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# Dynamics of stochastic gene rings

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## Abstract

The dynamics of gene rings are investigated in-silico using a recent gene regulatory network modeling scheme and a stochastic simulation algorithm. The modeling strategy was validated by obtaining known dynamics, such as limit cycle oscillations and bistability, in odd and even number gene rings respectively. An overview of deterministic and stochastic approaches in biochemical network modeling is provided. Low toggling rates between bistable states in even gene rings as coupling strength varied revealed that this network topology is robust to perturbations. Limit cycle, single steady state, and multiple steady state dynamics were observed in coupled gene rings. The biological relevance of these results is discussed.

## Introduction

A fundamental problem in the study of biological systems is to uncover the structure and logic of gene regulatory networks (GRNs) among thousands of genes and their RNA and protein products[1,2]. Stem cells and regenerative medicine, along with novel approaches to cancer differentiation therapy, are possible medical implications of this line of research[3,4,5].

One relevant network topology for investigating the dynamics of GRNs is a cyclic topology. Many biologically relevant phenomenons, including oscillations in gene expression levels or protein concentrations, can be modeled by the presence of at least one negative feedback loop in the regulatory network. Furthermore, rings with odd numbers of genes, where each gene represses their successor in the cycle, can be viewed as the source of non-trivial dynamical behavior in these networks, since without loops only a unique fixed point can be reached[6]. Processes that are assumed to be bistable, such as decision circuits in cellular differentiation[7], can also be modeled using rings of repression with an even number of genes. Modeling GRNs with a ring topology is therefore a useful approach for developing a deeper understanding of the dynamics generated by certain networks.

Recently, gene expression has been shown to be a stochastic process, as genes generally exist in low copy numbers in a cell[8,9,10]. Therefore, in order to model gene expression correctly, one has to use a realistic model where the dynamics of the system are driven by a stochastic simulation algorithm (SSA)[11,12].

This study will focus on ring networks, coupled by direct repression, and simulated using a SSA. Specifically, the resulting dynamics of coupling stochastic gene rings, as well as perturbations to bistable rings, will be investigated with an emphasis on the relevance to biotic systems.

## Background

Modeling biochemical networks requires a system of equations. Often, a deterministic approach in which a set of ordinary differential equations (ODEs) is used, where if there are N chemically active molecular species present, then the set will contain N differential equations[12]. Reaction constants here are reaction rates and molecular species concentrations are represented by continuous single valued functions of time. In this case, each equation accounts for the time rate of change in concentrations of one species (e.g. a particular mRNA or protein) as a function of the concentrations of all the species in the reaction network[12]. Models that use this approach are known as "rate equation models" and are appropriate for systems with large numbers of molecules where fluctuations are negligible[13].

However in GRNs, genes, mRNAs, and proteins often exist in low copy numbers and the fluctuations are non-negligible. To appropriately account for fluctuations in systems such as these, stochastic approaches, such as simulations of the chemical master equation (i.e. a single differential difference equation) must be used[13]. Here, reaction constants are taken as probabilities per unit time. In this stochastic formulation of chemical kinetics, which is has now also been shown to be necessary in the analysis of GRNs under conditions of high concentrations (e.g. when active proteins appear in high numbers)[14], the time evolution of the system is described by the chemical master equation[12]. This master equation measures the probability of finding various molecular populations at each instant of time and usually, if more than a few chemical species are present, the Markov process that it describes is numerically simulated via Monte Carlo techniques (rather than solved analytically) to obtain the dynamics of the system[12].

# Modelling strategy

Periodic boundary conditions are assumed, which here means that the proteins of gene $i$ repress gene $i + 1$ by direct binding. Each gene in the ring is represented by the following set of equations[11]:

$$RNAp + \Pr o_i \xrightarrow{k_i} \Pr o_i + RNAp + p_i \quad (1)$$

$$\Pr o_{i+1} + p_i \xrightarrow{k_{i+1,i}} \Pr o_{i+1} p_i \quad (2)$$

$$\Pr o_{i+1} p_i \xrightarrow{k_{i,i+1}} \Pr o_{i+1} + p_i \quad (3)$$

$$\Pr o_i p_i \xrightarrow{k_{d,i}} \Pr o_i \quad (4)$$

$$p_i \xrightarrow{k_{d,i}} 0 \quad (5)$$

The reactant side of Equation (1) is describing the binding of an RNA polymerase to the promoter region of the DNA of the gene in question. On the product side, the RNA polymerase is unbound from the promoter, and a protein is produced. Modeling these multiple events in a single reaction step reduces the computational time of the simulations.

