Biological simulations in drug discovery

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Simulation of biological processes, systems and organs is a necessary complement to genetic and molecular sequencing. Using the heart as an example, the authors describe the way in which such modelling can be used in drug discovery, development and assessment. The technology is readily applicable to other organs and systems as well; models of neurones and neuronal systems, the pancreas and the lungs have already been developed. Ultimately, a *virtual corpus* is feasible.

Genes can only specify the properties of the proteins they code for, and any integrative properties of the system must be 'computed' by their interactions –
Sydney Brenner

Sydney Brenner[1] meant not only that biological systems themselves 'compute' these interactions, but also that in order to understand them *we* need to compute them, and he concluded: 'this provides a framework for analysis by simulation'. The purpose of this article is to describe how far such simulation has progressed, what its prospects are and how it can already be used as a powerful, even necessary, tool in drug discovery, assessment and development.

Modelling biological processes has a long history, but biological simulation that models the activities of individual protein mechanisms such as channels and transporters began with the Nobel Prize-winning work of Hodgkin and Huxley[2] in 1952 on the squid giant axon. Soon after, early models of cardiac cells were also developed[3]. These models were limited not only by the information available about nerve, heart and other cells, but also by the sheer inadequacy of computing power. In consequence, they could not become rich enough either to simulate disease states or to form a basis for the analysis of drug actions, though it is worth noting that the squid nerve model did serve to analyse the effects of anaesthetics.

The situation today is totally transformed. Models of biological systems now contain sufficient detail not only to reconstruct normal functions but also to reconstruct major disease states. By incorporating the biochemical and biophysical properties of enzymes, receptors, ion channels and other gene products, the interactions of drugs with these cellular entities can be modelled, and simulations of drug action on normal and diseased tissue can be achieved.

There is an urgent need for such simulation. We will otherwise be unable to cope with the avalanche of new information being generated at the molecular and genetic levels. The Human Genome Project is revealing tens of thousands of genes for which the function of the encoded proteins is unknown. The molecular roles of these new gene products can only be determined by elucidating their enzymatic or receptor activities and determining how these proteins interact in whole cell and organ systems. Simulation is an essential tool in linking vertically from the genome to the functional level. Simulation of biological systems at the cellular and organ levels can provide crucial information concerning the role and physiological importance of new gene products.

The technology

Computing power has increased exponentially for many years. Even laptop machines now have the power to handle complex models of single cells, while models of organs can be reconstructed using parallel computers in which many processors work simultaneously to increase the speed of computation. Extrapolating these developments over the next few years, we have to envisage that desktop machines of the future will have even greater capability than the largest supercomputers of today. Moreover, the biological

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information available is now so rich, and the new generation of cell models so detailed, that it is possible even to represent the effects of single mutations (Noble, D. and Noble, P.J., submitted). This opens the way to studying the effects of multiple mutations, and so to studying the way in which drug actions will vary in different individuals. Patient stratification is achievable as a predictive tool.

In fact, the technology is developing so fast that it is becoming feasible in our lifetime for all experimentalists to have their virtual organ on hand as they design experiments. The Internet is also set to play a role, providing access to simulations and databases worldwide.

The models

Models are created by extracting the relevant functional information on the properties of enzymes, receptors, transporters and other gene products, and incorporating these data into systems of equations that represent the interactions between the proteins via their cell and tissue environment. The methods by which these equations can be solved depend on the system being modelled. Some properties can be represented as algebraic solutions to the equations. More often, systems of differential equations (or mixed differential and algebraic equations) are created. These are usually highly nonlinear, so full analytical solutions are hardly ever obtainable (but see Ref. 4). If the interactions can be represented as mediated by levels of ions, metabolites, etc. that are effectively uniformly distributed within each cell or subcellular compartment, then systems of ordinary differential equations are adequate. When diffusion gradients are important, or when cell models are incorporated into networks or whole organs, then we must resort to partial differential equations and the computational problems become much greater.

