Robustness in biological systems

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Abstract

The systems approach to biology aims to address problems using a holistic approach that focuses on emergent properties, namely those that cannot be attributed to a single part of a biological system, but rather, arise from complex, collective processes at the molecular level. An important feature of these emergent properties is robustness—the emergent phenomena are, to a considerable extent, insensitive to changes in the parameters of the microscopic processes that give rise to the phenomena in the first place. In this paper, we examine the general features of robustness as it appears in biology. We also present a model for adaptation in bacterial chemotaxis as an example of how robustness can influence the process of understanding systems-level phenomena by model building.

1 Introduction

The discovery in 1953 of the structure of DNA, the molecule of life, marked the beginning of an era in which molecular biology is the dominant approach to the study of life. Biology was previously primarily concerned with the classification of the forms in which life appears, and Darwin's theory of evolution by natural selection was successful in explaining why life appears in such a variety of forms. Molecular biology, on the other hand, aims to provide an explanation for life at the macroscopic level in terms of the underlying processes, down from the level of individual cells to the molecules that operate within cells. As such, it represents a paradigmatic departure from taxonomical biology, and is closer in spirit to the "top-down" approach that dominated physics in the first half of the twentieth century.

Furthermore, molecular biology attempts to describe *universal* phenomena exhibited by various living systems in terms of molecular processes. As a concrete example, we follow Kaneko [8] in considering the motor activity of an animal. A reductionist approach takes us from the muscles, to the muscle fibre cells, to the proteins in the cells that initiate motor activity. Two types of protein are involved: one called actin, which forms a rail, and another called myosin, which can move along the rail, consuming chemical energy and thus initiating further reactions that cause the muscle to contract. Having elucidated the biochemical reactions involved in this process, the molecular biologist looks to see how common this network is among different species, with the hope of claiming universality.

In the last half-century, biologists have found that the list of molecules that participate in various biochemical processes has grown to staggering proportions. This has led to various "-omic" projects, which aim to identify and enumerate the molecules involved in specific processes. For instance, the Human Genome Project successfully mapped the sequence of human DNA, thus enumerating the genes of the human genome. There are similar undertakings for the proteome (the set of proteins) and the metabolome (the molecules involved in metabolism).

The appearance of these large data sets has given impetus to attempts to understand biology at the "systems" level—that is, at a level in between the macroscopic and the molecular. While the term "systems biology" is increasingly used to describe some of the cutting-edge trends in modern biology, it is still young as field of study, and does not have a universally accepted set of goals and methodologies. A 2006 editorial piece [6] in *Nature* magazine expresses the opinion that systems biology describes the imminent transition in molecular cell biology from a "purely reductionist hypothesis-driven approach" to a new set of methodologies that embrace quantification and mathematical modelling, and are driven by high-throughput data acquisition.

While an understanding of microscopic processes at the level of genes and proteins remains important in systems biology, the focus is on understanding the system's structure and dynamics. Kitano [9] draws an analogy between systems biology and the design of an aeroplane, which serves to make this distinction clearer. Enumerating all the proteins and genes of a particular system is like listing all the parts of an aeroplane. Given a complete list of these parts, it is nevertheless impossible to reconstruct an aeroplane based just on this list, because of the complexity underlying the engineered object. Rather, we need to

to know the structure in which these parts are assembled relative to each other, and the interactions between the different parts and substructures; these correspond to a biological system's structure and dynamics respectively.

In this picture of systems biology, the "-omic" data sets represent a first step towards understanding system structures. The dynamics of a system must be understood through modelling, a good model being one that can provide a detailed understanding of the system's behaviour, and predict the response of the system to complex stimuli. In particular, systems-level description often includes *emergent properties*, namely macroscopic phenomena that arise from systems-level dynamics but cannot be attributed to any particular component.

While understanding structures and dynamics at the systems level is essential to gaining a deeper understanding of the network of processes involved in a biological phenomenon, systems biology is also concerned with identifying design principles that appear in all biological networks. One of the most ubiquitous design principles is that of *robustness*, which may be defined [10] most generally as a property that allows a system to maintain its functions against internal and external perturbations. In this paper, we will discuss robustness in some detail, explaining the methods used in biological networks to achieve robustness, and touching on the relationship between robustness and evolvability. We will provide several concrete examples of robustness in biology, and in section 3, we will examine a detailed robust model that arises in bacterial chemotaxis.

