Modelling microtubule patterns

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The cellular cytoskeleton is well studied in terms of its biological and physical properties, making it an attractive subject for systems approaches. Here, we describe the experimental and theoretical strategies used to study the collective behaviour of microtubules and motors. We illustrate how this led to the beginning of an understanding of dynamic cellular patterns that have precise functions.

Cell organization results from the collective behaviour of molecules. Therefore, knowing the sequence of whole genomes will prove useless in the absence of an understanding of the dynamics of molecular interactions and of how these are related to cell organization. In fact, the emergence of patterns and complex functions derive from dynamic networks of molecular interactions[1]. In this article we describe how a systems approach (theory) has been applied to the study of the self-organization of one subcellular compartment, the microtubule cytoskeleton, and how this provides a guideline to understand cell morphogenesis as a whole.

The main function of the cytoskeleton is to organize space on a scale much larger than individual proteins (typically several micrometers to a millimetre). The highly dynamic nature of most cytoskeletal structures requires the use of tools allowing the description of spatio-temporal processes. The polymers that constitute the cytoskeleton have remarkable mechano-chemical properties that facilitate transformation of chemical energy into order. To understand this, the effects of forces, transport and diffusion must be considered. None of these concepts are in the traditional curriculum of biologists and recently the field has benefited greatly from the contribution of physicists. Mixing temporal and spatial properties of the system certainly complicates the analysis. However, help is at hand: theoretical techniques have been developed in the past 30 years to address very similar problems — found especially in soft-condensed matter physics (polymers, gels, liquid-crystals). In addition, high-resolution video light-microscopy techniques are now routinely used with specific fluorescent tags to obtain quantitative spatio-temporal data about cellular processes. Consequently, the field is rapidly expanding on the premise that theoretical methods can be applied to cell biology that are guided and validated by quantitative data.

These approaches are new to biology and will probably be the key to our understanding of cell organization and behaviour. One important aspect of cells is that they represent the boundary of the molecular scale. Considering that molecules are subject to random thermal motions, how can a cell become organized and fulfil its biological function? To address this question, it is essential to understand the importance of stochastic effects (such as diffusion) and how a 'coherent' behaviour can emerge from such effects. The difficulty in approaching these problems is that causality implies that emergent properties all depend on each other in successive steps in scale, from molecules to cells and tissues (Fig. 1). However, it is impossible to build a single model spanning even a couple of scales. One problem is that we are unlikely to ever know the exact characteristics of all cellular components with high precision. Another problem is that at each scale, the theoretical description can be so complex as to preclude combining different scales. Therefore, theoreticians must choose a specific level of complexity and/or scale, define relevant assumptions and apply an appropriate theoretical method considering the available experimental data. Different types of data and methods are required at different scales.

In this review, we explain how experimental work carried out on the microtubule cytoskeleton has lead to the beginning of a vision of how to deal with such problems. We discuss how abstracting the complex properties of a system at one scale can be used to describe the next level of complexity, without incorporating all the details of the underlying levels. The important lesson is that to understand emergent properties from dynamic biophysical interactions, it is essential to precisely define the 'graining' of the model and to use the appropriate combination of theoretical tools and data.

FROM TUBULIN TO DYNAMIC TUBES

The problem of scales in studying the formation of large and complex biological structures is illustrated in Figure 1. The elucidation of the structure of the tubulin molecule has helped to understand the dynamic instability that occurs on the assembled microtubules. Microtubules are long tubes (up to 50 μm) with a diameter of 25 nm that are built of tubulin protofilaments with structural polarity (see Box 1). A key organizational property of microtubules is dynamic instability[2]. Individual microtubules nucleated either from a centrosome or randomly in the cytoplasm of living cells grow and transit stochastically between growth and shrinking phases[3]. Stochastic dynamics can be regulated by force[4,5], which allows microtubules to orient in cells. Because microtubules collapse when they contact the edge of the cell, their length automatically adjusts to cell size. However,

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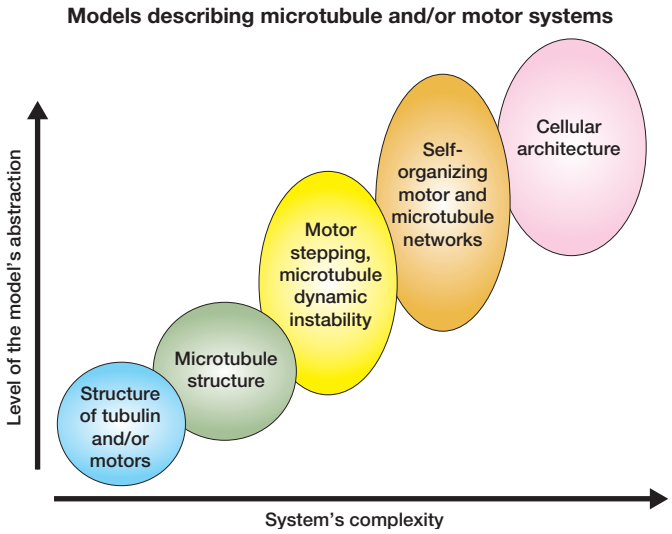

Figure 1 Different levels of modelling the microtubule motor system. The microtubule cytoskeleton is studied at different levels, ranging from the analysis of the three-dimensional structure of tubulin and motor proteins, to the intracellular architecture of living cells. As the scale and the complexity of the system under study increase, different modelling approaches, and different experimental techniques, have to be applied. At each level, models aim to explain the emergence of features of the cytoskeleton from the knowledge of the essential properties at the previous level.

forces are not the only regulator of microtubule length and the rates of tubulin assembly, disassembly and state transitions are regulated differently in almost every type of cell. For example, the mitotic spindle has a size that is cell and species specific, thus ensuring that chromosomes can be distributed accurately in cells with sizes ranging from a few microns to millimetres. Most of the components of the spindle are conserved and the adaptation of its size will have to be explained from quantitative differences in the reaction rates between components. Quantitative modelling will be necessary and this raises some interesting questions about what information a successful model requires: do we need to consider the three-dimensional structure of tubulin to understand dynamic instability and, in turn, do we need this knowledge to understand the morphogenetic properties of microtubules?

