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Systems biology: The reincarnation of systems theory applied in biology?

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Abstract

With the availability of quantitative data on the transcriptome and proteome level, there is an increasing interest in formal mathematical models of gene expression and regulation. International conferences, research institutes and research groups concerned with *systems biology* have appeared in recent years and systems theory, the study of organisation and behaviour *per se*, is indeed a *natural* conceptual framework for such a task. This is, however, not the first time that systems theory has been applied in modelling cellular processes. Notably in the 1960s systems theory and biology enjoyed considerable interest among eminent scientists, mathematicians and engineers. Why did these early attempts vanish from research agendas? Here we shall review the domain of systems theory, its application to biology and the lessons that can be learned from the work of Robert Rosen. Rosen emerged from the early developments in the 1960s as a main critic but also developed a new alternative perspective to living systems, a concept that deserves a fresh look in the post-genome era of bioinformatics.

INTRODUCTION

We see an ever-increasing move towards inter- and trans-disciplinary attacks upon problems in the life sciences. The reason is the diversity of organisation and behaviour in natural systems. The size of data sets and complexity of patterns hidden in them has led to a renewed interest in mathematical techniques that allows us to identify formal models of natural systems. The next step in the post-genome era is not simply assigning biological function to identified genes but to analyse the organisation and control of genetic pathways. These pathways are of course dynamic systems; non-linear, adaptive and anticipatory systems to be precise.

Systems biology is an emerging field of biological research that aims at a system-level understanding of genetic or metabolic pathways by investigating *interrelationships* (organisation or structure) and *interactions* (dynamics or behaviour) of genes, proteins and metabolites. Recently, international conferences, institutes,[1,2] research groups and articles,[2] focusing on systems biology, have appeared. The reason for this renewed interest in systems thinking is the rapid technological advance in the area of genomics. Genomics is the field of biological research taking us from the DNA sequence of a gene to a structure of the product for which it codes (usually a protein) to the activity of that protein and its function within a cell and, ultimately, the organism. Crossing several scale-layers from molecules to organisms, we find that organisms, cells, genes and proteins are defined as complex structures of *interdependent* and subordinate *components* whose relationships and properties are largely determined by their function in the whole. This definition coincides with the most general definition of a system as a set of components or objects and relations among them.[3] Systems theory is then the study of organisation and behaviour *per se* and a natural conclusion is therefore to consider systems biology as the application of systems theory to genomics.

The idea to use systems theory in biology is, however, not new; notably in the 1960s a number of eminent

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researchers took a systems approach to 'search for general biological laws governing the behavior and evolution of living matter in a way analogous to the relation of the physical laws and non-living matter'.[4–6] It was the transfer of ideas from physics to biology and the perception that biological systems are a special case of physical systems that led to criticism which cumulated in the most comprehensive discussion of the limitations of 'classical' systems biology in the work of Robert Rosen.[7–10] In the following sections, we review the need for mathematical modelling, the usual approaches to modelling biological systems and problems arising from them. In this paper we will focus on Rosen's relational biology, 'metabolic-repair' (M,R)-systems, his discussion of anticipatory behaviour and causality. We show that, for metabolism and repair defined as mappings, replication is implicitly defined. Anticipatory behaviour or intrinsic control is realised through the boundary conditions of the repair and replication map. Finally, it can be shown that the category that defines the (M,R)-system is rich enough in entailment to allow the repair and replication maps to be entailed by something and hence avoiding a finality argument when discussing causal entailment.

THE CASE FOR MATHEMATICAL MODELLING

The engineering sciences are a good example of how mathematics has been used effectively in a wide range of applications. One could argue that many biologists find themselves now in a similar situation to engineers about six decades ago when they were faced with the need to analyse and control complex dynamic systems for which empirical means are inappropriate. Also, both species, engineers and biologists, are not born as mathematicians. Engineers have learned to use mathematics towards their ends and a symbiosis of researchers from both areas should allow both to advance successfully. For the engineer, the underlying strategy is to represent the natural system by a set of random and/or state variables and then to investigate relationships among those variables within a formal system (Figure 1). This approach cumulates into a philosophy whereby, as Henri Poincaré suggested, 'the aim of science is not things in themselves but the relations between things; outside these relations there is no reality knowable.'[11]

The importance of what we now call systems biology was pointed out by Norbert Wiener in his book 'Cybernetics, on Control and Communication in the Animal and the Machine', published in 1948.[12] In 1970, cybernetics or feedback regulatory mechanism on a molecular level were described by Jacob and Monod[13,14] who investigated regulatory proteins and the interactions of allosteric enzymes in particular. Organisms as a whole are self-regulating, adaptive and anticipatory systems and numerous examples have been published. While the control of physiological mechanisms requires the processing of *information*, the actual processes are sustained by *energy*

