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Review

How computational models can help unlock biological systems

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ABSTRACT

With computation models playing an ever increasing role in the advancement of science, it is important that researchers understand what it means to model something; recognize the implications of the conceptual, mathematical and algorithmic steps of model construction; and comprehend what models can and cannot do. Here, we use examples to show that models can serve a wide variety of roles, including hypothesis testing, generating new insights, deepening understanding, suggesting and interpreting experiments, tracing chains of causation, doing sensitivity analyses, integrating knowledge, and inspiring new approaches. We show that models can bring together information of different kinds and do so across a range of length scales, as they do in multi-scale, multi-faceted embryogenesis models, some of which connect gene expression, the cytoskeleton, cell properties, tissue mechanics, morphogenetic movements and phenotypes. Models cannot replace experiments nor can they prove that particular mechanisms are at work in a given situation. But they can demonstrate whether or not a proposed mechanism is sufficient to produce an observed phenomenon. Although the examples in this article are taken primarily from the field of embryo mechanics, most of the arguments and discussion are applicable to any form of computational modelling.

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1. Introduction

Few things in the universe are as inspiring to behold as living systems, and one of the recurring mysteries about them is how their remarkable characteristics arise from interactions between relatively simple building blocks. For example, "How can collections of cells each of which is able to take only one of two states, on and off, allow human minds to think complex, meaningful thoughts?" or "How do the various organic and inorganic players in an ecosystem interact so as to produce long-term stability?" or "How do embryos acquire their increasingly complex and elegant forms?" These are profound mysteries.

As medical researchers and biologists strive to address questions of this kind with increasing rigour, they require tools that will allow them to gain insights into the complex interactions that occur in these systems, and one of the best currently-available tools is computational modelling [1–5]. There are many reasons that computational models are so effective in this setting, and a primary goal of this article is to highlight them, while at the same time recognizing their limitations. This article also aims to provide insight into how models work in general and show some of the specific ways that they can be used in the context of biological systems, especially those related to cell and tissue mechanics and embryology.

In general, the goal of a computational model is to replicate the behaviour of the system it parallels and to do so based on actual, known properties of the system components. Achieving this goal may require the model to span a range of length scales and incorporate information from multiple fields of endeavour. As this article argues, models that achieve this challenging goal can serve as an important complement to experimental and theoretical studies, and can provide valuable knowledge.

Before the advent of computers, one could write force balance equations describing equilibrium of forces at a single triple junction and volume constancy equations for single cells. However, studying interactions between meaningful numbers of cells by hand was impractical due to the large number of equations that had to be constructed and solved. To make matters worse, as the cells moved, their geometries changed and the equations had to be re-derived and re-solved for each small increment of motion.

When computers became available to university researchers in the early 1970s, they ushered in a revolution. With the advent of computers, code could be written to automatically construct and solve these equations and to do so repeatedly for multiple times steps. The time course of the cell movements could then be predicted and new things could be learned about how cells in model aggregates behaved [1]. Thus, computers provided a new way for researchers to investigate interactions between different systems elements.

Interest in the mechanics of cell–cell interactions was growing at the time, and there was debate about the nature of cellular forces and how they could drive collective phenomena such as cell sorting and aggregate rounding [6,7]. Some of the earliest computer programs were written to investigate the mechanics of cell–cell interactions and thereby tackle these intriguing questions. Even though many of those early studies were rudimentary by current standards, they were instrumental in defining the field of computational modelling and they unlocked important mysteries about how cells interact with each other [1].

Researchers quickly realized that they could change the properties of the virtual cells in their models and the rules that governed their interactions at will, and that by doing so they could test hypotheses, understand which features gave rise to particular outcomes and carry out almost any kind of virtual experiment that crossed their minds. Over time, the algorithms they used improved and became more reliable, stronger connections were forged between models and real-world experiments, and modelling ultimately entered the mainstream of biology. Indeed, computational models have now become a standard tool for assessing proposed new biological mechanisms, often considered essential even when the associated experimental evidence is strong.

Many of the computational advances needed for these models came out of the fields of engineering and physics. The reason is that during the 1970s, 80s and 90s, computational models came to play an increasingly central role in various branches of engineering, especially its structural, aerospace, mechanical, electromagnetic, fluid dynamics, chemical, control and electrical domains [8]. It was in these contexts that extensive algorithm development took place and that the mathematical theory needed to bring confidence to the calculations was developed. In engineering and physics, a particular technique called the finite element method (FEM) took shape during this period and became the most widely-accepted, general-purpose framework for studying phenomena that involve non-trivial geometries. Many modern cell and tissue models, as well as other kinds of models, draw on conceptual and computational developments associated with this method.

A large variety of computational models arose for studying cells and their interactions during this time, including lattice (Potts), vertex, centric, and finite element models (reviewed by Brodland [1]), and since then, even more models have arisen [2,3,9–13]. Multiple approaches continue to be used because each one has its own inherent strengths and challenges. In addition, several large computational packages have become generally available, including CompuCell, The Virtual Cell and Smoldyn [14–16].

As this article discusses, computational models are based on specific conceptual, mathematical and algorithmic assumptions, and while these presuppositions can bring power and efficiency to the models, they can also introduce differences between the model and the real world that it endeavours to parallel. Determining which model is most appropriate in a particular setting will depend on the focus and goals of the study, with options including deterministic versus stochastic approaches, agent (particle) versus continuum schemes, single- versus multiple-scale approaches and forward versus inverse approaches.

2. The process of modelling

2.1. What does it mean to model something?

In order to better understand what it means to model something, consider Fig. 1, which shows a rectangular box across the top and represents the physical world, where a particular real embryo exists. For purposes of this illustration, we will consider the process of neurulation in amphibian embryos. An axolotl embryo at the start of this process is shown in the upper left corner of the figure. Over time, its neural plate, which consists of most of the visible tissue, rolls up to form a tube – the precursor of the spinal cord and brain – as shown in the other frames in the upper box. The box at the bottom represents the virtual or "*in silico*" world, and there one hopes that a corresponding model embryo is undergoing the same processes. Only when rendered using computer graphics does the

