Mathematical modeling of the intracellular protein dynamics: the importance of active transport along microtubules.

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Bibliography:

- A. Cangiani and R. Natalini, A spatial model of cellular molecular trafficking including active transport along microtubules, *J. Theor. Biol.*, 267, **2010**

  The Authors presented a systems of PDEs considering diffusion process as well as active transport along microtubules.

- M. Sturrock, A.J. Terry, D.P. Xirodimas, A.M. Thompson, M.A.J. Chaplain, Spatiotemporal modelling of the Hes1 and p53-Mdm2 intracellular signalling pathways, *J. Theor. Biol.*, 273, **2011**

  The Authors proposed PDEs to capture the evolution of the species, mRNA and proteins, in the Hes1 and p53-Mdm2 systems.

Intracellular scale models

Concern the phenomena occurring inside a cell, such as:

- transmembrane transport processes
- transport processes inside cytoplasm and nucleus
- cascades of biochemical reactions

Signalling pathways constitute natural regulatory systems that:

- ensure cell resistance to random changes in its condition (i.e. to preserve cell homeostasis),
- ensure a proper response to external forcing (i.e. a direct response to the environment)

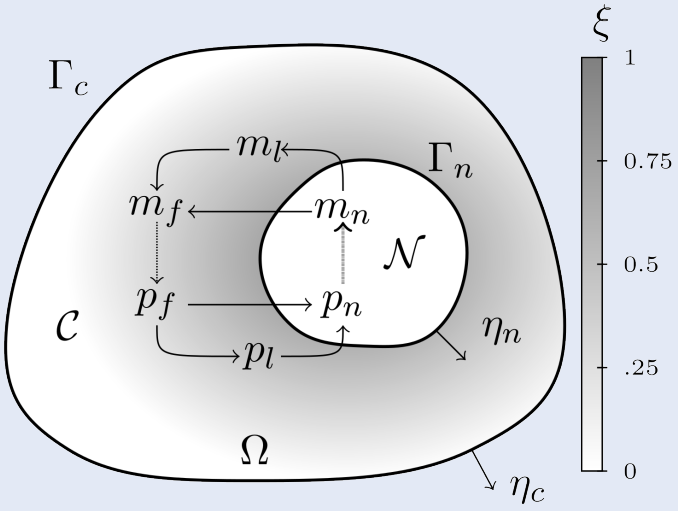
Objectives

- The main objective of our work is to contribute to a better understanding how spatial distribution influences the dynamics of protein synthesis.
- We look for a spatial explanation of the oscillatory dynamics sometimes observed in experiments.

The main assumptions of the model:

- Active transport along microtubules of both mRNA and proteins;
- Diffusion of both mRNA and proteins in the nucleus;
- Distinction between linked to microtubules and free molecules;
- No assumption on protein production localization;

