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Biological switches and clocks

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To introduce this special issue on biological switches and clocks, we review the historical development of mathematical models of bistability and oscillations in chemical reaction networks. In the 1960s and 1970s, these models were limited to well-studied biochemical examples, such as glycolytic oscillations and cyclic AMP signalling. After the molecular genetics revolution of the 1980s, the field of molecular cell biology was thrown wide open to mathematical modellers. We review recent advances in modelling the gene–protein interaction networks that control circadian rhythms, cell cycle progression, signal processing and the design of synthetic gene networks.

Keywords: bistability; oscillations; chemical reaction networks

1. INTRODUCTION

The living cell receives signals from its environment and its own internal state, processes the information, and initiates appropriate responses in terms of changes in gene expression, cell movement, and cell growth or death. Like a digital computer, information processing within cells is carried out by a complex network of switches and oscillators, but instead of being fabricated from silicon transistors and quartz crystals, the cell's computer is an evolved network of interacting genes and proteins. In the same way that computer design was made possible by a sophisticated theory of electronic circuitry, a basic understanding of cellular regulatory mechanisms will require a relevant theory of biomolecular circuitry. Although the 'engineering mindset' is sorely needed to make sense of the cell's circuitry, the squishy, sloppy, massively parallel, analogue nature of biochemistry is so different from the solid-state, precise, sequential, digital nature of computers that the mathematical tools and intellectual biases of the solid-state physicist/electrical engineer are not entirely appropriate to unravelling the molecular logic of cell physiology. New modelling paradigms and software tools are evolving to meet the challenges of the new 'systems biology' of the living cell.

In this context, we organized a six-week workshop on 'Biological switches and clocks' at the Kavli Institute for Theoretical Physics in Santa Barbara, CA, in summer 2007. The goal of the workshop was to bring together a diverse group of theorists and experimentalists, who shared a common interest in understanding the molecular mechanisms that control the physiological properties of living organisms. The programme was organized around a few main themes: circadian rhythms; signalling networks; cell cycle regulation; synthetic gene networks; and deterministic and stochastic modelling. This special issue of *Interface* comprised peer-reviewed contributions from the participants in the workshop.

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One contribution of 10 to a Theme Supplement 'Biological switches and clocks'.

2. HISTORICAL CONTEXT

The first 'theoretical biologists' to consider biological interactions as dynamical systems were Alfred Lotka (a physical chemist) and Vito Volterra (a mathematician), who studied the pair of nonlinear ordinary differential equations

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(x, y), \qquad \frac{\mathrm{d}y}{\mathrm{d}t} = g(x, y), \tag{2.1}$$

where x and y are the variables describing the time-dependent state of a chemical or biological system. Lotka (1924) and Volterra (1931) proposed models with periodic solutions (clocks) and multiple steady states (switches), which Volterra applied in the field of population biology to describe predator–prey oscillations and competition between species. For example, the Lotka–Volterra equations for prey (x) and predator (y) species are

$$\frac{\mathrm{d}x}{\mathrm{d}t} = ax - bxy, \qquad \frac{\mathrm{d}y}{\mathrm{d}t} = cxy - dy, \tag{2.2}$$

where a, b, c, ... are 'rate' constants. Equations (2.2) have a one-parameter family of nested periodic solutions given by a 'conservation of energy' condition

$$E(x, y) = a \ln y - by + d \ln x - cx = \text{const.}$$

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The Lotka–Volterra equations for two competing species (x and y) are

$$\left.\begin{aligned}\frac{\mathrm{d}x}{\mathrm{d}t} &= ax(K-x)-bxy,\\ \frac{\mathrm{d}y}{\mathrm{d}t} &= cy(L-y)-dxy.\end{aligned}\right\} \tag{2.3}$$

If $c/d<K/L<b/a$, then these differential equations have two stable steady-state solutions

$$(x=K,\ y=0) \quad \text{and} \quad (x=0,\ y=L),$$

separated by an unstable steady state at

$$\left(x=c\frac{bL-aK}{bd-ac},\ y=a\frac{dK-cL}{bd-ac}\right).$$

Dynamical systems (2.2) and (2.3) are paradigms of oscillations and bistability in a biological context.