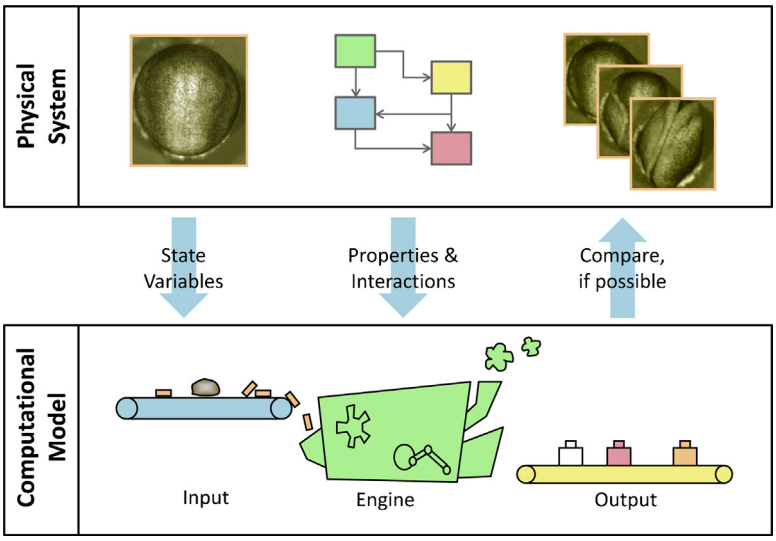

Fig. 1. The process of modelling. The rectangular box across the top represents the physical system while the lower box indicates the computational model. The arrows in between indicate how the state variables serve as input to the model while the properties and interactions instruct the function of the engine, where all calculations are done. The output of the engine is a set of numbers that can be rendered in various more readable forms and used to compare the model output with corresponding data in the physical system, where such exist. By comparing with physical data the model can be verified and validated (see text) and then used to predict behaviour where corresponding physical experiments may not exist, though care must be exercised if extrapolations are made.

model actually have any resemblance to the physical system it aims to represent, and when appropriately depicted, one hopes that it will appear very much like its archetype [17].

At the moment shown, the embryo is generally spherical in shape, and its neural ridges, which trace an inverted U-shape around the upper (cephalic) three-quarters of the embryo and bound the neural plate, are just visible. Physical experiments carried out over several decades showed that a number of features of the embryo are crucial to its ongoing development. These include its current bulk and cellular geometries, the cellular architecture of its cytoskeleton and of other force generating systems, and the genes expressed in each cell and the proteins to which they have given rise [18–20]. It is generally understood that changes to any of these quantities would threaten the future of the embryo.

The *in silico* embryo must match at least these features, and we call the numbers or other quantitative descriptors that embody them "state variables" because they describe the current state of the model embryo. An important part of the modelling process is to choose these state variables well. The set must include all quantities important to the embryo during the period to be modelled, but not be encumbered by too many unnecessary ones. Proper choice of these variables requires a certain amount of prior understanding of the system of interest. Measured or estimated values of these state variables – which in the case of the embryo shown included thousands of surface points that defined the exterior form of the embryo, gene expression patterns, cytoskeletal morphologies, and cell fabric (size, shape and directionality) information – serve as the input to the computational model.

Over time, the physical features of the embryo as captured by its state variables interact with each other, producing a specific change in the geometry of the embryo, hopefully the one necessary to form properly its neural tube. The model must simulate these interactions – some of which, like tissue force-elongation relationships, can be captured in the form of material properties [21]. The purpose of the computational "engine" is to faithfully reproduce these interactions using mathematical relationships and computational algorithms. In order to do this, most engines use the mathematical machinery developed for a particular approach – such as a finite element or Potts method – but sometimes custom approaches are used [9,10,22]. Much of the success of a model can depend on choosing an appropriate engine, and fine tuning its operational parameters, such as the size of the time step used for a particular simulation. Although some of the powerful commercial finite element packages may seem suitable for cell studies, they do not generally tolerate negative stiffnesses which some cellular components exhibit [1], nor are they designed to accommodate biologically-distinctive features such as cells or their neighbour changes [23].

As the engine runs, it reports the results of its calculations, which may include the current locations of all of the points used to define its geometry, regional cellular fabric (size, shape and directionality), internal stress values, and updated cytoskeletal and genomic information. Suitable graphics routines are essential for transforming the numerical findings into a more comprehensible form. The various components of the output can be compared to any corresponding values that are known for the physical system. If there is good agreement, it indicates that the chosen state variables and interactions are sufficient to explain the observed phenomenon. Unfortunately, agreement does not prove that the mechanism embodied in the model is the only possible explanation. In contrast, lack of agreement indicates that the variables and interactions assumed in the model are not sufficient to account for the physical observations. This outcome often gives rise to further thought, additional physical or numerical experiments, revised models and, hopefully, new understanding.

A broad range of factors can be considered in making these comparisons. In the model of neurulation discussed here, these comparisons included bulk tissue motions, regional strain rate profiles, maps of tissue stresses, tissue thicknesses and cross-sectional silhouettes, and cell shape details [21,24]. In other kinds of biological systems, one might consider factors such as stochastic fluctuations, system stability, time constants, phase transitions, bifurcations, or signatures that are useful for classifying system behaviour.

2.2. Relationships between experiments and computational models

Physical experiments make use of observations and may involve manipulations designed to expose how the system of interest functions. A key challenge in such experiments is finding ways to make

the required observations and manipulations without affecting the system in unintentional ways. Some techniques, such as laser ablation [25], are inherently destructive, while others such as confocal microscopy produce photo-bleaching, thus limiting the number of measurements that can be taken [26]. Still others, such as mechanical force measurement, necessarily perturb the system they aim to measure [27].

In order to properly interpret data from physical experiments, it is often necessary to make use of powerful mathematical techniques. One reason is that natural embryo-to-embryo variations can be quite substantial, often in the range of 30% [28], and so multiple replicates of experiments must typically be carried out and statistical methods used in order to infer causation. Another reason is that the systems may be quite complicated and direct and accurate interpretation of observed effects may not be possible [27]. The new force inference techniques offer yet another example of a useful interpretation approach that relies on a strong but largely hidden mathematical framework [29–31].

Computational models typically use observation and manipulation in the same ways as physical experiments, because their goals are often the same. Most physical experiments can be replicated using properly constructed models, and doing so can serve as a key ingredient in model validation. Models however, can avoid some of the experimental difficulties identified above, and they often permit kinds of experiments that are not currently experimentally feasible. Some of the features of virtual experiments carried out using computational models as opposed to physical experiments include these:

1. An arbitrary number of experiments can be run from the exact same starting configuration.
2. Embryo-to embryo variations can be totally eliminated if desired.
3. Observations can be made without any interference to the system or its components.
4. Observations can be made with arbitrary frequency and resolution without damage or deterioration to the model.
5. Observations can be ported directly to suitable graphical display or analysis software.
6. Any of the state variables can be manipulated (including ones that cannot be modified experimentally, and if desired, the manipulations can be statistical in nature).
7. The manipulations can be of arbitrary, user-defined magnitude, including very small (something generally not possible in experimental systems).

Although, as noted here, computational models have a number of advantages compared to experiments, they also are subject to challenges, as the following section shows.