Cell structure

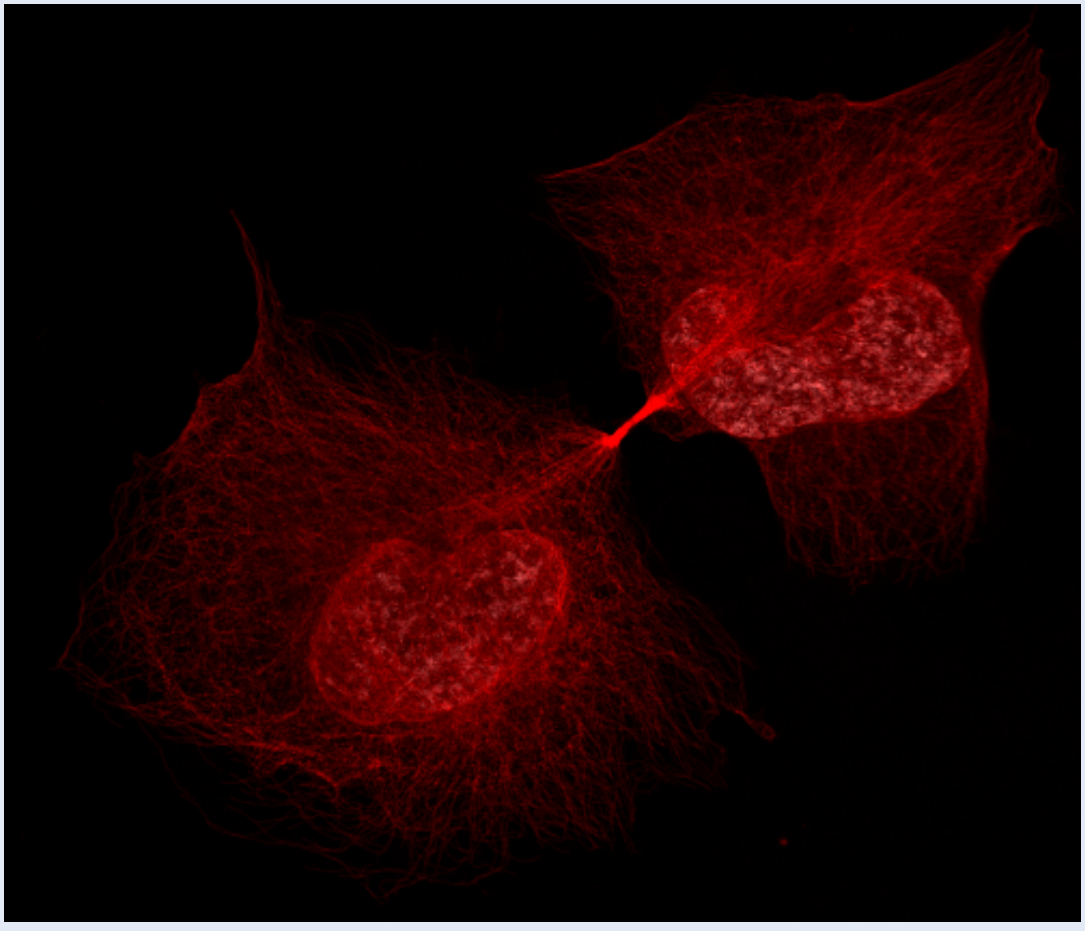


- Ω — convex bounded (smooth) domain in $\mathbb{R}^d$ — "the cell";
- Γ_c — "cell membrane" — its boundary;
- $\mathcal{N}$ — "nucleus" — bounded (smooth) sub-domain of Ω;
- Γ_n — nuclear envelope — boundary of the nucleus (we assume $\Gamma_n \cap \Gamma_c = \emptyset$);
- $\mathcal{C} = \Omega \backslash \mathcal{N}$ — "cytoplasm" — complement of the nucleus in the cell;
- ξ - microtubules density;

Model variables

- $m_n(t,x)$ and $p_n(t,x)$ — concentrations of mRNA and proteins in the nucleus;
- $m_l(t,x)$ and $p_l(t,x)$ — concentrations of mRNA and proteins in the cytoplasm, linked to the microtubules;
- $m_f(t,x)$ and $p_f(t,x)$ — concentrations of mRNA and proteins in the cytoplasm not linked (free) to the microtubules;

Microtubules



Human non-small cells lung carcinoma (H1299 cells) in the final stage of cell division with colored microtubules. Courtesy of Marta Małuszek from IIMCB.

