Stochastic Modelling and Simulation of Coupled Autoregulated Oscillators in a Multicellular Environment: The *her1/her7* Genes

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Abstract. Delays are an important feature in temporal models of genetic regulation due to slow biochemical processes such as transcription and translation. In this paper we show how to model intrinsic noise effects in a delayed setting. As a particular application we apply these ideas to modelling somite segmentation in zebrafish across a number of cells in which two linked oscillatory genes *her1* and *her7* are synchronized via Notch signalling between the cells.

Keywords: delay stochastic simulation algorithm, coupled regulatory systems, multicellular environment, multiscale modelling.

1 Introduction

Temporal models of genetic regulatory networks have to take account of time delays that are associated with transcription, translation and nuclear and cytoplasmic translocations in order to allow for more reliable predictions [1]. An important aspect of modelling biochemical reaction systems is intrinsic noise that is due to the uncertainty of knowing when a reaction occurs and which reaction it is. When modelling intrinsic noise we can identify three modelling regimes. The first regime corresponds to the case where there are small numbers of molecules in the system so that intrinsic noise effects dominate. In this regime the Stochastic Simulation Algorithm (SSA) [2] is the method of choice and it describes the evolution of a discrete nonlinear Markov process representing the number of molecules in the system. The intermediate regime is called the Langevin regime and here the framework for modelling chemical kinetics is that of a system of Itô stochastic differential equations. In this regime the numbers of molecules are such that we can talk about concentrations rather than individual numbers of molecules but the intrinsic noise effects are still significant. The final regime is the deterministic regime where there are large numbers of molecules for each species. This regime is given by the standard chemical kinetic rate equations that are described by ordinary differential equations. In some sense this third regime represents the mean behaviour of the kinetics in the other two regimes. It is vital to model the chemical kinetics of a system in the most appropriate regime otherwise the dynamics may be poorly represented.

In order to take proper account of both intrinsic randomness and time delays, we have developed the delay stochastic simulation algorithm (DSSA) [3]. This algorithm very naturally generalises the SSA in a delayed setting.

Transcriptional and translational time delays are known to drive genetic oscillators. There are many types of molecular clocks that regulate biological processes but apart from circadian clocks [4] these clocks are still relatively poorly characterised. Oscillatory dynamics are also observed for Notch signalling molecules such as *Hes1* and *Her1/Her7*. The *hes1* gene and the two linked genes *her1* and *her7* are known to play key roles as molecular clocks during somite segmentation in mouse and zebrafish, respectively.

In zebrafish the genes *her1* and *her7* are autorepressed by their own gene products and positively regulated by Notch signalling that leads to oscillatory gene expression with a period of about 30 min, generating regular patterns of somites (future segments of the vertebrate) [5]. In both cases the transcriptional and translational delays are responsible for the oscillatory behaviour.

In a recent set of experiments Hirata et al. [6] measured the production of *hes1* mRNA and *Hes1* protein in mouse. They measured a regular two hour cycle with a phase lag of approximately 15 minutes between the oscillatory profiles of mRNA and protein. The oscillations are not dependent on the stimulus but can be induced by exposure to cells expressing delta. This work led to a number of modelling approaches using the framework of Delay Differential Equations (DDEs) [1,7]. However, in a more recent work Barrio et al. used a discrete delay simulation algorithm that took into account intrinsic noise and transcriptional and translational delays to show that the *Hes1* system was robust to intrinsic noise but that the oscillatory dynamics crucially depended on the size of the transcriptional and translational delays.

In a similar setting Lewis [5] and Giudicelli and Lewis [8] have studied the nature of somite segmentation in zebrafish. In zebrafish it is well known that two linked oscillating genes *her1/her7* code for inhibitory gene regulatory proteins that are implicated in the pattern of somites at the tail end of the zebrafish embryo. The genes *her1* and *her7* code for autoinhibitory transcription factors *Her1* and *Her7* (see Fig. 1). The direct autoinhibition causes oscillations in mRNA and protein concentrations with a period determined by the transcriptional and translational delays.