2.3. Steps in building a computational model

Here we consider the steps involved in construction of a computational model. The first step requires formulation of a conceptual model, that is, a set of ideas about the basic operation of the system of interest, or at least a catalogue of the components that are involved and how they might interact with each other. In building a conceptual model of an early-stage embryo, some of the decisions that must be made include: Is the behaviour of interest fundamentally 3D or is a 2D approximation sufficient? Do individual cells need to be represented explicitly, or can their behaviour be adequately captured by a suitable cell-based constitutive equation? Do gene expression and gene regulatory networks (GRNs) need to be included because they or the proteins they make and regulate change over the period that will be modelled? Do cytoskeletal components or other force-generating structures need to be included, and if so, which ones? What interactions between these components need to be incorporated into the model, that is, which interactions are crucial to its operation? How important are stochastic factors? Each assumption that is made introduces another possible reason that the model will not match the physical system it strives to mimic. However, suitable assumptions can bring model simplification and clarity, and they are essential to effective model construction.

Selecting which simplifications to make in any particular situation and the choices involved in the other steps of model construction require knowledge and insight regarding the system as well as mathematical and computational skill. Details of these processes are beyond the scope of this article. However, it can be said that modelling is an advanced skill and that collaborations between modellers and biologists can be highly fruitful when they bring their respective expertise, learn to speak the language of the other and are well paired.

The second step involves translating the ideas in the conceptual model into mathematical form. The resulting mathematical model must include a full listing of all state variables and must indicate whether real numbers, integers, Boolean flags or some other kind of representation is appropriate for each. Often, matrices or other data structures are introduced. Each kind of interaction must also be expressed as a mathematical relationship. Construction of an appropriate mathematical model can take considerable time, and it often requires that new experiments be done, that additional assumptions be made or that the conceptual model be revised.

The final step of model construction involves converting the mathematical relationships found in the previous step into computer code. Choice of a suitable computational framework, such as that associated with finite elements or Potts formulations, and choice of suitable algorithms to implement the various aspects of these frameworks are crucial to this process. The list of possible choices is very large and for the sake of completeness we mention also phase-field, lattice-Boltzmann, boundary element, finite volume, Monte Carlo, Gillespie, molecular dynamics and dissipative particle dynamics approaches. Poor choices can be very costly in terms of time spent, frustration, and poor or faulty results, and so this step in the process should be done with diligence and due consultation.

The choice of computer language is also important. High level languages such as MATLAB allow complex code to be written quickly and easily, but runtimes are slower than in programs written in lower level languages such as C. Many labs use MATLAB to prototype their algorithms, and if they substantially achieve their anticipated goals, they rewrite them in C++ or a similar language. Standard libraries should be used when possible so as to save time and improve reliability. Languages such as Python are gaining broader acceptance and they are particularly useful for making code segments written in different languages function together.

Once a computational model is finally built, extensive testing is required to ensure that the code accurately embodies the mathematical relationships over the parameter ranges in which it will be used. This verification process is crucial to the success and reliability of the resulting computational model. Next, the verified model should, wherever possible, be used to simulate simple cases whose behaviour is known so that the model output and experimental findings can be compared. Agreement over a range of cases suggests that all three steps in the construction of the model (that is the various assumptions and simplifications introduced at each step) may be suitable, a process called validation. Biological systems that involve significant stochastic factors pose particular problems in this regard, and require extra care. Ongoing verification and validation are important so that the model can be used with confidence.

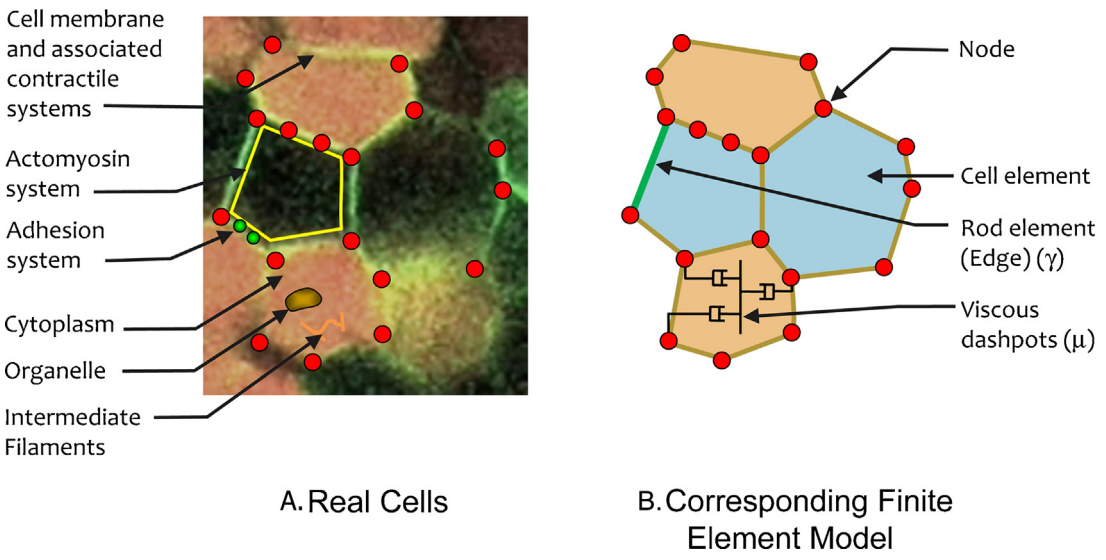

Fig. 2. Construction of a cell-based model. (A) shows an image of real cells in an epithelium (compliments of G. Krens and C.P. Heisenberg) while (B) shows the corresponding finite element model. The model is described in terms of its nodes and elements. The active components of the cells – such as the membrane and its associated contractile systems, actomyosin contractions and adhesions – contribute to the active forces that drive cell motions, and are represented in many models by a net interfacial tension γ and in a finite element implementation, are modelled using contractile rod elements. The passive components – such as the cytoplasm, organelles and intermediate filaments – are assumed to give rise to an effective viscosity μ, and are represented in some models using orthogonal dashpot systems, one of which is shown in (B).

The comments made in this section have close parallels to experimental work. For example, care must be exercised in protocol design; assumptions are made about the specimens studied and about the reagents and other materials used; and skill, art and experience are generally necessary for successful experiment design and execution.

Having mapped out the basic ingredients and function of computational models, we are now in a position to consider some of the specific ways in which they can be used.

3. Functions that models can serve

3.1. Test hypotheses

Certain kinds of hypotheses can be very difficult to test experimentally because multiple factors are at work, and it may be difficult or impossible to isolate the ones specific to the hypothesis. Consider, for example, convergent extension (CE), a canonical pattern of tissue narrowing and elongation that occurs during gastrulation and early neurulation in the dorsal epithelium and neural plate of vertebrates. Even though this tissue is typically a monolayer, its mechanics is quite complicated and difficult to figure out. The edges of the tissue are attached to other tissues which can exert forces on it, it slides with respect to subjacent tissues, its cells divide, and lamellipodia with medio-lateral orientations arise from its cells and contract. Experiments have shown that if this tissue is excised, it still narrows and elongates [32], while other experiments showed that cell division was not crucial to these motions in amphibians, though it is necessary in birds [33]. Although these experiments would seem to indicate that lamellipodium action must be driving CE, it was not clear from a mechanical point of view that it could cause the observed patterns of motion, especially given the associated boundary constraints.

