JOURNAL OF THE ROYAL SOCIETY Interface

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J. R. Soc. Interface 2009 **6**, 257-270
doi: 10.1098/rsif.2008.0205

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doi:10.1098/rsif.2008.0205
Published online 22 July 2008

Programming with models: modularity and abstraction provide powerful capabilities for systems biology

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Mathematical models are increasingly used to understand how phenotypes emerge from systems of molecular interactions. However, their current construction as monolithic sets of equations presents a fundamental barrier to progress. Overcoming this requires modularity, enabling sub-systems to be specified independently and combined incrementally, and abstraction, enabling generic properties of biological processes to be specified independently of specific instances. These, in turn, require models to be represented as programs rather than as data-types. Programmable modularity and abstraction enables libraries of modules to be created, which can be instantiated and reused repeatedly in different contexts with different components. We have developed a computational infrastructure that accomplishes this. We show here why such capabilities are needed, what is required to implement them and what can be accomplished with them that could not be done previously.

Keywords: model building; systems biology; modularity; abstraction

1. INTRODUCTION

Systems biology seeks to understand how physiology emerges from molecular interactions (Ideker *et al.* 2001; Kirschner 2005). Mathematical models are increasingly used to shed light on this (Kitano 2002; Longabaugh *et al.* 2005; Aldridge *et al.* 2006). The construction of such models presents unusual challenges, not previously encountered in physics or engineering, upon which this paper focuses.

Models may be static, as in constraint-based models (Becker *et al.* 2007), or explicitly incorporate time. The latter are typically some form of dynamical system: they specify a set of molecular states and how those states evolve in time and space. Depending on the type of model, the states may be represented in different ways as follows: discrete levels, as in Boolean models or forms of automata (Li *et al.* 2006; Fisher & Henzinger 2007); concentrations, as in ordinary or partial differential equation models (Schoeberl *et al.* 2002; Slepchenko *et al.* 2002); molecular numbers, as in stochastic models (Ramsey *et al.* 2005); or sets of individual molecules, as in agent-based models (Danos & Laneve 2004). Time and space may themselves be treated either continuously or discretely. While much of what follows may be broadly generalized, we focus here on dynamic models represented by ordinary differential equations,

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(\boldsymbol{x}; \boldsymbol{a}), \tag{1.1}$$

where $\boldsymbol{x} \in \mathbb{R}^n$ is a state vector of species concentrations; $\boldsymbol{a} \in \mathbb{R}^m$ is a vector of parameter values; and f expresses the balance between the rates of production and consumption of each species. In such models, time is continuous and space, if it is represented explicitly at all, is discretized as a finite set of cellular compartments. Such models are predominantly nonlinear. Although they may occasionally be analysed mathematically (Gunawardena 2005), they are usually simulated numerically, for which parameter values must be specified and initial conditions chosen.

Our motivation for the work described here comes from wanting to integrate model building into an experimental research programme. We seek to use models to reason rigorously about biological assumptions and thereby to guide understanding and experimental strategy. The resulting models can be very high dimensional in both states and parameters and a central concern in the field is how such complex models can be used when many of the parameter values are unknown. There are many perspectives on this problem: some properties of systems can be proved to be independent of parameter values (Feinberg 1995; Angeli *et al.* 2004); parameters can be estimated from data in statistically meaningful ways (Jaqaman & Danuser 2006); methods of dimensionality reduction can reduce complexity (Barbano *et al.* 2007); some properties of systems have been found empirically to be robust to parameter

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rsif.2008.0205 or via http://journals.royalsociety.org.

Received 15 May 2008
Accepted 20 June 2008

variation (Alon *et al.* 1999; von Dassow *et al.* 2000); and both theory and empirical results suggest that, for any given phenotypic behaviour, only an exponentially small number of parameters are significant (Rand *et al.* 2005; Gutenkunst *et al.* 2007). We note further that both pharmaceutical and biotechnology companies are adopting modelling in drug development (Bangs & Paterson 2003; Hendriks *et al.* 2006; Haberichter *et al.* 2007), suggesting that model complexity is not a barrier to usefulness. Methodologies for building complex models in a flexible and controlled manner therefore become all the more important. We assume in this paper that the parameter problem can be addressed and focus on the problem of building models which can formalize biological assumptions about molecules and cells.

A variety of modelling tools are available (Arkin 2001; Ramsey *et al.* 2005), standards and ontologies formulated (Hucka *et al.* 2003; Novére *et al.* 2005) and public model repositories established (Novére *et al.* 2006). With the right information to hand, it is straightforward to build a model. However, if model building is to be integrated into an experimental research programme, a single monolithic model is never sufficient. Simply to understand how a model works, it is usually essential to create it incrementally, adding a few ingredients at a time and exploring the effect of alternative assumptions. More importantly, feedback between experiments and models leads to corrections or new assumptions, links appear to systems studied by others and new knowledge continually emerges in the laboratory and in the broader scientific community. Furthermore, experiments may be carried out in a variety of cell types or mutational backgrounds, in which the configuration of the system under study may vary. Such differences are readily accommodated in the informal mental 'models' maintained by all biologists. Mathematical models lack such plasticity. Even simple biological changes can have profound effects on the equations, requiring new equations to be introduced or the modification of many existing equations and many terms in each. Beyond a certain level of model complexity, it is easier to build a new monolithic model from scratch.

For example, Huang & Ferrell (1996) constructed an influential model of the MAP kinase cascade, which shed light on the decision-making underlying maturation of Xenopus oocytes. Levchenko *et al.* (2000) subsequently elucidated the surprising effect of a scaffold protein on MAP kinase signalling. The second model contains exactly the same MAP kinase cascade as the first, based on identical assumptions, and differs only in the addition of one new component, the scaffold. Nevertheless, it was not obtained from the former by incremental extension; a new monolithic set of equations was constructed. At present, anyone wishing to build upon these prior contributions would have to do the same. The need to reinvent the wheel each time is a fundamental barrier to progress.

It also makes it difficult for model building to scale as systems become increasingly complex. Notwithstanding this complexity, much of molecular biology is built from general processes that operate on different components in broadly similar ways. For instance, the scaffold in the MAP kinase cascade behaves in much the same way as all scaffold proteins: it has no intrinsic enzymatic function but binds other proteins (Morrison & Davis 2003). Scaffolds may differ in the number of binding partners and in the behaviour of partners when bound but there is a core mechanism that must be incorporated in any model in which a scaffold participates. Model construction would become far easier if this core mechanism could be described once (by scaffolding experts, say) and this description reused repeatedly by instantiating it with the particular binding partners and binding assumptions that are relevant to the context being modelled. Many other molecular processes are generic, in the sense that the same core mechanism is used with different components in different contexts. For example, all receptor tyrosine kinase signalling pathways are built from the following generic processes: receptor dimerization; endo- and exocytosis; endosomal recycling; multisite post-translational modification (including phosphorylation and ubiquitination); scaffolding; GTPase switching; MAP kinase cascades; membrane localization; nuclear import and export; etc. Model construction would be revolutionized if models were built in a modular and incremental fashion from a library of expert descriptions of such generic processes. Model building would then begin to scale with increasing biological complexity and models could build upon each other, greatly increasing their scope and credibility.