Horikawa et al. [9] have performed a series of experiments in which they investigate the system-level properties of the segmentation clock in zebrafish. Their main conclusion is that the segmentation clock behaves as a coupled oscillator. The key element is the Notch-dependent intercellular communication which itself is regulated by the internal hairy oscillator and whose coupling of neighbouring cells synchronises the oscillations. In one particular experiment they replaced some coupled cells by cells that were out of phase with the remaining cells but showed that at a later stage they still became fully synchronised. Clearly the intercellular coupling plays a crucial role in minimising the effects of noise to maintain coherent oscillations.

Fig. 1. Diagram showing the inter- and intracellular Delta-Notch signalling pathway and the autoinhibition of her1 and her7 genes. DeltaC proteins in the neighboring cells activate the Notch signal within the cell.

Both Lewis and Horikawa have used a stochastic model to understand the above effects. But this model is very different from our approach. The Lewis model for a single cell and two coupled cells is generalised by Horikawa et al. to a one-dimensional array of cells. In both approaches they essentially couple a delay differential equation with noise associated with the uncertainty of proteins binding to the operator sites on the DNA. In our case we are rigorously applying the effects of intrinsic noise, in a delayed setting, at all stages of the chemical kinetics. We also note that this is the first stage in developing a truly multi-scaled approach to understanding the effects of delays in a multi-celled environment. Such a multi-scaled model will require us to couple together delay models in the discrete, stochastic and deterministic regimes - see, for example, attempts to do this in Burrage et al. [10].

Section 2 gives a brief description of our DSSA implementation along with a mathematical description of the coupled Her1/Her7 Delta-Notch system for a linear chain of cells. Section 3 presents the numerical results and the paper concludes with discussion on the significance of our approach.

2 Methods

The SSA describes the evolution of a discrete stochastic chemical kinetic process in a well stirred mixture. Thus assume that there are m reactions between N chemical species, and let $X(t) = (X_1(t), \dots, X_N(t))^T$ be the vector of chemical species where $X_i(t)$ is the number of species i at time t . The chemical kinetics is uniquely characterised by the m stoichiometric vectors $\nu_1, \dots, \nu_m$ and the propensity functions $a_1(X), \dots, a_m(X)$ that represent the unscaled probabilities of the reactions to occur. The underlying idea behind the SSA is that at each time step t a step size θ is determined from an exponential waiting time distribution such that at most one reaction can occur in the time interval $(t, t + \theta)$. If the most likely reaction, as determined from the relative sizes of the propensity functions, is reaction j say, then the state vector is updated as $X(t + \theta) = X(t) + \nu_j$.

Algorithm 1. DSSA

Data: reactions defined by reactant and product vectors, consuming delayed reactions are marked, stoichiometry, reaction rates, initial state $X(0)$, simulation time T , delays

Result: state dynamics

begin

while $t < T$ **do**

generate U_1 and U_2 as $U(0, 1)$ random variables

$a_0(X(t)) = \sum_{j=1}^m a_j(X(t))$

$\theta = \frac{1}{a_0(X(t))} \ln(1/U_1)$

select j such that

$\sum_{k=1}^{j-1} a_k(X(t)) < U_2 a_0(X(t)) \leq \sum_{k=1}^j a_k(X(t))$

if *delayed reactions are scheduled within* $(t, t + \theta]$ **then**

let k *be the delayed reaction scheduled next at time* $t + \tau$

if k *is a consuming delayed reaction* **then**

$X(t + \tau) = X(t) + \nu_k^p$ (update products only)

else

$X(t + \tau) = X(t) + \nu_k$

$t = t + \tau$

else

if j *is not a delayed reaction* **then**

$X(t + \theta) = X(t) + \nu_j$

else

 record time $t + \theta + \tau_j$ for delayed reaction j with delay τ_j

if j *is a consuming delayed reaction* **then**

$X(t + \theta) = X(t) + \nu_j^s$ (update reactants)

$t = t + \theta$

end

In a delayed setting, the SSA loses its Markov property and concurrent events become an issue as non-delayed instantaneous reactions occur while delayed reactions wait to be updated. In our implementation [3] (see Algorithm 1), the DSSA proceeds as the SSA as long as there are no delayed reactions scheduled in the next time step. Otherwise, it ignores the waiting time and the reaction that should be updated beyond the current update point and moves to the scheduled delayed reaction. Furthermore, in order to avoid the possibility of obtaining negative molecular numbers, reactants and products of delayed consuming reactions must be updated separately, namely when the delayed reaction is selected and when it is completed, respectively.