2 What is robustness?

2.1 Robustness as a design principle

Before we discuss biological robustness, it is useful to begin with an example of robust design in engineering, namely that of a simple automatic flight control system used in passenger aeroplanes [10]. As Figure 1 illustrates, an automatic flight control system operates the aeroplane's flight-control surfaces (rudder, flaps, etc.) and the propulsion system, based on some programmed instructions. It is composed of three computer modules that generally perform the same function, but are designed differently in order to avoid a common mode failure. External perturbations modify the real flight path relative to the desired path, but a feedback loop in the system ensures that the control system is aware of the perturbation, and can compensate for it accordingly. Thus, the function of the system—fixing the flight path—is robust against external perturbations. The system design also includes *redundancy* in the number of modules, and *heterogeneity* in the design of the modules, in order to avoid catastrophic failure.

Similar principles arise in the case of biological robustness. Specifically, the macroscopic biological phenomena associated with robustness may be classified [9] into three areas: (i) adaptation, i.e. the ability to cope with changes in the external environment; (ii) a degree of insensitivity to the particular values of the parameters that describe the underlying microscopic processes; and (iii) slow degradation of a system's function following damage (as opposed to catastrophic failure). It is important to remember that it is always the system's

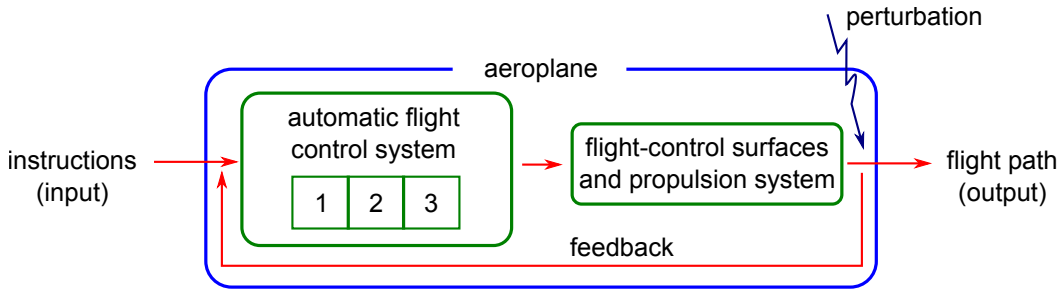

Figure 1: **Robustness in flight-path control**. The flight control system in the aeroplane operates the aeroplane's flight hardware. The system is comprised of three modules that perform the same function. A feedback loop is used to deal with external perturbations. Adapted from Ref. [10].

function, and not necessarily its *state*, that is robust to perturbations. Thus, robustness is associated with the preservation of function in both biological and engineered systems.

There are various mechanisms that are used to achieve robustness in a biological system. Kitano [10] lists the following categories:

1. *System control* involves the use of feedback loops to achieve dynamic response. Negative feedback is used to respond to perturbations, while positive feedback amplifies stimuli.

2. *Alternative mechanisms.* This refers to the use of multiple and different means to achieve a given operation. In the aeroplane example above, the use of three computers in the flight control system provides alternative pathways for the execution of the same function.

3. *Modularity.* This is the principle of restricting the effect of perturbations to small parts of the system. A module may be defined functionally, spatially or temporally.

4. *Decoupling,* which isolates variation at the level of genes and proteins from systems-level functionalities.

We will see specific instances of these mechanisms in the examples described below.

2.2 Examples of robustness in biology

Bacterial chemotaxis is perhaps the best understood example of a robust biological system, and we will present it in detail in section 3. Here we will briefly discuss three other manifestations of biological robustness.

First, there is the case of robust pattern formation in development. Development is the process by which a multi-cellular organism, consisting of cells with specialized functions and characteristics, forms from a single cell—a fertilized egg—that divides. While all the cells in the organism have the same genome, the specialization of structure and function

arises because different proteins are expressed within different cells during the process of development. In the case of spatial patterns, signalling molecules called morphogens provide positional information that determines which proteins are expressed [1]. In particular, spatial gradients of morphogen molecules lead to position-dependent protein expression, as cells detect local morphogen concentrations by means of receptors on the cell surface, and this in turn activates a biochemical process that leads to the production of specific proteins. Experiments [7] have shown that patterning is robust with respect to genetic and environmental perturbations that affect the microscopic details of the biochemical processes.