To accomplish such an attractive transformation requires new capabilities. Among them are modularity, which allows sub-systems to be specified independently and composed together, and abstraction, which allows generic properties of components or sub-systems to be specified independently of specific instances. These capabilities are familiar from engineering, where they are used to design and build artificial systems of significantly greater complexity than any current model in systems biology. However, their application to biological models is not straightforward. In particular, they require models to be treated as high-level programs within a computational infrastructure, leading to a new style of model building. Although the significance of these capabilities has been acknowledged, as will be reviewed in §4, none of the available modelling tools provide programmable modularity and abstraction. We have developed a computational infrastructure that does. It is open source and freely available (see §2). Here, our purpose is not to describe it in detail but rather to show why it is necessary, what is challenging about implementing modularity and abstraction and what can be accomplished with them that could not be done previously. We hope to show how these capabilities bring us closer to the transformation of model building described above.

2. METHOD

2.1. Computational infrastructure

Little b was developed within the LispWorks environment (LispWorks Ltd, Cambridge, UK). It is freely and publicly available from http://vcp.med.harvard.edu, http://littleb.org or http://sourceforge.net. The computational infrastructure compiles a biological

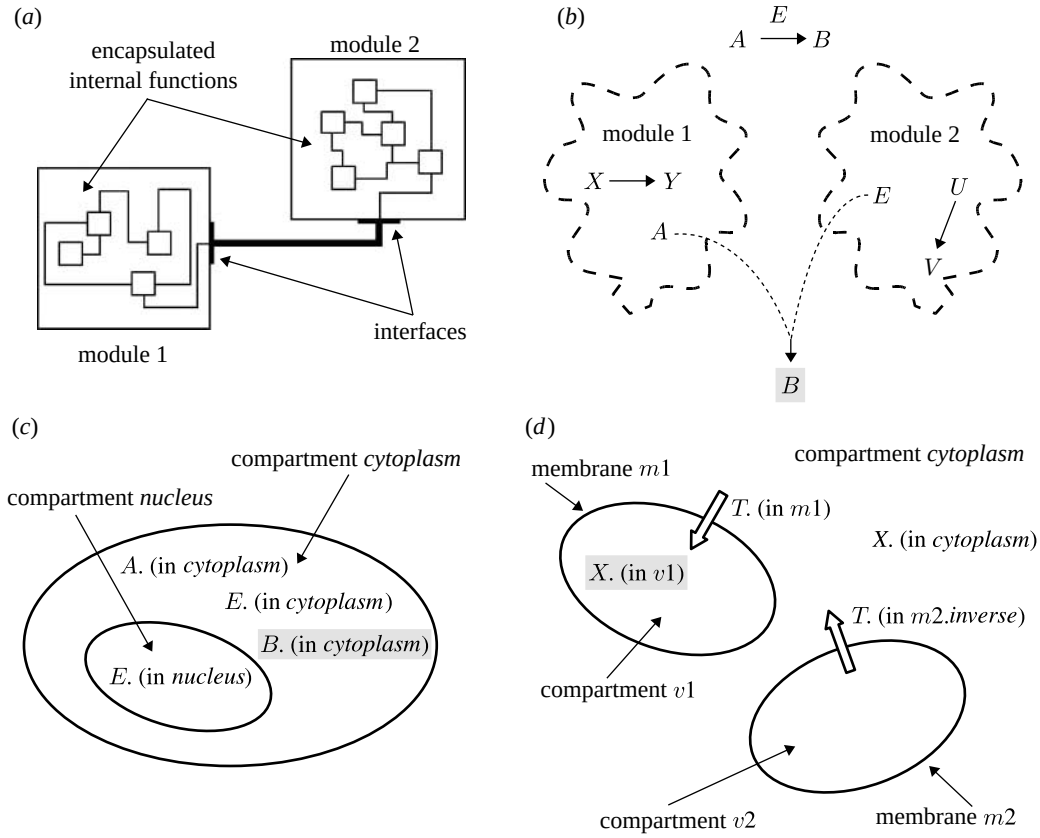


Figure 1. Modularity, inference and identity. (*a*) Engineering modularity exposes restricted functionality through interfaces, while hiding internal complexity behind barriers. (*b*) Modularity for biological models must allow for the possibility that any molecule may interact with any other molecule. E is an enzyme that converts A to B. Module 1 contains A but not E or B while module 2 contains E but not A or B. Composing modules 1 and 2 results in a new species, B, not previously present in either module. A module must work correctly in contexts determined by other modules whose characteristics are not known in advance of module composition. *Little b*'s computational infrastructure uses reasoning to infer the presence of the highlighted entities. (*c*) Unique identities must encode information about location. E converts A to B and is present in both the *cytoplasm* compartment and the *nucleus* compartment. However, A is only present in the *cytoplasm*. The system should infer that B is present in the *cytoplasm* but, in the absence of other information, should not infer that it is present in the *nucleus*. (*d*) Membranes are complex locations. T transports X unidirectionally across a membrane. T is oriented in membrane $m1$ of vesicle $v1$ to transport X into $v1$ but is oppositely oriented in membrane $m2$ of vesicle $v2$. If X is present in the *cytoplasm* compartment then the computational infrastructure should infer that it is in $v1$ but, in the absence of other information, should not infer that it is in $v2$. In *little b*, membranes encode information about their two adjacent volume compartments and molecules are oriented by locating them in either the standard (default) membrane or its *inverse*. The behaviour of T and X can be described once but then works correctly irrespective of T's location.

description expressed as a *little b* program into MATLAB (The MathWorks, Natick, MA) files. Rate equations are either automatically derived using mass-action assumptions or the user can provide phenomenological rate functions (for instance, the Hill functions used in §3.3). The symbolic mathematics sub-system (figure 2) can accommodate rational functions of several variables, with arbitrary real exponents. Dimensions and units are consistently handled. The correctness of the infrastructure was tested by the construction of a series of examples of increasing complexity, including four previously developed models (Huang & Ferrell 1996; Bhalla & Iyengar 1999; Levchenko *et al.* 2000; von Dassow *et al.* 2000). In addition to reproducing MATLAB results, the equations and their internal representations were checked. Lisp evaluation times range from 0.2 s for the multisite phosphorylation model in figure 3*d* to 11 min for the segment polarity model of the *Drosophila* lattice, which has 104 cells, 3439 species and 13 328 reactions. (Timings on an IBM T43p laptop, Pentium M, 2.1 GHz, 1 Gb RAM.) The latest release of *little b* incorporates the graph-based syntax pioneered in the BioNetGen system.