Modelling the heart

All organs and systems are capable of such simulation, but modelling has progressed most rapidly in the case of the heart. Most cell types in this organ have been simulated[5–17], and these simulations have been incorporated into large networks representing two- and three-dimensional tissue[18–23], and into accurate models of the whole ventricles[24–31]. In this case, therefore, a major part of a virtual organ already exists. It can be used not only to simulate normal functions, such as rhythmicity, contraction and blood flow, but also to reconstruct effects on the rest of the body in the form of the electrical changes that we call the electrocardiogram (ECG). Accurate models of the torso are also now available (Fig. 1).

These achievements led to the first use of such a simulation in the assessment of drug action on the ECG for the American Food and Drug Administration (FDA)[32]. The particular drug being assessed was the T-type Ca^{2+} channel blocker mibefradil, but the ECG perturbations concerned are in fact attributable to an action, at higher doses, on L-type Ca^{2+} channels. The results are therefore also applicable to commonly used drugs such as verapamil, a fact that had not previously been appreciated. Such drugs shorten the action potential, as would be expected from Ca^{2+} channel blockade, but they also broaden the T wave in a way that resembles the effect of drugs that prolong the action potential and thus might predispose to arrhythmias such as torsades de pointes. Hence the concern of the FDA. The whole ventricular model placed in a volume conductor to enable computation of the ECG was an ideal tool for resolving this apparent paradox. It was resolved by showing that closely similar ECG changes may have quite different cellular explanations and that the effects observed at the single cell level might be extrapolated to ECG prediction. We now have a powerful tool for assessing the ECG side effects of any new compound that might have cardiac actions. It is not too fanciful to expect that drug testing of this kind will one day become obligatory.

Models of arrhythmia

Another use of an anatomically detailed heart model is to create models of arrhythmia that can be used to screen for potentially therapeutic drug actions. A good example is the work at Johns Hopkins University, where Raimond Winslow's team has used experimental data on expression levels of various transporter proteins obtained by Eduardo Marban's team studying cells from congestive heart failure patients. When these changes are introduced into cell models, the characteristic prolongation of the action potential is well reproduced, as is the occurrence of arrhythmogenic after-depolarizations[33]. Introducing these congestive heart failure cell models into a whole ventricular model leads to the generation of multiple re-entrant arrhythmias, and the computed ECG resembles that of congestive heart failure patients. This model is now being used to search for beneficial drug actions.

Myocardial ischaemia

A further example of an important pathology that can be reproduced in the case of the heart is myocardial ischaemia. Here, it is essential to link the modelling of the cell electrophysiology to that of the key metabolic pathways. This work has led to the reconstruction of the cellular basis of ischaemic arrhythmias and of the arrhythmias of reperfusion. The modelling being developed by Denis Noble's team at Oxford University is based on the modelling of some of the processes that control metabolites and pH during total ischaemia (see Ref. 34 for details). The