Our second example concerns the life cycle of the λ-phage, a virus that infects bacterial cells. Once the virus enters the interior of a host cell, it goes into one of two possible modes: either the lytic cycle, in which its genome is expressed, causing it to form many copies of itself, thus destroying the host cell; or the lysogenic cycle, in which case it passively integrates its genetic material with that of the bacterium. In the latter mode, it remains latent until the host is placed under stress, at which point it expresses its genes and reproduces as before. The network of biochemical processes that determines which of the cycles a phage chooses is well understood, and it has been shown experimentally [12] that the decision-making process is robust against point mutations in individual genes involved in the network. Again, this indicates that the emergent behaviour is, to an extent, independent of the microscopic details. Rather, it is the presence of positive and negative feedback loops in the *structure* of the network that give rise to the behaviour [1, 10].

Finally, an unpleasant example of robustness in biology is the disease of cancer. The preserved "function" of the system of tumor cells in this case is survival and multiplication, and medical therapies may be thought of as perturbations to the system. The system all too often robustly maintains resistance to therapies by means of genetic diversity, feedback loops, and the use of alternative mechanisms to preserve its function [11].

2.3 Other aspects of robustness

So far, we have discussed manifestations of robustness, and the principles by which robustness is achieved, but we have not discussed why the phenomenon of robustness has arisen in the first place. It has been argued [10] that robustness occurs naturally in evolving, complex dynamic systems, i.e., that the requirements for the emergence of robustness and those for the property of *evolvability* are the same. This is linked to the structure of biological networks, which appears to usually assume the so-called *bow-tie architecture*. This consists of a set of highly conserved core processes (e.g. transcription and translation of DNA) that are linked with diverse inputs (protein signals or nutrient levels) and diverse outputs. The core processes (the knot in the bow-tie) consist of the most important functions and they are rigidly maintained; the inputs and output are both more numerous and more variable, and thus endow the system with the capacity to evolve.

The robustness of the bow-tie architecture arises naturally from the formation of a set of core processes, and it provides the structure with an evolutionary advantage. On the other hand, it has also been shown [5] that robustness against a variety of general perturbations is accompanied by extreme *fragility* with respect to a small set of perturbations. The aeroplane

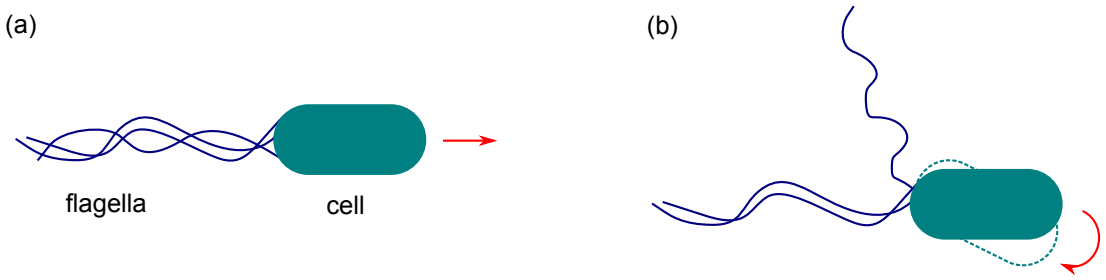

Figure 2: **The run-tumble paradigm for chemotaxis**. *E. coli* consists of a single cell with helical flagella attached. When the flagella rotate synchronously as in (a), they propel the bacterium forwards in a "running" motion. If a flagellum changes the direction of rotation as in (b), the cell "tumbles," i.e. rotates in place.

example can again be used to illustrate the point [10]. The primitive craft built by the Wright brothers did not include a control system to deal with turbulence in the atmosphere, and so it does not have the corresponding property of robustness that is present in modern aircraft. On the other hand, modern aeroplanes are also extremely fragile with respect to a total power failure, whereas a primitive aircraft does not suffer this defect, merely because it isn't electrically powered. Thus there is a trade-off between the acquisition of greater robustness against a large number general perturbations and fragility with respect to a small number of specific perturbations. In order to understand and quantify this trade-off, a universal quantitative measure of robustness itself must be established, and this remains an open challenge for future systems biologists.

3 Robustness in bacterial chemotaxis

3.1 Chemotaxis and exact adaptation

Bacterial chemotaxis refers to the response of bacteria to gradients in specific chemicals. In the case of chemical attractants (including nutrients), bacteria move up the concentration gradient, while they move down a gradient of chemical repellents. The model bacterium used in biological experiments is *Escherichia coli*, which is commonly found in the guts of warm-blooded animals, and the mechanics of how this organism swims towards or away from specific chemicals has been understood since the 1970's [4].