2.2. Segment polarization

Lattices were generated in MATLAB by choosing a set of points and using a Voronoi tessellation to produce polygonal cells. For the *Drosophila* lattice, the points were selected manually as the centres of the biological cells in the embryo photograph, as in figure 6*a*. A MATLAB script generates the (x, y) coordinates of the vertices of each cell, along with the cell areas and the lengths of the apposed membrane segments. The generic cellular lattice module then uses these data to construct the resulting compartments and membranes. Figure 6*b* shows the four lattices used for this study, on which are superimposed the pre-pattern (initial condition) of cells in which the levels of Wingless mRNA (wg) and protein (WG) and Engrailed mRNA (en) and

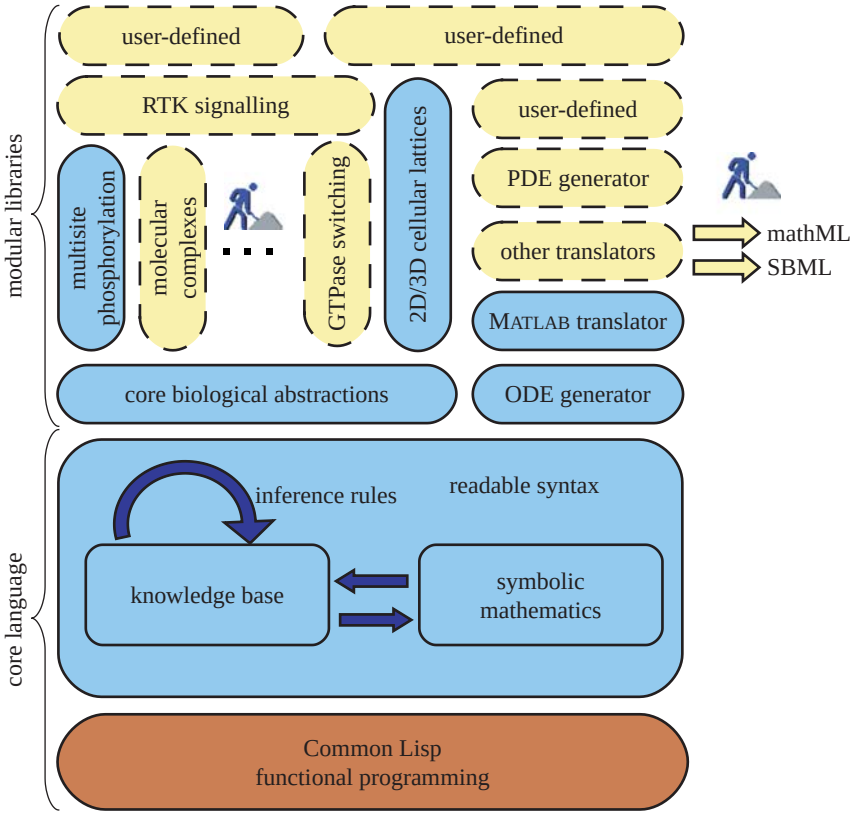


Figure 2. *Little b* provides an extensible architecture, permitting the development of new generic modules. The core language extends Common Lisp with new syntax, a reasoning system and symbolic mathematics. Modular libraries provide both biological and mathematical abstractions in a hierarchical fashion. A library of core biological abstractions defines reusable constructs for representing and reasoning about reactions, molecular complexes and biochemical locations. Higher order modules, such as 'multisite phosphorylation' and 'two-dimensional (2D)/three-dimensional (3D) cellular lattices' discussed in the text, can be programmed on top of the core abstractions. Users have access to all levels of the hierarchy and can build new modules that extend the biological or mathematical capabilities. Yellow dashed boxes indicate libraries that are envisaged or under development, while blue full boxes show the currently implemented *little b* computational infrastructure.

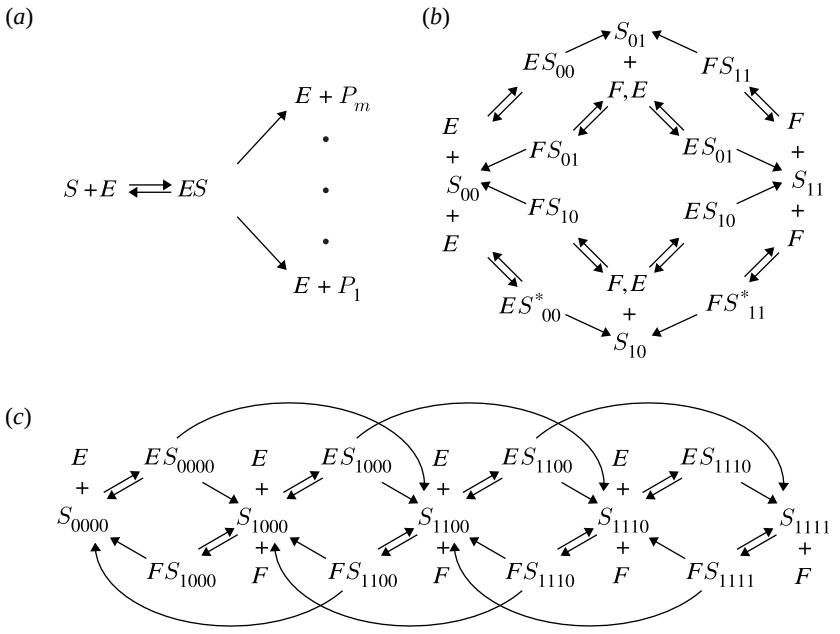
protein (EN) are set to normalized concentrations of 1, as previously (von Dassow *et al.* 2000). All other components are initially zero, with the exception of the basal activator of cid expression, which has normalized concentration of 0.4 in each cell, as previously (von Dassow *et al.* 2000). The four lattices are: *Hexagonal*, corresponding to the regular hexagonal lattice used previously (von Dassow *et al.* 2000); *Drosophila*, extracted from the embryo photograph; *Rectangular*, in which the cells are rectangular but come in two sizes, arranged in alternating columns; *Shifted rectangular*, in which the lattice is identical to the rectangular lattice but the pre-pattern is shifted to the right.

We used identical assumptions to von Dassow *et al* to represent the regulatory network in figure 5*a*, forgoing later modifications (Ingolia 2004). We did not wrap lattices onto a torus (von Dassow *et al.* 2000), as such double periodicity makes no sense for irregular lattices. We checked for edge effects by embedding one lattice inside a larger one; we found no evidence for major changes in behaviour. We chose two previously used parameter sets (von Dassow *et al.* 2000) but found that the high Hill coefficients gave rise to unphysiological oscillations in some components (electronic supplementary material, figure 2*a*). We were able to find lower Hill coefficients without jeopardizing correct segmentation on the hexagonal lattice. The parameter values used in figure 6*c* were derived in this way from the Yippee parameter set, while those in figure 6*d* were derived from parameter set four, as detailed in the electronic supplementary material, table 1. In running the simulations, we found occasional slow decays beyond the 1000 minutes used previously (von Dassow *et al.* 2000; electronic supplementary material, figure 2*b*). We therefore scored correct segmentation by simulating for 5000 simulated minutes, thresholding Wingless and Engrailed values as previously (von Dassow *et al.* 2000), and checking if the results agreed with the pre-pattern. Edge cells were ignored in scoring.