Microtubules

- Since microtubules are thin but numerous we propose a representation of them through the function $0 \leq \xi(x) \leq 1$ defined on $\mathcal{C}$
- In part of the domain where no microtubules are present: $\xi := 0$
- If the whole elementary volume is occupied by microtubules: $\xi := 1$
- The movement of molecules in the cytoplasm depends on whether the molecules are bound (using motor proteins) to the microtubules or not — protein and mRNA are divided into
  - associated with microtubules (linked molecules)
  - not associated (free molecules)

Nucleus

- Motion is governed by diffusion, with a diffusion coefficient $\kappa_n(x) > 0$
- Nucleus is surrounded by nuclear envelope, a double membrane that encloses the entire organelle and isolates its contents from the cellular cytoplasm
- The nuclear envelope has many small holes called nuclear pores that allow material to move in and out of the nucleus

The system of equations

On nucleus:

$$\text{on } \mathcal{N} \quad \begin{cases} \partial_t m_n - \nabla \cdot \left(\kappa_n \nabla m_n \right) = -\lambda_m m_n + \dfrac{\alpha_m}{1 + \left(\frac{p_n}{\overline{p}} \right)^{\gamma}} \\ \\ \partial_t p_n - \nabla \cdot \left(\kappa_n \nabla p_n \right) = -\lambda_p p_n \end{cases}$$

- $\kappa_n > 0$ - diffusion coefficient;
- $\lambda_m > 0$ - decay parameter, i.e. degradation of mRNA is proportional to λ_m;
- $\lambda_p > 0$ - decay parameter, i.e. degradation of protein is proportional to λ_p;
- $\alpha_m > 0$ - mRNA transcription rate;
- $\gamma > 0$ - Hill parameter governing the strength of inhibition;
- $\overline{p} > 0$ - critical concentration of protein above which an effective inhibition of transcription occurs [Sturrock at al. 2011];

Cytoplasm

- The free particles satisfy a reaction-diffusion equation with a diffusion coefficient $\kappa_c(x) > 0$
- The rate of translation is proportional to the free mRNA concentration with a factor $\alpha_p > 0$
- $\lambda_m > 0$ - decay parameter, i.e. degradation of mRNA is proportional to λ_m
- $\lambda_p > 0$ - decay parameter, i.e. degradation of protein is proportional to λ_p

Cytoplasm

- The linked proteins is drifted with the velocity v_p
- The linked mRNA is drifted at the velocity v_m
- Protein is drifted in direction of nucleus whereas mRNA is drifted in the opposite direction
- The functions $\beta_m(\xi) > 0$ model the release of mRNA and $\beta_p(\xi) > 0$ model the binding of proteins, respectively
- The chance for mRNA to be released from microtubules increases with the volume not occupied by microtubules, i.e. $\beta_m(\xi)$ is decreasing
- The chance for protein to be bound to the microtubules increase with the volume occupied by microtubules, i.e. $\beta_p(\xi)$ is an increasing function

The system of equations

On cytoplasm:

$$\text{on } \mathcal{C} \quad \begin{cases} \partial_t m_f - \nabla \cdot \left(\kappa_c \nabla m_f \right) = -\lambda_m m_f + \beta_m m_l \\ \partial_t m_l + \nabla \cdot \left(\nu_m m_l \, \mathbf{v} \right) = -\lambda_m m_l \; - \beta_m m_l \\ \partial_t p_f \; - \nabla \cdot \left(\kappa_c \nabla p_f \right) = -\lambda_p p_f \; - \beta_p p_f + \alpha_p m_f \\ \partial_t p_l - \nabla \cdot \left(\nu_p p_l \, \mathbf{v} \right) = -\lambda_p p_l \; + \beta_p p_f \end{cases}$$

- $\mathbf{v} : \mathcal{C} \in \mathbb{R}^d$ - unit vector field such that the microtubules are tangent to $\mathbf{v}$ and oriented from the nuclear envelope to the cell membrane.

Boundary conditions

On nuclear envelope for protein:

$$\text{on } \Gamma_n \begin{cases} \eta_n \cdot \left(\kappa_n \nabla p_n\right) = \mu_p p_f + \eta_n \cdot \mathbf{v}\, \nu_p p_l, \\ -\eta_n \cdot \left(\kappa_c \nabla p_f\right) = -\mu_p p_f, \end{cases}$$

- We assume that free protein molecules passes only through the nuclear envelope to enter the nucleus with speed $\mu_p > 0$ (term $\mu_p p_f$).
- We also take into account the proteins linked to microtubules p_l that are released directly in the nucleus (term $\eta_n \cdot \mathbf{v}\, \nu_p p_l$).
- η_n is the unit vector normal to the nuclear envelope outward of the nucleus.