In equations (2.2) and (2.3), x and y are interpreted as populations of interacting biological species in some common physical environment, but, in principle, they could just as well be interpreted as concentrations of reacting biochemical moieties in a cell. In the latter interpretation, the systems would model biochemical oscillations and bistability. But in the 1930s, no one was seriously thinking along these lines, because there were no known examples of biochemical clocks or switches. The field of cell biology was only beginning to find its roots in biochemistry and genetics.

To be sure, circadian rhythms and spontaneous oscillatory electrical impulses were recognized as interesting and important cellular phenomena, but the molecular bases of these activities were completely unknown. Embryonic development also inspired speculations about how biochemical reactions might spontaneously break symmetry and generate spatial patterns, but, aside from a singular paper by Turing (1952), no one had any idea on how 'biochemistry' might escape the boring fate of uniform chemical equilibrium. Classical biochemistry, with its linear pathways converting substrates A and B into end products Y and Z, seemed to offer little scope for exotic chemical dynamics.

This situation changed dramatically in the 1950s and the early 1960s, with the recognition of positive and negative feedback control of gene expression (Jacob & Monod 1961; Umbarger 1961) and with the discoveries of bistability in *lac* operon induction (Novick & Weiner 1957), oscillations in yeast glycolysis (Chance *et al.* 1964; Ghosh & Chance 1964) and periodic enzyme synthesis in bacteria (Masters & Donachie 1966). Biochemists began to appreciate the importance of switches and clocks in molecular cell biology. In 1965, Brian Goodwin presented a simple model of periodic enzyme synthesis based on the negative feedback of metabolic end products on gene transcription (Goodwin 1965), and in 1968 Joseph Higgins published a ground-breaking paper on the theory of biochemical oscillations in enzyme-catalysed reaction networks (Higgins 1967). The work by Field *et al.* (1972) and Field & Noyes (1974) on the oscillatory Belousov–Zhabotinsky reaction provided the first detailed and predictive mechanistic description of a reaction system showing bistability as well as temporal, spatial and chaotic oscillations (Field & Burger 1985; Epstein & Pojman 1998).

A particularly simple example of a chemical reaction network exhibiting exotic dynamics (oscillations, bistability, pattern formation, travelling waves) was the 'Brusselator' of Prigogine & Lefever (1968), whose temporal dynamics are governed by

$$\left.\begin{aligned}\frac{\mathrm{d}x}{\mathrm{d}t} &= a-bx+cx^2y-dx,\\ \frac{\mathrm{d}y}{\mathrm{d}t} &= bx-cx^2y-ey.\end{aligned}\right\} \tag{2.4}$$

For the special case $e=0$ (as originally proposed by Prigogine & Lefever), this dynamical system has a unique steady state at ($x=a/d$, $y=bd/ac$), which is unstable if $d^3-bd^2+ca^2<0$. When the steady state is unstable, it is surrounded by a unique, stable limit cycle oscillation (figure 1). For $e\neq 0$, it is possible for the Brusselator to exhibit bistability, as illustrated in figure 2. The regions of oscillations and bistability come together in a characteristic X-shaped two-parameter bifurcation diagram (figure 3). Oscillations and bistability in the Brusselator are based on the ternary autocatalytic reaction, $\mathrm{Y}+2\mathrm{X}\rightarrow 3\mathrm{X}$. Exploiting the exotic properties of this reaction, Sel'kov (1968) presented a simple, effective model of glycolytic oscillations, and Schlögl (1972) devised a simple, popular model of bistability.

The early theoretical work of Goodwin, Higgins, Prigogine and Lefever, Sel'kov and Schlögl (Goodwin 1965; Higgins 1967; Prigogine & Lefever 1968; Sel'kov 1968; Schlögl 1972) convinced biophysical chemists that oscillations and bistability were definitely to be expected in biochemical reaction systems with positive and negative feedbacks to destabilize steady-state solutions. Although a ternary autocatalytic reaction such as $\mathrm{Y}+2\mathrm{X}\rightarrow 3\mathrm{X}$ is unlikely in physical chemistry, high-order nonlinearities in substrate concentration can be achieved by multi-subunit enzymes of the type being discovered at that time to regulate biochemical pathways (Monod *et al.* 1965; Goldbeter & Lefever 1972).

