Experimental Physiology – *Paton Lecture*

Claude Bernard, the first systems biologist, and the future of physiology

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The first systems analysis of the functioning of an organism was Claude Bernard's concept of the constancy of the internal environment (*le milieu intérieur*), since it implied the existence of control processes to achieve this. He can be regarded, therefore, as the first systems biologist. The new vogue for systems biology today is an important development, since it is time to complement reductionist molecular biology by integrative approaches. Claude Bernard foresaw that this would require the application of mathematics to biology. This aspect of Claude Bernard's work has been neglected by physiologists, which is why we are not as ready to contribute to the development of systems biology as we should be. In this paper, I outline some general principles that could form the basis of systems biology as a truly multilevel approach from a physiologist's standpoint. We need the insights obtained from higher-level analysis in order to succeed even at the lower levels. The reason is that higher levels in biological systems impose boundary conditions on the lower levels. Without understanding those conditions and their effects, we will be seriously restricted in understanding the logic of living systems. The principles outlined are illustrated with examples from various aspects of physiology and biochemistry. Applying and developing these principles should form a major part of the future of physiology.

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Historical introduction

Claude Bernard was Sir William Paton's great physiological hero. When the Physiological Society celebrated its centenary in 1976, Bill contributed a paper to the historical part of the meeting concerning one of Bernard's experiments on curare and drawing attention to the important role his ideas played in the foundation of the Society in 1876 (Paton, 1976). The reasons for his admiration of Claude Bernard are not hard to find. Bernard was a superb experimentalist, as the history of his work on digestion shows (Holmes, 1974). He also displayed his skills in many other areas of physiology and he laid out the principles of his science in his highly influential *Introduction à l'étude de la Médecine Expérimentale* (Bernard, 1865, 1984), in which he revealed himself to be a great thinker as well as a great experimentalist. The theoretical problem he addressed is one that is very relevant both to my claim that he was the first systems biologist and to the challenge that physiology faces today.

What was Claude Bernard's problem? It was that the chemists had created 'organic' molecules. This was a major development, since people had thought since Lémery's *Cours de Chymie* (published in 1675) that there were three completely separate classes of compounds: mineral, vegetable and animal. The first break in this idea came from the work of Lavoisier (1784), who showed that all compounds from vegetable and animal sources always contained at least carbon and hydrogen, and frequently nitrogen and phosphorus. This work bridged the vegetable–animal chemical boundary, but it left intact the boundary between the living and non-living. In fact, Berzelius (1815) even proposed that organic compounds were produced by laws different from inorganic compounds; the idea that there was a specific vital force that could not operate outside living systems. In 1828, however, Wöhler succeeded in creating urea from ammonium cyanate. The distinction between organic and non-organic origins was further weakened by Kolbe who, in 1845, synthesized acetic acid from its elements. Many

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other discoveries of this kind (Finar, 1964) led to the idea that life itself could be reduced to chemistry and physics.

This was the challenge that physiologists such as Claude Bernard faced. His answer was precise. Neither vitalism nor chemical reductionism characterized living organisms. To the challenge that 'There are . . . chemists and physicists who . . . try to absorb physiology and reduce it to simple physico-chemical phenomena', Bernard responded, 'Organic individual compounds, though well defined in their properties, are still not active elements in physiological phenomena. They are only passive elements in the organism.' The reason, he explained, is that 'The living organism does not really exist in the *milieu extérieur* but in the liquid *milieu intérieur* . . .. a complex organism should be looked upon as an assemblage of simple organisms . . .. that live in the liquid *milieu intérieur*.'

His response to vitalism was equally robust: 'Many physicians . . .. assume a vital force in opposition to physico-chemical forces. I propose therefore to prove that the science of vital phenomena must have the same foundations as the science of the phenomena of inorganic bodies, and that there is no difference between the principles of biological science and those of physico-chemical science.'

By 'principles' here Bernard meant the laws governing the behaviour of the components. The control of the *milieu intérieur* meant not that the individual molecules did anything different from what they would do in non-living systems, but rather that the *ensemble* behaves in a controlled way, the controls being those that maintain the constancy of the internal environment. How could that be formalized? Could there be a theoretical physiology? Physical scientists had long since used mathematics to formalize their theories. Could that also be done in physiology? Bernard's answer to this question was 'yes, but not yet.' He cautioned, 'The most useful path for physiology and medicine to follow now is to seek to discover new facts instead of trying to reduce to equations the facts which science already possesses.' I believe that this view has been in part responsible for the broadly antitheoretical stance of British and American Physiology. It is important, therefore, to recognize that it represents only half of Bernard's views on the matter. For the emphasis in that statement should be on the word *now*. He also wrote that it was necessary to 'fix numerically the relations' between the components. He continued: 'This application of mathematics to natural phenomena is the aim of all science, because the expression of the laws of phenomena should always be mathematical.' His caution, therefore, was purely practical and temporal. In 1865 he saw, correctly of course, that physiology simply did not have enough data to make much mathematical application worthwhile *at that time*. But he clearly foresaw that the day would come when there would be sufficient data and that mathematical analysis would then become necessary.

The problem physiology faces today both resembles that faced by Bernard and differs from it. We face a new form of reductionism: that of genetic determinism, exemplified by the idea that there is a genetic program, what Jacob and Monod called '*le programme génétique*' (Monod & Jacob, 1961; Jacob, 1970). This challenge strongly resembles that of 'reducing life to physics and chemistry', the chemical being DNA. The major difference from Bernard's day is that we now have more facts than we can handle. There is a data explosion at all levels of biology. The situation is almost the reverse of that in Bernard's time. I have no doubt, therefore, that if he were alive today he would be championing his 'application of mathematics to natural phenomena.' I will illustrate why this is necessary and how it can be achieved by outlining some principles of systems biology from a physiologist's viewpoint. The principles are derived from my book on systems biology, *The Music of Life* (Noble, 2006), but their arrangement as a set of 10 was first presented by Noble (2007).