A detailed understanding of the molecular mechanisms responsible for microtubule dynamic instability requires knowledge at the atomic-molecular scale (see Box 1). This means that it is important to know the three-dimensional structure of tubulin and its intramolecular movements associated with GTP hydrolysis to explain why a growing tube, in which tubulin subunits are added at the tip, may stochastically switch to a disassembling mode[6–9] (Box 1). At present, we do not have a complete molecular model of dynamic instability; however, this knowledge is not necessary to understand the formation of complex microtubule patterns, such as the mitotic spindle. This is because microtubule dynamic instability can be mathematically formalized by modelling the length of microtubules using two states: growing and shrinking. Although additional states (such as pauses) do exist, this simplified model is often sufficient to describe the dynamic behaviour of microtubules, as it can be observed at the resolution of the light microscope[10–12]. Therefore, computers and a light microscope are often sufficient to describe how microtubules behave *in vitro* (Box 1). However, we cannot predict what types of large-scale microtubule patterns can form from only knowing the three-dimensional structure of tubulin, as many proteins, in addition to tubulin, are involved *in vivo*. These include all the microtubule-associated proteins that regulate dynamic instability, but also the effect of cortical contact and the physical confinement imposed by the cell boundary[13]. Knowledge of the three-dimensional structure of tubulin and conformational changes that occur when microtubules are assembled may further our understanding of how microtubule dynamics are regulated during the cell cycle and cell differentiation by regulatory molecules. The structural properties of tubulin endow microtubules with surprising dynamic properties, but this molecular information is not

BOX 1 MICROTUBULE DYNAMIC INSTABILITY

Molecular basis

Although the exact molecular details are still a matter of debate, it is becoming clear that a change in tubulin subunit conformation, coupled with polymer assembly, is fundamental to dynamic instability. The tubulin molecule is a dimer of approximately 8 nm, with one non-exchangeable GTP-binding site, and one site in which GTP is hydrolyzed on polymer assembly. Structural studies indicate that, in solution, the dimer is in a GTP state, with a straight conformation that favours the formation of relatively linear protofilaments[8]. These associate laterally to form a tube of approximately 25 nm in diameter. However, during assembly, the GTP is hydrolyzed into GDP, resulting in a more curved conformation of the tubulin. Because bonds between subunits have formed in the GTP state, constraints imposed during assembly maintain the interactions between the GDP dimer, but the tube is under tension[78]. The structure of the tube may be maintained as long as some GTP–tubulin subunits are being added at the microtubule tip[9]. If tube closure is faster than GTP–tubulin addition at the tip, the tension built into the tube leads to explosive disassembly ('dynamic instability')[3].

Mathematical description

Because of dynamic instability, a microtubule population can, in principle, grow infinitely (ignoring tubulin depletion) or reach a finite size distribution. This can be predicted mathematically[12], as the fraction of microtubules in the shrinking and growing states can be computed from the frequencies of catastrophe and rescue (f_{cat} and f_{res}). From this, the length variation averaged over the population can be derived, using the growth and shrinking rates (V_g and V_s). The sign of the resulting flux $J = (V_g f_{res} - V_s f_{cat})/(f_{res} + f_{cat})$, determines whether the population is either growing infinitely ($J > 0$) or reaching a steady state ($J < 0$). When J is negative, the length distribution is a decreasing exponential, with an average steady state length $L = V_g V_s/(V_s f_{cat} - V_g f_{res})$. These equations can be derived without knowing the molecular mechanism that determines microtubule dynamic instability. When details about individual microtubule are needed, stochastic models or simulations should be used.

necessary to build a model of dynamic instability that can be used to understand more complex processes involving microtubule self-organization in larger scale structures.

FROM MOLECULAR MOTORS TO ORDERED MOVEMENT

The microtubule cytoskeleton provides mechanical stability and tracks for molecular transport by motor proteins. Molecular motors (see Box 2) use the structural polarity of microtubules for unidirectional movement. Two structurally different families of motor proteins exist in cells that move along microtubules: kinesins[14] and dyneins[15]. Most kinesins move towards the microtubule plus end, whereas some kinesins and the dyneins move towards the minus end. The size of motors and tubulin are similar. However, the movement of motors along a microtubule can cover distances of a hundred times their own size. In addition to the polymerization of tubulin, the movement of motors is the second important element of cytoskeletal dynamics that translates structural and dynamic properties from the nanometre range into the micrometer range.

Theoretical models have addressed motility at different levels — most models aim to understand the stepping mechanism of motors. What do we need to know to understand motor motility at this level? Ideally, a combination of structural information at atomic resolution and kinetic information at substep temporal resolution should be incorporated into a single model. However, not all atomic structures for the states that a motor encounters during its mechanochemical cycle are known, nor are the kinetics of all substeps[16–18]. The wealth of available experimental information has, however, already reduced the number of possibilities of how molecular motors function. Essentially, two classes of kinetic cycle models have survived on the basis of which essential features of motor motility can be understood, even without a complete knowledge of all structural details of a motor (Fig. 2). Whether powerstroke models are more appropriate than ratchet models is still a matter of debate. Both types of models derive the overall kinetics of the mechano-chemical stepping cycle from assumptions about the properties of the transitions between the individual substates[19–22]. Given the differences between various motors, it is likely that, in the future, one type of model will be more appropriate for some motors, whereas the other model will better describe the stepping of others. Important outputs of both types of models are the relationships between the velocity of a motor and the available ATP concentration, between the velocity and the applied force, or between the processivity and the applied force.

How much detail about the stepping mechanism is needed to model the collective behaviour of motors at higher levels (for example, the transport of microtubules in so-called gliding assays[23])? It has been demonstrated that the details of the individual substeps of the motors do not need to be considered to capture some of the essential features of collective motor transport[24–26]. Duty ratio models, for example, simply consider the fraction of time a motor is bound to the microtubule during its biochemical cycle, yet this is sufficient for the calculation of the velocity of microtubule transport by several motors[27,28].

