Robustness and Evolvability of Multilayer Gene Regulatory Networks

Hyobin Kim and Hiroki Sayama

Department of Systems Science and Industrial Engineering
Center for Collective Dynamics of Complex Systems
Binghamton University, State University of New York, Binghamton, NY, USA
hkim240@binghamton.edu

Abstract

We present multilayer gene regulatory networks (GRNs) consisting of an intercellular layer and an intracellular layer. A network in an intercellular layer represents interactions between cells, and a network in an intracellular layer indicates interactions between genes. All the nodes of an intercellular network have identical random Boolean networks (RBNs) as intracellular GRNs. We introduce genetic perturbations (e.g., mutations) to the intracellular GRNs. Varying the properties of the intracellular GRNs from ordered, through critical, to chaotic regimes, we investigate how criticality of GRNs affects the robustness and evolvability of multilayer GRNs against the genetic perturbations. We found that the robust and evolvable multilayer GRNs were generated with the highest probability when intracellular GRNs were critical. Based on our findings, we conclude that the criticality of GRNs plays an important role in determining the robustness and evolvability of multilayer GRNs at a hierarchical level.

A few studies have been performed to elucidate relationship between properties of intracellular GRNs and properties of organisms at a multicellular level (Villani et al., 2006; Flann et al., 2013). We also developed a morphogenetic model to reveal how criticality of intracellular GRNs has an influence on pattern formation of multicellular organisms (Kim and Sayama, 2018). However, because our model was a particle-based model where cells continued to move by spring-mass-damper kinetics, it was not easy to assess the robustness and evolvability of the whole system. Here we present a more formal hierarchical network model and investigate how the criticality of GRNs affects the robustness and evolvability of the whole system at a hierarchical level.

We present multilayer GRNs consisting of an intercellular layer and an intracellular layer. A network in an intercellular layer represents interactions between cells, and a network in an intracellular layer represents interactions between genes (Fig. 1). All the nodes of an intercellular network have identical RBNs as intracellular GRNs, where a RBN has a random network topology and randomly assigned Boolean functions to each node. Our multilayer GRNs have cellular topologies that are randomly changed in each simulation run. The multilayer GRNs with such dynamic cellular topologies are modeled as a developing embryo based on biological evidence showing that the intercellular network topology of a developing embryo is constantly rearranged by cellular movements and cell growth (Jackson et al., 2017).

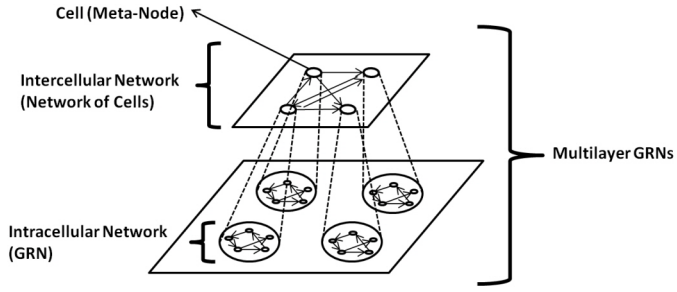

Figure 1: A schematic diagram showing example multilayer GRNs. (In actual simulations, the number of nodes of an intercellular network was 9, and the number of nodes of an intracellular GRN was 6.)

We generate multilayer GRNs that have ordered ($K = 1$), critical ($K = 2$), and chaotic ($K = 3$) intracellular GRNs by adjusting node in-degree (K) (Kauffman, 1969). The dynamics of multilayer GRNs as the whole system at a hierarchical level are determined by the dynamics of intracellular GRNs (the input nodes of each gene and the assigned Boolean functions to the genes) and the topology of the intercellular network (the neighboring cells for the interactions between cells). In our multilayer GRNs, cells interact with each other through cell signaling, which follows the cell-cell interactions of Villani et al.'s coupled RBN model (Villani et al., 2006). In an intracellular GRN, a certain gene is assigned to communicate with neighboring cells. This gene is called communicating gene. The communicating gene is activated if any of the communicating genes of neighboring cells is activated. The states of the other genes except for the communicating gene are updated by the input nodes of each gene and randomly assigned Boolean functions to the genes in the intracellular GRN.