The principles of systems biology

First principle: biological functionality is multilevel. I start with this principle because it is obviously true, all the other principles can be shown to follow from it, and it is therefore the basis on which a physiological understanding of the phenomenon of life must be based. It is also a more general statement of the insight contained in Claude Bernard's idea of the constancy of the internal environment. That functionality is attributable to the organism as a whole and it controls all the other levels. This is the main reason why I describe Bernard as the first systems biologist. It is hard to think of a more important overall systems property than the one Bernard first identified.

Yet, the language of modern reductionist biology often seems to deny this obvious truth. The enticing metaphor of the 'book of life' made the genome into the modern equivalent of the 'embryo-homunculus', the old idea that each fertilized egg contains within it a complete organism in miniature (Mayr, 1982; p. 106). That the miniature is conceived as a digital 'map' or 'genetic program' does not avoid the error to which I am drawing attention, which is the idea that the living organism is simply the unfolding of an already-existing program, fine-tuned by its interaction with its environment, to be sure, but in all essentials, already there in principle as a kind of zipped-up organism. In its strongest form, this view of life leads to gene-selectionism and to gene-determinism: 'They [genes] created us body and mind' (Dawkins, 1976).

Dawkins himself does not really believe that. In a more recent book, he entitles one chapter 'Genes aren't us' (Dawkins, 2003) and, even in *The Selfish Gene*, the bold, simple message of the early chapters is qualified at the

end. My reservations, however, go much further than his. For, in truth, the stretches of DNA that we now call genes do nothing on their own. They are simply databases used by the organism as a whole. This is the reason for replacing the metaphor of the 'selfish' gene by genes as 'prisoners' (Noble, 2006; chapter 1). As Maynard Smith & Szathmáry (1999) express it, 'Co-ordinated replication prevents competition between genes within a compartment, and forces co-operation on them. They are all in the same boat.' From the viewpoint of the organism, genes as DNA molecules are therefore captured entities, no longer having a life of their own independent of the organism.

Second principle: transmission of information is not one way. The central dogma of molecular biology (Crick, 1970) is that information flows from DNA to RNA, from RNA to proteins, which can then form protein networks, and so on up through the biological levels to that of the whole organism. Information does not flow the other way. This is the dogma that is thought to safeguard modern neo-Darwinian theory from the spectre of 'Lamarckism', the inheritance of acquired characteristics. Applied to all the levels, this view is illustrated in Fig. 1. It encourages the bottom-up view of systems biology, the idea that if we knew enough about genes and proteins we could reconstruct all the other levels. Bioinformatics alone would be sufficient.

There are two respects in which the dogma is at least incomplete. The first is that it defines the relevant information uniquely in terms of the DNA code, the sequence of C, G, A, T bases. But the most that this information can tell us is *which* protein will be made. It does not tell us *how much* of each protein will be made. Yet, this is one of the most important characteristics of any living cell. Consider the speed of conduction of a nerve or muscle impulse, which depends on the density of rapidly activated sodium channels: the larger the density, the greater the ionic current and the faster the conduction. But this relationship applies only up to a certain optimum density, since the channel gating also contributes to the cell capacitance, which itself slows conduction, so there is a point beyond which adding more channel proteins is counter-productive (Hodgkin, 1975; Jack *et al.* 1975; p. 432). A feedback mechanism must therefore operate between the electrical properties of the nerve and the expression levels of the sodium channel protein. We now refer to such feedback mechanisms in the nervous system, which take many forms, as electro-transcription coupling (e.g. Deisseroth *et al.* 2003).

Similar processes must occur in the heart (e.g. Bers & Guo, 2005) and all the other organs. One of the lessons I have learnt from many attempts to model cardiac electrophysiology (Noble, 2002) is that, during the slow phases of repolarization and pacemaker activity, the ionic currents are so finely balanced that it is inconceivable that nature arrives at the correct expression and activity levels without some kind of feedback control. We don't yet know what that control might be, but we can say that it must exist. Nature cannot be as fragile as our computer models are! Robustness is an essential feature of successful biological systems.

There is nothing new in the idea that such feedback control of gene expression must exist. It is, after all, the basis of cell differentiation. All nucleated cells in the body contain exactly the same genome (with the exception of course of the germ cells, with only half the DNA). Yet the expression pattern of a cardiac cell is completely different from, say, a hepatic or bone cell. Moreover, whatever is determining those expression levels is accurately inherited during cell division. This cellular inheritance process is robust; it depends on some form of gene marking. It is this information on relative gene expression levels that is critical in determining each cell type.

