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Robustness and evolvability in genetic regulatory networks

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Abstract

Living organisms are robust to a great variety of genetic changes. Gene regulation networks and metabolic pathways self-organize and reaccommodate to make the organism perform with stability and reliability under many point mutations, gene duplications and gene deletions. At the same time, living organisms are evolvable, which means that these kind of genetic perturbations can eventually make the organism acquire new functions and adapt to new environments. It is still an open problem to determine how robustness and evolvability blend together at the genetic level to produce stable organisms that yet can change and evolve. Here we address this problem by studying the robustness and evolvability of the attractor landscape of genetic regulatory network models under the process of gene duplication followed by divergence. We show that an intrinsic property of this kind of networks is that, after the divergence of the parent and duplicate genes, with a high probability the previous phenotypes, encoded in the attractor landscape of the network, are preserved and new ones might appear. The above is true in a variety of network topologies and even for the case of extreme divergence in which the duplicate gene bears almost no relation with its parent. Our results indicate that networks operating close to the so-called ''critical regime'' exhibit the maximum robustness and evolvability simultaneously.

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1. Introduction

Robustness and evolvability are two central properties of biological systems (Srtelling et al., 2004; de Visser et al., 2003; Kirschner and Gerhart, 1998; Nehaniv, 2003; Poole et al., 2003; Wagner, 2005a). Living organisms are robust since they can maintain performance under a broad range of random perturbations, ranging from temporary chemical or physical changes in the environment, to permanent genetic mutations. They are also evolvable since organisms eventually do change as a result of changes in their genetic material, acquiring new functions and adapting to new environments. Robustness and evolvability have been observed to occur at different levels of biological organization, going from gene regulation to organismal fitness. However, despite the central importance of these two concepts to the understanding of the functioning and evolution of biological systems, it is not clear yet what are the structural and dynamical mechanisms that generate complex structures that are both robust and evolvable. Furthermore, neither robustness nor evolvability have been defined unambiguously. Therefore, before addressing the problem of how robustness and evolvability emerge in genetic regulatory networks (GRN), we must start by defining them in this context.

Several definitions have been given depending upon the context and level of organization under consideration. Here we follow de Visser et al. (2003) and define *robustness as the invariance of phenotypes in the face of perturbation*. In this definition the word ''perturbation'' means anything

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that drives the system away from its wild-type state. However, throughout this work we will use perturbation as synonymous of permanent genetic change (i.e. mutations). On the other hand, we adopt the following definition for evolvability (Wagner, 2005b): *A biological system is evolvable if it can acquire novel functions* (*phenotypes*) *through genetic change* (*perturbations*), *functions that help the organism survive and reproduce*. Accordingly, in this work we seek to devise models for genetic networks that are robust in the sense that phenotypes are preserved in the presence of perturbations, and at the same time are evolvable in the sense that under such perturbations new phenotypes may also emerge. Note that *phenotype* and *perturbation* are two essential elements involved in the previous definitions. It is therefore important to specify what phenotypes are in our models and what kind of perturbations will be considered.

With regard to phenotypes, it has been a long standing hypothesis that the dynamical attractors of the genetic network correspond to cellular types or cellular fates (Kauffman, 1969, 1993). This hypothesis has been partially confirmed, both numerically and experimentally, in recent work where patterns of gene expression of real organisms have been identified with the dynamical attractors of properly constructed genetic network models (Albert and Othmer, 2003; Mendoza and Alvarez-Buylla, 2000; Espinosa-Soto et al., 2004; Huang and Ingber, 2000; Huang et al., 2005; Gardner et al., 2003). In the next sections we present the GRN model we will be working with and explain the occurrence of dynamical attractors and their biological significance. Here it suffices to mention that a dynamical attractor in this context can be considered as the stationary gene expression profile which the genetic network falls into after a transient time, starting out from a given initial condition (such as a heat shock). The work cited above provides evidence supporting the fact that some phenotypic traits, such as the cellular type or the cellular fate (apoptosis, quiescence, proliferation, differentiation) can be viewed as end programs encoded in the dynamical attractors of the GRN.

The *attractor landscape* of a genetic network, (namely, the set of all the attractors and their basins of attraction), is an emergent property that depends on the structural and dynamical organization of the entire network in the same way as phenotypes are emergent properties determined to a large extent by the organization of the underlying genetic network. Therefore, given the biological significance of the attractors, the problem of the robustness and evolvability of phenotypes can be addressed by studying the conservation and transformation of the *attractor landscape* of the GRN under perturbations. This takes us to the second important element involved in the definitions of robustness and evolvability: what kind of perturbations will be considered?

The only perturbation considered in this work is gene duplication and divergence. Nowadays it is widely accepted that one of the main mechanisms of genome growth and evolution is gene duplication followed by genetic divergence (Lynch and Conery, 2000; Lynch, 2002; Lynch and Katju, 2004; Teichmann and Babu, 2004; Zhang, 2003). Susumu Ohno was among the first who pointed out the importance of gene duplication, for it constitutes a remarkable source of material for functional gene novelty in organisms (Ohno, 1995). Rapidly after gene duplication, the gradual accumulation of mutations in one copy makes the parent and duplicate genes diverge (Lynch and Conery, 2000; Teichmann and Babu, 2004; Zhang, 2003). This divergence might consist of (i) non-functionalization, in which one of the copies becomes silenced; (ii) neofunctionalization, in which one copy develops a new function, whereas the other copy retains its original function; (iii) subfunctionalization, where the two copies acquire complementary functions that, added together, carry out the original function. In any case, "*changes of gene expression after gene duplication appear to be a general rule rather than exception, and these changes often occur quickly after gene duplication*." (Zhang, 2003). Indeed, the results presented here indicate that the duplication and divergence of a single gene can change the entire attractor landscape. This change may consist not only in the emergence of new phenotypes (attractors), but also in the reconfiguration of differentiation and gene expression pathways.

2. Genetic network models

The dynamics of GRN can be modeled using different approaches (De Jong, 2002; Smolen et al., 2000; Mason et al., 2004). In this work, as a test-bed for the study of robustness and evolvability in GRN, we choose to model gene activities by random Boolean networks (RBN) with different topologies. Since their proposal in 1969 (Kauffman, 1969), RBN have successfully described in a qualitative way several important aspects of the gene regulation and cell differentiation processes (Kauffman, 1993, 1995). The model consists of a set of N binary variables, $\sigma_1, \sigma_2, \ldots, \sigma_N$, each acquiring the values 0 or 1 corresponding to the two states of gene expression ("off" and "on," respectively). The state of each gene σ_n is regulated by a set of k_n other genes. In turn, σ_n can regulate the expression of l_n other genes. Note that the network is directed since, if σ_m regulates the expression of σ_n, the opposite does not necessarily occur. We will call the set of k_n genes that regulate the expression of σ_n the *inputs* or *regulators* of σ_n. Analogously, the set of l_n genes for which σ_n is an input will be referred to as the *outputs* or *targets* of σ_n.[1]

As in every directed network, the topology of the input connections need not be the same as the topology of the

[1]"Inputs" and "outputs" is the common terminology in the literature on complex networks. However, in the biological literature it is more common to refer to the inputs and outputs as "regulator genes" and "target genes", respectively. Here we will use these two terminologies indistinguishably.