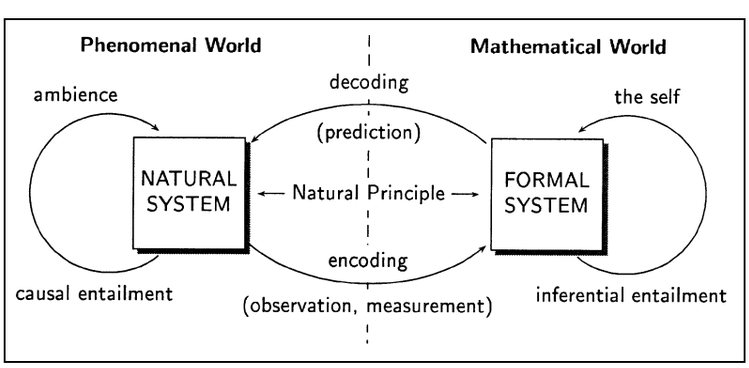

Figure 1: The modelling relation between a natural system *N* and a formal system *F*.[8] If the modelling relation brings both systems into congruence by suitable modes of encoding and decoding, it describes a *natural principle*. In this case *F* is a *model* of *N*, that is, *N* is a realisation of *F*

obtained from the environment. The acquisition, transfer and utilisation of energy have subsequently been seen as major components in the analysis of biological systems.[15] Systems biology has a past and the books by Ashby[4] and Bertalanffy[5] are a 'must read' for anyone attracted to the area of systems biology. Bertalanffy provides a general introduction of system theory but also reviews applications in biology with a discussion on models of open systems and organisms considered as physical systems. For an up-to-date account of the systems sciences, including a historical perspective, the reader is referred to Klir's book[3] and the Principia Cybernetica Web.[16] Specifically referring to applications in biology, the volume 'Systems Theory and Biology' edited by Mihajlo Mesarović[17] is valuable. Minhajlo Mesarović initiated and developed one of the most comprehensive mathematical systems theories.[18,19] The most extensive discussion of systems thinking in biology is James G. Miller's book on a 'general theory of living systems'.[20] Miller provides the most detailed account of living systems in eight levels of increasing complexity – from molecules to cells, organs, organisms and societies. Reality is described as a continuous dynamic process, best represented as a system of systems and natural systems are studied as a structure of processes evolving through spatio-temporal events. The conclusion is that despite the endless complexity of life, it can be organised and repeated patterns appear at different levels. Indeed, the fact that the incomprehensible presents itself as comprehensible has been a necessary condition for the sanity and salary of scientists.

CAUSING PROBLEMS

Causality

The principal purpose of mathematical models applied in the natural sciences is to identify sets of rules, statements about local *associations* or *dependencies* among variables. In genomics, mathematical models may be expected not only to *describe* associations but also to *explain* dependencies among genes. A 'causal law', which is not strictly bound to any specific philosophical perspective, is then understood as a 'causal dependency', a general proposition by virtue of which it is possible to infer the existence of an event from the existence of another. It is the explanatory aspect of mathematical modelling that leads us to the limits of systems biology but it is also the most exciting aspect of the developments in the post-genome area. We find that the 'causal problem' is an ontological, not a logical question, it cannot be reduced to logical terms but it can be analysed with the help of formal reasoning. In the words of Bertrand Russell: 'Inferences of science and common sense differ from those of deductive logic and mathematics in a very important respect, namely, when the premises are true and the reasoning correct, the conclusion is only *probable*.'[21]

The first comprehensive theory of causation was Aristotle's. It distinguishes four types of cause: the material cause (or stuff), the formal (formative) cause (or shape), the efficient cause (or force) and the final cause (or goal). For a formal logical system, given an 'effect', say proposition P, axioms correspond to the material cause of P, production rules are understood as the efficient cause of P and the specification of particular sequences of production rules or an algorithm is identified as the formal cause. For a dynamic system a state can itself be entailed only by a preceding state. If for a chronicle $\{(n, f(n))\}$ we ask *why* the nth entry gives the particular value $f(n)$, the answer is *because* of the initial condition $f(0)$, ie $f(0)$ is the material cause; and *because* of a state transition mapping T for which $f(n+1) = T[f(n)]$, ie T corresponds to the efficient cause; and *because* of exponent n from which $f(n)$ is obtained by iterating the transition map n times beginning with $f(0)$; ie n refers to the formal cause. As shall be discussed in further detail below, in Rosen's relational biology, for a component $f: A \rightarrow B$, such that $a \mapsto f(a)$, the question 'why $f(a)$?' is answered by 'because f' and

'because a'. In other words, 'a entails $f(a)$' or formally $f \Rightarrow (a \Rightarrow f(a))$. Here f corresponds to the efficient cause of ('effect $f(a)$'), and a refers to the material cause of $f(a)$. One of Rosen's achievements is that he introduced a formalism rich enough in entailment to allow final causation without implying teleology. The conceptual framework in which he developed his *relational biology* is category theory.[22,23]

TOWARDS A RELATIONAL BIOLOGY

The problems of applying systems theory in biology can be summarised by (a) the difficulty of building precise and yet general models, (b) the 'openness' of biological systems, the fact that these systems are hierarchical and highly interconnected, and (c) that models based on differential equations cannot represent anticipatory behaviour as present in cellular processes.

