The heart is already working

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Abstract

Understanding the logic of living systems requires knowledge of the mechanisms involved at the levels at which functionality is expressed. This information resides neither in the genome, nor even in the individual proteins that genes code for. No functionality is expressed at these levels. It emerges as the result of interactions between many proteins relating to each other in multiple cascades and in interaction with the cellular environment. There is therefore no alternative to copying nature and computing these interactions to determine the logic of healthy and diseased states. The rapid growth in biological databases, models of cells, tissues and organs and the development of powerful computing hardware and algorithms have made it possible to explore functionality in a quantitative manner all the way from the level of genes to the physiological function of whole organs and regulatory systems. I use models of the heart to demonstrate that we can now go all the way from individual genetic information (on mutations, for example) to exploring the consequences at a whole-organ level.

Systems biology?

I was asked recently by a medical journalist how it came about that, according to reports he had seen, I had been doing the 'new' subject of 'systems biology' for the last dozen years or so. 'You must be one of the founders of the subject!' I replied 'no, I have actually been doing it for over 40 years [1], and if you really want to identify the founders of my field you should look at the work of Hodgkin and Huxley [2] who first identified the system of interactions between cell electrical potential and ion channel activity that enabled them to formulate equations and reconstruct quantitatively the nerve impulse and its conduction.' The journalist did not even know that they had won the Nobel Prize (in 1963) for this achievement.

Yes, let's celebrate the emergence of Systems Biology as a discipline. The challenge posed by interpreting the genome and proteome results in terms of higher-level physiological function is now one of the greatest challenges for science in the 21st century. But let us also pay tribute to the foundations, which in some areas were laid long ago.

Will it work? Well, it all depends on what we are trying to do, how we interpret the challenge. Sydney Brenner, at a Novartis Foundation meeting [3] in 2001, said 'I know one approach that will fail, which is to start with genes, make proteins from them and to try to build things bottom-up.' What did he mean?

It is easy to show how impossible an exhaustive bottom-up would be. It will take the full power of the most powerful computer in the world (a mammoth called *Blue Gene*, being constructed by IBM at a cost of $100 million) calculating for a long time, probably months, to reconstruct the chemical processes involved in the folding of a single protein to form the three-dimensional structure that gives it function.

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Such a molecule is around 1 nm in size and the chemical processes involved take of the order of a millionth of a second. To get to the size of a cell, typically tens of μm in size (say 10^{-5} m), we would need to simulate the interactions of around 10^{12} such molecules, and to do so for many seconds, minutes, hours, days or even years, a span of time scales of around 10^{15}. An exhaustive molecular reconstruction of the activity of a single cell would require unimaginably large computational resources – around 10^{27} *Blue Genes*. There simply won't be enough stuff in the whole solar system to build such monsters. So far, this would be only the beginning of reconstructing the tissues, organs and systems of the body. Even the smallest of organs in the body has millions of cells. I don't think I need to labour the point. This approach fails on the first hurdle. It is unbelievably impractical.

The approach we have used in reconstructing the heart from genes to the whole organ is to use what Brenner called the 'middle-out' approach. We start a systems analysis at any of the levels at which we have sufficient quantitative data to feed into a simulation. Since all levels can be the starting point for a causal chain, any of them can be the starting point for successful simulation. In networks of interactions at many levels, there is in fact no alternative. Analysis must start somewhere. This might be at the level of gene–protein networks, or cell function, or organ structure – any of the biological levels can be a valid starting point. This is the 'middle' part of Brenner's metaphor. There can be many 'middles'. Brenner's would be different from mine. I start with cells, whereas he starts with genes. That doesn't matter. In the best of all systems biological worlds we will all eventually meet up anyway.

Then, when we have established sufficient understanding and success at our chosen level we can reach out (this is the 'out' part of the metaphor) to other levels. Ideally, we eventually reach right down to the level of genes, and right up to the level of the organism. This linking is necessary if

we are to interpret the genome in terms of its physiological function. Linking levels is part of what systems biology is about.