Our model is based on the chemical reaction models of Lewis and Horikawa et al. but our implementation is entirely different as intrinsic noise is represented

Table 1. Model parameters used for DDE and DSSA. Parameter values [5].

Parameter	Description	Rate constant
b_{h1}, b_{h7}	Her1/Her7 protein degradation rate	0.23 min^{-1}
b_d	DeltaC protein degradation rate	0.23 min^{-1}
c_{h1}, c_{h7}	her1/her7 mRNA degradation rate	0.23 min^{-1}
c_d	deltaC mRNA degradation rate	0.23 min^{-1}
a_{h1}, a_{h7}	Her1/Her7 protein synthesis rate (max.)	4.5 min^{-1}
a_d	DeltaC protein synthesis rate (max.)	4.5 min^{-1}
k_{h1}, k_{h7}	her1/her7 mRNA synthesis rate (max.)	33 min^{-1}
k_d	deltaC mRNA synthesis rate (max.)	33 min^{-1}
P_0	critical no. of Her1 + Her7 proteins/cell	40
D_0	critical no. of Delta proteins/cell	1000
τ_{h1m}, τ_{h7m}	time to produce a single her1/her7 mRNA molecule	12.0, 7.1 min
τ_{h1p}, τ_{h7p}	time to produce a single Her1/Her7 protein	2.8, 1.7 min
τ_{dm}	time to produce a single deltaC mRNA molecule	16.0 min
τ_{dp}	time to produce a single DeltaC protein	20.5 min

correctly for each reaction. In the initial state the number of molecules for each species is set to zero.

For the 5-cell model we get 30 different species and a set of 60 reactions. The corresponding rate constants are listed in Table 1. Denote by M_{h1} , M_{h7} , M_d , P_{h1} , P_{h7} and P_d the six species her1 mRNA, her7 mRNA, deltaC mRNA, Her1 protein, Her7 protein and deltaC protein in a particular cell i . For each cell we have 6 (non-delayed) degradations

with reaction rate constants c_{h1} , c_{h7} , c_d , b_{h1} , b_{h7} , and b_d , respectively, and propensities $a_{R_1} = c_{h1}M_{h1}$, $a_{R_2} = c_{h7}M_{h7}$, $a_{R_3} = c_dM_d$, $a_{R_4} = b_{h1}P_{h1}$, $a_{R_5} = b_{h7}P_{h7}$, and $a_{R_6} = b_dP_d$. The three translation reactions with delays τ_{h1p} , τ_{h7p} , and τ_{dp} are

with reaction rate constants a_{h1} , a_{h7} and a_d and propensities $a_{R_7} = a_{h1}M_{h1}$, $a_{R_8} = a_{h7}M_{h7}$, and $a_{R_9} = a_dM_d$. The three regulated transcription reactions with delays τ_{h1m} , τ_{h7m} , and τ_{dm} are

with reaction rate constants k_{h1} , k_{h7} , and k_d and corresponding propensities $a_{R_{10}} = k_{h1}f(P_{h1}, P_{h7}, \tilde{P}_D)$, $a_{R_{11}} = k_{h7}f(P_{h1}, P_{h7}, \tilde{P}_D)$, and $a_{R_{12}} = k_dg(P_{h1}, P_{h7})$. For cells 2 to 4 the Hill function f is defined by

$$f(P_{h1}, P_{h7}, \tilde{P}_D) = r_h \frac{1}{1 + (P_{h1}P_{h7})/P_0^2} + r_{hd} \frac{1}{1 + (P_{h1}P_{h7})/P_0^2} \frac{\tilde{P}_D/D_0}{1 + \tilde{P}_D/D_0}$$

with $\tilde{P}_D = (P_D^{n1} + P_D^{n2})/2$ (the average number of P_D for the two neighboring cells $n1$ and $n2$). The parameters r_h and r_{hd} are weight parameters that determine the balance of internal and external contribution of oscillating molecules. With $r_h + r_{hd} = 1$ the coupling strength r_{hd}/r_h can be defined. In our experiments we set $r_{hd} = 1$, that is, the coupling is 100% combinatorial. In accordance with the Horikawa model we used the Hill functions

$$f(P_{h1}, P_{h7}, P_D) = \frac{1}{1 + (P_{h1}P_{h7})/P_0^2} \frac{P_D/D_0}{1 + P_D/D_0},$$

$$f(P_{h1}, P_{h7}, P_D) = \frac{1}{1 + (P_{h1}P_{h7})/P_0^2} \frac{500/D_0}{1 + 500/D_0}$$

for cell 1 and 5, respectively. The Hill function g is given by $g(P_{h1}, P_{h7}) = \frac{1}{1 + (P_{h1}P_{h7})/P_0^2}$.