Modelling systems with sets of first order differential equations,

$$\frac{\mathrm{d}f_j}{\mathrm{d}t} = \phi_j(f_1, \ldots, f_r), \quad j = 1, \ldots, r$$

the rate of change of observable (state variable) f_j depends *only* on present and past states but cannot be dependent upon future states. In other words, these systems can only be *reactive* but not *anticipatory*.[8] The reactive paradigm embodies one of the most important assumptions of science: effects should not precede their causes. And yet simple biological systems suggest the notion of *self-reference*, an implicit model of knowledge of itself. The following example of a biosynthetic reaction network is due to Robert Rosen[8] (see also Casti[24]). Let metabolites A_i represent the substrates for the enzymes E_i that catalyses it at stage i. As illustrated in Figure 2, the initial substrate A_0 activates the enzyme E_n (ie increases its reaction rate). Under the foregoing hypotheses, with concentration A_0 at time t the concentration of A_n at some future time is predicted in order to maintain homeostasis in the pathway. The ambient concentration of A_0 serves as a *predictor*, which in effect 'tells' the enzyme E_n that there will be an increase in the concentration A_{n-1} of its substrate, and thereby pre-adapts the pathway so that it can deal with the expected changes.

The second problem faced by representing cellular processes with sets of linear differential equations is captured by Zadeh's uncertainty principle:[25]

> As the complexity of a system increases, our ability to make precise and yet significant statements about its behavior diminishes until a threshold is reached beyond which precision and significance (or relevance) become almost exclusive characteristics.

The problem is that perturbations to cells have multi-gene/multi-transcript/multi-protein responses, 'closing' the system, ie restricting the model to a small set of variables, inevitably leads to an often unacceptable level of uncertainty in the inference.

The tradition of describing cellular systems in terms of energy and masses with forces acting on them is rooted in the realm of Newtonian mechanics. In this context a system is closed by internalising external influences through added state variables and more parameters to the system. Take for example the simplest of dynamical systems, a single

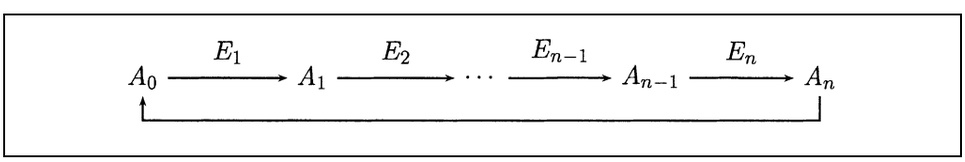

Figure 2: An anticipatory chemical reaction network

particle moving along a line under the action of a constant force. The motion is governed by Newton's Second Law, which defines the force F acting on a mass point m to be the rate of change of momentum (mv):

$$F = m\frac{dv}{dt} = m\frac{d^2x}{dt^2}$$

with v denoting the velocity which, in turn, is defined as rate of change of position or displacement x from some origin of coordinates. Conceptual closure amounts to the assumption of constancy for the external factors and the fact that external forces are described as a function of something inside the system:

$$F(x, v) = -\theta x$$

where θ is a parameter specific to the system under consideration. Rewritten as a set of first order differential equations, this system has two *state variables*, f_1 denoted by x and f_2 denoted by v, where

$$\frac{dx}{dt} = v \text{ and } \frac{dv}{dt} = -\frac{\theta}{m}x$$

Relational biology

The model is deterministic in that the object's state at time t is fully determined from the initial conditions (if known) and therefore permits the prediction of future states by integrating the set of differential equations. Newton's laws of motion, which state that the acceleration of an object is directly proportional to the force acting on it and inversely proportional to its mass, imply that the future behaviour of a system of bodies is determined completely and precisely for all time in terms of the initial positions and velocities of all the bodies at a given instant of time, and of the forces acting on the bodies. These forces may be *external forces*, which arise outside the system investigated, or they may be *internal forces* of interactions between the various bodies that make up the system in question. Rosen described the response of a system to forces as the 'inertial' aspect while the exertion of forces by the system corresponds to the system's 'gravitational' aspect. He suggested a shift attention from exclusively 'inertial', ie structural aspects such as the DNA molecule and its sequence, to 'gravitational' concepts. Instead of concerning us with material causation of behaviour, manifested in state sets, he suggested formal and efficient causations as the focus of attention. Such a shift of perspective is exemplified in category theory, Rosen's preferred language to discuss these problems in the abstract, by studying mappings between sets (of objects) rather than analysing the objects themselves.