By what principle could we possibly say that this is not relevant information? In the processes of differentiation and growth it is just as relevant as the raw DNA sequences. Yet, it is clear that this information *does* travel 'the other way'. The genes are told by the cells and tissues what to do, how frequently they should be transcribed and when to stop. There is 'downward causation' (Noble, 2006; chapter 4) from those higher levels that determines how the genome is 'played' in each cell (Fig. 2). Moreover, the possible number of combinations that could arise from so many gene components is so large (Feytmans *et al.* 2005) that there wouldn't be enough material in the whole universe for nature to have tried more than a small fraction

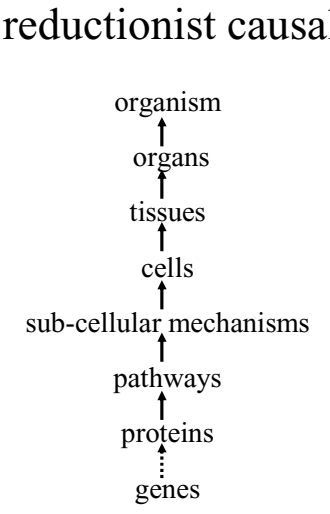


Figure 1. The reductionist 'bottom-up' causal chain (reproduced with permission from Noble, 2006)
This begins with the central dogma that information flows from DNA to proteins (bottom dotted arrow), never the other way, and extends the same concept through all the higher levels.

of the possible combinations even over the billions of years of evolution (Noble, 2006; chapter 2).

So the dogma is at least incomplete. But I also think it is incorrect in several important ways. Sure, protein sequences are not back-translated to form DNA sequences. In this limited original form, as formulated by Crick (1970), the central dogma is correct. But there is growing evidence from work on plants and microbes that environmental factors *do* change the genome, particularly by gene transfer (Goldenfeld & Woese, 2007). We cannot, therefore, use the original central dogma to exclude information transfer *into* the genome, determined by the organism and its environment.

Moreover, the DNA code itself is marked by the organism. This is the focus of the rapidly growing field of epigenetics (Qiu, 2006). At least two such mechanisms are now known at the molecular level: methylation of cytosine bases and control by interaction with the tails of histones around which the DNA is wound. Both of these processes modulate gene expression. The terminological question then arises: do we regard this as a form of code-modification? Is a cytosine, the C of the code, a kind of C* when it is methylated? That is a matter of definition of code, and one which I will deal with in the next section, but what is certain is that it is relevant information determining levels of gene expression, and that this information does flow against the direction of the central dogma. In fact, a form of inheritance of acquired characteristics (those of specific cell types) is rampant within all multicellular organisms with very different specialized cell types (Noble, 2006; chapter 7). At the least we have to say that, during the lifetime of the individual organism, transmission of information is far from being one way.

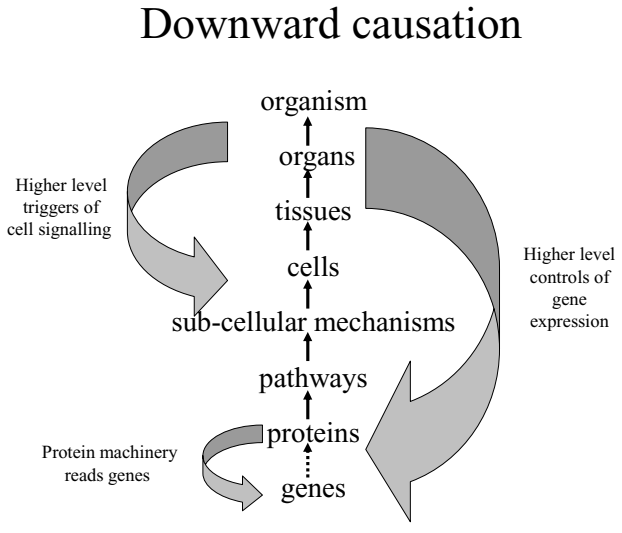


Figure 2. Figure 1 has been completed by adding the downward forms of causation, such as higher levels triggering cell signalling and gene expression
Note the downward-pointing arrow connecting from proteins to genes to indicate that it is protein machinery that reads and interprets gene coding. Loops of interacting downward and upward causation can be built between all levels of biological organization. Reproduced with permission from Noble (2006).

Third principle: DNA is not the sole transmitter of inheritance. The defenders of the original version of the central dogma would argue that, while my conclusions regarding the second principle are correct, what happens when information is transmitted to the next generation through the germ-line nevertheless involves wiping the slate clean of epigenetic effects. Methylation of cytosine bases and other forms of genome marking are removed. The genome is reset so that 'Lamarckism' is impossible.

But this is to put the matter the wrong way round. We need to explain *why* the genome (usually) reverts to an unmarked state. We don't explain that by appealing to the central dogma, for that dogma is simply a restatement of the same idea. We are in danger of circular logic here. Later, I will suggest a plausible reason why, at least most of the time, the resetting is complete, or nearly so. In order to do that, we first need to analyse the idea that genetics, as originally understood, is just about DNA.

This is not the original biological meaning of 'gene'. The concept of a gene has changed (Kitcher, 1982; Mayr, 1982; Dupré, 1993; Pichot, 1999). Its original biological meaning was an inheritable phenotype characteristic, such as eye/hair/skin colour, body shape and weight, number of legs/arms, to which we could perhaps add more complex traits like intelligence, personality, sexuality, etc. Genes, as originally conceived, are not just the same as stretches of DNA unless we subscribe to the view that the inheritance of all such characteristics is attributable entirely to DNA sequences. That is clearly false, since the egg cell is also inherited, together with any epigenetic characteristics transmitted by sperm (Anway *et al.* 2005), perhaps via RNA in addition to its DNA, and all the epigenetic influences of the mother and environment. Of course, the latter (environment) begins to be about 'nurture' rather than 'nature', but one of my points is that this distinction is fuzzy. The proteins that initiate gene transcription in the egg cell and impose an expression pattern on the genome are initially from the mother, and other such influences continue throughout development in the womb. Where we draw the line between nature and nurture is not at all obvious. There is an almost seamless transition from one to the other. 'Lamarckism', the inheritance of acquired characteristics, lurks in this fuzzy crack to a degree yet to be defined (Jablonka & Lamb, 1995, 2005). As the evolutionary geneticist Maynard Smith says, 'It [Lamarckism] is not so obviously false as is sometimes made out' (Maynard Smith, 1998).