3. RESULTS

3.1. Modularity and abstraction

Modularity is a fundamental method for building complex engineering systems. Modules are sub-systems that are encapsulated to hide their internal complexity and inter-module communication is only permitted through specified interfaces (figure 1*a*). By subdividing the design problem, modularity allows the engineer to subdue complexity. (Modularity is also used to describe a property of biological systems that may have been



(*d*)

```
1   (define E [species-type])
2   (define F [species-type])
3   (define S [[multisite-molecule] :num-sites 4])
4   [multisite-enzymatic-reaction-set
5       :enzyme E :substrate S :processivity 2 :mode :sequential]
6   [multisite-enzymatic-reaction-set
7       :enzyme F :substrate S :processivity 2 :mode :sequential :reverse T]
8   (define cell [compartment])
9   cell.(contains E)
10  cell.(contains F)
11  cell.(contains S.(s 0 0 0 0))
```

Figure 3. Reaction schemes for multisite phosphorylation. (*a*) The biochemical scheme for phosphorylation and dephosphorylation assumes a single enzyme–substrate complex and irreversible release of products, $P_1, \ldots, P_m$. Distributivity corresponds to $m=1$, processivity to $m>1$. ATP, ADP and phosphate are assumed held constant and the kinetics are given by mass action. (*b*) Distributive, non-sequential phosphorylation and dephosphorylation with $n=2$ sites. Phospho-forms are denoted by S_b, where b is a sequence of n bits indicating the presence or absence (1 or 0, respectively) of phosphate. E kinase, F phosphatase and S substrate. (*c*) Processive, sequential phosphorylation and dephosphorylation with $n=4$ and processivity degree 2. Enzyme release steps are omitted for clarity. (*d*) Example *little b* program using the generic module (lines 4/5 and 6/7) for multisite phosphorylation, which can generate any reaction scheme like (*b*, *c*). Bold blue text, object classes; bold italic pink text, keywords. The module is instantiated for (*c*) but can be instantiated for (*b*) by changing the number of sites to 2 (line 3), the processivity to 1 and the mode to non-sequential (lines 5/7). Model equations are generated after rate constants and initial conditions are specified (not shown). Despite appearances, *little b* code is Lisp code and is interpreted by the Lisp read-eval-print mechanism in the usual way. *Little b* uses reader macros to alter Lisp's default syntax to one more suited for describing biology. Lisp allows a new language to be created within itself, a capability that we find particularly useful for biology.

selected for by evolution (Hartwell *et al.* 1999). No such meaning is intended here; modularity for us is always a method of model construction.)

The kind of 'engineering modularity' just described was introduced into biological modelling in the ProMoT tool (Ginkel *et al.* 2003) and aspects of it are provided in other methodologies (Lloyd *et al.* 2004; Webb & White 2005). However, encapsulating a module and specifying its interface, at the time it is designed, restrict the module's interactions to situations envisaged at design time. Biological modules have no natural encapsulation other than membranes. In the absence of such physical separation, the components of one module may, in principle, always interact biochemically with the components of another. Moreover, biochemical interaction can create entirely new entities. For instance, figure 1*b* illustrates a situation in which module 1 contains A but neither E nor B, while module 2 contains E but neither A nor B. Suppose now that E is an enzyme that converts A to B. This information may have been present in the system when modules 1 and 2 were composed but not necessarily as part of either module 1 or module 2. It could also have been supplied subsequently, as a result, perhaps, of new experimental data. In either case, once that information is present, the composition of modules 1 and 2 implies that E has access to its substrate. Hence, B should also be present, despite the fact that it was not previously present in either module. Such new entities need to be identified and the corresponding mathematical variables introduced into the dynamical system. In engineering modularity, this process is effectively carried out by

the designer at design time. Modularity without encapsulation requires a computational infrastructure capable of reasoning over the information provided by the user and working out which new entities are required.

The creation of new entities leads to two further difficulties. Firstly, it may have knock-on consequences in other modules, requiring yet more entities to be created, and these may in turn have further consequences and so on. A similar issue arises in reasoning systems used in artificial intelligence, where, for instance, pattern–action rules, of the form $P \Rightarrow A$, specify actions (A) to be performed—including modification, removal or creation of entities—whenever there are entities matching the corresponding set of patterns (P). The execution of a rule can then trigger the execution of further rules. The widely used RETE algorithm (Forgy 1982) provides an efficient way of controlling the resulting chain of actions and ensuring that it terminates in a consistent way.

The second problem is that an entity that needs to be created may already exist in the system. If so, it is essential that a duplicate is not created. This may be achieved by giving each entity a unique identity. However, this identity cannot simply be a random tag. Figure 1*c* illustrates a situation in which enzyme E is present in both the cytoplasm and the nucleus of a cell, while its substrate, A, is also present but only in the cytoplasm. Biochemistry tells us that the enzymatic product B should be present in the cytoplasm but not in the nucleus. The computational infrastructure needs to be able to distinguish between A being present somewhere and A being present in the same location as the enzyme for which it acts as a substrate. Hence, identity needs to encode location. To describe the enzymatic conversion of A to B by E, it is then sufficient to say that A and E must have the same location, which is then inherited by the product B. Note that this is a simple instance of abstraction: the properties of a reaction are described independently of where it takes place. Whether the product is present or not is only determined once the reaction and its components are instantiated in some specific location. Such a facility becomes invaluable when there are many locations, as in a multicellular tissue, as will be seen in §3.3. Transmembrane reactions involve several locations (figure 1*d*) and their behaviour may depend on how the corresponding molecules are oriented within the membrane. Locations, therefore, have to be treated in general as structured entities, which describe the local hierarchy of membranes and compartments. Controlling the relationship between identities and locations is one of the crucial requirements for implementing modularity.

These considerations make it clear that modularity without encapsulation requires some form of programming, in order to infer which new entities need to be created and to generate and compare their identities. Programming also allows further levels of abstraction to be implemented, as will be seen in §3.2. We have designed a high-level programming language, *little b*, for this purpose. Biological information specified in a program is evaluated by the *little b* computational infrastructure and translated into equations expressed in MATLAB (figure 2). *Little b* is implemented as a macro language of Common Lisp (Graham 1996). Lisp is used in several biological applications (Krummenacker *et al.* 2005; Massar *et al.* 2005). We chose it for the following reasons: Common Lisp is open source; an open source implementation of the RETE algorithm is available in the Lisp-based Intelligent Software Agents (LISA) system; Lisp metaprogramming allows *little b* to present a readable, biologically meaningful, non-Lisp-like syntax to the user (figure 3*d*); and Lisp's symbolic processing capability provides powerful mechanisms for implementing modularity and abstraction (Sinclair & Moon 1991). The resulting capabilities for model building are explored in the two sections that follow.

3.2. *Multisite phosphorylation as a generic library module*

Phosphorylation and dephosphorylation on serine, threonine and tyrosine are ubiquitous and vital cellular regulatory mechanisms (Cohen 2001). One of their most striking features is the remarkable extent of multisite modification. The mammalian tumour suppressor p53 has at least 16 sites known to be functionally significant (Holmberg *et al.* 2002) while the microtubule-associated protein tau can become hyperphosphorylated on 39–45 sites in Alzheimer's disease (Hanger *et al.* 2007). This makes multisite phosphorylation a prime candidate for a generic library module, which abstracts current biological understanding and can be reused repeatedly to make different models in different contexts. The module discussed here has the capability to generate most of the models previously used in studies of multisite phosphorylation. We treat phosphorylation and dephosphorylation together, in keeping with a systems approach.

The current understanding of the mechanisms of multisite phosphorylation and dephosphorylation is typically complex. First, kinases and phosphatases may differ in the number of modifications made in a single encounter between enzyme molecule and substrate molecule. If at most a single modification is made, the enzyme will be distributive; if more than one is sometimes made, it will be processive (Huang & Ferrell 1996). The degree of processivity is the maximal number of modifications in a single encounter. Examples of both distributivity and processivity are known. Mek phosphorylation of Erk on its two activating sites is distributive (Burack & Sturgill 1997; Ferrell & Bhatt 1997), as is MKP3 dephosphorylation of Erk on the same sites (Zhao & Zhang 2001). The p38 MAP kinase distributively phosphorylates ATF2 on two sites (Waas *et al.* 2001) while Fcp1 distributively dephosphorylates three out of four sites on a tetra–heptad repeat segments of the RNA polymerase II carboxy-terminal domain (Hausmann *et al.* 2004). The phosphorylation of Pho4 on four sites by Pho80–Pho85 is processive with an average of 2.1 phosphorylations per molecular encounter (Jeffrey *et al.* 2001). The phosphorylation of the focal adhesion protein p130Cas by Src on 15 tyrosines is highly processive (Pellicena & Miller 2001). The alternative splicing factor ASF/SF2 is an SR protein, containing multiple serine/arginine repeat sequences, which is phosphorylated by the SR-specific protein kinase SRPK1 on 8–9 sites and by Clk/Sty on 20 sites, in both cases in a highly processive

manner (Aubol *et al.* 2003; Velazquez-Dones *et al.* 2005). Degrees of processivity have not been measured for either p130Cas or ASF/SF2. Processive phosphatases have not been identified but phosphatases have generally been less well studied than kinases.