Phenotypes are what we can observe dirctly about organisms. They are tangible, material properties that we can measure, can compare and experiment with. The phenotype is seen as being 'caused' or 'forced' by the genotype. As Rosen points out,[10] the phenotype–genotype dualism is allied to the Newtonian dualism between states and the forces that change the states. In Aristotelian language, the states represent material causation of behaviour, while the forces are an amalgam of formal and efficient causation. Biological phenotypes, considered as material systems, are open. They are open to 'forcing' by genes as well as open to interactions with their environment. To study an open system it is therefore necessary to consider the 'outside', the environment, in order to understand what is going on 'inside'. The Newtonian paradigm, underlying the traditional approach to modelling biological systems, is frequently seen as synonymous with reductionism and its failure to supply the whole from its parts. On the basis of this analysis and continuing the work of Rashevsky, Rosen argued his case for a new approach, called *relational biology*. He emphasised that we must look for principles that govern the way in which physical phenomena are organised, principles that govern the *organisation* of phenomena, rather than the phenomena themselves. Relational biology is therefore about organisation and describes entailment without states. The association of energy or matter, described by states

and dynamical laws, is to be replaced by the description of a system in terms of its components, their function and contribution to the organisation of the system. An example of this approach for molecular systems is Rosen's concept of metabolism-repair or (M,R)-systems.

METABOLISM-REPAIR SYSTEMS

Driven by technological advances and the sequencing of genomes, at present, more hypotheses are generated than tested. However, with the availability of data, biologists will soon return to refined biological questions, 'zooming in' to specific genetic pathways. With the boom in bioinformatics, the attempts to explain genetic systems are likely to proceed from the Cartesian metaphor, viewing organisms as performing computations, describing biological principles in the same way as *machines* are. This tradition has its roots in Newtonian mechanics and formal logic, embodied in reductionism. As we witness a shift of focus from molecular charaterisation to an understanding of functional activity in genomics, this strategy is prone to repeat historical failures as outlined in Rosen's 'Comprehensive inquiry into the nature, origin, and fabrication of life'.[9] As bioinformaticians dream of *in silico* models of cellular systems, Rosen developed a new biology *on paper*. Starting from the *modelling relation*, illustrated in Figure 1, he began by considering two natural systems N_1 and N_2 as analogues when they realise a common formalism F. This relation of analogy between natural systems is then independent of their material constitution. The formal system F is *relational*, consisting of a set of formal, interrelated, *components*. Any two natural systems that realise this formalism are said to manifest a common *organisation*. In relational biology a component is defined by a *mapping*

$$f : A \rightarrow B$$

where the 'identity' of the component is embodied in the mapping itself, while the influence of surrounding components of the natural system N and the external environment are embedded in the specific *arguments* in the domain A on which the mapping can operate.

(M, R)-systems

The previous section introduced the anticipatory character of biological systems. The basis for anticipatory behaviour is a form of self-reference on internal modelling. A *cell* is a good example of a self-referential system. We can describe a cell functionally as consisting of two major functional components, reflecting the morphological partition between nucleus (genome) and cytoplasm (phenome). The *metabolic* or ergonic component represents its basic chemical activity through the acquisition, transfer and utilisation of energy. The *repair* or cybernetic component ensures continued viability of the cell in the face of external disturbances. The latter requires the processing and utilisation of information to permit the control of what the cell does and characterising its temporal characteristics. Essential for the maintenance of life, both components are closely interrelated in jointly sustaining the steady state.[15] Rosen devised a class of relational cell models called metabolism-repair (M,R)-systems to characterise the minimal organisation a material system would have to manifest or realise what is called a *cell*.[9] The present section addresses Rosen's answer to the problems of causation and anticipatory behaviour described above. We are going to review Rosen's arguments and show (in the abstract) that the presence of 'metabolism' and 'repair' components imply the existence of a 'replication' principle. The key point is that replication comes without infinite regress in modelling and hence allows the discussion of final causation while avoiding the explanation of phenomena by the purpose they serve rather than by postulated causes (teleology). To achieve this, we require a conceptual framework rich enough in entailment – such as category theory.

Let A represent the set of environmental inputs to the cell, while B

is the set of outputs, ie products the cell is capable of producing. The mapping f could be described as an abstract 'enzyme', which converts substrate $a \in A$ into 'product' $b \in B$:

$$f : a \to B, \qquad f \in \mathcal{H}(A, B)$$
$$a \mapsto f(a) = b \tag{1}$$

Further, let $\mathcal{H}(A, B)$ be the *set* of metabolisms that are realisable by the cell, ie a set of mappings from A to B. As pointed out by Casti,[26] the set of physically realisable cellular metabolisms $\mathcal{H}(A, B)$ is determined by various physicochemical constraints and the classical Newtonian machinery has been used to capture many aspects of the cell's metabolic activity in respect of the mapping f above. However, both Rosen and subsequently Casti have argued that these formalisms lack a structure to account for *repair* and *replication*. The purpose of repair is to stabilise cellular metabolic activity against fluctuations and disturbances in both its environmental inputs and in the metabolic map f itself. In other words the repair is to copy f while we refer to replication as the process of copying the repair mechanism.

