location of the particles. Brighter spots correspond to higher concentration of mRNA.

Observations and Outlook

- Anchoring of motor-mRNA seems essential for localization. The mRNA is not fully localized after 24-48 hours, so that bidirectional transport should be considered in parameter estimation.
- This approach can be generalized to a model with any number of states, such as the one on the right.
- A two-dimensional spatial model would be able to predict the width of the accumulation area. This would also allow the introduction of the MT structure.
- The extension to a 4-state model (see right) is in progress.



Continuum Model

We consider the PDE model (1) for the states considered, with wave train ansatz (2):

$$(1)\quad \begin{cases} u_t = \ cu_y - \beta_1 u + \beta_2 v \\ v_t = Dv_{yy} + \beta_1 u - \beta_2 v \end{cases} \qquad (2)\quad \begin{pmatrix} u_k \\ v_k \end{pmatrix}(y,t) = e^{\lambda_k t} e^{\nu y} \begin{pmatrix} u_0 \\ v_0 \end{pmatrix}, \nu = ik\,.$$

λ are then eigenvalues of matrix $\begin{pmatrix} c\nu - \beta_1 & \beta_2 \\ \beta_1 & D\nu^2 - \beta_2 \end{pmatrix}$.
Taking expansion $\lambda = a_1\nu + a_2\nu^2 + \mathcal{O}(\nu^3)$, and initial conditions u_0, v_0 as Gaussians with width σ, the approximate solution is a spreading Gaussian at target time t with

$$(3a)\quad \text{mean particle position} = c\frac{\beta_2}{\beta_1+\beta_2}t\,,$$

$$(3b)\ \text{variance particle position} = 2a_2 t + \sigma^2 = 2\left(c^2\frac{\beta_1\beta_2}{(\beta_1+\beta_2)^3} + D\frac{\beta_1}{\beta_1+\beta_2}\right)t + \sigma^2$$

Renewal theory approach We consider the same set-up with times $T_i \sim Exp(\beta_1)$ in convection and $\tilde{T}_j \sim Exp(\beta_2)$ in diffusion. We let $S_i = T_i + \tilde{T}_i$, then $J_n = S_1 + S_2 + ... + S_n$ is the amount of time after n convection-diffusion cycles.
Then $X_t = \sup\{n : J_n \le t\}$ gives the number of state cycles by target time t.
We assign rewards $W_i = cT_i + G(0, 2D\tilde{T}_i)$ to each time cycle so that $Y_t = \sum_{i=1}^{X_t} W_i$ is the particle position at time t. Then the renewal theorems give $E[Y_t] \to$ (3a), $Var[Y_t] \to$ (3b) w.p. 1 as $t \to \infty$ with $\sigma = 0$, since the assumptions in [1] are satisfied.
Equations (3) are used to compare and confirm the discrete model predictions.

Optimization results

- Using FRAP data, we optimize model (1) for the key parameters using exponential time-differencing rk4 methods [3].
- The initial condition is a tanh function (bleach spot in FRAP).
- The data corresponds to evolution of $\int_{\text{bleach spot}}(u+v)(t)dxdy$ with time.
- Data from bleach spots in 3 regions in the cytoplasm is used to estimate parameters for different locations.
- Optimization results for the data below correspond to the area under the nucleus:

$c(\mu_m/s)$	$D(\mu_m^2/s)$	$\beta_1(1/s)$	$\beta_2(1/s)$
0.145	0.028	0.134	0.0063

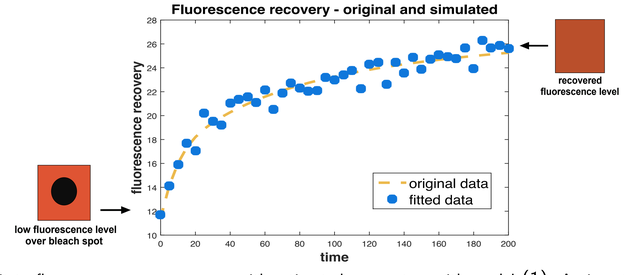


Figure: Fit to fluorescence recovery curve with estimated parameters with model (1). An inverted Laplacian fit to FRAP is done in [4] for two rate parameters in a reaction-diffusion system. That approach does not apply to equations (1).

References

[1] Gut Allan. Cumulative shock models. *Advanced Applied probability*, 22(2):504–507, 1990.
[2] James Gagnon, Jill Kreiling, Erin Powrie, Timothy Wood, and Kimberly Mowry. Directional transport is mediated by a dynein-dependent step in an rna localization pathway. *PLOS Biology*, 11:138–153, 2013.
[3] Aly-Khan Kassam and Lloyd Trefethen. Fourth-order time-stepping for stiff pde's. *SIAM Journal on Scientific Computing*, 26(4):1214–1233, 2005.
[4] Brian Sprague, Robert Pego, Diana Stavrera, and James McNally. Analysis of binding reactions by fluorescence recovery after photobleaching. *Biophysical Journal*, 86:3473–3495, 2004.