Bistability and oscillations were also recognized, at this time, as expected features of genetic regulatory systems with positive and negative feedbacks. A simple and elegant theoretical treatment of these cases was published by Griffith in 1968. For a positive feedback system (x=mRNA, y=protein),

$$\frac{\mathrm{d}x}{\mathrm{d}t}=\frac{a+y^2}{K^2+y^2}-bx, \qquad \frac{\mathrm{d}y}{\mathrm{d}t}=cx-dy, \tag{2.5}$$

Griffith showed that mRNA level can easily show bistable (low or high) expression as a function of the demand d for the protein (Griffith 1968*a*). In an accompanying paper, Griffith (1968*b*) showed that the corresponding negative feedback system,

$$\frac{\mathrm{d}x}{\mathrm{d}t}=\frac{a}{K^p+y^p}-bx, \qquad \frac{\mathrm{d}y}{\mathrm{d}t}=cx-dy, \tag{2.6}$$

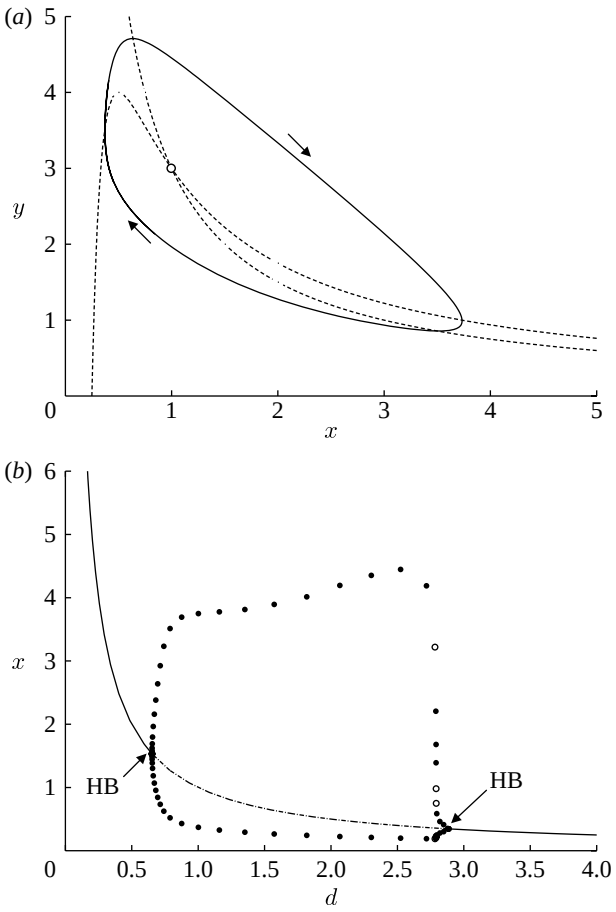

Figure 1. Oscillations in the Brusselator equation (2.4). Parameter values: $a=1$, $b=3$, $c=1$, $d=1$ and $e=0$. (*a*) Phase plane. Dashed lines are the x-nullcline, $y=[(b+d)x-a]/(cx^2)$, and the y-nullcline, $y=b/(cx)$. Solid line is the limit cycle oscillation, which encircles the unstable steady state (open circle). (*b*) One-parameter bifurcation diagram. Thick solid line is a locus of stable steady-state solutions, interrupted by (thin solid line) a locus of unstable steady-state solutions. The circles indicate how the amplitude of the limit cycle oscillation (maximum and minimum values of x) depends on d between the two Hopf bifurcation (HB) points, at $d=0.6527$ and 2.879.

has a unique, stable steady state (i.e. no bistability or oscillations). However, a negative feedback loop with n components,

$$\left.\begin{aligned}\frac{\mathrm{d}x_1}{\mathrm{d}t} &= \frac{a_0}{K^p + x_n^p} - b_1 x_1,\\ \frac{\mathrm{d}x_2}{\mathrm{d}t} &= a_1 x_1 - b_2 x_2,\\ &\cdots\\ \frac{\mathrm{d}x_n}{\mathrm{d}t} &= a_{n-1} x_{n-1} - b_n x_n,\end{aligned}\right\} \qquad (2.7)$$

can exhibit limit cycle oscillations, as Griffith proved, if the nonlinearity p of the feedback control is sufficiently strong: $p > \sec^n(\pi/n)$. These compelling results laid the foundation for a theory of genetic regulatory systems (Tyson & Othmer 1978).

