An alternative route to robustness: The relationship between assortativity, in-components, and characteristic path length in gene regulatory networks

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Abstract

Gene regulatory networks (GRNs) comprise the interacting genes and gene products that drive genetic regulation within the cell. Because of the vital role they play in producing cell function, GRNs are robust to a variety of perturbations, including genetic mutation. There are multiple underlying causes for this robustness, including topological properties of GRNs, such as their degree distribution. Another topological property, assortativity, has recently been attributed to the robustness of GRNs. Assortative GRNs were found to have smaller in-components (ICs) than their disassortative counterparts, and this led to increased robustness to multiple types of genetic mutation. However, some assortative GRNs lacked the distinctive small ICs, yet were still robust. This suggests that assortativity affects robustness via multiple mechanisms, and unraveling these is a necessary step for understanding which specific features of GRNs give rise to their robustness. Here, we uncover a separate route by which assortativity affects robustness, whereby assortativity influences the characteristic path length of the GRN, which in turn alters robustness.

Introduction

Gene expression produces the complex machinery necessary for cellular life, and its regulation is a crucial means by which cells can assume specific functions. For example, the regulation of gene expression enables cells to respond to different environments (Gasch et al., 2000; Causton et al., 2001) and navigate diverse paths of differentiation to produce distinct cell fates (Davidson, 2006; Huang et al., 2005).

One of the cell's implicit constructs that accomplishes this regulation is its network of gene-gene interactions, in which the product of one gene directly influences the expression of another gene, as happens with transcription factors. This network is referred to as a gene regulatory network (GRN). Because GRNs are often involved in critical biological functions, it is important that they are robust to genetic perturbation, such as gene knock-out (Jeong et al., 2001) or the rewiring of regulatory interactions (Isalan et al., 2008).

Several theoretical studies have attempted to elucidate the source of this robustness, and one source appears to be GRN topology. For example, GRNs that possess heavy-tailed degree distributions were shown to be more robust than those that have other degree distributions (Aldana and Cluzel, 2003). This observation has been supported by empirical findings that suggest real-world GRNs have heavy-tailed degree distributions (Babu et al., 2004).

Assortativity is another topological property that has been shown to vary in real-world networks (Newman, 2002; Foster et al., 2010). The assortativity of a GRN measures the tendency for genes to interact with other similar genes. One measure of assortativity considers whether pairs of interacting genes have similar numbers of connections in the GRN, and this is referred to as degree assortativity. Recently, we presented theoretical work which showed that the increased degree assortativity of a GRN produces increased robustness to a variety of genetic perturbations, including point mutations (Pechenick et al., 2012) and gene birth (Pechenick et al., 2013). This occurs via a reduction in the average size of the in-components (ICs) of a GRN. An IC of a gene is the set of all other genes in the GRN which can directly or indirectly influence that gene's expression. We observed that IC sizes shrink with increasing assortativity, and showed how this explains the increased robustness (Pechenick et al., 2012). However, this mechanism did not explain all the observed changes to robustness, as some GRNs displayed increased robustness with increased assortativity but did not exhibit corresponding changes to their IC sizes. This observation invites further inquiry: What is the alternative route by which increased assortativity leads to increased robustness?

In this study, we uncover an additional mechanism whereby assortativity influences the robustness of GRNs. We first show that IC sizes do not always shrink with increasing assortativity, and that the GRNs with unaffected ICs are still more robust than their less assortative counterparts. We then show that while ICs are unchanged in these GRNs, their characteristic path length increases with increasing assortativity. Finally, we demonstrate that characteristic path length generally affects the robustness of GRNs.