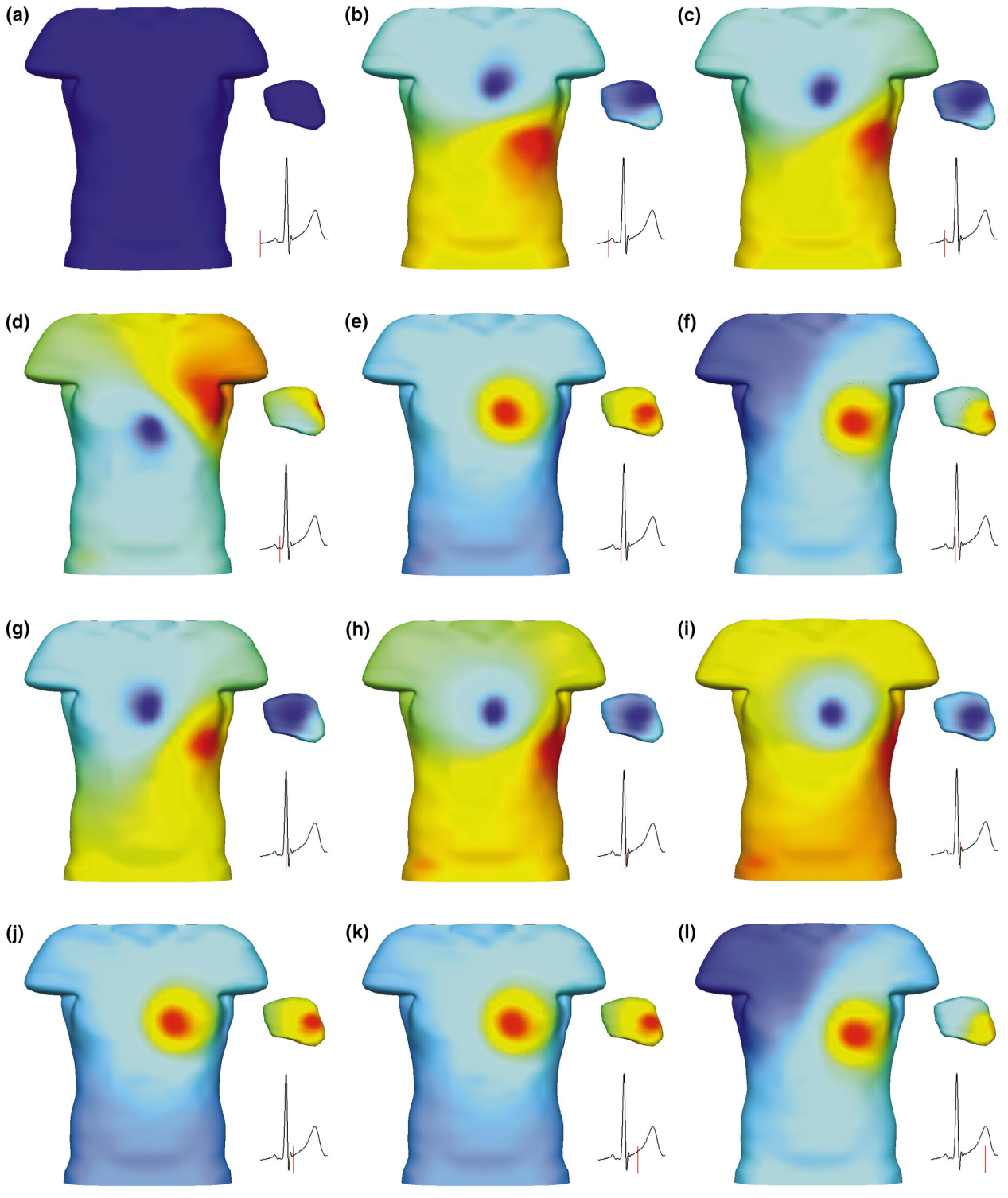

Figure 1. *Frames from a movie showing modelling of the heart within the torso, permitting accurate reconstruction of the electrocardiogram. (a) Resting state. (b) Beginning of P wave. (c) Peak of P wave. (d) Before QRS complex. (e) Beginning of QRS complex. (f) Rising phase of R wave. (g) Peak of R wave. (h) Decline of R wave. (i) End of QRS complex. (j) ST segment. (k) Beginning of T wave. (l) Peak of T wave. From Pullan and Hunter (1998).*

model predicts the cardioprotective action of drugs (such as HOE694) that block the Na^+–H^+ exchanger and some important counterintuitive predictions concerning the role of Na^+–Ca^{2+} exchange and drugs that act on this protein transporter[34] can also be made.

Broad application

There are many kinds of cardiac arrhythmia to which this approach is applicable. Modelling atrial fibrillation, for example, becomes feasible as human atrial and sinoatrial node cell models are added to the ventricular model to complete the virtual organ. The role of mechanoelectric feedback in the generation and suppression of arrhythmias has also been extensively studied and modelled[35–41], and counterintuitive predictions by those models have been confirmed with subsequent direct experimental investigations.

While creating a virtual heart is a project that is way ahead of progress with other organs, the same strategy is being applied to other biological systems. Models of many types of nerve cells and of the pancreas, liver, lung, muscle and kidney are all in development[42–45]. The same software tools can be used, so that much of the computer and programming experience in modelling the heart is readily transferable. Recall too that most of the genes that code for our brains also code for our genitals. Nature knows the same tricks: re-use components that work. This means that the database representing the proteins whose functionality the modelling reproduces is one that naturally cross-refers from one organ to another.

