Project 2

Speckle Formation of Photoreceptors

Introduction

There are two main reasons why light plays an important role for higher plants. Firstly, the light energy is transformed into chemical energy by photosynthesis. Secondly, light initiates and regulates growth and cellular differentiation. This process of *signal transduction* controls, for example, the growth of leaves, the development of a photosynthesis system, etc. Plants can even sense the intensity, the direction, and the quality of light.

Plants are equipped with various photoreceptors. For red light, the most important protein is *Phytochrome B* (phyB). It is generally assumed that phyB is involved in shade detection and avoidance, as well as day length detection.

Red light activates phyB proteins by changing their molecular conformation. This is the initial step of a signaling cascade which finally leads to physiological responses. After the activation, phyB molecules migrate to the cell nucleus, where they form large complexes, called *nuclear speckles*.

The exact biological function of nuclear speckles is still unknown, and one way of getting more insight is to analyse the mechanism that leads to speckle formation.

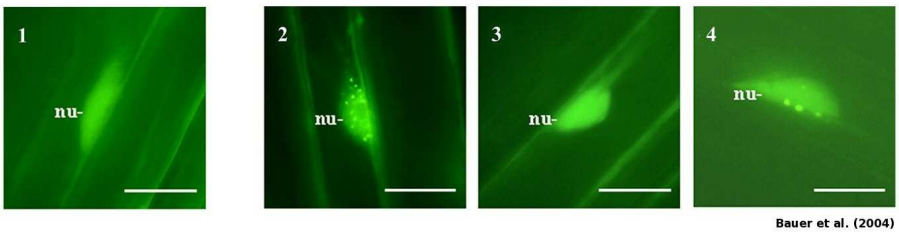

Figure 1: Nuclear speckles of phyB-GFP in wild type *A. Thaliana*. Uniform cellular distribution in the dark (1), speckle formation after 2 min (2), 1 h (3) and 6 h (4) of red light. Position of nucleus (nu) is indicated.

Experimental Data

The most popular and best understood organism in plant physiology is *Arabidopsis Thaliana*. It is possible to label phyB molecules in A. Thaliana with a fluorescent molecule and observe the translocation and complex formation of the molecules under a fluorescence microscope (cf. Figure 1). The images give information about the time, the number of speckles, and the size of the speckles.

Stochastic Model

We make the following model assumptions.

- We do not take into account spatial information, such as the positions of the molecules in space. We assume that the cell is a well-stirred mixture of molecules, that is, at each part of the cell the concentration of phyB (or phyB complexes) is the same.
- We do not consider changes of temperature or the cell volume.
- PhyB molecules collide randomly and may then form a complex.
- A complex of phyB molecules can only gain or loose single phyB molecules.

For $n \in \mathbb{N}$, we write s_n for a speckle that consists of n phyB molecules. Assume that initially all phyB molecules are not bound to a complex, that is, we have m chemical species of type s_1 in the volume and zero of type s_n for $n > 1$. A chemical reaction may occur if two molecules collide. Let $C_1, \ldots, C_{m-1}, D_1, \ldots, D_{m-1}$ denote the different types of chemical reactions that can occur. For $k \in \{1, \ldots, m-1\}$, the reactions C_k and D_k are given by

$$s_1 + s_k \to s_{k+1} \text{ and } s_{k+1} \to s_1 + s_k.$$

We describe the system as a stochastic process $(\vec{X}(t), t \geq 0)$, where

$$\vec{X}(t) = (X_1(t), \ldots, X_m(t))$$

is a vector of random variables. The entry $X_n(t)$ refers to the number of speckles of size n at time t. Thus, $\vec{X}(t)$ takes values $\vec{x} = (x_1, \ldots, x_m) \in \mathbb{N}_0^m$ with

$$\sum_{n=1}^{m} n \cdot x_n = m.$$

Let $\vec{c}_k = (c_{k1}, \ldots, c_{km}) \in \{-2, -1, 0, 1\}^m$ be the vector that describes the effect of reaction C_k on the current state, i.e., if $\vec{x}$ is the current state then $\vec{x} + \vec{c}_k$ is the state after reaction C_k has occured, where

$$c_{kn} = \begin{cases} -2 & \text{if} \quad n = 1 \text{ and } k = 1, \\ -1 & \text{if} \quad n \in \{1, k\} \text{ and } k > 1, \\ +1 & \text{if} \quad n = k + 1, \\ 0 & \text{otherwise.} \end{cases}$$

Since reaction D_k is the inverse of C_k, the effect of D_k is given by $-\vec{c}_k$. Assume that the probability of a reaction occuring in the next infinitesimal time interval $[t, t+h)$ is

$$\begin{array}{rcll} Pr\left(\vec{X}(t+h) = \vec{x} + \vec{c}_k \mid \vec{X}(t) = \vec{x}\right) & = & \alpha_k(\vec{x}) \cdot h & \text{for reaction } C_k, \\ Pr\left(\vec{X}(t+h) = \vec{x} - \vec{c}_k \mid \vec{X}(t) = \vec{x}\right) & = & \beta_k(\vec{x}) \cdot h & \text{for reaction } D_k. \end{array} \tag{1}$$

The functions α_k and β_k are called *propensity functions* and $\alpha_k(\vec{x})$ $(\beta_k(\vec{x}))$ is the *rate* of reaction C_k (D_k) in state $\vec{x}$, respectively. The more reactants are

available the more likely is a reaction. Hence, we assume that the reaction rates are linear in the number of distinct combinations of reactants. We define $\alpha_1(\vec{x}) = a_1 \cdot \binom{x_1}{2}$ and $\alpha_k(\vec{x}) = a_k \cdot x_1 \cdot x_k$ for $k > 1$, where $\vec{x} = (x_1, \ldots, x_m)$ and $a_k > 0$ is called *reaction rate constant*. For $k \in \{1, \ldots, m-1\}$, let $\beta_k(\vec{x}) = b_k \cdot x_{k+1}$, where b_k is the rate constant of reaction D_k.