3. INTERLUDE

Although these scientists in the 1960s and 1970s created a mathematical theory of switches and clocks

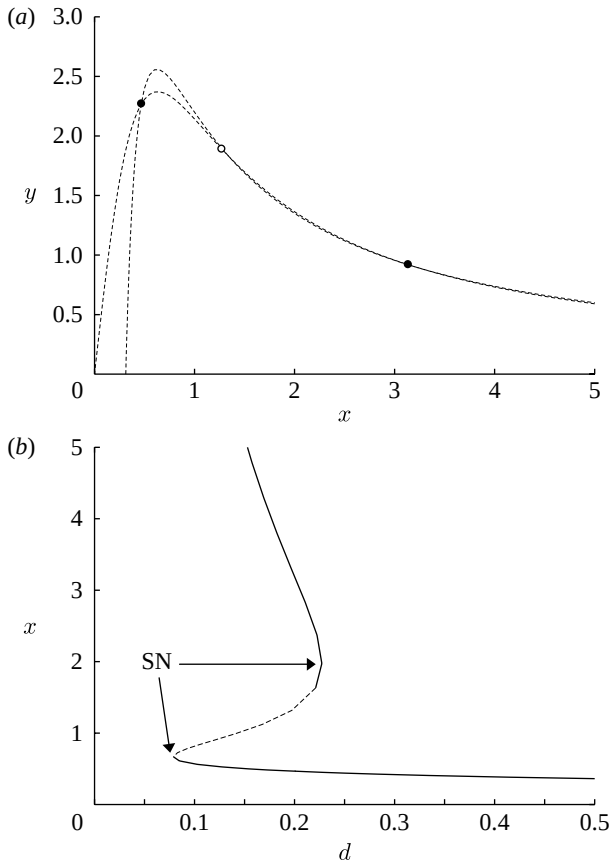

Figure 2. Bistability in the Brusselator equation (2.4). Parameter values: $a=1$, $b=3$, $c=1$, $d=0.2$ and $e=0.4$. (*a*) Phase plane. Dashed lines are the x- and y-nullclines as shown in figure 1. Solid circles, stable steady states; open circle, unstable steady state (saddle point). (*b*) One-parameter bifurcation diagram. The solid line is the locus of steady-state solutions. The upper and lower branches are stable steady states; the intermediate branch tracks unstable steady states. The stable and unstable branches come together at saddle-node (SN) bifurcation points, at $d=0.07894$ and 0.2273.

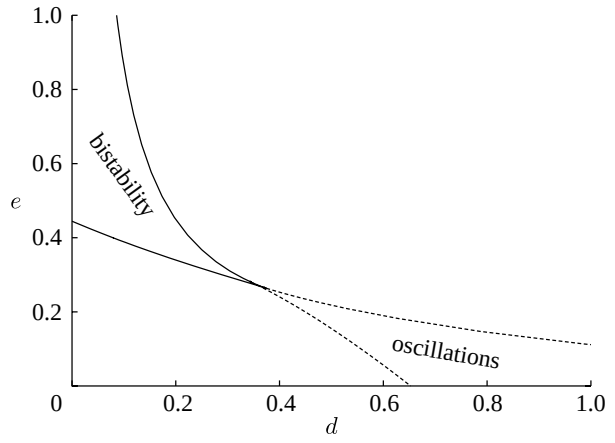

Figure 3. Two-parameter bifurcation diagram for the Brusselator. The solid lines track the loci of SN bifurcations in dependence on d and e. Note that, for example, for $e=0.4$, two SNs occur at $d=0.07894$ and 0.2273, as shown in figure 2*b*. The dashed lines track the HB points. For $e=0$, two HBs occur at $d=0.6527$ and 2.879, as shown in figure 1*b*.

in molecular cell biology, there were only a few examples of interesting cell physiology for which enough molecular machinery was known to apply the

Table 1. Molecular cell biologists recognizing a need for mathematical modelling.