Inheritance of the egg cell is important for two reasons. First, it is the egg cell DNA-reading machinery (a set of around 100 proteins and the associated cellular ribosome architecture) that enables the DNA to be used as a

template to make more proteins. Second, the set of other cellular elements, mitochondria, endoplasmic reticulum, microtubules, nuclear and other membranes, and a host of chemicals arranged specifically in cellular compartments, is also inherited. Most of this is not coded for by DNA sequences. Lipids certainly are not so coded. But they are absolutely essential to all the cell architecture. There would be no cells, nuclei, mitochondria, endoplasmic reticulum, ribosomes and all the other cellular machinery and compartments without the lipids. The specific details of all this cellular machinery matter. We can't make any old DNA do its thing in any old egg cell. Most attempts at interspecies cloning simply don't work. Invariably, a block occurs at an early stage in development. The only successful case so far is that of a wild ox (*Bos javanicus*) cloned in a domestic cow egg. The chances are that it will work only in very closely related species. The egg cell information is therefore also species specific.

Could epigenetic inheritance and its exclusion from the germ cell line be a requirement of multicellular harmony? The exact number of cell types in a human is debatable. It is partly a question of definition. A project that seeks to model all the cell types in the body, the Human Physiome Project (Crampin *et al.* 2004), estimates that there are around 200, all with completely different gene expression patterns. There would be even more if one took account of finer variations, such as those that occur in various regions of the heart and which are thought to protect the heart against fatal arrhythmias.

The precise number is not too important. The important fact is that it is large and that the range of patterns of gene expression is therefore also large and varied. Their patterns must also be harmonious in the context of the organism as a whole. They are all in the same boat; they sink or swim together. Disturbing their harmony would have serious consequences. It was arrived at after more than 2 billion years of experimentation.

Each cell type is so complex that the great majority of genes are expressed in many cell types. So it makes sense that all the cells in the body have the same gene complement, and that the coding for cell type is transmitted by gene marking, rather than by gene complement. I think that this gives the clue to the purpose of re-setting in germ-line inheritance. Consider what would happen if germ-line inheritance reflected adaptive changes in individual cell types. Given that all cell types derive ultimately from the fused germ-line cells, what would the effect be? Clearly, it would be to alter the patterns of expression in nearly all the cell types. There would be no way to transmit an improvement in, say, heart function to the next generation via gene marking of the germ cells without *also* influencing the gene expression patterns in many other types of cell in the body. And of course there is no guarantee that what is beneficial for a heart cell will be so in, say, a bone cell or a liver cell. On the contrary, the chances are that an adaptation beneficial in one cell type would be likely to be deleterious in another.

Much better, therefore, to let the genetic influences of natural selection be exerted on undifferentiated cells, leaving the process of differentiation to deal with the fine-tuning required to code for the pattern of gene expression appropriate to each type of cell. If this explanation is correct, we would not necessarily expect it to be 100% effective. It is conceivable that some germ-line changes in gene expression patterns might be so beneficial for the organism as a whole, despite deleterious effects on a few cell lines, that the result would favour selection. This could explain the few cases where germ-line 'Lamarckian' inheritance seems to have occurred. It also motivates the search for other cases. The prediction would be that it will occur in multicellular species only when beneficial to overall intercellular harmony. It might be more likely to occur in simpler species. That makes sense in terms of the few examples that we have so far found (Maynard Smith, 1998). Notice that, in contrast to the central dogma, this explanation is a systems level explanation.

Finally, in this section, I will comment on the concept of code. Applied to DNA, this is clearly metaphorical. It is also a useful metaphor, but we should beware of its limitations. One of these is to imply that only information that is coded is important, as in talk of the genome as the 'book of life'. The rest of cellular inheritance is not so coded; in fact, it is not even digital. The reason is very simple. The rest of the cellular machinery doesn't need to 'code for' or get 'translated into' anything else for the simple reason that it 'represents' itself; cells divide to form more cells, to form more cells, and so on. In this sense, germ-line cells are just as 'immortal' as DNA but a lot of this information is transmitted directly without having to be encoded. We should beware of thinking that only digitally 'coded' information is what matters in genetic inheritance.