A second aspect of multisite phosphorylation is that some kinases are known to be sequential, phosphorylating in a strict order of sites. For instance, GSK3, in its 'primed' phosphorylation mode (Roach 1991; Fiol & Roach 1996), phosphorylates (S/T)XXX(S/T) repeat motifs in a strict C-to-N order (Harwood 2001). The fibroblast growth factor receptor also autophosphorylates on five tyrosines in a specific order (Furdui *et al.* 2006). While these examples suggest that sequential phosphatases may also exist, none have yet been identified. Notwithstanding this, most models assume that both enzymes are sequential and that the phosphatase works in the reverse order to the kinase. In this case, only $n+1$ phospho-forms are produced in contrast to the 2^n phospho-forms present when the enzymes show no preference for site order.

A wide variety of networks of reactions can arise from these assumptions (figure 3*b*,*c*). To generate models from such networks, it is necessary to either impose an *ad hoc* rate function, such as Michaelis–Menten, or to make further assumptions about the biochemistry of phosphorylation and dephosphorylation and then calculate the rate function using mass-action. Either method can be programmed in *little b* but Michaelis–Menten is, at best, a dubious approximation (Ciliberto *et al.* 2007) and we will illustrate instead the use of mass action. Figure 3*a* shows a generic biochemical scheme that allows for enzyme processivity. S is a substrate phospho-form and E an enzyme. They reversibly associate to form an enzyme–substrate complex, ES, which irreversibly disassociates to release E and multiple product phospho-forms, $P_1, \ldots, P_m$. Such a scheme can cover both kinases and phosphatases, provided ATP, ADP and phosphate are assumed to be held at constant concentrations by some mechanism that is not explicitly modelled. This is usually true *in vivo* and is assumed in all models in the literature. The effects of ATP, ADP and phosphate are then effectively absorbed into the mass-action rate constants. The scheme in figure 3*a* applies to each relevant phospho-form, for both kinase and phosphatase.

A model can now be generated by using mass action to calculate the rates of production and consumption of each chemical species. Writing down such a model by hand for any given network of reactions is laborious and error prone. It quickly becomes infeasible as the number of sites increases. The program in figure 3*d* uses a generic *little b* module to generate models for a broad range of different assumptions, for any specified number of phosphorylation sites. It accommodates the following use cases: kinase and phosphatase are both sequential but operate in opposite order (figure 3*c*); kinase and phosphatase have no preference for site order (figure 3*b*); and either enzyme is processive to varying degree (figure 3*c* has degree 2 for both enzymes), with distributivity corresponding to a processivity degree of 1 (figure 3*b*). These different cases can be selected by merely setting the values of the corresponding attributes in the module. This covers most of the mass-action models known to us in the literature. Note that, although the enzyme–substrate complexes are part of the model, the user does not need to specify them independently. *Little b* creates them automatically according to the biochemical scheme in figure 3*a* and takes them into account when calculating the rate equations.

We used the program in figure 3*d* to study the decision-making capacity of multisite phosphorylation. A single substrate molecule with n sites can, in principle, encode 2^n different states. The state of a population of such molecules, however, is described by a frequency distribution giving the relative stoichiometry of each phospho-form. The decision-making capacity of this phospho-form distribution is quite different from that of a single molecule. It is determined not by the molecular structure but by the dynamics of interaction between the substrate and its cognate kinases and phosphatases. We have proved in recent work (Thomson & Gunawardena submitted) that, when the enzymes are distributive, there may be as many as $\lfloor (n+2)/2 \rfloor$ stable distributions of phospho-forms at steady state ($\lfloor x \rfloor$ being the greatest integer not greater than x). This suggests that increasing numbers of sites can support increasingly complex decision-making. To reveal this multistability experimentally, we sought different initial conditions that would lead to different stable phospho-form distributions. Although the steady states of such models can be analysed mathematically, the dynamics leading to them can only be studied by simulation, which needs to be undertaken for different numbers of sites and for sequential as well as non-sequential systems. The generic module in figure 3*d* makes this straightforward. Figure 4 shows two scenarios in which each of the stable phospho-form distributions can be reached by starting with some mixture of unphosphorylated and fully phosphorylated substrate. This behaviour was representative of the multistable systems we studied. These predictions from simulation suggest a simple method for detecting multistability experimentally, which we are now testing in the laboratory.

The multisite phosphorylation module shows that powerful abstractions for describing generic biological processes can be programmed from the core biological abstractions in *little b*. The value of a programmatic approach is further illustrated by considering how alternative assumptions can be dealt with. For example, there are situations in which it is helpful to explicitly model ATP. A number of important drugs are kinase inhibitors which compete for the ATP-binding pocket (Cohen 1999). If such drug effects are to be studied, the scheme in figure 3*a* has to be modified accordingly. (In fact, it must be altered in two different ways because the kinase uses ATP and produces ADP while the phosphatase only produces phosphate.) While these are entirely natural changes from a biological perspective, the recalculation of the rate functions is so awkward to undertake manually that all models would have to be reconstructed from scratch. Using the programmatic approach, it is only necessary to modify the generic module to operate with the new schemes in preference to that in figure 3*a*.

