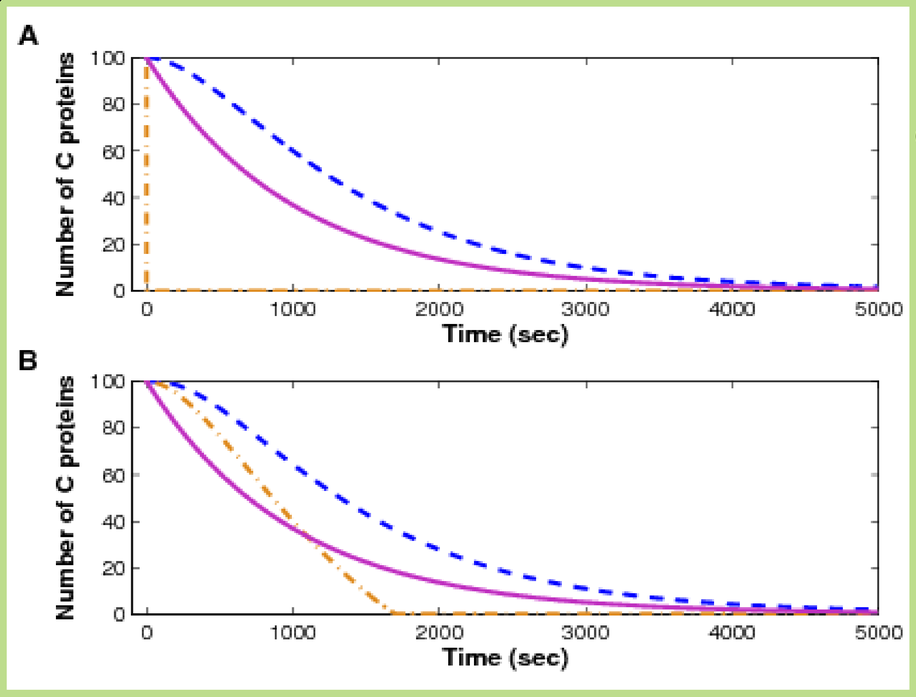
# Role of Small non-Coding RNA in Genetic Regulation Networks

Yishai Shimoni[1], Gilgi Friedlander[2], Guy Hetzroni[1], Gali Niv[2], Shoshy Altuvia[2], Ofer Biham[1], and Hanah Margalit[2]

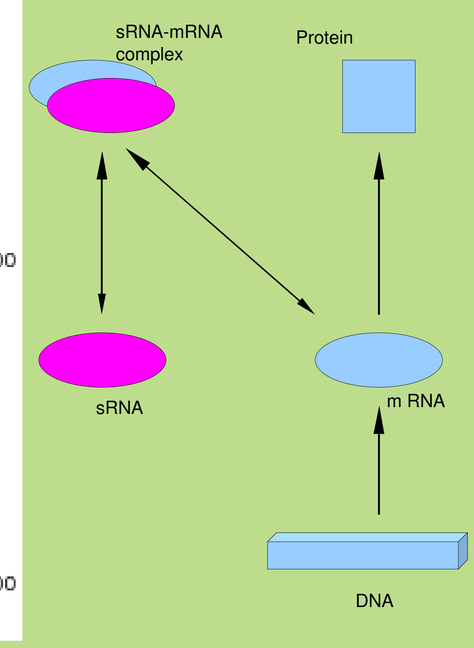
Racah Institute of Physics[1] and Faculty of Medicine[2], the Hebrew University of Jerusalem