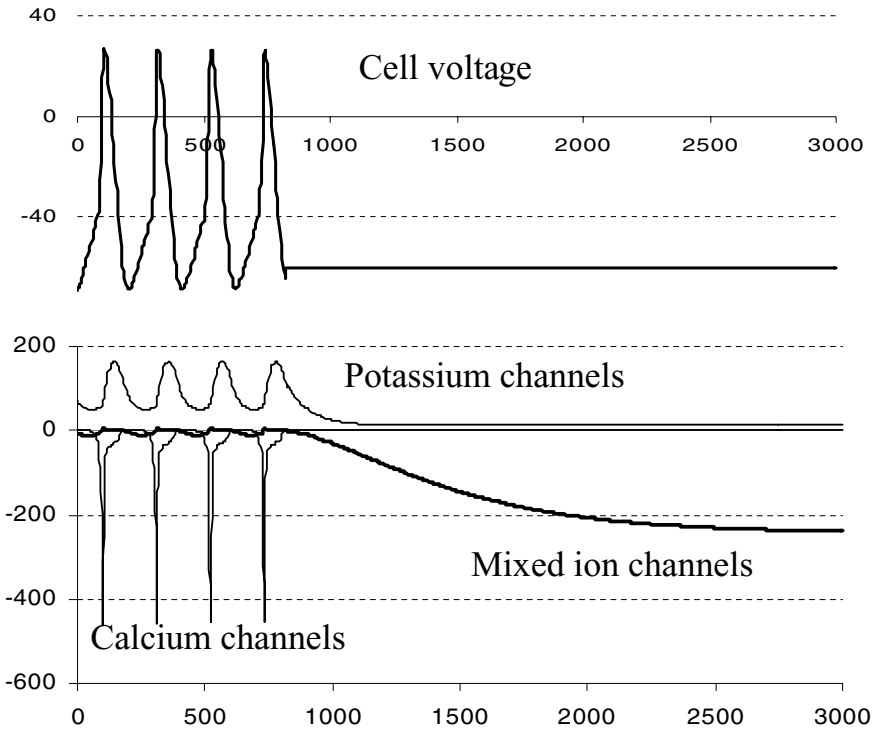
Fourth principle: the theory of biological relativity; there is no privileged level of causality. A fundamental property of systems involving multiple levels between which there are feedback control mechanisms is that there is no privileged level of causality. Consider, as an example, the cardiac pacemaker mechanism. This depends on ionic current generated by a number of protein channels carrying sodium, calcium, potassium and other ions. The activation, de-activation and inactivation of these channels proceed in a rhythmic fashion in synchrony with the pacemaker frequency. We might therefore be tempted to say that their oscillations generate that of the overall cell electrical potential, i.e. the higher-level functionality. But this is not the case. The kinetics of these channels varies with the electrical potential. There is therefore feedback between the higher-level property, the cell potential, and

the lower level property, the channel kinetics (Noble, 2006; chapter 5). This form of feedback was originally identified by Alan Hodgkin working on the nerve impulse, so it is sometimes called the Hodgkin cycle. If we remove the feedback, e.g. by holding the potential constant, as in a voltage clamp experiment, the channels no longer oscillate (Fig. 3). The oscillation is therefore a property of the system as a whole, not of the individual channels or even of a set of channels unless they are arranged in a particular way in the right kind of cell.

Nor can we establish any priority in causality by asking which comes first, the channel kinetics or the cell potential. This fact is also evident in the differential equations we use to model such a process. The physical laws represented in the equations themselves, and the initial and boundary conditions, operate *at the same time* (i.e. during every integration step, however infinitesimal), not sequentially.

It is simply a prejudice that inclines us to give some causal priority to lower-level, molecular events. The concept of level in biology is itself metaphorical. There is no literal sense in which genes and proteins lie *underneath* cells, tissues and organs. It is a convenient form of biological classification to refer to different levels, and we would find it very hard to do without the concept (Fig. 4). But we should not be fooled by the metaphor into thinking that 'high' and 'low' here have their normal meanings. From the metaphor itself, we can derive no justification for referring to one level of causality as privileged over others. That would be a misuse of the metaphor of level.

One of the aims of my book, *The Music of Life* (Noble, 2006), is to explore the limitations of biological metaphors. This is a form of linguistic analysis that is rarely applied in science, though a notable exception is Steven J. Gould's monumental work on the theory of evolution



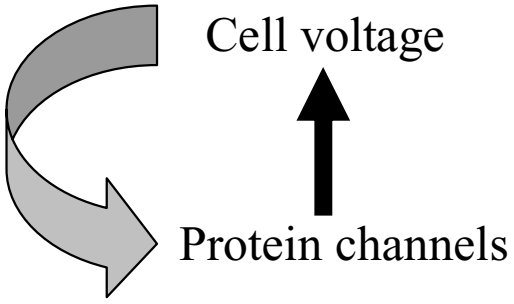


Figure 3. Computer model of pacemaker rhythm in the heart (reproduced with permission from Noble & Noble, 1984)
For the first four beats, the model is allowed to run normally and generates rhythm closely similar to a real heart. Then the feedback from cell voltage to protein channels is interrupted. All the protein channel oscillations then cease. They slowly change to steady, constant values. The diagram shows the causal loop involved. Protein channels carry current that changes the cell voltage (upward arrow), while the cell voltage changes the protein channels (downward arrow). In the simulation, this downward arrow was broken at 800 ms.

(Gould, 2002), in which he analyses the arguments for the multiplicity of levels at which natural selection operates.

These points can be generalized to any biological function. The only sense in which a particular level might be said to be privileged is that, in the case of each function, there is a level at which the function is integrated, and it is one of our jobs as biological scientists to determine what that level may be.

The idea that there is no privileged level of causality has a much wider range of applications than purely biological ones (Dupré, 1993; Cartwright, 1999; Keller, 2002), though the idea is rarely expressed in this bold, relativistic form. I use the word 'relativity' in formulating the principle because it shares certain features with theories of scale relativity proposed by some theoretical physicists, in particular the idea that there is no privileged scale, which is at the foundation of the theory of scale relativity (Nottale, 1993). There is an obvious correlation between scale and level, since lower and higher levels in any system operate at different scales. For this reason, some have proposed the application of the scale relativity theory framework and its associated mathematical tools to tackle the challenge of multiscale integration in systems biology (Nottale, 2000; Auffray & Nottale, 2008; Nottale & Auffray, 2008). But it is too early to judge whether this can provide a firm basis to a fully fledged theory of systems biology. Although the theory of scale relativity has already delivered a number of predictions in the realm of astrophysics which have been validated by subsequent observations, it still has to establish fully its position within theoretical physics. Nor is it possible yet to decide which principles are specific to systems biology and which are of general importance beyond the boundaries of biology.