E. coli consists of a single cell with several—typically four to six—helical flagella distributed isotropically on the cell body (see Figure 2). Each flagellum is attached to the cell membrane by means of a reversible rotary motor. When all the flagella rotate in a specific sense (say counterclockwise, CCW) they form a bundle that propels the bacterium forward in a straight path that is termed a "run." Occasionally, one or more of the flagella begins to turn clockwise (CW), causing it to come apart from the bundle; as a result, the cell reorients its direction of travel, and this is called a "tumble."

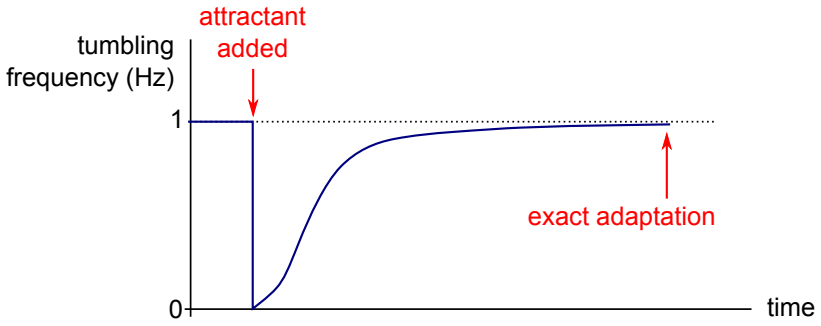

Figure 3: **Exact adaptation in chemotaxis**. The addition of an attractant uniformly throughout the system leads to a sharp drop in the tumbling frequency, but after some time has passed, the cell returns to the steady-state frequency. Adapted from Ref. [1].

In the absence of chemical gradients, the durations of runs and tumbles are distributed exponentially, with mean times of about 1.0 s and 0.1 s respectively. The motion of the bacterium can thus be modelled as a random walk, which effectively explores the surrounding space. If a chemical attractant is present nearby and the bacterium finds itself moving up the gradient, then the probability of the motors switching from CCW to CW is suppressed, resulting in runs being favoured over tumbles (relative to the uniform concentration state). In the random-walk model, this corresponds to the addition of a bias in the direction of the concentration gradient. Thus, the bacterium is able to move along gradients of chemical attractants and repellents.

Now, in the presence of a uniform chemical background, *E. coli* switches between runs and tumbles with an average steady-state tumbling frequency of about 1 Hz. Suppose that at a certain time, a chemical attractant is added abruptly and uniformly throughout the system. Since *E. coli* now senses an increase in the concentration of the attractant irrespective of the direction in which it is travelling, it responds by favouring runs over tumbles, and so the tumbling frequency drops sharply (see Figure 3). However, the cell eventually realizes that there is in fact *no* attractant gradient present, and it gradually returns to the steady-state tumbling frequency. This response is known as *exact adaptation*, and it is robust with respect to a wide range of concentration gradients and biochemical parameters of the response circuit. We now turn to the detailed chemotaxis circuit with the aim of understanding robust adaptation.

3.2 The chemotaxis protein circuit

Bacteria detect molecules using proteins called receptors, which are embedded in the cell membrane. Chemical attractants and repellents can bind to these receptors, and in this context they are referred to as ligands. Inside the cell, the receptors are attached to a kinase, an enzyme that catalyzes phosphorylation in other proteins; we will consider the

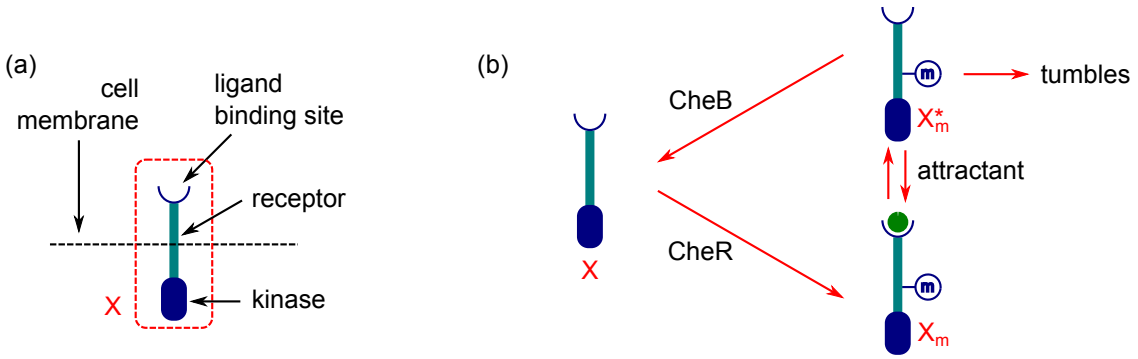

Figure 4: **The chemotaxis network**. (a) Bacteria detect ambient molecules (ligands) using a receptor embedded in the cell membrane. (b) The biochemical pathways in the Barkai-Leibler model. Adapted from Refs. [1, 3].

receptor-kinase system as one entity X, as indicated in Figure 4a.