To test hypotheses about CE and other cell-level phenomena, a cell-level computational model (Fig. 2) was built. This model focusses on a different length scale smaller than that used in the model shown in Fig. 1. In that model, the finite elements (the basic volumetic building blocks) represent groups of cells, while here the elements are smaller and represent individual cells and doing so in such a way as to capture their exact shapes.

A model was an ideal platform for studying CE because it allowed the user to construct virtual tissues having lamellipodia with user-selected frequencies of occurrence, orientations and contraction strengths, and it allowed the effects of various graduated boundary conditions to be investigated [34,35]. In essence, the model allowed a large number of hypotheses about force sets that might drive CE to be tested, and then parametric studies were carried out around the successful combinations so as to identify their effective ranges. Comparable experimental systems could not have been constructed *in vivo* because suitable mutants or blocking techniques were not available, and even if they had been, these technologies would not have produced finely graduated adjustments.

The model showed that contraction of a single lamellipodium produced mostly local tissue rearrangement along curves similar to a four-leaf clover, as one might expect [34]. However, each contraction also produced very slight narrowing and elongation of the tissue, values too small to identify in physical systems, if it were unconstrained. When many asynchronous lamellipodia contractions occurred, visible medio-lateral narrowing and cephalo-caudal elongation was produced. Furthermore, hypotheses about the role of boundary conditions could be tested and it was found that if the lateral edges of the tissues were partially or fully constrained, the cells became elongated in the medio-lateral direction. Subsequent parametric studies allowed various rate constants and mechanical descriptors to be established. These studies showed that lamellipodium contraction could indeed drive CE, but by their nature they could not prove that they did, any more than could a physical experiment.

Models have become a standard tool for testing hypotheses in biological settings, and today many top journals look for them, recognizing that they offer the possibility of a sanitized and "pure" setting within which to test hypotheses without concerns about interference from other factors. Not surprisingly, models have been used to test hypotheses about the mechanics of a wide range of processes including directed mitosis [36–38] neural tube closure [39], cardiac tube bending [40], gut tortuosity [41–43],

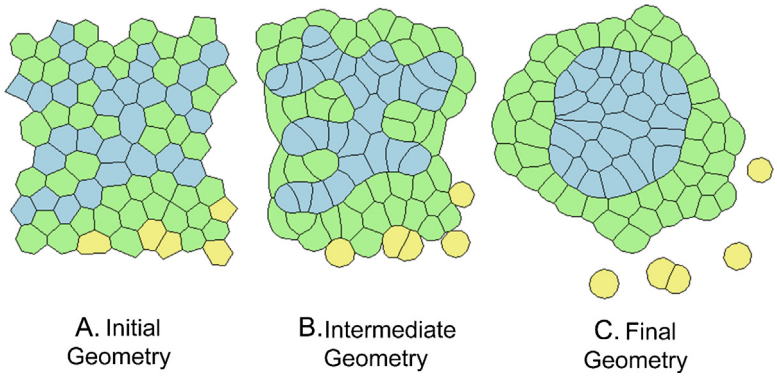

Fig. 3. A cell-level simulation. Three cell types were used in this simulation and their interfacial tensions were chosen in such a way [53] that they would demonstrate sorting between the green and blue cells and dissociation of the yellow cells from the green cells. ($\gamma_{GG} = \gamma_{BB} = \gamma_{YY} = \gamma_{GM} = \gamma_{YM} = 1$, $\gamma_{GB} = 5$, $\gamma_{GY} = 3$, $\gamma_{BM} = 10$).

convergent extension [44], cell migration [45–48] and wound healing [49].

3.2. Lead to new insights

When the first cell-level finite element models were constructed [23], it was necessary to test them on systems whose properties and behaviours were known. Cell sorting (Fig. 3) was one of the systems chosen because of its long experimental history [50,51]. In a typical experiment, cells of two histological types are mixed together and over time they sort, producing a compact mass consisting of one cell type surrounded completely by a mass of another type. Sometimes multiple interior masses form, and not every cell joins a group of its own type.

When early versions of the models reported in Reference [52] were run and properties suggested by earlier experiments and computational models were used, the model cells consistently moved to the wrong position, that is, the specific cell types that one might expect to be in the interior and exterior were exchanged. A single incorrect sign in one line of code could conceivably have caused this anomaly, and much time was spent searching for coding errors. In time, it was recognized that adhesions reduce the tension along a cell–cell interface, and that this effect reduces the energy associated with such surfaces rather than increasing it. Thus, the model predictions were actually correct. Instead, the explanation offered over the course of some forty years for cell sorting turned out to be incorrect. This surprizing discovery led to the Differential Interfacial Tension Hypothesis (DITH) and ultimately to a comprehensive explanation of the forces that drive cell sorting, checkerboard pattern formation, cell dissociation and a range of other well-known phenomena [1,53]. Although the DITH concept initially faced much opposition, today the basic ideas that it enunciated are widely accepted. Thus, we see an example of a computational model that led to a new insight.

3.3. Force us to think more deeply

More often than one might wish, computer simulations do not produce the results its users might expect. This oft disheartening outcome implies either a problem with the model or that the physical situation is different than originally thought. Although the model discussed in the previous section brought much understanding, it had one troubling feature that we could not resolve: FE models of sorting, and to a lesser extend Potts models of sorting, tend to produce multiple islands of cells [1,52,54] while experiments [51] tend to produce single large islands.

Careful examination of the simulations showed that chains of cells of one type acted as barriers, preventing separated groups of cells of the other type from making contact and fusing. The introduction of cell-to-cell tension variations and temporal variations, which are inherent in Potts models, did not fundamentally change the FE model predictions. The unresolved discrepancy raised questions about whether the models were missing a basic mechanical characteristic of real cells, or whether the difference arose because the experiments were three-dimensional and the models were only two-dimensional.

Resolving this question had to wait until 3D models were developed [1,55]. Those models showed that cells in 3D typically have 14 neighbours rather than the 6 neighbours that 2D cells have. They also showed that if any one cell type represents at least 30% of the cells in the mass, there is a high probability that almost all of the cells of that type will be connected to each other. Thus, although a cross section of the mass may show separated islands of cells with apparent barriers between them, those islands are actually connected to each other through chains that migrate through the third dimension. However, even if all of the cells of one type are connected, they cannot clump together because chains of other cell types course through them. These complex entanglements would seem to be sufficient to prevent sorting. However, the model also showed that in 3D, chains of cells spontaneously become unstable and break. In the presence of even gentle sorting forces, the entanglements break and cells can sort relatively easily and completely, often forming a single interior island and a continuous outside layer – just as in experiments.