Fifth principle: gene ontology will fail without higher-level insight. Genes, as defined by molecular genetics to be the coding regions of DNA, code for proteins. Biological function then arises as a consequence of multiple interactions between different proteins in the context of the rest of the cell machinery. Each function therefore depends on many genes, while many genes play roles in multiple functions. What then does it mean to give genes names in terms of functions? The only unambiguous labelling of genes is in terms of the proteins for which they code. Thus, the gene for the sodium–calcium exchange protein is usually referred to as *ncx*. Ion channel genes are also often labelled in this way, as in the case of sodium channel genes being labelled *scn*.

This approach, however, naturally appears unsatisfactory from the viewpoint of a geneticist, since the original question in genetics was not which proteins are coded for by which stretches of DNA [in fact, early ideas on where the genetic information might be found (Schrödinger, 1944) favoured the proteins], but rather what is responsible for higher-level phenotype characteristics. There is no one-to-one correspondence between genes or proteins and higher-level biological functions. Thus, there is no 'pacemaker' gene. Cardiac rhythm depends on many proteins interacting within the context of feedback from the cell electrical potential.

Let's do a thought experiment. Suppose we could knock out the gene responsible for L-type calcium channels and still have a living organism (perhaps because a secondary pacemaker takes over and keeps the organism viable – and something else would have to kick-in to enable excitation–contraction coupling, and so on throughout the body because L-type calcium channels are ubiquitous!). Since

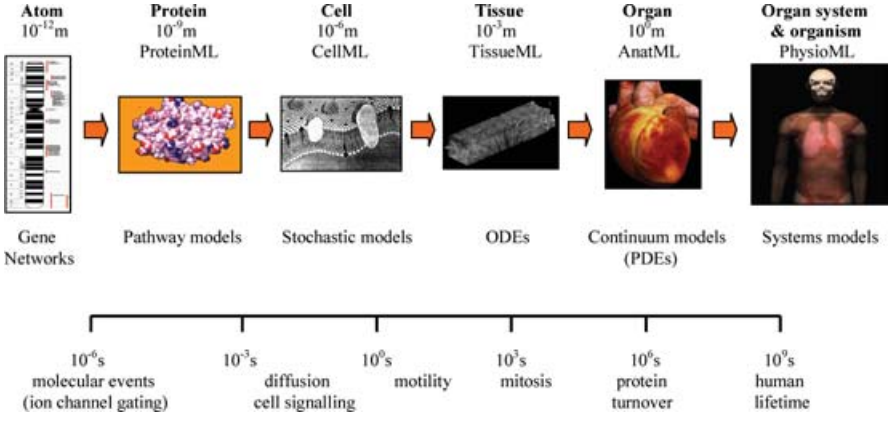


Figure 4. Spatial (top) and temporal (bottom) scales encompassed by the Human Physiome Project
The types of mathematical model appropriate to each spatial scale are also indicated. The last two images on the right in this figure, and all subsequent anatomical images, are from anatomically based models developed by the Auckland Bioengineering group. The tissue image is a three-dimensional confocal microscopy reconstruction of a transmural segment of rat heart by the Auckland group led by Peter Hunter (Hunter *et al.* 2002). Abbreviations: ML, markup language; ODE, ordinary differential equations; PDE, partial differential equations. Reproduced with Permission from Hunter *et al.* (2002).

L-type calcium current is necessary for the upstroke of the action potential in the SA node of most species, we would find that we had abolished normal pacemaker rhythm. Do we then call the gene for L-type calcium channels the 'pacemaker' gene? The reason why this is unsatisfactory, even misleading, to a systems-level biologist is obvious. Yet it is the process by which we label many genes with high-level functions. The steadily growing list of 'cancer genes' have been identified in this way, by determining which mutations (including deletions) change the probability of cancer occurring. We can be fairly sure though that this characteristic is not why they were selected during the evolutionary process. In this sense, there are no 'cancer genes'. As the Gene Ontology (GO) Consortium (http://geneontology.org/) puts it, 'oncogenesis is not a valid GO term because causing cancer is not the normal function of any gene'.

Another good example of this approach is the discovery of what are called clock genes, involved in circadian rhythm. Mutations in a single gene (now called the *period* gene) are sufficient to abolish the circadian period of fruit flies (Konopka & Benzer, 1971). This discovery of the first 'clock gene' was a landmark, since it was the first time that a single gene had been identified as playing such a key role in a high-level biological rhythm. The expression levels of this gene are clearly part of the rhythm generator. They vary (in a daily cycle) in advance of the variations in the protein for which they code. The reason is that the protein is involved in a negative feedback loop with the gene that codes for it (Hardin *et al.* 1990). The idea is very simple. The protein levels build up in the cell as the *period* gene is read to produce more protein. The protein then diffuses into the nucleus, where it inhibits further production of itself by binding to the promoter part of the gene sequence. With a time delay, the protein production falls off and the inhibition is removed so that the whole cycle can start again. So, we not only have a single gene capable of regulating the biological clockwork that generates circadian rhythm, it is itself a key component in the feedback loop that forms the rhythm generator.