Boundary conditions

On nuclear envelope for mRNA:

$$\text{on } \Gamma_n \begin{cases} \eta_n \cdot \left(\kappa_n \nabla m_n\right) = -\mu_m m_n, \\ -\eta_n \cdot \left(\kappa_c \nabla m_f\right) = \left(1 - \xi\right) \mu_m m_n, \\ \eta_n \cdot \mathbf{v}\, \nu_m m_l = \xi \mu_m m_n, \end{cases}$$

- We assume that mRNA passes only through the nuclear envelope to exit the nucleus with speed $\mu_m > 0$.
- mRNA population is split into the two sub-populations linked and free mRNA (resp. m_l and m_f) accordingly to the density of microtubules ξ close to the nuclear envelope.
- η_n is the unit vector normal to the nuclear envelope outward of the nucleus.

Boundary conditions

On cell membrane:

We apply no-flux boundary conditions across the cell membrane.

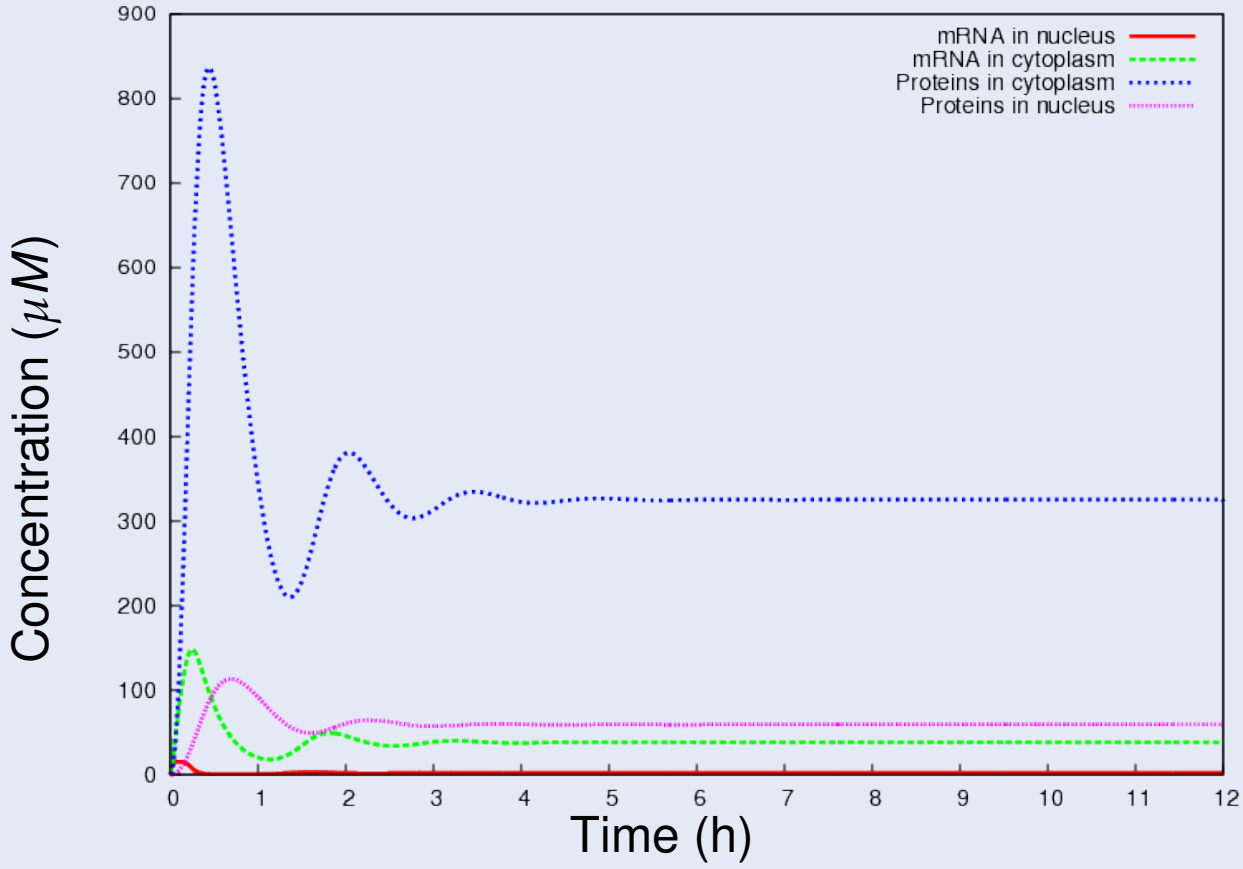
$$\text{on } \Gamma_c \begin{cases} \eta_c \cdot \left(\kappa_c \nabla m_f\right) = \eta_c \cdot \mathbf{v}\, \nu_m m_l, \\ \eta_c \cdot \left(\kappa_c \nabla p_f\right) = 0, \quad \text{and} \quad \eta_c \cdot \mathbf{v}\, \nu_p p_l = 0, \end{cases}$$