To arrive at a repair mechanism we consider the following diagram:

$$A \xrightarrow{f} B \xrightarrow{g} C \tag{2}$$

Causal entailment

In the diagram, a entails $f(a)$ and referring to the discussion in the section on 'causing problems' we can answer the question 'why $f(a)$?' in two ways: because a entails $f(a)$ and because f acting on a entails $f(a)$. We can summarise the entailment in the diagram by

$$\forall a \in A,\ f \Rightarrow (a \Rightarrow f(a))$$

and

$$g \Rightarrow (b \Rightarrow g(b)) \quad \forall b \in B$$

If an element $b \in B$ is entailed, then it must lie in the range of mapping f and we can write $f(a) = b$ for some element a in the domain of f and obtain

$$g \Rightarrow (f(a) \Rightarrow g(f(a)))$$

Suppose the set C in the diagram denotes the collection of mappings from A to B, $\mathcal{H}(A, B)$, we then find that g in fact generates a new f for any $b \in B$. In other words, $g(b)$ is itself a mapping such that g entails f:

$$g(f(a)) = f$$

In this case we denote this '*repair map*' by Φ and illustrate the repair process by the following augmented diagram:

$$A \xrightarrow{f} B \xrightarrow{\Phi} \mathcal{H}(A, B)$$

To allow some form of internal control, the repair map Φ converts the abstract products b into new versions of f:

$$\Phi : B \to \mathcal{H}(A, B) \tag{3}$$

For any specific activity, we denote the metabolism for which the cellular process is designed by f^*; ie in the absence of disturbances, given the environmental input $a^* \in A$, f^* produces the cellular output $b^* \in B$. If there is a disturbance to the metabolic function f^* or a change from the environment a^*, the cell 'repairs' the situation by generating new f^* for any b^*. The repair or control is implicit in the *boundary condition* of the repair map Φ. If there is neither a change from the metabolic map f^* nor from the environment a^*, then Φ ought to produce f^*:

$$\Phi_{f^*}(b^*) = f^*$$

stabilising the cell's metabolic behaviour in response to external influences and/or errors. While in the simple diagram (1), representing a *metabolism*, we could answer the question of 'why $f(a)$', f itself was unentailed. The finality argument would be to answer 'because f', f is to bring A into B and yet f is itself unentailed if we had not Φ in place. However, with the introduction of the *repair* function $\Phi \Rightarrow (f(a) \Rightarrow f)$, the question 'why f?' is answered 'because Φ', Φ being the efficient cause of f and 'because $f(a)$', where f is entailed by its value, the material cause.

The construction of the repair map

immediately poses the question to what replicates $\mathbf{\Phi}$? One solution is to add yet another function to the diagram (2) but this would lead to an infinite regress in the discussion of causal entailment. The cell's metabolic processing apparatus, through information stored in the DNA, allows replication and it was Rosen's major achievement to show that, using category theory,[22,23] replication is in fact already built into the scheme outlined in diagram (2). Although we can add a replication map to the diagram, we do not need to argue for this map through an addition to (2) as it already implicitly exists.

Category theory

To arrive at this conclusion, we view the quadruple $(A,\ B,\ f,\ \mathbf{\Phi})$ as a simple (M,R)-system on the *category* **C**. A category comprises a collection of *objects* such as $A,\ B$ and associated *arrows* (mappings) such as for example $f\colon A \to B$, where A is the *domain* of f and B its *co-domain*. The collection of all mappings with domain A and co-domain B is denoted $\mathcal{H}(A,\ B)$. We suppose that **C** is a concrete category, ie its objects are structured sets and its arrows are mappings compatible with their structure. If **C** is closed under cartesian products, ie if $A,\ B,\ C,\ D$ are objects of **C**, $f \in \mathcal{H}(A,\ B)$, and $g \in \mathcal{H}(A,\ B)$ are maps of **C**, then $A \times C$ and $B \times D$ are objects of **C**, and $f \times g \in \mathcal{H}(A \times C,\ B \times D)$, where $(f \times g)(a,\ c) = (f(a),\ g(c))$, then for $\mathcal{H}(A,\ B)$ defining an object in **C**, we introduce for this special case a new notation:

$$B^A = \{f \mid f : A \to B\}$$

In (3) above we have in fact assumed that $\mathcal{H}(A,\ B) = B^A$ is an object in the category to which A and B belong because only then can B^A be the range of another mapping in the category and hence can be entailed within the category. In other words, for $\mathbf{\Phi}$ to entail f, the *exponential* (function set or map object) B^A must exist. Note that B^A does not necessarily exist as an object in **C**; there is, for example, no analogous construction in the category of monoids. If B^A exists as an object of the category **C**, it is associated with the existence of a special *evaluation mapping e*:

$$e_f : (B^A \times A) \to B, \qquad (4)$$

$$(f,\ a) \mapsto e_f(f,\ a) = f(a)$$

Note that we use subscript f in e_f not to denote a dependency on f but to distinguish it from evaluations associated with maps other that f.