However, such rhythmic mechanisms do not work in isolation. There has to be some connection with light-sensitive receptors (including the eyes). Only then will the mechanism lock on to a proper 24 h cycle rather than free-running at say 23 or 25 h. In the mouse, for example, many other factors play a role. Moreover, the clock gene itself is involved in other functions. That is why Foster and Kreitzman have written 'What we call a clock gene may have an important function within the system, but it could be involved in other systems as well. Without a complete picture of all the components and their interactions, it is impossible to tell what is part of an oscillator generating rhythmicity, what is part of an input, and what is part of an output. In a phrase, it ain't that simple!' (Foster & Kreitzman, 2004).

Indeed not. The *period* gene has also been found to be implicated in embryonic development as the adult fly is formed over several days, and it is deeply involved in coding for the male love songs generated by wing-beat oscillations which are specific to each of around 5000 species of fruit fly and ensure that courtship is with the right species. Perhaps it should be renamed the 'fruit fly love gene'!

The point is obvious. We should not be misled by gene ontology. The first function a gene is found to be involved in is rarely, if ever, the only one and may not even be the most important one. Gene ontology will require higher-level insight to be successful in its mission. Moreover, current methods of relating genotype to phenotype suffer from a major methodological limitation: by determining the effects of *changes* (mutations) in the genome, we can say little *a priori* on the direct causal relations between wild-type genes and the phenotype. They reveal simply the *differences* produced as a result of the *change* in genotype. All the causal effects *common* to both the wild-type and the mutated gene are hidden. What is observed may be just the tip of the iceberg.

Gene ontology in its fullest sense, as originally conceived by geneticists to relate genes to high-level features, is therefore very difficult and subject to many traps for the unwary. This would explain why projects such as the GO Consortium are more limited in their scope. Thus, GO assigns three categories to a gene, namely molecular function, biological process and cellular component, which are not intended to deal with higher-level function. It specifically excludes protein domains or structural features, protein–protein interactions, anatomical or histological features above the level of cellular components, including cell types, and it excludes the environment, evolution and expression. In other words, it excludes virtually all of what we classically understand by physiology and most aspects of evolutionary biology.

Sixth principle: there is no genetic program. No genetic programs? Surely, they are all over the place! They are the crown jewels of the molecular genetic revolution, invented by none other than the famous French Nobel Prize winners, Monod and Jacob (Monod & Jacob, 1961; Jacob, 1970). Their enticing idea was born during the early days of electronic computing, when computers were fed with paper tape or punched cards coded with sequences of instructions. Those instructions were clearly separate from the machine itself that performed the operations. They dictated those operations. Moreover, the coding is digital. The analogy with the digital code of DNA is obvious. So, are the DNA sequences comparable to the instructions of a computer program?

An important feature of such computer programs is that the program is separate from the activities of the machine that it controls. Originally, the separation was

physically complete, with the program on the tape or cards only loaded temporarily into the machine. Nowadays, the programs are stored within the memory of the machine, and the strict distinction between the program, the data and the processes controlled may be breaking down. Perhaps computers are becoming more like living systems, but in any case the concept of a genetic program was born in the days when programs were separate, identifiable sets of instructions.

So, what do we find when we look for genetic programs in an organism? We find no genetic programs! There are no sequences of instructions in the genome that could possibly play a role similar to that of a computer program. The reason is very simple. A database, used by the system as a whole, is not a program. To find anything comparable to a program we have to extend our search well beyond the genome itself. Thus, as we have seen above, the sequence of events that generates circadian rhythm includes the *period* gene, but it necessarily also includes the protein for which it codes, the cell in which its concentration changes and the nuclear membrane across which it is transported with the correct speed to effect its inhibition of transcription. This is a gene–protein–lipid–cell network, not simply a gene network. The nomenclature matters. Calling it a gene network fuels the misconception of genetic determinism. In the generation of a 24 h rhythm, none of these events in the feedback loop is privileged over any other. Remove any of them, not just the gene, and you no longer have circadian rhythm.

Moreover, it would be strange to call this network of interactions a program. The network of interactions *is itself the circadian rhythm process*. As Enrico Coen, the distinguished plant geneticist, put it, 'Organisms are not simply manufactured according to a set of instructions. There is no easy way to separate instructions from the process of carrying them out, to distinguish plan from execution' (Coen, 1999). In short, the concept of a program here is completely redundant. It adds nothing to what a systems approach to such processes can reveal.

Seventh principle: there are no programs at any other level. I have introduced the analogy of the genome as a database and the metaphor of 'genes as prisoners' in order to provoke the change in mindset that is necessary for a fully systems approach to biology to be appreciated. The higher levels of the organism 'use the database' and 'play the genome' to produce functionality. If the genome can be likened to a huge pipe organ (Noble, 2006; chapter 2), then it seems correct to ask who is the player, who was the composer? If we can't find the program of life at the level of the genome, at what level do we find it? The answer is 'nowhere'!

We should view all such metaphors simply as ladders of understanding. Once we have used them we can, as it were, throw them away. This way of thinking can seem strange to some scientists for whom there must be just one correct answer to any scientific question. I explore this important issue in *The Music of Life* by analysing the 'selfish gene' and 'prisoner gene' metaphors linguistically to reveal that no conceivable experiment could decide which is correct (Noble, 2006; chapter 1). They highlight totally different aspects of the properties of genes. This philosophy is applied throughout the book as it answers questions like 'where is the program of life?' The conclusion is simply that there are no such programs at any level. At all levels, the concept of a program is redundant since, as with the circadian rhythm network, the networks of events that might be interpreted as programs are themselves the functions we are seeking to understand. Thus, there is no program for the heart's pacemaker separate from the pacemaker network itself.