The single cell, single-gene model consists only of 2 species (her1 mRNA and Her1 protein) and 4 reactions. The two degradation and the single translation reactions correspond to those in the 5-cell model. For the inhibitory regulation of transcription we assume a Hill function with Hill coefficient 2 (P_{h1} acts as a dimer). The Hill function takes the form $f(P_{h1}) = 1/(1 + (P_{h1}/P_0)^2)$.

3 Results and Discussion

In this section we present individual simulations of a system of 5 coupled cells, so that the dimension of the system is 30, in both the DSSA and DDE cases.

Figure 2 (a,b) shows the dynamics for a single cell. In the DDE case after an initial overshoot, the amplitudes are completely regular and the oscillatory period is approximately 40 minutes. In the intrinsic noise case there are still sustained oscillations but there is some irregularity in the profiles and the oscillatory period is closer to 50 minutes. The time lag (5-7 min) between protein and mRNA is about the same in both cases. In Fig. 2 (c,d) we present DSSA simulations of the 5 coupled cells and give the profiles for mRNA and protein at deltaC and her1 for cell 3. Now the period of oscillation is closer to 45 minutes and the lag between protein and mRNA is about 25 minutes for deltaC and about 7 minutes for her1. Thus we see that the coupling has some effect on the period of oscillation. In Fig. 3 we mimic an experiment by Horikawa et al. In both the DDE and the DSSA setting we disturb cell 3 after a certain time period (500 minutes in the DSSA case and 260 minutes in the DDE case). This is done by resetting all the values for cell 3 to zero at this point. This is meant to represent the experiment of Horikawa et al. in which some of the cells are replaced by oscillating cells that are out of phase. They then observed that nearly all the cells become resynchronized after three oscillations (90 min.). Interestingly, in the DDE setting it only takes about 60 minutes for the onset of resynchronization while in the DSSA setting it takes about 180 minutes. The difference can be partly due to the larger number of cells that are experimentally transplanted.

Fig. 2. (a) DDE solution and (b) single DSSA run for the Her1/Her7 single cell model. (c,d) DSSA simulation of five Delta-Notch coupled cells, showing the dynamics of deltaC mRNA and protein and her1 mRNA and protein in cell three.

Fig. 3. DSSA simulation result and DDE solution for the 5-cell array in the non-disturbed and disturbed setting. The graphs show the dynamics of deltaC and her1 mRNA in cell three. (a,c) DSSA and DDE results in the non-disturbed setting, respectively. (b,d) DSSA and DDE results in the disturbed setting. Initial conditions for cell 3 are set to zero. All other initial molecular numbers stem from the non-disturbed DSSA and DDE results in (a,c) after 500 and 260 minutes, respectively.

4 Conclusions

In this paper we have simulated Delta-Notch coupled *her1/her7* oscillators for 5 cells in both the deterministic (DDE) and delayed, intrinsic noise setting (DSSA). We have shown that there are some similarities between the dynamics of both but the intrinsic noise simulations do make some predictions that are different to the deterministic model (see Fig. 3) that can be verified experimentally. Thus it is important that both intrinsic noise delayed models and continuous deterministic delay models are simulated whenever insights into genetic regulation are being gleaned. However, since the time steps in the DSSA setting can be very small, there are considerable computational overheads in modelling even a chain of 5 cells. In fact, one simulation takes about 90 minutes on a Pentium 4 PC (3.06 GHz) using MatLab 7.2. If we wish to extend these ideas to large cellular systems then we need new multiscale algorithms which will still model intrinsic noise in a delayed setting but will overcome the issues of small stepsizes. This has been considered in the non-delay case by for example Tian and Burrage [11] through their use of τ -leap methods, and similar ideas are needed in the delay setting. This is the subject of further work, along with considerations on how to combine spatial and temporal aspects when dealing with the lack of homogeneity within a cell.

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