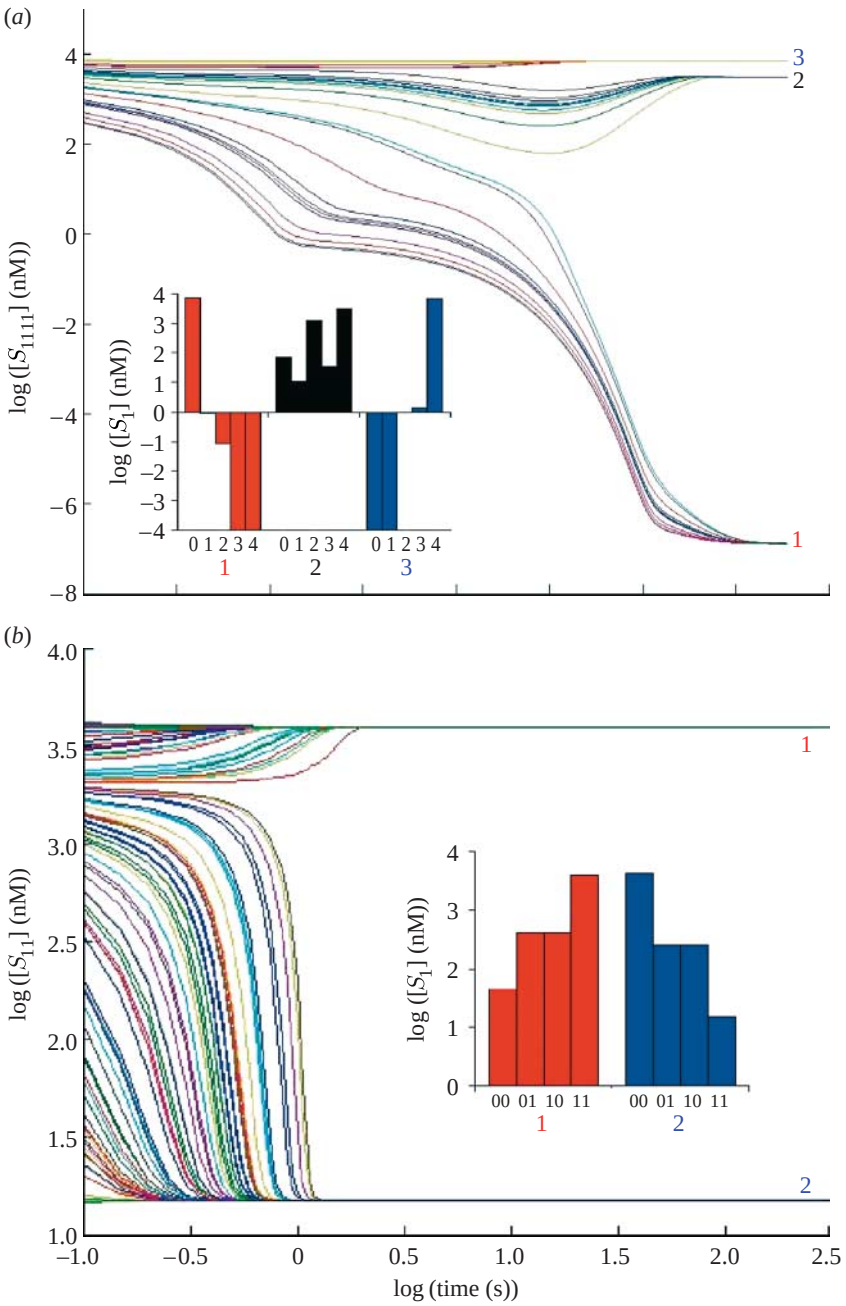


Figure 4. Multistability in multisite phosphorylation. Rate constants are given in figure 1 in the electronic supplementary material. (*a*) Distributive, sequential phosphorylation and dephosphorylation, with $n=4$, as figure 3*c* but with processivity degree 1. Substrate is initially present as $[S_{0000}]=\alpha[S_{\mathrm{tot}}]$, $[S_{1111}]=(1-\alpha)[S_{\mathrm{tot}}]$, where α is drawn randomly from the uniform distribution on [0,1] and $[S_{\mathrm{tot}}]$ is the total amount of substrate present. Square brackets denote concentration. Vertical axis, concentration of S_{1111}; horizontal axis, time; log scales on both. The initial conditions find the three stable phospho-form distributions shown in the inset, for appropriate values of α. In the inset, phospho-forms are designated 0, 1, 2, 3, 4 by number of phosphorylations. (*b*) Distributive, non-sequential phosphorylation and dephosphorylation with $n=2$, as figure 3*b*. Initial substrate is a random combination of S_{00} and S_{11}, as previously, leading to the two stable phospho-form distributions shown in the inset. Vertical axis, concentration of S_{11}; horizontal axis, time; log scales on both.

The corresponding models can then be generated with no greater difficulty than before.

3.3. Developmental patterning on realistic cellular lattices

During initial patterning of the *Drosophila* embryo, maternal mRNAs stimulate expression of gap genes, followed by pair rule genes, followed by segment polarity genes, thereby establishing the anterior–posterior pattern of parasegments (Lawrence 1992). The early stages of this process take place in the syncytial blastoderm but the segment polarity genes turn on after cellularization. Von Dassow *et al.* created a computational infrastructure, Ingeneue (Meir *et al.* 2002), for building models of gene regulatory networks in a two-dimensional lattice of regular hexagonal cells and used it to build a model of *Drosophila* segment polarization (von Dassow *et al.* 2000). The proposed segment polarity network (figure 5*b*) was able to correctly stabilize a pre-pattern of Wingless and

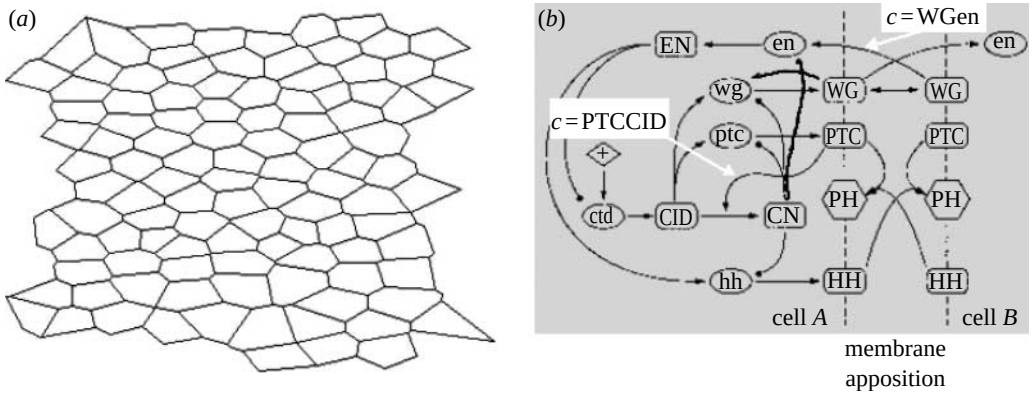


Figure 5. Modular construction of developmental networks in arbitrary cellular lattices. (*a*) Polygonal lattice of cells. The user provides the vertex coordinates to *little b*'s generic lattice module, which creates an internal representation of the lattice. (*b*) The segment polarity gene regulation network after von Dassow *et al.* (2000). (Adapted by permission from Macmillan Publishers Ltd: *Nature* **406**, 188–192, copyright 2001.) The positive feedback of Wingless protein (WG) on its mRNA (wg) and the repression of Engrailed mRNA (en) by cleaved Cubitus Interruptus (CN) are both included. Labels show the interactions (c=WGen and c=PTCCID) that are varied in figure 6. *Little b* can take any polygonal lattice and any network of reactions and put the two together in a modular way. Each cell acquires a copy of the regulatory network and two adjacent cells interact across their common membrane segment using the same mechanism as in von Dassow *et al.* (2000). The bookkeeping scheme required to identify each species in each location is automatically worked out by *little b* and used to build the model equations.