Early models of neurulation also gave rise to deeper thinking about the mechanics of this process on a number of occasions. Early cross-sectional models of neural plate closure showed that many concepts of how the tube forms considered plausible at the time were not consistent with the laws of mechanics [39,56,57]. Furthermore, when attempts were made to extend those models to 3D, they failed to allow the simultaneous rolling that occurs from the sides and cephalic end of real embryos, and thus failed to recapitulate tube closure. These failures led to a more careful examination of the in-plane properties of the epithelia involved and to the realization that in-plane cell motions made these tissues substantially more flexible and plastic in plane than they would otherwise be [58]. This and other cell-based studies showed that the cellular nature of tissues gives them important mechanical features not predicted by classical continuum formulations.

3.4. Suggest and refine experiments

In a perfect world, insights from experiments, theory and simulations (models) would be applied simultaneously to studies of biological systems, and they would be in continual dialogue, with each continuously informing the other. Two roles that

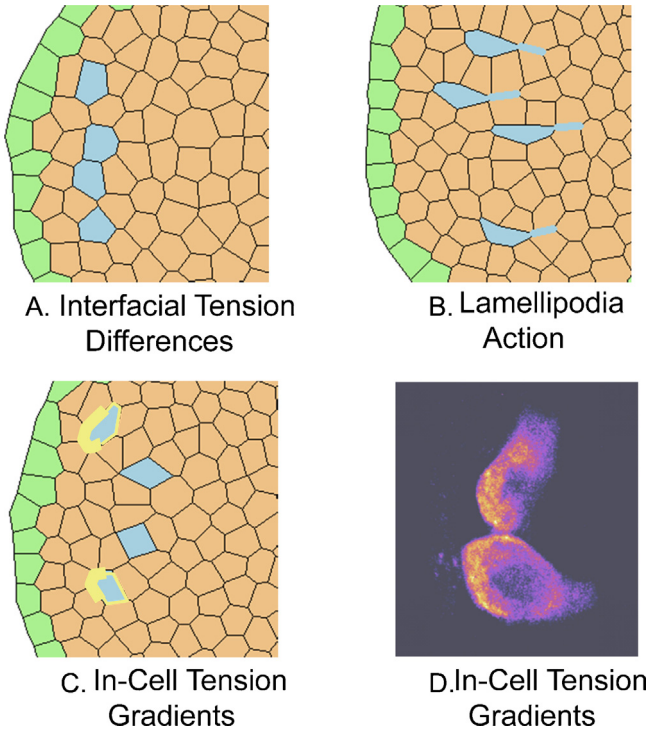

Fig. 4. Models and experiments. A. Interfacial tension difference were able to drive the mesoderm cells (in blue) only one cell row into the ectoderm (shown in orange). (b) Lamellipodia oriented away from the enveloping layer (EVL) shown in green, can drive ectoderm cells long distances, but elongate the cells in ways inconsistent with corresponding experiments. (C) Tension gradients in the mesoderm cells, strong nearer the EVL and weaker away from it, as suggested by the yellow lines can drive ongoing motions and do so without excessive cell elongation. (D) Based on the implications of (C), cells were stained for myosin, and it was found in patterns consistent with the model predictions. Figure part (D) courtesy of G. Krens and C.P. Heisenberg.

computational models can play in this exchange are to suggest new kinds of experiments and to provide a fast-track method to pre-test them.

While investigating the mechanics of single-cell migration, we used models to investigate a variety of possible scenarios (hypotheses) regarding why individual mesoderm cells at the involuting lip of the blastopore migrated deep into ectoderm as they did (Fig. 4). In this particular setting, the tissue-medium interface was covered by an enveloping cell layer (EVL) which is shown in green. Physical experiments suggested that interfacial tension differences could be involved, as could lamellipodia. The simulation in Fig. 4A showed that cell-type differences in interfacial tensions could drive mesoderm cells (shown in blue) through the ectoderm (orange), but only one cell diameter. Essentially, the presumptive mesoderm cells were simply being engulfed by the ectoderm cells. In contrast, lamellipodia could drive motions of arbitrary distances (Fig. 4B), but the models suggested that the cells would become elongated, and that model shapes were not consistent with those observed. Finally, when it was assumed that the tensions on one side of the migrating cells were different from those on the other (Fig. 4C), the model predicted ongoing motility and cells with egg or arrow shapes, consistent with those observed experimentally. This finding suggested experiments be carried out to label myosin, and those experiments confirmed that it was distributed in patterns consistent with those suggested by the model (Fig. 4D).

Computational models can also be used to pre-run proposed physical experiments. The reason is that they are often relatively easy to set up and run – requiring only changes to the input file or modest changes to the computational engine (coding changes). They can therefore be ideal for quickly testing ideas about how

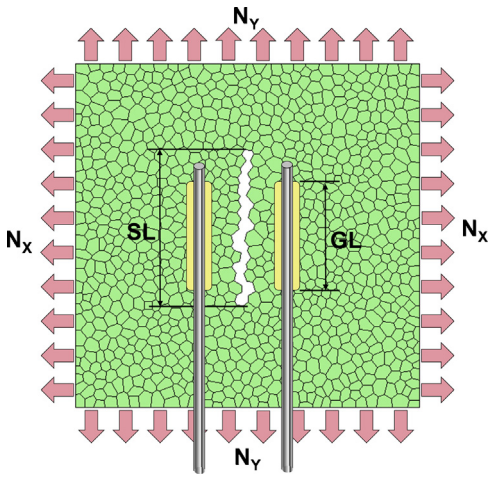

Fig. 5. Using a model to interpret an experiment. A cell-level model was used to model a slitting experiment in which wires had a portion of their length glued to the undisturbed tissue. That length is labelled GL in the figure. A slit of length SL was then made in both the real and model epithelia. The model made it possible to predict the load fraction carried by the wires and the fraction carried around the ends of the incision in the epithelium. In this way the model allowed the wire loads, and thus the experiment, to be properly interpreted.

certain cell motions might be driven or to test how changes to various system characteristics (model parameters) would affect system response. As a result, they can be used to verify whether the effects of proposed experimental interventions would be large enough or otherwise suitable to measure. As a result they can serve to test and fine tune experimental protocols. Whereas the time required to carry out model-based virtual experiments is often of the order of hours or days, the time required to develop genetic or biochemical tools for physical experiments and to carry out the requisite number of replicates and their associated data analyses is typically weeks to months.

3.5. *Interpret experiments*

Biological systems tend to be complicated, involving multiple length scales and complex geometries, and models can be very useful for interpreting experiments on them. One way to obtain an indication of the directional tension in an embryonic epithelium is to make a slit in it normal to the direction of interest [32,59]. The degree of opening depends on the slit length and on the relative compliance of the incised and adjacent tissues and on the ways in which the load that was carried across the slit tissue is redistributed and carried through alternate load paths. In order to reduce the large-deformation effects that occurred and more accurately measure the *in vivo* loads, the arrangement shown in Fig. 5 was devised. The wires were designed to limit tissue deformation and pick up most of the force relieved by the incision, with an unavoidable fraction being transferred to and carried by the tissue at the ends of the slit. So as to properly interpret the wire forces, simulations with a range of relevant parametric values were carried out and formulas for interpreting the experiments derived.