In fact, most of the proposed models that describe collective transport phenomena involving single microtubules, neglect all details of the biochemical cycle and assume processive motility. Typical elements of these models are a regular lattice of binding sites, a binding rate, a dissociation rate and a velocity for the motor (Fig. 3). Usually, potential cooperativity of motor attachment[29] is not considered. Such assumptions are suitable to model the occurrence of traffic jams of motors along their

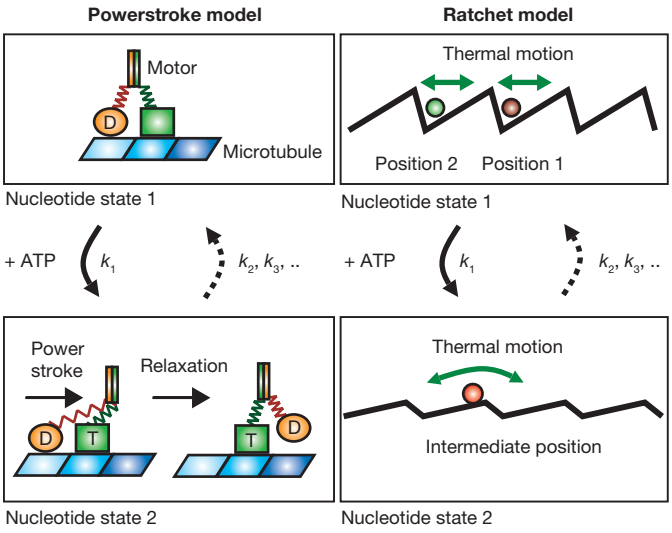

Figure 2 Models for the motor stepping mechanism. Two different classes of cycle models describe the stepping mechanism of molecular motors. Most models assume a powerstroke that is associated with one of the transitions[89,79,20]. The powerstroke is seen as a visco-elastic relaxation process of a strained molecule when the nucleotide state is changed. During this power stroke, a mechanical element of the motor is moved into a certain direction, determining the directionality of the motor. Only a few models explicitly include geometrical features, by associating different molecular geometries with different nucleotide states[90]. In these models, the attribution of certain transitions to ATP binding, or the assumption that one or several of the transitions are force-dependent, explains how the steady-state velocity of a motor depends on the ATP concentration, or on the applied force. A very different approach is used in ratchet models[91,92]. Molecular fluctuations due to thermal motion are not overcome by the motion of the powerstroke, but instead are a central element of the stepping mechanism. The interaction between the motor and the microtubule is described as a periodic potential representing the binding sites for the motor, in which the effect of external forces can be included. Specific interaction potentials are attributed to each nucleotide state of the motor. They alternate as ATP is consumed and this results in directed movement, if at least one of the potentials is asymmetric. D, ADP; T, ATP.

tracks if exclusion of a motor from an already occupied binding site is taken into account[30,31] — either the motors are treated as individual particles and their movement is simulated in Monte-Carlo type simulations (sometimes treating unbound motors simply as a homogenous reservoir), or, in less refined approaches, the behaviour of a population of motors is modelled at steady state using conservation equations (differential equations computing local concentrations depending on binding and/or unbinding rates and fluxes, with the constraint that the total number of motors is constant). To model the transport of cargo by multiple motors[32], or the extraction of membrane tubes from large vesicles by multiple motors[33], the effects of mechanical force on some of the rates need to be included in the model (for example, the velocity of the motor and its dissociation rate depend on the force; Fig. 3). These examples illustrate that, at this level of collective motor motility, the motors are modelled by simplified relationships that are relatively easy to handle. This is also true for modelling higher levels of organization.

FROM COLLECTIVE TUBE DYNAMICS TO PATTERNS

Several *in vitro* and *in vivo* experiments, and an increasing number of theoretical and simulation studies have addressed the organization of microtubules in space (or even in outer space![34]).

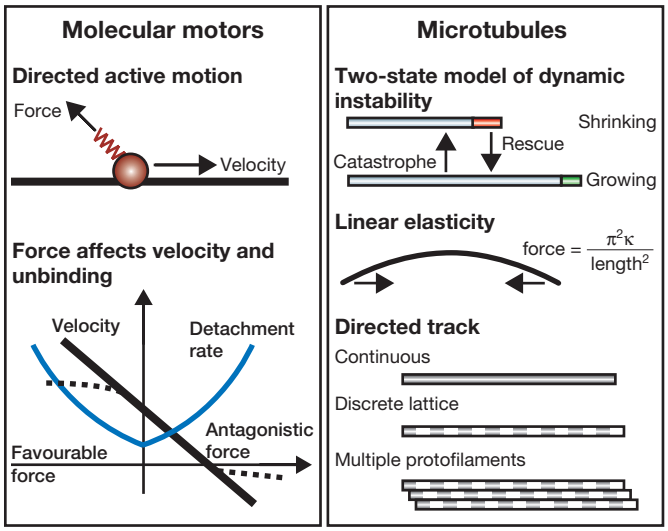

Figure 3 Standard model elements. Standard assumptions are used to describe microtubules and associated motors when the focus of the study is on the collective aspect of many such objects. Motors attach at a single position on the microtubule (the stepping is ignored) and move smoothly. They are linked to their load through springs, often of zero-length at rest, so that force f is linearly related to extension δ: $f = k\delta$, where k is the stiffness of the spring. The motion of kinesin is very well characterized[85] and its speed v varies linearly for a small (plain line) force f as $v = v_m(1-f/f_m)$, where v_m is the unloaded speed and f_m the stall force, and saturates for higher forces (dotted line). The dissociation rate k_{off} varies as $k_{off} = k_0 \exp(|f|/f_0)$, where the force f_0 is commensurate to the stall force f_m. Most authors use these relationships for all motors, adapting the parameters to model dynein, Ncd or Eg5, for example. Microtubule dynamic instability is described with a simple two-state model with stochastic rescues and catastrophes. Shrinkage occurs at constant speed and growing can be proportional to the amount of free tubulin, or depend on the force antagonistic to the polymerization. In the simplest models, the transition rates are constant, but this may be an oversimplification (for example, transition may depend on length[93], or microtubules may pause). Microtubules are usually thin lines rather than tubes. They mechanically behave as homogeneous elastic beams with known elasticity (κ). If the shape of the microtubule is not explicitly included, the buckling force can still be considered as an upper limit of the pushing force. The discrete lattice of tubulin is often ignored — motors may bind everywhere. They neither compete for binding sites, nor collide with obstacles or other motors. In special cases, where many motors bind to a microtubule, the lattice of binding sites may be included in the model. Note that individual studies usually only consider some of the elements listed, following an investigator's intuition. The simplifications that are made are often not rigorously justified.