While causality operates within and between all levels of biological systems, there are certain levels at which so many functions are integrated that we can refer to them as important levels of abstraction. Sydney Brenner wrote, 'I believe very strongly that the fundamental unit, the correct level of abstraction, is the cell and not the genome' (unpublished Lecture, Columbia University, 2003). He is correct, since the development of the eukaryotic cell was a fundamental stage in evolutionary development, doubtless requiring at least a billion years to be achieved. To systems physiologists though there are other important levels of abstraction, including whole organs and systems.

Eighth principle: there are no programs in the brain. In his book *The Astonishing Hypothesis*, Francis Crick proclaimed, 'You, your joys and your sorrows, your memories and your ambitions, your sense of personal identity and free will, are in fact no more than the behaviour of a vast assembly of nerve cells and their associated molecules' (Crick, 1994). This is a variation of the idea that in some sense or other, the mind is just a function of the brain. The pancreas secretes insulin, endocrine glands secrete hormones ... and the brain 'secretes' consciousness! All that's left is to find out how and where in the brain that happens. In one of his last statements, Crick has even hinted at where that may be: 'I think the secret of consciousness lies in the claustrum' (Francis Crick, 2004, quoted by V. S. Ramachanran, in *The Astonishing Francis Crick*, Edge, 18 October, 2004, http://www.edge.org/3rd_culture/crick04/crick04_index.html). This structure is a thin layer of nerve cells in the brain. It is very small and it has many connections to other parts of the brain, but the details are of no importance to the argument. The choice of brain location for the 'secret of consciousness' varies greatly according to the author. Descartes even thought that it was in the pineal gland. The mistake is always the same, which is to think that in some way or other the brain is a kind of performance space in which the world of perceptions is reconstructed

inside our heads and presented to us as a kind of Cartesian theatre. But that way of looking at the brain leaves open the question: where is the 'I', the conscious self that sees these reconstructions? Must that be another part of the brain that views these representations of the outside world?

We are faced here with a mistake similar to that of imagining that there must be programs in the genomes, cells, tissues and organs of the body. There are no such programs, even in the brain. The activity of the brain and of the rest of the body simply *is* the activity of the person, the self. Once again, the concept of a program is superfluous. When a guitarist plays the strings of his guitar at an automatic speed that comes from frequent practice, there is no separate program that is making him carry out this activity. The patterns and processes in his nervous system and the associated activities of the rest of his body simply *are* him playing the guitar. Similarly, when we deliberate intentionally, there is no nervous network 'forcing' us to a particular deliberation. The nervous networks, the chemistry of our bodies, together with all their interactions within the social context in which any intentional deliberation makes sense, *are* us acting intentionally. Looking for something in addition to those processes is a mistake.

Ninth principle: the self is not an object. In brief, the mind is not a separate object competing for activity and influence with the molecules of the body. Thinking in that way was originally the mistake of the dualists, such as Sherrington and Eccles, led by the philosophy of Descartes. Modern biologists have abandoned the separate substance idea, but many still cling to a materialist version of the same mistake (Bennett & Hacker, 2003), based on the idea that somewhere in the brain the self is to be found as some neuronal process. The reason why that level of integration is too low is that the brain, and the rest of our bodies which are essential for attributes such as consciousness to make sense (Noble, 2006; chapter 9), are tools (back to the database idea again) in an integrative process that occurs at a higher level involving social interactions. We cannot attribute the concept of self-ness to ourselves without also doing so to others (Strawson, 1959). Contrary to Crick's view, therefore, our selves are indeed much 'more than the behaviour of a vast assembly of nerve cells and their associated molecules' precisely because the social interactions are essential even to understanding what something like an intention might be. I analyse an example of this point in much more detail in chapter 9 of *The Music of Life*. This philosophical point is easier to understand when we take a systems view of biology, since it is in many ways an extension of that view to the highest level of integration in the organism.

Conclusions

Tenth principle: there are many more to be discovered; a genuine 'theory of biology' does not yet exist. Well, of course, choosing just 10 principles was too limiting. This last one points the way to many others of whose existence we have only vague ideas. We do not yet have a genuine theory of biology. The Theory of Evolution is not a theory in the sense in which I am using the term. It is more an historical account, itself standing in need of explanation. We don't even know yet whether it consists of events that are difficult, if not impossible, to analyse fully from a scientific perspective, or whether it was a process that would have homed in to the organisms we have, regardless of the conditions. My own suspicion is that it is most unlikely that, if we could turn the clock right back and let the process run again, we would end up with anything like the range of species we have today on earth (Gould, 2002).

But, whichever side of this particular debate you may prefer, the search for general principles that could form the basis of a genuine theory of biology is an important aim of systems biology. Can we identify the logic by which the organisms we find today have succeeded in the competition for survival? In searching for that logic, we should not restrict ourselves to the lower levels. Much of the logic of living systems is to be found at the higher levels, since these are often the levels at which selection has operated (Keller, 1999; Gould, 2002) and determined whether organisms live or die. This is the level at which physiology works. Physiology therefore has a major contribution to make to systems biology.

In conclusion, I return to the theme with which this article began. Claude Bernard's concept of the constancy of the internal environment was the first example of multilevel functionality. It was critical in defining physiology as a subject distinct from the applications of physics and chemistry. The challenge we face today resembles that faced by Bernard in the mid-nineteenth century, but the chemistry involved is that of the molecule DNA. The answer though should be much the same. Higher-level control cannot be reduced to lower-level databases like the genome. A major part of the future of physiology surely lies in returning to our roots. Higher-level systems biology is, I suggest, classical physiology by another name.

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