A similar approach was used to interpret laser ablation experiments on *Drosophila* embryos [60]. In those experiments, designed to determine the forces driving germ band retraction, circular ablations were made in each distinct segment of the germ band and cell-level models were used to interpret the resulting elliptical wounds. Interpretation was complicated because anisotropies existed in the shapes of the cells in the unwounded germ band tissues and in the tensions that acted along the individual cell edges. Only by using suitable simulations could the experiments be properly interpreted.

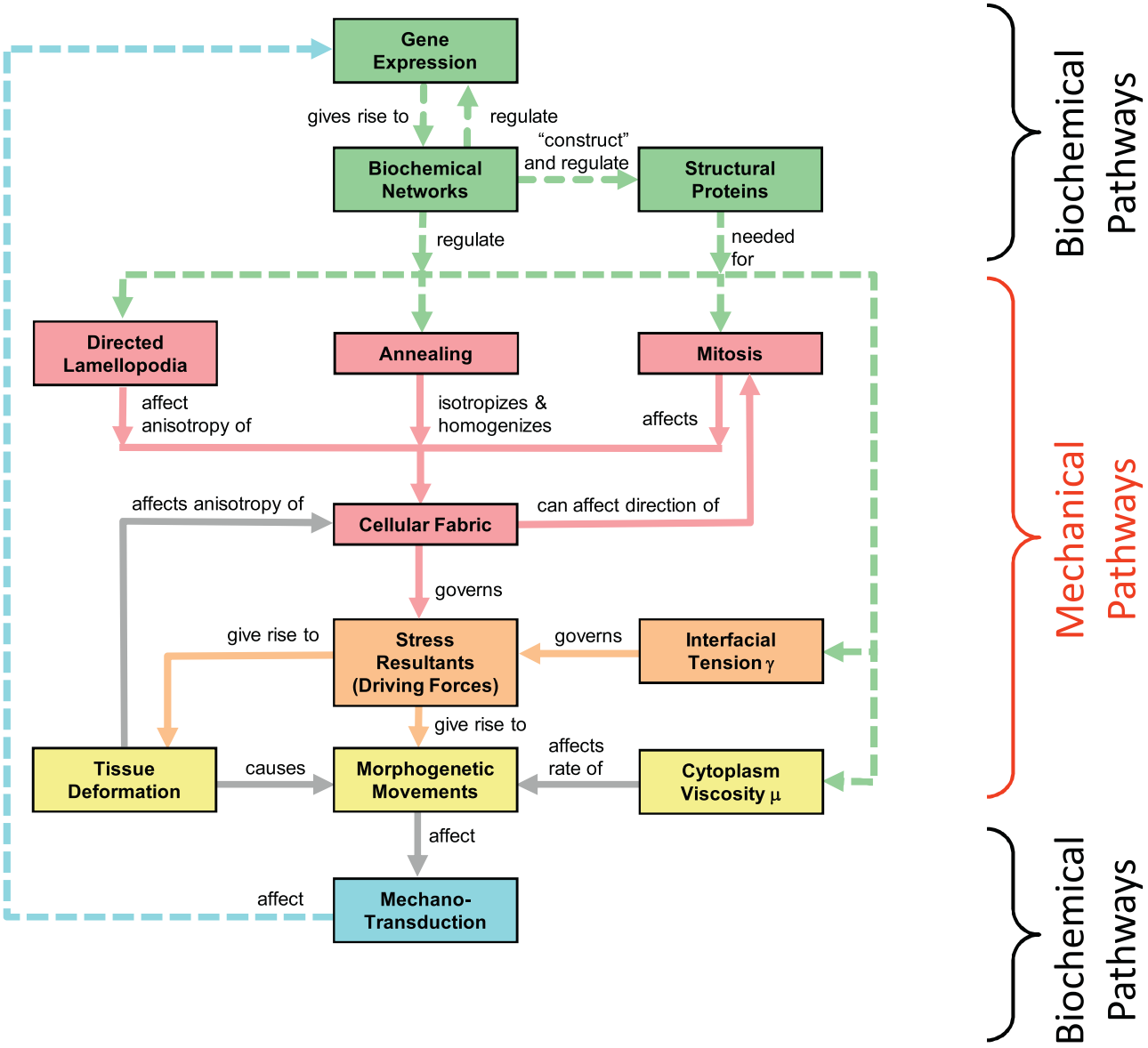

Fig. 6. A network of inferences. Each box represents a single or related set of state variables and each arrow a relationship. Relationships known quantitatively are shown with solid arrows. The bars on the right indicate relationships that would be considered biochemical pathways and mechanical ones, and the figure emphasizes how they interact with each other.

3.6. *Trace chains of causation*

Several characteristics of simulations make them ideal for tracing chains of causation. As noted in Section 2.2, unlike real-world experiments, they allow an unlimited number of simulations to be run from starting configurations that are the exact same down to the smallest scale of the simulation, thus allowing the effects of variation between natural specimens to be completely eliminated. This is an important factor because in physical experiments these variations widen the confidence interval of any measurements, increase the number of replicates required to obtain data of specified precision, and limit the size of the smallest differences confidently discernible between sets of experiments. Secondly, simulations allow the user to make changes of arbitrary kinds and sizes in any state variable at any time during a simulation and to do so in any chosen region. Finally, they provide unlimited access to internal state variables and allow them to be read with virtually any desired precision and temporal and spatial resolution.

At the same time, care must be exercised so as not to read more into simulation results than is warranted. The properties on which simulations are based have finite accuracy, the process of representing properties using mathematical and computer models introduces further error, and algorithms and their numerical implementations introduce error. In addition, biological systems have inherent stochastic features arising from natural variations at the genetic, ultrastructural, cellular, and tissue levels, and for computational results to be meaningful, they must hold over the natural range of expected variability of the physical systems they aim to describe.

Many examples exist where these features made it possible to trace causal sequences that could not be practically found using current experimental techniques. For example, studies of mitoses in model epithelia showed that they tend to reduce cell-shape anisotropy if they occur along the long axis of the cell as is common in real epithelia. In contrast, globally-oriented mitoses can produce tissue reshaping and, if sufficiently restrained at the tissue boundary, increase shape anisotropy [36]. Oriented mitoses in

unconstrained tissues causes elongation in the direction in which the daughter cells are oriented, as one might expect. However, intuition struggles to know whether these same tissues would narrow, maintain their width or widen. Since physical systems cannot yet be constructed with these properties, it is only through models that this question can be answered, and the mathematical reasons the tissue width remains constant found.

Thousands of simulations were run to investigate how cellular fabric interacted with various mitosis patterns, with applied boundary stress, with tissue deformation and with other factors. The ultimate result was an interaction diagram as shown in Fig. 6. Many of the interactions shown in that diagram were later described mathematically, and allowed a system of constitutive equations to be derived for cellular materials [58].