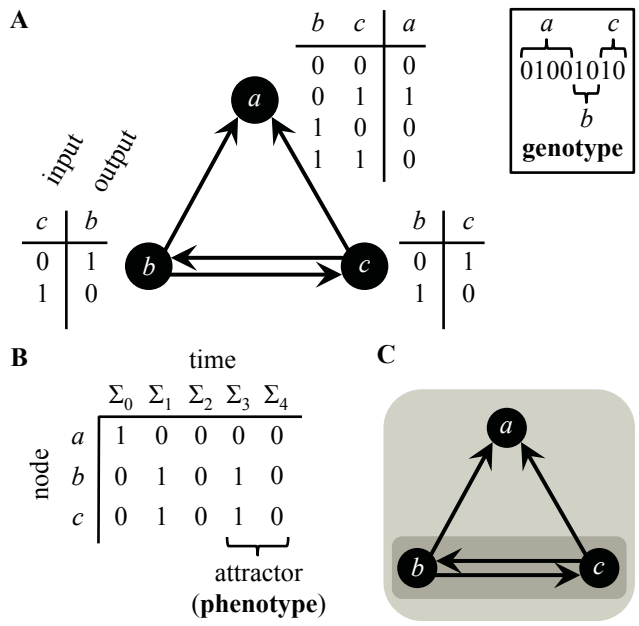

Figure 1: A small Boolean network example. (A) This network is composed of 3 nodes and 4 directed edges. Each node possesses a look-up table with the *cis*-regulatory logic that determines the dynamics of the Boolean network. This logic defines the expression state of the node at time $t+1$ as a function of the states of its inputs at time t. For example, the logic for node a shows how each possible combination of expression states $\sigma_b(t)$ and $\sigma_c(t)$ of the inputs at time t dictate the expression state $\sigma_a(t+1)$. (A; box) The *cis*-regulatory logic for the entire network is its genotype. (B) Starting with the initial configuration Σ_0 at time $t=0$, the states are updated according to the genotype until they repeat, forming an attractor that is analogous to a phenotype. Here, the attractor length is two. (C) The in-component (IC) of node a is the set $\{a,b,c\}$ (light grey), which includes a and all other nodes that directly or indirectly provide input to a. The ICs for b and c are both $\{b,c\}$ (dark grey).

Methods

The Model

Random Boolean networks were used to computationally model genetic regulation (Kauffman, 1969), and will herein be referred to as gene regulatory networks (GRNs). In these GRNs, nodes represent genes and edges represent directed regulatory interactions between genes where the regulator regulates the target gene (Figure 1A). Binary gene expression is represented by the Boolean state of each gene, which is dictated at discrete time points by the Boolean functions that define the possible outcomes of the regulatory interactions. Boolean functions represent the *cis*-regulatory logic for each gene, and are commonly encoded as truth tables (Figure 1A). The state of a gene $\sigma_i(t+1)$ is determined by a Boolean function f which considers the states of the k_{in} regulators of node i at time t:

$$\sigma_i(t+1) = f_i(\sigma_{i_1}(t), \ldots, \sigma_{i_{k_{\text{in},i}}}(t)), \tag{1}$$

where $\sigma_{i_1}, \ldots, \sigma_{i_{k_{\text{in},i}}}$ are the states of the $k_{\text{in},i}$ regulators of node i. This function is deterministic, and thus the states of all the genes that exist at the initial time point 0, referred to as configuration Σ_0, will invariably produce the subsequent configuration Σ_1 at the next time point. In combination with the finite number of possible configurations (2^N, where N is the number of genes in the GRN), this determinism guarantees that once a configuration is reencountered, a sequence of configurations will repeat indefinitely:

$$\Sigma_0 \to \cdots \to \Sigma_t \to \cdots \to \Sigma_{t+l-1} \to \Sigma_t \to \cdots, \tag{2}$$

where l is the number of configurations in the repeated sequence, called an attractor of length l (Figure 1B). This represents a stable gene expression pattern for the GRN (Huang et al., 2005). Although general and abstract, these models have successfully recapitulated cellular responses in the yeast *Saccharomyces cerevisiae* (Serra et al., 2004), the fly *Drosophila melanogaster* (Albert and Othmer, 2003), the plant *Arabidopsis thaliana* (Espinosa-Soto et al., 2004), and the sea urchin *Strongylocentrotus purpuratus* (Peter et al., 2012).

Genotype-to-Phenotype Mapping

In order to study how these GRNs maintain their function in the face of genetic perturbation, it is first necessary to explicitly define their mapping of genotype-to-phenotype. The *cis*-regulatory logic of the entire GRN was considered its genotype (Pechenick et al., 2012; Payne et al., 2013) (Figure 1A; box), as it dictates the overall dynamics of the GRN. The attractor that results from this genotype and some initial configuration Σ_0 represents a phenotype of the GRN (Figure 1B) (Huang et al., 2005).

Robustness

Upon establishing the genotype-to-phenotype mapping of these GRNs, the robustness of a phenotype to genetic perturbation must be defined in terms of a specific perturbation (Wagner, 2005). We considered point mutations to the genotype of the GRN, which represent mutations to the *cis*-regulatory regions of the genes in the GRN (Pechenick et al., 2012; Payne et al., 2013). The functional impact of such mutations has been demonstrated in a number of biological contexts (Wray, 2007), such as the patterning of bristles on the fly larvae *Drosophila sechellia* (Sucena and Stern, 2000), the skeletal development of the fish *Gasterosteus aculeatus* (Shapiro et al., 2004), and the branching structure of maize *Zea mays* (Clark et al., 2006).