Patterning phenomena involving many microtubules can be generated *in vitro* with pure tubulin[35]. Patterning is faster in the presence of multiheaded motors that can crosslink, move and orient microtubules. It was observed that microtubule asters could form in cellular extracts[36], and *in vitro* experiments have shown that purified motors and microtubuless are indeed capable of organizing microtubules into large-scale patterns[37–39]. Mixtures of fibres and motors are now described as 'active gels', because they are crosslinked networks of polymers (gels) in which the crosslinks are motors (active). Stochastic simulations have been used to study the pattern formation and they emphasized the non-intuitive consequences of some kinetic parameters. The standard assumptions on which such simulations are built are illustrated in Figure 3. Despite all the simplifications, the approximately 15 parameters and slow execution precluded a thorough exploration of the possible regimes at the time.

Variables

Position and time

$\vec{x}$ t

Concentrations of molecular motors

Unbound motors $u(\vec{x}, t)$

Bound motors $b(\vec{x}, t)$

Density of fibres

$m(\vec{x}, t)$

Orientation of fibres

$\vec{T}(\vec{x}, t)$

Equations

Binding/unbinding kinetics

$c_{u\to b} = k_{on}\, um$

$c_{b\to u} = k_{off}\, b$

Diffusion of free motors

$\vec{J}_u = -D\vec{\nabla}u$

Transport of bound motors

$\vec{J}_b = vb\vec{T}$

Conservation

$$\frac{\partial u}{\partial t} = -\vec{\nabla}\cdot\vec{J}_u - c_{u\to b} + c_{b\to u}$$

$$\frac{\partial b}{\partial t} = -\vec{\nabla}\cdot\vec{J}_b - c_{b\to u} + c_{u\to b}$$

Alignment of fibres by motor activity

$$\frac{\partial \vec{T}}{\partial t} = \gamma b\vec{\nabla}^2\vec{T} + \ldots$$

Figure 4 Mean-field methods. The mean-field approach is based on the same concepts that are used to model the dynamics of chemical reactions. In kinetic theory, the variables considered are the concentrations of the different species as a function of time. For microtubule-associated motors, two concentrations are usually sufficient: the concentration of bound and unbound motors. The positions or orientations of individual particles are ignored, but concentration may still be a function of position, if the system is inhomogeneous in space (it is then a field of mean values, or a 'mean field'). To represent fibres, a concentration and an orientation field are needed. The latter is a vector field representing the local direction in which microtubules are aligned. If the fibres are not homogenous in length, another field may be introduced to represent their mean length. The power of the approach comes from the fact that these variables can be linked by equations that predict either the steady state of the system, or its dynamics. Motor binding and dissociation kinetics are modelled from the law of mass action, with parameters k_{on} and k_{off} characterizing the speed of each reaction. The flux due to diffusion of free motors is modelled using Fick's law, with diffusion coefficient D. The motion of attached motors creates a flux proportional to the transport speed v. The conservation equations link temporal variations with the divergence of the fluxes and binding and/or unbinding kinetics. Oligomeric motors affect fibre orientation, but there is no agreement on how this should be described mathematically. As an example[43], the orientation field is linked to the local concentration of bound motors, so that a high concentration of motors promotes a local alignment of the fibre, using an effective parameter γ.

Less refined theories were thus developed in which the variables are average quantities, such as local filament orientations and local concentrations[40–45]. As illustrated in Fig. 4, these theories are called 'mean field' in physics, because they share a formalism in which differential equations are written that govern the averaged quantities. These equations can then be solved (analytically or numerically) to obtain an overview of the possibilities of the system (or in a physicist's words, a phase diagram).

When choosing a particular theoretical approach[46], it is important to consider the scale of the problem. The strongest limitation of mean-field theories is that if there are few fibres, fluctuations may have an important role and average quantities may not faithfully represent the system. This is often the case for microtubules in simple eukaryotes — for example, the yeast *Schizzosaccaromyces pombe* only has approximately 20 microtubules in interphase, and methods in which the stochastic transitions are explicitly considered (for example, simulations) would be more appropriate than mean-field methods. The future probably lies in composite approaches: for example, tubulin, or other molecules found in large quantities, can be treated as a concentration field. Similarly, in

BOX 2 THE MECHANO-CHEMICAL CYCLE OF MOLECULAR MOTORS

A molecular motor is a mechano-chemical enzyme transforming the chemical energy of ATP hydrolysis into mechanical work[79]. During its biochemical cycle, a motor passes through a series of different nucleotide states, each having a different conformation and microtubule affinity. Conformational changes cause mechanical elements in the motor molecule to move their position and to change their flexibility. Therefore, while going through the biochemical cycle, a motor also goes through a mechanical cycle that can produce mechanical force. Processive motors are able to perform consecutive steps after binding to a microtuble, whereas non-processive motors detach from the microtubule after one step.

Several experimental approaches have provided information on the stepping mechanism of motors. Spectroscopic methods have been used in the past to measure the kinetic behaviour of ensembles of motors and classic steady-state enzyme parameters, such as k_{cat} and K_M values, were first measured. 'Synchronisation' of the activity of the motors in the ensemble, by stopped flow or uncaging methods, yielded information on the transition rates between the individual states of a motor's biochemical cycle[80,81]. Light microscopy has facilitated connecting biochemical rates with mechanical properties — in particular, single molecule imaging and optical interferometry have provided valuable information about the stepping mechanism[82–85]. Without the need for ensemble synchronisation, single molecule experiments provide detailed information about the kinetics of individual motors steps, as well as about the produced forces[86]. Distributions, in addition to ensemble averages, can also be measured. Single molecule measurements have thus provided strong constraints for models of the stepping mechanism — for example, the long-standing debate about whether conventional kinesin walks by an inchworm or a hand-over-hand mechanism could be solved in favour of the latter mechanism[19,87,88].

large cells, the actin network could be represented with mean fields, while microtubules are modelled as stochastic entities. Such hybrid models should be computationally efficient; however, their mean-field description will need to include filament turnover (disassembly and nucleation). Moreover, filament branching will need to be considered to model actin organization in motile cells and dynamic instability will need to be considered to model the mitotic spindle.