Heart cell models

Models of heart cells have become highly sophisticated and have benefited from four decades of iterative interaction between experimental and simulation work. Models of all the main types of cardiac myocytes exist (in many cases there are multiple models of the same cell type) and, most significantly, we are now able to represent the variations in gene expression levels, for example across the ventricular wall [4], between the centre and periphery of the sino-atrial node [5], and within the atrium [6]. These expression variations are of fundamental importance in understanding the global phenomena such as the electrocardiogram, and for analysing the way in which the cardiac rhythm is generated. They are also fundamental to understand the disease states, some of which, like heart failure, can be characterized by alterations in gene expression profiles [7].

Linking to genetics

An important strength of models based on reconstructing the functional properties of proteins is that it is possible for the models to reach down to the genetic level, for example by reconstructing the effects of particular mutations when these are characterized by known changes in protein function.

An example of this approach is the use of state-specific Markov models of the cardiac sodium channel [8] in which models of the wild-type and of a mutant sodium channel were formulated and validated. The simulated mutation was the ΔKPQ mutation, a three-amino-acid deletion that affects the channel inactivation and is associated with a congenital form of the long-QT syndrome, LQT3. The simulations showed that mutant channel reopenings from the inactivated state and channel bursting due to a transient failure of inactivation generate a persistent inward sodium current during the action potential plateau in the mutant cell. This causes major prolongation of repolarization and the development of arrhythmogenic early after-depolarizations at slow pacing rates, a behaviour that is consistent with the clinical presentation of bradycardia-related arrhythmogenic episodes during sleep or relaxation in LQT3 patients.

Another sodium-channel mutation that has been, at least partially, reconstructed is a mis-sense mutation that affects the voltage dependence of sodium channel inactivation and which is responsible for one form of idiopathic ventricular fibrillation. In this case, small shifts of the voltage dependence of inactivation generate early after-depolarizations that may underlie fatal arrhythmia [9].

Early after-depolarizations are also responsible for the arrhythmias of congestive heart failure. Winslow et al. [10] have modelled this process based on experimentally determined changes in gene expression levels for several of the transporter proteins involved.

Counterintuitive predictions

Complex systems are characterized by the fact that the results of modelling them are frequently counterintuitive. This is not surprising. Beyond a certain degree of complexity, armchair (qualitative) thinking is not only inadequate, it can even be misleading. A good example of this comes from the extension of cellular models to include some of the biochemical changes that occur during ischaemia [11]. This work succeeds in reconstructing arrhythmias attributable to delayed after-depolarizations that arise as a consequence of intracellular calcium oscillations in conditions of sodium–calcium overload. These oscillations generate an inward current carried by the sodium–calcium exchanger, which can lead to premature excitation. This work has led to some interesting counterintuitive predictions concerning up- and down-regulation of sodium–calcium exchange in disease states [12]. This transporter is currently a focus of anti-arrhythmia drug therapy. Simulation is playing an important role in clarifying and assessing the mechanism of action of such drugs.

Another area in which modelling has been rich in counter-intuitive results is that of mechano-electric feedback. Kohl and Sachs [13] describe the extent to which this feedback mechanism has been unravelled in elegant experimental and computational work. Some of the results, particularly on the actions of changes in cell volume (which are important in many disease states) are unexpected and have been responsible for determining the next stage in experimental work. Indeed, it is hard to see how such unravelling of complex physiological processes can occur without the iterative interaction between experiment and simulation.

Assessing and predicting drug actions

Drugs act on proteins such as receptors, channels, transporters and enzymes. Models that reach down to the protein level are therefore highly relevant to assessing and predicting drug actions. Simulations have already been used in assessing drug action by the Food and Drug Administration in the U.S.A. and we can expect this kind of use of biological models to increase greatly as their complexity and power grows. I have reviewed some of these developments fully elsewhere [14,15]. One obvious use in the case of the heart is in assessing the cardiac safety of drugs. It should be noted that half the drug withdrawals that have occurred in the U.S.A. post-launch since 1998 have been attributable to cardiac side-effects, often in the form of effects on the electrocardiogram and consequent arrhythmias. This is a large and very expensive form of attrition. Since virtually all the ion transporters involved in the cardiac repolarization are now modelled and that very realistic simulations of the T wave of the electrocardiogram can be obtained when these models are incorporated into three-dimensional cardiac tissue models, it is clearly becoming possible to use *in silico* screens for drug development. One of the reasons that this is necessary is that the electrocardiogram is, unfortunately, an unreliable indicator of potential arrhythmogenicity. Similar changes in

form of the QT interval and T waveform can be induced by very different molecular and cellular effects, some benign, others dangerous. We need to understand and predict the mechanisms all the way from individual channel properties through to the electrocardiogram. This goal is within reach, particularly as we acquire more experience of the incorporation of accurate cellular models into anatomically detailed organ models.