To estimate robustness, a random walk was conducted in the genotype space of a GRN to determine the robust-

ness of the corresponding phenotype to genotypic perturbation. First, an initial configuration Σ_0 and *cis*-regulatory logic were randomly selected for the GRN. This conservative approach eliminates any assumptions about the initial conditions of biological GRNs. Then, the genotype was subjected to a single bit flip. The attractor that resulted from Σ_0 and the mutated *cis*-regulatory logic was then compared to the original attractor, and if they matched the mutation was considered neutral and the new *cis*-regulatory logic was preserved as the starting point for the next step in the random walk. Otherwise, the new *cis*-regulatory logic was discarded, and the next step was attempted using the *cis*-regulatory logic from the previous step. Upon completing the random walk, the proportion of mutations that were neutral was used as an estimate of robustness. This estimate approximates the average genotypic robustness for all genotypes that comprise a single phenotype, which is the definition of phenotypic robustness proposed by Wagner (2008).

Construction of GRNs

GRNs with a heavy-tailed output degree distribution were constructed as follows: For each gene i in a GRN with N genes, its number of regulatory targets $k_{\text{out},i}$ was selected from a power-law distribution (Darabos et al., 2009):

$$p(k_{\text{out}}) = \frac{1}{Z(\gamma)} k_{\text{out}}^{-\gamma}, \tag{3}$$

where $Z(\gamma) = \sum_{j=1}^{N} j^{-\gamma}$ is the normalization constant. This generated an out-degree sequence $k_{\text{out},1} \ldots k_{\text{out},N}$, which was used to randomly select the $k_{\text{out},i}$ regulatory targets for each gene i. The resulting in-degree sequence $k_{\text{in},1} \ldots k_{\text{in},N}$ approximated a Poisson input degree distribution. The combination of Poisson input and power-law output degree distribution closely resembles empirical real-world GRN data, such as those from the microbes *E. Coli*, *B. Subtilis*, and *S. cerevisiae* (Aldana et al., 2007).

Assortativity

Degree assortativity (r), referred to here simply as assortativity, is a global network property that captures the tendency for nodes with similar degrees to share an edge between them. This property was defined by Newman (2002) and is calculated as

$$r = \frac{\frac{1}{M}\sum_i j_i k_i - (\frac{1}{M}\sum_i \frac{1}{2}(j_i + k_i))^2}{\frac{1}{M}\sum_i \frac{1}{2}(j_i^2 + k_i^2) - (\frac{1}{M}\sum_i \frac{1}{2}(j_i + k_i))^2}, \tag{4}$$

where M is the number of edges in the network, j_i and k_i are the degrees of the nodes at either end of edge i, and r resides in the domain $[-1, 1]$; -1 indicates maximum dissimilarity and 1 indicates maximum similarity between degrees of nodes that share an edge. In a directed network, j_i and k_i may each be one of two types of degree, in- and out-degree, which results in four possible types of assortativity (Foster et al., 2010). For the purposes of this study, only out-degree was considered, as in Pechenick et al. (2012, 2013).

In-Components (ICs) and Characteristic Path Length

An in-component (IC) is a local network property. The IC of a node a corresponds to the set of nodes that are capable of influencing a (Figure 1C). The mean size of the ICs of all nodes in a network provides a measure of the extent to which nodes can affect other nodes in that network, and is calculated as

$$\overline{S} = \frac{\sum_{i=1}^{N} S_i}{N}, \tag{5}$$

where S_i is the IC size of node i, and N is the number of nodes in the network.

The characteristic path length is a global network property that also captures the relative ease with which information can flow between nodes in a network (Watts and Strogatz, 1998). This property is calculated by determining the shortest directed path between all pairs of nodes, and taking the mean of all existing paths. If a path does not exist between a pair of nodes, that pair is not considered in the calculation.