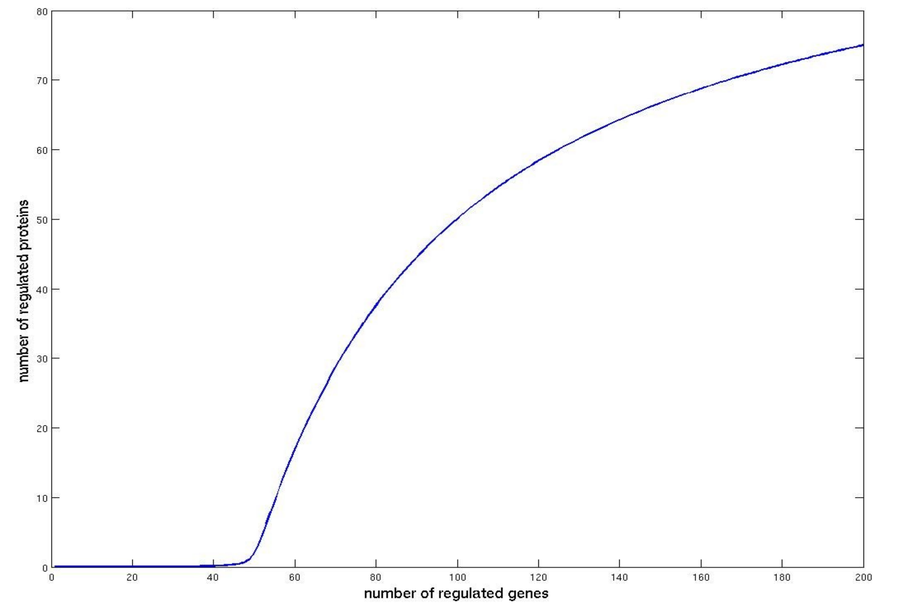
We show quantitatively that regulation by small RNA (sRNA) is advantageous when fast responses to external signals are needed, which is consistent with experimental data about its involvement in stress responses. We integrate the network of sRNA regulation in *E. coli* with the transcription regulation network, uncovering mixed regulatory circuits consisting of both transcriptional and post-transcriptional regulations. Analysis of one such regulatory circuit, a feed-forward loop of OmpR-MicF-*ompF*, demonstrates its advantages: tight repression, guaranteed by the combination of transcriptional and post-transcriptional regulations, and fast recovery upon the end of the external signal. Another regulatory circuit is the genetic mixed feedback loop, where gene *a* regulates gene *b* by transcriptional regulation, while gene *b* regulates gene *a* by either protein-protein interaction or small non-coding RNA-mRNA interaction. Mixed feedback loops tend to exhibit bi-stability or oscillations. These loops are analysed using deterministic and stochastic methods, shedding more light on the possible roles of sRNA regulation.

## Single Gene Regulation




Repression of a single gene. Shown is the copy number of a regulated protein C vs. time after applying negative regulation on gene *c* using transcriptional regulation (dashed line), post-translation regulation by protein-protein interaction (dashed-dotted line), and post-transcriptional regulation by sRNA (solid line). (A) In the initial state the products of both the regulating and the target genes are present in the cell. In this case the post-translation regulation by protein-protein interaction provides much faster response than the two other mechanisms. (B) In the initial state the protein product of the target gene is present but the regulator is not. In this case the response achieved by post-transcriptional regulation by sRNA is initially faster but eventually slower than that achieved by protein-protein interaction.



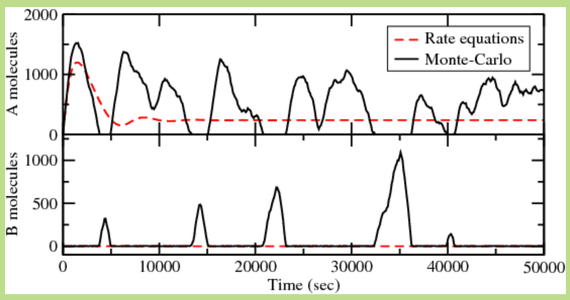

One sRNA gene may be responsible for the regulation of many genes. Shown is the copy number of each of the *n* protein types regulated by a single sRNA-producing gene vs. *n*. Here, the production of the sRNA is 50 times faster than that of each of the target mRNAs. In this case, as long as $n<50$, the regulation is effective. It gradually weakens as *n* exceeds 50, and the copy number of each of the target proteins increases.