The binding and unbinding of the repression protein from gene i to the promoter of gene $i + 1$, is described by Equations (2 and 3), respectively.

Equation (4) is required to allow the protein to decay while still bound to the promoter at the same rate as when not bound. Without this reaction, when a protein is bound to a promoter it would not decay, and therefore would serve as a "protection" against decay.

The final reaction, Equation (5), simply describes the decay of a protein due to use in some biological processes, or the unavailability of the protein due transport outside of the region containing the GRN under investigation.

For simplicity, only the symmetric case where each gene is identical (i.e. in rates constants, number of promoters, etc.) to the other genes in the loop is considered.

The dynamics of this model are simulated using SGN Sim[15], which here stochastically simulates Equations (1-5) of the rings for the number of genes in question.

1000 simulations were conducted for each case on a Pentium 4, at 1.73 GHZ with 1 GB of RAM for 5000 s of simulation time, and unless otherwise indicated, with the following parameters: $ki$ = 0.1 $s^{-1}$, $k_{i+1,i}$ = 10 $s^{-1}$, $k_{i,i+1}$ = 0.001 $s^{-1}$, $k_{d,i}$ = 0.1 $s^{-1}$.

Availability: All rings/reaction .g files are available at: http://www.ucalgary.ca/~dacharle/researchdocs.htm. SGN Sim is freely available for download[15].

## Coupling Strength

From the set of reactions in Equations (1-5) which describe the ring systems studied here, there is one reaction which is responsible for binding of the repressor protein, Equation (2) (with rate constant $k_{i+1,i}$), and another, Equation (3) (with rate constant $k_{i,i+1}$), for unbinding. The quantity, coupling strength (CS), is defined as a ratio of these two rate constants16:

$$CS = k_{i+1,i} / k_{i,i+1} \quad (6)$$

Coupling strength provides an approximation of the fraction of the time that a gene is expected to be repressed by the binding of a protein resulting from the expression of a previous gene in the cycle. The higher the coupling strength, the more coupled the genes are since their dynamics are more interdependent.

# Results and discussion

In rings with odd numbers of genes, limit cycle oscillations are obtained (Figure 1). However, in rings with even numbers of genes, steady states emerge in which either odd or even genes are expressed and the others repressed (Figure 2). The above results were previously obtained using a system of differential equations[17], as well as a stochastic approach[13], and are here matched qualitatively in order to validate the modeling strategy and parameters used in this study.

Rings with even numbers of genes were subject to a perturbation of 1000 proteins of gene 2 introduced into the system at 3000 s of simulation time. This perturbation resulted in toggling between two possible steady states (Figure 3) in a percentage of the runs for each case examined (Table 1). Below a CS of 10, the system began to converge to a single steady state

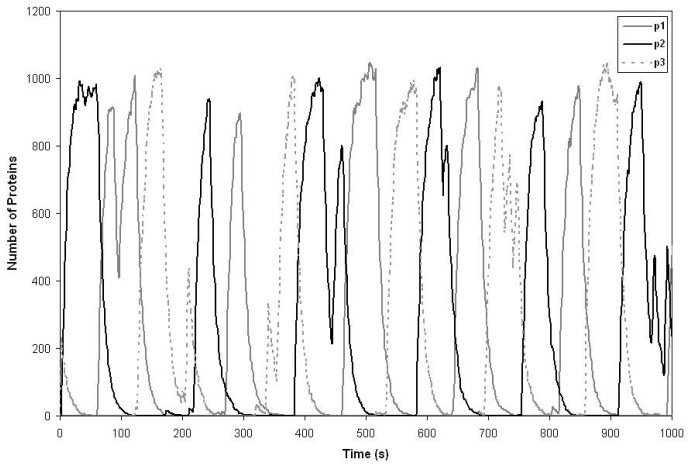


**Figure 1** Limit cycle oscillations in a 3 gene ring. As the proteins (p2) from the expression of gene 2 build up in the system, they repress the p3 expression of gene 3, which subsequently allows for p1 expression of gene 1, and so forth.