- We focus our attention on the intracellular transport neglecting the exchange between cell and surrounding environment.
- For proteins,this means that no particles can cross the cell membrane either by diffusion or by active transport along microtubules.
- Since the mRNA can not leave the cell, mRNA molecules, which are transported along microtubules are released into the cytoplasm before it reaches the cell membrane. This leads to the formation of free mRNA incoming flux so that the total number of mRNA molecules in the cell is preserved.
- η_c is the unit vector normal to the cell membrane outward of the cell.

Theorem

For the parameters smooth enough and non-negative initial data, there exists a global solution to the model. In addition the solution is non-negative.

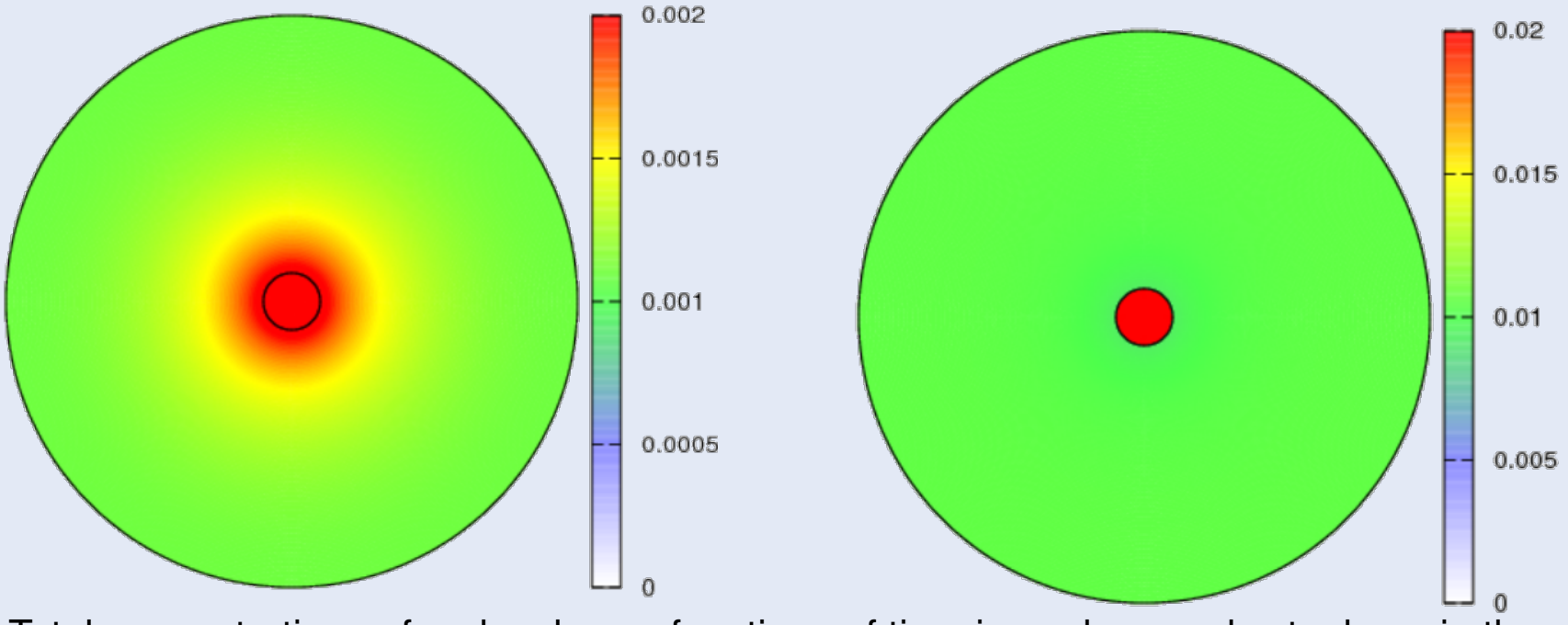
Numerical results without microtubules