## Deterministic Vs. Stochastic Simulation

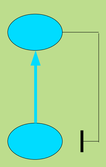
### Example: positive-negative mixed feedback loop

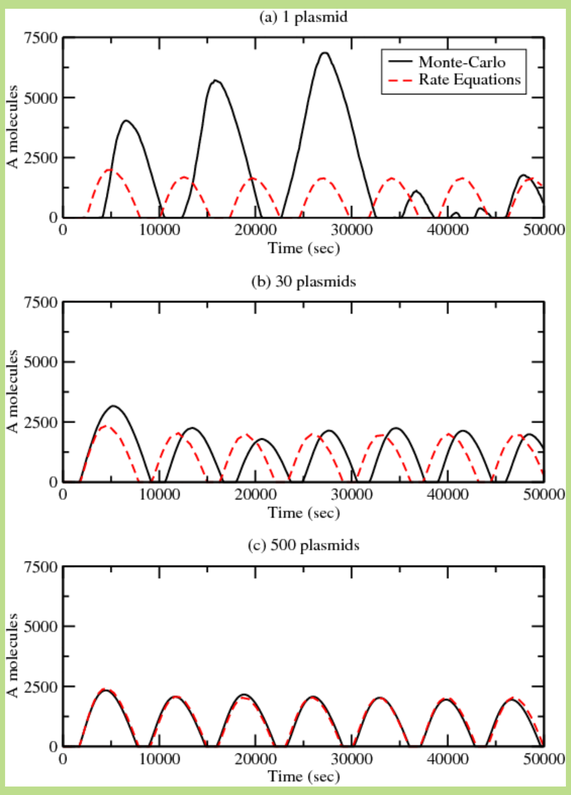
Rate equations represent the average concentration of different molecular species, but disregard the stochastic nature of the systems they represent, as well as the fact that the number of copies of any species is discrete. In order to take these properties into account, we apply two methods. The first is to use master equations, which are equations representing the time dependant probability of having a specific copy number for each species. The second method applied is Monte-Carlo simulations. This is an iterative simulation method, in which the state of the system is modelled as a function of time.

The copy number of *A* molecules in a positive-negative protein-protein mixed feedback loop, comparing between rate equation simulation (dashed) and Monte-Carlo simulation (solid) in the parameter range where rate equations give decaying oscillations, and Monte-Carlo simulations give irregular oscillations.
In this parameter range the disagreement between the two models is most pronounced.

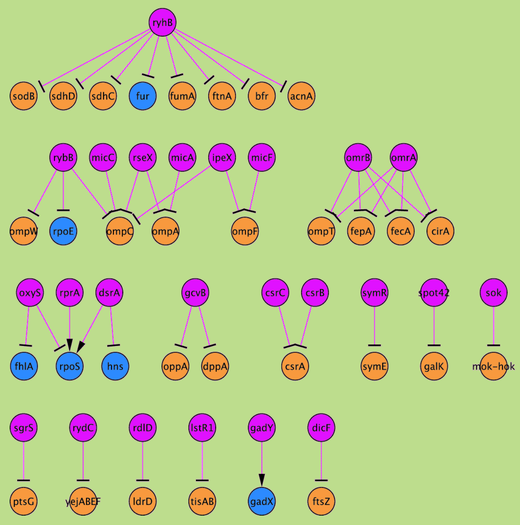



Oscillations in s stochastic system do not have a specific period and amplitude. In order to test the agreement between the stochastic and the deterministic models, we wish to find a limit in which both models give the same results.
This limit exists when all the species in the system appear in large numbers in any given time, and the stochastic nature of the system becomes less relevant. A convenient way to achieve this limit is to assume that the genetic circuit discussed is not present solely on the chromosomal DNA, but on many plasmids. This assumption causes a slight change in both models, which boils down to a change in the rate constants.
In the figure on the right the copy number of *A* molecules in a positive-negative protein-protein mixed feedback loop is displayed, comparing between rate equation simulation (dashed) and Monte-Carlo simulation (solid) in the parameter range where oscillations appear
(a) with a single plasmid;
(b) with 30 plasmids;
(c) with 500 plasmids.
It can be seen that in the limit of many plasmids the systems agree.