GRN Rewiring

Upon construction, a GRN with N genes has an out-degree sequence $k_{\text{out},1} \ldots k_{\text{out},N}$ and an in-degree sequence $k_{\text{in},1} \ldots k_{\text{in},N}$, and together these are referred to simply as its degree sequence. It is important to point out that different degree sequences can be drawn from the same degree distribution (Equation 3). In order to examine the effects of various topological properties on GRNs, it was desirable to vary those properties without altering the degree sequence of the GRN. An edge-swap algorithm was thus used to modify topology while keeping both the in- and out-degree of every gene intact (Milo et al., 2003). In each iteration of this algorithm, two edges $i \to j$ and $x \to y$ were selected, and the regulatory targets were swapped between the regulators to yield two new edges $i \to y$ and $x \to j$. If the new edges caused the GRN to be closer to a desired value for a particular topological property, or if no change was observed with respect to this property, then the new edges were kept. Otherwise, the new edges were discarded and the old edges were kept.

Simulation Design

To examine the relationship between assortativity, mean IC size, and characteristic path length, 2000 weakly connected GRNs with $N = 30$ and $\bar{k}_{\text{out}} = 4$ ($\gamma = 1.55$) were constructed. Self-loops were excluded because they trivially increase assortativity without changing mean IC size or characteristic path length, and such exclusion did not affect past results (Pechenick et al., 2013). GRNs with $\bar{k}_{\text{out}} = 4$ are in the chaotic dynamical regime, which was chosen because these GRNs tend to have large ICs and exhibit dramatic variation in robustness, whereas ordered and critical GRNs exhibit limited changes in robustness as assortativity varies (Pechenick et al., 2012, 2013). The 2000 degree sequences

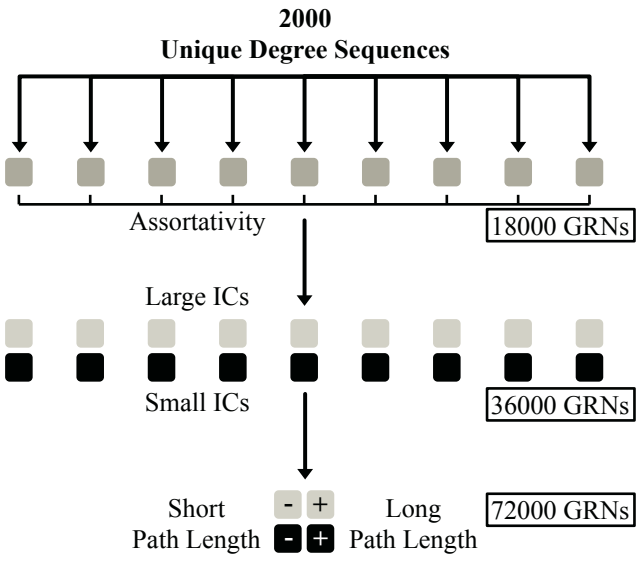

Figure 2: Simulation design flowchart. Beginning with 2000 unique degree sequences, every degree sequence was rewired (preserving degree sequence) to construct GRNs at each of 9 evenly spaced assortativity values. Each GRN was then rewired (preserving degree sequence and assortativity) to produce two new GRNs, one with small and one with large mean IC size. Next, each of those GRNs was rewired (preserving degree sequence, assortativity, and mean IC size), producing one GRN with short and one with long characteristic path length. This final step is only displayed once for clarity.

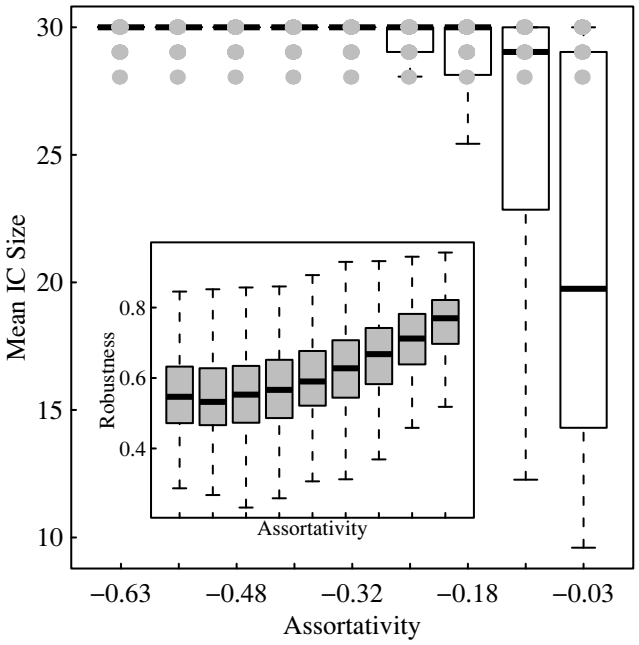

Figure 3: Mean in-component (IC) size vs. assortativity. The mean IC size of the 18000 GRNs decreases as assortativity increases ($p = 0.025$, Spearman's rank correlation on median values). However, 422 (21%) of the 2000 degree sequences do not exhibit a decrease in mean IC size (grey circles). (Inset) Robustness vs. assortativity. Robustness for the 422 degree sequences (3798 GRNs) increases as assortativity increases ($p \ll 0.001$, Spearman's rank correlation on median values). Outliers are omitted from plots for clarity.