Returning to our cellular (M,R)-system

$$A \overset{f}{\to} B \overset{\mathbf{\Phi}}{\to} B^A \qquad (5)$$

the map $\mathbf{\Phi}$ is an element of the set of mappings from B to the set of mappings from A to B:

$$\mathbf{\Phi} \in (B^A)^B$$

For this *set* to exist as an *object* in the category **C**, following the general model (4), there then must exist the evaluation map

$$e_{\mathbf{\Phi}} : ((B^A)^B \times B) \to B^A, \qquad (6)$$

$$(\mathbf{\Phi},\ b) \mapsto e_{\mathbf{\Phi}}(\mathbf{\Phi},\ b) = \mathbf{\Phi}(b)$$

The existence of the evaluation map $e_{\mathbf{\Phi}}$ can be explained as follows. The value $f = \mathbf{\Phi}(b)$ can be viewed as depending on *two* things: b as well as $\mathbf{\Phi}$. Although we do not usually think that way with functions, there is no reason why $\mathbf{\Phi}$ is fixed in the setting of (M,R)-ststems. We can express this dependency on both $\mathbf{\Phi}$ and b as a two-valued mapping

$$(\mathbf{\Phi},\ b) \mapsto f$$

Suppose we want to *evaluate* this map, which we denote by h for now: we can describe this as a two-step process which effectively turns the mapping $h(\mathbf{\Phi},\ b)$ of two variables into a map $H(\mathbf{\Phi})$ of one variable $\mathbf{\Phi}$ but with values $H(\mathbf{\Phi})$ which are a function of the second variable b. The formal definition of this map H reads

$$(H(\mathbf{\Phi}))(b) = h(\mathbf{\Phi},\ b) \qquad (7)$$

where $\mathbf{\Phi} \in (B^A)^B$ and $b \in B$.

Here each value $H(\Phi)$ is a function of b, hence an element of the exponential set:

$$(B^A)^B = \{\Phi \mid \Phi : B \to B^A\}$$

such that

$$H\colon B \to (B^A)^B$$

In formula (7) on the left-hand side the mapping $H(\Phi)$ is evaluated at argument b and h may therefore be called an *evaluation map* and denoted by e_Φ, leading us to the definition in (6).

Our reasoning so far can be summarised as follows. For Φ, the repair of f, being entailed by something (being replicated), it is required that the set of mappings from B to B^A exists as an object in **C**. Then, if such a map object (exponent) exists, it is associated with the evaluation map e_Φ. The evaluation map in turn was explained by the bijection

$$\frac{h : (B^A)^B \times B \to B^A}{H : B \to (B^A)^B}$$

between functions h in two variables and those H in one variable but which maps into $(B^A)^B$, the space in which Φ resides! In other words, given the metabolic function $f\colon A \to B$, and repair map $\Phi : B \to B^A$, these imply the replication of Φ. With replication of Φ in place, we can introduce a *replication map*, denoted Υ,

$$\Upsilon : B^A \to (B^A)^B \tag{8}$$

such that Φ is entailed by f. As previously defined for the repair map, the boundary condition for a stable operation is $\Upsilon(f) = \Phi_f$. The boundary conditions are important as they define the (M,R)-systems as a controlled process. In conventional control engineering the existence of a separate control component is assumed. The control action is an external influence on the process and we may refer to this type of control as extrinsic (exogenous). For (M,R)-systems there is no direct control input and the separation between controller and process is not recognisable (intrinsic or endogenous control). Instead the 'anticipatory regulation' is implicit in the boundary conditions for Φ and Υ. The boundary conditions imply an internal self-model of the cell. Given A, B and $\mathcal{H}(A, B)$, it is possible to directly construct the maps Φ_{f^*} and Υ_{f^*}, ie repair (of metabolism f) and replication (of the repair map Φ) emerge 'naturally' from the existence of an abstract metabolic component. An argument in support of theoretical or mathematical biology is that such results, abstract they may be, are neither the outcome of *in vivo*, *in vitro* or *in silico* analysis but can also be obtained, *on papyrus*. . ..