Hartwell *et al.* (1999)	'The best test of our understanding of cells will be to make quantitative predictions about their behaviour and test them. This will require detailed simulations of the biochemical processes taking place within [cells]. … We need to develop simplifying, higher-level models and find general principles that will allow us to grasp and manipulate the functions of [biochemical networks].'
Venter (1999)	'If we hope to understand biology, instead of looking at one little protein at a time, which is not how biology works, we will need to understand the integration of thousands of proteins in a dynamically changing environment. A computer will be the biologist's number one tool.'
Fraser & Harland (2000)	'[R]esults to date show a dizzying array of signalling systems acting within and between cells. … In such settings, intuition can be inadequate, often giving incomplete or incorrect predictions. … In the face of such complexity, computational tools must be employed as a tool for understanding.'
Nurse (2000)	'Perhaps a proper understanding of the complex regulatory networks making up cellular systems like the cell cycle will require a … shift from common sense thinking. We might need to move into a strange more abstract world, more readily analyzable in terms of mathematics than our present imaginings of cells operating as a microcosm of our everyday world.'

theory profitably. Notable successes involved theories of glycolytic oscillations, cyclic AMP signaling, and calcium oscillations and waves. For details, consult the books by Goldbeter (1996) and Winfree (2001). But other important problems—such as the origins of circadian rhythms, the regulation of fruit fly development, the coordination of cell growth and division or the chemotactic response of motile bacteria—lay beyond the grasp of theorists, because nothing was known about the genes and proteins which controlled these processes. Theoretical developments floundered in the 1980s, awaiting that decade's spectacular advances in molecular genetics. By the early 1990s, there were enough reliable clues about molecular mechanisms for mathematical cell biologists to polish up their equations and rev up their computers. Early successes included the works of Bray *et al.* (1993) on bacterial chemotaxis, Novak & Tyson (1993) on frog egg cell cycles, Goldbeter (1995) and Ruoff & Rensing (1996) on circadian rhythms in *Drosophila* and *Neurospora*, Reinitz *et al.* (1995) on fruit fly morphogenesis and McAdams & Shapiro (1995) on bacterial circuits.

By 2000, a major shift in opinion was developing in favour of quantitative modelling in molecular cell biology. This shift was powered by a growing number of significant theoretical contributions (Arkin *et al.* 1998; Sharp & Reinitz 1998; Van Dassow *et al.* 2000; Asthagiri & Lauffenburger 2001), an increasing recognition among molecular biologists of the field's need for mathematical models (table 1) and the commitment of key administrators at NIH, NSF, DOE and elsewhere to target research support to quantitative biology.

4. MODERN DEVELOPMENTS

Since 2000, the field of molecular systems biology has blossomed, with the founding of an International Society for Systems Biology (www.iscb.org), of new institutes, centres and departments of systems biology across the world, and of high-profile journals (*Molecular Systems Biology*, *PLoS Computational Biology*). It is not practical here to review all the great works that have been published in recent years, but we will mention some developments that are particularly relevant to the KITP workshop on biological switches and clocks.

Perhaps the most compelling proof of the relevance of quantitative modelling in molecular cell biology was the design of artificial genetic networks that function as toggle switches and oscillators, in Jim Collins' laboratory at Boston University (Gardner *et al.* 2000) and Stan Leibler's laboratory at Princeton University (Elowitz & Leibler 2000). Both circuits were designed first in computer models quite similar to equations (2.5) and (2.7) of Griffith. Building the circuits in bacteria was a standard exercise in genetic engineering; the tricky part was engineering the proteins to have the right kinetic rate constants for synthesis and degradation. The models predicted ranges for these rate constants that must be respected in order to observe the desired behaviour, and the circuits behaved exactly as predicted by the models. From these pioneering demonstrations has arisen a new and exciting technology called synthetic biology (Hasty *et al.* 2001).

An example of how modelling can be used to design gene regulatory networks of specific functionality is found in the article by Conrad *et al.* (2008) in this special issue. They are interested in networks that respond to an input pulse train as a 'resonator' (i.e. output signal only if the pulse train has a desired frequency) or as an 'integrator' (i.e. output signal only after a desired total number of pulses). For a variety of network topologies, they show that either functionality can be achieved, provided the kinetic rate constants in the model are chosen within an appropriate range of values. That is to say, functionality is tied not so much to topology as to rate constant values. Hence, in designing gene regulatory networks, it is essential to pick not only a suitable network topology but also to engineer the rate constants into the correct range for the desired behaviour.