This raises the prospect that when we are able to deliver the entire complement of organs they will be linked to provide an integrated model of the entire body – a *virtual corpus*. A team at Auckland University predicts that a whole chest, incorporating models of lungs, heart, circulation and torso, is feasible within three years. This project will become increasingly useful as more organs are added to it, because many problems in drug development arise from unexpected or unpredicted actions on organs other than the original target organ.

Drug discovery

In nearly every therapeutic area of drug discovery there is a need for change. This is a natural outgrowth of the evolution and understanding of disease processes, coupled with the increasing sophistication of the tools used in identifying causes of disease. The strategies adopted by pharmaceutical and biotechnology companies through the 1970s to the early 1990s are no longer viable.

In broad outline, the strategies of different therapeutic areas are similar, but adapted to the particular organ, tissue or body system and to the diseases to be studied. Biological simulations will increasingly play a central role in the discovery of new drugs for all therapeutic areas as additional organ and tissue models become available.

Discovering drug targets

The wealth of biochemical, cell biological and physiological information that already exists will be the foundations of these models. Data on the new gene products can also be readily incorporated into the models as these become available. Consequently, tissue and organ models can be used to search for new drug targets. A drug target is the protein, usually an enzyme or receptor, that the drug is designed to interact with to alter a metabolic process and produce a beneficial effect in a particular disease. '*In silico*' tissue and organ models are completely novel tools that allow drug discovery teams to search for possible drug targets in a facile and systematic manner. The search can be carried out in disease models as well as in models of physiologically normal tissue. Also, where necessary, models of test animal tissues and organs can be built to compare results directly with those obtained in human counterparts. Once possible drug candidates are identified, they will need to be validated experimentally. Thus, simulations will not eliminate, but might significantly reduce, the number of laboratory and animal experiments necessary to select a final drug target.

Evaluating drug candidates

Similarly, tissue and organ models can be used to examine the therapeutic value and possible toxicities of drug candidates, with the same benefits of increased efficiencies and cost reductions, and with greater predictive power.

These points are exemplified in the heart, where there is an acute and serious need for a new approach to drug discovery. Clinical trials of antiarrhythmic drugs have been disappointing or worse. Examples of these failures abound, from the severely disturbing results of CAST (the Cardiac Anti-Arrythmia Suppression Trial; see Ref. 46) through to the recent clinical trial of the class III antiarrhythmic Dofetilide. In retrospect, these failures are not too surprising. The paradigm of drug discovery on which they were based is deeply flawed. The idea was to identify the protein transporter carrying the arrhythmogenic current and then to block it. There are several problems with this approach. The first is that blocking the last (arrhythmic) stage in a biological cascade can have unexpected consequences earlier in the sequence of ischaemic events. There is no guarantee that these will be beneficial. Second, the transporter concerned might play more than one role in the functioning of the organ, and the key function need not be its electrogenic one. A good example of this kind of

problem is the role of the Na^+–Ca^{2+} exchanger, which has arrhythmogenic electrogenicity as a side effect of its central function to use the Na^+ gradient to pump Ca^{2+}. Interfering with this transporter in models of ischaemic arrhythmias has complex, even counterintuitive, actions[34]. The heart serves as a generalization for the approaches adopted in many of today's drug discovery programmes.

One of the consequences of the traditional approach to drug discovery is that the pharmaceutical industry has accepted the dogma that the overwhelming majority of drug discovery and development programmes are expected to fail. It is likely that the adoption of the new technologies described here will reverse this trend by making the drug discovery and development processes more predictive and further allow the industry to readdress complex diseases, such as cardiac arrhythmia, which represent a therapeutic need as well as a large global market.