Total concentrations of molecules as functions of time in nucleus and cytoplasm in the case without microtubules ($\xi = 0$). Simulation run for vanishing initial condition, i.e.:

$$m_n\left(0,x\right) = p_n\left(0,x\right) = m_f\left(0,x\right) = m_l\left(0,x\right) = p_f\left(0,x\right) = p_l\left(0,x\right) = 0.$$

Numerical results without microtubules



Total concentrations of molecules as functions of time in nucleus and cytoplasm in the case without microtubules ($\xi = 0$), mRNA on left hand side, protein on right hand side. Simulation run for vanishing initial condition, i.e.:
$m_n\left(0,x\right) = p_n\left(0,x\right) = m_f\left(0,x\right) = m_l\left(0,x\right) = p_f\left(0,x\right) = p_l\left(0,x\right) = 0$. Figure show the spacial distribution at the steady state (t=12h).

Numerical results with microtubules

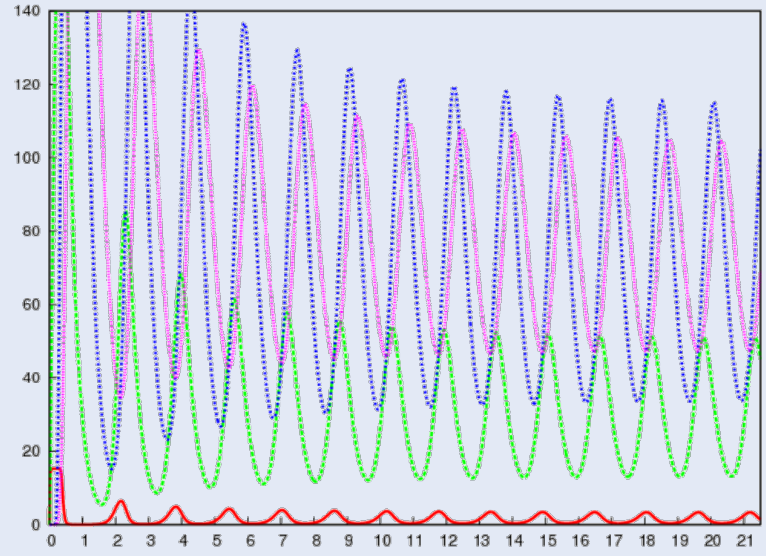
The density of microtubules ξ could be approximated as a linear decreasing function of the radius. We consider the following probability of linking