We can realise an (M,R)-system in different ways and initially automata theory was considered. However as demonstrated by John Casti,[26] since Rosen introduced the concept, considerable advances in the mathematical theory of dynamic systems should enable us to take his ideas further. Casti developed a theory of *linear* (M,R)-systems.[27] In the model above we can consider a as an input time-series leading to output b. The input/output space A and B are then finite-dimensional vector spaces whose elements are sequences of vectors from $\mathbb{R}^m$ and $\mathbb{R}^p$ respectively:

$$A = \{a\colon a = [u_0, u_1, \ldots, u_N]\}, \quad u_i \in \mathbb{R}^m$$
$$B = \{b\colon b = [\gamma_1, \gamma_2, \gamma_3 \ldots]\}, \quad \gamma_i \in \mathbb{R}^p$$

Mathematical causation is acknowledged by the fact that the first output appears one discrete time step after the first input. If f is further assumed to be linear and constant (autonomous), we can express the relationship between cellular inputs and outputs by the following equation:

$$\gamma_t = \sum_{i=0}^{t-1} A_{t-i} u_i, \qquad t = 1, 2, \ldots \tag{9}$$

where $A_k \in \mathbb{R}^{p \times m}$ denotes the coefficient matrix that characterises the process.

The (M,R)-system consists of $f\colon A \to B$ such that $f(a^*) = b^*$ plus $\Phi\colon B \to B^A$ such that $\Phi(b^*) = f^*$. With a linear realisation (9) we are now in a position to investigate how the (M,R)-system restores or stabilises disturbances in the cellular environment a and/or metabolic map f. A change in the

external environment, $a^* \rightarrow a$, for a fixed metabolic map f^* leads to $f^*(a) = b^{\text{new}}$ subsequently to $\Phi(f^*(a)) = f^{\text{new}}$. For a stable process, we require that $f^*(a) = f^*(a^*)$ or $f^{\text{new}}(a) = b^*$ for the cell to recover fully from the disturbance. The cell's metabolic activity would be permanently changed to f^{new} if $\Phi(f^{\text{new}}(a)) = f^{\text{new}}$. If we had $\Phi(f^{\text{new}}(a)) = f^*$, then the cell's metabolism would only undergo periodic changes cycling back and forth between f^* and f^{new}.

In case of a fixed environment a^*, fixed repair map Φ_{f^*} with a disturbance $f^* \rightarrow f$, we require $\Phi_{f^*}(f(a^*)) = f^*$ in order to restore the original design-metabolism f^*. This in fact describes a map $f \mapsto \Phi_{f^*}(f(a^*))$. Let us denote this map as follows

$$\Psi_{f^*,a^*} : B^A \rightarrow B^A$$

$$f \mapsto \Phi_{f^*}(f(a^*))$$

We may find that for disturbances f the repair mechanism stabilises the system to $\Phi_{f^*}(f(a^*)) = f^*$ but in some cases the system could settle for the new metabolism f such that $\Phi_{f^*}(f(a^*)) = f$. This situation is represented by fixed points of the map Ψ_{f^*,a^*}. One such fixed point is of course f^*, for which our basic system is working normally such that $\Phi_{f^*}(f^*(a^*)) = f^*$.

Casti addresses other biological questions such as mutations and Lamarckian inheritance.[24,26] We may conclude that Rosen's somewhat abstract formulation of (M,R)-systems, initially argued for by calling upon category theory and thereby allowing us to reason about more fundamental properties of cellular systems, has also more 'applied' formulations in the form of sequential machines and linear dynamic systems. The formal tools required for such an analysis are familiar to control engineers. John Casti described various properties of such systems and established further links of these ideas to a number of other areas of science and engineering.[24,27] The, for many, unexpected link between biological questions and engineering analysis should encourage control engineers in particular to take an interest in systems biology. We can expect that over the coming years new technology will allow us to measure gene expression in time. Similar approaches to those discussed here should then be developed to study gene interactions.

CONCLUSIONS AND DISCUSSION

The principal aim of systems biology is to provide both a conceptual basis and working methodologies for the scientific explanation of biological phenomena. System theory is not a collection of facts but a way of thinking, which can help biologists to decide which variables to measure and to validate their 'mental models'. Frequently it is the *process* of formal modelling rather than the mathematical model obtained that is the valuable outcome (Figure 3). In engineering it is a common experience that we often learn most from those models that fail. The purpose of a conceptual framework is therefore to help explain unknown relationships, to make predictions and to help design

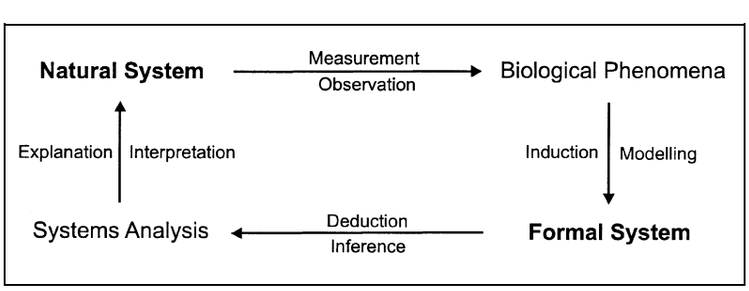

Figure 3: Systems biology: systems thinking in genomics

experiments, suggesting to us which variables to measure and why. Or, as the mathematician David Hilbert once noted, we might think that 'there is nothing more practical than a good theory'.