X assumes two states: an active state X* and an inactive state (just labelled X). In the active state, the kinase phosphorylates a protein known as CheY, and the phosphorylated protein CheY-P can bind to the flagellar motors to increase the probability of switching from CCW to CW rotation. Ligand binding has the effect of modifying the activity of X, that is, it changes the kinetics of the transition between X and X*. In general, attractants lower the activity of X, resulting in a lower concentration of Che-P proteins that bind to the motors to induce tumbles; repellents have the opposite effect.

This simple picture is the first step in understanding chemotactic response, but of course it cannot account for adaptation of any kind: a uniform increase in the concentration of attractant molecules leads to lowered activity of X, but this does not change with time. However, we will see that the addition of a simple alternative pathway to this model can give rise to robust, exact adaptation.

3.3 The Barkai-Leibler model for robust exact adaptation

We saw that the binding of ligands to the receptors directly affects the activity of X, but this alone cannot account for adaptation. There is in fact a second pathway that also modifies the activity of X, namely the process of methylation of the receptor. Barkai and Leibler [3] showed that exact adaptation arises if this process is included. The original model takes into account the fact that there are several sites on the receptor that can be methylated, and all of these affect the activity of X. Here, we will follow a simplified model [1] in which the receptor has just one methylation site; the methylated receptor-kinase complex is denoted X_m.

Figure 4b illustrates the reactions involved in our simplified model. X is methylated by an enzyme called CheR, which operates at a constant rate. Whereas X is always in the inactive state, the methylated form X_m transitions between active and inactive states, and the active state gives rise to tumbles as explained previously. The process of demethylation

is controlled by another enzyme, CheB, which *only* acts on the active state X_m^*. It is also assumed that CheB obeys the standard Michaelis-Menten equation for enzyme kinetics.

This leads to the following equation for the kinetics of the system:

$$\frac{\mathrm{d}}{\mathrm{d}t}\left([X_m] + [X_m^*]\right) = V_R[\mathrm{CheR}] - \frac{V_B}{K + [X_m^*]}[\mathrm{CheB}][X_m^*],$$

where square brackets indicate concentrations of substrates, V_B and V_R are rate constants, and K is a constant associated with Michaelis-Menten kinetics. The steady-state activity is obtained by setting the left-hand side equal to zero, and solving for the activity $[\mathrm{X}_\mathrm{m}^*]$. The response of the system following the abrupt, uniform addition of an attractant is governed by this differential equation (together with appropriate initial conditions), and the system displays exact adaptation as illustrated in Figure 3. Moreover, while precise value of the steady-state activity—and hence the steady-state tumbling frequency—is fine-tuned relative to the parameters K, V_B, V_R, [CheB] and [CheR], the occurrence of exact adaptation is not: that is, exact adaptation occurs in a large volume of the experimentally accessible parameter space.

The validity of this model ultimately rests on experiment. It has been shown [2] that exact adaptation in *E. coli* is in fact basically independent of the concentrations [CheR] and [CheB]. In particular, concentrations of [CheR] were varied from 0.5 to 50 times the naturally-occurring levels using generically engineered strains of *E. coli*, and exact adaptation was observed throughout.

4 Conclusion

We have attempted to present a summary of the status and importance of robustness in modern biology, particularly in the context of the emerging science of systems biology. At present, there exists no general theory of robustness in biological networks, and so the topic remains somewhat abstract: while we can isolate general principles that are associated with robustness, there is a need for more precision in the concepts and a greater degree of quantification. Nevertheless, we have seen that there are several examples in which robustness plays an important role. In particular, we saw that robustness can be used as an effective guide to model building in the case of bacterial chemotaxis. It remains to be seen if robustness will evolve into a universal concept that affects all of modern biology, and if it can be used in a systems approach to treating diseases such as cancer.

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