Models have a distinguished history of tracing causation quantitatively from one scale to another and from one kind of pathway to another [21,61,62]. In the case of neurulation, a morphogenetic process that has received much attention, the chain of causation was traced from specific gene expression patterns to the cytoskeletal components to which they gave rise, to net interfacial tensions along individual cell edges, to tissue properties, to mechanical interactions between tissues, to morphogenetic movements, to phenotypes [21]. Models of this kind have made it possible to better understand how specific genetic changes ultimately produce altered final geometries, including some consistent with birth defects [24,63,64].

3.7. Carry out sensitivity analyses

Sensitivity analysis is a somewhat mathematical idea that is closely related to the study of causation. In essence, it seeks to an answer the question, "How much does a change of a specified amount in an input variable affect a particular output variable?" Many ideas from control theory are closely related to this approach, including stochastic analysis, influence factors (gains), setpoints and system stability. Such ideas are particularly useful when complex inter-relationships are at work, as in Fig. 6.

One might, for example ask how much mitosis rates would have to be increased to change the cellular fabric at a particular rate. In this case, the answer depends on the current values of associated parameters, including mitosis and lamellipodia rates and fabric descriptors [58]. One might also use the model to investigate the consequences of an elevated mitosis rate or use it to test the effectiveness of proposed corrective interventions.

3.8. Investigate coupling and feedback

Coupling and feedback are also closely related to control theory and to modelling, and are two features of biological systems that have received comparatively little attention even though they are germane to how many such systems operate.

Two phenomena are said to be coupled if one influences the other. Thus, each of the single-ended arrows in Fig. 7A indicates a coupling relationship. Coupling can arise in unexpected ways, including through mechanics. For example, microfilaments may be active on the apical surface of an epithelium, as shown in cross-section in Fig. 7B. If the microfilament force is increased (Fig. 7C), it would cause the cell in which it acts to narrow. As the actin and myosin filaments increasingly intercalate, the cross-section of the bundle increases causing the contraction force to increase still further. These inter-relationships are shown in the figure, with plus and minus signs indicating the sense of the correlation. In the language of gene regulatory networks, a positive sign would correspond to up regulation and a negative one to down regulation. The diagram might be part of a larger figure that would include the effects of other factors that influence or are influenced by the ones shown.

Feedback is said to occur if one can trace one or more closed loops by following the directions of the influence arrows. If the product of the signs associated with the arrows is negative, a negative feedback loop is said to exist and such loops are generally associated with stability. If the product is positive, a positive feedback loop exists and if the gain of the loop as calculated by the product of the influence coefficients around the loop is greater than one, instability generally results. That is, any change will become amplified without bound, until one or more of the factors, such as cell width, reaches its maximum or minimum value. Unstable feedback can be useful in biological systems, and it has been postulated as a basis for both chemically- and mechanically-driven pattern formation [39,65].

Coupling can be an important and elusive effect in mechanical systems, and extra care must be exercised when strains or deflections are large [66]. For example, as the cells in the model neuroepithelium in Fig. 7B narrow, the strains are sufficiently large that their height increases visibly. One must then be careful in calculating stress to define whether it is per unit undeformed area or deformed area. Likewise one must be careful to define whether the strains are calculated on the basis of initial or current lengths. When deflections are large, which can happen without strains necessarily being large, coupling can arise between in-plane and bending mechanisms and these can be multiplicative in nature, not additive as implied by the factors shown in Fig. 7A. In such cases, computational models that use an updated Lagrangian formulation, and do all calculations and reporting in terms of the current configuration tend to properly account for these and the many other kinds of often-unexpected coupling mechanisms.

3.9. Integrate knowledge

Models are just beginning to serve as an interface for bringing knowledge of different kinds together. For example, Fig. 6 shows biochemical and mechanical pathways connected together, with specific causal connections between them. With time one can reasonably expect the number of such examples and the kinds of knowledge integrated to increase. Models also make it possible to connect affects across length scales, such as from the cytoskeletal components to cells to tissues to whole embryos [21,24,67–69].

3.10. Help to unify biology

Models can also be useful in showing commonality between different areas of biology, and one connection that has been receiving increased interest is the one between embryology and cancer [70–73]. A number of computational models have been applied successfully to both classes of problems with only minor modification [74–76], lending strength to the idea that the cell motions observed in both settings may share a common basis.

3.11. Lead to new approaches

Finally, models have led to a number of new approaches in biology, and one can expect that they will continue to do so. For many years the basic paradigm in mechanics modelling has been that one determines an initial geometry for the model, approximates the material properties and estimates the applied loads and uses a model to calculate the resulting displacements or motions (Fig. 8). This is the standard approach in the design of most structures and structural systems from buildings, to aircraft, to hip implants. The governing equations are often written in the form shown, with the column matrix $\{f\}$ being the applied forces, $\{u\}$ being the

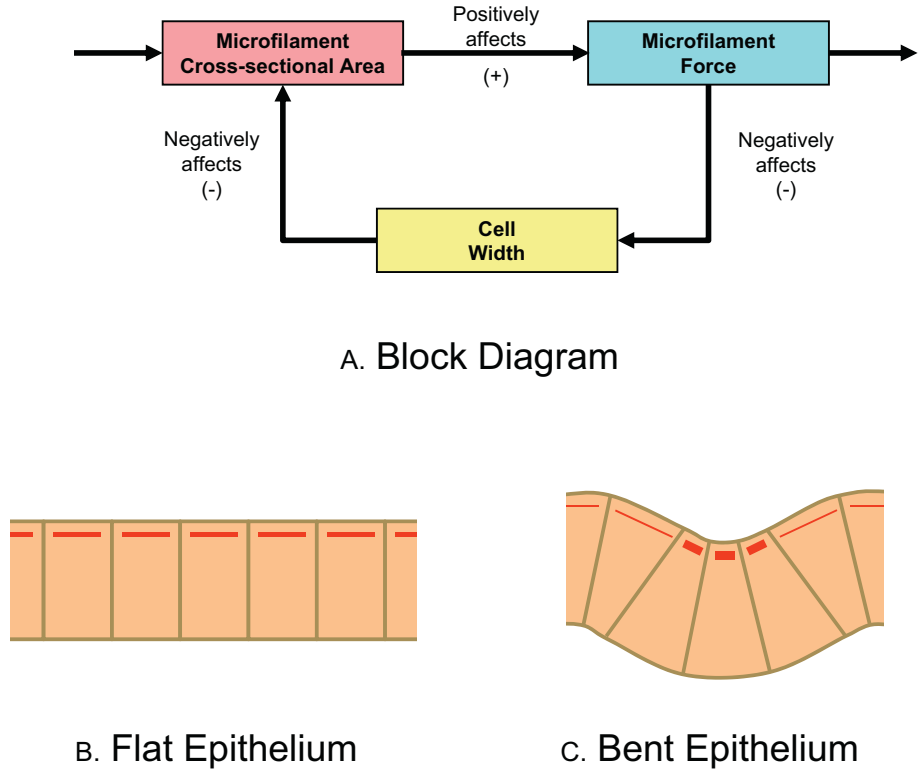

Fig. 7. Relational feedback. (A) shows a subset of the relationships between the state variables associated with epithelium bending. (B) shows a cross-section of a flat epithelium. Its apical surface is at the top of the figure. (C) shows the epithelium when it is bent and the text describes how bending produces positive feedback between the indicated variables.

displacements produced and [K] being a square matrix calculated on the basis of the geometry and material properties that relates them. Although the matrix [K] may imply linear elastic properties, that need not be the case [60]. One then solves the equations for {u} and from those nodal displacements can calculate stresses, strains and other secondary quantities of interest [8]. One variant on this approach is elastography, where the goal of the analysis is to extract regional mechanical properties, information of particular use, for example, in identification of breast tumours [77].