The mitotic spindle is a very striking manifestation of microtubule organization and it is, not surprisingly, the focus of many modelling approaches[47] — to date, mainly using stochastic methods. Currently, it is not possible to model the full spindle structure through the different mitotic stages, from prophase to metaphase and anaphase. Models have addressed one cell-cycle stage at a time, and only aspects of the structure that can be isolated: chromosome capture in prophase[48,49]; kinetochore positioning[50], kinetochore–microtubule interactions[51–53] , anti-parallel overlaps[54,55] and pole formation[56–58] in metaphase; and spindle elongation[59] and chromosome transport[60] in anaphase. The goal is to combine such studies in the future, aiming to fully reconstitute spindle function. For example, in a simulation one could combine several alternative assembly pathways to study the functional redundancy of the mitotic spindle. In this context, a major challenge is to reproduce, in a model, the surprising ability of mitotic spindles to recover from perturbations or obstructions.

Modelling approaches have also been used to study the interphase organization of microtubules in several cell types and cell states (for example, in plant cells[61,62], in fish keratocytes[63–65], or ciliated cells[66]). In the early embryo of the nematode worm *Caenorhabditis elegans*, pronuclear migration[67] and anaphase transverse oscillations[68] were modelled with stochastic methods. The oscillations were described as a tug-of-war between processive motors, similar to gliding assays with two motors of opposite polarity[69,70]. Another positioning mechanism can be obtained from microtubules pushing on the boundary of a cell, as demonstrated *in vitro* with purified components[71], and theoretically[72]. This mechanism is thought to be sufficient to position the nucleus in interphase in fission yeast[73]. From these studies, a major goal is to categorize and analyse all the mechanisms by which positioning could be achieved in cells[74].

POTENTIAL PITFALLS AND FUTURE DIRECTIONS

Cell organization arises from networks of kinetic interactions between molecules and theoretical approaches are essential to understand such phenomena. Mathematics will be required to determine whether a model is coherent, given the wealth of quantitative information produced by modern biology. Citing many studies of the microtubule system, we have described a classic pattern of scientific investigation: experiments provide information and quantitative measurements, and theories are used to understand how new properties 'emerge' from the interacting agents. A certain level of experimental knowledge is used, both to check the output of models addressing the lower scale, and to generate others at a larger scale. Conversely, once a problem of interest has been defined, finding the appropriate level of assumptions on which to build a model usually comes naturally. Individual models work only in a narrow range of levels of complexity, and inevitably make (a lot of) approximations. The resulting inaccuracies would accumulate if models were carelessly linked, thus, making the whole construction very unreliable. To avoid this, every model has to be based on sound biological knowledge, powered by accurate physics and, most importantly, should provide predictions that are experimentally validated. Only then can we hope to link the different organizational levels outlined in Fig. 1, and thereby understand cellular organization comprehensively. As we have shown, this goal is being pursued with some success in the case of the microtubule system.

The power of theory traditionally lies in simplicity: a model is successful if it can reproduce a lot of facts using few simple assumptions. Models with many free parameters are often suspicious, because their values may have been chosen to obtain the desired output. Reducing the number of parameters is not easy in biology, because most cellular processes involve diverse components and many parameters are necessary to characterize their interactions, all of which have their own specific rates. This pitfall can be avoided if the parameters can be directly constrained by measurement, or if their values have been fitted with confidence from previous independent studies. When this information is available, only a few free parameters will remain, and it will be possible to methodically evaluate their effect on the systems

and to identify the most important ones. This may provide fitted values that could be used for the next model, but foremost, it will indicate what should be measured next experimentally. Most importantly, models can predict the parameter space within which a given behaviour will be stable[38]. This will certainly be of utmost importance to understand how evolutionary processes have been constrained within a world of 'possibles'.

A prerequisite for a successful model is, therefore, a minimum knowledge of the molecular composition of the system under study and of the relevant biophysical and kinetic parameters — and herein lies the advantage of *in vitro* approaches. However, practical issues still limit the number of purified elements that can be mixed in a reconstituted self-organizing system. The key to understanding the *in vivo* situation will be to have an almost complete picture of the relevant players in the cellular context. Systematic inhibition studies promise to provide knowledge about the complete set of *in vivo* players[75,76], while new fluorescence microscopy-based methods will have to be used to measure their *in vivo* interactions, their affinities and kinetic properties[77]. This knowledge can then be used as the basis for more complete theoretical models to explain how complex cytoskeletal patterns emerge *in vivo* from the collective behaviour of many components, and their regulators. The present period is very exciting, as we still do not know the approximations that can be made, or which are the best theoretical methods, when it comes to modelling the microtubule cytoskeleton. Many methods are currently in use, but we should expect that a consensus will emerge in the not too distant future. It may take the form of standardized methods valid ated on a set of quantitatively precise 'classical' problems. Although day-to-day stimulation of experimental work is sufficient to justify spending some of our resources on theory, the field is heading towards a more ambitious goal: predicting the cytoskeletal response to new situation will be the first real test of the understanding gleaned from experiments. □