The probability in Equation 1 only depends on the length of the time interval, which means that the propensity functions are time-independent. Besides, the successor states $\vec{x} + \vec{c}_k$ and $\vec{x} - \vec{c}_k$ only depend on the current state, and neither on the specific time nor on the history of reactions that led to the current state. Hence, $(\vec{X}(t), t \geq 0)$ is a *time-homogeneous Markov chain*. We can represent $(\vec{X}(t), t \geq 0)$ as a finite state-transition graph with state space

$$S = \{\vec{x} = (x_1, \ldots, x_m) \in \mathbb{N}_0^m \mid \sum_{n=1}^{m} n \cdot x_n = m\}$$

and transitions

$$\vec{x} \xrightarrow{\alpha_k(\vec{x})} \vec{x} + \vec{c}_k \quad \text{if} \quad k > 1, x_1 > 0, x_k > 0 \text{ or } k = 1, x_1 > 1,$$

$$\vec{x} \xrightarrow{\beta_k(\vec{x})} \vec{x} - \vec{c}_k \quad \text{if} \quad x_{k+1} > 0.$$

The initial distribution of $(\vec{X}(t), t \geq 0)$ is such that

$$Pr\left(\vec{X}(0) = (m, 0, \ldots, 0)\right) = 1.$$

Given the initial number m of molecules and reaction rate constants a_k, b_k, what is the probability of finding i speckles of size n in the long run? Your task is to compute the limiting distribution $\lim_{t\to\infty} Pr(\vec{X}(t) = \vec{x})$ for $\vec{x} \in S$ and analyze its dependency on the parameters m, a_k, and b_k.

Simulation Algorithm

Algorithm 1 generates *trajectories* of the Markov chain $(\vec{X}(t), t \geq 0)$ based on repeated sampling of (pseudo) random numbers. The output $\vec{x}$ is a random sample of the states that are visited at time h with positive probability. The algorithm implements the so-called *race condition*. If at time t the current state is $\vec{u}$, with each outgoing transition of $\vec{u}$ we assign a random variable T, which represents the time until the transition is *enabled*. For a transition

$$\vec{u} \xrightarrow{\lambda} \vec{w},$$

the associated time T is exponentially distributed with parameter $-\lambda$. The transition that is enabled first is then taken, i.e., the next state is the target of the transition with the smallest time T.

Exercise:
Implement Algorithm 1 and test it with $m = 200$, $h \in \{50, 100, 150, 200\}$, and $a_k = b_k = 1$ for $k \in \{1, \ldots, m-1\}$. Estimate the expected speckle size for all four choices of h by averaging over $N = 100$ samples. How does the distribution of the speckle size change if a_k is size dependent (e.g. $a_k = k \cdot \exp(-0.01 \cdot k)$)?

Algorithm 1 Stochastic simulation algorithm for $(\vec{X}(t), t \geq 0)$.

Input: total number m of molecules, reaction rate constants a_k, b_k for $k \in \{1, \ldots, m-1\}$, and time horizon $h > 0$
Output: sample state $\vec{x}$

$\vec{x} = (m, 0, \ldots, 0)$; {initial state}
$t = 0$; {initial time}
while $t < h$ **do**
  $\tau = \infty$;
  for $k = 1$ to $m - 1$ **do** {sample time until next C_k-reaction occurs}
    $\lambda = \text{prop}(a_k, \vec{x})$; {compute propensity $\alpha_k(\vec{x})$}
    if $\lambda > 0$ **then** {reaction is possible}
      $r = \text{drawRand}$; {draw random number uniformly distributed on $[0, 1]$}
      $T = -\frac{\ln r}{\lambda}$; {$T$ is the time at which the next C_k-reaction occurs}
      if $T < \tau$ **then** {if this reaction happens earlier}
        $\tau = T$; {set time until next reaction}
        $\vec{z} = \vec{c}_k$; {set change vector}
      end if
    end if
  end for
  for $k = 1$ to $m - 1$ **do** {sample time until next D_k-reaction occurs}
    $\lambda = \text{prop}(b_k, \vec{x})$; {compute propensity $\beta_k(\vec{x})$}
    if $\lambda > 0$ **then** {reaction is possible}
      $r = \text{drawRand}$; {draw random number uniformly distributed on $[0, 1]$}
      $T = -\frac{\ln r}{\lambda}$; {$T$ is the time at which the next D_k-reaction occurs}
      if $T < \tau$ **then** {if this reaction happens earlier}
        $\tau = T$; {set time until next reaction}
        $\vec{z} = -\vec{c}_k$; {set change vector}
      end if
    end if
  end for
  $\vec{x} = \vec{x} + \vec{z}$; {update state}
  $t = t + \tau$; {update time}
end while