Newer variants assume that the motions are known, and solve for the acting loads [29,78]. In classical analyses of biological systems the loads tend to be the least well known, and so techniques that do not require them as input hold definite advantages. Although the value of a method to calculate forces from images

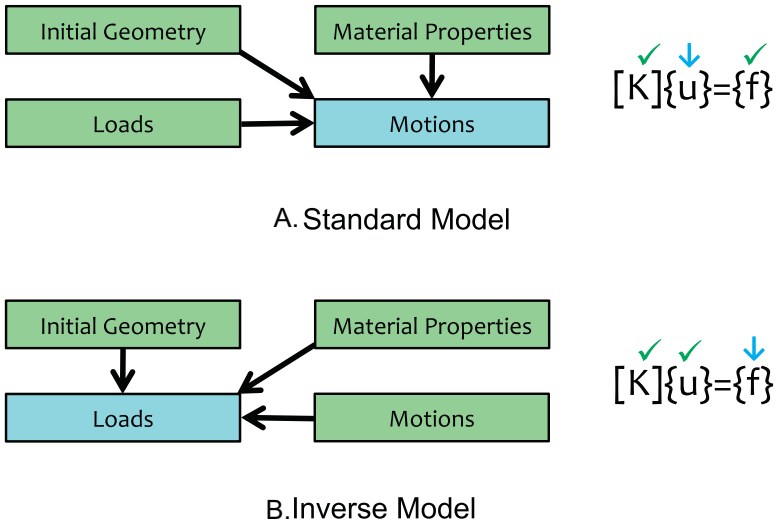

Fig. 8. How modelling gave rise to inverse (inference) methods. (A) In a traditional Potts or finite element model, an initial geometry is chosen, the relevant material properties are approximated and any applied loads (perhaps internal to the tissue or other system) are estimated. The model is then used to solve for the motions or displacements. In matrix terms, the driving force {f} is assumed to be known as is the matrix or matrices (here represented simply by [K]) that relate force and displacement {u}, and the equation shown is solved for the unknown displacements {u}. (B) The concept behind VFM, and to a lesser extend CellFIT, is that the initial geometry, material properties and current geometry or the motions giving rise to it are known from images or time-lapse movies. The matrix equations are then solved for the applied loads {f}. Additional steps, not shown are then used to convert the nodal forces {f} into edge loads.

or motions had been recognized for many years, it was not until recently [78] that the mathematical details necessary to make it work were found and Video Force Microscopy (VFM) became viable. VFM came directly out of modelling, with modelling providing the core governing matrix equations and indicating a strong direction for the further analysis that came to be known as VFM. More recently, another technique called CellFIT [29], has been devised, and it also came directly out of modelling, in this case, models in which the cell boundaries contained intermediate nodes and the cell edges were no longer constrained to be straight. The latter technique overcomes a number of limitations of VFM, and because it is based on equilibrium alone, eliminates the need to know the material properties required by VFM. These techniques overcome the challenges of estimating the loads acting in cells, tissues and embryos, and they allow detailed force maps to be calculated from time-lapse movies and images.

4. Limitations of models

4.1. Models cannot replace laboratory experiments

As George E.P. Box once said, "Essentially, all models are wrong, but some are useful." [79].

All model building occurs in the virtual world, and whenever the goal is engineering or science, as opposed to computer games or entertainment, it must be directly anchored to the real world, and the real-world data it requires must come from laboratory experiments and empirical measurements. Thus, models must not be seen as replacing laboratory experiments, but as complementary players that dialogue with them. Based on our experience, good computational models actually inspire new experiments, increase the number of experiments done, and inform experiments in one way or another, so as to enhance their value.

4.2. Models cannot prove mechanisms (but they can disprove them)

In spite of what many hard-working modellers and their associates would like to believe, models cannot prove that particular mechanisms are actually the cause of any observed result. Models can prove that particular mechanisms are sufficient to explain an observed result, but they cannot prove that that mechanism is the reason that the result is observed. Instead, some other mechanism, perhaps as yet unknown, might have caused it. Models can, however, disprove proposed mechanisms. In essence, they are disproving the hypothesis that a particular set of driving forces are sufficient to produce a particular result. Some of the early 2D models of neurulation served exactly this role [39,57].

Later 3D models sought to bring together genetic, cytoskeletal and mechanical property information and to identify the most probable mechanisms of tube closure. In constructing the model adjustments had to be made to the driving forces, within the range that experiments allowed, in order to match the observed motions. After this was done, the model predictions of regional strains and stresses and of cell shapes and neural plate cross-sections were compared with and found to agree well with corresponding experimental data, thus considerably strengthening the likelihood that the proposed model was substantially correct [21].

5. Discussion and conclusions

One of the overarching reasons that models can help to unlock biological systems is that they offer perspectives different than those provided by experiments and theory. Science could advance without models, but progress would be considerably slower and more circuitous, just as it would be if experiments or theory were not available. Models have value because they allow their users to peer at deadbolt mechanisms from a different vantage point, sometimes even from inside. The additional knowledge and new perspectives they bring are often enough when added to experimental and theoretical contributions to allow the bolt to finally be drawn back and the door that barred a previously-hidden vista to finally be pushed open.

Through examples, this article has sought to demonstrate some of these perspectives and highlight their value. It has also aimed to show the limitations of models. Although the application focus here has been biological systems, most of the arguments made apply to modelling of any system.

Models are becoming a standard feature of scientific investigations, and proposed findings are often not accepted on the basis of experiments and theory alone. Indeed, computational modelling is transitioning into mainstream science in much the same way that statistics did many years ago. Today, computational models are becoming nearly obligatory, especially when a study argues for a new mechanism or functional relationship. Given the increasing role that computational models are playing, it is incumbent on scientists of all stripes to acquire a clear understanding of what models actually are and of how they can legitimately be used. Computational modelling clearly has a very bright future, and those who use models, collaborate with those who do, or wish to evaluate studies that include models must be equipped with this knowledge.

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