We introduce genetic perturbations (e.g., mutations) to the intracellular GRNs. We perturb an intracellular GRN in one

of nine cells by adding, deleting, or switching one regulatory link between a pair of genes. We assess the robustness and evolvability of both intracellular GRNs at a single cell level and multilayer GRNs at a hierarchical level. If the mutated intracellular GRN and the multilayer GRNs containing the mutated intracellular GRN conserve existing attractors and simultaneously create new attractors after the genetic perturbation, they are considered as *robust and evolvable intracellular GRN/multilayer GRNs* (Aldana et al., 2007).

When finding the attractors of the mutated intracellular GRN, we explored all the state space of the GRN (state space size = 2^6). Meanwhile, in the case of multilayer GRNs, we focused on the attractors with the largest basins of attraction to keep computational loads reasonable. Because the state space size of our multilayer GRNs is 2^{54} (= $2^{9\times6}$), it is not feasible to explore all the state space. Thus, we used 10,000 randomly chosen initial states to find the attractors with the largest basins. The number of the initial states was determined based on studies identifying the attractors of large-scale Boolean networks (Aldana et al., 2007).

We performed 1,000 independent simulation runs for each value of K (from 1 to 3), in which the topology of an intercellular network was randomly determined based on the number of links randomly chosen between 1 and 81. Investigating the attractors of the intracellular GRNs and the multilayer GRNs against the genetic perturbations, we obtained proportions of robust and evolvable intracellular GRN and multilayer GRNs (Fig. 2). We found that the critical intracellular GRNs yielded maximum robustness and evolvability at a single cell and hierarchical level.

Fig. 3 shows how the robustness and evolvability of multilayer GRNs composed of critical intracellular GRNs are varied depending on the number of links of an intercellular network. For 1,000 independent simulation runs, we measured the robustness and evolvability of multilayer GRNs, increasing the number of links of an intercellular network from 10 to 80 by 10. As the number of links of an intercellular network grew, the proportions did not monotonically increase or decrease but fluctuated. In addition, when compared to $K = 1$ and $K = 3$, the robust and evolvable multilayer GRNs were produced with the higher probability at $K = 2$. Especially, the value reached the maximum when the number of links of an intercellular network was around 40 (e.g., link density ≈ 0.5). It means that the degree of interactions between cells can maximize the generation of robust and evolvable multilayer GRNs by amplifying the effect of the criticality of GRNs. Based on the findings, we conclude that the criticality of GRNs plays an important role in determining the robustness and evolvability of multilayer GRNs at a hierarchical level.

This study has a limitation. Using RBNs as GRNs, we examined the robustness and evolvability of multilayer GRNs. To obtain findings more relevant to real biological systems, we are planning to use empirically obtained biological Boolean networks such as a segment polarity Boolean network in a *Drosophila melanogaster* embryo.

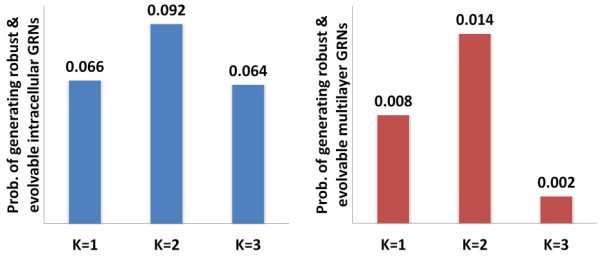

Figure 2: Robustness and evolvability at a single cell and hierarchical level. The blue graphs represent intracellular GRNs and red ones represent multilayer GRNs. (Pearson's chi-squared test: *p-value* < 0.05)

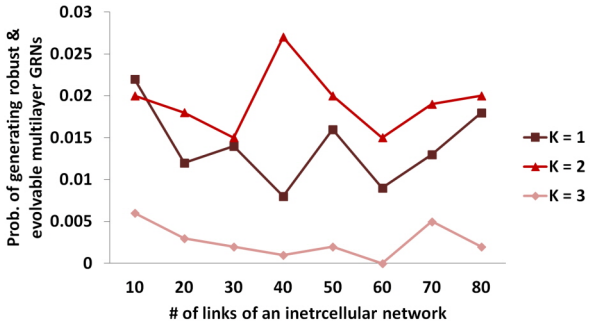

Figure 3: Robustness and evolvability of multilayer GRNs depending on the number of links of an intercellular network. (Pearson's chi-squared test: *p-value* < 0.05)

Acknowledgements

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