Genomics

The issues of dealing with the inherent complexity of biology in drug discovery represent an equally daunting challenge. Genomics, the discovery and study of new genes, opens new and exciting possibilities to develop novel drug targets. It is estimated that the human genome contains 120,000 genes. It is further estimated that the great majority of these will be fully sequenced within the next three years. Nearly every major drug discovery programme has begun to source the available data on such genes. While huge amounts of data have been generated on the expression of genes in disease and normal states, until now no tools have been developed to decipher the biological information within those data. Most of these data focus on the analysis of particular genes, searches for homologous genes and identification of associations (largely phenomenological) between genes and particular disease states. Many of these genes, for example genes encoding structural proteins, are of little use in drug discovery. Further, as our knowledge of disease states evolves, it has become clear that many, if not most, diseases result from a complex interplay of genes, mediated through the interactions between the proteins they code for. Most drug discovery programmes are overwhelmed with such data and cannot effectively use what is likely to be a treasure trove of critical information. It can be argued that without addressing the problem of complex relationships between genes and their expression at the organ level in a rational fashion, the full potential of genomics will not be realized. The tools of modelling permit such exploration of the basic biology of a single gene product in the context of multiple other genes and also in the setting of the physiological organ (a good example is the congestive heart failure model referred to above). This process, once automated, will open the possibility to fully exploit much of the data being generated in the extensive databases of the Human Genome Project.

As an example of the potential benefit, consider the problem of the failure of so many attempts at cardiac antiarrhythmia drug discovery. Because susceptibility to most cardiovascular diseases probably has a multigenic basis, genomics linked to functional interpretation through modelling will have a very positive impact in determining the roles of the genes involved.

Linking to other biological databases

Clearly, the underlying basis of all tissue and organ models is databases that contain complex sets of information, such as properties of individual gene products, anatomical data and so on. In the case of modelling transporters, these databases contain the relevant constants to be used in the equations for ion-channel gating, activation by ion concentrations and metabolites, interaction with drug receptors, and cell and intercell signalling systems. The anatomical models into which the cell models are inserted also correspond to databases on the relevant structural detail. The construction of such databases and the techniques for data mining remain important issues. Until recently these appeared to be formidable problems. However, newer techniques emerging from information technology (IT) are applicable to these problems and offer new opportunities for biologists to collaborate with their IT colleagues.

The quality of data for the construction of models is another issue. Although the magnitude of the problem remains to be defined, it is likely that enormous amounts of new data will be required. The Physiome Project was recently formed by the International Union of Physiological Sciences (IUPS) to address many of these issues, as well as to provide a forum for scientists to exchange information and to cooperate in building models. It will bring together scientists from the relevant disciplines to coordinate and guide work in this rapidly growing field (see Ref. 47 for further details).

Beyond creating links to databases for model construction, other links are being developed that will greatly enhance the usefulness of models. Links to functional genomics databases will provide automatic assessment of the physiological significance of the newly discovered gene products and a rapid assessment of their potential usefulness as drug targets. Links to genetics databases and to databases collating differential gene expression in normal tissues and organs as well as in disease states offer a myriad of opportunities. For example, with form analyses of SNPs (single nucleotide polymorphisms), information on genetic predisposition to disease can be incorporated into models, as can patient

response to drug therapy (pharmacogenomics). With tissue and organ models, automatic and early assessment of drug toxicities will be a reality in multiple species (see above).

These links are not without their problems though. A major gap still occurs at the stage of predicting function from protein sequence and structure. We simply do not yet have the theoretical chemical tools to achieve this (see, for example, the discussion of Williams[48]), although the US DoE has initiated a programme whose stated goal is to provide the three-dimensional structure of all known protein folds.

Side effects and animal models

Most drugs brought to clinic that subsequently fail, do so through encountering unexpected side effects. Efficacy is also an issue. Drugs can work in animal models but fail in clinical trials, which raises the issue of the predictive nature of animal models. It is therefore essential to have a tool that will help determine the hierarchical significance of any one molecule in a pathway, evaluate the role of that molecule in the generation or suppression of a disease process at the cellular and organ levels, determine the specificity of the molecular process in disease and then rapidly scan the actions of suppressing or activating that molecule on all organs and systems for possible side effects. The data to do this, however, must be based on human biology.