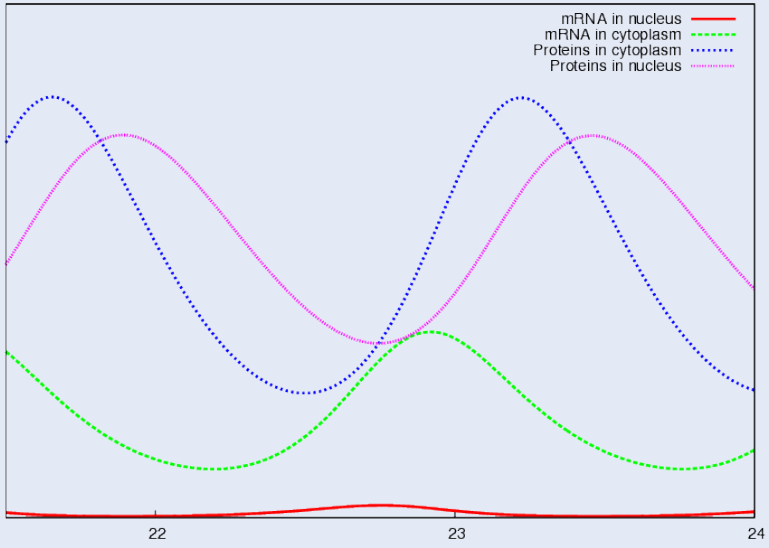
$$\beta_m\left(\xi\right) = \frac{1-\xi}{\xi}\,\overline{\beta}_m \qquad \text{with} \qquad \overline{\beta}_m = 10^{-4}s^{-1}$$

and

$$\beta_p\left(\xi\right) = \frac{\xi}{1-\xi}\,\overline{\beta}_p \qquad \text{with} \qquad \overline{\beta}_p = 10^{-7}s^{-1}$$