Quantitative modelling has also had a major impact on our understanding of the molecular basis of circadian rhythms. As new genes and proteins have been discovered and their roles in circadian physiology worked out, mathematical models of the regulatory network have become more sophisticated and accurate (Leloup & Goldbeter 2000; Forger & Peskin 2003; Locke *et al.* 2005; Ruoff *et al.* 2005*a*). Because the physiology and molecular biology of circadian rhythms are sufficiently different in diverse organisms (bread moulds, plant cells, fruit flies, mammals), models must be tailored to specific organisms, while also laying bare some of the unifying principles of circadian control in eukaryotes. Circadian rhythms of cyanobacteria, on the other hand, are controlled by a completely different

molecular mechanism that has provided a challenging problem for mathematical modellers (Emberly & Wingreen 2006; Mehra *et al.* 2006; Mori *et al.* 2007; van Zon *et al.* 2007). Many crucial properties of circadian physiology, such as temperature compensation, synchronization and phase resetting, are inherently dynamical in nature and require precise theoretical descriptions to be understood and managed (Ruoff 1992; Rand *et al.* 2004; Stelling *et al.* 2004; Ruoff *et al.* 2005*b*; Kurosawa & Iwasa 2005; Bagheri *et al.* 2007; Hong *et al.* 2007). In this special issue, Bagheri *et al.* (2008) used systems-theoretic tools (mathematical control theory) to explore the phase response characteristics of a noisy circadian clock model, in order to understand how populations of oscillators entrain one another to generate a robust rhythm and how the rhythm re-synchronizes to an external 24 hours light–dark cycle from an initial 8 hours phase shift (say, flying between Chicago and Paris).

The molecular machinery controlling cell growth and division in yeast cells, embryos and mammalian somatic cells received a great deal of attention from molecular biologists in the 1990s, with a few theoretical groups trying to keep pace with the flood of genetic and biochemical data (Obeyesekere *et al.* 1996; Kohn 1998; Aguda & Tang 1999; Chen *et al.* 2000; Qu *et al.* 2004). Although the experimentalists paid little attention to modellers during this time, in recent years there have appeared many influential papers that self-consciously test (and confirm) predictions of the models (Cross *et al.* 2002, 2005; Cross 2003; Pomerening *et al.* 2003; Sha *et al.* 2003) or bring modelling to bear on experimental design and interpretation (Pomerening *et al.* 2005; Queralt *et al.* 2006). All eukaryotes use the same basic mechanism, based on cyclin-dependent kinases, to regulate the progression of DNA synthesis, mitosis and cell division (Csikasz-Nagy *et al.* 2006), but the idiosyncrasies of specific cell types require specifically tailored mathematical models (Chen *et al.* 2004; Calzone *et al.* 2007). The cell cycles of bacteria are regulated by a completely different set of genes and proteins, unrelated to the eukaryotic control system, and modellers have focused on specific details of the gene–protein interaction network in α-proteobacteria (Shen *et al.* 2007; Li *et al.* 2008), as well as convergent evolution of the dynamical properties of the two distinct control systems (Brazhnik & Tyson 2006).

Signalling networks have also provided a rich testing ground for mathematical modelling of molecular regulatory networks. The classic example, bistability in the *lac* operon, has received thorough attention over the years, as reviewed by Santillán & Mackey (2008) in this issue. In the late 1990s, Ferrell and colleagues published a series of papers on the MAP kinase pathway in *Xenopus* eggs, in which they experimentally demonstrated bistability of its response to progesterone signals, and used modelling to suggest that this bistability is due to positive feedback in the signalling pathway (Ferrell & Machelder 1998; Ferrell & Xiong 2001). Recently, Kholodenko and others have shown theoretically that positive feedback in the MAP kinase pathway is not necessary for bistability but can arise subtly from the multisite phosphorylation reactions that are ubiquitous features of these kinase cascades (Markevich *et al.* 2004; Gunawardena 2005; Chickarmane *et al.* 2007). A major emphasis of the workshop was modelling of signalling networks, for example, pheromone signalling in yeast (Behar *et al.* 2007), bacterial chemotaxis (Keymer *et al.* 2006), regulatory circuits in the AIDS virus (Weinberger & Shenk 2007; Weinberger *et al.* 2008) and osmo-adaptation in yeast (Mettetal *et al.* 2008). In this special issue, the relevant contributions are by Csikasz-Nagy & Soyer (2008) on 'adaptation' in a simple biochemical network and by Jang & Gomer (2008) on size regulation in *Dictyostelium*.