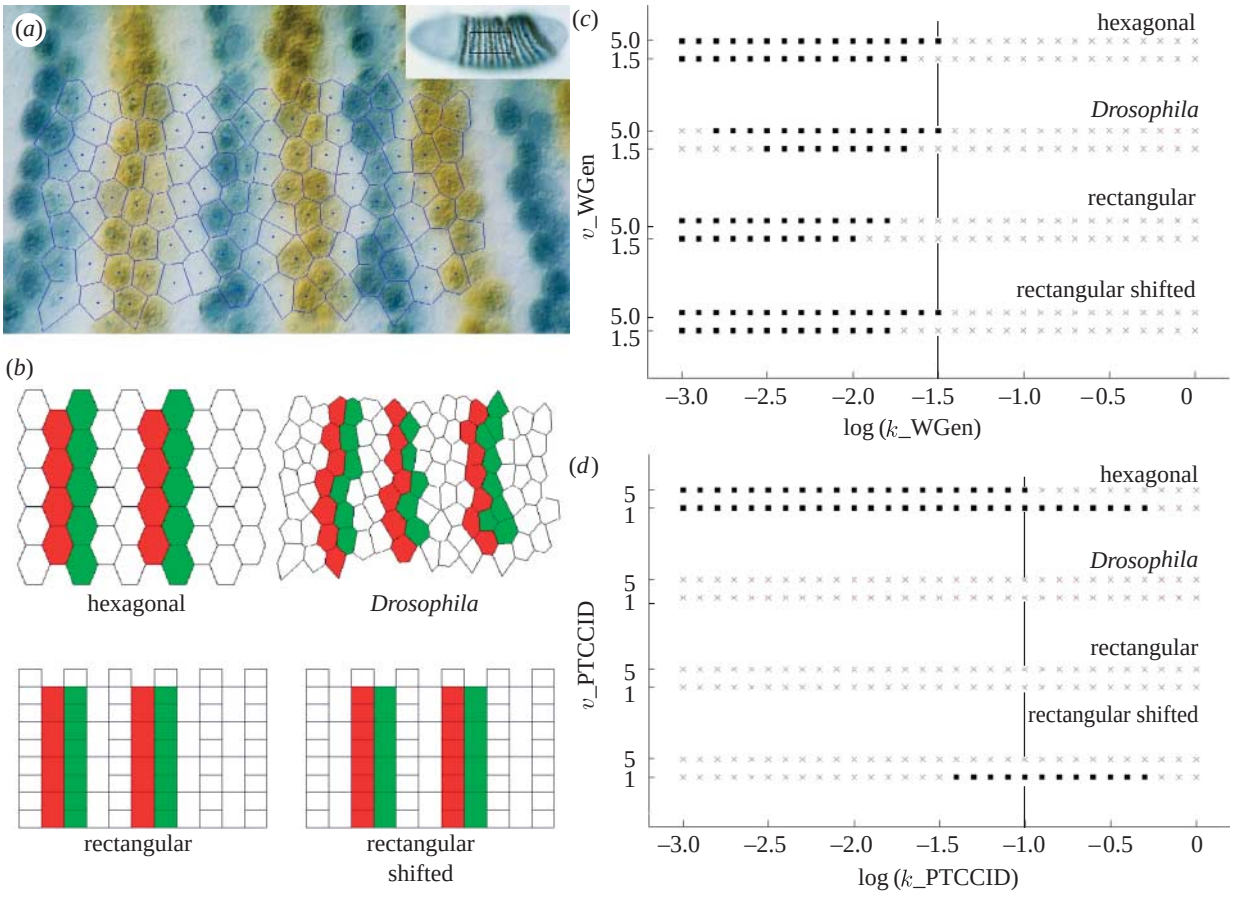


Figure 6. Segment polarization in different cellular lattices. (*a*) Image of a *Drosophila* embryo, with the extracted cellular lattice superimposed. (*b*) Four lattices showing the pre-pattern where Wingless (red) and Engrailed (green) are high. For correct segmentation, the regulatory network must stabilize this pre-pattern, starting from the pre-pattern as initial condition. (*c*,*d*) Correct (filled square) or incorrect (cross) segmentation for the lattices listed on the right. The half-maximal value, k_c (horizontal axis), and the Hill coefficient, v_c (vertical axis), of a Hill function, $x^{v\_c}/((k\_c)^{v\_c}+x^{v\_c})$, describing one of the connections c in figure 5*b*, are varied. The half-maximal value varies horizontally on a log scale, while the Hill coefficient takes either a low (1 or 1.5) or high (5) value. The parameter values other than v_c and k_c are obtained from two previously defined parameter sets (von Dassow *et al.* 2000), as described in §2, and are listed in the electronic supplementary material, table 1. (*c*) Intercellular transcriptional activation of Engrailed by Wingless (c=WGen). (*d*) Intracellular cleavage of Cubitus Interruptus by Patched (c=PTCCID).

Engrailed expression over a wide range of different parameter values, suggesting that such robustness might be an evolutionary criterion for selecting network designs. This idea stimulated much interest (Wolpert 2001).

In reality, *Drosophila* embryo epithelia are not regular hexagonal lattices of cells (figure 6*a*). Cells may be of different sizes and shapes and have different numbers of neighbours. Indeed, in proliferating animal epithelia, approximately 29% of the cells resemble pentagons, 46% resemble hexagons and 21% resemble heptagons (Gibson *et al.* 2006). The segment polarity network should produce the right patterning irrespective of which lattice emerges from cellularization. Robustness to lattice variation is a more stringent requirement than robustness to parameter variation. The latter involves a mere numerical change in parameters without any change to the structure of the underlying equations; the former involves a restructuring of the equations themselves because the pattern and rates of cell–cell communication are altered. Robustness to lattice variation may thus exert greater selective pressure on network designs than does robustness to parameter variation.

Ingeneue provides computational support for building reactions in regular hexagonal cells, with cell–cell communication being modelled by interaction across apposed membrane segments. It keeps track of each apposed pair of membrane segments and all the molecular interactions across each apposition. The bookkeeping required is substantial but straightforward for a regular hexagonal lattice and such functionality is hard-wired into Ingeneue. Each irregular lattice, however, needs its own bookkeeping scheme and the equations need to be rewritten to reflect each new scheme. Although the robustness to lattice variation seems a relevant and important question, Ingeneue has no capability to address it, nor does any existing tool.

We implemented a generic cellular lattice module in *little b* for two-dimensional polygonal (or three-dimensional polyhedral) cells. The lattice module reads a list of coordinates of the vertices of the lattice, supplied by the user, and creates an internal representation of the cellular lattice. This module may be composed with any regulatory or protein interaction network module, using the same membrane apposition assumption for cell–cell communication as used in Ingeneue (figure 5*b*). *Little b* builds the resulting model automatically. The bookkeeping scheme appropriate to the chosen lattice emerges automatically from modular composition, the location abstraction (see §3.1) instantiates all the species and reactions in each cell of the lattice and the intercellular links are wired together according to the membrane appositions. Programmable modularity and abstraction enables the modelling of any gene regulatory network in any lattice of polygonal cells, subsuming and greatly extending the currently available functionality for multicellular modelling.

We used this module to examine the behaviour of figure 5*b* in four lattices, including a physiologically realistic lattice extracted from a *Drosophila* embryo image (figure 6*a*,*b*). The parameter robustness observed for the regular hexagonal lattice arises from a combination of ultrasensitive Hill functions and feedback (von Dassow & Odell 2002; Ingolia 2004). We chose two previously defined sets of parameter values (von Dassow *et al.* 2000), as described in §2, and varied in one set the Hill function controlling an intercellular activation and in the other set an intracellular negative feedback loop (both labelled in figure 5*b*). The intercellular activation showed a range of parameter values for which all four lattices produced correct patterning (figure 6*c*), although the range was substantially larger for the hexagonal lattice alone. However, the intracellular negative feedback showed no parameter values for which all four lattices work correctly (figure 6*d*). Indeed, the *Drosophila* lattice never produces the correct pattern, despite a substantial range in which the regular hexagonal lattice works. The regulatory network in figure 5*b* seems to be highly sensitive to lattice geometry.