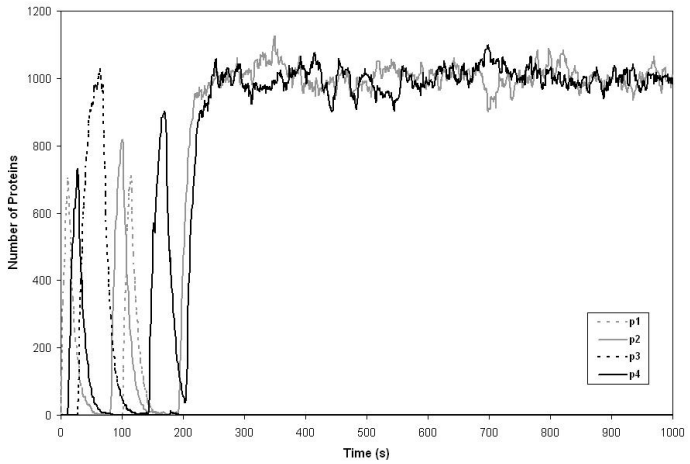


**Figure 2** Even gene steady state in a 4 gene ring. Protein expression (p1 and p3) of the odd genes is completely repressed by expression (p2 and p4) of the even genes. Once in the steady state (at approximately 200 s), the system remains in this state for the duration of the simulation.

where all genes were being expressed at around the same level and were not affected by systematic perturbations, and in trials above a CS of 1000, the system toggled in less than 10% of runs (data not shown). Here, toggling due to the perturbation was only considered for the odd gene steady state because the perturbation chosen adds additional proteins associated with gene 2, whose protein products repress gene 3. This perturbation results in strengthening the even genes' repression of the odd genes and therefore toggling never occurred from an even to odd gene expression state. Because of the this, the percentage of toggles for a given CS for an even gene ring would be half the values shown in Table 1 if both states were considered.

Coupling of bistable gene rings (Figure 4) yielded the following dynamics: coupling of 2 odd gene rings resulted in limit cycle oscillations, even-odd coupling in a single steady state, and even-even coupling in multiple steady states. For all runs involving coupled odd gene rings, limit cycle oscillations with the following pattern resulted: genes 2 and 4 "up" (i.e. high protein expression levels) and the others repressed, followed by gene 1 up, followed by genes 3 and 5 up. In all simulations for the even-odd coupled gene rings, a single steady state in which genes 2,4,6, are highly expressed, was obtained. In the final case, involving even-even coupling, the system was observed to reach one of 3 steady states (Figure 5) after a small transient, where the following genes were highly expressed (and the others repressed): genes 1 and 3 (96% of runs), genes 2, 5, and 7 (3% of runs), and genes 2,4, and 5 (1% of runs). Note that in 7% of instances when the system was in the genes 1 and 3 up state, gene 6 was expressed for roughly the first 1000 s and then was abruptly repressed to a zero level of expression. Since the expression of gene 6 was always associated with the gene 1 and 3 up state, where these genes continued to be expressed even after gene 6 was repressed, it was not considered to be a unique steady state of the system.

# Conclusions

Studying GRNs in a cyclic topology where the system's dynamics are stochastically simulated is a useful approach for investigating genetically controlled behavior.

In the case where rings with an even number of genes were subject to systematic perturbations for a wide range of CSs, it was found that these bistable rings of repression only toggled from one steady to another in 3% to 15% (as a function of CS) of simulations. This result lends support to the

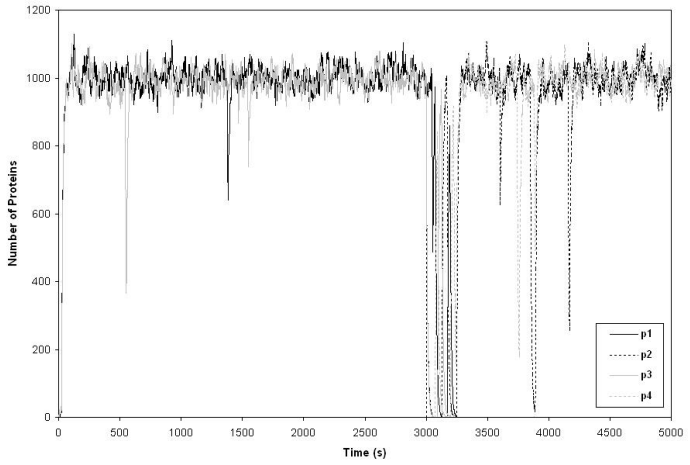


**Figure 3** A perturbation of 1000 proteins (p2) introduced at 3000 s results in toggling of the steady state in a 4 gene ring. The system starts off in the odd steady state (i.e. odd genes expressing and even genes repressed) but toggles to the even steady state as a result of the perturbation.