Such a tool becomes increasingly useful as more organs are added: the effect is a high-power multiplier of efficacy in searching for possible toxicity. While dramatic efforts are being made to create more effective animal models using the tools offered by transgenic technologies, it remains clear that animal surrogates can never fully simulate the human. Cures for cancer abound in rats and mice but are disappointingly few in humans. Again, computer modelling offers the only reasonable methodology to supplement animal testing effectively and in so doing cut back on such testing while increasing the effectiveness of preclinical assessment of compounds. The hope must be that we can progressively reduce animal experimentation and use those experiments that are necessary more efficiently and effectively.

One of the consequences of early evaluation of side effects will be that there will be fewer compounds entering trials, but the goal is that there should be a much higher proportion of successes.

Validation

An important question is how reliable are the models, and how do we assess this? This is a complex question, but the short answer is that reliability can only come from progressive iterative interaction between simulation and experimentation. This is exactly how already well-established fields of modelling have earned their spurs. Aircraft simulation, for example, was once an adjunct to wind-tunnel experiments. Now the simulation is so good that the wind-tunnels are no longer used, and the methodology reliable enough for the recent successful design of a car to break the sound barrier to be totally dependent on simulation. No prior experiment was feasible in this case.

We are not yet at this stage with biological modelling, of course, but it is important to note that there is already around two decades of iteration between modelling and experiment. Some parts of biological modelling are therefore themselves already well established. The second important point to note is that modelling becomes useful, even essential, long before it becomes 'perfect'. All models are, of course, only partial representations of reality (the only perfect computation, as Sydney Brenner[1] indicated, is provided, by definition, by the biological system itself). The utility expands greatly, though, as increasingly large areas of reality are brought within the models. It is important also to note the role of multiple models of the same process. This is not, usually, a matter of redundancy: it is itself of great value. By determining whether predictions are strongly model-dependent we can guard against the possibility that we are investigating only the properties of particular models.

It is important too not to underestimate the difficulties involved. Biological data need expert interpretation: they are not there to be taken blindly out of automated databases. Experimental conditions can vary significantly, yet data from different laboratories are often used in pasting models together. Moreover, someone has to decide which parts of a complex system are relevant. Models of metabolic processes, for example, range from ambitious attempts to database very large numbers of enzyme reactions[34] to selecting functional subsets[35]. The balance in selecting the level of modelling, however, must always be judged by the ability to develop models that are predictive and have emergent properties rather than overwhelming amounts of data. It is this balance which will determine the success of metabolic pathway modelling, particularly as information from the genomic and biochemical analysis will in the future permit us to incorporate tens of thousands of proteins into such models.

A sure indicator of how far along the validation path particular models have progressed is to ask how readily the models can be adapted to disease states by introducing the molecular and cellular changes known to occur in these states. A good example of this in the case of the heart model is that sodium overload, which is known to occur in ischaemia, is sufficient, by itself, to induce the conditions of arrhythmia. The models require no other input. Similarly, changing the levels of expression of protein transporters to

correspond to those in congestive heart failure is sufficient to reproduce torsades de pointes. These examples mean that the rest of the system, which forms the complex context in which these disease states develop, is robustly modelled. The correct predictions concerning well-established drug actions also form part of the validation. The process of validation is thus a piecemeal one: a little like completing a jigsaw. Once enough pieces are correctly in place the picture becomes clear. There is no rigorous algorithm by which this can be assessed, but it becomes obvious once it has been achieved.

Conclusions

Biological simulation is not just a luxury for those few biologists who like mathematics or computing, even though it may have appeared so in the early days when access to computing power and knowledge of the strengths of simulation were both very limited and restricted to an elite. As the quotation from Sydney Brenner at the beginning of this article makes clear, biological computation is a natural following of nature herself. Genes code for the sequences of a very large number of proteins. The functionality emerges through the interactions between these proteins, which can therefore be viewed as a computational machine. No amount of soul-searching through the gene sequences themselves will throw light on how this is done. The 'logic of life' (see Ref. 49) does not reside at this level. It is to be found at the level of the interactions, and at very many different levels of these interactions, all the way from subcellular systems to whole organisms, and even their environment. If we wish to understand these interactions, and how they might be interfered with to produce effects of therapeutic benefit, there really is no alternative to following in nature's own footsteps and doing the simulations ourselves.

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