were then rewired to 9 evenly spaced assortativity values in the range $[-0.64, -0.02] \pm 0.01$ (Figure 2), where the edge-swap algorithm proceeded until assortativity was within 0.01 of the desired value, as in Pechenick et al. (2013). This resulted in 18000 GRNs, where every degree sequence was represented at every assortativity value. Note that heavy-tailed degree distributions are inherently negatively assortative (Johnson et al., 2010), and the domain of the assortativity values considered here was entirely negative.

To isolate the effects of mean IC size, GRNs were rewired to low and high values for mean IC size. The edge-swap algorithm was allowed to minimize or maximize mean IC size for each GRN until no desired change in mean IC size had been observed for 10000 iterations, and assortativity was constrained within ± 0.02 of the original 9 evenly spaced values. Here, every degree sequence was represented twice at every assortativity value. We refer to these two classes of GRNs as "small ICs" and "large ICs."

Then, to isolate the effects of characteristic path length for each of the two classes of GRNs, GRNs were rewired to low and high values for characteristic path length. As before, the edge-swap algorithm was allowed to proceed until the desired decrease or increase was not observed for 10000 iterations. Assortativity was constrained within ± 0.02, as before, and mean IC size was not allowed to vary at all from its starting value. Here, every degree sequence was represented four times at every assortativity value for every combination of low and high mean IC size and characteristic path length. For more on rewiring networks to obtain multiple desired topological properties, see Holme and Zhao (2007).

The robustness of a GRN was estimated by taking the average of the outcomes for 100 random walks with different initial configurations Σ_0 and *cis*-regulatory logic, where each random walk consisted of 500 attempted steps. These parameters were selected as a compromise between accuracy and computational efficiency (Pechenick et al., 2012).

Results

Small mean IC size is not solely responsible for increased robustness.

Consistent with previous observations (Pechenick et al., 2012, 2013), the mean IC sizes of the 18000 GRNs at 9 assortativity values tended to decrease with increasing assortativity (Figure 3). This decrease in mean IC size was impli-

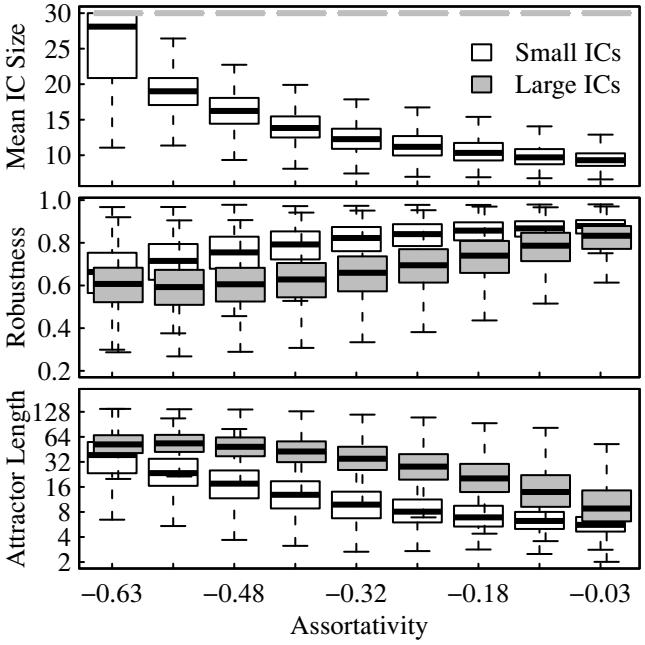

Figure 4: Mean in-component (IC) size, robustness, and attractor length vs. assortativity for GRNs with either small or large ICs. At every assortativity value, (top) GRNs with large ICs have significantly larger mean IC size, (middle) lower robustness, and (bottom) longer attractors than GRNs with small ICs (all $p \ll 0.001$, Wilcoxon rank sum test). Outliers are omitted from plots for clarity.