Another use of simulation in drug discovery will be in screening drugs for multiple actions. Very few drugs that act on the heart bind to just one receptor. It is much more common for 2, 3 or even more receptors or channels to be affected. An important point to realize here is that multisite action may actually be beneficial. The reviews referred to above give examples of multireceptor drug actions that would be expected to be beneficial. I would predict that this will in fact be one of the ways in which more rational discovery of anti-arrhythmic drugs may occur. In regulating cardiac function, nature has developed many multiple-action processes, particularly those regulated by G-protein coupled receptors. In seeking for more 'natural' ways of intervening in disease states, we should also be seeking to play the orchestra of proteins in more subtle ways. We need simulation to guide us through the complexity and to understand multiple action functionality.

Incorporation of cellular models into anatomically detailed models of the whole organ

In modelling the heart, we have benefited from the fact that, in addition to the data-rich cellular level, there has also been data-rich modelling of the three-dimensional geometry of the whole organ [16,17]. Connecting these two levels has been an exciting venture. Anatomically detailed models of the ventricles, including fibre orientations and sheet structure, have been used to incorporate the cellular models in an attempt to reconstruct the electrical and mechanical behaviour of the whole organ.

For example, we can use such whole organ models to reconstruct the spread of the activation wavefront [18]. This is heavily influenced by cardiac ultra-structure, with preferential conduction along the fibre-sheet axes, and the result corresponds well with that obtained from multielectrode recording from dog hearts *in situ*. I referred earlier to work that reconstructs the later phases of the electrocardiogram using detailed reconstruction of the variations in gene expression levels in different parts of the ventricle. Accurate reconstruction of the depolarization wavefront promises to provide reconstruction of the early phases, i.e. the QRS complex, and as the sinus node, atrium and conducting system are incorporated into this whole heart (on which work is in progress) we can look forward to the first example of reconstruction of a complete physiological process from the level of protein function right up to routine clinical observation. The whole ventricular model has already been incorporated into a virtual torso [19], including the electrical conducting properties of the different tissues, to extend the external field computations to reconstruction of multiple-lead chest and limb recording.

Coronary circulation

Ischaemic heart disease is a major cause of serious incapacity and mortality. It is also a good example of the fact that most disease states are multifactorial. Very few diseases are attributable to single gene or protein malfunction. As noted above, cellular reconstructions of the metabolic and electrophysiological processes that occur following deprivation of the energy supply to cardiac cells have already advanced to the point at which some arrhythmic mechanisms can be reproduced. The initiating process in such energy deprivation is restriction or block of coronary arteries. This is another example where modelling at different data-rich levels is holding out the prospect of very exciting integration of function [20]. The simulations included the deformation that occurs as mechanical events influence blood flow.

This model has already been used to investigate the changes in blood flow that occur following constriction or block of one of the main arterial branches, and work is in progress to connect this to the modelling of ischaemia at the cell and tissue level. If we can also connect the cellular mechanisms of arrhythmia to the processes by which regular excitation breaks down into the multiple wavelets of ventricular fibrillation [21] then yet another 'grand challenge' for integrative physiological computation will come within range: the full-scale reconstruction of a coronary heart attack.

This is a suitable point at which to note that I chose the term 'grand challenge' deliberately. This kind of work requires massive computer power. The whole organ simulations require many hours of computation using supercomputers. In contrast, the single cell models can be run faster than in real time on a PC or laptop! Future progress will be determined partly by the availability of computing capacity.

The future: from genome to proteome to physiome

Computer modelling of biological systems is an important technique for organizing and integrating vast amounts of biological information. Although this paper has focused on modelling of the heart, it is important to note that biological simulation is now being done for a wide range of pathways, cells and systems (see, e.g. http://www.afcs.org). The role of *in silico* biology in medical and pharmaceutical research is likely to become increasingly prominent as we seek to exploit the data generated through rapid gene sequencing and proteomic mapping through to creating the physiome (see http://www.physiome.org and http://www.physiome.org.nz).

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