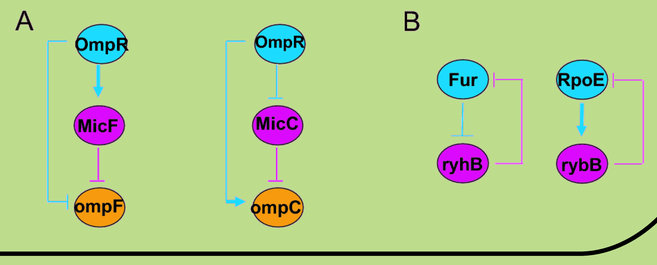





## Network Modules Involving sRNA Regulation

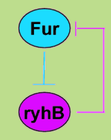


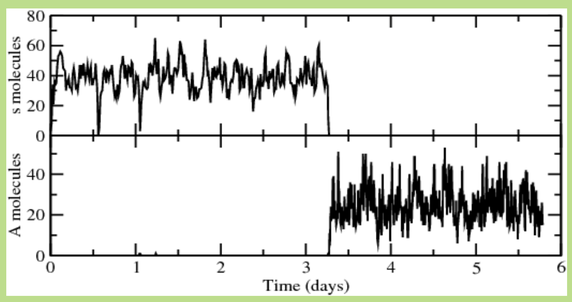

On the left, the sRNA-target network is shown. Nodes represent sRNAs and their experimentally proven targets (see Supplementary Material for references). sRNAs are in pink circles, protein coding genes in orange circles and genes coding transcriptional regulators in blue circles. Arrows represent activation while truncated arrows represent inhibition.
Below, examples of interesting mixed regulatory circuits extracted from the complete network, involving transcriptional regulation and post-transcriptional regulation by sRNA.
(A) Shows a feed-forward loop. Under high osmolarity, OmpR activates transcription of the sRNA gene *micF*, which represses the translation of the porin coding gene *ompF*. OmpR also inhibit directly the transcription of *ompF* (left). Under the same conditions, OmpR represses transcription of the sRNA gene *micC*, which inhibits the translation of the porin coding gene *ompC*. OmpR also activates directly the transcription of *ompC* (right).
(B) Presents two mixed feedback loops. The transcription factor Fur inhibits transcription of the sRNA gene *rhyB*, which in turn inhibits Fur's translation (left). RpoE activates transcription of the sRNA gene *rybB*, which in turn represses RpoE synthesis (right).

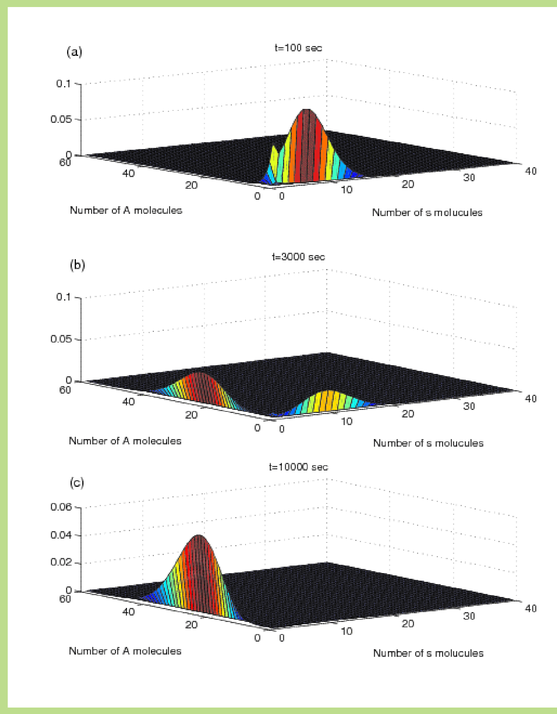



## Stochastic Timer

Monte-Carlo (bottom) and master equation (right) simulation of a double negative sRNA interaction mixed feedback loop. In the parameter range shown here, the system acts as a stochastic switch: At first, the number of *s* molecules (a below) is seen to fluctuate around some meta-stable state, while the number of *A* molecules (b below) remains close to zero. After a random amount of time - in this case after three days - the system shifts to a state where the number of *A* molecules fluctuates around a stable value, while the number of *s* molecules remains close to zero. On the right the joint probability distribution of sRNA molecules and protein molecules for a double negative sRNA MFL is displayed, at times of 100 seconds (a), 3000 seconds (b), and 10000 seconds (c). The initial state is a system with no sRNA molecules and no proteins.
In systems in which three days are more than the lifetime expectancy of a single cell, or more than the time between cell division this is effectively a switch.




The Hebrew University of Jerusalem