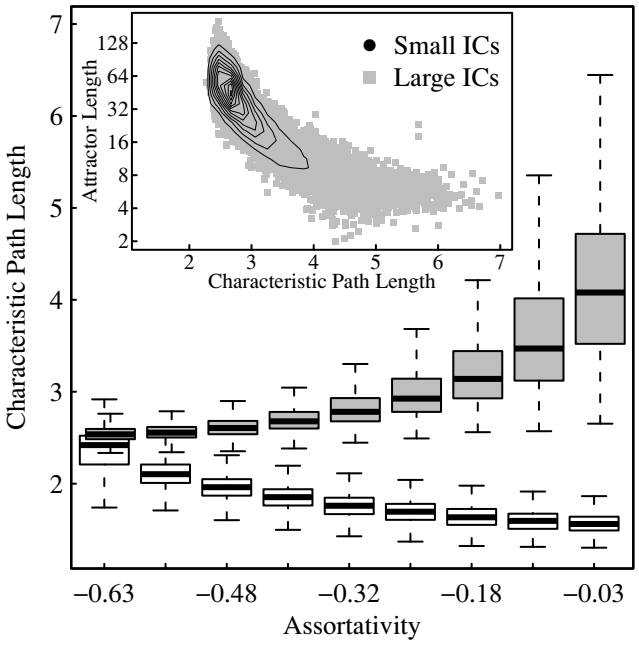

Figure 5: Characteristic path length vs. assortativity. The characteristic path length of GRNs with large ICs increases as assortativity increases, whereas it decreases for GRNs with small ICs (both $p \ll 0.001$, Spearman's rank correlation on median values). Outliers are omitted from the plot for clarity. (Inset) Attractor length vs. characteristic path length. The attractor length of GRNs with large ICs decreases as characteristic path length increases, whereas it increases for GRNs with small ICs (both $p \ll 0.001$, Spearman's rank correlation on all values). Contour lines are provided as a visual guide for the relative density of points.

cated in a corresponding increase in robustness (Pechenick et al., 2012); however, some degree sequences produced GRNs with identical mean IC sizes at every assortativity value (Figure 3; grey circles), and these GRNs also showed increased robustness with increased assortativity (Figure 3; inset). Therefore, a topological mechanism distinct from mean IC size is required to explain the observed increase in robustness for these GRNs.

Both small and large mean IC sizes are possible for the same degree sequences.

One possible explanation for the two types of GRNs observed in Figure 3 is that certain degree sequences are simply incapable of rewiring in such a way that results in smaller ICs. Likewise, some degree sequences may be forced to form smaller ICs as assortativity increases. If this were true, not only would multiple mechanisms be needed to explain the observed increases in robustness, but these mechanisms would act exclusively on certain degree sequences and not others.

In order to test whether this is indeed the case, we rewired each degree sequence at each assortativity value to try to obtain two new GRNs with a small and large mean IC size (preserving assortativity), respectively (Figure 4; top). At the lowest assortativity value, where GRNs almost exclusively have large ICs, 47% of degree sequences failed to produce GRNs with smaller ICs. However, this dropped to 13% at the second lowest assortativity value, 2% at the third, and $< 1\%$ for all other assortativity values. For the top three assortativity values, every degree sequence was able to produce distinct GRNs with either small or large ICs. Therefore, while multiple mechanisms are still necessary to understand how assortativity influences robustness, these respective mechanisms are not restricted to only certain degree sequences. We then estimated robustness for these two classes of GRNs and found that for every assortativity value GRNs with small ICs were significantly more robust than their counterparts with large ICs (Figure 4; middle). This suggests that the previously described mechanism for how small ICs can lead to increased robustness (Pechenick et al., 2012) is generally relevant for GRNs across different degree sequences and at a wide range of assortativity values.

However, since GRNs with large ICs displayed increased robustness with increased assortativity (Figure 4; middle),