The need for mathematical models becomes apparent as we begin to analyse the organisation and control of genetic pathways. The complexity of molecular processes combined with the difficulties in observing them and measuring quantitative data lead inevitably to uncertainty in their analysis. Mathematical models, providing sufficiently accurate numerical predictions, are possible in some cases as demonstrated in the areas of metabolic engineering and control. With applications in biotechnology the inner structure of models in this area is less important than the ability to replicate observable phenomena in simulations. If however, on the other hand, we are trying to answer more fundamental questions regarding the mechanisms, principles or causal entailment in genetic pathways, we find that the ancient problem of causality haunts us once again.

Differential equations may be used to model a specific form of causal entailment in natural systems; the equations by themselves, however, do not state that changes are *produced* by anything, but only that they are either *accompanied* or *followed* by certain other changes. Considering $\mathrm{d}f/\mathrm{d}t = \phi(t)$ or equivalently $\mathrm{d}f = \phi(t)\mathrm{d}t$, it merely asserts that the change $\mathrm{d}f$ undergone during the time interval $\mathrm{d}t$ equals $\phi(t)\mathrm{d}t$. The notion of causality is not a syntactic problem but a semantic one; it has to do with the interpretation rather than with the formulation of theories or formal systems. In other words, hypothesising causal entailment in general, and gene/protein interactions in particular, remains a task of the biologist, possibly supported by his or her *choice* of mathematical model (conceptual framework). As problems of genomics become conceptual as well as empirical, and models are expected to explain principles rather than just simulating them, we are therefore likely to witness interesting debates on the merits of alternative theories.

Scientific theories deal with concepts not with reality, and mathematical models are representations, not reflecting what things are in themselves. All theoretical results are derived from certain formal assumptions in a deductive manner. In the biological sciences, as in the physical sciences, the theories are formulated as to correspond in some useful sense to the real world, whatever that may mean. Energy or matter is the primary object of physics. Its study in the phenomenal world is based on changes and for anything to be different from anything else, either space or time has to be presupposed, or both. Immanuel Kant identified the concepts of space, time and causality as *a priori* and therefore conditional for experience. Changes in space and time are the essence of causal entailment and as the philosopher Arthur Schopenhauer discovered, the subjective correlative of matter or causality, for the two are one and the same, is the *understanding*. 'To know causality is the sole function of the understanding and its only power. Conversely, all causality, hence all matter, and consequently the whole of reality, is only for the understanding, through the understanding, in the understanding'.[28] In his famous essay 'What is life?',[29] the physicist Erwin Schrödinger comes to the conclusion that 'our sense perceptions constitute our sole knowledge about things. This objective world remains a hypothesis, however natural', echoing Albert Einstein's observation that 'as far as the laws of mathematics refer to reality, they are not certain; and as far as they are certain, they do not refer to reality.'

The work of Robert Rosen is important in that he not only identified the weaknesses of our common approach to represent natural systems but he also outlined a possible way to transcend the reactive paradigm in order to obtain representations of anticipatory systems. Rosen was looking for ways to characterise molecular and genetic systems

in a general way and quite independently of their physical or chemical constitution. His (M,R)-systems, which are reviewed here, are unlikely to become a methodology that is *useful* to biologists. However, they serve as an example of a mathematical study of basic biological principles. His conceptual framework arose from a criticism of the transfer of principles of Newtonian physics to biology. It is in this context that his work deserves renewed interest in the post-genome era of biology and bionformatics.

One of the challenges for the emerging field of systems biology is then to link abstract mathematical models, like for example (M,R)-systems, to specific current problems of genomics. An important difference from the 1960s is the availability of three types of gene expression data at different levels: genome level (sequences), transcriptome level (microarrays) and proteome level (mass spectroscopy, gel techniques). In particular with microarrays we can now conduct time course experiments, generating data suitable for time-series analysis. With the shift of focus from molecular characterisation to an understanding of functional activity in genomics, systems biology can provide us with methodologies to study the organisation and dynamics of complex multivariable genetic pathways. What are then the conditions for systems biology to succeed?

Systems biology

Mihajlo Mesarović wrote in 1968 that 'in spite of the considerable interest and efforts, the application of systems theory in biology has not quite lived up to expectations . . . one of the main reasons for the existing lag is that systems theory has not been directly concerned with some of the problems of vital importance in biology.' His advice for the biologists was that progress could be made by more direct and stronger interactions with system scientists. 'The real advance in the application of systems theory to biology will come about only when the biologists start *asking questions* which are based on the system-theoretic concepts rather than using these concepts to represent in still another way the phenomena which are already explained in terms of biophysical or biochemical principles . . . then we will not have the ''application of engineering principles to biological problems'' but rather a field of *systems biology* with its own identity and in its own right.'[6]

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