Table 1 Glossary

Active gels	Such gels were constructed using oligomeric motors that can bind two or more cytoskeletal filaments, which use ATP as the energy source to walk along the fibres.
Conservation equation	Equation expressing the conservation of a certain quantity, for example, the number of molecules in a closed system.
Catastrophe	Transition from a growing to a shrinking microtubule; the opposite of rescue.
Centrosome	Organelle found at the spindle poles that have the ability to nucleate microtubules.
Cytoskeleton	Fibres and associated molecules that establish the internal organization of the cell and most of its mechanical properties.
Duty ratio model	Model of molecular motors where the fraction of time spent bound to and unbound from the microtubule is used to derive the efficiency of transport by many motors.
Dynamic instability	Succession of growing and shrinking phases of an individual microtubule that are separated by stochastic transitions (catastrophes and rescues). On the level of a population, this describes the coexistence of growing and shrinking microtubules under identical conditions.
Emergent properties	Properties that arise from the interactions of many components and that were not apparent (that is, hard to predict) when considering the properties of component.
Fick's law	Linear relationship, derived by Adolf Fick, between the flux produced by diffusing molecules and the gradient of concentration of these molecules (Fig. 4).
Gliding assay	Microscopic experiment in which motors immobilized on a coverslip propel microtubules on the glass surface. *In vivo,* the opposite usually occurs: motors move along relatively immobile microtubules.
Graining	The amount of details considered in a model. A 'fine-grained' model has many details; a 'coarse-grained' model has few.
Hooke's law	Linear relationship, derived by Robert Hooke, between the magnitude of the force applied to a spring and the resulting stretch (Fig. 3).
Kinetochore	Protein complex formed on the centromeric DNA that connects the chromosome to the microtubules of the spindle. The kinetochore is an essential mechanical link during chromosome segregation.
Mean field	Quantity representing an averaged property of the system that depends on position, for example, a concentration field.
Molecular motor	A protein complex whose function is to generate motion (and force) using an energy source. In the cytoskeleton, molecular motors use two heads to walk along fibres (rotary motors also exist in other cellular modules). The stepping is not perfect and the motor may stochastically miss a step and unbind from the fibre completely. The processivity is the average number of successive steps a motor makes before unbinding.
Monte-Carlo simulation	Simulation in which the intrinsically random behaviour of the system's elements (for example, the time of motor stepping, binding and unbinding) are modelled using random numbers generated by the computer.
Phase diagram	An outline of the regimes in which a system can be found, as a function of its parameters (for example, the information that a motors movement along a microtubule is fluid or jammed, as a function of the motor concentration).
Power stroke	A conformational change of a molecular motor that translates chemical energy into directional movement. The power stroke of the attached head swivels the unbound head forward.
Spindle	Structure made of chromosomes, microtubules and numerous associated proteins, which segregates the duplicated chromosomes. To physically separate the chromosomes in two equal groups, the spindle adopts a bipolar configuration. In metazoans, the chromosomes are at the centre of the structure and microtubules minus-ends are focused in two poles, in which the centrosomes are found.
Stall force	Amount of antagonistic force needed to stop a motor.
Stochastic	Synonymous with 'random' and opposite to 'deterministic'. A stochastic event may not be predicted, but many events of the same nature can be statistically characterised by a probability distribution.

COMPETING FINANCIAL INTERESTS
The authors declare that they have no competing financial interests.