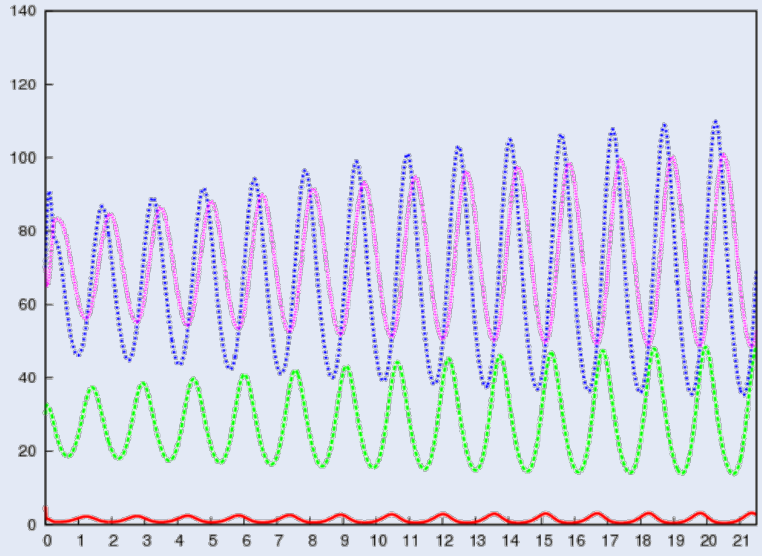
Numerical results with microtubules

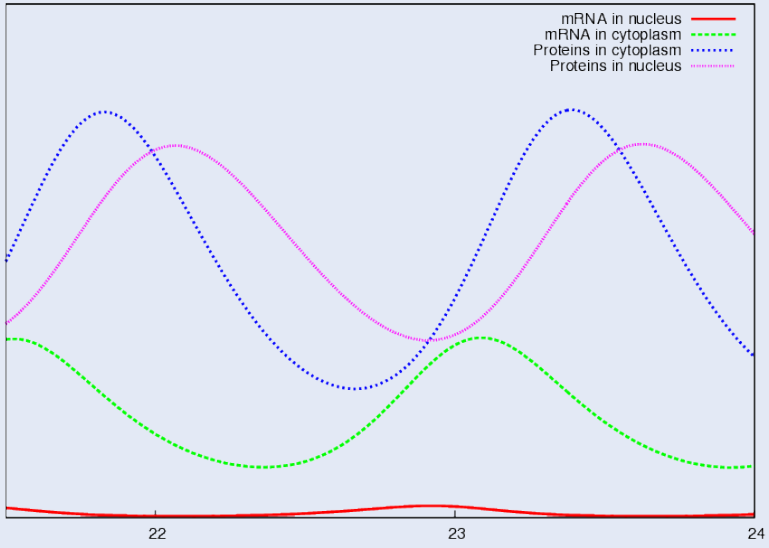




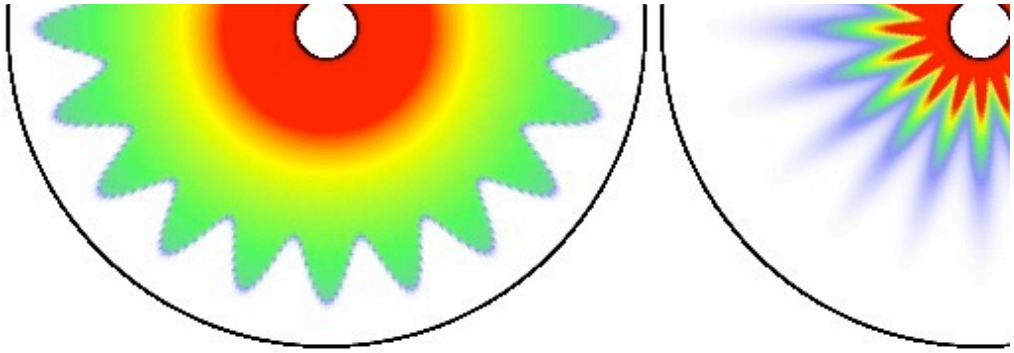
Total concentrations of molecules as functions of time in nucleus and cytoplasm in the case with microtubule, with vanishing initial condition. The red solid line stands for the concentration of mRNA in the nucleus and the green dashed line stands for the concentration of mRNA in the cytoplasm. The blue dotted line stands for the concentration of protein in the cytoplasm and the pink thinly dotted line stands for the concentration of protein in the nucleus.

Numerical results with microtubules





Total concentrations of molecules as functions of time in nucleus and cytoplasm in the case with microtubule, with homogeneous initial condition. The red solid line stands for the concentration of mRNA in the nucleus and the green dashed line stands for the concentration of mRNA in the cytoplasm. The blue dotted line stands for the concentration of protein in the cytoplasm and the pink thinly dotted line stands for the concentration of protein in the nucleus.



Conclusions

- We proposed a generic model of intracellular protein dynamics that accounts for the active transport of molecules (both mRNA and protein) along the microtubules.
- The model assumes that the regulation of protein synthesis acts as a negative feedback, i.e. protein produced in the cytoplasm in the process of translation, acts also as self-inhibitor.
- On the basis of the numerical simulations we performed, we formulate a hypothesis on the oscillations of protein and mRNA concentrations.
- We believe that the presence of microtubules and active transport of mRNA from the nucleus to the cytoplasm explain the existence of oscillations in the concentration of protein and mRNA.

Possible extensions of the model

- To consider the mRNA production not in the whole nucleus, but at a certain place inside the nucleus which would correspond to the mRNA production in a specific site on DNA.
- Further extensions that will bring the model closer to the reality may account for the rearrangements in microtubules structure. This extension may particularly play a role when half life of the microtubules is in the same range as the half life of considered molecules.
- The proposed model is generic and the main purpose of that work was to provide a methodology for future models of specific regulatory networks.

Bibliography:

- Szymańska, Z., Parisot, M., Lachowicz, M., ”Mathematical modeling of the intracellular protein dynamics: the importance of active transport along microtubules”, *J. Theor. Biol.*, 363:118-128, **2014**;
- Lachowicz, M., Parisot, M., Szymańska, Z., ”Intracellular protein dynamics as a mathematical problem” - submitted;