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Modular genetic regulatory networks increase organization during pattern formation

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ABSTRACT

Studies have shown that genetic regulatory networks (GRNs) consist of modules that are densely connected subnetworks that function quasi-autonomously. Modules may be recognized motifs that comprise of two or three genes with particular regulatory functions and connectivity or be purely structural and identified through connection density. It is unclear what evolutionary and developmental advantages modular structure and in particular motifs provide that have led to this enrichment. This study seeks to understand how modules within developmental GRNs influence the complexity of multicellular patterns that emerge from the dynamics of the regulatory networks. We apply an algorithmic complexity to measure the organization of the patterns. A computational study was performed by creating Boolean intracellular networks within a simulated epithelial field of embryonic cells, where each cell contains the same network and communicates with adjacent cells using contact-mediated signaling. Intracellular networks with random connectivity were compared to those with modular connectivity and with motifs. Results show that modularity effects network dynamics and pattern organization significantly. In particular: (1) modular connectivity alone increases complexity in network dynamics and patterns; (2) bistable switch motifs simplify both the pattern and network dynamics; (3) all other motifs with feedback loops increase multicellular pattern complexity while simplifying the network dynamics; (4) negative feedback loops affect the dynamics complexity more significantly than positive feedback loops.

1. Introduction

Multicellular organisms contain a large variety of cellular patterns. For instance, Fig. 1 illustrates a *Drosophila melanogaster* embryo in which muscle and nervous system structures interconnect through sensory and activation signaling. These patterns are formed during development and are a consequence of genetic regulatory networks (GRNs) that operate within cells and that respond to communication between cells (Lander, 2007, 2011; Flann et al., 2013). GRNs are networks of interacting genes where the expression or non-expression of genes determines the expression state of other genes. The dynamics of GRNs determine the gene expression profile for each cell leading to spatial patterns of cellular differentiation. This process is repeated to implement an organism's body plan, and subsequent morphology (Davidson, 2010).

GRNs contain subnetworks of genes referred to as modules. Smaller modules, usually consisting two or three genes with specific functioning, are known as motifs (Shen-Orr et al., 2002; Milo et al., 2002). Motifs are detected at a higher frequency than would be expected in random networks. Computational biologists have hypothesized that motifs play a determinative role in cell function (Barabasi and Oltvai, 2004; Ghaffarizadeh et al., 2014). However, their influence on pattern formation during development is poorly understood. This work presents a computational study aimed at understanding how the presence of motifs within intracellular networks changes the GRN dynamics and the emergent multicellular patterns.

In particular, this study measures the complexity of the organization of the dynamics and patterns. As can be seen in Fig. 1, patterns can involve complex arrangements of specialized regions and interconnections that develop later, or earlier patterns such as simple segmentation (Mazumdar and Mazumdar, 2002) or mosaic

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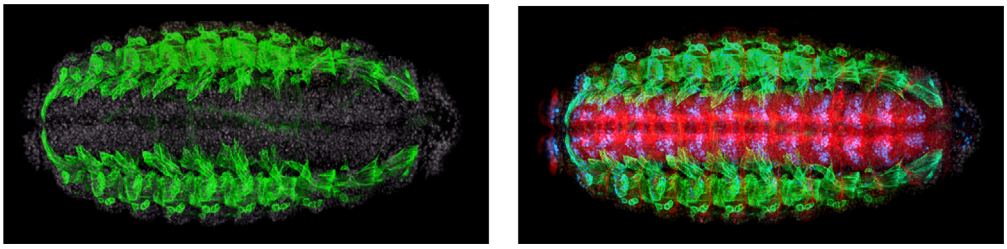

Fig. 1. Ventral view of stage 16 *Drosophila melanogaster* embryo immunostained for tropomyosin (green; a protein expressed in muscle), Pax 3/7 (blue; a regulatory protein expressed in central nervous system nuclei and ectoderm), and HRP (red; neurons). All nuclei shown in gray (DAPI). Courtesy of Julieta María Acevedo and Lucas Leclere, Marine Biological Laboratory, Woods Hole, www.mbl.edu/dev.biologists.org/. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

arrangements (Goodyear and Richardson, 1997; Podgorski et al., 2007). To quantify the level of organization we employ Kolmogorov complexity, also known as algorithmic complexity (Kolmogorov, 1965). Such methods measure the information contained within an object, such as a cellular pattern, by considering the size of the algorithm needed to generate the object, hence the term algorithmic complexity. The smaller the algorithm, the simpler the pattern. The calculations of Kolmogorov complexity is detailed in the methods section following.