1. Kauffman, S. *At home in the universe.* (Oxford University Press, New York, 1995).
2. Desai, A. & Mitchison, T. J. *Microtubule polymerization dynamics. Annu. Rev. Cell Dev. Biol.* **13,** 83–117 (1997).
3. Kirschner, M. & Mitchison, T. Beyond self-assembly: from microtubules to morphogenesis. *Cell* **45,** 329–342 (1986).
4. Janson, M. E. & Dogterom, M. Scaling of microtubule force-velocity curves obtained at different tubulin concentrations. *Phys. Rev. Lett.* **92,** 248101 (2004).
5. Janson, M. E., de Dood, M. E. & Dogterom, M. Dynamic instability of microtubules is regulated by force. *J. Cell Biol.* **161,** 1029–1034 (2003).
6. Molodtsov, M. I. *et al.* A molecular-mechanical model of the microtubule. *Biophys. J.* **88,** 3167–3179 (2005).
7. VanBuren, V., Cassimeris, L. & Odde, D. J. Mechanochemical model of microtubule structure and self-assembly kinetics. *Biophys. J.* **89,** 2911–2926 (2005).
8. Wang, H. W. & Nogales, E. Nucleotide-dependent bending flexibility of tubulin regulates microtubule assembly. *Nature* **435,** 911–915 (2005).
9. Nogales, E. & Wang, H. W. Structural intermediates in microtubule assembly and disassembly: how and why? *Curr. Opin. Cell Biol.* **18,** 179–184 (2006).
10. Hill, T. L. *Linear aggregation theory in cell biology.* xiv, p305 (Springer-Verlag, New York, 1987)
11. Verde, F. *et al.* Control of microtubule dynamics and length by cyclin A- and cyclin B-dependent kinases in *Xenopus* egg extracts. *J. Cell Biol.* **118,** 1097–1108 (1992).
12. Dogterom, M. & Leibler, S. Physical aspects of the growth and regulation of microtubule structures. *Phys. Rev. Lett.* **70,** 1347–1350 (1993).
13. Janulevicius, A., van Pelt, J. & van Ooyen, A. Compartment volume influences microtubule dynamic instability: a model study. *Biophys. J.* **90,** 788–798 (2006).
14. Miki, H., Okada, Y. & Hirokawa, N. Analysis of the kinesin superfamily: insights into structure and function. *Trends Cell Biol.* **15,** 467–476 (2005).
15. Sakato, M. & King, S. M. Design and regulation of the AAA+ microtubule motor dynein. *J. Struct. Biol.* **146,** 58–71 (2004).
16. Marx, A., Muller, J. & Mandelkow, E. The structure of microtubule motor proteins. *Adv. Protein Chem.* **71,** 299–344 (2005).
17. Mandelkow, E. & Hoenger, A. Structures of kinesin and kinesin-microtubule interactions. *Curr. Opin. Cell Biol.* **11,** 34–44 (1999).
18. Burgess, S. A. & Knight, P. J. Is the dynein motor a winch? *Curr. Opin. Struct. Biol.* **14,** 138–146 (2004).
19. Schief, W. R. *et al.* Inhibition of kinesin motility by ADP and phosphate supports a hand-over-hand mechanism. *Proc. Natl Acad. Sci. USA* **101,** 1183–1188 (2004).
20. Thomas, N. *et al.* Kinesin: a molecular motor with a spring in its step. *Proc. Biol. Sci.* **269,** 2363–2371 (2002).
21. Cross, R. A. The kinetic mechanism of kinesin. *Trends Biochem. Sci.* **29,** 301–309 (2004).
22. Fisher, M. E. & Kolomeisky, A. B. Simple mechanochemistry describes the dynamics of kinesin molecules. *Proc. Natl Acad. Sci. USA* **98,** 7748–7753 (2001).
23. Chowdhury, D., Schadschneider, A. & Nishinari, K. Physics of transport and traffic phenomena in biology: from molecular motors and cells to organisms. *Phys. Life Rev.* **2,** 318–352 (2005).
24. Bourdieu, L. *et al.* Spiral defects in motility assays: A measure of motor protein force. Phys. Rev. Lett. **75,** 176–179 (1995).
25. Vilfan, A., Frey, E. & Schwabl, F. Elastically coupled molecular motors. *European Physical Journal B* **3,** 535–546 (1998).
26. Gibbons, F. *et al.* A dynamical model of kinesin-microtubule motility assays. *Biophys. J.* **80,** 2515–2526 (2001).
27. Howard, J. Molecular motors: structural adaptations to cellular functions. *Nature* **389,** 561–567 1997.
28. Leibler, S. & Huse, D. A. Porters versus rowers: a unified stochastic model of motor proteins. *J. Cell Biol.* **121,** 1357–1368 (1993).
29. Vilfan, A. *et al.* Dynamics and cooperativity of microtubule decoration by the motor protein kinesin. *J. Mol. Biol.* **312,** 1011–1026 (2001).
30. Parmeggiani, A., Franosch, T. & Frey, E. Phase coexistence in driven one-dimensional transport. *Phys. Rev. Lett.* **90,** 086601 (2003).
31. Klumpp, S., Nieuwenhuizen, T. M. & Lipowsky, R. Self-organized density patterns of molecular motors in arrays of cytoskeletal filaments. *Biophys. J.* **88,** 3118–3132 (2005).
32. Klumpp, S. & Lipowsky, R. Cooperative cargo transport by several molecular motors. *Proc. Natl Acad. Sci. USA* **102,** 17284–17289 (2005).
33. Leduc, C. *et al.* Cooperative extraction of membrane nanotubes by molecular motors. *Proc. Natl Acad. Sci. USA* **101,** 17096–17101 (2004).
34. Papaseit, C., Pochon, N. & Tabony, J. Microtubule self-organization is gravity-dependent. *Proc. Natl Acad. Sci. USA* **97,** 8364–8368 (2000).
35. Glade, N., Demongeot, J. & Tabony, J. Microtubule self-organisation by reaction-diffusion processes causes collective transport and organisation of cellular particles. *BMC Cell Biol.* **5,** 23 (2004).
36. Verde, F. *et al.* Taxol induced microtubule asters in mitotic extracts of *Xenopus* eggs: requirement for phosphorylated factors and cytoplasmic dynein. *J. Cell Biol.* **112,** 1177–1187 (1991).
37. Urrutia, R. *et al.* Purified kinesin promotes vesicle motility and induces active sliding between microtubules *in vitro*. *Proc. Natl Acad. Sci. USA* **88,** 6701–6705 (1991).
38. Surrey, T. *et al.* Physical properties determining self-organization of motors and microtubules. *Science* **292,** 1167–1171 (2001).
39. Nedelec, F. J. *et al.* Self-organization of microtubules and motors. *Nature* **389,** 305–308 (1997).
40. Bassetti, B. L., Cosetino, M. & Jona, P. A Model for the self-organzation of microtubule driven by molecular motors. *Eur. Phys. J. E.* **15,** 483–492 (1999).
41. Lee, H. Y. & Kardar, M. Macroscopic equations for pattern formation in mixtures of microtubules and molecular motors. *Phys. Rev. E.* **64,** 056113 (2001).
42. Kruse, K. *et al.* Asters, vortices, and rotating spirals in active gels of polar filaments. *Phys. Rev. Lett.* **92,** 078101 (2004).
43. Sankararaman, S., Menon, G. I. & Kumar, P. B. Self-organized pattern formation in motor-microtubule mixtures. *Phys. Rev. E.* **70,** 031905 (2004).
44. Aranson, I. S. & Tsimring, L. S. Pattern formation of microtubules and motors: inelastic interaction of polar rods. Phys. Rev. E. **71,** 050901 (2005).
45. Ahmadi, A., Liverpool, T. B. & Marchetti, M. C. Nematic and polar order in active filament solutions. *Phys. Rev. E.* **72,** 060901 (2005).
46. Mogilner, A., Wollman, R. & Marshall, W. F. Quantitative modeling in cell biology: what is it good for? *Dev. Cell* **11,** 279–287 (2006).
47. Mogilner, A. *et al.* Modeling mitosis. *Trends Cell Biol.* 16, 88–96 (2006).
48. Holy, T. E. & Leibler, S. Dynamic instability of microtubules as an efficient way to search in space. *Proc. Natl Acad. Sci. USA* **91,** 5682–5685 (1994).
49. Wollman, R. *et al.* Efficient chromosome capture requires a bias in the 'search-and-capture' process during mitotic-spindle assembly. *Curr Biol.* **15,** 828–832 (2005).
50. Sprague, B. L. *et al.* Mechanisms of microtubule-based kinetochore positioning in the yeast metaphase spindle. *Biophys. J.* **84,** 3529–3546 (2003).
51. Khodjakov, A., Gabashvili, I. S. and Rieder, C. L. "Dumb" versus "smart" kinetochore models for chromosome congression during mitosis in vertebrate somatic cells. *Cell Motil. Cytoskeleton* **43,** 179–185 (1999).
52. Joglekar, A. P. & Hunt, A. J. A simple, mechanistic model for directional instability during mitotic chromosome movements. *Biophys. J.* **83,** 42–58 (2002).
53. Civelekoglu-Scholey, G. *et al.* Model of chromosome motility in *Drosophila* embryos: adaptation of a general mechanism for rapid mitosis. *Biophys. J.* **90,** 3966–3982 (2006).
54. Nedelec, F. Computer simulations reveal motor properties generating stable antiparallel microtubule interactions. *J. Cell Biol.* **158,** 1005–1015 (2002).
55. Cytrynbaum, E. N., Scholey, J. M. & Mogilner, A. A force balance model of early spindle pole separation in *Drosophila* embryos. *Biophys. J.* **84,** 757–769 (2003).
56. Chakravarty, A., Howard, L. & Compton, D. A. A mechanistic model for the organization of microtubule asters by motor and non-motor proteins in a mammalian mitotic extract. *Mol. Biol. Cell* **15,** 2116–2132 (2004).
57. Goshima, G., Nedelec, F. & Vale, R. D. Mechanisms for focusing mitotic spindle poles by minus end-directed motor proteins. *J. Cell Biol.* **171,** 229–240 (2005).
58. Schaffner, S. C. & Jose, J. V. Biophysical model of self-organized spindle formation patterns without centrosomes and kinetochores. *Proc. Natl Acad. Sci USA* **103,** 11166–11171 (2006).
59. Brust-Mascher, I. *et al.* Model for anaphase B: role of three mitotic motors in a switch from poleward flux to spindle elongation. *Proc. Natl Acad. Sci. USA* **101,** 15938–15943 (2004).
60. Raj, A. & Peskin, C. S. The influence of chromosome flexibility on chromosome transport during anaphase A. *Proc. Natl Acad. Sci. USA* **103,** 5349–5354 (2006).
61. Dixit, R. & Cyr, R. Encounters between dynamic cortical microtubules promote ordering of the cortical array through angle-dependent modifications of microtubule behavior. *Plant Cell* **16,** 3274–3284 (2004).
62. Zumdieck, A. *et al.* Continuum description of the cytoskeleton: ring formation in the cell cortex. *Phys. Rev. Lett.* **95,** 258103 (2005).
63. Maly, I. V. & Borisy, G. G. Self-organization of treadmilling microtubules into a polar array. *Trends Cell Biol.* **12,** 462–465 (2002).
64. Cytrynbaum, E. N., Rodionov, V. & Mogilner, A. Computational model of dynein-dependent self-organization of microtubule asters. *J. Cell Sci.* **117,** 1381–1397 (2004).
65. Malikov, V. *et al.* Centering of a radial microtubule array by translocation along microtubules spontaneously nucleated in the cytoplasm. *Nature Cell Biol.* **7,** 1213–1218 (2005).
66. Kruse, K., Camalet, S. & Julicher, F. Self-propagating patterns in active filament bundles. *Phys. Rev. Lett.* **87,** 138101 (2001).
67. Kimura, A. & Onami, S. Computer simulations and image processing reveal length-dependent pulling force as the primary mechanism for *C. elegans* male pronuclear migration. *Dev. Cell* **8,** 765–775 (2005).
68. Grill, S. W., Kruse, K. and Julicher, F. Theory of mitotic spindle oscillations. *Phys. Rev. Lett.* **94,** 108104 (2005).
69. Vale, R. D., Malik, F. & Brown, D. Directional instability of microtubule transport in the presence of kinesin and dynein, two opposite polarity motor proteins. *J. Cell Biol.* **119,** 1589–1596 (1992).
70. Badoual, M., Julicher, F. and Prost, J. Bidirectional cooperative motion of molecular motors. *Proc. Natl Acad. Sci. USA* **99,** 6696–6701 (2002).
71. Holy, T. E. *et al.* Assembly and positioning of microtubule asters in microfabricated chambers. *Proc. Natl Acad. Sci. USA* **94,** 6228–6231 (1997).
72. Yurke, M. D.a.B. Microtubule Dynamics and the Positioning of Microtubule Organizing Center. *Phys. Rev. Lett.* **81,** 485–488 (1998).
73. Tran, P. T. *et al.* A mechanism for nuclear positioning in fission yeast based on microtubule pushing. *J. Cell Biol.* **153,** 397–411 (2001).
74. Dogterom, M. *et al.* Force generation by dynamic microtubules. *Curr. Opin. Cell Biol.* **17,** 67–74 (2005).
75. Cottingham, F. R. *et al.* Novel roles for saccharomyces cerevisiae mitotic spindle motors. *J. Cell Biol.* **147,** 335–350 (1999).
76. Goshima, G. & Vale, R. D. The roles of microtubule-based motor proteins in mitosis: comprehensive RNAi analysis in the *Drosophila* S2 cell line. *J. Cell Biol.* **162,** 1003–1016 (2003).