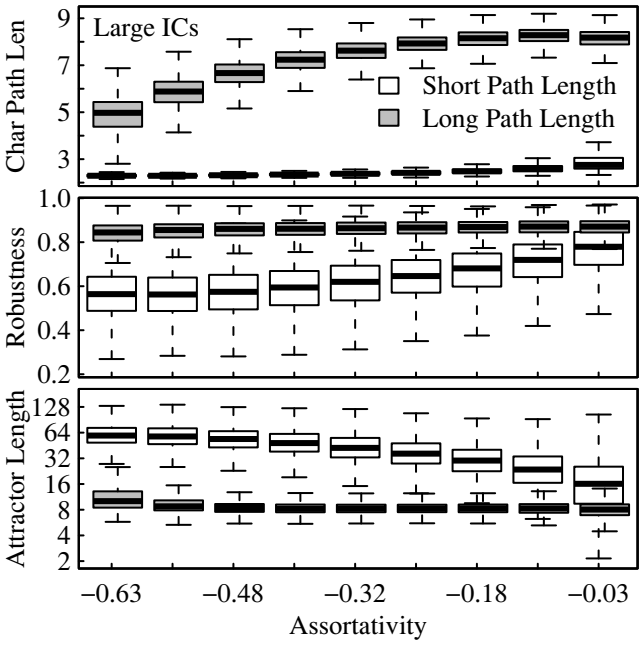

Figure 6: For GRNs with large ICs: Characteristic path length, robustness, and attractor length vs. assortativity for GRNs with either short or long characteristic path length. At every assortativity value, (top) GRNs with long characteristic path length have significantly longer paths, (middle) higher robustness, (bottom) and shorter attractors than GRNs with short characteristic path length (all $p \ll 0.001$, Wilcoxon rank sum test). Outliers are omitted from plots for clarity.

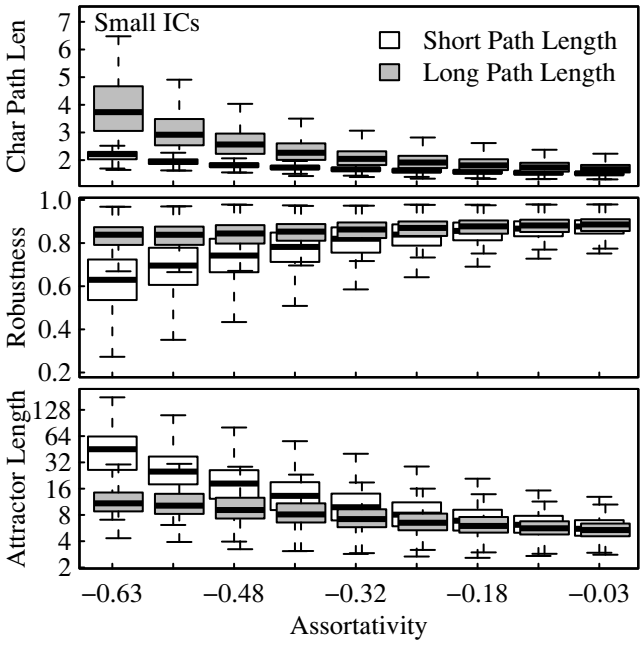

Figure 7: For GRNs with small ICs: Characteristic path length, robustness, and attractor length vs. assortativity for GRNs with either short or long characteristic path length. At every assortativity value, GRNs with long characteristic path length are significantly different from GRNs with short characteristic path length, as in Figure 6 (all $p \ll 0.001$, Wilcoxon rank sum test). Outliers are omitted from plots for clarity.

another general mechanism besides mean IC size is required to explain the effect assortativity has on robustness. In Pechenick et al. (2012), we proposed that mean IC size affects robustness by altering the attractor lengths of GRNs, and we observed here that attractor length decreases with assortativity for both classes of GRNs (Figure 4; bottom). This is consistent with a negative relationship between robustness and attractor length, and suggests that the alternative mechanism producing highly robust GRNs with large ICs is doing so by reducing attractor length.

Characteristic path length changes with assortativity.

For the GRNs with large ICs, mean IC size does not change with assortativity (Figure 4; top), so another topological property must be influencing the attractor lengths of these GRNs. We found that the characteristic path length of GRNs changes with assortativity in a manner that is dependent on which of the two GRN classes is being considered (Figure 5). For GRNs with small ICs, characteristic path length shrinks with increasing assortativity, and appears to be associated with a shrinking mean IC size (Figure 4; top). However, for GRNs with large ICs, characteristic path length *grows* with increasing assortativity. These opposing trends are accompanied by two additional opposing trends: For GRNs with small ICs, attractor length is positively correlated with characteristic path length, whereas for GRNs with large ICs, attractor length is negatively correlated with characteristic path length (Figure 5; inset). This is consistent with the decreases in attractor length as assortativity increases for both GRNs with small and large ICs (Figure 4; bottom).

Long characteristic path length contributes to robustness.