To understand how GRNs regulate biological events, scientists have developed mathematical and computational models to generate predictions and explain experimental observations. Among these modeling approaches is to represent a GRN as a Boolean network in which the activity of a gene is either on or off, determined by a set of logical functions over the activity of other genes (Kauffman, 1969). It is this modeling framework that we apply in this study.

To evaluate the influence of the modular structure of networks and in particular motifs on network dynamics and patterns, we design GRNs that are embedded into cells arranged in a 2D grid, simulating an epithelium. Such abstractions of the epithelium have been employed successfully in many developmental systems, such as the cellularized *Drosophila* embryo (Mazumdar and Mazumdar, 2002), and the sensory epithelia of the developing vertebrate retina (Eglen and Willshaw, 2002) and the inner ear (Goodyear and Richardson, 1997). Each cell contains the same Boolean network, referred to as an intracellular network. We explore the impact on network dynamics and multicellular patterns of modules with random connectivity and regulatory functions, and when the modules are explicitly recognized motifs.

2. Modular structure and motifs within gene regulatory networks

Networks are structurally modular if they contain highly connected clusters of genes that are linked by sparser connections than those within the modules. Fig. 2(a) and (b) shows a small network with a modular structure compared to randomly connected networks. Evidence suggests that modular network structure increases the functional modularity of gene regulatory networks by performing relatively independent tasks (Kim et al., 2012; Clune et al., 2013) such as decision making, signal processing, and communication. The modular organization of biological structure is supported by experimental studies from pathogen structure, gene networks, and protein–protein interaction networks (Lorenz et al., 2012). For example, Kim et al. (2012) considered the connected subset of protein networks in protein–protein interaction data for budding yeast. Their analysis suggests that the yeast protein network is significantly modular, and it contains various motifs.

We study two kinds of modularity in this work: structural modularity when the modules within the intracellular network are connected randomly and functional modularity when the subnetworks are recognized motifs. Motifs frequently occur and consist of few interacting genes (Ghaffarizadeh et al., 2014) and were first noted in *Escherichia coli*, where they were detected at a higher frequency than would be expected in random networks. Since then multiple motifs have been identified in bacteria and yeast (Alon, 2007), the immune system (Singh et al., 2015), and *Drosophila* (Kim et al., 2012). This finding suggests that motifs are building blocks of transcription networks and that they may have evolved to achieve specific regulatory behaviors in cellular transcription networks (Kim et al., 2008). Regulatory motifs have been found in regulatory networks that perform two distinct functions: (1) developmental networks that guide differentiation and cell fate determination by transducing signals into irreversible cell-fate decisions (Levine and Davidson, 2005; Swiers et al., 2006) and (2) sensory networks that respond to signals such as stresses and nutrients rapidly and make reversible decisions (Shen-Orr et al., 2002).

The motifs that are associated with developmental networks are commonly comprised of feedback loops. Positive-feedback loops are most common and are made up of two transcription factors that regulate each other. There are two kinds of positive-feedback loops, a double excitatory loop (Fig. 2(b)) and a double-inhibitory loop (Fig. 2(a)). The regulatory dynamics of these positive feedback loops often results in two or more steady states and is referred to as

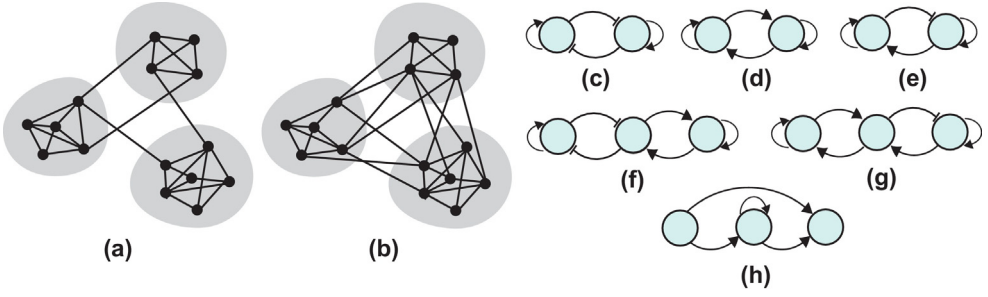

Fig. 2. This figure illustrates networks with modular structures and example of variety of motifs. (a) A network with modular structure where intra-modular connectivity is higher than inter-module connectivity. (b) A randomly connected network. (c) A positive feedback loop (a double inhibitory loop with two positive autoregulatory loops). (d) A positive feedback loop (a double excitatory loop with two positive autoregulatory loops). (e) A negative feedback loop Kim et al. (2008) with two positive autoregulatory loops. (f) Coupled positive–positive feedback loops. (g) Coupled positive–negative feedback loops. (h) The type-1 coherent feed-forward loop Kalir et al. (2005).