77. Wallrabe, H. & Periasamy, A. Imaging protein molecules using FRET and FLIM microscopy. *Curr. Opin. Biotechnol.* **16,** 19–27 (2005).
78. Chretien, D., Fuller, S. D. & Karsenti, E. Structure of growing microtubule ends: two-dimensional sheets close into tubes at variable rates. *J. Cell. Biol.* **129,** 1311–1328 (1995).
79. Vale, R. D. & Milligan, R. A. The way things move: looking under the hood of molecular motor proteins. *Science* **288,** 88–95 2000.
80. Hackney, D. D. Highly processive microtubule-stimulated ATP hydrolysis by dimeric kinesin head domains. *Nature* **377,** 448–450 (1995).
81. Gilbert, S. P., Moyer, M. L. & Johnson, K. A. Alternating site mechanism of the kinesin ATPase. *Biochemistry* **37,** 792–799 (1998).
82. Howard, J., Hudspeth, A. J. & Vale, R. D. Movement of microtubules by single kinesin molecules. *Nature* **342,** 154–158 (1989).
83. Vale, R. D. *et al.* Direct observation of single kinesin molecules moving along microtubules. *Nature* **380,** 451–453 (1996).
84. Svoboda, K. *et al.* Direct observation of kinesin stepping by optical trapping interferometry. *Nature* **365,** 721–727 (1993).
85. Carter, N. J. & Cross, R. A. Mechanics of the kinesin step. *Nature* **435,** 308–312 (2005).
86. Svoboda, K. & Block, S. M Force and velocity measured for single kinesin molecules. *Cell* **77,** 773–784 (1994).
87. Asbury, C. L., Fehr, A. N. & Block, S. M. Kinesin moves by an asymmetric hand-over-hand mechanism. *Science* **302,** 2130–2134 (2003).
88. Yildiz, A. *et al.* Kinesin walks hand-over-hand. Science **303,** 676–678 (2004).
89. Peskin, C. S. & Oster, G. Coordinated hydrolysis explains the mechanical behavior of kinesin. *Biophys. J.* **68,** S202–S210 (1995).
90. Shao, Q. & Gao, Y. Q. On the hand-over-hand mechanism of kinesin. *Proc. Natl Acad. Sci. USA* **103,** 8072–8077 (2006).
91. Jülicher, F., Ajdari, A. & Prost, J. Modeling molecular motors. *Rev. Modern Phys.* **69,** 1269–1281 (1997).
92. Astumian, R. D. The role of thermal activation in motion and force generation by molecular motors. Philos. Trans. R. Soc. Lond. B. **355,** 511–522 (2000).
93. Dogterom, M. *et al.* Influence of M-phase chromatin on the anisotropy of microtubule asters. *J. Cell Biol.* **133,** 125–140 (1996).