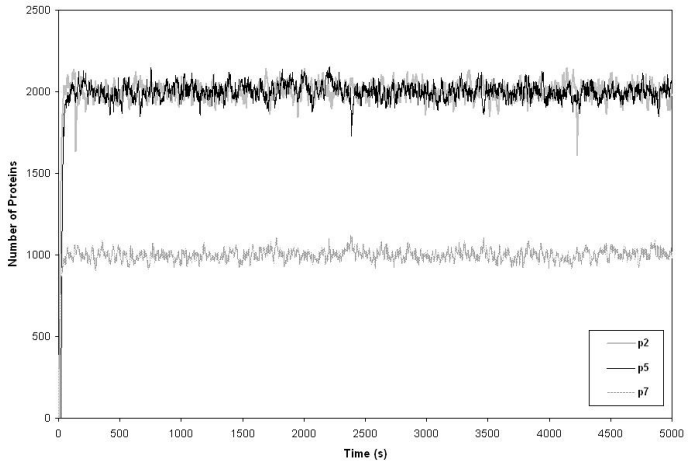


**Figure 5** One of 3 possible steady states of an even-even coupled gene ring. In this steady state, genes 2 and 5 are expressing at twice the level of the gene 7, and the other genes are completely repressed.

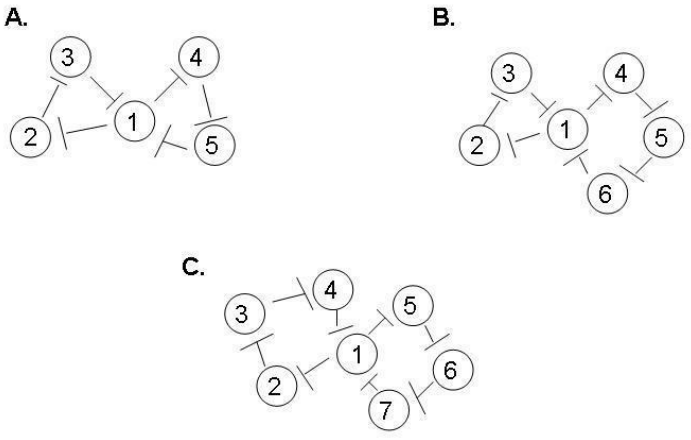


**Figure 4** Coupling diagrams of a) odd-odd gene rings, b) odd-even gene rings, and c) even-even gene rings. The flat-headed arrows denote negative regulation resulting from the binding of a protein from the expression of the previous ring in the cycle.

| Coupling Strength | Toggling (%) |
|---|---|
| 10 | 15 |
| 15 | 6 |
| 20 | 14 |
| 35 | 3 |
| 50 | 3 |
| 100 | 4 |
| 1000 | 7 |

**Table 1** Toggling between steady state percentage in an even gene ring of repression as a function of coupling strength. 1000 simulations were conducted for each case.

hypothesis that bistable genetic subcircuits that control downstream genes are stable, and therefore robust to noise and perturbations, allowing them to act as cellular memory unit once a "decision" (e.g. which path a stem cell will follow into a distinct cell type) is made[10]. Note that these results were obtained within an appropriate context, as the stochasticity in the initial decision, and the biochemical dynamics of the system afterwards, were accounted for by the SSA.

Uncovering the dynamics resulting from the coupling of genetic oscillators is important for understanding rhythmic phenomenon in living organisms and has many potential applications in bioengineering[18]. The results for coupled gene rings in this study show that distinct dynamics, such as limit cycle oscillations, monostability, and tristability, can result from the coupling of GRNs (which could occur, for example, in cell-cell communication where signaling molecules produced from GRNs are able to move between neighboring cells via gap junctions or bind directly to a surface cell receptor (as occurs in the yeast *Saccharomyces cerevisiae* when some cells send a peptide signal in order to induce other members of the population to prepare for mating[19]) and is another step towards understanding more complex GRN dynamics.

Directions for future work involve investigating the effects of cross links within gene rings as well as research focused around system transient times; other relevant topologies for investigating GRN dynamics are also being considered.

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# Glossary

**Gap junction**. A channel present in some cell types which allows ions and small molecules to flow between adjacent cells.

**Promoter**. A DNA sequence to which an RNA polymerase molecule initially binds during the initiation steps of transcription.

**Transcription**. The biosynthesis of an RNA copy of a DNA template strand catalyzed by RNA polymerase.

**Translation**. The biosynthesis of a protein from messenger RNA catalyzed by ribosomes.

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