To determine whether characteristic path length is directly responsible for changes in robustness and attractor length, we varied the characteristic path length of GRNs to their high and low bounds while preserving both assortativity and mean IC size for GRNs with large ICs (Figure 6) and small ICs (Figure 7). For GRNs with large ICs, the maximum characteristic path length that was achievable increased with increasing assortativity (Figure 6; top), which echoes the relationship between these two properties observed in Figure 5. However, these results go on to show that GRNs with longer characteristic path length have higher robustness

(Figure 6; middle) and shorter attractors (Figure 6; bottom) than their counterparts with shorter characteristic path length that possess the same assortativity and mean IC size. These results argue for a direct positive role for characteristic path length in affecting the robustness of GRNs with large ICs at a wide range of assortativity values.

For GRNs with small ICs, although changes in characteristic path length seemed to have the opposite effect on attractor length, and therefore robustness, as GRNs with large ICs (Figure 5; inset), it was unclear whether this represented a direct relationship between the two properties. In particular, for GRNs with small ICs, increasing assortativity produces decreases in both characteristic path length (Figure 5) and mean IC size (Figure 4; top). Since a decrease in mean IC size leads to increased robustness (Figure 4; middle), it is difficult to disentangle the effects of characteristic path length from those caused by changes in mean IC size. By varying characteristic path length independently of both assortativity and mean IC size, we can directly address this.

In contrast to GRNs with large ICs, for GRNs with small ICs, the maximum characteristic path length that was achievable decreased with increasing assortativity (Figure 7; top), likely reflecting a positive association between characteristic path length and mean IC size. However, consistent with the results for GRNs with large ICs, GRNs with small ICs that possess long characteristic path length exhibit higher robustness (Figure 7; middle) and shorter attractors (Figure 7; bottom) than their counterparts with short characteristic path length with the same assortativity and mean IC size. Taken together, these results indicate that although the ability to vary the characteristic path length of a GRN depends on both its mean IC size and its assortativity, adopting a longer characteristic path length leads to higher robustness in a manner that is independent of these other two properties. Therefore, as the assortativity of a GRN increases, one of two things tends to occur that can result in higher robustness. Its mean IC size may shrink, which leads to higher robustness in a manner that dominates the effects of the associated shrinking of characteristic path length. Or, its mean IC size may not shrink, in which case characteristic path length will tend to grow and lead to higher robustness.

Discussion

We have presented an alternative mechanism by which assortativity influences the robustness of GRNs to mutations in their *cis*-regulatory logic. It is often the case that an increase in assortativity results in a decrease in mean IC size, which increases the robustness of the GRN. However, even when mean IC size does not change, robustness nonetheless increases. We have found that in this case, an increase in assortativity leads to an increase in characteristic path length, which is associated with increased robustness. Furthermore, this effect was not limited to GRNs with large mean IC sizes. The assortativity and mean IC size of a GRN does constrain its characteristic path length. Nevertheless, we have shown that a GRN with a long characteristic path length is on average more robust than a GRN with similar assortativity and mean IC size, but with a shorter characteristic path length.

These results complement previous theoretical work that showed that the characteristic path length of network models influences their dynamics. In contrast to the inverse relationship between characteristic path length and attractor length that we observed, Serra and Villani (2002) showed that decreasing the characteristic path length of cellular automata (CA) led to simpler dynamics. This result is likely due to their use of the majority update rule, which took advantage of the shorter paths to more easily achieve uniform behavior across the network. In line with what we have shown, Lizier et al. (2011) observed that an increase in the characteristic path length of random Boolean networks led to greater information storage and less information transfer, which are properties that they found associated with the simpler dynamics typically found in the ordered dynamical regime.

As we gather more data about the structure of biological GRNs, the results presented in this work will provide a theoretical basis for searching for specific topological features that are indicative of robustness. Indeed, high assortativity would imply robustness, yet its absence would not discount it. A relatively small mean IC size could suggest robustness at a range of assortativity values, and yet it too is not exclusively necessary. In the absence of a small mean IC size, a long characteristic path length would signal robustness that could rival the robustness of a GRN that did possess a small mean IC size. We have shown that any one or a combination of these properties contributes to highly robust GRNs. Furthermore, this study exclusively considered out-degree assortativity, and further work will be necessary to determine how the other types of degree assortativity are involved (Foster et al., 2010). As we continue to map and examine biological GRNs, it will be informative to catalog their topological properties in an attempt to understand whether they depict common or varied evolutionary strategies of achieving robustness.

Acknowledgements

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