It is conceivable that a relatively small change in the regulatory network will generate robust patterning for realistic lattices. Alternatively, significant control loops may be missing from our current understanding of this system. The point we wish to make in this paper is that *little b* allows the exploration of these scientifically interesting questions, which were entirely out of reach previously.

4. DISCUSSION

The multisite phosphorylation example shows that a generic biological process can be abstracted into the succinct module used in figure 3*d*, while the *Drosophila* segmentation example shows the power of modular composition. These capabilities lay the foundation for the vision of model building articulated in §1. This needs to be set in the context of currently available tools and methodologies for model building.

Many excellent ones are available. Some focus on particular biological processes, such as gene regulation (Ramsey *et al.* 2005), some favour specific biological domains, such as immunology (Meier-Schellersheim *et al.* 2006) or neuroscience (Bower & Beeman 1998; Carnevale & Hines 2006), and some provide specialized capabilities for simulation (Stiles & Bartol 2001; Lok & Brent 2005; Meier-Schellersheim *et al.* 2006) or analysis (Ermentrout 2002).

What makes *little b* different from existing tools is its programming language for modularity and abstraction. A language allows its user to describe novel situations not previously envisaged by the language designer. Most tools support model building through some form of 'template', accessible through a menu of available templates. Hierarchical templates can be an efficient means of providing access to the kinds of features discussed here. However, while such templates are implemented in a programming language, this language is not itself accessible to the user, who cannot define new templates. For instance, while users of Ingeneue (§3.3) are restricted to the template for a regular, hexagonal cellular lattice, *little b*'s programmability allowed us to define a polygonal cell in terms of pre-existing definitions of locations, compartments and membranes, and define a polygonal lattice in terms of polygonal cells. These hierarchically defined components could have been devised by any users of the language. Indeed, further

studies in developmental patterning or cellular physiology might be aided by more detailed abstractions describing tissues having particular arrangements of cell types with distinct morphologies—for example, a generic neuron parametrized by axon length, dendrite number, etc., or a stratified epithelium parametrized by cell types, cell numbers or other properties. These too can be implemented by individual users as required and made available to all other users as generic modules. The programmatic approach supports a decentralized approach, not just to model building, but also to creating the abstractions and generic modules that allow model building to scale with increasing biological complexity.

The Systems Biology Markup Language (SBML) marked a watershed in biological modelling (Hucka *et al.* 2003). Although nominally a language, it is an Extensible Markup Language, not a programming language. SBML treats a model as a datatype, not as a program. It provides a syntax for describing the model's components, thereby enabling a model constructed by one tool to be read and understood by another. This has helped to nucleate the model-building community and create a de facto standard for model curation (Novére *et al.* 2005, 2006). Software that is SBML compliant can provide facilities for analysing SBML models and, in principle, for composing them. However, generic abstractions, such as the tissue abstractions suggested above, would have to be incorporated within the ontology provided by SBML. This ontology is, in effect, agreed upon by the SBML community and cannot be altered by any one user; it has to be updated by the developers with each release of SBML. As we have seen, *little b* allows the user to create and share new abstractions at will in a decentralized manner. The two approaches may become complementary: once novel abstractions have been introduced and rigorously tested within a programming language community, they can be codified in an updated ontology and thereby made accessible to a broader biological community, who need not necessarily be conversant with the programming language itself.

The BioNetGen system introduced an elegant graph-based syntax for specifying molecular species in terms of their interaction domains (Blinov *et al.* 2004) and similar ideas were independently developed in other systems (Danos & Laneve 2004; Lok & Brent 2005; Meier-Schellersheim *et al.* 2006). Biochemical reactions can then be specified as graph-rewriting rules, leading to rule-based languages for model building (Hlavacek *et al.* 2006). Sets of rules may be composed, providing a modular approach to model building, as well as access to new analytical capabilities (Danos *et al.* 2007, 2008). However, these languages are low level, akin to computer assembly languages, and lack the control and data structures commonplace in high-level programming languages. In the context of multisite phosphorylation (§3.2), for instance, it would be necessary to provide different rules for a substrate with one site, two sites, three sites, etc. The functionality of figure 3*d*, in which the number of sites is a parameter, could only be implemented within the rule-based language by first creating the machinery for high-level programming. While this might be possible in principle (since rule-based languages are usually Turing powerful), the effort required would be prohibitive. Rule-based languages are naturally complementary to *little b*: the former provide a low-level syntax for molecular complexes while the latter provides higher level abstractions.

In contrast to rule-based languages, Cellerator (Shapiro *et al.* 2003) is a model building tool that uses MATHEMATICA to assemble models. The functionality of figure 3*d* could certainly be implemented in MATHEMATICA or other high-level programming languages such as Perl or C, either on their own or in conjunction with a rule-based language. However, this would solve only a single isolated problem—that of multisite phosphorylation on varying numbers of sites. This solution could not be composed with others in a modular way, as the lattice module of §3.3 can be composed with any regulatory network module. To do this would require also implementing the core functionality of *little b* (identity, pattern–action rules, etc.) within a unified linguistic framework.

Graphical languages have also been proposed for biological specification and can provide synoptic summaries of much complex biological information (Kitano *et al.* 2005; Kohn *et al.* 2006). Experience in engineering design suggests that while they are excellent for describing structure, such as the layout of an integrated circuit, function is best described through textual languages, as in VERILOG or VHDL. Accordingly, we anticipate that tools based on graphical languages will come to take advantage of textual languages like *little b* 'under the hood'.

We have focused in this paper on the transition from monolithic to modular models and on the computational infrastructure needed to support this. Not only is this required to build models more effectively, but it is also essential for their credibility. Models, particularly complex ones, are usually published as supplementary information. Even the most conscientious reviewer is unlikely to be able to subject such a model to the same level of scrutiny as a published experimental method or mathematical proof. Models may sometimes be submitted to a public repository but few others are likely to want to use an existing model without also wanting to change it, with all the attendant difficulties noted previously. Accordingly, monolithic models may have been closely studied only by their creators, a situation of some concern in an emerging discipline. Modular models, by contrast, can be pulled apart and their component modules evaluated, modified and recombined. Generic library modules, such as that for multisite phosphorylation, could be developed and refined by experts and made available to all model builders, thereby creating a scientific 'commons' for model building. *Little b*'s extensible open source architecture allows all users to develop novel abstractions of their particular biological domain and contribute these back to the community, allowing the field to evolve in a decentralized manner and enabling us to build upon each other's work rather than recreate it. The models that result from this may be more complex but their credibility, reliability and usefulness will be more easily established. Programmable model

building will provide a more robust foundation for systems biology.

We are grateful to the Bauer Center for Genomics Research and to Craig Muir for support during the initial phase of this work. We thank, especially, Steve Harrison, Ed Harlow, Marc Kirschner and Rebecca Ward of the Harvard Medical School for enabling this work to come to fruition through their support for the Virtual Cell Program. We thank Peter Lawrence for the *Drosophila* image in figure 6*a*, Radhika Nagpal for access to material in press, David Young for his open source implementation of LISA and Dave Fox of LispWorks for his assistance with the Lisp environment. We thank Carl Pabo, Brian Seed and Rebecca Ward for their insightful comments and the other members of the Virtual Cell Program for their assistance. The authors declare that they have no competing financial interests. Correspondence should be addressed to J.G. (email: jeremy@hms.harvard.edu) and A.M. (email: aneilbaboo@gmail.com).

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