In all these cases, the molecular reaction networks are known to be very complex and full of regulatory signals (feedback and feed-forward). The networks generate complex dynamical activity that is crucial to the survival, behaviour, development and reproduction of the living cell. Correlating the physiology of cells to the underlying molecular networks is no longer possible by intuitive biochemical reasoning, and mathematical models now play a central role in uncovering these correlations, framing our mechanistic hypotheses, and testing their implications. This new wave of realistic modelling and rigorous testing requires a new generation of computational tools for model building, model composition, simulation of real experimental protocols, comparison of simulations to a large array of experimental data, estimation of kinetic parameters from the data, statistically significant tests of model predictions, assessments of model sensitivity and robustness, and systematic analysis of mechanistic alternatives. Much progress has been made on this front, as demonstrated by the sophisticated tools now available to modellers.

— Virtual Cell, http://www.nrcam.uchc.edu/
— Copasi (Complex Pathway Simulator), http://www.copasi.org/tiki-index.php
— Systems Biology Workbench, http://www.sys-bio.org/research/sbwintro.htm
— E-Cell, http://www.e-cell.org/ecell/
— JigCell, http://jigcell.biol.vt.edu/
— Cell Designer, http://www.celldesigner.org/
— Silicon Cell, http://homepages.cwi.nl/~gollum/SiC/

Nonetheless, the computational challenges confronting the field are great, and much work is desperately needed in the area of tool development. In this regard, the paper by Rand (2008) in this special issue presents a sophisticated and promising new approach to the thorny problem of parameter estimation in complex network models with many parameters.

Modelling by discrete networks and stochastic processes were two other foci of the workshop. Discrete (Boolean) models, pioneered by Kauffman, Glass and Thomas (Kauffman 1969; Thomas 1973; Glass 1975), have been used to advantage recently in a variety of areas, including development (Albert & Othmer 2003; Chaves *et al.* 2005), immunology (Thakar *et al.* 2007) and cell cycle (Li *et al.* 2004; Faure *et al.* 2006; Davidich & Bornholdt 2008). This approach is nicely represented by two articles in this special issue. Chaves & Albert (2008) used Boolean models to study the effects of cell division on expression patterns of the segment-polarity

genes during *Drosophila* embryonic development, and Bornholdt (2008) reviewed the strengths and limitations of Boolean modelling of cell cycle regulation. Stochastic models of signalling networks (Ozbudak *et al.* 2002; Thattai & Van Oudenaarden 2004; Acar *et al.* 2008) and circadian rhythms (Forger & Peskin 2005; Yi & Jia 2005; Gonze & Goldbeter 2006) were discussed in some detail. In this special issue, Gonze *et al.* (2008) presented a stochastic version of their model of stress-induced oscillatory shuttling of Msn2 (a yeast transcription factor) between nucleus and cytoplasm.

Finally, the participants engaged in some lively discussions about if, when and how to introduce mathematical modelling into molecular cell biology classes at the undergraduate level. The majority opinion was that undergraduate students should be introduced to quantitative modelling and that this might reasonably be done with the aid of 'teaching modules' designed to complement standard textbooks. These ideas are elaborated in an article by Sible and Holmes ('Teaching at the interface: does computational cell biology fit in the undergraduate curriculum') to be published elsewhere.

5. LOOKING FORWARD

The KITP workshop brought together theoreticians and experimentalists to discuss successful collaborations, gripe about existing hurdles and forge new collaborations at the interface of math and cell biology. This special issue documents some of these success stories and problematic areas. The field of computational cell biology is here to stay, and the future looks very bright indeed (Goldbeter 2002).

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